



**Joana Rafael Matzen
Neves da Silva**

**BIODIVERSIDADE E EVOLUÇÃO MOLECULAR DA
CLASSE MALACOSTRACA**

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MALACOSTRACA**



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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica da Professora Doutora Maria Marina Ribeiro Pais da Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro, Doutor Filipe José Oliveira Costa, Professor Auxiliar da Universidade do Minho e do Professor Doutor Gary Robert Carvalho, Professor do Departamento de Biologia da Universidade de Bangor, País de Gales, Reino Unido.

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It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living.

David Attenborough

To my greatest source of love, my parents Gisela and Rafael; the greatest source of friendship, my sisters Püppi and Catarina; and the greatest source of strength, my grandmothers Ilse and Irene.

o júri

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palavras-chave

Malacostraca, Decapoda, Amfipoda, Isopoda, ADN código de barras, biodiversidade, filogenia, COI, 16S, 28S, *numts*

resumo

No actual cenário de perda acelerada de biodiversidade, o nosso conhecimento dos ecossistemas marinhos, apesar da sua extensão e complexidade, continua muito inferior ao dos ecossistemas terrestres. A classe Malacostraca (Arthropoda, Crustacea), um grupo dos mais representativos nos ecossistemas marinhos, apresenta um elevado nível de diversidade morfológica e ecológica, mas difícil sua identificação ao nível de espécie requer frequentemente a ajuda de especialistas em taxonomia. A utilização recente do “barcoding” (código de barras do ADN), revelou ser um método rápido e eficaz para a identificação de espécies em diversos grupos de metazoários, incluindo os Malacostraca. No âmbito desta tese foi construída uma base de dados de código de barras de ADN envolvendo 132 espécies de Malacostraca vários locais de amostragem no Atlântico Nordeste e Mediterrâneo. As sequências de ADN mitocondrial provenientes de 601 espécimes formaram, em 95% dos casos, grupos congruentes com as identificações baseadas em características morfológicas. No entanto, foi detectado polimorfismo em seis casos e a divergência intra-específica foi elevada em exemplares pertencentes a duas espécies morfológicas, sugerindo, neste caso, a ocorrência de especiação críptica. Este estudo confirma a utilidade do código de barras de ADN para a identificação de Malacostraca marinhos. Apesar do sucesso obtido, este método apresenta alguns problemas, como por exemplo a possível amplificação de pseudogenes. A ocorrência de pseudogenes e as possíveis abordagens para a detecção e resolução deste tipo de problemas são discutidas com base em casos de estudo: análises dos códigos de barras ADN na espécie *Goneplax rhomboides* (Crustacea, Decapoda). A análise dos códigos de barras ADN revelou ainda grupos prioritários de decápodes para estudos taxonómicos e sistemáticos, nomeadamente os decápodes dos géneros *Plesionika* e *Pagurus*. Neste âmbito são discutidas as relações filogenéticas entre espécies seleccionadas dos géneros *Plesionika* e *Pagurus*. Este trabalho aponta para várias questões no âmbito da biodiversidade e evolução molecular da classe Malacostraca que carecem de um maior esclarecimento, podendo ser considerado como a base para estudos futuros. Análises filogenéticas adicionais integrando dados morfológicos e moleculares de um maior número de espécies e de famílias deverão certamente conduzir a uma melhor avaliação da biodiversidade e da evolução dentro da classe.

Keywords

Malacostraca, Decapoda, Amphipoda, Isopoda, DNA barcode, biodiversity, phylogeny, COI, 16S, 28S, *numts*

Abstract

The biodiversity of many habitats is under threat and although seas cover the majority of our planet's surface, far less is known about the biodiversity of marine environments than that of terrestrial systems. The complexity of its species and ecosystems is immense.

Marine malacostraca are known as a group with a high level of morphological and ecological diversity but are difficult to identify by traditional approaches and usually require the help of highly trained taxonomists. A faster identification method, DNA barcoding, was found to be an effective tool for species identification in many metazoan groups including some malacostraca.

Moreover, the generation of a larger comparative database allows additional insights into the tempo and mode of molecular evolution. Indeed, examination of diversity at the COI region yields an informative framework to identify and explore priority issues, demanding in turn a fully integrative approach utilising additional molecular, distributional and ecological information. Here we expand the DNA barcode database with a case study involving more than 132 malacostracan species from the Northeast Atlantic Ocean and Mediterranean Sea. DNA sequences from around 601 specimens grouped into clusters corresponding to known morphological species in 95% of cases. However shared polymorphism between sister-species was detected in six species. Intraspecific divergence was high in specimens belonging to two morphological species, suggesting the occurrence of cryptic speciation, allowing a rapid assessment of taxon diversity in groups that have until now received limited morphological and systematic examination. We highlight taxonomic groups or species with unusual nucleotide composition or evolutionary rates. Such data are relevant to strategies for conservation of existing decapod biodiversity, as well as elucidating the mechanisms and constraints shaping the patterns observed. This study reconfirms the usefulness of DNA barcoding for the identification of marine malacostraca, despite complexities that sometimes arise due to pseudogenes (*numts*). Here, we study the effect of *numts* on DNA barcoding based on barcoding analyses in decapoda species: *Goneplax rhomboides*. DNA barcodes reveal priority groups for taxonomic and systematic focus of decapods. Here we discussed two cases of phylogenetic relationships among selected species of *Plesionika* and *Pagurus*, respectively.

Issues relating to the molecular biodiversity and evolution of the Malacostraca arising from this study allow identification of future priorities. Further phylogenetic analyses including morphological and molecular data of selected families is required, especially encompassing broad geographic and ecological coverage, will lead to an improved evaluation of the biodiversity and evolution among selected Malacostraca species.

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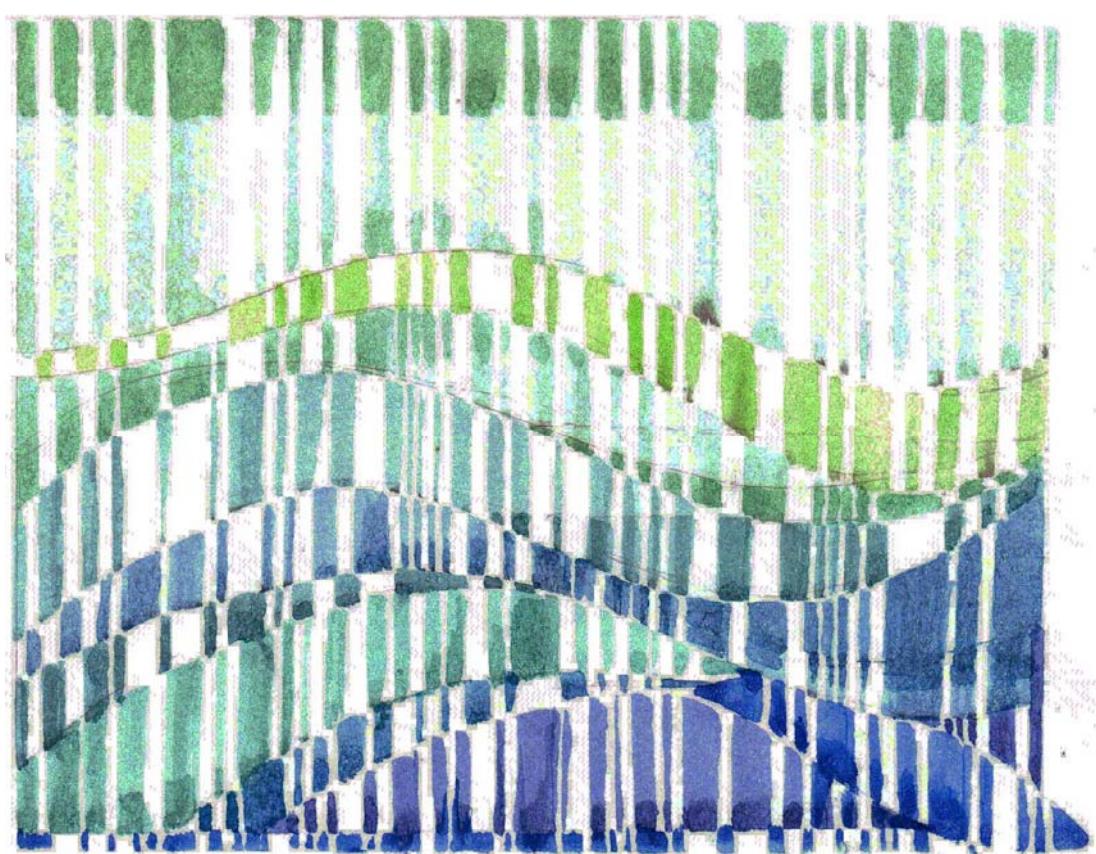
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Section 1. GENERAL INTRODUCTION

“(…) *The sea turned suddenly very young and
very old
Revealing beaches
And a people
Of just-created men still the colour of clay
Still naked still in awe”*

(*Discovery*, Sophia de Mello Breyner Anderson)

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1.1 Marine biodiversity and evolution

Life on Earth originated in the primordial ocean and for billions of years evolved in this aquatic environment (Snelgrove, 2011). The variety of life in many habitats is under threat and although the oceans cover the majority of our planet's surface, far less is known about the biodiversity of marine environments than that of terrestrial systems (Ormond *et al.*, 1999). Moreover we know that marine taxa have been evolving for up to 2.7 billion years longer than terrestrial counterparts (Carvalho *et al.*, 2011). The oceans are still far richer in major groups of animals than freshwater or terrestrial environments (Table 1.1): 33 of the 36 major phyla of multicellular animals occur in the sea, and 18 of them are marine endemics (Carvalho *et al.*, 2011; Roff and Zacharias, 2011). High species and phyletic diversity is commensurate with corresponding overabundance of life-styles from floaters and swimmers, to those notwithstanding partial aerial exposure in intertidal zones or inhabiting deep sea hydrothermal vents at > 3,500 m (Carvalho *et al.*, 2011). The size of marine organisms ranges a thousand billion fold, from drifting bacteria through blue whales and life time from hours (e.g., bacteria) to 4000 years (e.g., corals) and the complexity of ecosystems is immense (e.g., bay muds, cold seeps, coral reefs, seamounts, hydrothermal vents, estuaries, intertidal flats, etc) (Snelgrove, 2011). The origin of life on Earth is estimated to have occurred at about 3.5-4.0 billion years ago but metazoans did not begin markedly to diversify until approximately 600 Myr ago (Gaston and Spicer, 2003). Profound changes in the biodiversity and biocomplexity of marine life occurred during the early to mid-Ordovician through an interval of some

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25 Myr (Figure 1.1) (Harper, 2006). During this event, most marine higher taxa diversified at a faster rate than at any other time in the Phanerozoic. Biodiversity increased twice at the ordinal level, about three times at the family level, and nearly four times at the level of genus (Harper, 2006; Van Roy *et al.*, 2010). Although many taxa counts are available through 45 million years of the Ordovician Period, there are relatively few studies on the ecological and environmental aspects of this diversification (Bottjer *et al.*, 2001 *in* Harper 2006). Moreover the causes of the event, and its relationship to both intrinsic (biological) and extrinsic (environmental) factors, are far from clear (Harper, 2006). There is a pattern of overall increase in biodiversity through time but over the history of life on Earth, in excess of 90% of all species are estimated to have become extinct. Natural extinctions tend to be taxonomically clumped and the intensity of extinctions has also varied markedly over time with low levels during the majority of periods and some short periods with mass extinctions (Figure 1.1) (Gaston and Spicer, 2003). The big five mass extinctions are believed to have had rather different causes (Erwin, 2001 *in* Gaston and Spicer, 2003). Globally, the present rate of species extinction is comparable to the extinctions at the end of the Cretaceous period 65 million years ago when the long dominance of the dinosaurs came to an end (Brosing, 2008).

In 1992, the Convention on Biological Diversity (www.biodiv.org) defined biological diversity as the “*variability among living organisms from all sources including, inter alia, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems*”. From a practical viewpoint species are generally the units of biodiversity. Although, the increasing recognition by naturalists, genetics and evolutionists over the past 200 years that species occur as reproductively isolated

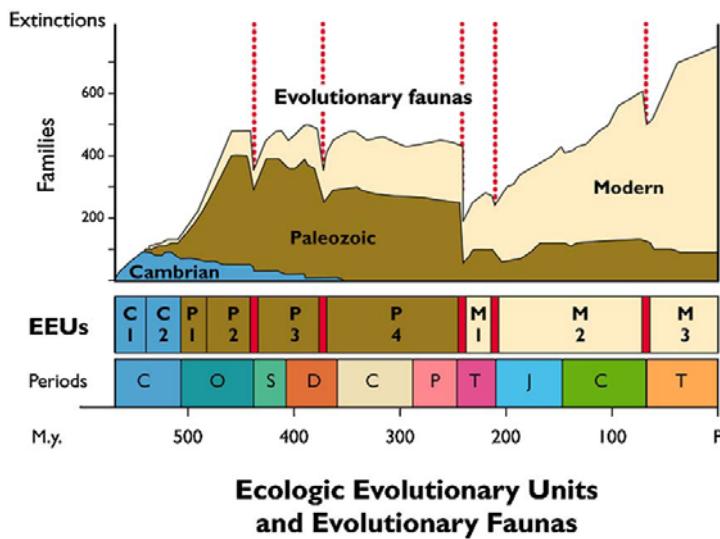


Figure 1.1: Family diversity of skeletonized marine invertebrates during the Phanerozoic (modified from Sheehan, 1996 *in* Harper 2006). The diagram includes evolutionary ecologic units (EEUs), together with an indication of the five large extinction events (red lines); and ten specified geological periods: Cambrian (C (blue)), Ordovician (O), Silurian (S), Devonian (D), Carboniferous (C (green)), Permian (P), Triassic (T (pink)), Jurassic (J), Cretaceous (C (green box)), Tertiary (T (orange box)).

natural entities in the field led to the various species concepts (see review in Table 1.2). As a result, the impact of species concept on biodiversity studies of the same group of organisms can produce not only different species identities but also different species range and number of individuals. An important consequence of the biological species concept is the recognition of reproductively isolated sibling species that show no clear morphological differentiation but which are reproductively isolated (Claridge *et al.*, 1997). The biological species concept can only be applied to organisms that regularly exchange genetic material. Also application of this species concept to populations isolated in space (allopatry) is difficult and usually subjective (Fitzhugh, 2005). These difficulties and the desire to apply cladistic techniques at the species level have lead to widespread rejection of the biological species concept by the scientific community in favour of a broad phylogenetic species concept (Hey, 2006).

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However, a cosmopolitan marine bryozoan study showed that divergent clusters may indeed correspond to reproductively isolated groups, providing a link between these two species concepts (Gomez *et al.*, 2007). There is a clear common ground between these two general concepts for describing biological diversity and together they can form a unitary taxonomic entity (Agapow *et al.*, 2004; Hey, 2006; Hey *et al.*, 2003).

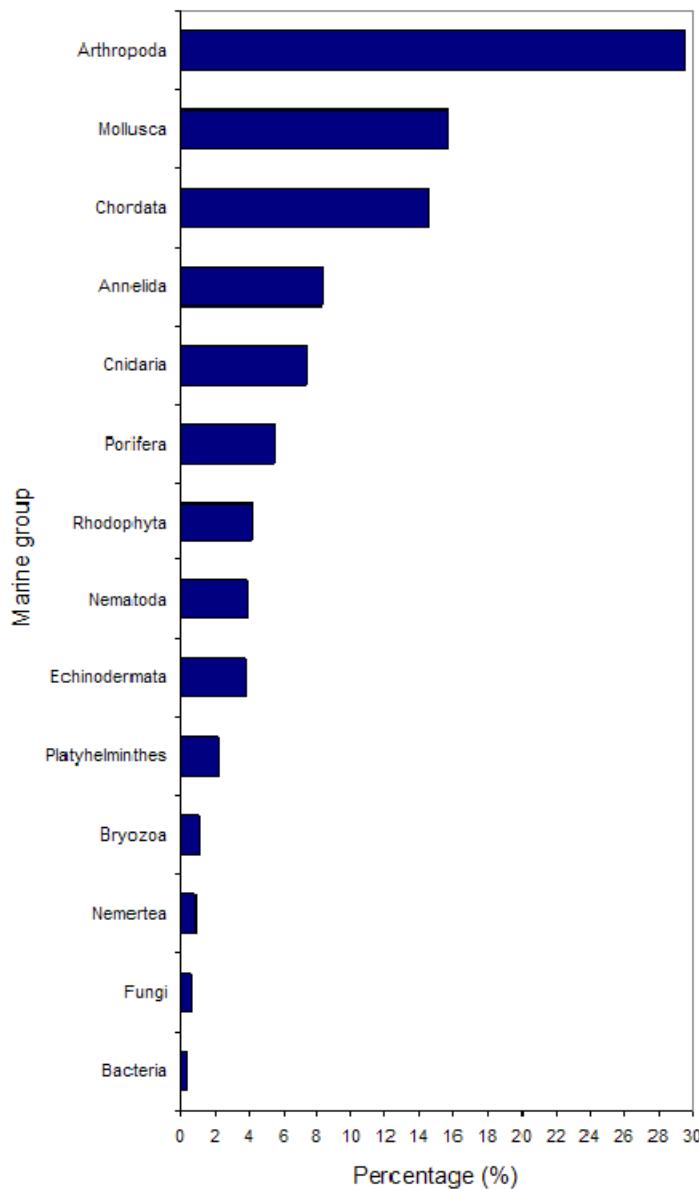


Figure 1.2: Contribution of each marine group (> 0.3%) for the total number of 150,891 (February 2010) valid species according to WoRMS (see Radulovici *et al.*, 2010).

Table 1.1: List of major higher taxonomic groups of marine, freshwater and terrestrial flora and fauna. Modified from Roff and Zacharias, 2011.

	Marine	Freshwater	Terrestrial	Marine	Freshwater	Terrestrial
The major Kingdom or phylum of unicellular forms						
Archaea	+	+	+	+	+	+
Eubacteria	+	+	+	+	+	+
Fungi	+	+	+	+	+	+
Prokista	+	+	+	+	+	+
The major division of algae						
Bacilliarophyta	+	+	+	+	+	+
Charophyta	+	+	+	+	+	+
Chloroachniophyta	+	+	+	+	+	+
Chlorophyta	+	+	+	+	+	+
Chrysophyta	+	+	+	+	+	+
Cryptomoads	+	+	+	+	+	+
Cryptophyta	+	+	+	+	+	+
Cyanophyta	+	+	+	+	+	+
Dinophyta	+	+	+	+	+	+
Euglenophyta	+	+	+	+	+	+
Eustigmatophyta	+	+	+	+	+	+
Glaucophyta	+	+	+	+	+	+
Haplophyta	+	+	+	+	+	+
Phaeophyta	+	+	+	+	+	+
Prasinophyta	+	+	+	+	+	+
Rhodophyta	+	+	+	+	+	+
Xanthophyta	+	+	+	+	+	+
The major divisions of “higher” plants						
Anthocerotophyta	+	+	+	+	+	+
Bryophyte	+	+	+	+	+	+
Marchantiophyta	+	+	+	+	+	+
Lycopodiophyta	+	+	+	+	+	+
Pteridophyta	+	+	+	+	+	+
Pteridospermatophyta	+	+	+	+	+	+
Coniferophyta	+	+	+	+	+	+
Cyadophyta	+	+	+	+	+	+
Ginkgophyta	+	+	+	+	+	+
Gnetophyia	+	+	+	+	+	+
Anthophyta or Magnoliophyta	+	+	+	+	+	+
The major phyla of multicellular animals						
Acanthocephala	+	+	+	+	+	+
Acoelomorpha	+	+	+	+	+	+
Annelida	+	+	+	+	+	+
Anthropoda	+	+	+	+	+	+
Brachiopoda	+	+	+	+	+	+
Bryozoa	+	+	+	+	+	+
Chaetognatha	+	+	+	+	+	+
Chordate	+	+	+	+	+	+
Cnidaria	+	+	+	+	+	+
Ctenophora	+	+	+	+	+	+
Cyclophora	+	+	+	+	+	+
Echinodermata	+	+	+	+	+	+
Echiura	+	+	+	+	+	+
Entoprocta	+	+	+	+	+	+
Gastrotricha	+	+	+	+	+	+
Gnathostomulida	+	+	+	+	+	+
Hemichordate	+	+	+	+	+	+
Kinorhyncha	+	+	+	+	+	+
Loricifera	+	+	+	+	+	+
Micrognathozoa	+	+	+	+	+	+
Mollusca	+	+	+	+	+	+
Nematode	+	+	+	+	+	+
Nematomorpha	+	+	+	+	+	+
Nemertea	+	+	+	+	+	+
Onychophora	+	+	+	+	+	+
Orthonecidia	+	+	+	+	+	+
Phoronida	+	+	+	+	+	+
Placozoa	+	+	+	+	+	+
Platyhelminthes	+	+	+	+	+	+
Porifera	+	+	+	+	+	+
Priapulida	+	+	+	+	+	+
Rhombozoa	+	+	+	+	+	+
Rotifera	+	+	+	+	+	+
Sipuncula	+	+	+	+	+	+
Tartigrada	+	+	+	+	+	+
Xenoturbellida	+	+	+	+	+	+

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Most prominently, it is widely thought that the use of a phylogenetic species concept may lead to the recognition of a far greater number of species and an associated decrease in population size and range (Agapow *et al.*, 2004). The selection of different species concepts can have serious consequences for conservation, including an apparent change in the number of endangered species, potential political fallout, and the difficulty of deciding what should be conserved.

Table 1.2: Species concepts and their strengths and weaknesses (Claridge *et al.*, 1997; Gosling, 1994).

Species (sp) concept	Definition	Strengths/ weaknesses	Practical application
1- Biological sp	1- A group of interbreeding natural populations that do not successfully mate or reproduce with other such groups	1- Popular, irrelevant to asexual or fossil taxa, complicated by natural hybridization, polyploidy, etc	1- Difficult
2- Cohesion sp	2- The smallest group of cohesive individuals that share intrinsic cohesive mechanisms (e.g., interbreeding ability, gene flow)	2- Cohesion is difficult to recognize and suffer from the same limitation as the biological species concept	2- Difficult
3- Ecological sp	3- A lineage which occupies an adaptive zone different in some way from that of any other lineage in its range and which evolves separately from all lineages outside its range	3- Adaptive zones difficult to define, assumes two species cannot occupy same niche for even a short period	3- Difficult
4- Evolutionary sp	4- A single lineage of ancestral descendant populations which is distinct from other such lineages and which has its own evolutionary tendencies and historical fate	4- Criteria vague and difficult to observe	4- Difficult
5- Morphological sp	5- The smallest natural populations permanently separated from each other by a distinct discontinuity in heritable characteristics (e.g., morphology, behaviour, biochemistry)	5- Morphological criteria may not reflect actual links that hold organisms together into a natural unit	5- Common
6- Phylogenetic sp	6- The smallest group of organisms that is diagnostically distinct from other such clusters and within which there is a pattern of ancestry and descent (i.e., monophyletic). It is diagnosed on the basis of one or more genetic traits	6- Will give rise to recognition of many more species and deals only with the manifestation of cohesion rather than the evolutionary mechanisms that define them ignoring the possibility of sibling species	6- Increasing
7- Recognition sp	7- A group of organisms that recognize each other for the purpose of mating and fertilization	7- It is difficult to define traits to identify potential mates	7- Difficult

It is impossible to imagine modern biology without naming and classifying the living systems. In a concept as wide as biodiversity, similar organizational frameworks are also required: defined as the totality of different organisms, the genes they contain, and the ecosystems they form. Table 1.3 summarises the components of

marine biodiversity, wherein the compositional elements of the hierarchy and corresponding structural and some functional components at the genetic, population, community and ecosystem levels are listed (Gaston and Spicer, 2003; Roff and Zacharias, 2011).

The belief that oceans are a homogeneous environment in which speciation is not a common process (Ormond *et al.*, 1999) rose from the relatively limited availability of scientific studies. Particular challenges in the assessment of biodiversity in the marine environment arise from limited access to certain habitats, communities and the patchy distribution of species in pelagic environments (Carvalho, 1998). The ocean is not our natural environment and only recently the naval and marine technological development has provided us access to a wider ecosystem variability, e.g., canyons, seamounts, depths and plains. To date, approximately 229,602 marine species have been described (Bouchet, 2006; Jones *et al.*, 2007) but according to the World Register of Marine Species (WoRMS; <http://www.marinespecies.org>) only 150,891 (February 2010) are valid species (Figure 1.2). Our assumptions about total modern marine biodiversity (described and undescribed) are based on broad estimates from different methods. Examples include extrapolations from quantitative marine samples from specific ecosystems (e.g., the deep sea (Grassle and Maciolek, 1992)), extrapolations from the described fauna from better known regions or groups (e.g., European seas or Brachyura (Bouchet, 2006)), or comparisons with estimates of terrestrial biodiversity (e.g., tropical rain forests (Reaka-kudla, 1997)). These have led to estimates of total marine species diversity spanning three orders of magnitude, from 5×10^5 to 10^8 (Grassle and Maciolek, 1992). These extrapolations have created much controversy (Lambson and Boucher, 2003) as there is no easy and straightforward way of estimating global marine biodiversity.

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reliably, e.g., for marine invertebrates, the extent of taxonomic knowledge depends on the size of the taxonomic community studying various groups (Bouchet, 2006) (see Figure 1.3).

Table 1.3: Compositional, structural and functional attributes of biodiversity for marine environments (Gaston and Spicer, 2009; Roff and Zacharias, 2010).

	Genetic diversity	Organismal diversity	Ecological diversity
Compositional	Nucleotides, Genes, Chromosomes	Individuals	Biomes, Ecosystems, Habitats
Structural	Population	Population	Population/ Community
Functional	Genetic process (e.g., mutation, gene flow, natural selection)	Demographic process (e.g., migration, dispersal, fitness)	Physicochemical process/ habitat relationship (e.g., currents, biogeochemical cycles/ predation, succession)

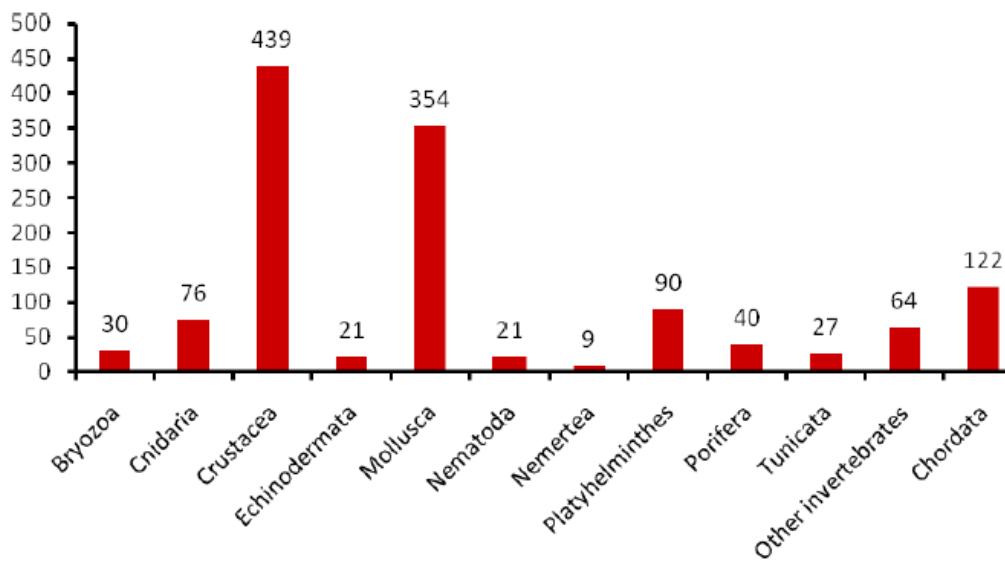


Figure 1.3: Average number of marine species described per taxon every year until 2010. Adapted from Radulovici *et al.*, 2010.

1.1.1 Biodiversity and its conservation

Uncovering and understanding the main marine biodiversity temporal, spatial and biological patterns is crucial to support sound conservation policies in the increasingly threatened marine realm. The widespread and growing concern about the reduction of biological diversity in our planet resulted in the establishment of the Convention of Biological Diversity in 1992 (www.biodiv.org). Within this international treaty nations are committed to develop strategies for the conservation and sustainable use of biological diversity. Lately, an increasing awareness of these problems in the scientific community has led to several large-scale initiatives and research projects to catalogue marine biodiversity. These include the: Census of Marine life: CoML (<http://www.coml.org/>); Consortium for the Barcode of Life: MarBOL-Marine Barcode of Life (<http://www.marinebarcoding.org/>) and FISH-BOL-Fish Barcode of Life Initiative (<http://www.fishbol.org/>); MARBEF - Marine Biodiversity and Ecosystem Functioning (<http://www.marbef.org/>); SESAME – Southern European Seas: Assessing and Modeling Ecosystem changes (<http://www.marbef.org/>); ECOMARG- Study of the Continental Margin Ecosystem and the Impact of Fisheries (<http://www.sesame-ip.eu/>); HERMES – Hotspot Ecosystem Research on the Margins of European Seas (<http://www.eu-hermes.net/>); HERMIONE - Hotspot Ecosystem Research and Man's Impact on European Seas (<http://www.eu-hermione.net/>). With these few examples a special effort has been undertaken to assess the diversity (how many different kinds), distribution (where they live), and abundance (how many) of marine life—a task never before attempted on such a global scale.

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The term “marine conservation” is defined by Roff and Zacharias (2010) as “preservation of the components of marine biodiversity, including structures and processes, in a natural state”. Preservation of the marine biodiversity entails the establishment and management of Marine Protective Areas (MPAs) and restricts human influences on them (Gray, 1997). The definition of conservation units within species is fundamental to prioritize and conduct successful management of MPAs (Daugherty *et al.*, 1990). A distinction between two types of conservation units has been suggested (Moritz, 1994): management unit (MUs), representing sets of populations demographically independent, which may be combined for the purposes of achieving a desired conservation aim (Rubinoff, 2006); and evolutionarily significant units (ESUs) that represent historically isolate sets of populations, that together include the evolutionary diversity of a taxon (Blaxter *et al.*, 2005). Criticisms to these operational definitions include the facts that they only apply when the populations under study are subdivided, and it is difficult to decide objectively on what is a diagnosable distinct clade and the risk of ignoring introgressive hybridization and incomplete lineage sorting (Joly *et al.*, 2009; Maddison and Knowles, 2006).

Until we have a robust idea of the diversity of marine habitats and what controls it, we have little hope of conserving biodiversity efficiently, or determining the impact of human activities such as marine aquaculture, fishing, dumping of waste, pollution and the climatic changes. Only less than three percent of the marine surface areas from the Eastern margin of North Atlantic Ocean are under protection, and significantly less than the 10% minimum required by the Convention on Biological Diversity (<http://www.ospar.org/>).

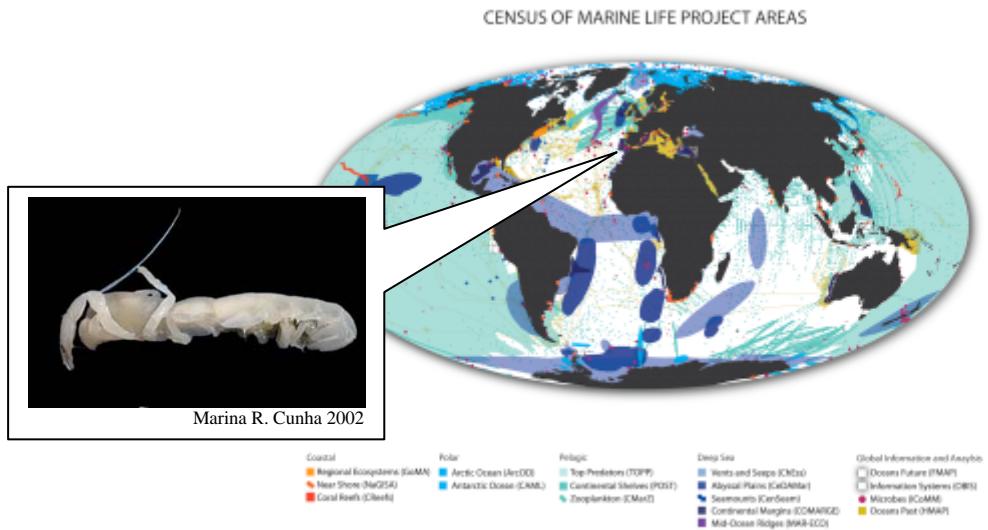


Figure 1.4: A new species of ghost shrimp (*Vulcanocalliax arutyunovi*) found associated with mud volcanoes in the Gulf of Cadiz from the Eastern margin of North Atlantic is the second recorded thalassinidean crustacean from deep-sea chemoautotrophic communities (Dworschak and Cunha, 2007). Census of Marine Life project areas map adapted from <http://www.coml.org/>.

No part of the oceans is now removed from human influence. Efforts to conserve our oceans are now vital, not just for the benefit of local human environmental and socio-economic health but because globally we are all connected. Fortunately, several recent initiatives (mention in this first paragraph of this section) have been providing the basis for understanding the causes and consequences of changes in the diversity of life in marine waters: ecology and evolution of deep-sea communities showed much higher diversity than previously thought (Figure 1.4); many undescribed species were discovered both in relatively poorly-studied and well known environments; novel habitats have been described (e.g.: hydrothermal vents, whale carcasses and hydrocarbon seepage areas); high levels of intraspecific variability of seagrass and multispecies complexes in commercial species (including oyster *Crassotrea*, shrimp *Penaeus* and stone crab *Menippe*) which reveal a critical significance to expand the use of ecosystem principles (i.e., biodiversity, population

control, nutrient recycling, reliance on solar energy) to promote the implementation of community-based integrated ecosystems management plans that achieve local, national and global benefits (Roff and Zacharias, 2011).

*“Conservation is based on emotion.
It comes from the heart and one should never forget that.”*
(George Schaller, 2005)

1.1.2 Biodiversity and evolution of marine Malacostraca

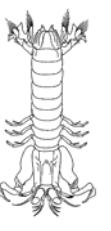
For anyone with interest in a group of organisms as large and diverse as the Malacostraca, it is difficult to grasp the enormity of the entire taxon at one time. Those who work on crustaceans usually specialize in only one small part of the field, i.e., some researchers assumed that they can just profess some special knowledge about only a relatively few species in one or two families (Martin *et al.*, 2009). The total number of categories that humans have erected to contain and order this group is indicative of the incredible amount of morphological diversity they exhibit (Table 1.4 and Figure 1.5). The Malacostraca is a very speciose and morphologically and ecologically highly diverse taxon (Barnes *et al.*, 1996). The Malacostraca (Greek: "soft shell") are the largest class of crustaceans and include most of the animals that non-experts recognize as crustaceans, e.g., including decapods (such as crabs, lobsters and shrimp), stomatopods (mantis shrimp) and euphausiids (krill). They also include the amphipods and the only substantial group of land-based crustaceans, the isopods (woodlice and related species). Even if we could understand all the taxonomic (morphology) diversity as presented in Malacostraca classification (Table 1.4) such knowledge would shed no light on the actual biology of these fascinating animals:

their behaviour, feeding, locomotion, reproduction; their relationships to other organisms; their adaptations to the environment; and other facets of their existence that fall under the heading of biodiversity (Martin and Davis, 2001). With more than 40,000 members, this group represents two thirds of all crustacean species and contains all the larger forms (Brusca and Brusca, 2003).

1.1.2.1 Synopsis: Malacostraca morphology

The Malacostraca are divided in three subclasses: the Phyllocarida, Hoplocarida and Eumalacostraca (Table 1.4). The first includes a single superorder (Leptostraca) while the latter is further subdivided into three superorders: Eucarida, Peracarida and Syncarida (Appeltans *et al.*, 2011; Barnes *et al.*, 1996; Martin and Davis, 2001). The Phylocarida represents the most primitive malacostracan conditions (5-8-7 body segments plus telson) (Brusca and Brusca, 2003). Eumalacostraca (see selected specimens in Figure 1.5) and Hoplocaridae share similarities (e.g., 5-8-6, plus telson, arrangement of the body segments) being recently separated because hoplocarids possess several striking apomorphies (Appeltans *et al.*, 2011; Martin and Davis, 2001). Many other characteristic features are present but their presence varies amongst lineages; one notable ancestral feature which varies is the carapace, which may be absent, reduced or well developed covering the whole cephalothorax (see selected specimens in Figure 1.5).

Table 1.4: Class Malacostraca down to the Order level, according to Martin and Davis (2001). Figures modified from Barnes *et al.*, 1996.

Subclass	Superorder	Order	
Phyllocarida (Packard, 1879)	Leptostraca (Claus, 1880)	Nebaliacea †Archaeostraca Claus, 1888 Canadaspidida Novozilov in Orlov, 1960 Thoplostraca Schram, 1973	
Eumalacostraca (Grobben, 1892)	Syncarida (Packard, 1885)	Anaspidacea Calman, 1904 Bathynellacea Chappuis, 1915 †Palaeocandacea Brooks, 1979	
	Peracarida (Calman, 1904)	Amphipoda Latreille, 1816 Bochusacea Gutu & Iliffe, 1998 Cumacea Krøyer, 1846 Isopoda Latreille, 1817 Lophogastrida Sars, 1870 Mictacea Bowman, Garner, Hessler, Iliffe & Sanders, 1985 Mysida Haworth, 1825 Spelaeogriphacea Gordon, 1957 Tanaidacea Dana, 1849 Themosbaenacea Monod, 1927 †Pygocephalomorpha Gordon, 1957	
	Eucarida (Calman, 1904)	Amphionidae Williamson, 1973 Decapoda Latreille, 1802 Euphausiacea Dana, 1852	
	Hoplocarida (Calman, 1904)	Stomatopoda Latreille, 1817 †Aeschonectida Schram, 1969 †Archaeostomatopoda Schram, 1969	

† Fossil only

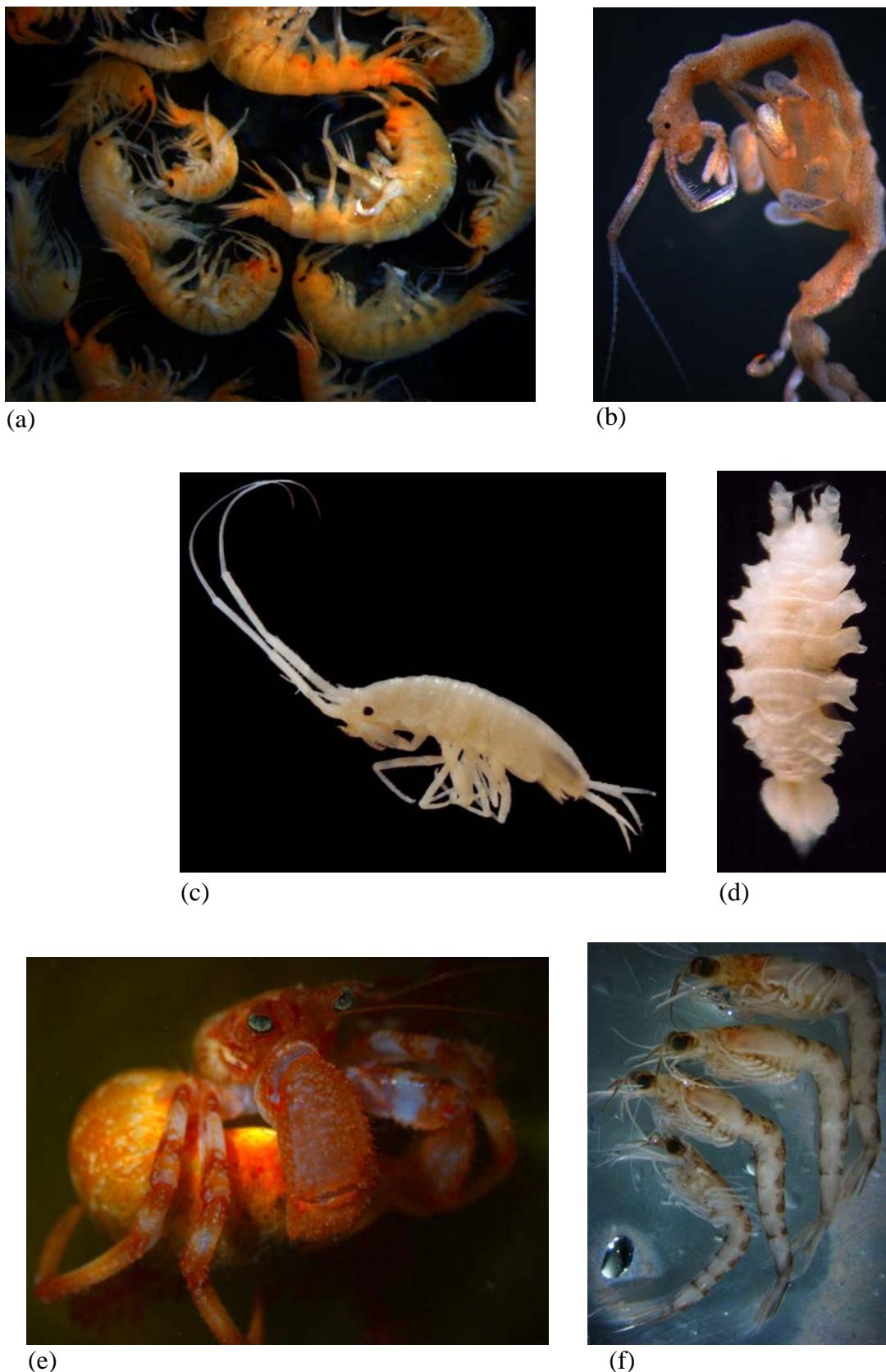


Figure 1.5: Eumalacostraca diversity. (a-b) Amphipoda; (c-d) Isopoda; (e) Decapoda; (f) Euphasiacea (photo credits J Matzen da Silva (a,b,d,e) and MR Cunha (c,d)).

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The following paragraph summarizes the major morphological traits of Malacostraca by (Brusca and Brusca, 2003) (Figure 1.6):

“Body of 19-20 segments, including 5-segmented cephalon, 8-segmented thorax, and 6-segmented pleon (7-segmented in leptostracans), plus telson; with or without caudal rami; carapace covering part or all torax, or reduced, or absent; 0-3 pairs of maxillipeds; thoracopods primitively biramous, uniramous in some groups, phyllopodous only in members of the subclass Phyllocaridae; antennules and antennae usually biramous; abdomen (pleon) usually with 5 pairs of biramous pleopods and 1 pair of biramous uropods; eyes usually present, compound, stalked or sessile, mostly dioecious; female gonopores on sixth, and male pores on eighth thoracomeres. When uropods are present, they are often broad and flat, lying alongside the broad telson form a tail fan.”

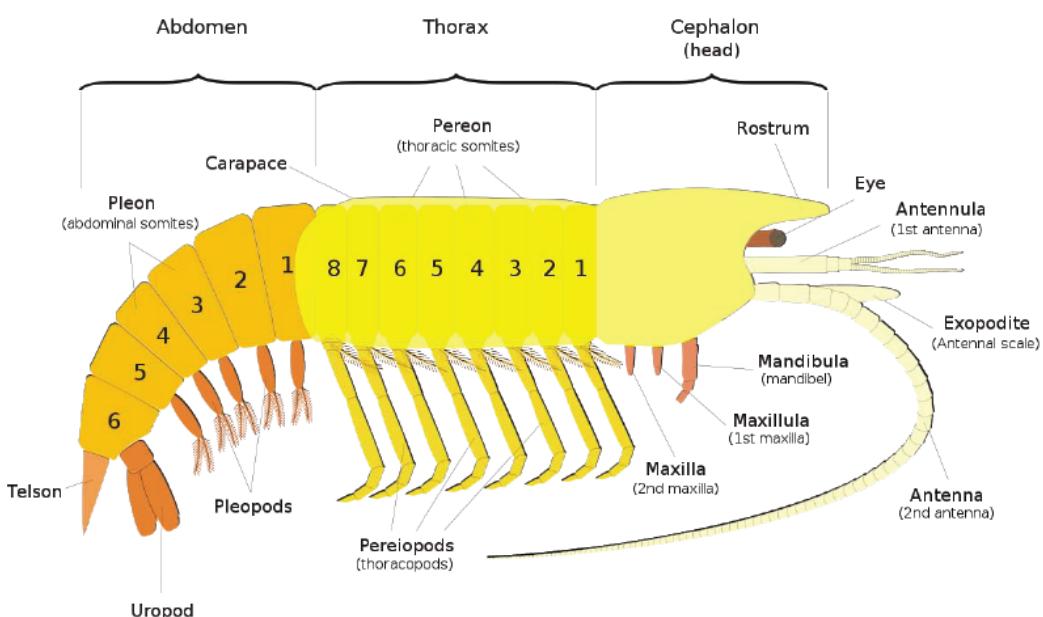


Figure 1.6: General Malacostraca morphological characteristics: the head has 6 segments, with a pair of antennules and a pair of antennae, as well as mouthparts; stalked or sessile eyes; 8 pairs of thoracic legs, of which several pairs are often modified into feeding appendages, maxillipeds; 8 thoracic segments; 6 abdominal segments (http://zipcodezoo.com/Key/Animalia/Malacostraca_Class.asp).

1.1.2.2 Synopsis of Malacostraca phylogeny

The first Malacostraca appeared more than 500 Myr ago, during the Cambrian (Schram, 1974) period (see Figure 1.1). The traditional view places the Malacostraca in the Crustacea (Richter and Scholtz, 2001). However, the relationships of the Malacostraca to other crustacean taxa are highly controversial (Richter, 2002) and restricted to the limited molecular data available per order (Koenemann *et al.*, 2010). A sister-group relationship between the Malacostraca and Insecta has also been proposed (Wilson *et al.*, 2000) and recently discussed in “*Arthropod phylogeny revisited*” (Koenemann *et al.*, 2010) (Figure 1.7).

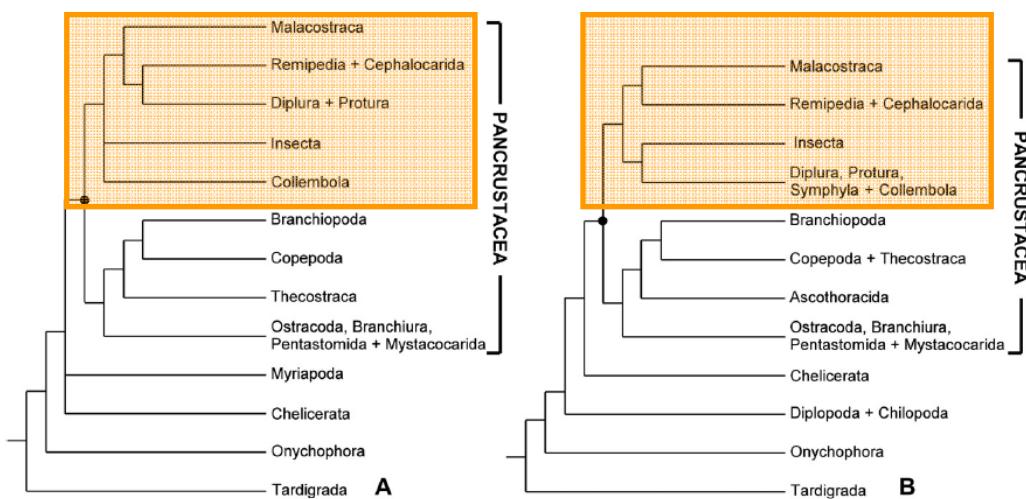


Figure 1.7: Arthropoda phylogenetic trees (A and B inferred by bayesian and likelihood analysis, respectively) based two mitochondrial markers, 16S rDNA and cytochrome c oxidase subunit I (COI), and the nuclear ribosomal gene 18S rDNA (adapted from Koenemann *et al.* 2010). The variance positions of the Malacostraca sister groups is highlighted with orange boxes. In view of the methodological variations encompassed by Koenemann *et al.*, (2010) study, it was unable to resolve the highest level relationships within Arthropoda due the limited number of species and molecular data.

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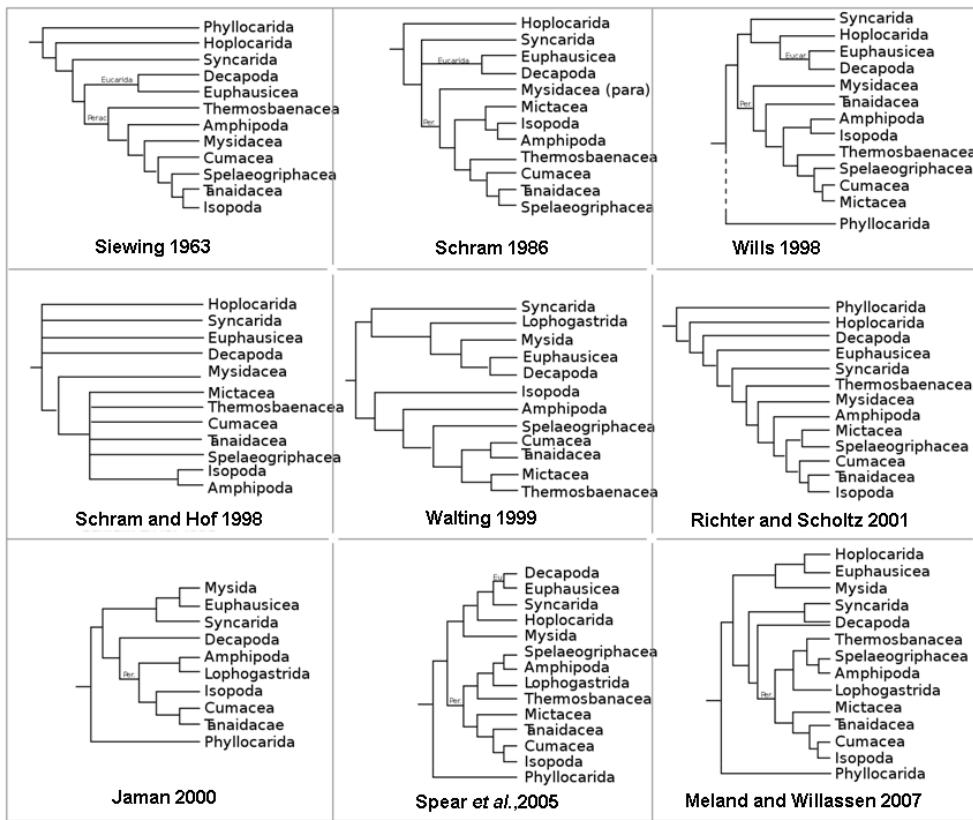


Figure 1.8: Malacostraca phylogenetic trees proposed by different studies. The first six trees were based on morphological characters whereas the last three were obtained with molecular data (adapted from Spears (Jarman *et al.*, 2000; Meland and Willassen, 2007; Spears *et al.*, 2005). E.g., look upon the evolution of the phylogenetic positions of two orders Amphipoda and Isopoda among all the 9 trees.

The monophyly of the Malacostraca is supported by several congruent morphological and molecular studies. Although this class is united by a number of well defined and documented features, the phylogenetic relationship (the evolutionary tree, see Figure 1.8) of the orders included in this class is unclear due to the vast diversity present in their morphology, incomplete species representation, restricted selected data and variations in methodological approaches (Jarman *et al.*, 2000; Meland and Willassen, 2007; Richter and Scholtz, 2001; Spears *et al.*, 2005). For instance, recent molecular studies on 18S (Meland and Willassen, 2007) and 28S rRNA genes sequences

(Jarman *et al.*, 2000) have even disputed the monophyly of the Peracarida by removing the Mysida and the authors have disproven the monophyly of the Edriophthalma (Isopoda and Amphipoda) and the Mysidacea (Mysida, Lophogastrida and Pygocephalomorpha) groups.

1.2 Molecular diversity

The introduction of molecular tools into the biological sciences added a new dimension to the study of marine biodiversity (Féral, 2002). Genetic diversity is a critical component of biodiversity. The genetic information carried by the DNA, controls all morphological and physiological characters of organisms. Ultimately, any mutational change in these phenotypic characters is due to some change in the DNA molecule (Nei and Kumar, 2000). With the advances in the methods for screening at the molecular level it is now, more than ever before, possible to directly understand these processes. The information content of a genome is enormous. A typical metazoan genome consists of some billion nucleotide pairs arranged in a complex sequence. Each genome truly is an assemblage of information, not only encoding the ribonucleic acids and proteins that are central to the working machinery of the cellular life, but also, it retains within its nucleotide sequence a detailed historical record of the phylogenetic links to other forms of life (Murphy *et al.*, 2001).

Variation in genes is necessary to allow organisms to adapt to constantly changing environments. Alleles are different versions of the same gene that can be expressed as different phenotypes. Genetic diversity is reflected in many ways from

differences among individuals in characters such as body pigmentation or commensalism behaviour, for example, to differences in protein and DNA sequences (Frankham *et al.*, 2002). The number of alleles of a gene can vary with new alleles appearing in a population by the process mutation. The frequency of occurrence of an allele changes as the net result of mutation (additional variability) and natural selection, or genetic drift after a population bottleneck (both contributing to genetic variability decrease). Drift is observed to be more severe in small populations and results in changes that do not need to be adaptive (Wright, 1931). In any population only a fraction of all possible zygotes become mature adults, and therefore the transmission of the alleles from one generation to the next as stochastic process is open to discussion (Lowe and Allendorf, 2010). In small populations the effects of genetic drift can be rapid and significant, sweeping out gene variants in a short time (erosion of genetic variability). Genetic drift has the opposite effect of the mutation process (that introduces novel gene variants to the population) and gene variants can be lost rapidly under conditions of drift. The application of DNA based markers in diversity studies has proved to be helpful to design adequate genetic studies (DeSalle *et al.*, 2005).

1.2.1 Molecular methods

Protein based molecular markers were the first approaches widely used to document patterns of genetic diversity in populations and molecular systematic studies (Avise, 2004), such as allozymes (enzymes coded by alleles at the same locus that differ in electrophoretic mobility). Allozymes were a major tool from mid 1960s

until mid-1980s and beyond, and although there are many limitations because this technique only detects nonsynonymous substitutions, the revealed polymorphism was often low they were enormously important and effective in shaping our ideas about natural genetic diversity in wild populations (e.g., mammals, reptiles, invertebrates, amphibians, plants) and in humans (Page and Holmes, 2007). With the advance of polymerase chain reaction (PCR) determining nucleotide sequences is arguably one of the strongest measures of genetic diversity, although a large number of other techniques involving DNA analysis are also prevalent (Gaston and Spicer, 2003). The methods that have been developed include DNA hybridization, species-specific polymerase chain reaction (PCR) primers, restriction fragment length polymorphism (RFLP) analysis, single strand conformational polymorphism (SSCP) analysis, random amplified polymorphic DNA (RAPD) analysis, and PCR product sequencing (Wong and Hanner, 2008) (see Table 1.5), and their usage is dependent on the precise question being addressed. The revolution in molecular genetics has dramatically changed the potential for scientists to make progress in genetic diversity in populations, molecular systematic and phylogeny studies and lately in molecular taxonomy. In a recent review of molecular identification methods (Teletchea, 2009) ten different techniques were described to fish species identification in the past decade (i.e. PCR-RFLP; PCR-sequencing; PCR-specific primers; PCR-SSCP; Real time PCR; PCR-RAPD; PCR-DGGE; PCR-AFLP; cloning and sequencing and microarrays (Telechea, 2009 and references therein in Table 1). Molecular tools of universal implementation, such as the recently proposed DNA barcodes (“a rigorously standardized sequence of a minimum length and quality from an agreed-upon gene, deposited in a major sequence database, and attached to a voucher specimen whose origins and current status are recorded”) provides a simple, yet robust system to

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unambiguously identify not only whole individuals, but eggs, larvae and body fragments (Carvalho, 1998). Such approaches necessarily include a range of molecular identification strategies based on the analysis of homologous gene regions (e.g. COI, 16S, 18S and ITS) for delimiting species boundaries and in their discovery (Carvalho, 1998).

DNA barcoding (Table 1.5), in particular, has the potential to speed the identification of described species (Hebert *et al.*, 2003), but its use to estimate ESUs (Evolutionary Significant Units) numbers, regardless of their formal taxonomic state, is probably even more important for understanding biodiversity patterns, trends and the process of speciation (Lane, 2009). DNA barcode has been both admired and attacked for its simplicity because part of mitochondrial gene (658 nucleotide base pair) responsible for the final step of cell respiration was chosen as the global standard for identifying species. So far research projects, organization and individuals devoted to developing DNA barcoding have described almost 65,000 species (Lindahl *et al.*, 1997). Due the importance of Barcode of Life Initiative an interred Chapter 2.1 is dedicated to discuss the concept of DNA barcoding.

Table 1.5: A comparison of DNA-based species identification techniques. Modified from Wong and Hanner (2008).

	Applicable to degraded material	Low DNA requirement	Simple protocol	Mixture Detection	Time efficient	No prior knowledge required	Reproducible between labs	Standardized across broad taxa
Hybridization	X			X				
Species-specific primer	X	X	X	X	X		X	
RFLP		X	X		X	X	X	
SSCP		X	X		X			
Chain - termination or Sanger sequencing	X*	X	X		X	X	X	
DNA barcoding 2nd generation sequencing	X*	X	X		X	X	X	X

“X” Techniques exhibit the corresponding feature and “X *” only applies to small fragments in the case of severely degraded samples.

The kind of molecular approaches described in the next subsections have major applications for marine conservation and management strategies, because they bring the scope of evolutionary history to complement species diversity and endemism in the establishment of significant units for conservation, and consequently to the design of marine protected areas (Avise, 1998; Palumbi, 2003).

1.2.2 DNA sequencing

The majority of changes at the DNA level are considered selectively neutral or of little or no functional consequence to the organism (Kimura, 1983). In the neutral theory of molecular evolution, genetic drift is the main force changing allele frequencies (Kimura, 1983; Ohta, 1992). Therefore, most of the variation within species and differences between species are the result of neutral mutations (Ayala, 2000). Although the adaptive variability controls the capacity of individuals and therefore populations to adapt, neutral genetic markers are extensively used in conservation genetics. Neutral DNA variation, or the degree of polymorphisms of a neutral marker, is proportional to the underlying rate of mutation (Drake *et al.*, 1998). They are very informative and are at the basis for the analysis of phylogenetic relationships, population structure, gene flow, parental assignment, introgressive hybridization (Wakeley, 2004). Phylogenies based on DNA sequences, reconstruct the history of mutation events describing some of the genealogical structure that occurred in the coalescent tree across an immense span of time, from a geological instant through about 4 billion years of the history of life (Figure 1.1). If a genetic study is limited to a trade-off between increasing the number of sequences or

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increasing the length of DNA sequenced, the important factor to consider for improving the informativeness of a tree is the number of mutations scored. The larger the number of mutations scored, the easier it is to deduce where, and when in the original tree the coalescence events occurred that unite the various lineages represented in the sample of DNA sequences (Harding, 2002). Interspecific phylogeny studies are crucial for obtaining information on the course of evolution and provide the basis for taxonomy and classification. However interspecific phylogenies are also proposed to directly study other topics such as speciation (Machordom and Macpherson, 2004; Schwenk *et al.*, 1995; Witt *et al.*, 2006) phylogeography (Audzijonyte *et al.*, 2006; Pfenninger and Schwenk, 2007; Schwenk *et al.*, 1998), co-speciation (Bush and Butlin, 2004). The phylogenetic relationships between related species are also relevant to understand the historical diversification in behaviour, ecology, physiology (Brooks and McLennan, 1991).

The mitochondrial and nuclear genes encoding ribosomal RNA have been particularly important for inferring species phylogenies because they are easily accessible, collectively demonstrated a wide range of evolutionary rates, and therefore they have the potential to provide resolution across a time scale (Hillis, 1987). They can be used to study many systematic problems, from studies of evolutionary processes to the phylogeny of life. Next sections will describe in more detail the nature of mitochondrial and nuclear genes and their diversity.

1.2.2.1 Mitochondrial DNA

Mitochondrial DNA has a number of specific biological properties, which should make it an appropriate marker of molecular biodiversity. First, its inheritance is clonal (uni-parental, usually maternal in animals), which means that the whole genome behaves as a single, non recombining locus, all sites share a common genealogy (Avise, 2004; Avise *et al.*, 1987). This considerably simplifies the representation and analysis of within-species variation data (Galtier *et al.*, 2009). Secondly mtDNA, which has been supposed to evolve in a nearly neutral manner, it is less likely to be involved in adaptive processes because it is involved in basic metabolic functions (e.g. oxidative phosphorylation) (Avise, 2004; Avise *et al.*, 1987; Bazin *et al.*, 2006; Lane, 2009). Finally, the evolutionary rate of mtDNA has been frequently assumed to be clock, like, in the absence of any mutation spreading through positive selection, only neutral (and slightly deleterious) mutations accumulate in time, so that mtDNA divergence levels should approximately reflect divergence times (Galtier *et al.*, 2009). Evolutionary rates (molecular clock) across taxa of “ 2% per million year” are often considered as a reasonable reference in the absence of relevant fossil data (Galtier *et al.*, 2009).

Most metazoan mitochondrial genomes are covalently closed circular molecules, approximately 16 kb in size, containing 37 genes: 13 for protein-coding genes, two for ribosomal RNA genes, and 22 for transfer RNA genes (Boore, 1999) and a non-coding segment of about 1,000 bp long, called the control region, which contains the ‘d’ or displacement loop (D-loop), that initiates replication and transcription (Harrison, 1989). For example, during the last few years 40 complete mitochondrial (mt) genome

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sequences of Crustacea: Malacostraca: Eumalacostraca were determined (<http://www.ncbi.nlm.nih.gov/>). Specimens from Amphipoda have the lowest circular molecule of 14.113 bp (GenBank accession number: NC_013032) in length and Decapoda the highest length with 18.197bp (GenBank accession number: NC_007379.). The following schematic Figure 1.9 shows the structure and gene organization of a general metazoan mtDNA. Mitochondrial DNA sequence comparisons between individuals enable the detection of differences in the basic units of biological information, allowing the maximum resolution by direct sequencing. Therefore it is considered a very highly informative molecular marker (Avise, 2004). Several factors have contributed to its popularity, such as the non-recombining, uniparental pattern of inheritance, providing information about the source-parent lineage only (Avise, 2004); few insertion/deletion/duplication events; and a relatively conserved gene order and number (Avise *et al.*, 1987). Also it shows high levels of genetic variation, a consequence of either a low selective pressure (or high mutation rate and due in part to inefficient mutation repair mechanisms (Taylor and Lehmann, 1998), making mtDNA a useful microevolutionary phylogenetic marker (Avise, 2004).

MtDNA can be found in multiple (hundreds to thousands) copies of a given sample due to the presence of up to several hundred mitochondria per cell (Avise, 2004). Haploid genomes, such as mitochondrial, are considerably easier to survey than diploid nuclear DNA because without multiple heterozygous sites there are no ambiguous linkage relationships. This is one of several reasons why population genetic studies have favoured mtDNA analyses (although nuclear sequences data for large samples of individuals are rapidly becoming available) (Harding, 2002).

MtDNA has been a marker extensively used to examine the evolutionary relationships among species and is a powerful tool for estimating levels of genetic

diversity, phylogenetic structure and recent demographic history within taxa. The presence of different regions evolving at different rates allows the selection of the most suitable DNA regions in accordance to the time frame of a particular phylogenetic question. For example, the slowly evolving sequences of ribosomal RNA genes (12S and 16S) have been extremely informative in reconstructing deep branches among different species. The cytochrome oxidase I (COI), for example, is generally used to study close to moderately deep interspecific relationships (Avise, 2004; Avise and Walker, 1999), although it is also informative for intraspecific analyses (Hebert *et al.*, 2003; Meyran *et al.*, 1997). Rapidly evolving mtDNA sequences, such as the D-loop that evolves about 4-5 times faster than the rest of the mtDNA molecule (Taberlet, 1996) revolutionized population structure and phylogeographic studies of animals at the intraspecific (Belay and Mori, 2006). Possibly the most important reason to use mitochondrial genes is the availability of universal mtDNA primers sets that have minimized laboratory time in the initial setting up of a project (Toon *et al.*, 2009).

Although the many qualities of the mtDNA, there are several drawbacks, that should be considered (Galtier *et al.*, 2009). Its maternal mode of inheritance may constitute a disadvantage when males mediated gene flow is very strong (Hurst and Jiggins, 2005). Outstanding recurrent mutation, heteroplasmy must be considered which may constitute both a technical problem and a risk to do its utility as a marker in phylogenetics (Rubinof *et al.*, 2006). Due to the presence of mtDNA like copies in the nuclear genome (Schubart, 2009), mtDNA sequences retrieved by PCR, may potentially lead to highly incorrect conclusions (see Chapter 2.2), if segments of real mtDNA are not certified (Bensasson *et al.*, 2001; Zhang and Hewitt, 1996). For instance Galtier *et al.* (2009) reviewed cases that mtDNA is not immune from either

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recombination (e.g., mussel, butterfly, scorpion, fish), or positive selection, (i.e. synonymous/nonsynonymous > 1 detected more in invertebrates than in vertebrates) hence failing to support the theoretical relationship between population size and genetic diversity. MtDNA is under a complex mutation processes variable across species in space and time. The interpretation of within species mtDNA genealogies should take into account such phenomenon especially when strong homoplasy is observed. Finally everybody knows that mtDNA is not functional neutral, it has been assumed that mitochondrial genome essentially undergoes neutral or deleterious selection (Galtier *et al.*, 2009). The neutrality assumption was recently challenged by Bazin *et al* (2006) when mtDNA and nuclear diversity was compared across invertebrates and vertebrates from marine and terrestrial environments among different organisms and population sizes. They found that nuclear loci diversity was consistent with the theoretical relationship between population size and genetic diversity in contrast with mitochondrial diversity results (Bazin *et al.*, 2006). The mitochondrial specific lack of population size effect was explained by recurrent selective sweeps in large populations suggesting that adaptive evolution might significantly impact mtDNA variation patterns (Galtier *et al.*, 2009). Recently Lane (2009) discussed that likelihood high mutation rate in mitochondrial, coupled to a development filter that gets rid of the less adaptive mutations before they have chance to undermine the health of an animal´s offspring, will have a role in speciation.

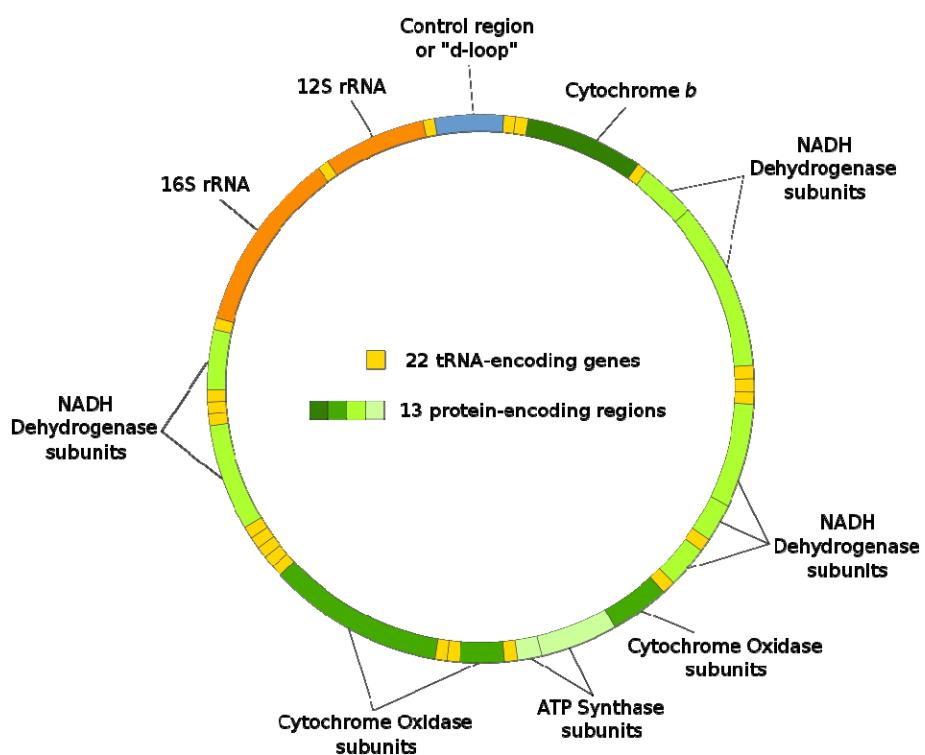


Figure 1.9: Schematic representation of the structure and gene organization of a general metazoan (Mitochondrial_DNA_de.svg). MtDNA genome contains 13 protein-coding genes (ATP6, ATP8, CO1, CO2, CO3, Cytb, ND1, ND2, ND3, ND4L, ND4, ND5 and ND6), 22 transfer RNA genes, two ribosomal RNA genes (12S and 16S), and a non-coding segment called the control region or “d-loop” (Harrison, 1989).

1.2.2.2 Nuclear loci

Given the vast complexity of the nuclear genome, many nuclear genes are useful for inferring deeper phylogenetic relationships (Friedlander *et al.*, 1994). Use of nuclear loci for inter-specific studies has received far less attention than has the use of mtDNA (Figure 1.10) however, studies of nuclear rRNA genes and coding sequences appear promising for such applications. The best example of the broad utility of nuclear genes is the rDNA repeat unit, e.g. 18S rDNA and 28S rDNA. The ribosomal nuclear genes 18S rDNA and 28S rDNA have been extensively used in arthropoda systematics (Colgan *et al.*, 2000; Mardulyn and Whitfield, 1999; McArthur and Koop, 1999; Mitsuhashi *et al.*, 2007; Porter *et al.*, 2005; Toon *et al.*, 2009). Rates of evolution vary among and within these genes, making them valuable phylogenetic tools at different taxonomic levels. As an example of a good case study, Tong *et al.*, (2009) found that divergences for 18S were consistently moderate among species (5.8 – 7.2%) and among infraorders (5.6%) within Pleocyemata but were higher among the suborders Pleocyemata and Dendrobranchiata (12.8% and 14.1%). The ribosomal nuclear genes 28S divergence estimates were higher than 18S among species (9.1 – 11.6%), within Pleocyemata (11.3%), and among the suborders (20.8 – 21.8%). Levels of divergence were lower for the intermediate taxon levels, among genera (3.4 – 8.0%) and among families (7.3 – 9.9%). Because nuclear protein coding genes are highly conserved, they are useful for comparisons among widely divergent taxa and have been used in arthropod systematics, as for example the genes of histone 3 (H3) (Porter *et al.*, 2005) or elongation factor 2 (EF-2) (Regier and Shultz, 2001).

Use of nuclear genes in addition to mitochondrial genes adds to the number of independent markers in a dataset, thus increasing the chances of reconstructing the true species phylogeny. In addition, a larger effective population size, and on average, a lower substitution rate (Moriyama and Powell, 1997), results in nuclear genes evolving slower than mitochondrial genes. Consequently, they may be better at resolving deeper phylogenetic nodes.

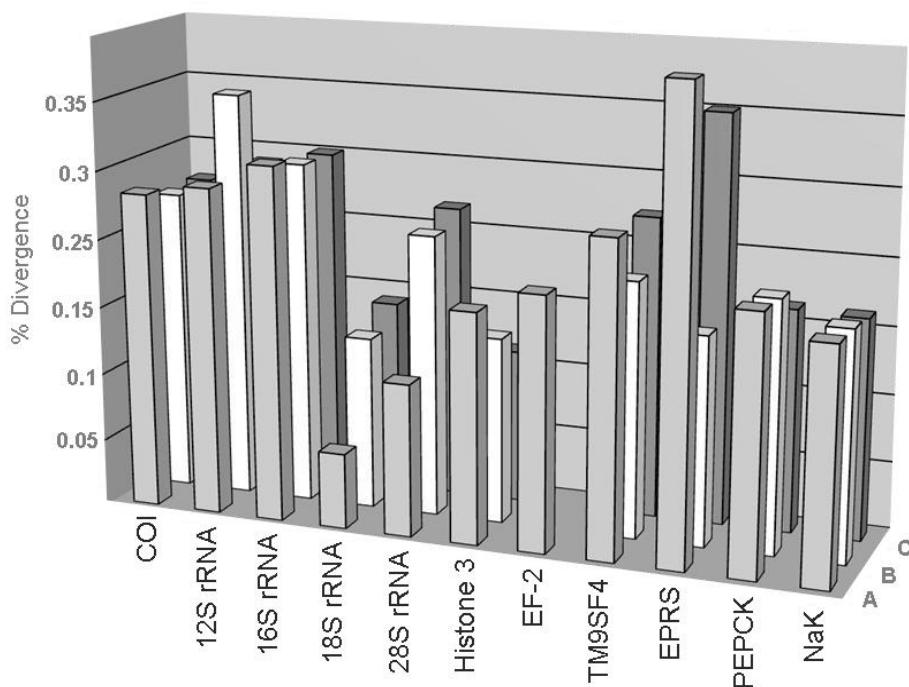


Figure 1.10: Pairwise interspecific divergence among three different non congeneric decapods species pairs (A; B; C) for mitochondrial (COI; 12S rRNA; 16S rRNA) and nuclear genes (18S rRNA; 28S rRNA; Histone 3; EF-2; Transmembrane 9 superfamily protein member 4 (TM9SF4); Glutamyl-prolyl-tRNA synthetase (EPRS); Transcriptional repression of the gluconeogenic (PEPCK); sodium-potassium ATPase o-subunit (NaK). Figure transformed from Toon *et al.*, 2009.

1.2.3 Phylogenetic inference methods

1.2.3.1 Models of DNA sequence

Ideally, the inference method used will extract the maximum amount of information available in the sequence data, will combined this with deal models parameters whose values are not known a priori (Felsenstein, 2004). Models describing the rate of change from one nucleotide to another will depend on the kind of data under evaluation (Nei, 1996; Nei and Kumar, 2000). Some commonly used, nested models of DNA sequence evolution are listed as follows from the least complex to the most parameter rich: Jukes and Cantor (Jukes and Cantor, 1969), which assumes equal base frequencies at equilibrium and equal rates for all substitution types (Felsenstein, 1981) model assumes variable base frequencies, all substitutions equally likely, and Kimura 2-parameter (Kimura, 1980), which assumes equal base frequencies at equilibrium but a transition bias in substitution rates, are the simplest. Hasegawa-Kishino-Yano (Hasegawa *et al.*, 1985) a “K2P” but with unequal base frequencies; Tamura-Nei (Tamura and Nei, 1993) with unequal base frequencies at equilibrium and two classes of transition rates; Kimura 3-parameter (Kimura (Kimura, 1980) variable base frequencies, equal transition frequencies, variable transversion frequencies; Transition Model (TIM) variable base frequencies, variable transitions, transversions equal; Transversion Model (TVM) variable base frequencies, variable transversions, transitions equal; Symmetrical Model (SYM) (Zharkikh, 1994) equal base frequencies, symmetrical substitution matrix; General Time Reversible (GTR, Number of Substitution Types (nst = 6)) (Lanave *et al.*, 1984) variable base

frequencies, symmetrical substitution matrix. Gamma distributed rate variation among sites (G) and the Proportion of Invariable Sites (I) are additional parameters that describe the rate variation among sites in a sequence. Goodness of fit statistics are used to measure the fit of the observed data to the chosen model of evolution (Swofford *et al.*, 1996). The choice of a certain model must be based on a statistical test that assesses its fit. The comparison of different nested models of DNA substitutions can be performed in a hierarchical hypothesis-testing framework (Posada, 2003; Posada and Buckley, 2004). Several statistics are available, such as, Likelihood Ratio Tests (LRT), the Akaike Information Criterion (AIC), the Bayesian Information Criterion (BIC) (Posada, 2008), and the Decision Theory-Model Selection (DT-ModSel) (Minin *et al.*, 2003).

Molecular phylogenies present an estimate of the evolutionary relationship of extant or extinct, taxa or individuals. Nowadays there are an increasing number of approaches (Table 1.6) in order to map gene phylogenies or gene networks (such as mtDNA haplotypes). The major inference methods for molecular phylogenetics are maximum likelihood, bayesian inference, distance methods, and maximum parsimony. Maximum likelihood (ML) is a popular statistical method used to make inferences about parameters of the underlying probability distribution of a given data set. Likelihood-based approaches have proven especially powerful for inferring phylogenetic trees (Felsenstein, 2004), but are computer demanding. Likelihood methods for phylogenies were introduced by Edwards and Cavalli-Sforza (1964) for gene frequency data and are currently used for sequence data. Bayesian inference (BI) phylogenetics has been proposed more recently as a powerful method for the analysis of large phylogenetic trees and complex evolutionary models (Holder and Lewis, 2003). In this analysis the probability of a correct tree is the posterior probability of

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that tree. This posterior probability is dependent on how well represented are the data and the model of evolution proposed. It differs from the ML methods in that, BI analysis seek the tree that maximizes the probability of the tree, given the data and the model of evolution. Calculations can be approximated by the Markov Chain Monte Carlo (MCMC) procedure, lessening the computation time (Huelsenbeck and Rannala, 2004). MCMC method can be seen as a set of independent searches that occasionally exchange information. Distance matrix methods calculate a measure of the distance between each pair of species and then find a tree that predicts the observed set of distances as closely as possible (Felsenstein, 2004). There are several distance matrix methods available in the literature. Two examples are minimum evolution and neighbour joining. The Minimum Evolution (ME) methods seeks to find the tree with the shortest overall branch lengths (Palero and Crandall, 2009). The Neighbour-Joining (NJ) algorithm developed by Saitou and Nei (1987) is considered a simplified version of the Minimum Evolution method. The NJ method searches for pairs of Operational Taxonomic Units (OTU) that minimizes the total branch length at each stage of OTU's, clustering beginning with a star tree. This star tree is produced under the assumption that there is no clustering of taxa. The first step is to find the first pair of OTU's that show the lowest branch length. Once the first pair of neighbours are identified they are combined into one unit and this procedure is repeated until the final tree is produced. The NJ algorithm does not assume that all lineages evolve at the same rate and produces only one unrooted tree that may minimize its total length. The NJ performance is dependent on the underlying model of molecular evolution selected to create the pairwise distance matrix between sequences used for tree construction. The tree obtained is additive, meaning that the distance between any two terminal nodes is the sum of the branch lengths connecting them. Backward and parallel

mutations do occur in real data but these are not contemplated in the construction of the NJ tree. Although the NJ is a fast and reliable method of producing the correct topology (Gascuel, 1997), statistical tests to assess the tree reliability are necessary. The NJ method is appropriate for large data sets and capable of conducting rapid bootstrap resampling tests – a nonparametric statistical analysis of support for phylogenetic trees (Holder and Lewis, 2003). As referred by Felsenstein (2004), computer simulations studies have shown that NJ performs quite well. Parsimony or maximum parsimony (MP), is a popular technique to infer a phylogenetic tree for a set of taxa on the basis of the similarities and differences among taxa. MP scores the minimum number of changes, between different character states to explain the observed data given the tree. Parsimony follows the general principle in science known as Ockham's razor – there is no need to make more assumptions than necessary to explain the observations. It is a character based method and the information contained in the sequences is not transformed into a distance matrix. Parsimony makes a direct use of the character state, and the optimality criterion is the minimum tree length. The preferred trees are the ones presenting the smaller number of evolutionary steps (shortest lengths) required for explaining a given data set (most parsimonious) (Swofford *et al.*, 1996). In this process more than one minimal tree may be found. In order to guarantee that the best possible tree is found, an exhaustive evaluation of all possible tree topologies has to be performed. MP does not use all the sequence information, it only uses the informative sites (those sites that have at least two different kinds of bases in two different sequences), and does not correct for multiple mutations (it does not introduce a model of evolution). Homoplastic events by back and parallel mutations, introduces uncertainty in phylogenetic inference and the MP method may produce an erroneous tree. There is a strong bias in parsimony

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methods when some lineages have experienced rapid rates of change. In general, long branches tend to “attract” each other (Hall, 2008). This long-branch attraction phenomenon refers to the tendency of taxa at the ends of long branches to be made artificially close to each other and MP is much more sensitive to the Long Branch Attraction (LBA) artefact than is ML (Philippe *et al.*, 2005).

The NJ tree is often treated as the starting point for a computationally intensive search for the best phylogeny and its success is dependent on the sequence divergences being adequately corrected for multiple hits (or the tree being so easy to infer that poor distance estimates are sufficient). To perform a tree search, a standard must be used for comparing trees — an optimality criterion, in the terminology of phylogenetics (Holder and Lewis, 2003) (see for more details Table 1.7).

Table 1.6: Comparison of phylogenetic inference methods. Modified from Holder and Lewis (2003) and Hall (2008).

Methods	Advantages	Disadvantages	Program
NJ	Fast	Information is lost in compressing sequences into distances; reliable estimates of pairwise distances can be hard to obtain for divergent sequences	ClustalX Geneious PAUP MEGA PHYLIP Phylo_win SplitsTree
MP	Fast enough for the analysis of hundreds of sequences; robust if branches are short (closely related sequences or dense sampling)	Can perform poorly if there is substantial variation in branch lengths	PAUP MEGA PHYLIP Phylo_win
ME	Uses models to correct for unseen changes	Distance corrections can break down when distances are large	PAUP MEGA PHYLIP
ML	The likelihood fully captures what the data tell us about the phylogeny under a given model	Can be prohibitively slow (depending on the thoroughness of the search and access to computational resources)	Geneious PALM PAUP PAML PHYLIP Phylo_win PHYML Treefinder
BI	Has a strong connection to the maximum likelihood method; might be a faster way to assess support for trees than maximum likelihood bootstrapping	The prior distributions for parameters must be specified: it can be difficult to determine whether the Markov chain Monte Carlo (MCMC) approximation has run for long enough	Bali-Phy Mr.Bayes BAMBE Geneious PAML Phase

1.2.3.2 Assessing node confidence in phylogenetic trees

Measures of nodal support provide a useful summary of how well data support the relationships defined by a tree. Most measures of nodal support attempt to estimate the degree to which an analysis has converged on a stable result, e.g., Bremer support in MP approach; the parsimony – based method of partitioned branch support (PBS) when general genes are included in the analysis; partitioned likelihood support (PLS) can be obtained for each dataset under likelihood – based approach (Palero and Crandall, 2009).

Table 1.7: Tree construction and tree searching methods. Adapted from Holder and Lewis (2003).

Method	Description	Advantages	Disadvantages
<i>Tree construction methods</i>			
Stepwise addition	Builds a complete tree, starting with three sequences and attaching new sequences one at a time to the branch that yields the optimum tree at each step	Fast; later steps can reverse earlier pairing decisions	Yields one tree, often not global optimum; alternative additional sequences might yield different trees; not as fast as neighbour-joining
Star decomposition	Builds a completely resolved tree, starting with all sequences connected to a single ‘hub’ node. At each step, two lineages attached to the hub node are joined becoming neighbours. Neighbours are chosen so that tree is optimal at each step	Fast; addition sequences irrelevant	Yields one tree, often not global optimum; neighbours cannot be dismantled at later steps; ties broken arbitrarily by some implementations
Neighbour joining	A star-decomposition method that uses an approximation to the minimum-evolution optimality criterion	One of the fastest of all tree construction methods	The same as those listed for star decomposition
<i>Tree searching methods</i>			
Heuristic search	Given a starting tree containing all sequences of interest performs branch swapping to generate alternative trees in an attempt to find a better tree under a given optimality criterion. Strict hill-climber: if a better tree is found, the process begins again, stopping only if a local optimum is attained. Typically uses a stepwise addition or NJ trees as the starting tree	Faster than exact searches	Can miss the global optimal tree
Exact search	Exhaustive searches examine every possible tree and are guaranteed to return the best tree. Branch-and-bound techniques can eliminate some bad trees from consideration and still guarantee that they will return the best tree	The only methods that are guaranteed to find the best trees	Time-consuming: only practical for a few sequences (<20)

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Currently, the nonparametric bootstrap (Efron and Tibshirani, 1993) is one of the most widely used method for assessing nodal support (introduced to phylogenetics by Felsenstein in 1985) but others methods, such as Bayesian posterior probabilities and the Approximate Likelihood Ratio Test (aLRT) (Anisimova and Gascuel, 2006) are also becoming more widely used (Hall, 2008). It is important to highlight that these methods estimate reproducibility, not accuracy. In the case of bootstrapping applied to phylogenetics, a phylogenetic tree is constructed from each resampled data (Felsenstein, 1985). As referred by Nei and Kumar (2000), the test is not for examining the reliability of the tree obtained from the original data set, but for examining the reliability of a bootstrap consensus tree, produced by considering all the trees generated by bootstrap resampling. A number of replicates (typically at least 1000) of the original characters (e.g., sites of DNA sequence alignment) are randomly produced with replacement, obtaining a new dataset. The fraction of times a particular clade appears on the analyses of the bootstrapped data sets, represents the confidence level for that grouping of OTU's (Huelsenbeck and Rannala, 2004). Since its description, the bootstrap has been a frequent choice for estimating the reliability of the internodes of cladograms and the bootstrap values are usually summarized in a consensus tree (Zander, 2004). The computation time is generally very long and that is why this method is mostly applied to the NJ trees and prohibitive to MP or ML trees, obtained from numerous sequences (Huelsenbeck and Rannala, 2004). Version 2.45 of PHYML is 50-100 times faster than most implementations of ML and introduced a new test for tree reliability, the aLRT (Anisimova and Gascuel, 2006), which is a thousand times faster than the ML bootstrap. The likelihoods of the different arrangements around the branch are calculated during the process of

estimating the ML tree, so little additional time is required to estimate the most likely arrangement (Hall, 2008).

Hillis and Bull (1993) consider bootstrap method conservative because the statistical theory requires, in contrast to the statistical confidence of interior branches in a BI analysis - the posterior probabilities, that all positions of an alignment are independently and identically distributed, and this assumption does not apply to nucleotide or amino acid sequences (Palero and Crandall, 2009). Additionally, there is a lack of information about the sensitiveness of this method to misspecification of the model of evolution (Huelsenbeck and Rannala, 2004). The weakness of this method has led researchers to seek other methods of estimating nodal support.

Bayesian posterior probability has been the most popular alternative and the nodal BI is the probability that a given node is found in the true tree, conditional on the observed data, and the model (Palero and Crandall, 2009). It is most of the times a value much higher than the maximum likelihood bootstrapping (the most similar analogue to the bayesian analysis of phylogenetic), thus occurring over-credibility (Suzuki *et al.*, 2002). There is a widely used conservative rule that a group is supported significantly if it appears in at least 95% of bootstrap trees (Felsenstein, 2004). The great advantage of the Bayesian posterior probability is that this statistic is drawn from the same distribution that determines the best estimate of the tree topology, as opposed to a bootstrap analysis that requires 1000 reruns of the analysis (Palero and Crandall, 2009).

1.3 Aim and output of the Thesis

The main aim of this thesis is to contribute to the understanding of the patterns of genetic variation and evolution in Malacostraca. This will be achieved by a study encompassing 132 Malacostraca species from the Eastern margin of North Atlantic Ocean and Mediterranean Sea using an integrated approach with emphasis on molecular techniques. Besides the introduction (Section 1) and final remarks (Section 4) the results are presented in two main sections with specific objectives:

Section 2: Molecular Biodiversity.

In this section I intend to explore the biodiversity of selected Malacostraca specimens from Amphipoda, Decapoda and Isopoda analysing patterns of COI diversity among these three orders especially because they represent differences in life style and dispersal ability. To increase the accuracy of the analysis I combined novel data produced in this study with selected public data. Chapter 2.1 provide a well annotated reference library, matching known species phenotypes with respective DNA barcodes of marine malacostracan. This chapter is a show case of the global barcoding protocol, describing key aspects of the methods and protocols to create a reference library of DNA barcodes.

After providing a brief background on DNA barcoding I analysed patterns of molecular divergence within and among taxa and highlighted putative cryptic species where such analysis will advance the understanding of evolution in selected members of this Class. I also investigated how molecular studies can change the practical application of species concepts in biodiversity assessments

of a given taxonomic assemblage, and confirmed how taxonomic groups are recognized from broad range of characteristics, its relationship with species delineation, and concluded that the traditional knowledge and skills of specialists in various groups are thus indispensable. In this section I also mention a particular problematic of DNA barcoding (and of mtDNA in general), the accidental amplification of non-functional copies of mtDNA present in the nuclear DNA that is discussed in more detail in Chapter 2.2.

Section 3: Molecular evolution and shallow phylogenies.

Geographic and temporal patterns of morphological and behavioural diversification among species have been associated with sequence variation in DNA. The Malacostraca barcode project extended our understanding of evolution and species in varied ways, i.e. flagging taxonomic groups or species with unusual nucleotide composition or evolutionary rates. Through, most well representative novel Decapoda COI data combined with published genbank data, systematic and evolutionary insights are discussed in Chapter 3.1. Here I emphasized from different geographic sources and ecosystems the correlation of molecular diversity with evolutionary processes of species, genus, and families of decapods, highlighting both the apparent taxonomic ambiguity among certain groups, and the need for an accelerated and independent (i.e., integrate) taxonomic approach. The scale of divergence at each taxonomic level appears to vary extensively between families. To understand with greater detail the molecular evolution of mitochondrial DNA and its relationship with species delineation, two priority groups were selected among decapods for taxonomic

and multi-locus systematic focus (*Plesionika* and *Pagurus* discussed in Chapter 3.2 and Chapter 3.3, respectively).

This thesis includes two published paper and one in press:

Matzen da Silva J, S Creer, A dos Santos, AC Costa, MR Cunha, FO Costa, GR Carvalho (2011) Systematic and Evolutionary Insights Derived from mtDNA COI Barcode Diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE* 6(5), e19449. doi:10.1371/journal.pone.0019449

Matzen da Silva, J, A dos Santos, MR Cunha, FO Costa, S Creer, GR Carvalho (2001) Testing the utility of partial COI and 16S sequences for phylogenetic appraisals of selected *Plesionika* (Decapoda: Pandalidae) from the Eastern margin of North Atlantic and Mediterranean Sea. *Marine Ecology: An Evolution Perspective* **in press**.

Matzen da Silva, J, A dos Santos, MR Cunha, FO Costa, S Creer, GR Carvalho (2011) Multigene molecular systematics confirm species status of morphologically convergent *Pagurus* hermit crabs. *PLoS ONE* 6(12): e28233. doi:10.1371/journal.pone.0028233.

References

- Agapow PM, Bininda-Emonds ORP, Crandall KA, Gittleman JL, Mace GM, Marshall JC, Purvis A (2004) The impact of species concept on biodiversity studies. *The Quarterly Review of Biology* **79**, 161-179.
- Anisimova M, Gascuel O (2006) Approximate Likelihood-ratio Test for branches: A fast, accurate, and powerful alternative. *Systematic Biology* **55**, 539-552.
- Appeltans W, Bouchet P, Boxshall GA, Fauchald K, Gordon DP, Hoeksema BW, *et al.* (2011) World Register of Marine Species. In: <http://www.marinespecies.org>

- Audzijonyte A, Daneliya ME, Vainola R (2006) Comparative phylogeography of Ponto-Caspian mysid crustaceans: isolation and exchange among dynamic inland sea basins. *Molecular Ecology* **15**, 2969–2984.
- Avise JC (1998) The history and purview of phylogeography: a personal reflection. *Molecular Ecology* **7**, 371-379.
- Avise JC (2004) *Molecular markers, natural history and evolution*, 2nd edn. Sinauer Associates, Massachusetts.
- Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, et al. (1987) Intraspecific phylogeography - the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489-522.
- Avise JC, Walker DE (1999) Species realities and numbers in sexual vertebrates: Perspectives from an asexually transmitted genome. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 992-995.
- Ayala FJ (2000) Neutralism and Selectionism: the molecular clock. *Gene* **261**, 27-33.
- Barnes RSK, Calow P, Olive PJW (1996) Invertebrates with legs. The Arthropods and similar groups. In: *The invertebrates a new synthesis* (eds. Barnes R. S. K., Calow P., Olive P. J. W.), pp. 207-211. University Press, Cambridge.
- Bazin E, Glemin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science* **312**, 570-572.
- Belay G, Mori A (2006) Intraspecific phylogeographic mitochondrial DNA (D-loop) variation of Gelada baboon, *Theropithecus gelada*, in Ethiopia. *Biochemical Systematics and Ecology* **34**, 554-561.
- Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution* **16**, 314-321.
- Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R, Abebe E (2005) Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1935–1943.
- Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Research* **27**, 1767–1780.
- Bouchet P (2006) The magnitude of marine biodiversity. In: *The exploration of marine biodiversity* (ed. Duarte C.M.), p. 69. Natural History Museum, Paris.

Section 1. General introduction

- Brooks DR, McLennan DA (1991) *Phylogeny, Ecology and Behavior: A Research Program in Comparative Biology* University of Chicago Press, Chicago.
- Brosing A (2008) A reconstruction of an evolutionary scenario for the Brachyura (Decapoda) in the context of the Cretaceous-Tertiary boundary. *Crustaceana* **81**, 271-287.
- Brusca RC, Brusca GJ (2003) Phylum Arthropoda: The Crustacea. In: *Invertebrates*, p. 936. Sinauer Associates, Inc., Massachusetts.
- Bush GL, Butlin RK (2004) Sympatric Speciation in Insects. In: *Adaptive Speciation* (eds. Dieckman U., Doeblei M., Metz J. A. J., Tautz D.), pp. 229-247. Cambridge University Press, Cambridge.
- Carvalho GR (1998) Molecular ecology: Origins and approach. In: *Advances in Molecular Ecology*, p. 313. Carvalho, G. R. , Amsterdam.
- Carvalho GR, Creer S, Allen MJ, Costa FO, Tsigenopoulos CS, Goff-Vitry ML, et al. (2011) Genomics in the discovery and monitoring of marine biodiversity. In: *Introduction to Marine Genomics* (eds. Cock J.M., Tessmar-Raible K., Boyen C., Viard F.), p. 399.
- Claridge MF, Dawah AH, Wilson MR (1997) Practical approaches to species concepts for living organisms. In: *Species: The Units of Biodiversity* (eds. Claridge MF , Dawah AH , Wilson MR), p. 441. NHBS Environment Bookstore.
- Colgan DJ, Ponder WF, Eggler PE (2000) Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta* **29**, 29-63.
- Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of Tuatara (Sphenodon). *Nature* **347**, 177-179.
- DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1905-1916.
- Drake JW, Charlesworth B, Charlesworth D, Crow JF (1998) Rates of spontaneous mutation. *Genetics* **148**, 1667-1686.
- Dworschak PC, Cunha MR (2007) A new subfamily, Vulcanocalliacinae n.subfam., for *Vulcanocalliax arutyunovi* n.gen., n.sp. from a mud volcano in the Gulf of Cádiz (Crustacea, Decapoda, Callianassidae). *Zootaxa* **1460**, 35-46.

- Effron B, Tibshirani RJ (1993) *An introduction to the bootstrap. Monographs on statistics and applied probability* Chapman and Hall, London, U.K.
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal Molecular Evolution* **17**, 368-376.
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783-791.
- Felsenstein J (2004) *Inferring Phylogenies* Sinauer Associates, Sunderland.
- Féral JP (2002) How useful are the genetic markers in attempts to understand and manage marine biodiversity? *Journal of Experimental Marine Biology and Ecology* **268**, 121-145.
- Fitzhugh K (2005) The inferential basis of species hypotheses: the solution to defining the term ‘species’. *Marine Ecology* **26**, 155–165.
- Frankham R, Ballou JD, Briscoe D (2002) *Introduction to Conservation Genetics* Cambridge University Press, Cambridge.
- Friedlander TP, C. RJ, Mitter C (1994) Phylogenetic information content of five nuclear gene sequences in animals: initial assessment of character sets from concordance and divergence studies. *Systematic Biology* **43**, 511-525.
- Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* **18**, 4541-4550.
- Gascuel O (1997) BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* **14**, 685-695.
- Gaston KJ, Spicer JI (2003) *Biodiversity: An introduction*, 2nd edn. Blackwell Science, Oxford.
- Gomez A, Wright PJ, Lunt DH, Cancino JM, Carvalho GR, Hughes RN (2007) Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. *Proceedings of the Royal Society B-Biological Sciences* **274**, 199-207.
- Gosling EM (1994) Speciation and wide - scale genetic differentiation. In: *Genetics and evolution of aquatic organisms* (ed. Beaumont A. R.), p. 543. Chapman and Hall, London.
- Grassle JF, Maciolek NJ (1992) Deep-sea species richness: Regional and local diversity estimates from quantitative bottom samples. *The American Naturalist* **132**, 313-341.

Section 1. General introduction

- Gray JS (1997) Marine biodiversity: patterns, threats and conservation needs. *Biodiversity and Conservation* **6**, 153-175.
- Hall BG (2008) *Phylogenetic trees made easy*, 3rd edn. Sinauer Associates, Inc., Massachusetts.
- Harding RM (2002) New phylogenies: an introductory look at the coalescent In: *New uses for new phylogenies* (eds. Harvey P. H, Brown A. J. L., Smith J. M., Nee S.), pp. 15-22. Oxford University Press, Oxford.
- Harper DAT (2006) The Ordovician biodiversification: Setting an agenda for marine life. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232**, 148-166.
- Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in a population and evolutionary biology. *Tree* **4**, 6-11.
- Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal Molecular Evolution* **22**, 160-174.
- Hebert PDN, Ratnasingham S, deWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S96-S99.
- Hey J (2006) On the failure on modern species concepts. *Trends in Ecology and Evolution* **21**, 447-450.
- Hey J, Waples RS, Arnold ML, Butlin RK, Harrison RG (2003) Understanding and confronting species uncertainty in biology and conservation. *Trends in Ecology and Evolution* **18**, 597-603.
- Hillis DM (1987) Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics* **18**, 23-42.
- Holder M, Lewis OO (2003) Phylogeny estimation: traditional and bayesian approaches. *Nature Reviews Genetics* **4**, 275-284.
- Huelsenbeck JP, Rannala B (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53**, 905-913.
- Hurst GDD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society of London Series B-Biological Sciences* **272**, 1525-1534.

- Jarman SN, Nicol S, Elliot NG, McMinn A (2000) 28S rDNA Evolution in the Eumalacostraca and the phylogenetic position of krill. *Molecular Phylogenetics and Evolution* **17**, 26-36.
- Joly S, McLenachan PA, Lockhart PJ (2009) A statistical approach for distinguishing hybridization and incomplete lineage sorting. *The American Naturalist* **174**, E54-E70.
- Jones GP, Srinivasan M, Almany G (2007) Population connectivity and conservation of marine biodiversity. *Oceanography* **20**, 100-111.
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro H.N.), pp. 21-132. Academic Press, New York.
- Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **15**, 111-120.
- Kimura M (1983) Rare variant alleles in the light of the Neutral Theory. *Molecular Biology and Evolution* **1**, 84-93.
- Koenemann S, Jenner RA, Hoenemann M, Stemme T, von Reumont BM (2010) Arthropod phylogeny revisited, with a focus on crustacean relationships. *Arthropod Structure and Development* **39**, 88-110.
- Lambsead PJD, Boucher G (2003) Marine nematode deep-sea biodiversity - hyperdiverse or hype? *Journal of Biogeography* **30**, 475-485.
- Lanave C, Preparata G, Saccone C, Serio G (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20**, 86-93.
- Lane N (2009) On the origin of barcodes. *Nature* **462**, 272-274.
- Lindahl T, Karran P, Wood RD (1997) DNA excision repair pathways. *Current Opinion in Genetics and Development* **7**, 158-169.
- Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology* **19**, 3038-3051.
- Machordom A, Macpherson E (2004) Rapid radiation and cryptic speciation in squat lobsters of the genus Munida (Crustacea, Decapoda) and related genera in the South West Pacific: molecular and morphological evidence. *Molecular Phylogenetics and Evolution* **33**, 259-279.
- Maddison WP, Knowles LL (2006) Inferring Phylogeny Despite Incomplete Lineage Sorting. *Systematic Biology* **51**, 21-30.

Section 1. General introduction

- Mardulyn P, Whitfield JB (1999) Phylogenetic signal in COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): Evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics and Evolution* **12**, 282-294.
- Martin JW, Crandall KA, Felder DL (2009) *Decapod Crustacean Phylogenetics*. Taylor and Francis Group, New York.
- Martin JW, Davis GE (2001) An updated classification of the recent Crustacea. *Natural History Museum of Los Angeles* **39**, 1-124.
- McArthur AG, Koop BF (1999) Partial 28S rDNA sequences and the antiquity of hydrothermal vent endemic gastropods. *Molecular Phylogenetics and Evolution* **13**, 255-274.
- Meland K, Willlassen E (2007) The disunity of "Mysidacea" (Crustacea). *Molecular Phylogenetics and Evolution* **44**, 1083-1104.
- Meyran J-C, Monnerot M, Taberlet P (1997) Taxonomic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **8**, 1-10.
- Minin V, Abdo Z, Joyce P, Sullivan J (2003) Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology* **52**, 1-10.
- Mitsuhashi M, Sin YW, Lei HC, Chan YY, Chu KH (2007) Systematic status of the caridean families Gnathophylidae Dana and Hymenoceridae Ortmann (Crustacea: Decapoda): a preliminary examination based on nuclear rDNA sequences. *Invertebrate Systematics* **21**, 613-622.
- Moritz C (1994) Defining evolutionary significant units for conservation. *Trends in Ecology and Evolution* **9**, 373-375.
- Moriyama EN, Powell JR (1997) Synonymous substitution rates in *Drosophila*: mitochondrial versus nuclear genes. *Journal Molecular Evolution* **45**, 378-391.
- Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ruyder OA, O'Brien SJ (2001) Molecular phylogenetics and the origins of placental mammals. *Nature* **409**, 614-618.
- Nei M (1996) Molecular Evolutionary Genetics. *Ann. Rev. Genet.* **30**, 371-403.
- Nei M, Kumar S (2000) *Molecular evolution and phylogenetics* Oxford University Press, New York.

- Ohta T (1992) The nearly neutral theory of molecular evolution. *Annual Review of Ecology and Systematics* **23**, 263-286.
- Ormond RFG, Gage JD, Angel MV (1999) *Marine biodiversity: Patterns and processes*. Cambridge University Press, Cambridge
- Page RDM, Holmes EC (2007) *Molecular evolution: a phylogenetic approach*. Blackwell Science Ltd.
- Palero F, Crandall KA (2009) Phylogenetic inference using molecular data. In: *Decapod Crustacean Phylogenetics* (ed. Martin A.P., Crandall, K.A., and Felder, D.L.), pp. 67 - 88. Taylor and Francis Group, New York.
- Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* **13**, 146–158.
- Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* **7**, 121.
- Philippe H, Zhou Y, Brinkmann H, Rodrigue N, Delsuc F (2005) Heterotachy and long-branch attraction in phylogenetics. *BMC Evolutionary Biology* **5**, 1-8.
- Porter ML, Pérez-Losada M, Crandall KA (2005) Model-based multi-locus estimation of decapod phylogeny and divergence times. *Molecular Phylogenetics and Evolution* **37**, 335-369.
- Posada D (2003) Using Modeltest and PAUP to select a model of nucleotide substitution. In: *Current Protocols in Bioinformatics* (ed. Baxevanis A.D. Davison D. B., Page R. D. M., Petsko G. A., Stein L. D., and Stormo G. D.), pp. 6.5.1-6.5.14. Wiley J. and Sons, Inc., New York.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.
- Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**, 793-808.
- Reaka-kudla ML (1997) The global biodiversity of coral reefs: a comparison with rain forests. In: *Biodiversity II* (eds. Reaka-Kudla M. L. , Wilson D. E. , Wilson E. O.), pp. 83-108. Joseph Henry Press, Washington.
- Regier JC, Shultz JW (2001) Elongation factor-2: a useful gene for arthropod phylogenetics. *Molecular Phylogenetics and Evolution* **20**, 136-148.

Section 1. General introduction

- Richter S (2002) The Tetraconata concept: hexapod-crustacean relationships and the phylogeny of Crustacea. *Organisms, Diversity and Evolution* **2**, 217-237.
- Richter S, Scholtz G (2001) Phylogenetic analysis of the Malacostraca (Crustacea). *Journal of Zoological Systematics and Evolutionary Research* **39**, 113-136.
- Roff J, Zacharias M (2011) *Marine Conservation Ecology* Earthscan Ltd., London.
- Rubinof D, Cameron S, Will K (2006) A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. *Journal of Heredity* **97**, 581-594.
- Rubinoff D (2006) Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology* **20**, 1026-1033.
- Schram FR (1974) Convergences between Late Paleozoic and Modern Caridoid Malacostraca. *Systematic Zoology* **23**, 323-332.
- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Schwenk K, Ender A, Streit B (1995) What can molecular markers tell us about the evolutionary history of Daphnia species complexes? . *Hydrobiologia* **307**, 1-7.
- Schwenk K, Sand A, Boersma M, Brehm M, Mader E, Offerhaus D, Spaak P (1998) Genetic markers, genealogies and biogeographic patterns in the cladocera. *Aquatic Ecology* **32**, 37–51.
- Snelgrove PVR (2011) *Discoveries of the CENSUS OF MARINE LIFE*. Cambridge University Press, New York.
- Spears T, DeBry RW, Abele LG, Chodyla K (2005) Peracarid monophyly and interordinal phylogeny inferred from nuclear small-subunit ribosomal DNA sequences (Crustacea: Malacostraca: Peracarida). *Proceedings of the Biological Society of Washington* **118**, 117-157.
- Suzuki Y, Glazko GV, Nei M (2002) Over credibility of molecular phylogenies obtained by Bayesian phylogenetics. *PNAS* **99**, 16138-16143.
- Swofford DL, Olsen G, Waddell P, Hillis D (1996) Phylogenetic inference. In: *Molecular Systematics* (eds. Hillis D., Moritz C., Mable B.), pp. 407-514. Sinauer Associates, Sunderland.

- Taberlet P (1996) The use of mitochondrial DNA control region sequencing in conservation genetics. In: *Molecular Genetic Approaches in Conservation* (eds. Smith T. B., Wayne R.K.). Oxford University Press, New York.
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.
- Taylor EM, Lehmann AR (1998) Conservation of eukaryotic DNA repair mechanisms. *International Journal of Radiation Biology* **74**, 277-286.
- Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. *Reviews in Fish Biology and Fisheries* **19**, 265-293.
- Toon A, Finley M, Staples J, Crandall KA (2009) Decapod phylogenetics and molecular evolution. In: *Decapod Crustacean Phylogenetics* (ed. Martin A.P., Crandall, K. A., and Felder, D. L.), pp. 15-30. Taylor and Francis Group, New York.
- Van Roy P, Orr PJ, Botting JP, Muir LA, Vinther J, Lefebvre B, et al. (2010) Ordovician faunas of Burgess Shale type. *Nature* **465**, 215-218.
- Wakeley J (2004) Metapopulation models for historical inference. *Molecular Ecology* **13**, 865-875.
- Wilson K, Cahill V, Ballment E, Benzie J (2000) The complete sequence of the mitochondrial genome of the crustacean Penaeus monodon: are malacostracan crustaceans more closely related to insects than to branchiopods? . *Molecular Biology and Evolution* **17**, 863-874.
- Witt JDS, Threlloff DL, Hebert PDN (2006) DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology* **15**, 3073–3082.
- Wong EHK, Hanner RH (2008) DNA barcoding detects market substitution in North American seafood. *Food Research International* **41**, 828-837.
- Wright S (1931) Evolution in mendelian populations. *Genetics* **16**, 97-159.
- Zander RH (2004) Minimal values for reability of bootstrap and Jackknife propositions, decay index and bayesian posterior probabilities. *PhyloInformatics* **2**, 1-13.
- Zhang D-X, Hewitt GM (1996) Nuclear integrations: challenges for mitochondria DNA markers. *Tree* **11**, 247-251.

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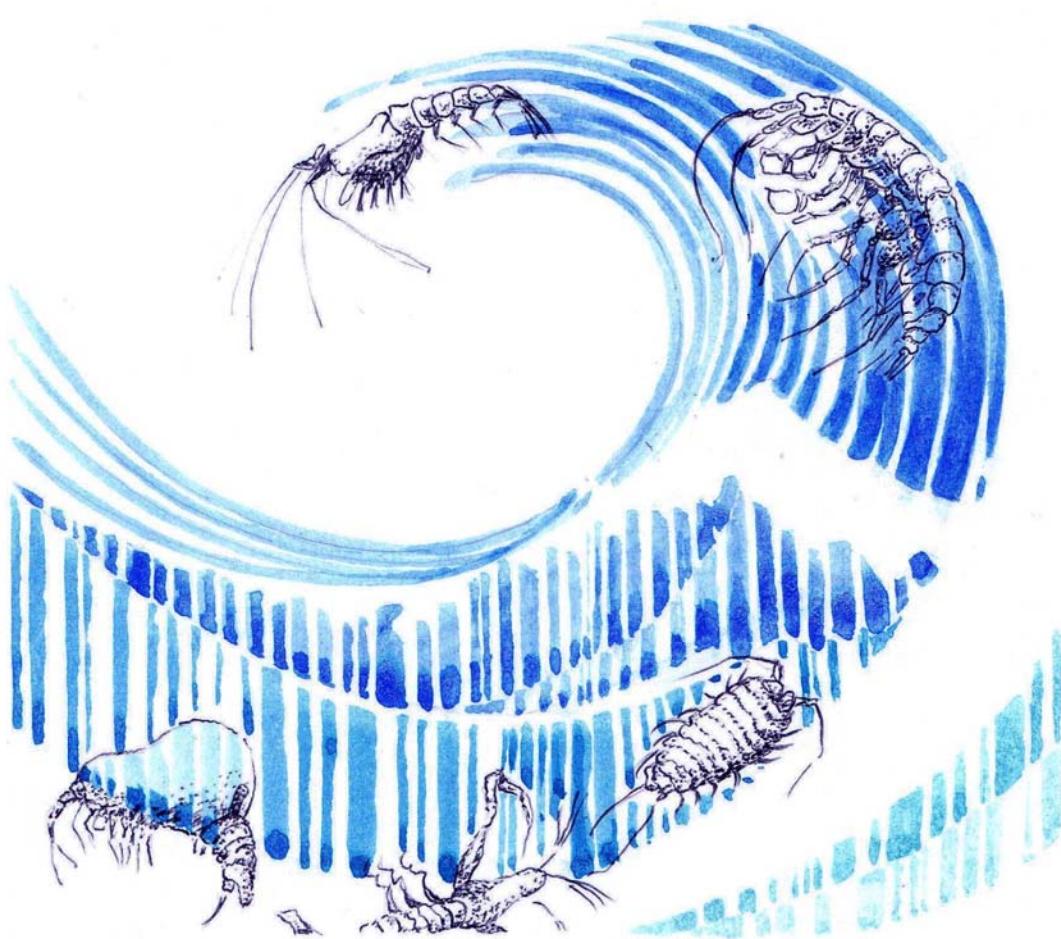
Zharkikh A (1994) Estimation of evolutionary distances between nucleotide sequences. *Journal Molecular Evolution* **39**, 315-329.

Section 2. MOLECULAR BIODIVERSITY

“Biodiversity is the core of the sustenance of life; which currently hangs in delicate balance. A sign of the growing importance of this issue is the United Nations’ declaration marking 2010 as the International Year of Biodiversity (...) thus communication have an integral place in promoting behavioural change. (...) it is imperative that it invokes the audience to think, analyse and question their existing behaviour.”

Sir David Attenborough
(The Global Green Awards for Creativity in Sustainability, 2010)

Section 2. Molecular biodiversity



Section 2. Molecular biodiversity

**CHAPTER 2.1. DNA barcodes for biodiversity as a case study of
Malacostraca**

Abstract

Last decades showed that marine biodiversity has been severely underestimated at all levels. In order to investigate diversity patterns and underlying processes an emerging tool for species identification - DNA barcoding - was proposed as a new approach based on DNA sequence variation to help to advance our knowledge of biodiversity. Despite previous crustacean studies based on DNA barcode the aim of this chapter is to test the DNA barcode concept and examine whether DNA barcoding captures species boundaries and allows species identification. For the first time three well represented orders, i.e for Amphipoda with 83 species, Decapoda with 103 and Isopoda with 151 species were analysed in conjunction. It was possible to identify initial trends of molecular biodiversity, which generate core questions for subsequent priority analysis. With a greater need for biological inventories and studies of biodiversity than ever, the complexity of Malacostraca and the lack of classical taxonomic expertise demand such an approach.

Keywords: Malacostraca; DNA barcoding; molecular biodiversity; Amphipoda; Decapoda; Isopoda

2.1.1 Introduction

With a greater need for biological inventories and studies of biodiversity, and fewer researchers to do the job, the diagnosis of biodiversity is exacerbated by various peculiarities of current taxonomic protocols (Costa and Carvalho, 2007) and by the controversy of the species concept (discussed in General Introduction). It is thus important that new approaches are explored (Schander and Willassen, 2005). More than two centuries ago, the taxonomist Carl Linnaeus introduced the binominal species nomenclature focusing mainly on morphology, and in 1942 Ernst Mayer proposed the biological species concept, which provides a framework for contemporary molecular approaches to taxonomy. Genetic information, specifically DNA sequences, has been suggested to serve as a criterion, or at least a complement, for taxonomic identification (Blaxter, 2003; Blaxter *et al.*, 2004; Mallet and Willmott, 2007; Mallet, 2006; Savolainen *et al.*, 2005; Tautz *et al.*, 2003) allowing to generate large data sets that can be used to explore basic principles of molecular evolution. Hebert *et al.* (2003a) suggested that a DNA-based identification system that employ DNA sequences as taxon 'barcodes', based on the mitochondrial gene cytochrome c oxidase subunit 1 (COI), can aid the completion of biological inventories and studies of biodiversity across the animal kingdom. Hebert *et al* (2003a,b) results indicate that sequence divergences in COI regularly enable the discrimination of closely allied species in all animal phyla except the Cnidaria. DNA barcoding reveals only a tiny segment of each species genome, but because it examines the same core region, such target sequences can be compared across all species, revealing how given sequences have changed from species to species and

over evolutionary time (Costa and Carvalho, 2010). It is expected that large data sets generated by DNA barcoding surveys will help refine such analyses and identify possible ecological or biological correlates, providing insights into what drives selective sweeps (Costa and Carvalho, 2010). Additionally, DNA barcode sequence data can prove phylogenetically informative, highlighting preliminary patterns of genomic diversity and even bridging gaps in resolution between species-level tips and deeper tree branches (Hajibabaei *et al.*, 2007b).

Given the high diversity and dramatic phenotypic changes during the development of Malacostracan species, taxonomic identification is not an easy task. Hence, the development of reliable and universal molecular tags constitutes a major requirement for taxonomy, systematic, evolution, ecological studies and for economic and conservation strategies (e.g., sustainable fisheries). The aim of this chapter is to explore how DNA barcoding is sensitive to capture species boundaries and species diversity of representative Malacostraca orders using comparative information collected among a large data set of amphipods, decapods and isopods species. Despite the inclusion of only a small proportion of representative Malacostraca orders, it is possible to identify initial trends, which generate core questions, for subsequent priority. The key here will be to focus on how such an approach might be taken forward.

The concept of DNA barcoding: utility and applications

The basic idea is simple: a small amount of DNA is extracted from the specimen, amplified and sequenced. The DNA barcode hypothesis is that the gene region sequenced is nearly identical among individuals of the same species, but clearly different between species, and therefore its sequence, can serve as an identification tag for the species, the DNA barcode. This “matching hypothesis” (Costa and Carvalho,

2007) constitutes the key starting point for launching and implementing the new bio-identification system where a database linking a given species and respective DNA barcode array will be built. However, ambiguities may also signal the presence of potentially unrecognised species that were overlooked by conventional methods, i.e. identification of different life stages, sexual dimorphism or species with large phenotypic plasticity, and finally cryptic species that are considered widely distributed in marine systems (Knowlton, 1993; Pfenninger and Schwenk, 2007). It is precisely in this type of setting that DNA barcoding can be of great assistance (Blaxter, 2004; Costa and Carvalho, 2010; Gomez *et al.*, 2007; Hebert *et al.*, 2004a; Stoeckle, 2003) and it provides a molecular basis to test species hypotheses when data are not congruent with known species boundaries (Gomez *et al.*, 2007; Hebert *et al.*, 2004a). As one example, some bivalve species in the genus *Thyasira* are distinguished only by sperm and egg morphology, which is impractical in most circumstances (Little and Stevenson, 2007). DNA barcoding is particularly valuable when taxa are difficult to raise in the laboratory, and several studies have recognized the benefit of short DNA sequences in associating immature stages with adult counterparts (Barrett and Hebert, 2005; Hebert *et al.*, 2004a; Paquin and Hedin, 2004; Vences *et al.*, 2005). As new species are discovered and identifications revisited by experts, voucher specimen identifications and the global reference database - Barcode of Life Data System (BOLD: <http://www.boldsystems.org> (Ratnasingham and Hebert, 2007) - can be updated and immediately effective. This step is in contrast to most molecular studies conducted, which do not have the possibility of specimen recovery for sequences deposited in public databases (Genbank), therefore making it difficult or impossible to verify ambiguities retrospectively (Harris, 2003; Pleijel *et al.*, 2008). In addition BOLD database is freely accessible, it can be used as an online workbench that aids the management, quality assurance. Furthermore all data can

be download after publication, analyzed directly in BOLD with distance methods and future taxonomic updates are always possible (Radulovici *et al.*, 2010).

As stated in General introduction (in Section 1) mitochondrial DNA (mtDNA) genes have long dominated the field of molecular systematics because of their maternal inheritance, limited recombination, rapid evolution, and the robustness of mtDNA against degradation, making them ideal markers for many species-level questions (Avise *et al.*, 1987). The proposal of COI as the target gene for DNA barcoding was not an arbitrary choice since decades of research showed a useful phylogenetic signal for both above- and below-species level and that ‘universal’ primers were capable to recover the 5’end of COI in most metazoan phyla (Ball *et al.*, 2005; Costa *et al.*, 2007; Hajibabaei *et al.*, 2005; Hajibabaei *et al.*, 2007b; Hebert *et al.*, 2003b; Hogg and Hebert, 2004; Kaila and Stahls, 2006; Monaghan *et al.*, 2006; Radulovici *et al.*, 2009; Smith *et al.*, 2005; Tang *et al.*, 2010; Wells *et al.*, 2001; Wells and Sperling, 2001; Wong *et al.*, 2009). Initially, the 5’ end of COI a 658 base pair fragment of the 5’ end of COI (Hebert *et al.*, 2003a; Hebert *et al.*, 2003b) was established as the barcode region for most Metazoa because it is flanked by two “universal” primers that work for a range of metazoans (Folmer *et al.*, 1994). The need to use widely applicable primers is understandable, but examination of the DNA barcoding literature reveals that the majority of projects actually rely on taxon-specific primers, rather than universal primers, in order to optimize PCR performance (Barrett and Hebert, 2005; Costa *et al.*, 2007; Hebert and Gregory, 2005; Hebert *et al.*, 2004a; Hebert *et al.*, 2004b; Ivanova *et al.*, 2005; Ivanova *et al.*, 2006), particularly with degraded material (Lambert *et al.*, 2005). In addition, some DNA barcoding projects have used even small fragments (< 400 bp) of COI (Hajibabaei *et al.*, 2007a; Hajibabaei *et al.*, 2006; Page *et al.*, 2005; Whiteman *et al.*, 2004).

Various DNA barcoding candidates gene regions have been employed for species-level biosystematics such 16S-rDNA, cytochrome *b*, 18S-rDNA, ITS1-rDNA, ITS2-rDNA, etc (Hajibabaei *et al.*, 2007a,b). For instance, different rRNA genes have been proposed to be efficient DNA barcodes in nematodes (Floyd *et al.*, 2002), tardigrades (Blaxter *et al.*, 2004), amphibians (Vences *et al.*, 2005) and hydrozoa (Moura *et al.*, 2008). Different fragments of the nuclear ribosomal DNA genes (LSU, SSU or ITS) have been extensively tested as DNA barcodes genes, i.e., for plants (Kress *et al.*, 2005), Protists (Aranda da Silva *et al.*, 2006), meiobenthic organisms (Markmann and Tautz, 2005), beetles (Monaghan *et al.*, 2005), parasitoid flies (Smith *et al.*, 2006), etc. Several combined regions of plastid DNA (e.g., rpoC1+rpoB+matK or rpoC1+matK+trnH-psbA, see for review Hollingsworth *et al.*, 2011) have been tested for plant barcoding (Hollingsworth *et al.*, 2011) and it was suggested by CBOL Plant Working group two plastid coding regions candidates, rbcL+matK, as barcode genes and when required, complemented by additional markers (Hollingsworth *et al.*, 2009).

The pursuit of regions of the genome appropriate for use as DNA barcodes in other eukaryotic life forms like fungus was proposed recently as a barcode candidates the full NADH dehydrogenase subunit 6 (ND6) gene due to their rarity of introns and to their length, above 400 bp, comparable to the lower end size of the length range of barcodes successfully used for most metazoan (Santamaria *et al.*, 2009). For decade ITS of nuclear DNA has been selected as a target for analyzing fungal diversity in environmental samples and recently selected as barcode candidate (Bellemain *et al.*, 2010).

As underlined by Costa and Carvalho (2007), the barcode classification of a given species is initially a hypothesis to be checked against the conventional classification. Using the DNA barcode as an identification key, just as the Linnean classification did

before, may provide a new and interesting source of hypotheses for species identity (Holm, 2007; Rubinoff, 2006; Rubinoff *et al.*, 2006). Where there appears to be an uncoupling between observed ecological, behavioural or morphological heterogeneity and reproductive or molecular divergence, DNA barcoding can facilitate testing alternative hypotheses or the application of alternative species concepts (Costa and Carvalho, 2007).

Although, there is no consensus on the best method to analyze DNA barcoding data because the “best method” is case related (Casiraghi *et al.*, 2010) due to variable mutation rate (Galtier *et al.*, 2009) or incomplete taxa sampling (Ekrem *et al.*, 2007; Meyer and Paulay, 2005), COI sequence clustering by neighbour joining (NJ) has been suggested as an effective and suitable way to recognize and identify animal species (Hebert *et al.*, 2003a; Hebert *et al.*, 2003b; Hebert *et al.*, 2004b) and to discover cryptic taxa (Gomez *et al.*, 2007; Hebert *et al.*, 2004a; Pfenninger *et al.*, 2007).

According to the barcoding approach, species can be identified based on a ‘barcoding gap’ between intra- and interspecific genetic distances by using a threshold value of 2.7% or a 10-fold value for species delimitation in a case study of birds (Hebert *et al.*, 2004b). Nielsen and Matz (2006) assumed that this approach seems to be too simplistic and provide some guidelines towards the use of statistics in DNA barcoding experiments. The objective of DNA barcoding analyses is to assign each query sequence to a set of referenced sequences extracted (tagged-specimen). In order to reach this aim, different bioinformatic approaches have been developed. A summary of the classification methods used in DNA barcoding approach and ways to present results is listed in Table 2.1.1 (Casiraghi *et al.*, 2010).

Table 2.1.1: Bioinformatics of DNA barcoding: examples of methods used in DNA barcode analyses and ways to present the results. Modified from Casiraghi *et al.*, (2010).

Tipology	Methods	Software/tool(s)*
Threshold (distance)	Similarity	Blastall-BLASTn; BLAT; Blastall-megaBLAST
	Pairwise distance	TaxI; TaxonDNA
	K2P distance	MUSCLE; MEGA; BOLD-IDS
Phylogenetic	Patristic distance	MrBayes; PALIP; APE; Perl scripts
	Neighbour joining	MUSCLE; MEGA
	Maximum Parsimony	MUSCLE; TNT
	Maximum likelihood	MUSCLE; SPRI; PHYLM2
	Bayesian inference	SAP
	Coalescent based	COALESCENCE; FLUCTUATE; PAUP; MESQUITE
Character based	Diagnostic	CAOS; MATLAB; Perl scripts; DNA-BAR; DOME ID
Combined	Coalescence	TCS; MEGA; Arlequin; PAUP; Phylip

* (Casiraghi *et al.*, 2010 and references therein)

In some aspects, DNA barcoding and classical (morphology-based) taxonomy suffer from similar impediments for completing biodiversity inventories. Some of these impediments are: i) the need to rely on pre-existing taxonomic hypotheses (Kerr *et al.*, 2007; Seberg *et al.*, 2003); ii) low resolution in some cases (Vences *et al.*, 2005) (*i.e.*, hybrids, recently diverged species, species complexes or slow evolving groups); iii) incomplete geographic or taxonomic sampling (Wheeler, 2004; Zang *et al.*, 2010). As with any other method, DNA barcoding has limitations and revealed low sensitivity for species identification/delineation in cases when: potential introgression of mtDNA occurs (Bachtrog *et al.*, 2006); nuclear sequences of mitochondrial origin (*numts*) are present (Song *et al.*, 2008); and when contaminations are amplified with “universal” primers (Siddall *et al.*, 2009). DNA barcoding does not substitute current taxonomic research protocols, and therefore it can (and should) be fully integrated into taxonomic

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practice (Padial and de la Riva, 2007) instead of replacing classical taxonomy (Will and Rubinoff, 2004).

Advances in sequencing and computational technologies enabled the accomplishment of many ambitions of the Barcode of Life initiative. The International Consortium for the Barcode of Life (CBOL, <http://barcoding.si.edu>) launched in May 2004 seeks the implementation of a universal system for species identification (Costa and Carvalho, 2007), and currently includes more than 200 organizations (museums, herbaria, zoos, biodiversity research institutes, universities, conservation organizations, government agencies and private companies) from 50 nations (CBOL, <http://barcoding.si.edu>). The largest project currently operational is the International Barcode of Life (iBOL, <http://www.ibol.org>), launched in October 2010, with the goal of acquiring DNA barcodes for 500,000 species until 2015 (Radulovici *et al.*, 2010). To meet these challenges, CBOL initiated a dialogue with the major genomics repositories (e.g. National Center for Biotechnology Information (NCBI)), biodiversity organizations (e.g. Global Biodiversity Information Facility (GBIF)), major barcoding centres and the multiple taxonomic communities (Costa and Carvalho, 2007; Padial and de la Riva, 2007). The integrated bioinformatics platform that supports all phases of the analytical pathway from specimen collection to tightly validated barcode library provided by the Barcode of Life Data System (BOLD, <http://www.barcodinglife.org>) is described here in the Material and Methods section. Barcoding allows for relatively rapid (~ 12 h) and accurate identification by non-specialists of listed taxa at customs checkpoints (Little and Stevenson, 2007) with many practical and fundamental applications.

Practical applications of DNA barcoding

These new technologies have already made a large impact in most fields of biology, not least in systematics, and many recent taxonomic revisions are based on insights from DNA barcoding. There are several more ordinary applications (Table 2.1.2) that may also impact society, not only in certain scientific fields (Costa and Carvalho, 2007), but also in a range of social and economic activities (Hollingsworth, 2007). Alongside long-term, ultimate goals, such as democratisation of access to taxonomic knowledge many professions would benefit from access to automated identification systems (Hollingsworth, 2007). More pragmatically, DNA barcodes have proved useful in:

- Biosecurity: for surveillance of disease vectors (Besansky *et al.*, 2003) and invasive insects (Armstrong and Ball, 2005);
- Laws Protecting Endangered Wildlife: primates and other protected species endangered by bush meat hunting (Dawnay *et al.*, 2007; Lorenz *et al.*, 2005)) and fisheries (Ward *et al.*, 2009);
- Environmental protection: benefits of treating the meiofaunal taxa defined by barcodes without reference to their correspondence to 'species', and facilitates access to taxon groups that are not accessible to other methods of enumeration and classification (Stribling, 2006);
- Toxinological research: identification of species utilizing mitochondrial DNA from toxins (Creer, 2005; Powell *et al.*, 2006);
- Fisheries food industries and stock management: mislabelling of species in food markets (Smith *et al.*, 2008; Wong and Hanner, 2008);
- Conservation agencies: enabling the rapid and inexpensive identification for biodiversity inventories (Savolainen *et al.*, 2005);

- Analysis into Tree of Life studies (Sirovich *et al.*, 2009).

Practical application for the Malacostraca

Malacostraca constitute a group for which a robust DNA-based taxonomy would be highly valuable due to morphological convergences leading to important biodiversity under-estimation (Lefébure *et al.*, 2006; Proudlove and Wood, 2003) (see General Introduction). For this reason some groups are better investigated than others, resulting in unbalanced availability of keys and species lists. But surprisingly, many organisms, in most faunal investigations are only determined to genus, family or some higher taxon (Schander and Willassen, 2005). A compilation of 138 reports and inventories published from the North Atlantic, the Baltic Sea and the Mediterranean Sea between 1960 and 2004 showed that the percentages of specimens determined to species vary greatly between different organisms groups, but in total about one third of the specimens are not determined to species (Schander and Willassen, 2005). A search of COI sequence records in BOLD for “Malacostraca” (June 2011) retrieved - among GenBank Animals (COI) and MarBoL campaign - 893 species for three selected orders, Amphipoda, Decapoda and Isopoda. However, 13.33% of the data include species previously described but not formally named, and half of the contribution derives from Isopoda.

Cryptic species are common in crustaceans and the barcode approach helps to understand the process of cryptic speciation in ecological and evolutionary scopes (Moura *et al.*, 2008; Whiteman *et al.*, 2004). So far, 137 cryptic species (~2,6 % of total species know) of Crustacea have been discovered based on 52,000 species reported in 45,669 articles (Pfenninger and Schwenk, 2007).

Table 2.1.2: Some users of taxonomic information and their potential interest in DNA-based identification (adapted from Hollingsworth, 2007).

User	Identification need	Typical source of information for identification
Taxonomist	Assessments of diversity and distributions	Specialised literature, museum collections, field guides, databases, colleagues
Ecologist	Assessments of diversity and distributions, verification of research sample identity	Specialised literature, museum collections, field guides, databases, taxonomists, colleagues
Conservationist	Assessments of diversity and distributions, identification of specimens to conserve	Field guides, images, databases, taxonomists, colleagues
Legal	Identification of based on fragmentary material, forensic samples, wildlife crime/illicit trade	Field guides, images, target key, databases, taxonomists
Human/animal health	Identification of species with harmful attributes or medical properties	Field guides, images, target key, databases, taxonomists
Environmental protection	Identification of indicator species, identification of invasive/pest species	Field guides, images, target key, databases, specialist colleagues, taxonomists
Biodiversity utilisation (agriculture, fish management, forestry)	Identification of species with useful attributes, identification of species that impede utilisation (pests/invasive, etc)	Field guides, images, target key, databases, specialist colleagues, taxonomists
Amateur naturalist	Assessments of distributions and diversity	Specialised literature, museum collections, field guides, databases, taxonomists
Public	Occasional curiosity driven interest	Field guides, images

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The current study builds on previous barcoding work on crustaceans in both marine and freshwater species (Bucklin *et al.*, 2007; Costa *et al.*, 2007; Radulovici *et al.*, 2009). We chose to focus mainly on the orders Amphipoda (~7000 species described species), Decapoda (~15,000 described species) and Isopoda (~10,000 described species) from Class Malacostraca containing over 28,000 extant species (Richter and Scholtz, 2001) which represent the most speciose crustacean orders (i.e. with a broad disparity in morphology, anatomy, embryology, behaviour and ecology). Additionally they are an important component of marine food webs (Thorp and Covich, 2001) and specially the decapods are economically important by providing large harvests and high income to World wide, and ecologically relevant as top predators in the marine benthic ecosystem. This study adds to existing databases a large number of specimens sampled across a vast geographical area for a better representation of intraspecific variation. The geographical region of this study covers a wide range of physiographic, oceanographic and bathymetric characteristics. Although COI divergence and species identification success has been previously assessed for some marine malacostraca (Costa *et al.*, 2007; Radulovici *et al.*, 2009), the average divergence found among Malacostraca is still far from being representative of the Class (i.e., only a total of 66 (Costa *et al.*, 2007) and 87 (Radulovici *et al.*, 2009) species belonging to the Class Malacostraca, respectively, were analysed. Given their high diversity and dramatic phenotypic changes during development, malacostracan species identification is not an easy task. Hence, the development of reliable and universal molecular tags constitutes a major requirement for biodiversity, conservation, management and fisheries/aquacultures strategies and the right to refuse taxa revision (Flegel, 2007,2008) involving such emblematic and complex species.

2.1.2 Material and Methods

A protocol for creation of reference libraries of DNA barcodes (described in Figure 2.1.1) was utilised as workbench of management and analysis of specimens data (see, <http://www.barcodinglife.org>). In this section we describe two main features of the management and analysis system in BOLD used in this study.

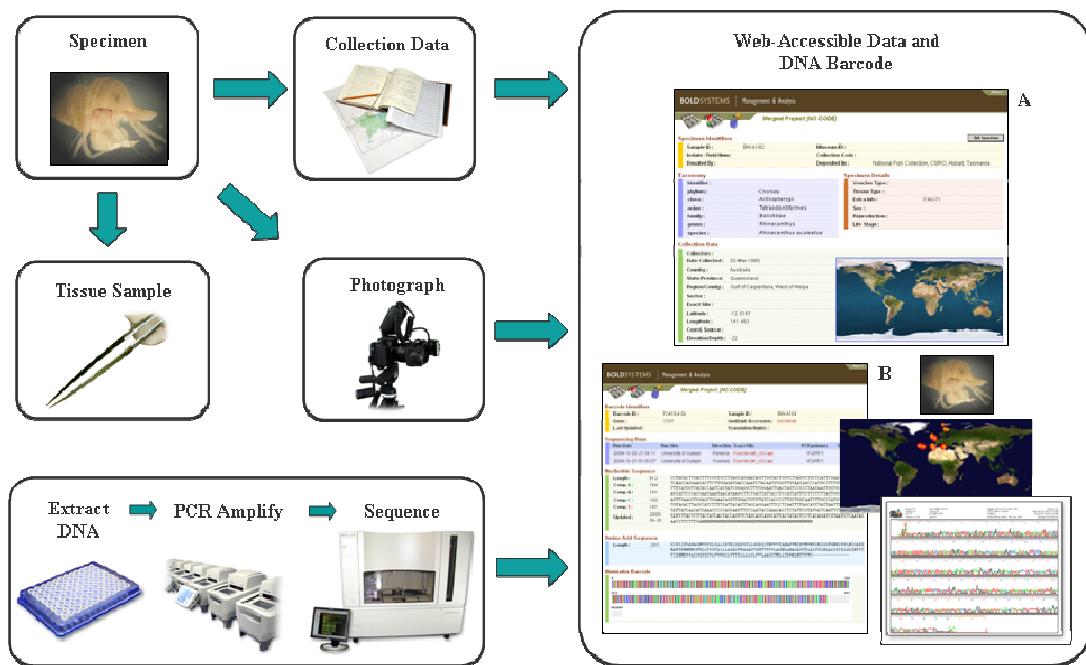


Figure 2.1.1: DNA barcode analytical chain: (A) Specimen page and (B) sequence page (figure provided personally by F.O. Costa adapted from P.D.N.Hebert).

Project establishment and data standards

Through the completion of a short online form (<http://www.barcodinglife.org/views/newuserapp.php>) we created two campaigns, Marine Life (MarBOL) and Portugal–Aquatic Life, including a total of eleven projects

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(see Table 2.1.3). The BOLD database currently host specimen records for which essentially, seven data elements are listed (Ratnasingham and Hebert, 2007):

1. Species name;
2. Voucher data;
3. Collection record;
4. Identifier of the specimen;
5. COI sequence of at least 500 base pair (bp);
6. PCR primers used to generate the amplicon;
7. Trace files.

The platform data records in BOLD consists of two main pages: “specimen page” and “sequence page” (Figure 2.1.1). The access of these pages is possible through a direct link in the project console that includes a comprehensive list of all specimens included in the project. The information found in the specimen page is illustrated in Figure 2.1.1 (A) where varied specimen data including Specimen Identifiers, Taxonomy, Specimen details, Collection Data, and Photography are deposited. Each specimen page is coupled to a sequence page (B in Figure 2.1.1) that records the barcode sequence (FASTA format), PCR primers and trace files, amino acid translation, and ultimately the GenBank accession number as well.

Specimen and metadata acquisition

We collected 926 specimens from the North East of the Atlantic Ocean and the Mediterranean Sea between 2001 and 2008 (Figure 2.1.1). The specimens comprised 173 described species of the orders Amphipoda, Decapoda, and Isopoda. Deep-water specimens were collected by the INRB - Instituto Nacional de Recurso Biológicos, I.P -

IPIMAR with trawling nets and by the IOC-UNESCO Training through Research programme and the EU funded project Hotspot Ecosystem Research on the Margins of European Seas (HERMES) using dredges, TV guided grabs and box-cores. Littoral specimens were collected at low tide using dip nets, baited traps and scuba diving along the coast of Sicily Island (Mediterranean Sea), Anglesey Island (Irish Sea), Azores Islands (North Atlantic Ocean), Svalbard Island (Greenland Sea), and Tromsøya Island (Norwegian Sea). Samples were stored in 70% ethanol (2001-2006) and in 96% ethanol (2006-2008). Morphological identifications were done by experts based on morphological criteria (meristic, morphometric and colouration) currently recognized in recent monographs from North Atlantic Ocean and Mediterranean Sea for Decapoda (De Grave *et al.*, 2009) and for Peracaridea (various monographs and numerous scattered scientific papers). Taxonomy lists followed the scientific names accepted in the World Register of Marine Species database (<http://www.marinespecies.org/>). For each specimen, detailed geographic information and where possible, reference specimens were deposited as vouchers in publicly accessible collections. In most cases, the whole specimen was stored as a morphological voucher for future reference. For a few large decapod species, only tissue (legs or abdominal muscle) was obtained for barcoding, and the samples were stored as tissue vouchers, accompanied by photographs taken prior to DNA extraction (Figure 2.1.1). All details regarding taxonomy, vouchers and collection sites with geographical coordinates can be found in the Barcode of Life Data System website (BOLD, www.barcodinglife.org) under the Marine Life (MarBOL) and Portugal – Aquatic Life campaigns (see Table 2.1.3). In order to ensure adequate geographical coverage, multiple specimens (at least two per site) from different geographical areas were examined, whenever possible, for each target species.

Table 2.1.3: DNA barcoding projects designed in this study under two campaigns of Barcode of Life Database.

Campaigns	Project name	Code	Specimens	Species
Marine Life (MarBOL)	Decapods of Mediterranean Sea	JSDME	64	23
	Decapods of Norway	JSDN	20	3
	Decapods of Svalbard	JSDSV	6	3
	Decapods of U.K (Scotland)	JSDSC	33	12
	Decapods of U.K (Wales and England)	JSDUK	72	23
Portugal - Aquatic Life	Amphipods of Portugal - Hermes	FCAPH	40	13
	Decapods of Portugal - Hermes	FCDPH	46	12
	Isopods of Portugal - Hermes	FCIOP	12	5
	Decapods of Portugal - Azores	JSDAZ	77	20
	Decapods of Portugal - IPIMAR	FCDOP	175	45
	Decapods of Portugal - IPIMAR X	JSDPX	63	25

Molecular analyses

Total genomic DNA was extracted from small amounts of tissue (1 mm^3 muscle tissue or whole legs for small specimens) using the Chelex dry release (Hajibabaei *et al.*, 2005) or QIAGEN DNeasy tissue extraction kits (QIAGEN) for older or less well preserved samples. Prior to DNA extraction, the sample was washed overnight in $50\text{ }\mu\text{l}$ of QIAGEN Buffer AE (10 mM Tris-Cl; 0.5 mM EDTA; pH 9.0) in order to rehydrate the tissue. For the Chelex dry release extraction method tissue samples were added to $120\text{ }\mu\text{l}$ of a 10:2 mixture of Chelex buffer with Proteinase K (Sigma), incubated at 55°C for 8-12 hours and subsequently heated to 95°C for 20 minutes. The barcode region was amplified with alternative sets of primers depending on PCR reaction success. The primers used are listed in Table 2.1.4. A cocktail of primers with M13 tails (Ivanova *et al.*, 2007) was used when amplification failed using the first set of primers.

All PCRs were performed in a 25 µl volume containing 1 X PCR buffer, 3 mM MgCl₂, 0.1- 0.2 mM dNTP, 1U TAQ polymerase (Promega), 5 – 10 pmol of each primer, and 2 - 10 ng of DNA template. The thermal cycling conditions consisted of 94°C for 60 s; 35-40 cycles of 94°C for 30 s, 48 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. Alternative thermal cycling conditions was consisted of 94°C for 60 s; 5 cycles of 94°C for 30 s, 45°C for 90 s, and 72°C for 60 s; 35 cycles of 94°C for 30 s, 50 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. The thermal cycling was identical for all primer except the CrustF2/HCO primer set, which was as follows: one cycle of 94°C for 60 s; 35 cycles of 94°C for 30 s, 42°C for 90 s, and 72°C for 60 s; followed by a final extension of 5 min at 72°C. PCR products were visualized on precast 1% agarose gels using the E-gel 96 system (Invitrogen). Prior to sequencing 15 µl PCR products were cleaned with 1U shrimp alkaline phosphatase (Promega) to dephosphorylate residual deoxynucleotides and 0.5 U Exonuclease I (Promega) to degrade excess primers (Werle *et al.*, 1994). The purification thermal conditions consisted of 37°C for 45 min and 80°C for 15 min. Bidirectional sequencing was performed using BigDye termination chemistry on an Applied Biosystems® 3730 sequencer by Macrogen Inc. (www.macrogen.com, South Korea). Sequences were manually checked for ambiguities and assembled in CodonCode Aligner version 1.3.0 (<http://www.codoncode.com/>). Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) implemented in MEGA 4 (Tamura *et al.*, 2007) and the amino acid translation were examined to ensure that no gaps or stop codons were present in the alignment. BLAST searches were performed for all sequences via interrogating GenBank's online nucleotide database using the megablast algorithm.

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Table 2.1.4: PCR primer sets or cocktails used to amplify COI. M13 tails are in italic when present.

Name	Ratio	Cocktail name/Primer sequence 5'- 3'	Reference
Forward			
LCO1490		GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> , 1994
CrustF1		TTTTCTACAAATCATAAAGACATTGG	Costa <i>et al.</i> , 2007
CrustF2		GGTTCTCTCCACCAACCACAARGAYATHGG	Costa <i>et al.</i> , 2007
CrustDf1		GGTCWACAAAYCATAAAGAYATTGG	Radulovici <i>et al.</i> , 2009
COL6		TYTCHACAAAYCATAAAGAYATYGG	Schubart (2009)
Reverse			
HCO2198		TAACCTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> , 1994
CrustDr1		TAAACYTCAGGRTGACCRAARAAYCA	Radulovici <i>et al.</i> , 2009
CrustR2		GGTAGAATTAGAATATACACTT	*
COH6		TADACTCDGGRTGDCAAARAAYCA	Schubart (2009)
COI-III (Forward)			
M13LCO1490	1	<i>GTAAAACGACGCCAGTGGTCAACAAATCATAAAGATATTGG</i>	This study
M13CrustF1	1	<i>GTAAAACGACGCCAGTTTCTACAAATCATAAAGACATTGG</i>	This study
LepF1_t1	1	<i>GTAAAACGACGCCAGTATTCAACCAATCATAAAGATATTGG</i>	Hebert <i>et al.</i> , 2004
COI-III (Reverse)			
M13HCO2198	1	<i>CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA</i>	This study
M13CrustR2	1	<i>CAGGAAACAGCTATGACGGTAGAATTAGAATATACACTT</i>	This study
LepR1_t1	1	<i>CAGGAAACAGCTATGACTAAACTCTGGATGTCCAAAAATCA</i>	Hebert <i>et al.</i> , 2004
M13(-21)		<i>GTAAAACGACGCCAGT</i>	Messing (1983)
M13(-27)		<i>CAGGAAACAGCTATGAC</i>	Messing (1983)

*Primer designed within the context of the BOLD- FCDPA project

All the sequences have been deposited in Genbank and accession numbers for the barcodes, specimen and collection data, sequences, trace files and primers details are available within the project files in BOLD (<http://www.barcodinglife.org>). The Consortium for the Barcode of Life, in cooperation with GenBank and the other members of the International Nucleotide Sequence database Collaboration (INSDC), have created and implemented the BARCODE data standard. “BARCODE” denomination it is just applied for records in an INSDC database when they include the following data (Hubert *et al.*, 2008):

1. Bi-directional sequences of at least 500 bp containing no ambiguous sites;
2. Links to electropherogram trace files available in the NCBI Trace Archive;

3. Sequences for the forward and reverse PCR amplification primers;
4. Species names that refer to documental names in a taxonomic publication or other documentation of the species concept used;
5. Links to voucher specimens using the approved format of institutional acronym, collection code, and catalogue ID number.

GenBank data

To reduce the bias effect of species sampling and to provide a comprehensive sister-species coverage and survey of intraspecific variation additional sequences were included from public projects of the BOLD website (<http://www.barcodinglife.org/>). Only Amphipoda and Isopoda (Peracaridea) sequences were imported from public projects (see supplement Table S 2.1.1 in 2.1 Annex) due to the unbalanced representation of our data compared with Decapoda. The BOLD platform provides to all publicly accessible COI barcode sequences, as for example the GenBank Animals (COI) collection and MarBoL campaign. Sequences were omitted if they a) were not assigned to a species, b) suspected of being derived from misidentified, mislabelled species, c) displayed intraspecific distances > 10% exhibited stop codons or indels inducing reading frame shift and d) were less than 500 bp in length within the COI barcode region. Finally 266 sequences from 147 species, 66 genera, 22 families from Amphipoda and 70 species, 42 genera and 26 families from Isopoda were included with sufficient length and quality according to the stringent criteria described. Standardization was made for comparisons of a maximum of five individuals per species based on the average number of five specimens per species in novel data (in this study).

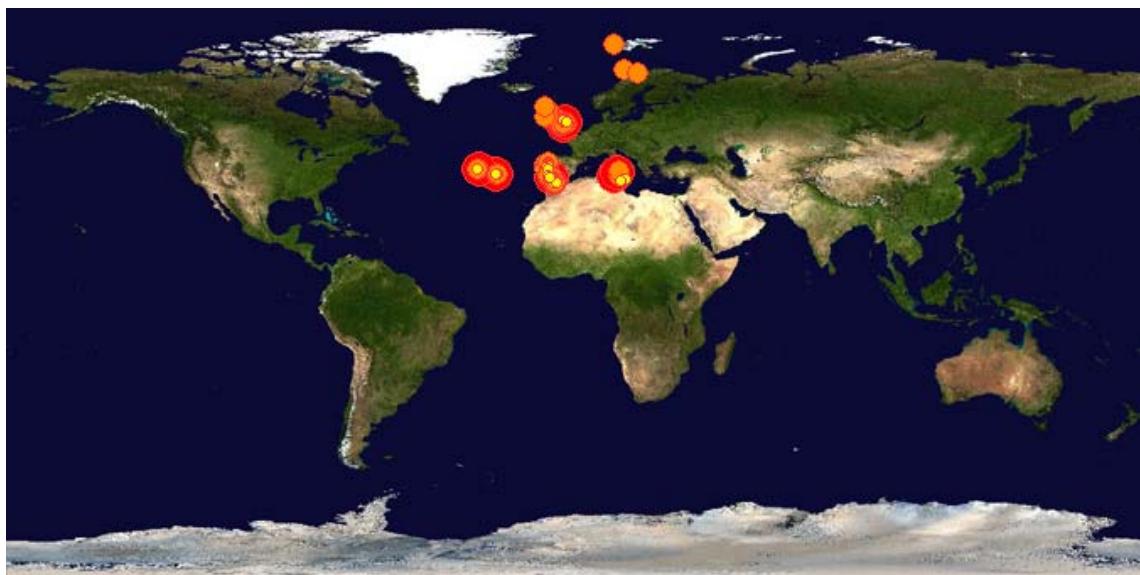


Figure 2.1.2: Sampling sites of Malacostraca specimens from North East Atlantic Ocean and Mediterranean Sea (2001 to 2008). The ranges of specimens collected per species are represented by yellow (1 to 5), orange (5 to 50) and red balls (> 50), respectively.

Analytical tools

Sequence divergence was calculated using the Kimura 2-parameter (K2P) model (Kimura, 1980) and the mid-point rooted Neighbour-joining (NJ) tree of K2P distances was created to provide a graphic representation of the species divergence (Saitou and Nei, 1987) as implemented in the sequence “Analysis module” in BOLD. The distance to the nearest neighbour for each of the species in the list of specimens was summarized as implemented in “Nearest Neighbour Summary” in BOLD platform. Distances are highlighted if the distance to the nearest neighbor is less than 2%, or when the distance is less than the intra-specific distance (<http://www.boldsystems.org>). All sequences were aligned and a Neighbour Joining tree (Saitou and Nei, 1987) with 1000 bootstrap value produced using MEGA 4 (Tamura *et al.*, 2007). Tree sequences clustering far from their known taxonomic or phylogenetic position, and non-monophyletic, putative

cryptic species and congeneric species with distance values lower than 2% were recorded.

2.1.3 Results

A total of 926 specimens have been sampled, successful amplifications of the barcode region were obtained for 132 out of 173 species examined in this study. The primer sets used (Figure 2.1.3) amplified successfully the COI region of 601 specimens, belonging to 132 species, 96 genera and 61 families. Amplification failed in specimens stored in 70% ethanol before 2006 or having a proximately body size smaller than 1 mm³. Short or low quality sequences (double peaks, background noise) obtained from 40 specimens and possibly representing *numts* (see Chapter 2.2) were discarded. Only 30% of our sequences matched with other in the GenBank because most species in our study had not been COI sequenced before. Additionally, BLASTs of barcode sequences of the isopod *Gnathia* sp. (n=3) and *Metacirolana hansenii* (Bonnier, 1896) (n=2) did not return a crustacean as nearest match, due the under representation of this order. The number of sequences per species varied between 1-33, with a mean of 5, and an average length of 625 base pairs (bp). All of the GenBank COI records created by this project only 65% carry the BARCODE keyword due to the high occurrence of reverse trace files with low quality from our group of primers sets.

Well defined genetic variation through increasing taxonomic levels was observed, supporting a marked change of genetic divergence at the species boundaries (Figure 2.1.3).

Table 2.1.5: List of sequences retrieved from GenBank displaying conflicting species assignments or unusually high intraspecific divergences when compared against other published mitochondrial barcodes.

Taxon	Species	Data source	GenBank no.	Mismatches
Amphipoda				
Ampithoidae	<i>Ampithoe longimana</i> Smith, 1873	Macdonald <i>et al.</i> , 2005	AY926653	18% divergence when compared with conspecific in Radulovici <i>et al.</i> , 2009
Crangonyctidae	<i>Crangonyx floridanus</i> (Bousfield, 1963)	Johanna <i>et al.</i> , 2010	AJ968909	23% divergence when compared with other conspecific specimens from the same region
	<i>Acanthogammarus victori</i> Dybowsky, 1874	Oju <i>et al.</i> , 2009 (unpublished)	FJ756298	75% divergence when compared with conspecific in Macdonal <i>et al.</i> , 2005
	<i>Acanthogammarus victori</i> Dybowsky, 1874	Macdonald <i>et al.</i> , 2005	AY926652	14% divergence when compared with conspecific AY061799 in Vainola <i>et al.</i> , 2001
Decapoda				
Grapsidae	<i>Pachygrapsus maurus</i> (Lucas, 1846)	Matzen da Silva <i>et al.</i> , 2011	no.	<2% divergence with <i>P. marmoratus</i>
Inachidae	<i>Macropodia longipes</i> (A. Milne-Edwards & Bouvier, 1899)	Matzen da Silva <i>et al.</i> , 2011	no.	<2% divergence with <i>M. tenuirostris</i>
Isopoda				
Asellidae	<i>Lirceolus cocyetus</i> Lewis, 2001	Krejca 2005 (unpublished)	AY566527-28; 42-44	>20% divergence when compared with conspecific from different region
	<i>Lirceolus hardeni</i> Lewis and Bowman, 1996	Krejca 2005 (unpublished)	AY566534-40	>12% divergence when compared with conspecific from different region
	<i>Proasellus annautovici</i> (Remy, 1932, 1941)	Wysocka <i>et al.</i> , 2006 (unpublished)	DQ305141	75% divergence when compared with conspecific
	<i>Proasellus annautovici</i> (Remy, 1932, 1941)	Wysocka <i>et al.</i> , 2006 unpublished	DQ305138-40	>12% divergence when compared with conspecific from different region
	<i>Proasellus remyi</i> (Monod, 1932)	Wysocka <i>et al.</i> , 2006 (unpublished)	DQ305129-30	>12% divergence when compared with conspecific from different region
Cirolanidae	<i>Cirolana rugicauda</i> Heller, 1861	Wetzer 2001	AF255788	>40% divergence when compared with conspecific (AF260839-40)
Cymothoidae	<i>Olencira praegustator</i> (Latrebe, 1802)	Wetzer 2001	AF255791	75% divergence when compared with conspecific in Wetzer 2001
Janiidae	<i>Ianiropsis epilitoralis</i> Menzies, 1952	Osborn 2009	EF682303	75% divergence when compared with conspecific in Wetzer 2001
Ligiidae	<i>Ligia exotica</i> Roux, 1828	Markow and Pfeiler 2010	GU270929	75% divergence when compared with conspecific AY051320 in Arnedo <i>et al.</i> 2001 (unpublished)
	<i>Ligia howensis</i> Dana, 1853	Arnedo <i>et al.</i> , 2001 (unpublished)	AY051324-28	>10% divergence when compared with conspecific from different region
	<i>Ligia pertensi</i> (Dollfus, 1900)	Arnedo <i>et al.</i> , 2001 (unpublished)	AY051332-36	>10% divergence when compared with conspecific from different region
	<i>Ligidium beieri</i> Strohal, 1928	Klossa-Kilia <i>et al.</i> , 2006	DQ182809; 820-823	>20% divergence when compared with conspecific from different region
	<i>Ligidium germanicum</i> Verhoeff, 1901	Klossa-Kilia <i>et al.</i> , 2006	DQ182795-99	>20% divergence when compared with conspecific from different region
	<i>Ligidium gligii</i> Arcangeli, 1928	Klossa-Kilia <i>et al.</i> , 2006	DQ182824-26; 38-39	>20% divergence when compared with conspecific from different region
	<i>Ligidium wernerii</i> Strohal, 1937	Klossa-Kilia <i>et al.</i> , 2006	DQ182827-30	>20% divergence when compared with conspecific from different region

Table 2.1.5: Continued

Taxon	Species	Data source	GenBank no.	Mismatches
Munnopsidae	<i>Acanthocope galatheae</i> Wolff, 1962	Osborn 2008	EF682285	<2% divergence with Lipomerinae genus (EF682297)
	<i>Munnopsis</i> sp.		EF682272	Cluster with Gamma proteobacteria species (Siddall <i>et al.</i> , 2009)
Munnopsidae	<i>Acanthocope galatheae</i> Wolff, 1962	Osborn 2009	EF682285	<2% divergence with Lipomerinae genus (EF682297)

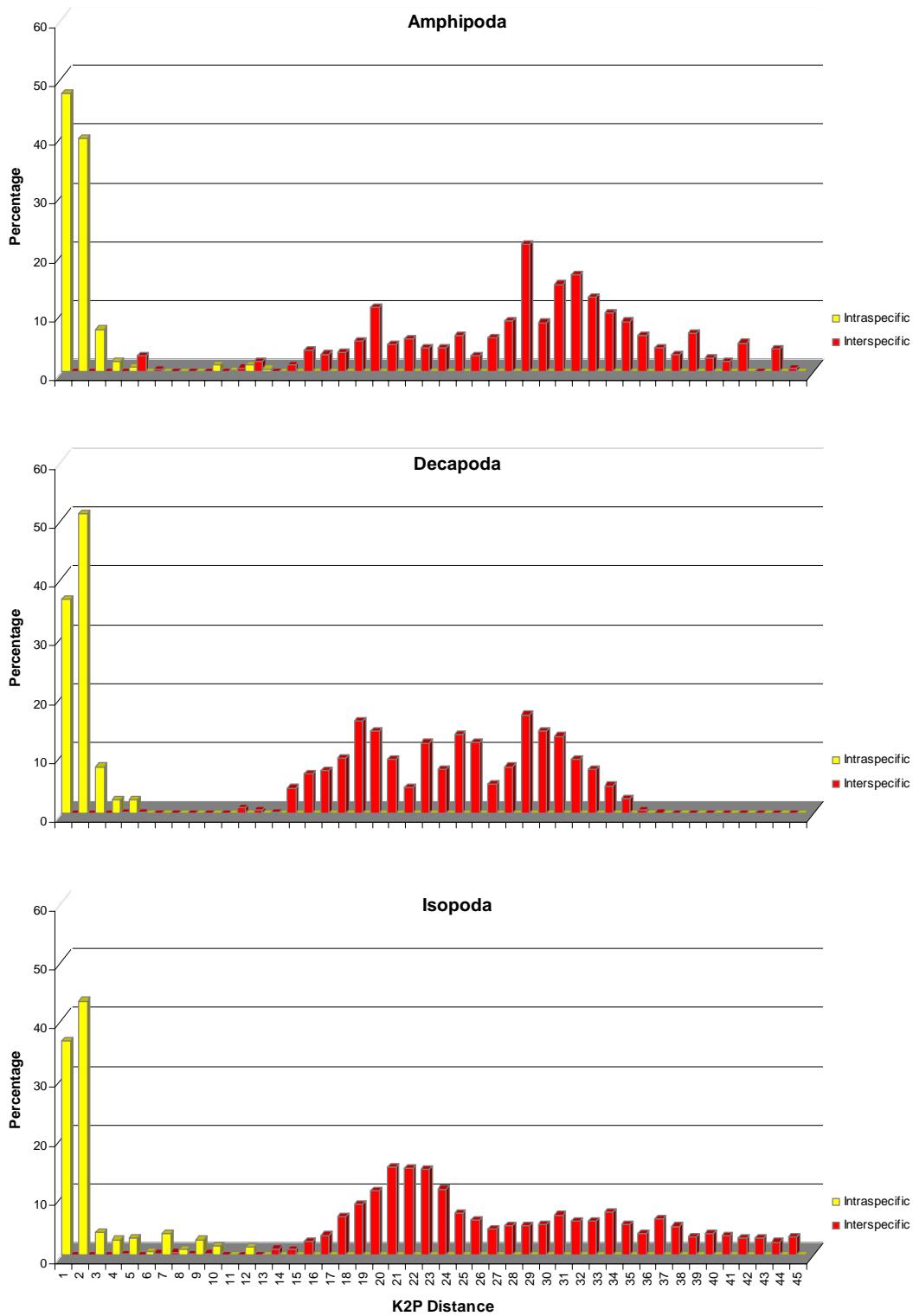


Figure 2.1.3: Frequency distribution of intraspecific and interspecific COI barcode distances (K2P) from pairwise comparisons among members of the order Amphipoda, Decapoda and Isopoda (see Table 2.1.6).

Morphological species were represented by individual clusters containing highly similar sequences in 95.45% of cases. However, within this newly-generated dataset, five species barcodes conflicted with the assigned morphological taxonomic identification: one case of high intraspecific divergence, greater than 3%, were observed in *Xantho hydrophylus* (Herbst, 1790) and two cases of low interspecific divergence were observed in *Macropodia longipes* (A. Milne-Edwards & Bouvier, 1899) and *M. tenuirostris*, *Pachygrapsus maurus* (Lucas, 1846) and *P. marmoratus* (Fabricius, 1787), respectively.

Data analysis

Using the combined COI barcode projects from BOLD (see Table S 2.1.2), we provide the most comprehensive COI data set so far examined for the Malacostraca. The combined dataset includes GenBank published sequences, COI barcode projects from the BOLD and new data generated herein (Table 2.1.3). Ambiguities were present: indels in the COI sequence, high intraspecific divergence and odd sequences were identified, excluded from the final alignment and listened in Table 2.1.5. Collectively, the combined dataset provide barcoding coverage for 902 sequences of 335 species, 202 genera, and 101 families. The entire K2P/NJ tree derived from this study is available in 2.1 Annex, Figure S 2.1.1.

A gradual increase of genetic variation through increasing taxonomic levels was observed (Figure 2.1.3) however partially overlapped as K2P distances ranged from 0% to 11.1% among conspecifics and 2.29% to 46.70% among congeneric species.

The analysis of the distribution of the nearest-neighbour distance (NND), namely the minimum genetic distance between species and its closest congeneric relative revealed that 95.5% of the NND was higher than 10% (Figure 2.1.4) and only 7.46% of the

maximum intraspecific was between 3 to 12% divergence values and 1.2% of the NND (four cases) were lower than 2.7% (Table 2.1.6).

NND average 18.67%, which was 30-fold higher than the mean within species distance of around 0.61% and the 15-fold higher than the mean maximum intraspecific distance of 1.26%. Overlap in the distribution of the genetic distances between conspecifics individuals and congeneric species is represented by 10.9% of the results (see Figure 2.1.4).

In few cases we detected deep divergences (>10%) among individuals that had been assigned to species (Table 2.1.6). This suggests that a careful reassessment of the current taxonomy for these groups could prove informative.

Table 2.1.6: Summary of the genetic divergence (K2P) for increasing taxonomic levels. Data are from 902 sequences from 335 species, 202 genera, 101 families and three orders.

Taxon ^a	no. sequences	Taxa	Comparisons	Min. distance	Mean distance ^b	Max. distance
Class Malacostraca						
Within Species	752	201	1228	0	0.76 ± 0.045	11.1
Within Genus	840	145	3842	2.29	19.99 ± 0.084	46.70
Within Family	890	90	2697	9.66	30.09 ± 0.142	61.44
Within Order	902	3	129795	14.90	30.80 ± 0.018	66.02
Within Class	902	1	268355	16.17	35.10 ± 0.011	70.23
Order Amphipoda						
Within Species	234	59	403	0	0.63 ± 0.087	11.1
Within Genus	250	42	838	3.13	22.31 ± 0.235	44.04
Within Family	259	26	713	9.66	30.89 ± 0.194	43.87
Within Order	265	1	32737	18.96	34.29 ± 0.035	63.93
Order Decapoda						
Within Species	338	85	588	0	0.54 ± 0.03	3.8
Within Genus	350	66	746	2.68	20.96 ^c ± 0.221	32.75
Within Family	359	43	839	12.49	23.65 ± 0.167	34.36
Within Order	362	1	63056	14.90	26.66 ± 0.015	40.99
Order Isopoda						
Within Species	180	57	237	0	1.53 ± 0.156	10.28
Within Genus	240	37	2258	2.29	18.81 ± 0.075	46.70
Within Family	272	21	1145	9.80	33.19 ± 0.217	61.44
Within Order	275	1	34002	17.60	34.89 ± 0.035	66.02

^a Number of species with more than one sequence and number of sequence analysed are show in column “no. sequences”; ^b Data reported as K2P distance (%) ± standard error (SE); ^c Putative ambiguities were present in three genera and when removed from the analysis the mean distance value drops to 17.55%; (see discussion).

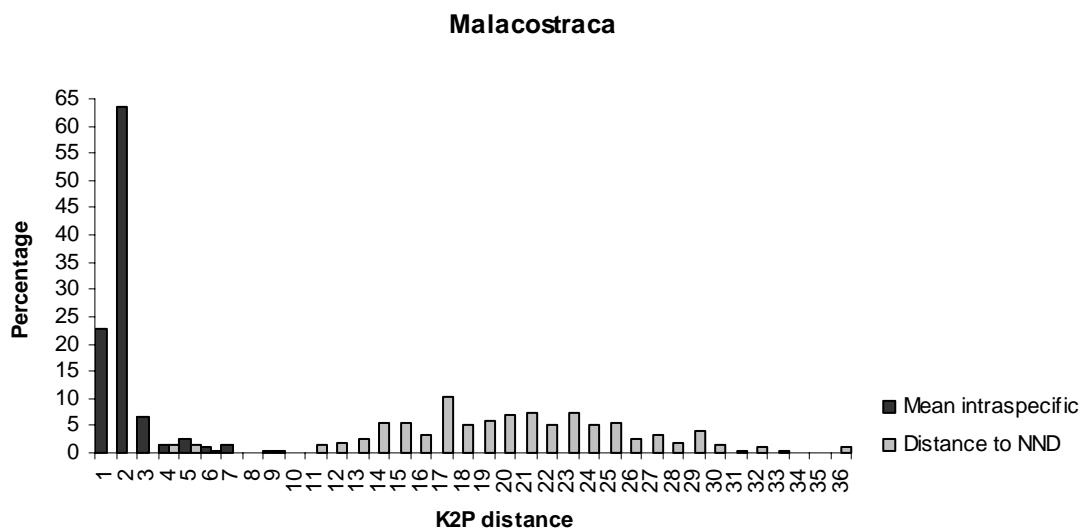


Figure 2.1.4: Distribution of the genetic distances to the nearest-neighbour and mean intra-specific distance at COI sequences for 335 Malacostraca species compared.

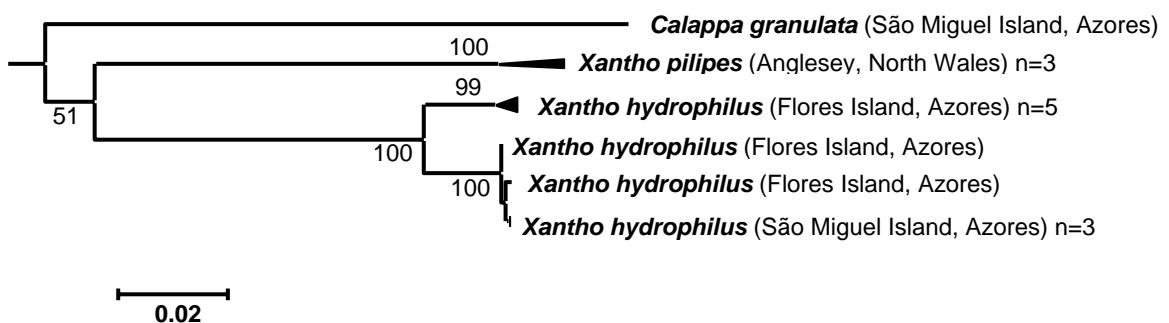


Figure 2.1.5: Branches of the neighbour-joining tree (K2P model) highlighting the putative species complexes (and related species) found in decapods in this study. Bootstrap values based on 1000 replications are included.

2.1.4 Discussion

Our analysis shows a general increase of the molecular divergence of COI with taxonomic rank, a trend that suggests that morphological taxonomy is roughly in agreement with DNA evolution (Table 2.1.6). This is due the fact that diversification within species is driven by mutation at a rate higher than speciation within lineages leading to a “barcoding gap” in the distribution of the pairwise distance between conspecific individuals and between species (Meyer and Paulay, 2005). Yet, this relationship is not entirely consistent, and the distribution of divergence at different taxonomic scales sometimes overlaps. The present study confirms that, in the vast majority of taxa examined here the barcoding gap was observed in a frequency distribution of: 1) intraspecific and interspecific COI barcode (Figure 2.1.3); and 2) genetic distances to the nearest-neighbour and mean intra-specific distance at COI sequences for 335 Malacostraca species (Figure 2.1.4. and Table 2.1.7). Among the set of 335 species, few species exhibited barcode sequences for which distance values were shared or overlapped with those of other species and three main factors have been suggested (Joly *et al.*, 2009; Meyer and Paulay, 2005) and described below. First, on coalescence theory point of view the amount of time elapsed between the introduction of a mutation and the arising of a particular allele or gene distribution in a population was incomplete (Joly *et al.*, 2009; Tajima, 1983). Second, the taxa may share polymorphism due to introgressive hybridization (Joly *et al.*, 2009). If it is that case it will be detected due the presence of two divergent clusters, each hybrid specimen being found predominantly in one species or the other (Hubert *et al.*, 2008). Finally, COI barcodes may reveal cryptic speciation. Although a uniform threshold (species can be

identified based on a ‘barcoding gap’ between intra- and interspecific genetic distances by using a threshold value of 2.7% (Hebert *et al.*, 2004b) may be a source of error leading to erroneous assignment of individuals to species (Hickerson *et al.*, 2006; Hubert *et al.*, 2008; Meier *et al.*, 2006). Hubert *et al.*, (2008) reported that 24 species from Canadian freshwater with monophyletic COI lineages would have been overlooked with 1% threshold. Alternatives for statistical analysis with more realistic models under a coalescent tools framework have been suggested increasing the statistical power of individual assignment through the use of a single gene (Abdo and Golding, 2007; Nielsen and Matz, 2006). Although such methods are computationally expensive, especially in the case of large sample sizes and large number of nucleotide patterns (minimum number of mutation per site) (Abdo and Golding, 2007). In the largest barcoding studies conducted so far on Malacostraca (see Table S1 in Radulovici *et al.*, 2009) the average observed distances were: within species 0.51% , within genus 22.66% , within family 28.64% , within order 31.73%, and finally within Malacostraca Class 34.73%. The average distance values reported in Table 2.1.6 revealed a pattern markedly higher than in Radulovici *et al.* (2009) studies. In addition this result confirms the observation reported in Costa *et al.*, (2007) in which the levels of sequence divergence among congeneric species of crustacean have the highest value yet reported: e.g, 6.1% for lepidopterans (Hebert *et al.*, 2003a); 7.9% for birds (Hebert *et al.*, 2004b); 8.3% for freshwater fishes (Hubert *et al.*, 2008); and 9.9% for marine fishes (Ward *et al.*, 2005).

Section 2. Molecular biodiversity

Table 2.1.7: Summary of the malacostraca diversity and distribution of the genetic distance of each of 132 species analysed to the nearest-neighbour at COI (K2P distance).

Order	Family	Number of Species	Number		
			< 0.1	0.1 - 1.0	1.0 - 2.7
Amphipoda	Acanthogammaridae	1			1
	Ampeliscidae	4			4
	Ampithoidae	2			2
	Brachyscelidae	1			1
	Calliopiidae	2			2
	Caprellidae	5			5
	Carangoliopsidae	1			1
	Crangonyctidae	3			3
	Cyamidae	3			3
	Cystisomatidae	2			2
	Epimeriidae	1			1
	Eusiridae	3			3
	Gammarellidae	1			1
	Gammaridae	10			10
	Hyalidae	2			2
	Hyperiidae	3			3
	Ischyroceridae	2			2
	Liljeborgiidae	1			1
	Lysianassidae	8			8
	Melitidae	2			2
	null	1			1
	Oedicerotidae	4			4
	Oxycephalidae	2			2
	Paramelitidae	6			6
	Phoxocephalidae	1			1
	Pleustidae	1			1
	Pontoporeiidae	2			2
	Scinidae	1			1
Decapoda	Sebidae	1			1
	Talitridae	3			3
	Uristidae	3			3
	Vibiliidae	1			1
	Alpheidae	2			2
	Aristeidae	2			2
	Atelecyclidae	1			1
	Calappidae	1			1
	Callianassidae	1			1
	Cancridae	2			2
	Carcinidae	4			4
	Corystidae	1			1
	Crangonidae	5			5
	Cymonomidae	1			1
	Diogenidae	4			4
	Dorippidae	1			1
	Dromiidae	1			1
	Epialtidae	2			2
	Eriphiidae	1			1
	Galatheidae	4			4

Table 2.1.7: Continued

Order	Family	Number			
		of Species	< 0.1	0.1 - 1.0	1.0 - 2.7
Decapoda	Geryonidae	1			1
	Goneplacidae	1			1
	Grapsidae	1			1
	Hippolytidae	2			2
	Homolidae	1			1
	Inachidae	4			4
	Laomediidae	1			1
	Latreilliidae	1			1
	Leucosiidae	1			1
	Lithodidae	1			1
	Macropipidae	3			3
	Majidae	2			2
	Nephropidae	2			2
	Oplophoridae	5			5
	Oregoniidae	2		2	
	Paguridae	6			6
	Palaemonidae	2			2
	Palinuridae	1			1
	Pandalidae	10			10
	Pasiphaeidae	4			4
	Penaeidae	4			4
	Pilumnidae	2			2
	Plagusiidae	1			1
	Polychelidae	2			2
	Porcellanidae	1			1
	Processidae	1			1
	Scyllaridae	1			1
	Sergestidae	2			2
	Solenoceridae	2			2
	Xanthidae	3			3
Isopoda	Aegidae	2			2
	Amphisopodidae	4			4
	Armadillidiidae	2			2
	Asellidae	10			10
	Cirolanidae	9			9
	Cymothoidae	2			2
	Gnathiidae	1			1
	Idoteidae	4			4
	Ischnomesidae	1			1
	Janirellidae	1			1
	Janiridae	1			1
	Ligiidae	5			5
	Munnopsidae	34			34
	Philosciidae	1			1
	Phreatoicidae	23		2	21
	Porcellionidae	2			2
	Santiidae	2			2
	Scyphacidae	23			23

Table 2.1.7: Continued

Order	Family	Number of Species	Number			
			< 0.1	0.1 - 1.0	1.0 - 2.7	> 2.7
Isopoda	Tainisopidae	12				12
	Serolidae	3				3
	Sphaeromatidae	4				4
	Stenasellidae	2				2
	Tainisopidae	12				12
	Trachelipodidae	1				1
	Tylidae	2				2
Total		335	0	0	4	331

Sequence indels in COI gene

Indels are much less common in protein-coding genes because they ordinarily lead to a shift in the reading frame, savaging the gene product but they are not unknown in the COI-5' segment (i.e., COI barcode region) (Ketmaier *et al.*, 2008; Wang *et al.*, 2008).

In some cases, all members of a major taxonomic group share a 3bp indel, suggesting its occurrence early in evolution of the lineage (http://www.dnabarcoding.ca/primer/Data_Analysis.html). In this study amphipods species *Brachyscelus crusculum* Bate, 1861 and *Calamorhynchus pellucidus* Streets, 1878, showed two 3bp insertions at nucleotide positions 122 and 361, one 3bp insertion at nucleotide position 122 for *Cranocephalus scleroticus* (Street, 1878) in contrast with 3bp deletion at nucleotide position 474 for *Vibilia viatrix* Bovallius, 1887 (for a COI barcode region with 658bp length). In other cases, indels have a much narrower taxonomic distribution, suggesting their recent origin. It was suggested by Ketmaier *et al.* (2008) that the indels may be result of natural selection or genetic drift, depending on how they alter the overall functionality of the COI gene. Up to now, only members of the phylum Aschelminthes (vermiform animal, closely associated with the Platyhelminthes) were found to possess a 3bp insert at nucleotide position 78

(http://www.dnabarcoding.ca/primer/Data_Analysis.html). Nevertheless, to understand why this phenomenon only happens in this group of species and how the indels are generated in COI gene, a full-scale sequence analysis of species populations as well as functional studies on the deleterious/insertions proteins are required.

Decapoda data

The COI sequences of one morphological species, i.e. *Xantho hydrophylus* (Herbst, 1790), revealed maximum intraspecific distances higher than 3%, where *X. hydrophylus* grouped into 2 clusters (Figure 2.1.5) that diverged by 2.8%, suggesting either the presence of cryptic species or nuclear mitochondrial pseudogenes (see Chapter 2.2). The occurrence of sequences with high divergence, many ambiguity codes, and aberrant nucleotide composition and/or phylogenetic trees with unusually long branches and nonsensical systematic relationships have been attributed to the presence of *numts* in COI data (Buhay, 2009; Song *et al.*, 2008). The occurrence of stop codons was not registered and the proportion of adenine – thymine and amino acids composition did not differ between specimens. A growing concern regarding *numts* (Schubart, 2009) and DNA barcoding is that, if undetected (for more details see Chapter 2.2), *numts* might lead to an overestimation of species richness (Song *et al.*, 2008). Intraspecific clusters were not related to geography because specimens collected from São Miguel and Flores islands with approximate distance of 360 miles were found in both clusters. Consequently, we suggest that the decapod *X. hydrophylus* may represent cryptic species. Additional taxonomic, ecological and molecular work is required to investigate the full extent of cryptic speciation for this case.

Oregoniidae family in Table 2.1.7 showed low levels of interspecific diversity. This family is represented only by two species that has been debated to be recently evolved (Matzen da Silva *et al.*, 2011a; Radulovici *et al.*, 2009).

High COI divergence within genus (> 20%) in: (1) *Plesionika* Bate, 1888 (six species); (2) *Pontocaris* Bate, 1888 (two species); and (3) *Systellaspis* (Faxon, 1893) (two species) indicates that species are genetically distinct and systematically very diverse in contrast with other congeneric species of Decapoda. In the first case the extensive diversification in the genus *Plesionika* with 92 extant described species (De Grave *et al.*, 2009) certainly lead to a need of systematic clarifications and it is discussed with more detail in chapter 3.3 of this study. The second and third cases, despite few numbers of representative species, may indicate a systematic conflict of morphological taxonomy approach, e.g., *Pontocaris lacazei* and *P. cataphracta* have been replaced to the genus *Aegaeon* by Chan (1996) (d'Udekem d'Acoz, 1999). Additional insights on systematic affinities can be obtained for *Plesionika*, *Pontocaris* and *Systellaspis* if a combination with additional taxa and genes will be analysed.

Peracaridae data

Amphipods and isopods are members of the superorder Peracarida, and a synapomorphy of the superorder is sharing an dispersive life style with no free-living larvae, direct development with young emerging with the adult morphology, and thus there is purported poor dispersal ability (Wetzer, 2001). A larger geographic coverage and the inclusion of groups with lower potential for dispersal brought interesting results in this study. In this study Peracarida revealed a consistent pattern in which particular lineages always had relatively large sequence divergences within and between species (Figure 2.1.3 and Table 2.1.6), whereas other lineages had consistently smaller

divergences. Overlap in the distribution of the genetic distances between conspecific individuals and congeneric species may originate from deep intraspecific divergences, low sister-species divergence (Figure 2.1.3 and 2.5) or simply revealing a systematic (i.e., for amphipods and isopods 33.8% and 61.05% of genbank COI data, respectively include species previously described but not formally named) and taxonomic conflict among these species (Table 2.1.5 lists deep intraspecific divergence found in GenBank's data set). Barcoding studies have the ability to screen large sample sizes and flag cases of intraspecific deep divergence (i.e., revealing species complexes or phylogeographic patterns). Although most of the studies included here do not belong to global barcode projects, the intraspecific deep divergence encountered in amphipods and isopods data reveal interesting patterns. Deep divergence within the amphipod species *Amphithoe longimana* Smith, 1873 (3.74%) reflected the different geographic origin of the data, Lake Baikal (Macdonald *et al.*, 2005) and St. Lawrence Gulf (Radulovici *et al.*, 2009), respectively. An interesting study by Kaliszewska *et al.* (2005) about right whale *Cyamus* host (amphipods) reveals high levels of nucleotide diversity between northern and southern oceans (8 to 11%) but almost no population structure within oceans. Mitochondrial clock calibrations suggested that these divergences occurred around 6 million years ago (Ma) (Kaliszewska *et al.*, 2005). Deep intraspecific divergence (< 11%) have been found among isopods in relation to geography, specially among subterranean species *Androlana lira* (Hutchins *et al.*, 2010), freshwater *Cirolanides texensis* J. E. Benedict, 1896 (unpublished) and marine species *Cirolana harfordi* (Lockington, 1877) (Wetzer, 2001) highlighting the importance of considering each geographic clade a distinct management unit in order to preserve the total genetic diversity for the species. Terrestrial *Ligidium* species (Klossa-Kilia *et al.*, 2006) revealed much higher deep intraspecific divergence than observed in

the literature (> 20%, see Table 2.1.5). Although this genus occurs exclusively in terrestrial environments it is very interesting in a perspective of an ecological and evolutionary point of view to mention as example. In this case the authors attributed the high deep intraspecific divergence observed to the strict ecological specialization (e.g., woodlouse, the external environmental temperature relates directly to their rate of respiration, females will keep fertilised eggs in a marsupium on the underside of her body) of these animals that leads to increased levels of isolation even between populations that are in close proximity. As a consequence, *Ligidium* populations, especially those present on islands, are unique genetic pools and extremely vulnerable to extinction (Klossa-Kilia *et al.*, 2006). Although there are cases where patterns of differentiation and evolutionary relationships among freshwater isopods populations were unrelated to geographic dispersal or even with patterns of isolation by distance but driven by stochastic population crashes and genetic bottlenecks (caused by seasonal habitat fluctuations), coupled with genetic drift (Gouws *et al.*, 2005). High genetic differentiation in the rocky intertidal isopod *Ligia occidentalis* Dana, 1853 from Pacific revealed 15 highly-divergent clades ranging from 13.2% to 26.7% average sequence divergence (K2P distance). These results complement those of other authors who have found an emerging pattern of substantial genetic divergence and putative cryptic speciation among geographically separated populations of marine isopods, including *Glyptonotus antarcticus* Eights, 1853 (Held and Wägele, 2005), *Idotea balthica* (Pallas, 1772) (Wares, 2001; Wares *et al.*, 2007) and *Serolis paradoxa* (Fabricius, 1775) (Leese *et al.*, 2008). These results show values similar to interspecific genetic distances found in a wide variety of crustaceans (Costa *et al.*, 2007; Markow and Pfeiler, 2010; Radulovici *et al.*, 2009), suggesting that genetic K2P divergence higher than 12% could represent cryptic species.

In Table 2.1.7 we have one case of congeneric species of Isopods with divergence lower than 2.7%, i.e., two freshwater endemic species (*Eophreatoicus* sp). This result needs to be carefully discussed because it belongs to a group of new distinctive morphotypes species identified by Wilson *et al.* (2009) with distribution ranges of few kilometres. The authors suggested that these lineages may have overlapped geographically sometime in the past, during which time a brief period of hybridisation took place. Subsequently, the mtDNA haplotype might have become fixed as it has in most of the lineages, without re-establishment of gene flow or significant introgression between the lineages (Wilson *et al.*, 2009).

The speciose superorder Peracarida, wrought with the highest levels of homoplasy, can be the nuisance of a morphological systematist's existence (Richter and Scholtz, 2001; Wetzer, 2001). On a molecular level, these same groups may also have the most divergent mitochondrial nucleotide sequences and they could be used as model organisms to understand cryptic speciation, rates of molecular evolution and patterns of molecular divergence within species. Despite the attractiveness of a standardized temporal scheme of biological classification for extant species (see General Introduction) such schemes do not account for highly unequal evolutionary rates among lineages. These differences in rates have been attributed to variation in biochemical mechanisms such as DNA replication and DNA repair that can be differentially active among taxa (Caccone and Powell, 1990). Caccone and Powell (1990) calculated absolute rates of change in *Drosophila* DNA as ca. 5 – 10 times faster than what is found in most vertebrates, and this holds for the more conservative part of the nuclear genome. They point out that morphological similarity, chromosomal similarity, and/or ability to form interspecific hybrids is often associated with quite high levels of single copy DNA divergence in insects as compared to mammals and birds. Sequence

divergence for mitochondrial genes among crustaceans has been reported to be highly variable, e.g., among decapod infraorder Astacidea and Anomura for a 350 bp fragment of the 16S rRNA gene, it was found 26.1% sequence divergence (Tam and Kornfield, 1998), whereas Matzen da Silva *et al.* (2011) illustrated a range of sequence divergence between 6.7% to 36.8% among 11 decapod families for a 500 bp fragment of the COI gene (see Chapter 3.1). Based on global barcoding protocol for Peracarida it will be possible to investigate how peracarid data generated from different geographic and ecosystems areas are correlated through phylogenetic studies at the genus, family and order level as it was investigated for Decapoda Order in Chapter 3.1 and for selected genera in Chapter 3.2 and 3.3.

2.1.5 Conclusions

The present data set coupled with the functionality in BOLD provides a tool that is already operational for molecular assisted identification of the malacostraca species. Despite previous Crustacean studies based on barcode data, the current study identified for the first time initial trends of nucleotide substitution patterns of three well represented orders (until the date), i.e for Amphipoda with 83 species, Decapoda with 103 and Isopoda with 151 species, which generated core questions for subsequent priorities studies (e.g., high molecular diversity *vs* homoplasy of Peracarida species; systematic issues of *Plesionika*, *Pontocaris* and *Systellaspis* genera; putative cryptic speciation of *Xantho hydrophylus*). DNA barcoding is a tool for species identification and for flagging priority cases for systematic studies (see Table 2.1.7) and for that

reason modern taxonomy and systematics is increasingly incorporating COI sequences as additional data into their fields.

It is obvious that different organism groups are not equally well represented. The COI combined data shows that the percentage of specimens assigned to species varies greatly between different orders, i.e. 49% for isopods, 66.2% for amphipods and 89.6% for decapods. These numbers must not be taken as absolute, and the true part of undetermined species is probably higher. Peracarida species are good examples of organisms with more difficult anatomy from the identification viewpoint in this group of data. A common reason for not being able to determine species fully (e.g., *Ampelisca*; *Notopoma* and *Harpinia* from amphipods specimens, and *Gnathia*; *Disconectes* and *Natatola* from isopods specimens) is also that samples get damaged during collection, so that the diagnostic characters are lost or are represented by deep sea less/unknown species. Individuals may also be in a life history stage or of sex that does not have the diagnostic morphological characters to separate it from other species. DNA barcodes can be incorporated into large taxon-specific biodiversity and systematic studies (Matzen da Silva *et al.*, 2011a), phylogenetic approach for order-level assignments of crustacean (Costa *et al.*, 2007), inferring preliminary phylogeographic patterns (Costa *et al.*, 2009), and applied for shallow phylogenies studies (Matzen (Matzen da Silva *et al.*, 2011b). In the present study, in which the phylogenetic relationships are unknown, combining multiple congruent data partitions would not only have the effect of increasing the size of data sets and thereby phylogenetic signal, but for a previously unstudied group within Malacostraca some recommendations can be numbered: (1) collection of a minimum two to four species or genera thought to be most divergent (this may include taxa which are the most speciose, have unusual lifestyles, morphology, complex taxonomic history); (2) obtain as equal a representation of taxa

across the group as possible; (3) survey two to three genes; (4) check data for nucleotide diversity. Additionally a global campaign for all Malacostraca split by orders has a lot to offer when it comes to the identification of organisms that are beyond the reach of morphological demarcation and diagnostics. The association of sex and life stages is a prerequisite to understanding the biology and the diversity of the species. An increased geographic scale and the inclusion of marine groups with lower potential for dispersal, e.g. Peracarida species, will bring interesting results as it was described here in this chapter with freshwater and terrestrial specimens. Additionally to geographic horizontal distribution, it is now possible with the advance of robotic technology to increase the sampling among vertical distribution leading to the discovery of many new species. Although most of the data reviewed here did not flag a high amount of cryptic speciation, DNA barcoding is a crucial method to explore the incidence of cryptic species and to understand the role of cryptic species in ecological and evolutionary processes (Whiteman *et al.*, 2004). Moreover, with a greater need for biological inventories and studies of biodiversity than ever, the complexity of this class and the lack of taxonomists demand such an approach. Additional studies with a wider inclusion of families will better increase taxon specific primers in order to optimize PCR performance and to reduce the effect of the taxonomic impediment for biological inventories and studies of biodiversity. Such data are relevant to strategies for conservation of existing malacostraca biodiversity, as well as elucidating the mechanisms and constraints shaping the patterns observed.

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References

- Abdo A, Golding GB (2007) A step toward barcoding life: a model-based, decision-theoretic method to assign genes to preexisting species groups. *Systematic Biology* **56**, 44-56.
- Aranda da Silva A, Pawlowski J, Gooday A (2006) High diversity of deep-sea Gromia from the Arabian Sea revealed by small subunit rDNA sequence analysis. . *Marine Biology* **148**, 769-777.
- Armstrong KF, Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1813-1823.
- Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, *et al.* (1987) Intraspecific phylogeography - the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489-522.
- Bachtrog D, Thornton K, Clark A, Andolfatto P (2006) Extensive introgression of mitochondrial DNA relative to nuclear genes in the *Drosophila yakuba* species group. *Evolution* **60**, 292-302.
- Ball SL, Hebert PDN, Burian SK, Webb JM (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society* **24**, 508-524.
- Barrett RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **83**, 481-491.
- Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) Research article ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. *BMC Microbiology* **10**, doi:10.1186/1471-2180-1110-1189.

- Besansky NJ, Severson DW, Ferdig MT (2003) DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. *Trends in Parasitology* **19**, 545-546.
- Blaxter M (2003) Molecular systematics - Counting angels with DNA. *Nature* **421**, 122-124.
- Blaxter M, Elsworth B, Daub J (2004) DNA taxonomy of a neglected animal phylum: an unexpected diversity of tardigrades. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, S189-S192.
- Blaxter ML (2004) The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society B-Biological Sciences* **359**, 669–679.
- Bucklin A, Wiebe PH, Smolenack SB, Copley NJ, Beaudet JG, Bonner KG, et al. (2007) DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *Journal of Plankton Research* **29**, 483-493.
- Buhay JE (2009) "COI-like" sequences are becoming problematic in molecular systematics and DNA barcoding studies. *Journal of Crustacean Biology* **20**, 96-110.
- Caccone A, Powell JR (1990) Extreme rates and heterogeneity in insect DNA evolution. *Marine Biology* **101**, 655-664.
- Casiraghi M, Labra M, Ferri E, Galimberti A, De Mattia F (2010) DNA barcoding: a six-question tour to improve users' awareness about the method. *Briefings in Bioinformatics* **11**, 440-453.
- Costa FO, Carvalho GR (2007) The Barcode of Life Initiative: synopsis and prospective societal impacts of DNA barcoding of fish. *Genomics, Society and Policy* **3**, 52-56.
- Costa FO, Carvalho GR (2010) New insights into molecular evolution: prospects from the Barcode of Life Initiative (BOLI). *Theory in Biosciences* **129**, 149-157.
- Costa FO, deWaard JR, Boutilier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272-295.
- Costa FO, Henzler CM, Lunt DH, Whiteley NM, Rock J (2009) Probing marine Gammarus (Amphipoda) taxonomy with DNA barcodes. *Systematics and Biodiversity* **7**, 365-379.

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- Creer S (2005) On the application of molecular barcodes in toxinological research. *Toxicon* **46**, 709-710.
- d'Udekem d'Acoz C (1999) *Inventaire et distribution des crustacés décapodes de l'Atlantique nord - oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25°N* Collection Patrimoines Naturels Paris.
- Dawnay N, Oqden R, McRewing R, Carvalho GR, Thorpe RS (2007) Validation of the barcoding gene COI for use in forensic genetic species identification. *Forensic Science International* **173**, 1-6.
- De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, *et al.* (2009) A classification of living and fossil genera of decapod Crustaceans. *Raffles Bulletin of Zoology* **1**, 1-109.
- Ekrem T, Willassen E, Stur E (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution* **43**, 530-542.
- Flegel T (2007) The right to refuse revision in the genus *Penaeus*. *Aquaculture* **264**, 2-8.
- Flegel T (2008) Confirmation of the right to refuse revision in the genus *Penaeus*. *Aquaculture* **280**, 1-2.
- Floyd R, Abebe E, Papert A, Black M (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* **11**, 836-850.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294-299.
- Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* **18**, 4541-4550.
- Gomez A, Wright PJ, Lunt DH, Cancino JM, Carvalho GR, Hughes RN (2007) Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. *Proceedings of the Royal Society B-Biological Sciences* **274**, 199-207.
- Gouws G, Stewart BA, Matthee CA (2005) Lack of taxonomic differentiation in an apparently widespread freshwater isopod morphotype (Phreatoicidea: Mesamphisopidae: *Mesamphisopus*) from South Africa. *Molecular Phylogenetics and Evolution* **37**, 289-305.

- Hajibabaei M, DeWaard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, *et al.* (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1959-1967.
- Hajibabaei M, Singer GAC, Clare EL, Hebert PDN (2007a) Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. *BMC Biology* **5**, 1-7.
- Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007b) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* **23**, 167-172.
- Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* **6**, 959-964.
- Harris DJ (2003) Can you bank on GenBank? *Trends in Ecology and Evolution* **18**, 317-319.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 313-321.
- Hebert PDN, Gregory TR (2005) The Promise of DNA Barcoding for Taxonomy. *Systematic Biology* **54**, 852-859.
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004a) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 14812-14817.
- Hebert PDN, Ratnasingham S, deWaard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S96-S99.
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004b) Identification of birds through DNA barcodes. *PLoS Biology* **2**, 1657-1663.
- Held C, Wägele J-W (2005) Cryptic speciation in the giant Antarctic isopod *Glyptonotus antarcticus* (Isopoda: Valvifera: Chaetiliidae). *Scientia Marina* **69**, 175-181.

Section 2. Molecular biodiversity

- Hickerson MJ, Meyer CP, Moritz C (2006) DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* **55**, 729-739.
- Hogg ID, Hebert PDN (2004) Biological identification of springtails (Hexapoda : Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **82**, 749-754.
- Hollingsworth PM (2007) DNA barcoding: potential users (Commissioned Responses to Costa & Carvalho). *Genomics, Society and Policy* **3**, 44-47.
- Hollingsworth PM, Forresta LL, Spougeb JL, Hajibabaeic M, Ratnasinghamc S, van der Bankd M, et al. (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences* **106**, 12794–12797.
- Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. *PLoS ONE* **6**, e19254.
- Holm P (2007) The Book of Life goes online *Genomics, Society and Policy* **3**, 48-51.
- Hubert N, Hanner R, Holm E, Mandrak NE, Taylor EM, Burridge M, et al. (2008) Identifying Canadian Freshwater Fishes through DNA Barcodes. *PLoS ONE* **3**, e2490.
- Hutchins B, Fong DW, Carlini DB (2010) Genetic Population Structure of the Madison Cave Isopod, *Antrolana lira* (Cymothoida: Cirolanidae) in the Shenandoah Valley of the Eastern United States. *Journal of Crustacean Biology* **30**, 312-322.
- Ivanova NV, deWaard JR, Hajibabaei M, Hebert PDN (2005) Protocols for High-Volume DNA Barcode Analysis. *Molecular Ecology Notes* **6**, 998-1002.
- Ivanova NV, Deward JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* **6**, 998-1002.
- Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 1-5.
- Joly S, McLenaghan PA, Lockhart PJ (2009) A statiscal approach for distinguishing hybridization and incomplete lineage sorting. *The American Naturalist* **174**, E54-E70.
- Kaila L, Stahls G (2006) DNA barcodes: Evaluating the potential of COI to differentiate closely related species of Elachista (Lepidoptera : Gelechioidea : Elachistidae) from Australia. *Zootaxa* **1170**, 1-26.

- Kerr KCR, Stoeckle MY, Dove CJ, Weigt LA, Francis CM, Hebert PDN (2007) Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes* **7**, 535-543.
- Ketmaier V, Joyce DA, Horton T, Mariani S (2008) A molecular phylogenetic framework for the evolution of parasitic strategies in cymothoid isopods (Crustacea). *Journal of Zoological Systematics and Evolutionary Research* **46**, 19-23.
- Kimura M (1980) A simple method for estimating evolutionary rate of base: substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **15**, 111-120.
- Klossa-Kilia E, Kilias G, Tryfonopoulos G, Koukou K, Sfenthourakis S, Parmakelis A (2006) Molecular phylogeny of the Greek populations of the genus *Ligidium* (Isopoda, Oniscidae) using three mtDNA gene segments. *Zoologica Scripta* **35**, 459-472.
- Knowlton N (1993) Sibling species in the sea. *Annual Review of Ecology and Systematics* **24**, 186-216.
- Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 8369-8374.
- Lambert DM, Baker A, Huynen L, Haddrath O, Hebert PDN, Millar CD (2005) Is a large-scale DNA-based inventory of ancient life possible? *Journal of Heredity* **96**, 279-284.
- Leese F, Kop A, Wägele J-W, Held C (2008) Cryptic speciation in a benthic isopod from Patagonian and Falkland Island waters and the impact of glaciations on its population structure. *Frontiers in Zoology* **5**.
- Lefébure T, Douady CJ, Gouy M, Gibert J (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* **40**, 435–447.
- Little DP, Stevenson DW (2007) A comparison of algorithms for the identification of specimens using DNA barcodes: examples from gymnosperms. *Cladistics* **23**, 1-21.

Section 2. Molecular biodiversity

- Lorenz JG, Jackson WE, Beck JC, Hanner R (2005) The problems and promise of DNA barcodes for species diagnosis of primate biomaterials. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1869-1877.
- Macdonald KS, Yampolsky L, Duffy JE (2005) Molecular and morphological evolution of the amphipod radiation of Lake Baikal. *Molecular Phylogenetics and Evolution* **35**, 323-343.
- Mallet J, Willmott K (2007) Taxonomy: renaissance or Tower of Babel? *Trends in Ecology and Evolution* **18**, 57-59.
- Mallet KKDaJ (2006) DNA barcodes: recent successes and future prospects. *Heredity*, 1-2.
- Markmann M, Tautz D (2005) Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1917-1924.
- Markow TA, Pfeiler E (2010) Mitochondrial DNA evidence for deep genetic divergences in allopatric populations of the rocky intertidal isopod *Ligia occidentalis* from the eastern Pacific. *Molecular Phylogenetics and Evolution* **56**, 468-473.
- Matzen da Silva J, Creer S, Dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR (2011a) Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Malacostraca). *PLoS ONE* **6**, e19449.
- Matzen da Silva J, dos Santos A, Cunha MR, Costa FO, Creer S, Carvalho GR (2011b) Multigene molecular systematics confirm species status of morphologically convergent Pagurus hermit crabs. *PLoS One* **6**, e28233.
- Meier R, Shiyang K, Vaidya G, Ng PKL (2006) DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success. *Systematic Biology* **55**, 715-728.
- Meyer CP, Paulay G (2005) DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* **3**, e422.
- Monaghan MT, Balke M, Gregory TR, Vogler AP (2005) DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1925-1933.

- Monaghan MT, Balke M, Pons J, Vogler AP (2006) Beyond barcodes: complex DNA taxonomy of a south pacific island radiation. *Proceedings of the Royal Society B-Biological Sciences* **273**, 887-893.
- Moura CJ, Harris DJ, Cunha MR, Rogers AD (2008) DNA barcoding reveals cryptic diversity in marine hydrozoans (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zoologica Scripta* **37**, 93-100.
- Nielsen R, Matz M (2006) Statistical approaches for DNA barcoding. *Systematic Biology* **55**, 162-169.
- Padial JMa, de la Riva I (2007) Integrative taxonomists should use and produce DNA barcodes. *Zootaxa* **1586**, 67-68.
- Page TJ, Choy SC, Hughes JM (2005) The taxonomic feedback loop: symbiosis of morphology and molecules. *Biology Letters* **1**, 139-142.
- Paquin P, Hedin M (2004) The power and perils of 'molecular taxonomy': a case study of eyeless and endangered Cicurina (Araneae : Dictynidae) from Texas caves. *Molecular Ecology* **13**, 3239-3255.
- Pfenninger M, Nowak C, Kley C, Steinke D, Streit B (2007) Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology* **16**, 1957-1968.
- Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* **7**, 121.
- Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander B, et al. (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* **48**, 369-371.
- Powell RL, Reyes SR, Lannutti DI (2006) Molecular barcoding, DNA from snake venom, and toxinological research: Considerations and concerns. *Toxicon* **48**, 1095-1097.
- Proudlove G, Wood PJ (2003) The blind leading the blind: cryptic subterranean species and DNA taxonomy. *Trends in Ecology and Evolution* **18**, 272-273.
- Radulovici AE, Archambault P, Dufresne F (2010) DNA barcodes for marine biodiversity: Moving fast forward? *Diversity* **2**, 450-472.

- Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. *Molecular Ecology Resources* **9**, 181-187.
- Ratnasingham S, Hebert PDN (2007) Barcoding BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.
- Richter S, Scholtz G (2001) Phylogenetic analysis of the Malacostraca (Crustacea). *Journal of Zoological Systematics and Evolutionary Research* **39**, 113-136.
- Rubinoff D (2006) DNA barcoding evolves into the familiar. *Conservation Biology* **20**, 1548-1549.
- Rubinoff D, Cameron S, Will K (2006) Are plant DNA barcodes a search for the Holy Grail? *Trends in Ecology and Evolution* **21**, 1-2.
- Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution* **4**, 406-425.
- Santamaria M, Vicario S, Pappadà G, Scioscia G, Scazzocchio C, Saccone C (2009) Towards barcode markers in Fungi: an intron map of Ascomycota mitochondria. *BMC Bioinformatics* **10**, 1-13.
- Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1805-1811.
- Schander C, Willlassen E (2005) What can biological barcoding do for marine biology? *Marine Biology Research* **1**, 79-83.
- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Seberg O, Humphries CJ, Knapp S, Stevenson DW, Petersen G, Scharff N, Andersen NM (2003) Shortcuts in systematics? A commentary on DNA-based taxonomy. *Trends in Ecology and Evolution* **18**, 63-65.
- Siddall ME, Fontanella FM, Watson SC, Kvist S, Erséus C (2009) Barcoding Bamboozled by Bacteria: Convergence to Metazoan Mitochondrial Primer Target by Marine Microbes. *Systematic Biology* **58**, 445-451.
- Sirovich L, Stoeckle M, Zhang Y (2009) A scalable method for analysis and display of DNA sequences. *PLoS ONE* **4**, e7051.

- Smith MA, Fisher BL, Hebert PDN (2005) DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1825-1834.
- Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera : Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America* **103**, 3657-3662.
- Smith PJ, McVeagh SM, Steinke D (2008) DNA barcoding for the identification of smoked fish products. *Journal of Fish Biology* **72**, 464-471.
- Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**, 13486-13491.
- Stoeckle M (2003) Taxonomy, DNA, and the bar code of life. *Bioscience* **53**, 796-797.
- Stribling JB (2006) Environmental protection using DNA barcodes or taxa? *Bioscience* **56**, 878-879.
- Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. *Genetics* **105**, 437-460.
- Tam YK, Kornfield I (1998) Phylogenetic relationships of clawed lobster genera (Decapoda: Nephropidae) based on mitochondrial 16S rRNA gene sequences. *Journal of Crustacean Biology* **18**, 138-146.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Tang RWK, Yau C, Ng W-C (2010) Identification of stomatopod larvae (Crustacea: Stomatopoda) from Hong Kong waters using DNA barcodes. *Molecular Ecology Resources* **10**, 439-448.
- Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP (2003) A plea for DNA taxonomy. *Trends in Ecology and Evolution* **18**, 70-74.
- Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-4680.

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- Thorp JH, Covich AP (2001) Introduction to the subphylum crustacea. In: *Ecology and Classification of North American Freshwater Invertebrates* (eds. Thorp J. H. , Covich A. P.), pp. 777-809. Academic Press, San Diego.
- Vences M, Thomas M, Bonett RM, Vieites DR (2005) Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1859-1868.
- Wang W, Luo Q, Guo H, Bossier P, Van Stappen G, Sorgeloos P (2008) Phylogenetic Analysis of Brine Shrimp (*Artemia*) in China Using DNA Barcoding. *Genomics, Proteomics and Bioinformatics* **6**, 155-162.
- Ward RD, Hanner R, Hebert PDN (2009) The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* **74**, 329–356.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1847-1857.
- Wares JP (2001) Intraspecific variation and geographic isolation in *Idotea balthica* (Isopoda: Valvifera). *Journal of Crustacean Biology* **21**, 1007-1013.
- Wares JP, Daley S, Wetzer R, Toonen RJ (2007) An evaluation of cryptic lineages of *Idotea balthica* (Isopoda: Idoteidae): morphology and microsatellites. *Journal of Crustacean Biology* **27**, 643–648.
- Wells JD, Introna F, Di Vella G, Campobasso CP, Hayes J, Sperling FAH (2001) Human and insect mitochondrial DNA analysis from maggots. *Journal of Forensic Sciences* **46**, 685-687.
- Wells JD, Sperling FAH (2001) DNA-based identification of forensically important Chrysomyinae (Diptera : Calliphoridae). *Forensic Science International* **120**, 110-115.
- Werle E, Schneider C, Renner M, Volker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* **22**, 4354-4355.
- Wetzer R (2001) Hierarchical analysis of mtDNA variation and the use of mtDNA for isopod (Crustacea: Peracarida: Isopoda) systematics. *Contributions to Zoology* **70**, 23-39.

- Wheeler QD (2004) Taxonomic triage and the poverty of phylogeny. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 571-583.
- Whiteman NK, Santiago-Alarcon D, Johnson KP, Parker PG (2004) Differences in straggling rates between two genera of dove lice (Insecta : Phthiraptera) reinforce population genetic and cophylogenetic patterns. *International Journal for Parasitology* **34**, 1113-1119.
- Will KW, Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* **20**, 47-55.
- Wilson GDF, Humphrey CL, Colgan DJ, Gray K-A, Johnson RN (2009) Monsoon-influenced speciation patterns in a species flock of *Eophreatoicus* Nicholls (Isopoda; Crustacea). *Molecular Phylogenetics and Evolution* **51**, 349-364.
- Wong EHK, Hanner RH (2008) DNA barcoding detects market substitution in North American seafood. *Food Research International* **41**, 828-837.
- Wong EHK, Shivji MS, Hanner RH (2009) Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach. *Molecular Ecology Resources* **9**, 243-256.
- Zang AB, He LJ, Crozier RH, Muster C, Zhu C-D (2010) Estimating sample sizes for DNA barcoding. *Molecular Phylogenetics and Evolution* **54**, 1035-1039.

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2.1.6 ANNEX

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Table S 2.1.1: List of Peracaridea imported COI sequeneces from public project from the Barcode of Life Data Systems website (<http://www.barcdoingslife.org/>)

Order	GenBank no.	Species	Genus	Family
Amphipoda	U92668	<i>Abyssorchromene sp. 1143-2</i>	<i>Abyssorchromene</i>	Lysianassidae
	U92669	<i>Abyssorchromene sp. SCSN</i>	<i>Abyssorchromene</i>	Lysianassidae
	AY061800	<i>Acanthogammarus flavus</i>	<i>Acanthogammarus</i>	Gammaridae
	EF989700	<i>Acanthoscina acanthodes</i>	<i>Acanthoscina</i>	Scinidae
	L169AR1-01	<i>Americorchestia longicornis</i>	<i>Americorchestia</i>	Talitridae
	L169AR1-02	<i>Americorchestia longicornis</i>	<i>Americorchestia</i>	Talitridae
	L169AR1-03	<i>Americorchestia longicornis</i>	<i>Americorchestia</i>	Talitridae
	L169AR1-04	<i>Americorchestia longicornis</i>	<i>Americorchestia</i>	Talitridae
	L169AR1-05	<i>Americorchestia longicornis</i>	<i>Americorchestia</i>	Talitridae
	L197AR2-01	<i>Americorchestia megalophthalma</i>	<i>Americorchestia</i>	Talitridae
	L215AR1-01	<i>Americorchestia megalophthalma</i>	<i>Americorchestia</i>	Talitridae
	L211AR1-01	<i>Americorchestia megalophthalma</i>	<i>Americorchestia</i>	Talitridae
	L230AR1-02	<i>Americorchestia megalophthalma</i>	<i>Americorchestia</i>	Talitridae
	GSL11-01	<i>Ampelisca eschrichtii 1</i>	<i>Ampelisca</i>	Ampeliscidae
	GSL11-02	<i>Ampelisca eschrichtii 1</i>	<i>Ampelisca</i>	Ampeliscidae
	BSM07T4-01	<i>Ampelisca eschrichtii 2</i>	<i>Ampelisca</i>	Ampeliscidae
	BSM07T7-01	<i>Ampelisca eschrichtii 2</i>	<i>Ampelisca</i>	Ampeliscidae
	BSM07T13-01	<i>Ampelisca eschrichtii 2</i>	<i>Ampelisca</i>	Ampeliscidae
	BSM07T13-02	<i>Ampelisca eschrichtii 2</i>	<i>Ampelisca</i>	Ampeliscidae
	BSM08P6-01	<i>Ampelisca eschrichtii 2</i>	<i>Ampelisca</i>	Ampeliscidae
	AY926653	<i>Ampithoe longimana</i>	<i>Ampithoe</i>	Ampithoidae
	L191AR1-04	<i>Ampithoe longimana</i>	<i>Ampithoe</i>	Ampithoidae
	L191AR1-05	<i>Ampithoe longimana</i>	<i>Ampithoe</i>	Ampithoidae
	L191AR1-08	<i>Ampithoe longimana</i>	<i>Ampithoe</i>	Ampithoidae
	BSM07T1-01	<i>Ampithoe rubricata</i>	<i>Ampithoe</i>	Ampithoidae
	BSM07T1-02	<i>Ampithoe rubricata</i>	<i>Ampithoe</i>	Ampithoidae
	BSM07T1-04	<i>Ampithoe rubricata</i>	<i>Ampithoe</i>	Ampithoidae
	PEI08D3-08	<i>Ampithoe rubricata</i>	<i>Ampithoe</i>	Ampithoidae
	PEI08D3-10	<i>Ampithoe rubricata</i>	<i>Ampithoe</i>	Ampithoidae
	BSM07T1-13	<i>Anonyx makarovi</i>	<i>Anonyx</i>	Uristidae
	BSM07T1-14	<i>Anonyx makarovi</i>	<i>Anonyx</i>	Uristidae
	GSL10-01	<i>Anonyx makarovi</i>	<i>Anonyx</i>	Uristidae
	TE005T079-01	<i>Anonyx makarovi</i>	<i>Anonyx</i>	Uristidae
	TE005T080-01	<i>Anonyx makarovi</i>	<i>Anonyx</i>	Uristidae
	L47AR1-13	<i>Anonyx sarsi</i>	<i>Anonyx</i>	Uristidae
	L42AR2-11	<i>Anonyx sarsi</i>	<i>Anonyx</i>	Uristidae
	L47AR1-09	<i>Anonyx sarsi</i>	<i>Anonyx</i>	Uristidae
	L47AR1-10	<i>Anonyx sarsi</i>	<i>Anonyx</i>	Uristidae
	L47AR1-11	<i>Anonyx sarsi</i>	<i>Anonyx</i>	Uristidae
	FJ037788	<i>Apohyale pugettensis</i>	<i>Apohyale</i>	Hyalidae
	EF989658	<i>Brachyscelus crusculum</i>	<i>Brachyscelus</i>	Brachyscelidae
	AY926654	<i>Brandtia lata</i>	<i>Brandtia</i>	Acanthogammaridae
	EF989649	<i>Calamorhynchus pellucidus</i>	<i>Calamorhynchus</i>	Oxycephalidae
	L90AR12-09	<i>Calliopius laeviusculus</i>	<i>Calliopius</i>	Calliopiiidae
	CNCU27-01	<i>Calliopius laeviusculus</i>	<i>Calliopius</i>	Calliopiiidae
	L51AR2-02	<i>Calliopius laeviusculus</i>	<i>Calliopius</i>	Calliopiiidae
	L84AR1-02	<i>Calliopius laeviusculus</i>	<i>Calliopius</i>	Calliopiiidae
	EMK64-02	<i>Calliopius laeviusculus</i>	<i>Calliopius</i>	Calliopiiidae
	BSM07T6-05	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae

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Order	GenBank no.	Species	Genus	Family
Amphipoda	BSM07T6-06	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae
	BSM07T1-07	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae
	BSM08T9-01	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae
	BSM08T9-02	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae
	BSM08T9-03	<i>Caprella linearis</i>	<i>Caprella</i>	Caprellidae
	GSL22-01	<i>Caprella mutica</i>	<i>Caprella</i>	Caprellidae
	GSL22-02	<i>Caprella mutica</i>	<i>Caprella</i>	Caprellidae
	L232F19-01	<i>Caprella mutica</i>	<i>Caprella</i>	Caprellidae
	L232F36-01	<i>Caprella mutica</i>	<i>Caprella</i>	Caprellidae
	L42AR2-10	<i>Caprella septentrionalis</i>	<i>Caprella</i>	Caprellidae
	MCNCM5-01	<i>Caprella septentrionalis</i>	<i>Caprella</i>	Caprellidae
	HCND15-01	<i>Caprella septentrionalis</i>	<i>Caprella</i>	Caprellidae
	EGNVALLEA-01	<i>Caprella septentrionalis</i>	<i>Caprella</i>	Caprellidae
	EMK14-01	<i>Caprella septentrionalis</i>	<i>Caprella</i>	Caprellidae
	EF989681	<i>Caprellidae sp. KBH 25</i>		Caprellidae
	AY926657	<i>Chaetogammarus stoerensis</i>	<i>Chaetogammarus</i>	Gammaridae
	DQ838017	<i>Chydaekata acuminata</i>	<i>Chydaekata</i>	Paramelitidae
	DQ838018	<i>Chydaekata acuminata</i>	<i>Chydaekata</i>	Paramelitidae
	DQ838019	<i>Chydaekata acuminata</i>	<i>Chydaekata</i>	Paramelitidae
	DQ838020	<i>Chydaekata acuminata</i>	<i>Chydaekata</i>	Paramelitidae
	DQ838021	<i>Chydaekata acuminata</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256007	<i>Chydaekata sp. A TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256008	<i>Chydaekata sp. A TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256009	<i>Chydaekata sp. A TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256010	<i>Chydaekata sp. A TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256011	<i>Chydaekata sp. A TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255990	<i>Chydaekata sp. B TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255991	<i>Chydaekata sp. B TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255992	<i>Chydaekata sp. B TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255993	<i>Chydaekata sp. B TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255994	<i>Chydaekata sp. B TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256012	<i>Chydaekata sp. D TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256013	<i>Chydaekata sp. D TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256014	<i>Chydaekata sp. D TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ256015	<i>Chydaekata sp. D TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255963	<i>Chydaekata sp. E TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255964	<i>Chydaekata sp. E TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255965	<i>Chydaekata sp. E TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255966	<i>Chydaekata sp. E TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ255967	<i>Chydaekata sp. E TLF-2008</i>	<i>Chydaekata</i>	Paramelitidae
	DQ679981	<i>Chydaekata sp. TLF-2005</i>	<i>Chydaekata</i>	Paramelitidae
	DQ679982	<i>Chydaekata sp. TLF-2005</i>	<i>Chydaekata</i>	Paramelitidae
	DQ679983	<i>Chydaekata sp. TLF-2005</i>	<i>Chydaekata</i>	Paramelitidae
	DQ679984	<i>Chydaekata sp. TLF-2005</i>	<i>Chydaekata</i>	Paramelitidae
	DQ679985	<i>Chydaekata sp. TLF-2005</i>	<i>Chydaekata</i>	Paramelitidae
	AJ968910	<i>Crangonyx floridanus</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968911	<i>Crangonyx floridanus</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968893	<i>Crangonyx pseudogracilis</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968894	<i>Crangonyx pseudogracilis</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968895	<i>Crangonyx pseudogracilis</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968896	<i>Crangonyx pseudogracilis</i>	<i>Crangonyx</i>	Crangonyctidae
	AJ968897	<i>Crangonyx pseudogracilis</i>	<i>Crangonyx</i>	Crangonyctidae

Order	GenBank no.	Species	Genus	Family
Amphipoda	AY926658	<i>Crangonyx serratus</i>	<i>Crangonyx</i>	Crangonyctidae
	EF989648	<i>Cranocephalus scleroticus</i>	<i>Cranocephalus</i>	Oxycephalidae
	DQ095150	<i>Cyamus boopis</i>	<i>Cyamus</i>	Cyamidae
	DQ095106	<i>Cyamus erraticus</i>	<i>Cyamus</i>	Cyamidae
	DQ095107	<i>Cyamus erraticus</i>	<i>Cyamus</i>	Cyamidae
	DQ095127	<i>Cyamus erraticus</i>	<i>Cyamus</i>	Cyamidae
	DQ095128	<i>Cyamus erraticus</i>	<i>Cyamus</i>	Cyamidae
	DQ095129	<i>Cyamus erraticus</i>	<i>Cyamus</i>	Cyamidae
	DQ095048	<i>Cyamus gracilis</i>	<i>Cyamus</i>	Cyamidae
	DQ095049	<i>Cyamus gracilis</i>	<i>Cyamus</i>	Cyamidae
	DQ095050	<i>Cyamus gracilis</i>	<i>Cyamus</i>	Cyamidae
	DQ095078	<i>Cyamus gracilis</i>	<i>Cyamus</i>	Cyamidae
	DQ095079	<i>Cyamus gracilis</i>	<i>Cyamus</i>	Cyamidae
	EF989691	<i>Cyllopus lucasii</i>	<i>Cyllopus</i>	Hyperidae
	EF989690	<i>Cyllopus magellanicus</i>	<i>Cyllopus</i>	Hyperidae
	EF989675	<i>Cystisoma gershwiniae</i>	<i>Cystisoma</i>	Cystisomatidae
	EF989676	<i>Cystisoma pellucida</i>	<i>Cystisoma</i>	Cystisomatidae
	AY529049	<i>Dikerogammarus haemobaphes</i>	<i>Dikerogammarus</i>	Gammaridae
	AY529048	<i>Dikerogammarus villosus</i>	<i>Dikerogammarus</i>	Gammaridae
	EF570297	<i>Dikerogammarus villosus</i>	<i>Dikerogammarus</i>	Gammaridae
	EU761577	<i>Diporeia hoyi</i>	<i>Diporeia</i>	Pontoporeiidae
	EU761576	<i>Diporeia hoyi</i>	<i>Diporeia</i>	Pontoporeiidae
	EU761575	<i>Diporeia hoyi</i>	<i>Diporeia</i>	Pontoporeiidae
	EU761574	<i>Diporeia hoyi</i>	<i>Diporeia</i>	Pontoporeiidae
	EU761573	<i>Diporeia hoyi</i>	<i>Diporeia</i>	Pontoporeiidae
	EAV01	<i>Echinogammarus ischnus</i>	<i>Echinogammarus</i>	Gammaridae
	EAV02	<i>Echinogammarus ischnus</i>	<i>Echinogammarus</i>	Gammaridae
	EAVS3-01	<i>Echinogammarus ischnus</i>	<i>Echinogammarus</i>	Gammaridae
	EAVS3-02	<i>Echinogammarus ischnus</i>	<i>Echinogammarus</i>	Gammaridae
	GSL9-01	<i>Eusirus cuspidatus</i>	<i>Eusirus</i>	Eusiridae
	TE005T173-02	<i>Eusirus cuspidatus</i>	<i>Eusirus</i>	Eusiridae
	ASPM09-03	<i>Gammarellus angulosus</i>	<i>Gammarellus</i>	Gammarellidae
	L186AR1-01	<i>Gammarus duebeni</i>	<i>Gammarus</i>	Gammaridae
	L186AR1-02	<i>Gammarus duebeni</i>	<i>Gammarus</i>	Gammaridae
	L44AR2-01	<i>Gammarus duebeni</i>	<i>Gammarus</i>	Gammaridae
	L44AR2-02	<i>Gammarus duebeni</i>	<i>Gammarus</i>	Gammaridae
	L44AR2-03	<i>Gammarus duebeni</i>	<i>Gammarus</i>	Gammaridae
	L42AR1-04	<i>Gammarus lawrencianus</i>	<i>Gammarus</i>	Gammaridae
	L90AR11-04	<i>Gammarus lawrencianus</i>	<i>Gammarus</i>	Gammaridae
	L44AR1-03	<i>Gammarus lawrencianus</i>	<i>Gammarus</i>	Gammaridae
	GSL32-01	<i>Gammarus lawrencianus</i>	<i>Gammarus</i>	Gammaridae
	L192AR2-14	<i>Gammarus lawrencianus</i>	<i>Gammarus</i>	Gammaridae
	L176AR1-05	<i>Gammarus mucronatus</i>	<i>Gammarus</i>	Gammaridae
	L192AR2-09	<i>Gammarus mucronatus</i>	<i>Gammarus</i>	Gammaridae
	L185AR2-02	<i>Gammarus mucronatus</i>	<i>Gammarus</i>	Gammaridae
	L218AR1-02	<i>Gammarus mucronatus</i>	<i>Gammarus</i>	Gammaridae
	L212AR1-04	<i>Gammarus mucronatus</i>	<i>Gammarus</i>	Gammaridae
	GF01CN0106	<i>Gammarus oceanicus</i>	<i>Gammarus</i>	Gammaridae
	GF02CN0106	<i>Gammarus oceanicus</i>	<i>Gammarus</i>	Gammaridae
	L196AR2-02	<i>Gammarus oceanicus</i>	<i>Gammarus</i>	Gammaridae
	L169AR2-02	<i>Gammarus tigrinus</i>	<i>Gammarus</i>	Gammaridae
	EAVS2-01	<i>Gammarus tigrinus</i>	<i>Gammarus</i>	Gammaridae

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Order	GenBank no.	Species	Genus	Family
Amphipoda	EAVS12-01	<i>Gammarus tigrinus</i>	<i>Gammarus</i>	Gammaridae
	EAVS12-05	<i>Gammarus tigrinus</i>	<i>Gammarus</i>	Gammaridae
	L212AR1-03	<i>Gammarus tigrinus</i>	<i>Gammarus</i>	Gammaridae
	EAVS25-03	<i>Halirages fulvocinctus</i>	<i>Halirages</i>	Callioipiidae
	EAVS25-04	<i>Halirages fulvocinctus</i>	<i>Halirages</i>	Callioipiidae
	L90AR7-03	<i>Hyale prevostii</i>	<i>Hyale</i>	Hyalidae
	STAUG-01	<i>Ischyrocerus anguipes</i>	<i>Ischyrocerus</i>	Ischyroceridae
	H52-02	<i>Ischyrocerus anguipes</i>	<i>Ischyrocerus</i>	Ischyroceridae
	PHILL-01	<i>Ischyrocerus anguipes</i>	<i>Ischyrocerus</i>	Ischyroceridae
	ASPM09-08	<i>Ischyrocerus anguipes</i>	<i>Ischyrocerus</i>	Ischyroceridae
	EMK14-02	<i>Ischyrocerus anguipes</i>	<i>Ischyrocerus</i>	Ischyroceridae
	H52-01	<i>Ischyrocerus anguipes 2</i>	<i>Ischyrocerus</i>	Ischyroceridae
	MENIER-01	<i>Ischyrocerus anguipes 2</i>	<i>Ischyrocerus</i>	Ischyroceridae
	ASPM09-02	<i>Ischyrocerus anguipes 2</i>	<i>Ischyrocerus</i>	Ischyroceridae
	ASPM09-07	<i>Ischyrocerus anguipes 2</i>	<i>Ischyrocerus</i>	Ischyroceridae
	ASPM11-01	<i>Ischyrocerus anguipes 2</i>	<i>Ischyrocerus</i>	Ischyroceridae
	BSM07T1-15	<i>Melita dentata</i>	<i>Melita</i>	Melitidae
	BSM07T1-16	<i>Melita dentata</i>	<i>Melita</i>	Melitidae
	BSM07T10-01	<i>Melita formosa</i>	<i>Melita</i>	Melitidae
	BSM07T10-02	<i>Melita formosa</i>	<i>Melita</i>	Melitidae
	BSM07T13-05	<i>Melita formosa</i>	<i>Melita</i>	Melitidae
	MI02CN0306	<i>Monoculodes intermedius</i>	<i>Monoculodes</i>	Oedicerotidae
	MI01CN0306	<i>Monoculodes intermedius</i>	<i>Monoculodes</i>	Oedicerotidae
	L90AR6-01	<i>Monoporeia sp.</i>	<i>Monoporeia</i>	Pontoporeiidae
	L90AR6-03	<i>Monoporeia sp.</i>	<i>Monoporeia</i>	Pontoporeiidae
	L90AR12-04	<i>Oediceros saginatus</i>	<i>Oediceros</i>	Oedicerotidae
	L90AR12-10	<i>Oediceros saginatus</i>	<i>Oediceros</i>	Oedicerotidae
	L90AR11-01	<i>Oediceros saginatus</i>	<i>Oediceros</i>	Oedicerotidae
	L90AR11-02	<i>Oediceros saginatus</i>	<i>Oediceros</i>	Oedicerotidae
	L90AR11-05	<i>Oediceros saginatus</i>	<i>Oediceros</i>	Oedicerotidae
	L90AR12-06	<i>Onisimus litoralis 3</i>	<i>Onisimus</i>	Uristidae
	L90AR4-02	<i>Onisimus litoralis 3</i>	<i>Onisimus</i>	Uristidae
	L90AR2-04	<i>Onisimus litoralis 3</i>	<i>Onisimus</i>	Uristidae
	L90AR2-05	<i>Onisimus litoralis 3</i>	<i>Onisimus</i>	Uristidae
	L90AR1-04	<i>Orchomenella minuta</i>	<i>Orchomenella</i>	Lysianassidae
	L90AR1-05	<i>Orchomenella minuta</i>	<i>Orchomenella</i>	Lysianassidae
	L90AR2-01	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	L90AR2-03	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	BSM07T6-09	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	BSM07T1-21	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	BSM07T1-22	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	BSM07T1-42	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	BSM07T1-43	<i>Orchomenella pinguis</i>	<i>Orchomenella</i>	Lysianassidae
	TE005T072-01	<i>Paramphithoe hystrix</i>	<i>Paramphithoe</i>	Epimeriidae
	L185AR1-02	<i>Platorchestia platensis 1</i>	<i>Platorchestia</i>	Talitridae
	L185AR1-03	<i>Platorchestia platensis 1</i>	<i>Platorchestia</i>	Talitridae
	L184AR1-02	<i>Platorchestia platensis 1</i>	<i>Platorchestia</i>	Talitridae
	BSM07T1-05	<i>Pleustes panoplus</i>	<i>Pleustes</i>	Pleustidae
	BSM08T13-01	<i>Pleustes panoplus</i>	<i>Pleustes</i>	Pleustidae
	BSM08T13-02	<i>Pleustes panoplus</i>	<i>Pleustes</i>	Pleustidae
	L90AR1-08	<i>Pontogeneia inermis</i>	<i>Pontogeneia</i>	Eusiridae
	L90AR5-05	<i>Pontogeneia inermis</i>	<i>Pontogeneia</i>	Eusiridae

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Amphipoda	BSM07T6-11	<i>Pontogeneia inermis</i>	<i>Pontogeneia</i>	Eusiridae
	L42AR2-03	<i>Psammonyx nobilis</i>	<i>Psammonyx</i>	Lysianassidae
	L42AR2-04	<i>Psammonyx nobilis</i>	<i>Psammonyx</i>	Lysianassidae
	L42AR2-12	<i>Psammonyx nobilis</i>	<i>Psammonyx</i>	Lysianassidae
	L42AR2-13	<i>Psammonyx nobilis</i>	<i>Psammonyx</i>	Lysianassidae
	BSM07T1-33	<i>Psammonyx terranova</i>	<i>Psammonyx</i>	Lysianassidae
	BSM07T1-34	<i>Psammonyx terranova</i>	<i>Psammonyx</i>	Lysianassidae
	BSM07T1-35	<i>Psammonyx terranova</i>	<i>Psammonyx</i>	Lysianassidae
	BSM07T1-39	<i>Psammonyx terranova</i>	<i>Psammonyx</i>	Lysianassidae
	BSM07T1-08	<i>Psammonyx terranova</i>	<i>Psammonyx</i>	Lysianassidae
	RA02CN0306	<i>Rhachotropis aculeata</i>	<i>Rhachotropis</i>	Eusiridae
	BSM07T13-04	<i>Rhachotropis aculeata</i>	<i>Rhachotropis</i>	Eusiridae
	TE-004T69-01	<i>Rhachotropis aculeata</i>	<i>Rhachotropis</i>	Eusiridae
	GSL31-07	<i>Rhachotropis aculeata</i>	<i>Rhachotropis</i>	Eusiridae
	TE-004T21-40-04	<i>Rhachotropis aculeata</i>	<i>Rhachotropis</i>	Eusiridae
	THL01GS105	<i>Themisto libellula</i>	<i>Themisto</i>	Hyperidae
	THL02GS105	<i>Themisto libellula</i>	<i>Themisto</i>	Hyperidae
	BSM08T16-04	<i>Themisto libellula</i>	<i>Themisto</i>	Hyperidae
	TE005T081-02	<i>Themisto libellula</i>	<i>Themisto</i>	Hyperidae
	TE005T201-01	<i>Themisto libellula</i>	<i>Themisto</i>	Hyperidae
Isopoda	EF682265	<i>Acanthamunnopsis longicornis</i>	<i>Acanthamunnopsis</i>	Munnopsidae
	EF682261	<i>Acanthamunnopsis milleri</i>	<i>Acanthamunnopsis</i>	Munnopsidae
	EF682262	<i>Acanthamunnopsis sp. 2 A17</i>	<i>Acanthamunnopsis</i>	Munnopsidae
	EF682264	<i>Acanthamunnopsis sp. 3 A26</i>	<i>Acanthamunnopsis</i>	Munnopsidae
	EF682263	<i>Acanthamunnopsis sp. 4 A20</i>	<i>Acanthamunnopsis</i>	Munnopsidae
	EF682286	<i>Acanthocope sp. MB H2</i>	<i>Acanthocope</i>	Munnopsidae
	TE-004T81-02	<i>Aega psora</i>	<i>Aega</i>	Aegidae
	EF670478	<i>Antrolana lira</i>	<i>Antrolana</i>	Cirolanidae
	EF670477	<i>Antrolana lira</i>	<i>Antrolana</i>	Cirolanidae
	EF670476	<i>Antrolana lira</i>	<i>Antrolana</i>	Cirolanidae
	EF670475	<i>Antrolana lira</i>	<i>Antrolana</i>	Cirolanidae
	EF670474	<i>Antrolana lira</i>	<i>Antrolana</i>	Cirolanidae
	EF027716	<i>Armadillidium lobocurvum</i>	<i>Armadillidium</i>	Armadillidiidae
	EF027715	<i>Armadillidium lobocurvum</i>	<i>Armadillidium</i>	Armadillidiidae
	EF027714	<i>Armadillidium lobocurvum</i>	<i>Armadillidium</i>	Armadillidiidae
	EU364630	<i>Armadilloniscus ellipticus</i>	<i>Armadilloniscus</i>	Scyphacidae
	AY566523	<i>Bathynomus giganteus</i>	<i>Bathynomus</i>	Cirolanidae
	EF682292	<i>Betamorpha africana</i>	<i>Betamorpha</i>	Munnopsidae
	EF682291	<i>Betamorpha fusiformis</i>	<i>Betamorpha</i>	Munnopsidae
	AY566522	<i>Caecidotea reddelli</i>	<i>Caecidotea</i>	Asellidae
	AF260834	<i>Caecidotea sp. RW96.018.184</i>	<i>Caecidotea</i>	Asellidae
	AF255778	<i>Caecidotea sp. RW96.026.181</i>	<i>Caecidotea</i>	Asellidae
	EU597422	<i>Ceratoserolis trilobitoides</i>	<i>Ceratoserolis</i>	Serolidae
	EU597421	<i>Ceratoserolis trilobitoides</i>	<i>Ceratoserolis</i>	Serolidae
	EU597420	<i>Ceratoserolis trilobitoides</i>	<i>Ceratoserolis</i>	Serolidae
	EU597419	<i>Ceratoserolis trilobitoides</i>	<i>Ceratoserolis</i>	Serolidae
	EU597418	<i>Ceratoserolis trilobitoides</i>	<i>Ceratoserolis</i>	Serolidae
	EF989646	<i>Cilicaea sp. 72</i>	<i>Cilicaea</i>	Sphaeromatidae
	AF255787	<i>Cirolana harfordi</i>	<i>Cirolana</i>	Cirolanidae
	AF260838	<i>Cirolana harfordi</i>	<i>Cirolana</i>	Cirolanidae
	AY566470	<i>Cirolanides texensis</i>	<i>Cirolanides</i>	Cirolanidae
	AY566471	<i>Cirolanides texensis</i>	<i>Cirolanides</i>	Cirolanidae

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Order	GenBank no.	Species	Genus	Family
Isopoda	AY566473	<i>Cirolanides texensis</i>	<i>Cirolanides</i>	Cirolanidae
	AY566474	<i>Cirolanides texensis</i>	<i>Cirolanides</i>	Cirolanidae
	AY566475	<i>Cirolanides texensis</i>	<i>Cirolanides</i>	Cirolanidae
	AF255775	<i>Colubotelson thomsoni</i>	<i>Colubotelson</i>	Phreatoicidae
	EF682302	<i>Coperonus sp. 1 G20</i>	<i>Coperonus</i>	Munnopsidae
	EF682301	<i>Coperonus sp. 5 G19</i>	<i>Coperonus</i>	Munnopsidae
	AF255776	<i>Crenoicus buntiae</i>	<i>Crenoicus</i>	Phreatoicidae
	EU364625	<i>Deto marina</i>	<i>Deto</i>	Scyphacidae
	EF682293	<i>Disconectes antarctica</i>	<i>Disconectes</i>	Munnopsidae
	EF682294	<i>Dubinectes acutitelson</i>	<i>Dubinectes</i>	Munnopsidae
	L90AR5-01	<i>Edotia triloba</i>	<i>Edotia</i>	Idoteidae
	L90AR5-07	<i>Edotia triloba</i>	<i>Edotia</i>	Idoteidae
	L90AR5-09	<i>Edotia triloba</i>	<i>Edotia</i>	Idoteidae
	EU263143	<i>Eophreatoicus sp. 02</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263142	<i>Eophreatoicus sp. 02</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263145	<i>Eophreatoicus sp. 03</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263147	<i>Eophreatoicus sp. 04</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263146	<i>Eophreatoicus sp. 04</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263150	<i>Eophreatoicus sp. 08</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263149	<i>Eophreatoicus sp. 08</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263148	<i>Eophreatoicus sp. 08</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263152	<i>Eophreatoicus sp. 09</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263151	<i>Eophreatoicus sp. 09</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263153	<i>Eophreatoicus sp. 10</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263154	<i>Eophreatoicus sp. 11</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263156	<i>Eophreatoicus sp. 12</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263159	<i>Eophreatoicus sp. 13</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263158	<i>Eophreatoicus sp. 13</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263162	<i>Eophreatoicus sp. 20</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263161	<i>Eophreatoicus sp. 20</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263166	<i>Eophreatoicus sp. 21</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263165	<i>Eophreatoicus sp. 21</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263164	<i>Eophreatoicus sp. 21</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263163	<i>Eophreatoicus sp. 21</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263170	<i>Eophreatoicus sp. C2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263179	<i>Eophreatoicus sp. Gb</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263178	<i>Eophreatoicus sp. Gb</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263177	<i>Eophreatoicus sp. Gb</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263180	<i>Eophreatoicus sp. J1</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263183	<i>Eophreatoicus sp. J2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263181	<i>Eophreatoicus sp. J2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263188	<i>Eophreatoicus sp. M1</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263187	<i>Eophreatoicus sp. M1</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263193	<i>Eophreatoicus sp. M2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263192	<i>Eophreatoicus sp. M2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263191	<i>Eophreatoicus sp. M2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263190	<i>Eophreatoicus sp. M2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263189	<i>Eophreatoicus sp. M2</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263197	<i>Eophreatoicus sp. S1</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263196	<i>Eophreatoicus sp. S1</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263200	<i>Eophreatoicus sp. Zi</i>	<i>Eophreatoicus</i>	Phreatoicidae
	EU263199	<i>Eophreatoicus sp. Zr</i>	<i>Eophreatoicus</i>	Phreatoicidae

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Isopoda	EU263202	<i>Eophreatoicus</i> sp. Zu	<i>Eophreatoicus</i>	Phreatoicidae
	EU263135	<i>Eremisopus</i> sp. Faraway	<i>Eremisopus</i>	Amphisopodidae
	EF682281	<i>Eurycope complanata</i>	<i>Eurycope</i>	Munnopsidae
	EF682280	<i>Eurycope glabra</i>	<i>Eurycope</i>	Munnopsidae
	EF682279	<i>Eurycope</i> sp. G9	<i>Eurycope</i>	Munnopsidae
	EF682282	<i>Eurycope</i> sp. MB I31	<i>Eurycope</i>	Munnopsidae
	AF260841	<i>Exciorlana chiltoni</i>	<i>Exciorlana</i>	Cirolanidae
	DQ351361	<i>Exosphaeroma hylecoetes</i>	<i>Exosphaeroma</i>	Sphaeromatidae
	DQ351360	<i>Exosphaeroma hylecoetes</i>	<i>Exosphaeroma</i>	Sphaeromatidae
	DQ351355	<i>Exosphaeroma hylecoetes</i>	<i>Exosphaeroma</i>	Sphaeromatidae
	DQ351354	<i>Exosphaeroma hylecoetes</i>	<i>Exosphaeroma</i>	Sphaeromatidae
	DQ351353	<i>Exosphaeroma hylecoetes</i>	<i>Exosphaeroma</i>	Sphaeromatidae
	AF255781	<i>Glyptidotea lichtensteini</i>	<i>Glyptidotea</i>	Idoteidae
	AF260845	<i>Gnorimosphaeroma oregonense</i>	<i>Gnorimosphaeroma</i>	Sphaeromatidae
	EU364626	<i>Haloniscus anophthalmus</i>	<i>Haloniscus</i>	Scyphacidae
	EU364585	<i>Haloniscus longiantennatus</i>	<i>Haloniscus</i>	Scyphacidae
	EU364584	<i>Haloniscus longiantennatus</i>	<i>Haloniscus</i>	Scyphacidae
	EU364583	<i>Haloniscus longiantennatus</i>	<i>Haloniscus</i>	Scyphacidae
	EU364578	<i>Haloniscus longiantennatus</i>	<i>Haloniscus</i>	Scyphacidae
	EU364621	<i>Haloniscus searlei</i>	<i>Haloniscus</i>	Scyphacidae
	EU364620	<i>Haloniscus searlei</i>	<i>Haloniscus</i>	Scyphacidae
	EU364619	<i>Haloniscus searlei</i>	<i>Haloniscus</i>	Scyphacidae
	EU364618	<i>Haloniscus searlei</i>	<i>Haloniscus</i>	Scyphacidae
	EU364617	<i>Haloniscus searlei</i>	<i>Haloniscus</i>	Scyphacidae
	EU364563	<i>Haloniscus</i> sp. 1 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364600	<i>Haloniscus</i> sp. 11 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364599	<i>Haloniscus</i> sp. 11 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364598	<i>Haloniscus</i> sp. 11 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364597	<i>Haloniscus</i> sp. 11 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364596	<i>Haloniscus</i> sp. 11 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364603	<i>Haloniscus</i> sp. 12 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364602	<i>Haloniscus</i> sp. 12 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364601	<i>Haloniscus</i> sp. 12 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364604	<i>Haloniscus</i> sp. 13 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364608	<i>Haloniscus</i> sp. 14 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364607	<i>Haloniscus</i> sp. 14 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364605	<i>Haloniscus</i> sp. 14 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364606	<i>Haloniscus</i> sp. 15 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364609	<i>Haloniscus</i> sp. 16 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364610	<i>Haloniscus</i> sp. 17 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364611	<i>Haloniscus</i> sp. 18 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364613	<i>Haloniscus</i> sp. 19 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364612	<i>Haloniscus</i> sp. 19 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364568	<i>Haloniscus</i> sp. 2 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364567	<i>Haloniscus</i> sp. 2 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364566	<i>Haloniscus</i> sp. 2 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364565	<i>Haloniscus</i> sp. 2 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364564	<i>Haloniscus</i> sp. 2 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364615	<i>Haloniscus</i> sp. 20 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364614	<i>Haloniscus</i> sp. 20 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364569	<i>Haloniscus</i> sp. 3 SJBC-2008	<i>Haloniscus</i>	Scyphacidae
	EU364570	<i>Haloniscus</i> sp. 4 SJBC-2008	<i>Haloniscus</i>	Scyphacidae

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Order	GenBank no.	Species	Genus	Family
Isopoda	EU364575	<i>Haloniscus sp. 5 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364573	<i>Haloniscus sp. 5 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364572	<i>Haloniscus sp. 5 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364571	<i>Haloniscus sp. 5 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364577	<i>Haloniscus sp. 6 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364576	<i>Haloniscus sp. 6 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364574	<i>Haloniscus sp. 6 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364580	<i>Haloniscus sp. 7 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364579	<i>Haloniscus sp. 7 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364587	<i>Haloniscus sp. 9 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	EU364586	<i>Haloniscus sp. 9 SJBC-2008</i>	<i>Haloniscus</i>	Scyphacidae
	L182AR1-08	<i>Idotea balthica</i>	<i>Idotea</i>	Idoteidae
	EF682298	<i>Ilyarachna triangulata</i>	<i>Ilyarachna</i>	Munnopsidae
	EF682304	<i>Ischnomesid sp. MB L1</i>	<i>Ischnomesid</i>	Ischnomesidae
	L90AR1-01	<i>Jaera albifrons</i>	<i>Jaera</i>	Janiridae
	EU364629	<i>Laevophiloscia yalgoensis</i>	<i>Laevophiloscia</i>	Philosciidae
	DQ182858	<i>Ligia italica</i>	<i>Ligia</i>	Ligiidae
	DQ182859	<i>Ligia italica</i>	<i>Ligia</i>	Ligiidae
	DQ182860	<i>Ligia italica</i>	<i>Ligia</i>	Ligiidae
	DQ182861	<i>Ligia italica</i>	<i>Ligia</i>	Ligiidae
	DQ442914	<i>Ligia oceanica</i>	<i>Ligia</i>	Ligiidae
	NC_008412	<i>Ligia oceanica</i>	<i>Ligia</i>	Ligiidae
	DQ182780	<i>Ligidium hypnorum</i>	<i>Ligidium</i>	Ligiidae
	DQ182781	<i>Ligidium hypnorum</i>	<i>Ligidium</i>	Ligiidae
	DQ182810	<i>Ligidium hypnorum</i>	<i>Ligidium</i>	Ligiidae
	DQ182814	<i>Ligidium sp. ADR3</i>	<i>Ligidium</i>	Ligiidae
	DQ182804	<i>Ligidium sp. ZA5</i>	<i>Ligidium</i>	Ligiidae
	AY566531	<i>Lirceolus bisetus</i>	<i>Lirceolus</i>	Asellidae
	AY566532	<i>Lirceolus bisetus</i>	<i>Lirceolus</i>	Asellidae
	AY566533	<i>Lirceolus bisetus</i>	<i>Lirceolus</i>	Asellidae
	AY566530	<i>Lirceolus sp. Dandridge Spring</i>	<i>Lirceolus</i>	Asellidae
	AY566545	<i>Lirceolus sp. Preserve Cave</i>	<i>Lirceolus</i>	Asellidae
	AY566546	<i>Lirceolus sp. Preserve Cave</i>	<i>Lirceolus</i>	Asellidae
	AY566547	<i>Lirceolus sp. Rattlesnake Cave</i>	<i>Lirceolus</i>	Asellidae
	AY566548	<i>Lirceolus sp. Rattlesnake Cave</i>	<i>Lirceolus</i>	Asellidae
	AY566479	<i>Lirceolus sp. Slaughter Bend</i>	<i>Lirceolus</i>	Asellidae
	AF255790	<i>Lironeca vulgaris</i>	<i>Lironeca</i>	Cymothoidae
	AF260842	<i>Lironeca vulgaris</i>	<i>Lironeca</i>	Cymothoidae
	AF260843	<i>Lironeca vulgaris</i>	<i>Lironeca</i>	Cymothoidae
	AY948306	<i>Mesamphisopus penicillatus</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948307	<i>Mesamphisopus sp. 5-GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948290	<i>Mesamphisopus sp. GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948291	<i>Mesamphisopus sp. GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948292	<i>Mesamphisopus sp. GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948293	<i>Mesamphisopus sp. GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY948294	<i>Mesamphisopus sp. GG-2005</i>	<i>Mesamphisopus</i>	Amphisopidae
	AY566478	<i>Mexistenasellus coahuila</i>	<i>Mexistenasellus</i>	Stenasellidae
	EF682275	<i>Munneurycope murrayi</i>	<i>Munneurycope</i>	Munnopsidae
	EF682276	<i>Munneurycope sp. 2 B17</i>	<i>Munneurycope</i>	Munnopsidae
	EF682283	<i>Munneurycope sp. ABE G1</i>	<i>Munneurycope</i>	Munnopsidae
	EF682268	<i>Munnopsisinae sp. D10</i>	<i>Munnopsisinae</i>	Munnopsidae
	EF682273	<i>Munnopsis abyssalis</i>	<i>Munnopsis</i>	Munnopsidae

Order	GenBank no.	Species	Genus	Family
Isopoda	EF682271	<i>Munnopsoides sp. MB C12</i>	<i>Munnopsoides</i>	Munnopsidae
	EF682289	<i>Munnopsurus sp. 1 G4</i>	<i>Munnopsurus</i>	Munnopsidae
	EF682288	<i>Munnopsurus sp. MB K2</i>	<i>Munnopsurus</i>	Munnopsidae
	EF682300	<i>Notopais magnifica</i>	<i>Notopais</i>	Munnopsidae
	AF255791	<i>Olencira praegustator</i>	<i>Olencira</i>	Cymothoidae
	AF260844	<i>Olencira praegustator</i>	<i>Olencira</i>	Cymothoidae
	EF682267	<i>Paramunnopsis sp. 1 D11</i>	<i>Paramunnopsis</i>	Munnopsidae
	EF682270	<i>Paramunnopsis sp. 2 D6</i>	<i>Paramunnopsis</i>	Munnopsidae
	EF682266	<i>Paramunnopsis sp. 5 D15</i>	<i>Paramunnopsis</i>	Munnopsidae
	AF255783	<i>Paridotea ungulata</i>	<i>Paridotea</i>	Idoteidae
	EF682287	<i>Paropsurus giganteus</i>	<i>Paropsurus</i>	Munnopsidae
	DQ305142	<i>Porcellio scaber</i>	<i>Porcellio</i>	Porcellionidae
	EU364627	<i>Porcellionides pruinosus</i>	<i>Porcellionides</i>	Porcellionidae
	DQ305136	<i>Proasellus gjorgjevici</i>	<i>Proasellus</i>	Asellidae
	DQ305135	<i>Proasellus gjorgjevici</i>	<i>Proasellus</i>	Asellidae
	DQ305134	<i>Proasellus gjorgjevici</i>	<i>Proasellus</i>	Asellidae
	DQ305133	<i>Proasellus gjorgjevici</i>	<i>Proasellus</i>	Asellidae
	EU107639	<i>Pygolabis eberhardi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107638	<i>Pygolabis eberhardi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107637	<i>Pygolabis gascoynensis</i>	<i>Pygolabis</i>	Tainisopidae
	EU364628	<i>Pygolabis humphreysi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107580	<i>Pygolabis humphreysi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107579	<i>Pygolabis humphreysi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107578	<i>Pygolabis humphreysi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107577	<i>Pygolabis humphreysi</i>	<i>Pygolabis</i>	Tainisopidae
	EU107636	<i>Pygolabis paraburdo</i>	<i>Pygolabis</i>	Tainisopidae
	EU107635	<i>Pygolabis paraburdo</i>	<i>Pygolabis</i>	Tainisopidae
	EU107634	<i>Pygolabis paraburdo</i>	<i>Pygolabis</i>	Tainisopidae
	EU107633	<i>Pygolabis paraburdo</i>	<i>Pygolabis</i>	Tainisopidae
	EU107632	<i>Pygolabis paraburdo</i>	<i>Pygolabis</i>	Tainisopidae
	EU107646	<i>Pygolabis sp. 1 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107642	<i>Pygolabis sp. 2 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107643	<i>Pygolabis sp. 3 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107647	<i>Pygolabis sp. 5 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107645	<i>Pygolabis sp. 6 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107644	<i>Pygolabis sp. 6 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107641	<i>Pygolabis sp. 7 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107640	<i>Pygolabis sp. 7 TLF-2007</i>	<i>Pygolabis</i>	Tainisopidae
	EU107618	<i>Pygolabis weeliwolli</i>	<i>Pygolabis</i>	Tainisopidae
	EU107617	<i>Pygolabis weeliwolli</i>	<i>Pygolabis</i>	Tainisopidae
	EU107616	<i>Pygolabis weeliwolli</i>	<i>Pygolabis</i>	Tainisopidae
	EU107615	<i>Pygolabis weeliwolli</i>	<i>Pygolabis</i>	Tainisopidae
	EU107614	<i>Pygolabis weeliwolli</i>	<i>Pygolabis</i>	Tainisopidae
	AY566472	<i>Remasellus parvus</i>	<i>Remasellus</i>	Asellidae
	AY857829	<i>Santia sp. A1</i>	<i>Santia</i>	Santiidae
	AY857828	<i>Santia sp. B1</i>	<i>Santia</i>	Santiidae
	EU597357	<i>Septemserolis septemcarinata</i>	<i>Septemserolis</i>	Serolidae
	EU597356	<i>Septemserolis septemcarinata</i>	<i>Septemserolis</i>	Serolidae
	EU597355	<i>Septemserolis septemcarinata</i>	<i>Septemserolis</i>	Serolidae
	EU597354	<i>Septemserolis septemcarinata</i>	<i>Septemserolis</i>	Serolidae
	EU597353	<i>Septemserolis septemcarinata</i>	<i>Septemserolis</i>	Serolidae
	AF255786	<i>Serolina bakeri</i>	<i>Serolina</i>	Serolidae

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Order	GenBank no.	Species	Genus	Family
Isopoda	AY566524	<i>Speocirolana hardeni</i>	<i>Speocirolana</i>	Cirolanidae
	AY566525	<i>Speocirolana hardeni</i>	<i>Speocirolana</i>	Cirolanidae
	AY566526	<i>Speocirolana thermydronis</i>	<i>Speocirolana</i>	Cirolanidae
	AY566518	<i>Sphaerolana affinis</i>	<i>Sphaerolana</i>	Cirolanidae
	AY566519	<i>Sphaerolana affinis</i>	<i>Sphaerolana</i>	Cirolanidae
	AF255785	<i>Sphaeroma quadridentatum</i>	<i>Sphaeroma</i>	Sphaeromatidae
	AY028589	<i>Stenasellus racovitzai</i>	<i>Stenasellus</i>	Stenasellidae
	EF682295	<i>Syneurycope heezeni</i>	<i>Syneurycope</i>	Munnopsidae
	CA01NFG106	<i>Syscenus infelix</i>	<i>Syscenus</i>	Aegidae
	CA02NFG106	<i>Syscenus infelix</i>	<i>Syscenus</i>	Aegidae
	TE-004T88-01	<i>Syscenus infelix</i>	<i>Syscenus</i>	Aegidae
	TE-004T161-01	<i>Syscenus infelix</i>	<i>Syscenus</i>	Aegidae
	TE-004T161-02	<i>Syscenus infelix</i>	<i>Syscenus</i>	Aegidae
	EU107666	<i>Tainisopus sp. 1 TLF-2007</i>	<i>Tainisopus</i>	Tainisopidae
	EU107665	<i>Tainisopus sp. 1 TLF-2007</i>	<i>Tainisopus</i>	Tainisopidae
	EF659963	<i>Trachelipus sp. AP-2007</i>	<i>Trachelipus</i>	Trachelipodidae
	EF659962	<i>Trachelipus sp. AP-2007</i>	<i>Trachelipus</i>	Trachelipodidae
	EF659957	<i>Trachelipus sp. AP-2007</i>	<i>Trachelipus</i>	Trachelipodidae
	EF659956	<i>Trachelipus sp. AP-2007</i>	<i>Trachelipus</i>	Trachelipodidae
	EU364624	<i>Tylos neozelanicus</i>	<i>Tylos</i>	Tylidae
	EF027456	<i>Tylos ponticus</i>	<i>Tylos</i>	Tylidae
	EF027455	<i>Tylos ponticus</i>	<i>Tylos</i>	Tylidae
	EF027454	<i>Tylos ponticus</i>	<i>Tylos</i>	Tylidae
	EF682290	<i>Tytthocope sp. 3 G8</i>	<i>Tytthocope</i>	Munnopsidae
	EF682284	<i>Vanhoeffenura sp. MB E1</i>	<i>Vanhoeffenura</i>	Munnopsidae

Table S 2.1.2: Accession numbers from BOLD for the sequences used in this study. Specimens' list of 902 COI sequences from 337 species, 202 genera and 101 families

SampleID	Order	Family	Species
FJ581472	Amphipoda	Talitridae	<i>Americorchestia longicornis</i>
FJ581471	Amphipoda	Talitridae	<i>Americorchestia longicornis</i>
FJ581470	Amphipoda	Talitridae	<i>Americorchestia longicornis</i>
FJ581469	Amphipoda	Talitridae	<i>Americorchestia longicornis</i>
FJ581468	Amphipoda	Talitridae	<i>Americorchestia longicornis</i>
FJ581474	Amphipoda	Talitridae	<i>Americorchestia megalophthalma</i>
FJ581477	Amphipoda	Talitridae	<i>Americorchestia megalophthalma</i>
FJ581476	Amphipoda	Talitridae	<i>Americorchestia megalophthalma</i>
FJ581475	Amphipoda	Talitridae	<i>Americorchestia megalophthalma</i>
FJ581484	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 1</i>
FJ581483	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 1</i>
FJ581489	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 2</i>
FJ581488	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 2</i>
FJ581487	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 2</i>
FJ581485	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 2</i>
FJ581481	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca eschrichtii 2</i>
FJ581492	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe longimana</i>
FJ581491	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe longimana</i>
FJ581490	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe longimana</i>
FJ581497	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe rubricata</i>
FJ581496	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe rubricata</i>
FJ581495	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe rubricata</i>
FJ581494	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe rubricata</i>
FJ581493	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe rubricata</i>
FJ581503	Amphipoda	Uristidae	<i>Anonyx makarovi</i>
FJ581505	Amphipoda	Uristidae	<i>Anonyx makarovi</i>
FJ581504	Amphipoda	Uristidae	<i>Anonyx makarovi</i>
FJ581501	Amphipoda	Uristidae	<i>Anonyx makarovi</i>
FJ581500	Amphipoda	Uristidae	<i>Anonyx makarovi</i>
FJ581513	Amphipoda	Uristidae	<i>Anonyx sarsi</i>
FJ581517	Amphipoda	Uristidae	<i>Anonyx sarsi</i>
FJ581516	Amphipoda	Uristidae	<i>Anonyx sarsi</i>
FJ581515	Amphipoda	Uristidae	<i>Anonyx sarsi</i>
FJ581514	Amphipoda	Uristidae	<i>Anonyx sarsi</i>
FJ581558	Amphipoda	Calliopiidae	<i>Calliopius laeviusculus</i>
FJ581555	Amphipoda	Calliopiidae	<i>Calliopius laeviusculus</i>
FJ581552	Amphipoda	Calliopiidae	<i>Calliopius laeviusculus</i>
FJ581547	Amphipoda	Calliopiidae	<i>Calliopius laeviusculus</i>
FJ581544	Amphipoda	Calliopiidae	<i>Calliopius laeviusculus</i>
FJ581574	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581573	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581572	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581570	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581569	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581568	Amphipoda	Caprellidae	<i>Caprella linearis</i>
FJ581579	Amphipoda	Caprellidae	<i>Caprella mutica</i>
FJ581578	Amphipoda	Caprellidae	<i>Caprella mutica</i>
FJ581577	Amphipoda	Caprellidae	<i>Caprella mutica</i>
FJ581576	Amphipoda	Caprellidae	<i>Caprella mutica</i>

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FJ581589	Amphipoda	Caprellidae	<i>Caprella septentrionalis</i>
FJ581585	Amphipoda	Caprellidae	<i>Caprella septentrionalis</i>
FJ581584	Amphipoda	Caprellidae	<i>Caprella septentrionalis</i>
FJ581583	Amphipoda	Caprellidae	<i>Caprella septentrionalis</i>
FJ581582	Amphipoda	Caprellidae	<i>Caprella septentrionalis</i>
FJ581623	Amphipoda	Gammaridae	<i>Echinogammarus ischnus</i>
FJ581622	Amphipoda	Gammaridae	<i>Echinogammarus ischnus</i>
FJ581621	Amphipoda	Gammaridae	<i>Echinogammarus ischnus</i>
FJ581620	Amphipoda	Gammaridae	<i>Echinogammarus ischnus</i>
FJ581637	Amphipoda	Eusiridae	<i>Eusirus cuspidatus</i>
FJ581636	Amphipoda	Eusiridae	<i>Eusirus cuspidatus</i>
FJ581638	Amphipoda	Gammarellidae	<i>Gammarellus angulosus</i>
FJ581644	Amphipoda	Gammaridae	<i>Gammarus duebeni</i>
FJ581643	Amphipoda	Gammaridae	<i>Gammarus duebeni</i>
FJ581642	Amphipoda	Gammaridae	<i>Gammarus duebeni</i>
FJ581641	Amphipoda	Gammaridae	<i>Gammarus duebeni</i>
FJ581640	Amphipoda	Gammaridae	<i>Gammarus duebeni</i>
FJ581661	Amphipoda	Gammaridae	<i>Gammarus lawrencianus</i>
FJ581659	Amphipoda	Gammaridae	<i>Gammarus lawrencianus</i>
FJ581654	Amphipoda	Gammaridae	<i>Gammarus lawrencianus</i>
FJ581652	Amphipoda	Gammaridae	<i>Gammarus lawrencianus</i>
FJ581649	Amphipoda	Gammaridae	<i>Gammarus lawrencianus</i>
FJ581674	Amphipoda	Gammaridae	<i>Gammarus mucronatus</i>
FJ581672	Amphipoda	Gammaridae	<i>Gammarus mucronatus</i>
FJ581670	Amphipoda	Gammaridae	<i>Gammarus mucronatus</i>
FJ581668	Amphipoda	Gammaridae	<i>Gammarus mucronatus</i>
FJ581666	Amphipoda	Gammaridae	<i>Gammarus mucronatus</i>
FJ581677	Amphipoda	Gammaridae	<i>Gammarus oceanicus</i>
FJ581676	Amphipoda	Gammaridae	<i>Gammarus oceanicus</i>
FJ581675	Amphipoda	Gammaridae	<i>Gammarus oceanicus</i>
FJ581685	Amphipoda	Gammaridae	<i>Gammarus tigrinus</i>
FJ581682	Amphipoda	Gammaridae	<i>Gammarus tigrinus</i>
FJ581690	Amphipoda	Gammaridae	<i>Gammarus tigrinus</i>
FJ581686	Amphipoda	Gammaridae	<i>Gammarus tigrinus</i>
FJ581678	Amphipoda	Gammaridae	<i>Gammarus tigrinus</i>
FJ581692	Amphipoda	Calliopiidae	<i>Halirages fulvocinctus</i>
FJ581691	Amphipoda	Calliopiidae	<i>Halirages fulvocinctus</i>
FJ581698	Amphipoda	Hyalidae	<i>Hyale prevostii</i>
FJ581732	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes</i>
FJ581731	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes</i>
FJ581719	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes</i>
FJ581726	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes</i>
FJ581723	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes</i>
FJ581733	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes 2</i>
FJ581720	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes 2</i>
FJ581727	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes 2</i>
FJ581724	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes 2</i>
FJ581734	Amphipoda	Ischyroceridae	<i>Ischyrocerus anguipes 2</i>
FJ581758	Amphipoda	Melitidae	<i>Melita dentata</i>
FJ581757	Amphipoda	Melitidae	<i>Melita dentata</i>
FJ581763	Amphipoda	Melitidae	<i>Melita formosa</i>
FJ581762	Amphipoda	Melitidae	<i>Melita formosa</i>
FJ581761	Amphipoda	Melitidae	<i>Melita formosa</i>

FJ581765	Amphipoda	Oedicerotidae	<i>Monoculodes intermedius</i>
FJ581764	Amphipoda	Oedicerotidae	<i>Monoculodes intermedius</i>
FJ581767	Amphipoda	Pontoporeiidae	<i>Monoporeia sp.</i>
FJ581766	Amphipoda	Pontoporeiidae	<i>Monoporeia sp.</i>
FJ581794	Amphipoda	Oedicerotidae	<i>Oediceros saginatus</i>
FJ581793	Amphipoda	Oedicerotidae	<i>Oediceros saginatus</i>
FJ581792	Amphipoda	Oedicerotidae	<i>Oediceros saginatus</i>
FJ581791	Amphipoda	Oedicerotidae	<i>Oediceros saginatus</i>
FJ581790	Amphipoda	Oedicerotidae	<i>Oediceros saginatus</i>
FJ581798	Amphipoda	Uristidae	<i>Onisimus litoralis 3</i>
FJ581797	Amphipoda	Uristidae	<i>Onisimus litoralis 3</i>
FJ581796	Amphipoda	Uristidae	<i>Onisimus litoralis 3</i>
FJ581795	Amphipoda	Uristidae	<i>Onisimus litoralis 3</i>
FJ581800	Amphipoda	Lysianassidae	<i>Orchomenella minuta</i>
FJ581799	Amphipoda	Lysianassidae	<i>Orchomenella minuta</i>
FJ581807	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581806	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581804	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581803	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581811	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581810	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581809	Amphipoda	Lysianassidae	<i>Orchomenella pinguis</i>
FJ581852	Amphipoda	Epimeriidae	<i>ParAmphipodahithoe hystrix</i>
FJ581858	Amphipoda	Talitridae	<i>Platorchestia platensis 1</i>
FJ581857	Amphipoda	Talitridae	<i>Platorchestia platensis 1</i>
FJ581856	Amphipoda	Talitridae	<i>Platorchestia platensis 1</i>
FJ581861	Amphipoda	Pleustidae	<i>Pleustes panoplus</i>
FJ581860	Amphipoda	Pleustidae	<i>Pleustes panoplus</i>
FJ581859	Amphipoda	Pleustidae	<i>Pleustes panoplus</i>
FJ581863	Amphipoda	Eusiridae	<i>Pontogeneia inermis</i>
FJ581862	Amphipoda	Eusiridae	<i>Pontogeneia inermis</i>
FJ581864	Amphipoda	Eusiridae	<i>Pontogeneia inermis</i>
FJ581868	Amphipoda	Lysianassidae	<i>Psammonyx nobilis</i>
FJ581867	Amphipoda	Lysianassidae	<i>Psammonyx nobilis</i>
FJ581870	Amphipoda	Lysianassidae	<i>Psammonyx nobilis</i>
FJ581869	Amphipoda	Lysianassidae	<i>Psammonyx nobilis</i>
FJ581878	Amphipoda	Lysianassidae	<i>Psammonyx terranova</i>
FJ581877	Amphipoda	Lysianassidae	<i>Psammonyx terranova</i>
FJ581876	Amphipoda	Lysianassidae	<i>Psammonyx terranova</i>
FJ581875	Amphipoda	Lysianassidae	<i>Psammonyx terranova</i>
FJ581874	Amphipoda	Lysianassidae	<i>Psammonyx terranova</i>
FJ581881	Amphipoda	Eusiridae	<i>Rhachotropis aculeata</i>
FJ581885	Amphipoda	Eusiridae	<i>Rhachotropis aculeata</i>
FJ581883	Amphipoda	Eusiridae	<i>Rhachotropis aculeata</i>
FJ581880	Amphipoda	Eusiridae	<i>Rhachotropis aculeata</i>
FJ581886	Amphipoda	Eusiridae	<i>Rhachotropis aculeata</i>
FJ581920	Amphipoda	Hyperiidae	<i>Themisto libellula</i>
FJ581919	Amphipoda	Hyperiidae	<i>Themisto libellula</i>
FJ581918	Amphipoda	Hyperiidae	<i>Themisto libellula</i>
FJ581917	Amphipoda	Hyperiidae	<i>Themisto libellula</i>
FJ581916	Amphipoda	Hyperiidae	<i>Themisto libellula</i>
U92668	Amphipoda	Lysianassidae	<i>Abyssorchromene sp. 1143-2</i>
U92669	Amphipoda	Lysianassidae	<i>Abyssorchromene sp. SCSN</i>

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AY061800	Amphipoda	Gammaridae	<i>Acanthogammarus flavus</i>
EF989700	Amphipoda	Scinidae	<i>Acanthoscina acanthodes</i>
AmphipodaH022.1	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca dalmatina</i>
AmphipodaH045.1	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca</i>
AmphipodaH045.2	Amphipoda	Amphipodaeliscidae	<i>Amphipodaelisca</i>
AY926653	Amphipoda	Amphipodaithoidae	<i>Amphipodaithoe longimana</i>
FJ037788	Amphipoda	Hyalidae	<i>Apohyale pugettensis</i>
AY926654	Amphipoda	Acanthogammaridae	<i>Brandtia lata</i>
AmphipodaH017.1	Amphipoda	Amphipodaeliscidae	<i>Byblis cf. guernei</i>
EF989681	Amphipoda		<i>Caprellidae sp. KBH 25</i>
AmphipodaH040.6	Amphipoda	Carangoliopsidae	<i>Carangoliopsis cf. spinulosa</i>
AmphipodaH040.7	Amphipoda	Carangoliopsidae	<i>Carangoliopsis cf. spinulosa</i>
AY926657	Amphipoda	Gammaridae	<i>Chaetogammarus stoevensis</i>
DQ838017	Amphipoda	Paramelitidae	<i>Chydaekata acuminata</i>
DQ838018	Amphipoda	Paramelitidae	<i>Chydaekata acuminata</i>
DQ838019	Amphipoda	Paramelitidae	<i>Chydaekata acuminata</i>
DQ838020	Amphipoda	Paramelitidae	<i>Chydaekata acuminata</i>
DQ838021	Amphipoda	Paramelitidae	<i>Chydaekata acuminata</i>
DQ256007	Amphipoda	Paramelitidae	<i>Chydaekata sp. A TLF-2008</i>
DQ256008	Amphipoda	Paramelitidae	<i>Chydaekata sp. A TLF-2008</i>
DQ256009	Amphipoda	Paramelitidae	<i>Chydaekata sp. A TLF-2008</i>
DQ256010	Amphipoda	Paramelitidae	<i>Chydaekata sp. A TLF-2008</i>
DQ256011	Amphipoda	Paramelitidae	<i>Chydaekata sp. A TLF-2008</i>
DQ255990	Amphipoda	Paramelitidae	<i>Chydaekata sp. B TLF-2008</i>
DQ255991	Amphipoda	Paramelitidae	<i>Chydaekata sp. B TLF-2008</i>
DQ255992	Amphipoda	Paramelitidae	<i>Chydaekata sp. B TLF-2008</i>
DQ255993	Amphipoda	Paramelitidae	<i>Chydaekata sp. B TLF-2008</i>
DQ255994	Amphipoda	Paramelitidae	<i>Chydaekata sp. B TLF-2008</i>
DQ256015	Amphipoda	Paramelitidae	<i>Chydaekata sp. D TLF-2008</i>
DQ256012	Amphipoda	Paramelitidae	<i>Chydaekata sp. D TLF-2008</i>
DQ256013	Amphipoda	Paramelitidae	<i>Chydaekata sp. D TLF-2008</i>
DQ256014	Amphipoda	Paramelitidae	<i>Chydaekata sp. D TLF-2008</i>
DQ255963	Amphipoda	Paramelitidae	<i>Chydaekata sp. E TLF-2008</i>
DQ255964	Amphipoda	Paramelitidae	<i>Chydaekata sp. E TLF-2008</i>
DQ255965	Amphipoda	Paramelitidae	<i>Chydaekata sp. E TLF-2008</i>
DQ255966	Amphipoda	Paramelitidae	<i>Chydaekata sp. E TLF-2008</i>
DQ255967	Amphipoda	Paramelitidae	<i>Chydaekata sp. E TLF-2008</i>
DQ679981	Amphipoda	Paramelitidae	<i>Chydaekata sp. TLF-2005</i>
DQ679982	Amphipoda	Paramelitidae	<i>Chydaekata sp. TLF-2005</i>
DQ679983	Amphipoda	Paramelitidae	<i>Chydaekata sp. TLF-2005</i>
DQ679984	Amphipoda	Paramelitidae	<i>Chydaekata sp. TLF-2005</i>
DQ679985	Amphipoda	Paramelitidae	<i>Chydaekata sp. TLF-2005</i>
AJ968910	Amphipoda	Crangonyctidae	<i>Crangonyx floridanus</i>
AJ968911	Amphipoda	Crangonyctidae	<i>Crangonyx floridanus</i>
AJ968896	Amphipoda	Crangonyctidae	<i>Crangonyx pseudogracilis</i>
AJ968897	Amphipoda	Crangonyctidae	<i>Crangonyx pseudogracilis</i>
AJ968893	Amphipoda	Crangonyctidae	<i>Crangonyx pseudogracilis</i>
AJ968894	Amphipoda	Crangonyctidae	<i>Crangonyx pseudogracilis</i>
AJ968895	Amphipoda	Crangonyctidae	<i>Crangonyx pseudogracilis</i>
AY926658	Amphipoda	Crangonyctidae	<i>Crangonyx serratus</i>
DQ095150	Amphipoda	Cyamidae	<i>Cyamus boopis</i>
DQ095106	Amphipoda	Cyamidae	<i>Cyamus erraticus</i>
DQ095107	Amphipoda	Cyamidae	<i>Cyamus erraticus</i>

DQ095127	Amphipoda	Cyamidae	<i>Cyamus erraticus</i>
DQ095128	Amphipoda	Cyamidae	<i>Cyamus erraticus</i>
DQ095129	Amphipoda	Cyamidae	<i>Cyamus erraticus</i>
DQ095048	Amphipoda	Cyamidae	<i>Cyamus gracilis</i>
DQ095049	Amphipoda	Cyamidae	<i>Cyamus gracilis</i>
DQ095050	Amphipoda	Cyamidae	<i>Cyamus gracilis</i>
DQ095078	Amphipoda	Cyamidae	<i>Cyamus gracilis</i>
DQ095079	Amphipoda	Cyamidae	<i>Cyamus gracilis</i>
EF989691	Amphipoda	Hyperiidae	<i>Cyllopus lucasii</i>
EF989690	Amphipoda	Hyperiidae	<i>Cyllopus magellanicus</i>
EF989675	Amphipoda	CystIsopodamatidae	<i>CystIsopodama gershwiniae</i>
EF989676	Amphipoda	CystIsopodamatidae	<i>CystIsopodama pellucida</i>
AmphipodaH019.1	Amphipoda	Oedicerotidae	<i>Deflexilodes acutipes</i>
AmphipodaH019.2	Amphipoda	Oedicerotidae	<i>Deflexilodes acutipes</i>
AY529049	Amphipoda	Gammaridae	<i>Dikerogammarus haemobaphes</i>
EF570297	Amphipoda	Gammaridae	<i>Dikerogammarus villosus</i>
AY529048	Amphipoda	Gammaridae	<i>Dikerogammarus villosus</i>
EU761573	Amphipoda	Pontoporeiidae	<i>Diporeia hoyi</i>
EU761574	Amphipoda	Pontoporeiidae	<i>Diporeia hoyi</i>
EU761575	Amphipoda	Pontoporeiidae	<i>Diporeia hoyi</i>
EU761576	Amphipoda	Pontoporeiidae	<i>Diporeia hoyi</i>
EU761577	Amphipoda	Pontoporeiidae	<i>Diporeia hoyi</i>
AmphipodaH028.1	Amphipoda	Phoxocephalidae	<i>Harpinia truncata</i>
AmphipodaH028.2	Amphipoda	Phoxocephalidae	<i>Harpinia truncata</i>
AmphipodaH028.3	Amphipoda	Phoxocephalidae	<i>Harpinia truncata</i>
AmphipodaH044.1	Amphipoda	Phoxocephalidae	<i>Harpinia</i>
AmphipodaH044.2	Amphipoda	Phoxocephalidae	<i>Harpinia</i>
AmphipodaH024.1	Amphipoda	Lysianassidae	<i>Hippomedon massiliensis</i>
AmphipodaH024.2	Amphipoda	Lysianassidae	<i>Hippomedon massiliensis</i>
AmphipodaH008.1	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.2	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.3	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.4	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.5	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.6	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH008.7	Amphipoda	Liljeborgiidae	<i>Idunella excavata</i>
AmphipodaH031.1	Amphipoda	Lysianassidae	<i>Lysianassa plumosa</i>
AmphipodaH031.2	Amphipoda	Lysianassidae	<i>Lysianassa plumosa</i>
AmphipodaH031.3	Amphipoda	Lysianassidae	<i>Lysianassa plumosa</i>
AmphipodaH014.1	Amphipoda	Ischyroceridae	<i>Notopoma</i>
AmphipodaH014.2	Amphipoda	Ischyroceridae	<i>Notopoma</i>
AmphipodaH002.1	Amphipoda	Oedicerotidae	<i>Oedicroides pilosa</i>
AmphipodaH002.2	Amphipoda	Oedicerotidae	<i>Oedicroides pilosa</i>
AmphipodaH001	Amphipoda	Hyperiidae	<i>Parathemisto</i>
AmphipodaH012.1	Amphipoda	Caprellidae	<i>Parvipalpus linea</i>
AmphipodaH012.2	Amphipoda	Caprellidae	<i>Parvipalpus linea</i>
AmphipodaH030.3	Amphipoda	Caprellidae	<i>Phtisica marina</i>
AmphipodaH003.1	Amphipoda	Sebidae	<i>Seba aloe</i>
AmphipodaH003.2	Amphipoda	Sebidae	<i>Seba aloe</i>
AmphipodaH003.3	Amphipoda	Sebidae	<i>Seba aloe</i>
AmphipodaH009.2	Amphipoda	Vibiliidae	<i>Vibilia viatrix</i>
AmphipodaH009.3	Amphipoda	Vibiliidae	<i>Vibilia viatrix</i>
AmphipodaH009.4	Amphipoda	Vibiliidae	<i>Vibilia viatrix</i>

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AmphipodaH009.5	Amphipoda	Vibiliidae	<i>Vibilia viatrix</i>
EF989658	Amphipoda	Brachyscelidae	<i>Brachyscelus crusculum</i>
EF989649	Amphipoda	Oxycephalidae	<i>Calamorhynchus pellucidus</i>
EF989648	Amphipoda	Oxycephalidae	<i>Cranocephalus scleroticus</i>
JSDUKdeep_36	Decapoda	Oplophoridae	<i>Acanthephyra pelagica</i>
JSDUKdeep_37	Decapoda	Oplophoridae	<i>Acanthephyra pelagica</i>
JSDUKdeep_38	Decapoda	Oplophoridae	<i>Acanthephyra pelagica</i>
JSDPX63-03	Decapoda	Oplophoridae	<i>Acanthephyra purpurea</i>
JSDPX63-02	Decapoda	Oplophoridae	<i>Acanthephyra purpurea</i>
JSDMe59	Decapoda	Epialtidae	<i>Acanthonyx lunulatus</i>
JSDMe61	Decapoda	Epialtidae	<i>Acanthonyx lunulatus</i>
FCFOPC047-03	Decapoda	Alpheidae	<i>Alpheus glaber</i>
FCFOPC043-05	Decapoda	Alpheidae	<i>Alpheus glaber</i>
FCFOPC044-05	Decapoda	Alpheidae	<i>Alpheus glaber</i>
FCFOPC047-05	Decapoda	Alpheidae	<i>Alpheus glaber</i>
FCFOPC044-06	Decapoda	Alpheidae	<i>Alpheus glaber</i>
JSDMe36	Decapoda	Aristeidae	<i>Aristaeomorpha foliacea</i>
JSDPXA40-01	Decapoda	Aristeidae	<i>Aristaeomorpha foliacea</i>
JSDMe35	Decapoda	Aristeidae	<i>Aristaeomorpha foliacea</i>
JSDMe84	Decapoda	Aristeidae	<i>Aristaeomorpha foliacea</i>
FCFOPC055-05	Decapoda	Aristeidae	<i>Aristaeomorpha foliacea</i>
JSDMe75	Decapoda	Aristeidae	<i>Aristeus antennatus</i>
JSDUK01	Decapoda	Atelecyclidae	<i>Atelecyclus rotundatus</i>
JSDPX22-01	Decapoda	Atelecyclidae	<i>Atelecyclus rotundatus</i>
FCDOPB077-04	Decapoda	Atelecyclidae	<i>Atelecyclus rotundatus</i>
JSDUK02	Decapoda	Atelecyclidae	<i>Atelecyclus rotundatus</i>
JSDUK06	Decapoda	Alpheidae	<i>Athanas nitescens</i>
JSDUK07	Decapoda	Alpheidae	<i>Athanas nitescens</i>
JSDUK05	Decapoda	Alpheidae	<i>Athanas nitescens</i>
JSDUK03	Decapoda	Alpheidae	<i>Athanas nitescens</i>
JSDUK04	Decapoda	Alpheidae	<i>Athanas nitescens</i>
FCFOPC041-22	Decapoda	Macropipidae	<i>Bathynectes maravigna</i>
JSDUKdeep_48	Decapoda	Macropipidae	<i>Bathynectes maravigna</i>
FCFOPC041-20	Decapoda	Macropipidae	<i>Bathynectes maravigna</i>
FCDPHM2_109	Decapoda	Macropipidae	<i>Bathynectes maravigna</i>
FCDPH12-388Gr68	Decapoda	Macropipidae	<i>Bathynectes maravigna</i>
JSDAz218	Decapoda	Calappidae	<i>Calappa granulata</i>
JSDAz51	Decapoda	Diogenidae	<i>Calcinus tubularis</i>
JSDAz66	Decapoda	Diogenidae	<i>Calcinus tubularis</i>
JSDAz52	Decapoda	Diogenidae	<i>Calcinus tubularis</i>
JSDAz133	Decapoda	Diogenidae	<i>Calcinus tubularis</i>
JSDAz67	Decapoda	Diogenidae	<i>Calcinus tubularis</i>
JSDPX44-10	Decapoda	Cancridae	<i>Cancer bellianus</i>
JSDUK10	Decapoda	Cancridae	<i>Cancer pagurus</i>
JSDUK11	Decapoda	Cancridae	<i>Cancer pagurus</i>
JSDMe58	Decapoda	Carcinidae	<i>Carcinus aestuarii</i>
JSDUK19	Decapoda	Carcinidae	<i>Carcinus maenas</i>
JSDN01	Decapoda	Carcinidae	<i>Carcinus maenas</i>
JSDN03	Decapoda	Carcinidae	<i>Carcinus maenas</i>
JSDN02	Decapoda	Carcinidae	<i>Carcinus maenas</i>
JSDUK16	Decapoda	Carcinidae	<i>Carcinus maenas</i>
JSDMe23	Decapoda	Pandalidae	<i>Chlorotocus crassicornis</i>
JSDMe24	Decapoda	Pandalidae	<i>Chlorotocus crassicornis</i>

JSDMe67	Decapoda	Diogenidae	<i>Clibanarius erythropus</i>
JSDAz115	Decapoda	Diogenidae	<i>Clibanarius erythropus</i>
JSDMe63	Decapoda	Diogenidae	<i>Clibanarius erythropus</i>
JSDMe66	Decapoda	Diogenidae	<i>Clibanarius erythropus</i>
JSDUK22	Decapoda	Corystidae	<i>Corystes cassivelaunus</i>
JSDUK23	Decapoda	Corystidae	<i>Corystes cassivelaunus</i>
JSDUK24	Decapoda	Corystidae	<i>Corystes cassivelaunus</i>
FCDPHMSM241_105	Decapoda	Cymonomidae	<i>Cymonomus granulatus</i>
FCDPH15-581Gr59	Decapoda	Cymonomidae	<i>Cymonomus granulatus</i>
FCDPHMSM241_106	Decapoda	Cymonomidae	<i>Cymonomus granulatus</i>
FCDPH15-581Gr57	Decapoda	Cymonomidae	<i>Cymonomus granulatus</i>
FCDPH15-581Gr56	Decapoda	Cymonomidae	<i>Cymonomus granulatus</i>
JSDMe47	Decapoda	Diogenidae	<i>Dardanus arrosor</i>
JSDMe48	Decapoda	Diogenidae	<i>Dardanus arrosor</i>
JSDAz217	Decapoda	Diogenidae	<i>Dardanus arrosor</i>
FCFOP64-02	Decapoda	Diogenidae	<i>Dardanus arrosor</i>
FCFOP64-04	Decapoda	Diogenidae	<i>Dardanus arrosor</i>
JSDAz46	Decapoda	Diogenidae	<i>Dardanus calidus</i>
JSDAz47	Decapoda	Diogenidae	<i>Dardanus calidus</i>
JSDAz48	Decapoda	Diogenidae	<i>Dardanus calidus</i>
JSDAz207	Decapoda	Diogenidae	<i>Dardanus calidus</i>
JSDAz208	Decapoda	Diogenidae	<i>Dardanus calidus</i>
JSDUKdeep_51	Decapoda	Pandalidae	<i>Dichelopandalus bonnieri</i>
JSDUKdeep_53	Decapoda	Pandalidae	<i>Dichelopandalus bonnieri</i>
JSDAz130	Decapoda	Dromiidae	<i>Dromia personata</i>
JSDAz199	Decapoda	Dromiidae	<i>Dromia personata</i>
FCDPHMSM241_103	Decapoda	Leucosiidae	<i>Ebalia nux</i>
FCDPHMSM321_97	Decapoda	Leucosiidae	<i>Ebalia nux</i>
FCDPHMSM241_104	Decapoda	Leucosiidae	<i>Ebalia nux</i>
FCDPH14-565B64	Decapoda	Leucosiidae	<i>Ebalia nux</i>
FCDPH15-569Gr66	Decapoda	Leucosiidae	<i>Ebalia nux</i>
FCFOPC051-07	Decapoda	Inachidae	<i>Ergasticus clouei</i>
FCFOPC051-08	Decapoda	Inachidae	<i>Ergasticus clouei</i>
JSDAz31	Decapoda	Eriphiidae	<i>Eriphia verrucosa</i>
JSDAz220	Decapoda	Eriphiidae	<i>Eriphia verrucosa</i>
JSDAz12	Decapoda	Eriphiidae	<i>Eriphia verrucosa</i>
JSDAz89	Decapoda	Eriphiidae	<i>Eriphia verrucosa</i>
JSDAz90	Decapoda	Eriphiidae	<i>Eriphia verrucosa</i>
JSDSv17	Decapoda	Hippolytidae	<i>Eualus gaimardi</i>
JSDPX42(05)-01	Decapoda	Penaeidae	<i>Funchalia villose</i>
JSDPX42(05)-02	Decapoda	Penaeidae	<i>Funchalia villose</i>
JSDUK30	Decapoda	Galatheidae	<i>Galathea dispersa</i>
JSDPX44-011	Decapoda	Geryonidae	<i>Geryon longipes</i>
JSDUKdeep_32	Decapoda	Geryonidae	<i>Geryon longipes</i>
FCFOPC052-06	Decapoda	Geryonidae	<i>Geryon longipes</i>
JSDPX44-012	Decapoda	Geryonidae	<i>Geryon longipes</i>
JSDMe82	Decapoda	Geryonidae	<i>Geryon longipes</i>
JSDUK41	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>
FCFOPC041-27	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>
JSDPX63-05	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>
FCDOPB083-08	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>
JSDMe22	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>
FCDPH15-575B63	Decapoda	Goneplacidae	<i>Goneplax rhombooides</i>

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JSDAz30	Decapoda	Grapsidae	<i>Grapsus adscensionis</i>
JSDAz27	Decapoda	Grapsidae	<i>Grapsus adscensionis</i>
JSDAz29	Decapoda	Grapsidae	<i>Grapsus adscensionis</i>
JSDAz28	Decapoda	Grapsidae	<i>Grapsus adscensionis</i>
JSDAz87	Decapoda	Epialtidae	<i>Herbstia condylata</i>
JSDAz88	Decapoda	Epialtidae	<i>Herbstia condylata</i>
JSDAz201	Decapoda	Homolidae	<i>Homola barbata</i>
MSM01-03-242_95	Decapoda	Homolidae	<i>Homola barbata</i>
JSDPXP21-01	Decapoda	Homolidae	<i>Homola barbata</i>
JSDPXM34-01	Decapoda	Homolidae	<i>Homola barbata</i>
JSDSv07	Decapoda	Oregoniidae	<i>Hyas araneus</i>
JSDSv08	Decapoda	Oregoniidae	<i>Hyas araneus</i>
JSDUK176	Decapoda	Oregoniidae	<i>Hyas coarctatus</i>
JSDUK175	Decapoda	Oregoniidae	<i>Hyas coarctatus</i>
JSDPX40-01	Decapoda	Solenoceridae	<i>Hymenopenaeus debilis</i>
JSDPX42(05)-06	Decapoda	Solenoceridae	<i>Hymenopenaeus debilis</i>
JSDPX39-01	Decapoda	Solenoceridae	<i>Hymenopenaeus debilis</i>
JSDPX39-02	Decapoda	Solenoceridae	<i>Hymenopenaeus debilis</i>
JSDPX42(05)-04	Decapoda	Solenoceridae	<i>Hymenopenaeus debilis</i>
FCFOPC045-10	Decapoda	Inachidae	<i>Inachus dorsettensis</i>
JSDUK46	Decapoda	Inachidae	<i>Inachus dorsettensis</i>
FCFOP42-19	Decapoda	Inachidae	<i>Inachus dorsettensis</i>
FCDOPB074-01	Decapoda	Inachidae	<i>Inachus dorsettensis</i>
FCDOPB074-02	Decapoda	Inachidae	<i>Inachus dorsettensis</i>
FCFOPC057-06	Decapoda	Inachidae	<i>Inachus leptochirus</i>
JSDPX23-04	Decapoda	Laomediidae	<i>Jaxea nocturna</i>
JSDPX41-01	Decapoda	Latreilliidae	<i>Latreillia elegans</i>
JSDMe18	Decapoda	Latreilliidae	<i>Latreillia elegans</i>
FCFOPC049-04	Decapoda	Latreilliidae	<i>Latreillia elegans</i>
JSDPX44-08	Decapoda	Latreilliidae	<i>Latreillia elegans</i>
JSDMe02	Decapoda	Carcinidae	<i>Liocarcinus depurator</i>
FCFOPC041-24	Decapoda	Carcinidae	<i>Liocarcinus depurator</i>
JSDUK51	Decapoda	Carcinidae	<i>Liocarcinus depurator</i>
FCDOPB077-07	Decapoda	Carcinidae	<i>Liocarcinus depurator</i>
JSDUK53	Decapoda	Carcinidae	<i>Liocarcinus depurator</i>
JSDPX9-02	Decapoda	Macropipidae	<i>Macropipus tuberculatus</i>
FCFOP42-23	Decapoda	Macropipidae	<i>Macropipus tuberculatus</i>
JSDMe45	Decapoda	Macropipidae	<i>Macropipus tuberculatus</i>
JSDPX9-01	Decapoda	Macropipidae	<i>Macropipus tuberculatus</i>
FCFOPC041-33	Decapoda	Macropipidae	<i>Macropipus tuberculatus</i>
JSDUK107	Decapoda	Inachidae	<i>Macropodia rostrata</i>
JSDUK110	Decapoda	Inachidae	<i>Macropodia rostrata</i>
JSDUK108	Decapoda	Inachidae	<i>Macropodia rostrata</i>
JSDAz202	Decapoda	Majidae	<i>Maja brachydactyla</i>
JSDAz205	Decapoda	Majidae	<i>Maja brachydactyla</i>
JSDUK121	Decapoda	Majidae	<i>Maja brachydactyla</i>
JSDUK123	Decapoda	Majidae	<i>Maja brachydactyla</i>
JSDUK122	Decapoda	Majidae	<i>Maja brachydactyla</i>
JSDPXA21-01	Decapoda	Majidae	<i>Maja goltzianna</i>
JSDPX18-01	Decapoda	Majidae	<i>Maja goltzianna</i>
JSDMe13	Decapoda	Dorippidae	<i>Medorippe lanata</i>
JSDMe81	Decapoda	Dorippidae	<i>Medorippe lanata</i>
JSDMe12	Decapoda	Dorippidae	<i>Medorippe lanata</i>

JSDPX23-01	Decapoda	Penaeidae	<i>Melicertus kerathurus</i>
JSDPX23-02	Decapoda	Penaeidae	<i>Melicertus kerathurus</i>
JSDPX23-03	Decapoda	Penaeidae	<i>Melicertus kerathurus</i>
FCDPHM16ABx_115	Decapoda	Xanthidae	<i>Monodaeus couchii</i>
FCDPH14-550D10	Decapoda	Xanthidae	<i>Monodaeus couchii</i>
JSDPX24-01	Decapoda	Xanthidae	<i>Monodaeus couchii</i>
FCDPHM21Bx_110	Decapoda	Xanthidae	<i>Monodaeus couchii</i>
FCDPH14-528Gr77	Decapoda	Xanthidae	<i>Monodaeus couchii</i>
FCDPHM52_118	Decapoda	Galatheidae	<i>Munida intermedia</i>
FCDOPB084-04	Decapoda	Galatheidae	<i>Munida rugosa</i>
JSDMe53	Decapoda	Galatheidae	<i>Munida rutllanti</i>
JSDMe54	Decapoda	Galatheidae	<i>Munida rutllanti</i>
FCFOPC045-03	Decapoda	Galatheidae	<i>Munida rutllanti</i>
FCFOPC045-01	Decapoda	Galatheidae	<i>Munida rutllanti</i>
FCFOPC045-02	Decapoda	Galatheidae	<i>Munida rutllanti</i>
JSDXP1-04	Decapoda	Macropipidae	<i>Necora puber</i>
JSDXP1-01	Decapoda	Macropipidae	<i>Necora puber</i>
JSDXP1-02	Decapoda	Macropipidae	<i>Necora puber</i>
JSDUK127	Decapoda	Macropipidae	<i>Necora puber</i>
JSDUK124	Decapoda	Macropipidae	<i>Necora puber</i>
JSDUKdeep_01	Decapoda	Lithodidae	<i>Neolithodes grimaldii</i>
JSDUKdeep_02	Decapoda	Lithodidae	<i>Neolithodes grimaldii</i>
JSDUKdeep_03	Decapoda	Lithodidae	<i>Neolithodes grimaldii</i>
FCDOPB090-02	Decapoda	Nephropidae	<i>Nephrops norvegicus</i>
FCDOPB090-03	Decapoda	Nephropidae	<i>Nephrops norvegicus</i>
FCDOPB090-04	Decapoda	Nephropidae	<i>Nephrops norvegicus</i>
FCFOPC041-06	Decapoda	Nephropidae	<i>Nephrops norvegicus</i>
FCFOPC041-07	Decapoda	Nephropidae	<i>Nephrops norvegicus</i>
JSDUKdeep_18	Decapoda	Nephropidae	<i>Nephropsis atlantica</i>
JSDUKdeep_19	Decapoda	Nephropidae	<i>Nephropsis atlantica</i>
JSDUKdeep_20	Decapoda	Nephropidae	<i>Nephropsis atlantica</i>
FCFOPC053-08	Decapoda	Oplophoridae	<i>Oplophoridae</i>
FCFOPC057-03	Decapoda	Oplophoridae	<i>Oplophoridae</i>
FCFOPC052-15	Decapoda	Oplophoridae	<i>Oplophorus spinosus</i>
FCFOPC052-16	Decapoda	Oplophoridae	<i>Oplophorus spinosus</i>
JSDPX41-02	Decapoda	Oplophoridae	<i>Oplophorus spinosus</i>
JSDPX41-03	Decapoda	Oplophoridae	<i>Oplophorus spinosus</i>
JSDPX41-04	Decapoda	Oplophoridae	<i>Oplophorus spinosus</i>
FCDPHMSM274_94	Decapoda	Paguridae	<i>Pagurus</i>
FCDPHMSM242_96	Decapoda	Paguridae	<i>Pagurus</i>
FCFOPC054-06	Decapoda	Paguridae	<i>Pagurus alatus</i>
FCFOPC047-04	Decapoda	Paguridae	<i>Pagurus alatus</i>
FCFOPC047-02	Decapoda	Paguridae	<i>Pagurus alatus</i>
FCFOPC047-01	Decapoda	Paguridae	<i>Pagurus alatus</i>
FCFOPC052-11	Decapoda	Paguridae	<i>Pagurus alatus</i>
FCDOPB089-05	Decapoda	Paguridae	<i>Pagurus bernhardus</i>
JSDUK149	Decapoda	Paguridae	<i>Pagurus bernhardus</i>
JSDUK154	Decapoda	Paguridae	<i>Pagurus bernhardus</i>
JSDUK145	Decapoda	Paguridae	<i>Pagurus bernhardus</i>
JSDUK146	Decapoda	Paguridae	<i>Pagurus bernhardus</i>
JSDAz65	Decapoda	Paguridae	<i>Pagurus cuanensis</i>
JSDAz197	Decapoda	Paguridae	<i>Pagurus cuanensis</i>
FCDOPB076-17	Decapoda	Paguridae	<i>Pagurus excavatus</i>

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JSDMe03	Decapoda	Paguridae	<i>Pagurus excavatus</i>
JSDMe04	Decapoda	Paguridae	<i>Pagurus excavatus</i>
FCFOP42-11	Decapoda	Paguridae	<i>Pagurus excavatus</i>
FCFOP42-12	Decapoda	Paguridae	<i>Pagurus excavatus</i>
FCDOPB071-8	Decapoda	Paguridae	<i>Pagurus prideauxi</i>
JSDUK157	Decapoda	Paguridae	<i>Pagurus prideauxi</i>
FCFOP42-16	Decapoda	Paguridae	<i>Pagurus prideauxi</i>
JSDMe52	Decapoda	Paguridae	<i>Pagurus prideauxi</i>
JSDN08	Decapoda	Paguridae	<i>Pagurus prideauxi</i>
JSDN14	Decapoda	Paguridae	<i>Pagurus pubescens</i>
JSDN28	Decapoda	Paguridae	<i>Pagurus pubescens</i>
JSDSv01	Decapoda	Paguridae	<i>Pagurus pubescens</i>
JSDN22	Decapoda	Paguridae	<i>Pagurus pubescens</i>
JSDSv03	Decapoda	Paguridae	<i>Pagurus pubescens</i>
JSDAz186	Decapoda	Palaemonidae	<i>Palaemon elegans</i>
JSDUK162	Decapoda	Palaemonidae	<i>Palaemon elegans</i>
JSDUK164	Decapoda	Palaemonidae	<i>Palaemon elegans</i>
JSDUK161	Decapoda	Palaemonidae	<i>Palaemon elegans</i>
JSDUK163	Decapoda	Palaemonidae	<i>Palaemon elegans</i>
JSDUK166	Decapoda	Palaemonidae	<i>Palaemon serratus</i>
JSDUK165	Decapoda	Palaemonidae	<i>Palaemon serratus</i>
JSDUK167	Decapoda	Palaemonidae	<i>Palaemon serratus</i>
FCDOPB088-13	Decapoda	Palaemonidae	<i>Palaemon serratus</i>
FCDOPB088-14	Decapoda	Palaemonidae	<i>Palaemon serratus</i>
JSDAz228	Decapoda	Palinuridae	<i>Palinurus elephas</i>
JSDAz229	Decapoda	Palinuridae	<i>Palinurus elephas</i>
JSDUK172	Decapoda	Pandalidae	<i>Pandalus montagui</i>
JSDUK171	Decapoda	Pandalidae	<i>Pandalus montagui</i>
JSDMe39	Decapoda	Penaeidae	<i>Parapenaeus longirostris</i>
JSDMe38	Decapoda	Penaeidae	<i>Parapenaeus longirostris</i>
JSDMe37	Decapoda	Penaeidae	<i>Parapenaeus longirostris</i>
FCFOPC042-06	Decapoda	Penaeidae	<i>Parapenaeus longirostris</i>
JSDPX63-01	Decapoda	Pasiphaeidae	<i>Pasiphaea hoplocerca</i>
JSDUKdeep_27	Decapoda	Pasiphaeidae	<i>Pasiphaea multidentata</i>
JSDUKdeep_26	Decapoda	Pasiphaeidae	<i>Pasiphaea multidentata</i>
FCFOPC041-19	Decapoda	Pasiphaeidae	<i>Pasiphaea sivado</i>
FCFOPC041-18	Decapoda	Pasiphaeidae	<i>Pasiphaea sivado</i>
FCFOPC041-17	Decapoda	Pasiphaeidae	<i>Pasiphaea sivado</i>
FCFOPC041-16	Decapoda	Pasiphaeidae	<i>Pasiphaea sivado</i>
FCFOPC041-15	Decapoda	Pasiphaeidae	<i>Pasiphaea sivado</i>
JSDUKdeep_11	Decapoda	Pasiphaeidae	<i>Pasiphaea tarda</i>
JSDUKdeep_13	Decapoda	Pasiphaeidae	<i>Pasiphaea tarda</i>
JSDUKdeep_12	Decapoda	Pasiphaeidae	<i>Pasiphaea tarda</i>
FCFOPC050-01	Decapoda	Penaeidae	<i>Penaeopsis serrata</i>
JSDAz41	Decapoda	Plagusiidae	<i>Percnon gibbesi</i>
JSDAz75	Decapoda	Plagusiidae	<i>Percnon gibbesi</i>
JSDAz39	Decapoda	Plagusiidae	<i>Percnon gibbesi</i>
JSDAz77	Decapoda	Plagusiidae	<i>Percnon gibbesi</i>
JSDAz40	Decapoda	Plagusiidae	<i>Percnon gibbesi</i>
FCFOPC041-52	Decapoda	Crangonidae	<i>Philoceras</i>
FCDPHMSM241_101	Decapoda	Crangonidae	<i>Philoceras monocanthus</i>
JSDUK173	Decapoda	Pilumnidae	<i>Pilumnus hirtellus</i>
JSDAz135	Decapoda	Pilumnidae	<i>Pilumnus hirtellus</i>

JSDUK174	Decapoda	Pilumnidae	<i>Pilumnus hirtellus</i>
FCDPH14-550D69	Decapoda	Pilumnidae	<i>Pilumnus inermis</i>
FCFOPC047-09	Decapoda	Pandalidae	<i>Plesionika</i>
JSDPX41-12	Decapoda	Pandalidae	<i>Plesionika acanthonotus</i>
JSDPX41-13	Decapoda	Pandalidae	<i>Plesionika acanthonotus</i>
FCFOPC057-05	Decapoda	Pandalidae	<i>Plesionika acanthonotus</i>
FCFOPC049-09	Decapoda	Pandalidae	<i>Plesionika antigai</i>
FCFOPC051-04	Decapoda	Pandalidae	<i>Plesionika antigai</i>
FCFOPC065-02	Decapoda	Pandalidae	<i>Plesionika antigai</i>
JSDMe74	Decapoda	Pandalidae	<i>Plesionika edwardsii</i>
JSDMe73	Decapoda	Pandalidae	<i>Plesionika edwardsii</i>
JSDMe70	Decapoda	Pandalidae	<i>Plesionika edwardsii</i>
JSDMe71	Decapoda	Pandalidae	<i>Plesionika edwardsii</i>
FCFOPC047-12	Decapoda	Pandalidae	<i>Plesionika heterocarpus</i>
FCDOPB093-01	Decapoda	Pandalidae	<i>Plesionika heterocarpus</i>
FCFOP70-20	Decapoda	Pandalidae	<i>Plesionika heterocarpus</i>
JSDMe29	Decapoda	Pandalidae	<i>Plesionika heterocarpus</i>
JSDMe30	Decapoda	Pandalidae	<i>Plesionika heterocarpus</i>
FCFOPC041-47	Decapoda	Pandalidae	<i>Plesionika martia</i>
FCFOPC043-04	Decapoda	Pandalidae	<i>Plesionika martia</i>
FCFOPC041-45	Decapoda	Pandalidae	<i>Plesionika martia</i>
FCFOPC041-46	Decapoda	Pandalidae	<i>Plesionika martia</i>
FCFOPC042-07	Decapoda	Pandalidae	<i>Plesionika martia</i>
FCFOPC046-02	Decapoda	Pandalidae	<i>Plesionika narval</i>
FCFOPC049-07	Decapoda	Pandalidae	<i>Plesionika narval</i>
FCFOPC049-06	Decapoda	Pandalidae	<i>Plesionika narval</i>
JSDMe78	Decapoda	Pandalidae	<i>Plesionika narval</i>
FCFOPC042-09	Decapoda	Pandalidae	<i>Plesionika narval</i>
JSDUK74	Decapoda	Carcinidae	<i>Polybius henslowii</i>
JSDUK103	Decapoda	Carcinidae	<i>Polybius henslowii</i>
FCDOPB083-11	Decapoda	Carcinidae	<i>Polybius henslowii</i>
FCDOPB083-10	Decapoda	Carcinidae	<i>Polybius henslowii</i>
FCFOPC041-01	Decapoda	Carcinidae	<i>Polybius henslowii</i>
FCFOPC052-01	Decapoda	Polychelidae	<i>Polycheles typhlops</i>
JSDPX15-15	Decapoda	Polychelidae	<i>Polycheles typhlops</i>
JSDPX15-16	Decapoda	Polychelidae	<i>Polycheles typhlops</i>
JSDUKdeep_56	Decapoda	Polychelidae	<i>Polycheles typhlops</i>
JSDUKdeep_58	Decapoda	Polychelidae	<i>Polycheles typhlops</i>
JSDMe15	Decapoda	Crangonidae	<i>Pontocaris cathanphracta</i>
JSDMe16	Decapoda	Crangonidae	<i>Pontocaris cathanphracta</i>
JSDMe17	Decapoda	Crangonidae	<i>Pontocaris cathanphracta</i>
FCFOPC044-09	Decapoda	Crangonidae	<i>Pontocaris lacazei</i>
FCDPH15-575B51	Decapoda	Crangonidae	<i>Pontocaris lacazei</i>
FCFOPC044-07	Decapoda	Crangonidae	<i>Pontocaris lacazei</i>
FCFOPC041-51	Decapoda	Crangonidae	<i>Pontocaris lacazei</i>
FCFOPC044-08	Decapoda	Crangonidae	<i>Pontocaris lacazei</i>
FCFOPC048-01	Decapoda	Crangonidae	<i>Pontophilus spinosus</i>
JSDUK178	Decapoda	Porcellanidae	<i>Porcellana platycheles</i>
JSDUK182	Decapoda	Porcellanidae	<i>Porcellana platycheles</i>
JSDUK179	Decapoda	Porcellanidae	<i>Porcellana platycheles</i>
JSDUK181	Decapoda	Porcellanidae	<i>Porcellana platycheles</i>
JSDUK180	Decapoda	Porcellanidae	<i>Porcellana platycheles</i>
JSDPX41-14	Decapoda	Processidae	<i>Processa modica</i>

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JSDPX41-15	Decapoda	Processidae	<i>Processa modica</i>
FCFOPC041-40	Decapoda	Processidae	<i>Processa modica</i>
FCFOPC041-41	Decapoda	Processidae	<i>Processa modica</i>
FCFOPC041-42	Decapoda	Processidae	<i>Processa modica</i>
JSDUKdeep_06	Decapoda	Crangonidae	<i>Sabinea hystrix</i>
JSDUKdeep_08	Decapoda	Crangonidae	<i>Sabinea hystrix</i>
JSDUKdeep_07	Decapoda	Crangonidae	<i>Sabinea hystrix</i>
JSDAz236	Decapoda	Scyllaridae	<i>Scyllarides latus</i>
JSDAz37	Decapoda	Scyllaridae	<i>Scyllarides latus</i>
JSDAz36	Decapoda	Scyllaridae	<i>Scyllarides latus</i>
JSDAz240	Decapoda	Scyllaridae	<i>Scyllarides latus</i>
JSDAz235	Decapoda	Scyllaridae	<i>Scyllarides latus</i>
FCFOPC048-03	Decapoda	Sergestidae	<i>Sergestes arcticus</i>
FCFOPC069-03	Decapoda	Sergestidae	<i>Sergia robusta</i>
JSDPX4-03	Decapoda	Sergestidae	<i>Sergia robusta</i>
JSDPX79-05	Decapoda	Sergestidae	<i>Sergia robusta</i>
JSDPX71-01	Decapoda	Sergestidae	<i>Sergia robusta</i>
JSDUKdeep_23	Decapoda	Sergestidae	<i>Sergia robusta</i>
JSDMe20	Decapoda	Solenoceridae	<i>Solenocera membranacea</i>
FCFOPC041-12	Decapoda	Solenoceridae	<i>Solenocera membranacea</i>
FCFOPC041-14	Decapoda	Solenoceridae	<i>Solenocera membranacea</i>
FCFOP66-11	Decapoda	Solenoceridae	<i>Solenocera membranacea</i>
FCDOPB083-07	Decapoda	Solenoceridae	<i>Solenocera membranacea</i>
JSDUKdeep_41	Decapoda	Polychelidae	<i>Stereomastis grimaldi</i>
JSDUKdeep_43	Decapoda	Polychelidae	<i>Stereomastis grimaldi</i>
FCFOPC047-08	Decapoda	Pandalidae	<i>Stylopandalus richardi</i>
JSDPX42(05)-07	Decapoda	Oplophoridae	<i>Systellaspis debilis</i>
JSDPX42(05)-08	Decapoda	Oplophoridae	<i>Systellaspis debilis</i>
JSDPX42(05)-09	Decapoda	Oplophoridae	<i>Systellaspis debilis</i>
JSDPX42-01	Decapoda	Oplophoridae	<i>Systellaspis pellucida</i>
JSDPX42-03	Decapoda	Oplophoridae	<i>Systellaspis pellucida</i>
JSDUK183	Decapoda	Hippolytidae	<i>Thoralus cranchii</i>
JSDUK184	Decapoda	Hippolytidae	<i>Thoralus cranchii</i>
JSDUK185	Decapoda	Hippolytidae	<i>Thoralus cranchii</i>
FCDPH12-399Gr4	Decapoda	Callianassidae	<i>Vulcanocalliax arutyunovi</i>
JSDAz22	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz23	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz24	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz25	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz26	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz80	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz81	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz94	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz152	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDAz245	Decapoda	Xanthidae	<i>Xantho hydrophilus</i>
JSDUK187	Decapoda	Xanthidae	<i>Xantho pilipes</i>
JSDUK188	Decapoda	Xanthidae	<i>Xantho pilipes</i>
JSDUK186	Decapoda	Xanthidae	<i>Xantho pilipes</i>
FJ581463	Isopoda	Aegidae	<i>Aega psora</i>
FJ581626	Isopoda	Idoteidae	<i>Edotia triloba</i>
FJ581625	Isopoda	Idoteidae	<i>Edotia triloba</i>
FJ581624	Isopoda	Idoteidae	<i>Edotia triloba</i>
FJ581714	Isopoda	Idoteidae	<i>Idotea balthica</i>

FJ581736	Isopoda	Janiridae	<i>Jaera albifrons</i>
FJ581915	Isopoda	Aegidae	<i>Syscenus infelix</i>
FJ581914	Isopoda	Aegidae	<i>Syscenus infelix</i>
FJ581913	Isopoda	Aegidae	<i>Syscenus infelix</i>
FJ581912	Isopoda	Aegidae	<i>Syscenus infelix</i>
FJ581911	Isopoda	Aegidae	<i>Syscenus infelix</i>
IsopodaH022.1	Isopoda	Munnopsidae	<i>Disconectes sp022</i>
IsopodaH0G3	Isopoda	Gnathiidae	<i>Gnathia spD</i>
IsopodaH0G2	Isopoda	Gnathiidae	<i>Gnathia spD</i>
IsopodaH0G1	Isopoda	Gnathiidae	<i>Gnathia spD</i>
IsopodaH028.4	Isopoda	Janirellidae	<i>Janirella cf. abyssicola</i>
IsopodaH028.3	Isopoda	Janirellidae	<i>Janirella cf. abyssicola</i>
IsopodaH053.8	Isopoda	Cirolanidae	<i>Metacirolana hansenii</i>
IsopodaH053.7	Isopoda	Cirolanidae	<i>Metacirolana hansenii</i>
IsopodaH70.1	Isopoda	Cirolanidae	<i>Natatolana</i>
EF682265	Isopoda	Munnopsidae	<i>Acanthamunnopsis longicornis</i>
EF682261	Isopoda	Munnopsidae	<i>Acanthamunnopsis milleri</i>
EF682262	Isopoda	Munnopsidae	<i>Acanthamunnopsis sp. 2 A17</i>
EF682264	Isopoda	Munnopsidae	<i>Acanthamunnopsis sp. 3 A26</i>
EF682263	Isopoda	Munnopsidae	<i>Acanthamunnopsis sp. 4 A20</i>
EF682286	Isopoda	Munnopsidae	<i>Acanthocope sp. MB H2</i>
EF670478	Isopoda	Cirolanidae	<i>Antrolana lira</i>
EF670477	Isopoda	Cirolanidae	<i>Antrolana lira</i>
EF670476	Isopoda	Cirolanidae	<i>Antrolana lira</i>
EF670475	Isopoda	Cirolanidae	<i>Antrolana lira</i>
EF670474	Isopoda	Cirolanidae	<i>Antrolana lira</i>
EF027716	Isopoda	Armadillidiidae	<i>Armadillidium lobocurvum</i>
EF027715	Isopoda	Armadillidiidae	<i>Armadillidium lobocurvum</i>
EF027714	Isopoda	Armadillidiidae	<i>Armadillidium lobocurvum</i>
EU364630	Isopoda	Scyphacidae	<i>Armadilloniscus ellipticus</i>
AY566523	Isopoda	Cirolanidae	<i>Bathynomus giganteus</i>
EF682292	Isopoda	Munnopsidae	<i>Betamorpha africana</i>
EF682291	Isopoda	Munnopsidae	<i>Betamorpha fusiformis</i>
AY566522	Isopoda	Asellidae	<i>Caecidotea reddelli</i>
AF260834	Isopoda	Asellidae	<i>Caecidotea sp. RW96.018.184</i>
AF255778	Isopoda	Asellidae	<i>Caecidotea sp. RW96.026.181</i>
EU597422	Isopoda	Serolidae	<i>Ceratoserolis trilobitooides</i>
EU597421	Isopoda	Serolidae	<i>Ceratoserolis trilobitooides</i>
EU597420	Isopoda	Serolidae	<i>Ceratoserolis trilobitooides</i>
EU597419	Isopoda	Serolidae	<i>Ceratoserolis trilobitooides</i>
EU597418	Isopoda	Serolidae	<i>Ceratoserolis trilobitooides</i>
EF989646	Isopoda	Sphaeromatidae	<i>Cilicaea sp. 72</i>
AF255787	Isopoda	Cirolanidae	<i>Cirolana harfordi</i>
AF260838	Isopoda	Cirolanidae	<i>Cirolana harfordi</i>
AY566470	Isopoda	Cirolanidae	<i>Cirolanides texensis</i>
AY566471	Isopoda	Cirolanidae	<i>Cirolanides texensis</i>
AY566473	Isopoda	Cirolanidae	<i>Cirolanides texensis</i>
AY566474	Isopoda	Cirolanidae	<i>Cirolanides texensis</i>
AY566475	Isopoda	Cirolanidae	<i>Cirolanides texensis</i>
AF255775	Isopoda	Phreatoicidae	<i>Colubotelson thomsoni</i>
EF682302	Isopoda	Munnopsidae	<i>Coperonus sp. 1 G20</i>
EF682301	Isopoda	Munnopsidae	<i>Coperonus sp. 5 G19</i>
AF255776	Isopoda	Phreatoicidae	<i>Crenoicus buntiae</i>

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EU364625	Isopoda	Scyphacidae	<i>Doto marina</i>
EF682293	Isopoda	Munnopsidae	<i>Disconectes antarctica</i>
EF682294	Isopoda	Munnopsidae	<i>Dubinectes acutitelson</i>
EU263143	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 02</i>
EU263142	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 02</i>
EU263145	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 03</i>
EU263147	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 04</i>
EU263146	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 04</i>
EU263150	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 08</i>
EU263149	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 08</i>
EU263148	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 09</i>
EU263152	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 09</i>
EU263151	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 09</i>
EU263153	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 10</i>
EU263154	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 11</i>
EU263156	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 12</i>
EU263159	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 13</i>
EU263158	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 13</i>
EU263162	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 20</i>
EU263161	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 20</i>
EU263166	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 21</i>
EU263165	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 21</i>
EU263164	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 21</i>
EU263163	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. 21</i>
EU263170	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. C2</i>
EU263179	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Gb</i>
EU263178	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Gb</i>
EU263177	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Gb</i>
EU263180	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. J1</i>
EU263183	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. J2</i>
EU263181	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. J2</i>
EU263188	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M1</i>
EU263187	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M1</i>
EU263193	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M2</i>
EU263192	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M2</i>
EU263191	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M2</i>
EU263190	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M2</i>
EU263189	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. M2</i>
EU263197	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. S1</i>
EU263196	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. S1</i>
EU263200	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Zi</i>
EU263199	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Zr</i>
EU263202	Isopoda	Phreatoicidae	<i>Eophreatoicus sp. Zu</i>
EU263135	Isopoda	Amphipodahlsopodapodidae	<i>EremIsopodapus sp. Faraway</i>
EF682281	Isopoda	Munnopsidae	<i>Eurycope complanata</i>
EF682280	Isopoda	Munnopsidae	<i>Eurycope glabra</i>
EF682279	Isopoda	Munnopsidae	<i>Eurycope sp. G9</i>
EF682282	Isopoda	Munnopsidae	<i>Eurycope sp. MB I31</i>
AF260841	Isopoda	Cirolanidae	<i>Excirolana chiltoni</i>
DQ351361	Isopoda	Sphaeromatidae	<i>Exosphaeroma hylecoetes</i>
DQ351360	Isopoda	Sphaeromatidae	<i>Exosphaeroma hylecoetes</i>
DQ351355	Isopoda	Sphaeromatidae	<i>Exosphaeroma hylecoetes</i>
DQ351354	Isopoda	Sphaeromatidae	<i>Exosphaeroma hylecoetes</i>

DQ351353	Isopoda	Sphaeromatidae	<i>Exosphaeroma hylecoetes</i>
AF255781	Isopoda	Idoteidae	<i>Glyptidotea lichtensteini</i>
AF260845	Isopoda	Sphaeromatidae	<i>Gnorimosphaeroma oregonense</i>
EU364626	Isopoda	Scyphacidae	<i>Haloniscus anophthalmus</i>
EU364585	Isopoda	Scyphacidae	<i>Haloniscus longiantennatus</i>
EU364584	Isopoda	Scyphacidae	<i>Haloniscus longiantennatus</i>
EU364583	Isopoda	Scyphacidae	<i>Haloniscus longiantennatus</i>
EU364578	Isopoda	Scyphacidae	<i>Haloniscus longiantennatus</i>
EU364621	Isopoda	Scyphacidae	<i>Haloniscus searlei</i>
EU364620	Isopoda	Scyphacidae	<i>Haloniscus searlei</i>
EU364619	Isopoda	Scyphacidae	<i>Haloniscus searlei</i>
EU364618	Isopoda	Scyphacidae	<i>Haloniscus searlei</i>
EU364617	Isopoda	Scyphacidae	<i>Haloniscus searlei</i>
EU364600	Isopoda	Scyphacidae	<i>Haloniscus sp. 11 SJBC-2008</i>
EU364599	Isopoda	Scyphacidae	<i>Haloniscus sp. 11 SJBC-2008</i>
EU364598	Isopoda	Scyphacidae	<i>Haloniscus sp. 11 SJBC-2008</i>
EU364597	Isopoda	Scyphacidae	<i>Haloniscus sp. 11 SJBC-2008</i>
EU364596	Isopoda	Scyphacidae	<i>Haloniscus sp. 11 SJBC-2008</i>
EU364603	Isopoda	Scyphacidae	<i>Haloniscus sp. 12 SJBC-2008</i>
EU364602	Isopoda	Scyphacidae	<i>Haloniscus sp. 12 SJBC-2008</i>
EU364601	Isopoda	Scyphacidae	<i>Haloniscus sp. 12 SJBC-2008</i>
EU364604	Isopoda	Scyphacidae	<i>Haloniscus sp. 13 SJBC-2008</i>
EU364608	Isopoda	Scyphacidae	<i>Haloniscus sp. 14 SJBC-2008</i>
EU364607	Isopoda	Scyphacidae	<i>Haloniscus sp. 14 SJBC-2008</i>
EU364605	Isopoda	Scyphacidae	<i>Haloniscus sp. 14 SJBC-2008</i>
EU364606	Isopoda	Scyphacidae	<i>Haloniscus sp. 15 SJBC-2008</i>
EU364609	Isopoda	Scyphacidae	<i>Haloniscus sp. 16 SJBC-2008</i>
EU364610	Isopoda	Scyphacidae	<i>Haloniscus sp. 17 SJBC-2008</i>
EU364611	Isopoda	Scyphacidae	<i>Haloniscus sp. 18 SJBC-2008</i>
EU364613	Isopoda	Scyphacidae	<i>Haloniscus sp. 19 SJBC-2008</i>
EU364612	Isopoda	Scyphacidae	<i>Haloniscus sp. 19 SJBC-2008</i>
EU364563	Isopoda	Scyphacidae	<i>Haloniscus sp. 1 SJBC-2008</i>
EU364615	Isopoda	Scyphacidae	<i>Haloniscus sp. 20 SJBC-2008</i>
EU364614	Isopoda	Scyphacidae	<i>Haloniscus sp. 20 SJBC-2008</i>
EU364568	Isopoda	Scyphacidae	<i>Haloniscus sp. 2 SJBC-2008</i>
EU364567	Isopoda	Scyphacidae	<i>Haloniscus sp. 2 SJBC-2008</i>
EU364566	Isopoda	Scyphacidae	<i>Haloniscus sp. 2 SJBC-2008</i>
EU364565	Isopoda	Scyphacidae	<i>Haloniscus sp. 2 SJBC-2008</i>
EU364564	Isopoda	Scyphacidae	<i>Haloniscus sp. 2 SJBC-2008</i>
EU364569	Isopoda	Scyphacidae	<i>Haloniscus sp. 3 SJBC-2008</i>
EU364570	Isopoda	Scyphacidae	<i>Haloniscus sp. 4 SJBC-2008</i>
EU364575	Isopoda	Scyphacidae	<i>Haloniscus sp. 5 SJBC-2008</i>
EU364573	Isopoda	Scyphacidae	<i>Haloniscus sp. 5 SJBC-2008</i>
EU364572	Isopoda	Scyphacidae	<i>Haloniscus sp. 5 SJBC-2008</i>
EU364571	Isopoda	Scyphacidae	<i>Haloniscus sp. 5 SJBC-2008</i>
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EU364574	Isopoda	Scyphacidae	<i>Haloniscus sp. 6 SJBC-2008</i>
EU364580	Isopoda	Scyphacidae	<i>Haloniscus sp. 7 SJBC-2008</i>
EU364579	Isopoda	Scyphacidae	<i>Haloniscus sp. 7 SJBC-2008</i>
EU364587	Isopoda	Scyphacidae	<i>Haloniscus sp. 9 SJBC-2008</i>
EU364586	Isopoda	Scyphacidae	<i>Haloniscus sp. 9 SJBC-2008</i>
EF682298	Isopoda	Munnopsidae	<i>Ilyarachna triangulata</i>

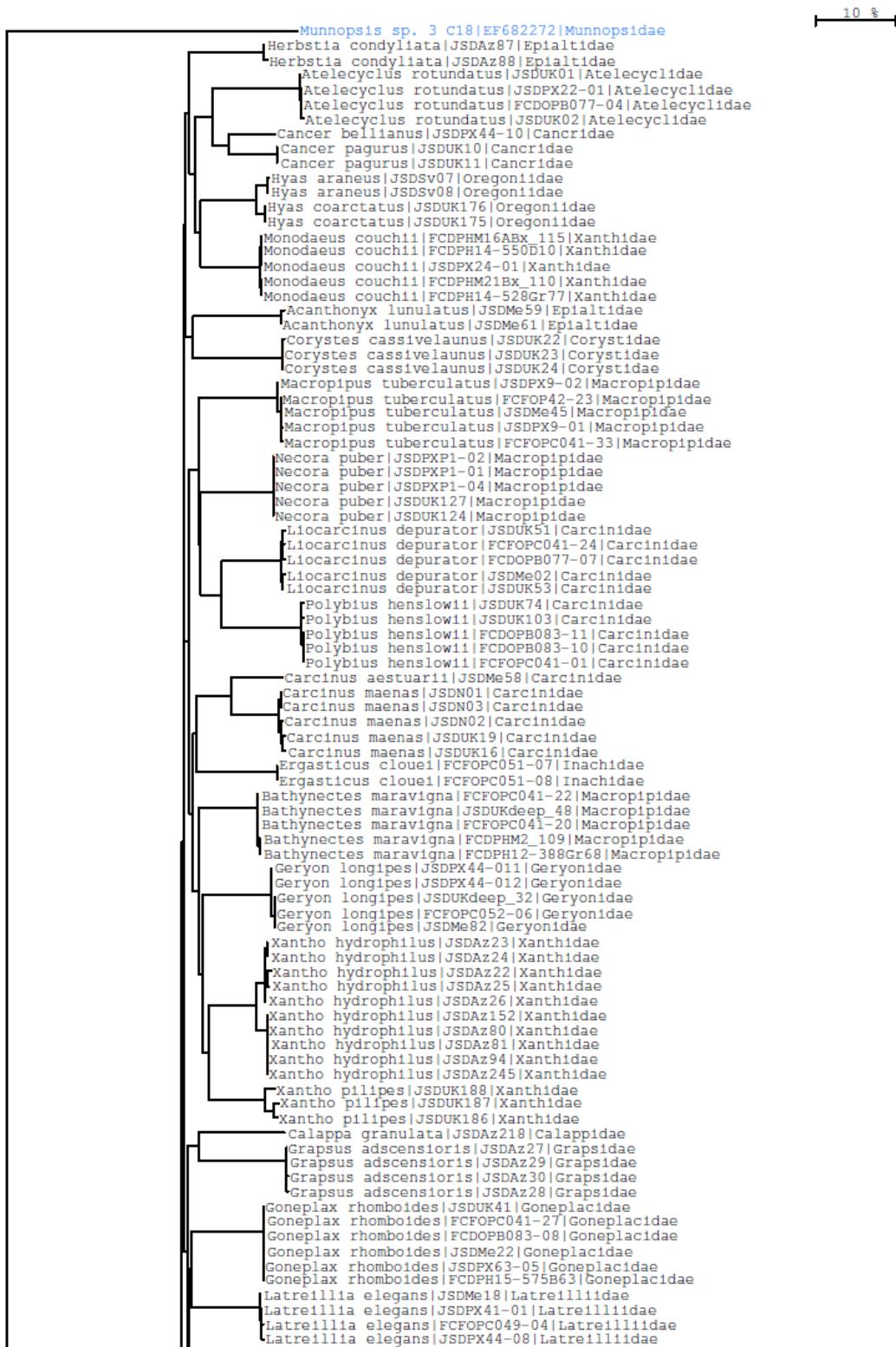
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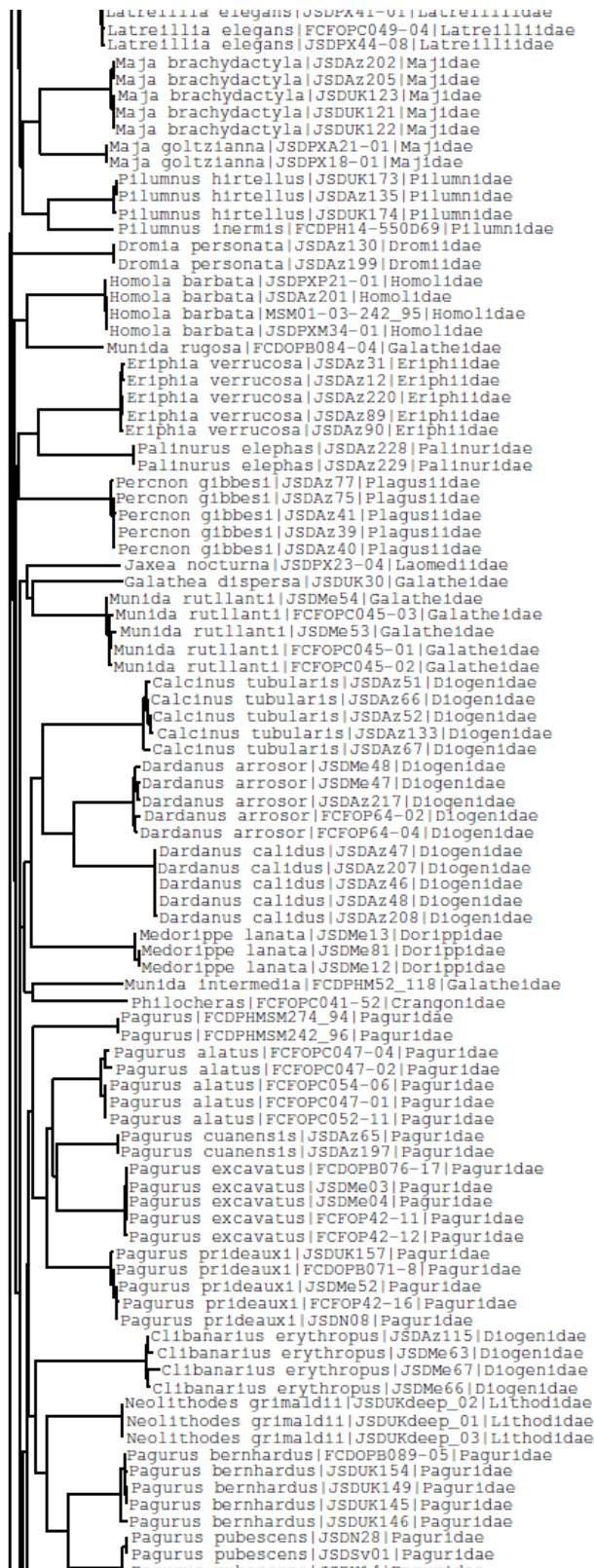
EF682304	Isopoda	Ischnomesidae	<i>Ischnomesid sp. MB L1</i>
EU364629	Isopoda	Philosciidae	<i>Laevophiloscia yalgoensis</i>
DQ182858	Isopoda	Ligiidae	<i>Ligia italica</i>
DQ182859	Isopoda	Ligiidae	<i>Ligia italica</i>
DQ182860	Isopoda	Ligiidae	<i>Ligia italica</i>
DQ182861	Isopoda	Ligiidae	<i>Ligia italica</i>
NC_008412	Isopoda	Ligiidae	<i>Ligia oceanica</i>
DQ442914	Isopoda	Ligiidae	<i>Ligia oceanica</i>
DQ182780	Isopoda	Ligiidae	<i>Ligidium hypnorum</i>
DQ182781	Isopoda	Ligiidae	<i>Ligidium hypnorum</i>
DQ182804	Isopoda	Ligiidae	<i>Ligidium sp. ZA5</i>
DQ182810	Isopoda	Ligiidae	<i>Ligidium hypnorum</i>
DQ182814	Isopoda	Ligiidae	<i>Ligidium sp. ADR3</i>
AY566531	Isopoda	Asellidae	<i>Lirceolus bisetus</i>
AY566532	Isopoda	Asellidae	<i>Lirceolus bisetus</i>
AY566533	Isopoda	Asellidae	<i>Lirceolus bisetus</i>
AY566530	Isopoda	Asellidae	<i>Lirceolus sp. Dandridge Spring</i>
AY566545	Isopoda	Asellidae	<i>Lirceolus sp. Preserve Cave</i>
AY566546	Isopoda	Asellidae	<i>Lirceolus sp. Preserve Cave</i>
AY566547	Isopoda	Asellidae	<i>Lirceolus sp. Rattlesnake Cave</i>
AY566548	Isopoda	Asellidae	<i>Lirceolus sp. Rattlesnake Cave</i>
AY566479	Isopoda	Asellidae	<i>Lirceolus sp. Slaughter Bend</i>
AF255790	Isopoda	Cymothoidae	<i>Lironeca vulgaris</i>
AF260842	Isopoda	Cymothoidae	<i>Lironeca vulgaris</i>
AF260843	Isopoda	Cymothoidae	<i>Lironeca vulgaris</i>
AY948306	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus penicillatus</i>
AY948307	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus sp. 5-GG-2005</i>
AY948290	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus sp. GG-2005</i>
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AY948292	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus sp. GG-2005</i>
AY948293	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus sp. GG-2005</i>
AY948294	Isopoda	AmphipodahIsopodapus	<i>MesAmphipodahIsopodapus sp. GG-2005</i>
AY566478	Isopoda	Stenasellidae	<i>Mexistenasillus coahuila</i>
EF682275	Isopoda	Munnopsidae	<i>Munneurycope murrayi</i>
EF682276	Isopoda	Munnopsidae	<i>Munneurycope sp. 2 B17</i>
EF682283	Isopoda	Munnopsidae	<i>Munneurycope sp. ABE G1</i>
EF682268	Isopoda	Munnopsidae	<i>Munnopsisinae sp. D10</i>
EF682273	Isopoda	Munnopsidae	<i>Munnopsis abyssalis</i>
EF682271	Isopoda	Munnopsidae	<i>Munnopsoides sp. MB C12</i>
EF682289	Isopoda	Munnopsidae	<i>Munnopsurus sp. 1 G4</i>
EF682288	Isopoda	Munnopsidae	<i>Munnopsurus sp. MB K2</i>
EF682300	Isopoda	Munnopsidae	<i>Notopais magnifica</i>
AF255791	Isopoda	Cymothoidae	<i>Olencira praegustator</i>
AF260844	Isopoda	Cymothoidae	<i>Olencira praegustator</i>
EF682267	Isopoda	Munnopsidae	<i>Paramunnopsis sp. 1 D11</i>
EF682270	Isopoda	Munnopsidae	<i>Paramunnopsis sp. 2 D6</i>
EF682266	Isopoda	Munnopsidae	<i>Paramunnopsis sp. 5 D15</i>
AF255783	Isopoda	Idoteidae	<i>Paridotea unguilata</i>
EF682287	Isopoda	Munnopsidae	<i>Paropsurus giganteus</i>
EU364627	Isopoda	Porcellionidae	<i>Porcellionides pruinosus</i>
DQ305142	Isopoda	Porcellionidae	<i>Porcellio scaber</i>
DQ305136	Isopoda	Asellidae	<i>Proasellus gjorgjevici</i>
DQ305135	Isopoda	Asellidae	<i>Proasellus gjorgjevici</i>

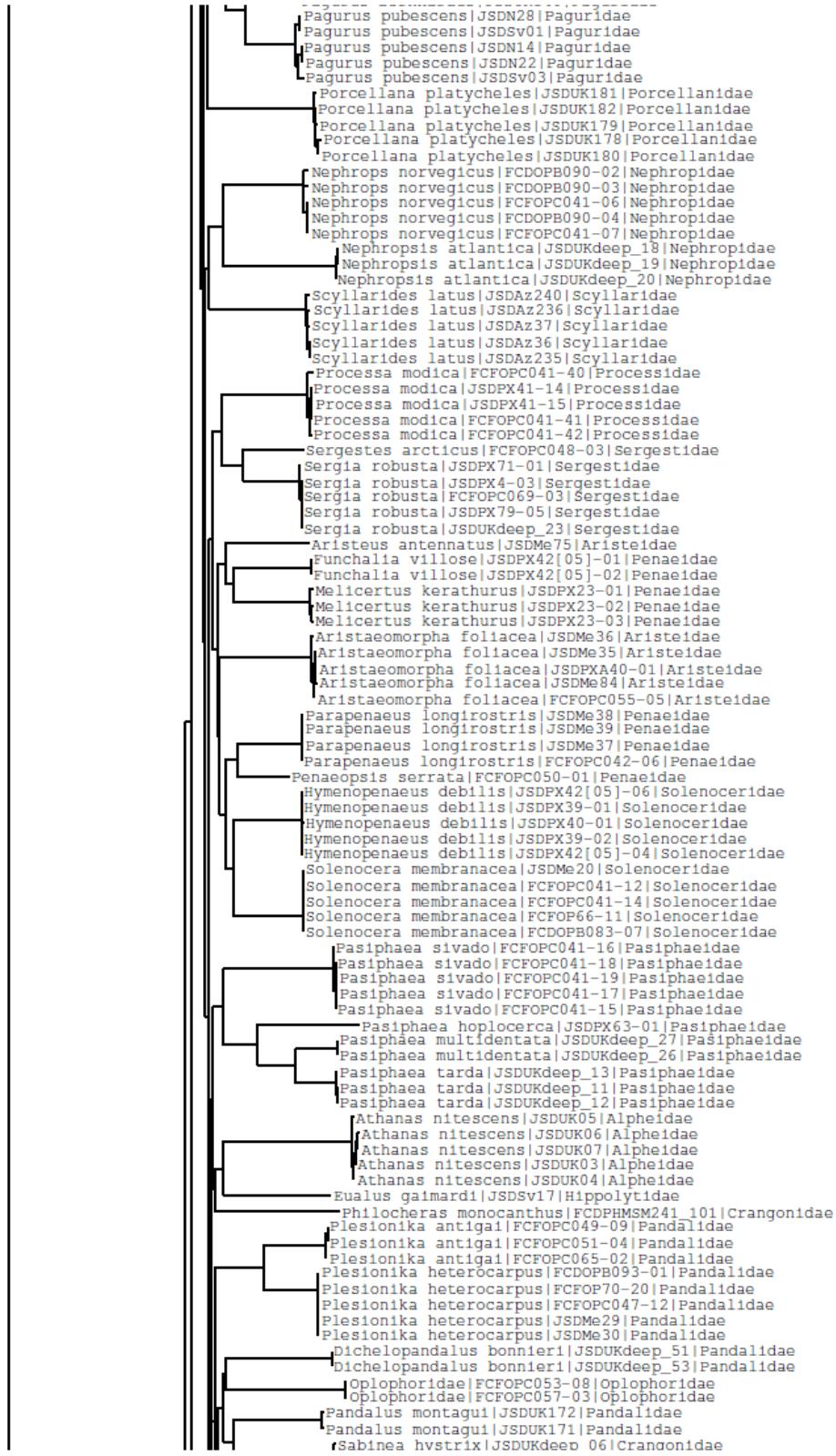
DQ305134	Isopoda	Asellidae	<i>Proasellus gjorgjevici</i>
DQ305133	Isopoda	Asellidae	<i>Proasellus gjorgjevici</i>
EU107639	Isopoda	TainIsopodapidae	<i>Pygolabis eberhardi</i>
EU107638	Isopoda	TainIsopodapidae	<i>Pygolabis eberhardi</i>
EU107637	Isopoda	TainIsopodapidae	<i>Pygolabis gascoynensis</i>
EU364628	Isopoda	TainIsopodapidae	<i>Pygolabis humphreysi</i>
EU107580	Isopoda	TainIsopodapidae	<i>Pygolabis humphreysi</i>
EU107579	Isopoda	TainIsopodapidae	<i>Pygolabis humphreysi</i>
EU107578	Isopoda	TainIsopodapidae	<i>Pygolabis humphreysi</i>
EU107577	Isopoda	TainIsopodapidae	<i>Pygolabis humphreysi</i>
EU107636	Isopoda	TainIsopodapidae	<i>Pygolabis paraburdo</i>
EU107635	Isopoda	TainIsopodapidae	<i>Pygolabis paraburdo</i>
EU107634	Isopoda	TainIsopodapidae	<i>Pygolabis paraburdo</i>
EU107633	Isopoda	TainIsopodapidae	<i>Pygolabis paraburdo</i>
EU107632	Isopoda	TainIsopodapidae	<i>Pygolabis paraburdo</i>
EU107646	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 1 TLF-2007</i>
EU107642	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 2 TLF-2007</i>
EU107643	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 3 TLF-2007</i>
EU107647	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 5 TLF-2007</i>
EU107645	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 6 TLF-2007</i>
EU107644	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 6 TLF-2007</i>
EU107641	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 7 TLF-2007</i>
EU107640	Isopoda	TainIsopodapidae	<i>Pygolabis sp. 7 TLF-2007</i>
EU107618	Isopoda	TainIsopodapidae	<i>Pygolabis weeliwolli</i>
EU107617	Isopoda	TainIsopodapidae	<i>Pygolabis weeliwolli</i>
EU107616	Isopoda	TainIsopodapidae	<i>Pygolabis weeliwolli</i>
EU107615	Isopoda	TainIsopodapidae	<i>Pygolabis weeliwolli</i>
EU107614	Isopoda	TainIsopodapidae	<i>Pygolabis weeliwolli</i>
AY566472	Isopoda	Asellidae	<i>Remasellus parvus</i>
AY857829	Isopoda	Santiidae	<i>Santia sp. A1</i>
AY857828	Isopoda	Santiidae	<i>Santia sp. B1</i>
EU597357	Isopoda	Serolidae	<i>Septemserolis septemcarinata</i>
EU597356	Isopoda	Serolidae	<i>Septemserolis septemcarinata</i>
EU597355	Isopoda	Serolidae	<i>Septemserolis septemcarinata</i>
EU597354	Isopoda	Serolidae	<i>Septemserolis septemcarinata</i>
EU597353	Isopoda	Serolidae	<i>Septemserolis septemcarinata</i>
AF255786	Isopoda	Serolidae	<i>Serolina bakeri</i>
AY566524	Isopoda	Cirolanidae	<i>Speocirolana hardeni</i>
AY566525	Isopoda	Cirolanidae	<i>Speocirolana hardeni</i>
AY566526	Isopoda	Cirolanidae	<i>Speocirolana thermydronis</i>
AY566518	Isopoda	Cirolanidae	<i>Sphaerolana affinis</i>
AY566519	Isopoda	Cirolanidae	<i>Sphaerolana affinis</i>
AF255785	Isopoda	Sphaeromatidae	<i>Sphaeroma quadridentatum</i>
AY028589	Isopoda	Stenasellidae	<i>Stenasellus racovitzai</i>
EF682295	Isopoda	Munnopsidae	<i>Syneurycope heezeni</i>
EU107666	Isopoda	TainIsopodapidae	<i>TainIsopodapus sp. 1 TLF-2007</i>
EU107665	Isopoda	TainIsopodapidae	<i>TainIsopodapus sp. 1 TLF-2007</i>
EF659963	Isopoda	Trachelipodidae	<i>Trachelipus sp. AP-2007</i>
EF659962	Isopoda	Trachelipodidae	<i>Trachelipus sp. AP-2007</i>
EF659957	Isopoda	Trachelipodidae	<i>Trachelipus sp. AP-2007</i>
EF659956	Isopoda	Trachelipodidae	<i>Trachelipus sp. AP-2007</i>
EU364624	Isopoda	Tylidae	<i>Tylos neozelanicus</i>
EF027456	Isopoda	Tylidae	<i>Tylos ponticus</i>

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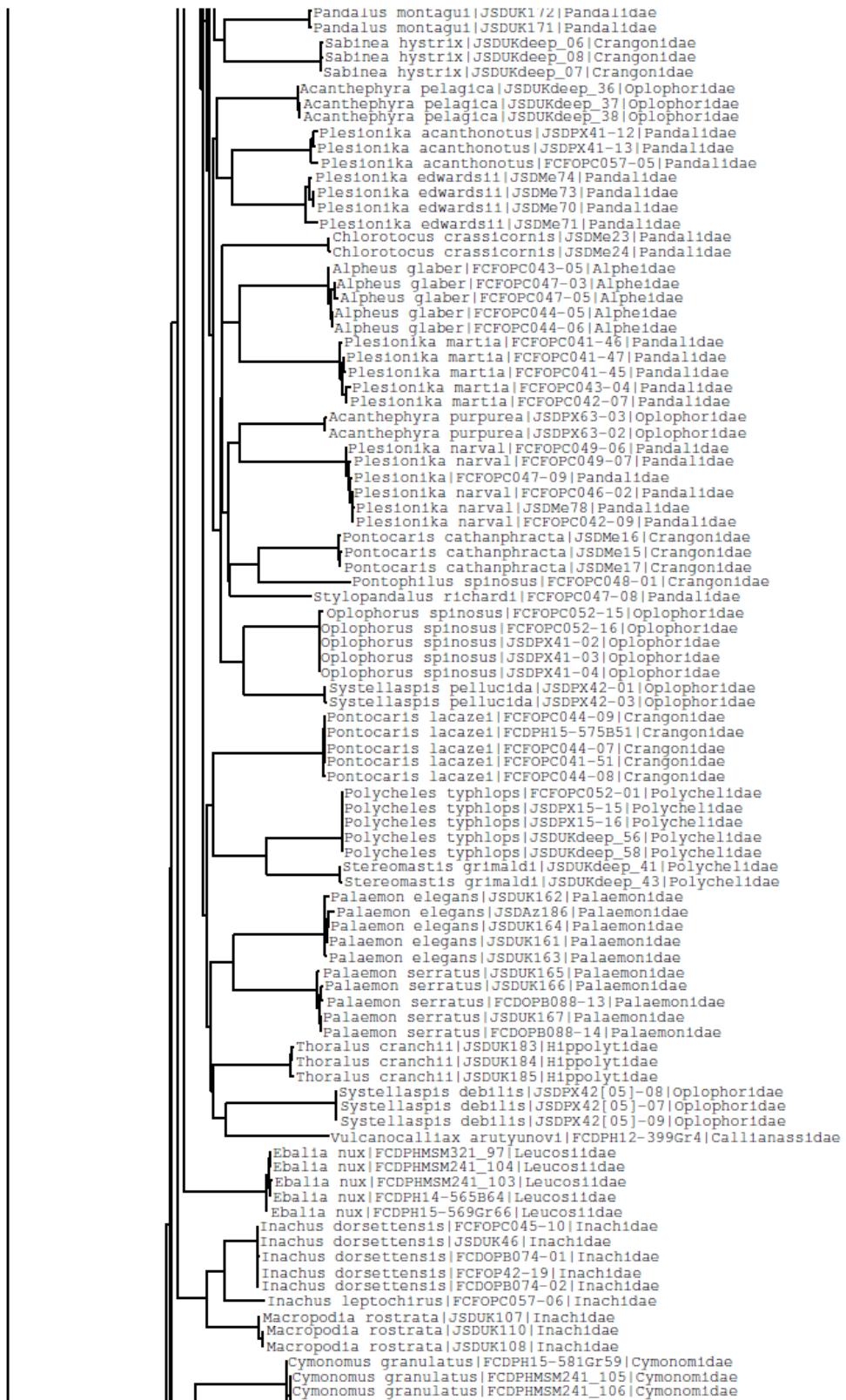
EF027455	Isopoda	Tylidae	<i>Tylos ponticus</i>
EF027454	Isopoda	Tylidae	<i>Tylos ponticus</i>
EF682290	Isopoda	Munnopsidae	<i>Tytthocope sp. 3 G8</i>
EF682284	Isopoda	Munnopsidae	<i>Vanhoeffenura sp. MB E1</i>
EF643519	Isopoda	Armadillidiidae	<i>Armadillidium vulgare</i>

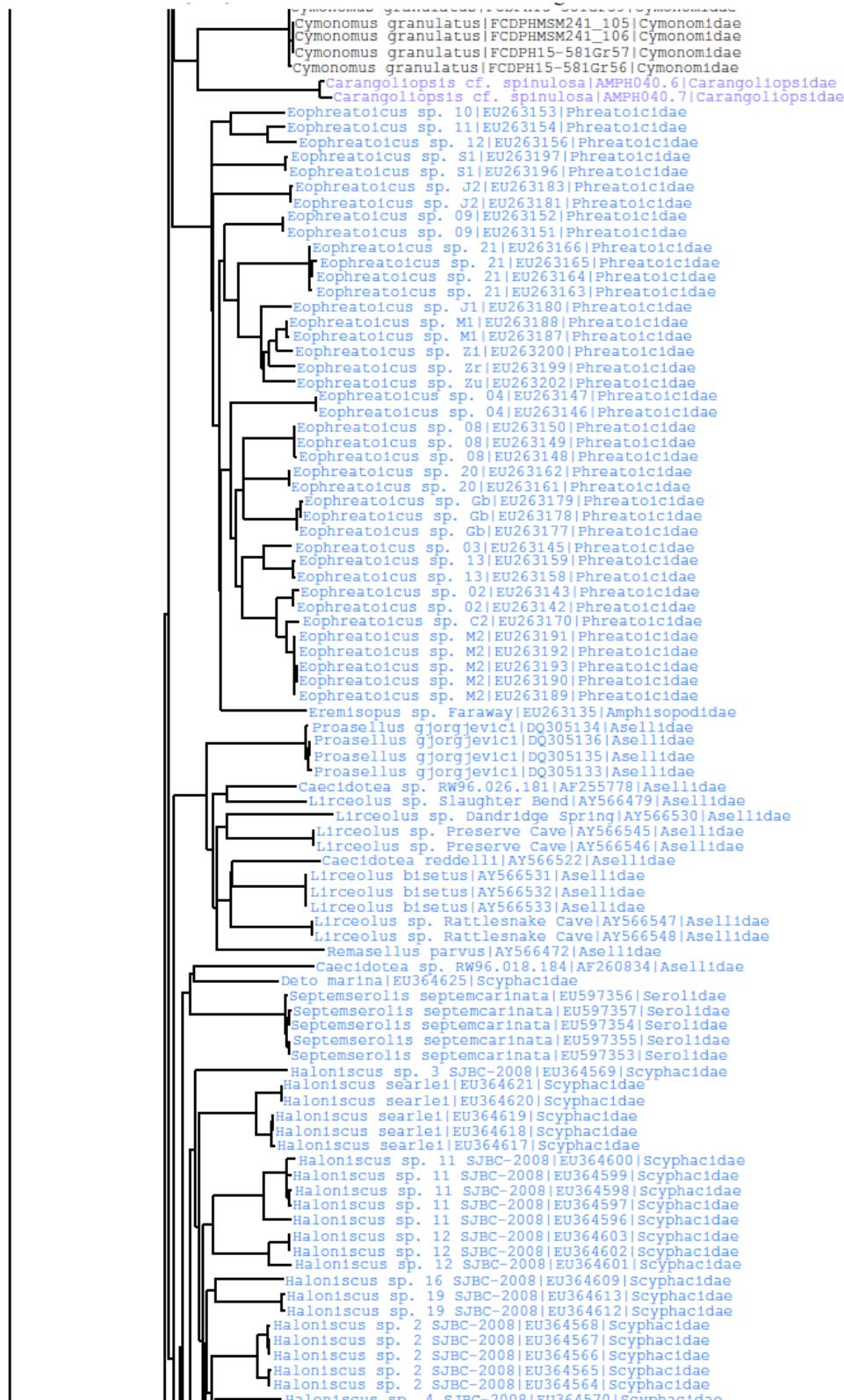


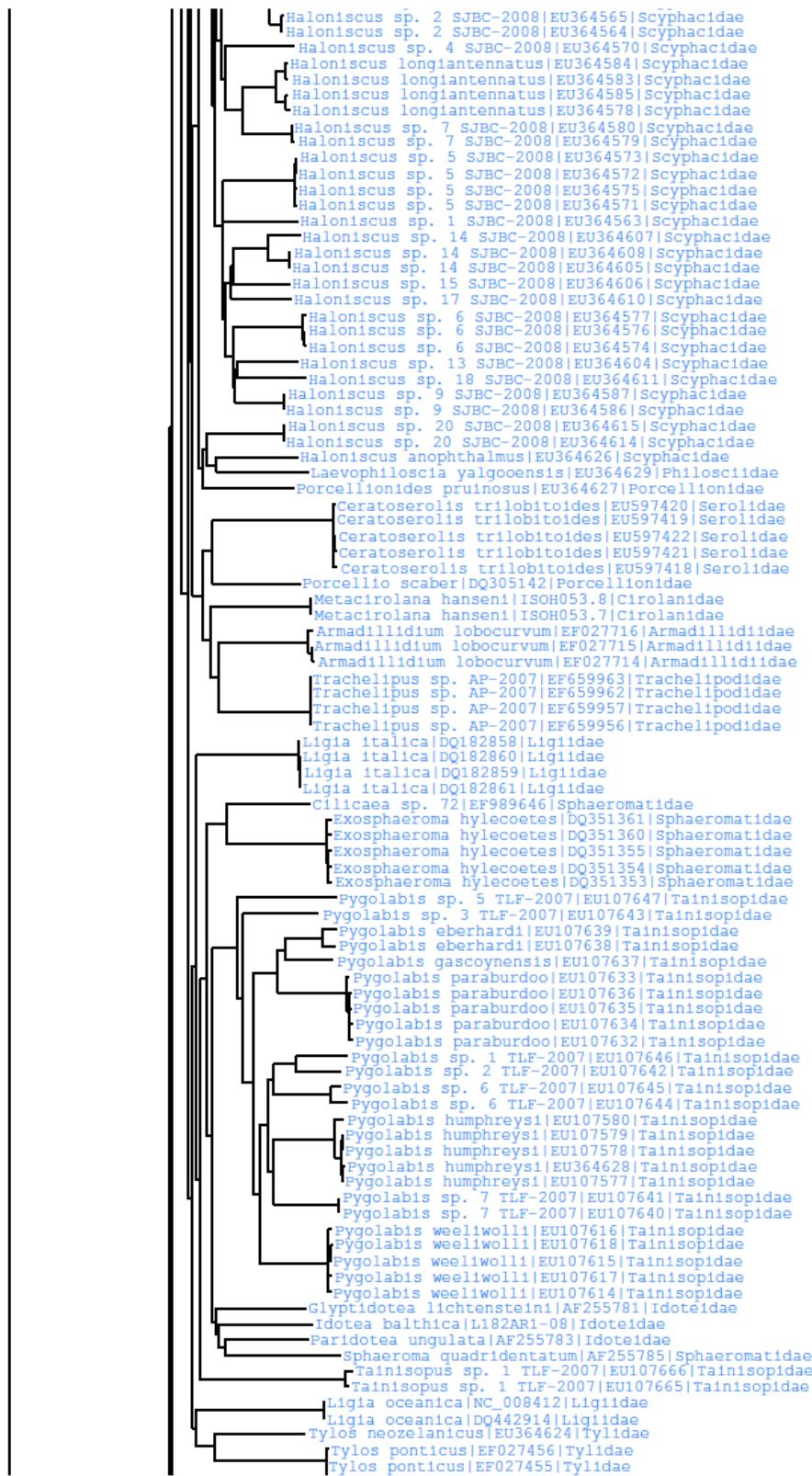


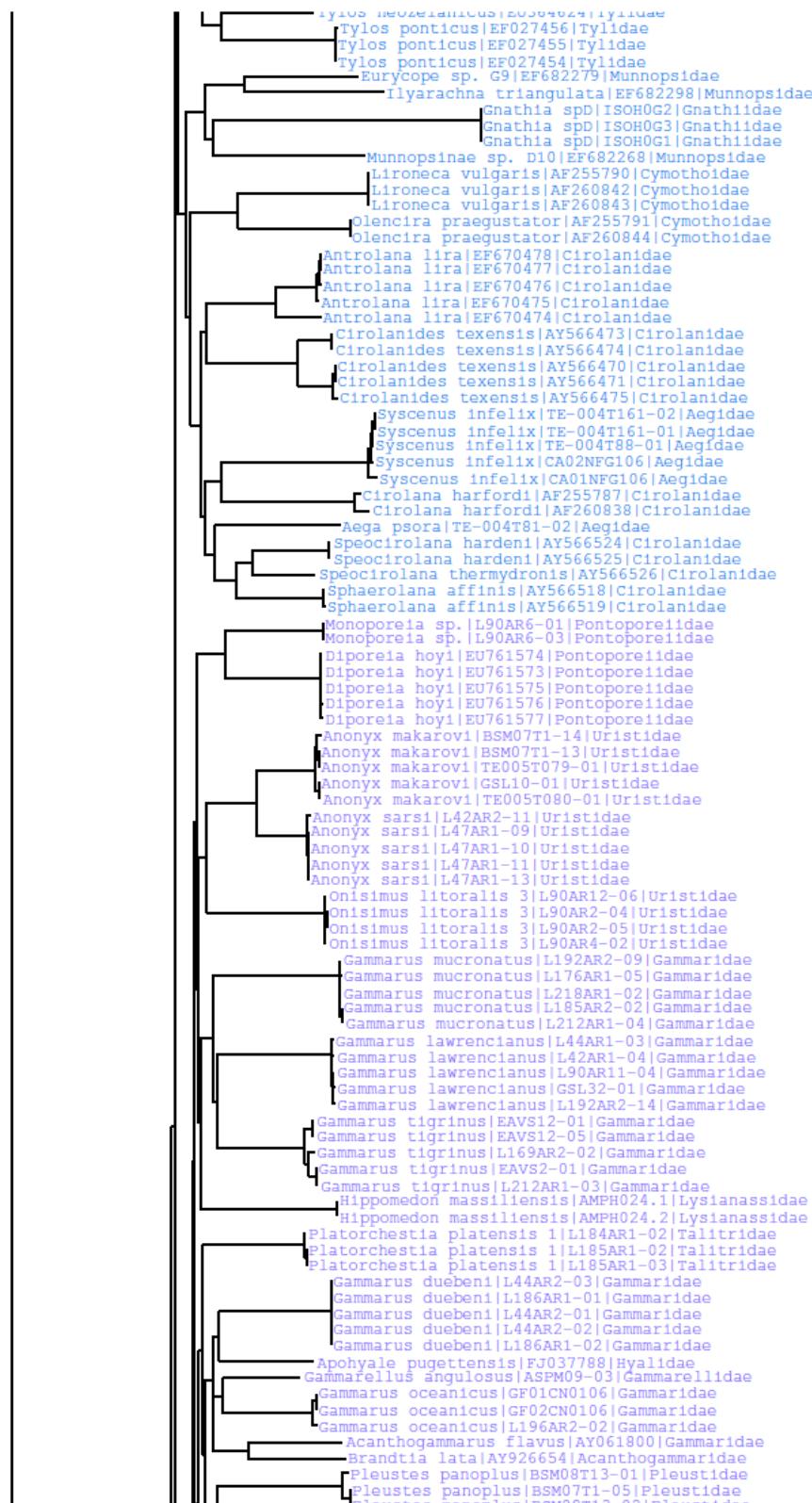


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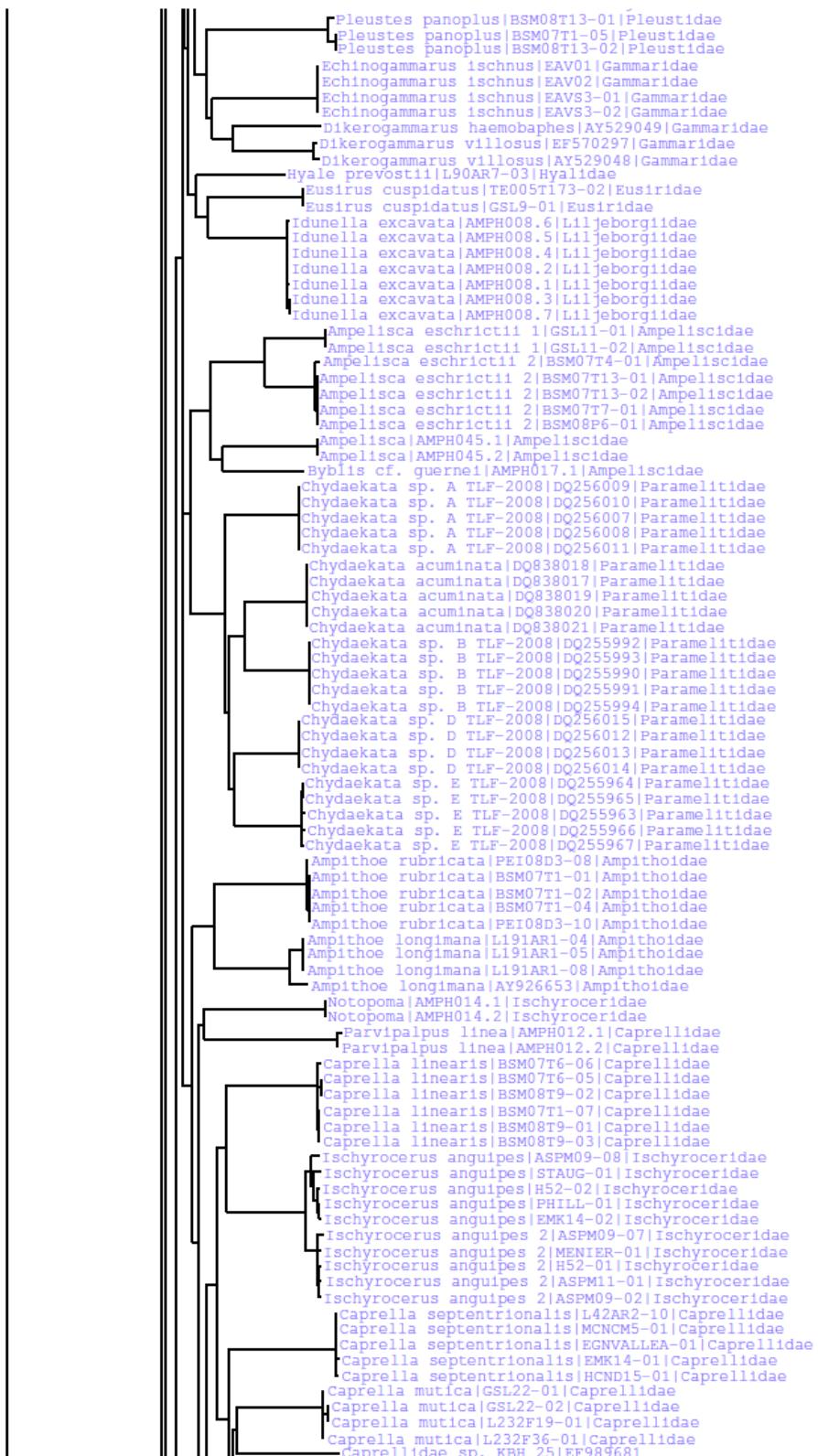


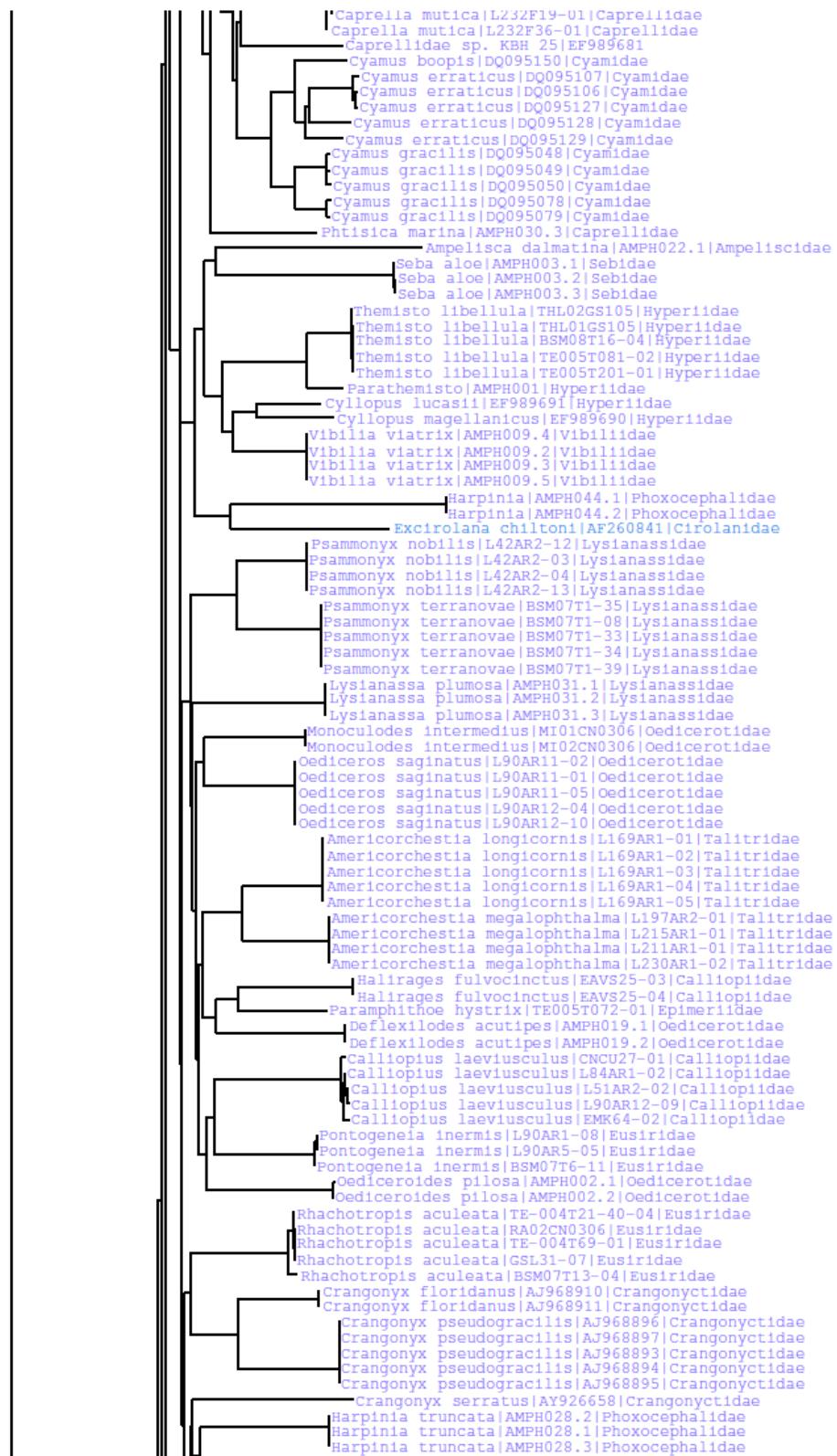






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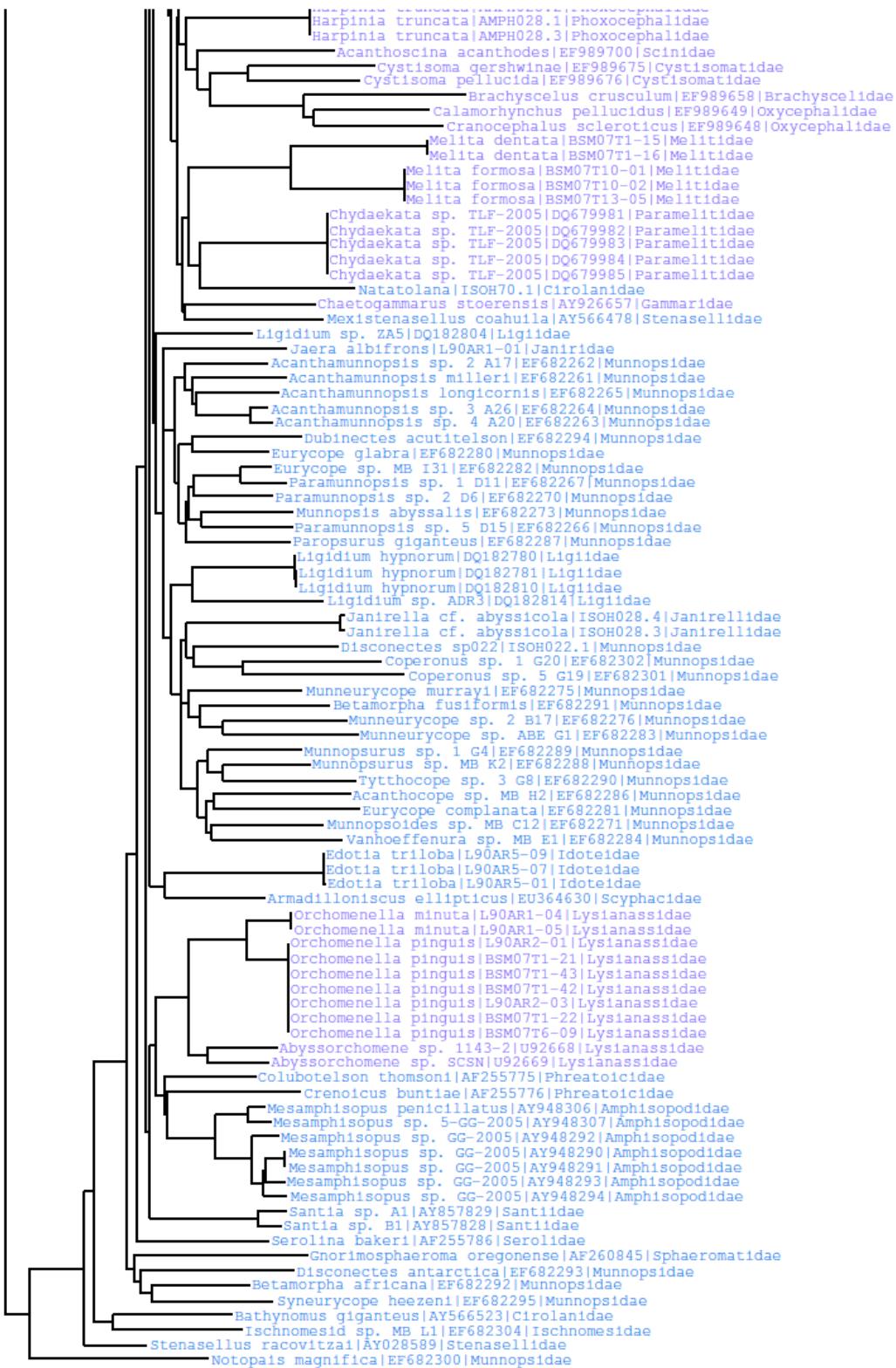
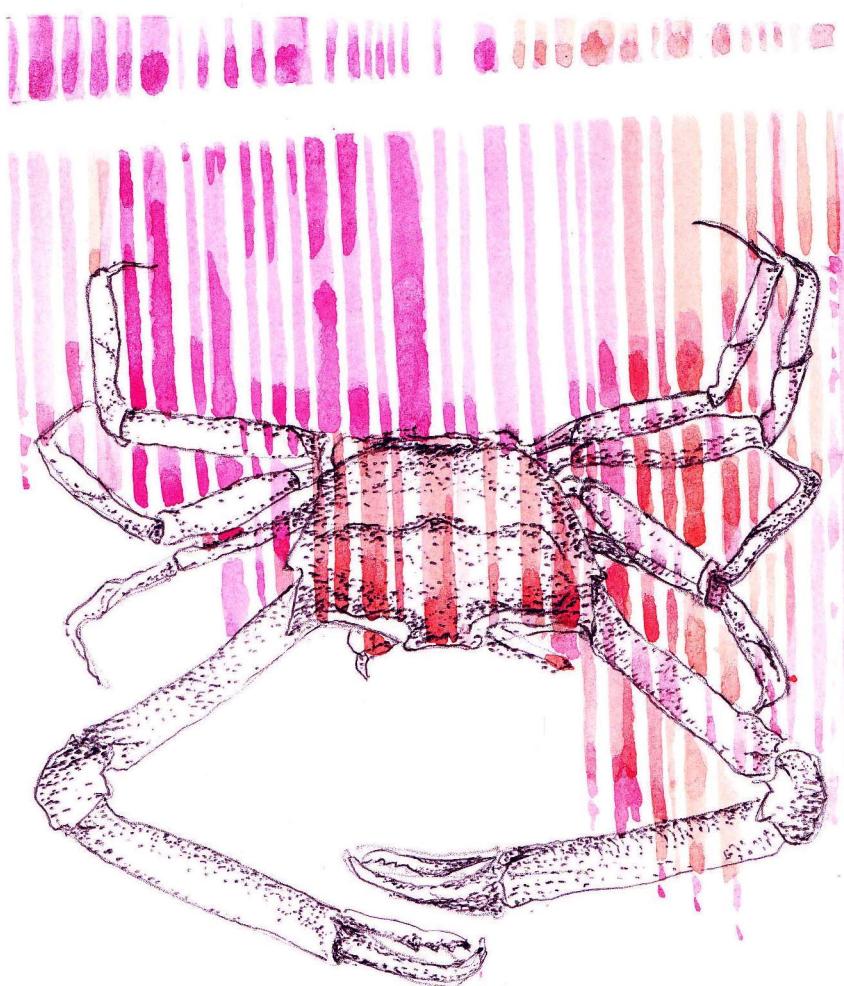


Figure S 2.1.1: Taxon ID Tree of Malacostraca generated by BOLD. Neighbour Joining tree (Kimura 2-parameter, uniform rates among sites, pairwise deletion) combining COI data from public BOLD projects and present study. A total number of 902 sequences from 337 species, 202 genera and 101 families were used (30 of June 2011). See Table S 2.1.2 for taxon list.



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CHAPTER 2.2 Identifying putative nuclear sequences of mitochondrial origin (*numts*) from *Goneplax rhomboides* (Linnaeus, 1758) based on COI amino acid variability

Abstract

COI has been the gene most commonly used for species identification/delineation due to the traditionally accepted advantages of mitochondrial DNA (mtDNA). However, here we discuss one of the most important disadvantages, the consequences of misinterpretation caused by the inclusion of nuclear sequence of mitochondrial origin (*numts*). *Numts* have been found in major clades of eukaryotic organisms and recently in decapods. During recent years it has been proposed that a high number of quality control steps should be implemented to avoid or flag *numts* during the processing of COI barcode data. Here we follow some of the steps proposed, using 20 individuals of *Goneplax rhomboides* (Linnaeus, 1758). We discuss the importance of analysing translated amino acid sequences as a new step to add for screening *numts*. Our study confirms that DNA barcode protocols and sequence data must be carefully and comprehensively screened for detection of possible *numt* amplification despite the good quality of the chromatograms observed. Failure to do so may result in erroneous taxonomic inferences, particularly in the case of *numts* that have diverged only a little from orthologous sequences.

Keywords: *Numt*; DNA barcoding; COI amino acid; *Goneplax rhomboides*

Matzen da Silva J, S Creer, A dos Santos, MR Cunha, FO Costa, GR Carvalho (2011)
Identifying putative nuclear sequences of mitochondrial origin
(*numts*) from *Goneplax rhomboides* (Linnaeus, 1758) based on COI
amino acid variability. *Paper in preparation*.

2.2.1 Introduction

Mitochondrial DNA is commonly used as a marker for molecular systematics, phylogeny and for species diagnosis (“DNA barcoding”). COI has been the DNA barcode of choice for species identification/delineation due the traditionally accepted advantages of mitochondrial DNA (mtDNA) (Hebert *et al.*, 2003). Nuclear sequences of mitochondrial origin (*numts*) are a challenge in using mitochondria for these purposes. It was suggested that because of *numts*, the barcoding approach is unreliable, at least in primates (Thalmann *et al.*, 2004). Recently, DNA barcoding among arthropods was found to overestimate the number of species when *numts* are co-amplified (Hazkani-Covo *et al.*, 2010), showing that *numts* introduce serious ambiguity into the DNA barcoding program as arthropods are very speciose and one the major phyla studied in taxonomy. Because the DNA barcoding initiative attempts to barcode all life forms, the *numts* issue cannot simply be ignored. Initially *numts* were considered non-functional copies of mtDNA in the nucleus originally derived from functional genes (Vanin, 1985) having stop codons and frameshift mutations that prevent their expression (Balakirev and Ayala, 2003). It is therefore assumed that all *numt* mutations are selectively neutral and have equal probability to become fixed in the population. Recently, however, some evidence for functional roles have been obtained, such as gene expression, gene regulation, generation of genetic diversity in *Drosophila* (Balakirev and Ayala, 2003), mice (Hirotsune *et al.*, 2003) and in human and chimpanzee (Svensson *et al.*, 2006) genes.

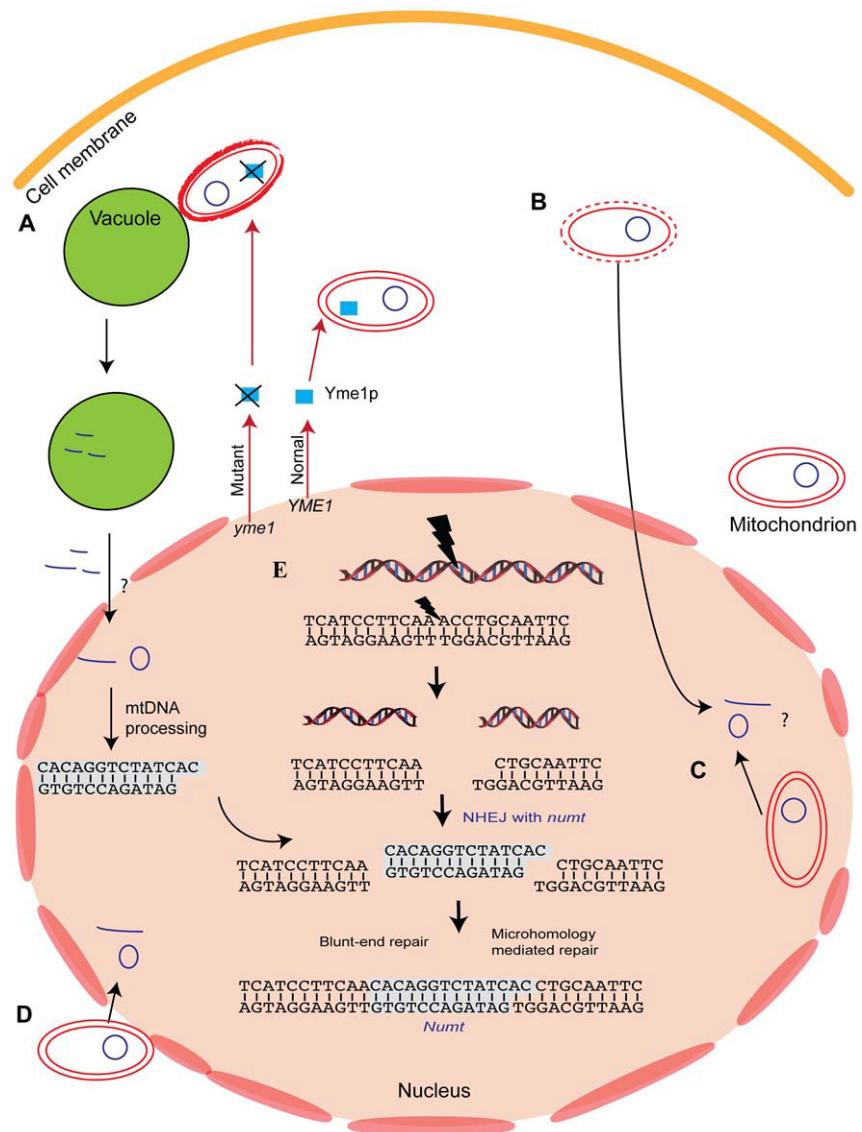


Figure 2.2.1: Mechanism of *numts* insertion (figure adapted from Hazkani-Covo *et al.*, 2010). Different mechanisms are here suggested: (A) The degradation of abnormal mitochondrial (Campbell and Thorsness, 1998). Mutant nuclear gene (*yme1*) have protein that are mitochondrial associated (Yme1p) and it has been suggested that perturbation in mitochondrial functions due to the alteration of gene products affect mitochondrial integrity by the vacuole, and this degradation increases mtDNA escape to nucleus in a process known as mitophagy and are more frequently than the wild-type strain (*YME1*); (B) lysis of mitochondrial compartment; (C) encapsulation of mitochondrial DNA inside the nucleus; (D) direct physical association between the mitochondria and the nucleus and membrane fusions; (E) MtDNA integrated into the chromosome during the repair of double strand breaks (DSBs) in a mechanism known as non-homologous end-joining (NHEJ). The insertion involves two DSB repair events. Each can be repair with or without the involvement of short microhomology and the base-pair complements are available between the *numt* and the chromosome ends. Processes that occur after *numt* insertion, such as duplication or deletions of *numts* will depend on the fate of the genome as a whole (see for review Hazkani-Covo *et al.* (2010)).

Numts can be generated in different ways (Figure 2.2.1). For *numts* to persist in nuclear genomes, mtDNA must first physically reach the nucleus, then it must become integrated into the nuclear chromosome, with intragenomic dynamics of amplification, mutation, or deletion following (Hazkani-Covo *et al.*, 2010) . To understand the process by which mtDNA penetrates the nucleus, a sensitive assay was developed by Campbell and Thorsness (1998) that allows identification and analysis of genes that affect this process in yeast (case A in Figure 2.2.1).

Numts have been found in major clades of eukaryotic organisms, e.g., arthropods (Bensasson *et al.*, 2001; Song *et al.*, 2008), crustaceans (Buhay, 2009) and additional taxa in decapods (Nguyen *et al.*, 2002; Schubart, 2009; Williams and Knowlton, 2001). Their proportion very greatly depending on the organism, life style, and on the genome properties i.e., size (Figure 2.2.2), duplication rates, mutation, deletion, retrotransposition to chromosomes (i.e. , a portion of the mRNA transcript of a gene is spontaneously reverse transcribed back into DNA and inserted into chromosomal DNA) (Balakirev and Ayala, 2003; Bensasson *et al.*, 2001; Gerstein and Zheng, 2006; Hazkani-Covo *et al.*, 2010; Zhang and Hewitt, 1996). In animals, *numts* lose their function upon transfer into the nucleus during the repair of chromosomal breaks by non homologous recombination. Therefore they are ideal loci that can be used to understand genome dynamics, molecular ecology and evolution (Bensasson *et al.*, 2001; Keller *et al.*, 2007; Lafontaine and Dujon, 2010; Zhang and Hewitt, 1996). Moreover, they are not under the effects of natural selection (noncoding genes), they are abundant in many taxa (Bensasson *et al.*, 2001) and distributed widely throughout nuclear genome (Keller *et al.*, 2007).

Recently publications about the concern and the effect of *numts* on DNA barcoding projects have produced interesting papers in crustacean systematic studies

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and population levels (Buhay, 2009; Song *et al.*, 2008; Williams and Knowlton, 2001).

Numt sequences can be highly divergent from the orthologous COI sequences.

Additionally, high genetic divergences are used to indicate possible new species that may be nested within species complexes. The occurrence of sequences with high divergence, and aberrant nucleotide composition and/or phylogenetic trees with unusually long branches and nonsensical systematic relationships have been attributed to the presence of *numts* in COI data (Buhay, 2009; Song *et al.*, 2008). Buhay (2009) reported a list of potential cases of *numts* in Crustacea (e.g., krill, crab, amphipod, crayfish, squat lobster, shrimp, isopod, barnacle, and copepoda) when Buhay found reading frame problems but without the occurrence of stop codons and no significant variance of proportion of adenine – thymine among specimens (*numts* have a significantly lower AT% compared with the orthologous mtDNA (Song *et al.*, 2008)). A growing concern regarding *numts* and DNA barcoding (Schubart, 2009) is that, if undetected, *numts* might lead to an overestimation of species richness (Song *et al.*, 2008). Methods for identifying *numts* have been now tested (see Figure 2.2.3), and several recent studies demonstrate the potential utility of *numts* sequences in evolutionary studies (Bensasson *et al.*, 2001; Gerstein and Zheng, 2006; Keller *et al.*, 2007; Schubart, 2009; Svensson *et al.*, 2006). Also other studies showed that *numts* had been inadvertently amplified instead of the target 16S and cytochrome *b* genes in crabs and freshwater crayfish (Nguyen *et al.*, 2002; Schubart, 2009). An intensive analysis of four closely related crayfish species (*Orconectes* spp.) also highlighted the existence of COI-derived *numts* and additionally illustrated how barcoding methods may incorrectly infer that single individuals may belong to multiple, unique species (Song *et al.*, 2008).

Characterization of *numts* is important to understand genome dynamics and evolution, and its significance increases when several genomes of related organisms can be

compared. Thus, it is important that *numt* sequences should not be discarded, but recognized, appropriately annotated and submitted as such (Buhay, 2009; Schubart, 2009).

In the case of barcoding, COI *numts* can be highly divergent from the actual COI sequences and can lead to false inferences of divergent clades, if only molecular data are scrutinised (Buhay, 2009). The main aim here is to delineate and analyse putative *numt* sequences using methods proposed recently by Song *et al* (2008), Buhay (2009) and Schubart (2009). We also discuss the occurrence of unexpected intraspecific amino acid changes within the highly conserved COI gene among *Goneplax rhomboides* (Linnaeus, 1758), specimens from Atlantic Ocean and Mediterranean Sea. A description of the taxonomic implication related with *Goneplax rhomboides* is described in the next paragraph.

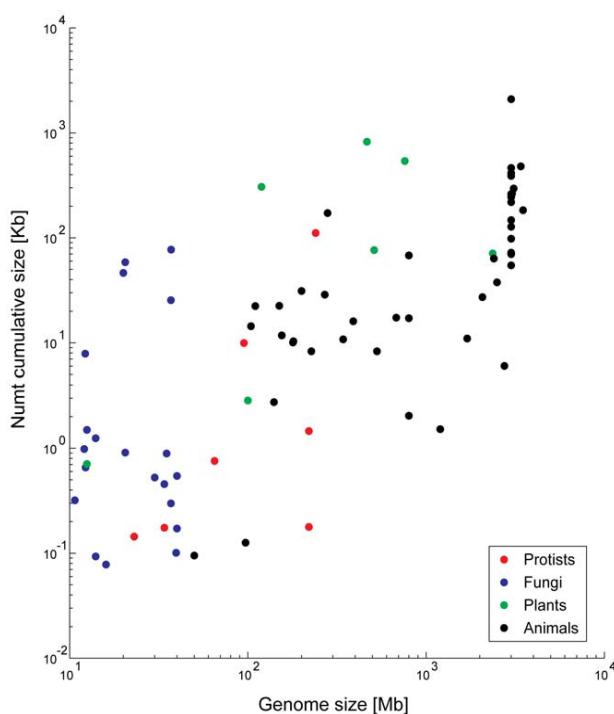


Figure 2.2.2: *Numt* content is correlated to genome size (adapted from Hazkani-Covo *et al.*, 2010).

Synopsis: Species status of *G. rhomboides*

Goneplax rhomboides (Linnaeus, 1758), a senior synonym of *Cancer angulata* Pennant, 1777, has been regarded as a single species inhabiting the Mediterranean Sea and the Atlantic Ocean from the North Sea to southern Africa and the Indian Ocean coast of South Africa. It is a common species, found from the intertidal to 700 m, and commonly known as the angular crab (Guinot and Castro, 2007). Confusion has long existed over the status of two species, *Goneplax rhomboides*, for the Atlantic populations and *G. angulata* (Pennant, 1777) for individuals with a weak or absent anterolateral tooth and found mostly in the Mediterranean. On account of numerous transitions shown by the anterolateral tooth of the North Atlantic and Mediterranean *Goneplax*, several authors (see for reference Guinot and Castro, 2007), concluded that this character was highly variable, clearly not species-specific, and suggested synonymising the two species. However, the larval evidence itself cannot be regarded as conclusive because closely related congeners often have very similar, almost identical larval stages (Taishaku and Konishi, 1995) or incomplete description of each larvae stage respectively. In Guinot and Castro (2007) study, only one species referred to as *G. rhomboides* is recognized for the North Atlantic and Mediterranean Sea (Figure 2.2.3). The available material and published evidence demonstrate that the form of the anterolateral teeth (second tooth acute, weak or absent) is variable, and there is no obvious morphological basis to recognise more than one taxon across its widespread distribution (Atlantic and Mediterranean). However, Guinot and Castro do not discount that a detailed molecular analysis might cast new ideas on the identity of both Atlantic and Mediterranean populations. Although they inferred a hypothesis that *G. rhomboides* is replaced in the south Atlantic by *G. clevai* n. sp based on previous characters reported above (Figure 2.2.4).



Figure 2.2.3: Photography took in this study of representative morphotype specimens *G. rhomboides* (*G. romboidea* 01) collected from South Portuguese coast and sequenced twice with two different COI primers set COI-III and COLH (Table 2.2.1).

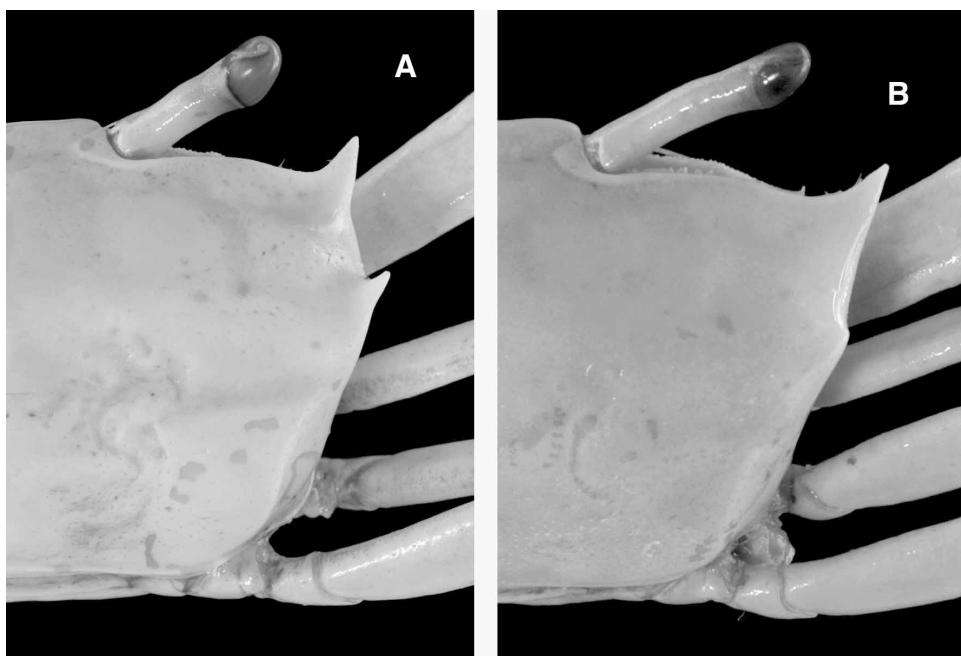


Figure 2.2.4: Eye and anterolateral border of the carapace: (A) *Goneplax clevai* n. sp and (B) *G. rhomboides* (Linnaeus, 1758) (photography adapted from Guinot and Castro 2007).

2.2.2 Material and Methods

Samples

Twenty specimens of *Goneplax rhomboides* (Brachyura; Goneplacidae) from England, Portugal, the Gulf of Cadiz and Sicily were analysed to discuss the impact of possible *numt* amplification (despite the good quality of the chromatograms observed) in erroneous taxonomic inferences in this species, particularly in the case of *numts* that have diverged little from orthologous sequences (Table 2.2.1).

Sequences

Twenty five sequences (*G. rhomboides* 01 to 20) from 20 specimens were analysed. Five individuals (*G. rhomboides* 01 to 05) were sequenced twice with a different set of primers. Here based on previous observation of different distinct lineages based on barcoding threshold criteria of determining candidate taxa (Hebert *et al.*, 2003) of five individuals amplified with M13-tailed primer cocktails (*G.rhomboideis* 01 to 05 COI-III) we re-amplified the same five individuals with the “universal decapod primer set” COL6 (Schubart, 2009) and COH6 (Schubart and Huber, 2006), i.e., *G. rhomboides* 01 to 05 COLH (see Table 2.2.1).

For more details about DNA isolation and COI polymerase chain reaction (PCR) protocols please see section “Material and Methods” in Chapter 2.1. The use of other genes has been recommended by Rubinoff (2006) to help determine the source of the error, whether it is *numts*, contaminated DNA, or poor sequence quality and editing. Here we amplified all samples with an additional marker; the partial mitochondrial sequences of the large ribosomal subunit 16S with primer pair 16Sar and 16Br-Dr (Palumbi *et al.*, 1991). Amplification of 16S was performed in a 25 µl volume

containing 1 X PCR buffer, 3 mM MgCl₂, 0.1 mM dNTP, 1U TAQ polymerase, 5 pmol of each primer, and 2 - 10 ng of DNA template. The thermal cycling conditions consisted of 94°C for 3 min; 40 cycles of 94°C for 45 s, 58°C for 60 s, and 72°C for 60 s; followed by a final extension of 72°C for 10 mins (Schubart *et al.*, 2006). Following amplification, PCR products were cleaned by incubation with 10 U Exonuclease I (New England Biolabs ®) and 1 U Shrimp Alkaline Phosphate (Promega ®) at 37°C for 1 hour, followed by heating at 80 °C for 5 min. Samples were sequenced by Macrogen Inc (South Korea) using an Applied Biosystems ® 3730 sequencer.

Screening questionable *numts*

Recent studies have questioned the universal utility of the Folmer region (Folmer *et al.*, 1994) in DNA barcoding (Burns *et al.*, 2007), and especially when a large number of *numts* are suspected in this region (Song *et al.*, 2008), additional primers and markers than COI gene have been analysed. A number of steps proposed by Song *et al* 2008 (Figure 2.2.5) were taken into account to help avoid and identify *numts* in DNA barcoding analysis, i.e., the use of mtDNA rich tissue (muscle), performing PCRs with different set of primers and markers (see below), taking into account the observation of PCR ghost bands or extra bands, translating sequences to check for indels and stop codons, identifying polymorphic sites, including if they are encountered when sequencing from both strands, checking for frameshift mutations, cross-checking clean sequences with COI sequences from the published mitochondrial genome of the most closely-related xanthida crab *Pseudocarcinus gigas* (Lamarck, 1818) (NC_006891) to our study group and examining compositional biases (Bensasson *et al.*, 2001; Song *et al.*, 2008). All sequences were translated using the invertebrate mitochondrial code in Mega 4 (Tamura *et al.*, 2007).

Tabel 2.2.1: Selected *G. rhomboides* with respective sample name, museum catalogue number, site of collection, COI primer set tested and genetic database accession numbers (Genbank).

Samples names	Catalogue No. ¹	Collection site	COI primers set		Reference	Genbank accession No.
			COI	16S		
1 <i>G. rhomboides</i> 01 COI-III	MB89000356	South coast of Portugal	cocktail COI-III		This study ²	JN591693
2 <i>G. rhomboides</i> 02 COI-III	MB89000357	South coast of Portugal	cocktail COI-III		This study ²	JN591694
3 <i>G. rhomboides</i> 03 COI-III	MB89000358	South coast of Portugal	cocktail COI-III		This study ²	JN591695
4 <i>G. rhomboides</i> 04 COI-III	MB89000359	South coast of Portugal	cocktail COI-III		This study ²	JN591696
5 <i>G. rhomboides</i> 05 COI-III	MB89000360	South coast of Portugal	cocktail COI-III		This study ²	JN591698
6 <i>G. rhomboides</i> 01 COLH	MB89000356	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591659
7 <i>G. rhomboides</i> 02 COLH	MB89000357	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591655
8 <i>G. rhomboides</i> 03 COLH	MB89000358	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591674
9 <i>G. rhomboides</i> 04 COLH	MB89000359	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591675
10 <i>G. rhomboides</i> 05 COLH	MB89000360	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591676
11 <i>G. rhomboides</i> 06 CrustD	DBUA803.01	Gulf of Cadiz	CrustDF1/CrustDRI		Radulovici <i>et al.</i> , 2009	JN591678
12 <i>G. rhomboides</i> 07 Folmer	MB89000362	Northwest coast of Portugal	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591661
13 <i>G. rhomboides</i> 08 Folmer	MB89000363	Northwest coast of Portugal	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591680
14 <i>G. rhomboides</i> 09 COI-III	MB8900097	Northwest coast of Sicily	cocktail COI-III		This study ²	JN591681
15 <i>G. rhomboides</i> 10 Folmer	MB89000494	Irish Sea, England	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591682
16 <i>G. rhomboides</i> 11 Folmer	MB89000495	Irish Sea, England	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591683
17 <i>G. rhomboides</i> 12 Folmer	MB89000496	Irish Sea, England	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591684
18 <i>G. rhomboides</i> 13 Folmer	MB89000497	Irish Sea, England	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591685
19 <i>G. rhomboides</i> 14 Folmer	MB98000243	Irish Sea, England	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591686
20 <i>G. rhomboides</i> 15 Folmer	MB89000320	Northwest coast of Portugal	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591687
21 <i>G. rhomboides</i> 16 CrustD	MB89000321	Northwest coast of Portugal	CrustDF1/CrustDRI		Radulovici <i>et al.</i> , 2009	JN591688
22 <i>G. rhomboides</i> 17 CrustD	MB89000498	South coast of Portugal	CrustDF1/CrustDRI		Radulovici <i>et al.</i> , 2009	JN591689
23 <i>G. rhomboides</i> 18 CrustD	MB89000499	South coast of Portugal	CrustDF1/CrustDRI		Radulovici <i>et al.</i> , 2009	JN591690
24 <i>G. rhomboides</i> 19 Folmer	MB89000500	South coast of Portugal	LCO1490/HCO2198		Folmer <i>et al.</i> , 1994	JN591691
25 <i>G. rhomboides</i> 20 COLH	MB89000501	South coast of Portugal	COL6/COH6		Schubart, 2009	JN591692

¹Samples starting with code number (No.) MB89 and DBUA were archived in the Natural and History Museum and University of Aveiro of Portugal, respectively.² See Chapter II in *Material and Methods*. Numbers a bold represents the groups of specimens that were sequenced twice with different set of primers.

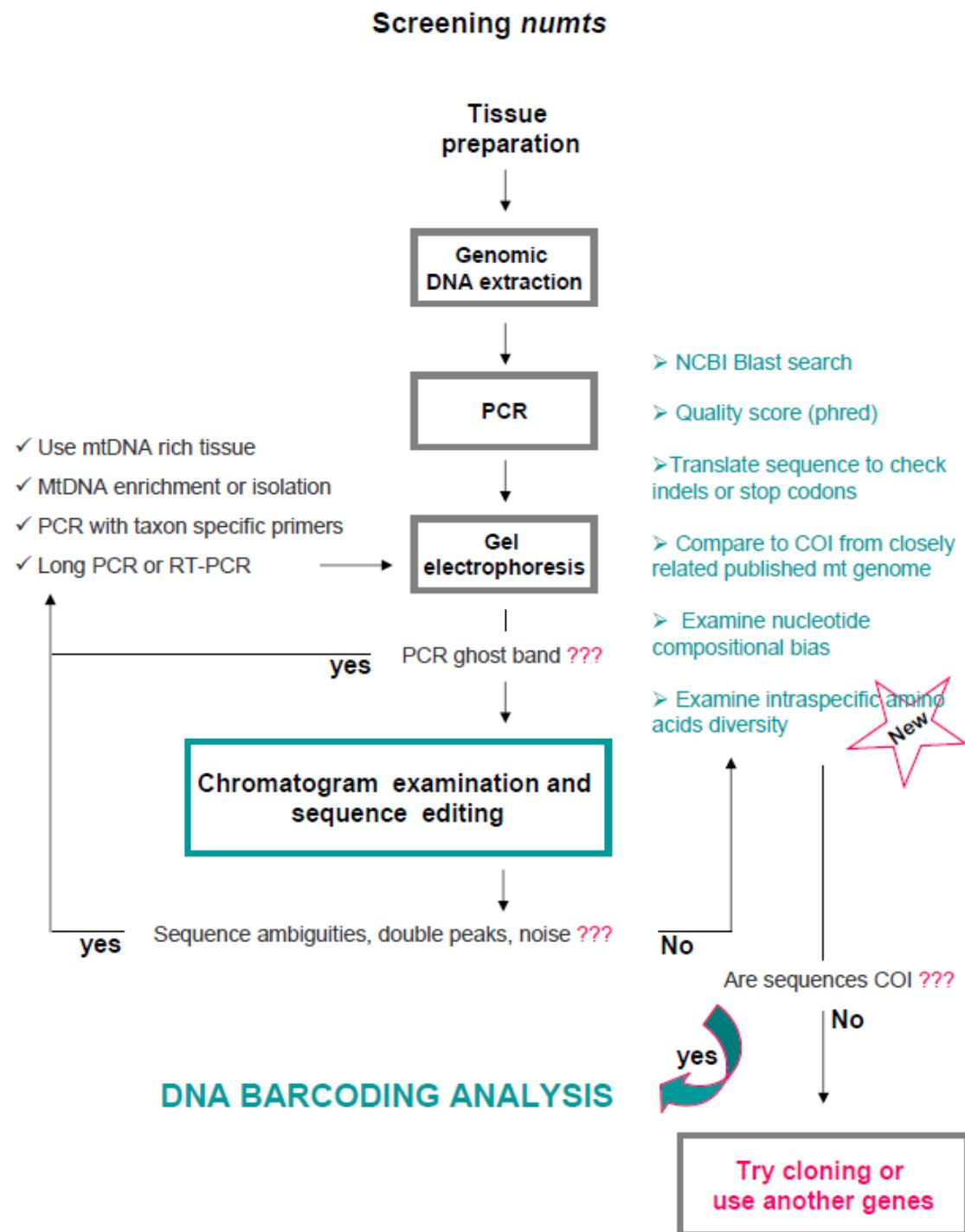


Figure 2.2.5: Steps to help avoid and identify *numts* contamination in DNA barcoding (figure modified from Song *et al.* 2008). A new step is proposed in this study to be added on the previous protocol when the chromatogram examinations pass to the quality control.

Chromatograms

The simultaneous amplification of *numts* and COI will most times result in difficult to score chromatograms because many different PCR products are sequenced simultaneously. Here we compared the quality of ten chromatograms from five selected samples (*G. rhomboides* 01 to 05 COI-III and COLH) amplified with two different primers set (Table 2.2.2) in CodonCode (<http://www.codoncode.com/>). COI is a very conserved gene with respect to amino acid composition and despite differences in the nucleotides (mostly at third position), those differences often do not translate into amino acid changes (Buhay, 2009). To help to screen our questionable *numts* sequences we compared within each individual the reading frame of orthologous COI and the questionable pseudogene sequences (e.g., *G. rhomboides* 01 COLH vs *G. rhomboides* 01 COI-III) having as a COI reference the closest species founded, *P. gigas* (NC_006891). Although all of these methods are effective, none are guaranteed (see <http://www.pseudogene.net>).

Data analysis

Sequences were aligned with Mega 4 (Tamura *et al.*, 2007). To study divergence patterns of our COI data amplified with different set of primers we performed phylogenetic analyses using distance (NJ), parsimony (MP) and Bayesian (BI) frameworks (Buhay, 2009; Schubart, 2009; Song *et al.*, 2008). The appropriate model of evolution was Kishino and Yano model (HKY) (Hasegawa *et al.*, 1985), determined by jModeltest (Posada, 2008) under the Akaike information criterion criteria (AIC) (Akaike, 1973) and those parameters were set in MrBayes (Huelsenbeck and Ronquist, 2001). BI analyses consisted of running four simultaneous chains for 5×10^6 generations every 1000 generations. The nodal support was assessed by using the posterior

probability generated from a consensus tree of the sampled trees past burn-in determined by using Tracer v1.4 (Drummond and Rambaut, 2007). The MP analysis was carried out with 2000 bootstrap pseudoreplicates in Mega 4 (Schubart, 2009). To analyse how the presence of questionable *numts* might influence the inferences from DNA barcoding, we performed a distance-based reconstruction with a neighbour-joining (NJ) analysis under K2P model, typically used in barcoding studies (Hebert *et al.*, 2003; Hebert *et al.*, 2004), and it was carried out with 1000 bootstrap replications too with COI and 16S sequences each from all samples (Table 2.2.1). The COI alignment was then split into separate datasets (apparently legitimate COI sequences and questionable *numts* sequences) and we calculated the sequence divergence among haplotypes of each data set in Mega 4 (Table 2.2.2).

2.2.3 Results

Sequence quality analysis

Clean sequences of all individuals were obtained from freshly preserved specimens from the North Atlantic and Mediterranean Sea (see Methods). None of our PCR results showed double products as an indication of potential *numt* amplification (see Schubart 2009). Twenty COI sequences and five questionable COI sequences were individually examined for nucleotide and amino acid composition after each chromatogram was inspected using CodonCode Aligner. No stop codons and indels, prevented translation in our 25 sequences analysed and our chromatograms were of good quality (as an example of analysed chromatograms, see Figure 2.2.6).



Figure 2.2.6: Screenshot example of 5'COI sequences, translation and chromatograms from our representative *G. rhomboides* 01 COLH and five questionable COI sequences amplified with M13-tailed cocktail primers (COI-III). The variety of colours along the sequence text, each colour represents a different amino acid translated along 564 bp of COI sequence. No stop codons and indels have prevented translation in our data set.

Each individual is numbered from 01 to 20 with respectively amplified primers, i.e., Folmer (Folmer *et al.*, 1994), CrustD (Radulovici *et al.*, 2009), COLH (Schubart, 2009) and finally COI-III (M13-tailed cocktail primers *in Chapter II*).

The aligned region of the COI fragment of the *G. rhombooides* consisted of 564 base pairs (bp), of which 30 were variable and 27 parsimony-informative. When five questionable *numts* sequences were excluded from the alignment only one variable region was founded along the aligned sequences (C/T in position 39).

Phylogenetic analyses

Phylogenetic analyses with three reconstruction methods (BI, MP, NJ) revealed the evolutionary path of our sequences from species *G. rhombooides* amplified with different set of primers. The tree topologies were most phylogenetic informative for the BI analysis, which was therefore selected for representation, even if most of the interior branches were not significantly supported. These topologies are not in conflict with the ones produced by MP and NJ. In all trees, the questionable *numts* sequences do not represent the closest related sequences to the orthologous COI of the corresponding species and thus they would likely confuse phylogenetic relationships if not identified bioinformatically. In fact, the putative questionable sequences are mainly basal as reported in other studies (Buhay, 2009; Schubart, 2009; Williams and Knowlton, 2001). The phylogenetic tree shows a clear separation (BI posterior probabilities 100%; MP and NJ bootstrap 98%) of the COI sequences, corresponding to five individuals (*G. rhombooides* 01 to 05) of 20 individuals in total from different populations.

Phylogeny reconstruction of all individuals of the species *G. rhombooides* for which COI is available is presented as a radiation tree (unrooted) in Figure 2.2.7. This form of representation better demonstrates the phylogenetic position of the questionable

numts of the COI from *G. rhombooides* individuals. Overall it appears that the evolution of the questionable *numts* predates the separation of the COI of our twenty individuals involved. Differences among the sequences of *numts* from the same individual can reach levels up to 20% (Williams and Knowlton 2001). Here the distances K2P values varied from 0.4% to 5.0% between members of the two different groups (highlighted with orange colour in Table 2.2.2) defined by phylogenetic methods (Figure 2.2.7). No nucleotide diversity among 16S sequences was found within our 20 sequences.

Nucleotide and amino acid composition

The results of nucleotide and amino acid analyses showed an obvious difference between the apparently legitimate COI sequences and the questionable ones. COI sequences revealed low nucleotide and no amino acid diversity when compared with the questionable *numts* sequences (*G. rhombooides* 01 to 05 COI-III).

Nucleotide composition among 30 positions was much higher in the questionable sequences (*G. rhombooides* 01 to 05 COI-III) than within orthologous COI sequences (see Table 2.2.3). Despite no significant AT% differences being found between the five questionable COI sequences (AT% = 64.30 ± 0.474) when compared with our 20 COI sequences (AT% = 65.14 ± 0.082) the standard deviations was much higher in the first case.

The majority of mutations were silent (occurred in third codon position) and the transition C↔ T was particularly frequent (60%). Although positions 4 (A→C), 103 (T→G), 133 (G→A) and 454 (T→G) revealed missense mutations, i.e., the new nucleotide alters the codon so as to produce an altered amino acid in the protein product. The results of amino acid analyses showed an obvious difference between members of the questionable COI vs orthologous COI sequences compared to the *P. gigas*

(NC_06891) sequence as reference (Figure 2.2.8). Figure 2.2.8 shows the positions with bars to show the amino acid variation between five questionable *numts* and two COI sequences. COI did not show variation between NC_06891 and the representative *G. rhomboides* COI sequence (*G. rhomboides* 01 COLH) among 564 bp showing only two variation in codon position 253 and 463 (“ * ” in Figure 2.2.8). The composition of amino acids revealed that the mitochondrial COI gene is highly conserved even when compared to a different family and that the analysis of COI sequence composition is a valuable tool for separating *numts* from representative COI (Figure 2.2.8).

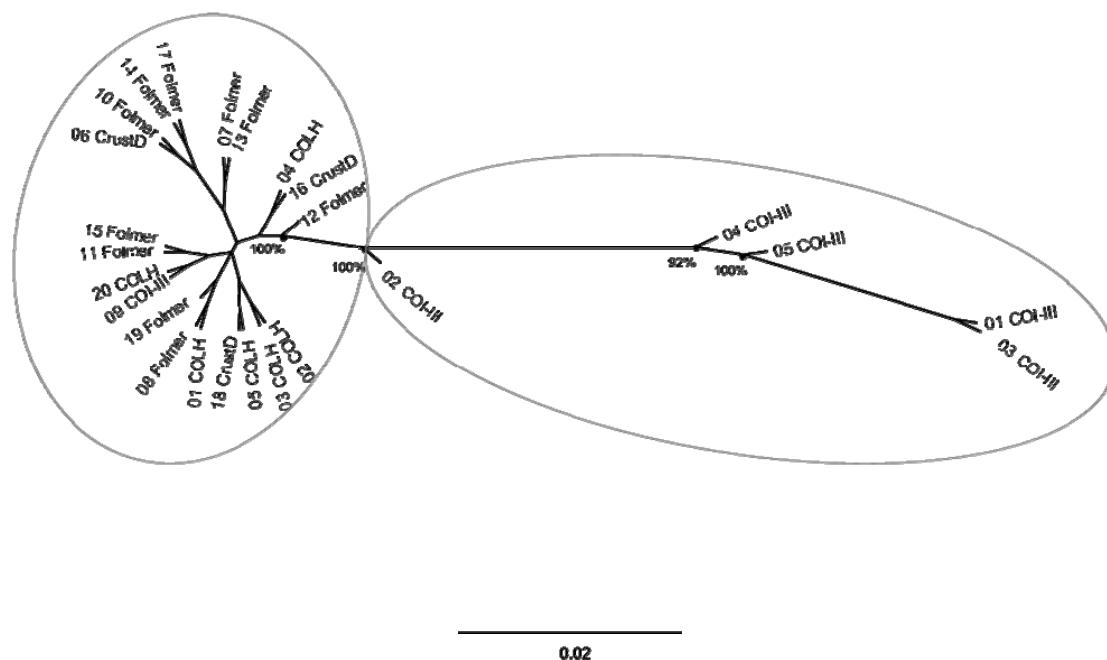


Figure 2.2.7: Radiation tree (BI) of representatives from 20 *G. rhomboides* (01 to 20) amplified with different primer set (see Table 2.2.1: Folmer; CrustD; COLH; COI-III). Posterior probabilistic BI values > 75% are represented in each node of the tree. Two groups are highlighted: the questionable *numts* sequences (on the right) and the orthologous COI sequences (on the left).

Table 2.2.2: Pairwise COI barcode nucleotide divergences for *G. rhomboides* using K2P distances (%).

Samples names	Distances (%)																								
	Between samples																								
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1 <i>G. rhomboides</i> 01 CO1-III																									
2 <i>G. rhomboides</i> 02 CO1-III	3.7																								
3 <i>G. rhomboides</i> 03 CO1-III	0.2	4.3																							
4 <i>G. rhomboides</i> 04 CO1-III	1.3	1.6	1.8																						
5 <i>G. rhomboides</i> 05 CO1-III	1.1	1.8	1.4	2																					
6 <i>G. rhomboides</i> 01 COLH	4.8	0.4	5.4	2.7	2.9																				
7 <i>G. rhomboides</i> 02 COLH	5.0	0.4	5.6	2.9	3.1	0.2																			
8 <i>G. rhomboides</i> 03 COLH	4.8	0.4	5.4	2.7	2.9	0	0.2																		
9 <i>G. rhomboides</i> 04 COLH	4.8	0.4	5.4	2.7	2.9	0	0.2	0																	
10 <i>G. rhomboides</i> 05 COLH	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0																
11 <i>G. rhomboides</i> 06 CrustD	5.0	0.4	5.6	2.9	3.1	0.2	0	0.2	0.2	0.2															
12 <i>G. rhomboides</i> 07 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0														
13 <i>G. rhomboides</i> 08 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2													
14 <i>G. rhomboides</i> 09 CO1-III	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0												
15 <i>G. rhomboides</i> 10 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0											
16 <i>G. rhomboides</i> 11 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0	0										
17 <i>G. rhomboides</i> 12 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0	0	0									
18 <i>G. rhomboides</i> 13 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0	0	0	0								
19 <i>G. rhomboides</i> 14 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0	0	0	0	0							
20 <i>G. rhomboides</i> 15 Folmer	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	
21 <i>G. rhomboides</i> 16 CrustD	4.8	0.4	5.4	2.7	2.9	0	0.2	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
22 <i>G. rhomboides</i> 17 CrustD	5.0	0.4	5.6	2.9	3.1	0.2	0	0.2	0.2	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
23 <i>G. rhomboides</i> 18 CrustD	5.0	0.4	5.6	2.9	3.1	0.2	0	0.2	0.2	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
24 <i>G. rhomboides</i> 19 Folmer	5.0	0.4	5.6	2.9	3.1	0.2	0	0.2	0.2	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0	0	
25 <i>G. rhomboides</i> 20 COLH	4.8	0.4	5.4	2.7	3.1	0	0.2	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2	0.2	

In orange it is highlighted the impact of questionable *numts* sequences in distances values based on previous observation of more than one distinct lineages and in bold are the repeated specimens sequenced with cocktail primers and “universal decapods primers”.

Table 2.2.3: Nucleotide diversity composition founded in 30 positions among 564 bp of 25 sequences of *G. rhombooides*.

Positions	*		*		*		*		*		*		*		*		*		*		*		*		*		*		
	4	30	39	105	108	111	117	132	133	150	153	204	229	241	253	270	291	294	303	324	336	348	396	423	429	444	447	454	471
<i>G. rhombooides</i> 01 COLH	A	C	C	T	T	A	G	A	G	C	G	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 02 COLH	A	C	T	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 03 COLH	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 04 COLH	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 05 COLH	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 06 CrustD	A	C	T	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 07 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 08 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 09 COI-III	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 10 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 11 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 12 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 13 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 14 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 15 Folmer	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 16 CrustD	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 17 CrustD	A	C	T	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 18 CrustD	A	C	T	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 19 Folmer	A	C	T	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 20 COLH	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 01 COI-III	A	T	C	T	C	G	C	C	G	C	A	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 02 COI-III	A	C	C	T	T	A	G	A	G	A	C	T	C	C	A	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 03 COI-III	C	T	C	G	C	G	C	G	C	A	C	T	C	C	G	T	C	T	T	C	T	T	C	C	A	G	C	T	C
<i>G. rhombooides</i> 04 COI-III	A	T	C	G	C	G	C	G	C	A	C	T	C	C	G	T	C	T	T	C	T	T	C	C	A	G	T	T	C
<i>G. rhombooides</i> 05 COI-III	A	T	C	G	C	G	C	G	C	A	C	T	C	C	G	T	C	T	T	C	T	T	C	C	A	G	C	T	C

A, C, T, G represent the four nucleotides composition of COI with 504 bp long. A bold are highlighted five transversion mutations and * represents the non synonymous mutations.



Figure 2.2.8: Amino acid variation among five questionable *nutilus* (G. rhom 01 to 05 COI-III), one representative of our COI sequences (G. rhom 01 COILH) and NC_06891 as orthologous COI reference. Positions with amino acid variations are marked with bars. Symbol “*” highlight variation between NC_06891 and the reference G. rhom 01 COILH

2.2.4 Discussion

Initially Bensasson *et al.* (2001) demonstrated that *numts* are a common phenomenon in Metazoa and simultaneously Williams and Knowlton (2001) confirmed that they are often present in more than one copy in Decapoda taxa. Our results strongly suggested that five sequences of *G. rhomboides* 01 to 05 COI-III are potentially *numts* when amplified with M13-tailed cocktail primers which can be identified by phylogenetic analyses, by the presence of nucleotide composition (point mutations) and amino acid intraspecific diversity among the same group of individuals amplified twice with different set of primers.

Erroneous taxonomic inferences

“However, we do not discount that a detailed molecular analysis might cast new ideas on the identity of both Atlantic and Mediterranean populations”, (Guinot and Castro, 2007).

Even for Guinot and Castro the idea of the existence of two species of *Goneplax* in the Atlantic Ocean and Mediterranean Sea was possible. It is true that based on morphological characteristics there were no doubts about the identification of the specimens analysed in this study. The next step was to test, from a molecular systematic perspective, the validation of morphological identification with other two genes. Universal decapods primers for COI amplification developed by Schubart (2009) were choose to accept or not the hypotheses of having *numts* in COI sequences data. Once again barcoding concept has been an important tool in clarifying systematic issues highlighting the importance of using multiple sources of evidence for robust systematic

assessment (e.g. barcode, morphological and biogeographical data) but also to have corresponding voucher numbers of the stored specimens (Pleijel *et al.*, 2008). The latter always facilitates the confirmation of previous identification and/or allows the collection of new tissue for DNA extraction.

Chromatograms of *Numts*

Despite the fact that most coamplified *numts* can be easily considered non-functional because of the presence of in-frame stop codons, and the generation of difficult to score chromatograms, in this study these assertions were not applicable. Some *numts* have no indels, no in-frame stop codons, and a highly similar AT% to the orthologs (Song *et al.*, 2008). It is possible of course that in-frame stop codons and indels may lie downstream beyond the region amplified by the universal primers, but on average, a randomly mutating stretch of DNA would expect to generate stop codons every 64 bases (4^3). Alternative hypotheses to explain these apparent mitochondrial copies are that the individuals are actually heteroplasmic, or that some error or contamination took place in the laboratory (Brower, 2006). Phylogeny, nucleotide composition and amino acid variability showed to be an effective filter for identifying potential *numts*. Using Sequin in the NCBI database (<http://www.ncbi.nlm.nih.gov/Sequin/index.html>) the questionable sequences were marked as “genomic DNA” with a note of “*numt*” under the source section of “FEATURES” (Buhay, 2009).

Primers

The best way to avoid pseudogenes is most likely to use optimized primers (Schubart, 2009). Schubart (2009) reported that the occurrence of *numts* strongly

decrease when using taxon-specific primers and he had designed a sensitive number of specific primers for a number of selected decapod species but also “universal decapods primers” used here in this study, forward COL6 (Schubart, 2009) and reverse COH6 (Schubart and Huber, 2006) primers, respectively (for review see Table 4 in Schubart 2009). He had demonstrated that the Folmer *et al* (1994) primers are not optimized for decapods due the fact that decapod sequences show variability at the third and sixth position from the 3'end of forward and reverse primer sequences. The COL6/COH6 (COLH) primers were tested after the use of the Folmer and cocktail primers (COI-III) and in this case we confirm the added suitability of COLH primers for decapod species (as denoted by the high success of COI amplification with COLH for number of diverse species that we were unable to amplify with the Folmer and COI-III primers set).

Phylogeny analyses

The COI and *numts* sequences were amplified with different primer sets and some of the sequences exhibit over 2% divergence, indicative in certain instances of two distinct species lineages (Hebert *et al.*, 2004). In such cases a typical and good quality barcoding study with additional morphological and biogeographically studies could suggest the presence of two species as it was initially suggested (see review of species status of *G. rhomboides* in Introduction).

It appears that *numts* sequences are mainly basal and often represent sister lineages to functional COI sequences (Buhay, 2009; Schubart, 2009). The BI tree showed (Figure 2.2.7) that *G. rhomboides* 02 COI-III could invoke questions regarding the systematic placing of the sequence, because it exhibits similar nucleotide homology with orthologous COI sequences, but high amino acid diversity (Figure 2.2.8). In the same tree three *numt* sequences cluster together with 100% posterior probabilistic BI value.

The tree is unable to delineate the wider systematic implications of the evolution of the numts, because we lack a wider alignment of ancestral and derived taxa in the present study. The present putative *numts* have unusually high numbers of point mutations (n=30) suggesting that COI-III cocktail primers preferentially amplified *numts* because of their similarity with ancestral sequence or divergence within the specific primer regions of the orthologous decapods mtDNA (Song *et al.*, 2008).

Therefore, acquiring robust phylogenetic inference from the COI gene for a systematic and phylogenetic study is not as simple as aligning sequences from clean chromatograms. Additional markers tested confirmed that it is important to be very careful when analysing sequences derived from PCR products amplified with conserved primers. Our data suggest that in some taxa, conserved primers may be more efficient in amplifying *numts* than orthologous COI genes, burdening species delineation with systematic confusion (see for review Guinot and Castro 2007).

2.2.5 Conclusions

Careful sequence examination does not eliminate all incorrect inference examination because some *numts* as shown here can be of the expected length, possess no in-frame stop codons and are not readily detectable from the orthologous mtDNA. Despite the enormous success of the Ivanova *et al* (2007) primer-cocktail barcoding protocol (applied mostly in fish) our results show that M13-tailed primer cocktails should be used with caution. An examination of the nucleotide and amino acid composition, and application of phylogenetic methods for sequence analysis may serve

as an effective and economical filter for identifying divergent *numts*. Such approaches easily can detect different compositional biases/mutation rates between mtDNA and nuclear DNA in the face of clean, non-ambiguous chromatograms. This study confirms the idea that coamplification of *numts* is not only, even easily detected, a limitation of DNA barcoding but, left undetected, also has significant taxonomic implications.

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References

- Akaike H (1973) Information theory and an extension of maximum likelihood principle. In: *Second International Symposium on Information Theory* (ed. Petrov B.N and Csaki, F.), pp. 267-281. Akademiai Kiado, Budapest
- Balakirev ES, Ayala FJ (2003) Pseudogenes: Are they “junk” or functional DNA? *Annual Review of Genetics* **37**, 123-151.
- Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution* **16**, 314-321.
- Brower AVZ (2006) Problems with DNA barcodes for species delimitation: "ten species" of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). *Systematics and Biodiversity* **4**, 127-132.
- Buhay JE (2009) "COI-like" sequences are becoming problematic in molecular systematics and DNA barcoding studies. *Journal of Crustacean Biology* **20**, 96-110.

- Burns J, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PDN (2007) DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper Butterflies (*Hesperiidae*) can differ by only one to three nucleotides. *Journal of the Leptidopterists' Society* **61**, 138-153.
- Campbell CL, Thorsness PE (1998) Escape of mitochondrial DNA to the nucleus in *ymel* yeast is mediated by vacuolar-dependent turnover of abnormal mitochondrial compartments. *Journal of Cell Science* **111**, 2455-2464.
- Drummond AJ, Rambaut A (2007) A beast: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 1-8.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294-299.
- Gerstein M, Zheng D (2006) The real life of pseudogenes. *Scientific American* **295**, 48-55
- Guinot D, Castro P (2007) A new species of *Goneplax* Leach, 1814 (Crustacea, Decapoda, Brachyura, Goneplacidae) from the south Atlantic and the western limits of the Indo-West Pacific region, long confused with *G. rhomboides* (Linnaeus, 1758). *Zootaxa* **1577**, 17-31.
- Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal Molecular Evolution* **22**, 160-174.
- Hazkani-Covo E, Zeller RM, Martin W (2010) Molecular poltergeists: Mitochondrial DNA copies (*numts*) in sequenced nuclear genomes. *PLoS Genetics* **6**, e1000834.
- Hebert PDN, Ratnasingham S, deWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S96-S99.
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. *PLoS Biology* **2**, 1657-1663.
- Hirotsune S, Yoshida N, Chen A, Garrett L, Sugiyama F, Takahashi S, et al. (2003) An expressed pseudogene regulates the messenger-RNA stability of its homologous coding gene. *Nature* **423**, 91-96.
- Huelsenbeck JP, Ronquist F (2001) Mr Bayes:Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.
- Keller I, Bensasson D, Nichols RA (2007) Transition-transversion bias is not universal: A counter example from grasshopper pseudogenes. *PLoS Genetics* **3**, 185-191.
- Lafontaine I, Dujon B (2010) Origin and fate of pseudogenes in Hemiascomycetes: a comparative analysis. *BMC Genomics* **11**, 1-15.
- Nguyen TTT, Murphy NP, Austin CM (2002) Amplification of multiple copies of mitochondrial cytochrome *b* gene fragments in the Australian freshwater crayfish, *Cherax destructor* Clark (Parastacidae; Decapoda) *Animal Genetics* **33**, 304-308.
- Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice L, Grabowski G (1991) *The simple fool's guide to PCR. A collection of PCR protocols*, 2nd edn. University of Hawaii, Honolulu.
- Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander B, et al. (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* **48**, 369-371.

- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.
- Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. *Molecular Ecology Resources* **9**, 181-187.
- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Schubart CD, Cannicci S, Vannini M, Fratini S (2006) Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. *Journal of Zoological Systematics and Evolutionary Research* **44**, 193-199.
- Schubart CD, Huber MGJ (2006) Genetic comparations of German population of the stone crayfish, *Austropotamius torrentium* (Crustacea: Astacidae). *Bulletin Francais de la Peche et de la Pisciculture* **380-381**, 1019-1028.
- Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**, 13486-13491.
- Svensson O, Arvestad L, Lagergren J (2006) Genome-wide survey for biologically functional pseudogenes. *PLoS Computational Biology* **2**, 358-369.
- Taishaku H, Konishi K (1995) Zoeas of *Calappa* species with special reference to larval characters of the Family Calappidae (Crustacea, Brachyura). *Zoological Science* **12**, 649-654.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Thalmann O, Hebler J, Poinar HN, Paabo S, Vigilant L (2004) Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other great apes. *Molecular Ecology* **13**, 321-335.
- Vanin EF (1985) Processed pseudogenes: characteristics and evolution. *Annual Review of Genetics* **19**, 253-272.
- Williams ST, Knowlton N (2001) Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution* **18**, 1484-1493.
- Zhang D-X, Hewitt GM (1996) Nuclear integrations: challenges for mitochondria DNA markers. *Tree* **11**, 247-251.

Section 3. MOLECULAR EVOLUTION AND SHALLOW PHYLOGENIES

*“The time will come I believe, though
I shall not live it, when we shall have
fairly true genealogical trees of each
great Kingdom of nature.”*

Darwin (1857)



**CHAPTER 3.1. Systematic and evolutionary insights derived from
mtDNA COI barcode diversity in the Decapoda
(Crustacea: Malacostraca)**

Abstract

Decapods are the most recognizable of all crustaceans and comprise a dominant group of benthic invertebrates of the continental shelf and slope, including many species of economic importance. Of the 17635 morphologically described Decapoda species, only 5.4% are represented by COI barcode region sequences. It therefore remains a challenge to compile regional databases that identify and analyse the extent and patterns of decapod diversity throughout the world. We contributed 101 decapod species from the North East Atlantic, the Gulf of Cadiz and the Mediterranean Sea, of which 81 species represent novel COI records. Within the newly-generated dataset, 3.6% of the species barcodes conflicted with the assigned morphological taxonomic identification, highlighting both the apparent taxonomic ambiguity among certain groups, and the need for an accelerated and independent taxonomic approach. Using the combined COI barcode projects from the Barcode of Life Database, we provide the most comprehensive COI data set so far examined for the Order (1572 sequences of 528 species, 213 genera, and 67 families). Patterns within families show a general predicted molecular hierarchy, but the scale of divergence at each taxonomic level appears to vary extensively between families. The range values of mean K2P distance observed were: within species 0.285% to 1.375%, within genus 6.376% to 20.924% and within family 11.392% to 25.617%. Nucleotide composition varied greatly across decapods, ranging from 30.8 % to 49.4 % GC content. Decapod biological diversity was quantified by identifying putative cryptic species allowing a rapid assessment of taxon diversity in groups that have until now received limited morphological and

systematic examination. We highlight taxonomic groups or species with unusual nucleotide composition or evolutionary rates. Such data are relevant to strategies for conservation of existing decapod biodiversity, as well as elucidating the mechanisms and constraints shaping the patterns observed.

Keywords: COI barcode; Decapoda; nucleotide composition; diversity; evolution.

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3.1 Introduction

In recent decades, the loss of biodiversity has been recognized as a major global environmental problem, with much effort being targeted at biodiversity conservation (Blaxter, 2003; Butchart *et al.*, 2010; Wilson, 2003a,2003b,2003c). Yet, a major obstacle in studying the human impact on the biosphere is what has often been referred to as the ‘taxonomic impediment’: a lack of taxonomic expertise in many groups of living organisms (Minelli, 2003) and also the morphological variability associated with such phenotypic plasticity (Jong, 2004; Sánchez *et al.*, 2007) or dimorphism (Pérez-Barros *et al.*, 2008). Biodiversity assessments that are based primarily on morphological characters not only are labour intensive, but risk also under – or over-estimation of biodiversity (Lefébure *et al.*, 2006). To overcome such problems, a short, standardized 650 bp sequence of the cytochrome *c* oxidase subunit 1 (COI) mitochondrial DNA (mtDNA) has been proposed as a barcoding tool, or at least to confirm species delimitation for taxonomic, ecological and evolutionary studies (Costa and Carvalho, 2007; Ebach and Holdrege, 2005; Gregory, 2005; Hebert *et al.*, 2003a; Miller, 2007; Radulovici *et al.*, 2010; Schindel and Miller, 2005). The NCBI GenBank molecular database demonstrates that, amongst others (e.g. 16S, with >7000 entries), COI is one of the most frequently used genes (> 10 000 nucleotides entries) for ecological and evolutionary studies of Decapoda, and augmenting these records will enhance the comparative value of such standardised approaches. Specifically, COI as a barcoding tool helps to identify an organism based on DNA sequence variability and assignment to a certain species previously

described (Lefébure *et al.*, 2006). Also the DNA barcode sequences can be used as a DNA taxonomy tool to perform prediction and classification of potentially new species. Although the approach remains controversial, (Boero, 2010; Ebach and Holdrege, 2005; Hickerson *et al.*, 2006; Meier *et al.*, 2008; Roe and Sperling, 2007; Will *et al.*, 2005), barcoding datasets are rapidly accumulating as part of the worldwide campaign for inventories of global biodiversity (Hajibabaei *et al.*, 2007a; Hajibabaei *et al.*, 2007b; Hebert *et al.*, 2003a; Hebert *et al.*, 2003b; Meusnier *et al.*, 2008; Savolainen *et al.*, 2005). The impacts of DNA barcoding is extended well beyond biodiversity science. By assembling sequence information for a single gene region from all species, in contrast to the usual focus of large scale genomics projects which acquire sequence information for all genes in single taxa, DNA barcodes can provide a quick preview of recent evolutionary history (Min and Hickey, 2007). For example, data have revealed key features of the mitochondrial genome with implications on the role of selection, as well as highlighting taxonomic groups or species with unusual nucleotide composition or evolutionary rates (Clare *et al.*, 2008; Min and Hickey, 2007). The growing volume of barcode records has revealed that sequence variability within species is generally much lower than divergence among species, commonly referred to as the “barcoding gap”, a pattern that occurs in diverse lineages, suggesting a pervasive evolutionary process (Hebert *et al.*, 2003a; Hebert *et al.*, 2003b; Radulovici *et al.*, 2010). The barcode region is a genomic sentinel; shifts in the nucleotide composition of the barcode region in the animal kingdom closely mirror those in the rest of the mitochondrial genome. The classical pairwise distance method such as Neighbour Joining (NJ) based on Kimura 2-parameter distance (K2P) is currently the predominant approach used to analyse patterns of diversity with COI barcode region. It has been informative at the species-level discrimination across a

variety of groups from terrestrial, marine and freshwater environments (Costa *et al.*, 2007; Hajibabaei *et al.*, 2006; Puillandre *et al.*, 2009; Rock *et al.*, 2008; Smith *et al.*, 2008; Summerbell *et al.*, 2005). The accuracy of such results depends especially on the delineation between intraspecific variation and interspecific DNA sequence divergence, (Hebert *et al.*, 2004; Meyer and Paulay, 2005b). A threshold barcoding gap was proposed to define species boundaries of around 10 times the mean value for within species variation for the focal group (Casiraghi *et al.*, 2010; Frézal and Lebloids, 2008; Hebert *et al.*, 2004). More specifically, the proposed threshold value of 2% COI sequence divergence (Hebert *et al.*, 2003a), and 0.16 patristic distances for species delimitation in Crustacea (Lefébure *et al.*, 2006) may, however, be problematic in some cases (i.e., heteroplasmy, hybridization, incomplete lineage sorting, nuclear introgression of mtDNA (Bachtrog *et al.*, 2006; Casiraghi *et al.*, 2010; Frézal and Lebloids, 2008; Mallet, 2005; Mallet and Willmott, 2007)) because DNA barcoding follows the typological species approach and species are entities continue to evolve. To cope with such limitations, DNA barcode sequences have been analysed based on other species concepts (Casiraghi *et al.*, 2010), and referred to as Recognizable Taxonomic Units (Oliver and Beattie, 1993), or Molecular Operational Taxonomic Units (Floyd *et al.*, 2002).

Barcode sequences can be used to flag species whose mitochondrial genomes show unusual nucleotide composition and rates of amino acid change, thereby identifying lineages that merit more investigation. Other approaches of diversity assessment involve the examination of variation of nucleotide GC content across taxonomic groups to detect unusual variation in mitochondrial GC content (Clare *et al.*, 2008; Costa *et al.*, 2007; Min and Hickey, 2007). However, the question of the functional significance of this GC variation remains controversial. It is not clear if it

has adaptive significance, a by-product of neutral evolutionary processes or if it has actually any significant impact on the phenotype (Sémon *et al.*, 2005).

Decapods are the most recognizable of all crustaceans (De Grave *et al.*, 2009; Martin *et al.*, 2009), and include the “true” crabs (Brachyura), hermit crabs and their relatives (Anomura), shrimps (Dendrobranchiata, Caridea and Stenopodidea), and lobsters (Astacidae, Thalassinidea), among other lesser known groups (Martin *et al.*, 2009). Establishing a robust DNA barcoding framework for decapods is particularly relevant because the order contains over 17,000 species (De Grave *et al.*, 2009), some of which support seafood and marine industries worth billions of dollars each year to the global economy. Estimates by the Food and Agriculture Organization of the United Nations (FAO), indicated that landings of crustaceans represented about 7% of the total marine fish production in 2007, of which 83% were marine decapods.

Conservation and management of decapods have long been entirely focused on crustacean fisheries (Calado, 2006; Jones *et al.*, 2007), but they also form a dominant functional group of megabenthic invertebrates on the Atlantic continental shelf and slope (Cartes and Carrasson, 2007; García-Castrillo and Olaso, 1995; Macpherson and Duarte, 1991; Markle *et al.*, 1988; Olaso and Rodriguez-Marin, 1995), encompassing a wide range of trophic levels (Polunin *et al.*, 2001) and a variety of feeding habits (Cartes *et al.*, 2002). In view of their collective ecological importance and potential community interactions, the unambiguous delimitation of species becomes even more urgent.

Of the 17635 morphologically described freshwater and marine extant species (De Grave *et al.*, 2009), only 5.4% are represented by COI barcode region sequences. There is no global campaign yet to barcode crustaceans or decapods, as exists for other animal groups (e.g., fish, birds and lepidopterans). It therefore remains a

challenge to compile regional databases that enable analysis of the extent and patterns of decapod diversity throughout the world. Here, using the most comprehensive COI data set for decapods so far examined, we analyse patterns of COI variability partitioning within and among species, genera and families. The combined dataset includes GenBank published sequences, COI barcode projects from the Barcode of Life Database (BOLD), (Ratnasingham and Hebert, 2007) and new data generated herein (Table 3.1.1). Collectively, the combined dataset provide barcoding coverage for 1572 sequences of 528 species, 213 genera, and 67 families. Our molecular systematic assessment affords an opportunity to examine the utility of COI DNA barcodes for species recognition in a taxonomically complex and ecologically important group of organisms. We encompass in our study specimens with a range of different shapes (shrimp, lobsters, crayfish and crabs) and sizes (e.g., small crab (Porcellanidae: *Petrolisthes* spp) and big crab (Majidae: *Hyas* spp). Comprehensive biogeographic representation of species was achieved by including species from continental freshwater (e.g., Atyidae and Parastacidae family), brackish (e.g., Palaemonidae and Panopeidae) and marine realms with a high range of latitudinal distribution. On the basis of their latitudinal distribution, decapods from temperate or cold (e.g., Lithodidae: *Lithodes* spp) to tropical waters (e.g., Xiphocarididae: *Xiphocaris* spp) across a range of depth distribution (e.g., Galatheidae) were compared. Species with diverse ecological habits, including such sex reversal (e.g., Palaemonidae), association of shrimps (e.g., Palaemonidae) and crabs (e.g., Pagurus) with other animals and dispersal behaviour (e.g., Pandalidae and Portunidae) were also represented in our analysis. Despite the relatively small proportion of decapods that are considered here, the samples analysed collectively encompass the breadth of morphological and ecological diversity of the order.

Table 3.1.1: Combined data set derived from new data generated herein and publicly available DNA barcoding projects from the Barcode of Life Database.

Projects	Code	No. of sequences	Species	Citation
BOLD public projects title				
Genbank Crustacea Malac - Decapoda	GBCMD	894	349	GenBank
Genbank Crustacea Malac - Decapoda - Atyidae	GBCDA	85	23	GenBank
Genbank Crustacea Malac - Decapoda - Palaemonidae	GBCDP	89	39	GenBank
Genbank Crustacea Malac - Decapoda - Parastacidae	GBCPA	161	59	GenBank
Crustaceans of the St. Lawrence Gulf	WWGSL	130	30	Radulovici <i>et al</i> 2009
Decapods of Pacific and Atlantic	FCDPA	118	57	Costa <i>et al</i> 2007
Campaign Marine Life (MarBOL)				
Decapods of Norway, Svalbard, U.K (Scotland), U.K (Wales and England), Mediterranean Sea	JSDN; JSDSV; JSDSC; JSDUK; JSDME	159	52	This study
Campaign Portugal – Aquatic Life				
Decapods of Portugal (Hermes, Iipimar, IipimarX, Azores)	FCDPH; FCDOP; JSDPX; JSDAZ	270	82	This study

3.1 Material and Methods

Data sampling

We collected 516 decapods specimens from the North East of the Atlantic, the Gulf of Cadiz and the Mediterranean Sea between 2005 and 2008. The specimens encompassed 101 species in 74 genera from 42 families of the order Decapoda. Deep-water specimens were collected by the National Institute of Biological Resources (INRB-IPIMAR) with nets and by the IOC-UNESCO Training through Research programme and the EU funded project Hotspot Ecosystem Research on the Margins of European Seas (HERMES) with two dredges and three box-cores. Littoral specimens were collected at low tide using dip nets, baited traps and scuba diving. Samples were stored in 70% ethanol (2001-2005) and in 100% ethanol (2006-2008).

Morphological identifications were undertaken and confirmed by taxonomists. Scientific names followed the Integrated Taxonomic Information System (www.itis.gov). In most cases, the whole specimen was stored as a morphological voucher for future reference (see supplement Table S 3.1.1 in 3.1 Annex). For some large decapod species, only tissue (legs or abdominal muscle) was obtained for barcoding and the samples were stored as tissue vouchers, accompanied by photographs taken prior to DNA extraction. All details regarding taxonomy, vouchers and collection sites with geographical coordinates can be found in the Barcode of Life Data System website (BOLD, www.barcodinglife.org) under two campaigns, Marine Life (MarBOL) and Portugal – Aquatic Life (Table 3.1.1). In order to ensure adequate geographical coverage, multiple specimens (at least two per site) from different geographical areas of target species were examined.

Total genomic DNA was extracted from small amounts of tissue (1 mm³ muscle tissue or whole legs for small specimens) using the Chelex dry release (Hajibabaei *et al.*, 2005) or QIAGEN DNeasy tissue extraction kits (QIAGEN) for older or less well preserved samples. Prior to DNA extraction, the sample was washed overnight in 50 µl of QIAGEN Buffer AE (10 mM Tris-Cl; 0.5 mM EDTA; pH 9.0) in order to rehydrate the tissue. For the Chelex dry release extraction method tissue samples were added to 120 µl of a 10:2 mixture of Chelex buffer with Proteinase K (Sigma), incubated at 55°C for 8-12 hours and subsequently heated to 95°C for 20 minutes. The barcode region was amplified with alternative sets of primers depending on PCR reaction success. The primers used with forward direction were LCOI490 (Folmer *et al.*, 1994), CrustDF1 (Radulovici *et al.*, 2009), CrustF1 (Costa *et al.*, 2007), CrustF2 (Costa *et al.*, 2007), and COL6 (Schubart, 2009) and with the reverse primers HCO2198 (Folmer *et al.*, 1994); CrustDR1 (Radulovici *et*

al., 2009); CrustR2 (5'- GGT AGA ATT AGA ATA TAC ACT T – 3', designed within the context of the BOLD- FCDPA project), COH6 (Schubart, 2009). A cocktail of primers with M13 tails (Ivanova *et al.*, 2007) was used with two forward and two reverse primers LCOI490; CrustF1; HCO2198; and CrustR2. All PCRs were performed in a 25 µl volume containing 1 X PCR buffer, 3 mM MgCl₂, 0.1- 0.2 mM dNTP, 1U TAQ polymerase (Promega), 5 – 10 pmol of each primer, and 2 - 10 ng of DNA template. The thermal cycling conditions consisted of 94°C for 60 s; 35-40 cycles of 94°C for 30 s, 48 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. Alternative thermal cycling conditions was consisted of 94°C for 60 s; 5 cycles of 94°C for 30 s, 45°C for 90 s, and 72°C for 60 s; 35 cycles of 94°C for 30 s, 50 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. The thermal cycling was identical for all primer except the CrustF2/HCO primer set, which was as follows: one cycle of 94°C for 60 s; 35 cycles of 94°C for 30 s, 42°C for 90 s, and 72°C for 60 s; followed by a final extension of 5 min at 72°C. PCR products were visualized on precast 1% agarose gels using the E-gel 96 system (Invitrogen). Prior to sequencing 15 µl PCR products were cleaned with 1U shrimp alkaline phosphatase (Promega) to dephosphorylate residual deoxynucleotides and 0.5 U Exonuclease I (Promega) to degrade excess primers (Werle *et al.*, 1994). The purification thermal conditions consisted of 37°C for 45 min and 80°C for 15 min. Bidirectional sequencing was performed using BigDye Termination chemistry on an Applied Biosystems® 3730 sequencer by Macrogen Inc. (www.macrogen.com, South Korea). Sequences were manually checked for ambiguities and assembled in CodonCode Aligner version 1.3.0 (<http://www.codoncode.com/>). Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) implemented in MEGA4 (Tamura K, 2007) and the amino

acid translation was examined to ensure that no gaps or stop codons were present in the alignment. BLAST searches were performed for all sequences via interrogating GenBank's online nucleotide database using the megablast algorithm.

Genbank data set

To provide a comprehensive sister-species coverage and survey of intraspecific variation, our data set was complemented by COI sequences from GenBank, as available on 4th June 2009. Additional sequences were included from the Barcode of Life Data Systems website (<http://www.barcdoingslife.org/>, as accessed on 4th June 2009). The BOLD platform allows us in our Project List page to have access not just to our full list of personal projects, but also all publicly accessible projects on BOLD, e.g., GenBank Animals (COI) and MarBOL compains. The BOLD system archives sequences located in COI barcode region from samples identified only to genus and species level being less than half of the COI entries in NCBI GenBank database. Sequences were omitted in our study if they were not allocated to a species, were from taxa with multiple denominations or taxonomic ranks, and suspected of being derived from misidentified, mislabelled species or putative pseudogenes (when found intraspecific distances > 10%, aberrant nucleotide composition, unusually long branches in our NJ tree and nonsensical systematic relationships (Buhay, 2009; Song *et al.*, 2008)), exhibited stop codons or indels, were less than 500 bp in length within the COI barcode region and finally sequences that were not reported in scientific journals to avoid potential misidentifications that could possibly be derived from GenBank (Harris, 2003), we submitted these to a rigorous quality control. From public projects (Ratnasingham and Hebert, 2007) we downloaded 5052 comprising 856 species from 249 genera and 83 families only 3187 COI barcode region

sequences were selected from 520 species, 178 genera and 53 families with sufficient length and quality according to our stringent criteria.

Combined data set: sequence selection and data validation

Two main factors may bias divergence assessments. First, disequilibrium in the representation of some taxa could skew divergence distributions. Here we standardized taxon comparisons to maximum of 10 individuals per species (Matz and Nielsen, 2005a) randomly selected reducing to 1906 sequences from 603 species, 225 genera and 68 families were included in the total data analyses. To test how patterns of genetic divergence at COI correspond to morphological species concepts, species diversity was estimated based on the similarity and clustering pattern in their COI barcodes independent of taxonomic assignments. A threshold of 2% sequence divergence was employed to draw boundaries for barcode haplotype clusters. This arbitrary threshold was selected based on the observation that intraspecific divergences observed in a variety of groups rarely exceed this value (Ebach and Holdrege, 2005; Gregory, 2005; Hebert *et al.*, 2003a; Miller, 2007; Schindel and Miller, 2005).

Secondly, the taxonomic classification may be incorrect or uncertain. Most common problems will result from cryptic species, and paraphyletic or polyphyletic taxa (Lefébure *et al.*, 2006; Meyer and Paulay, 2005a). All sequences were aligned and a Neighbour Joining tree produced using BOLD platform. We identified, in this tree, all sequences clustering far from their known taxonomic or phylogenetic position, and removed the non-monophyletic, putative cryptic species and congeneric species with distance values lower than 2% evaluated from the literature. After such selective removal, we proceeded to analyse 1572 sequences from 528 species, 213

genera, and 67 families. Additionally we tested the possible artefact attributable to biased species representation by computing within mean species divergence and the influence of presumably non-monophyletic taxa on the divergence distribution. An assumption-free statistical test was proposed by Lefébure *et al.*, (2006) to directly measure the overlap between raw data (highly represented taxa have more impact than weakly represented ones) and a second set of data where each taxa was given the same weight by computing mean divergence through distance values. Comparing the frequency of intraspecific distances values (< 3%) between the raw data and the mean data will indicate whether or not the divergence assessment is a result of a strong disequilibrium in the representation of some taxa. The divergence distribution was tested within species diversity between the initially dataset with 1906 sequences (case A) and the validate data set with 1572 sequences (case B). To obtain the first statistical indication of the overlap between divergence distributions, Mann-Whitney U Test were performed (Figure 3.1.1) among (Lefébure *et al.*, 2006): raw data A_R vs B_R , mean data A_M vs B_M ; and between different data A_R vs A_M and B_R vs B_M with the SPSS software version 16.0.2 (Levesque, 2008).

Decapoda diversity assessment

The diversity assessments for the decapods and for the most represented families were analysed from the data set with 1572 sequences from 528 species, 213 genera, and 67 families (B_R). For statistical purposes only, families containing at least 50 sequences (Lefébure *et al.*, 2006; Meyer and Paulay, 2005a) with more than 5 species were compared (Lefébure *et al.*, 2006). Nucleotide divergences of COI and variation in GC content were analysed between the 11 most representative families (Table 3.1.2).

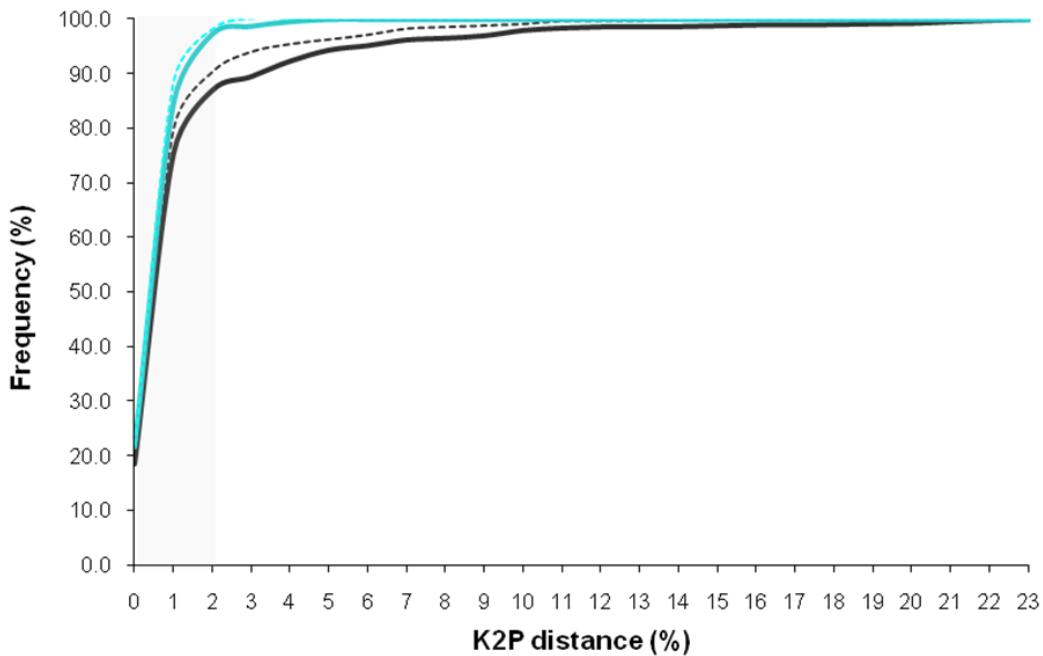


Figure 3.1.1: Intraspecific diversity assessment: the effect of sampling bias, non-monophyletic clades, putative cryptic species and congeneric species with low genetic distance. Solid lines represent the raw data for the total data set (A_R , black lines) and for the dataset in which non-monophyletic clades, putative cryptic species and congeneric species with low genetic distance were removed (B_R , blue lines). The dashed lines represent results for the data in which all taxa have the same weight (mean values of genetic distance), for the total (black, A_M) and trimmed (blue, B_M) datasets respectively.

The K2P has become the metric most widely used in barcoding studies and is deployed here. Genetic distances between specimens were calculated for each intraspecies (S), intragenus (G) and intrafamily (F) with the ‘Distance Summary’ command implemented by BOLD. Although distance distributions within families are not independent from each other, we performed the Kruskal–Wallis one-way analysis of variance between S, G, and F distributions to obtain a first statistical indication of the overlap between divergence distributions with GenStat (Payne, 2009).

In order to investigate the sensitivity of results to variations in matrices distances methods, Patristic distances were computed using the program PATRISTIC (Fourment and Gibbs, 2006).

Our second line of investigation examined the diversity in GC content across multiple taxonomic groups. To ensure homology with the BOLD data (because the sequences are heterogeneous in length), all sequences (1572) were trimmed to 500 bases and GC content and nucleotide composition were calculated for 11 families using MEGA 4 (Tamura K, 2007).

3.1 Results

Data acquisition: new sequencing

Here we created new COI sequences of 497 specimens from a total of 101 species, 72 genera and 46 families (Table S 3.1.1), of which 81 species, 48 genera and 13 families are exclusive of our generated data. The number of sequences per species varied between 1-32, with a mean of 5, and an average length of 620 base pairs (bp). Within this newly-generated dataset, 3.6% of the species barcodes conflicted with the assigned morphological taxonomic identification. Such cases were distributed throughout the Decapoda, including the long legged crabs *Macropodia longipes* (A. Milne Edwards & Bouvier, 1899) and *M. tenuirostris* (Leach, 1814) (Brachyura:Majidae) and the marbled rock crabs *Pachygrapsus maurus* (Lucas, 1846) and *P. marmoratus* (Fabricius, 1787) (Brachyura:Grapsidae) are represented by two “mixed” clades in a NJ tree (see supplement Figure S 3.1.1 in 3.1 Annex).

Data validation

From a theoretical point of view, two main factors may bias our intraspecific assessment of COI divergence: disequilibrium in the representation of some taxa or incorrect taxonomic classification (i.e., cryptic (morphological indistinguishable, but genetically distinct), or non-monophyletic species). The analysis of the combined GenBank and our novel data (Table S 3.1.2) indicated the existence of sample bias ($p < 0.05$) as shown in Figure 3.1.1 (case A_R , for the entire symbols definition see Methods section). However when putative cryptic (16 species), non-monophyletic (47 species) and con-generic species with unusually low genetic distance (13 species under 2% K2P) were removed from the dataset (case B_R) (Table S3), the sample bias was lost ($p > 0.05$). Assuming a intraspecific barcode threshold of maximum 2% (K2P), the success of achieving congruent species assignments (Table S 3.1.4) was 97.3 % and 98% when mean intraspecific divergences values were compared (in case B_R vs B_M , Figure 3.1.1).

In order to reduce the impact of artefacts in our divergence assessment, statistical tests were performed among: raw data A_R vs B_R , mean data A_M vs B_M and between different data A_R vs A_M and B_R vs B_M proposed by Lefébure *et al* (2006). The first three comparisons revealed sampling bias due to incorrect taxonomic classification and non-monophyletic taxa ($p > 0.05$) and B_R vs B_M showed no sample bias ($p < 0.05$) indicating a balanced design.

COI divergence assessment

COI barcode nucleotide divergences were calculated for the validated dataset from 1572 sequences of 528 species, 213 genera, and 67 families (B_R) (Table S 3.1.4) to reduce the impact of artefacts in our divergence assessment. Sample sizes and

mean divergences at various taxonomic levels are given in Table 3.1.2. As expected, genetic divergence increased with higher taxonomic rank: 0% to 4.6% within species, 2.5% to 32.7% within genera, and 6.6% to 48.3% within families. Although these ranges overlap, intraspecific (S), intragenus (G) and intrafamily (F) distances (Figures 3.1.2 and 3.1.3), were significantly different ($p < 0.001$). Patterns within families (Table 3.1.3 and Figure 3.1.3) show a general predicted molecular hierarchy, but the scale of divergence at each taxonomic level appears to vary extensively between families. The range values of mean K2P distance observed were: within species 0.285% to 1.375%, within genus 6.376% to 20.924% and within family 11.392% to 25.617%. The Galatheidae showed the lowest divergence within species (0.285 %), and Lithodidae showed the lowest divergence within genus (6.376%) and within family (11.392%) distances: the highest values were observed within the Pandalidae (within genus: 20.924%) and Parastacidae (within species: 1.375% and within family: 25.617%). The Crangonidae showed the highest range of divergences within a family, the Pandalidae within genus and Parastacidae within species (Figure 3.1.3). No sample bias was detected in the within family analysis ($p > 0.05$). The Parastacidae was the only family exhibiting sample bias ($p < 0.05$), arising from the unbalanced distribution of data with 53% of the sequences being derived from the *Euastacus*, and 21% from the *Cherax* genera.

The majority (97%) of mean distance values within species were less than 2%, though the scale of divergence appears to vary extensively between species: all 10 specimens of *Goneplax rhomboides* (Linnaeus, 1758) (Brachyura: Goneplacidae) share the same haplotype, and *Cherax preissii* (Erichson, 1846) (Astacidea: Parastacidae) exhibited the highest mean intraspecific value of 2.61 ± 0.193 K2P distance.

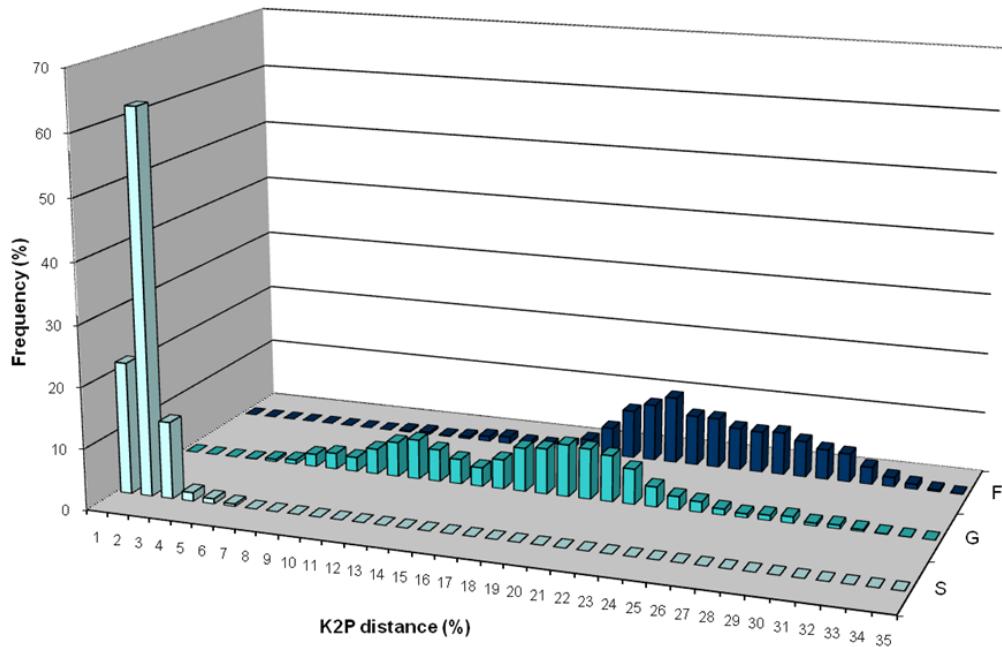


Figure 3.1.2: Frequency distribution of COI K2P distances (%) intraspecies (S), intragenus (G), and intrafamily (F) from 302 species, 154 genera, and 58 families.

Table 3.1.2: Pairwise COI barcode nucleotide divergences for the Decapoda using K2P distances (%).

Decapoda ^a (1572 seq., 528 sp., 213 gen., 67 fam.)	No. of comparisons	Min Dist	Mean Dist ^b	Max Dist
Intraspecies	3577	0	0.541±0.01	4.605
Intragenus	18077	2.509	15.49±0.04	32.75
Intrafamily	35422	6.694	22.325±0.023	48.348
Intraorder	1176159	8.509	26.07±0.003	54.994

^aNumber of sequences, species (sp), genera (gen) and families (fam) are shown in parentheses

^bData reported as K2P distances (%) ± SE.

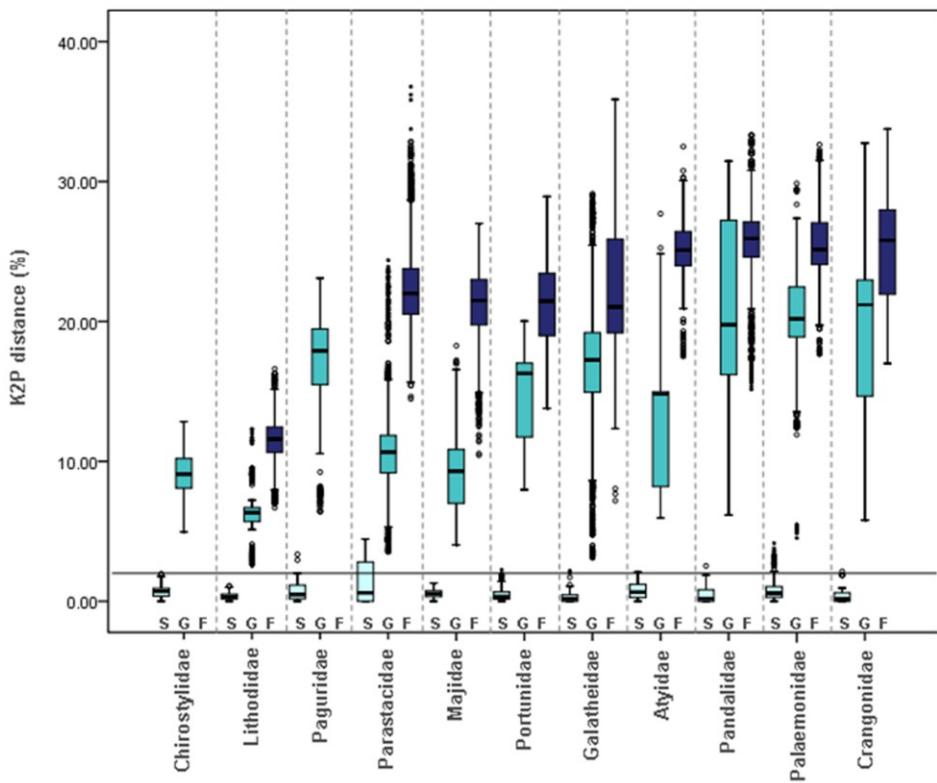


Figure 3.1.3: Boxplot distribution of 11 selected families of the Decapoda order intraspecies (S), intragenus (G), and intrafamily (F) COI K2P distances (%). The plot summarises median (central bar), position of the upper and lower quartiles (called Q1 and Q3, central box), extremes of the data (dots) and very extreme points of the distribution that can be considered as outliers (stars). Points are considered as outliers when they exceed $Q3 + 1.5(Q3-Q1)$ for the lower part, where $(Q3-Q1)$ is the inter quartile range. The number of sequences, species, and genera per family are given in Table 3.1.3. Mean K2P distance (%) \pm SE within taxa are: Chirostylidae S=0.701 \pm 0.028 and G=8.999 \pm 0.039; Lithodidae S=0.416 \pm 0.021, G=6.376 \pm 0.137 and F=11.392 \pm 0.063; Paguridae S=0.686 \pm 0.045 and G=17.173 \pm 0.084; Parastacidae S=1.375 \pm 0.131, G=11.017 \pm 0.078 and F=22.681 \pm 0.064; Majidae S=0.547 \pm 0.028, G=9.643 \pm 0.214 and F=21.084 \pm 0.061; Portunidae S=0.453 \pm 0.024, G=14.826 \pm 0.311 and F=28.929 \pm 0.047; Galatheidae S=0.285 \pm 0.017, G=16.839 \pm 0.04 and F=22.355 \pm 0.033; Atyidae S=0.758 \pm 0.041, G=13.475 \pm 0.352 and F=25.218 \pm 0.056; Pandalidae S=0.49 \pm 0.042, G=20.924 \pm 0.213 and F=25.617 \pm 0.07; Palaemonidae S=0.812 \pm 0.055, G=20.157 \pm 0.108 and F=25.398 \pm 0.048; Crangonidae S=0.344, G=19.991 \pm 0.514 and F=25.241 \pm 0.103.

Table 3.1.3: Number of Decapoda sequences, species, genera and families analyzed in the present study.

Family	Species	Genus	Sequences
Atyidae	16	9	59
Chirostylidae	13	1	66
Crangonidae	16	7	58
Galatheidae	84	10	220
Lithodidae	12	6	52
Majidae	24	14	67
Paguridae	11	1	57
Palaemonidae	32	5	87
Pandalidae	19	5	74
Parastacidae	43	8	98
Portunidae	22	10	90

Table 3.1.4: Variation of GC content in the COI barcode region and codon position among the Decapoda and from 11 selected families.

Taxon	Min.	Mean	Max.	Codon position		
				1st	2nd	3rd
Order						
Decapoda (1572 seq, 528 sp, 213 gen, 67 fam)	30.8	39.39 ± 0.085	49.4	50.90 ± 0.062	42.92 ± 0.021	21.73 ± 0.213
Families						
Atyidae (59 seq, 16 sp, 9 gen)	35.30	42.40 ± 0.346	47.00	52.76 ± 0.170	43.51 ± 0.099	30.83 ± 0.958
Chirostylidae (66 seq, 13 sp, 1 gen)	31.50	33.05 ± 0.139	35.60	49.54 ± 0.129	42.78 ± 0.508	6.81 ± 0.328
Crangonidae (58 seq, 16 sp, 7 gen)	34.60	39.33 ± 0.361	47.60	50.46 ± 0.227	43.17 ± 0.083	24.36 ± 0.904
Galatheidae (220 seq, 84 sp, 10 gen)	33.20	37.24 ± 0.169	45.50	50.56 ± 0.148	43.03 ± 0.037	18.11 ± 0.493
Lithodidae (52 seq, 12 sp, 6 gen)	34.40	36.31 ± 0.226	40.40	48.82 ± 0.107	43.76 ± 0.097	16.36 ± 0.621
Majidae (67 seq, 24 sp, 14 gen)	30.80	35.37 ± 0.253	38.70	48.29 ± 0.244	42.22 ± 0.066	15.61 ± 0.574
Paguridae (57 seq, 24 sp, 14 gen)	32.40	36.34 ± 0.197	41.00	49.80 ± 0.262	43.07 ± 0.051	16.14 ± 0.608
Palaemonidae (87 seq, 32 sp, 5 gen)	36.40	41.01 ± 0.404	48.60	52.70 ± 0.311	43.83 ± 0.094	26.51 ± 0.884
Pandalidae (74 seq, 19 sp, 5 gen)	35.20	41.93 ± 0.274	49.40	51.39 ± 0.163	43.32 ± 0.066	31.06 ± 0.740
Parastacidae (98 seq, 43 sp, 8 gen)	37.30	40.39 ± 0.186	48.60	51.25 ± 0.110	43.34 ± 0.063	26.53 ± 0.550
Portunidae (90 seq, 22 sp, 10 gen)	31.90	38.31 ± 0.334	44.20	50.44 ± 0.243	41.87 ± 0.056	22.60 ± 0.866

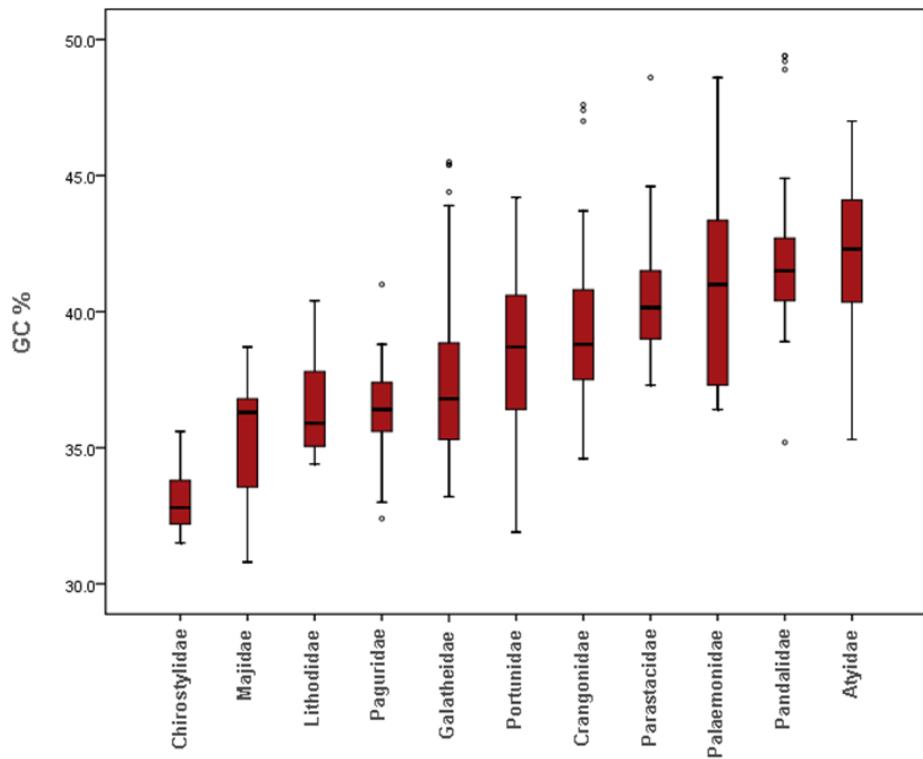


Figure 3.1.4: Boxplot distribution of ascending GC content (%) from 11 selected families. The number of sequences, species, and genera per family is indicated in Table 3.1.3 and statistic values in Table 3.1.4.

GC content divergence assessment

Our second line of inquiry involved assessing the GC content in diverse lineages as a measure of nucleotide diversity. The frequency of the occurrence of GC-content can be a useful metric for understanding species diversity and evolutionary processes (Romiguier *et al.*, 2010). Nucleotide composition varied greatly, ranging from 30.8 % to 49.4 % of GC content (Table 3.1.4). In all cases, GC content

decreased from the first to the third codon position with mean values of 50.90%, 42.92% to 21.73% respectively. The pattern of variance (standard error) confirmed that the highest range in GC content was observed in the third codon position: the second position displayed the least variation (Table 3.1.4). The proportion of nucleotides throughout 1572 sequences in case B_R was T=34.7%, C=20.1%, A=26.9%, and G=18.3%, respectively. Nucleotide bias did not occur at the first codon position (1st), though at the second codon position (2nd), there was marked bias in T and C, and favouring A against C at the third codon position (3rd). The average frequency (R) of transitional (A/C and C/T) and transversional (A/T; A/C; C/G; G/T) rates are: COI barcode region R= 1.02; for 1st codon R = 2.7, for 2nd codon R = 1 for and for 3rd codon position R = 0.8.

Our observations reveal considerable variation in the range of GC values within and among decapod families (Figure 3.1.4). Such variation leads to a zone of overlap covering even the most GC rich values in Pandalidae (49.4 % GC), and the lowest values in Chirostylidae (35.6% GC). The highest GC% content was observed in the Atyidae with a mean value of 42.40 ± 0.3465 , and the lowest in Chirostylidae of 33.05 ± 0.1392 (Figure 3.1.4), mostly reflecting a marked difference at the third codon base with 30.83 ± 0.9582 and 6.811 ± 0.3289 . All 11 families examined were significantly different ($p < 0.05$), but with considerable overlap (Figure 3.1.4). No sample bias effect was observed ($p > 0.05$), except for the Palaemonidae ($p < 0.05$), which also exhibited the highest standard error variation (SE) value.

3.1 Discussion

The COI gene appears to be an informative molecular marker at several taxonomic scales, but particularly at the species level. Our analysis shows a general increase in the molecular divergence of COI with taxonomic rank, a trend that suggests that morphological taxonomy is roughly in agreement with DNA evolution. Yet, this relationship is not entirely consistent, and the distribution of divergences at different taxonomic scales sometimes overlaps. The COI gene tree was used in this study to present our results and to allow comparison with previously defined species groups within decapods. However other genes and phylogenetic methods are required to evaluate the evolution information contained in the barcode region of COI (DeSalle *et al.*, 2005). It is worthwhile emphasizing that it was not within the scope here to generate new insights into decapods species evolutionary relationships, but rather to analyse patterns of COI variability among decapods.

New data acquisition

Our data further supports the validity of DNA barcoding for species identification in marine decapods. The ratio of within species to between species variation (21X) was much higher than the threshold (10X) proposed by Hebert *et al.*, (Hebert *et al.*, 2004) as a potential species' boundary. Therefore, assigning specimens to species was usually straightforward with no overlap between within species – and between species distance (95% of the cases).

COI divergence assessment

It has been discussed whether COI barcoding sequence variation will defer yield new insights into the evolutionary relationships among different taxonomic metazoan groups, once complete barcode data are available. Whereas each family apparently coincides with the expected molecular hierarchy, the scale of divergence at each taxonomic level appears to vary extensively between and within families.

The highest values of F (Figure 3.1.3) belong to families of infraorder Caridea (Atyidae, Pandalidae, Palaemonidae, Crangonidae), representative of the currently recognized natant lineages of the suborder Pleocymata (Porter *et al.*, 2005). Such high values of genetic distance reflect possibly the remarkable range of adaptation and biological diversity within the infraorder Caridea (Bracken *et al.*, 2009; De Grave *et al.*, 2008; De Grave *et al.*, 2009; Martin and Davis, 2001). Many caridean families inhabit both shallow and deep water marine environments (Martin and Davis, 2001), hydrothermal vents (Herring and Dixon, 1998), freshwater lakes and mountain streams (De Grave *et al.*, 2008), caves (Zakšek *et al.*, 2009), and commonly establish temporary or lifelong associations with other taxa (Marin *et al.*, 2005; Silliman *et al.*, 2003; Stevens and Anderson, 2000). The phylogeny of the infraorder Caridea based on mitochondrial and nuclear genes has suggested that the Caridea is monophyletic (Bracken *et al.*, 2009), underpinned by a possible radiation in the Triassic period (Porter *et al.*, 2005). Apparent polyphyletic and paraphyletic compositions of some Caridean families have, however, been reported by morphological and molecular studies (Bracken *et al.*, 2009). Also multi-locus genes, including both mitochondrial and nuclear genomes and additional taxa, will need to be analysed to provide informative characters to resolve the phylogeny among Caridean groups.

The economically important Lithodidae and Pandalidae exhibit markedly contrasting patterns of intrafamily divergence (Figure 3.1.3). The typically cold-water Lithodidae king crab comprises weakly divergent species, suggesting either that the family represents an extreme situation of rapid morphological diversification, and/or slow molecular evolution, reflecting a slow metabolism found in organisms that inhabit cold environments (Bucciarelli *et al.*, 2002; Childress, 1995), or possessing larger body sizes (Martin and Palumbi, 1993a; Ostrow *et al.*, 2007) or both (Gillooly and Allen, 2007; Gillooly *et al.*, 2005; Gillooly *et al.*, 2001). Moreover, distribution, and therefore opportunities for population differentiation, in these groups remains constrained by the stressful effects of temperature extremes on early life-history stages (Hall and Thatje, 2009). However, the phylogeny of the family Lithodidae is controversial (Tsang *et al.*, 2009; Tsang *et al.*, 2008), and molecular, and adult and larval morphological data remain equivocal (Cunningham *et al.*, 1992; Zaklan, 2002).

The Oregoniidae also exhibits very low mean divergences within taxa ($S=0.66\%$; $G=5.56\%$; $F=12.96\%$), here represented by five deep water species from two genera. Nucleotide substitution rate is the ultimate source of genetic variation and it is the substrate for molecular evolution. The metabolic rate hypothesis (Martin *et al.*, 1992; Martin and Palumbi, 1993b) has been proposed to explain mtDNA substitution rate variations in animals. Correlation between metabolic rate and nucleotide substitution may be mediated by (i) the mutagenic effects of oxygen radicals that are abundant by-products of aerobic respiration, and (ii) increased rates of DNA synthesis and nucleotide replacement in organisms with higher metabolic rates (Martin and Palumbi, 1993b). The general hypothesis assumes that deep-sea animals exhibit hypo-metabolism (Company and Sardà, 1998; Seibel and Childress,

2000; Seibel *et al.*, 1997), which is characterised by abnormally low level metabolic rates. The theory holds that limited light with depth reduces visual predation pressure and selects for reduced locomotory ability and metabolic capacity (Childress *et al.*, 1990). Although this theory applies predominantly to pelagic animals, deep-sea benthic animals (including crustaceans) exhibit metabolic rates also typically an order of magnitude lower than their shallow-water counterparts (Childress, 1995; Childress *et al.*, 1990). While this phenomenon in deep-sea benthic crustaceans may simply be a function of very low temperatures at depth in areas of steep thermal gradients (Childress *et al.*, 1990), reduced metabolic rates observed in deep-sea benthic crustaceans may still be ecologically relevant to their rate of molecular evolution.

The Pandalidae is one of the most species-rich families due to extensive diversification in the genus *Plesionika*. Our data set showed the highest nucleotide divergences within the genus, represented by the genera *Plesionika*, *Pandalus*, *Pandalopsis* and the monospecific *Dichelopandalus* and *Stylopandalus*. The genus *Pandalus* (Leach, 1814) is retained as a possible paraphyletic group (Komai, 1999), and the phylogeny of *Pandalopsis* remains to be described. The phylogenetic relationship between members of the genus *Plesionika* is still to be established and in spite of recent taxonomic revisions (Chan, 2004; Chan and Yu, 1991, 1998), our data endorse the need for additional effort.

Taxonomic classification

One of the factors that may bias our divergence assessment is the possibility of incorrect or uncertain taxonomic classification. The COI barcodes grouped together the two spider crab specimens (0% distance) *Macropodia longipes* and *M. tenuirostris* (Leach, 1814). Such genetic similarity, if generally supported,

emphasizes the idea that these two species should be considered as one based on similar morphological characteristics of adults and larval stages (d'Udekem d'Acoz, 1999; Garcia-Raso, 1987; Gonzalez-Gorillo and Rodriguez, 2001; Guerao and Abelló, 1997; Noël, 1992). Combined data presented herein suggests that *M. longipes* is in fact a synonym of *M. tenuirostris*.

Low divergence levels were observed (0.065%) also between *Pachygrapsus maurus* and *P. marmoratus*. *Pachygrapsus marmoratus* can be distinguished from related species *P. maurus* by the presence of two lateral post-orbital teeth, whereas *P. maurus* possesses one (Zariquiey-Alvarez, 1968). *Pachygrapsus marmoratus* and *P. maurus* are considered sister species and are genetically clearly distinct to other species of the genus (Cuesta and Rodríguez, 2000).

Ecologically, these two species share the same rocky intertidal area and were collected from Flores Island in the Azores Archipelago. Our data might indicate hybridization or a misidentification. In our study *P. maurus* was represented by two juvenile specimens, and in spite of the evident differences in adult morphology (Schubart and Cuesta, 1996) the diagnostic features can be hard to distinguish in juvenile specimens. Further molecular (e.g. AFLP or microsatellites (Arif and Hkhan, 2009)) and morphological analyses should be combined to identify species and between species hybrids within the *Pachygrapsus* species.

Cryptic and young species

For decapods, COI resolves relationships among the more closely related species within genus, and can be used to address the question of whether species groups based on morphological, ecological and biogeographical characters represent evolutionary lineages. The described levels of intraspecific variation must be

considered preliminary, since several species were characterized based on only up to ten specimens – sufficient for a valid barcode, but not sufficient to accurately capture genetic diversity of the species. However pairwise sequence differences derived from 10 specimens per species reflected differences in the range of diversity. DNA sequences for additional specimens collected across the geographic ranges of additional species are needed to test and validate this result. In some cases, higher levels of intraspecific variation may reflect underlying population structure. For example, the freshwater crayfish *Cherax preissii* (Erichson, 1846) (Astacidea: Parastacidae) showed highest divergence values with a maximum of 4.45% genetic distance (0.5 patristic distance) between two main populations from the North and South of Australia. A recent systematic study of the genus *Cherax* suggested that the taxonomy of *C. preissii* should be re-examined [88], even if the diversity between Australian populations reveals evidence of contemporary, but not ongoing, gene flow during pluvial Pleistocene periods [89]. However, extensive cryptic species have been documented in freshwater crayfish taxa, concurring with the increased discovery of diversification in freshwater taxa (Apte *et al.*, 2007; Gouws *et al.*, 2006; Liu *et al.*, 2007; Marin *et al.*, 2005; Munasinghe *et al.*, 2003; Shih *et al.*, 2007). Another example is the species *Macrobrachium nipponense* (De Haan, 1849) (Caridea: Palaemonidae) with a maximum distance of 4.15% (0.4 patristic distance). The genus *Macrobrachium* has more than 100 species described, distributed exclusively in freshwater and brackish habitats (except *M. intermedium* (Stimpson, 1860)) (Murphy and Austin, 2004; Williamson, 1972). The species of this genus exhibits significant intra-population and intra-individual variation in egg size (Mashiko and Numachi, 2000) and larval characters (Alekhnovich and Kulesh, 2001). *Macrobrachium nipponense* exhibits high tolerance of variation in water parameters, having the ability

to change in three generations to full freshwater (Wong and McAndrew, 1990), and together with its popularity in the aquarium trade, renders it an effective invasive species (De Grave and Ghane, 2006; Salman *et al.*, 2006). Taxonomic complexity is associated with morphological plasticity of taxonomically important (e.g., the rostrum and/or the second periopod) changes in relation to growth (Holthuis, 1950) and environmental variation (Dimmock *et al.*, 2004). The morphological characters are extremely conservative and molecular systematic data from the genus *Macrobrachium* suggests that the uses of traditional morphological characters and molecular data are essential to diagnose accurately natural species groups (Liu *et al.*, 2007).

It seems likely that cryptic species will be discovered among geographically widespread decapods species. Here, two shrimp species *Palaemon elegans* (Rathke, 1837) and *Pasiphaea tarda* (Kreyer, 1845) from the Northeast Atlantic Ocean showed non-monophyletic patterns when compared with their con-specifics from other oceanographic regions. For the first example, *P. elegans*, the mean distance within species was 5.296% (0.530 patristic distances). Previously, three morphological types for the cosmopolitan species *P. elegans*, have been suggested (see for review (d'Udekem d'Acoz, 1999)), supported by high allozymic divergence within the Mediterranean Sea (Fortunato and Sbordoni, 1998). This species is adapted to extremely variable salinities, temperatures and oxygen (Kirkpatrick and Jones, 1985; Taylor and Spicer, 1987). A surprisingly complex population structure within *P. elegans* has been recently discovered comprising three haplogroups (Reuschel *et al.*, 2010) from: Atlantic and Alboran Sea, Mediterranean Sea and the Black Sea, Caspian and Baltic Sea. The Baltic Sea population revealed high levels of nucleotide divergence suggesting the existence of a cryptic species that originated in the late

Miocene period when ancestral Baltic populations of *P. elegans* were isolated from Atlantic populations (Reuschel *et al.*, 2010) [42]. It is likely, however, that the occurrence of this species in the Baltic Sea represents an introduced invasive species rather than an effect of natural expansion (Grabowski, 2006; Reuschel *et al.*, 2010). Based on such a scenario, it is possible that specimens from the Baltic Sea from Costa *et al.* (Costa *et al.*, 2007) represent a cryptic species, or that hybridization is taking place between *P. elegans* and *P. intermedius* (Reuschel *et al.*, 2010). Interestingly we had only found difference in one amino acid positions between Northeast Atlantic Ocean and Baltic populations. Although this difference cannot be considered as indicating species separation, it does suggest the need for a re-examination of specimens (Chang *et al.*, 2009).

Most marine species the preponderance of pelagic larval stages and the absence of obvious distribution barriers suggests a high level of gene flow with populations predicted to be genetically homogeneous (Palumbi, 2003). However high levels of genetic differentiation between populations over small spatial scales were described (Baratti *et al.*, 2005; Mathews, 2007) suggesting that marine ecosystems may not be as interconnected as they seem (Bierne *et al.*, 2003; Mathews and Anker, 2009). *Pasiphae tarda* revealed a maximum intraspecific distance of 4.913 % (0.509 patristic distance). In relation to the data presented here for the cosmopolitan species *P. tarda*, it is possible that limited larval dispersal/gene flow is associated with deep genetic breaks between populations between the North Pacific Ocean and the North Atlantic. Several comparative phylogeography studies in marine taxa, including corals, decapods and bryozoans, have suggested various ages of the genetic discontinuities, ranging from the Miocene to the Pliocene during episodic marine regressions (Barber *et al.*, 2006; Cuesta and Schubart, 1998; Gomez *et al.*, 2007; Harrison, 2004; Pfeiler

et al., 2005). These authors showed concordance of genetic structure across multiple taxa combined with temporal discordance suggesting that regional genetic structures have arisen from common physical processes operating over extended time periods. The presence of intraspecific genetic structure, as well as deeply divergent lineages, strongly suggests that such overarching processes promote lineage diversification (Barber *et al.*, 2006; Cuesta and Schubart, 1998; Gomez *et al.*, 2007; Harrison, 2004; Pfeiler *et al.*, 2005).

The presence of intraspecific genetic structure is furthermore supported by high amino acid diversity within species showing variation in four amino acid positions between Pacific and Atlantic populations.

Whether *C. preissii*, *M. nipponeuse*, *P. elegans* and *P. tarda* exhibit taxonomically significant geographic variation and/ or comprise cryptic species should be reviewed with additional morphological, as well as population genetic and molecular systematic studies with multi-locus genes. Based on the taxonomic incongruence identified here, such approaches can explore further the levels of cryptic speciation and reproductive isolation across putative species (Brökeland and Raupach, 2008).

The utility of COI as a tool for rapid identification depends on the genetic variation among species exceeds intraspecific variation to such an extent that a clear “bacoording gap” exists. However, the gap might be absent in younger species (incomplete lineage sorting) and species with hybrid zones because of the insufficient variation to be determined as distinctly different using only barcodes (Meier *et al.*, 2006; Meyer and Paulay, 2005b). Our data further support the incomplete lineage sorting of the genus *Hyas* reported between *H. araneus* (Linnaeus, 1758) and *H. coarctatus* (Leach, 1815), (Radulovici *et al.*, 2009). These species are

morphologically distinct from larval stages to adulthood (Hultgren and Stachowicz, 2008), indicating that misidentification is highly unlikely, and incomplete lineage sorting is more plausible. We found low levels of divergence (0.778%) between one specimen of *Hyas lyratus* (Dana, 1851) from Costa *et al.*, (Costa *et al.*, 2007) and *H. coarctatus* supporting the recent evolution of the genus. However additional analyses among nuclear rDNA genes will be necessary to confirm the hypothesis of recent evolution and identification or delineation at to species of the genus *Hyas*.

Nuclear mitochondrial pseudogenes (*numts*)

COI has been the preference for species identification/delineation due the traditionally accepted advantages of mtDNA. However, it is also well recognised that analysis of mtDNA sequence variation can be distorted by the inclusion of nuclear mitochondrial pseudogenes (*numts*). Because the DNA barcoding initiative attempts to barcode all life forms, the potential impact of *numts* issue cannot be ignored (Buhay, 2009; Song *et al.*, 2008; Williams and Knowlton, 2001). *Numts* are non-functional copies of mtDNA in the nucleus that have been found in major clades of eukaryotic organisms, e.g., arthropods (Bensasson *et al.*, 2001; Song *et al.*, 2008), crustaceans (Buhay, 2009) and decapods (Nguyen *et al.*, 2002; Schubart, 2009; Williams and Knowlton, 2001). Their proportion varies greatly depending on the organism, life style, and on the genome properties (i.e., rates duplication, mutation, deletion, and retrotransposition, see (Balakirev and Ayala, 2003; Gerstein and Zheng, 2006) for review). *Numt* sequence can be highly divergent from the orthogous COI sequences. Additionally, high genetic divergences are used to indicate possible new species that may be nested within species complexes. Buhay (Buhay, 2009) reported a list of potential cases of *numts* in Crustacea when she found reading frame problems

without the occurrence of stop codons. Even though the proportion of adenine – thymine (*numts* have a significantly lower AT% compared with the orthologous mtDNA (Song *et al.*, 2008)) did not differ between specimens, there is increasing concern about the potential overestimation of species richness (Song *et al.*, 2008) by inclusion of *numts*. Here, we have discussed the occurrence of high nucleotide divergences within species, e.g., *Cherax preissii* (Gouws *et al.*, 2006). As an example here, we cannot ignore the possibility of dealing with numts sequences even if our quality controls failed to detect them (see Methods). Also other studies showed that mitochondrial cytochrome *b* gene fragments in the freshwater crayfish, *Cherax destructor* (Clark 1936) had *numts* (Nguyen *et al.*, 2002). They reported of four closely related crayfish species (*Orconectes* spp.) the presence of *numts* of the COI gene and how barcoding methods would incorrectly infer single individuals belonging to multiple, unique species (Song *et al.*, 2008). Moreover, we found high amino acid diversity among *C. preissii* species showing difference from three amino acid positions. More than two amino acid intraspecific changes could represent a radical change (Taylor, 1986) at highly conserved COI gene and as they are likely caused by sequencing error (Chang *et al.*, 2009). Especially when *numts* were already reported for this genus or even for members of the family Parastacidae, it is worthwhile for the scientific community to analyse additional morphological characters and molecular markers other than mitochondrial genes. Characterization of *numts* is important to understand genome dynamics and evolution, and their significant increases when several genomes of related organisms can be compared. It is thereby important to ensure that *numts* sequences are not discarded, but recognized, labelled, and submitted as such (Buhay, 2009; Schubart, 2009).

GC content divergence assessment

For decapods, substantially more nucleotide changes were observed at the 3rd codon position than the 1st, and more at the 1st than the 2nd: the SE of the GC % of the 3rd, 1st and 2nd bases of Decapoda were 0.213, 0.062 and 0.021, respectively (Table 3.1.4). Such values indicate the fact that most synonymous mutations occur at the 3rd position, with a few at the 1st position and none at the 2nd as also observed in Australian fish (Ward *et al.*, 2005).

Despite the commonly held view that invertebrate mitochondria are AT-rich, while chordate mitochondria are GC-rich (Clare *et al.*, 2008; Hebert *et al.*, 2003a; Mooers and Holmes, 2000) with a mean value up to 45% GC content (Clare *et al.*, 2008), our observations reveal considerable variation in the range of GC values (31 – 50% GC) within decapods (Figure 3.1.4), with a mean value of 38%. Similar values have been reported in independent Decapoda COI assessments, but also for total mtDNA diversity within the order (Clare *et al.*, 2008). Appraising a wider taxonomic breadth, Clare *et al.*, (Clare *et al.*, 2008) also detected large shifts in GC content (up to 8%) even at the generic level in the Insecta, highlighting that heterogeneity in mtDNA GC content is not restricted to our current observations.

The wide range of GC content in some families in our analysis is intriguing, though observations here must be treated cautiously as most sequences originated from GenBank, a source where sequencing error and misidentifications have been well documented (Buhay, 2009). Nevertheless, the wide range among families was largely due to 3rd codon positions as also observed in fish species (Ward *et al.*, 2005). Several explanations for genome shifts in nucleotide composition exist, which can be categorized into theories of mutational bias (observation that purine to purine or pyrimidine to pyrimidine changes -transitional- occur with greater frequency than

purine to pyrimidine or vice versa - transversional (Banerjee *et al.*, 2005) and natural selection (Mooers and Holmes, 2000)). There remains a strong interest in exploring the environmental context of such shifts, including fluctuations in temperature, salinity, pressure (Bjedov *et al.*, 2003; Chandor *et al.*, 2007; Cinzia *et al.*, 2006; Friedberg, 1995; Gillooly and Allen, 2007; Hebert *et al.*, 2002; Sicot *et al.*, 2000; Somero, 2003), and biological factors such as population size, generation time, body size, larval dispersal, mutation rate and parasite behaviour (Baer *et al.*, 2007; Drake, 2006; Drake *et al.*, 1998; Gillooly *et al.*, 2005; Hassanin, 2006; Ho *et al.*, 2005; Maki, 2002; Ohta, 1992; Page *et al.*, 1998). It is important to underline that the families with higher GC values belong to the oldest Pleocymata lineage Caridea (Porter *et al.*, 2005). It is known that DNA sequences with similar GC content may be grouped together if phylogenetic analysis is performed on DNA sequences (Foster *et al.*, 1997). GC-rich DNA is assumed to produce a more heat-stable helix (Bernardi, 1995) and thus can be selectively advantageous in animals with high metabolic regulation induced by environmental drivers such as light, temperature, salinity, oxygen, and pH. Recently a study (Banerjee *et al.*, 2005) showed the existence of a strong positive correlation between hydrophobicity and genomic GC content in prokaryotic organisms. Although the importance of hydrophobicity on the stability of proteins has been observed in most of the protein families (Dill, 1990), GC increment may be related to the structural and functional changes of the encoded proteins (Banerjee *et al.*, 2005) in Caridea, suggesting that natural selection is the main force influencing mutation patterns.

Sample size and geographical coverage for species diversity assessment

Early in the DNA barcode initiative the question of how many specimens are needed to create a reliable reference for specimen identification and diversity assessment remained largely unresolved. A sample size of 12 individuals per species was proposed by (Matz and Nielsen, 2005b), but it has been correctly asserted that a reference sequence sample for all species seems pointless without taking the evolutionary characteristics of each species into account (Zang *et al.*, 2010). Zang *et al.* (2010) showed that there is no significant correlation between samples size and the percentage of the total number of haplotypes observed, and the effort of finding new haplotypes varies considerably over different species/populations. In our data the pattern of diversity found among species is very diverse, but it remains unclear how representative it is as an estimate of genetic/variation diversity based on a sample of 10 individuals. As an example we have the species *G. rhomboides* represented by 7 individuals from the Portuguese west coast and three from Great Britain sharing a unique haplotype. Such data suggest that we should have better randomized sampling from the whole geographical distribution of a species in DNA barcoding projects to better encompass the diversity of the species. Nevertheless, the trends disclosed, together with the high levels of concordance overall between previous indications of taxonomic anomalies and links to coarse environmental features, does suggest that data presented here are broadly representative of contemporary biodiversity patterns. Indeed, examination of diversity at the COI region yields an informative framework to identify and explore priority issues, demanding in turn a fully integrative approach utilising additional molecular, distributional and ecological information.

3.1 Conclusions

Although our study is limited to decapods, and the sampling is limited to a small proportion of the entire order (5.4% of the 17635 extant species described), it is unlikely that the general patterns observed have been biased by our sampling or taxonomic coverage. Here with our range of molecular data we have contributed to the assessment of decapods biodiversity in several ways, including: revealing putative cryptic species (e.g., *Palaemon elegans*); assigning correct species names of taxa with different life history stages (*Pachygrapsus marmoratus*); confirming the existence of the synonymy names (*Macropodia tenuirostris*); facilitating a rapid assessment of taxon diversity in groups that have until now received limited morphological and systematic examination (*Macropodia*), and we also flag taxonomic groups (Caridea; Lithodidae and Pandalidae) with unusual nucleotide composition or evolutionary rates. Intraspecific genetic diversity has a fundamental role in delimiting species boundaries. The burgeoning record of barcode records, in conjunction with additional ecological and molecular approaches, is likely to enhance understanding of the history and evolutionary trajectory of decapod species. It has become essential that species are accurately delineated, cryptic species are identified and/or conservation units are proposed on the basis of sound phylogenetic and phylogeographic variation in space and time. Efforts to conserve biodiversity should work to preserve both existing biodiversity as well as the evolutionary processes shaping genetic diversity, the core determinant evolutionary potential for adaptation to changing environments.

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References

- Alekhnovich AV, Kulesh VF (2001) Variation in the parameters of the life cycle in prawns of the genus *Macrobrachium* Bate (Crustacea, Palaemonidae). *Russian Journal of Ecology* **32**, 420-425.
- Apte S, Smith PJ, Wallis GP (2007) Mitochondrial phylogeography of New Zealand freshwater crayfishes, *Paranephrops* spp. *Molecular Ecology* **16**, 1897-1908.

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- Arif IA, Hkhan HA (2009) Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Animal Biodiversity and Conservation* **32**, 9-17.
- Bachtrog D, Thornton K, Clark A, Andolfatto P (2006) Extensive introgression of mitochondrial DNA relative to nuclear genes in the *Drosophila yakuba* species group. *Evolution* **60**, 292-302.
- Baer CF, Miyamoto MM, Denver DR (2007) Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nature Reviews Genetics* **8**, 619-631.
- Balakirev ES, Ayala FJ (2003) Pseudogenes: Are they “junk” or functional DNA? *Annual Review of Genetics* **37**, 123-151.
- Banerjee T, Gupta SK, Ghosh TC (2005) Role of mutational bias and natural selection on genome-wide nucleotide bias in prokaryotic organisms. *BioSystems* **81**, 11-18.
- Baratti M, Goti E, Messana G (2005) High level of genetic differentiation in the marine isopod *Sphaeroma terebrans* (Crustacea: Isopoda: Sphaeromatidae) as inferred by mitochondrial DNA analysis. *J. Exp. Mar. Biol. Ecol.* **315**, 225–234.
- Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution* **60**, 1825-1839.
- Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution* **16**, 314-321.
- Bernardi G (1995) The human genome: organization and evolutionary history. *Annual Review of Genetics* **29**, 445-476.
- Bierne N, Bonhomme F, David P (2003) Habitat preference and the marine - speciation paradox. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 1399-1406.
- Bjedov I, Olivier Tenailleau, Benedicte Gerard, Valeria Souza, Erick Denamur, Miroslav Radman, *et al.* (2003) Stress-Induced Mutagenesis in Bacteria. *Science* **300**, 1404-1409.
- Blaxter M (2003) Molecular systematics - Counting angels with DNA. *Nature* **421**, 122-124.

- Boero F (2010) The study of species in the Era of biodiversity: A tale of stupidity. *Diversity* **2**, 115-126.
- Bracken HD, De Grave S, Felder DL (2009) Phylogeny of the infraorder Caridea based on mitochondrial and nuclear genes (Crustacea: Decapoda). In: *Decapod Crustacean Phylogenetics. Crustacean Issues*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 281–305. Taylor and Francis Group, New York.
- Brökeland W, Raupach MJ (2008) A species complex within the isopod genus *Haploniscus* (Crustacea: Malacostraca: Peracarida) from the Southern Ocean deep sea: a morphological and molecular approach. *Zoological Journal of the Linnean Society* **152**, 655-706.
- Bucciarelli G, Bernardi G, Bernardi G (2002) An ultracentrifugation analysis of fish genomes. *Gene* **295**, 153-162.
- Buhay JE (2009) "COI-like" sequences are becoming problematic in molecular systematics and DNA barcoding studies. *Journal of Crustacean Biology* **20**, 96-110.
- Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA, et al. (2010) Global biodiversity: Indicators of recent declines. *Science* **328**, 1164-1168.
- Calado R (2006) Marine ornamental species from European waters: a valuable overlooked resource or a future threat for the conservation of marine ecosystems? *Scientia Marina* **70**, 389-398.
- Cartes JE, Abelló P, Lloris D, Carbonell A, Torres P, Maynou F, Gil de Sola L (2002) Analysis of feeding guilds of fish and decapod crustaceans during the MEDITS-99 cruise along the Iberian Peninsula Mediterranean coasts. *Scientia Marina* **66**, 209–220.
- Cartes JE, Carrasson M (2007) Influence of trophic variables on the depth-range distributions and zonation rates of deep-sea megafauna: the case of the Western Mediterranean assemblages. *Deep-Sea Research I*, 263-279.
- Casiraghi M, Labra M, Ferri E, Galimberti A, De Mattia F (2010) DNA barcoding: a six-question tour to improve users' awareness about the method. *Briefings in Bioinformatics* **11**, 440-453.

- Chan T-Y (2004) The “*Plesionika rostricrescentis* (Bate, 1888)” and “*P. lophotes* Chace, 1985” species groups of *Plesionika* Bate, 1888, with descriptions of five new species (Crustacea: Decapoda: Pandalidae). In: *Tropical Deep-Sea Benthos* (eds. B. Marshal, B. Richer de Forges), pp. 293-318. Muséum national d’Histoire naturelle, Paris.
- Chan T-Y, Yu H-P (1991) Two similar species: *Plesionika edwardsii* (Brandt, 1851) and *Plesionika crosnieri*, new species (Crustacea: Decapoda: Pandalidae). *Proceedings of the Biological Society of Washington* **104**, 545-555.
- Chan T-Y, Yu H-P (1998) A new deep-sea shrimp of the genus *Plesionika* Bate, 1888 (Crustacea: Decapoda: Pandalidae) from Taiwan. *International Symposium on Marina Biology in Taiwan*, 119-127.
- Chandor A, Douki T, Gasparutto D, Gambarelli S, Sanakis Y, Nicolet Y, Ollagnier-de-Choudens S (2007) Characterization of the DNA repair spore photoproduct lyase enzyme from *Clostridium acetobutylicum*: A radical-SAM enzyme. *Comptes Rendus Chimie* **10**, 756-765.
- Chang C-H, Rougerie R, Chen J-H (2009) Identifying earthworms through DNA barcodes: Pitfalls and promise. *Pedobiologia* **52**, 171-180.
- Childress JJ (1995) Are the physiological and biochemical adaptations of metabolism in deep-sea animals? *Tree* **10**, 30-36.
- Childress JJ, Cowles DL, Favuzzi JA, Mickel TJ (1990) Metabolic rates of benthic deep-sea decapod crustaceans decline with increasing depth primarily due to the decline in temperature. *Deep-Sea Research* **37**, 926-949.
- Cinzia V, Vergara A, Giordano D, Mazzarella L, Prisco G (2006) The Root effect- a structural and evolutionary perspective. *Antarctic Science* **19**, 271-278.
- Clare EL, Kerr KCR, von Konigslow TE, Wilson JJ, Hebert PDN (2008) Diagnosing mitochondrial DNA diversity: Applications of a sentinel gene approach. *Journal Molecular Evolution* **66**, 362-367.
- Company JB, Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. *Deep-Sea Research I*, 1861-1880.
- Costa FO, Carvalho GR (2007) The Barcode of Life Initiative: synopsis and prospective societal impacts of DNA barcoding of fish. *Genomics, Society and Policy* **3**, 52-56.

- Costa FO, deWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272-295.
- Cuesta JA, Rodríguez A (2000) Zoeal stages of the intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787) (Brachyura, Grapsidae) reared in the laboratory. *Hydrobiologia* **439**, 119-130.
- Cuesta JA, Schubart CD (1998) Morphological and molecular differentiation between three allopatric populations of the littoral crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). *Journal of Natural History* **32**, 1499 - 1508.
- Cunningham CW, Blackstone NW, Buss LW (1992) Evolution of king crabs from hermit crab ancestors. *Nature* **355**, 539-542.
- d'Udekem d'Acoz C (1999) *Inventaire et distribution des crustacés décapodes de l'Atlantique nord - oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25°N*, 1st edn. Collection Patrimoines Naturels, Paris.
- De Grave S, Cai Y, Anker A (2008) Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. *Hydrobiologia* **595**, 287-293.
- De Grave S, Ghane A (2006) The establishment of the oriental river prawn, *Macrobrachium nipponense* (de Haan, 1849) in Anzali Lagoon, Iran. *Aquatic Invasions* **1**, 204-208.
- De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, et al. (2009) A classification of living and fossil genera of decapod Crustaceans. *Raffles Bulletin of Zoology* **1**, 1-109.
- DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1905-1916.
- Dill KA (1990) Dominant forces in protein folding. *Biochemistry* **29**, 7133-7155.
- Dimmock A, Williamson I, Mather PB (2004) The influence of environment on the morphology of *Macrobrachium australiense* (Decapoda: Palaemonidae). *Aquaculture International* **12**, 435-456.
- Drake JW (2006) Chaos and order in spontaneous mutation. *Genetics* **173**, 1-8.

- Drake JW, Charlesworth B, Charlesworth D, Crow JF (1998) Rates of spontaneous mutation. *Genetics* **148**, 1667-1686.
- Ebach MC, Holdrege C (2005) DNA barcoding is no substitute for taxonomy. *Nature* **434**, 697-697.
- Floyd R, Abebe E, Papert A, Black M (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* **11**, 836-850.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294-299.
- Fortunato C, Sbordoni V (1998) Allozyme variation in the Mediterranean rockpool prawn (*Palaemon elegans*): environmental vs. historical determinants. In: *Proceedings and Abstracts of Fourth International Crustacean Congress* (ed. Congress Fourth international Crustacean), Amsterdam. (Abstract:12).
- Foster PG, Jermin LS, Hickey DA (1997) Nucleotide composition bias affects amino acid content in proteins coded by animal mitochondria. *Journal Molecular Evolution* **44**, 282–288.
- Fourment M, Gibbs MJ (2006) Patristic: a program for calculation patristic distances and graphically comparing the components of genetic change. *BMC Evolutionary Biology* **6**, 1-5.
- Frézal L, Lebloids R (2008) Four years of DNA barcoding: Current advances and prospects. *Infection, Genetics and Evolution*, 727-736.
- Friedberg EC (1995) Out of the Shadows and into the Light - the Emergence of DNA-Repair. *Trends in Biochemical Sciences* **20**, 381-381.
- García-Castrillo G, Olaso I (1995) Composition and structure of the invertebrate megabenthos on the shelf of the Cantabrian Sea. *ICES Marine Science Symposia* **199**, 151-156.
- Garcia-Raso JE (1987) Carideos ibéricos (Crustacea, Decapoda): síntesis. *Miscellánea Zoológica* **11**, 113-120.
- Gerstein M, Zheng D (2006) The real life of pseudogenes. *Scientific American* **295**, 48-55
- Gillooly JF, Allen AP (2007) Linking global patterns in biodiversity to evolutionary dynamics using metabolic theory. *Ecology* **88**, 1890-1894.

- Gillooly JF, Allen AP, West GB, Brown JH (2005) The rate of DNA evolution: Effects of body size and temperature on the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 140-145.
- Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251.
- Gomez A, Wright PJ, Lunt DH, Cancino JM, Carvalho GR, Hughes RN (2007) Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. *Proceedings of the Royal Society B-Biological Sciences* **274**, 199-207.
- Gonzalez-Gorillo JI, Rodriguez A (2001) The complete larval development of the spider, *Macropodia parva* (Crustacea, Decapoda, Majidae) from laboratory culture. *Invertebrate Reproduction and Development* **39**, 165-142.
- Gouws G, Stewart BA, Daniels SR (2006) Phylogeographic structure of a freshwater crayfish (Decapoda: Parastacidae: *Cherax preissii*) in south-western Australia. *Marine and Freshwater Research* **57**, 837-848.
- Grabowski M (2006) Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquatic invasions* **1**, 116-123.
- Gregory TR (2005) DNA barcoding does not compete with taxonomy. *Nature* **434**, 1067.
- Guerao G, Abelló P (1997) Larval development of the spider crab *Macropodia*. *Journal of Crustacean Biology* **17**, 459-471.
- Hajibabaei M, DeWaard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, et al. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1959-1967.
- Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 968-971.
- Hajibabaei M, Singer GAC, Clare EL, Hebert PDN (2007a) Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. *BMC Biology* **5**, 1-7.

- Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007b) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* **23**, 167-172.
- Hall S, Thatje S (2009) Global bottlenecks in the distribution of marine Crustacea: temperature constraints in the family Lithodidae. *Journal of Biogeography* **36**, 2125-2135.
- Harris DJ (2003) Can you bank on GenBank? *Trends in Ecology and Evolution* **18**, 317-319.
- Harrison JS (2004) Evolution, biogeography, and the utility of mitochondrial 16S and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda: Pinnotheridae). *Molecular Phylogenetics and Evolution* **30** 743–754.
- Hassanin A (2006) Phylogeny of Arthropoda inferred from mitochondrial sequences: Strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. *Molecular Phylogenetics and Evolution* **38**, 100-116.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 313-321.
- Hebert PDN, Ratnasingham S, deWaard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S96-S99.
- Hebert PDN, Remigio EA, Colbourne JK, Taylor DJ, Wilson CC (2002) Accelerated molecular evolution in halophilic crustaceans. *Evolution* **56**, 909-926.
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. *PLoS Biology* **2**, 1657-1663.
- Herring PJ, Dixon DR (1998) Extensive deep-sea dispersal of postlarval shrimp from a hydrothermal vent. *Deep-Sea Research I*, 2105-2118.
- Hickerson MJ, Meyer CP, Moritz C (2006) DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* **55**, 729-739.
- Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution* **22**, 1561-1568.

- Holthuis LB (1950) Subfamily Palaemoninae. The Palaemonidae collected by the Siboga Snellius Expeditions with remarks on other species. The Decapoda of the Siboga Expedition. Part 10. In: *Siboga Expedition Monograph*, pp. 1-268.
- Hultgren KM, Stachowicz JJ (2008) Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. *Molecular Phylogenetics and Evolution* **48**, 986-996.
- Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 1-5.
- Jones GP, Srinivasan M, Almany G (2007) Population connectivity and conservation of marine biodiversity. *Oceanography* **20**, 100-111.
- Jong Gd (2004) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytologist* **166**, 101-118.
- Kirkpatrick K, Jones MB (1985) Salinity tolerance and osmoregulation of a prawn, *Palaemon affinis* Milne Edwards (Caridea: Palaemonidae). *Journal of experimental Marine Biology and Ecology* **93**, 61-70.
- Komai T (1999) A revision of the genus *Pandalus* (Crustacea: Decapoda: Caridea: Pandalidae). *Journal of Natural History* **33**, 1265-1372.
- Lefébure T, Douady CJ, Gouy M, Gibert J (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* **40**, 435–447.
- Levesque R (2008) SPSS Programming and Data Management for SPSS 16.0: A Guide for SPSS and SAS Users. SPSS Inc., Chicago.
- Liu M-Y, Cai Y-X, Tzeng C-S (2007) Molecular systematics of the freshwater prawn genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) inferred from mtDNA sequences, with emphasis on East Asian species. *Zoological Studies* **46**, 272-289.
- Macpherson E, Duarte CM (1991) Bathymetric trends in demersal fish size: is there a general relationship? *Marine Ecology Progress Series* **71**, 103-112.
- Maki H (2002) Origins of spontaneous mutations: Specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses. *Annual Review of Genetics* **36**, 279-303.

- Mallet J (2005) Hybridization as an invasion of the genome. *Trends in Ecology and Evolution* **20**, 229-237.
- Mallet J, Willmott K (2007) Taxonomy: renaissance or Tower of Babel? *Trends in Ecology and Evolution* **18**, 57-59.
- Marin IN, Anker A, Britayev TA, Palmer AR (2005) Symbiosis between the alpheid shrimp, *Athanas ornithorhynchus* Banner and Banner, 1973 (Crustacea: Decapoda), and the brittle star, *Macrophiothrix longipeda* (Lamarck, 1816) (Echinodermata: Ophiuroidea). *Zoological Studies* **44**, 234-241.
- Markle DF, Dadswell MJ, Halliday RG (1988) Demersal fish and decapod crustacean fauna of the upper continental slope off Nova Scotia from La Have to Sr. Pierre Banks. *Canadian Journal of Zoology* **66**, 1952-1960.
- Martin AP, Naylor GJP, Palumbi SR (1992) Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* **357**, 153-155.
- Martin AP, Palumbi SR (1993a) Body Size, Metabolic-Rate, Generation Time, and the Molecular Clock. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 4087-4091.
- Martin AP, Palumbi SR (1993b) Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 4087-4091.
- Martin JW, Crandall KA, Felder DL (2009) *Decapod Crustacean Phylogenetics*. Taylor and Francis Group, New York.
- Martin JW, Davis GE (2001) Un updated classification of the recent Crustacea. *Natural History Museum of Los Angeles* **39**, 1-124.
- Mashiko K, Numachi K (2000) Derivation of populations with different - aized eggs in the Palaemonid prawn *Macrobrachium nipponense*. *Journal of Crustacean Biology* **20**, 118-127.
- Mathews LM (2007) Evidence for restricted gene flow over small spatial scales in a marine snapping shrimp *Alpheus angulosus*. *Marine Biology* **152**, 645–655.
- Mathews LM, Anker A (2009) Molecular phylogeny reveals extensive ancient and ongoing radiations in a snapping shrimp species complex (Crustacea, Alpheidae, *Alpheus armillatus*). *Molecular Phylogenetics and Evolution* **50**, 268-281.

- Matz MV, Nielsen R (2005a) A likelihood ratio test for species membership based on DNA sequence data. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1969-1974.
- Matz MV, Nielsen R (2005b) Taxonomy - Will DNA barcodes breathe life into classification? *Science*, 1073.
- Meier R, Shiyang K, Vaidya G, Ng PKL (2006) DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success. *Systematic Biology* **55**, 715-728.
- Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the "Barcode gap" and leads to misidentification. *Systematic Biology* **57**, 809-813.
- Meusnier I, Singer GAC, Landry J-F, Hickey DA, Hebert PDN, Hajibabaei M (2008) A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics* **9**, 1-4.
- Meyer CP, Paulay G (2005a) DNA barcodes perform best with well-characterized taxa. *PLoS Biology* **3**, e435.
- Meyer CP, Paulay G (2005b) DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* **3**, e422.
- Miller SE (2007) DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences* **104**, 4775-4776.
- Min XJ, Hickey DA (2007) DNA barcodes provide a quick preview of mitochondrial genome composition. *PLoS ONE* **2**, e325.
- Minelli A (2003) The status of taxonomic literature. *Trends in Ecology and Evolution* **18**, 75-76.
- Mooers AO, Holmes EC (2000) The evolution of base composition and phylogenetic inference. *Trends in Ecology and Evolution* **15**, 365-369.
- Munasinghe DHN, Murphy NP, Austin CM (2003) Utility of mitochondrial DNA sequences from four gene regions for systematic studies of Australian freshwater crayfish of the genus *Cherax* (Decapoda:Parastacidae). *Journal of Crustacean Biology* **23**, 402-417.
- Murphy NP, Austin CM (2004) Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda:

- Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. *Zoologica Scripta* **34**, 187-197.
- Nguyen TTT, Murphy NP, Austin CM (2002) Amplification of multiple copies of mitochondrial cytochrome *b* gene fragments in the Australian freshwater crayfish, *Cherax destructor* Clark (Parastacidae; Decapoda) *Animal Genetics* **33**, 304-308.
- Noël PY (1992) Clé préliminaire d'identification des Crustacea Decapoda de France et des principales autres espèces d'Europe. In: *Collection Patrimoines Naturels* (ed. Naturelle Museum National d'Histoire), pp. 1-145.
- Ohta T (1992) The nearly neutral theory of molecular evolution. *Annual Review of Ecology and Systematics* **23**, 263-286.
- Olaso I, Rodriguez-Marin E (1995) Decapod crustaceans in the diets of demersal fish in the Cantabrian Sea. *ICES Marine Science Symposia* **199**, 209-221.
- Oliver L, Beattie AJ (1993) A possible method for the rapid assessment of biodiversity. *Conservation Biology* **7**, 562-568.
- Ostrow D, Phillips N, Avalos A, Blanton D, Boggs A, Keller T, *et al.* (2007) Mutational Bias for body Size in Rhabditid nematodes. *Genetics* **176**, 1653-1661.
- Page RDM, Lee PLM, Becher SA, Griffiths R, Clayton DH (1998) A different tempo of mitochondrial DNA evolution in Birds and their parasitic life. *Biology Letters* **1**, 139-142.
- Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* **13**, 146–158.
- Payne RW (2009) GenStat. *Computational Statistics* **1**, 255-258.
- Pérez-Barros P, d'Amato ME, Guzmán NV, Lovrich GA (2008) Taxonomic status of two South American sympatric squat lobsters, *Munida gregaria* and *Munida subrugosa* (Crustacea: Decapoda: Galatheidae), challenged by DNA sequence information. *Biological Journal of the Linnean Society* **94**, 421-434.
- Pfeiler E, Hurtado LA, Knowles LL, Torre-Cosío J, Bourillón-Moreno L, Márquez-Farías JF, Montemayor-López G (2005) Population genetics of the swimming crab *Callinectes bellicosus* (Brachyura: Portunidae) from the eastern Pacific Ocean. *Marine Biology* **146**, 559–569.

- Polunin NVC, Morales-Nin B, Herod W, Cartes JE, Pinnegar JK, Moranta J (2001) Feeding relationships in Mediterranean bathyal assemblages elucidated by carbon and nitrogen stable-isotope data. *Marine Ecology Progress Series* **220**, 13–23.
- Porter ML, Pérez-Losada M, Crandall KA (2005) Model-based multi-locus estimation of decapod phylogeny and divergence times. *Molecular Phylogenetics and Evolution* **37**, 335–369.
- Puillandre N, Strong EE, Bouchet P, Boissellier MC, Couloux A, Samadi S (2009) Identifying gastropod spawn from DNA barcodes: possible but not yet practicable. *Molecular Ecology Resources* **9**, 1311–1221.
- Radulovici AE, Archambault P, Dufresne F (2010) DNA barcodes for marine biodiversity: Moving fast forward? *Diversity* **2**, 450–472.
- Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. *Molecular Ecology Resources* **9**, 181–187.
- Ratnasingham S, Hebert PDN (2007) Barcoding BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.
- Reuschel S, Cuesta JA, Schubart CD (2010) Marine biogeographic boundaries and human introduction along the European coast revealed by phylogeography of the prawn *Palaemon elegans*. *Molecular Phylogenetics and Evolution*, 765–775.
- Rock J, Costa FO, Walker DI, North AW, Hutchinson WF, Carvalho GR (2008) DNA barcodes of fish of the Scotia Sea, Antarctica indicate priority groups for taxonomic and systematics focus. *Antarctic Science* **20**, 253–262.
- Roe AD, Sperling FAH (2007) Patterns of evolution of mitochondrial cytochrome c oxidase I and II DNA and implications for DNA barcoding. *Molecular Phylogenetics and Evolution* **44**, 325–345.
- Romiguier J, Ranwez V, Douzery EJP, Galtier N (2010) Contrasting GC-content dynamics across 33 mammalian genomes: Relationship with life-history traits and chromosome sizes. *Genome Research* **20**, 1001–1009.
- Salman DS, P. TJ, N. MD, Ama'al GY (2006) The invasion of *Macrobrachium nipponense* (De Haan, 1849) (Caridea: Palaemonidae) into the Southern Iraqi Marshes. *Aquatic invasions* **1**, 109–115.

- Sánchez JA, Aguilar C, Dorado D, Manrique N (2007) Phenotypic plasticity and morphological integration in a marine modular invertebrate. *BMC Evolutionary Biology* **7**, 1-9.
- Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1805-1811.
- Schindel DE, Miller SE (2005) DNA barcoding a useful tool for taxonomists. *Nature* **435**, 17-17.
- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Schubart CD, Cuesta JA (1996) Phylogeny of North Atlantic and Mediterranean species of *Pachygrapsus* (Brachyura: Grapsidae) and intraspecific variation among localities. *6th Colloquium Crustacea Decapoda Mediterranea*, 83 - 84.
- Seibel BA, Childress JJ (2000) Metabolism of benthic octopods (Cephalopoda) as a function of habitat depth and oxygen concentration. *Deep-Sea Research I*, 1247-1260.
- Seibel BA, Thuesen EV, Childress JJ (1997) Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. *Biological Bulletin* **192**, 262-278.
- Sémon M, Mouchiroud D, Duret L (2005) Relationship between gene expression and GC-content in mammals: statistical significance and biological relevance. *Human Molecular Genetics* **14**, 421-427.
- Shih H-T, Ng PKL, Schubart CD, Chang H-W (2007) Phylogeny and phylogeography of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) in southwestern Taiwan based on two mitochondrial genes. *Zoological Science* **24**, 57-66.
- Sicot FX, Mesnage M, Masselot M, Exposito JY, Garrone R, Deutsch J, Gaill F (2000) Molecular adaptation to an extreme environment: Origin of the thermal stability of the Pompeii worm collagen. *Journal Molecular Evolution* **302**, 811-820.

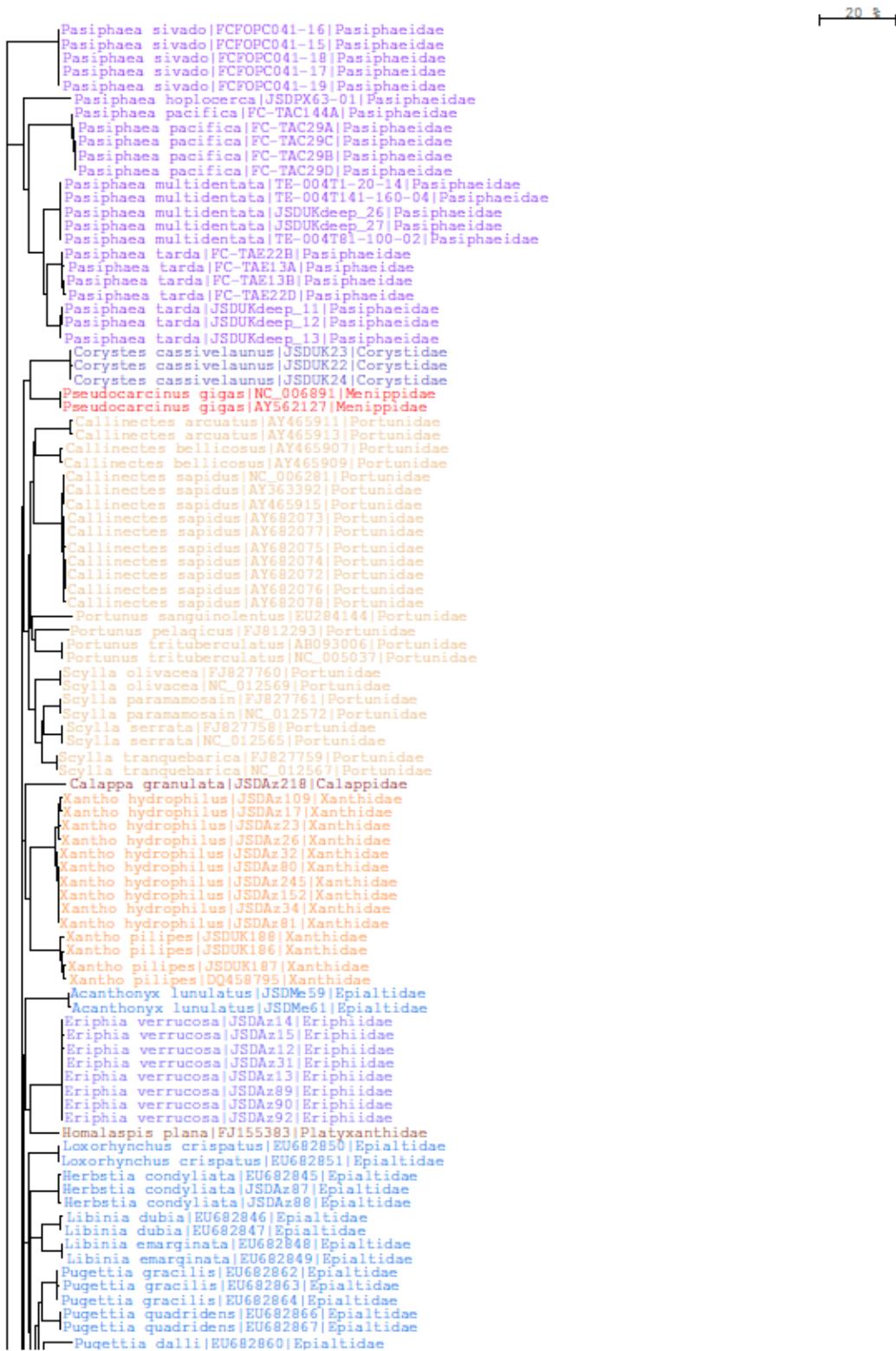
- Silliman BR, Layman CA, Altieri AH (2003) Symbiose between and alpheid shrimp and xanthoid crab in salt marshes of mid-atlantic states, U.S.A. *Journal of Crustacean Biology* **23**, 876-879.
- Smith MA, Poyarkov JR NA, Hebert PDN (2008) CO1 DNA barcoding amphibians: take the chance, meet the challenge. *Molecular Ecology Resources* **8**, 235-246.
- Somero GN (2003) Protein adaptations to temperature and pressure: complementary roles of adaptive changes in amino acid sequence and internal milieu. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **136**, 577-591.
- Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**, 13486-13491.
- Stevens BG, Anderson PJ (2000) An association between the anemone, Cribripinopsis fernaldi, and shrimps of the families Hippolytidae and Pandalidae. *Journal of Northwest Atlantic Fishery Science* **27**, 77-82.
- Summerbell RC, Lévesque CA, Seifert KA, Bovers M, Fell JW, Diaz MR, et al. (2005) Microcoding: the second step in DNA barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1897-1903.
- Tamura K DJ, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Taylor AC, Spicer JI (1987) Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* (Crustacea: Decapoda) to acute hypoxia and anoxia. *Marine Biology*, 521-539.
- Taylor WR (1986) The classification of amino acid conservation. *Journal of Theoretical Biology* **119**, 205-218.
- Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-4680.
- Tsang LM, Chan T-Y, Cheung MK, Chu KH (2009) Molecular evidence for the Southern Hemisphere origin and deep sea diversification of spiny lobsters

- (Crustacea: Decapoda: Palinuridae). *Molecular Phylogenetics and Evolution*, 1-13.
- Tsang LM, Ma KY, Ahyong ST, Chan T-Y, Chu KH (2008) Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, 359-368.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1847-1857.
- Werle E, Schneider C, Renner M, Volker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* **22**, 4354-4355.
- Will KW, Mishler BD, Wheeler QD (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54**, 844-851.
- Williams ST, Knowlton N (2001) Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution* **18**, 1484-1493.
- Williamson DI (1972) Larval development in a marine and a freshwater species of *Macrobrachium* (Decapoda, Palaemonidae). *Crustaceana* **23**, 282-298.
- Wilson EO (2003a) Biodiversity in the information age. *Issues in Science and Technology* **19**, 45-46.
- Wilson EO (2003b) The encyclopedia of life. *Trends in Ecology and Evolution* **18**, 77-80.
- Wilson EO (2003c) On global biodiversity estimates. *Paleobiology* **29**, 14-14.
- Wong JTY, McAndrew BJ (1990) Selection for the larval freshwater tolerance in *Macrobrachium nipponense* (de Hann). *Aquaculture* **88**, 151-156.
- Zaklan SD (2002) Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): distribution, biology, and fisheries. In: *Crabs in cold water regions: biology, management, and economics* (ed. MacIntosh R.A.), pp. 751–845. Anchorage College, Anchorage.
- Zakšek V, Sket B, Gottstein S, Franjević D, Trontelj P (2009) The limits of cryptic diversity in groundwater: phylogeography of the cave shrimp *Troglocaris anophthalmus* (Crustacea: Decapoda: Atyidae). *Molecular Ecology* **18**, 931-946.

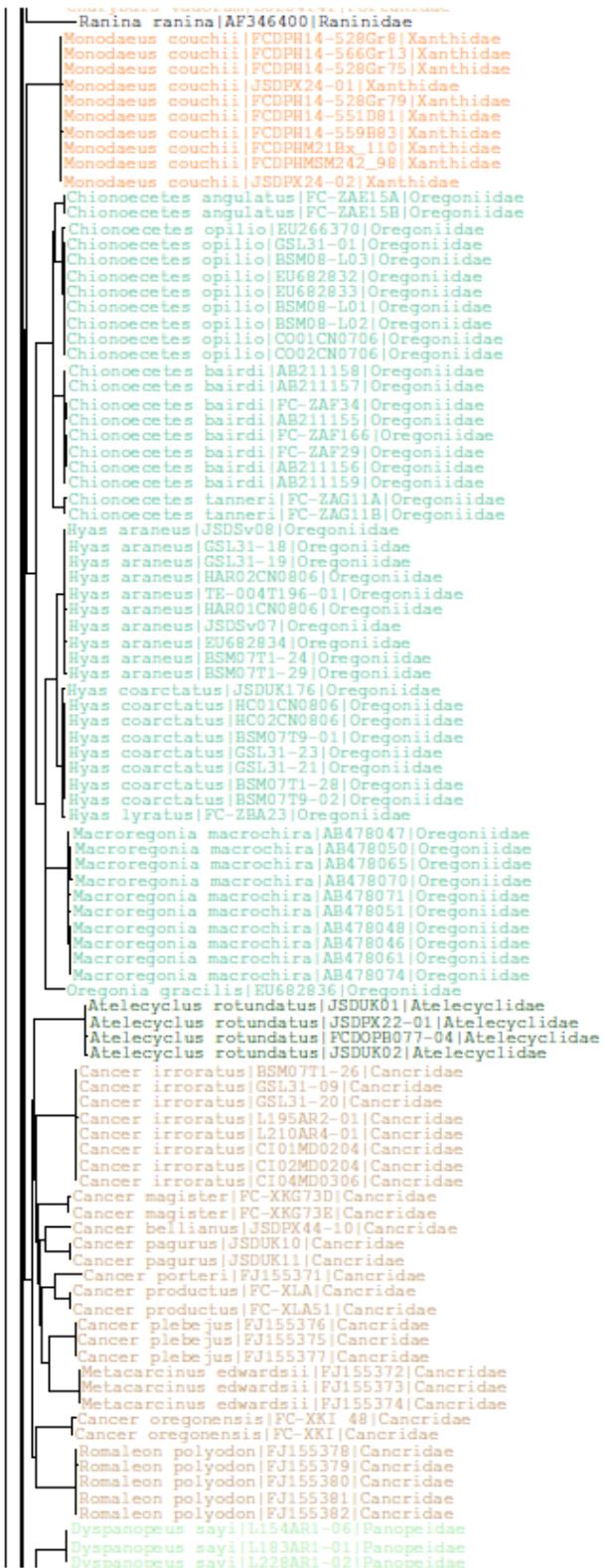
Zang AB, He LJ, Crozier RH, Muster C, Zhu C-D (2010) Estimating sample sizes for DNA barcoding. *Molecular Phylogenetics and Evolution* **54**, 1035-1039.

Zariquey-Alvarez R (1968) Crustáceos decápodos ibéricos. *Investigacione Pesquera* **32**, 1-510.

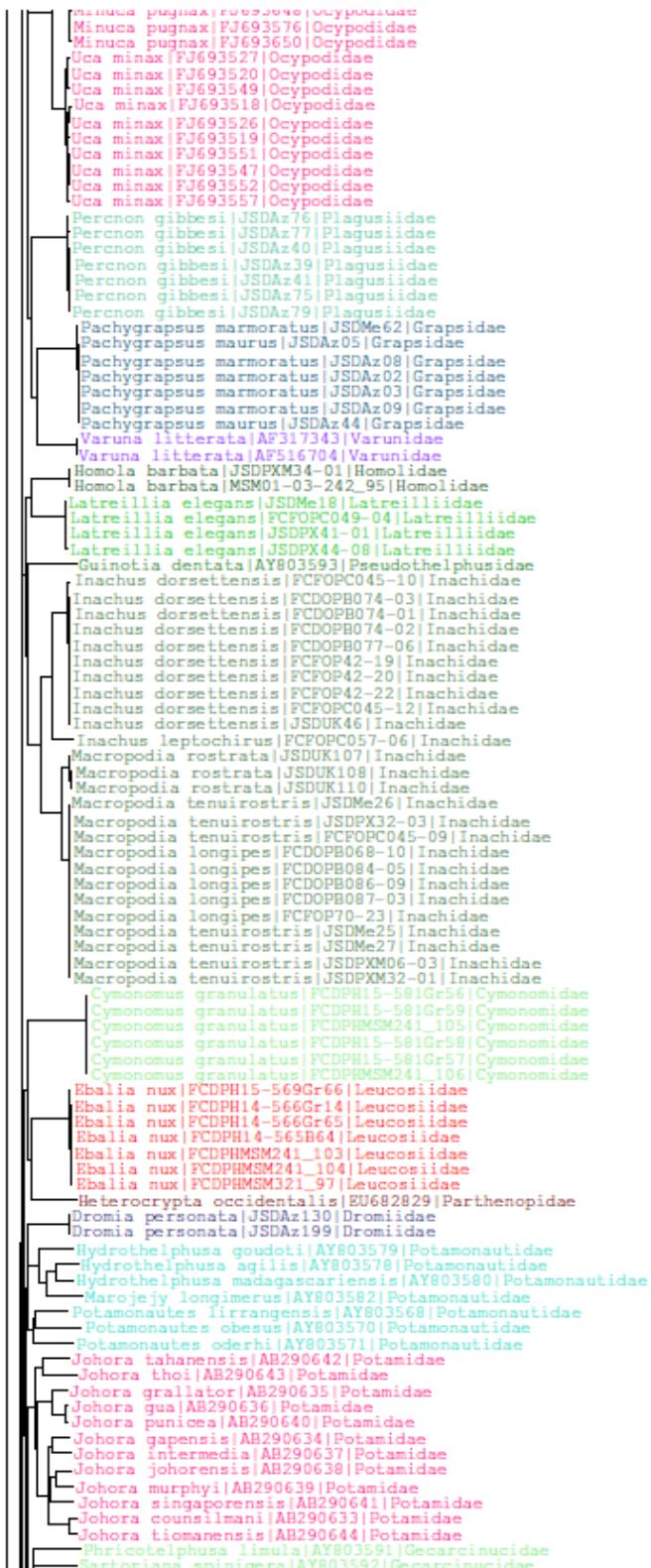
3.1 ANNEX



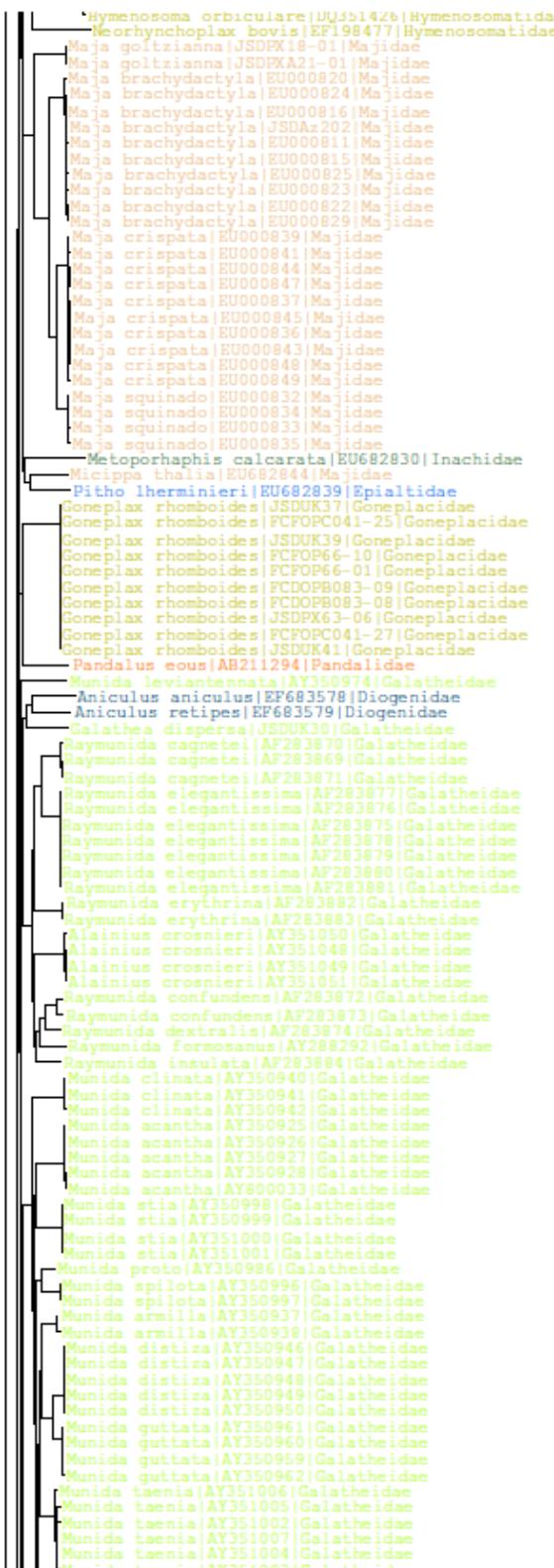


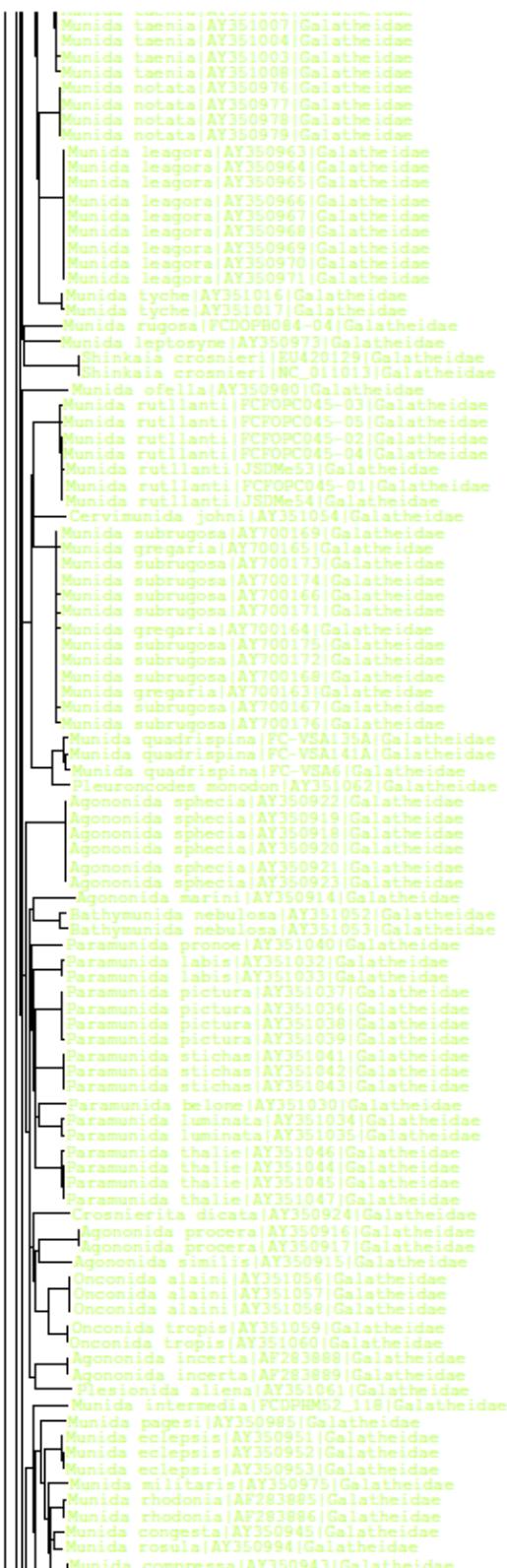




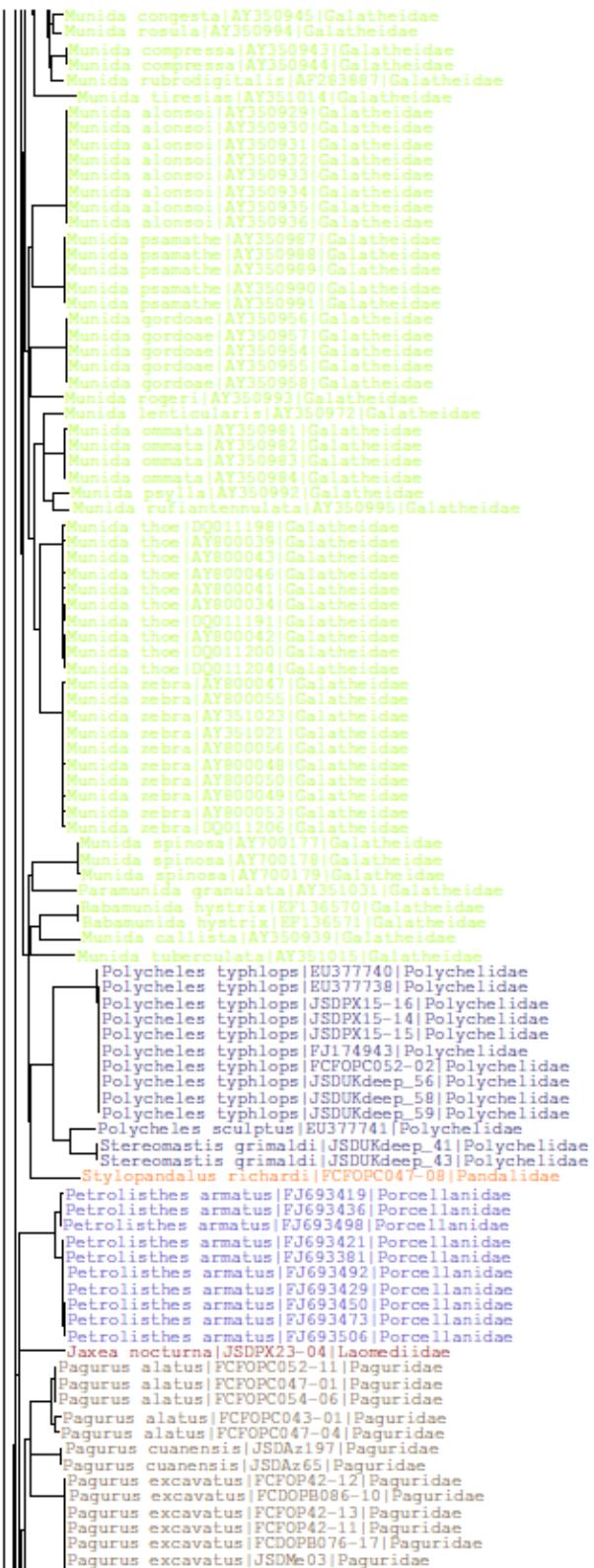






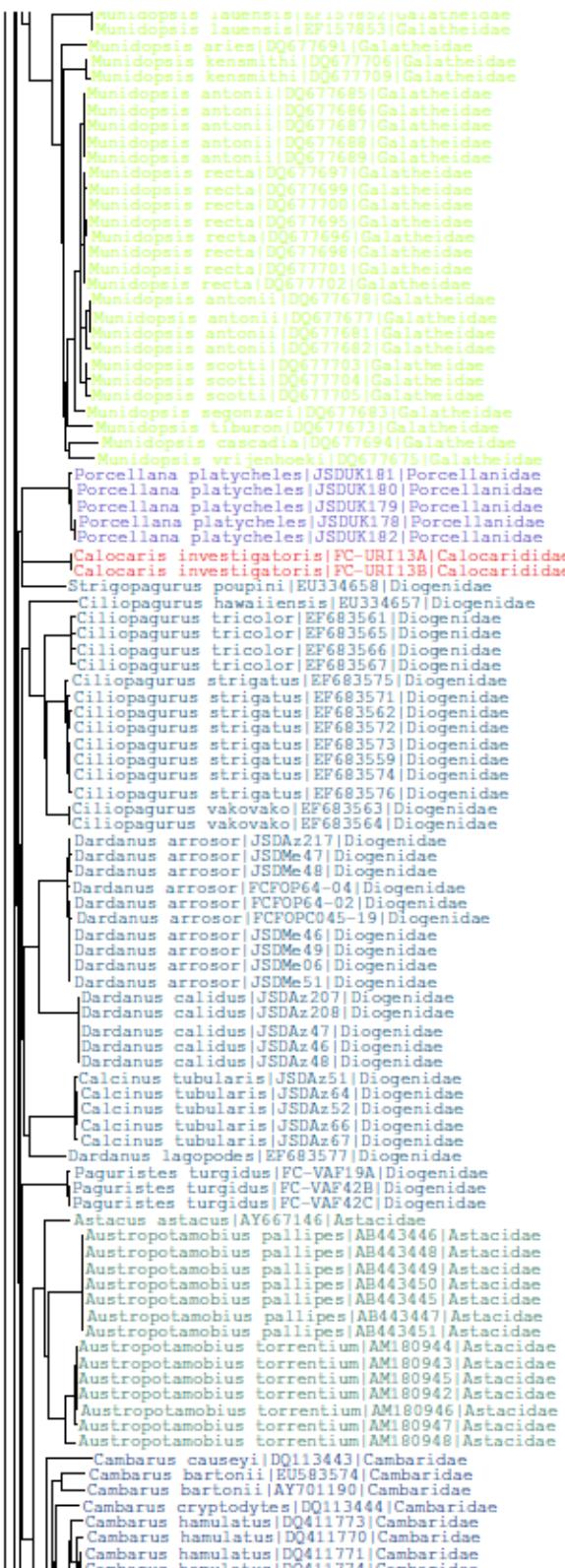


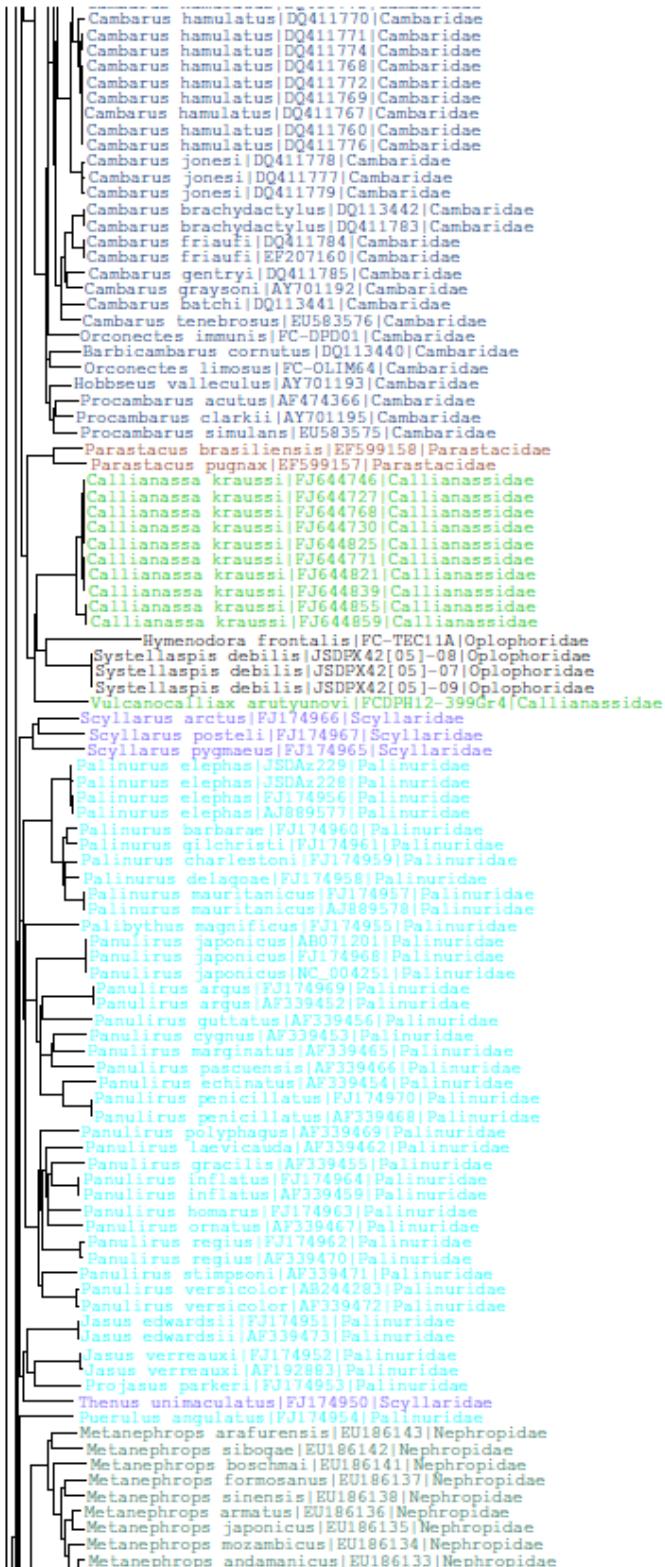
Section 3. Molecular evolution and shallow phylogenies



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Pagurus excavatus | FCOPB076-17 | Paguridae
Pagurus excavatus | JSDMe03 | Paguridae
Pagurus excavatus | JSDMe04 | Paguridae
Pagurus excavatus | JSDMe05 | Paguridae
Pagurus prideauxi | FCOPB071-10 | Paguridae
Pagurus prideauxi | FCOPB074-14 | Paguridae
Pagurus prideauxi | JSDUK147 | Paguridae
Pagurus prideauxi | JSDUK111 | Paguridae
Pagurus prideauxi | FCOPB042-18 | Paguridae
Pagurus prideauxi | FCOPB074-06 | Paguridae
Pagurus prideauxi | FCOPB071-12 | Paguridae
Pagurus prideauxi | FCOPB071-8 | Paguridae
Pagurus prideauxi | JSDUK159 | Paguridae
Upogebia africana | DQ351382 | Upogebiidae
Upogebia africana | DQ351380 | Upogebiidae
Upogebia africana | DQ351379 | Upogebiidae
Upogebia africana | DQ351381 | Upogebiidae
Upogebia africana | DQ351383 | Upogebiidae
Upogebia africana | DQ351384 | Upogebiidae
Upogebia africana | DQ351385 | Upogebiidae
Upogebia africana | DQ351386 | Upogebiidae
Upogebia africana | DQ351387 | Upogebiidae
Upogebia africana | DQ351388 | Upogebiidae
Clibanarius erythropus | JSDAz115 | Diogenidae
Clibanarius erythropus | JSDMe63 | Diogenidae
Clibanarius erythropus | JSDMe66 | Diogenidae
Clibanarius erythropus | JSDMe67 | Diogenidae
Pagurus longicarpus | L209AAR1-01 | Paguridae
Pagurus longicarpus | L154AAR1-08 | Paguridae
Pagurus longicarpus | L190AAR1-01 | Paguridae
Pagurus longicarpus | L183AAR1-03 | Paguridae
Pagurus longicarpus | L190AAR1-02 | Paguridae
Pagurus longicarpus | L183AAR1-02 | Paguridae
Pagurus longicarpus | L210AAR4-04 | Paguridae
Pagurus longicarpus | L228AAR1-01 | Paguridae
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Sumunida laevimana | EU243508 | Chirostyliidae
Sumunida laevimana | EU243509 | Chirostyliidae
Sumunida laevimana | EU243510 | Chirostyliidae
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Sumunida capillata | EU243343 | Chirostyliidae
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Sumunida annulosa | EU243496 | Chirostyliidae
Sumunida multilinearata | EU243546 | Chirostyliidae
Sumunida picta | EU243557 | Chirostyliidae
Sumunida picta | EU243558 | Chirostyliidae
Sumunida picta | EU243556 | Chirostyliidae
Sumunida similior | EU243490 | Chirostyliidae
Sumunida squamifera | EU243559 | Chirostyliidae
Sumunida squamifera | EU243560 | Chirostyliidae
Sumunida spinosa | EU243535 | Chirostyliidae
Sumunida spinosa | EU243534 | Chirostyliidae
Sumunida spinosa | EU243501 | Chirostyliidae
Sumunida spinosa | EU243537 | Chirostyliidae
Sumunida spinosa | EU243500 | Chirostyliidae
Sumunida spinosa | EU243540 | Chirostyliidae
Sumunida spinosa | EU243539 | Chirostyliidae
Sumunida spinosa | EU243536 | Chirostyliidae
Sumunida spinosa | EU243533 | Chirostyliidae
Sumunida spinosa | EU243542 | Chirostyliidae
Sumunida keijii | EU243337 | Chirostyliidae
Sumunida keijii | EU243338 | Chirostyliidae
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Sumunida keijii | EU243514 | Chirostyliidae
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Sumunida sternomaculata | AY800029 | Chirostyliidae
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Sumunida sternomaculata | EU243425 | Chirostyliidae
Sumunida sternomaculata | EU243483 | Chirostyliidae
Sumunida treguieri | EU243352 | Chirostyliidae
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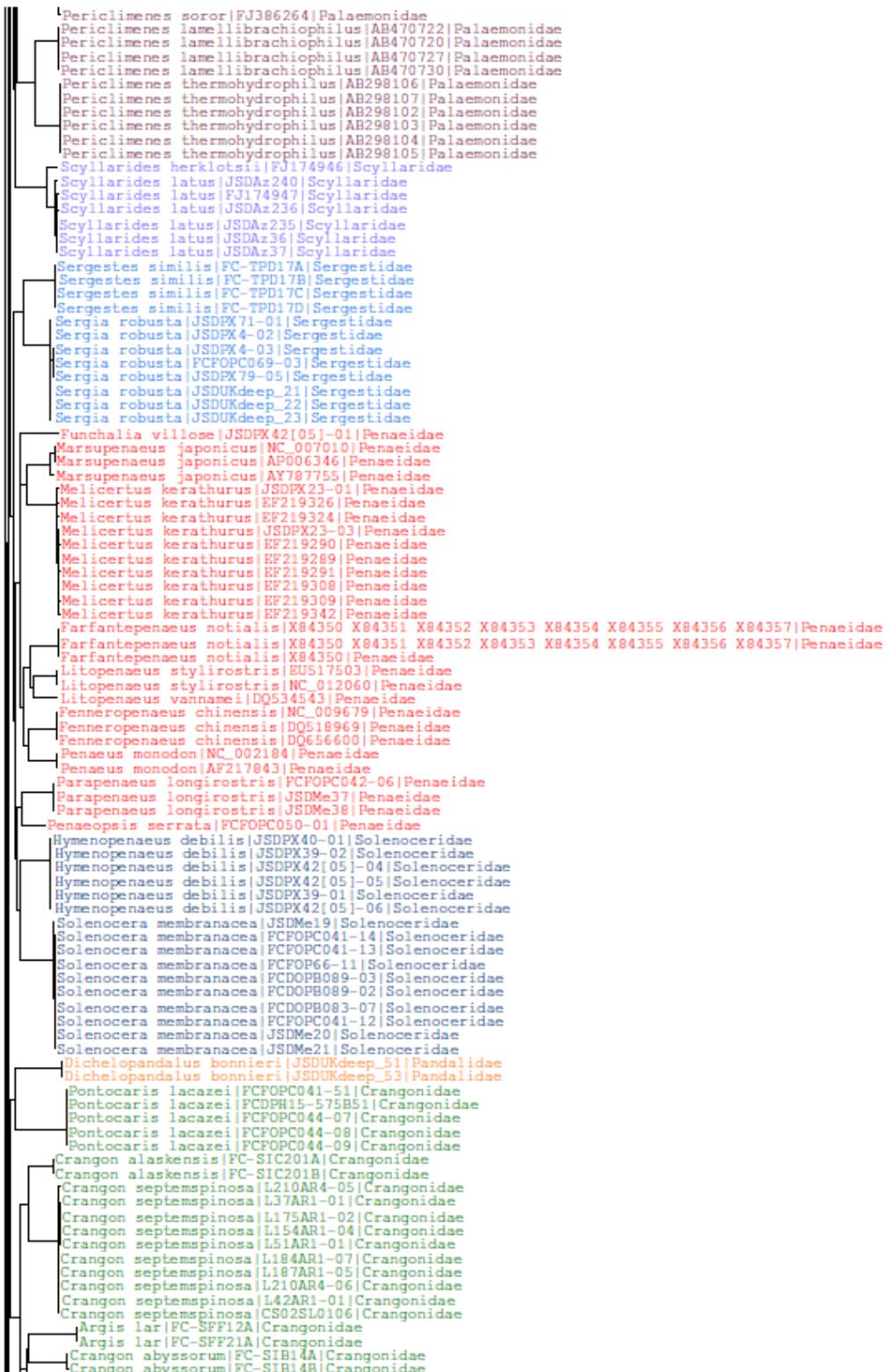




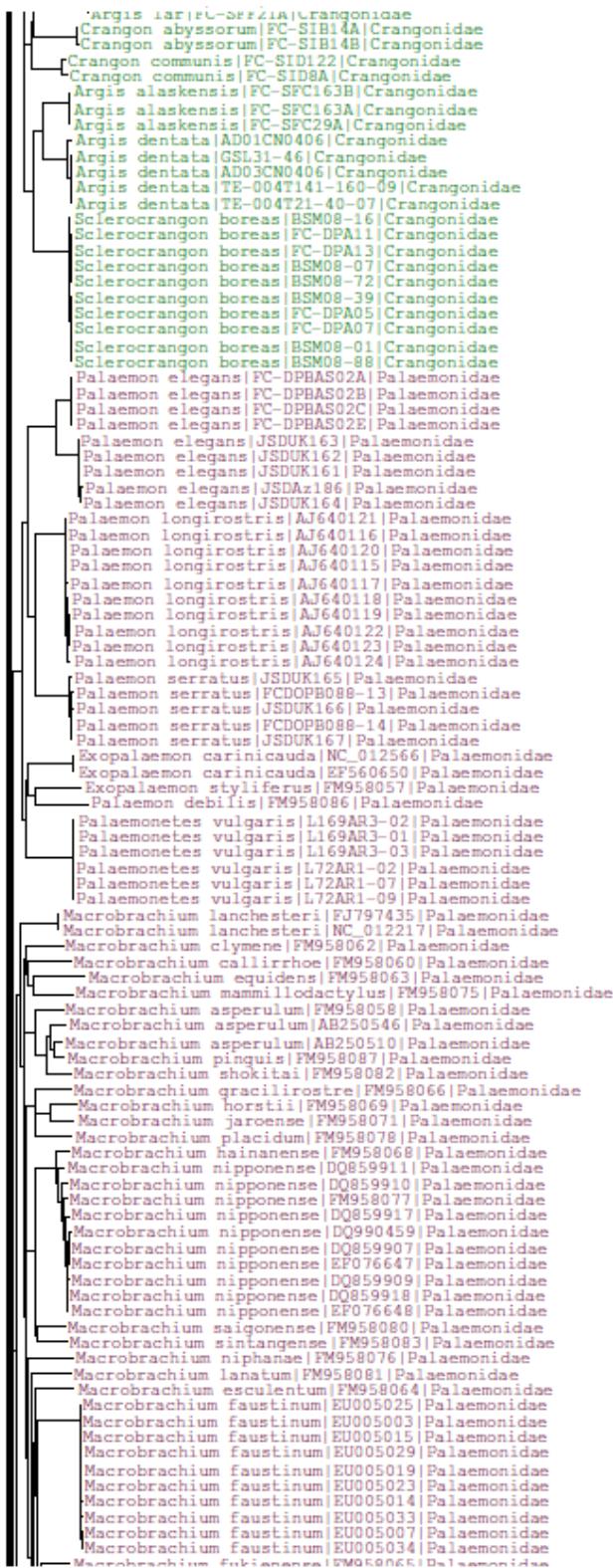


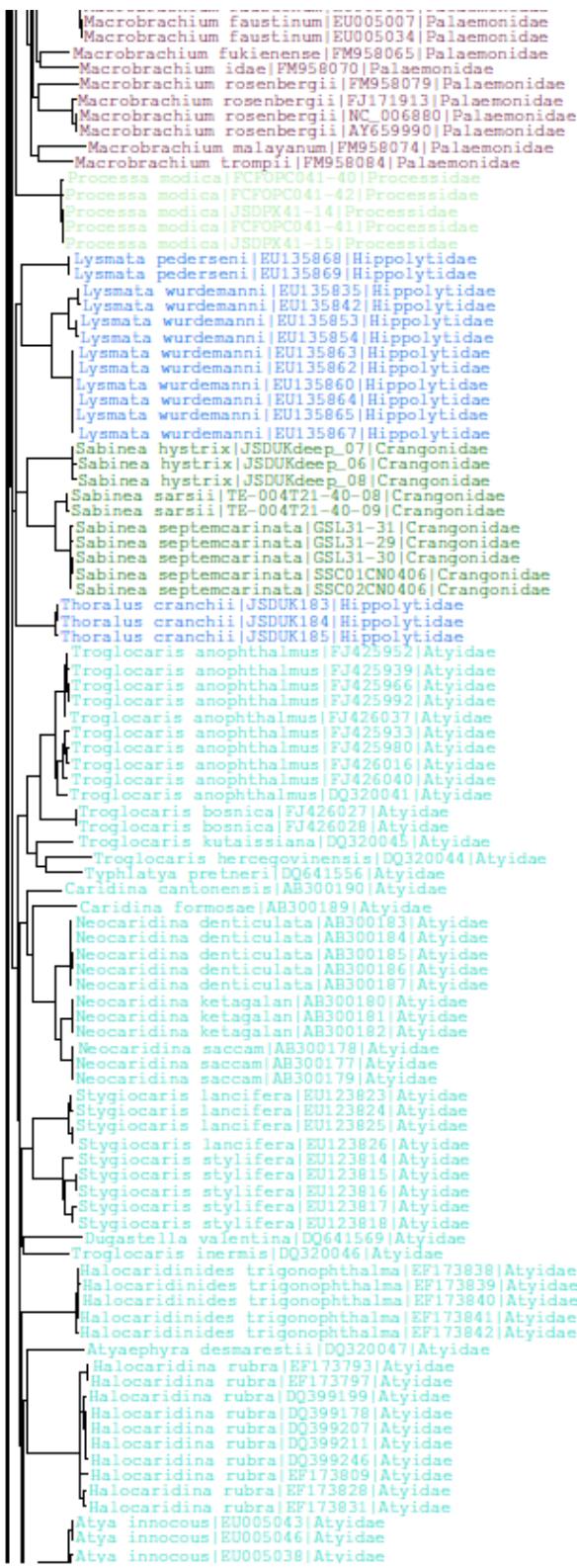
Section 3. Molecular evolution and shallow phylogenies

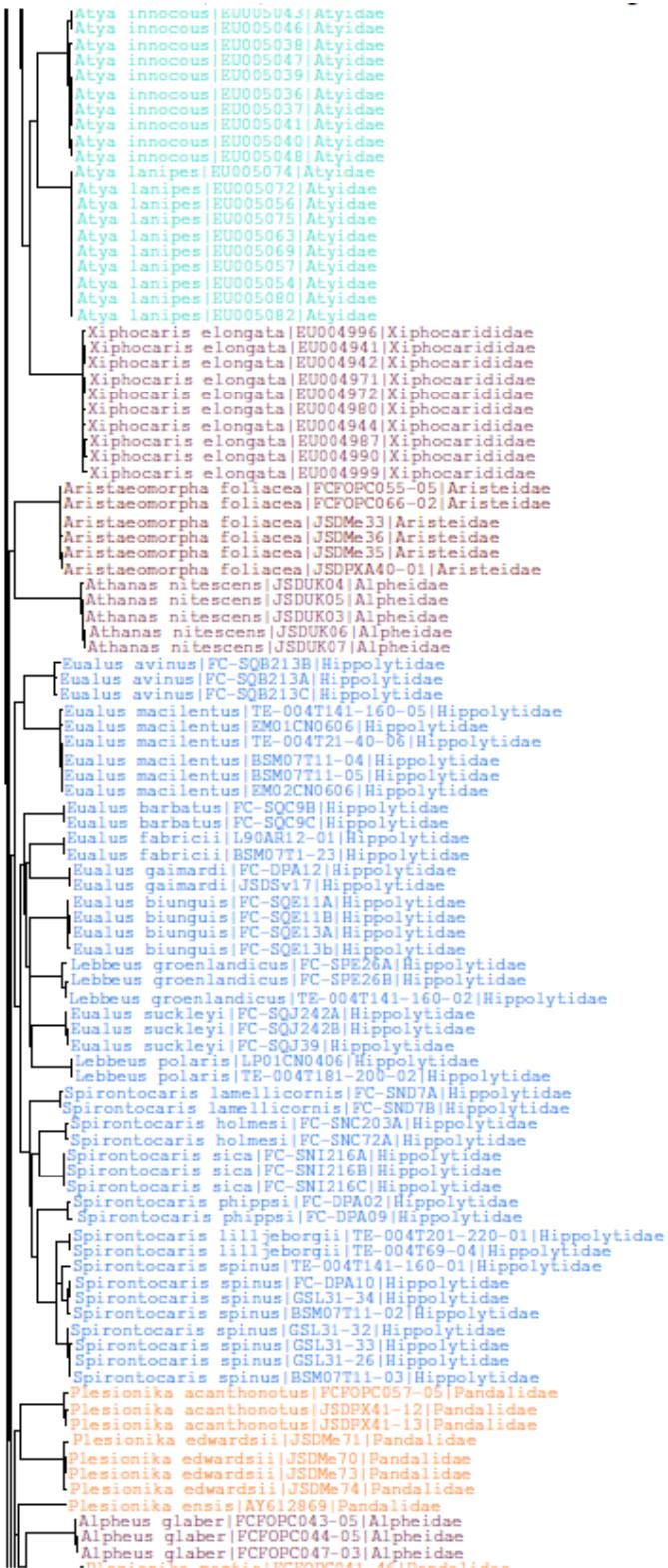




Section 3. Molecular evolution and shallow phylogenies







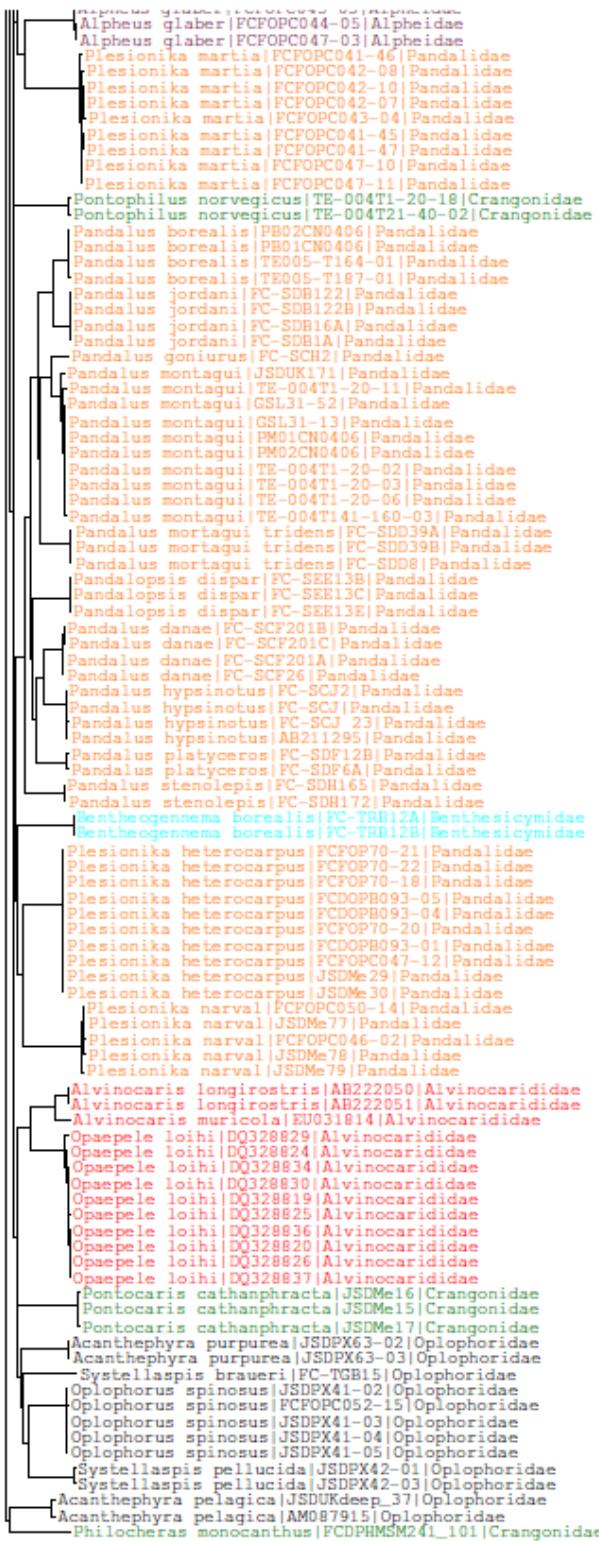


Figure S 3.1.1: Taxon ID Tree of Decapoda generated by BOLD. Neighbour Joining tree (Kimura 2-parameter, uniform rates among sites, pairwise deletion) combining COI data from public BOLD projects and present study. A total number of 1906 sequences from 603 species, 225 genera and 71 families were used (7 of November 2010).

Section 3. Molecular evolution and shallow phylogenies

Table S 3.1.1: Novel COI decapod barcodes generated by the present study.

Process ID	Family	Species	Region	Lat	Lon	Depth	COI
JSDSC022-09	Oplophoridae	<i>Acanthephyra pelagica</i>	North West St Kilda, Scotland	58.3	-9	-1700	656
JSDPX109-08	Oplophoridae	<i>Acanthephyra purpurea</i>	South coast Portugal	36.5	-9.1	-589	627
JSDPX110-08	Oplophoridae	<i>Acanthephyra purpurea</i>	South coast Portugal	36.5	-9.1	-589	604
JSDME059-08	Majidae	<i>Acanthonyx lunulatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	569
JSDME061-08	Majidae	<i>Acanthonyx lunulatus</i>	Stretto di Sicilia, Sicily	37.3	13.5	-1	657
FCDOP164-07	Alpheidae	<i>Alpheus glaber</i>	South coast Portugal	36.8	-7.6	-538	548
FCDOP171-07	Alpheidae	<i>Alpheus glaber</i>	South coast Portugal	36.9	-7.5	-425	579
FCDOP199-07	Alpheidae	<i>Alpheus glaber</i>	South coast Portugal	36.8	-7.5	-538	520
FCDOP283-07	Aristeidae	<i>Aristeomorpha foliacea</i>	South coast Portugal	36.5	-7.8	-742	651
FCDOP301-07	Aristeidae	<i>Aristeomorpha foliacea</i>	South coast Portugal	37	-9	-515	651
JSDME033-08	Aristeidae	<i>Aristeomorpha foliacea</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	664
JSDME035-08	Aristeidae	<i>Aristeomorpha foliacea</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	627
JSDME036-08	Aristeidae	<i>Aristeomorpha foliacea</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	532
JSDPX142-08	Aristeidae	<i>Aristeomorpha foliacea</i>	South coast Portugal	37.3	-9.2	-719	587
FCDOP051-07	Atelecyclidae	<i>Atelecyclus rotundatus</i>	West coast Portugal	41	-10	-81	617
JSDPX028-08	Atelecyclidae	<i>Atelecyclus rotundatus</i>	South coast Portugal	37.2	-9	-140	608
JSDUK001-08	Atelecyclidae	<i>Atelecyclus rotundatus</i>	Irish Sea, England	53.6	-3.4	-16	554
JSDUK002-08	Atelecyclidae	<i>Atelecyclus rotundatus</i>	Irish Sea, England	53.6	-3.4	-16	630
JSDUK003-08	Alpheidae	<i>Athanas nitescens</i>	West coast Anglesey, Wales	53.2	-4.5	-1	655
JSDUK004-08	Alpheidae	<i>Athanas nitescens</i>	West coast Anglesey, Wales	53.2	-4.5	-1	656
JSDUK005-08	Alpheidae	<i>Athanas nitescens</i>	West coast Anglesey, Wales	53.2	-4.5	-1	602
JSDUK006-08	Alpheidae	<i>Athanas nitescens</i>	West coast Anglesey, Wales	53.2	-4.5	-1	536
JSDUK007-08	Alpheidae	<i>Athanas nitescens</i>	West coast Anglesey, Wales	53.2	-4.5	-1	599
FCDPH001-07	Portunidae	<i>Bathynectes maravigna</i>	Gulf of Cadiz	36.1	-7.8	-1086	640
FCDPH039-07	Portunidae	<i>Bathynectes maravigna</i>	Gulf of Cadiz	36.1	-7.4	-1080	640
FCDPH040-07	Portunidae	<i>Bathynectes maravigna</i>	Gulf of Cadiz	36.1	-7.4	-1080	621
FCDPH078-07	Portunidae	<i>Bathynectes maravigna</i>	Gulf of Cadiz				607
FCDOP108-07	Portunidae	<i>Bathynectes maravigna</i>	South coast Portugal	36.8	-7.8	-553	540
FCDOP110-07	Portunidae	<i>Bathynectes maravigna</i>	South coast Portugal	36.8	-7.8	-553	519
JSDSC027-09	Portunidae	<i>Bathynectes maravigna</i>	South Donegal, Scotland	55.8	-10	-500	656
JSDSC028-09	Portunidae	<i>Bathynectes maravigna</i>	South Donegal, Scotland	55.8	-10	-500	656
JSDSC029-09	Portunidae	<i>Bathynectes maravigna</i>	South Donegal, Scotland	55.8	-10	-500	522
JSDAZ204-08	Calappidae	<i>Calappa granulata</i>	North coast San Miguel, Azores	37.8	-26	-100	632
JSDAZ051-08	Diogenidae	<i>Calcinus tubularis</i>	East coast Flores, Azores	39.5	-31	-1	607
JSDAZ052-08	Diogenidae	<i>Calcinus tubularis</i>	East coast Flores, Azores	39.5	-31	-1	633
JSDAZ064-08	Diogenidae	<i>Calcinus tubularis</i>	East coast Flores, Azores	39.5	-31	-1	677
JSDAZ066-08	Diogenidae	<i>Calcinus tubularis</i>	East coast Flores, Azores	39.5	-31	-1	612
JSDAZ067-08	Diogenidae	<i>Calcinus tubularis</i>	East coast Flores, Azores	39.5	-31	-1	643
JSDPX107-08	Cancridae	<i>Cancer bellianus</i>	South coast Portugal	37.5	-9.2	-612	614
JSDUK010-08	Cancridae	<i>Cancer pagurus</i>	Irish Sea, England	54.2	-3.4	-9	662
JSDUK011-08	Cancridae	<i>Cancer pagurus</i>	Irish Sea, England	54.4	-3.6	-19	674
JSDN001-08	Portunidae	<i>Carcinus maenas</i>	Tromso, Norway	69.6	18.9	-1	629
JSDN002-08	Portunidae	<i>Carcinus maenas</i>	Tromso, Norway	69.6	18.9	-1	663
JSDN003-08	Portunidae	<i>Carcinus maenas</i>	Tromso, Norway	69.6	18.9	-1	645
JSDUK016-08	Portunidae	<i>Carcinus maenas</i>	Irish Sea, England	54.6	-3.7	-19	690
JSDUK019-08	Portunidae	<i>Carcinus maenas</i>	Irish Sea, England	54.6	-3.7	-19	580
JSDAZ115-08	Diogenidae	<i>Clibanarius erythropus</i>	South coast San Miguel, Azores	37.7	-26	-1	596
JSDME063-08	Diogenidae	<i>Clibanarius erythropus</i>	Scala dei Turrchi, Sicily	37.3	13.5	-1	619
JSDME066-08	Diogenidae	<i>Clibanarius erythropus</i>	Scala dei Turrchi, Sicily	37.3	13.5	-1	656
JSDME067-08	Diogenidae	<i>Clibanarius erythropus</i>	Scala dei Turrchi, Sicily	37.3	13.5	-1	591
JSDUK022-08	Corystidae	<i>Coryistes cassivelaunus</i>	Irish Sea, England	53.7	-3.2	-13	636
JSDUK023-08	Corystidae	<i>Coryistes cassivelaunus</i>	Irish Sea, England	54.3	-3.7	-31	661
JSDUK024-08	Corystidae	<i>Coryistes cassivelaunus</i>	Irish Sea, England	54.3	-3.7	-31	662
FCDPH028-07	Cymonomidae	<i>Cymonomus granulatus</i>	Gulf of Cadiz	34.6	-7.4	-700	640
FCDPH029-07	Cymonomidae	<i>Cymonomus granulatus</i>	Gulf of Cadiz	34.6	-7.4	-700	614
FCDPH030-07	Cymonomidae	<i>Cymonomus granulatus</i>	Gulf of Cadiz	34.6	-7.4	-700	640

FCDPH031-07	Cyromonomidae	<i>Cyromonomus granulatus</i>	Gulf of Cadiz	34.6	-7.4	-700	597
FCDPH074-07	Cyromonomidae	<i>Cyromonomus granulatus</i>	Gulf of Cadiz	35.2	-6.4	-353	510
FCDPH075-07	Cyromonomidae	<i>Cyromonomus granulatus</i>	Gulf of Cadiz	35.2	-6.4	-353	600
FCDOP015-07	Diogenidae	<i>Dardanus arrosor</i>	West coast Portugal	41	-8.9	-46	658
FCDOP017-07	Diogenidae	<i>Dardanus arrosor</i>	West coast Portugal	41	-8.9	-46	658
FCDOP191-07	Diogenidae	<i>Dardanus arrosor</i>	South coast Portugal	37	-7.5	-109	619
JSDAZ203-08	Diogenidae	<i>Dardanus arrosor</i>	North coast San Miguel, Azores	37.8	-26	-100	656
JSDME006-08	Diogenidae	<i>Dardanus arrosor</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME007-08	Diogenidae	<i>Dardanus arrosor</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME046-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	657
JSDME047-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	619
JSDME048-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	621
JSDME049-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	656
JSDME050-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	656
JSDME051-08	Diogenidae	<i>Dardanus arrosor</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	656
JSDAZ193-08	Diogenidae	<i>Dardanus calidus</i>	South East coast San Miguel, Azores	37.7	-25	-40	667
JSDAZ194-08	Diogenidae	<i>Dardanus calidus</i>	South East coast San Miguel, Azores	37.7	-25	-20	663
JSDAZ046-08	Diogenidae	<i>Dardanus calidus</i>	South coast Flores, Azores	39.4	-31	-1	610
JSDAZ047-08	Diogenidae	<i>Dardanus calidus</i>	East coast Flores, Azores	39.5	-31	-1	620
JSDAZ048-08	Diogenidae	<i>Dardanus calidus</i>	East coast Flores, Azores	39.5	-31	-1	620
JSDSC030-09	Pandalidae	<i>Dichelopandalus bonnieri</i>	Central Vidal, Scotland	56.1	-9	-500	656
JSDSC032-09	Pandalidae	<i>Dichelopandalus bonnieri</i>	Central Vidal, Scotland	56.1	-9	-500	656
JSDAZ116-08	Dromiidae	<i>Dromia personata</i>	South coast San Miguel, Azores	37.7	-26	-1	672
JSDAZ185-08	Dromiidae	<i>Dromia personata</i>	South East coast San Miguel, Azores	37.7	-25	-30	672
FCDPH036-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.2	-6.5	-544	613
FCDPH014-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.3	-6.7	-413	640
FCDPH037-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.2	-6.4	-414	658
FCDPH038-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.2	-6.4	-358	622
FCDPH072-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.2	-6.4	-353	558
FCDPH073-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	35.2	-6.4	-353	595
FCDPH066-07	Leucosiidae	<i>Ebalia nux</i>	Gulf of Cadiz	34.6	-7	-732	591
JSDAZ012-08	Xanthidae	<i>Eriphia verrucosa</i>	East coast Flores, Azores	39.5	-31	-1	609
JSDAZ013-08	Xanthidae	<i>Eriphia verrucosa</i>	East coast Flores, Azores	39.5	-31	-1	637
JSDAZ014-08	Xanthidae	<i>Eriphia verrucosa</i>	East coast Flores, Azores	39.5	-31	-1	635
JSDAZ015-08	Xanthidae	<i>Eriphia verrucosa</i>	East coast Flores, Azores	39.5	-31	-1	611
JSDAZ031-08	Xanthidae	<i>Eriphia verrucosa</i>	East coast Flores, Azores	39.5	-31	-1	600
JSDAZ089-08	Xanthidae	<i>Eriphia verrucosa</i>	South coast San Miguel, Azores	37.7	-26	-1	671
JSDAZ090-08	Xanthidae	<i>Eriphia verrucosa</i>	South coast San Miguel, Azores	37.7	-26	-1	671
JSDAZ092-08	Xanthidae	<i>Eriphia verrucosa</i>	South coast San Miguel, Azores	37.7	-26	-1	674
JSDSV017-09	Hippolytidae	<i>Eualus gaimardi</i>	Kongsfjorden, Svalbard	78.9	11.9	-14	671
JSDPX073-08	Benthescycimidae	<i>Funchalia villosa</i>	South coast Portugal	36.4	-7.6	-714	610
JSDUK030-08	Galatheidae	<i>Galathea dispersa</i>	Irish Sea, England	54.4	-5.1	-116	670
FCDOP246-07	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	36.6	-8	-752	656
FCDOP247-07	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	36.6	-8	-752	651
FCDOP248-07	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	36.6	-8	-752	652
FCDOP249-07	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	36.6	-8	-752	656
FCDOP250-07	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	36.6	-8	-752	651
JSDME082-08	Geryonidae	<i>Geryon longipes</i>	Castelammare del Golfo, Sicily	35.7	14	-605	672
JSDME083-08	Geryonidae	<i>Geryon longipes</i>	Castelammare del Golfo, Sicily	35.7	14.1	-670	673
JSDPX095-08	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	37.5	-9.2	-612	586
JSDPX096-08	Geryonidae	<i>Geryon longipes</i>	South coast Portugal	37.5	-9.2	-612	671
JSDSC018-09	Geryonidae	<i>Geryon longipes</i>	North West St Kilda, Scotland	58.2	-9	-600	619
JSDSC019-09	Geryonidae	<i>Geryon longipes</i>	North West St Kilda, Scotland	58.2	-9	-600	591
JSDSC020-09	Geryonidae	<i>Geryon longipes</i>	North West St Kilda, Scotland	58.2	-9	-600	619
FCDOP056-07	Goneplacidae	<i>Goneplax rhomboides</i>	West coast Portugal	41.3	-9	-79	620
FCDOP057-07	Goneplacidae	<i>Goneplax rhomboides</i>	West coast Portugal	41.3	-9	-79	621
FCDPH035-07	Goneplacidae	<i>Goneplax rhomboides</i>	Gulf of Cadiz	35.2	-6.4	-355	640
FCDOP019-07	Goneplacidae	<i>Goneplax rhomboides</i>	West coast Portugal	40.8	-9.2	-133	658
FCDOP020-07	Goneplacidae	<i>Goneplax rhomboides</i>	West coast Portugal	41	-10	-81	658
FCDOP113-07	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.8	-7.8	-553	604

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FCDOP114-07	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.8	-7.8	-553	597
FCDOP115-07	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.8	-7.8	-553	538
FCDOP116-07	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.8	-7.8	-553	602
FCDOP117-07	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.8	-7.8	-553	603
JSDME022-08	Goneplacidae	<i>Goneplax rhomboides</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	638
JSDPX035-08	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.4	-7.5	-751	582
JSDPX036-08	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.4	-7.5	-751	610
JSDPX112-08	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.5	-9.1	-589	556
JSDPX113-08	Goneplacidae	<i>Goneplax rhomboides</i>	South coast Portugal	36.5	-9.1	-589	591
JSDUK037-08	Goneplacidae	<i>Goneplax rhomboides</i>	Irish Sea, England	53.7	-3.2	-13	605
JSDUK038-08	Goneplacidae	<i>Goneplax rhomboides</i>	Irish Sea, England	53.7	-3.2	-13	623
JSDUK039-08	Goneplacidae	<i>Goneplax rhomboides</i>	Irish Sea, England	53.7	-3.2	-13	603
JSDUK040-08	Goneplacidae	<i>Goneplax rhomboides</i>	Irish Sea, England	53.7	-3.2	-13	605
JSDUK041-08	Goneplacidae	<i>Goneplax rhomboides</i>	Irish Sea, England	54.3	-3.7	-31	513
JSDAZ028-08	Grapsidae	<i>Grapsus adscensionis</i>	East coast Flores, Azores	39.5	-31	-1	657
JSDAZ029-08	Grapsidae	<i>Grapsus adscensionis</i>	East coast Flores, Azores	39.5	-31	-1	604
JSDAZ030-08	Grapsidae	<i>Grapsus adscensionis</i>	East coast Flores, Azores	39.5	-31	-1	557
JSDAZ087-08	Majidae	<i>Herbstia condylata</i>	South coast San Miguel, Azores	37.7	-26	-1	618
JSDAZ088-08	Majidae	<i>Herbstia condylata</i>	South coast San Miguel, Azores	37.7	-26	-1	648
FCDPH064-07	Homolidae	<i>Homola barbata</i>	Gulf of Cadiz	35.2	-6.4	-350	579
JSDPX149-08	Homolidae	<i>Homola barbata</i>	South coast Portugal	37.3	-9.1	-376	625
JSDSV007-09	Majidae	<i>Hyas araneus</i>	Kongsfjorden, Svalbard	78.9	11.9	-14	615
JSDSV008-09	Majidae	<i>Hyas araneus</i>	Kongsfjorden, Svalbard	78.9	11.9	-14	677
JSDUK175-08	Majidae	<i>Hyas coarctatus</i>	North coast Anglesey, Wales	53.4	-4.3		650
JSDUK176-08	Majidae	<i>Hyas coarctatus</i>	North coast Anglesey, Wales	53.4	-4.3		614
JSDPX038-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	37.3	-9.1	-342	610
JSDPX039-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	37.3	-9.1	-342	610
JSDPX043-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	36.5	-8	-706	522
JSDPX076-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	36.4	-7.5	-751	610
JSDPX077-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	36.4	-7.5	-751	610
JSDPX078-08	Solenoceridae	<i>Hymenopenaeus debilis</i>	South coast Portugal	36.4	-7.5	-751	608
FCDOP040-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	39.8	-9.2	-76	658
FCDOP041-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP042-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP053-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	41	-10	-81	658
FCDOP009-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	41	-8.9	-46	658
FCDOP010-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	41	-8.9	-46	658
FCDOP012-07	Inachidae	<i>Inachus dorsettensis</i>	West coast Portugal	41	-8.9	-46	658
FCDOP185-07	Inachidae	<i>Inachus dorsettensis</i>	South coast Portugal	37	-7.5	-109	617
FCDOP187-07	Inachidae	<i>Inachus dorsettensis</i>	South coast Portugal	37	-7.5	-109	653
JSDUK046-08	Inachidae	<i>Inachus dorsettensis</i>	Irish Sea, England	53.3	-5.5	-83	634
FCDOP292-07	Inachidae	<i>Inachus leptochirius</i>	South coast Portugal	36.6	-7.8	-758	606
JSDPX032-08	Laomediidae	<i>Jaxea nocturna</i>	Coast of Portugal				664
FCDOP216-07	Latreilliidae	<i>Latreillia elegans</i>	South coast Portugal	36.6	-7.6	-543	661
JSDME018-08	Latreilliidae	<i>Latreillia elegans</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	607
JSDPX056-08	Latreilliidae	<i>Latreillia elegans</i>	South coast Portugal	37.6	-9.1	-548	603
JSDPX105-08	Latreilliidae	<i>Latreillia elegans</i>	South coast Portugal	37.5	-9.2	-612	661
FCDOP054-07	Portunidae	<i>Liocarcinus depurator</i>	West coast Portugal	41	-10	-81	658
FCDOP111-07	Portunidae	<i>Liocarcinus depurator</i>	South coast Portugal	36.8	-7.8	-553	657
FCDOP112-07	Portunidae	<i>Liocarcinus depurator</i>	South coast Portugal	36.8	-7.8	-553	612
JSDME001-08	Portunidae	<i>Liocarcinus depurator</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME002-08	Portunidae	<i>Liocarcinus depurator</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	605
JSDUK051-08	Portunidae	<i>Liocarcinus depurator</i>	Irish Sea, England	53.7	-3.2	-13	661
JSDUK052-08	Portunidae	<i>Liocarcinus depurator</i>	Irish Sea, England	53.7	-3.2	-13	662
JSDUK053-08	Portunidae	<i>Liocarcinus depurator</i>	Irish Sea, England	53.7	-3.2	-13	670
FCDOP013-07	Portunidae	<i>Macropipus tuberculatus</i>	West coast Portugal	41	-8.9	-46	516
FCDOP182-07	Portunidae	<i>Macropipus tuberculatus</i>	South coast Portugal	37	-7.5	-109	554
JSDME009-08	Portunidae	<i>Macropipus tuberculatus</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	529
JSDME041-08	Portunidae	<i>Macropipus tuberculatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	597
JSDME042-08	Portunidae	<i>Macropipus tuberculatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	618

JSDME043-08	Portunidae	<i>Macropipus tuberculatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	576
JSDME044-08	Portunidae	<i>Macropipus tuberculatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	572
JSDME045-08	Portunidae	<i>Macropipus tuberculatus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	520
JSDPX136-08	Portunidae	<i>Macropipus tuberculatus</i>	South coast Portugal	37.4	-9.1	-472	600
FCDOP033-07	Majidae	<i>Macropodia longipes</i>	West coast Portugal	41.7	-9.3	-173	633
FCDOP064-07	Majidae	<i>Macropodia longipes</i>	West coast Portugal	41.7	-9.3	-173	658
FCDOP066-07	Majidae	<i>Macropodia longipes</i>	West coast Portugal	41.6	-9	-78	658
FCDOP068-07	Majidae	<i>Macropodia longipes</i>	West coast Portugal	41.5	-9.2	-132	658
FCDOP030-07	Majidae	<i>Macropodia longipes</i>	West coast Portugal	41.3	-9	-79	658
JSDUK107-08	Majidae	<i>Macropodia rostrata</i>	Irish Sea, England	53.7	-3.2	-13	542
JSDUK108-08	Majidae	<i>Macropodia rostrata</i>	Irish Sea, England	54.6	-3.7	-19	622
JSDUK110-08	Majidae	<i>Macropodia rostrata</i>	Irish Sea, England	54.6	-3.7	-19	620
FCDOP184-07	Majidae	<i>Macropodia tenuirostris</i>	South coast Portugal	37	-7.5	-109	608
JSDME025-08	Majidae	<i>Macropodia tenuirostris</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME026-08	Majidae	<i>Macropodia tenuirostris</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	555
JSDME027-08	Majidae	<i>Macropodia tenuirostris</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	626
JSDPX037-08	Majidae	<i>Macropodia tenuirostris</i>	South coast Portugal	37.3	-9.1	-342	609
JSDPX145-08	Majidae	<i>Macropodia tenuirostris</i>	South coast Portugal	37.4	-9.1	-548	627
JSDPX146-08	Majidae	<i>Macropodia tenuirostris</i>	South coast Portugal	37.4	-9.1	-423	609
JSDAZ188-08	Majidae	<i>Maja brachydactyla</i>	South East coast San Miguel, Azores	37.7	-25	-30	580
JSDAZ191-08	Majidae	<i>Maja brachydactyla</i>	South East coast San Miguel, Azores	37.7	-25	-80	631
JSDUK121-08	Majidae	<i>Maja brachydactyla</i>	Irish Sea, England	54.4	-3.6	-19	672
JSDUK122-08	Majidae	<i>Maja brachydactyla</i>	Irish Sea, England	53.3	-5.5	-83	664
JSDUK123-08	Majidae	<i>Maja brachydactyla</i>	Irish Sea, England	53.3	-5.5	-83	664
JSDPX019-08	Majidae	<i>Maja goltzianna</i>	South coast Portugal	36.6	-7.4	-176	622
JSDPX141-08	Majidae	<i>Maja goltzianna</i>	South coast Portugal	37.3	-8.6	-131	611
JSDME012-08	Dorippidae	<i>Medorippe lanata</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME013-08	Dorippidae	<i>Medorippe lanata</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	589
JSDME081-08	Dorippidae	<i>Medorippe lanata</i>	Castelammare del Golfo, Sicily	36.4	15.2	-91	633
JSDPX029-08	Penaeidae	<i>Melicertus kerathurus</i>	Coast of Portugal				610
JSDPX030-08	Penaeidae	<i>Melicertus kerathurus</i>	Coast of Portugal				611
JSDPX031-08	Penaeidae	<i>Melicertus kerathurus</i>	Coast of Portugal				650
FCDPH047-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.3	-6.4	-489	614
FCDPH051-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.3	-6.4	-489	601
FCDPH008-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.4	-6.7	-483	640
FCDPH053-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.4	-6.3	-393	640
FCDPH055-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.2	-6.3	-552	640
FCDPH013-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.3	-6.7	-457	600
FCDPH079-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.3	-6.8	-498	613
FCDPH067-07	Xanthidae	<i>Monodaeus couchii</i>	Gulf of Cadiz	35.2	-6.4	-350	628
JSDPX033-08	Xanthidae	<i>Monodaeus couchii</i>	South coast Portugal	36.5	-7.4	-561	613
JSDPX034-08	Xanthidae	<i>Monodaeus couchii</i>	South coast Portugal	36.5	-7.4	-561	619
FCDPH087-07	Galatheidae	<i>Munida intermedia</i>	Gulf of Cadiz	35.2	-6.5	-622	547
FCDOP063-07	Galatheidae	<i>Munida rugosa</i>	West coast Portugal	41.7	-9.3	-173	640
FCDOP176-07	Galatheidae	<i>Munida rutillanti</i>	South coast Portugal	37	-7.5	-109	648
FCDOP177-07	Galatheidae	<i>Munida rutillanti</i>	South coast Portugal	37	-7.5	-109	648
FCDOP178-07	Galatheidae	<i>Munida rutillanti</i>	South coast Portugal	37	-7.5	-109	627
FCDOP179-07	Galatheidae	<i>Munida rutillanti</i>	South coast Portugal	37	-7.5	-109	656
FCDOP180-07	Galatheidae	<i>Munida rutillanti</i>	South coast Portugal	37	-7.5	-109	656
JSDME053-08	Galatheidae	<i>Munida rutillanti</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	541
JSDME054-08	Galatheidae	<i>Munida rutillanti</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	570
JSDPX151-08	Portunidae	<i>Necora puber</i>	West coast Portugal	39.2	-9.3	-33	608
JSDPX152-08	Portunidae	<i>Necora puber</i>	West coast Portugal	39.2	-9.3	-33	610
JSDPX153-08	Portunidae	<i>Necora puber</i>	West coast Portugal	39.2	-9.3	-33	610
JSDPX154-08	Portunidae	<i>Necora puber</i>	West coast Portugal	39.2	-9.3	-33	582
JSDPX155-08	Portunidae	<i>Necora puber</i>	West coast Portugal	39.2	-9.3	-33	611
JSDUK124-08	Portunidae	<i>Necora puber</i>	West coast Anglesey, Wales	53.2	-4.5	-1	655
JSDUK127-08	Portunidae	<i>Necora puber</i>	Irish Sea, England	54.6	-3.7	-19	623
JSDSC001-09	Lithodidae	<i>Neolithodes grimaldii</i>	Central Vidal, Scotland	56.1	-9	-1500	655
JSDSC002-09	Lithodidae	<i>Neolithodes grimaldii</i>	Central Vidal, Scotland	56.1	-9	-1500	656

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JSDSC003-09	Lithodidae	<i>Neolithodes grimaldii</i>	Central Vidal, Scotland	56.1	-9	-1500	656
FCDOP079-07	Nephropidae	<i>Nephrops norvegicus</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP080-07	Nephropidae	<i>Nephrops norvegicus</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP081-07	Nephropidae	<i>Nephrops norvegicus</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP094-07	Nephropidae	<i>Nephrops norvegicus</i>	South coast Portugal	36.8	-7.8	-553	658
FCDOP095-07	Nephropidae	<i>Nephrops norvegicus</i>	South coast Portugal	36.8	-7.8	-553	658
FCDOP096-07	Nephropidae	<i>Nephrops norvegicus</i>	South coast Portugal	36.8	-7.8	-553	658
FCDOP097-07	Nephropidae	<i>Nephrops norvegicus</i>	South coast Portugal	36.8	-7.8	-553	658
JSDSC010-09	Nephropidae	<i>Nephropsis atlantica</i>	Central Vidal, Scotland	56.2	-9	-1000	585
JSDSC011-09	Nephropidae	<i>Nephropsis atlantica</i>	Central Vidal, Scotland	56.2	-9	-1000	649
JSDSC012-09	Nephropidae	<i>Nephropsis atlantica</i>	Central Vidal, Scotland	56.2	-9	-1000	649
FCDOP256-07	Ophophoridae	<i>Ophophorus spinosus</i>	South coast Portugal	36.6	-8	-752	571
JSDPX057-08	Ophophoridae	<i>Ophophorus spinosus</i>	South coast Portugal	37.6	-9.1	-548	584
JSDPX058-08	Ophophoridae	<i>Ophophorus spinosus</i>	South coast Portugal	36.4	-7.6	-714	614
JSDPX059-08	Ophophoridae	<i>Ophophorus spinosus</i>	South coast Portugal	36.4	-7.6	-714	614
JSDPX060-08	Ophophoridae	<i>Ophophorus spinosus</i>	South coast Portugal	36.4	-7.6	-714	609
JSDAZ002-08	Grapsidae	<i>Pachygrapsus marmoratus</i>	East coast Flores, Azores	39.5	-31	-1	628
JSDAZ003-08	Grapsidae	<i>Pachygrapsus marmoratus</i>	East coast Flores, Azores	39.5	-31	-1	600
JSDAZ008-08	Grapsidae	<i>Pachygrapsus marmoratus</i>	East coast Flores, Azores	39.5	-31	-1	632
JSDAZ009-08	Grapsidae	<i>Pachygrapsus marmoratus</i>	East coast Flores, Azores	39.5	-31	-1	620
JSDME062-08	Grapsidae	<i>Pachygrapsus marmoratus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	626
JSDAZ005-08	Grapsidae	<i>Pachygrapsus maurus</i>	East coast Flores, Azores	39.5	-31	-1	674
JSDAZ044-08	Grapsidae	<i>Pachygrapsus maurus</i>	West coast Flores, Azores	39.5	-31	-1	639
FCDOP160-07	Paguridae	<i>Pagurus alatus</i>	South coast Portugal	36.8	-7.6	-538	614
FCDOP197-07	Paguridae	<i>Pagurus alatus</i>	South coast Portugal	36.8	-7.5	-538	659
FCDOP200-07	Paguridae	<i>Pagurus alatus</i>	South coast Portugal	36.8	-7.5	-538	530
FCDOP252-07	Paguridae	<i>Pagurus alatus</i>	South coast Portugal	36.6	-8	-752	659
FCDOP280-07	Paguridae	<i>Pagurus alatus</i>	South coast Portugal	36.5	-7.8	-735	520
JSDUK145-08	Paguridae	<i>Pagurus bernhardus</i>	Irish Sea, England	54.4	-3.7	-30	675
JSDUK146-08	Paguridae	<i>Pagurus bernhardus</i>	Irish Sea, England	54.2	-4	-23	659
JSDUK149-08	Paguridae	<i>Pagurus bernhardus</i>	Irish Sea, England	53.3	-5.5	-83	619
JSDUK150-08	Paguridae	<i>Pagurus bernhardus</i>	North coast Anglesey, Wales	53.4	-4.3	676	
JSDUK151-08	Paguridae	<i>Pagurus bernhardus</i>	North coast Anglesey, Wales	53.4	-4.3	676	
JSDUK152-08	Paguridae	<i>Pagurus bernhardus</i>	North coast Anglesey, Wales	53.4	-4.3	675	
JSDUK153-08	Paguridae	<i>Pagurus bernhardus</i>	North coast Anglesey, Wales	53.4	-4.3	676	
JSDUK154-08	Paguridae	<i>Pagurus bernhardus</i>	North coast Anglesey, Wales	53.4	-4.3	626	
JSDAZ183-08	Paguridae	<i>Pagurus cuanensis</i>	South coast San Miguel, Azores	37.7	-26	-24	632
JSDAZ065-08	Paguridae	<i>Pagurus cuanensis</i>	West coast Flores, Azores	39.5	-31	-1	629
FCDOP067-07	Paguridae	<i>Pagurus excavatus</i>	West coast Portugal	41.6	-9	-78	658
FCDOP001-07	Paguridae	<i>Pagurus excavatus</i>	West coast Portugal	40.1	-9.4	-135	658
FCDOP002-07	Paguridae	<i>Pagurus excavatus</i>	West coast Portugal	40.4	-9.1	-77	658
FCDOP003-07	Paguridae	<i>Pagurus excavatus</i>	West coast Portugal	40.8	-9.1	-85	658
JSDME003-08	Paguridae	<i>Pagurus excavatus</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	628
JSDME004-08	Paguridae	<i>Pagurus excavatus</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	649
JSDME005-08	Paguridae	<i>Pagurus excavatus</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	649
FCDOP032-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41.7	-9.3	-173	626
FCDOP036-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41.6	-9	-78	658
FCDOP037-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41.5	-9.2	-132	654
FCDOP038-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.8	-9.2	-76	605
FCDOP039-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.8	-9.2	-76	658
FCDOP034-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41.8	-9	-86	555
FCDOP035-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41.6	-9	-78	658
FCDOP044-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.7	-9.4	-76	658
FCDOP045-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.7	-9.5	-143	658
FCDOP046-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.3	-9.9	-297	658
FCDOP048-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.3	-9.9	-297	658
FCDOP052-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	41	-10	-81	658
FCDOP076-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	39.8	-9.2	-76	658
FCDOP004-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	40.8	-9.1	-85	658
FCDOP005-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	40.8	-9.1	-85	658

FCDOP006-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	40.8	-9.1	-85	571
FCDOP007-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	40.8	-9.1	-85	658
FCDOP008-07	Paguridae	<i>Pagurus prideauxi</i>	West coast Portugal	40.8	-9.1	-85	658
JSDME010-08	Paguridae	<i>Pagurus prideauxi</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	655
JSDME011-08	Paguridae	<i>Pagurus prideauxi</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	657
JSDME052-08	Paguridae	<i>Pagurus prideauxi</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	620
JSDN008-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN009-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN011-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN012-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN013-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN025-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN026-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDN027-08	Paguridae	<i>Pagurus prideauxi</i>	Barents sea, Norway	71	15		659
JSDUK147-08	Paguridae	<i>Pagurus prideauxi</i>	Irish Sea, England	54.2	-4	-23	659
JSDUK157-08	Paguridae	<i>Pagurus prideauxi</i>	Irish Sea, England	53.7	-3.2	-13	570
JSDUK158-08	Paguridae	<i>Pagurus prideauxi</i>	Irish Sea, England	53.7	-3.2	-13	604
JSDUK159-08	Paguridae	<i>Pagurus prideauxi</i>	Irish Sea, England	54.3	-3.7	-31	572
JSDN005-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN014-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		603
JSDN015-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN016-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN018-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN020-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN022-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		627
JSDN023-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		659
JSDN028-08	Paguridae	<i>Pagurus pubescens</i>	Barents sea, Norway	71	15		610
JSDSV001-09	Paguridae	<i>Pagurus pubescens</i>	Kongsfjorden, Svalbard	78.9	11.9	-15	610
JSDSV002-09	Paguridae	<i>Pagurus pubescens</i>	Kongsfjorden, Svalbard	78.9	11.9	-15	679
JSDSV003-09	Paguridae	<i>Pagurus pubescens</i>	Kongsfjorden, Svalbard	78.9	11.9	-15	628
JSDAZ172-08	Palaemonidae	<i>Palaemon elegans</i>	South coast San Miguel, Azores	37.7	-26	-1	582
JSDUK161-08	Palaemonidae	<i>Palaemon elegans</i>	West coast Anglesey, Wales	53.2	-4.5	-1	663
JSDUK162-08	Palaemonidae	<i>Palaemon elegans</i>	West coast Anglesey, Wales	53.2	-4.5	-1	630
JSDUK163-08	Palaemonidae	<i>Palaemon elegans</i>	West coast Anglesey, Wales	53.2	-4.5	-1	663
JSDUK164-08	Palaemonidae	<i>Palaemon elegans</i>	West coast Anglesey, Wales	53.2	-4.5	-1	634
FCDOP072-07	Palaemonidae	<i>Palaemon serratus</i>	West coast Portugal	41.4	-8.8	-37	658
FCDOP073-07	Palaemonidae	<i>Palaemon serratus</i>	West coast Portugal	41.4	-8.8	-37	658
JSDUK165-08	Palaemonidae	<i>Palaemon serratus</i>	North coast Anglesey, Wales	53.4	-4.3		661
JSDUK166-08	Palaemonidae	<i>Palaemon serratus</i>	North coast Anglesey, Wales	53.4	-4.3		621
JSDUK167-08	Palaemonidae	<i>Palaemon serratus</i>	North coast Anglesey, Wales	53.4	-4.3		666
JSDAZ214-08	Palinuridae	<i>Palinurus elephas</i>	South East coast San Miguel, Azores	37.7	-25	-90	613
JSDAZ215-08	Palinuridae	<i>Palinurus elephas</i>	South East coast San Miguel, Azores	37.7	-25	-100	648
JSDUK171-08	Pandalidae	<i>Pandalus montagui</i>	Irish Sea, England	54.6	-3.7	-19	663
JSDUK172-08	Pandalidae	<i>Pandalus montagui</i>	Irish Sea, England	54.6	-3.7	-19	621
FCDOP154-07	Penaeidae	<i>Parapenaeus longirostris</i>	South coast Portugal	36.8	-7.7	-561	576
JSDME037-08	Penaeidae	<i>Parapenaeus longirostris</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	572
JSDME038-08	Penaeidae	<i>Parapenaeus longirostris</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	522
JSDPX108-08	Pasiphaeidae	<i>Pasiphaea hoplocerca</i>	South coast Portugal	36.5	-9.1	-589	565
JSDSC017-09	Pasiphaeidae	<i>Pasiphaea multidentata</i>	Rosemary Bank, Scotland	59.3	-10	-850	656
JSDSC016-09	Pasiphaeidae	<i>Pasiphaea multidentata</i>	Rosemary Bank, Scotland	59.3	-10	-850	656
FCDOP103-07	Pasiphaeidae	<i>Pasiphaea sivado</i>	South coast Portugal	36.8	-7.8	-553	658
FCDOP104-07	Pasiphaeidae	<i>Pasiphaea sivado</i>	South coast Portugal	36.8	-7.8	-553	705
FCDOP105-07	Pasiphaeidae	<i>Pasiphaea sivado</i>	South coast Portugal	36.8	-7.8	-553	630
FCDOP106-07	Pasiphaeidae	<i>Pasiphaea sivado</i>	South coast Portugal	36.8	-7.8	-553	621
FCDOP107-07	Pasiphaeidae	<i>Pasiphaea sivado</i>	South coast Portugal	36.8	-7.8	-553	579
JSDSC007-09	Pasiphaeidae	<i>Pasiphaea tarda</i>	Rosemary Bank, Scotland	59.2	-10	-600	560
JSDSC008-09	Pasiphaeidae	<i>Pasiphaea tarda</i>	Rosemary Bank, Scotland	59.2	-10	-600	656
JSDSC009-09	Pasiphaeidae	<i>Pasiphaea tarda</i>	Rosemary Bank, Scotland	59.2	-10	-600	573
FCDOP223-07	Penaeidae	<i>Penaeopsis serrata</i>	South coast Portugal	36.6	-7.4	-471	565
JSDAZ039-08	Grapsidae	<i>Percnon gibbesi</i>	East coast Flores, Azores	39.5	-31	-5	617

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JSDAZ040-08	Grapsidae	<i>Percnon gibbesi</i>	East coast Flores, Azores	39.5	-31	-5	656
JSDAZ041-08	Grapsidae	<i>Percnon gibbesi</i>	East coast Flores, Azores	39.5	-31	-5	520
JSDAZ075-08	Grapsidae	<i>Percnon gibbesi</i>	South coast San Miguel, Azores	37.7	-26	-1	600
JSDAZ076-08	Grapsidae	<i>Percnon gibbesi</i>	South coast San Miguel, Azores	37.7	-26	-1	657
JSDAZ077-08	Grapsidae	<i>Percnon gibbesi</i>	South coast San Miguel, Azores	37.7	-26	-1	638
JSDAZ079-08	Grapsidae	<i>Percnon gibbesi</i>	South coast San Miguel, Azores	37.7	-26	-1	657
FCDPH070-07	Crangonidae	<i>Philocheirus monocanthus</i>	Gulf of Cadiz	35.2	-6.4	-353	617
JSDAZ121-08	Pilumnidae	<i>Pilumnus hirtellus</i>	South coast San Miguel, Azores	37.7	-25		606
JSDUK173-08	Pilumnidae	<i>Pilumnus hirtellus</i>	West coast Anglesey, Wales	53.2	-4.5	-1	578
JSDUK174-08	Pilumnidae	<i>Pilumnus hirtellus</i>	West coast Anglesey, Wales	53.2	-4.5	-1	663
FCDPH041-07	Pilumnidae	<i>Pilumnus inermis</i>	Gulf of Cadiz	35.4	-6.3	-392	614
FCDOP291-07	Pandalidae	<i>Plesionika acanthonotus</i>	South coast Portugal	36.6	-7.8	-758	657
JSDPX067-08	Pandalidae	<i>Plesionika acanthonotus</i>	South coast Portugal	36.4	-7.6	-714	657
JSDPX068-08	Pandalidae	<i>Plesionika acanthonotus</i>	South coast Portugal	36.4	-7.6	-714	657
JSDME070-08	Pandalidae	<i>Plesionika edwardsii</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	575
JSDME071-08	Pandalidae	<i>Plesionika edwardsii</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	657
JSDME073-08	Pandalidae	<i>Plesionika edwardsii</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	574
JSDME074-08	Pandalidae	<i>Plesionika edwardsii</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	568
FCDOP084-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	39.1	-9.6	-297	573
FCDOP085-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	39.1	-9.6	-297	658
FCDOP086-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	39.1	-9.6	-297	658
FCDOP087-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	39.1	-9.6	-297	658
FCDOP088-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	39.1	-9.6	-297	658
FCDOP025-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	41.3	-9	-79	658
FCDOP026-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	41.3	-9	-79	658
FCDOP027-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	41.3	-9	-79	582
FCDOP028-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	41.3	-9	-79	658
FCDOP029-07	Pandalidae	<i>Plesionika heterocarpus</i>	West coast Portugal	41.3	-9	-79	658
FCDOP208-07	Pandalidae	<i>Plesionika heterocarpus</i>	South coast Portugal	36.8	-7.5	-538	567
JSDME029-08	Pandalidae	<i>Plesionika heterocarpus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	657
JSDME030-08	Pandalidae	<i>Plesionika heterocarpus</i>	Stretto di Sicilia, Sicily	37.3	11.8	-200	657
FCDOP133-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.8	-553	657
FCDOP134-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.8	-553	657
FCDOP135-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.8	-553	574
FCDOP155-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.7	-561	657
FCDOP156-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.7	-561	657
FCDOP158-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.7	-561	657
FCDOP163-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.6	-538	615
FCDOP206-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.5	-538	657
FCDOP207-07	Pandalidae	<i>Plesionika martia</i>	South coast Portugal	36.8	-7.5	-538	657
FCDOP195-07	Pandalidae	<i>Plesionika narval</i>	South coast Portugal	36.9	-7.5	-356	600
FCDOP232-07	Pandalidae	<i>Plesionika narval</i>	South coast Portugal	36.6	-7.4	-471	657
JSDME077-08	Pandalidae	<i>Plesionika narval</i>	Isola di Ustica, Sicily	38.7	13.3	-200	657
JSDME078-08	Pandalidae	<i>Plesionika narval</i>	Isola di Ustica, Sicily	38.7	13.3	-200	598
JSDME079-08	Pandalidae	<i>Plesionika narval</i>	Isola di Ustica, Sicily	38.7	13.3	-200	657
FCDOP058-07	Portunidae	<i>Polybius henslowii</i>	West coast Portugal	41.3	-9	-79	658
FCDOP059-07	Portunidae	<i>Polybius henslowii</i>	West coast Portugal	41.3	-9	-79	660
FCDOP060-07	Portunidae	<i>Polybius henslowii</i>	West coast Portugal	41.3	-9	-79	658
FCDOP061-07	Portunidae	<i>Polybius henslowii</i>	West coast Portugal	41.3	-9	-79	658
FCDOP062-07	Portunidae	<i>Polybius henslowii</i>	West coast Portugal	41.3	-9	-79	658
FCDOP089-07	Portunidae	<i>Polybius henslowii</i>	South coast Portugal	36.8	-7.8	-553	658
JSDUK102-08	Portunidae	<i>Polybius henslowii</i>	Irish Sea, England	53.6	-3.4	-16	636
JSDUK103-08	Portunidae	<i>Polybius henslowii</i>	Irish Sea, England	53.6	-3.4	-16	668
JSDUK074-08	Portunidae	<i>Polybius henslowii</i>	Irish Sea, England	54.3	-3.7	-31	675
JSDUK078-08	Portunidae	<i>Polybius henslowii</i>	Irish Sea, England	54.6	-3.7	-19	581
FCDOP242-07	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	36.6	-8	-752	586
FCDOP243-07	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	36.6	-8	-752	658
FCDOP245-07	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	36.6	-8	-752	513
JSDPX014-08	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	37.4	-9.2	-627	663
JSDPX015-08	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	37.4	-9.2	-627	628

JSDPX016-08	Polychelidae	<i>Polycheles typhlops</i>	South coast Portugal	37.4	-9.2	-627	663
JSDSC033-09	Polychelidae	<i>Polycheles typhlops</i>	South West St Kilda, Scotland	57.3	-9	-500	656
JSDSC035-09	Polychelidae	<i>Polycheles typhlops</i>	South West St Kilda, Scotland	57.3	-9	-500	656
JSDSC036-09	Polychelidae	<i>Polycheles typhlops</i>	South West St Kilda, Scotland	57.3	-9	-500	656
JSDME015-08	Crangonidae	<i>Pontocaris cathanphracta</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	576
JSDME016-08	Crangonidae	<i>Pontocaris cathanphracta</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	651
JSDME017-08	Crangonidae	<i>Pontocaris cathanphracta</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	661
FCDPH023-07	Crangonidae	<i>Pontocaris lacazei</i>	Gulf of Cadiz	35.2	-6.4	-355	565
FCDOP139-07	Crangonidae	<i>Pontocaris lacazei</i>	South coast Portugal	36.8	-7.8	-553	599
FCDOP173-07	Crangonidae	<i>Pontocaris lacazei</i>	South coast Portugal	36.9	-7.5	-425	584
FCDOP174-07	Crangonidae	<i>Pontocaris lacazei</i>	South coast Portugal	36.9	-7.5	-425	615
FCDOP175-07	Crangonidae	<i>Pontocaris lacazei</i>	South coast Portugal	36.9	-7.5	-425	564
JSDUK178-08	Porcellanidae	<i>Porcellana platycheles</i>	West coast Anglesey, Wales	53.2	-4.5	-1	567
JSDUK179-08	Porcellanidae	<i>Porcellana platycheles</i>	West coast Anglesey, Wales	53.2	-4.5	-1	628
JSDUK180-08	Porcellanidae	<i>Porcellana platycheles</i>	West coast Anglesey, Wales	53.2	-4.5	-1	690
JSDUK181-08	Porcellanidae	<i>Porcellana platycheles</i>	West coast Anglesey, Wales	53.2	-4.5	-1	661
JSDUK182-08	Porcellanidae	<i>Porcellana platycheles</i>	West coast Anglesey, Wales	53.2	-4.5	-1	620
FCDOP128-07	Processidae	<i>Processa modica</i>	South coast Portugal	36.8	-7.8	-553	656
FCDOP129-07	Processidae	<i>Processa modica</i>	South coast Portugal	36.8	-7.8	-553	656
FCDOP130-07	Processidae	<i>Processa modica</i>	South coast Portugal	36.8	-7.8	-553	656
JSDPX069-08	Processidae	<i>Processa modica</i>	South coast Portugal	36.4	-7.6	-714	661
JSDPX070-08	Processidae	<i>Processa modica</i>	South coast Portugal	36.4	-7.6	-714	696
JSDSC004-09	Crangonidae	<i>Sabinea hystrix</i>	North West St Kilda, Scotland	58.2	-9	-1500	626
JSDSC005-09	Crangonidae	<i>Sabinea hystrix</i>	North West St Kilda, Scotland	58.2	-9	-1500	656
JSDSC006-09	Crangonidae	<i>Sabinea hystrix</i>	North West St Kilda, Scotland	58.2	-9	-1500	626
JSDAZ221-08	Scyllaridae	<i>Scyllarides latus</i>	North coast San Miguel, Azores	37.8	-26	-40	633
JSDAZ222-08	Scyllaridae	<i>Scyllarides latus</i>	North coast San Miguel, Azores	37.8	-26	-40	569
JSDAZ226-08	Scyllaridae	<i>Scyllarides latus</i>	South East coast San Miguel, Azores	37.7	-25	-20	630
JSDAZ036-08	Scyllaridae	<i>Scyllarides latus</i>	East coast Flores, Azores	39.5	-31	-1	613
JSDAZ037-08	Scyllaridae	<i>Scyllarides latus</i>	East coast Flores, Azores	39.5	-31	-1	607
FCDOP307-07	Sergestidae	<i>Sergia robusta</i>	South coast Portugal	37	-9	-653	528
JSDPX051-08	Sergestidae	<i>Sergia robusta</i>	South coast Portugal	36.4	-8	-759	661
JSDPX052-08	Sergestidae	<i>Sergia robusta</i>	South coast Portugal	37.6	-9.1	-548	576
JSDPX114-08	Sergestidae	<i>Sergia robusta</i>	South coast Portugal	36.5	-9.1	-570	621
JSDPX129-08	Sergestidae	<i>Sergia robusta</i>	South coast Portugal	37.3	-9.2	-659	579
JSDSC013-09	Sergestidae	<i>Sergia robusta</i>	Rosemary Bank, Scotland	59.3	-10	-850	656
JSDSC014-09	Sergestidae	<i>Sergia robusta</i>	Rosemary Bank, Scotland	59.3	-10	-850	656
JSDSC015-09	Sergestidae	<i>Sergia robusta</i>	Rosemary Bank, Scotland	59.3	-10	-850	656
FCDOP055-07	Solenoceridae	<i>Solenocera membranacea</i>	West coast Portugal	41.3	-9	-79	658
FCDOP074-07	Solenoceridae	<i>Solenocera membranacea</i>	West coast Portugal	39.8	-9.2	-76	658
FCDOP075-07	Solenoceridae	<i>Solenocera membranacea</i>	West coast Portugal	39.8	-9.2	-76	658
FCDOP021-07	Solenoceridae	<i>Solenocera membranacea</i>	West coast Portugal	41	-10	-81	658
FCDOP100-07	Solenoceridae	<i>Solenocera membranacea</i>	South coast Portugal	36.8	-7.8	-553	642
FCDOP101-07	Solenoceridae	<i>Solenocera membranacea</i>	South coast Portugal	36.8	-7.8	-553	658
FCDOP102-07	Solenoceridae	<i>Solenocera membranacea</i>	South coast Portugal	36.8	-7.8	-553	658
JSDME019-08	Solenoceridae	<i>Solenocera membranacea</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	605
JSDME020-08	Solenoceridae	<i>Solenocera membranacea</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	613
JSDME021-08	Solenoceridae	<i>Solenocera membranacea</i>	Castelammare del Golfo, Sicily	38.1	12.9	-200	605
JSDSC024-09	Polychelidae	<i>Stereomastis grimaldi</i>	North West St Kilda, Scotland	58.3	-9	-1700	589
JSDSC026-09	Polychelidae	<i>Stereomastis grimaldi</i>	North West St Kilda, Scotland	58.3	-9	-1700	595
FCDOP204-07	Pandalidae	<i>Stylopandalus richardi</i>	South coast Portugal	36.8	-7.5	-538	575
JSDPX079-08	Ooplophoridae	<i>Systellaspis debilis</i>	South coast Portugal	36.4	-7.5	-751	611
JSDPX080-08	Ooplophoridae	<i>Systellaspis debilis</i>	South coast Portugal	36.4	-7.5	-751	629
JSDPX081-08	Ooplophoridae	<i>Systellaspis debilis</i>	South coast Portugal	36.4	-7.5	-751	657
JSDPX082-08	Ooplophoridae	<i>Systellaspis pellucida</i>	South coast Portugal	37.2	-9.1	-542	660
JSDPX084-08	Ooplophoridae	<i>Systellaspis pellucida</i>	South coast Portugal	37.2	-9.1	-542	661
JSDUK183-08	Hippolytidae	<i>Thoralus cranchii</i>	West coast Anglesey, Wales	53.2	-4.5	-1	608
JSDUK184-08	Hippolytidae	<i>Thoralus cranchii</i>	West coast Anglesey, Wales	53.2	-4.5	-1	610
JSDUK185-08	Hippolytidae	<i>Thoralus cranchii</i>	West coast Anglesey, Wales	53.2	-4.5	-1	661
FCDPH004-07	Callianassidae	<i>Vulcanocalliax arutyunovi</i>	Gulf of Cadiz	35.7	-7.3	-1319	603

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JSDAZ104-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	591
JSDAZ108-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	610
JSDAZ109-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	615
JSDAZ138-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	623
JSDAZ17-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	578
JSDAZ18-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	591
JSDAZ19-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	578
JSDAZ22-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	603
JSDAZ23-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	558
JSDAZ24-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	636
JSDAZ231-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	650
JSDAZ025-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	601
JSDAZ026-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	582
JSDAZ032-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	622
JSDAZ034-08	Xanthidae	<i>Xantho hydrophilus</i>	East coast Flores, Azores	39.5	-31	-1	620
JSDAZ080-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	673
JSDAZ081-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	632
JSDAZ094-08	Xanthidae	<i>Xantho hydrophilus</i>	South coast San Miguel, Azores	37.7	-26	-1	618
JSDUK186-08	Xanthidae	<i>Xantho pilipes</i>	West coast Anglesey, Wales	53.2	-4.5	-1	672
JSDUK187-08	Xanthidae	<i>Xantho pilipes</i>	West coast Anglesey, Wales	53.2	-4.5	-1	627
JSDUK188-08	Xanthidae	<i>Xantho pilipes</i>	West coast Anglesey, Wales	53.2	-4.5	-1	635

Table S 3.1.2: Accession numbers for the sequences used in this study. Specimens' list of 1906 COI sequences from 603 species, 225 genera and 71 families.

Process ID	Identification	Family	BOLD project name
JSDSC022-09	<i>Acanthephyra pelagica</i>	Ophelidae	This study
GBCMD1845-08	<i>Acanthephyra pelagica</i>	Ophelidae	Genbank Crustacea Malac.
JSDPX109-08	<i>Acanthephyra purpurea</i>	Ophelidae	This study
JSDPX110-08	<i>Acanthephyra purpurea</i>	Ophelidae	This study
FCDPA135-04	<i>Acantholithodes hispidus</i>	Lithodidae	Decapods of Pacific and Atlantic
JSDME059-08	<i>Acanthonyx lunulatus</i>	Majidae	This study
JSDME061-08	<i>Acanthonyx lunulatus</i>	Majidae	This study
GBCMD3342-09	<i>Acanthonyx petiverii</i>	Majidae	Genbank Crustacea Malac.
GBCMD3341-09	<i>Acanthonyx petiverii</i>	Majidae	Genbank Crustacea Malac.
GBCMD0079-06	<i>Agononida incerta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0080-06	<i>Agononida incerta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0193-06	<i>Agononida marinii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0195-06	<i>Agononida procerii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0196-06	<i>Agononida procerii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0194-06	<i>Agononida similis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0197-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0198-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0199-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0200-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0201-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0202-06	<i>Agononida sphecia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0327-06	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0328-06	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0329-06	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0330-06	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
FCDOP164-07	<i>Alpheus glaber</i>	Alpheidae	This study
FCDOP171-07	<i>Alpheus glaber</i>	Alpheidae	This study
FCDOP199-07	<i>Alpheus glaber</i>	Alpheidae	This study
GBCMD0029-06	<i>Alvinocaris longirostris</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD0030-06	<i>Alvinocaris longirostris</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD3419-09	<i>Alvinocaris muricola</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD3975-09	<i>Amarinus paralacustris</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD904-07	<i>Aniculus aniculus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD903-07	<i>Aniculus retipes</i>	Diogenidae	Genbank Crustacea Malac.
FCDPA020-04	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA021-04	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA106-04	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
WWGSL077-08	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW123-07	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW124-07	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WWGSL131-08	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WWGSL101-08	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
FCDPA022-04	<i>Argis lar</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA023-04	<i>Argis lar</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDOP283-07	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study
FCDOP301-07	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study
JSDME033-08	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study
JSDME035-08	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study

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JSDME036-08	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study
JSDPX142-08	<i>Aristaeomorpha foliacea</i>	Aristeidae	This study
GBCMD0548-06	<i>Astacopsis gouldi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0549-06	<i>Astacopsis tricornis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0550-06	<i>Astacopsis tricornis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0458-06	<i>Astacus astacus</i>	Astacidae	Genbank Crustacea Malac.
FCDOP051-07	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDPX028-08	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDUK001-08	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDUK002-08	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDUK003-08	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK004-08	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK005-08	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK006-08	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK007-08	<i>Athanas nitescens</i>	Alpheidae	This study
GBCMD2197-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2196-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2195-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2194-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2193-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2192-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2190-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2187-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2186-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2185-08	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2179-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2177-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2176-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2170-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2164-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2161-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2159-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2158-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2153-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2151-08	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1204-07	<i>Atyaephyra desmarestii</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2300-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2299-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2298-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2297-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2296-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2295-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD2294-08	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
GBCMD1223-07	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD1222-07	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD1221-07	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD3559-09	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD3558-09	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD3557-09	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD3556-09	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
GBCMD1842-08	<i>Babamunida hystrix</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1841-08	<i>Babamunida hystrix</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1687-08	<i>Barbicambarus cornutus</i>	Cambaridae	Genbank Crustacea Malac.

GBCMD0331-06	<i>Bathymunida nebulosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0332-06	<i>Bathymunida nebulosa</i>	Galatheidae	Genbank Crustacea Malac.
FCDPH001-07	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH039-07	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH040-07	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH078-07	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDOP108-07	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDOP110-07	<i>Bathynectes maravigna</i>	Portunidae	This study
JSDSC027-09	<i>Bathynectes maravigna</i>	Portunidae	This study
JSDSC028-09	<i>Bathynectes maravigna</i>	Portunidae	This study
JSDSC029-09	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPA050-04	<i>Bentheogennema borealis</i>	Benthesicymidae	Decapods of Pacific and Atlantic
FCDPA051-04	<i>Bentheogennema borealis</i>	Benthesicymidae	Decapods of Pacific and Atlantic
JSDAZ204-08	<i>Calappa granulata</i>	Calappidae	This study
JSDAZ051-08	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAZ052-08	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAZ064-08	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAZ066-08	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAZ067-08	<i>Calcinus tubularis</i>	Diogenidae	This study
GBCMD3953-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3950-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3934-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3912-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3909-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3859-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3855-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3841-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3825-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD3821-09	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
GBCMD0368-06	<i>Callinectes arcuatus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0369-06	<i>Callinectes arcuatus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0366-06	<i>Callinectes bellicosus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0367-06	<i>Callinectes bellicosus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0893-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0343-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0370-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0461-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0462-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0463-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0464-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0465-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0466-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0467-06	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
FCDPA052-04	<i>Calocaris investigatoris</i>	Calocarididae	Decapods of Pacific and Atlantic
FCDPA053-04	<i>Calocaris investigatoris</i>	Calocarididae	Decapods of Pacific and Atlantic
GBCMD3370-09	<i>Cambarus bartonii</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD0469-06	<i>Cambarus bartonii</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1686-08	<i>Cambarus batchi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1685-08	<i>Cambarus brachydactylus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1545-07	<i>Cambarus brachydactylus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1684-08	<i>Cambarus causeyi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1683-08	<i>Cambarus cryptodytes</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1544-07	<i>Cambarus friaufi</i>	Cambaridae	Genbank Crustacea Malac.

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GBCMD1812-08	<i>Cambarus friaufi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1543-07	<i>Cambarus gentryi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD0470-06	<i>Cambarus graysoni</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1568-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1561-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1560-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1559-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD3447-09	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD3446-09	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD3445-09	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD3444-09	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1554-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1552-07	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1551-07	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1550-07	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD1549-07	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD3368-09	<i>Cambarus tenebrosus</i>	Cambaridae	Genbank Crustacea Malac.
JSDPX107-08	<i>Cancer bellianus</i>	Cancridae	This study
WW867-08	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WW878-08	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WWGSL364-08	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WWGSL334-08	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WW648-08	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WW093-07	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WW094-07	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
WW117-07	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
FCDPA150-04	<i>Cancer magister</i>	Cancridae	Decapods of Pacific and Atlantic
FCDPA151-04	<i>Cancer magister</i>	Cancridae	Decapods of Pacific and Atlantic
FCDPA062-04	<i>Cancer oregonensis</i>	Cancridae	Decapods of Pacific and Atlantic
FCDPA063-04	<i>Cancer oregonensis</i>	Cancridae	Decapods of Pacific and Atlantic
JSDUK010-08	<i>Cancer pagurus</i>	Cancridae	This study
JSDUK011-08	<i>Cancer pagurus</i>	Cancridae	This study
GBCMD4318-09	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4317-09	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4316-09	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4322-09	<i>Cancer porteri</i>	Cancridae	Genbank Crustacea Malac.
FCDPA064-04	<i>Cancer productus</i>	Cancridae	Decapods of Pacific and Atlantic
FCDPA166-05	<i>Cancer productus</i>	Cancridae	Decapods of Pacific and Atlantic
GBCMD1040-07	<i>Candidiopotamon Rathbunae</i>	Potamidae	Genbank Crustacea Malac.
GBCMD0394-06	<i>Carcinus aestuarii</i>	Portunidae	Genbank Crustacea Malac.
JSDN001-08	<i>Carcinus maenas</i>	Portunidae	Crustaceans of the St. Lawrence Gulf
JSDN002-08	<i>Carcinus maenas</i>	Portunidae	Crustaceans of the St. Lawrence Gulf
JSDN003-08	<i>Carcinus maenas</i>	Portunidae	This study
JSDUK019-08	<i>Carcinus maenas</i>	Portunidae	This study
WWGSL137-08	<i>Carcinus maenas</i>	Portunidae	This study
WW664-08	<i>Carcinus maenas</i>	Portunidae	This study
GBCMD0390-06	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0870-06	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0872-06	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0873-06	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
GBCMD1847-08	<i>Caridina cantonensis</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1848-08	<i>Caridina formosae</i>	Atyidae	Genbank Crustacea Malac.
GBCMD0333-06	<i>Cervimunida johni</i>	Galatheidae	Genbank Crustacea Malac.

GBCMD3595-09	<i>Chaceon affinis</i>	Geryonidae	Genbank Crustacea Malac.
GBCMD1863-08	<i>Charybdis acuta</i>	Portunidae	Genbank Crustacea Malac.
GBCMD1866-08	<i>Charybdis feriatus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD2347-08	<i>Charybdis japonica</i>	Portunidae	Genbank Crustacea Malac.
GBCMD1865-08	<i>Charybdis vadorum</i>	Portunidae	Genbank Crustacea Malac.
GBCMD1753-08	<i>Chasmagnathus convexus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1752-08	<i>Chasmagnathus convexus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0141-06	<i>Cherax crassimanus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0900-06	<i>Cherax destructor</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0365-06	<i>Cherax destructor</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0551-06	<i>Cherax parvus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0139-06	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1540-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD3601-09	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1535-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1534-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1532-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1531-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1530-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1529-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1527-07	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0552-06	<i>Cherax quadricarinatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0135-06	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0136-06	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0137-06	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0138-06	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0142-06	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0143-06	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0144-06	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0145-06	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0146-06	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
FCDPA065-04	<i>Chionoecetes angulatus</i>	Oregoniidae	Decapods of Pacific and Atlantic
FCDPA066-04	<i>Chionoecetes angulatus</i>	Oregoniidae	Decapods of Pacific and Atlantic
FCDPA138-04	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
FCDPA067-04	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
FCDPA068-04	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD0011-06	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD0012-06	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD0013-06	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
GBCMD0014-06	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
GBCMD0015-06	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
GBCMD3431-09	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
GBCMD3364-09	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
GBCMD3363-09	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
WW859-08	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
WW GSL086-08	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
WW GSL087-08	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
WW GSL088-08	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
WW095-07	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
WW118-07	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
FCDPA069-04	<i>Chionoecetes tanneri</i>	Oregoniidae	Decapods of Pacific and Atlantic
FCDPA070-04	<i>Chionoecetes tanneri</i>	Oregoniidae	Decapods of Pacific and Atlantic
FCDPA139-04	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic

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FCDPA072-04	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic
FCDPA073-04	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic
GBCMD1844-08	<i>Ciliopagurus hawaiiensis</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD923-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD920-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD911-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD910-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD909-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD908-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD907-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD906-07	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD921-07	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD3452-09	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD3451-09	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD3450-09	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD919-07	<i>Ciliopagurus vakovako</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD918-07	<i>Ciliopagurus vakovako</i>	Diogenidae	Genbank Crustacea Malac.
JSDAZ115-08	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDME063-08	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDME066-08	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDME067-08	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDUK022-08	<i>Corystes cassivelaunus</i>	Corystidae	This study
JSDUK023-08	<i>Corystes cassivelaunus</i>	Corystidae	This study
JSDUK024-08	<i>Corystes cassivelaunus</i>	Corystidae	This study
FCDPA024-04	<i>Crangon abyssorum</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA025-04	<i>Crangon abyssorum</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA116-04	<i>Crangon alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA117-04	<i>Crangon alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA026-04	<i>Crangon communis</i>	Crangonidae	Decapods of Pacific and Atlantic
FCDPA027-04	<i>Crangon communis</i>	Crangonidae	Decapods of Pacific and Atlantic
WW609-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW663-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW596-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW672-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WWGSL338-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WWGSL339-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW696-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW697-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW698-08	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW086-07	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GBCMD0203-06	<i>Crosnierita dicata</i>	Galatheidae	Genbank Crustacea Malac.
FCDPH028-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH029-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH030-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH031-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH074-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH075-07	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDOP015-07	<i>Dardanus arrosor</i>	Diogenidae	This study
FCDOP017-07	<i>Dardanus arrosor</i>	Diogenidae	This study
FCDOP191-07	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDAZ203-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDME006-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDME046-08	<i>Dardanus arrosor</i>	Diogenidae	This study

JSDME047-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDME048-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDME049-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDME051-08	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDAZ193-08	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAZ194-08	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAZ046-08	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAZ047-08	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAZ048-08	<i>Dardanus calidus</i>	Diogenidae	This study
GBCMD905-07	<i>Dardanus lagopodes</i>	Diogenidae	Genbank Crustacea Malac.
JSDSC030-09	<i>Dichelopandalus bonnieri</i>	Pandalidae	This study
JSDSC032-09	<i>Dichelopandalus bonnieri</i>	Pandalidae	This study
JSDAZ116-08	<i>Dromia personata</i>	Dromiidae	This study
JSDAZ185-08	<i>Dromia personata</i>	Dromiidae	This study
GBCMD1572-07	<i>Dugastella valentina</i>	Atyidae	Genbank Crustacea Malac.
WW639-08	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
WW658-08	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
WWGSL318-08	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
FCDPH036-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH014-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH037-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH038-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH072-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH073-07	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH066-07	<i>Ebalia nux</i>	Leucosiidae	This study
GBCMD3982-09	<i>Elamena producta</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD0149-06	<i>Engaeus strictifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0031-06	<i>Erimacrus isenbeckii</i>	Atelecyclidae	Genbank Crustacea Malac.
GBCMD3647-09	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0048-06	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0049-06	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0082-06	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0171-06	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
GBCMD2945-09	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD2654-09	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0083-06	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0084-06	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0172-06	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3665-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3660-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3658-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3656-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3653-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD2655-09	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0403-06	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0406-06	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0409-06	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0415-06	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0081-06	<i>Eriocheir leptognathus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0174-06	<i>Eriocheir leptognathus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3649-09	<i>Eriocheir ogasawaraensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3648-09	<i>Eriocheir ogasawaraensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0087-06	<i>Eriocheir rectus</i>	Varunidae	Genbank Crustacea Malac.

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GBCMD2944-09	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3673-09	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3671-09	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3670-09	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3669-09	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0124-06	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0128-06	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0129-06	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0397-06	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0400-06	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
JSDAZ012-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ013-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ014-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ015-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ031-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ089-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ090-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAZ092-08	<i>Eriphia verrucosa</i>	Xanthidae	This study
FCDPA123-04	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA124-04	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA125-04	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA035-04	<i>Eualus barbatus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA140-04	<i>Eualus barbatus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA036-04	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA037-04	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA038-04	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA039-04	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
WW1014-08	<i>Eualus fabricii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW433-08	<i>Eualus fabricii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FCDPA087-04	<i>Eualus gaimardi</i>	Hippolytidae	This study
JSDSV017-09	<i>Eualus gaimardi</i>	Hippolytidae	Decapods of Pacific and Atlantic
WWGSL127-08	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WWGSL100-08	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW728-08	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW729-08	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW088-07	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW089-07	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FCDPA126-04	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA127-04	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA040-04	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
GBCMD0553-06	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0554-06	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0555-06	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0556-06	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0557-06	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0558-06	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0559-06	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0560-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0561-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0562-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0563-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0564-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0565-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.

GBCMD0566-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0567-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0569-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0570-06	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0574-06	<i>Euastacus bindal</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0150-06	<i>Euastacus bispinosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0575-06	<i>Euastacus bispinosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0576-06	<i>Euastacus brachythorax</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0577-06	<i>Euastacus brachythorax</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0578-06	<i>Euastacus clarkae</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0579-06	<i>Euastacus clarkae</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0363-06	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0580-06	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0581-06	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0582-06	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0583-06	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0584-06	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0585-06	<i>Euastacus dangadi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0586-06	<i>Euastacus dangadi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0364-06	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0587-06	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0588-06	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0589-06	<i>Euastacus diversus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0590-06	<i>Euastacus diversus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0591-06	<i>Euastacus fleckeri</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0592-06	<i>Euastacus fleckeri</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0593-06	<i>Euastacus gamilaroi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0594-06	<i>Euastacus gamilaroi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0595-06	<i>Euastacus gumar</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0596-06	<i>Euastacus gumar</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0597-06	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0598-06	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0599-06	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0600-06	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0601-06	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0602-06	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0603-06	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0604-06	<i>Euastacus jagara</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0605-06	<i>Euastacus jagara</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0606-06	<i>Euastacus kershawi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0607-06	<i>Euastacus kershawi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0608-06	<i>Euastacus maida</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0609-06	<i>Euastacus maida</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0610-06	<i>Euastacus mirangudjin</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0611-06	<i>Euastacus mirangudjin</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0612-06	<i>Euastacus monteithorum</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0613-06	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0614-06	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0615-06	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0616-06	<i>Euastacus polysetosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0617-06	<i>Euastacus polysetosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0618-06	<i>Euastacus reductus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0619-06	<i>Euastacus rieki</i>	Parastacidae	Genbank Crustacea Malac.

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GBCMD0620-06	<i>Euastacus rieki</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0621-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0622-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0623-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0624-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0625-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0626-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0627-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0628-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0629-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0630-06	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0632-06	<i>Euastacus setosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0633-06	<i>Euastacus setosus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0636-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0637-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0638-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0639-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0640-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0641-06	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0642-06	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0643-06	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0644-06	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0645-06	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0647-06	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0648-06	<i>Euastacus suttoni</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0649-06	<i>Euastacus suttoni</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0652-06	<i>Euastacus valentulus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0653-06	<i>Euastacus valentulus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0654-06	<i>Euastacus woiwuru</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0655-06	<i>Euastacus woiwuru</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0656-06	<i>Euastacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0657-06	<i>Euastacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0658-06	<i>Euastacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0659-06	<i>Euastacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0660-06	<i>Euastacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0661-06	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0662-06	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0663-06	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0664-06	<i>Euastacus yigara</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0665-06	<i>Euastacus yigara</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0485-06	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD0487-06	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD0673-06	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD0674-06	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3293-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3289-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3238-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3189-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3180-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3154-09	<i>Eumunida annulosa</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3309-09	<i>Eumunida capillata</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3308-09	<i>Eumunida capillata</i>	Chiostyliidae	Genbank Crustacea Malac.
GBCMD3307-09	<i>Eumunida capillata</i>	Chiostyliidae	Genbank Crustacea Malac.

GBCMD3313-09	<i>Eumunida keiji</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3312-09	<i>Eumunida keiji</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3310-09	<i>Eumunida keiji</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3136-09	<i>Eumunida keiji</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3153-09	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3142-09	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3141-09	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3140-09	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3107-09	<i>Eumunida marginata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3148-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3103-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3102-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3101-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3100-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3099-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3098-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3097-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3096-09	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3104-09	<i>Eumunida multilineata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3093-09	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3092-09	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3094-09	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3152-09	<i>Eumunida similior</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3150-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3149-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3117-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3116-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3115-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3114-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3113-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3111-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3110-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3108-09	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3091-09	<i>Eumunida squamifera</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3090-09	<i>Eumunida squamifera</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD0493-06	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD0494-06	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3254-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3225-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3224-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3217-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3200-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3192-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3174-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3167-09	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3088-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3298-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3292-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3291-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3139-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3138-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3134-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
GBCMD3133-09	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.

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GBCMD3643-09	<i>Exopalaemon carinicauda</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD3817-09	<i>Exopalaemon carinicauda</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2474-09	<i>Exopalaemon styliferus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2443-08	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3087-09	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD0901-06	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD924-07	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD902-07	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD1943-08	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
JSDPX073-08	<i>Funchalia villose</i>	Benthesicymidae	This study
GBCMD0091-06	<i>Gaetice depressus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0176-06	<i>Gaetice depressus</i>	Varunidae	Genbank Crustacea Malac.
JSDUK030-08	<i>Galathea dispersa</i>	Galatheidae	This study
GBCMD0148-06	<i>Geocharax falcata</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD969-07	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
GBCMD968-07	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
GBCMD967-07	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
GBCMD966-07	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
GBCMD965-07	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
GBCMD981-07	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
GBCMD980-07	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3492-09	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1001-07	<i>Geothelphusa aramotoi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1007-07	<i>Geothelphusa bicolor</i>	Potamidae	Genbank Crustacea Malac.
GBCMD2940-09	<i>Geothelphusa candidiensis</i>	Potamidae	Genbank Crustacea Malac.
GBCMD2939-09	<i>Geothelphusa candidiensis</i>	Potamidae	Genbank Crustacea Malac.
GBCMD0898-06	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
GBCMD0004-06	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1041-07	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1009-07	<i>Geothelphusa ferruginea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1002-07	<i>Geothelphusa miyazakii</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3495-09	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3493-09	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD992-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD991-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD990-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD989-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD987-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD985-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD984-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD983-07	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3491-09	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3490-09	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3489-09	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3488-09	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD974-07	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD973-07	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD972-07	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD971-07	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD970-07	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1000-07	<i>Geothelphusa sakamotoana</i>	Potamidae	Genbank Crustacea Malac.
GBCMD2943-09	<i>Geothelphusa siasi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD2942-09	<i>Geothelphusa siasi</i>	Potamidae	Genbank Crustacea Malac.

GBCMD2941-09	<i>Geothelphusa siasi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD2935-09	<i>Geothelphusa tali</i>	Potamidae	Genbank Crustacea Malac.
GBCMD964-07	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3502-09	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3501-09	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3500-09	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3499-09	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3498-09	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1010-07	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
FCDOP247-07	<i>Geryon longipes</i>	Geryonidae	This study
FCDOP248-07	<i>Geryon longipes</i>	Geryonidae	This study
FCDOP250-07	<i>Geryon longipes</i>	Geryonidae	This study
JSDME082-08	<i>Geryon longipes</i>	Geryonidae	This study
JSDME083-08	<i>Geryon longipes</i>	Geryonidae	This study
JSDPX095-08	<i>Geryon longipes</i>	Geryonidae	This study
JSDPX096-08	<i>Geryon longipes</i>	Geryonidae	This study
JSDSC018-09	<i>Geryon longipes</i>	Geryonidae	This study
JSDSC019-09	<i>Geryon longipes</i>	Geryonidae	This study
JSDSC020-09	<i>Geryon longipes</i>	Geryonidae	This study
FCDOP056-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOP057-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOP019-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOP020-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOP113-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOP115-07	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDPX113-08	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDUK037-08	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDUK039-08	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDUK041-08	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDAZ028-08	<i>Grapsus adscensionis</i>	Grapsidae	This study
JSDAZ029-08	<i>Grapsus adscensionis</i>	Grapsidae	This study
JSDAZ030-08	<i>Grapsus adscensionis</i>	Grapsidae	This study
GBCMD0547-06	<i>Guinotia dentata</i>	Pseudothelphusidae	Genbank Crustacea Malac.
GBCMD3977-09	<i>Halicarinus cookii</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD3978-09	<i>Halicarinus innominatus</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD3979-09	<i>Halicarinus ovatus</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD3976-09	<i>Halicarinus varius</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD0783-06	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD0804-06	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD0812-06	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD0816-06	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD0851-06	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1478-07	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1474-07	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3461-09	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1443-07	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1440-07	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1433-07	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1432-07	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1431-07	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1430-07	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3456-09	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1754-08	<i>Helicana doerjesi</i>	Varunidae	Genbank Crustacea Malac.

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GBCMD1756-08	<i>Helicana japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1755-08	<i>Helicana japonica</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1758-08	<i>Helicana wuana</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1757-08	<i>Helicana wuana</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1765-08	<i>Helice formosensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1764-08	<i>Helice formosensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1763-08	<i>Helice latimera</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1762-08	<i>Helice tientsinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1761-08	<i>Helice tientsinensis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1760-08	<i>Helice tridens</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1759-08	<i>Helice tridens</i>	Varunidae	Genbank Crustacea Malac.
GBCMD0092-06	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1891-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1886-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1883-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1880-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1877-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1873-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1872-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1871-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD1870-08	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD2475-09	<i>Hemigrapsus sexdentatus</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3351-09	<i>Herbstia condylata</i>	Majidae	This study
JSDAZ087-08	<i>Herbstia condylata</i>	Majidae	This study
JSDAZ088-08	<i>Herbstia condylata</i>	Majidae	Genbank Crustacea Malac.
GBCMD3367-09	<i>Heterocrypta occidentalis</i>	Parthenopidae	Genbank Crustacea Malac.
GBCMD1038-07	<i>Himalayapotamon atkinsonianum</i>	Potamidae	Genbank Crustacea Malac.
GBCMD0471-06	<i>Hobbseus valleculus</i>	Cambaridae	Genbank Crustacea Malac.
GBCMD4310-09	<i>Homalaspis plana</i>	Platyxanthidae	Genbank Crustacea Malac.
GBCMD2637-09	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
WW869-08	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
WW870-08	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
WW874-08	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
WW098-07	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
WW099-07	<i>Homarus americanus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4295-09	<i>Homarus gammarus</i>	Nephropidae	Genbank Crustacea Malac.
JSDPX149-08	<i>Homola barbata</i>	Homolidae	This study
FCDPH064-07	<i>Homola barbata</i>	Homolidae	This study
GBCMD3362-09	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW876-08	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW877-08	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW096-07	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW119-07	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
JSDSV007-09	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
JSDSV008-09	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW858-08	<i>Hyas araneus</i>	Majidae	This study
WW644-08	<i>Hyas araneus</i>	Majidae	This study
WW651-08	<i>Hyas araneus</i>	Majidae	Genbank Crustacea Malac.
WW879-08	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW881-08	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW097-07	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW120-07	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
JSDUK176-08	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf

WW650-08	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW646-08	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
WW647-08	<i>Hyas coarctatus</i>	Majidae	This study
FCDPA071-04	<i>Hyas lyratus</i>	Majidae	Decapods of Pacific and Atlantic
GBCMD0532-06	<i>Hydrothelphusa agilis</i>	Potamonautesidae	Genbank Crustacea Malac.
GBCMD0533-06	<i>Hydrothelphusa goudotii</i>	Potamonautesidae	Genbank Crustacea Malac.
GBCMD0534-06	<i>Hydrothelphusa madagascariensis</i>	Potamonautesidae	Genbank Crustacea Malac.
FCDPA130-04	<i>Hymenodora frontalis</i>	Oplophoridae	Decapods of Pacific and Atlantic
JSDPX038-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX039-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX043-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX076-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX077-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX078-08	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
GBCMD3980-09	<i>Hymenosoma depressum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1786-08	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1785-08	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1784-08	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1783-08	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1782-08	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD3974-09	<i>Hymenosoma hodgkini</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1409-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD3439-09	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1402-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1397-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1396-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1383-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1379-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1378-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1375-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
GBCMD1373-07	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
FCDOP040-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP041-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP042-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP053-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP009-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP010-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP012-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP185-07	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP187-07	<i>Inachus dorsettensis</i>	Inachidae	This study
JSDUK046-08	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOP292-07	<i>Inachus leptochirus</i>	Inachidae	This study
GBCMD2630-09	<i>Jasus edwardsii</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0115-06	<i>Jasus edwardsii</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2629-09	<i>Jasus verreauxi</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0057-06	<i>Jasus verreauxi</i>	Palinuridae	Genbank Crustacea Malac.
JSDPX032-08	<i>Jaxea nocturna</i>	Laomediidae	This study
GBCMD1056-07	<i>Johora counsilmani</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1055-07	<i>Johora gapensis</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1054-07	<i>Johora grallator</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3517-09	<i>Johora gua</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3516-09	<i>Johora intermedia</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3515-09	<i>Johora johorensis</i>	Potamidae	Genbank Crustacea Malac.

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GBCMD3514-09	<i>Johora murphyi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3513-09	<i>Johora punicea</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1048-07	<i>Johora singaporensis</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1047-07	<i>Johora tahanensis</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1046-07	<i>Johora thoi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1045-07	<i>Johora tiomanensis</i>	Potamidae	Genbank Crustacea Malac.
FCDOP216-07	<i>Latreillia elegans</i>	Latreilliidae	This study
JSDME018-08	<i>Latreillia elegans</i>	Latreilliidae	This study
JSDPX056-08	<i>Latreillia elegans</i>	Latreilliidae	This study
JSDPX105-08	<i>Latreillia elegans</i>	Latreilliidae	This study
FCDPA033-04	<i>Lebbeus groenlandicus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FCDPA032-04	<i>Lebbeus groenlandicus</i>	Hippolytidae	Decapods of Pacific and Atlantic
WW GSL124-08	<i>Lebbeus groenlandicus</i>	Hippolytidae	Decapods of Pacific and Atlantic
WW121-07	<i>Lebbeus polaris</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW GSL135-08	<i>Lebbeus polaris</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
GBCMD0334-06	<i>Leiogalathea laevirostris</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3973-09	<i>Leptomithrax sternocostulatus</i>	Majidae	Genbank Crustacea Malac.
GBCMD3350-09	<i>Libinia dubia</i>	Majidae	Genbank Crustacea Malac.
GBCMD3349-09	<i>Libinia dubia</i>	Majidae	Genbank Crustacea Malac.
GBCMD3348-09	<i>Libinia emarginata</i>	Majidae	Genbank Crustacea Malac.
GBCMD3347-09	<i>Libinia emarginata</i>	Majidae	Genbank Crustacea Malac.
FCDOP054-07	<i>Liocarcinus depurator</i>	Portunidae	This study
FCDOP111-07	<i>Liocarcinus depurator</i>	Portunidae	This study
FCDOP112-07	<i>Liocarcinus depurator</i>	Portunidae	This study
GBCMD2633-09	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDME001-08	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDME002-08	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDUK051-08	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDUK052-08	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDUK053-08	<i>Liocarcinus depurator</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0868-06	<i>Liocarcinus depurator</i>	Portunidae	Genbank Crustacea Malac.
GBCMD2632-09	<i>Liocarcinus maculatus</i>	Portunidae	Genbank Crustacea Malac.
FCDPA054-04	<i>Lithodes couesi</i>	Lithodidae	Decapods of Pacific and Atlantic
FCDPA055-04	<i>Lithodes couesi</i>	Lithodidae	Decapods of Pacific and Atlantic
GBCMD4351-09	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4350-09	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4349-09	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4348-09	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4347-09	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
WW882-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW883-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW1091-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW1092-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW854-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW856-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
WW855-08	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
GBCMD1840-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1838-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1834-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1833-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1831-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1826-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1824-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.

GBCMD1822-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1821-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1815-08	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4370-09	<i>Litopenaeus stylirostris</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3810-09	<i>Litopenaeus stylirostris</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD963-07	<i>Litopenaeus vannamei</i>	Penaeidae	Genbank Crustacea Malac.
FCDPA056-04	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
FCDPA136-04	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
FCDPA148-04	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
GBCMD3346-09	<i>Loxorhynchus crispatus</i>	Majidae	Genbank Crustacea Malac.
GBCMD3345-09	<i>Loxorhynchus crispatus</i>	Majidae	Genbank Crustacea Malac.
GBCMD1689-08	<i>Lysmata pederseni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1688-08	<i>Lysmata pederseni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1722-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1715-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1704-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1703-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1697-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1695-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1694-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1693-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1692-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1690-08	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD2473-09	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD1266-07	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD1230-07	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2471-09	<i>Macrobrachium callirrhoe</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2469-09	<i>Macrobrachium clymene</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2468-09	<i>Macrobrachium equidens</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2467-09	<i>Macrobrachium esculentum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2230-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2226-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2219-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2218-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2214-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2210-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2208-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2204-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2200-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2199-08	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2466-09	<i>Macrobrachium fukienense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2465-09	<i>Macrobrachium gracilirostre</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2463-09	<i>Macrobrachium hainanense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2462-09	<i>Macrobrachium horstii</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2461-09	<i>Macrobrachium idae</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2460-09	<i>Macrobrachium jaroense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2450-09	<i>Macrobrachium lanatum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4288-09	<i>Macrobrachium lanchesteri</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4011-09	<i>Macrobrachium lanchesteri</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2457-09	<i>Macrobrachium malayanum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2456-09	<i>Macrobrachium mammillodactylus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2455-09	<i>Macrobrachium niphanae</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2454-09	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.

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GBCMD0875-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0877-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0878-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0879-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0885-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0886-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0889-06	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD1570-07	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD1569-07	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2444-09	<i>Macrobrachium pinguis</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2453-09	<i>Macrobrachium placidum</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD3322-09	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2452-09	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0894-06	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0417-06	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2451-09	<i>Macrobrachium saigonense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2449-09	<i>Macrobrachium shokitai</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2448-09	<i>Macrobrachium sintangense</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2447-09	<i>Macrobrachium trompii</i>	Palaemonidae	Genbank Crustacea Malac.
FCDOP013-07	<i>Macropipus tuberculatus</i>	Portunidae	This study
FCDOP182-07	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME009-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME041-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME042-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME043-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME044-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDME045-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDPX136-08	<i>Macropipus tuberculatus</i>	Portunidae	This study
FCDOP033-07	<i>Macropodia longipes</i>	Majidae	This study
FCDOP064-07	<i>Macropodia longipes</i>	Majidae	This study
FCDOP066-07	<i>Macropodia longipes</i>	Majidae	This study
FCDOP068-07	<i>Macropodia longipes</i>	Majidae	This study
FCDOP030-07	<i>Macropodia longipes</i>	Majidae	This study
JSDUK107-08	<i>Macropodia rostrata</i>	Majidae	This study
JSDUK108-08	<i>Macropodia rostrata</i>	Majidae	This study
JSDUK110-08	<i>Macropodia rostrata</i>	Majidae	This study
FCDOP184-07	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDME025-08	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDME026-08	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDME027-08	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDPX037-08	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDPX145-08	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDPX146-08	<i>Macropodia tenuirostris</i>	Majidae	This study
GBCMD4344-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4343-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4342-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4340-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4339-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4329-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4325-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4367-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4366-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
GBCMD4363-09	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.

JSDAZ188-08	<i>Maja brachydactyla</i>	Majidae	This study
GBCMD1682-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1678-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1677-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1673-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1671-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1670-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1669-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1668-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1664-08	<i>Maja brachydactyla</i>	Majidae	Genbank Crustacea Malac.
GBCMD1657-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1656-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1654-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1652-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1650-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1649-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1648-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1646-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1645-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
GBCMD1644-08	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
JSDPX019-08	<i>Maja goltziana</i>	Majidae	This study
JSDPX141-08	<i>Maja goltziana</i>	Majidae	This study
GBCMD1661-08	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
GBCMD1660-08	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
GBCMD1659-08	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
GBCMD1658-08	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
GBCMD0536-06	<i>Marojejy longimerus</i>	Potamonautesidae	Genbank Crustacea Malac.
GBCMD0897-06	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD0190-06	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD0474-06	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
JSDME012-08	<i>Medorippe lanata</i>	Dorippidae	This study
JSDME013-08	<i>Medorippe lanata</i>	Dorippidae	This study
JSDME081-08	<i>Medorippe lanata</i>	Dorippidae	This study
JSDPX029-08	<i>Melicertus kerathurus</i>	Penaeidae	This study
JSDPX031-08	<i>Melicertus kerathurus</i>	Penaeidae	This study
GBCMD3592-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3591-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3590-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3587-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3586-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3585-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3583-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3581-09	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD3339-09	<i>Menaethius monoceros</i>	Majidae	Genbank Crustacea Malac.
GBCMD4321-09	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4320-09	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4319-09	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4307-09	<i>Metanephrops andamanicus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4297-09	<i>Metanephrops arafurensis</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4304-09	<i>Metanephrops armatus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4299-09	<i>Metanephrops boschmai</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4303-09	<i>Metanephrops formosanus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4305-09	<i>Metanephrops japonicus</i>	Nephropidae	Genbank Crustacea Malac.

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GBCMD4306-09	<i>Metanephrops mozambicus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4309-09	<i>Metanephrops sagamiensis</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4298-09	<i>Metanephrops sibogae</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4302-09	<i>Metanephrops sinensis</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD4308-09	<i>Metanephrops velutinus</i>	Nephropidae	Genbank Crustacea Malac.
GBCMD3366-09	<i>Metoporhaphis calcarata</i>	Inachidae	Genbank Crustacea Malac.
GBCMD3352-09	<i>Micippa thalia</i>	Mithracidae	Genbank Crustacea Malac.
GBCMD3353-09	<i>Microphrys bicornutus</i>	Majidae	Genbank Crustacea Malac.
GBCMD4100-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4099-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4096-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4091-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4090-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4088-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4021-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4016-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4015-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4014-09	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD3355-09	<i>Mithraculus sculptus</i>	Mithracidae	Genbank Crustacea Malac.
FCDPH047-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH051-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH008-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH053-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH055-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH013-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH079-07	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH067-07	<i>Monodaeus couchii</i>	Xanthidae	This study
JSDPX033-08	<i>Monodaeus couchii</i>	Xanthidae	This study
JSDPX034-08	<i>Monodaeus couchii</i>	Xanthidae	This study
GBCMD0204-06	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0205-06	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0206-06	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0207-06	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0498-06	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0208-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0209-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0210-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0211-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0212-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0213-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0214-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0215-06	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0216-06	<i>Munida armilla</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0217-06	<i>Munida armilla</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0218-06	<i>Munida callista</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0219-06	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0220-06	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0221-06	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0222-06	<i>Munida compressa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0223-06	<i>Munida compressa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0224-06	<i>Munida congesta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0225-06	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0226-06	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.

GBCMD0227-06	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0228-06	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0229-06	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0230-06	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0231-06	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0232-06	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0233-06	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0234-06	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0235-06	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0236-06	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0237-06	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2318-08	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2317-08	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2316-08	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0238-06	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0239-06	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0240-06	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0241-06	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
FCDPH087-07	<i>Munida intermedia</i>	Galatheidae	This study
GBCMD0242-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0243-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0244-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0245-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0246-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0247-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0248-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0249-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0250-06	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0251-06	<i>Munida lenticularis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0252-06	<i>Munida leptosyne</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0253-06	<i>Munida leviantennata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0254-06	<i>Munida militaris</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0255-06	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0256-06	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0257-06	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0258-06	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0259-06	<i>Munida ofella</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0260-06	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0261-06	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0262-06	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0263-06	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0264-06	<i>Munida pagesi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0265-06	<i>Munida proto</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0266-06	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0267-06	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0268-06	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0269-06	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0270-06	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0271-06	<i>Munida psylla</i>	Galatheidae	Genbank Crustacea Malac.
FCDPA058-04	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic
FCDPA059-04	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic
FCDPA060-04	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic
GBCMD0076-06	<i>Munida rhodonia</i>	Galatheidae	Genbank Crustacea Malac.

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GBCMD0077-06	<i>Munida rhodonia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0272-06	<i>Munida rogeri</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0273-06	<i>Munida rosula</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0078-06	<i>Munida rubrodigitalis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0274-06	<i>Munida rufiantennulata</i>	Galatheidae	Genbank Crustacea Malac.
FCDOP063-07	<i>Munida rugosa</i>	Galatheidae	This study
FCDOP176-07	<i>Munida rutillanti</i>	Galatheidae	This study
FCDOP177-07	<i>Munida rutillanti</i>	Galatheidae	This study
FCDOP178-07	<i>Munida rutillanti</i>	Galatheidae	This study
FCDOP179-07	<i>Munida rutillanti</i>	Galatheidae	This study
FCDOP180-07	<i>Munida rutillanti</i>	Galatheidae	This study
JSDME053-08	<i>Munida rutillanti</i>	Galatheidae	This study
JSDME054-08	<i>Munida rutillanti</i>	Galatheidae	This study
GBCMD0275-06	<i>Munida spilota</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0276-06	<i>Munida spilota</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2304-08	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2303-08	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2302-08	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0277-06	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0278-06	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0279-06	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0280-06	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2315-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2314-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2313-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2312-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2310-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2309-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2308-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2307-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2306-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2305-08	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0281-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0282-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0283-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0284-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0285-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0286-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0287-06	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0499-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0504-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0506-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0507-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0508-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0511-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0679-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0686-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0688-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0692-06	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0293-06	<i>Munida tiresias</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0294-06	<i>Munida tuberculata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0295-06	<i>Munida tyche</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0296-06	<i>Munida tyche</i>	Galatheidae	Genbank Crustacea Malac.

GBCMD0300-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0302-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0512-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0513-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0514-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0515-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0518-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0520-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0521-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0694-06	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3483-09	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD957-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD954-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD953-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD950-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD949-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD948-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD947-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD946-07	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3482-09	<i>Munidopsis aries</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3479-09	<i>Munidopsis cascadia</i>	Galatheidae	Genbank Crustacea Malac.
WW101-07	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
WW849-08	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
WW848-08	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
GBCMD930-07	<i>Munidopsis kensmithi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD927-07	<i>Munidopsis kensmithi</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1520-07	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3598-09	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3597-09	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3596-09	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3475-09	<i>Munidopsis polymorpha</i>	Galatheidae	Genbank Crustacea Malac.
FCDPA137-04	<i>Munidopsis quadrata</i>	Galatheidae	Decapods of Pacific and Atlantic
GBCMD3478-09	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3477-09	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD939-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD938-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD937-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD936-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD935-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD934-07	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD933-07	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD932-07	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD931-07	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD952-07	<i>Munidopsis segonzaci</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3487-09	<i>Munidopsis tiburon</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD3485-09	<i>Munidopsis vrijenhoeki</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1017-07	<i>Nanhaiapotamon pingyuanense</i>	Potamidae	Genbank Crustacea Malac.
GBCMD4008-09	<i>Necora puber</i>	Portunidae	This study
GBCMD4001-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD4000-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3999-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3998-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3996-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.

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GBCMD3994-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3991-09	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
JSDPX151-08	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0867-06	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
GBCMD1854-08	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1853-08	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1852-08	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1851-08	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1850-08	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1857-08	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1856-08	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1855-08	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1860-08	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1859-08	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1858-08	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3981-09	<i>Neohymericus pubescens</i>	Hymenosomatidae	Genbank Crustacea Malac.
JSDSC001-09	<i>Neolithodes grimaldii</i>	Lithodidae	This study
JSDSC002-09	<i>Neolithodes grimaldii</i>	Lithodidae	This study
JSDSC003-09	<i>Neolithodes grimaldii</i>	Lithodidae	This study
GBCMD1787-08	<i>Neorhynchoplax bovis</i>	Hymenosomatidae	Genbank Crustacea Malac.
FCDOP079-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP080-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP081-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP094-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP095-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP096-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOP097-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
GBCMD2636-09	<i>Nephrops norvegicus</i>	Nephropidae	Genbank Crustacea Malac.
JSDSC010-09	<i>Nephropsis atlantica</i>	Nephropidae	This study
JSDSC011-09	<i>Nephropsis atlantica</i>	Nephropidae	This study
JSDSC012-09	<i>Nephropsis atlantica</i>	Nephropidae	This study
GBCMD0335-06	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0336-06	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0337-06	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0338-06	<i>Onconida tropis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0339-06	<i>Onconida tropis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1807-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1806-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1802-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1801-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1800-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1797-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1796-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1792-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1790-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
GBCMD1789-08	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
FCDOP256-07	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX057-08	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX058-08	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX059-08	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX060-08	<i>Oplophorus spinosus</i>	Oplophoridae	This study
FCDPA089-04	<i>Orconectes immunis</i>	Cambaridae	Decapods of Pacific and Atlantic
FCDPA075-04	<i>Orconectes limosus</i>	Cambaridae	Decapods of Pacific and Atlantic

GBCMD3360-09	<i>Oregonia gracilis</i>	Majidae	Genbank Crustacea Malac.
JSDAZ002-08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAZ003-08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAZ008-08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAZ009-08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDME062-08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAZ005-08	<i>Pachygrapsus maurus</i>	Grapsidae	This study
JSDAZ044-08	<i>Pachygrapsus maurus</i>	Grapsidae	This study
FCDPA134-04	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
FCDPA146-04	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
FCDPA147-04	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
WW GSL343-08	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW100-07	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW125-07	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
GBCMD0131-06	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW766-08	<i>Pagurus acadianus</i>	Paguridae	Genbank Crustacea Malac.
FCDOP160-07	<i>Pagurus alatus</i>	Paguridae	This study
FCDOP197-07	<i>Pagurus alatus</i>	Paguridae	This study
FCDOP252-07	<i>Pagurus alatus</i>	Paguridae	This study
FCDOP280-07	<i>Pagurus alatus</i>	Paguridae	This study
FCDOP200-07	<i>Pagurus alatus</i>	Paguridae	This study
WW437-08	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW438-08	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW439-08	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
GBCMD0134-06	<i>Pagurus armatus</i>	Paguridae	Genbank Crustacea Malac.
FCDOP077-07	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK146-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK149-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK150-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK151-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK152-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK153-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK154-08	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDAZ183-08	<i>Pagurus cuanensis</i>	Paguridae	This study
JSDAZ065-08	<i>Pagurus cuanensis</i>	Paguridae	This study
FCDOP050-07	<i>Pagurus excavatus</i>	Paguridae	This study
FCDOP067-07	<i>Pagurus excavatus</i>	Paguridae	This study
FCDOP001-07	<i>Pagurus excavatus</i>	Paguridae	This study
FCDOP002-07	<i>Pagurus excavatus</i>	Paguridae	This study
FCDOP003-07	<i>Pagurus excavatus</i>	Paguridae	This study
JSDME003-08	<i>Pagurus excavatus</i>	Paguridae	This study
JSDME004-08	<i>Pagurus excavatus</i>	Paguridae	This study
JSDME005-08	<i>Pagurus excavatus</i>	Paguridae	This study
WW779-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW774-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW775-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW772-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW773-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW GSL351-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW GSL337-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW GSL317-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
GBCMD0133-06	<i>Pagurus ochetensis</i>	Paguridae	Genbank Crustacea Malac.
FCDOP036-07	<i>Pagurus prideauxi</i>	Paguridae	This study

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FCDOP038-07	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOP034-07	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOP045-07	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOP004-07	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOP008-07	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDN009-08	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDN011-08	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDUK147-08	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDUK159-08	<i>Pagurus prideauxi</i>	Paguridae	This study
WW862-08	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW863-08	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
WW864-08	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
JSDN005-08	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN014-08	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN015-08	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN022-08	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN023-08	<i>Pagurus pubescens</i>	Paguridae	This study
JSDSV001-09	<i>Pagurus pubescens</i>	Paguridae	This study
JSDSV002-09	<i>Pagurus pubescens</i>	Paguridae	This study
GBCMD2445-09	<i>Palaemon debilis</i>	Paguridae	Genbank Crustacea Malac.
FCDPA157-05	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FCDPA158-05	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FCDPA159-05	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FCDPA161-05	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
JSDAZ172-08	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK161-08	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK162-08	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK163-08	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK164-08	<i>Palaemon elegans</i>	Palaemonidae	This study
GBCMD0178-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0179-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0180-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0181-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0182-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0183-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0184-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0185-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0186-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD0187-06	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
FCDOP072-07	<i>Palaemon serratus</i>	Palaemonidae	This study
FCDOP073-07	<i>Palaemon serratus</i>	Palaemonidae	This study
JSDUK165-08	<i>Palaemon serratus</i>	Palaemonidae	This study
JSDUK166-08	<i>Palaemon serratus</i>	Palaemonidae	This study
JSDUK167-08	<i>Palaemon serratus</i>	Palaemonidae	This study
WWGSL319-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
WWGSL320-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
WWGSL321-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
WW642-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
WWGSL089-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
WWGSL091-08	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
GBCMD2626-09	<i>Palibythus magnificus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2621-09	<i>Palinurus barbareae</i>	Synaxidae	Genbank Crustacea Malac.
GBCMD2622-09	<i>Palinurus charlestoni</i>	Palinuridae	Genbank Crustacea Malac.

GBCMD2623-09	<i>Palinurus delagoae</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2625-09	<i>Palinurus elephas</i>	Palinuridae	Genbank Crustacea Malac.
JSDAZ214-08	<i>Palinurus elephas</i>	Palinuridae	This study
JSDAZ215-08	<i>Palinurus elephas</i>	Palinuridae	This study
GBCMD0188-06	<i>Palinurus elephas</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2620-09	<i>Palinurus gilchristi</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2624-09	<i>Palinurus mauritanicus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0189-06	<i>Palinurus mauritanicus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD3594-09	<i>Pandalopsis coccinata</i>	Pandalidae	Genbank Crustacea Malac.
FCDPA018-04	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA019-04	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA105-04	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
WW103-07	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WW104-07	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL370-08	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL369-08	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
FCDPA113-04	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA114-04	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA115-04	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA001-04	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
GBCMD0018-06	<i>Pandalus eous</i>	Pandalidae	Genbank Crustacea Malac.
FCDPA002-04	<i>Pandalus goniurus</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA003-04	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA004-04	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA005-04	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
GBCMD0019-06	<i>Pandalus hypsinotus</i>	Pandalidae	Genbank Crustacea Malac.
FCDPA006-04	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA007-04	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA008-04	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA009-04	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
WW871-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL083-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
JSDUK171-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WW091-07	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WW092-07	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL105-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL106-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL109-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL114-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
WWGSL125-08	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
FCDPA010-04	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA011-04	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA012-04	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA153-04	<i>Pandalus platyceros</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA014-04	<i>Pandalus platyceros</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA016-04	<i>Pandalus stenolepis</i>	Pandalidae	Decapods of Pacific and Atlantic
FCDPA017-04	<i>Pandalus stenolepis</i>	Pandalidae	Decapods of Pacific and Atlantic
GBCMD2612-09	<i>Panulirus argus</i>	Pandalidae	Genbank Crustacea Malac.
GBCMD0094-06	<i>Panulirus argus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0095-06	<i>Panulirus cygnus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0096-06	<i>Panulirus echinatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0097-06	<i>Panulirus gracilis</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0098-06	<i>Panulirus guttatus</i>	Palinuridae	Genbank Crustacea Malac.

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GBCMD2618-09	<i>Panulirus homarus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2617-09	<i>Panulirus inflatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0101-06	<i>Panulirus inflatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0001-06	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2613-09	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0891-06	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0104-06	<i>Panulirus laevicauda</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0107-06	<i>Panulirus marginatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0109-06	<i>Panulirus ornatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0108-06	<i>Panulirus pascuensis</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2611-09	<i>Panulirus penicillatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0110-06	<i>Panulirus penicillatus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0111-06	<i>Panulirus polypagrus</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2619-09	<i>Panulirus regius</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0112-06	<i>Panulirus regius</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0113-06	<i>Panulirus stimsoni</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0032-06	<i>Panulirus versicolor</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0114-06	<i>Panulirus versicolor</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD3971-09	<i>Paragrappus laevis</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD0021-06	<i>Paralithodes brevipes</i>	Grapsidae	Genbank Crustacea Malac.
GBCMD0022-06	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0023-06	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0024-06	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0027-06	<i>Paralithodes camtschaticus</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0025-06	<i>Paralithodes platypus</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0026-06	<i>Paralithodes platypus</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0020-06	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
FCDPA057-04	<i>Paralomis multispina</i>	Lithodidae	Decapods of Pacific and Atlantic
GBCMD2320-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD2319-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1986-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1985-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1984-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1982-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1981-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD1980-08	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4355-09	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4354-09	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4353-09	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD4352-09	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0309-06	<i>Paramunida belone</i>	Lithodidae	Genbank Crustacea Malac.
GBCMD0310-06	<i>Paramunida granulata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0311-06	<i>Paramunida labis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0312-06	<i>Paramunida labis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0313-06	<i>Paramunida luminata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0314-06	<i>Paramunida luminata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0315-06	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0316-06	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0317-06	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0318-06	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0319-06	<i>Paramunida pronoe</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0320-06	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0321-06	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.

GBCMD0322-06	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0323-06	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0324-06	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0325-06	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0326-06	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD1157-07	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1155-07	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1120-07	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1104-07	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1098-07	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD2002-08	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD2000-08	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1999-08	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1997-08	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1993-08	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD0668-06	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1116-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD3522-09	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1085-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD3518-09	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1070-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1069-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1066-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1062-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
GBCMD1057-07	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
FCDOP154-07	<i>Parapenaeus longirostris</i>	Penaeidae	This study
JSDME037-08	<i>Parapenaeus longirostris</i>	Penaeidae	This study
JSDME038-08	<i>Parapenaeus longirostris</i>	Penaeidae	This study
GBCMD1926-08	<i>Parastacus brasiliensis</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD1927-08	<i>Parastacus pugnax</i>	Parastacidae	Genbank Crustacea Malac.
JSDPX108-08	<i>Pasiphaea hoplocerca</i>	Parastacidae	This study
JSDSC017-09	<i>Pasiphaea multidentata</i>	Pasiphaeidae	This study
JSDSC016-09	<i>Pasiphaea multidentata</i>	Pasiphaeidae	This study
WWGSL117-08	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
WWGSL126-08	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
WWGSL133-08	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
FCDPA128-04	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA041-04	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA042-04	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA043-04	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA129-04	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDOP103-07	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCDOP104-07	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCDOP105-07	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCDOP106-07	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCDOP107-07	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCDPA044-04	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA045-04	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA047-04	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCDPA048-04	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
JSDSC007-09	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study
JSDSC008-09	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study
JSDSC009-09	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study

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FCDOP223-07	<i>Penaeopsis serrata</i>	Pasiphaeidae	This study
GBCMD0890-06	<i>Penaeus monodon</i>	Penaeidae	Genbank Crustacea Malac.
GBCMD0058-06	<i>Penaeus monodon</i>	Penaeidae	Genbank Crustacea Malac.
JSDAZ039-08	<i>Percnon gibbesi</i>	Penaeidae	This study
JSDAZ040-08	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAZ041-08	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAZ075-08	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAZ076-08	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAZ077-08	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAZ079-08	<i>Percnon gibbesi</i>	Grapsidae	This study
GBCMD2644-09	<i>Periclimenes lamellibrachiophilus</i>	Grapsidae	Genbank Crustacea Malac.
GBCMD2642-09	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2649-09	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2646-09	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2810-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2793-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2781-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2760-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2745-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2730-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2720-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2717-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2701-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD2670-09	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4290-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4289-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4294-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4293-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4292-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4291-09	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4283-09	<i>Petrolisthes armatus</i>	Palaemonidae	Genbank Crustacea Malac.
GBCMD4245-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4243-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4235-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4228-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4214-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4191-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4172-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4166-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD4158-09	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FCDPH070-07	<i>Philoheras monocanthus</i>	Porcellanidae	This study
GBCMD0545-06	<i>Phricotelphusa limula</i>	Crangonidae	Genbank Crustacea Malac.
JSDAZ121-08	<i>Pilumnus hirtellus</i>	Parathelphusidae	This study
JSDUK173-08	<i>Pilumnus hirtellus</i>	Pilumnidae	This study
JSDUK174-08	<i>Pilumnus hirtellus</i>	Pilumnidae	This study
FCDPH041-07	<i>Pilumnus inermis</i>	Pilumnidae	This study
GBCMD3357-09	<i>Pitho lherminieri</i>	Pilumnidae	Genbank Crustacea Malac.
GBCMD0340-06	<i>Plesionika aliena</i>	Majidae	Genbank Crustacea Malac.
FCDOP291-07	<i>Plesionika acanthonotus</i>	Galatheidae	This study
JSDPX067-08	<i>Plesionika acanthonotus</i>	Pandalidae	This study
JSDPX068-08	<i>Plesionika acanthonotus</i>	Pandalidae	This study
JSDME070-08	<i>Plesionika edwardsii</i>	Pandalidae	This study
JSDME071-08	<i>Plesionika edwardsii</i>	Pandalidae	This study

JSDME073-08	<i>Plesionika edwardsii</i>	Pandalidae	This study
JSDME074-08	<i>Plesionika edwardsii</i>	Pandalidae	This study
GBCMD0385-06	<i>Plesionika ensis</i>	Pandalidae	Genbank Crustacea Malac.
FCDOP084-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP087-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP088-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP025-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP027-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP028-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP029-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP208-07	<i>Plesionika heterocarpus</i>	Pandalidae	This study
JSDME029-08	<i>Plesionika heterocarpus</i>	Pandalidae	This study
JSDME030-08	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOP133-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP134-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP135-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP155-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP156-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP158-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP163-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP206-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP207-07	<i>Plesionika martia</i>	Pandalidae	This study
FCDOP195-07	<i>Plesionika narval</i>	Pandalidae	This study
FCDOP232-07	<i>Plesionika narval</i>	Pandalidae	This study
JSDME077-08	<i>Plesionika narval</i>	Pandalidae	This study
JSDME078-08	<i>Plesionika narval</i>	Pandalidae	This study
JSDME079-08	<i>Plesionika narval</i>	Pandalidae	This study
GBCMD0341-06	<i>Pleuroncodes monodon</i>	Pandalidae	Genbank Crustacea Malac.
GBCMD3365-09	<i>Podochela hemphillii</i>	Pandalidae	Genbank Crustacea Malac.
FCDOP058-07	<i>Polybius henslowii</i>	Galatheidae	This study
FCDOP059-07	<i>Polybius henslowii</i>	Inachidae	This study
FCDOP060-07	<i>Polybius henslowii</i>	Portunidae	This study
FCDOP061-07	<i>Polybius henslowii</i>	Portunidae	This study
FCDOP062-07	<i>Polybius henslowii</i>	Portunidae	This study
FCDOP089-07	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK102-08	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK103-08	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK074-08	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK078-08	<i>Polybius henslowii</i>	Portunidae	This study
GBCMD4371-09	<i>Polycheles sculptus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD4374-09	<i>Polycheles typhlops</i>	Portunidae	Genbank Crustacea Malac.
GBCMD4372-09	<i>Polycheles typhlops</i>	Polychelidae	Genbank Crustacea Malac.
FCDOP243-07	<i>Polycheles typhlops</i>	Polychelidae	This study
GBCMD2638-09	<i>Polycheles typhlops</i>	Polychelidae	Genbank Crustacea Malac.
JSDPX014-08	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDPX015-08	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDPX016-08	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDSC033-09	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDSC035-09	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDSC036-09	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDME015-08	<i>Pontocaris cathanphracta</i>	Polychelidae	This study
JSDME016-08	<i>Pontocaris cathanphracta</i>	Polychelidae	This study
JSDME017-08	<i>Pontocaris cathanphracta</i>	Crangonidae	This study

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FCDPH023-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCDOP139-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCDOP173-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCDOP174-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCDOP175-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
WWGSL121-08	<i>Pontophilus norvegicus</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WWGSL096-08	<i>Pontophilus norvegicus</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
JSDUK178-08	<i>Porcellana platycheles</i>	Crangonidae	This study
JSDUK179-08	<i>Porcellana platycheles</i>	Crangonidae	This study
JSDUK180-08	<i>Porcellana platycheles</i>	Crangonidae	This study
JSDUK181-08	<i>Porcellana platycheles</i>	Porcellanidae	This study
JSDUK182-08	<i>Porcellana platycheles</i>	Porcellanidae	This study
GBCMD3972-09	<i>Portunus pelagicus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD1862-08	<i>Portunus sanguinolentus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD0002-06	<i>Portunus trituberculatus</i>	Porcellanidae	Genbank Crustacea Malac.
GBCMD0892-06	<i>Portunus trituberculatus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0538-06	<i>Potamon fluviatilis</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0522-06	<i>Potamonautes lirrangensis</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0524-06	<i>Potamonautes obesus</i>	Portunidae	Genbank Crustacea Malac.
GBCMD0525-06	<i>Potamonautes oderhi</i>	Potamidae	Genbank Crustacea Malac.
GBCMD0130-06	<i>Procambarus acutus</i>	Potamonautidae	Genbank Crustacea Malac.
GBCMD0472-06	<i>Procambarus clarkii</i>	Potamonautidae	Genbank Crustacea Malac.
GBCMD3369-09	<i>Procambarus simulans</i>	Potamonautidae	Genbank Crustacea Malac.
FCDOP128-07	<i>Processa modica</i>	Cambaridae	This study
FCDOP129-07	<i>Processa modica</i>	Cambaridae	This study
FCDOP130-07	<i>Processa modica</i>	Cambaridae	This study
JSDPX069-08	<i>Processa modica</i>	Processidae	This study
JSDPX070-08	<i>Processa modica</i>	Processidae	This study
GBCMD2628-09	<i>Projasus parkeri</i>	Processidae	Genbank Crustacea Malac.
GBCMD0895-06	<i>Pseudocarcinus gigas</i>	Processidae	Genbank Crustacea Malac.
GBCMD0371-06	<i>Pseudocarcinus gigas</i>	Processidae	Genbank Crustacea Malac.
GBCMD1751-08	<i>Pseudohelice subquadrata</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD2627-09	<i>Puerulus angulatus</i>	Eriphiidae	Genbank Crustacea Malac.
GBCMD3336-09	<i>Pugettia dalli</i>	Eriphiidae	Genbank Crustacea Malac.
GBCMD3334-09	<i>Pugettia gracilis</i>	Varunidae	Genbank Crustacea Malac.
GBCMD3333-09	<i>Pugettia gracilis</i>	Palinuridae	Genbank Crustacea Malac.
GBCMD3332-09	<i>Pugettia gracilis</i>	Majidae	Genbank Crustacea Malac.
GBCMD3330-09	<i>Pugettia quadridens</i>	Majidae	Genbank Crustacea Malac.
GBCMD3329-09	<i>Pugettia quadridens</i>	Majidae	Genbank Crustacea Malac.
GBCMD3326-09	<i>Pugettia richii</i>	Majidae	Genbank Crustacea Malac.
GBCMD3325-09	<i>Pugettia richii</i>	Majidae	Genbank Crustacea Malac.
GBCMD0116-06	<i>Ranina ranina</i>	Majidae	Genbank Crustacea Malac.
GBCMD0060-06	<i>Raymunida cagnetei</i>	Majidae	Genbank Crustacea Malac.
GBCMD0061-06	<i>Raymunida cagnetei</i>	Majidae	Genbank Crustacea Malac.
GBCMD0062-06	<i>Raymunida cagnetei</i>	Raninidae	Genbank Crustacea Malac.
GBCMD0063-06	<i>Raymunida confundens</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0064-06	<i>Raymunida confundens</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0065-06	<i>Raymunida dextralis</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0066-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0067-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0068-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0069-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0070-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.

GBCMD0071-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0072-06	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0073-06	<i>Raymunida erythrina</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0074-06	<i>Raymunida erythrina</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0192-06	<i>Raymunida formosanus</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD0075-06	<i>Raymunida insulata</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2610-09	<i>Rhithropanopeus harrisii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2555-09	<i>Rhithropanopeus harrisii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2537-09	<i>Rhithropanopeus harrisii</i>	Galatheidae	Genbank Crustacea Malac.
GBCMD2496-09	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD2493-09	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD2487-09	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD2485-09	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD2484-09	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD0701-06	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD0708-06	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD4315-09	<i>Romaleon polyodon</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD4314-09	<i>Romaleon polyodon</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD4313-09	<i>Romaleon polyodon</i>	Panopeidae	Genbank Crustacea Malac.
GBCMD4312-09	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
GBCMD4311-09	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
GBCMD1039-07	<i>Ryukyum yaeyamense</i>	Cancridae	Genbank Crustacea Malac.
JSDSC004-09	<i>Sabinea hystrix</i>	Cancridae	This study
JSDSC005-09	<i>Sabinea hystrix</i>	Cancridae	This study
JSDSC006-09	<i>Sabinea hystrix</i>	Potamidae	This study
WW GSL102-08	<i>Sabinea sarsi</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL103-08	<i>Sabinea sarsi</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL060-08	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL061-08	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL062-08	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW083-07	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW084-07	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GBCMD1925-08	<i>Samastacus spinifrons</i>	Crangonidae	Genbank Crustacea Malac.
GBCMD0546-06	<i>Sartoriana spinigera</i>	Crangonidae	Genbank Crustacea Malac.
GBCMD0544-06	<i>Sayamia sexpunctata</i>	Crangonidae	Genbank Crustacea Malac.
FCDPA080-04	<i>Sclerocrangon boreas</i>	Parastacidae	Decapods of Pacific and Atlantic
FCDPA082-04	<i>Sclerocrangon boreas</i>	Gecarcinucidae	Decapods of Pacific and Atlantic
FCDPA086-04	<i>Sclerocrangon boreas</i>	Parathelphusidae	Decapods of Pacific and Atlantic
FCDPA088-04	<i>Sclerocrangon boreas</i>	Crangonidae	Decapods of Pacific and Atlantic
WW GSL160-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL161-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL162-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL180-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL163-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
WW GSL164-08	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GBCMD3984-09	<i>Scylla olivacea</i>	Crangonidae	Genbank Crustacea Malac.
GBCMD3645-09	<i>Scylla olivacea</i>	Crangonidae	Genbank Crustacea Malac.
GBCMD3983-09	<i>Scylla paramamosain</i>	Crangonidae	Genbank Crustacea Malac.
GBCMD3644-09	<i>Scylla paramamosain</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3986-09	<i>Scylla serrata</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3646-09	<i>Scylla serrata</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3985-09	<i>Scylla tranquebarica</i>	Portunidae	Genbank Crustacea Malac.
GBCMD3642-09	<i>Scylla tranquebarica</i>	Portunidae	Genbank Crustacea Malac.

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GBCMD2635-09	<i>Scyllarides herklotsii</i>	Portunidae	Genbank Crustacea Malac.
GBCMD2634-09	<i>Scyllarides latus</i>	Portunidae	Genbank Crustacea Malac.
JSDAZ221-08	<i>Scyllarides latus</i>	Portunidae	This study
JSDAZ222-08	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAZ226-08	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAZ036-08	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAZ037-08	<i>Scyllarides latus</i>	Scyllaridae	This study
GBCMD2615-09	<i>Scyllarus arctus</i>	Scyllaridae	Genbank Crustacea Malac.
GBCMD2614-09	<i>Scyllarus posteli</i>	Scyllaridae	Genbank Crustacea Malac.
GBCMD2616-09	<i>Scyllarus pygmaeus</i>	Scyllaridae	Genbank Crustacea Malac.
GBCMD3344-09	<i>Scyra acutifrons</i>	Scyllaridae	Genbank Crustacea Malac.
GBCMD3343-09	<i>Scyra acutifrons</i>	Scyllaridae	Genbank Crustacea Malac.
FCDPA133-04	<i>Sergestes similis</i>	Scyllaridae	Decapods of Pacific and Atlantic
FCDPA142-04	<i>Sergestes similis</i>	Pisidae	Decapods of Pacific and Atlantic
FCDPA143-04	<i>Sergestes similis</i>	Pisidae	Decapods of Pacific and Atlantic
FCDPA144-04	<i>Sergestes similis</i>	Sergestidae	Decapods of Pacific and Atlantic
FCDOP307-07	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX051-08	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX052-08	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX114-08	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX129-08	<i>Sergia robusta</i>	Sergestidae	This study
JSDSC013-09	<i>Sergia robusta</i>	Sergestidae	This study
JSDSC014-09	<i>Sergia robusta</i>	Sergestidae	This study
JSDSC015-09	<i>Sergia robusta</i>	Sergestidae	This study
GBCMD2301-08	<i>Shinkaia crosnieri</i>	Sergestidae	Genbank Crustacea Malac.
GBCMD1979-08	<i>Shinkaia crosnieri</i>	Sergestidae	Genbank Crustacea Malac.
FCDOP055-07	<i>Solenocera membranacea</i>	Sergestidae	This study
FCDOP074-07	<i>Solenocera membranacea</i>	Galatheidae	This study
FCDOP075-07	<i>Solenocera membranacea</i>	Galatheidae	This study
FCDOP021-07	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOP100-07	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOP101-07	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOP102-07	<i>Solenocera membranacea</i>	Solenoceridae	This study
JSDME019-08	<i>Solenocera membranacea</i>	Solenoceridae	This study
JSDME020-08	<i>Solenocera membranacea</i>	Solenoceridae	This study
JSDME021-08	<i>Solenocera membranacea</i>	Solenoceridae	This study
GBCMD3504-09	<i>Somanniathelphusa amoyensis</i>	Solenoceridae	Genbank Crustacea Malac.
GBCMD1018-07	<i>Somanniathelphusa qiongshanensis</i>	Solenoceridae	Genbank Crustacea Malac.
GBCMD3507-09	<i>Somanniathelphusa taiwanensis</i>	Solenoceridae	Genbank Crustacea Malac.
GBCMD3506-09	<i>Somanniathelphusa taiwanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD3505-09	<i>Somanniathelphusa taiwanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD1022-07	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD1021-07	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD1019-07	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD3503-09	<i>Somanniathelphusa zhangpuensis</i>	Parathelphusidae	Genbank Crustacea Malac.
GBCMD1020-07	<i>Somanniathelphusa zhapoensis</i>	Parathelphusidae	Genbank Crustacea Malac.
FCDPA119-04	<i>Spirontocaris holmesi</i>	Parathelphusidae	Decapods of Pacific and Atlantic
FCDPA028-04	<i>Spirontocaris holmesi</i>	Parathelphusidae	Decapods of Pacific and Atlantic
FCDPA030-04	<i>Spirontocaris lamellicornis</i>	Parathelphusidae	Decapods of Pacific and Atlantic
FCDPA031-04	<i>Spirontocaris lamellicornis</i>	Hippolytidae	Decapods of Pacific and Atlantic
WWGSL136-08	<i>Spirontocaris lilljeborgii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW853-08	<i>Spirontocaris lilljeborgii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf

FCDPA077-04	<i>Spirontocaris phippsi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA084-04	<i>Spirontocaris phippsi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA120-04	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA121-04	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA122-04	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
FCDPA085-04	<i>Spirontocaris spinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
WWGSL057-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WWGSL063-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WWGSL064-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WWGSL065-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WWGSL123-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW726-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
WW727-08	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
JSDSC024-09	<i>Stereomastis grimaldi</i>	Hippolytidae	This study
JSDSC026-09	<i>Stereomastis grimaldi</i>	Hippolytidae	This study
GBCMD1044-07	<i>Stoliczka chaseni</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD1843-08	<i>Strigopagurus pouponi</i>	Polychelidae	Genbank Crustacea Malac.
GBCMD1769-08	<i>Stygiocaris lancifera</i>	Polychelidae	Genbank Crustacea Malac.
GBCMD1768-08	<i>Stygiocaris lancifera</i>	Potamidae	Genbank Crustacea Malac.
GBCMD1767-08	<i>Stygiocaris lancifera</i>	Diogenidae	Genbank Crustacea Malac.
GBCMD1766-08	<i>Stygiocaris lancifera</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1778-08	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1777-08	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1776-08	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1775-08	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1774-08	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
FCDOP204-07	<i>Stylopandalus richardi</i>	Atyidae	This study
FCDPA049-04	<i>Systellaspis braueri</i>	Atyidae	Decapods of Pacific and Atlantic
JSDPX079-08	<i>Systellaspis debilis</i>	Atyidae	This study
JSDPX080-08	<i>Systellaspis debilis</i>	Atyidae	This study
JSDPX081-08	<i>Systellaspis debilis</i>	Pandalidae	This study
JSDPX082-08	<i>Systellaspis pellucida</i>	Pandalidae	This study
JSDPX084-08	<i>Systellaspis pellucida</i>	Oplophoridae	This study
GBCMD3324-09	<i>Taliepus dentatus</i>	Oplophoridae	Genbank Crustacea Malac.
GBCMD3323-09	<i>Taliepus nuttallii</i>	Oplophoridae	Genbank Crustacea Malac.
GBCMD1542-07	<i>Telmessus acutidens</i>	Oplophoridae	Genbank Crustacea Malac.
GBCMD0028-06	<i>Telmessus cheiragonus</i>	Oplophoridae	Genbank Crustacea Malac.
GBCMD1043-07	<i>Terrapotamon abbotti</i>	Oplophoridae	Genbank Crustacea Malac.
GBCMD2631-09	<i>Thenus unimaculatus</i>	Epialtidae	Genbank Crustacea Malac.
JSDUK183-08	<i>Thoralus cranchii</i>	Epialtidae	This study
JSDUK184-08	<i>Thoralus cranchii</i>	Atelecyclidae	This study
JSDUK185-08	<i>Thoralus cranchii</i>	Atelecyclidae	This study
GBCMD3359-09	<i>Tiarinia cornigera</i>	Potamidae	Genbank Crustacea Malac.
GBCMD3358-09	<i>Tiarinia spinigera</i>	Scyllaridae	Genbank Crustacea Malac.
GBCMD3783-09	<i>Troglocaris anophthalmus</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD3777-09	<i>Troglocaris anophthalmus</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD3764-09	<i>Troglocaris anophthalmus</i>	Hippolytidae	Genbank Crustacea Malac.
GBCMD3750-09	<i>Troglocaris anophthalmus</i>	Majidae	Genbank Crustacea Malac.
GBCMD3736-09	<i>Troglocaris anophthalmus</i>	Majidae	Genbank Crustacea Malac.
GBCMD3724-09	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3700-09	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3679-09	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3676-09	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.

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GBCMD1210-07	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3689-09	<i>Troglocaris bosnica</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3688-09	<i>Troglocaris bosnica</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3552-09	<i>Troglocaris hercegovinensis</i>	Atyidae	Genbank Crustacea Malac.
GBCMD1205-07	<i>Troglocaris inermis</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3551-09	<i>Troglocaris kutaißiana</i>	Atyidae	Genbank Crustacea Malac.
GBCMD3603-09	<i>Typhlatya pretneri</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4146-09	<i>Uca minax</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4145-09	<i>Uca minax</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4144-09	<i>Uca minax</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4138-09	<i>Uca minax</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4137-09	<i>Uca minax</i>	Atyidae	Genbank Crustacea Malac.
GBCMD4117-09	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4115-09	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4113-09	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4112-09	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD4107-09	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1420-07	<i>Upogebia africana</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1419-07	<i>Upogebia africana</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1418-07	<i>Upogebia africana</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1417-07	<i>Upogebia africana</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1416-07	<i>Upogebia africana</i>	Ocypodidae	Genbank Crustacea Malac.
GBCMD1415-07	<i>Upogebia africana</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1414-07	<i>Upogebia africana</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1413-07	<i>Upogebia africana</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1412-07	<i>Upogebia africana</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1411-07	<i>Upogebia africana</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD0093-06	<i>Varuna litterata</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD0177-06	<i>Varuna litterata</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1929-08	<i>Virilastacus retamali</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1928-08	<i>Virilastacus retamali</i>	Upogebiidae	Genbank Crustacea Malac.
GBCMD1930-08	<i>Virilastacus rucapihuelensis</i>	Upogebiidae	Genbank Crustacea Malac.
FCDPH004-07	<i>Vulcanocalliax arutyunovi</i>	Varunidae	This study
JSDAZ109-08	<i>Xantho hydrophilus</i>	Varunidae	This study
JSDAZ138-08	<i>Xantho hydrophilus</i>	Parastacidae	This study
JSDAZ017-08	<i>Xantho hydrophilus</i>	Parastacidae	This study
JSDAZ023-08	<i>Xantho hydrophilus</i>	Parastacidae	This study
JSDAZ231-08	<i>Xantho hydrophilus</i>	Callianassidae	This study
JSDAZ026-08	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAZ032-08	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAZ034-08	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAZ080-08	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAZ081-08	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDUK186-08	<i>Xantho pilipes</i>	Xanthidae	This study
JSDUK187-08	<i>Xantho pilipes</i>	Xanthidae	This study
JSDUK188-08	<i>Xantho pilipes</i>	Xanthidae	This study
GBCMD0866-06	<i>Xantho pilipes</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2292-08	<i>Xiphocaris elongata</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2291-08	<i>Xiphocaris elongata</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2289-08	<i>Xiphocaris elongata</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2262-08	<i>Xiphocaris elongata</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2261-08	<i>Xiphocaris elongata</i>	Xanthidae	Genbank Crustacea Malac.
GBCMD2253-08	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.

GBCMD2246-08	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2243-08	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2237-08	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
GBCMD2234-08	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.

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Table S 3.1.3: Accession numbers for the sequences removed from the decapod diversity assessment analysis. Specimens' list of 340 COI sequences from 79 species, 30 genera and 19 families.

Sample ID	Identification	Family	BOLD project name
AM180945	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180946	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180947	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180948	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180942	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180943	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180944	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AY351053	<i>Bathymunida nebulosa</i>	Galatheidae	Genbank Crustacea Malac.
AY351052	<i>Bathymunida nebulosa</i>	Galatheidae	Genbank Crustacea Malac.
FJ644727	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644730	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644746	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644768	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644771	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644821	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644825	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644839	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644855	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
FJ644859	<i>Callianassa kraussi</i>	Callianassidae	Genbank Crustacea Malac.
EU583574	<i>Cambarus bartonii</i>	Cambaridae	Genbank Crustacea Malac.
AY701190	<i>Cambarus bartonii</i>	Cambaridae	Genbank Crustacea Malac.
DQ411770	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411771	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411772	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411773	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411760	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411767	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411768	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411769	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411774	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411776	<i>Cambarus hamulatus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411777	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
DQ411778	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
DQ411779	<i>Cambarus jonesi</i>	Cambaridae	Genbank Crustacea Malac.
FJ455506	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
NC_011598	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
AF516699	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
AF317328	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
AF317327	<i>Eriocheir hepuensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750314	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
FJ750319	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
FJ750321	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
FJ750323	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
FJ750326	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
NC_011597	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
AY640101	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
AY640095	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
AY640092	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.

AY640089	<i>Eriocheir japonica</i>	Varunidae	Genbank Crustacea Malac.
AF317332	<i>Eriocheir rectus</i>	Varunidae	Genbank Crustacea Malac.
FJ750306	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750308	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750309	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750310	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ455507	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AY640086	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AY640083	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435119	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435118	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435114	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
DQ006297	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006296	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006295	<i>Euastacus armatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006312	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006311	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006309	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006308	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006307	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006306	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006305	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006304	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006303	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006302	<i>Euastacus balanensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006326	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006325	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006324	<i>Euastacus crassus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006332	<i>Euastacus diversus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006331	<i>Euastacus diversus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006344	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006342	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006362	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006361	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006358	<i>Euastacus neohirsutus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006377	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006376	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006375	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006374	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006373	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006372	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006371	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006370	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006369	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006368	<i>Euastacus robertsi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006396	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006394	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006393	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006392	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006391	<i>Euastacus sulcatus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006412	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006411	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006410	<i>Euastacus yarraensis</i>	Parastacidae	Genbank Crustacea Malac.

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DQ006414	<i>Euastacus yigara</i>	Parastacidae	Genbank Crustacea Malac.
DQ006413	<i>Euastacus yigara</i>	Parastacidae	Genbank Crustacea Malac.
AB266292	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
AB266293	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
AB266294	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
AB266295	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
AB266296	<i>Geothelphusa albogilva</i>	Potamidae	Genbank Crustacea Malac.
AB266280	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
AB266281	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
AB266282	<i>Geothelphusa ancylophallus</i>	Potamidae	Genbank Crustacea Malac.
AB266312	<i>Geothelphusa aramotoi</i>	Potamidae	Genbank Crustacea Malac.
AB266306	<i>Geothelphusa bicolor</i>	Potamidae	Genbank Crustacea Malac.
AB453221	<i>Geothelphusa candidiensis</i>	Potamidae	Genbank Crustacea Malac.
AB453222	<i>Geothelphusa candidiensis</i>	Potamidae	Genbank Crustacea Malac.
AB290648	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
NC_007379	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
AB187570	<i>Geothelphusa dehaani</i>	Potamidae	Genbank Crustacea Malac.
AB266304	<i>Geothelphusa ferruginea</i>	Potamidae	Genbank Crustacea Malac.
AB266311	<i>Geothelphusa miyazakii</i>	Potamidae	Genbank Crustacea Malac.
AB266269	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266270	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266271	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266272	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266274	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266276	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266277	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266278	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266266	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266268	<i>Geothelphusa olea</i>	Potamidae	Genbank Crustacea Malac.
AB266287	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266288	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266289	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266290	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266291	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266283	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266284	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266285	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266286	<i>Geothelphusa pingtung</i>	Potamidae	Genbank Crustacea Malac.
AB266313	<i>Geothelphusa sakamotoana</i>	Potamidae	Genbank Crustacea Malac.
AB453218	<i>Geothelphusa siasiat</i>	Potamidae	Genbank Crustacea Malac.
AB453219	<i>Geothelphusa siasiat</i>	Potamidae	Genbank Crustacea Malac.
AB453220	<i>Geothelphusa siasiat</i>	Potamidae	Genbank Crustacea Malac.
AB453226	<i>Geothelphusa tali</i>	Potamidae	Genbank Crustacea Malac.
AB266297	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266298	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266299	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266300	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266301	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266302	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
AB266303	<i>Geothelphusa tawu</i>	Potamidae	Genbank Crustacea Malac.
EF173809	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
EF173793	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
EF173797	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.

EF173828	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
EF173831	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
DQ399246	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
DQ399211	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
DQ399207	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
DQ399199	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
DQ399178	<i>Halocaridina rubra</i>	Atyidae	Genbank Crustacea Malac.
AB334543	<i>Helice formosensis</i>	Varunidae	Genbank Crustacea Malac.
AB334544	<i>Helice formosensis</i>	Varunidae	Genbank Crustacea Malac.
AB334545	<i>Helice latimera</i>	Varunidae	Genbank Crustacea Malac.
AB334546	<i>Helice tientsinensis</i>	Varunidae	Genbank Crustacea Malac.
AB334547	<i>Helice tientsinensis</i>	Varunidae	Genbank Crustacea Malac.
AB334548	<i>Helice tridens</i>	Varunidae	Genbank Crustacea Malac.
AB334549	<i>Helice tridens</i>	Varunidae	Genbank Crustacea Malac.
GSL31-19	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
GSL31-18	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
TE-004T196-01	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
BSM07T1-29	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
BSM07T1-24	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
HAR02CN0806	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
HAR01CN0806	<i>Hyas araneus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
JSDSv08	<i>Hyas araneus</i>	Majidae	This study
JSDSv07	<i>Hyas araneus</i>	Majidae	This study
EU682834	<i>Hyas araneus</i>	Majidae	Genbank Crustacea Malac.
GSL31-23	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
GSL31-21	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
BSM07T1-28	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
BSM07T9-02	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
BSM07T9-01	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
HC02CN0806	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
HC01CN0806	<i>Hyas coarctatus</i>	Majidae	Crustaceans of the St. Lawrence Gulf
JSDUK176	<i>Hyas coarctatus</i>	Majidae	This study
FC-ZBA23	<i>Hyas lyratus</i>	Majidae	Decapods of Pacific and Atlantic
DQ351392	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351390	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351397	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351402	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351403	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351416	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351420	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351421	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351424	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
DQ351426	<i>Hymenosoma orbiculare</i>	Hymenosomatidae	Genbank Crustacea Malac.
AB290633	<i>Johora cunislmani</i>	Potamidae	Genbank Crustacea Malac.
AB290636	<i>Johora gua</i>	Potamidae	Genbank Crustacea Malac.
AB290640	<i>Johora punicea</i>	Potamidae	Genbank Crustacea Malac.
AB290644	<i>Johora tiomanensis</i>	Potamidae	Genbank Crustacea Malac.
EU135835	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135842	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135853	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135854	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135860	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135862	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.

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EU135863	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135864	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135865	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135867	<i>Lysmata wurdemanni</i>	Hippolytidae	Genbank Crustacea Malac.
AB250510	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
AB250546	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
FJ171913	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
FM958079	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
NC_006880	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
AY659990	<i>Macrobrachium rosenbergii</i>	Palaemonidae	Genbank Crustacea Malac.
FM958082	<i>Macrobrachium shokitai</i>	Palaemonidae	Genbank Crustacea Malac.
FCDOPB087-03	<i>Macropodia longipes</i>	Majidae	This study
FCDOPB086-09	<i>Macropodia longipes</i>	Majidae	This study
FCDOPB084-05	<i>Macropodia longipes</i>	Majidae	This study
FCDOPB068-10	<i>Macropodia longipes</i>	Majidae	This study
FCFOP70-23	<i>Macropodia longipes</i>	Majidae	This study
EU186133	<i>Metanephrops andamanicus</i>	Nephropidae	Genbank Crustacea Malac.
EU186136	<i>Metanephrops armatus</i>	Nephropidae	Genbank Crustacea Malac.
EU186135	<i>Metanephrops japonicus</i>	Nephropidae	Genbank Crustacea Malac.
EU186131	<i>Metanephrops sagamiensis</i>	Nephropidae	Genbank Crustacea Malac.
EU186132	<i>Metanephrops velutinus</i>	Nephropidae	Genbank Crustacea Malac.
AY700163	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
AY700164	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
AY700165	<i>Munida gregaria</i>	Galatheidae	Genbank Crustacea Malac.
AY700166	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700167	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700168	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700169	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700171	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700172	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700173	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700174	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700175	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700176	<i>Munida subrugosa</i>	Galatheidae	Genbank Crustacea Malac.
DQ677678	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677681	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677682	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677685	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677686	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677687	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677688	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677689	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
DQ677677	<i>Munidopsis antonii</i>	Galatheidae	Genbank Crustacea Malac.
JSDMe62	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAz09	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAz08	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAz03	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAz02	<i>Pachygrapsus marmoratus</i>	Grapsidae	This study
JSDAz44	<i>Pachygrapsus maurus</i>	Grapsidae	This study
JSDAz05	<i>Pachygrapsus maurus</i>	Grapsidae	This study
JSDUK164	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK163	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDUK162	<i>Palaemon elegans</i>	Palaemonidae	This study

JSDEUK161	<i>Palaemon elegans</i>	Palaemonidae	This study
JSDAz186	<i>Palaemon elegans</i>	Palaemonidae	This study
FC-DPBAS02E	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FC-DPBAS02C	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FC-DPBAS02B	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
FC-DPBAS02A	<i>Palaemon elegans</i>	Palaemonidae	Decapods of Pacific and Atlantic
AF339467	<i>Panulirus ornatus</i>	Palinuridae	Genbank Crustacea Malac.
EF680488	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
EF680490	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
EF680491	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
EF680493	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
EF680497	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394182	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394184	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394219	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394235	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394241	<i>Paranephrops planifrons</i>	Parastacidae	Genbank Crustacea Malac.
DQ394250	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394268	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394223	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394254	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394269	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394270	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394273	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394277	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ394282	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006417	<i>Paranephrops zealandicus</i>	Parastacidae	Genbank Crustacea Malac.
JSDEUKdeep_13	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study
JSDEUKdeep_12	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study
JSDEUKdeep_11	<i>Pasiphaea tarda</i>	Pasiphaeidae	This study
FC-TAE22D	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAE22B	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAE13B	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAE13A	<i>Pasiphaea tarda</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
AB265242	<i>Somanniathelphusa amoyensis</i>	Pandalidae	Genbank Crustacea Malac.
AB265248	<i>Somanniathelphusa qiongshanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265239	<i>Somanniathelphusa taiwanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265240	<i>Somanniathelphusa taiwanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265241	<i>Somanniathelphusa taiwanensis</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265244	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265245	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265247	<i>Somanniathelphusa zanklon</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265243	<i>Somanniathelphusa zhangpuensis</i>	Parathelphusidae	Genbank Crustacea Malac.
AB265246	<i>Somanniathelphusa zhapoensis</i>	Parathelphusidae	Genbank Crustacea Malac.
TE-004T141-160-01	<i>Spirontocaris spinus</i>	Parathelphusidae	Crustaceans of the St. Lawrence Gulf
GSL31-34	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
GSL31-33	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
GSL31-32	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
GSL31-26	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
BSM07T11-03	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
BSM07T11-02	<i>Spirontocaris spinus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FC-DPA10	<i>Spirontocaris spinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
EU123814	<i>Stygiocaris stylifera</i>	Hippolytidae	Genbank Crustacea Malac.

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EU123815	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
EU123816	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
EU123817	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
EU123818	<i>Stygiocaris stylifera</i>	Atyidae	Genbank Crustacea Malac.
FCFOPC047-06	<i>Stylopandalus richardi</i>	Atyidae	This study
FCFOPC046-02	<i>Stylopandalus richardi</i>	Pandalidae	This study
FJ425933	<i>Troglocaris anophthalmus</i>	Pandalidae	Genbank Crustacea Malac.
FJ425939	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ425952	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ425966	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ425980	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ425992	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ426016	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ426037	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
FJ426040	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
DQ320041	<i>Troglocaris anophthalmus</i>	Atyidae	Genbank Crustacea Malac.
DQ641556	<i>Typhlatya pretneri</i>	Atyidae	Genbank Crustacea Malac.
JSDAz245	<i>Xantho hydrophilus</i>	Atyidae	This study
JSDAz152	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz109	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz81	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz80	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz34	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz32	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz26	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz23	<i>Xantho hydrophilus</i>	Xanthidae	This study
JSDAz17	<i>Xantho hydrophilus</i>	Xanthidae	This study

Table S 3.1.4: Accession numbers for the sequences used for assessment of decapod diversity.

Specimens' list of 1572 COI sequences from 528 species, 213 genera and 67 families.

Genbank ID	Identification	Family	BOLD project name
JSDUKdeep_37	<i>Acanthephryra pelagica</i>	Oplophoridae	This study
AM087915	<i>Acanthephryra pelagica</i>	Oplophoridae	Genbank Crustacea Malac.
JSDPX63-03	<i>Acanthephryra purpurea</i>	Oplophoridae	This study
JSDPX63-02	<i>Acanthephryra purpurea</i>	Oplophoridae	This study
FC-VLC197	<i>Acantholithodes hispidus</i>	Lithodidae	Decapods of Pacific and Atlantic
JSDMe61	<i>Acanthonyx lunulatus</i>	Majidae	This study
JSDMe59	<i>Acanthonyx lunulatus</i>	Majidae	This study
EU682854	<i>Acanthonyx petiverii</i>	Majidae	Genbank Crustacea Malac.
EU682855	<i>Acanthonyx petiverii</i>	Majidae	Genbank Crustacea Malac.
AF283889	<i>Agononida incerta</i>	Galatheidae	Genbank Crustacea Malac.
AF283888	<i>Agononida incerta</i>	Galatheidae	Genbank Crustacea Malac.
AY350914	<i>Agononida marinii</i>	Galatheidae	Genbank Crustacea Malac.
AY350917	<i>Agononida procera</i>	Galatheidae	Genbank Crustacea Malac.
AY350916	<i>Agononida procera</i>	Galatheidae	Genbank Crustacea Malac.
AY350915	<i>Agononida similis</i>	Galatheidae	Genbank Crustacea Malac.
AY350923	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY350922	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY350921	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY350920	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY350919	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY350918	<i>Agononida sphicia</i>	Galatheidae	Genbank Crustacea Malac.
AY351051	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
AY351050	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
AY351049	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
AY351048	<i>Alainius crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
FCFOPC047-03	<i>Alpheus glaber</i>	Alpheidae	This study
FCFOPC044-05	<i>Alpheus glaber</i>	Alpheidae	This study
FCFOPC051-02	<i>Alpheus glaber</i>	Alpheidae	This study
AB222051	<i>Alvinocaris longirostris</i>	Bresiliidae	Genbank Crustacea Malac.
AB222050	<i>Alvinocaris longirostris</i>	Bresiliidae	Genbank Crustacea Malac.
EU031814	<i>Alvinocaris muricola</i>	Bresiliidae	Genbank Crustacea Malac.
FJ812290	<i>Amarinus paralacustris</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF683578	<i>Aniculus aniculus</i>	Diogenidae	Genbank Crustacea Malac.
EF683579	<i>Aniculus retipes</i>	Diogenidae	Genbank Crustacea Malac.
FC-SFC29A	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SFC163B	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SFC163A	<i>Argis alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
TE-004T141-160-09	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
TE-004T21-40-07	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GSL31-46	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
AD03CN0406	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
AD01CN0406	<i>Argis dentata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
FC-SFF21A	<i>Argis lar</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SFF12A	<i>Argis lar</i>	Crangonidae	Decapods of Pacific and Atlantic
JSDPXA40-01	<i>Aristeomorpha foliacea</i>	Aristeidae	This study
JSDMe36	<i>Aristeomorpha foliacea</i>	Aristeidae	This study
JSDMe35	<i>Aristeomorpha foliacea</i>	Aristeidae	This study
JSDMe33	<i>Aristeomorpha foliacea</i>	Aristeidae	This study

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FCFOPC066-02	<i>Aristeomorpha foliacea</i>	Aristeidae	This study
FCFOPC055-05	<i>Aristeomorpha foliacea</i>	Aristeidae	This study
DQ006289	<i>Astacopsis gouldi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006291	<i>Astacopsis tricornis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006290	<i>Astacopsis tricornis</i>	Parastacidae	Genbank Crustacea Malac.
AY667146	<i>Astacus astacus</i>	Astacidae	Genbank Crustacea Malac.
JSDUK02	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDUK01	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDPX22-01	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
FCDOPB077-04	<i>Atelecyclus rotundatus</i>	Atelecyclidae	This study
JSDUK07	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK06	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK05	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK04	<i>Athanas nitescens</i>	Alpheidae	This study
JSDUK03	<i>Athanas nitescens</i>	Alpheidae	This study
EU005036	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005037	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005038	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005039	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005040	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005041	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005043	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005046	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005047	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005048	<i>Atya innocous</i>	Atyidae	Genbank Crustacea Malac.
EU005054	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005056	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005057	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005063	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005069	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005072	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005074	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005075	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005080	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
EU005082	<i>Atya lanipes</i>	Atyidae	Genbank Crustacea Malac.
DQ320047	<i>Atyaephyra desmarestii</i>	Atyidae	Genbank Crustacea Malac.
AB443445	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443446	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443447	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443448	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443449	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443450	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AB443451	<i>Austropotamobius pallipes</i>	Astacidae	Genbank Crustacea Malac.
AM180945	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180946	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180947	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180948	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180942	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180943	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
AM180944	<i>Austropotamobius torrentium</i>	Astacidae	Genbank Crustacea Malac.
EF136570	<i>Babamunida hystrix</i>	Galatheidae	Genbank Crustacea Malac.
EF136571	<i>Babamunida hystrix</i>	Galatheidae	Genbank Crustacea Malac.
DQ113440	<i>Barbicambarus cornutus</i>	Cambaridae	Genbank Crustacea Malac.

JSDUKdeep_48	<i>Bathynectes maravigna</i>	Portunidae	This study
JSDUKdeep_47	<i>Bathynectes maravigna</i>	Portunidae	This study
JSDUKdeep_46	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPHM2_109	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH12-388Gr68	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH12-388Gr67	<i>Bathynectes maravigna</i>	Portunidae	This study
FCDPH11-339D1	<i>Bathynectes maravigna</i>	Portunidae	This study
FCFOPC041-22	<i>Bathynectes maravigna</i>	Portunidae	This study
FCFOPC041-20	<i>Bathynectes maravigna</i>	Portunidae	This study
FC-TRB12B	<i>Bentheogennema borealis</i>	Benthescymidae	Decapods of Pacific and Atlantic
FC-TRB12A	<i>Bentheogennema borealis</i>	Benthescymidae	Decapods of Pacific and Atlantic
JSDAz218	<i>Calappa granulata</i>	Calappidae	This study
JSDAz67	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAz66	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAz64	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAz52	<i>Calcinus tubularis</i>	Diogenidae	This study
JSDAz51	<i>Calcinus tubularis</i>	Diogenidae	This study
AY465913	<i>Callinectes arcuatus</i>	Portunidae	Genbank Crustacea Malac.
AY465911	<i>Callinectes arcuatus</i>	Portunidae	Genbank Crustacea Malac.
AY465909	<i>Callinectes bellicosus</i>	Portunidae	Genbank Crustacea Malac.
AY465907	<i>Callinectes bellicosus</i>	Portunidae	Genbank Crustacea Malac.
NC_006281	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682078	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682077	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682076	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682075	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682074	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682073	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY682072	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY465915	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
AY363392	<i>Callinectes sapidus</i>	Portunidae	Genbank Crustacea Malac.
FC-URI13B	<i>Calocaris investigatoris</i>	Calocarididae	Decapods of Pacific and Atlantic
FC-URI13A	<i>Calocaris investigatoris</i>	Calocarididae	Decapods of Pacific and Atlantic
DQ113441	<i>Cambarus batchi</i>	Cambaridae	Genbank Crustacea Malac.
DQ113442	<i>Cambarus brachydyactylus</i>	Cambaridae	Genbank Crustacea Malac.
DQ411783	<i>Cambarus brachydyactylus</i>	Cambaridae	Genbank Crustacea Malac.
DQ113443	<i>Cambarus causeyi</i>	Cambaridae	Genbank Crustacea Malac.
DQ113444	<i>Cambarus cryptodytes</i>	Cambaridae	Genbank Crustacea Malac.
EF207160	<i>Cambarus friaufi</i>	Cambaridae	Genbank Crustacea Malac.
DQ411784	<i>Cambarus friaufi</i>	Cambaridae	Genbank Crustacea Malac.
DQ411785	<i>Cambarus gentryi</i>	Cambaridae	Genbank Crustacea Malac.
AY701192	<i>Cambarus graysoni</i>	Cambaridae	Genbank Crustacea Malac.
EU583576	<i>Cambarus tenebrosus</i>	Cambaridae	Genbank Crustacea Malac.
JSDPX44-10	<i>Cancer bellianus</i>	Cancridae	This study
L195AR2-01	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
L210AR4-01	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
GSL31-20	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
GSL31-09	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
BSM07T1-26	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
CI04MD0306	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
CI02MD0204	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
CI01MD0204	<i>Cancer irroratus</i>	Cancridae	Crustaceans of the St. Lawrence Gulf
FC-XKG73E	<i>Cancer magister</i>	Cancridae	Decapods of Pacific and Atlantic

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FC-XKG73D	<i>Cancer magister</i>	Cancridae	Decapods of Pacific and Atlantic
FC-XKI	<i>Cancer oregonensis</i>	Cancridae	Decapods of Pacific and Atlantic
FC-XKI 48	<i>Cancer oregonensis</i>	Cancridae	Decapods of Pacific and Atlantic
JSDUK11	<i>Cancer pagurus</i>	Cancridae	This study
JSDUK10	<i>Cancer pagurus</i>	Cancridae	This study
FJ155375	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
FJ155376	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
FJ155377	<i>Cancer plebejus</i>	Cancridae	Genbank Crustacea Malac.
FJ155371	<i>Cancer porteri</i>	Cancridae	Genbank Crustacea Malac.
FC-XLA51	<i>Cancer productus</i>	Cancridae	Decapods of Pacific and Atlantic
FC-XLA	<i>Cancer productus</i>	Cancridae	Decapods of Pacific and Atlantic
AB290649	<i>Candidiopotamom rathbunae</i>	Potamidae	Genbank Crustacea Malac.
AY616445	<i>Carcinus aestuarii</i>	Portunidae	Genbank Crustacea Malac.
L174AR1-09	<i>Carcinus maenas</i>	Portunidae	Crustaceans of the St. Lawrence Gulf
L180AR1-01	<i>Carcinus maenas</i>	Portunidae	Crustaceans of the St. Lawrence Gulf
JSDUK19	<i>Carcinus maenas</i>	Portunidae	This study
JSDN03	<i>Carcinus maenas</i>	Portunidae	This study
JSDN02	<i>Carcinus maenas</i>	Portunidae	This study
JSDN01	<i>Carcinus maenas</i>	Portunidae	This study
DQ523686	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
DQ523685	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
DQ523683	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
AY616441	<i>Carcinus maenas</i>	Portunidae	Genbank Crustacea Malac.
AB300190	<i>Caridina cantonensis</i>	Atyidae	Genbank Crustacea Malac.
AB300189	<i>Caridina formosae</i>	Atyidae	Genbank Crustacea Malac.
AY351054	<i>Cervimunida johni</i>	Galatheidae	Genbank Crustacea Malac.
AB290212	<i>Chaceon affinis</i>	Geryonidae	Genbank Crustacea Malac.
EU284143	<i>Charybdis acuta</i>	Portunidae	Genbank Crustacea Malac.
EU284140	<i>Charybdis feriatus</i>	Portunidae	Genbank Crustacea Malac.
EU586120	<i>Charybdis japonica</i>	Portunidae	Genbank Crustacea Malac.
EU284141	<i>Charybdis vadorum</i>	Portunidae	Genbank Crustacea Malac.
AB334555	<i>Chasmagnathus convexus</i>	Varunidae	Genbank Crustacea Malac.
AB334556	<i>Chasmagnathus convexus</i>	Varunidae	Genbank Crustacea Malac.
AF493625	<i>Cherax crassimanus</i>	Parastacidae	Genbank Crustacea Malac.
NC_011243	<i>Cherax destructor</i>	Parastacidae	Genbank Crustacea Malac.
AY383557	<i>Cherax destructor</i>	Parastacidae	Genbank Crustacea Malac.
DQ006293	<i>Cherax parvus</i>	Parastacidae	Genbank Crustacea Malac.
EF118810	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118808	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118813	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118814	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118816	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118817	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118818	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118819	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
EF118821	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
AF493622	<i>Cherax preissii</i>	Parastacidae	Genbank Crustacea Malac.
DQ006294	<i>Cherax quadricarinatus</i>	Parastacidae	Genbank Crustacea Malac.
AF493621	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
AF493620	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
AF493619	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
AF493618	<i>Cherax quinquecarinatus</i>	Parastacidae	Genbank Crustacea Malac.
AF493630	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.

AF493629	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
AF493628	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
AF493627	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
AF493626	<i>Cherax tenuimanus</i>	Parastacidae	Genbank Crustacea Malac.
FC-ZAE15B	<i>Chionoecetes angulatus</i>	Oregoniidae	Decapods of Pacific and Atlantic
FC-ZAE15A	<i>Chionoecetes angulatus</i>	Oregoniidae	Decapods of Pacific and Atlantic
AB211159	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
AB211158	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
AB211157	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
AB211156	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
AB211155	<i>Chionoecetes bairdi</i>	Oregoniidae	Genbank Crustacea Malac.
FC-ZAF166	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
FC-ZAF34	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
FC-ZAF29	<i>Chionoecetes bairdi</i>	Oregoniidae	Decapods of Pacific and Atlantic
BSM08-L03	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
BSM08-L02	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
BSM08-L01	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
GSL31-01	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
CO02CN0706	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
CO01CN0706	<i>Chionoecetes opilio</i>	Oregoniidae	Crustaceans of the St. Lawrence Gulf
EU266370	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
EU682832	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
EU682833	<i>Chionoecetes opilio</i>	Oregoniidae	Genbank Crustacea Malac.
FC-ZAG11B	<i>Chionoecetes tanneri</i>	Oregoniidae	Decapods of Pacific and Atlantic
FC-ZAG11A	<i>Chionoecetes tanneri</i>	Oregoniidae	Decapods of Pacific and Atlantic
FC-ZGE124	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic
FC-ZGE48	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic
FC-ZGE18A	<i>Chorilia longipes</i>	Majidae	Decapods of Pacific and Atlantic
EU334657	<i>Ciliopagurus hawaiiensis</i>	Diogenidae	Genbank Crustacea Malac.
EF683559	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683562	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683571	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683572	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683573	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683574	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683575	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683576	<i>Ciliopagurus strigatus</i>	Diogenidae	Genbank Crustacea Malac.
EF683561	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
EF683565	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
EF683566	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
EF683567	<i>Ciliopagurus tricolor</i>	Diogenidae	Genbank Crustacea Malac.
EF683563	<i>Ciliopagurus vakovako</i>	Diogenidae	Genbank Crustacea Malac.
EF683564	<i>Ciliopagurus vakovako</i>	Diogenidae	Genbank Crustacea Malac.
JSDMe67	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDMe66	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDMe63	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDAz115	<i>Clibanarius erythropus</i>	Diogenidae	This study
JSDUK24	<i>Coryistes cassivelaunus</i>	Corystidae	This study
JSDUK23	<i>Coryistes cassivelaunus</i>	Corystidae	This study
JSDUK22	<i>Coryistes cassivelaunus</i>	Corystidae	This study
FC-SIB14B	<i>Crangon abyssorum</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SIB14A	<i>Crangon abyssorum</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SIC201B	<i>Crangon alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic

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FC-SIC201A	<i>Crangon alaskensis</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SID8A	<i>Crangon communis</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-SID122	<i>Crangon communis</i>	Crangonidae	Decapods of Pacific and Atlantic
L210AR4-06	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L210AR4-05	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L51AR1-01	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L42AR1-01	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L37AR1-01	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L187AR1-05	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L175AR1-02	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L154AR1-04	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
L184AR1-07	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
CS02SL0106	<i>Crangon septemspinosa</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
AY350924	<i>Crosnierita dicata</i>	Galatheidae	Genbank Crustacea Malac.
FCDPHMSM241_106	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPHMSM241_105	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH15-581Gr59	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH15-581Gr58	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH15-581Gr57	<i>Cymonomus granulatus</i>	Cymonomidae	This study
FCDPH15-581Gr56	<i>Cymonomus granulatus</i>	Cymonomidae	This study
JSDMe51	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDMe49	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDMe48	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDMe47	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDMe46	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDMe06	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDAz217	<i>Dardanus arrosor</i>	Diogenidae	This study
FCFOPC045-19	<i>Dardanus arrosor</i>	Diogenidae	This study
FCFOP64-04	<i>Dardanus arrosor</i>	Diogenidae	This study
FCFOP64-02	<i>Dardanus arrosor</i>	Diogenidae	This study
JSDAz208	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAz207	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAz48	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAz47	<i>Dardanus calidus</i>	Diogenidae	This study
JSDAz46	<i>Dardanus calidus</i>	Diogenidae	This study
EF683577	<i>Dardanus lagopodes</i>	Diogenidae	Genbank Crustacea Malac.
JSDUKdeep_53	<i>Dichelopandalus bonnieri</i>	Pandalidae	This study
JSDUKdeep_51	<i>Dichelopandalus bonnieri</i>	Pandalidae	This study
JSDAz199	<i>Dromia personata</i>	Dromiidae	This study
JSDAz130	<i>Dromia personata</i>	Dromiidae	This study
DQ641569	<i>Dugastella valentina</i>	Atyidae	Genbank Crustacea Malac.
L228AR1-02	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
L183AR1-01	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
L154AR1-06	<i>Dyspanopeus sayi</i>	Panopeidae	Crustaceans of the St. Lawrence Gulf
FCDPHMSM241_104	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPHMSM241_103	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPHMSM321_97	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH15-569Gr66	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH14-566Gr65	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH14-565B64	<i>Ebalia nux</i>	Leucosiidae	This study
FCDPH14-566Gr14	<i>Ebalia nux</i>	Leucosiidae	This study
FJ812283	<i>Elamena producta</i>	Hymenosomatidae	Genbank Crustacea Malac.
AF493633	<i>Engaeus strictifrons</i>	Parastacidae	Genbank Crustacea Malac.

AB241420	<i>Erimacrus isenbeckii</i>	Atelecyclidae	Genbank Crustacea Malac.
FJ750332	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
AF516698	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
AF317326	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
AF105250	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
AF105249	<i>Eriocheir formosa</i>	Varunidae	Genbank Crustacea Malac.
AF516701	<i>Eriocheir leptognathus</i>	Varunidae	Genbank Crustacea Malac.
AF316537	<i>Eriocheir leptognathus</i>	Varunidae	Genbank Crustacea Malac.
FJ750330	<i>Eriocheir ogasawaraensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750331	<i>Eriocheir ogasawaraensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750306	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750308	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750309	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ750310	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
FJ455507	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AY640086	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AY640083	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435119	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435118	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
AF435114	<i>Eriocheir sinensis</i>	Varunidae	Genbank Crustacea Malac.
JSDAz92	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz90	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz89	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz31	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz15	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz14	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz13	<i>Eriphia verrucosa</i>	Xanthidae	This study
JSDAz12	<i>Eriphia verrucosa</i>	Xanthidae	This study
FC-SQB213C	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQB213B	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQB213A	<i>Eualus avinus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQC9C	<i>Eualus barbatus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQC9B	<i>Eualus barbatus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQE13b	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQE13A	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQE11B	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQE11A	<i>Eualus biunguis</i>	Hippolytidae	Decapods of Pacific and Atlantic
BSM07T1-23	<i>Eualus fabricii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
L90AR12-01	<i>Eualus fabricii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
JSDSv17	<i>Eualus gaimardi</i>	Hippolytidae	This study
FC-DPA12	<i>Eualus gaimardi</i>	Hippolytidae	Decapods of Pacific and Atlantic
TE-004T141-160-05	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
TE-004T21-40-06	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
BSM07T11-05	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
BSM07T11-04	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
EM02CN0606	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
EM01CN0606	<i>Eualus macilentus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FC-SQJ242B	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQJ242A	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SQJ39	<i>Eualus suckleyi</i>	Hippolytidae	Decapods of Pacific and Atlantic
DQ006301	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006300	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006299	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.

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DQ006298	<i>Euastacus australasiensis</i>	Parastacidae	Genbank Crustacea Malac.
DQ006316	<i>Euastacus bindal</i>	Parastacidae	Genbank Crustacea Malac.
DQ006317	<i>Euastacus bispinosus</i>	Parastacidae	Genbank Crustacea Malac.
AF493634	<i>Euastacus bispinosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006319	<i>Euastacus brachythorax</i>	Parastacidae	Genbank Crustacea Malac.
DQ006318	<i>Euastacus brachythorax</i>	Parastacidae	Genbank Crustacea Malac.
DQ006321	<i>Euastacus clarkae</i>	Parastacidae	Genbank Crustacea Malac.
DQ006320	<i>Euastacus clarkae</i>	Parastacidae	Genbank Crustacea Malac.
DQ006323	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
DQ006322	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
AY380479	<i>Euastacus claytoni</i>	Parastacidae	Genbank Crustacea Malac.
DQ006328	<i>Euastacus dangadi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006327	<i>Euastacus dangadi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006330	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006329	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
AY380480	<i>Euastacus dharawalus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006337	<i>Euastacus fleckeri</i>	Parastacidae	Genbank Crustacea Malac.
DQ006336	<i>Euastacus fleckeri</i>	Parastacidae	Genbank Crustacea Malac.
DQ006339	<i>Euastacus gamilaroi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006338	<i>Euastacus gamilaroi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006341	<i>Euastacus gumar</i>	Parastacidae	Genbank Crustacea Malac.
DQ006340	<i>Euastacus gumar</i>	Parastacidae	Genbank Crustacea Malac.
DQ006345	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006343	<i>Euastacus guwinus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006348	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006347	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006346	<i>Euastacus hystricosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006350	<i>Euastacus jagara</i>	Parastacidae	Genbank Crustacea Malac.
DQ006349	<i>Euastacus jagara</i>	Parastacidae	Genbank Crustacea Malac.
DQ006352	<i>Euastacus kershawi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006351	<i>Euastacus kershawi</i>	Parastacidae	Genbank Crustacea Malac.
DQ006354	<i>Euastacus maidae</i>	Parastacidae	Genbank Crustacea Malac.
DQ006353	<i>Euastacus maidae</i>	Parastacidae	Genbank Crustacea Malac.
DQ006356	<i>Euastacus mirangudjin</i>	Parastacidae	Genbank Crustacea Malac.
DQ006355	<i>Euastacus mirangudjin</i>	Parastacidae	Genbank Crustacea Malac.
DQ006357	<i>Euastacus monteithorum</i>	Parastacidae	Genbank Crustacea Malac.
DQ006364	<i>Euastacus polysetosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006363	<i>Euastacus polysetosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006365	<i>Euastacus reductus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006367	<i>Euastacus rieki</i>	Parastacidae	Genbank Crustacea Malac.
DQ006366	<i>Euastacus rieki</i>	Parastacidae	Genbank Crustacea Malac.
DQ006380	<i>Euastacus setosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006379	<i>Euastacus setosus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006390	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006389	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006388	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006387	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006386	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006385	<i>Euastacus spinifer</i>	Parastacidae	Genbank Crustacea Malac.
DQ006398	<i>Euastacus Suttoni</i>	Parastacidae	Genbank Crustacea Malac.
DQ006397	<i>Euastacus Suttoni</i>	Parastacidae	Genbank Crustacea Malac.
DQ006402	<i>Euastacus valentulus</i>	Parastacidae	Genbank Crustacea Malac.
DQ006401	<i>Euastacus valentulus</i>	Parastacidae	Genbank Crustacea Malac.

DQ006404	<i>Eustacus woiwuru</i>	Parastacidae	Genbank Crustacea Malac.
DQ006403	<i>Eustacus woiwuru</i>	Parastacidae	Genbank Crustacea Malac.
DQ006409	<i>Eustacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
DQ006408	<i>Eustacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
DQ006407	<i>Eustacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
DQ006406	<i>Eustacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
DQ006405	<i>Eustacus yanga</i>	Parastacidae	Genbank Crustacea Malac.
EU243357	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243361	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243412	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243461	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243470	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243496	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
DQ011186	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
DQ011185	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
AY800021	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
AY800019	<i>Eumunida annulosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243341	<i>Eumunida capillata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243342	<i>Eumunida capillata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243343	<i>Eumunida capillata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243337	<i>Eumunida keijii</i>	Chirostylidae	Genbank Crustacea Malac.
EU243338	<i>Eumunida keijii</i>	Chirostylidae	Genbank Crustacea Malac.
EU243340	<i>Eumunida keijii</i>	Chirostylidae	Genbank Crustacea Malac.
EU243514	<i>Eumunida keijii</i>	Chirostylidae	Genbank Crustacea Malac.
EU243497	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
EU243508	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
EU243509	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
EU243510	<i>Eumunida laevimana</i>	Chirostylidae	Genbank Crustacea Malac.
EU243543	<i>Eumunida marginata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243502	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243547	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243548	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243549	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243550	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243551	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243552	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243553	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243554	<i>Eumunida minor</i>	Chirostylidae	Genbank Crustacea Malac.
EU243546	<i>Eumunida multilineata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243556	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
EU243557	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
EU243558	<i>Eumunida picta</i>	Chirostylidae	Genbank Crustacea Malac.
EU243498	<i>Eumunida similior</i>	Chirostylidae	Genbank Crustacea Malac.
EU243500	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243501	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243533	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243534	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243535	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243536	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243537	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243539	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243540	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.
EU243542	<i>Eumunida spinosa</i>	Chirostylidae	Genbank Crustacea Malac.

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EU243559	<i>Eumunida squamifera</i>	Chirostylidae	Genbank Crustacea Malac.
EU243560	<i>Eumunida squamifera</i>	Chirostylidae	Genbank Crustacea Malac.
EU243396	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243425	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243426	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243433	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243450	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243458	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243476	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243483	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
AY800029	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
AY800028	<i>Eumunida sternomaculata</i>	Chirostylidae	Genbank Crustacea Malac.
EU243352	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243358	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243359	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243511	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243512	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243516	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243517	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EU243562	<i>Eumunida treguieri</i>	Chirostylidae	Genbank Crustacea Malac.
EF560650	<i>Exopalaemon carinicauda</i>	Palaemonidae	Genbank Crustacea Malac.
NC_012566	<i>Exopalaemon carinicauda</i>	Palaemonidae	Genbank Crustacea Malac.
FM958057	<i>Exopalaemon styliferus</i>	Palaemonidae	Genbank Crustacea Malac.
X84350 X84351			
X84352 X84353			
X84354 X84355			
X84356 X84357	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
X84350 X84351			
X84352 X84353			
X84354 X84355			
X84356 X84357	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
X84350	<i>Farfantepenaeus notialis</i>	Penaeidae	Genbank Crustacea Malac.
NC_009679	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
DQ518969	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
DQ656600	<i>Fenneropenaeus chinensis</i>	Penaeidae	Genbank Crustacea Malac.
JSDPX42(05)-01	<i>Funchalia villose</i>	Benthescycmidae	This study
AF516703	<i>Gaetice depressus</i>	Varunidae	Genbank Crustacea Malac.
AF317339	<i>Gaetice depressus</i>	Varunidae	Genbank Crustacea Malac.
JSDUK30	<i>Galathea dispersa</i>	Galatheidae	This study
AF493632	<i>Geocharax falcata</i>	Parastacidae	Genbank Crustacea Malac.
JSDUKdeep_33	<i>Geryon longipes</i>	Geryonidae	This study
JSDUKdeep_32	<i>Geryon longipes</i>	Geryonidae	This study
JSDUKdeep_31	<i>Geryon longipes</i>	Geryonidae	This study
JSDPX44-012	<i>Geryon longipes</i>	Geryonidae	This study
JSDPX44-011	<i>Geryon longipes</i>	Geryonidae	This study
JSDMe83	<i>Geryon longipes</i>	Geryonidae	This study
JSDMe82	<i>Geryon longipes</i>	Geryonidae	This study
FCFOPC052-09	<i>Geryon longipes</i>	Geryonidae	This study
FCFOPC052-07	<i>Geryon longipes</i>	Geryonidae	This study
FCFOPC052-06	<i>Geryon longipes</i>	Geryonidae	This study
JSDUK41	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDUK39	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDUK37	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDPX63-06	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCFOPC041-27	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCFOPC041-25	<i>Goneplax rhomboides</i>	Goneplacidae	This study

FCDOPB083-09	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCDOPB083-08	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCFOP66-10	<i>Goneplax rhomboides</i>	Goneplacidae	This study
FCFOP66-01	<i>Goneplax rhomboides</i>	Goneplacidae	This study
JSDAz30	<i>Grapsus adscensionis</i>	Grapsidae	This study
JSDAz29	<i>Grapsus adscensionis</i>	Grapsidae	This study
JSDAz28	<i>Grapsus adscensionis</i>	Grapsidae	This study
AY803593	<i>Guinotia dentata</i>	Pseudothelphusidae	Genbank Crustacea Malac.
FJ812288	<i>Halicarcinus cookii</i>	Hymenosomatidae	Genbank Crustacea Malac.
FJ812287	<i>Halicarcinus innominatus</i>	Hymenosomatidae	Genbank Crustacea Malac.
FJ812286	<i>Halicarcinus ovatus</i>	Hymenosomatidae	Genbank Crustacea Malac.
FJ812289	<i>Halicarcinus varius</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF173842	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
EF173838	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
EF173839	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
EF173840	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
EF173841	<i>Halocaridinides trigonophthalma</i>	Atyidae	Genbank Crustacea Malac.
AB334554	<i>Helicana doerlesi</i>	Varunidae	Genbank Crustacea Malac.
AB334552	<i>Helicana japonica</i>	Varunidae	Genbank Crustacea Malac.
AB334553	<i>Helicana japonica</i>	Varunidae	Genbank Crustacea Malac.
AB334550	<i>Helicana wuana</i>	Varunidae	Genbank Crustacea Malac.
AB334551	<i>Helicana wuana</i>	Varunidae	Genbank Crustacea Malac.
EU169901	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169906	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169909	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169912	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169915	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169919	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169920	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169921	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
EU169922	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
AF317340	<i>Hemigrapsus sanguineus</i>	Varunidae	Genbank Crustacea Malac.
FJ518783	<i>Hemigrapsus sexdentatus</i>	Varunidae	Genbank Crustacea Malac.
JSDAz88	<i>Herbstia condylata</i>	Majidae	This study
JSDAz87	<i>Herbstia condylata</i>	Majidae	This study
EU682845	<i>Herbstia condylata</i>	Majidae	Genbank Crustacea Malac.
EU682829	<i>Heterocrypta occidentalis</i>	Parthenopidae	Genbank Crustacea Malac.
AB290651	<i>Himalayapotamon atkinsonianum</i>	Potamidae	Genbank Crustacea Malac.
AY701193	<i>Hobbsius valleculus</i>	Camaridae	Genbank Crustacea Malac.
FJ155383	<i>Homalaspis plana</i>	Platyxanthidae	Genbank Crustacea Malac.
GSL31-16	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
GSL31-12	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
GSL31-11	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
HA02MD0106	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
HA01MD0106	<i>Homarus americanus</i>	Nephropidae	Crustaceans of the St. Lawrence Gulf
FJ174944	<i>Homarus americanus</i>	Nephropidae	Genbank Crustacea Malac.
EU186145	<i>Homarus gammarus</i>	Nephropidae	Genbank Crustacea Malac.
JSDPXN34-01	<i>Homola barbata</i>	Homolidae	This study
MSM01-03-242_95	<i>Homola barbata</i>	Homolidae	This study
AY803578	<i>Hydrothelphusa agilis</i>	Potamonautesidae	Genbank Crustacea Malac.
AY803579	<i>Hydrothelphusa goudotii</i>	Potamonautesidae	Genbank Crustacea Malac.
AY803580	<i>Hydrothelphusa madagascariensis</i>	Potamonautesidae	Genbank Crustacea Malac.
FC-TEC11A	<i>Hymenodora frontalis</i>	Oplophoridae	Decapods of Pacific and Atlantic

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JSDPX42(05)-06	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX42(05)-05	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX42(05)-04	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX40-01	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX39-02	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
JSDPX39-01	<i>Hymenopenaeus debilis</i>	Solenoceridae	This study
FJ812285	<i>Hymenosoma depressum</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF198478	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF198479	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF198480	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF198481	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
EF198482	<i>Hymenosoma geometricum</i>	Hymenosomatidae	Genbank Crustacea Malac.
FJ812291	<i>Hymenosoma hodgkini</i>	Hymenosomatidae	Genbank Crustacea Malac.
JSDUK46	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOPC045-12	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOPC045-10	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOPB077-06	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOPB074-03	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOPB074-02	<i>Inachus dorsettensis</i>	Inachidae	This study
FCDOPB074-01	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOP42-22	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOP42-20	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOP42-19	<i>Inachus dorsettensis</i>	Inachidae	This study
FCFOPC057-06	<i>Inachus leptochirus</i>	Inachidae	This study
FJ174951	<i>Jasus edwardsii</i>	Palinuridae	Genbank Crustacea Malac.
AF339473	<i>Jasus edwardsii</i>	Palinuridae	Genbank Crustacea Malac.
FJ174952	<i>Jasus verreauxi</i>	Palinuridae	Genbank Crustacea Malac.
AF192883	<i>Jasus verreauxi</i>	Palinuridae	Genbank Crustacea Malac.
JSDPX23-04	<i>Jaxea nocturna</i>	Laomediidae	This study
AB290634	<i>Johora gapensis</i>	Potamidae	Genbank Crustacea Malac.
AB290635	<i>Johora grallator</i>	Potamidae	Genbank Crustacea Malac.
AB290637	<i>Johora intermedia</i>	Potamidae	Genbank Crustacea Malac.
AB290638	<i>Johora johorensis</i>	Potamidae	Genbank Crustacea Malac.
AB290639	<i>Johora murphyi</i>	Potamidae	Genbank Crustacea Malac.
AB290641	<i>Johora singaporense</i>	Potamidae	Genbank Crustacea Malac.
AB290642	<i>Johora tahanensis</i>	Potamidae	Genbank Crustacea Malac.
AB290643	<i>Johora thoi</i>	Potamidae	Genbank Crustacea Malac.
JSDPX44-08	<i>Latreillia elegans</i>	Latreilliidae	This study
JSDPX41-01	<i>Latreillia elegans</i>	Latreilliidae	This study
JSDMe18	<i>Latreillia elegans</i>	Latreilliidae	This study
FCFOPC049-04	<i>Latreillia elegans</i>	Latreilliidae	This study
TE-004T141-160-02	<i>Lebbeus groenlandicus</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FC-SPE26A	<i>Lebbeus groenlandicus</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SPE26B	<i>Lebbeus groenlandicus</i>	Hippolytidae	Decapods of Pacific and Atlantic
TE-004T181-200-02	<i>Lebbeus polaris</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
LP01CN0406	<i>Lebbeus polaris</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
AY351055	<i>Leiogalathea laevirostris</i>	Galatheidae	Genbank Crustacea Malac.
FJ812292	<i>Leptomithrax sternocostulatus</i>	Majidae	Genbank Crustacea Malac.
EU682846	<i>Libinia dubia</i>	Majidae	Genbank Crustacea Malac.
EU682847	<i>Libinia dubia</i>	Majidae	Genbank Crustacea Malac.
EU682848	<i>Libinia emarginata</i>	Majidae	Genbank Crustacea Malac.
EU682849	<i>Libinia emarginata</i>	Majidae	Genbank Crustacea Malac.
JSDUK53	<i>Liocarcinus depurator</i>	Portunidae	This study

JSDUK52	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDUK51	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDMe02	<i>Liocarcinus depurator</i>	Portunidae	This study
JSDMe01	<i>Liocarcinus depurator</i>	Portunidae	This study
FJ174948	<i>Liocarcinus depurator</i>	Portunidae	Genbank Crustacea Malac.
DQ480363	<i>Liocarcinus depurator</i>	Portunidae	Genbank Crustacea Malac.
FCFOPC041-24	<i>Liocarcinus depurator</i>	Portunidae	This study
FCFOPC045-07	<i>Liocarcinus depurator</i>	Portunidae	This study
FCDOPB077-07	<i>Liocarcinus depurator</i>	Portunidae	This study
FJ174949	<i>Liocarcinus maculatus</i>	Portunidae	Genbank Crustacea Malac.
FC-VMD11B	<i>Lithodes couesi</i>	Lithodidae	Decapods of Pacific and Atlantic
FC-VMD11A	<i>Lithodes couesi</i>	Lithodidae	Decapods of Pacific and Atlantic
AB476813	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
AB476814	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
AB476815	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
AB476816	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
AB476817	<i>Lithodes longispina</i>	Lithodidae	Genbank Crustacea Malac.
GSL31-25	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
GSL31-24	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
TE-004T22-01	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
TE-004T88-03	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
TE-004T153-01	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
TE-004-02	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
TE-004-01	<i>Lithodes maja</i>	Lithodidae	Crustaceans of the St. Lawrence Gulf
AB375131	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375133	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375137	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375138	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375140	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375145	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375147	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375149	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375150	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
AB375156	<i>Lithodes nintokuiae</i>	Lithodidae	Genbank Crustacea Malac.
EU517503	<i>Litopenaeus stylirostris</i>	Penaeidae	Genbank Crustacea Malac.
NC_012060	<i>Litopenaeus stylirostris</i>	Penaeidae	Genbank Crustacea Malac.
DQ534543	<i>Litopenaeus vannamei</i>	Penaeidae	Genbank Crustacea Malac.
FC-VMH38E	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
FC-VMH38D	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
FC-VMH38A	<i>Lopholithodes foraminatus</i>	Lithodidae	Decapods of Pacific and Atlantic
EU682850	<i>Loxorhynchus crispatus</i>	Majidae	Genbank Crustacea Malac.
EU682851	<i>Loxorhynchus crispatus</i>	Majidae	Genbank Crustacea Malac.
EU135868	<i>Lysmata pederseni</i>	Hippolytidae	Genbank Crustacea Malac.
EU135869	<i>Lysmata pederseni</i>	Hippolytidae	Genbank Crustacea Malac.
FM958058	<i>Macrobrachium asperulum</i>	Palaemonidae	Genbank Crustacea Malac.
FM958060	<i>Macrobrachium callirrhoe</i>	Palaemonidae	Genbank Crustacea Malac.
FM958062	<i>Macrobrachium clymene</i>	Palaemonidae	Genbank Crustacea Malac.
FM958063	<i>Macrobrachium equidens</i>	Palaemonidae	Genbank Crustacea Malac.
FM958064	<i>Macrobrachium esculentum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005003	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005007	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005014	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005015	<i>Macrobrachium faustinum</i>	Palaemonidae	Genbank Crustacea Malac.

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EU005019	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005023	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005025	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005029	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005033	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
EU005034	<i>Macrobrachium faustum</i>	Palaemonidae	Genbank Crustacea Malac.
FM958065	<i>Macrobrachium fukienense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958066	<i>Macrobrachium gracilirostre</i>	Palaemonidae	Genbank Crustacea Malac.
FM958068	<i>Macrobrachium hainanense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958069	<i>Macrobrachium horstii</i>	Palaemonidae	Genbank Crustacea Malac.
FM958070	<i>Macrobrachium idae</i>	Palaemonidae	Genbank Crustacea Malac.
FM958071	<i>Macrobrachium jaroense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958081	<i>Macrobrachium lanatum</i>	Palaemonidae	Genbank Crustacea Malac.
FJ797435	<i>Macrobrachium lanchesteri</i>	Palaemonidae	Genbank Crustacea Malac.
NC_012217	<i>Macrobrachium lanchesteri</i>	Palaemonidae	Genbank Crustacea Malac.
FM958074	<i>Macrobrachium malayanum</i>	Palaemonidae	Genbank Crustacea Malac.
FM958075	<i>Macrobrachium mammillodactylus</i>	Palaemonidae	Genbank Crustacea Malac.
FM958076	<i>Macrobrachium niphanae</i>	Palaemonidae	Genbank Crustacea Malac.
FM958077	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
EF076647	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
EF076648	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ990459	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859918	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859917	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859911	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859910	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859909	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
DQ859907	<i>Macrobrachium nipponense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958087	<i>Macrobrachium pinguis</i>	Palaemonidae	Genbank Crustacea Malac.
FM958078	<i>Macrobrachium placidum</i>	Palaemonidae	Genbank Crustacea Malac.
FM958080	<i>Macrobrachium saigonense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958083	<i>Macrobrachium sintangense</i>	Palaemonidae	Genbank Crustacea Malac.
FM958084	<i>Macrobrachium trompii</i>	Palaemonidae	Genbank Crustacea Malac.
JSDPX9-01	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe45	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe44	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe43	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe42	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe41	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDMe09	<i>Macropipus tuberculatus</i>	Portunidae	This study
FCFOPC041-30	<i>Macropipus tuberculatus</i>	Portunidae	This study
FCFOP42-23	<i>Macropipus tuberculatus</i>	Portunidae	This study
JSDUK110	<i>Macropodia rostrata</i>	Majidae	This study
JSDUK108	<i>Macropodia rostrata</i>	Majidae	This study
JSDUK107	<i>Macropodia rostrata</i>	Majidae	This study
JSDPXM32-01	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDPXM06-03	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDPX32-03	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDMe27	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDMe26	<i>Macropodia tenuirostris</i>	Majidae	This study
JSDMe25	<i>Macropodia tenuirostris</i>	Majidae	This study
FCFOPC045-09	<i>Macropodia tenuirostris</i>	Majidae	This study
AB478070	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.

AB478071	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478074	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478046	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478047	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478048	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478050	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478051	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478061	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
AB478065	<i>Macroregonia macrochira</i>	Oregoniidae	Genbank Crustacea Malac.
JSDAz202	<i>Maja brachyactyla</i>	Majidae	This study
EU000811	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000815	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000816	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000820	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000822	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000823	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000824	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000825	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000829	<i>Maja brachyactyla</i>	Majidae	Genbank Crustacea Malac.
EU000836	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000837	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000839	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000841	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000843	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000844	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000845	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000847	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000848	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
EU000849	<i>Maja crispata</i>	Majidae	Genbank Crustacea Malac.
JSDPXA21-01	<i>Maja goltziana</i>	Majidae	This study
JSDPX18-01	<i>Maja goltziana</i>	Majidae	This study
EU000832	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
EU000833	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
EU000834	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
EU000835	<i>Maja squinado</i>	Majidae	Genbank Crustacea Malac.
AY803582	<i>Marojejy longimerus</i>	Potamonautesidae	Genbank Crustacea Malac.
NC_007010	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
AY787755	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
AP006346	<i>Marsupenaeus japonicus</i>	Penaeidae	Genbank Crustacea Malac.
JSDMe81	<i>Medorippe lanata</i>	Dorippidae	This study
JSDMe13	<i>Medorippe lanata</i>	Dorippidae	This study
JSDMe12	<i>Medorippe lanata</i>	Dorippidae	This study
JSDPX23-03	<i>Melicertus kerathurus</i>	Penaeidae	This study
JSDPX23-01	<i>Melicertus kerathurus</i>	Penaeidae	This study
EF219289	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219290	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219291	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219308	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219309	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219324	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219326	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EF219342	<i>Melicertus kerathurus</i>	Penaeidae	Genbank Crustacea Malac.
EU682857	<i>Menaethius monoceros</i>	Majidae	Genbank Crustacea Malac.

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FJ155372	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
FJ155373	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
FJ155374	<i>Metacarcinus edwardsii</i>	Cancridae	Genbank Crustacea Malac.
EU186143	<i>Metanephrops arafurensis</i>	Nephropidae	Genbank Crustacea Malac.
EU186141	<i>Metanephrops boschmai</i>	Nephropidae	Genbank Crustacea Malac.
EU186137	<i>Metanephrops formosanus</i>	Nephropidae	Genbank Crustacea Malac.
EU186134	<i>Metanephrops mozambicus</i>	Nephropidae	Genbank Crustacea Malac.
EU186142	<i>Metanephrops sibogae</i>	Nephropidae	Genbank Crustacea Malac.
EU186138	<i>Metanephrops sinensis</i>	Nephropidae	Genbank Crustacea Malac.
EU682830	<i>Metoporhaphis calcarata</i>	Inachidae	Genbank Crustacea Malac.
EU682844	<i>Micippa thalia</i>	Mithracidae	Genbank Crustacea Malac.
EU682843	<i>Microphrys bicornutus</i>	Majidae	Genbank Crustacea Malac.
FJ693564	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693565	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693568	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693573	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693574	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693576	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693643	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693648	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693649	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693650	<i>Minuca pugnax</i>	Ocypodidae	Genbank Crustacea Malac.
EU682841	<i>Mithraculus sculptus</i>	Mithracidae	Genbank Crustacea Malac.
JSDPX24-02	<i>Monodaeus couchii</i>	Xanthidae	This study
JSDPX24-01	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPHM21Bx_110	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPHMSM242_98	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-559B83	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-551D81	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-528Gr79	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-528Gr75	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-566Gr13	<i>Monodaeus couchii</i>	Xanthidae	This study
FCDPH14-528Gr8	<i>Monodaeus couchii</i>	Xanthidae	This study
AY800033	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
AY350928	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
AY350927	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
AY350926	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
AY350925	<i>Munida acantha</i>	Galatheidae	Genbank Crustacea Malac.
AY350936	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350935	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350934	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350933	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350932	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350931	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350930	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350929	<i>Munida alonsoi</i>	Galatheidae	Genbank Crustacea Malac.
AY350938	<i>Munida armilla</i>	Galatheidae	Genbank Crustacea Malac.
AY350937	<i>Munida armilla</i>	Galatheidae	Genbank Crustacea Malac.
AY350939	<i>Munida callista</i>	Galatheidae	Genbank Crustacea Malac.
AY350942	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
AY350941	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
AY350940	<i>Munida clinata</i>	Galatheidae	Genbank Crustacea Malac.
AY350944	<i>Munida compressa</i>	Galatheidae	Genbank Crustacea Malac.

AY350943	<i>Munida compressa</i>	Galatheidae	Genbank Crustacea Malac.
AY350945	<i>Munida congesta</i>	Galatheidae	Genbank Crustacea Malac.
AY350950	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
AY350949	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
AY350948	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
AY350947	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
AY350946	<i>Munida distiza</i>	Galatheidae	Genbank Crustacea Malac.
AY350953	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
AY350952	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
AY350951	<i>Munida eclepsis</i>	Galatheidae	Genbank Crustacea Malac.
AY350958	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
AY350957	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
AY350956	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
AY350955	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
AY350954	<i>Munida gordoae</i>	Galatheidae	Genbank Crustacea Malac.
AY350962	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
AY350961	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
AY350960	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
AY350959	<i>Munida guttata</i>	Galatheidae	Genbank Crustacea Malac.
FCDPHM52_118	<i>Munida intermedia</i>	Galatheidae	This study
AY350971	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350970	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350969	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350968	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350967	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350966	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350965	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350964	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350963	<i>Munida leagora</i>	Galatheidae	Genbank Crustacea Malac.
AY350972	<i>Munida lenticularis</i>	Galatheidae	Genbank Crustacea Malac.
AY350973	<i>Munida leptosyne</i>	Galatheidae	Genbank Crustacea Malac.
AY350974	<i>Munida leviantennata</i>	Galatheidae	Genbank Crustacea Malac.
AY350975	<i>Munida militaris</i>	Galatheidae	Genbank Crustacea Malac.
AY350979	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
AY350978	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
AY350977	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
AY350976	<i>Munida notata</i>	Galatheidae	Genbank Crustacea Malac.
AY350980	<i>Munida ofella</i>	Galatheidae	Genbank Crustacea Malac.
AY350984	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
AY350983	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
AY350982	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
AY350981	<i>Munida ommata</i>	Galatheidae	Genbank Crustacea Malac.
AY350985	<i>Munida pagesi</i>	Galatheidae	Genbank Crustacea Malac.
AY350986	<i>Munida proto</i>	Galatheidae	Genbank Crustacea Malac.
AY350991	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
AY350990	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
AY350989	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
AY350988	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
AY350987	<i>Munida psamathe</i>	Galatheidae	Genbank Crustacea Malac.
AY350992	<i>Munida psylla</i>	Galatheidae	Genbank Crustacea Malac.
FC-VSA6	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic
FC-VSA141A	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic
FC-VSA135A	<i>Munida quadrispina</i>	Galatheidae	Decapods of Pacific and Atlantic

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AF283886	<i>Munida rhodonia</i>	Galatheidae	Genbank Crustacea Malac.
AF283885	<i>Munida rhodonia</i>	Galatheidae	Genbank Crustacea Malac.
AY350993	<i>Munida rogeri</i>	Galatheidae	Genbank Crustacea Malac.
AY350994	<i>Munida rosula</i>	Galatheidae	Genbank Crustacea Malac.
AF283887	<i>Munida rubrodigitalis</i>	Galatheidae	Genbank Crustacea Malac.
AY350995	<i>Munida rufiantennulata</i>	Galatheidae	Genbank Crustacea Malac.
FCDOPB084-04	<i>Munida rugosa</i>	Galatheidae	This study
JSDMe54	<i>Munida rutllanti</i>	Galatheidae	This study
JSDMe53	<i>Munida rutllanti</i>	Galatheidae	This study
FCFOPC045-05	<i>Munida rutllanti</i>	Galatheidae	This study
FCFOPC045-04	<i>Munida rutllanti</i>	Galatheidae	This study
FCFOPC045-03	<i>Munida rutllanti</i>	Galatheidae	This study
FCFOPC045-02	<i>Munida rutllanti</i>	Galatheidae	This study
FCFOPC045-01	<i>Munida rutllanti</i>	Galatheidae	This study
AY350997	<i>Munida spilota</i>	Galatheidae	Genbank Crustacea Malac.
AY350996	<i>Munida spilota</i>	Galatheidae	Genbank Crustacea Malac.
AY700177	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700178	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
AY700179	<i>Munida spinosa</i>	Galatheidae	Genbank Crustacea Malac.
AY351001	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
AY351000	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
AY350999	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
AY350998	<i>Munida stia</i>	Galatheidae	Genbank Crustacea Malac.
AY351008	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351007	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351006	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351005	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351004	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351003	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
AY351002	<i>Munida taenia</i>	Galatheidae	Genbank Crustacea Malac.
DQ011204	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
DQ011200	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
DQ011198	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
DQ011191	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800046	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800043	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800042	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800041	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800039	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY800034	<i>Munida thoe</i>	Galatheidae	Genbank Crustacea Malac.
AY351014	<i>Munida tiresias</i>	Galatheidae	Genbank Crustacea Malac.
AY351015	<i>Munida tuberculata</i>	Galatheidae	Genbank Crustacea Malac.
AY351017	<i>Munida tyche</i>	Galatheidae	Genbank Crustacea Malac.
AY351016	<i>Munida tyche</i>	Galatheidae	Genbank Crustacea Malac.
DQ011206	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800056	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800055	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800053	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800050	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800049	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800048	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY800047	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
AY351023	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.

AY351021	<i>Munida zebra</i>	Galatheidae	Genbank Crustacea Malac.
DQ677691	<i>Munidopsis aries</i>	Galatheidae	Genbank Crustacea Malac.
DQ677694	<i>Munidopsis cascadia</i>	Galatheidae	Genbank Crustacea Malac.
TE-004T169-01	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
TE-004T88-02	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
MC01NFG106	<i>Munidopsis curvirostra</i>	Galatheidae	Crustaceans of the St. Lawrence Gulf
DQ677706	<i>Munidopsis kensmithi</i>	Galatheidae	Genbank Crustacea Malac.
DQ677709	<i>Munidopsis kensmithi</i>	Galatheidae	Genbank Crustacea Malac.
EF157851	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
EF157852	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
EF157853	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
EF157850	<i>Munidopsis lauensis</i>	Galatheidae	Genbank Crustacea Malac.
DQ860146	<i>Munidopsis polymorpha</i>	Galatheidae	Genbank Crustacea Malac.
FC-VSB12A	<i>Munidopsis quadrata</i>	Galatheidae	Decapods of Pacific and Atlantic
DQ677697	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677698	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677699	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677700	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677701	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677702	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677695	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677696	<i>Munidopsis recta</i>	Galatheidae	Genbank Crustacea Malac.
DQ677703	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
DQ677704	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
DQ677705	<i>Munidopsis scotti</i>	Galatheidae	Genbank Crustacea Malac.
DQ677683	<i>Munidopsis segonzaci</i>	Galatheidae	Genbank Crustacea Malac.
DQ677673	<i>Munidopsis tiburon</i>	Galatheidae	Genbank Crustacea Malac.
DQ677675	<i>Munidopsis vrijenhoeki</i>	Galatheidae	Genbank Crustacea Malac.
AB265249	<i>Nanhaiptamon pingyuanense</i>	Potamidae	Genbank Crustacea Malac.
JSDPXP1-01	<i>Necora puber</i>	Portunidae	This study
FJ755621	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755628	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755629	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755630	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755631	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755633	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755635	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
FJ755638	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
DQ480362	<i>Necora puber</i>	Portunidae	Genbank Crustacea Malac.
AB300183	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
AB300184	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
AB300185	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
AB300186	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
AB300187	<i>Neocaridina denticulata</i>	Atyidae	Genbank Crustacea Malac.
AB300180	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
AB300181	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
AB300182	<i>Neocaridina ketagalan</i>	Atyidae	Genbank Crustacea Malac.
AB300177	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
AB300178	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
AB300179	<i>Neocaridina saccam</i>	Atyidae	Genbank Crustacea Malac.
FJ812284	<i>Neohy menicus pubescens</i>	Hymenosomatidae	Genbank Crustacea Malac.
JSDUKdeep_03	<i>Neolithodes grimaldii</i>	Lithodidae	This study
JSDUKdeep_02	<i>Neolithodes grimaldii</i>	Lithodidae	This study

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JSDUKdeep_01	<i>Neolithodes grimaldii</i>	Lithodidae	This study
EF198477	<i>Neorhynchoplax bovis</i>	Hymenosomatidae	Genbank Crustacea Malac.
FJ174945	<i>Nephrops norvegicus</i>	Nephropidae	Genbank Crustacea Malac.
FCFOPC041-09	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCFOPC041-08	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCFOPC041-07	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCFOPC041-06	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOPB090-04	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOPB090-03	<i>Nephrops norvegicus</i>	Nephropidae	This study
FCDOPB090-02	<i>Nephrops norvegicus</i>	Nephropidae	This study
JSDUKdeep_20	<i>Nephropsis atlantica</i>	Nephropidae	This study
JSDUKdeep_19	<i>Nephropsis atlantica</i>	Nephropidae	This study
JSDUKdeep_18	<i>Nephropsis atlantica</i>	Nephropidae	This study
AY351058	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
AY351057	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
AY351056	<i>Onconida alaini</i>	Galatheidae	Genbank Crustacea Malac.
AY351060	<i>Onconida tropis</i>	Galatheidae	Genbank Crustacea Malac.
AY351059	<i>Onconida tropis</i>	Galatheidae	Genbank Crustacea Malac.
DQ328819	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328820	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328824	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328825	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328826	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328829	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328830	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328834	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328836	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
DQ328837	<i>Opaepele loihi</i>	Bresiliidae	Genbank Crustacea Malac.
JSDPX41-05	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX41-04	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX41-03	<i>Oplophorus spinosus</i>	Oplophoridae	This study
JSDPX41-02	<i>Oplophorus spinosus</i>	Oplophoridae	This study
FCFOPC052-15	<i>Oplophorus spinosus</i>	Oplophoridae	This study
FC-DPD01	<i>Orconectes immunis</i>	Cambaridae	Decapods of Pacific and Atlantic
FC-OLIM64	<i>Orconectes limosus</i>	Cambaridae	Decapods of Pacific and Atlantic
EU682836	<i>Oregonia gracilis</i>	Majidae	Genbank Crustacea Malac.
FC-VAF42C	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
FC-VAF42B	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
FC-VAF19A	<i>Paguristes turgidus</i>	Diogenidae	Decapods of Pacific and Atlantic
L215AR1-03	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
BSM07T1-31	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
PA02MD0306	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
PA01MD0306	<i>Pagurus acadianus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
AF483156	<i>Pagurus acadianus</i>	Paguridae	Genbank Crustacea Malac.
FCFOPC054-06	<i>Pagurus alatus</i>	Paguridae	This study
FCFOPC052-11	<i>Pagurus alatus</i>	Paguridae	This study
FCFOPC043-01	<i>Pagurus alatus</i>	Paguridae	This study
BSM07T3-03	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
BSM07T3-02	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
BSM07T3-01	<i>Pagurus arcuatus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
AF483159	<i>Pagurus armatus</i>	Paguridae	Genbank Crustacea Malac.
JSDUK154	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK153	<i>Pagurus bernhardus</i>	Paguridae	This study

JSDUK152	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK151	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK150	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK149	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDUK146	<i>Pagurus bernhardus</i>	Paguridae	This study
FCDOPB089-05	<i>Pagurus bernhardus</i>	Paguridae	This study
JSDAz197	<i>Pagurus cuanensis</i>	Paguridae	This study
JSDAz65	<i>Pagurus cuanensis</i>	Paguridae	This study
JSDMe05	<i>Pagurus excavatus</i>	Paguridae	This study
JSDMe04	<i>Pagurus excavatus</i>	Paguridae	This study
JSDMe03	<i>Pagurus excavatus</i>	Paguridae	This study
FCFOP42-13	<i>Pagurus excavatus</i>	Paguridae	This study
FCFOP42-12	<i>Pagurus excavatus</i>	Paguridae	This study
FCFOP42-11	<i>Pagurus excavatus</i>	Paguridae	This study
L209AR1-01	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L210AR4-04	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L228AR1-01	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L154AR1-08	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L183AR1-03	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L183AR1-02	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L190AR1-02	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
L190AR1-01	<i>Pagurus longicarpus</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
AF483158	<i>Pagurus ochotensis</i>	Paguridae	Genbank Crustacea Malac.
JSDUK159	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDUK147	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDN11	<i>Pagurus prideauxi</i>	Paguridae	This study
JSDN09	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOPB074-06	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOPB071-12	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOPB071-10	<i>Pagurus prideauxi</i>	Paguridae	This study
FCDOPB071-8	<i>Pagurus prideauxi</i>	Paguridae	This study
FCFOP42-18	<i>Pagurus prideauxi</i>	Paguridae	This study
FCFOP42-14	<i>Pagurus prideauxi</i>	Paguridae	This study
GSL31-06	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
GSL31-05	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
GSL31-04	<i>Pagurus pubescens</i>	Paguridae	Crustaceans of the St. Lawrence Gulf
JSDSv02	<i>Pagurus pubescens</i>	Paguridae	This study
JSDSv01	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN23	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN22	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN15	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN14	<i>Pagurus pubescens</i>	Paguridae	This study
JSDN05	<i>Pagurus pubescens</i>	Paguridae	This study
FM958086	<i>Palaemon debilis</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640124	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640123	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640122	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640121	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640120	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640119	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640118	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640117	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
AJ640116	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.

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AJ640115	<i>Palaemon longirostris</i>	Palaemonidae	Genbank Crustacea Malac.
JSDUK167	<i>Palaemon serratus</i>	Palaemonidae	This study
JSDUK166	<i>Palaemon serratus</i>	Palaemonidae	This study
JSDUK165	<i>Palaemon serratus</i>	Palaemonidae	This study
FCDOPB088-14	<i>Palaemon serratus</i>	Palaemonidae	This study
FCDOPB088-13	<i>Palaemon serratus</i>	Palaemonidae	This study
L169AR3-03	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
L169AR3-02	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
L169AR3-01	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
L72AR1-09	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
L72AR1-07	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
L72AR1-02	<i>Palaemonetes vulgaris</i>	Palaemonidae	Crustaceans of the St. Lawrence Gulf
FJ174955	<i>Palibythus magnificus</i>	Synaxidae	Genbank Crustacea Malac.
FJ174960	<i>Palinurus barbareae</i>	Palinuridae	Genbank Crustacea Malac.
FJ174959	<i>Palinurus charlestoni</i>	Palinuridae	Genbank Crustacea Malac.
FJ174958	<i>Palinurus delagoae</i>	Palinuridae	Genbank Crustacea Malac.
JSDAz229	<i>Palinurus elephas</i>	Palinuridae	This study
JSDAz228	<i>Palinurus elephas</i>	Palinuridae	This study
FJ174956	<i>Palinurus elephas</i>	Palinuridae	Genbank Crustacea Malac.
AJ889577	<i>Palinurus elephas</i>	Palinuridae	Genbank Crustacea Malac.
FJ174961	<i>Palinurus gilchristi</i>	Palinuridae	Genbank Crustacea Malac.
FJ174957	<i>Palinurus mauritanicus</i>	Palinuridae	Genbank Crustacea Malac.
AJ889578	<i>Palinurus mauritanicus</i>	Palinuridae	Genbank Crustacea Malac.
AB290213	<i>Pandalopsis coccinata</i>	Pandalidae	Genbank Crustacea Malac.
FC-SEE13E	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SEE13C	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SEE13B	<i>Pandalopsis dispar</i>	Pandalidae	Decapods of Pacific and Atlantic
TE005-T164-01	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
TE005-T187-01	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
PB02CN0406	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
PB01CN0406	<i>Pandalus borealis</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
FC-SCF201C	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SCF201B	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SCF201A	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SCF26	<i>Pandalus danae</i>	Pandalidae	Decapods of Pacific and Atlantic
AB211294	<i>Pandalus eous</i>	Pandalidae	Genbank Crustacea Malac.
FC-SCH2	<i>Pandalus goniurus</i>	Pandalidae	Decapods of Pacific and Atlantic
AB211295	<i>Pandalus hypsinotus</i>	Pandalidae	Genbank Crustacea Malac.
FC-SCJ2	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SCJ	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SCJ 23	<i>Pandalus hypsinotus</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDB1A	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDB16A	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDB122B	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDB122	<i>Pandalus jordani</i>	Pandalidae	Decapods of Pacific and Atlantic
TE-004T141-160-03	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
TE-004T1-20-11	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
TE-004T1-20-06	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
TE-004T1-20-03	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
TE-004T1-20-02	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
GSL31-52	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
GSL31-13	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
PM02CN0406	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf

PM01CN0406	<i>Pandalus montagui</i>	Pandalidae	Crustaceans of the St. Lawrence Gulf
JSDUK171	<i>Pandalus montagui</i>	Pandalidae	This study
FC-SDD8	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDD39B	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDD39A	<i>Pandalus mortagui tridens</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDF12B	<i>Pandalus platyceros</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDF6A	<i>Pandalus platyceros</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDH172	<i>Pandalus stenolepis</i>	Pandalidae	Decapods of Pacific and Atlantic
FC-SDH165	<i>Pandalus stenolepis</i>	Pandalidae	Decapods of Pacific and Atlantic
FJ174969	<i>Panulirus argus</i>	Palinuridae	Genbank Crustacea Malac.
AF339452	<i>Panulirus argus</i>	Palinuridae	Genbank Crustacea Malac.
AF339453	<i>Panulirus cygnus</i>	Palinuridae	Genbank Crustacea Malac.
AF339454	<i>Panulirus echinatus</i>	Palinuridae	Genbank Crustacea Malac.
AF339455	<i>Panulirus gracilis</i>	Palinuridae	Genbank Crustacea Malac.
AF339456	<i>Panulirus guttatus</i>	Palinuridae	Genbank Crustacea Malac.
FJ174963	<i>Panulirus homarus</i>	Palinuridae	Genbank Crustacea Malac.
FJ174964	<i>Panulirus inflatus</i>	Palinuridae	Genbank Crustacea Malac.
AF339459	<i>Panulirus inflatus</i>	Palinuridae	Genbank Crustacea Malac.
FJ174968	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
NC_004251	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
AB071201	<i>Panulirus japonicus</i>	Palinuridae	Genbank Crustacea Malac.
AF339462	<i>Panulirus laevicauda</i>	Palinuridae	Genbank Crustacea Malac.
AF339465	<i>Panulirus marginatus</i>	Palinuridae	Genbank Crustacea Malac.
AF339466	<i>Panulirus pascuensis</i>	Palinuridae	Genbank Crustacea Malac.
FJ174970	<i>Panulirus penicillatus</i>	Palinuridae	Genbank Crustacea Malac.
AF339468	<i>Panulirus penicillatus</i>	Palinuridae	Genbank Crustacea Malac.
AF339469	<i>Panulirus polyphagus</i>	Palinuridae	Genbank Crustacea Malac.
FJ174962	<i>Panulirus regius</i>	Palinuridae	Genbank Crustacea Malac.
AF339470	<i>Panulirus regius</i>	Palinuridae	Genbank Crustacea Malac.
AF339471	<i>Panulirus stimpsoni</i>	Palinuridae	Genbank Crustacea Malac.
AF339472	<i>Panulirus versicolor</i>	Palinuridae	Genbank Crustacea Malac.
AB244283	<i>Panulirus versicolor</i>	Palinuridae	Genbank Crustacea Malac.
FJ812294	<i>Paragrapuspis laevis</i>	Grapsidae	Genbank Crustacea Malac.
AB211300	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
AB211299	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
AB211298	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
AB211297	<i>Paralithodes brevipes</i>	Lithodidae	Genbank Crustacea Malac.
AB211303	<i>Paralithodes camtschaticus</i>	Lithodidae	Genbank Crustacea Malac.
AB211302	<i>Paralithodes platypus</i>	Lithodidae	Genbank Crustacea Malac.
AB211301	<i>Paralithodes platypus</i>	Lithodidae	Genbank Crustacea Malac.
AB375544	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB375545	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428436	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428437	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428438	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428440	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428441	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB428442	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
AB211296	<i>Paralomis multispina</i>	Lithodidae	Genbank Crustacea Malac.
FC-VOG11	<i>Paralomis multispina</i>	Lithodidae	Decapods of Pacific and Atlantic
AB476747	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
AB476748	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
AB476749	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.

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AB476750	<i>Paralomis pacifica</i>	Lithodidae	Genbank Crustacea Malac.
AY351030	<i>Paramunida belone</i>	Galatheidae	Genbank Crustacea Malac.
AY351031	<i>Paramunida granulata</i>	Galatheidae	Genbank Crustacea Malac.
AY351033	<i>Paramunida labis</i>	Galatheidae	Genbank Crustacea Malac.
AY351032	<i>Paramunida labis</i>	Galatheidae	Genbank Crustacea Malac.
AY351035	<i>Paramunida luminata</i>	Galatheidae	Genbank Crustacea Malac.
AY351034	<i>Paramunida luminata</i>	Galatheidae	Genbank Crustacea Malac.
AY351039	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
AY351038	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
AY351037	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
AY351036	<i>Paramunida pictura</i>	Galatheidae	Genbank Crustacea Malac.
AY351040	<i>Paramunida pronoe</i>	Galatheidae	Genbank Crustacea Malac.
AY351043	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.
AY351042	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.
AY351041	<i>Paramunida stichas</i>	Galatheidae	Genbank Crustacea Malac.
AY351047	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
AY351046	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
AY351045	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
AY351044	<i>Paramunida thalie</i>	Galatheidae	Genbank Crustacea Malac.
JSDMe38	<i>Parapenaeus longirostris</i>	Penaeidae	This study
JSDMe37	<i>Parapenaeus longirostris</i>	Penaeidae	This study
FCFOPC042-03	<i>Parapenaeus longirostris</i>	Penaeidae	This study
EF599158	<i>Parastacus brasiliensis</i>	Parastacidae	Genbank Crustacea Malac.
EF599157	<i>Parastacus pugnax</i>	Parastacidae	Genbank Crustacea Malac.
JSDPX63-01	<i>Pasiphaea hoplocreta</i>	Pasiphaeidae	This study
TE-004T81-100-02	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
TE-004T141-160-04	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
TE-004T1-20-14	<i>Pasiphaea multidentata</i>	Pasiphaeidae	Crustaceans of the St. Lawrence Gulf
JSDUKdeep_26	<i>Pasiphaea multidentata</i>	Pasiphaeidae	This study
JSDUKdeep_27	<i>Pasiphaea multidentata</i>	Pasiphaeidae	This study
FC-TAC29D	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAC144A	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAC29C	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAC29B	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FC-TAC29A	<i>Pasiphaea pacifica</i>	Pasiphaeidae	Decapods of Pacific and Atlantic
FCFOPC041-19	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCFOPC041-18	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCFOPC041-17	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCFOPC041-16	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCFOPC041-15	<i>Pasiphaea sivado</i>	Pasiphaeidae	This study
FCFOPC050-01	<i>Penaeopsis serrata</i>	Penaeidae	This study
NC_002184	<i>Penaeus monodon</i>	Penaeidae	Genbank Crustacea Malac.
AF217843	<i>Penaeus monodon</i>	Penaeidae	Genbank Crustacea Malac.
JSDAz79	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz77	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz76	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz75	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz41	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz40	<i>Percnon gibbesi</i>	Grapsidae	This study
JSDAz39	<i>Percnon gibbesi</i>	Grapsidae	This study
AB470727	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB470730	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB470720	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.

AB470722	<i>Periclimenes lamellibrachiophilus</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386124	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386141	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386153	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386174	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386189	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386204	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386214	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386217	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386233	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
FJ386264	<i>Periclimenes soror</i>	Palaemonidae	Genbank Crustacea Malac.
AB298102	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB298103	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB298104	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB298105	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB298106	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
AB298107	<i>Periclimenes thermohydrophilus</i>	Palaemonidae	Genbank Crustacea Malac.
FJ693381	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693419	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693421	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693429	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693436	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693450	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693473	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693492	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693498	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FJ693506	<i>Petrolisthes armatus</i>	Porcellanidae	Genbank Crustacea Malac.
FCDPHMSM241_101	<i>Philocheras monocanthus</i>	Crangonidae	This study
AY803591	<i>Phricothelphusa limula</i>	Parathelphusidae	Genbank Crustacea Malac.
JSDUK174	<i>Pilumnus hirtellus</i>	Pilumnidae	This study
JSDUK173	<i>Pilumnus hirtellus</i>	Pilumnidae	This study
JSDAz135	<i>Pilumnus hirtellus</i>	Pilumnidae	This study
FCDPH14-550D69	<i>Pilumnus inermis</i>	Pilumnidae	This study
EU682839	<i>Pitho lherminieri</i>	Majidae	Genbank Crustacea Malac.
AY351061	<i>Plesionida aliena</i>	Galatheidae	Genbank Crustacea Malac.
JSDPX43-02	<i>Plesionika acanthonatus</i>	Pandalidae	This study
JSDPX42(05)-03	<i>Plesionika acanthonatus</i>	Pandalidae	This study
JSDPX41-13	<i>Plesionika acanthonotus</i>	Pandalidae	This study
JSDPX41-12	<i>Plesionika acanthonotus</i>	Pandalidae	This study
FCFOPC057-05	<i>Plesionika acanthonotus</i>	Pandalidae	This study
JSDMe74	<i>Plesionika edwardsii</i>	Pandalidae	This study
JSDMe73	<i>Plesionika edwardsii</i>	Pandalidae	This study
JSDMe71	<i>Plesionika edwardsii</i>	Pandalidae	This study
JSDMe70	<i>Plesionika edwardsii</i>	Pandalidae	This study
AY612869	<i>Plesionika ensis</i>	Pandalidae	Genbank Crustacea Malac.
JSDMe30	<i>Plesionika heterocarpus</i>	Pandalidae	This study
JSDMe29	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCFOPC047-12	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOPB093-05	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOPB093-04	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCDOPB093-01	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCFOP70-22	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCFOP70-21	<i>Plesionika heterocarpus</i>	Pandalidae	This study

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FCFOP70-20	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCFOP70-18	<i>Plesionika heterocarpus</i>	Pandalidae	This study
FCFOPC047-11	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC047-10	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC043-04	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC042-10	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC042-08	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC042-07	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC041-47	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC041-46	<i>Plesionika martia</i>	Pandalidae	This study
FCFOPC041-45	<i>Plesionika martia</i>	Pandalidae	This study
JSDMe79	<i>Plesionika narval</i>	Pandalidae	This study
JSDMe78	<i>Plesionika narval</i>	Pandalidae	This study
JSDMe77	<i>Plesionika narval</i>	Pandalidae	This study
FCFOPC050-14	<i>Plesionika narval</i>	Pandalidae	This study
AY351062	<i>Pleuroncodes monodon</i>	Galatheidae	Genbank Crustacea Malac.
EU682831	<i>Podochela hemphillii</i>	Inachidae	Genbank Crustacea Malac.
JSDUK103	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK102	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK78	<i>Polybius henslowii</i>	Portunidae	This study
JSDUK74	<i>Polybius henslowii</i>	Portunidae	This study
FCFOPC041-01	<i>Polybius henslowii</i>	Portunidae	This study
FCDOPB083-14	<i>Polybius henslowii</i>	Portunidae	This study
FCDOPB083-13	<i>Polybius henslowii</i>	Portunidae	This study
FCDOPB083-12	<i>Polybius henslowii</i>	Portunidae	This study
FCDOPB083-11	<i>Polybius henslowii</i>	Portunidae	This study
FCDOPB083-10	<i>Polybius henslowii</i>	Portunidae	This study
EU377741	<i>Polycheles sculptus</i>	Polychelidae	Genbank Crustacea Malac.
JSDUKdeep_59	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDUKdeep_58	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDUKdeep_56	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDPX15-16	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDPX15-15	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDPX15-14	<i>Polycheles typhlops</i>	Polychelidae	This study
EU377738	<i>Polycheles typhlops</i>	Polychelidae	Genbank Crustacea Malac.
EU377740	<i>Polycheles typhlops</i>	Polychelidae	Genbank Crustacea Malac.
FJ174943	<i>Polycheles typhlops</i>	Polychelidae	Genbank Crustacea Malac.
FCFOPC052-02	<i>Polycheles typhlops</i>	Polychelidae	This study
JSDMe17	<i>Pontocaris cathanphracta</i>	Crangonidae	This study
JSDMe16	<i>Pontocaris cathanphracta</i>	Crangonidae	This study
JSDMe15	<i>Pontocaris cathanphracta</i>	Crangonidae	This study
FCDPH15-575B51	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCFOPC044-09	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCFOPC044-08	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCFOPC044-07	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCFOPC041-51	<i>Pontocaris lacazei</i>	Crangonidae	This study
FCFOPC041-52	<i>Pontophilus echinulatus</i>	Crangonidae	This study
TE-004T1-20-18	<i>Pontophilus norvegicus</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
TE-004T21-40-02	<i>Pontophilus norvegicus</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
JSDUK182	<i>Porcellana platycheles</i>	Porcellanidae	This study
JSDUK181	<i>Porcellana platycheles</i>	Porcellanidae	This study
JSDUK180	<i>Porcellana platycheles</i>	Porcellanidae	This study
JSDUK179	<i>Porcellana platycheles</i>	Porcellanidae	This study

JSDUK178	<i>Porcellana platycheles</i>	Porcellanidae	This study
FJ812293	<i>Portunus pelagicus</i>	Portunidae	Genbank Crustacea Malac.
EU284144	<i>Portunus sanguinolentus</i>	Portunidae	Genbank Crustacea Malac.
NC_005037	<i>Portunus trituberculatus</i>	Portunidae	Genbank Crustacea Malac.
AB093006	<i>Portunus trituberculatus</i>	Portunidae	Genbank Crustacea Malac.
AY803584	<i>Potamon fluviatilis</i>	Potamidae	Genbank Crustacea Malac.
AY803568	<i>Potamonautes irrangensis</i>	Potamonautidae	Genbank Crustacea Malac.
AY803570	<i>Potamonautes obesus</i>	Potamonautidae	Genbank Crustacea Malac.
AY803571	<i>Potamonautes oderhi</i>	Potamonautidae	Genbank Crustacea Malac.
AF474366	<i>Procambarus acutus</i>	Cambaridae	Genbank Crustacea Malac.
AY701195	<i>Procambarus clarkii</i>	Cambaridae	Genbank Crustacea Malac.
EU583575	<i>Procambarus simulans</i>	Cambaridae	Genbank Crustacea Malac.
JSDPX41-15	<i>Processa modica</i>	Processidae	This study
JSDPX41-14	<i>Processa modica</i>	Processidae	This study
FCFOPC041-42	<i>Processa modica</i>	Processidae	This study
FCFOPC041-41	<i>Processa modica</i>	Processidae	This study
FCFOPC041-40	<i>Processa modica</i>	Processidae	This study
FJ174953	<i>Projasus parkeri</i>	Palinuridae	Genbank Crustacea Malac.
NC_006891	<i>Pseudocarcinus gigas</i>	Eriphiidae	Genbank Crustacea Malac.
AY562127	<i>Pseudocarcinus gigas</i>	Eriphiidae	Genbank Crustacea Malac.
AB334557	<i>Pseudohelice subquadrata</i>	Varunidae	Genbank Crustacea Malac.
FJ174954	<i>Puerulus angulatus</i>	Palinuridae	Genbank Crustacea Malac.
EU682860	<i>Pugettia dalli</i>	Majidae	Genbank Crustacea Malac.
EU682862	<i>Pugettia gracilis</i>	Majidae	Genbank Crustacea Malac.
EU682863	<i>Pugettia gracilis</i>	Majidae	Genbank Crustacea Malac.
EU682864	<i>Pugettia gracilis</i>	Majidae	Genbank Crustacea Malac.
EU682866	<i>Pugettia quadridens</i>	Majidae	Genbank Crustacea Malac.
EU682867	<i>Pugettia quadridens</i>	Majidae	Genbank Crustacea Malac.
EU682870	<i>Pugettia richii</i>	Majidae	Genbank Crustacea Malac.
EU682871	<i>Pugettia richii</i>	Majidae	Genbank Crustacea Malac.
AF346400	<i>Ranina ranina</i>	Raninidae	Genbank Crustacea Malac.
AF283871	<i>Raymunida cagnetei</i>	Galatheidae	Genbank Crustacea Malac.
AF283870	<i>Raymunida cagnetei</i>	Galatheidae	Genbank Crustacea Malac.
AF283869	<i>Raymunida cagnetei</i>	Galatheidae	Genbank Crustacea Malac.
AF283873	<i>Raymunida confundens</i>	Galatheidae	Genbank Crustacea Malac.
AF283872	<i>Raymunida confundens</i>	Galatheidae	Genbank Crustacea Malac.
AF283874	<i>Raymunida dextralis</i>	Galatheidae	Genbank Crustacea Malac.
AF283881	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283880	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283879	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283878	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283877	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283876	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283875	<i>Raymunida elegantissima</i>	Galatheidae	Genbank Crustacea Malac.
AF283883	<i>Raymunida erythrina</i>	Galatheidae	Genbank Crustacea Malac.
AF283882	<i>Raymunida erythrina</i>	Galatheidae	Genbank Crustacea Malac.
AY288292	<i>Raymunida formosanus</i>	Galatheidae	Genbank Crustacea Malac.
AF283884	<i>Raymunida insulata</i>	Galatheidae	Genbank Crustacea Malac.
FJ517398	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517453	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517471	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517512	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517515	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.

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FJ517521	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517523	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ517524	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
DQ094800	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
DQ094793	<i>Rhithropanopeus harrisii</i>	Panopeidae	Genbank Crustacea Malac.
FJ155378	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
FJ155379	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
FJ155380	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
FJ155381	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
FJ155382	<i>Romaleon polyodon</i>	Cancridae	Genbank Crustacea Malac.
AB290650	<i>Ryukyum yaeyamense</i>	Potamidae	Genbank Crustacea Malac.
JSDUKdeep_08	<i>Sabinea hystrix</i>	Crangonidae	This study
JSDUKdeep_07	<i>Sabinea hystrix</i>	Crangonidae	This study
JSDUKdeep_06	<i>Sabinea hystrix</i>	Crangonidae	This study
TE-004T21-40-09	<i>Sabinea sarsi</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
TE-004T21-40-08	<i>Sabinea sarsi</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GSL31-31	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GSL31-30	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
GSL31-29	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
SSC02CN0406	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
SSC01CN0406	<i>Sabinea septemcarinata</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
EF599160	<i>Samastacus spinifrons</i>	Parastacidae	Genbank Crustacea Malac.
AY803592	<i>Sartoriana spinigera</i>	Gecarcinucidae	Genbank Crustacea Malac.
AY803590	<i>Sayamia sexpunctata</i>	Parathelphusidae	Genbank Crustacea Malac.
BSM08-39	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
BSM08-88	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
BSM08-72	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
BSM08-16	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
BSM08-07	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
BSM08-01	<i>Sclerocrangon boreas</i>	Crangonidae	Crustaceans of the St. Lawrence Gulf
FC-DPA13	<i>Sclerocrangon boreas</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-DPA11	<i>Sclerocrangon boreas</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-DPA07	<i>Sclerocrangon boreas</i>	Crangonidae	Decapods of Pacific and Atlantic
FC-DPA05	<i>Sclerocrangon boreas</i>	Crangonidae	Decapods of Pacific and Atlantic
FJ827760	<i>Scylla olivacea</i>	Portunidae	Genbank Crustacea Malac.
NC_012569	<i>Scylla olivacea</i>	Portunidae	Genbank Crustacea Malac.
FJ827761	<i>Scylla paramamosain</i>	Portunidae	Genbank Crustacea Malac.
NC_012572	<i>Scylla paramamosain</i>	Portunidae	Genbank Crustacea Malac.
FJ827758	<i>Scylla serrata</i>	Portunidae	Genbank Crustacea Malac.
NC_012565	<i>Scylla serrata</i>	Portunidae	Genbank Crustacea Malac.
FJ827759	<i>Scylla tranquebarica</i>	Portunidae	Genbank Crustacea Malac.
NC_012567	<i>Scylla tranquebarica</i>	Portunidae	Genbank Crustacea Malac.
FJ174946	<i>Scyllarides herklotsii</i>	Scyllaridae	Genbank Crustacea Malac.
JSDAz240	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAz236	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAz235	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAz37	<i>Scyllarides latus</i>	Scyllaridae	This study
JSDAz36	<i>Scyllarides latus</i>	Scyllaridae	This study
FJ174947	<i>Scyllarides latus</i>	Scyllaridae	Genbank Crustacea Malac.
FJ174966	<i>Scyllarus arctus</i>	Scyllaridae	Genbank Crustacea Malac.
FJ174967	<i>Scyllarus posteli</i>	Scyllaridae	Genbank Crustacea Malac.
FJ174965	<i>Scyllarus pygmaeus</i>	Scyllaridae	Genbank Crustacea Malac.
EU682852	<i>Scyra acutifrons</i>	Pisidae	Genbank Crustacea Malac.

EU682853	<i>Scyra acutifrons</i>	Pisidae	Genbank Crustacea Malac.
FC-TPD17D	<i>Sergestes similis</i>	Sergestidae	Decapods of Pacific and Atlantic
FC-TPD17C	<i>Sergestes similis</i>	Sergestidae	Decapods of Pacific and Atlantic
FC-TPD17B	<i>Sergestes similis</i>	Sergestidae	Decapods of Pacific and Atlantic
FC-TPD17A	<i>Sergestes similis</i>	Sergestidae	Decapods of Pacific and Atlantic
JSDUKdeep_23	<i>Sergia robusta</i>	Sergestidae	This study
JSDUKdeep_22	<i>Sergia robusta</i>	Sergestidae	This study
JSDUKdeep_21	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX79-05	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX71-01	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX4-03	<i>Sergia robusta</i>	Sergestidae	This study
JSDPX4-02	<i>Sergia robusta</i>	Sergestidae	This study
FCFOPC069-03	<i>Sergia robusta</i>	Sergestidae	This study
EU420129	<i>Shinkaia crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
NC_011013	<i>Shinkaia crosnieri</i>	Galatheidae	Genbank Crustacea Malac.
JSDMe21	<i>Solenocera membranacea</i>	Solenoceridae	This study
JSDMe20	<i>Solenocera membranacea</i>	Solenoceridae	This study
JSDMe19	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCFOPC041-14	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCFOPC041-13	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCFOPC041-12	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOPB089-03	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOPB089-02	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCDOPB083-07	<i>Solenocera membranacea</i>	Solenoceridae	This study
FCFOP66-11	<i>Solenocera membranacea</i>	Solenoceridae	This study
FC-SNC203A	<i>Spirontocaris holmesi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SNC72A	<i>Spirontocaris holmesi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SND7B	<i>Spirontocaris lamellicornis</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SND7A	<i>Spirontocaris lamellicornis</i>	Hippolytidae	Decapods of Pacific and Atlantic
TE-004T201-220-01	<i>Spirontocaris lilljeborgii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
TE-004T69-04	<i>Spirontocaris lilljeborgii</i>	Hippolytidae	Crustaceans of the St. Lawrence Gulf
FC-DPA09	<i>Spirontocaris phippsi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-DPA02	<i>Spirontocaris phippsi</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SNI216C	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SNI216B	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
FC-SNI216A	<i>Spirontocaris sica</i>	Hippolytidae	Decapods of Pacific and Atlantic
JSDUKdeep_43	<i>Stereomastis grimaldi</i>	Polychelidae	This study
JSDUKdeep_41	<i>Stereomastis grimaldi</i>	Polychelidae	This study
AB290645	<i>Stoliczka chaseni</i>	Potamidae	Genbank Crustacea Malac.
EU334658	<i>Strigopagurus poupinii</i>	Diogenidae	Genbank Crustacea Malac.
EU123823	<i>Stygiocaris lancifera</i>	Atyidae	Genbank Crustacea Malac.
EU123824	<i>Stygiocaris lancifera</i>	Atyidae	Genbank Crustacea Malac.
EU123825	<i>Stygiocaris lancifera</i>	Atyidae	Genbank Crustacea Malac.
EU123826	<i>Stygiocaris lancifera</i>	Atyidae	Genbank Crustacea Malac.
FC-TGB15	<i>Systellaspis braueri</i>	Oplophoridae	Decapods of Pacific and Atlantic
JSDPX42(05)-09	<i>Systellaspis debilis</i>	Oplophoridae	This study
JSDPX42(05)-08	<i>Systellaspis debilis</i>	Oplophoridae	This study
JSDPX42(05)-07	<i>Systellaspis debilis</i>	Oplophoridae	This study
JSDPX42-03	<i>Systellaspis pellucida</i>	Oplophoridae	This study
JSDPX42-01	<i>Systellaspis pellucida</i>	Oplophoridae	This study
EU682872	<i>Taliepus dentatus</i>	Epiatlidae	Genbank Crustacea Malac.
EU682873	<i>Taliepus nuttallii</i>	Epiatlidae	Genbank Crustacea Malac.
AB278153	<i>Telmessus acutidens</i>	Atelecyclidae	Genbank Crustacea Malac.

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AB211304	<i>Telmessus cheiragonus</i>	Atelecyclidae	Genbank Crustacea Malac.
AB290646	<i>Terrapotamon abbotti</i>	Potamidae	Genbank Crustacea Malac.
FJ174950	<i>Thenus unimaculatus</i>	Scyllaridae	Genbank Crustacea Malac.
JSDUK185	<i>Thoralus cranchii</i>	Hippolytidae	This study
JSDUK184	<i>Thoralus cranchii</i>	Hippolytidae	This study
JSDUK183	<i>Thoralus cranchii</i>	Hippolytidae	This study
EU682837	<i>Tiarinia cornigera</i>	Majidae	Genbank Crustacea Malac.
EU682838	<i>Tiarinia spinigera</i>	Majidae	Genbank Crustacea Malac.
FJ426027	<i>Troglocaris bosnica</i>	Atyidae	Genbank Crustacea Malac.
FJ426028	<i>Troglocaris bosnica</i>	Atyidae	Genbank Crustacea Malac.
DQ320044	<i>Troglocaris hercegovinensis</i>	Atyidae	Genbank Crustacea Malac.
DQ320046	<i>Troglocaris inermis</i>	Atyidae	Genbank Crustacea Malac.
DQ320045	<i>Troglocaris kutaissiana</i>	Atyidae	Genbank Crustacea Malac.
FJ693518	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693519	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693520	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693526	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693527	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693547	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693549	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693551	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693552	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
FJ693557	<i>Uca minax</i>	Ocypodidae	Genbank Crustacea Malac.
AF516704	<i>Varuna litterata</i>	Varunidae	Genbank Crustacea Malac.
AF317343	<i>Varuna litterata</i>	Varunidae	Genbank Crustacea Malac.
EF599154	<i>Virilastacus retamali</i>	Parastacidae	Genbank Crustacea Malac.
EF599155	<i>Virilastacus retamali</i>	Parastacidae	Genbank Crustacea Malac.
EF599150	<i>Virilastacus rucapihuelensis</i>	Parastacidae	Genbank Crustacea Malac.
FCDPH12-399Gr4	<i>Vulcanocalliax arutyunovi</i>	Callianassidae	This study
JSDUK188	<i>Xantho pilipes</i>	Xanthidae	This study
JSDUK187	<i>Xantho pilipes</i>	Xanthidae	This study
JSDUK186	<i>Xantho pilipes</i>	Xanthidae	This study
DQ458795	<i>Xantho pilipes</i>	Xanthidae	Genbank Crustacea Malac.
EU004941	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004942	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004944	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004971	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004972	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004980	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004987	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004990	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004996	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.
EU004999	<i>Xiphocaris elongata</i>	Atyidae	Genbank Crustacea Malac.



**CHAPTER 3.2 Testing the utility of partial COI and 16S sequences for
phylogenetic appraisals of selected *Plesionika*
(Decapoda: Pandalidae) from the Northeast Atlantic
and Mediterranean Sea.**

Abstract

Despite the high species and ecological diversity of pandalid shrimps, there has been no previous attempt to resolve evolutionary relationships from several genera using molecular tools. Although mitochondrial DNA cytochrome oxidase I (COI) is widely used in barcoding studies to delimit species boundaries, additional insights on phylogenetic affinities can be obtained, especially when used in combination with data from additional genes. The knowledge of molecular diversity is essential to understand phylogenetic relationships and will help systematic clarifications. Based on partial fragments of the 16S and COI genes, we have specifically focused on addressing the phylogenetic position of the economically and ecologically important shrimp genus *Plesionika* within a framework of five genera from within the Pandalidae. Our results showed that species within *Plesionika* are substantially divergent when compare other genera, exhibiting the highest average nucleotide divergence with 11.23% and 8.46% in COI and 16S genes respectively. In addition sequence divergence was found to vary greatly within *Plesionika* genus (COI/16S respectively): 2.47/0.16% between *P. antigai* and *P. heterocarpus* and 16.16/9.83% between *P. heterocarpus* and *P. edwardsii*. We did not find amino acid sequences divergence between *P. heterocarpus* and *P. antigai* as when compared between *P. heterocarpus* and *P. edwardsii* (8.10%). Three species of *Plesionika* (*P. antigai*, *P. heterocarpus*, and *P. scopifera*) appear well separated from other *Plesionika* species in both maximum likelihood and Bayesian analysis. The present study reveals the utility of COI over 16S as genetic marker to further resolve relationships between different species of *Plesionika* from the Northeast Atlantic and

Mediterranean Sea, in addition to species delimitation. Findings highlight the need to further review paraphyly within *Plesionika* in an attempt to recognize a concordance in the evolutionary history of *Plesionika* with major ecological and geological events.

Keywords: *Plesionika*, molecular phylogeny, Pandalidae, COI, 16S,

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Testing the utility of partial COI and 16S sequences for phylogenetic appraisals
of selected *Plesionika* (Decapoda: Pandalidae) from the Northeast Atlantic and
Mediterranean Sea. *Marine Ecology: An Evolution Perspective* **in press**.

3.2.1 Introduction

Most systematic studies of the marine shrimps, Pandalidae, have been based solely on morphology; molecular tools have been rarely applied to solve questions of species status or to determine lower level phylogenetic relationships. Although previous studies on phylogenetic relationships within taxa of the infraorder Caridea exist (Baeza *et al.*, 2009; Bracken *et al.*, 2009; Page *et al.*, 2008; Shank *et al.*, 1999), limited studies attempted to understand pandalid molecular phylogeny and biogeographical relationships between the different genera. The Pandalidae is among the most species-rich families due to extensive diversification in the genus *Plesionika* Bate, 1888 with 92 extant described species (De Grave *et al.*, 2009). Among the pandalid shrimps found in the Northeast Atlantic and Mediterranean Sea, the species of the genus *Plesionika* have a subtropical and tropical distribution, with the Bay of Biscay region as the northernmost limit (Vafidis *et al.*, 2005). Members of *Plesionika* comprise small to intermediate sized shrimps (6-29 mm carapace length) with a benthic or nektobenthic occurrence, distributed on the shelf and slope (Puig *et al.*, 2001; Vafidis *et al.*, 2005). A clear segregation of size and depth of *Plesionika* spp. has been described and congeneric assemblages of *Plesionika* species exhibit specific bathymetric distributions, where each species has a preferred depth range (Carbonell *et al.*, 2003; Puig *et al.*, 2001). Species play an important ecological trophic role among megabenthic ecosystems (Cartes, 1998; Cartes *et al.*, 2002; Fanelli and Cartes, 2004), both as predators and prey. They feed on mesopelagic, suprabenthic, epibenthic, and infaunal organisms, and are considered non-migratory macroplankton feeders (Cartes *et al.*, 2002). Furthermore, they constitute a large part of the diet of demersal fish and cephalopods (Vafidis *et al.*, 2005). In the

Mediterranean demersal fisheries, some species form a by-catch of the trawl fishery, reaching prices similar to those of target species (Vafidis *et al.*, 2005).

The evolutionary history of *Plesionika* is complex, and despite revisions (Chan and Yu, 1991, 1998), its phylogeny, phylogeography and ontogeny remain ambiguous, especially for the species found in the Atlantic Ocean and Mediterranean Sea. Recently, the analysis of COI barcode diversity among 68 families of decapods (Matzen da Silva *et al.*, 2011) revealed high molecular diversity within *Plesionika* and non-monophyly among species. There has been no previous attempt to resolve evolutionary relationships among any *Plesionika* species using molecular systematic approaches. We here have specifically focused on addressing the phylogenetic position of the genus *Plesionika* within a framework of five genera from within the Pandalidae.

Systematic and phylogenetic studies of decapods have recently focused on mitochondrial genes sequences (Schubart, 2009): slowly evolving sequences of ribosomal subunit 16S rRNA (16S) have been extremely informative in reconstructing deep relationships (Schubart *et al.*, 2000), whereas the popular DNA barcoding mtDNA marker, cytochrome oxidase I (COI), is generally used to study shallow to moderately deep relationships among crustaceans (Groeneveld *et al.*, 2007; Mathews and Anker, 2009; Schubart *et al.*, 2005; Shih *et al.*, 2007; von Rintelen *et al.*, 2007). Here, to increase the robustness of the phylogenetic analysis of *Plesionika* we combined partial fragments of the 16S and COI data with the few available molecular data in GenBank among six genera (*Plesionika*, *Heterocarpus*, *Chlorotocus*, *Stylopandalus*, *Pandalus* and *Pandalopsis*) of the family Pandalidae (see Table 3.2.1), plus an outgroup *Macropodia* (Brachyura: Inachidae). There are eight morphological species of the genus *Plesionika* in European waters, from the Mediterranean and Northeast Atlantic waters, which can be easily distinguished, based on rostrum length and number of teeth, pleon

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features, and traits of the second length and number of teeth, pleon features, and traits of the second eight

Table 3.2.1: Pandalidae shrimps used for the *Plesionika* phylogeny reconstruction and outgroup taxa

Taxon	Region	GenBank nos.	
		COI	16S
<i>Chlorotocus crassicornis</i> (Costa, A., 1871)	Mediterranean Sea (Italy)	JN412722 ¹	JN412679 ²
<i>Dichelopandalus bonnieri</i> Caullery, 1896	Northeast Atlantic (Scotland)	JN412723 ¹	-
<i>Pandalopsis dispar</i> Rathbun, 1902	Northwest Pacific (Canada)	DQ882107	-
<i>Pandalus borealis</i> Krøyer, 1838	Northeast Atlantic (Sweden)	FJ403244	FJ403244
<i>Pandalus danae</i> Stimpson, 1857	Northeast Pacific (Canada)	DQ882112	-
<i>Pandalus goniurus</i> Stimpson, 1860	Northeast Pacific (Canada)	DQ882113	-
<i>Pandalus hypsinotus</i> Brandt, 1851	Northeast Pacific (Canada)	DQ882116	-
<i>Pandalus jordani</i> Rathbun, 1902	Northeast Pacific (Canada)	DQ882118	-
<i>Pandalus montagui</i> Leach, 1814 in Leach, 1813-1814	Northwest Atlantic (Canada)	FJ581851	EU868698
<i>Pandalus platyceros</i> Brandt, 1851	Northeast Pacific (Canada)	DQ882125	-
<i>Pandalus stenolepsis</i> Rathbun, 1902	Northeast Pacific (Canada)	DQ882128	-
<i>Heterocarpus amacula</i> Crosnier, 1988	Southeast Pacific (French Polynesian)	AY612856	AY612870
<i>Heterocarpus calmani</i> Crosnier, 1988	Indian Ocean (Madagascar)	AY612857	AY612871
<i>Heterocarpus ensifer</i> A.Milne-Edwards, 1881	Northwest Pacific (Palau)	AY612859	AY612873
<i>Heterocarpus gibbosus</i> Bate, 1888	Northwest Pacific (Taiwan)	AY612861	AY612876
<i>Heterocarpus hayashii</i> Crosnier, 1988	Northwest Pacific (Taiwan)	AY612863	AY612877
<i>Heterocarpus intermedius</i> Crosnier, 1999	Southwest Pacific (Fiji)	AY612864	AY612878
<i>Heterocarpus laevigatus</i> Bate, 1888	Northwest Pacific (Taiwan)	AY612865	AY612879
<i>Heterocarpus parvispina</i> de Man, 1917	Northwest Pacific (Taiwan)	AY612866	AY612880
<i>Heterocarpus sibogae</i> de Man, 1917	Northwest Pacific (Taiwan)	AY612867	AY612881
<i>Heterocarpus woodmasoni</i> Alcock, 1901	Southwest Pacific (Solomon Island)	AY612868	AY612882
<i>Plesionika antigai</i> Zariquiey Alvarez, 1955	Northeast Atlantic (Portugal)	JN412724 ¹	JN412682 ²
<i>Plesionika acanthonotus</i> (Smith, 1882)	Northeast Atlantic (Portugal)	JN412725 ¹	JN412680 ²
<i>Plesionika edwardsii</i> (Brandt, 1851)	Mediterranean Sea (Italy)	JN412726 ¹	JN412683 ²
<i>Plesionika heterocarpus</i> (Costa, A., 1871)	Northeast Atlantic (Portugal)	JN412727 ¹	JN412685 ²
<i>Plesionika martia</i> (A. Milne-Edwards, 1883)	Northeast Atlantic (Portugal)	JN412728 ¹	JN412685 ²
<i>Plesionika narval</i> (Fabricius, J.C., 1787)	Northeast Atlantic (Portugal)	JN412729 ¹	JN412689 ²
<i>Plesionika scopifera</i> Chan, 2004	Could not be determined	HQ241552	HQ241519
<i>Stylopandalus richardii</i> Richard, 1905	Northeast Atlantic (Portugal)	JN412730 ¹	-
<i>Macropodia longipes</i> * (A. Milne-Edwards & Bouvier, 1899)	Northeast Atlantic (Portugal)	JN107573 ¹	JN412692 ²

* Outgroup taxa

¹ Matzen da Silva *et al.*, 2011

² This study

European *Plesionika* reported pereiopod (e.g. Zariquey-Alvarez 1968; Lagardère 1971). Here, we analysed six of the eight reported European *Plesionika* species: *Plesionika acanthonotus* (S. I. Smith, 1882), *P. antigai* Zariquey (Alvarez, 1955), *P. edwardsii* (Brandt, 1851), *P. heterocarpus* (A. Costa, 1871), *P. martia* (A. Milne-Edwards, 1883) and *P. narval* (J. C. Fabricius, 1787). This study provides the first DNA sequence data that can be used to accurately identify *Plesionika* species from the North Atlantic Ocean and Mediterranean Sea. Moreover, since there has been no previous attempt to resolve evolutionary relationships among these species with molecular tools, we analyzed whether phylogenetic groupings based on mitochondrial COI and 16S genes, support or refute previously proposed morphological divisions. This molecular systematic assessment of *Plesionika* species facilitates for the first time an opportunity to examine the usefulness of two different mitochondrial markers for species recognition in a taxonomically complex and ecologically important group of marine shrimps.

3.2.2 Material and Methods

Sample collection

Decapods were collected from the Portuguese west coast, Mediterranean Sea and East Coast of Scotland between 2006 and 2008 (Figure 3.2.1). From the Portuguese coast, specimens were harvested as by-catch from rough ground bottom trawls by National Institute of Biological Resources of Portugal (INRB-IPIMAR) research vessels. Specimens were also collected opportunistically from survey work associated

with fisheries from Sicily and from Aberdeen Fisheries Research Services from Scotland. Decapods were stored in a freezer at -20°C and later identified. Morphological identification was assigned to the lowest possible taxonomic category, which, in the majority of cases, was to species level using Zariquiey-Alvarez (1968), Lagardère (1971) and Crosnier and Forest (1973). A sample of muscle was collected after taxonomic identification and preserved in 95% ethanol and stored at -20°C.

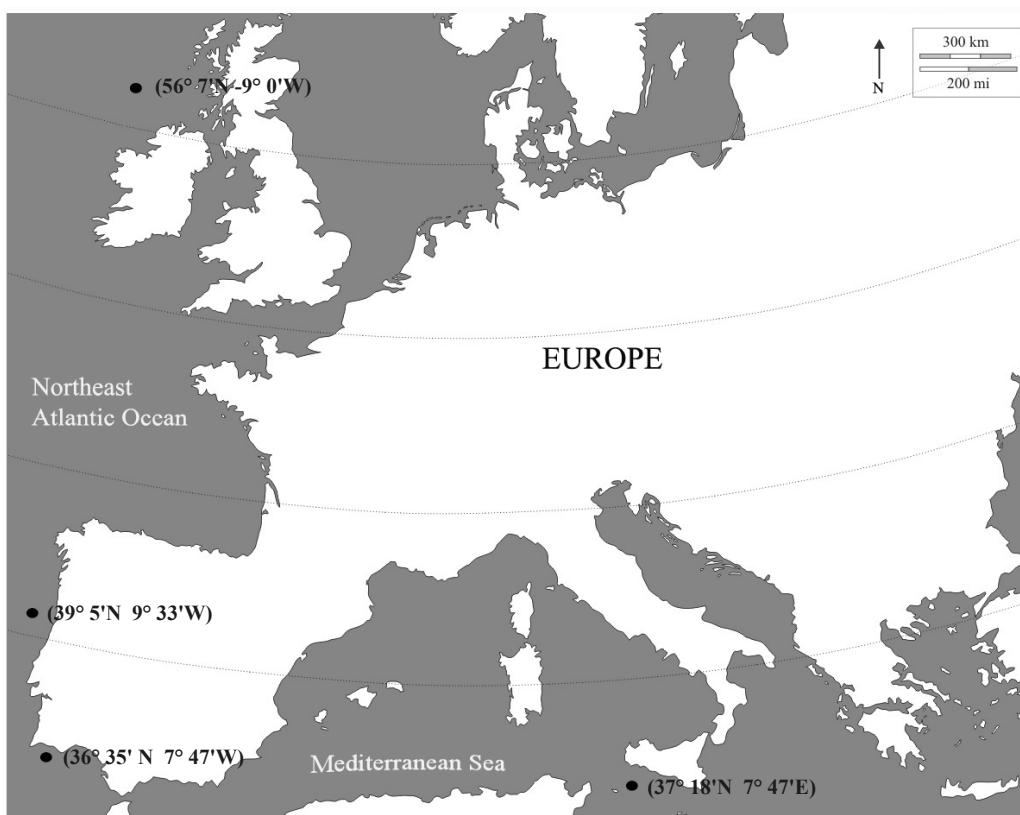


Figure 3.2.1: Pandalidae shrimps sampling locations from our study (see Table 3.2.1).

DNA isolation, amplification and sequencing

Total genomic DNA was extracted from small amounts of tissue (1 mm³ muscle tissue) using Chelex dry release method (Hajibabaei *et al.*, 2005). A 10:1 mixture of

Chelex buffer with Proteinase K (Sigma ®) was prepared and 110 µl of the mixture was placed in each 96-well plate. A small sample of tissue from each specimen was put into the extraction mixture. Extraction plates were then incubated at 55°C for 12 hours and subsequently heated to 95°C for 20 min. Extraction plates were centrifuged at 1000 rpm for 5 min immediately before setting up the polymerase chain reaction (PCR). The COI gene was amplified with alternative sets of primers depending on PCR reaction success following the protocol by Costa *et al.* (2007). Forward primer sequences were LCOI490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3') (Folmer *et al.*, 1994), CrustF1 (5'- TTT TCT ACA AAT CAT AAA GAC ATT GG-3') (Costa *et al.*, 2007), and the reverse primer HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.*, 1994). All PCRs were performed in a 25 µl volume containing 1 X PCR buffer, 3 mM MgCl₂, 0.1- 0.2 mM dNTP, 1U TAQ polymerase, 5 – 10 pmol of each primer, and 2 - 10 ng of DNA template. The thermal cycling conditions consisted of 94°C for 60 s; 35-40 cycles of 94°C for 30 s, 48 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. Alternative thermal cycling conditions consisted of 94°C for 60 s; 5 cycles of 94°C for 30 s, 45°C for 90 s, and 72°C for 60 s; 35 cycles of 94°C for 30 s, 50 - 56°C for 90 s, and 72°C for 60 s; followed by a final extension of 72°C for 5 mins. Amplification of 16S was performed in a 25 µl volume containing 1 X PCR buffer, 1.5-3 mM MgCl₂, 0.05- 0.1 mM dNTP, 1U TAQ polymerase, 5-10 pmol of each primer, and 2 - 10 ng of DNA template. Forward primer was 16SL2 (5'-TGC CTG TTT ATC AAA AAC AT-3') (Mathews *et al.*, 2002) and the reverse 16S 1472 (5'-AGA TAG AAA CCA ACC TGG-3') (Schubart *et al.*, 2000). The thermal cycling conditions consisted of 94°C for 10 min; 35 cycles of 98°C for 60 s, 48°C for 120 s, and 72°C for 120 s; followed by a final extension of 72°C for 2 mins. Following amplification, PCR products were cleaned by incubation with 10 U

Exonuclease I (New England Biolabs ®) and 1 U Shrimp Alkaline Phosphate (Promega ®) at 37°C for 1 hour, followed by heating at 80 °C for 5 min. Samples were sequenced by Macrogen Inc (South Korea) using an Applied Biosystems ® 3730 sequencer.

Sequence analyses

COI and 16S sequences were manually checked for ambiguous base calls and assembled in CodonCode Aligner version 1.3.0, and aligned using ClustalX in Mega 4 (Tamura *et al.*, 2007). The amino acid translation of COI was examined to ensure that no gaps or stop codons were present. BLAST searches were performed for all sequences using GenBank's online nucleotide database using the megablast algorithm (Zhang *et al.*, 2000). Nucleotide composition and substitution frequencies were calculated in PAUP v3.1(Swofford, 1993). Pairwise distances were calculated in PAUP v3.1 with the best-fit model of nucleotide substitution, GTR+G+I and TrN+G for COI and 16S respectively, indicated by Akaike information criterion (Akaike, 1973) using jModel test (Posada, 2008). To evaluate the range of intrageneric sequence identity found among *Plesionika* species, we compared pairwise distances of COI and 16S. For comparisons to other publications Kimura 2-parameter (K2P) distances of COI and 16S were also calculated.

The amount of phylogenetic signal versus noise in the concatenated alignment (ingroup only) was assessed with DAMBE (Xia and Xie, 2001). The program DAMBE was used to examine saturation of the mitochondrial COI and 16S sequences and to calculate transition (*ts*) versus transversion (*tv*) nucleotide substitutions. The I_{ss} values provide a measure of substitution saturation in molecular phylogenetic datasets, and were calculated for the whole dataset and for each of the codon position separately. I_{ss} is derived from the amount of entropy in the data and needs to be compared to critical

values, below which simulation studies showed decreased accuracy (Xia *et al.*, 2003). The DAMBE software was used to calculate I_{ss} values and compare them against critical $I_{ss,c}$ values for symmetric and asymmetric topologies (Xia *et al.*, 2003) for both data sets, respectively.

COI phylogenetic analyses

To provide a comprehensive sister-species coverage and survey of between species variation of pandalid shrimps, sequences for target species from GenBank were merged with our data (Table 3.2.1). To reconstruct phylogenetic relationships within each genus, the corresponding mitochondrial sequence data were analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Due to the absence of information about the phylogenetic relationship among pandalid genera and molecular data, we chose a decapod crab specimen, *Macropodia longipes* (A. Milne-Edwards & Bouvier, 1899) (Brachyura:Inachidae) as an outgroup. Ten independent ML analyses were conducted using GTR+I+G with invariant sites (I) and gamma distributed rates (G) (Yang, 1994) (see below) as the model (using the GTR+CAT setting) with 4 categories of rate variation (500 bootstrap replicates were undertaken for estimation of node support) for each partition on combined data. In order to find the ML tree, 10 independent runs of RAxML 7.0.4 were conducted. jModelTest (Posada, 2008) identified the model (Hasegawa *et al.*, 1985) as best indicated by Akaike Information Criterion (AIC) (Akaike, 1973), however, since this model is not implemented in the current version of RaxML, the GTR + I + G model was selected as the closest matching alternative. Since different substitution rates among the four nucleotides and among different nucleotide sites in mitochondrial protein-coding genes has been reported (Kumar, 1996; Yang *et al.*, 2000; Yang and Yoder, 1999) a

phylogenetic tree was constructed with MrBayes 3 (Huelsenbeck and Ronquist, 2001), with the best-fit model of each codon position indicated by the likelihood ratio test of jModeltest (Posada, 2008): the models TIM3ef+I (Posada, 2003), F81+ I (Felsenstein, 1981), and TIM3+G (Posada and Buckley, 2004), were used for the 1st, 2nd, and 3rd codon position of COI respectively (see species analyzed in Table 3.2.1).

Combined COI and 16S data phylogenetic analysis

A combined analysis was conducted with concatenated sequences from the two genes with 21 specimens (Table 3.2.1). As best indicated models by AIC (Akaike, 1973) were TIM3ef+I+G (Posada, 2003), TIM 3+G (Posada, 2003), and TIM 1+G (Posada, 2003) for 1st, 2nd, and 3rd codon position of COI, respectively.

The TrN+G (Tamura and Nei, 1993) model was used for the 16S sequence. ML analysis was conducted using the closest matching GTR+I+G model for each partition on combined data since the AIC selected models are not implemented in the current version of RaxML. In the BI analyses, the two genes regions were partitioned separately according to the previously determined model parameters. Minor gaps in 16S sequences were treated as fifth character-state in subsequent BI phylogenetic analyses. First, each gene was analysed separately using MrBayes. The BI analysis comprised two independent Markov Chain Monte Carlo (MCMC) runs were conducted for 5×10^6 generations under substitution model for each gene. After discarding the first 1 million generations (20%) cycles as the burn-in, we monitored the fluctuating value of the likelihood graphically with Tracer v1.4 (Drummond and Rambaut, 2007) to check that the generations had been reached stationary values. The consensus tree was selected and visualized using FigTree V.1.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

3.2.3 Results

Diversity

Gene sequences of approximately 600 base pair (bp) of COI and 500 bp of 16S were amplified successfully from all specimens and sequences of both genes were deposited in GenBank (Table 3.2.1). Following the addition of GenBank sequences, our resulting data set comprised 540bp of COI and 463bp of 16S.

The COI alignment contained 239 variable characters, of which 220 were parsimony informative. There was also evidence for base composition bias in the sequences, notably a pronounced underrepresentation of guanine at the third codon positions (mean frequency of nucleotides of 30 Pandalidae species: 27.26% A; 22.08% C; 18.41% G; 32.24% T) a phenomenon commonly observed in metazoan mitochondrial sequences (Wolstenholme, 1992). The pattern of nucleotide substitutions (excluding the outgroup) favours transition over transversions, yielding a *ts:tv*= ratio of 1.2. The number of variable sites was much greater at the third (179/180) than at the first (53/180) and second (7/180) positions. Previously, it has been shown that when third codon positions are analysed separately, saturation can prevent reliable phylogenetic inference (Tong *et al.*, 2000). Results from the substitution saturation analysis showed an $I_{ss} < I_{ss.c}$ ($p < 0.001$, in all three data sets), indicating that sequences are highly useful in phylogenetic reconstruction (Xia *et al.*, 2003).

The 16S alignment showed that the pandalida sequences were highly conserved for the group of 21 species of this family in some regions with 199/463 variable characters, of which 157/463 were parsimony informative. The 16S sequences are AT-rich (67.1%), indicating a moderate compositional bias. The pattern of nucleotide

substitution was also biased in favour of transitions over transversions, yielding a $ts:tv$ ratio=1.2.

The mean divergence distance (ML distance with substitution model GTR+I+G) within 30 species was 11.64%. The *Plesionika* genus (represented here by eight species) is substantially divergent from the other genera and showed the highest average nucleotide divergence with 11.23% (range 2.47-16.16%) and 8.46% (range 0.16-11.16%) within COI and 16S among Pandalidae genera. For example, *Heterocarpus*, the most representative genus in our data set (with 11 species), exhibited nucleotide divergence of 6.20% (range 0.02-10.23 %) and 1.67% (range 0.00-3.08%), respectively. On the other hand, sequence divergence was found to vary greatly within *Plesionika* (COI/16S respectively): 2.47/0.16% between *P. antigai* and *P. heterocarpus* and 16.16/9.83% between *P. heterocarpus* and *P. edwardsii* (Table 3.2.2). In addition, using the genetic code for *Drosophila* mtDNA the lowest and the highest distance in the amino acid sequences divergence (Poisson correction) observed was between *P. heterocarpus* and *P. antigai* (0.00%) and between *P. heterocarpus* and *P. edwardsii* (8.10%), respectively. No amino acid divergence was also observed between *Heterocarpus amacula* (Crosnier, 1988), *H. hayashii* (Crosnier, 1988), *H. sibogae* (De Man, 1917), *H. parvispina* (De Man, 1917), and *H. ensifer* (A. Milne-Edwards, 1881) species.

Phylogeny

Phylogenetic analyses using the ML and BI analysis based on 21 species of COI data set resolved the same grouping patterns in similar tree topologies as the combined data set (Figure 3.2.2 and 3.2.3). The 16S data were unable to resolve relationships among the *Plesionika* species with any significant support.

Combining data increased the cladistic separation between *P. antigai*, *P. heterocarpus* and *P. scopifera* from the rest of the *Plesionika* species, which are separated into two distinct clades by 26.6%. This also improved bootstrap and probability support values between the two distinct clades of the *Heterocarpus* genus (Figure 3.2.3). Both BI trees (see Fig. 3.2.2 and 3.2.3) had high levels of support delineating a robust relationship between two groups: group A defined to represent species with preference of subtropical and temperate zones and group B inhabiting temperate and frigid zones. When amino acid divergence was compared within the COI data set, group A showed a mean amino acid divergence of 3.5% against 0.9% for the group B. The number of variable and parsimony informative sites was much greater for the sequences in group A (27/180; 13/180) than in the group B (15/180; 3/180). *Plesionika antigai* and *P. heterocarpus* showed an absence of amino acid divergence in contrast with the remainder *Plesionika* species with an average of amino acid divergence of 3.40%. The amino acid distance between the two *Plesionika* groups was 7.20%. The paraphyly of the genus *Plesionika* was supported by high bootstrap support and Bayesian Posterior Probabilities (ML/BI), notably 99% in ML and 100% in BI analyses (Figure 3.2.3). The two distinct *Heterocarpus* clades highlighted by stars in each node in figures 3.2.2 and 3.2.3 represent species sampled from North Hemisphere (dark star) and from South Hemisphere (grey star), respectively. The Northern Hemisphere group showed an absence of amino acid divergence in contrast to samples from the Southern Hemisphere Indian and Pacific Oceans group, with an average amino acid distance of 2.00%.

BLAST search

A simple BLAST search of the nucleotide and protein sequences revealed that four sequences from GenBank of Pandalidae family are apparently completely misleading. The sequences of *Plesionika ensis* (AY612869; AY612883) show high similarity with *Heterocarpus* spp; *Pandalopsis coccinata* (Uruta, 1941) (AB290213) apparently doesn't belong to Caridea suborder, and *Pandalus eos* (Makarov, 1935) (AB211294) it is not even a Decapoda. Thus, these sequences were not included in our analyses.

3.2.4 Discussion

Species diversity

The genus *Plesionika* is distributed globally, consisting mainly of morphologically similar deepwater shrimps (Ohtomi, 1997; Vafidis *et al.*, 2005). However, this does not necessarily mean that there is no genetic heterogeneity among taxa, as the rate of evolution of morphological features is known to differ from rates of molecular evolution (Tong *et al.*, 2000). Genetic divergence of COI (K2P distance) within *Plesionika* species (mean 25.08%) was slightly higher than the mean congeneric divergences compared to two other pandalid shrimps, *Heterocarpus* (15.79%) and *Pandalus* (15.80%), one alvinocaridid shrimps *Alvonicaris* (16.60%) (Shank *et al.*, 1999), two hyppolitid shrimps *Spirontocaris* (15.40%) and *Eualus* (15.20%), one crangonid shrimp *Crangon* (18.91%) (Costa *et al.*, 2007) and three palaemonid shrimps

Table 3.2.2: Nucleotide ML distances estimated from 16S with TrN+G model (above diagonal) and COI (below diagonal) with GTR+G+I model in Pandalidae spp and outgroup. All values are expressed as percentage.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1 <i>Macropodia longipes</i>	21.39	24.66	22.63	23.95	22.57	23.08	22.15	20.53	23.08	23.38	21.28	19.73	23.14	22.43	26.11	25.52	22.91	23.98	30.60	24.42	
2 <i>Chlorotocus crassicornis</i>	24.18	2.76	4.43	2.98	3.00	2.91	4.72	3.30	2.91	3.97	5.67	6.33	4.44	9.87	5.35	9.89	6.06	6.13	10.95		
3 <i>Heterocarpus amacula</i>	11.13	8.91	2.84	0.82	2.05	0.80	1.73	0.80	0.78	2.81	4.30	4.19	3.83	8.19	4.64	7.68	3.53	3.92	8.47		
4 <i>Heterocarpus calmani</i>	17.68	12.52	7.99	2.32	2.25	2.35	0.35	2.45	2.35	2.27	0.24	5.83	6.44	4.15	10.58	5.36	10.23	4.80	5.75	9.85	
5 <i>Heterocarpus ensifer</i>	11.10	8.67	0.21	7.98	1.63	0.02	2.52	1.50	0.02	0.02	2.53	4.35	4.15	3.53	9.70	4.15	9.69	3.94	4.25	9.15	
6 <i>Heterocarpus gibbosus</i>	19.85	14.16	8.03	5.99	8.17	1.70	2.52	1.46	1.70	1.58	2.34	4.57	4.81	3.91	9.81	5.21	9.36	3.81	5.25	9.90	
7 <i>Heterocarpus hayashii</i>	11.10	8.81	0.02	7.92	0.23	7.96	2.56	1.57	0.00	0.04	2.57	4.26	4.06	3.46	9.51	4.06	9.50	3.86	4.17	8.82	
8 <i>Heterocarpus intermedius</i>	20.17	13.25	10.39	1.28	10.24	6.97	10.36	2.66	2.56	2.47	0.41	5.74	6.34	3.67	11.51	5.18	11.14	4.38	5.44	10.59	
9 <i>Heterocarpus laevigatus</i>	14.88	10.83	8.58	6.51	8.57	6.13	8.52	7.50	1.57	1.46	2.55	5.61	5.25	3.29	9.16	4.48	8.80	3.33	4.39	9.08	
10 <i>Heterocarpus parvispina</i>	11.05	8.73	0.25	7.62	0.32	7.89	0.28	10.37	8.16	0.04	2.57	4.26	4.06	3.46	9.51	4.06	9.50	3.86	4.17	8.82	
11 <i>Heterocarpus sibogae</i>	10.88	8.79	0.11	7.60	0.21	8.13	0.13	10.20	8.33	0.21	2.47	4.38	4.17	3.55	9.44	4.27	9.44	3.87	4.28	8.90	
12 <i>Heterocarpus woodmasoni</i>	23.46	12.73	10.93	1.15	10.91	6.58	10.85	1.76	6.18	10.56	10.71	5.84	6.70	4.11	10.16	5.15	9.82	5.04	5.87	9.77	
13 <i>Pandalus borealis</i>	14.73	16.09	8.46	11.97	8.61	12.27	8.38	13.15	15.23	8.43	8.23	14.24	0.47	3.89	9.67	6.38	9.50	5.57	6.81	8.84	
14 <i>Pandalus montagui</i>	15.41	15.29	10.56	13.72	11.01	15.01	10.52	15.12	14.00	10.57	10.35	12.90	2.15	4.07	10.76	6.32	10.91	5.12	6.57	10.33	
15 <i>Plesionika acanthonotus</i>	16.61	13.52	8.22	11.00	8.25	10.77	8.31	12.40	11.88	8.31	8.20	15.31	14.51	18.32	9.12	3.14	9.11	2.70	4.59	8.94	
16 <i>Plesionika antigai</i>	13.95	16.01	12.89	11.09	12.90	15.41	12.80	12.85	12.09	12.51	12.66	11.83	8.92	11.41	14.22	10.57	0.16	10.84	10.74	1.19	
17 <i>Plesionika edwardsii</i>	16.26	16.76	12.07	12.23	11.89	8.84	12.04	12.90	11.51	11.98	14.02	13.67	16.48	6.04	13.73	9.83	4.56	6.48	11.16		
18 <i>Plesionika heterocarpus</i>	12.49	14.26	10.77	11.52	10.35	13.79	10.69	12.90	12.36	10.94	10.36	12.09	11.51	12.70	13.90	2.47	16.16	9.92	10.12	1.08	
19 <i>Plesionika maria</i>	18.01	17.79	17.60	10.01	17.26	11.40	17.50	10.22	8.32	17.68	17.37	9.57	10.34	12.01	14.15	13.03	12.95	14.02	5.01	10.47	
20 <i>Plesionika narial</i>	20.57	16.69	9.88	10.80	9.96	13.93	9.85	11.51	12.42	10.24	9.84	12.63	13.29	14.78	14.62	12.59	12.42	8.13	13.18	9.52	
21 <i>Plesionika scopifera</i>	13.77	14.96	10.72	7.94	11.04	12.03	10.68	10.40	10.72	10.12	10.26	9.27	11.70	11.16	11.38	4.19	12.93	2.82	12.08	10.80	

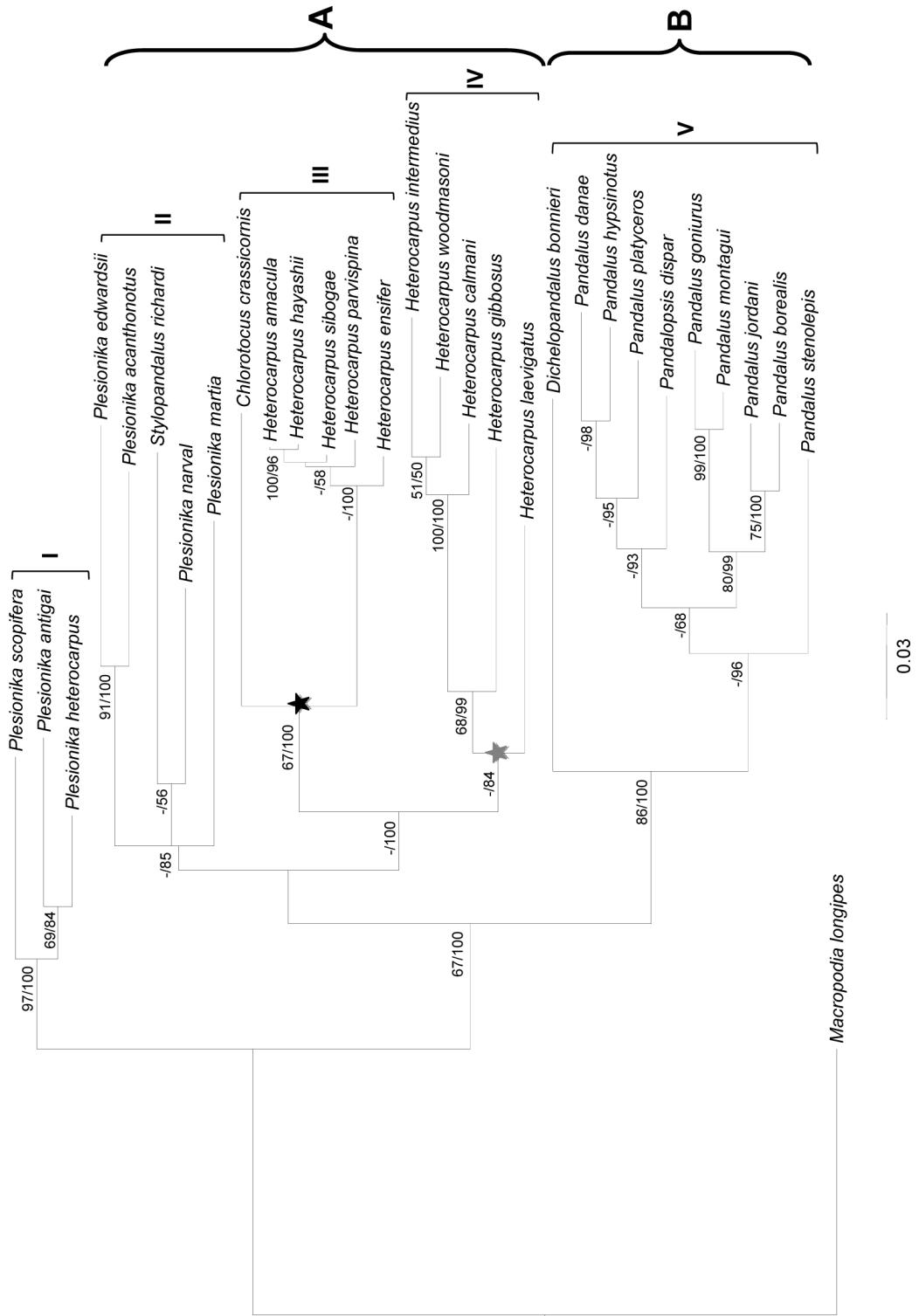


Figure 3.2.2: Bayesian phylogeny analyses of Pandalidae species for COI gene. A similar tree topology was obtained by Maximum likelihood (ML). The small numerals represent levels of support based on 500 bootstrap replicates /Bayesian Posterior Probabilities (ML/BI) expressed as percent. Values <50% are not show. Right braces indicate two groups: group A represent species with preference of subtropical and temperate zones and group B with preference of temperate and frigid zones. The black star indicates deep sea species samples from North Hemisphere and grey star species samples from South Hemisphere from Indic Pacific oceans.

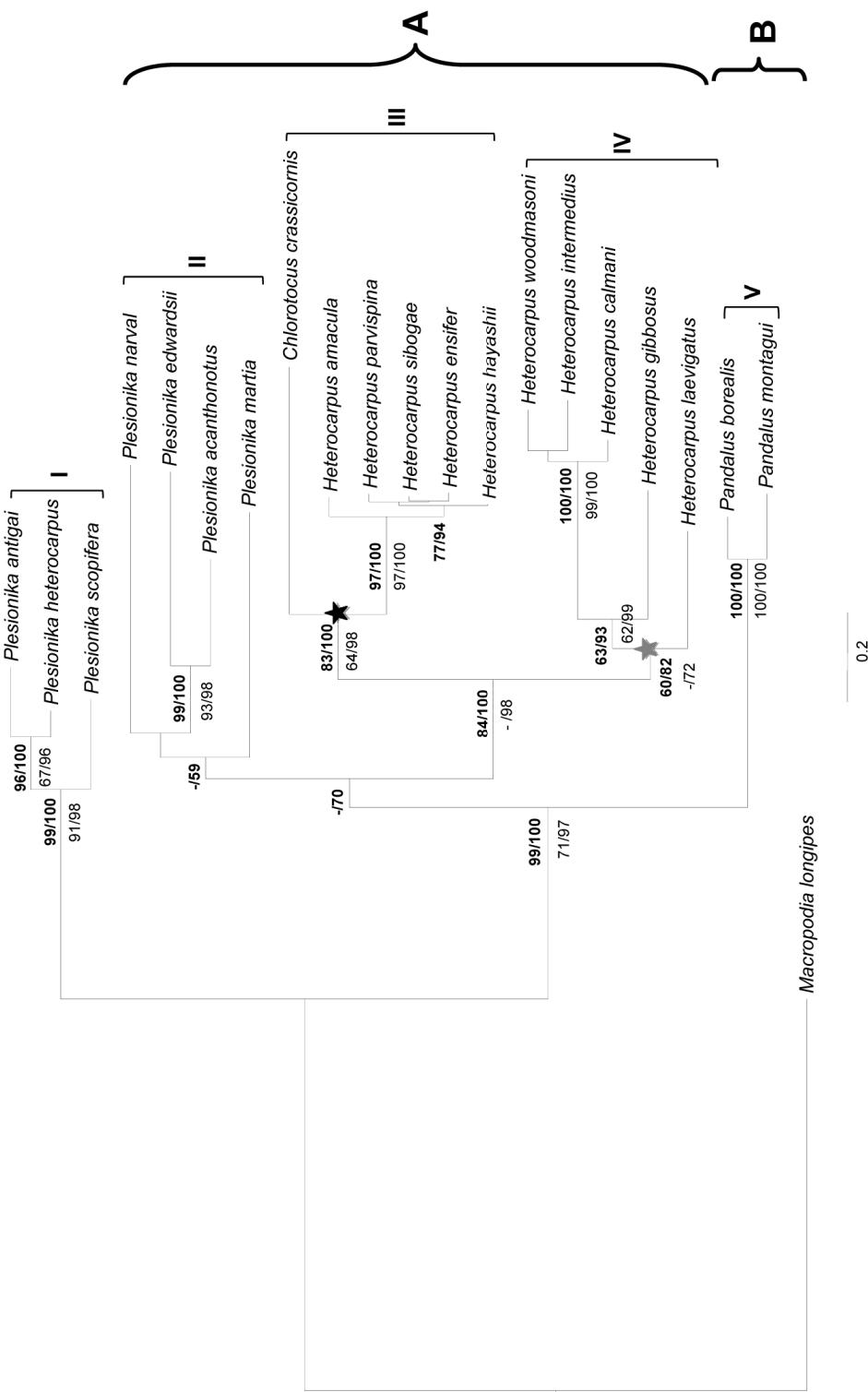


Figure 3.2.3: Bayesian phylogeny analyses of 21 Pandalidae from the combined COI and 16S data. A similar tree topology was obtained by Maximum likelihood (ML). The small numerals represent levels of support based on 500 bootstrap replicates /Bayesian Posterior Probabilities (ML/BP) expressed as percent (values below the nodes represent levels of support only for COI). Right braces indicate two groups: group A represent species with preference of subtropical and temperate zones and group B with preference of temperate and frigid zones. The black star indicates deep sea species samples from North Hemisphere and grey star species samples from South Hemisphere from Indic Pacific oceans.

Macrobrachium (20.30%), *Palaemon* (18.11%) and *Periclimenes* (20.19%) (Matzen da Silva *et al.*, 2011). Thus, it may be suggested from the present study that the relationship between molecular and morphological evolution is likely to exhibit high variance in *Plesionika* shrimps, a feature that has been reported in other crustaceans, e.g., Amphipoda (Macdonald *et al.*, 2005) and Copepoda (Rocha-Olivares *et al.*, 2001). The high variability observed can raise two major questions: 1) whether molecules and morphology yield strongly supported, conflicting hypotheses of relationships, or 2) are the observations attributable to undersampling of phylogenetic characters in one or both types of data (morphological vs molecular). The *Plesionika* average sequence divergence (K2P) for 16S was 22.20%. Similar studies for penaeid shrimp species have reported an average (K2P) of 6.4% and 11% nucleotide divergence in partial 16S sequences among seven *Metapenaeopsis* and 13 species of *Penaeus* genus, respectively (Garcia-Machado *et al.*, 1993; Tong *et al.*, 2000).

The present data show 89.50% and 95.66% similarity at the amino acid sequences of COI among *Plesionika* spp. and *Heterocarpus* spp., respectively. Very similar levels of similarity were observed among *Metapenaeopsis* with 98% (Tong *et al.*, 2000). Despite considerable conservation at amino acid sequences, large differences at silent sites of COI were observed in both *Plesionika* (61.4%) and *Heterocarpus* (44.2%) genera.

The range of COI and 16S divergence was found to vary greatly within *Plesionika* (Table 3.2.2). The closest observed distance between *P. antigai* and *P. heterocarpus* supports the morphological similarity between these two species (Zariquiey-Alvarez 1968, Lagardère 1971, Crosnier & Forest 1973). Studies with different molecular markers in decapods revealed considerable allozyme differentiation among morphologically similar species (Mulley and Latter, 1980; Stewart *et al.*, 2003;

Tam and Chu, 1993; Zaslavskaya *et al.*, 2007) suggesting that there is no conflict between molecular and morphological phylogenetic hypotheses among *Plesionika* species.

Molecular phylogeny

Results from the substitution saturation analysis among our COI and 16S data set showed that sequences are highly informative for phylogenetic reconstruction (Xia *et al.*, 2003). Although when the number of species analysed for COI was with 21 species without outgroup, the phylogenetic signal ($I_{ss}/I_{ss.c}$) of COI decreased only slightly. Comparing both markers, COI is shown to be more useful in phylogenetic reconstruction of *Plesionika* than 16S. A sensible approach for tackling this problem was to use an appropriate nucleotide substitution model of evolution that incorporates multiple mutations at the same site for each gene, in order to correct the observed distance for the multiple hits. The COI and the combined mitochondrial dataset phylogenetic analysis identified several well-supported groupings within the Pandalidae species. Both ML and BI analyses recovered identical relationships regarding the molecular systematic biogeography, life history and ecological implications of selected species of pandalid shrimps.

Molecular systematics of *Plesionika* and ecological/life history implications

COI sequence divergence between species in clade I and clade II (Figure 3.2.3) (13.07%) and within (3.16% and 11.24%, respectively) indicates that *Plesionika* genus are genetically distinct respect to other in contrast with other genera of Pandalidae.

Recently, Komai *et al.* (2010) reported a pattern of molecular variation between two bathyal hydrothermal Palaemonidae shrimps, *Periclimenes*, *P. thermohydrophilus*

and *P. cannaphilus* (inter-specific variability 11.3-19.9%) reflecting their isolated geographical distribution without partial overlap, which suggest a parapatric speciation event. The study reported a pattern of distribution reflecting the isolated geographical distribution of the two *P. thermohydrophilus* and *P. cannaphilus* without even partial overlap, suggesting a parapatric speciation event. The two *Periclimenes* species appear restricted to the tube worm habitat at rather shallow hydrothermal vents (shallower than 500 m), and therefore larval dispersal and opportunity for settlement can be rather limited (Komai *et al.*, 2010). The observed phylogenetic patterns of *Plesionika* do not reflect isolated geographical distributions of the two clades with overlapping ranges. It was recently reported that members of the *Plesionika* genus have preference for divided bathymetric space according to the season, sex, and life history among species (Carbonell *et al.*, 2003; Puig *et al.*, 2001). *Plesionika heterocarpus* and *P. antigai* co-habit comparatively shallower depths (clade I) being found in different proportions together between 380 and 500 m in the Mediterranean Sea (Campisi *et al.*, 1998) whilst *P. acanthonotus* occupies the deepest bathymetric distribution (clade II) (Carbonell *et al.*, 2003; Puig *et al.*, 2001). Here, the shallow water species *P. antigai*, *P. heterocarpus* and *P. scopifera* are paraphyletic to the deeper water species (depth > 400m) found in clade II. Similar observations have been recently reported for COI data from the annelid genus *Osedax* study (Vrijenhoek *et al.*, 2009), and for 16S data derived from deep-sea amphipods *Eurythenes gryllus*, indicating monophyly among sites within the same depth zone at the scale of ocean basins (France and Kocher, 1996). Similarly, using COI and 16S data of penaeid *Metapenaeopsis* spp, it was found by Tong *et al.* (2009) that species with deeper water preferences clustered together in phylogenetic analyses.

Plesionika are benthic species of moderate locomotory ability. To date, there have been no descriptions of adults showing diel water column migrational behaviour

(Cartes, 1993). Because so many benthic marine animals move little in adulthood, movements by larvae have been expected to be responsible for most dispersal and gene flow between populations and can reveal historical and contemporary patterns of connectivity (Hellberg, 2009), including the identification of phylogeographic breaks. Such putative boundaries are usually assumed to be the result of long-term barriers to gene flow. Recently however, it was showed that phylogeographic breaks can be related to the decrease in average dispersal distance and population size in the absence of barriers to gene flow (Irwin, 2002). To date, only five *Plesionika* species (*P. heterocarpus*, *P. edwardsii*, *P. gigliolii*, *P. martia*, and *P. acanthonotus*) from 92 extant species, have yielded insights into the relationship between population structure of adults and juveniles in relation to life history and oceanography (Puig *et al.*, 2001). Related taxa are often located within the same broad geographical or habitat types. The depth of maximum abundance of *P. heterocarpus*, *P. edwardsii* and *P. martia* juveniles exhibited strong bathymetric preference (100; 400; and 600 m depth, respectively) in contrast to *P. acanthonotus* that showed an absence of any spatial trend for juveniles (700-1000 m). Puig *et al.*, (2001) suggested a trophic linkage between availability of food and the population structure of the *Plesionika* spp (larvae, juvenile and adult stage). The original paradigm of high connectivity in marine taxa of infinite population size is increasingly contested: there are numerous studies (reviewed *in* Hauser and Carvalho, 2008), that show more restricted dispersal and gene flow, and high levels of self-recruitment (Sponaugle *et al.*, 2002). Also species with life history and oceanographical similarities are cluster together. Data on *Plesioinka* indicate an increasing seasonality in reproductive periods from the shallowest species, *P. heterocarpus* and *P. antigai* (clade I) with ovigerous females present throughout the year (Campisi *et al.*, 1998), to the deepest species (clade II), *P. acanthonotus* with

ovigerous females present only in late spring and early summer months (Company and Sardà, 1998). To date, morphological descriptions of larvae are available for few species; only the first larvae stage (zoea) of *P. acanthonus* species (Bourdillon-Casanova, 1960); all larvae stages of *P. narval* (Lebour, 1940; Landeira *et al.*, 2009a), and the first seven larval stages (zoea) of *P. edwardsii* (Landeira *et al.*, 2009b). No information is available on the distribution and trophic behaviour of these stages. Variation in larval developmental modes, and the expected variation in gene flow between populations that arise from the dispersal potential of different developmental modes, has important consequences over longer ecological and evolutionary timescales (Hart and Marki, 2010). Accordingly, there is still research to be done on the description of larvae before we can assign precise identification to extant cohorts in space and time in the water column. These observations suggest that ecological and physical conditions are important isolating mechanisms that may lead to speciation in *Plesionika* shrimps, but the addition of comparative sequence data from other shallow-dwelling taxa may confirm or refute this hypothesis in a more robust manner. Otherwise, no clear evolutionary pattern of depth utilization is apparent among the major *Plesionika* clades. The unique phylogenetic distance observed among our data however, does provide an extraordinary opportunity to study population evolution and larval distribution of *Plesionika* spp and its phylogenetic relationships with other genera within the family Pandalidae.

Molecular systematics of *Stylopandalus* and *Chlorotocus*

The monotypic genus *Stylopandalus* appears to have close affinities to *Plesionika* spp (clade II, Figure 3.2.2). However, we recommend the analysis of an increased number of species, molecular markers and morphological data to confirm true

monophyly. Theoretically observed uncertainty in phylogenetic estimation can result from a combination of factors: the convergence of morphological characters from similar ecological forces; multiple substitutions in genetic data (Swofford *et al.*, 1996); failure to use a sufficient quantity of data (Van de Peer, 2003); use of data that are inappropriate for the level of divergence that is being analysed (de Queiroz *et al.*, 1995); rapid evolutionary radiations among taxa (Toon *et al.*, 2009) or the use of genes with rates of divergence that are inappropriate for the levels of divergence between the taxa (Mardulyn and Whitfield, 1999). It is important to highlight that the *Stylopandalus* species has been subjected to systematic controversy, belonging previously to the genus *Parapandalus* (d'Udekem d'Acoz, 1999). Our data suggest perhaps a conflict between morphological and molecular data. We believe that adding more morphological and molecular characters (multi-locus data) in future phylogenetic analyses will help to clarify the real systematic affinities of this genus within Pandalidae shrimps. At the same time, the single species of the genus *Chlorotocus* showed putative statistical support (Figure 3.2.2 and 3.2.3) to being closely affiliated to *Heterocarpus* spp (clade III).

Molecular Systematic of selected Pandalidae species and ecological implications

All deep sea Pandalidae species were well supported in the same Clade V (Figures 3.2.2 and 3.2.3). This clade was further split into two main groups that we further recognized as deep sea species with preference: of subtropical and temperate waters (A) and of temperate and frigid waters (B). The group B showed high support values for both ML and BI supporting the idea that the genus *Dichelopandalus* is closely related from *Pandalus* genus (Chan and Yu, 1991; Komai, 1999). *Dichelopandalus bonnieri* (Caullery, 1896) has the most southern limit distribution of

the temperate and frigid water species. Whereas *Pandalus* species have their southern limit in the English Channel, *D. bonnieri* seems to have its limit of southern distribution on the northwest Portuguese coast based on reported larval occurrence (Calado and Narciso, 2002; dos Santos *et al.*, 2008).

The pandalid shrimp genus *Pandalopsis* (Figure 3.2.2) are readily distinguished from other pandalid genera by their broad ischial laminar expansion of the first pereiopod, however, a comprehensive revisionary study of the genus has not been published (Komai, 1994).

Biogeography of *Heterocarpus*

All the *Heterocarpus* were recovered in a single (paraphyletic) clade (Clade III and IV in Fig. 3.2.2 and 3.2.3). The *Heterocarpus* clade was further split into Northern (including Clade III) and Southern Hemisphere (Clade IV), from Indian and Pacific Oceans (Figure 3.2.2 and 3.2.3). Members of Clade III revealed a very low COI nucleotide (0.18%) and amino acid (0%) divergence. Two species from the Clade III, e.g *H. hayashii* and *H. sibogae* (0.13% divergence) are very similar morphologically (Li and Komai, 2003) suggesting a potential misidentification or incomplete lineage sorting. Incomplete lineage sorting has been reported before for other decapod species of genus *Hyas* (Radulovici *et al.*, 2009). *Hyas araneus* and *H. coarctatus* are species which are morphologically distinct from larval stages to adulthood (Hultgren and Stachowicz, 2008), but exhibit low interspecific nucleotide divergence (<3% K2P distance). Supporting the recent evolution of the genus *Hyas*, low levels of divergence have been reported between *Hyas lyratus* and *H. coarctatus* (0.79% K2P distance in Matzen da Silva *et al.*, 2011). The distance between clade III (North Hemisphere) and clade IV (South Hemisphere) was relatively small (COI=2.6%, see Figure 3.2.2). It is

well known that Southeast Asia and the Western Pacific are on the border of two palaeocontinents (Simanjuntak, 2006) with complex tectonic plate composition (Hall, 1998). During the Pleistocene period sea-level fluctuations and the different regime patterns of ocean circulation in both hemispheres likely affected the distribution and diversity of many fractions of marine life (Hall, 1998). Both historical oceanographic and geological changes are therefore strong contributing factors towards genetic population structuring and biodiversity present today in this biogeographic region (Harpending *et al.*, 1998; Lessa *et al.*, 2003). Temporary losses of the coastal habitat or a shift in the hydrological regime can alter population sizes and distribution ranges, causing extinction and recolonisation in certain regions (Fauvelot *et al.*, 2003). Although it was not our focal aim to analyse the phylogeography of the genus *Heterocarpus* genus, such interesting phylogenetic signals revealed in both the COI and combined mitochondrial data set highlight future opportunities for phylogeographical studies in this group of shrimps, as has previously been developed for other groups of decapods (Cassone and Boulding, 2006; Chen *et al.*, 2009; de Bruyn *et al.*, 2005; de Bruyn *et al.*, 2004; He *et al.*, 2011).

3.2.5 Conclusions

The present study reveals the enhanced utility of COI over 16S as genetic markers to further resolve relationships among species of *Plesionika* from the Northeast Atlantic and Mediterranean Sea. Large genetic distances between morphologically similar taxa may either be due to elevated mutation rates or could reflect divergent

lineages. We suggest that the latter could be true for *Plesionika* and that morphological similarity between species is masking a long history of evolution maintained by stabilizing selection, which has lead to phenotypic stasis. Among the Pandalidae, *Plesionika* is one of the most speciose genera, and it would appear warranted to perform further systematic clarification within this group, based on future and robust phylogenetic frameworks. Given the high degree of morphological convergence among allopatric species of *Plesionika* in the Atlantic and Pacific Oceans and the Mediterranean Sea, we believe that molecular data (with more extensive sequencing of various genetic loci) would enhance our understanding of phylogenetic relationships, compared to using morphology alone. Our findings underscore the need for comprehensive studies of the molecular systematic nature of paraphyly within *Plesionika* and *Stylopandalus* throughout the Northeast Atlantic and the Mediterranean Sea, in an attempt to recognize a concordance in the evolutionary history of *Plesionika* with major ecological and geological events.

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References

- Akaike H (1973) Information theory and an extension of maximum likelihood principle. In: *Second International Symposium on Information Theory* (ed. Petrov B.N and Csaki, F.), pp. 267-281. Akademiai Kiado, Budapest
- Baeza JA, Schubart CD, Zillner P, Fuentes S, Bauer RT (2009) Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of protandric simultaneous hermaphroditism and social monogamy. *Biological Journal of the Linnean Society* **96**, 415-424.
- Bracken HD, De Grave S, Felder DL (2009) Phylogeny of the infraorder Caridea based on mitochondrial and nuclear genes (Crustacea: Decapoda). In: *Decapod Crustacean Phylogenetics. Crustacean Issues*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 281–305. Taylor and Francis Group, New York.
- Calado R, Narciso LFC (2002) *Camarões e Lagostas da Costa Continental Portuguesa* Câmara Municipal de Cascais, Cascais.
- Campisi S, Mura M, Cau A (1998) Biological aspects of *Plesionika antigai* (Zariquiey Alvarez, 1955) (Crustacea: Decapoda: Pandalidae) in central-western Mediterranean. *Journal of Natural History* **32**, 1453-1462.

- Carbonell A, Palmer M, Abelló P, Torres P, Alemany R, Gil de Sola L (2003) Mesoscale geographical patterns in the distribution of pandalid shrimps *Plesionika* spp. in the western Mediterranean. *Marine Ecology Progress Series* **247**, 151-158.
- Cartes JE (1993) Feeding habitats of pasiphaeid shrimps close to the bottom on the western Mediterranean slope. *Marine Biology* **112**, 459-468.
- Cartes JE (1998) Dynamics of the bathyal benthic boundary layer in the northwestern Mediterranean: depth and temporal variations in macrofaunal - megafaunal communities and their possible connections within deep-sea trophic webs. *Progress in Oceanography* **41**, 111-139.
- Cartes JE, Abelló P, Lloris D, Carbonell A, Torres P, Maynou F, Gil de Sola L (2002) Analysis of feeding guilds of fish and decapod crustaceans during the MEDITS-99 cruise along the Iberian Peninsula Mediterranean coasts. *Scientia Marina* **66**, 209–220.
- Cassone BJ, Boulding EG (2006) Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Marine Biology* **149**, 213-226.
- Chan T-Y, Yu H-P (1991) Two similar species: *Plesionika edwardsii* (Brandt, 1851) and *Plesionika crosnieri*, new species (Crustacea: Decapoda: Pandalidae). *Proceedings of the Biological Society of Washington* **104**, 545-555.
- Chan T-Y, Yu H-P (1998) A new deep-sea shrimp of the genus *Plesionika* Bate, 1888 (Crustacea: Decapoda: Pandalidae) from Taiwan. *International Symposium on Marina Biology in Taiwan*, 119-127.
- Chen RT, Tsai CF, Tzeng WN (2009) Freshwater prawns (*Macrobrachium* Bate, 1868) of Taiwan with special references to their biogeographical origins and dispersion routes. *Journal of Crustacean Biology* **29**, 232-244.
- Company JB, Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. *Deep-Sea Research I*, 1861-1880.
- Costa FO, deWaard JR, Boutilier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272-295.

- d'Udekem d'Acoz C (1999) *Inventaire et distribution des crustacés décapodes de l'Atlantique nord - oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25°N* Collection Patrimoines Naturels Paris.
- de Bruyn M, Nugroho E, Hossain MM, Wilson JC, Mather PB (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: the Isthmus of Kra Seaway. *Heredity* **94**, 370-378.
- de Bruyn M, Wilson JC, Mather PB (2004) Reconciling geography and genealogy: Phylogeography of giant freshwater prawns from the Lake Carpentaria region. *Molecular Ecology* **13**, 3515-3526.
- De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, *et al.* (2009) A classification of living and fossil genera of decapod Crustaceans. *Raffles Bulletin of Zoology* **1**, 1-109.
- de Queiroz A, Donoghue MJ, Junhyong K (1995) Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics* **26**.
- dos Santos A, Santos AMP, Conway DVP, Bartilotti C, Lourenço P, Queiroga H (2008) Diel vertical migration of decapod larvae in the Portuguese coastal upwelling ecosystem: implications for offshore transport. *Marine Ecology Progress Series* **359**, 171-183.
- Drummond AJ, Rambaut A (2007) A beast: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 1-8.
- Fanelli E, Cartes JE (2004) Feeding habitats of pandalid shrimps in the Alboran Sea (SW Mediterranean): influence of biological and environmental factors. *Marine Ecology Progress Series* **280**, 227 - 238.
- Fauvelot C, Bernardi G, Planes S (2003) Reductions in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from Holocene sea-level change. *Evolution* **57**, 1571–1583.
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal Molecular Evolution* **17**, 368-376.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294-299.

- France SC, Kocher TD (1996) Geographic and bathymetric patterns of mitochondrial 16S rRNA sequence divergence among deep-sea amphipods, *Eurythenes gryllus*. *Marine Biology* **126**, 633-643.
- Garcia-Machado E, Dennebouy EN, Oliva-Suarez M, Mounolou JC, Monnerot M (1993) Mitochondrial 16S-rRNA gene of two species of shrimps: sequence variability and secondary structure. *Crustaceana* **65**, 279-286.
- Groeneveld JC, Gopal K, George RW, Matthee CA (2007) Molecular phylogeny of the spiny lobster genus *Palinurus* (Decapoda: Palinuridae) with hypotheses on speciation in the NE Atlantic/Mediterranean and SW Indian Ocean. *Molecular Phylogenetics and Evolution* **45**, 102–110.
- Hajibabaei M, DeWaard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, *et al.* (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1959-1967.
- Hall R (1998) Faunal and floral migrations and evolution in SE Asia-Australasia. In: *The plate tectonics of Cenozoic SE Asia and the distribution of land and sea* (ed. Metcalfe I Smith JMB, Morwood M, and Davidson ID), pp. 35-56. Swets and Zeitlinger Publisher, Lisse.
- Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherr ST (1998) Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences* **95**, 1961–1967.
- Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal Molecular Evolution* **22**, 160-174.
- He L, Zhang A, Zhu A, Weese D, Qiao Z (2011) Phylogeography of the mud crab (*Scylla serrata*) in the Indo-West Pacific reappraised from mitochondrial molecular and oceanographic clues: transoceanic dispersal and coastal sequential colonization. *Marine Ecology* **32**, 52-64.
- Hellberg ME (2009) Gene flow and isolation among populations of marine animals. *Annual Review of Ecology and Systematics* **40**, 291-310.
- Huelsenbeck JP, Ronquist F (2001) Mr Bayes:Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.

- Hultgren KM, Stachowicz JJ (2008) Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. *Molecular Phylogenetics and Evolution* **48**, 986-996.
- Irwin DE (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution* **56**, 2383-2394.
- Komai T (1994) Deep-sea shrimps of the genus *Pandalopsis* (Decapoda: Caridea: Pandalidae) from the Pacific coast of eastern Hokkaido, Japan, with the description of two new species. *Journal of Crustacean Biology* **14**, 538-559.
- Komai T (1999) A revision of the genus *Pandalus* (Crustacea: Decapoda: Caridea: Pandalidae). *Journal of Natural History* **33**, 1265-1372.
- Komai T, Nemoto S, Tsuchida S (2010) *Periclimenes cannaphilus*, new species, the second palaemonid shrimp (Crustacea: Decapoda: Caridea) associated with siboglinid tube worm inhabiting hydrothermal vents. *Journal of the Marine Biological Association of the United Kingdom* **90**, 799-808.
- Kumar S (1996) Patterns of nucleotide substitution in mitochondrial protein coding genes of vertebrates. *Genetics* **143**, 537-548.
- Lessa EP, Cook JA, Patton JL (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the late Quaternary. *Proceedings of the National Academy of Sciences* **100**, 10331-10334.
- Li X, Komai T (2003) Pandaloid shrimps from the Northern South China Sea, with description of a new species of *Plesionika* (Crustacea: Decapoda: Caridea). *The Raffles Bulletin of Zoology* **51**, 257-275.
- Macdonald KS, Yampolsky L, Duffy JE (2005) Molecular and morphological evolution of the amphipod radiation of Lake Baikal. *Molecular Phylogenetics and Evolution* **35**, 323-343.
- Mardulyn P, Whitfield JB (1999) Phylogenetic signal in COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): Evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics and Evolution* **12**, 282-294.
- Mathews LM, Anker A (2009) Molecular phylogeny reveals extensive ancient and ongoing radiations in a snapping shrimp species complex (Crustacea, Alpheidae, *Alpheus armillatus*). *Molecular Phylogenetics and Evolution* **50**, 268-281.

- Mathews LM, Schubart CD, Neigel JE, Felder DL (2002) Genetic, ecological, and behavioural divergence between two sibling snapping shrimp species (Crustacea: Decapoda:*Alpheus*). *Molecular Ecology* **11**, 1427–1437.
- Matzen da Silva J, Creer S, Dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR (2011) Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Malacostraca). *PLoS ONE* **6**, e19449.
- Mulley JC, Latter BDH (1980) Genetic variation and evolutionary relationships within a group of thirteen species of penaeid prawns. *Evolution* **34**, 904-916.
- Ohtomi J (1997) Reproductive biology and growth of the deep-water pandalid shrimp *Plesionika semilaevis* (Decapoda: Caridea). *Journal of Crustacean Biology* **17**, 81-89.
- Page TJ, Short JW, Humphrey CL, Hillyer MJ, Hughes JM (2008) Molecular systematics of the Kakaducarididae (Crustacea: Decapoda: Caridea). *Molecular Phylogenetics and Evolution* **46**, 1003-1014.
- Posada D (2003) Using Modeltest and PAUP to select a model of nucleotide substitution. In: *Current Protocols in Bioinformatics* (ed. Baxevanis A.D. Davison D. B., Page R. D. M., Petsko G. A., Stein L. D., and Stormo G. D.), pp. 6.5.1-6.5.14. Wiley J. and Sons, Inc., New York.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.
- Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**, 793-808.
- Puig P, Company JB, Sardà F, Palanques A (2001) Responses of deep-water shrimp populations to intermediate nepheloid layer detachments on the northwestern Mediterranean continental margin. *Deep-Sea Research* **48**, 2195-2207.
- Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. *Molecular Ecology Resources* **9**, 181-187.
- Rocha-Olivares A, Fleeger JW, Foltz DW (2001) Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic harpacticoid copepod. *Molecular Phylogenetics and Evolution* **18**, 1088-1102.

- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Schubart CD, Cuesta JA, Felder DL (2005) Phylogeography of *Pachygrapsus transversus* (Gibbes, 1850): The effect of the American continent and the Atlantic Ocean as gene flow barriers and recognition of *Pachygrpsus socius* Stimpson 1871 as a valid species. *Nauplius* **12**, 99 - 113.
- Schubart CD, Neigel JE, Felder DL (2000) Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* **12**, 817–830.
- Shank TM, Black MB, Halanych KM, Lutz RA, Vrijenhoek RC (1999) Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea: Bresiliidae): Evidence from mitochondrial cytochrome oxidase subunit I. *Molecular Phylogenetics and Evolution* **13**, 244-253.
- Shih H-T, Ng PKL, Schubart CD, Chang H-W (2007) Phylogeny and phylogeography of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) in southwestern Taiwan based on two mitochondrial genes. *Zoological Science* **24**, 57-66.
- Simanjuntak T (2006) Indonesia-Southeast Asia: Climates, settlements, and cultures in Late Pleistocene. *Comptes Rendus Palevol* **5**, 371-379.
- Stewart BA, Gouws G, Daniels SR, Matthee CA (2003) Delimitation of morphologically similar sponge crab species of the genus *Pseudodromia* (Crustacea, decapoda, Dromiidae) from South Africa. *Zoologica Scripta* **33**, 45-55.
- Swofford DL (1993) Phylogenetic analysis using parsimony. Smithsonian Institution, Washington.
- Swofford DL, Olsen G, Waddell P, Hillis D (1996) Phylogenetic inference. In: *Molecular Systematics* (eds. Hillis D., Moritz C., Mable B.), pp. 407-514. Sinauer Associates, Sunderland.
- Tam YK, Chu KH (1993) Electrophoretic study on the phylogenetic relationships of some species of *Penaeus* and *Metapenaeus* (Decapoda: Penaeidae) from the South China Sea. *Journal of Crustacean Biology* **13**, 697-705.

- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.
- Tong JG, Chan T-Y, Chu KH (2000) A preliminary phylogenetic analysis of *Metapenaeopsis* (Decapoda:Penaeidae) based on mitochondrial DNA sequences of selected species from the Indico-West Pacific. *Journal of Crustacean Biology* **20**, 541-549.
- Toon A, Finley M, Staples J, Crandall KA (2009) Decapod phylogenetics and molecular evolution. In: *Decapod Crustacean Phylogenetics* (ed. Martin A.P., Crandall, K. A., and Felder, D. L.), pp. 15-30. Taylor and Francis Group, New York.
- Vafidis D, Chrissi-Yianna P, Carbonell A, Company JB (2005) A review of the biology and Fisheries of the genus *Plesionika* Bate, 1888 (Decapoda, Caridea, Pandalidae) in European waters. *Crustaceana* **78**, 335-352.
- Van de Peer Y (2003) Phylogeny inference based on distance methods. In: *The Phylogenetic Handbook. A Practical Approach to DNA and Protein Phylogeny* (eds. Salemi M., Vandamme A-M), pp. 101-119. Cambridge University Press, New York.
- von Rintelen K, von Rintelen T, Glaubrecht M (2007) Molecular phylogeny and diversification of freshwater shrimps (Decapoda, Atyidae, Caridina) from ancient Lake Poso (Sulawesi, Indonesia) - The importance of being colourful. *Molecular Phylogenetics and Evolution* **45**, 1033-1041.
- Vrijenhoek RC, Johnson SB, Rouse GW (2009) A remarkable diversity of bone-eating worms (*Osedax*; Siboglinidae). *BMC Biology* **7**, 1-13.
- Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. *International Review of Cytology* **141**, 173-216.
- Xia X, Xie Z (2001) DAMBE: Software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92**, 371-373.
- Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26**, 1-7.

- Yang Z, Nielsen R, Goldman N, Krabbe Pedersen A-M (2000) Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics* **155**, 431-449.
- Yang ZH (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution* **39**, 306-314.
- Yang ZH, Yoder AD (1999) Estimation of the transition/transversion rate bias and species sampling. *Journal of Molecular Evolution* **48**, 274-283.
- Zaslavskaya NI, Kornienko ES, Korn OM (2007) Genetic differences between two spider crabs *Pisoides bidentatus* (A. Milne-Edwards, 1873) and *Pugettia quadridentata* (de Haan, 1839) (Decapoda: Brachyura: Majoidea) from the Sea of Japan. *Biochemical Systematics and Ecology* **35**, 750-756.
- Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**, 203-214.



**CHAPTER 3.3 Multigene molecular systematics confirm species status
of morphologically convergent *Pagurus* hermit crabs**

Abstract

In spite of contemporary morphological taxonomy appraisals, apparent high morphological similarity raises uncertainty about the species status of certain *Pagurus* hermit crabs. This is exemplified between two European species, *Pagurus excavatus* (Herbst, 1791) and *Pagurus alatus* (Fabricius 1775), whose species status is still difficult to resolve using morphological criteria alone. To address such ambiguities, we used combinations of Maximum Likelihood (ML) and Bayesian Inference (BI) methods to delineate species boundaries of *P. alatus* and *P. excavatus* and formulate an intermediate *Pagurus* phylogenetic hypothesis, based upon single and concatenated mitochondrial (cytochrome oxidase I and 16S ribosomal RNA) and nuclear (28S ribosomal RNA) gene partitions. The molecular data supported the species status of *P. excavatus* and *P. alatus* and also clearly resolved two divergent clades within hermit crabs from the Northeast Atlantic Ocean and the Mediterranean Sea. Despite the abundance and prominent ecological role of hermit crabs, *Pagurus*, in North East Atlantic Ocean and Mediterranean Sea ecosystems, many important aspects of their taxonomy, biology, systematics and evolution remain poorly explored. The topologies presented here should be regarded as hypotheses that can be incorporated into the robust and integrated understanding of the systematic relationships within and between species of the genus *Pagurus* inhabiting the Northeast Atlantic Ocean and the Mediterranean Sea.

Keywords: *Pagurus*, systematics, COI, 16S, 28S, Northeast Atlantic Ocean and Mediterranean Sea.

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3.3.1 Introduction

Although hermit crabs are one of the most morphologically and ecologically diverse group of decapod crustaceans, their evolutionary history at many taxonomic levels is far from being resolved (Mantelatto *et al.*, 2009; McLaughlin, 2003; McLaughlin and Lemaitre, 1997; McLaughlin *et al.*, 2007). More than 1000 species, 127 genera and 6 families are currently reported for the superfamily Paguroidea (De Grave *et al.*, 2009), that inhabit diverse biotopes from intertidal to deep seas (d'Udekem d'Acoz, 1999). However, the true taxonomic and ecological heterogeneity associated with hermit crabs is likely to be underestimated because many species and life-histories appear to be undescribed (Mantelatto *et al.*, 2009). One of the most diverse groups belong to the family Paguridae, with species distributed widely through all oceans (De Grave *et al.*, 2009).

The genus *Pagurus* Fabricius, 1775 is considered to be ancient, with fossils being assigned to the genus as early as the lower Cretaceous (Cunningham *et al.*, 1992) and from the Cenozoic (Jagt *et al.*, 2003). *Pagurus* is the least complex of all paguroids, sharing a reduced or virtually nonexistent rostrum, no sexually modified appendages other than the regular female egg-bearing pleopods, and having no penis (or sexual tubes) (McLaughlin and Lemaitre, 1997). Despite its comparatively morphological conservatism, *Pagurus* exhibits a high degree of species proliferation. Recently, 172 species (which five species are fossil records) are recognised (McLaughlin *et al.*, 2010), that possess specialized adaptations for housing stability, relying upon gastropod shells for protection. Such commensalism has constrained morphological evolution over 150 million years (Cunningham *et al.*, 1992; Jagt *et al.*, 2003) by requiring a decalcified

asymmetrical abdomen capable of looping into gastropod shells. Despite the abundance of hermit crabs in the North East Atlantic and Mediterranean Sea (d'Udekem d'Acoz, 1999) many important aspects of their taxonomy (Ingle, 1985), biology (Appel and Elwood, 2009; Elwood and Glass, 1981; Elwood and Stewart, 1985; Jackson and Elwood, 1989a, 1989b; Lancaster, 1988; Neil and Elwood, 1985; Rivera, 2006), ecology (Ellis *et al.*, 2000; Kellogg, 1976; Lancaster, 1988; Taylor, 1981, 1982), systematics and evolution (Jagt *et al.*, 2003; McLaughlin and Lemaitre, 1997; Young *et al.*, 2002) are poorly documented.

Systematic problems remain among the Paguridae, such as the polyphyletic genus *Pagurus* Fabricius, 1775 (Mantelatto *et al.*, 2009). To date, most systematic studies on hermit crabs have been based on morphology with relatively few studies utilising molecular tools to resolve species status (Mantelatto *et al.*, 2009; Mantelatto *et al.*, 2006; Scelzo *et al.*, 2010; Zaslavskaya *et al.*, 2009) or to determine phylogenetic relationships among major taxa (Mantelatto *et al.*, 2009; McLaughlin *et al.*, 2007; Young *et al.*, 2002). Within the region of the Northeast Atlantic Ocean and Mediterranean Sea *Pagurus* is represented by 23 species (d'Udekem d'Acoz, 1999) and high morphological similarity among some species has resulted in the recognition of two groups characteristic of the North Atlantic and Mediterranean Sea (Ingle, 1985). In some cases, morphology suggests very close relationships between congeners, raising uncertainty about their independent species status. Such a situation exists between the "alatus" group, *Pagurus excavatus* (Herbst, 1791) and *Pagurus alatus* (Fabricius, 1775) that is still difficult to resolve using morphology alone (Table 3.3.1) (d'Udekem d'Acoz, 1999; Ingle, 1985). In spite of the prevailing taxonomic challenges, Ingle (Ingle, 1985) has recognized both species, based mainly from the differences observed in the dorsal aspects of shield, antennular peduncle, the shape of the right cheliped, the

length of larger pereiopod and male pleopods. Furthermore, due to a lack of life history studies there has always been considerable confusion regarding ongoing synonymies that are assigned among *P. alatus*, *P. excavatus* and *P. variabilis* (A. Milne-Edwards & Bouvier, 1892) (Ingle, 1985; Spivak and Schubart, 2003). As currently recognised, *P. excavatus* is distributed southwards from the southern part of the Bay of Biscay and into the Mediterranean Sea. *Pagurus alatus* extends northwards into Iceland waters, but remains sympatric with *P. excavatus* in many southern regions of the area (Ingle, 1985). Here, we use molecular phylogenetic analyses of mitochondrial cytochrome oxidase I (COI), mitochondrial 16S ribosomal RNA (16S), and nuclear 28S ribosomal RNA (28S) DNA partitions to reconstruct the systematics of selected *Pagurus* species, in order to make inferences on taxonomic status of *P. alatus* and *P. excavatus*.

3.3.2 Material and Methods

Sampling

Twenty nine hermit crabs of six species, *Pagurus alatus* (Fabricius, 1775), *P. bernhardus* (Linnaeus, 1758), *P. cuanensis* (Bell, 1845), *P. excavatus* (Herbest, 1791), *P. prideaux* (Leach, 1815), and *P. pubescens* (Krøyer, 1838), were collected from the Portugal (Costa Algarvia, Costa de Prata, and Azores), United Kingdom (North Wales), Norway (Bear Island Slide and Svalbard) and Italy (Sicily) between 2006 and 2008. Specimens were harvested as by-catch from rough ground bottom trawls from INRB-IPIMAR, Sicilian fisheries survey and collected by physical searches. Species were identified (Ingle, 1985) prior to the excision and preservation of muscle tissue in 95%

ethanol (stored at -20°C) and whole body storage at -20°C. In order to accurately assign specimens to currently accepted (female) species of *P. alatus* and *P. excavatus* we used morphological criteria based upon four main groups of characters: the dorsal aspects of shield (Table 3.3.1), antennular peduncle, the shape of the right cheliped, length of larger pereiopod and male pleopods (Ingle 1985).

DNA isolation, amplification and sequencing

Total genomic DNA was extracted from approximately 1 mm³ of muscle tissue or whole legs for small specimens using the Chelex dry release method (Hajibabaei *et al.*, 2005). The COI gene was amplified with alternative sets of primers depending on PCR reaction success following by the protocol develop by Costa *et al.* 2007. All PCRs were performed in a 25 µl final volume containing 1 X PCR buffer, 3-4 mM MgCl₂, 0.1- 0.3 mM dNTP, 1U *Taq* polymerase, 5 – 10 pmol of each primer, and 2 - 10 ng of DNA template (Table 3.3.2). The thermal cycling conditions are listed in Table 3.3.2 for each set of primers. Following amplification, PCR reactions were cleaned by incubation with 10 U Exonuclease I (New England Biolabs ®) and 1 U Shrimp Alkaline Phosphate (Promega ®) at 37°C for 1 hour, followed by heating at 80 °C for 5 min. Samples were sequenced by Macrogen Inc. (South Korea) using an Applied Biosystems® 3730 sequencer.

All empirically derived sequences were manually checked for ambiguities in CodonCode Aligner version 1.3.0, aligned using the ClustalX plugin embedded within Mega 4 (Tamura *et al.*, 2007), prior to manual quality control and megablast annotation.

Table 3.3.1: Selected morphological characters by Ingle (1985) to distinct *Pagurus alatus* (Fabricius 1775) vs *Pagurus excavatus* (Herbst 1791). Words in bold are highlighting the solely differences between species and “SL” is the abbreviation of shield length.

<i>Pagurus alatus</i> (Fabricius 1775)	<i>Pagurus excavatus</i> (Herbst 1791)
Segment 1 of antennular peduncle, outer distal margin with one, two or sometimes three spines.	Segment 1 of antennular peduncle, outer distal margin without or with small obtuse spines at the most.
Outer dorso-lateral process of antenna (of large circa 80 mm SL, specimens) reaching just beyond distal margin of segment 4 and acicle reaching well beyond distal extremity of cornea; breadth of cornea slightly exceeding 1\2 length of eye.	Outer dorso-lateral process of antenna (of large specimens circa 80 mm SL) not reaching to distal margin of segment 4 and acicle reaching only to extremity of cornea; breadth of cornea slightly less than 1\2 length of eye.
Outer (particularly upper) surface of right cheliped palm not strikingly concave.	Outer upper (and sometimes lower) surface of right cheliped palm strikingly concave.
Dorsal aspect of shield and associated appendages	Larger pereiopod 3, dactyl (of large specimens, circa 10 mm SL) as long as combined lengths of propodus + carpus and noticeably curved; males with 3 unpaired pleopods.

Morphological selected characters by Ingle (1985)

Table 3.3.2: Primers sequences and thermocycling conditions for the amplification reactions.

Locus and primers	Sequences	Reference	Cycling conditions
COI			
Forward			
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer <i>et al.</i> , 1994	Denaturation 94°C/60 s;
CrustF1	5'-TTTTCTACAAATCATAAGACATTGG-3'	Costa <i>et al.</i> , 2007	35-40 cycles at 94°C/30 s,
Reverse			48-56°C/90 s, 72°C/60 s;
HCO2198	5'-TAACTTCAGGGTGACCAAAAAATCA-3'	Folmer <i>et al.</i> , 1994	Final extension at 72°C/ 5 min.
			Denaturation 94°C/60 s;
			5 cycles at 94°C/30 s,
			45°C/90 s, 72°C/60 s;
			35-40 cycles at 94°C/30 s,
			50-56°C/90 s, 72°C/60 s;
			Final extension at 72°C/ 5 min.
16S			
Forward			
16SL2	5'-TGCCTGTTATCAAAAACAT-3'	Mathews <i>et al.</i> , 2002	Denaturation 94°C/3 min.;
16Sar	5'-CGCCTGTTATCAAAAACAT-3'	Palumbi <i>et al.</i> , 1991	35-40 cycles at 94°C/30 s,
Reverse			50-55°C/60 s, 72°C/2 min.;
16S-1472	5'-AGATAGAACCAACCTGG-3'	Schubart <i>et al.</i> , 2000	Final extension at 72°C/ 5 min.
16SBr-Dr	5'-CCGGTTGAACTCAGATCATG-3'	Palumbi <i>et al.</i> , 1991	
28S			
Forward			
28S-rD1.2a	5'- CCCSSGTAAATTAAAGCATATTA-3'	Whiting <i>et al.</i> , 2002	Denaturation 94°C/5 min.;
Reverse			35 cycles at 95°C/30 s,
28SRd3.2b1	5'-TYAACGGTTCACGTRCTMTTGA-3'	This study	50°C/45 s, 72°C/60 s;
			1 cycle at 95°C/30 s,
			45°C/45 s and final extension at 72°C/ 5 min.

Phylogenetic relationships

The most popular barcode marker COI is generally used to study close to moderately deep interspecific taxon relationships of crustaceans (Groeneveld *et al.*, 2007; Mathews and Anker, 2009; Schubart *et al.*, 2005; Shih *et al.*, 2007; von Rintelen *et al.*, 2007). To provide a comprehensive sister-species coverage and assessment of interspecific variation, *Pagurus* COI sequences from GenBank were merged with our data (Table 3.3.3). In that propose we use using Kimura 2-parameters (K2P) genetic

distances within and among species implemented in Mega 4.1, and compared to literature data (Table 3.3.4 and 3.3.5). Amino acid translations of the target genes were examined to ensure that no gaps or stop codons were present in the alignment.

To identify phylogenetic groups among the resulting eleven putative *Pagurus* species, the 46 COI sequences comprising 513 bp was analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic reconstruction methods. The crab *Macropodia longipes* (Milne-Edwards & Bouvier, 1899) (Brachyura:Inachidae) and the most phylogenetically closest related species, *Dardanus arrosor* (Herbst, 1796) (Anomura: Diogenidae) were used as outgroups.

Since Bayesian posterior probability support values (bpp) can often be inflated for certain clades, relative to ML bootstrap values (Huelsenbeck and Rannala, 2004), we constructed trees using both Bayesian approaches and ML. For these searches, we set the substitution model parameters calculated by jModeltest in RAxML 7.0.4 (Stamatakis, 2008) and in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001), respectively. Ten independent ML analyses were conducted using GTR+I+G with invariant sites (I) and gamma distributed rates (G) (Yang, 1994) (see below) as the model (using the GTR+CAT setting) with 4 categories of rate variation (500 bootstrap replicates were undertaken for estimation of node support) for each partition on combined data. In order to find the ML tree, 10 independent runs of RAxML 7.0.4 were conducted. ModelTest (Posada, 2008) identified the HKY+I+G model (Hasegawa *et al.*, 1985) as best indicated by Akaike Information Criterion (AIC) (Akaike, 1973), however, since this model is not implemented in the current version of RAxML, the GTR + I + G model was selected as the closest matching alternative. In MrBayes, two independent Markov chain Monte Carlo (MCMC) analyses were run using four chains for 5×10^6 generations with the initial 1 million generations (20%) cycles discarded as

burn-in. To check that stationarity had been reached, we monitored the fluctuating value of the likelihood graphically with Tracer v1.4 (Drummond and Rambaut, 2007). Once the parameters reach stationarity, a 50% majority rule consensus tree was obtained from the remaining saved trees. The consensus tree was selected from the posterior distribution and visualized using FigTree V.1.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Since substitution rates among the four nucleotides and among different nucleotide sites in mitochondrial protein-coding genes as been reported (Kumar, 1996; Yang *et al.*, 2000; Yang and Yoder, 1999), codon models of substitution TrNef + G (Tamura and Nei, 1993), F81(Felsenstein, 1981) and HKY+G (Hasegawa *et al.*, 1985) were implemented in our BI analyses for first, second and third codon positions respectively.

Molecular systematic analyses

Use of nuclear genes in addition to mitochondrial genes adds to the number of independent markers in a dataset, thus increasing the chances to understand the systematic relationships between and within *P. alatus* and *P. excavatus* (Table 3.3.6). Here we analysed partial sequences of nuclear 28S (385 bp), mitochondrial 16S (462 bp), and the barcode region of COI (540 bp). The three gene regions were partitioned separately according to the previously determined model parameters (Table 3.3.7) in subsequent BI analyses. Gaps in 16S and 28S sequences were treated as a fifth character-state. BI analysis was conducted for each gene data set and the concatenated partition (CON) with the three gene regions partitioned separately according to the previously determined model parameters (Table 3.3.7) as described before. To evaluate the range of intrageneric sequence identity found among *Pagurus* species, we compared pairwise distances of COI and 16S (Table 3.3.8).

Section 3. Molecular evolution and shallow phylogenies

Table 3.3.3: *Pagurus* and outgroup specimens used for the COI phylogenetic reconstructions

Species	Collection site	GenBank accession No.
<i>Pagurus acadianus</i> (Benedict, 1901)	Maine (United States)	AF483156
<i>Pagurus acadianus</i> (Benedict, 1901)	Prince Edward Island (Canada)	FJ581812
<i>Pagurus acadianus</i> (Benedict, 1901)	Quebec (Canada)	FJ581815
<i>Pagurus acadianus</i> (Benedict, 1901)	Quebec (Canada)	FJ581814
<i>Pagurus acadianus</i> (Benedict, 1901)	Quebec (Canada)	FJ581813
<i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	JN107574
<i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	JN107575
<i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	JN107576
<i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	JN107577
<i>Pagurus arcuatus</i> (Squires, 1964)	Quebec (Canada)	FJ581818
<i>Pagurus arcuatus</i> (Squires, 1964)	Quebec (Canada)	FJ581817
<i>Pagurus arcuatus</i> (Squires, 1964)	Quebec (Canada)	FJ581816
<i>Pagurus armatus</i> (Dana, 1851)	Nova Scotia (Canada)	AF483159
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	Wales (United Kingdom)	JN107580
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	Wales (United Kingdom)	JN107581
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	Wales (United Kingdom)	JN107582
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	Costa de Prata (Portugal)	JN107583
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	England (United Kingdom)	JN107578
<i>Pagurus bernhardus</i> (Linnaeus, 1758)	England (United Kingdom)	JN107579
<i>Pagurus cuanensis</i> (Bell, 1845)	Azores (Portugal)	JN107584
<i>Pagurus cuanensis</i> (Bell, 1845)	Azores (Portugal)	JN107585
<i>Pagurus excavatus</i> (Herbst, 1791)	Costa de Prata (Portugal)	JN107586
<i>Pagurus excavatus</i> (Herbst, 1791)	Costa de Prata (Portugal)	JN107587
<i>Pagurus excavatus</i> (Herbst, 1791)	Costa de Prata (Portugal)	JN107590
<i>Pagurus excavatus</i> (Herbst, 1791)	Costa de Prata (Portugal)	JN107591
<i>Pagurus excavatus</i> (Herbst, 1791)	Sicily (Italy)	JN107588
<i>Pagurus excavatus</i> (Herbst, 1791)	Sicily (Italy)	JN107589
<i>Pagurus longicarpus</i> (Say, 1817)	New Brunswick (Canada)	FJ581825
<i>Pagurus longicarpus</i> (Say, 1817)	New Brunswick (Canada)	FJ581824
<i>Pagurus longicarpus</i> (Say, 1817)	New Brunswick (Canada)	FJ581823
<i>Pagurus longicarpus</i> (Say, 1817)	New Brunswick (Canada)	FJ581822
<i>Pagurus longicarpus</i> (Say, 1817)	New Brunswick (Canada)	FJ581826
<i>Pagurus longicarpus</i> (Say, 1817)	Nova Scotia (Canada)	FJ581820
<i>Pagurus ochotensis</i> (Brandt, 1851)	Alaska (United States)	AF483158
<i>Pagurus prideauxi</i> (Leach, 1815)	England (United Kingdom)	JN107597
<i>Pagurus prideauxi</i> (Leach, 1815)	Costa de Prata (Portugal)	JN107595
<i>Pagurus prideauxi</i> (Leach, 1815)	Costa de Prata (Portugal)	JN107596
<i>Pagurus prideauxi</i> (Leach, 1815)	Sicily (Italy)	JN107592
<i>Pagurus prideauxi</i> (Leach, 1815)	Sicily (Italy)	JN107593
<i>Pagurus prideauxi</i> (Leach, 1815)	Bear Island Slide (Norway)	JN107594
<i>Pagurus pubescens</i> (Krøyer, 1838)	Quebec (Canada)	FJ581829
<i>Pagurus pubescens</i> (Krøyer, 1838)	Bear Island Slide (Norway)	JN107598
<i>Pagurus pubescens</i> (Krøyer, 1838)	Bear Island Slide (Norway)	JN107599
<i>Pagurus pubescens</i> (Krøyer, 1838)	Svalbard (Norway)	JN107600
<i>Pagurus pubescens</i> (Krøyer, 1838)	Svalbard (Norway)	JN107601
<i>Pagurus pubescens</i> (Krøyer, 1838)	Svalbard (Norway)	JN107602
<i>Dardanus arrosor</i> (Herbst, 1796)	Sicily (Italy)	JN107572
<i>Macropodia longipes</i> (Milne-Edwards & Bouvier, 1899)	Costa de Prata (Portugal)	JN107573

3.3.3 Results

Pagurus diversity

The variation in COI diversity was examined among 11 species (Table 3.3.4 and 3.2.5). For each species, one to six representative individuals were analysed, and where possible, from different geographical areas, yielding a total of 46 sequences. No insertions, deletions, stop codons or sequences indicative of pseudogenes (Schubart, 2009; Song *et al.*, 2008) were observed, and BLAST searches confirmed that the sequences corresponded to decapod mtDNA COI. There was also evidence for base composition bias in the sequences, notably a pronounced underrepresentation of guanine at the third codon positions (35.3% T; 18.1% C; 28.5% A; 18.1%G) a phenomenon commonly observed in metazoan mitochondrial (Wolstenholme, 1992). The COI alignment contained a total of 513 bp with 177 variable characters, of which 170 were parsimony informative (33.13%). The high observed percentage of parsimony-informative character suggests that COI is sufficiently diverse for intrageneric phylogeny and clearly resolved all eleven *Pagurus* species examined in the present study (Figure 3.3.2) (Cruickshank, 2002; Schwentner *et al.*, 2009). The 11 species comprise a monophyletic clade (Figure 3.3.1) with an average between genetic species distance of 17.01% (Table 3.3.4). Among the *Pagurus* species, *P. acadianus* is most closely related to *P. bernhardus* (6.80%) and in contrast, *P. arcuatus* exhibits the least genetic divergence to *P. bernhardus* (6.80%) and in contrast, *P. arcuatus* and *P. excavatus* exhibited the highest genetic distances (23.10%) (Table 3.3.5). *P. pubescens* exhibited the highest average distance values (Table 3.3.5) with a range of 11.50-21.70% (see also Figure 3.3.1).

Table 3.3.4: Pairwise COI nucleotide divergences for *Pagurus* spp using K2P distances (%).

Pairwise divergences comparisons	n	Taxa	Min Dist(%)	Mean Dist(%)	Max Dist(%)	SE Dist(%)
<i>Pagurus</i> (11 species)						
Within a species*	44	9	0	0,6332	2,002	0,055
Between species	46	1	6,432	17,019	23,086	0,106

* Number of specimens with more than 1 sequences analysed.

Table 3.3.5: Pairwise COI nucleotide divergences for each selected *Pagurus* spp using K2P distances (%).

Species	Distances (%)	Between species									
		1	2	3	4	5	6	7	8	9	10
1 <i>Pagurus acadianus</i> (Benedict, 1910)	0,2										
2 <i>Pagurus alatus</i> (Fabricius, 1775)	0,5	17,70									
3 <i>Pagurus arcuatus</i> (Squires, 1964)	1,2	13,80	17,50								
4 <i>Pagurus bernhardus</i> (Linnaeus, 1758)	0,7	6,80	16,70	14,00							
5 <i>Pagurus cuanensis</i> (Bell, 1845)	0	17,80	13,40	19,50	18,00						
6 <i>Pagurus excavatus</i> (Herbst, 1791)	0,1	19,50	14,80	23,10	20,00	13,00					
7 <i>Pagurus prideauxi</i> (Leach, 1815)	0,4	18,20	13,70	20,50	17,30	15,00	15,60				
8 <i>Pagurus pubescens</i> (Krøyer, 1838)	1,6	11,50	17,70	13,90	12,70	18,90	21,70	21,20			
9 <i>Pagurus longicarpus</i> (Say, 1817)	0,6	19,30	16,20	19,80	18,80	20,50	21,30	18,70	19,80		
10 <i>Pagurus armatus</i> (Dana, 1851)	-	12,20	21,40	19,80	12,90	21,10	22,80	21,30	17,80	21,70	
11 <i>Pagurus orchofensis</i> (Brandt, 1851)	-	13,50	20,00	16,90	14,90	20,90	21,20	21,50	19,30	21,10	9,20

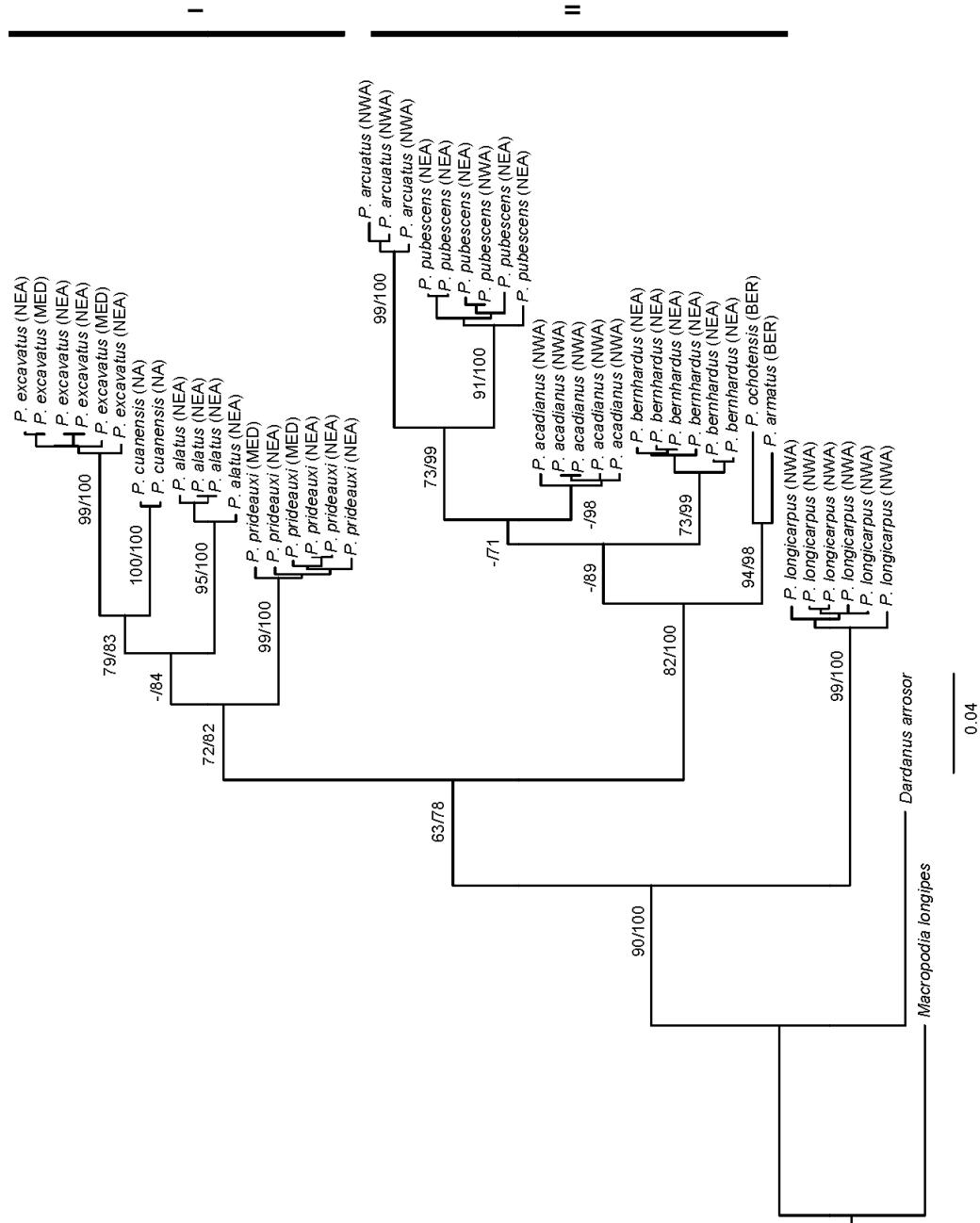


Figure 3.3.1: BI phylogram of the 46 COI sequences of 11 *Pagurus* species selected. The numbers on branches are ML bootstrap values and posterior probabilities of BI <50% (ML/BI percentages values, respectively). Each oceanographic region/specimens are defined: Northeast Atlantic Ocean (NEA), Northwest Atlantic Ocean (NWA), North Atlantic Ocean (NA), Mediterranean Sea (MED) and Bering Sea (BER) (see Table 3.3.3 for complement information). Two major clades have been roman numbered, I and II: represent two groups defined previously by Ingel (1985) based on adult and larvae morphological characters.

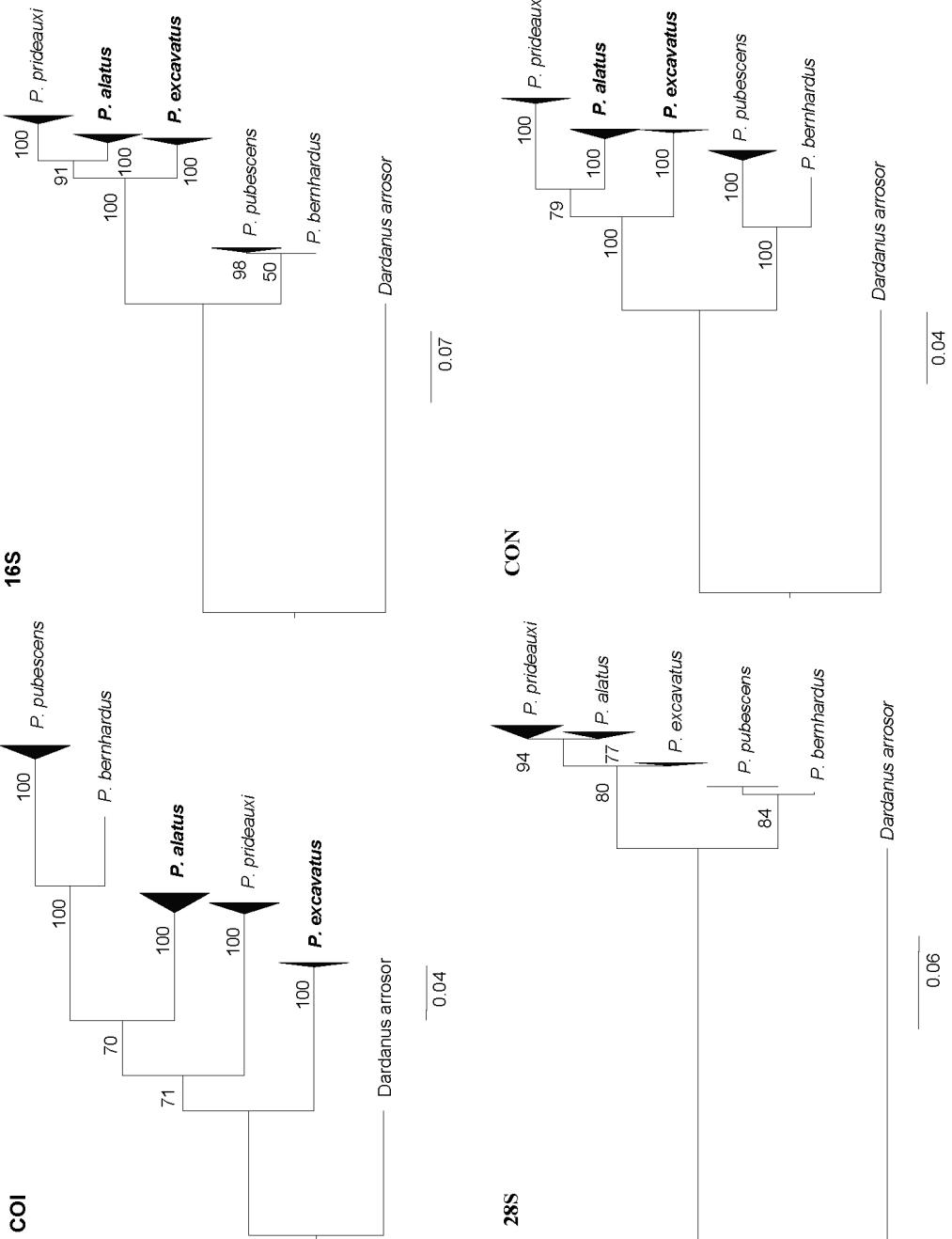


Figure 3.3.2: BI phylogenograms of each individual gene (COI, 16S, 28S) and concatenated data set (CON= COI+16S+28S). The numbers on branches are posterior probabilities >50% of BI (in percentage). Species *P. alatus* and *P. excavatus* are highlighted in bold.

Phylogenetic relationships

Overall, the phylogenetic algorithms (ML, BI) resulted in congruent topologies, delimiting the designated true *Pagurus* species (Figure 3.3.1). The resulting molecular phylogeny agrees in several respects with the current morphologically based classification of all species. All analyses support the basal placement of *P. longicarpus* and identify two main clades (Figure 3.3.1). In contrast, the relationships among inner clades of *Pagurus* were poorly resolved. Clade I is represented by four the Northeast Atlantic Ocean and Mediteranean Sea species and clade II by six species of the North Atlantic Ocean (East and West coasts) and Bering Sea specimens.

Molecular systematic assignments

The three independent genes revealed concordant phylogenetic differences between *P. alatus* and *P. excavatus*. *Pagurus alatus* is substantially divergent from *P. excavatus*, with a mean divergence of 14.9% and 5.1% for COI and 16S sequences respectively (Table 3.3.8). The 16S alignment was more conserved than the COI partition yielding 84/462 variable characters, of which 75/462 were parsimony informative. The 16S sequences are AT-rich (71.86%), indicating a moderate compositional bias. The pattern of nucleotide substitution was also biased in favour of transitions over transversions, yielding a ts:tv=1.1 and for 28S a ts:tv=3.2.

In the BI analysis, systematic positions of five species were not stable (Figure 3.3.2 A), but the trees underpinned by the 16S, 28S and the concatenated data partitions were broadly congruent with the COI hypothesis (Figure 3.3.1). The combined molecular analysis, based on strong posterior probabilities values (100%; in Figure 3.3.2, CON), clearly reveals the two independent lineages here discussed.

Table 3.3.6: Selected Northeast Atlantic Ocean and Mediterranean Sea *Pagurus* species and outgroup *Dardanus* species for molecular systematic reconstructions with respective date and site of collection, museum catalogue number, and genetic database accession numbers (Genbank).

Species	Collection site	Catalogue No.	Genbank accession No.		
			COI	16S	28S
<i>Pagurus alatus</i>	Costa Algarvia (Portugal)	MB89000415	JN107574	JN107604	JN107621
<i>Pagurus alatus</i>)	Costa Algarvia (Portugal)	MB89000418	JN107575	JN107606	JN107622
<i>Pagurus alatus</i>	Costa Algarvia (Portugal)	MB89000450	JN107576	JN107607	JN107619
<i>Pagurus alatus</i>	Costa Algarvia (Portugal)	MB89000463	JN107577	JN107605	JN107620
<i>Pagurus bernhardus</i>	Wales (United Kingdom)	MB89000491	JN107579	JN107608	JN107623
<i>Pagurus excavatus</i>	Costa de Prata (Portugal)	MB89000268	JN107586	JN107609	JN107626
<i>Pagurus excavatus</i>	Sicily (Italy)	MB89000078	JN107588	JN107610	JN107627
<i>Pagurus excavatus</i>	Sicily (Italy)	MB89000079	JN107589	JN107611	JN107628
<i>Pagurus prideauxi</i>	Costa de Prata (Portugal)	MB89000311	JN107596	JN107614	JN107631
<i>Pagurus prideauxi</i>	Sicily (Italy)	MB89000086	JN107593	JN107615	JN107632
<i>Pagurus prideauxi</i>	Wales (United Kingdom)	MB89000492	JN107597	JN107613	JN107630
<i>Pagurus prideauxi</i>	Bear Island Slide (Norway)	MB89000493	JN107594	JN107612	JN107629
<i>Pagurus pubescens</i>	Svalbard (Norway)	MB89000489	JN107601	JN107616	JN107633
<i>Pagurus pubescens</i>	Svalbard (Norway)	MB89000490	JN107602	JN107617	JN107633
<i>Dardanus arrosor</i>	Sicily (Italy)	MB89000494	JN107572	JN107603	JN107618

3.3.4 Discussion

Pagurus diversity

Systematics work to date in *Pagurus* has primarily been in the area of morphological taxonomy, with few hypotheses presented regarding species relationships. Generating such inferences has been difficult because of generally remarkable similarities in morphology among members of the genus. These similarities are perhaps surprising given the degree of the genetic divergence observed in this study between the most phenotypically closest species, *P. alatus* and *P. excavatus* (14.8%). A similar pattern has been observed in penaied shrimps (Palumbi and Benzie, 1991), porcelanids crabs (Hiller *et al.*, 2006), diogenid hermit crabs (Malay and Paulay, 2009)

Table 3.3.7: Substitution models for the molecular systematic analyses of selected *Pagurus* species from Northeast Atlantic Ocean and Mediterranean Sea.

Gene	Substitution model	Among-site rate variation*		Base frequencies			
		I	Γ	A	C	G	T
COI**	TIM2	0.454	0.41	0.3104	0.1484	0.1625	0.3787
1st codon	TrN	0.629	-	0.2901	0.1667	0.307	0.2363
2nd codon	F81	-	-	0.1306	0.2565	0.1688	0.4441
3rd codon	TPM3uf	-	0.622	0.4405	0.0937	0.0724	0.3935
16S	TVM	-	0.382	0.3532	0.1048	0.1766	0.3654
28S	TrN	0.327	0.258	0.1889	0.2976	0.2282	0.2852

*I proportion of invariant sites; Γ , gamma distribution shape parameter. I and Γ values refer to the AIC.

**COI partition represented by first (1st), second (2nd) and third (3rd) codon positions.

Table 3.3.8: Sequence identity matrix estimated from 16S with TVM+G model (above diagonal) and COI (below diagonal) with TIM2+I+G model between selected *Pagurus* species of Northeast Atlantic Ocean and Mediterranean Sea. All values are expressed as percentage.

Species	Collection site	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	0.02	0.02	0.04	1.81	0.61	0.57	0.57	0.5	0.53	0.5	0.5	1.92	1.83	
2 <i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	0.1	0	0.02	1.77	0.59	0.55	0.55	0.47	0.5	0.47	0.47	1.87	1.79	
3 <i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	0	0.1	0.02	1.77	0.59	0.55	0.55	0.47	0.5	0.47	0.47	1.87	1.79	
4 <i>Pagurus alatus</i> (Fabricius, 1775)	Costa Algarvia (Portugal)	0	0.1	0	1.81	0.61	0.57	0.57	0.5	0.53	0.5	0.5	1.83	1.83	
5 <i>Pagurus bernhardus</i> (Linnaeus, 1758)	Wales (United Kingdom)	5.35	4.69	5.35	5.35	1.81	1.74	1.74	1.9	1.89	1.9	1.9	0.56	0.51	
6 <i>Pagurus excavatus</i> (Herbst, 1791)	Sicily (Italy)	3.82	3.59	3.82	3.82	7.04	0.02	0.02	0.8	0.77	0.8	0.8	2.05	1.97	
7 <i>Pagurus excavatus</i> (Herbst, 1791)	Sicily (Italy)	3.82	3.59	3.82	3.82	7.04	0	0	0.76	0.73	0.76	0.76	1.98	1.9	
8 <i>Pagurus excavatus</i> (Herbst, 1791)	Costa de Prata (Portugal)	3.82	3.59	3.82	3.82	7.04	0	0	0.76	0.73	0.76	0.76	1.98	1.9	
9 <i>Pagurus prideauxi</i> (Leach, 1815)	Bear Island Slide (Norway)	3.56	3.39	3.56	3.56	4.99	4.05	4.05	4.05	0.02	0	0	2.01	1.93	
10 <i>Pagurus prideauxi</i> (Leach, 1815)	Wales (United Kingdom)	3.65	3.48	3.65	3.65	5.1	4.15	4.15	4.15	0.02	0.02	0.02	2	1.92	
11 <i>Pagurus prideauxi</i> (Leach, 1815)	Costa Algarvia (Portugal)	3.65	3.48	3.65	3.65	5.03	4.15	4.15	4.15	0.04	0.02	0	2.01	1.93	
12 <i>Pagurus prideauxi</i> (Leach, 1815)	Sicily (Italy)	3.65	3.48	3.65	3.65	5.1	4.15	4.15	4.15	0.02	0	0.02	2.01	1.93	
13 <i>Pagurus pubescens</i> (Krøyer, 1838)	Svalbard (Norway)	5.33	5.02	5.33	5.33	2.7	7.12	7.12	7.12	7.29	7.44	7.44	0.04	0.04	
14 <i>Pagurus pubescens</i> (Krøyer, 1838)	Svalbard (Norway)	5.64	5.26	5.64	5.64	2.85	7.07	7.07	7.07	7.17	7.32	7.32	0.18	0.18	

and has been attributed to stabilizing selection on morphological/ecological characters, or that they are on independent evolutionary trajectories. Likewise, strong stabilizing selection acting on morphological differentiation in *Austixa* crabs has been associated with life as a commensal of thalassinidean shrimps, accompanied by neutral molecular divergence (Harrison, 2004).

The smallest mean intraspecific divergence values observed (Table 5) are possibly underestimated, because samples were obtained from a single locality. Global-scale phylogeography surveys of COI sequence diversity have estimated average intraspecific diversity values of less than 1% within crustaceans, whereas interspecific values typically are greater than 4% among congeneric species (Costa *et al.*, 2007; Lefébure *et al.*, 2006; Radulovici *et al.*, 2009) and especially among decapods that can exhibit congeneric divergence values greater than 15% (Matzen da Silva *et al.*, 2011). Elsewhere, five species of the genus *Pagurus* from Sea of Japan exhibited lower levels of genetic identity when compared with the genera *Metapenaeus* and *Penaeus* (Zaslavskaya *et al.*, 2009). Here, the high genetic diversity observed among the pagurid species is in line with the observed morphological variability in informal morphological groups among adults and larvae described by Ingle (1985) and McLaughlin and Gore (1988), respectively.

Phylogenetic relationships

Ingle (Ingle, 1985) delineated two main groups of Northeast Atlantic Ocean and Mediterranean Sea *Pagurus* based on three adults, and additional larval morphological characters (Ingle, 1985) that are fully congruent with the current molecular systematic analysis. Furthermore, *P. armatus*, *P. ochotensis* and *P. bernhardus* that were assumed

to be most related, based on morphology (Ingle, 1985), share the most basal position in clade II. In addition, all species from clade I and II agree with two distinct larvae groups described by McLaughlin and Gore (McLaughlin and Gore, 1988) based on the characteristics and species assigned. The inconsistent phylogenetic position of *P. bernhardus*, observed in Mantelatto *et al.* (2009), was still unresolved here (Figure 3.3.1), represented by the weak support of the two focal nodes (< 50% bootstrap support) within clade II. However, the observed COI molecular divergence does support a sister group relationship between *P. acadianus* and *P. bernhardus*, a taxonomic relationship derived also from morphological comparisons (Cunningham *et al.*, 1992; d'Udekem d'Acoz *et al.*, 2001). In summary, the phylogenetic patterns observed for *Pagurus* are consistent with morphological groups established by Ingle (1985) and McLaughlin and Gore (1988) but further analyses would be required to establish the precise cladistic relationships within the genus as a whole.

Molecular systematic assignments

Concordance across molecular and morphological characters provides a reliable indicator of longstanding evolutionary independence and consequently provides an operational criterion for species recognition (Avise and Wollenberg, 1997). In the present study, morphological differences between *P. alatus* and *P. excavatus* were evident when two morphological variants were compared directly (Table 3.3.1), supporting molecular findings that justify separation at the species level. It is important to mention that a restricted collection from one biogeographical region of a species with a wide distribution cannot represent the complete range of morphological variation that is often found in decapods (Cuesta and Schubart, 1998; Mantelatto *et al.*, 2009; Spivak and Schubart, 2003). We also know that phenotypic plasticity in morphological traits

(especially organisms with commensal behaviours) might be strongly influenced by environmental factors between different areas (Agrawal, 2001; Brian *et al.*, 2006; Price *et al.*, 2003). The precise cladistic relationships among the five species were not stable (Figure 3.3.2, A), most likely due to lower taxon sampling and also the inability of COI to accurately resolve deep nodes. However, the combined molecular analysis (Figure 3.3.2, CON) clearly reveals the evidence for long-standing evolutionary independence between *P. excavatus* and *P. alatus*.

3.3.5 Conclusions

Molecular data have not been used before to investigate systematic relationships among *Pagurus* of the Northeast Atlantic Ocean and Mediterranean Sea. Despite the perceived limitations regarding the use of morphological characters for inferring evolutionary relationships among commensal species, our molecular data support the morphological taxonomy. Our data may indicate the possible existence of two monophyletic groups (clade I and II), supporting previous assertions based on larval and adult morphological criteria. However, the current data confirm the complexity of the relationships within *Pagurus*, highlighting the absence of complete and integrated morphological descriptions for the diverse and heterogeneous members of the genus. Since the present taxonomic and geographic coverage is incomplete, the topologies presented here should be regarded as working hypotheses. *Pagurus* have diversified into a wide variety of marine habitats and exemplify classic commensal, anti-predator evolutionary traits. Thus, the group provides an excellent model for studying the

interplay between speciation, neutral molecular divergence and potential stabilising selection on body form.

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References

- Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* **294**, 321-326.
- Akaike H (1973) Information theory and an extension of maximum likelihood principle. In: *Second International Symposium on Information Theory* (ed. Petrov B.N and Csaki, F.), pp. 267-281. Akademiai Kiado, Budapest
- Appel M, Elwood RW (2009) Gender differences, responsiveness and memory of a potentially painful event in hermit crabs. *Animal Behaviour* **78**, 1373-1379.

- Avise JC, Wollenberg K (1997) Phylogenetics and the origin of species. *PNAS* **94**, 7748-7755.
- Brian JV, Fernandes T, Ladle RJ, Todd PA (2006) Patterns of morphological and genetic variability in UK populations of the shore crab, *Carcinus maenas* Linnaeus, 1758 (Crustacea: Decapoda: Brachyura). *Journal of Experimental Marine Biology and Ecology* **329**, 47-54.
- Costa FO, deWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272-295.
- Cruickshank RH (2002) Molecular markers for the phylogenetics of mites and ticks. *Systematic and Applied Acarology* **7**, 3-14.
- Cuesta JA, Schubart CD (1998) Morphological and molecular differentiation between three allopatric populations of the littoral crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). *Journal of Natural History* **32**, 1499 - 1508.
- Cunningham CW, Blackstone NW, Buss LW (1992) Evolution of king crabs from hermit crab ancestors. *Nature* **355**, 539-542.
- d'Udekem d'Acoz C (1999) *Inventaire et distribution des crustacés décapodes de l'Atlantique nord - oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25°N* Collection Patrimoines Naturels Paris.
- d'Udekem d'Acoz C, Pinho MR, Melo O, Gonçalves J, Martins H (2001) New records and notes on little know shrimps (Crustacea, decapoda) from Azorean Waters. *Arquipélago* **18A**, 61-64.
- De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, et al. (2009) A classification of living and fossil genera of decapod Crustaceans. *Raffles Bulletin of Zoology* **1**, 1-109.
- Drummond AJ, Rambaut A (2007) A beast: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 1-8.
- Ellis JR, Rogers SI, Freeman SM (2000) Demersal assemblages in the Irish Sea, St George's channel and Bristol channel. *Estuarine, Coastal and Shelf Science* **51**, 299-315.

- Elwood RW, Glass CW (1981) Negotiation or aggression during shell fights of the hermit crab *Pagurus bernhardus*? *Animal Behaviour* **29**, 1239-1244.
- Elwood RW, Stewart A (1985) The timing of decisions during shell investigation by the hermit crab, *Pagurus bernhardus*. *Animal Behaviour* **33**, 620-627.
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal Molecular Evolution* **17**, 368-376.
- Groeneveld JC, Gopal K, George RW, Matthee CA (2007) Molecular phylogeny of the spiny lobster genus *Palinurus* (Decapoda: Palinuridae) with hypotheses on speciation in the NE Atlantic/Mediterranean and SW Indian Ocean. *Molecular Phylogenetics and Evolution* **45**, 102–110.
- Hajibabaei M, DeWaard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, et al. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **360**, 1959-1967.
- Harrison JS (2004) Evolution, biogeography, and the utility of mitochondrial 16S and COI genes in phylogenetic analysis of the crab genus *Austinixia* (Decapoda: Pinnotheridae). *Molecular Phylogenetics and Evolution* **30** 743–754.
- Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal Molecular Evolution* **22**, 160-174.
- Hiller A, Kraus H, Almon M, Werding B (2006) The *Petrolisthes galathinus* complex: Species boundaries based on color pattern, morphology and molecules, and evolutionary interrelationships between this complex and other Porcellanidae (Crustacea: Decapoda: Anomura). *Molecular Phylogenetics and Evolution* **40**, 547-569.
- Huelsenbeck JP, Ronquist F (2001) Mr Bayes:Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.
- Huelsenbeck JP, Rannala B (2004) Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53**, 905-913.
- Ingle RW (1985) Northeastern Atlantic and Mediterranean hermit crabs (Crustacea: Anomura: Paguroidea: Paguridae). The genus *Pagurus* Fabricius, 1775. *Journal of Natural History* **19**, 745 - 769.

- Jackson NW, Elwood RW (1989a) How animals make assessments: information gathering by the hermit crab *Pagurus bernhardus*. *Animal Behaviour* **38**, 951-957.
- Jackson NW, Elwood RW (1989b) Memory of information gained during shell investigation by the hermit crab, *Pagurus bernhardus*. *Animal Behaviour* **37**, 529-534.
- Jagt JWM, van Bakel BWM, Fraaije RHB, Neumann C (2003) In situ fossil hermit crabs (Paguroidea) from northwest Europe and Russia. Preliminary data on new records. *Revista Mexicana de Ciencias Geológicas* **23**, 364-369.
- Kellogg CW (1976) Gastropod shells: A potentially limiting resource for hermit crabs. *Journal of Experimental Marine Biology and Ecology* **22**, 101-111.
- Kumar S (1996) Patterns of nucleotide substitution in mitochondrial protein coding genes of vertebrates. *Genetics* **143**, 537-548.
- Lancaster I (1988) *Pagurus bernhardus* (L.) - an introduction to the natural history of hermit crabs. *Field Studies* **7**, 189-238.
- Lefébure T, Douady CJ, Gouy M, Gibert J (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: Proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* **40**, 435–447.
- Malay MCMD, Paulay G (2009) Peripatric speciation drives diversification and distributional pattern of reef hermit crabs (Decapoda: Diogenidae: *Calcinus*). *Evolution* **64**, 634-662.
- Mantelatto FL, Pardo LM, Pileggi LG, Felder DL (2009) Taxonomic re-examination of the hermit crab species *Pagurus forceps* and *Pagurus comptus* (Decapoda: Paguridae) by molecular analysis. *Zootaxa* **2133**, 20-32.
- Mantelatto FL, Robles R, Biagi R, Felder DL (2006) Molecular analysis of the taxonomic and distributional status for the hermit crab genera *Loxopagurus* Forest, 1964 and *Isocheles* Stimpson, 1858 (Decapoda, Anomura, Diogenidae). *Zoosystema* **28**, 495-506.
- Mathews LM, Anker A (2009) Molecular phylogeny reveals extensive ancient and ongoing radiations in a snapping shrimp species complex (Crustacea, Alpheidae, *Alpheus armillatus*). *Molecular Phylogenetics and Evolution* **50**, 268-281.

- Matzen da Silva J, Creer S, Dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR (2011) Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Malacostraca). *PLoS ONE* **6**, e19449.
- McLaughlin PA (2003) Illustrated keys to families and genera of the superfamily Paguroidea (Crustacea: Decapoda: Anomura), with diagnoses of genera of Paguridae. *Memoirs of Museum Victoria* **60**, 111-144.
- McLaughlin PA, Gore RH (1988) Studies on the Provenzanoi and other pagurid groups: I. The larval stages of *Pagurus maclaughlinae* García-Gómez, 1982 (Decapoda: Anomura: Paguridae), reared under laboratory conditions. *Journal of Crustacean Biology* **8**, 262-282.
- McLaughlin PA, Komai T, Lemaitre R, Rahayu DL (2010) Annotated checklist of anomuran decapod crustaceans of the world (exclusive of the Kiwaoidea and families Hirostylidae and Galatheidae of the Galathedoidea) Part I - Lithoidea, Lomisoidea and Paguroidea. *The Raffles Bulletin of Zoology* **23**, 5-107.
- McLaughlin PA, Lemaitre R (1997) Carcinization in the Anomura - fact or fiction? I. Evidence from adult morphology. *Contributions to Zoology* **67**, 79-123.
- McLaughlin PA, Lemaitre R, Sorhannus U (2007) Hermit crab phylogeny: a reappraisal and its "fall-out". *Journal of Crustacean Biology* **27**, 97–115.
- Neil SJ, Elwood RW (1985) Behavioural modifications during egg-brooding in the hermit crab, *Pagurus bernhardus* L. *Journal of Experimental Marine Biology and Ecology* **94**, 99-114.
- Palumbi SR, Benzie J (1991) Large mitochondrial differences between morphologically similar penaeid shrimp. *Molecular Marine Biology and Biotechnology* **1**, 27-34.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.
- Price TD, Qvarnstrom A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. Lond. B* **270**, 1433-1440.
- Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. *Molecular Ecology Resources* **9**, 181-187.
- Rivera JM (2006) Morphology of the first zoea of the hermit crab *Pagurus edwardsi* (Dana, 1852), (Decapoda: Anomura: Paguridae) obtained in the laboratory. *Revista de Biología Marina y Oceanografía* **41**, 239-244.

- Scelzo MA, Fantucci MZ, Mantelatto FL (2010) Spermatophore and Gonopore Morphology of the Southwestern-Atlantic Hermit Crab *Pagurus exilis* (Benedict, 1982) (Anomura, Paguridae). *Zoological Studies* **49**, 421-433.
- Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. In: *Decapod Crustacean Phylogenetics*. (ed. Martin J.W., Crandall, K.A., and Felder, D.L.), pp. 47-65. Taylor and Francis Group, New York.
- Schubart CD, Cuesta JA, Felder DL (2005) Phylogeography of *Pachygrapsus transversus* (Gibbes, 1850): The effect of the American continent and the Atlantic Ocean as gene flow barriers and recognition of *Pachygrpsus socius* Stimpson 1871 as a valid species. *Nauplius* **12**, 99 - 113.
- Schwentner M, Timms BV, Bastrop R, Richter S (2009) Phylogeny of Spinicaudata (Branchiopoda, Crustacea) based on three molecular markers – An Australian origin for *Limnadiopsis*. *Molecular Biology and Evolution* **53**, 716-725.
- Shih H-T, Ng PKL, Schubart CD, Chang H-W (2007) Phylogeny and phylogeography of the genus *Geothelphusa* (Crustacea: Decapoda, Brachyura, Potamidae) in southwestern Taiwan based on two mitochondrial genes. *Zoological Science* **24**, 57-66.
- Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**, 13486-13491.
- Spivak ED, Schubart CD (2003) Species status in question: a morphometric and molecular comparison of *Cyrtograpsus affinis* and *C. altimanus* (Decapoda, Brachyura, Varunidae). *Journal of Crustacean Biology* **23**, 212-222.
- Stamatakis A (2008) The RAxML. The Exelixis Lab, Department of Computer Science, Munchen.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512-526.

- Taylor PR (1981) Hermit crab fitness: The effect of shell condition and behavioral adaptations on environmental resistance. *Journal of Experimental Marine Biology and Ecology* **52**, 205-218.
- Taylor PR (1982) Environmental resistance and the ecology of coexisting hermit crabs: Thermal tolerance. *Journal of Experimental Marine Biology and Ecology* **57**, 229-236.
- von Rintelen K, von Rintelen T, Glaubrecht M (2007) Molecular phylogeny and diversification of freshwater shrimps (Decapoda, Atyidae, Caridina) from ancient Lake Poso (Sulawesi, Indonesia) - The importance of being colourful. *Molecular Phylogenetics and Evolution* **45**, 1033-1041.
- Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. *International Review of Cytology* **141**, 173-216.
- Yang Z, Nielsen R, Goldman N, Krabbe Pedersen A-M (2000) Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics* **155**, 431-449.
- Yang ZH (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *Journal of Molecular Evolution* **39**, 306-314.
- Yang ZH, Yoder AD (1999) Estimation of the transition/transversion rate bias and species sampling. *Journal of Molecular Evolution* **48**, 274-283.
- Young AM, Torres C, Mack JE, Cunningham CW (2002) Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. *Marine Biology* **140**, 1059-1066.
- Zaslavskaya NI, Kornienko ES, Korn OM (2009) Genetic similarity between *Pagurus gracilipes* (Stimpson, 1858) (Decapoda: Paguridae) and another pagurid and diogenid hermit crabs from Russian waters of the sea of Japan. *Invertebrate Zoology* **6**, 147-155.

Section 4. FINAL REMARKS

“The sea is a safe value for the future (...). As sustainability of the sea becomes a value in itself, the subjective though positive valuation of this value by the public opinion will confer more importance to the sea in the eyes of the decisions makers.”

Tiago Pitta e Cunha
(Portugal e o Mar, 2011)

Section 4: Final remarks



Section 4: Final remarks

4.1. Overview

Much of the large-scale diversity patterns that we observe today in both terrestrial and aquatic biota have been shaped by the climatic changes observed during the Last Glacial Maximum (LGM; 21 000 years ago) (Svenning *et al.*, 2008). In the last 200 years, and more intensely during the last 50 years, human activity has disrupted the balance that is so central to life, it has put at risk many species, and in the worst cases their habitats too, resulting in the well-acknowledged biodiversity crisis (Hoag, 2010).

In the last two decades, the consciousness of decision-makers and scientists for the importance of preserving ecosystems and their biodiversity, and the fact that there is a tremendous urgency to economically characterize and evaluate these ecosystems has been greater than ever before. Moreover, we have become aware of our lack of knowledge of extant biodiversity, and also acknowledged the scarcity of taxonomic specialists that can characterize and identify the existence of life around us (also known as the “taxonomic impediment”) (Evenhuis, 2007). Biodiversity loss has been recognized as a major global environmental problem and much effort has been targeted towards biodiversity studies in general (ecological, molecular, taxonomy, systematic, phylogeny, phylogeography, modelling, conservation, management, policies, etc). Nevertheless, there is still a long way to go as biodiversity conservation only became an officially funded research field in 1992 through the Convention on Biological Diversity (CBD) (<http://www.cbd.int>). Due CBD, biodiversity became a familiar feature of news programmes and newspapers, environment groups, political decision-makers, economists and ordinary citizens alike. Most commonly, biodiversity studies investigate the present diversity of life and not the tremendous variety of all organisms that have

ever lived. However, I believe that to trace the origins of the present biodiversity we need look into the past through fossil records description and phylogenetic studies of presently extant species. Genetic diversity is a critical component of biodiversity and determining nucleotide sequences is arguably one of the strongest measures of genetic diversity, although a large number of other techniques are also prevalent with their utility being dependent on the precise questions being addressed (Carvalho *et al.*, 2011). The primary goal of this Thesis was to uncover Malacostracan molecular biodiversity (Section 2) and elucidating evolutionary processes that have affected the genetic diversity of representative taxa (Section 3). Below it is discussed the main global conclusions and technical shortcomings identified, as well as some open questions for future research.

4.1.1 Molecular biodiversity

The Malacostraca are a very complex group of organisms with multifaceted life histories, morphology and ecological diversity and they are difficult to identify by traditional approaches that usually requires extensive taxonomy training. The role of a faster identification method, DNA barcoding, was investigated as an effective tool for species identification in 132 Malacostraca species (Chapter 2.1). Sequences from 601 specimens grouped into clusters corresponding to known morphological species in 95.45% of the cases. Two cases of high intraspecific divergences were found among individuals belonging to an apparently single morphological taxon, suggesting the occurrence of cryptic speciation. Our study reconfirms the usefulness of DNA barcoding for the identification of marine Malacostraca, and that the present cytochrome c oxidase

subunit 1 (COI) library can be used for subsequent applications in ecology and systematics. Some major organism groups (e.g., the Phylum Porifera, Superclasses of Fish and the Order Lepidoptera) have been the focus of global campaigns to build a complete DNA barcode database (Radulovici *et al.*, 2010). Due to the complexity of Malacostracan organisms I believe that global campaigns by specific taxa, i.e., by Order or in better cases by Family, would better increase the interest and confidence of each research group around the world to change and standardize sampling, taxonomy, molecular and technical information. Also as an alternative to global campaigns that are likely to be very difficult to co-ordinate, fund and achieve in the short term, regional campaigns would allow different groups of expertise to understand the connectivity, relationships, evolution and distribution of the diversity of species among Malacostraca taxa. Despite the general success of barcode projects (e.g. adherence to the concept by the scientific and general public in the molecular identification field, and standardization offers immense applications for various users) (Teletchea, 2010) there are various technical aspects that should be improved to increase the number - and mainly the quality - of deposited sequences. The present study strongly recommends more investment from each specialized research team by taxa to improve the quality of sample preservation and increase the number of specific taxa primers for barcode markers to avoid both contamination and *numts*. It was discussed the most important weaknesses of using COI for species identification and to contribute to species delineation, in particular the consequences of misinterpretation due to the accidental amplification and assumption of *numts* as valid sequences (Chapter 2.2). During recent years a additional quality control steps have been proposed to avoid or flag *numts* during the processing COI barcode data. This study adhered to some of these steps (for example among 20 individuals from *Gonepelax rhomboides*), but also proposes a new step consisting on the

analysis of intraspecific amino acid variability for screening putative *numts*. Failure to analyse this may result in erroneous taxonomic inferences, particularly in the case of *numts* that diverge little from orthologous sequences. The effectiveness of specific taxon primers to avoid contamination and also the inadvertent amplification of *numts* was also demonstrated in some decapod species. The identification of *numt* occurrence can be very useful in animals as they can lose or change their function upon transfer to the nucleus. The increase in barcoding projects and awareness of “unexpected” *numts* will encourage the characterization of *numts* to understand genome dynamics, molecular ecology and evolution among related organisms (Hazkani-Covo *et al.*, 2010).

4.1.2 Molecular evolution and shallow phylogenies

In the last seven years DNA barcoding projects have complemented current research in molecular phylogenetics and population genetics providing background information needed in the selection of taxa for further analyses. The utility of DNA barcoding as a complementary research tool in evolutionary insights was explored (Chapter 3.1), especially in selected decapod species. The taxonomic diversity and relationships of the decapod assemblages was compared using one of the most comprehensive COI datasets so far examined for the Order (1,910 sequences of 604 species, 225 genera and 68 families). This study contributed barcodes sequences from 101 decapod species from the North East Atlantic, the Gulf of Cadiz and the Mediterranean Sea (7.7% of the 1506 species described for this geographical area), of which 81 species represent novel COI records. Patterns within families showed a general predicted molecular hierarchy, but the scale of divergence at each taxonomic level

varied extensively between families. Although the sampling is limited to a small proportion of the entire order (5.4% of the 17,635 extant species described), the range of molecular data employed here has contributed to the assessment of decapod biodiversity in several ways including: i) revealing putative cryptic species (e.g., *Palaemon elegans*); ii) assigning correct species names to specimen samples originated from different life history stages (*Pachygrapsus marmoratus*); iii) confirming the existence of synonymous names (*Macropodia tenuirostris*); iv) facilitating a rapid assessment of taxon diversity in groups that have until now received limited morphological and systematic examination (*Macropodia*); v) and flagging taxonomic groups (Caridea; Lithodidae and Pandalidae) with unusual nucleotide composition or evolutionary rates. The increasing database of barcode records, in conjunction with additional ecological and molecular approaches, is likely to enhance understanding of the history and evolutionary trajectory of decapod species. It has become essential that species are accurately delineated, cryptic species are identified and/or conservation units are proposed on the basis of sound phylogenetic and phylogeographic variation in space and time. Despite the extensive investigations in this order, there are still several families, ecosystems, and geographic areas for which the decapod fauna remains poorly known and underwater surveys will likely yield new records and new species. Seamounts and canyons which are abundant topographical features along the Portuguese coast and Portuguese archipelago islands, are such barely explored ecosystem which may yield substantial amounts of information of marine ecosystems, informing us of the potential levels of richness and connectivity of members of this order. Furthermore, undetected cryptic species may compromise scientific areas for which decapods have been an increasing target group to understand the processes of anthropogenic ocean acidification and its impact on calcifying organisms (Orr *et al.*, 2005). Still, the concept of cryptic species is complex and it is not easily understood. In

many occasions, cryptic species-like patterns can be explained by: a) confusion with species complexes; b) incomplete species description of morphological characters (i.e., problems with the identification of all life stages like eggs and larvae); c) sexually dimorphic species; d) species with particularly strong phenotypic plasticity, or where the misleading phenotype (e.g., behaviour) is not included in species delimitation. The increase in molecular information will help to clarify many ambiguities in traditional taxonomy, to understand the evolutionary processes shaping different species with the same putative morphotype and how the environment and behaviour delineates the evolution of species.

To further deepen our knowledge on the ecological and historical processes responsible for the geographical distribution of species and their systematic history thesis examined the phylogenetic structure of the two of the most representative genera in this study: *Plesionika* (Chapter 3.2) and *Pagurus* (Chapter 3.3). Despite the limitations of using COI data alone for phylogenetic inference, the utility of such data here for these two genera has been demonstrated. COI data was combined with mitochondrial 16S ribosomal RNA (16S) and nuclear 28S ribosomal RNA (28S) to understand the systematic position of species. Despite the abundance and prominent ecological role of *Plesionika* and *Pagurus* in North East Atlantic and Mediterranean ecosystems, many important aspects of their taxonomy, biology, systematics and evolution remain poorly explored. The phylogenetic inferences presented here should be regarded as hypotheses to be tested in future studies with additional taxa and/or additional sources of molecular and morphological data to recognize a concordance in the evolution with major ecological and geological events. Consequently, a new project has been developed by Cátia Bartilotti from the Portuguese Institute of Marine Research (IPIMAR), proposing to investigate the larval ecology and taxonomy of decapods from

North Atlantic Ocean (the eastern and western parts). Bartilotti is studying the genus *Plesionika*, which have both ecological and economic importance (they constitute a by-catch of the trawl fishery, but can reach similar market prices to those of target species), by means of using conventional and molecular taxonomic techniques to investigate the phylogeny, phylogeography of *Plesionika* in connection with detailed ontogenetic data.

4.2 Future perspectives

An accurate delimitation of species is essential as species are the basic units for distributional and habitat studies in biodiversity assessment, therefore providing the framework for conservation strategies. Since Darwin's theory of evolution by natural selection (Darwin, 1859), the major advances in our understanding of animal evolution have been made in recent years (Telford and Littlewood, 2008) by the subsequent advances in comparative taxonomic and phylogenetic systematic studies. In my view, the application of molecular phylogenetic systematics as a biodiversity assessment tool can be only be achieved using combined evidence provided by genotype, phenotype and an understanding of the link between them through comparative embryology ("*Ontogeny recapitulates phylogeny*") and evolutionary developmental studies. Given this line of thinking, the modern age of species concepts lies between various approaches that clearly are intended to belong to all species (e.g., morphological species, phylogenetic species) and those concepts that treat species as real entities and that summarize the fundamental nature of their existence (e.g., evolutionary species) (Fitzhugh, 2005; Velasco, 2008). An effect of the vigorous debate on species concepts

is the often repeated claim that there can be no single species concept, and I agree that some kind of pluralistic view of species concept should be adopted according to “*No one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species.*” (Darwin, 1859). This study emphasizes the different kinds of methods and criteria that can be used for species identification/delineation, i.e., morphological and phylogenetic species concepts. Although, these two main concepts offer limitations (e.g., such as morphological criteria may not reflect actual links that hold together natural units and that phylogenetic criteria deals only with the manifestation of cohesion rather than evolutionary mechanisms (Hey, 2006)), the complementary aspects of traditional morphological taxonomy and modern molecular taxonomy (i.e. "integrative taxonomy"), will in most cases reveal an accurate species delineation . Thanks to a strong dialogue between taxonomists and end-users of taxonomical results (Teletchea, 2010) in my opinion DNA barcoding has been and will continue to be an excellent *eye-catching* tool for promoting “integrative taxonomy” between researchers able to understand the specific goals and inherent limits of taxonomy concepts.

Understanding species boundaries, dispersal abilities and distributions of marine fauna is particularly critical in the Malacostraca where the complexity of the marine environment lead to specialized life history traits, and convergence of form contributes to similar morphologies among taxa. Management strategies greatly benefit from DNA barcoding in understanding the processes involved in promoting and maintaining biodiversity of both Malacostraca and resources. Barcoding studies are still taxon-oriented but the concept has been used as a catapult for developing new alternatives for reliable identification, i.e. DNA microarrays for fish (Kochzius *et al.*, 2010), and next generation sequencing for identification of environmental samples (i.e., water,

sediment) (Creer *et al.*, 2010) and species diets, allowing a faster and multi-dimensional view of the relationship between trophic levels and biodiversity of a selected ecosystem. Despite the advantages of new molecular techniques for the identification/delineation of species in the last seven years, barcoding projects have consistently underlined that traditional taxonomists play a vital role in completing a global reference library. More than ever, the need for proper taxonomic description has been requested due to new species being revealed and cryptic species being discovered by divergent barcode clusters. We are in a new era of taxonomy (“Taxonomy’s Third Wave” by Bert Carelli (2010) *in* <http://taxodiary.com/>) and it is vital to establish concordance between established taxonomic methods and DNA barcoding (i.e. “renaissance of taxonomy” (Miller, 2007)) to encourage new generations of scientists to adhere to new approaches to taxonomy research. I think one way to achieve these goals is to align more closely the scientific community to the general society and I believe that the best places to do this is through museums, aquariums and zoos with public education institutions. That places can disseminate by excellence the latest scientific advances of techniques to know more about the biodiversity of ecosystems and the importance of their preservation because the public have a unique opportunity to be in directly contact with the beauty of the representative ecosystems or organisms in study or in danger. Developing barcoding projects directly with these agencies through scientific community (e.g. High Schools, Universities) is in my opinion the best democratic way to educate and to achieve a better understanding of the biodiversity conservation importance of our planet by all of us.

Despite the high levels of extant species diversity, marine systems are exposed to excessive and accelerating threats from environmental change, i.e. the consequences of climate change for the last ten years (Hoag, 2010), and human activity (Carvalho *et al.*,

2011). In an evolutionary-adaption perspective of species to environmental change Lane (2009) cited a phrase by the geneticist Doug Wallace: “*Reproductive success requires adapting to different food sources — carbohydrates, proteins or fats — and climates from icy cold to intense heat or humidity. A lot of this adaptation goes on in the mitochondria (...)*”. Mitochondrial genes mutate rapidly, generating variation that is subject to selection whenever environmental conditions change (Lane, 2009) despite being assumed to evolve in a nearly neutral manner (see Section 1 in 1.2.2.1 Mitochondrial DNA). For example, zooplankton species diversity and distribution have been used as an important measure of environmental change in the Arctic Ocean (e.g. temperature, acidification, etc) and may be used as biological marker to detect climate-induced changes in marine ecosystems biodiversity through DNA barcoding analysis (Bucklin *et al.*, 2007; Hunt *et al.*, 2010). DNA barcoding can be an excellent tool for flagging potential new species, to help delimiting species boundaries and also possibly to help understanding the evolutionary processes of adaptation, speciation and functional capacity.

Since the establishment of the Convention of Biological Diversity in 1992 the increasing numbers of several large-scale initiatives and research projects to inventory marine biodiversity (e.g. Census of Marine life (CoML) and the Marine Barcode of Life (MarBOL)) have generated a massive amount of information that we are still only beginning to understand regarding the biological complexity of the marine environment and the lack of awareness of the importance of the marine ecosystems by the decision makers (Pitta e Cunha, 2011). The goals that the European Commission acknowledged ten years ago to reverse the decline of biodiversity until 2012 within the Union it will not be accomplished, pointing now to the date of 2020 for achieving it. One of the factors that have contributed to the failure of the battle against marine biodiversity loss

is because it is exclusively related to environmental preservation and is not yet fully understood also as a matter of economic and even political nature. The environment and biodiversity are providers of ecosystem services that we depend for to guaranty our quality of life (Pitta e Cunha, 2011). By combining bioinformatics' advances in the analyzes of enormous amounts of nucleotide sequences data with largest open access data sharing (e.g. BOLD (<http://www.boldsystems.org>)), I anticipate that DNA barcoding might also become a *diplomatic* tool (i.e. through mathematics as universal language), helping scientists and decision-makers in devising and implementing sustainable development actions to preserve and understand the role of the global marine biodiversity now and into the future.

References

- Bucklin A, Wiebe PH, Smolenack SB, Copley NJ, Beaudet JG, Bonner KG, *et al.* (2007) DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *Journal of Plankton Research* **29**, 483-493.
- Carvalho GR, Creer S, Allen MJ, Costa FO, Tsigenopoulos CS, Goff-Vitry ML, *et al.* (2011) Genomics in the discovery and monitoring of marine biodiversity. In: *Introduction to Marine Genomics* (eds. Cock J.M., Tessmar-Raible K., Boyen C., Viard F.), p. 399.
- Creer S, Fonseca VG, Porazinska DL, Giblin-Davis RM, Sung W, Power DM, *et al.* (2010) Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology* **19**, 4-20.
- Darwin C (1859) *On the origin of species*, 1st edn. Murray, J., London.
- Evenhuis NL (2007) Helping solve the "other" taxonomic impediment: Completing the eight steps to total enlightenment and taxonomic nirvana. *Zootaxa* **1047**, 3-12.

Section 4: Final remarks

- Fitzhugh K (2005) The inferential basis of species hypotheses: the solution to defining the term ‘species’. *Marine Ecology* **26**, 155–165.
- Hazkani-Covo E, Zeller RM, Martin W (2010) Molecular poltergeists: Mitochondrial DNA copies (*numts*) in sequenced nuclear genomes. *PLoS Genetics* **6**, e1000834.
- Hey J (2006) On the failure on modern species concepts. *Trends in Ecology and Evolution* **21**, 447-450.
- Hoag H (2010) Confronting the biodiversity crisis. *Nature Reports Climate Change* **4**, 51-54.
- Hunt B, Strugnell J, Bednarsek N, Linse K, Nelson RJ, Pakhomov E, et al. (2010) Poles apart: The “bipolar” pteropod species *Limacina helicina* is genetically distinct between the Arctic and Antarctic Oceans. *PLoS ONE* **5**, e9835.
- Kochzius M, Seidel C, Antoniou A, Botla SK, Campo D, Cariani A, et al. (2010) Identifying fishes through DNA barcodes and microarrays. *PLoS ONE* **5**, e12620.
- Lane N (2009) On the origin of barcodes. *Nature* **462**, 272-274.
- Miller SE (2007) DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences* **104**, 4775-4776.
- Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, et al. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.
- Pitta e Cunha T (2011) *Portugal e o Mar. À Redescoberta da Geografia* Fundação Francisco Manuel dos Santos, Lisboa.
- Radulovici AE, Archambault P, Dufresne F (2010) DNA barcodes for marine biodiversity: Moving fast forward? *Diversity* **2**, 450-472.
- Svenning J-C, MNormand S, Kageyama M (2008) Glacial refugia of temperate trees in Europe: insights from species distribution modelling. *Journal of Ecology* **96**, 1117-1127.
- Teletchea F (2010) After 7 years and 1000 citations: Comparative assessment of the DNA barcoding and the DNA taxonomy proposals for taxonomists and non-taxonomists. *Mitochondrial DNA* **21**, 206-226.

- Telford MJ, Littlewood DTJ (2008) The evolution of the animals: introduction to a Linnean tercentenary celebration. *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**, 1421-1424.
- Velasco JD (2008) Species concepts should not conflict with evolutionary history, but often do. *Studies in History and Philosophy of Biological and Biomedical Sciences* **39**, 407-414.