Integrative taxonomy of decapod crustaceans with traditional and modern methods

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

Vorgelegt von

Dipl.-Biol. Roland Meyer,
München 2014

Cover picture: well camouflaged decapod crab *Eurypodius sp.* (upper left). SEM-picture of the first zoea larva of *Gnathophyllum elegans* (Risso, 1816) in lateral view (upper right). Larvae of *Inachus phalangium* (Fabricius, 1775) orientated on a SEM stub after critical point drying (lower left). *Acanthocyclus albatrossis* Rathbun, 1898 in the intertidal zone (lower right). All images by the author.

Erstgutachter: Prof. Dr. Roland R. Melzer
Zweitgutachter: Prof. Dr. Gerhard Haszprunar
Tag der mündlichen Prüfung: 24.06.2014

Eidesstattliche Erklärung

Diese Dissertation wurde im Sinne von § 12 der Promotionsordnung von Prof. Dr. Roland R. Melzer betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

München, den 04.02.2014 _____

Dipl.-Biol. Roland Meyer

Ehrenwörtliche Versicherung

Ich versichere hiermit ehrenwörtlich, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt wurde.

München, den 04.02.2014 _____

Dipl.-Biol. Roland Meyer

List of publications

Article I

Meyer R, Wehrtmann IS, Melzer RR (2006) Morphology of the first zoeal stage of *Portunus acuminatus*, Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. Crustaceana 70(2): 261-270.

Article II

Meyer R, Lehmann T, Melzer RR, Geiselbrecht H (2014) Morphology of the first zoeal stage of the mediterranean bumblebee shrimp *Gnathophyllum elegans* (Risso,1816) studied with light microscopy and scanning EM. Journal of the Marine Biological Association of the United Kingdom 94(1): 151-158.

Article III

Lerosey-Aubril R and **Meyer R** (2013) The sensory dorsal organs of crustaceans. Biological Reviews 88: 406-426.

Article IV

Meyer R, Martin J, Melzer RR (2010) Nucleus patterns of zoea larvae (Crustacea: Decapoda) in the context of taxonomy. Zootaxa 2422: 31-42.

Article V

Meyer R, Weis A, Melzer RR (2013) Decapoda of southern Chile: DNA barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* and *Eurypodius*. Systematics and Biodiversity 11(3): 389-404.

Article VI

Meyer R, Lochner S, Melzer RR (2009) Decapoda - crabs, shrimps & lobsters. In: Häussermann, V. & Försterra, G. (eds.) 2009. Marine Benthic Fauna of Chilean Patagonia, Nature in Focus, Santiago de Chile, 1000 pp. Spanish version: Häussermann, V. & Försterra, G. (eds.) 2009. Fauna Marina Bentonica de la Patagonia Chilena, Nature in Focus, Santiago de Chile, 1000 pp. ISBN 978-956-332-243-9 spanish; 978-956-332-244-6 english.

Declaration of author's contribution

In this dissertation, I present the results from my doctoral research conducted from 2006 until 2014 in six chapters, carried out under the supervision of Prof. Dr. Roland R. Melzer at the Ludwig-Maximilians-University of Munich.

Contribution to Article I

Meyer R, Wehrtmann IS, Melzer RR (2006) Morphology of the first zoeal stage of *Portunus acuminatus*, Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. Crustaceana 70(2): 261-270.

Roland Meyer accomplished the data collection, performed morphological analyses (using light- and scanning electron microscope) and designed all figures. Zoea larvae of *Portunus acuminatus* were obtained during the cooperation with Dr. Ingo S. Wehrtmann, Universidad de Costa Rica. Roland Meyer led the manuscript writing under the guidance of Prof. Dr. Roland R. Melzer.

Contribution to Article II

Meyer R, Lehmann T, Melzer RR, Geiselbrecht H (2014) Morphology of the first zoeal stage of the mediterranean bumblebee shrimp *Gnathophyllum elegans* (Risso,1816) studied with light microscopy and scanning EM. Journal of the Marine Biological Association of the United Kingdom 94(1): 151-158.

Manuscript concept and writing was done by Roland Meyer under the guidance of Prof. Dr. Roland R. Melzer. Tobias Lehmann and Hannes Geiselbrecht were responsible for SEM and light microscopy data. Collection of specimens was done by Roland Meyer.

Contribution to Article III

Lerosey-Aubril R and **Meyer R** (2013) The sensory dorsal organs of crustaceans. Biological Reviews 88: 406-426.

Roland Meyer was responsible for sampling and preservation of zoea material. Morphological analyses of the sensory dorsal organ of different zoea stages with the SEM were carried out by Roland Meyer. Manuscript concept and writing was done by Rudy Lerosey-Aubril.

Contibution to Article IV

Meyer R, Martin J, Melzer RR (2010) Nucleus patterns of zoea larvae (Crustacea: Decapoda) in the context of taxonomy. Zootaxa 2422: 31-42.
Roland Meyer coordinated and conducted the sampling of zoea material. DAPI staining was and microscopy was done by Jana Martin and Roland Meyer. Manuscript writing was done by Jana Martin and Roland Meyer under the guidance of Prof. Dr. Roland R. Melzer.

Contibution to Article V

Meyer R, Weis A, Melzer RR (2013) Decapoda of southern Chile: DNA barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* and *Eurypodius*. Systematics and Biodiversity 11(3): 389-404.
Roland Meyer coordinated and conducted the sampling of decapod material. Andrea Weis and Roland Melzer sampled and provided specimens for the study. Roland Meyer carried out all morphological and phylogenetic analyses. Design, preparation of figures and diagrams as well the manuscript writing was done by Roland Meyer under the guidance of Prof. Dr. Roland R. Melzer.

Contibution to Article VI

Meyer R, Lochner S, Melzer RR (2009) Decapoda - crabs, shrimps & lobsters. In: Häussermann, V. & Försterra, G. (eds.) 2009. Marine Benthic Fauna of Chilean Patagonia, Nature in Focus, Santiago de Chile, 1000 pp. Spanish version: Häussermann, V. & Försterra, G. (eds.) 2009. Fauna Marina Bentonica de la Patagonia Chilena, Nature in Focus, Santiago de Chile, 1000 pp. ISBN 978-956-332-243-9 spanish; 978-956-332-244-6 english.
Roland Meyer coordinated and conducted the sampling and *in situ* documenting of decapod material. Roland Melzer and Roland Meyer took part in various expeditions to the southern Chilean fiord region to obtain decapod material. Stefanie Lochner was responsible for specimen photos. Manuscript writing was done by Roland Meyer under the guidance of Prof. Dr. Roland R. Melzer.

München, den 04.02.2014 _____

Dipl.-Biol. Roland Meyer

München, den 04.02.2014 _____

Zusammenfassung

Die als Zehnfußkrebse oder auch als Decapoda bezeichneten Arthropoden sind eine weltweit verbreitete, zum Teil hoch spezialisierte und vielseitig angepasste Gruppe, die in fast allen aquatischen Ökosystemen, aber auch in terrestrischen Habitaten zu finden ist. Die enorme Artenzahl von 17,635 rezent und fossil bekannten Arten (De Grave *et al.*, 2009) sowie das hohe Alter der Gruppe an sich erschwert die systematische Eingliederung einzelner Arten. Fossile Funde von Dekapoden wurden bis ins Devon (vor 415 bis 359,2 Millionen Jahren) datiert (Schram et al., 1978). Damit haben die rezenten Vertreter viele Millionen Jahre Evolution durchlaufen und die Ergebnisse dieses langwierigen Prozesses schlagen sich in einer hohen morphologischen Vielfalt zwischen den Arten nieder. Um eine zuverlässige Phylogenie aufstellen und Arten eindeutig charakterisieren zu können sind neue Merkmale, Methoden und Ansätze erforderlich. Eine zuverlässige Bestimmung und Einordnung der verschiedenen Arten bildet die Basis für verschiedene Datenbanken und Projekte wie z.B. GenBank, Barcoding of Life (BOLD), German Barcode of Life (GBOL) oder Barcoding Fauna Bavarica und zeigt, welch hohen Stellenwert die Taxonomie besitzt.

Ziel dieser kumulativen Dissertation ist es mit Einsatz von verschiedenen modernen morphologischen und molekularen Methoden wie der Rasterelektronenmikroskopie, der Fluoreszenzmikroskopie und der Analyse von mitochondrialen DNA-Sequenzen (Cytochrom-c-Oxydase) neue Merkmalssätze zur besseren Charakterisierung der verschiedenen Arten und deren Artabgrenzungen zu erarbeiten. Aber auch klassische Methoden wie das Abwägen von morphologischen Merkmalen, kommen in einem integrativen Ansatz zur Artabgrenzung zur Anwendung. Die in den Arbeiten angewandte Rasterelektronenmikroskopie erlaubt eine weitaus höhere Vergrößerung als die klassische Lichtmikroskopie bei gleichzeitig höherer Auflösung und Schärfentiefe. Somit konnten auch kleinste eidonomische (Bestimmungs-) Merkmale wie das Dorsalorgan oder einzelne Setae-Typen bei Zoea-Larven detailliert beschrieben und als neue oder früher wenig beachtete morphologischen Merkmale zur systematischen Einordnung herangezogen werden (Publikationen I, II und III). Des Weiteren konnte mit Hilfe der Fluoreszenzmikroskopie anhand von DAPI-Färbungen gezeigt werden, dass die Anordnung der Zellkerne von Zoea-Larven aus den verschiedenen Unterordnungen Caridea, Anomura und Brachyura charakteristische Muster aufweist. Dieses Kriterium wird als möglicher Merkmalssatz in der Taxonomie diskutiert (Publikation VI). Ein weiteres Feld

der modernen Taxonomie wird durch Publikation V abgedeckt: molekulare Analysen auf der Basis des mitochondrialen proteincodierenden Genes COI (cytochrome oxidase subunit 1) bzw „barcoding“-Gens. Zum ersten Mal für die südchilenische Fjordregion wurde mit dem Ansatz der integrativen Taxonomie die dortige Dekapoda-Fauna erfasst und analysiert. Nahe verwandte Arten der Gattungen *Eurypodius* Guérin, 1825 und *Acanthocyclus* Lucas, in H. Milne Edwards & Lucas, 1844, die morphologisch schwer zu trennen sind, konnten neu charakterisiert werden. In der Arbeit wurden klassische, morphologische Merkmale mit molekularen, morphologieunabhängigen Merkmalen kombiniert.
Durch eine vorherige Inventarisierung der südchilenischen Dekapodenfauna während zahlreicher Expeditionen in die Region konnte zudem die Basis für die taxonomische Arbeit (ca. 650 Samples sind in der Zoologischen Staatssammlung München hinterlegt) geschaffen werden. Eine ausführlichen Dokumentation mit verschiedenen bildgebenden Methoden wie der Verwendung von tiefenscharfen Aufnahmen und *in situ* Fotos der verschiedenen Arten dieser noch nahezu unerforschten Region bildet das Rückgrat der taxonomischen Arbeiten und ist als Kapitel in dem zweisprachigen (Spanisch und Englisch) Standardwerk für die Region publiziert (PublikationVI).

Summary

Decapod crustaceans are a highly diverse and well adapted group belonging to the phylum Arthropoda. Representatives can be found in most aquatic ecosystems and in terrestrial habitats. The huge number of species, about 17,635 recent and fossil species are known (De Grave et al., 2009), but also the old age of the group makes a systematic classification of single species difficult. Fossil decapods were dated back to the Devonian (about 415 Mya to 359,2 Mya) (Schram et al., 1978). Because of the old age of the group there has been ample time for evolution. The results of this ongoing process are reflected in an enormous morphological variety among the species. For a coherent classification of this group and species determination it could be essential to establish new morphological features and combine new methods. Furthermore a proper identification and classification of species forms the basis of various databanks and projects e.g. GenBank, Barcoding of Life (BOLD), German Barcode of Life (GBOL) and the Barcoding Fauna Bavarica show the high significance of taxonomist’s work.

The aim of this dissertation is to find and establish new features for the classification of decapods by various modern morphological methods i.e. scanning electron microscopy (SEM), fluorescence microscopy and morphology independent features like the analyses of gene sequences (cytochrome oxidase subunit 1). But additionally, classical methods like the use of morphological features in a combined, integrative approach are used for species delineation. In different publications we used SEM techniques which allow us in comparison to light microscopy a closer examination of morphological features (article I, II and III). It was possible to describe the dorsal organ and the different types of setae of zoea larvae in detail and use these features for systematic classification. Furthermore we used fluorescence microscopy and DAPI staining to describe and characterize nucleus patterns in various representatives of Decapoda of the Infraorders Caridea, Anomura and Brachyura. Results of these examinations show that nucleus patterns are characteristic for each Infraorder. In Article VI this feature is discussed as possible taxonomic criterion.

In recent times molecular taxonomy gains more and more in importance. Integrative taxonomy combines sequence analyses of the COI gene (cytochrome oxidase subunit 1) or "barcoding gene" with classic morphological features. It is used to characterize and analyze the decapod fauna of the southern Chilean fjord region (article V). Furthermore, on the basis of our data, it was possible to give exact species descriptions for closely related and not always clear to distinguish representatives of the genera *Eurypodius* Guérin, 1825 and *Acanthocyclus* Lucas, in H. Milne Edwards & Lucas, 1844.

As a backbone for this study serve the results of an intensive inventory of the southern Chilean fiord region. During various expeditions in that region about 650 samples of decapods were collected and are now deposited for further investigations at the Bavarian State Collection of Zoology. Samples are documented in detail using different imaging methods i.e. *in situ* pictures and high resolution photos of the different species of this unique and unexplored region are published as a chapter in the bilingual (Spanish and English) standard work for the Chilean fjord region (article VI).

Table of contents

1. General Introduction

1.1. Introduction to the order Decapoda

The order Decapoda Latreille, 1803 (Greek δέκα, *deca-*, "ten", and πούς / ποδός, *-pod*, "foot") consists of about 233 families containing 2,725 genera and an estimated 17,635 species (including both extant and fossil species) (De Grave et al., 2009). It contains shrimps, lobsters and crabs and therefore the range of morphological diversity among the extant decapods is immense: it ranges among others from the shrimp-like representatives of the infraorder Caridea to the highly variable representatives in the infraorder Anomura and the “true crabs” placed in the infraorder Brachyura. The morphological diversity is displayed in figure 1.

The wide variety of this group is not only shown in morphological aspects but also in the size of the animals. Commensal pea crabs (Pinnotheridae) such as the chilean crab *Pinnixa bahamondei* Garth, 1957, which inhabits tubes of the tube worm *Chaetopterus sp.* and with sizes of a few centimeters can be found in this group as also the giant Japanese spider crab *Macrocheira kaempferi* (Temminck, 1836) with legs spanning up to 3.7 meters (Bassler et al., 1931). This highly diverse group managed to colonize a wide variety of habitats: representatives can be found in most aquatic environments both, in fresh and saltwater and on all kinds of substrates (Abele, 1974, De Grave et al., 2008). Some representatives like the impressive and largest land living arthropod of the world, the coconut crab *Birgus latro* (Linnaeus, 1767) managed to conquer terrestrial habitats (Drew et al., 2010). Only the pelagic larval stages of this anomuran crab still depend on the aquatic environment (Brown et al., 1991). But in general the life history of Decapoda is complex and comprises different phases. For more information see chapter “Development”.

Figure 1: Different representatives illustrating the wide variety in the order Decapoda: **A** Harlequin shrimp (*Hymenocera picta* Dana, 1852), **B** Emperor shrimp (*Periclimenes imperator* Bruce, 1967) living commensally on the sea cucumber *Bohadschia argus* Jaeger, 1833, **C** Painted rock lobster (*Panulirus versicolor* (Latreille, 1804)), **D** Hairy squat lobster (*Lauriea siagiani* Baba, 1994), **E** Porcelain anemone crab (*Neopetrolisthes maculatus* (H. Milne Edwards, 1837)) **F** Jaiba mora (*Homalaspis plana* (H. Milne Edwards, 1834)). All photos by the author.

1.2. Morphology

The decapod exoskeleton is differentiated in two main sections: (1) the cephalothorax consisting of the fused head (cephalon) and trunk or pereion, and (2) the pleon. Appendages of the cephalothorax are the 1st and 2nd antennae (antennule and antenna), all mouthparts (mandible, 1st and 2nd maxilla, 1st to 3rd maxilliped), and the thoracic appendages (5 pairs of peraeopods). In many decapods the first peraeopods have enlarged pincers (chelae) and are therefore called chelipeds (Brachyura). The cephalothorax is covered by a protective carapace, which is divided in the frontal, hepatic, gastric, cardiac, branchial and intestinal regions. Further appendages are found on the 7-segmented pleon. Each segment carries a pair of biramous pleopods. The first pair of pleopods can be modified in the male as gonopods (e.g

the petasma). The last pleopods together with the telson form the tail fan and are called uropods. For the morphological nomenclature of the appendages see figure 2.

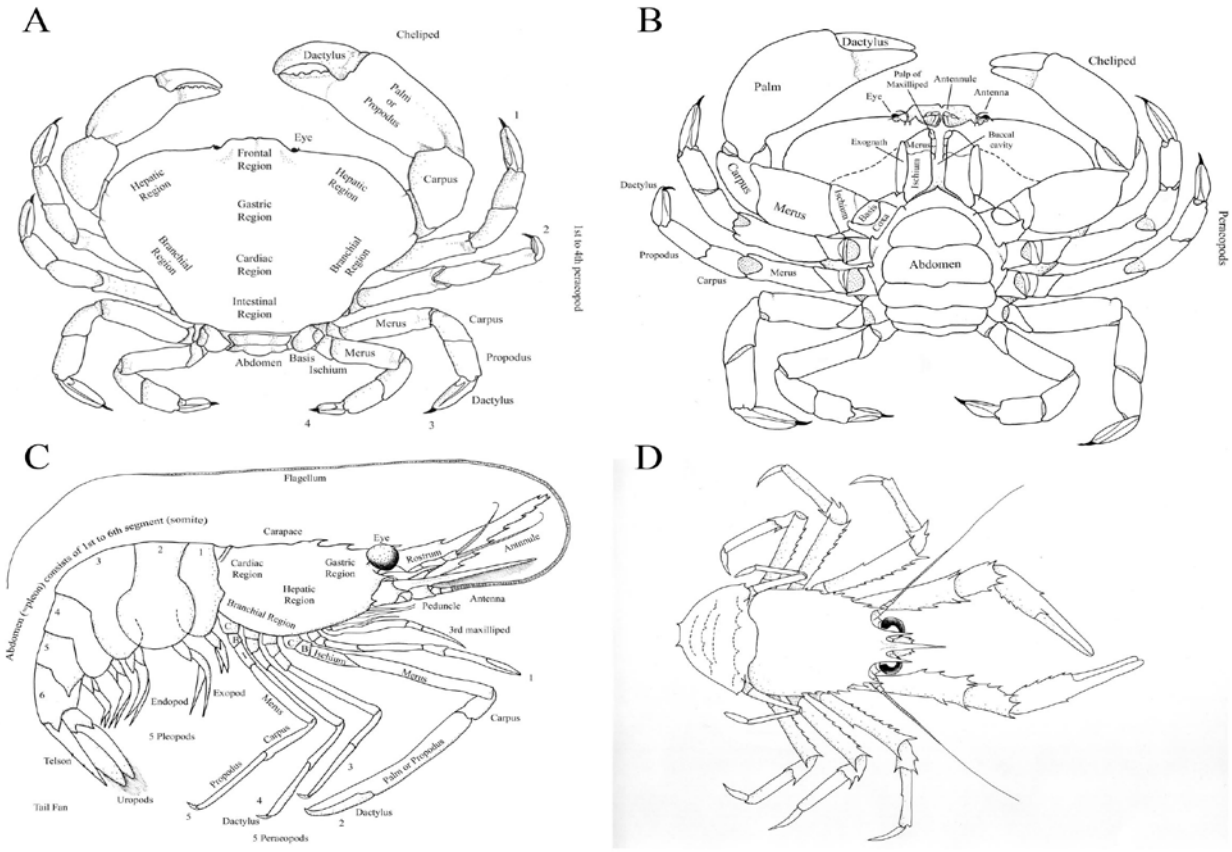


Figure 2: **A, B** Schematic drawings of a brachyuran crab in ventral and dorsal view, **C, D** caridean shrimp in lateral view and anomuran crab in dorsal view. from Meyer et al., (2009).

1.3. Phylogeny

The classification of Decapoda has a long history and has been revised several times in the last centuries: following the Challenger expedition, Bate (1888) erected the sub-order Macrura (Macrura refers to the long tail and well developed abdomen of most decapods) and three divisions within, the Trichobranchiata, the Dendrobranchiata and the Phyllobranchiata. Later, these divisions were no longer accepted and Borradaile (1907) divided the Decapoda into two sub-orders: the Natantia (“swimmers”) and the Reptantia (“crawlers”). The Natantia united all forms that swim in the water column i.e. penaeideans, carideans and stenopodids but showed up to be a non-monophyletic group. In recent times, the order Decapoda is divided into two monophyletic sub-orders: the ancestral group, Dendrobranchiata (prawns), and the Pleocyemata (shrimps, true crabs, lobsters etc.) after Burkenroad (1963). These two

suborders are distinguished by their gill structure, which is branched in Dendrobranchiata (dendro: tree; branchia: gill) and has a lamellary structure in Pleocyemata. All taxa of Pleocyemata share a number of synapomorphic features, the most important of which is that the fertilized eggs are incubated by the females and remain stuck to the pleopods until the zoea larvae are ready to hatch (see figure 3). This character gave the group its name. The Pleocyemata are subdivided into different infraorders: the Stenopodidea (Cleaner Shrimps), the Caridea (Shrimps, Coral Shrimps, Snapping Shrimps), the Astacidea (Freshwater Crayfish, True Lobsters, Reef Lobsters, Scampi), the Glypheidea, the Axiidea (Ghost Shrimps, Mud Shrimps, Sponge Shrimps), the Gebiidea, the Achelata (spiny lobsters, slipper lobsters), the Polychelida, the Anomura (Hermit Crabs, King Crabs, Squat Lobsters, Porcelain Crabs, Mole Crabs) and the Brachyura (True Crabs) (Martin & Davis, 2001).

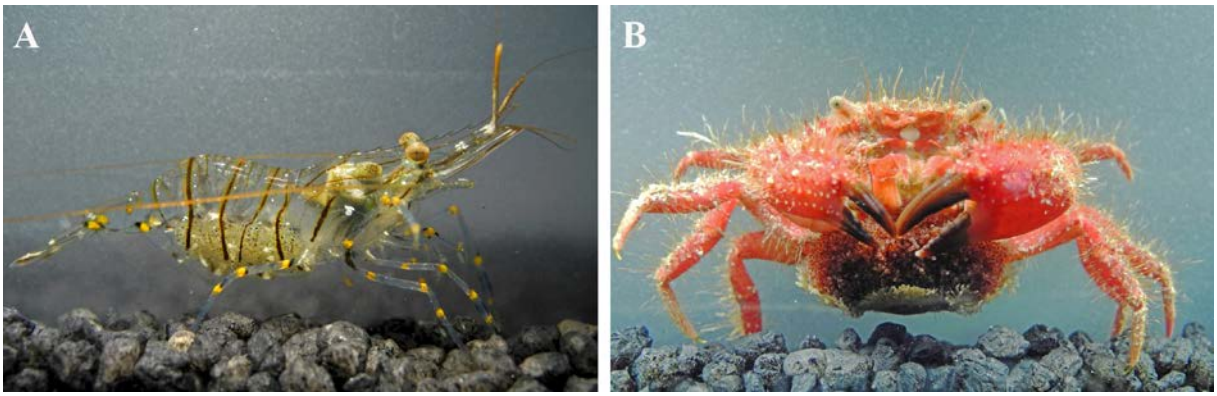


Figure 3: **A** Ovigerous female of the caridean shrimp *Palaemon elegans* Rathke, 1837 and **B** the brachyuran crab *Pilumnus spinifer* H. Milne Edwards, 1834. The fertilized eggs are stuck at the pleopods and incubated by the females till larvae hatch. Photos by A. Böttcher.

1.4. Development

The Pleocyemata undergo indirect development and have a pelago-benthic life cycle. This means that the larvae are planktontic and the adults live in the benthos (Anger, 2001). On the other hand, in the Dendrobranchiata all developmental stages live freely in the water column (i.e. they exhibit a holopelagic life cycle). In the Pleocyemata the fertilized eggs are carried on the female's pleopods, while in the Dendrobranchiata the eggs are set free into the water column. In the Dendrobranchiata the immatures hatch as nauplius larva. After 6 molts and anamorphic growth (i.e. the development of new segments at the posterior part of the larva) the nauplius develops into the zoea larva (see figure 4). The larvae of the Pleocyemata do not hatch until the zoea stage is reached, since the development of the nauplius occurs inside the

egg. While the zoea larvae of the Dendrobranchiata develop into the adult, pelagically-living prawn through several molts, the zoea larvae of the Pleocyemata pass through several morphologically different zoea stages. After a series of molts they develop into the 1st benthic stage (i.e. the megalopa) and after another molt they develop into the adult-shaped pleocyemate decapod (Gurney, 1942, Anger, 2001).

Both the indirect development and the ecological habitat separation of juvenile and adult forms (i.e. pelagic/benthic) allow Decapoda larvae to be distributed over vast distances by the ocean currents and to colonize new areas as adults.

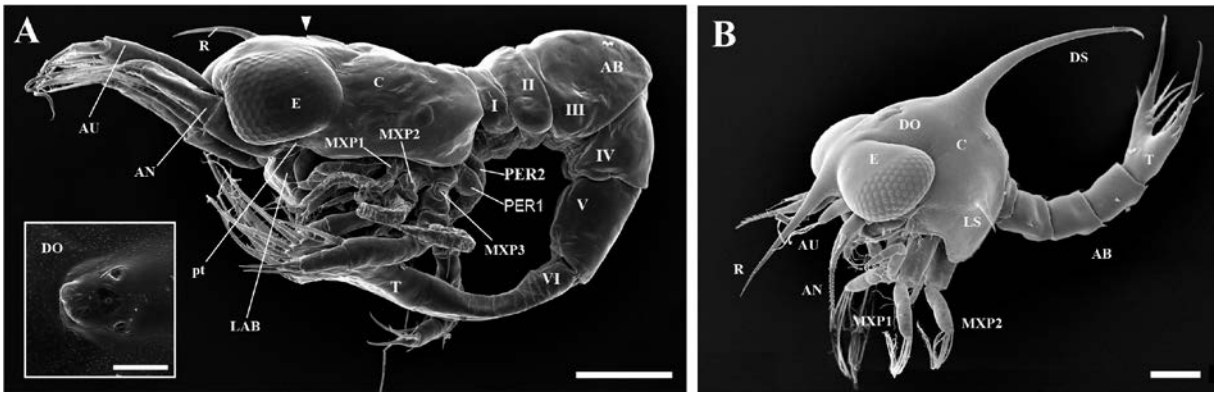


Figure 4: SEM-pictures of the first stage of zoea larva of **A** the caridean shrimp *Gnathophyllum elegans* and **B** the brachyuran crab *Portunus acuminatus*. Abbreviations: I-VI, abdominal segments; AB, abdomen; AN, antenna; AU, antennule; C, carapace; DO, dorsal organ; DS, dorsal spine; E, eye; LAB, labrum; LS, lateral spine; MXP 1-3, first to third maxillipeds; PER1&2, pereiopods 1&2; PT, pterygostomial spine; R, rostrum; T, telson. Scale bars: A, 200µm; insert 20µm; B, 100µm. (left picture from Meyer et al., (2013), right picture from Meyer et al., (2006)).

1.5. Morphological approach

The decapod´s exoskeleton with its external structure and organization offers a wealth of species specific features, which are traditionally used for species characterization. Therefore species descriptions of decapod crustaceans are mainly based on characteristic eidonomic features and species delimitation in this group is defined by comparative morphology. But especially the larval morphology of zoea larvae is very similar and thus new sets of data are helpful to distinguish larvae of different species from each other i.e. in plankton hauls. Classical works (Lebour, 1930, Aikawa, 1937, Gurney, 1938) have formed standards for

larvae descriptions and features have been established during recent decades and displayed as drawings as well as in textual presentations (Rice, 1980, Rice, 1981, Ingle, 1992).

By using modern methods in this thesis it was possible to analyze the external morphology of larval stages in greater detail and create additional sets of data i.e. the morphology of the sensory dorsal organ or the classification of different seta types described through the application of SEM. These additional results complement and support the morphology-based descriptions and can be set in taxonomic context (article I, II, III).

But also historic species descriptions of adult specimens can be sometimes confusing and not always clear in the characterization of features as displayed in article V within the 2 genera *Acanthocyclus* in H. Milne Edwards & Lucas, 1844 and *Eurypodius* Guérin, 1825. Furthermore the interpretation of morphological features can be subjective (Padial et al., 2010). Consequently the described morphospecies (=species based only on morphological features after Cain (1954)) should be confirmed by other, non-morphological data i.e. DNA sequencing in an approach of integrative taxonomy (Dayrat, 2005). In article V we combine both methods in an integrative approach to determine constant morphological features for species description and determination, and use DNA barcodes to check species delimitations.

1.6. Molecular analyses and integrative taxonomy

Molecular approaches are conquering the field of taxonomists work on a grand scale. Hebert et al. (2003) introduced the DNA barcode, an approximately 650-bp long segment of the mitochondrial cytochrome oxidase I gene, as a reliable tool for species identification. The morphology independent method is meanwhile established for various taxa, including common marine invertebrate groups like pycnogonids (Nielsen et al., 2009, Arabi et al., 2010, Krabbe et al., 2010, Masta et al., 2010, Dietz et al., 2011, Weis & Melzer, 2012), molluscs (Wilson et al., 2009, Joerger et al., 2010), echinoderms (Heimeier et al., 2010, Vardaro, 2010, Bribiesca-Contreras et al., 2013) and crustaceans (Lefebure et al., 2006, Costa et al., 2007, Pérez-Barros et al., 2008, Miguel Pardo et al., 2009).

This “tool” offers taxonomists new possibilities: cryptic species complexes that seem to be very common in the marine environments (Knowlton, 1986, Knowlton, 1993) and are hard to resolve on the basis of morphological data solely can be identified and documented by molecular analyses. It can also be applied where no morphological information is available for example degraded specimens or fragments of organism or even pieces of tissues so that the diagnostic characters are lost (Schander & Willassen, 2005). In the field of carcinology

this technique furthermore is useful to identify pelagic larvae of decapod crustaceans (Miguel Pardo et al., 2009, Tang et al., 2010) as many of these are yet not described in literature and morphological data thus are not available.

Barcoding was used in addition to traditional morphological methods for the investigation in the southern Chilean inventory of decapod crustaceans. In a case study, the approach of integrative taxonomy was useful to distinguish the single species of the genera *Acanthocyclus* (*A. gayi* Lucas, in H. Milne Edwards & Lucas, 1844, *A. hassleri* Rathbun, 1898, *A. albatrossis* Rathbun, 1898) and *Eurypodius* (*E.longirostris* Miers, 1886, *E. latreillii* Guérin, 1825) from each other and identify constant morphological species description characters.

But for the efficient use of the COI-data a worldwide species inventory, proper identification by taxonomists and barcoding of specimens are the backbone. Datasets of the yet barcoded species as reference data are published in various databases like BOLD (www.boldsystems.org) or GenBank (www.ncbi.nlm.nih.gov/genbank/).

With the inventory of decapod crustaceans of the southern Chilean fjord region and their proper identification based on morphological features (article VI) and the further barcoding of these species in cooperation with BOLD (article V) we made this data available for taxonomists worldwide.

2. Sampling and sourcing of specimens

2.1. Methods

Because of the worldwide distribution of decapods it was possible to include different geographic regions for this study and use given infrastructures for sampling i.e. biological stations and excursions. Sampling methods remained the same on all field trips and various techniques were used for collecting specimens including hand sampling while snorkeling or Scuba diving and trap or dredge collection. All specimens presented in the different publications were sampled from a variety of habitats in depths between 0 and 40 m and are deposited at the Bavarian State Collection of Zoology for reference purpose and further investigation.

To obtain identified zoea-larvae, ovigerous females were sampled, identified to species level and kept in aquarium enclosures till larvae hatched. To ensure a stable environment and good keeping conditions as well as appropriate conditions for the hatchery, we cooperated with Dr. Jens Bohn of the SEA LIFE Munich. Determination of the adult specimens was done using

external, eidonomical features such as the number of carapace spines or the shape of the legs and other appendages following various identification literature, e.g.(Rathbun, 1918, Rathbun, 1925, Rathbun, 1937, Haig, 1955, Garth, 1957, Haig, 1960, Zariquiey Álvarez, 1968, Retamal, 1981, Riedl, 1983). In addition, original species descriptions were checked.

2.2. Adriatic Sea and North Atlantic

Decapod larvae studied and published in article II, IV and partly in article III were obtained using the infrastructure of courses in marine biology of the Ludwig Maximilians Universität Munich at the "Station Biologique de Roscoff", Roscoff (France) and the "Institut Ruđer Bošković", Rovinj (Croatia).

First examinations on marine Decapoda larvae of the Rovinj-region were carried out by the work group "Arthropoda varia" in the year 2003. The results are presented in the form of my diploma theses "Decapoda-Larven aus der Nordadria: REM-Merkmalsanalyse und Atlas" and were published in Meyer et al. (2004) and Meyer and Melzer (2004). This research can be seen as preliminary work for the SEM-larvae analyses carried out in this thesis. During these projects it was possible to establish a zoea-larvae-collection at the Bavarian State Collection of Zoology for further investigations. Meanwhile several examinations have been conducted which were based on this collection i.e. Geiselbrecht and Melzer 2010 and Geiselbrecht and Melzer 2013.

2.3. Central Pacific Ocean

The material that was used in publication article I and partly in article III was obtained in the Gulf of Nicoya, Central Pacific Ocean in the framework of the research project "Desarrollo de estándares para una pesca sostenible del camarón camello (*Heterocarpus vicarious* Faxon, 1893) in the working group of Dr. Ingo Wehrtmann, Universidad de Costa Rica.

2.4. Southern Pacific Ocean

For samples of the research projects presented in article V and the book chapter "Decapoda - crabs, shrimps & lobsters" (article VI) we were able to use the infrastructure of the Huinay Scientific Field Station (42°22´ S, 72°24´ W), located at the Comau fjord, southern Chile (figure 5 A). Up to now, it is the only scientific field station in all the vast Chilean Patagonia.

The directorial staff (Dr. V. Häussermann and G. Försterra) is organising expeditions to remote fjord regions. Sampling areas and dive conditions are illustrated in figure 5 B and 5 C. Since 2003 several expeditions have been accompanied by members of the work group “Arthropoda varia”. To date about 650 specimens of decapods of the southern Chilean region are housed at the Bavarian State Collection of Zoology as result of this cooperation. This material served as basis for these research projects. I hope the material will serve for further investigations of the southern Chilean decapod fauna and will help to conserve this unique region.

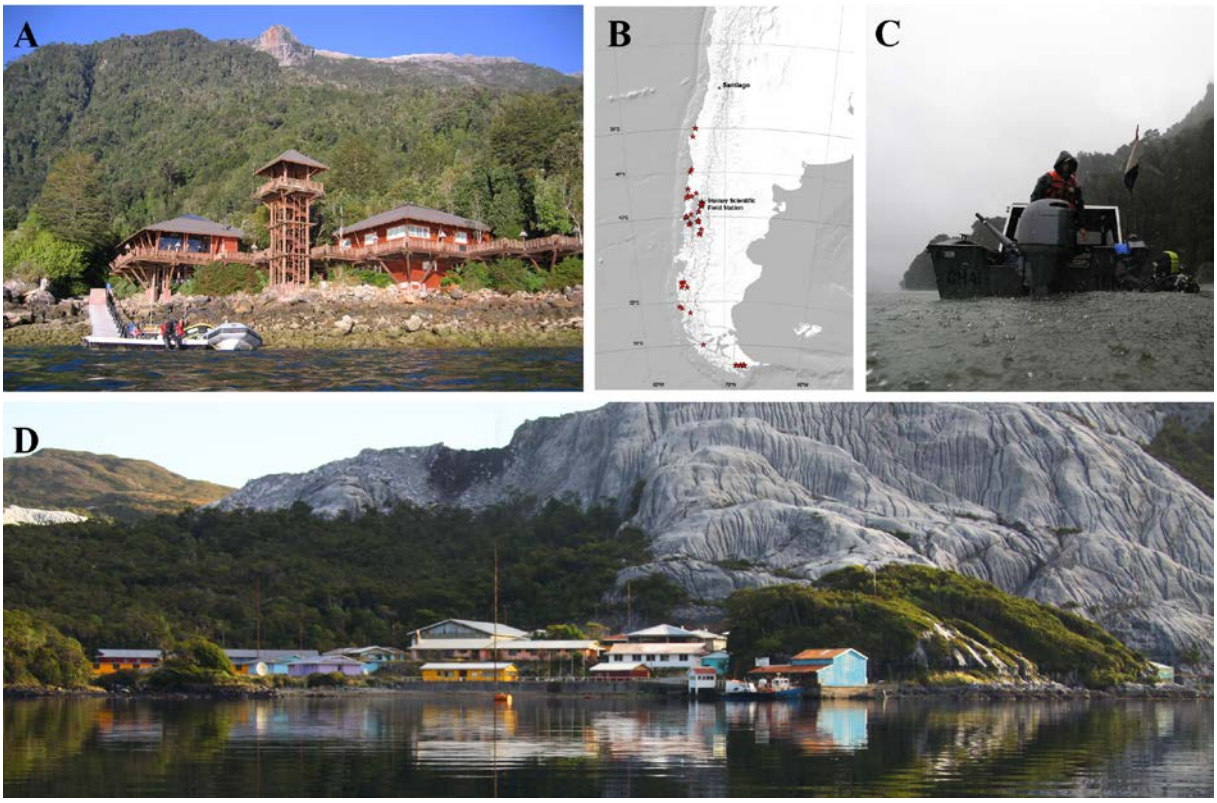


Figure 5: A The Huinay Scientific Field Station, Chile, B Sample region of the “Huinay Fiordos”- Expeditions in southern Chile, C typical Patagonian dive conditions. D Guarello Island (50°23′ S 75°20′ W), base of the HF 16 Expedition. All photos by the author.

3. Article I

Meyer R, Wehrtmann IS, Melzer RR (2006) Morphology of the first zoeal stage of *Portunus acuminatus*, Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. Crustaceana 70(2): 261-270.

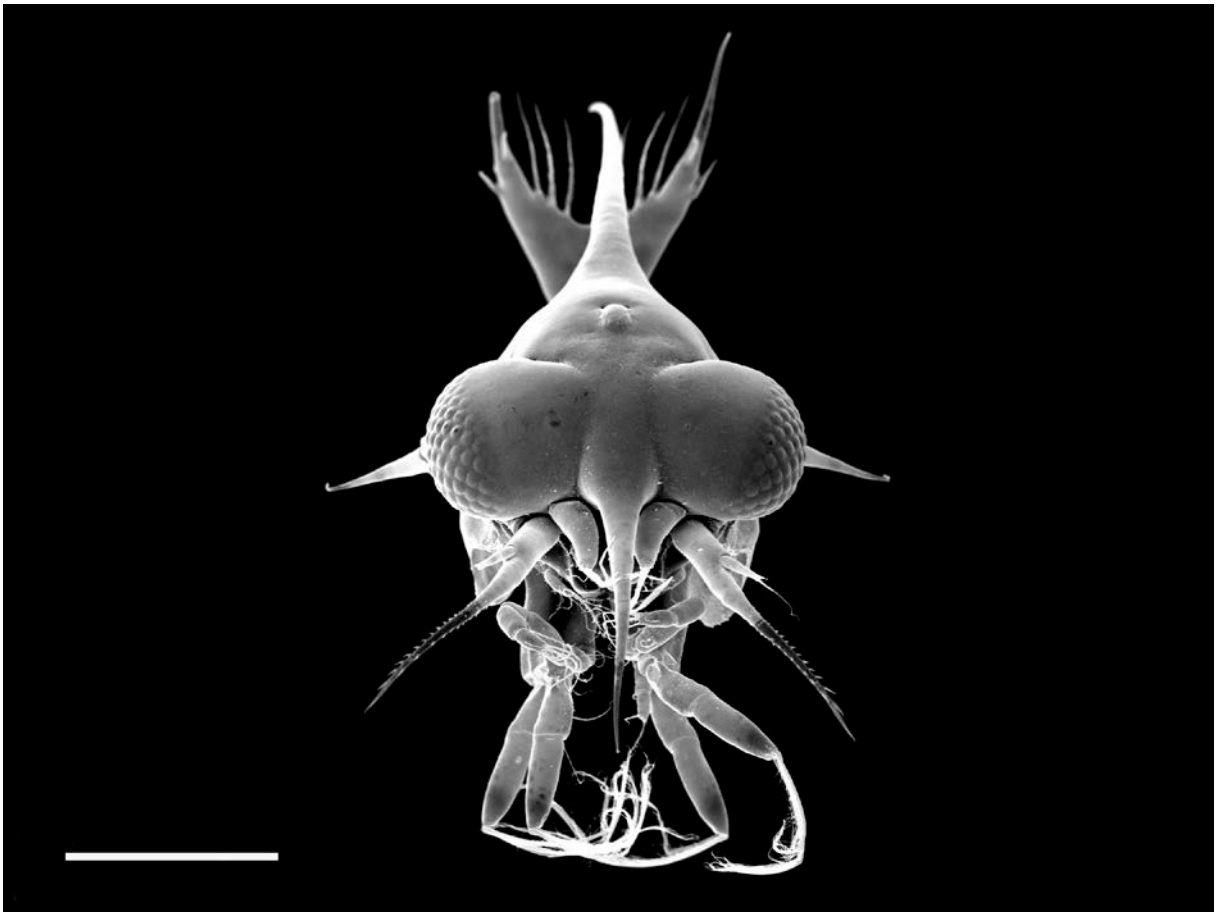


Figure 6: SEM-picture of the first zoea stage of *Portunus acuminatus* in frontal view (bar 200 µm). Photo by the author.

SCIENTIA MARINA 70 (2)
June 2006, 261-270, Barcelona (Spain)
ISSN: 0214-8358

Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory

ROLAND MEYER [1], INGO S. WEHRTMANN [2] and ROLAND R. MELZER [1]

[1] Zoologische Staatssammlung, Münchhausenstr. 21, D-81247 München.E-mail: kld1129@mail.lrz-muenchen.de
[2] Universidad de Costa Rica, Escuela de Biología, Museo de Zoología, 2060 San Pedro-San José, Costa Rica.

SUMMARY: Larvae of *Portunus acuminatus* (Stimpson, 1871) from one female, collected by trawling at a depth of 12 m in the Gulf of Nicoya, Pacific Costa Rica, Central America (090°48.899'N, 084°40.498'W) were hatched in the laboratory. The morphology of zoea I is described and illustrated for the first time and compared with known zoeae of other portunid species belonging to the subfamily Portuninae. We present a combination of three features which allows zoea I larvae of *P. acuminatus* to be distinguished from other described larvae of the genus. Descriptions are based on dissected larvae analysed by SEM and light microscopy.

Keywords: larval morphology, zoea, description, scanning electron microscope, Portunidae, Costa Rica.

RESUMEN: MORFOLOGÍA DE LA PRIMERA ZOEA DE *PORTUNUS ACUMINATUS* (STIMPSON, 1871) OBTENIDA EN EL LABORATORIO. – Se describe el primer estadio larvario del cangrejo *Portunus acuminatus*. Las larvas se obtuvieron en el laboratorio a partir de una hembra ovígera capturada en el Golfo de Nicoya (090°48.899'N, 084°40.498'W), Pacífico de Costa Rica. La descripción se ha realizado con la ayuda del microscopio electrónico de barrido y el microscopio óptico. Los caracteres morfológicos son comparados con los de otras especies de la subfamilia Portuninae. Presentamos una combinación de tres caracteres que permiten distinguir la primera zoea de *P. acuminatus* de otras larvas del género.

Palabras clave: morfología larval, zoea, SEM, Portunidae, Costa Rica, descripción.

INTRODUCTION

The swimming crab *Portunus acuminatus* (Stimpson, 1871) is a shallow water species distributed along the Pacific coast of America, from the Gulf of California (USA) to La Libertad (Ecuador). Sandy and/or muddy sediments are the habitat of *P. acuminatus*. Ovigerous females can be found from February to May (Garth and Stephenson, 1966).

The morphology of *P. acuminatus* zoeae has not been described yet. In the present study we describe and illustrate the first zoeal stage hatched in the laboratory and compare its morphology with described zoeae of other portunid species within the subfamily Portuninae. The study gives a detailed description of the larvae by analysing all morphological structures, by using a combination of SEM, light microscopy and dissection techniques. This includes an analysis of the inner, molar part of the mandibles with the SEM.

MATERIALS AND METHODS

Ovigerous females of *P. acuminatus* were trawled in April 2004 at a depth of 12 m in the Gulf of Nicoya, Pacific Costa Rica (90°48.899'N, 84°40.498'W). Individuals were transported to the

laboratory of the Universidad de Costa Rica, San José, and held in separate aquaria containing filtered seawater at ambient temperature and salinity (22±2°C, 33.0 psu). The females were identified according to Garth and Stephenson (1966). Water was changed daily. Ovigerous females were not fed, and kept under these conditions until the larvae hatched.

Recently hatched larvae were removed from the vials and fixed in a graded ethanol series (30%, 50%, 70%, 10 min. each) (see Meyer and Melzer, 2004). Fixed larvae were transported in August 2004 to the Zoologischen Staatssammlung München (Germany), where the SEM and light microscope preparation was done.

SEM preparation: fixed specimens were dehydrated in a graded acetone series (70%, 80%, 90%, 2 x 100%, 10 min. each). Larvae were either critical-point-dried in a Baltec CPD 030 or in HMDS (Hexamethyldisilazane) after Nation (1983) (see also Laforsch and Tollrian, 2000). After mounting on SEM stubs with self adhesive carbon stickers, individuals were dissected using a binocular and thin tungsten wires to make sure that all appendages were optimally orientated and separated for the scanning procedure. The dried specimens were coated with gold on a Polaron "Sputter Coater" and studied with a LEO 1430VP SEM at 10-15kV. To make sure that no setae on the appendages were removed or broken during the dissection, several appendages of each type were scanned and compared.

Light microscopy: ethanol fixed specimens were dissected in glycerine using a dissecting microscope and tungsten wires. For light microscopy, a Leica DM RBE and an Olympus SZX 12 equipped with a Visitron Spot Insight Colour digital camera were used.

It was not possible to dissect the complete set of appendages of a single, individual zoea. Therefore, many zoeae were prepared, and setae were counted from between 6 and 10 specimens of each type of appendage. The drawings of the maxillule and the maxilla were made with the aid of a camera lucida and then compared with the SEM data to analyse the different types of seta and smaller structures. For classification of the different types of setae we follow the terminology of Ingle (1992).

Measurements of the Zoea-I-larvae were done using LEO's SEM-User-Interface-Software. Carapace length (CL) was measured from the base of the rostrum to the posterior margin, carapace width (CW) as the distance between tips of lateral spines, the total length (TL) from the base of the rostrum to the tip of the furca, dorsal spine length (DS) from the base of the dorsal spine to the tip, rostral length (RL) from the base of the rostral spine to its tip, and the rostrodorsal length (RDL) as the distance between the tip of the dorsal spine and the tip of the rostral spine. Measurements are based on a total of 10 larvae.

The female and zoeae of *P. acuminatus* were deposited at the Zoologische Staatssammlung München under the registration numbers ZSMA 20050130 for the adult and ZSMA 20050131 for the larvae.

RESULTS

Description of the Zoea I

Dimensions [μm]: RDL = 996.61 ± 36.5, RL = 293.14 ± 6.7, DS = 427.1 ± 18.5, TL = 1041.3 ± 27.2, CW = 528.4 ± 19.5, CL = 363 ± 23.9.

General Characteristics (Fig. 1A-C)

Compound eyes sessile (Fig. 1A, B). Dorsal organ in anterio-median region of the carapace (Fig. 1A, B). Carapace surface covered with tuberculettes (Fig. 1A, insert), with posteriorly curved smooth dorsal spine and lateral spines (Fig. 1A). Dorso-lateral region, between dorsal and lateral spine, with a pair of pappose setae (Fig. 1A, B). Anterior part of rostral spine with small denticles (Fig. 1A). Abdominal segments 2-5 with dorso-marginally located setae (Fig. 1B).

Carapace (Fig. 4A): Group of pore-like structures located in the dorso-median region (Fig. 4A). Two rows of pores posterior to dorsal spine; anterior row with 4 pores, posterior row with 2. 2 pappose setae in the dorso-lateral region.

Antennule (Fig. 2A): Conical, unsegmented, with 2 aesthetascs and 2 single setae.

Antenna (Fig. 2A): Elongated spinous process bears on its proximal part setules (S) grading on the distal half in two rows of minute spines (D, inserts). Exopod unsegmented, with 2 terminal simple setae unequal in length.

Labrum (Fig. 4B): Posterior portion invested with numerous small denticulettes; labrum without setae.

Mandible (Fig. 4C, D): Left and right mandible dissimilar. Left mandible: outer margin of incisor process armed with 9 marginal spines; molar process a broad structure with 9 marginal and 2 sub-

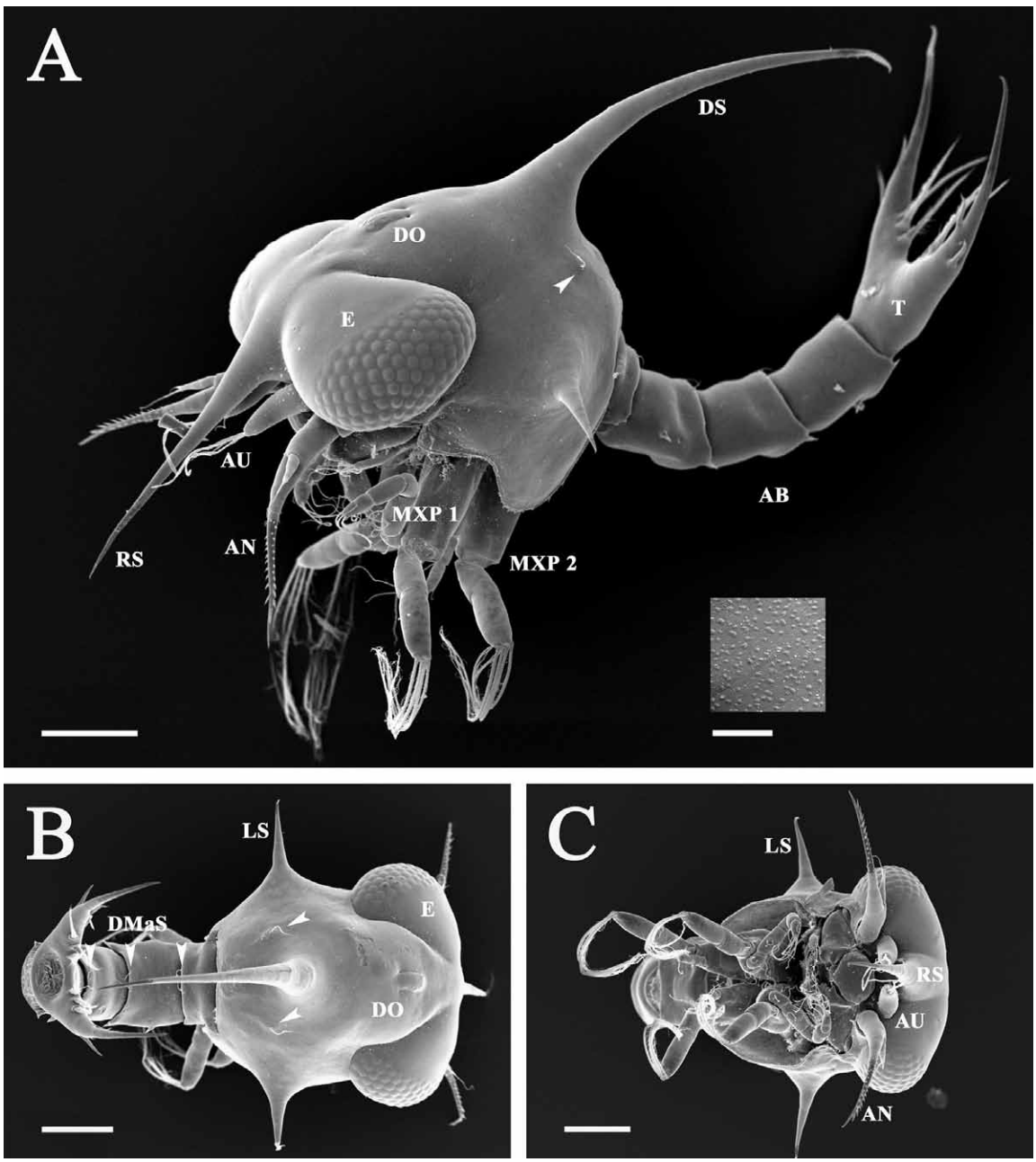


FIG. 1. – *Portunus acuminatus*, zoea I. – A general overview. A: Lateral view; insert shows carapace structure. B: Dorsal view. C: Ventral view. *AB*: abdominal segments, *AN*: antenna, *AU*: antennule, *DO*: dorsal organ, *DMaS*: dorso-marginal setae, *DS*: dorsal spine, *E*: eye, *LS*: lateral spine, *MXP1*: first maxilliped, *MXP2*: second maxilliped, *RS*: rostral spine, *T*: telson. Arrows show setae in dorso-lateral region and dorso-marginal setae. All scale bars 100 μm, insert 10 μm.

marginal spines. Right mandible: incisor process with two acute protrusions, inner margin of molar process with 8 marginal spines.

Maxillule (Fig. 4E, 5A): Coxal endite unsegmented with 6 plumodendiculate setae and one subterminal simple seta (s). Endopod 2-segmented; 4 terminal setae (one simple seta (s) and 3 thin plumodenticulate setae) and 2 subterminal thin plumodenticulate setae; proximal segment unarmed. Basial endite unsegmented; with one thin, subterminal plumodenticulate (p), two cuspidate (c) and two plumodenticulate (p) setae; microtrichia located on inner margin.

Maxilla (Fig. 4F, 5B): Coxal endite bilobed, with 3+3 plumodenticulate setae. Basial endite bilobed, with 4+4 plumodenticulate setae. Endopod unsegmented, bilobed, with 2 long setae on proximal and 3 on distal lobe; long microtrichiae on both margins

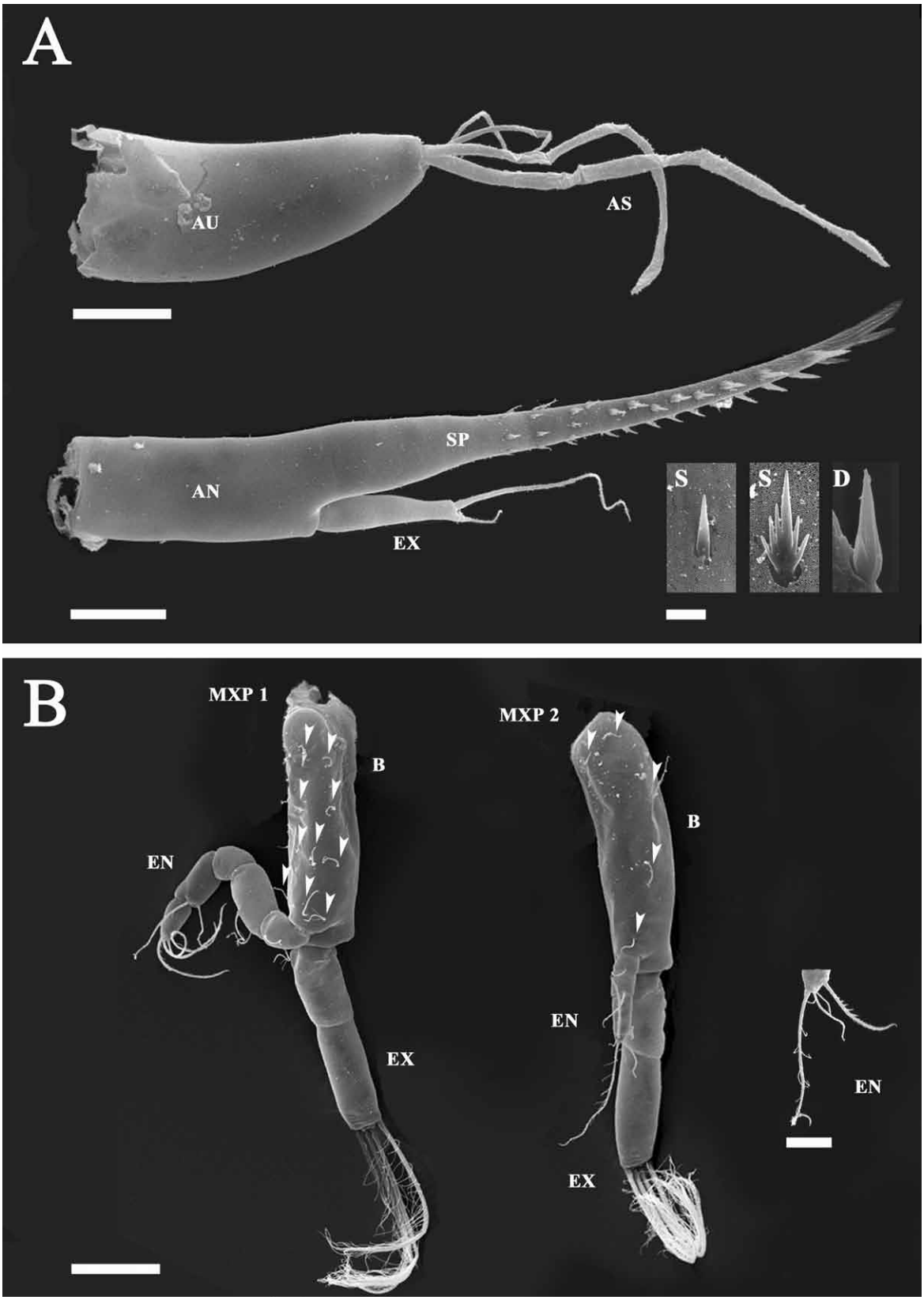


FIG. 2. – *Portunus acuminatus*, zoea I. – Appendages. A: Antennule (bar 20 μm) and antenna (bar 30 μm). Inserts: setules and denticles located on the spinous process of antenna (bar 2 μm). B: first and second maxilliped (arrows show setae arrangement on basis) (bar 60 μm) and distal part of endopod of maxiliped 2 (bar 20 μm). *AN*: antenna, *AS*: aesthetascs, *AU*: antennule, *B*: basis, *D*: denticle, *EN*: endopod, *EX*: exopod, *MXP1*: first maxilliped, *MXP2*: second maxilliped, *S*: setule, *SP*: spinous process.

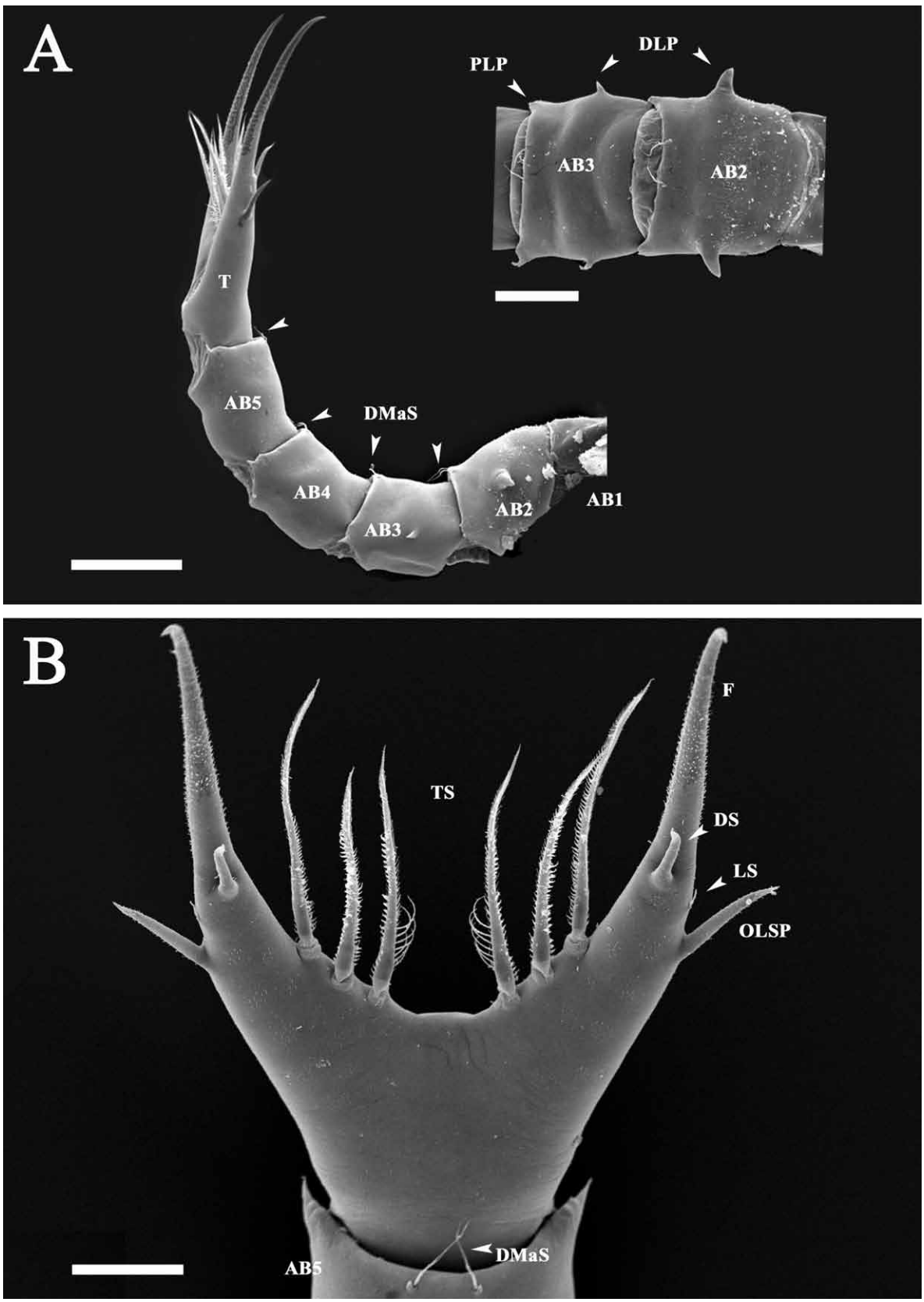


FIG. 3. – *Portunus acuminatus*, zoea I. – Appendages. A: Abdomen in lateral view (bar 100 μm) and abdominal segments 2-3 in dorsal view (bar 60 μm). B: Telson, dorsal view (bar 40 μm). *AB*: abdominal segments, *DLP*: dorso-lateral process, *DMaS*: dorso-marginal setae, *DS*: dorsal spine, *F*: furca, *LS*: lateral spine, *OLSP*: outer lateral spine, *PLP*: posterior-lateral process, *T*: telson, *TS*: telson setae.

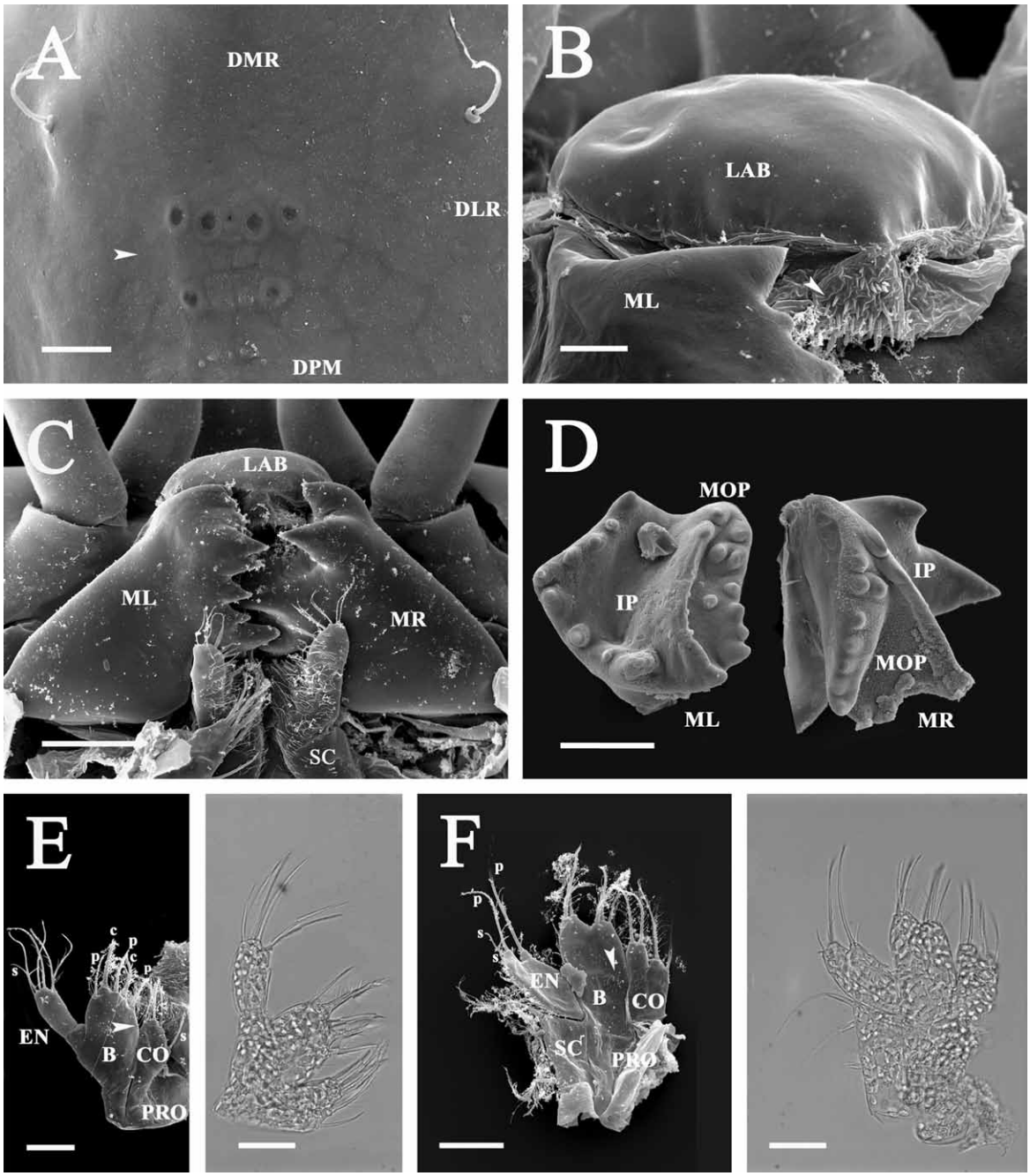


FIG. 4. – *Portunus acuminatus*, zoea I. – Appendages. A: carapace structure located at the dorso-median-region (bar 20 μm). B: Inner view on the labrum (bar 10 μm). C: Mandibles orientated in the zoea (maxillule and maxilla removed) (bar 40 μm). D: Inner view of the surface on dissected mandibles (bar 70 μm). E: Ventral view on the left maxillule; SEM and light microscope (bar 30 μm). F: Inner view of the right maxilla; SEM and light microscope (bar 30 μm). Arrows show microtrichia. *B*: basial endite, *c*: cuspidate seta, *CO*: coxal endite, *DLR*: dorso-lateral region, *DMR*: dorso-marginal region, *DPM*: dorso-posterior margin, *EN*: endopod, *IP*: incisor process, *LAB*: labrum, *ML*: left mandible, *MOP*: molar process *MR*: right mandible, *p*: plumodenticulate seta, *PRO*: protopod, *s*: simple seta, *SC*: scaphognathite

of the endopod. Scaphognathite (exopod) with 4 plumose marginal setae and a long distal stout process.

First maxilliped (Fig. 2B): Coxa without setae. Basis with 10 medial simple setae arranged 2+2+3+3 on inner side. Endopod 5-segmented, with 2,2,0,2,5 (1 subterminal and 4 terminal) sparsely plumose setae. Exopod 2-segmented; distal segment with 4 long plumose natatory setae.

Second maxilliped (Fig. 2B): Coxa without setae. Basis with 5 single setae arranged 2+1+1+1. Endopod 3-segmented, with 1,1,5 (2 plumodenticulate and 3 single setae). Exopod 2-segmented, distal segment with 4 plumose natatory setae.

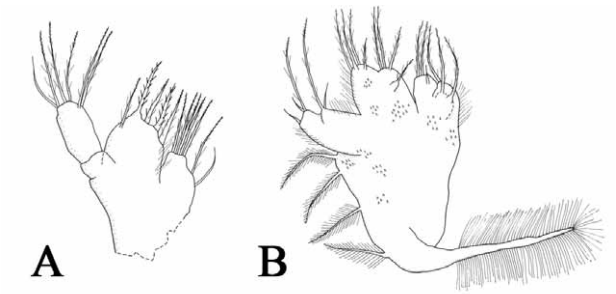


FIG. 5. – *Portunus acuminatus*, zoea I. A: maxillule (ventral view), B: maxilla (inner view).

Abdomen (Fig. 3A): 5-segmented; segments 2-5 with dorso-marginally located pair of single setae; segments 2 and 3 with dorso-lateral processes; segments 3-5 with posterio-lateral processes.

Telson (Fig. 3B): Posterio-external margins extended into furcae; inner margin with 6 plumodenticulate setae; the two innermost with broad medial setules; distal part of each branch with small denticles; each branch of furca with outer spines on proximal third.

DISCUSSION

The present description of *P. acuminatus* zoeae is based on a combination of SEM and light microscopical techniques applied to fixed larvae and dissected appendages. The advantage of this combination of techniques is that even minute setules or spines can be located and analysed using the SEM (e.g. Meyer *et al*. 2004). In addition, the three-dimensional structure of mouthparts can be studied in detail. However, it was not possible to get a complete SEM-preparation of the maxillule and the maxilla. Therefore light microscopy was used for an overview and SEM data were used for details to produce complete drawings of these two mouthparts.

Another advantage of our combined technique seems to be the fact that we could analyse in detail the inner part of the mandibles with the SEM. Ingle (1992) mentions that the left and right mandible in zoeae are usually slightly dissimilar and that details are not easy to resolve by light microscopy due to their gross three-dimensional structure. Using SEM combined with dissection allows a thorough analysis of mandibular structures, as shown by Greenwood and Fielder (1979) who described the mandibles of *Portunus rubromarginatus* using SEM. A comparison of the mandibular structures of *P. rubromarginatus* and *P. acuminatus* revealed differences between the species; thus, such analyses could give access to a relevant, and yet poorly studied set of characters for larval diagnosis.

Comparison of portunid zoeae

The family Portunidae Rafinesque, 1815, includes the following six subfamilies: Carcininae Macleay, 1838, Polybiinae (syn. Macropininae) Ortmann, 1893, Portuninae Rafinesque, 1815, Catoptrinae Borradaile, 1903, Caphyrinae Paul´son 1875 and Podophthalminae Dana, 1851 (Stephenson and Campbell, 1960). Larvae of only the first three of these were known when Rice and Ingle (1975) sought to survey their knowledge on portunid zoeae. They found distinctive features between the zoeae of the Carcininae, Polybininae and Portuninae subfamilies based on the presence or absence of carapace lateral spines, the number of abdominal segments with dorsolateral projections, the length of the posterio-lateral processes of abdominal segments 3 and 4, the telson fork armature, the number of setae of the telson's posterior border, and the armature of the middle segment of the endopod of the first maxilliped. Two of these characters can be studied in Zoea-I-larvae: (i) Carapace lateral spines are well developed in Polybiinae and Portuninae, but not in Carcininae. (ii) The middle segment of the endopod of the first maxilliped is armed in Polybiinae and unarmed in Portuninae (Rice and Ingle, 1975).

The morphological characters analysed in the present study correspond well with the subfamilial larval characters for the Portuninae established by Rice and Ingle (1975): **(1)** lateral carapace spines are well developed, **(2)** dorso-lateral projections are found on abdominal segments 2 and 3, **(3)** abdominal segments 3 to 5 bear posterior lateral processes, **(4)** the telson fork spine number is similar and **(5)** there is an unarmed endopod middle segment at the first maxilliped.

Since the publication of Rice and Ingle (1975) the larval stages of several species of Portuninae have been described, e.g. *Callinectes sapidus,* (Costlow and Bookhout, 1966), *Charybdis acuata,* (Kurata and Omi, 1969), *Portunus spinicarpus,* (Bookhout and Costlow, 1974), *Portunus gibbesii,* (Kurata, 1970), *Scylla serrata,* (Wear and Fielder, 1985), *Callinectes similis,* (Bookhout and Costlow, 1977), *Portunus rubromarginatus,* (Greenwood and Fielder, 1979), *Portunus pelagicus,* (Shinkarenko,

TABLE 1. – Morphological differences among Zoea-I-Larvae of selected representatives of genera and species of Portuninae (a: aesthetasc, DMaS: dorso-marginal setae, nd: no data, s: seta, (?): possibility of error, ≠ unequal in length). Characters shared by all Portuninae (antena exopod [2s≠], maxillule basial endite [5], maxillule distal segment [6], maxilla scaphognathite [4], exopod of first and second maxilliped [4], carapace lateral spines are present) are omitted.

Genera	*Portunus*								
Species	*P. acuminatus*	*P. pelagicus*	*P. pelagicus*	*P. pelagicus*	*P. gladiator*	*P. rubromarginatus*	*P. trituberculatus*	*P. spinicarpus*	*P. gibbesii*
Source	present study,	Shinkareko, 1979	Yatsuzuk and Sakai, 1980	Josileen and Menon, 2004	Terada, 1979	Greenwood and Fielder, 1979	Yatsuzuka and Sakai, 1982	Bookhout and Costlow, 1974	Kurata, 1970
Antennule (a+s)	2+2	2+1	2+1	2+2	nd	4+1	3+1	3+3	2+2
Maxillule									
Coxal endite	7	7	6	6	7	7	6	7	nd
Endopod Proximal seg.	0	1s	1s	1s	1s	1s	1s	0	nd
Maxilla									
Coxal endite	3+3	3+3	3+3	3+3	3+3	3+3	3+3	3+3	nd
Basial endite	4+4	4+4	4+4	3+3	4+4	4+4	4+3	4+4	nd
Endopod	3+2	4+2	4+2	4+2	4+2	3+2	4+2	4+2	3+2
Maxilliped 1									
Setation of the basis	2-2-3-3	2-2-3-3	2-2-3-3	6 (7) (?)	nd	2-3-2-2	2-2-3-3	2-2-2-2	nd
Endopod	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	1,1,0,2,5	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	nd
Maxilliped 2									
Setation of the basis	2-1-1-1	2-1-1-1	1-1-1-1	2 (3) ?	nd	1-1-1-1	1-1-1-1	1-1-1	nd
Endopod	1,1,5	1,1,5	1,1,5	1,1,1,5 (?)	nd	1,1,5	1,1,5	1,1,5	1,1,3
Abdomen									
DMaS on segments	2-5	0	0	0	2-5	2-5	0	2-5	2-5
Telson									
Spines on furca	3	2	3	2	2	3	2	2	3

Genera	*Charybdis*			*Cronius*		*Callinectes*		*Arenaeus*
Species	*C. japonica*	*C. bimaculata*	*C. helleri*	*C. helleri*	*C. ruber*	*C. tumidulus*	*C. sapidus*	*C. similis*	*A. cribrarius*
Source	Yatsuzuka *et al.*, 1984	Hwang, 1995	Negreiros-Fransozo 1996	Dineen *et al.*, 2001	Fransozo *et al.*, 2002	Fransozo *et al.*, 2002	Costlow and Bookhout, 1966	Bookhout and Costlow, 1977	Stuck and Truesdale, 1988
Antennule (a+s)	3+1	3+1	2+1	3+1	4+1	3+2	3+2	3+4	3+2
Maxillule									
Coxal endite	6	6	7	7	5	5	6	6	6
Endopod Proximal seg.	1s	1s	1s	1s	1s	0	0	0	0
Maxilla									
Coxal endite	2+2	3+3	3+5	3+3	4+3	4+3	3+3	3+4	2+2
Basial endite	4+4	4+4	3+5	4+4	5+4	4+4	4+4	4+4	4+4
Endopod	4+2	4+2	5(6)	4+2	4+2	4+2	4+2	4(5)+2	4+2
Maxilliped 1									
Setation of the basis	6 (?)	2-2-3-3	2-3-3-3	2-2-3-3	2-2-3-3	2-2-3-3	8 (?)	2-2-2-3	2-2-3-3
Endopod	2,1,0,2,3	2,2,0,2,5	2(3),2,0,2(3),5(6)	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5	2,2,0,2,5
Maxilliped 2									
Setation of the basis	1-1-1-1	1-1-1-1	nd	1-1-1-1	1-1-1-1	1-1-1-1-	0-1-1-1	1-0-1-1	1-1-1-1
Endopod	1,1,5	1,1,5	nd	1,1,5	1,1,5	1,1,5	1,1,4	1,1,5	1,1,5
Abdomen									
DMaS on segments	2-5	2-5	nd	2-5	2-5	2-5	2-5	2-5	2-5
Telson									
Spines on furca	2	3	nd	3	3	3	2	2	3

1979), *Portunus gladiator,* (Terada, 1979), *Thalamita danae,* (Fielder and Greenwood, 1979), *Charybdis callianassa,* (Greenwood and Fielder, 1980), *Portunus pelagicus,* (Yatsuzuka and Sakai, 1980), *Portunus trituberculatus,* (Yatsuzuka and Sakai, 1982), *Charybdis japonica,* (Yatsuzuka *et al*., 1984), *Arenaeus cribrarius,* (Stuck and Truesdale, 1988), *Thalamita prymna,* (Terada, 1986), *Thalamita crenata,* (Krishnan and Kannupandi, 1990), *Charybdis bimaculata,* (Hwang, 1995), *Charybdis helleri,* (Negreiros-Fransozo, 1996), *Callinectes danae,* (Sankarankutty *et al*., 1999), *Charybdis helleri,* (Dineen *et al*., 2001), *Cronius ruber* and *C. tumidulus,* (Fransozo *et al*., 2002) and *Portunus pelagicus,* (Josileen and Menon, 2004).

To include our findings in a generalised view on Portuninae zoeae and to find diagnostic features for *P. acuminatus*, we summarised the different morphological characters of Zoea-I-larvae of Portuninae (Table 1). It is concluded that all the described Zoea-I-larvae of this subfamily [Portuninae] share the following characteristics: (1) number of setae of the antenna exopod [2, unequal], (2) number of setae of the maxillula endopod [4 + 2], (3) the number of setae of scaphognathite of maxilla of the first zoea is 4, as in all non-majid zoeas, (4) unarmed middle segment of the endopod of the first maxilliped, (5) number of setae of maxilliped 2 [1-1-5, except *Callinectes sapidus*: 1-1-4], (6) number of natatory setae of exopods of maxilliped 1 and 2 is 4 as in all brachyuran zoeae, (7) presence of carapace lateral spines, (8) dorso-lateral processes on abdominal segments 2 and 3. These characteristics confirm the subfamily-classification established by Rice and Ingle (1975).

The distinction between the different subfamilies seems to be well established within the Portuninae. However, the comparison of the morphological characteristics of representatives of the four genera *Arenaeus*, *Callinectes*, *Cronius* and *Portunus* (Table 1) indicates that within the subfamily Portuninae all larvae have a very similar morphology that makes a diagnosis at the generic level based only on morphological data of the first zoeal stage impossible at the moment. Hence, using a combination of morphological and other characteristics like chromatophore-patterns, mandible structure and a comparison including all zoeal stages might lead to results (e.g. Terada, 1979). In addition, intraspecific variability hinders species distinction, as has been shown for *P. pelagicus* and *Charybdis helleri*, where differences between the setal numbers of various appendages and the telson morphology occur depending on the region of origin of the samples (Shinkarenko, 1979; Yatsuzuka and Sakai, 1980; Josileen and Menon, 2004). Stephenson (1972) explained this by the presence of undetected clines and subspecies (see also Meyer *et al.,* 2004). Furthermore, even larvae from the same location show differences (Wehrtmann and Albornoz, 1998; 2003).

Distinctive features of *P. acuminatus* zoeae

Nevertheless we found "good candidates" for species-specific features of *P. acuminatus* zoeae that have to be checked when new descriptions of other *Portunus* zoeae become available. At the present the Zoea-I-stage of *P. acuminatus* can be characterised and distinguished from other described larvae of the genus *Portunus* by the combination of the following three features: (1) absence of a seta on the proximal endopod segment of the maxillule, (2) the endopod setation of the maxilla and (3) the telson fork armature. As can be seen in Table 2, these features are also found in some other *Portunus* species, but not in this combination.

In addition, the larvae of *P. acuminatus* have two conspicuous carapace structures not well known in other portunid zoeae: the dorsal organ, located in the anterior median region and a cuticular pore organ located in the dorso-median region. The ultrastructure of the dorsal organ of other Decapoda is discussed in several papers (e.g. Laverack *et al*., 1996). We observed the presence of the posteriorly and dorsally situated organs in Zoea-I-larvae of different decapods (Meyer, pers. obs.). The presence or absence of these organs and their structure might become a useful character for larval diagnosis and also important for future phylogenetic studies.

ACKNOWLEDGEMENTS

This paper was written as part of cooperation (UCR-VI Project No. 808-A4-757) between the Universidad de Costa Rica (UCR) and the Zoologische Staatssammlung München (ZSM). We wish to thank the Centro de Investigación en Ciencias del Mar y Limnología (CIMAR) for providing us the rearing facilities and supporting us during the collection excursions. Special thanks go to Stefan Friedrich (Munich) for collection manage-

ment of the specimens and the "Freunde der Zoologischen Staatssammlung e.V." for supporting us with a grant for lab equipment. Furthermore, we would like to acknowledge Rita Vargas of the Museo de Zoología, Escuela de Biología, UCR, for helping us with the determination of the collected material, and Katharina Jörger (Munich) for providing us with the maxilla drawings. We are also thankful to two referees and Fernando Mantelatto for their valuable comments, which greatly improved the manuscript.

REFERENCES

Bookhout, C.G. and J.D. Costlow. – 1974. Larval development of *Portunus spinicarpus* reared in the laboratory. *Bull. Mar. Sci.*, 24: 20-51.

Bookhout, C.G. and J.D. Costlow. – 1977. Larval development of *Callinectes similis* reared in the laboratory. *Bull. Mar. Sci.*, 27: 704-728.

Costlow, J.D. and C.G. Bookhout Jr. – 1966. Larval development of *Callinectes sapidus* Rathbun reared in the laboratory. *Biol. Bull. (Woods Hole, Mass.).*, 116: 373-396.

Dineen, J.F., P.F. Clark, A.H. Hines, S.A. Reed and H.P. Walton. – 2001. Life history, larval description, and natural history of *Charybdis helleri* (Decapoda, Brachyura, Portunidae), an invasive crab in the Western Atlantic. *J. Crust. Biol.*, 21: 774-805.

Fielder, D.R. and J.G. Greenwood – 1979. Larval development of the swimming crab *Thalamita danae* Stimpson 1858 (Decapoda, Portunidae) reared in the laboratory. *Proc. R. Soc. Queensland,* 90: 13-20.

Fransozo, A., F.L.M. Mantelatto and G. Bertini. – 2002. The first zoeal stage of the genus *Cronius* (Brachyura, Portunidae) from the Brazilian coast, hatched in a laboratory. *J. Plankton Res.*, 24: 1237-1244.

Garth, J. and W. Stephenson. – 1966. *Brachyura of the Pacific Coast of America Brachyrincha: Portunidae*. Allan Hancock Fundation Monographs in Marine Biology. Allan Hancock Foundation, University of Southern California, Los Angeles, California.

Greenwood, J.G. and D.R. Fielder. – 1979. The zoeal stages and megalopa of *Portunus rubromarginatus* (Lanchester) (Decapoda: Portunidae), reared in the laboratory. *J. Plankton Res.*, 1: 191-205.

Greenwood, J.G. and D.R. Fielder. – 1980. The zoeal stages and megalopa of *Charybdis callinassa* (Herbst) (Decapoda: Portunidae), reared in the laboratory. *Proc. Royal Soc. Queensland.*, 91: 61-76.

Hwang, S.G. – 1995. Complete larval development of the swimming crab, *Charybdis bimaculata* (Miers, 1886) (Crustacea, Brachyura, Portunidae), reared in laboratory. *Korean J. Zool.*, 38(4): 465-482.

Ingle, R.W. – 1992. *Larval Stages of Northeastern Atlantic Crabs*. Chapman and Hall. London, New York, Tokyo, Melbourne, Madras.

Josileen, J. and N. Menon. – 2004. Larval stages of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura). *Crustaceana*, 77(7): 769-896.

Krishnan, T. and T. Kannupandi – 1990. Laboratory cultured zoea, megalopa and first crab of the estuarine crab *Thalamita crenata* (Latr.) A. Milne Edwards 1861 (Brachyura: Portunidae). *Mahasgar*, 23(2): 139-152.

Kurata, H. – 1970. *Studies on the life histories of decapod Crustacea of Georgia: Part III. Larvae of decapod Crustacea of Georgia*. Final Report. University of Georgia Marine Institution, Sapelo Island, Georgia.

Kurata, H. and H. Omi – 1969. The larval stage of a swimming crab, *Charybdis acuata. Bull. Tokai Reg. Fish. Res. Lab.*, 57: 129-136.

Laforsch, C. and R. Tollrian. – 2000. A new preparation technique of daphnids for Scanning Electron Microscopy using hexamethyldisilazane. *Arch. Hydrobiol.*, 149: 587-596.

Laverack, M.S., D.L. MacMillan, G. Ritchie and S.L. Sandow. – 1996. The Ultrastructure of the sensory dorsal organ of Crustacea. *Crustaceana*, 69(4): 636-651.

Meyer, R. and R.R. Melzer. – 2004. Scanning EM diagnosis of marine Decapoda larvae: A comparison of preparation techniques. *Crustaceana*, 77: 883-886.

Meyer, R., S. Friedrich and R.R. Melzer. – 2004. *Xantho poressa* (Olivi, 1792) and *Xantho pilipes* A. Milne-Edwards, 1867 larvae (Brachyura; Xanthidae): Scanning EM diagnosis. *Crustaceana*, 77: 997-1005.

Nation, J.L. – 1983. A new preparation method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technol.*, 58: 347-351.

Negreiros-Fransozo, M.L. – 1996. The zoea I of *Charybdis helleri* (A. Milne-Edwards, 1867) (Decapoda, Portunidae) obtained in laboratory. *Nauplius*, 4: 165-168.

Rice, A.L. and R.W. Ingle. – 1975. A comparative study of the larval morphology of the British portunid crabs *Macropipus puber* (L.) and *M. holsatus* (Fabricius), with a discussion of generic and sub-familial larval characters within the Portunidae. *Bul. British Mus. (Nat. Hist.) Zool.*, 28(4): 121-151.

Sankarankutty, C., S.Y. Hong and K.B. Kim. – 1999. Description of laboratory reared first zoea of *Callinectes danae* (Crustacea, Decapoda, Portunidae). *Rev. Bras. Zool.*, 16(suppl.2): 45-49.

Shinkarenko, L. – 1979. Development of the larval stages of the blue swimming crab *Portunus pelagicus* L. (Portunidae: Decapoda: Crustacea). *Aust. J. Mar. Freshwater Res.*, 30: 485-503.

Stephenson, W. and B. Campbell. – 1960. The Australian portunids (Crustacea: Portunidae). IV. Remaining genera. *Aust. J. Mar. Freshwater Res.*, 11: 73-122.

Stephenson, W. – 1972. An annoted check list and key to the Indo-west-Pacific swimming crabs (Crustacea: Decapoda: Portunidae). *Ray. Soc. New Zealand Bull.*, 10: 1-64.

Stuck, K.C. and F.M. Truesdale. – 1988. Larval development of the speckled swimming crab, *Arenaeus cribrarius* (Decapoda, Brachyura, Portunidae) reared in the laboratory. *Bull. Mar. Sci.*, 42: 101-132.

Terada, M. – 1979. A classification of zoea larvae in the subfamily Portuninae of the family Portunidae. *Zool. Mag. (Tokyo)*, 88(3): 254-268.

Terada, M. – 1986. Zoeal development of the swimming crab *Thalamita prymna* (Herbst); Portunidae, Portuninae. *Researches on Crustacea*, 15: 15-23.

Wear, R.G. and D.R. Fielder – 1985. The marine fauna of New Zealand: larvae of the Brachyuran (Crustacea, Decapoda). *N.Z. Ocean. Inst. Mem.*, 92: 1-89.

Wehrtmann, I. S. and L. Albornoz. – 1998. Larval development of *Nauticaris magellanica* (A. Milne Edwards, 1891) (Decapoda: Caridea: Hippolytidae), reared under laboratory conditions. *Bull. Mar. Sci.*, 62(1): 45-72.

Wehrtmann, I. S. and L. Albornoz. – 2003. Larvae of *Nauticaris magellanica* (Decapoda: Caridea: Hippolytidae) reared in the laboratory differ morphologically from those in nature. *J. Mar. Biol. Assoc. U.K.*, 83: 949-957.

Yatsuzuka, K. and K. Sakai. – 1980. The larvae and juvenile crabs of Japanese Portunidae (Crustacea, Brachyura): I *Portunus (Portunus) pelagicus* (Linne). *Rep. Usa Mar. Biol. Inst.*, 2: 25-41.

Yatsuzuka, K. and K. Sakai. – 1982. The larvae and juvenil crabs of Japanese Portunidae (Crustacea, Brachyura): II *Portunus (Portunus) trituberculatus* (Miers). *Rep. Usa Mar. Biol. Inst.*, 4: 9-26.

Yatsuzuka, K., K. Sakai, N. Del and R. Roman. – 1984. The larvae and juvenile crabs of Japanese Portunidae (Crustacea, Brachyura): III. *Charybdis japonica*, A. Milne Edward. *Rep. Usa Mar. Biol. Inst.*, 6: 23-40.

Scient. ed.: E. Macpherson
Received May 16, 2005. Accepted October 25, 2005.

4. Article II

Meyer R, Lehmann T, Melzer RR, Geiselbrecht H (2014) Morphology of the first zoeal stage of the mediterranean bumblebee shrimp *Gnathophyllum elegans* (Risso,1816) studied with light microscopy and scanning EM. Journal of the Marine Biological Association of the United Kingdom 94(1): 151-158.

Figure 7: The mediterranean bumblebee shrimp *Gnathophyllum elegans* in dorsal view. Photo by the author.

Journal of the Marine Biological Association of the United Kingdom, 2014, 94(1), 151–158.
doi:10.1017/S002531541300115X

Morphology of the first zoeal stage of the Mediterranean bumblebee shrimp *Gnathophyllum elegans* studied with both light microscopy and scanning EM

ROLAND MEYER, TOBIAS LEHMANN, ROLAND R. MELZER AND HANNES GEISELBRECHT
Zoologische Staatssammlung, Münchhausenstr. 21, D-81247 München, Germany

The morphology of the first zoea of Gnathophyllum elegans *is described from laboratory-reared material for the first time and analysed in detail with light and scanning electron microscopy. For systematic reasons, morphological characteristics in* G. elegans *are compared with those in* Gnathophyllum americanum, Periclimenes amethysteus*, a representative of the subfamily Pontoniinae and the closely related* Hymenocera picta *(Decapoda: Caridea: Hymenoceridae). We observed differences in the morphology of both* Gnathophyllum*-larvae, such as the number and arrangement of certain setae. Thus, larvae of the two* Gnathophyllum *species can be readily distinguished from each other. Further differential diagnosis with* P. amethysteus *confirms a high similarity between* Gnathophyllum*-larvae and larvae in the subfamily Pontoniinae, as already mentioned in earlier publications. The systematic relationships of the Gnathophyllidae, Hymenoceridae and Pontoniinae are discussed based on zoeal characters.*

Keywords: larval morphology, first zoea, scanning EM, Decapoda, Pontoniinae, Gnathophyllidae, *Gnathophyllum elegans*

Submitted 23 January 2013; accepted 29 July 2013; first published online 4 September 2013

INTRODUCTION

The Mediterranean bumblebee shrimp *Gnathophyllum elegans* (Risso, 1816) is widely distributed throughout the Mediterranean Sea (Adriatic Sea, Aegean Sea, Alboran Sea, Ionian Sea and Israelean coast) and adjacent Atlantic regions (Azores, Canary Islands, Madeira and Moroccan Atlantic) (d'Udekem d'Acoz, 1999; Türkay, 2001). As a nocturnal species it can be found in shallow coastal waters (0–35 m) (Denitto *et al.*, 2009; Pipitone & Vaccaro, 2011), hidden under stones and pebbles at daytime. Zariquiey Álvarez (1955) mentioned that at night the species can be dredged in *Posidonia oceanica* Delile, 1813 meadows. Occasionally it is associated with the sea anemone *Telmatactis cricoides* (Duchassaing, 1850) (Wirtz, 1997). In our sampling area we did not observe any kind of association and found specimens obviously free living. However, when transferred in a community aquarium we monitored the bumblebee shrimp associated with different echinoderms, e.g. *Echinaster (Echinaster) sepositus* (Retzius, 1783) and *Arbacia lixula* (Linnaeus, 1758).

The family Gnathophyllidae includes five genera with a total of 14 species (De Grave & Fransen, 2011). The larval development of this family is poorly documented; only the first stage zoea in *Gnathophyllum americanum* Guérin-Méneville, 1855 (in Guérin-Méneville, 1855–1856) is described so far (Bruce, 1986). Also the systematic classification of the genera is under discussion. There are notions of a close relationship between the Gnathophyllidae Dana, 1852, Hymenoceridae Ortmann, 1890 and members of the palaemonid subfamily Pontoniinae Kingsley, 1879. To put further arguments into this question, in the present study we describe and illustrate the first zoea in *G. elegans* and compare the first zoeal characteristics of *G. elegans* with those of *G. americanum, Hymenocera picta* Dana, 1852 and *Periclimenes amethysteus* (Risso, 1827).

Corresponding author:
R. Meyer
Email: rolandmeyer@hotmail.de

MATERIALS AND METHODS

On May 2012, one ovigerous female of *Gnathophyllum elegans* was collected in a depth of 2 m while night-snorkelling west of the isle of Šolta (43°23′00″N16°13′47″E), Croatia. The specimen was identified according to Zariquiey Álvarez (1968) and kept in an aquarium with a temperature of 20–23°C and salinity of 35 psu at the Zoologische Staatssammlung München (Germany) till larvae hatched. About 200 first zoeas were released on 20 June 2012. Few larvae were observed and photographed in the egg integument and instantly after hatching in order to document natural pigmentation. Larvae were fixed immediately in a graded ethanol series (30%, 50%, 70% for 10 min each, see Meyer & Melzer (2004)) and then dissected using a stereomicroscope and thin tungsten wires. Of each type of appendage about 20 pieces were dissected and kept separately in small glass vials containing 70% ethanol. For scanning electron microscopy (SEM) dissected appendages and entire larvae were dehydrated in a graded acetone series (70%, 80%, 90%, 2 × 100%, 10 min each) and then critical-point-dried in a Baltec CPD 030. After mounting

on SEM stubs with self-adhesive carbon stickers, specimens were coated with gold on a Polaron 'Sputter Coater' (Quorum Technologies, UK) and studied with a LEO 1430VP (Zeiss, Germany) SEM at 10–15 kV. Several appendages were scanned and compared to identify the exact number of setae. For classification of the different types of setae the terminology of Ingle (1992) was followed.

Measurements of the first zoeas were made using LEO's SEM-User-Interface-Software (N = 4). Total length (TL) was measured from the base of the rostrum to the posterior end of the telson, carapace length (CL) from the base of the rostrum to the posterior margin of the carapace and carapace width (CW) between the lateral margins of carapace directly behind the eyes. Furthermore the rostrum length (RL) measured from the tip of the rostrum to the antennule peduncle and the antenna endopod length (AEL) measured to the scale of the antenna are given in this description.

Complete larvae and dissected maxillipeds were also studied with light microscopy (LM) using the Leica DM 5000 B microscope (Leica, Germany) equipped with the CCD Camera ProgRes SpeedXTcore 5 (Jenaoptik, Germany). Drawings of the mandibles and the telson were prepared by interpretation of several SEM and LM images.

The female and the zoea larvae are deposited at the Zoologische Staatssammlung München under the registration numbers ZSMA 20120316 and ZSMA 20120317. Additionally the DNA barcode (cytochrome oxidase I sequence) of a different specimen of *Gnathophyllum elegans* sampled in the same region (ZSMA 20111534) is available on the Boldsystems website (www.boldsystems.org) under the number CFAD190-11.

RESULTS

First zoea of *Gnathophyllum elegans*

Dimension (mm): TL = 1.860 + 0.044; CL = 0.446 + 0.020; CW = 0.459 + 0.038; RL = 0.185 + 0.016; AEL = 0.173 + 0.023.

Carapace (Figure 1A, B): with short, slender and unarmed rostral process; epigastric tubercle with dorsal organ present; carapace armed with pterygostomial spine, otherwise absence of spines. Compound eyes sessile.

Anterior sensory dorsal organ (SDO) (Figure 1B): small protrusion composed of four small cuticular depressions

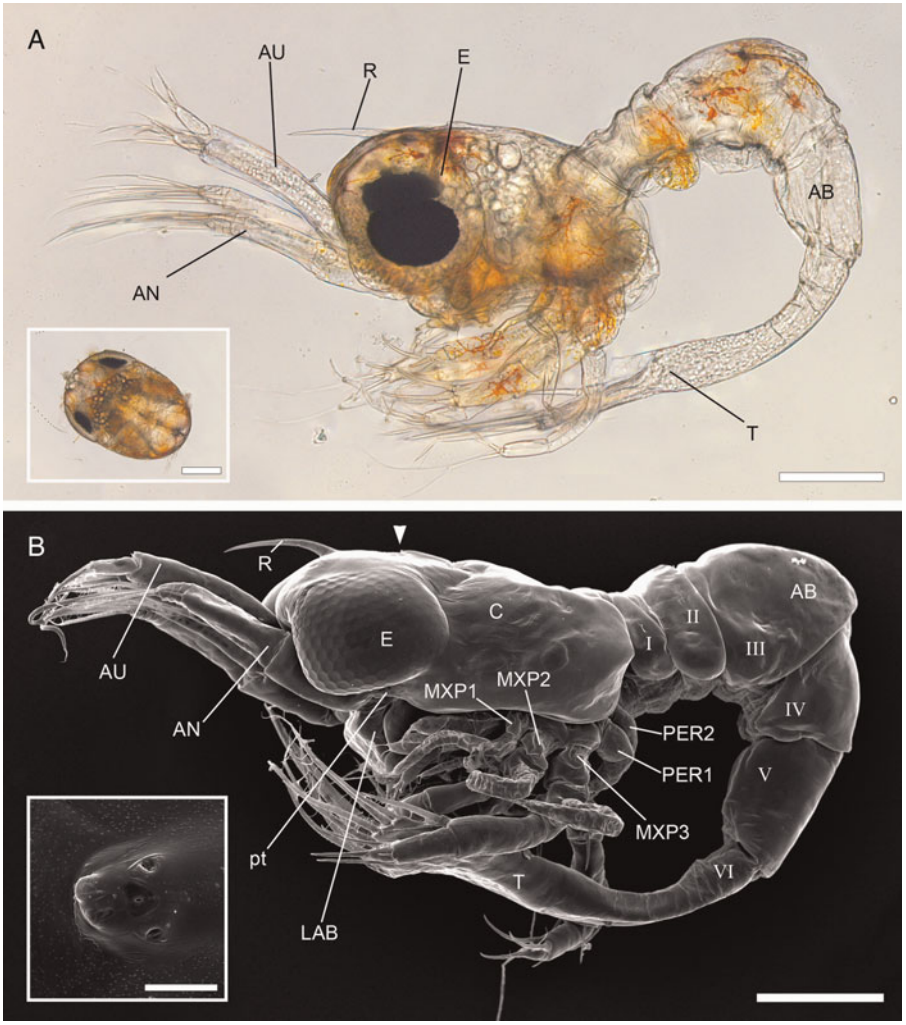


Fig. 1. *Gnathophyllum elegans*, first zoea: (A) LM image, lateral view, natural pigmentation visible; insert showing egg just before hatching; (B) SEM image, lateral view, arrowhead points to dorsal organ; insert showing detail of dorsal organ. Abbreviations: I–VI, abdominal segments; AB, abdomen; AN, antenna; AU, antennule; C, carapace; E, eye; LAB, labrum; MXP 1–3, first to third maxillipeds; PER1&2, pereiopods 1 and 2; PT, pterygostomial spine; R, rostrum; T, telson. Scale bars: A, 200 μm; B, 200 μm, insert 20 μm.

disposed as the corners of a square with the centre occupied by a pore.

Antennule (Figures 1A, B & 2A): subcylindrical, unsegmented peduncle with distal plumose seta; flagellum with four aesthetascs and plumose seta.

Antenna (Figures 1A, B & 2B): biramous; protopod unsegmented, with medio-terminal small spine; endopod apically with plumose seta and short spine; scaphocerite with four segmentations distally and ten plumose setae plus simple seta distomedially, first and last reduced, with small tubercle proximally on medial border; small seta on the outer side of the proximal article and small distal plumose seta, small simple seta on the apex.

Mandibles (Figures 2C, D & 5A): right and left mandibles almost identical; mandibular palps absent. Incisor process with marginal protrusion bearing row of three acute spines ventrally and serrated 'lacinia mobilis', molar process slender, bearing group of small spines. Right mandible with clearly separate submarginal spine between incisor process and 'lacinia mobili'. Left mandible with submarginal spine grown together with ventral row of spines.

Maxillule (Figure 2E): coxal endite with four simple distal setae and slightly serrated proximal located seta; basal endite with two stout and slender plumose setae and two simple setae distally; endopod compressed, stout, distally acute with preterminal simple seta; exopod absent.

Maxilla (Figure 2F): coxal endite with three simple setae, basal endite bilobed, proximal lobe with simple seta, and distal lobe with two simple setae, endopod unsegmented with long simple seta and microtrichia on inner margin. Exopod with five marginal plumose setae.

First maxilliped (Figure 3A, B): basis with three simple setae. Endopod 3-segmented with 0, 1, 1 + 3 setae; exopod partially crossed by six incisions, the last one armed laterally with simple seta, distally four long plumose setae arranged in a row.

Second maxilliped (Figure 3C, E): basis with two simple setae. Endopod 3-segmented, proximal segment largest and unarmed, intermediate segment with distomedial serrated and distolateral simple seta distally, distal segment with small simple seta located proximally and three simple setae plus strong serrate seta distally. Exopod partially crossed by nine incisions, setae arranged as in first maxilliped.

Third maxilliped (Figure 3D, F, G): basis with two simple setae and small proximomedial tubercle. Endopod

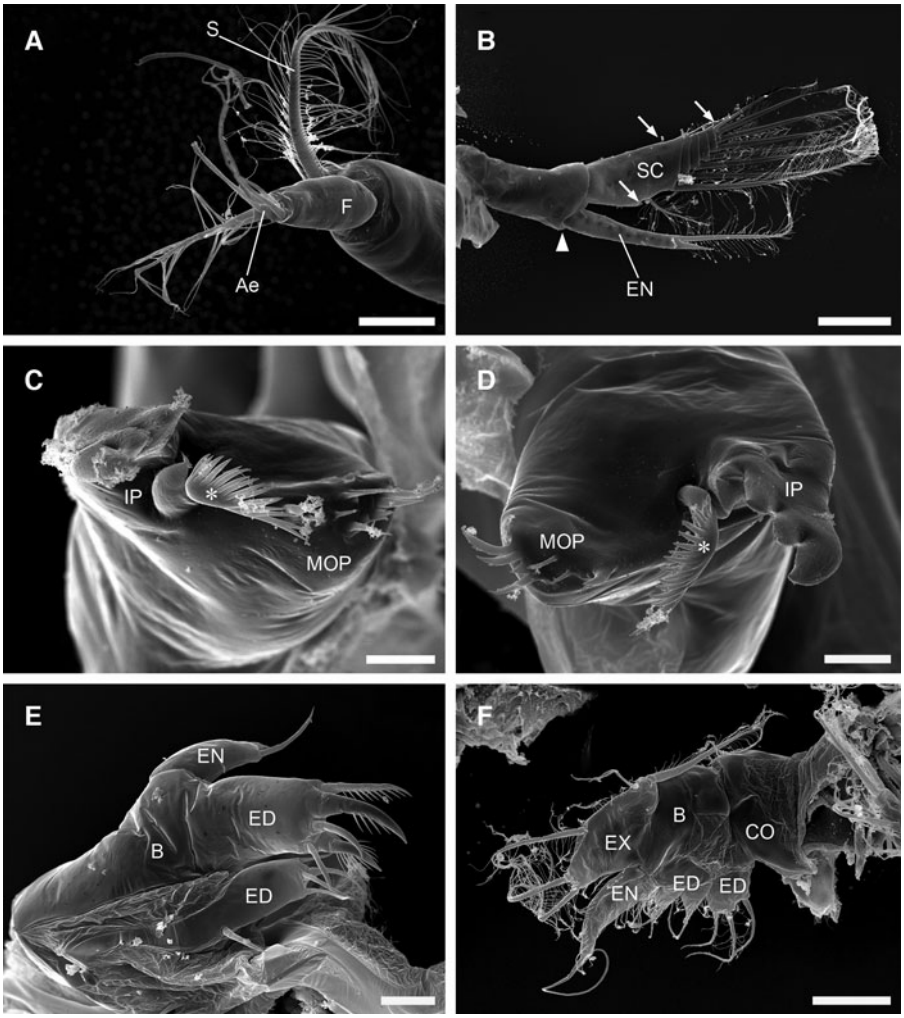


Fig. 2. *Gnathophyllum elegans*, Zoea I, SEM images showing appendages of cephalothorax and mouthparts: (A) antennule; (B) antenna, arrowhead = small spine; arrows = small setae and tubercle; (C) right mandible, asterisk = 'lacinia mobilis'; (D) left mandible, asterisk = 'lacinia mobilis'; (E) maxillule; (F) maxilla. Abbreviations: AE, aesthetasc; B, basis; CO, coxa; ED, endite; EN, endopod; EX, exopod; F, flagellum; IP, incisor process; MOP, molar process; S, seta; SC, scaphocerite. Scale bars: A, 40 μm; B, 100 μm; C, 10 μm; D, 10 μm; E, 20 μm; F, 40 μm.

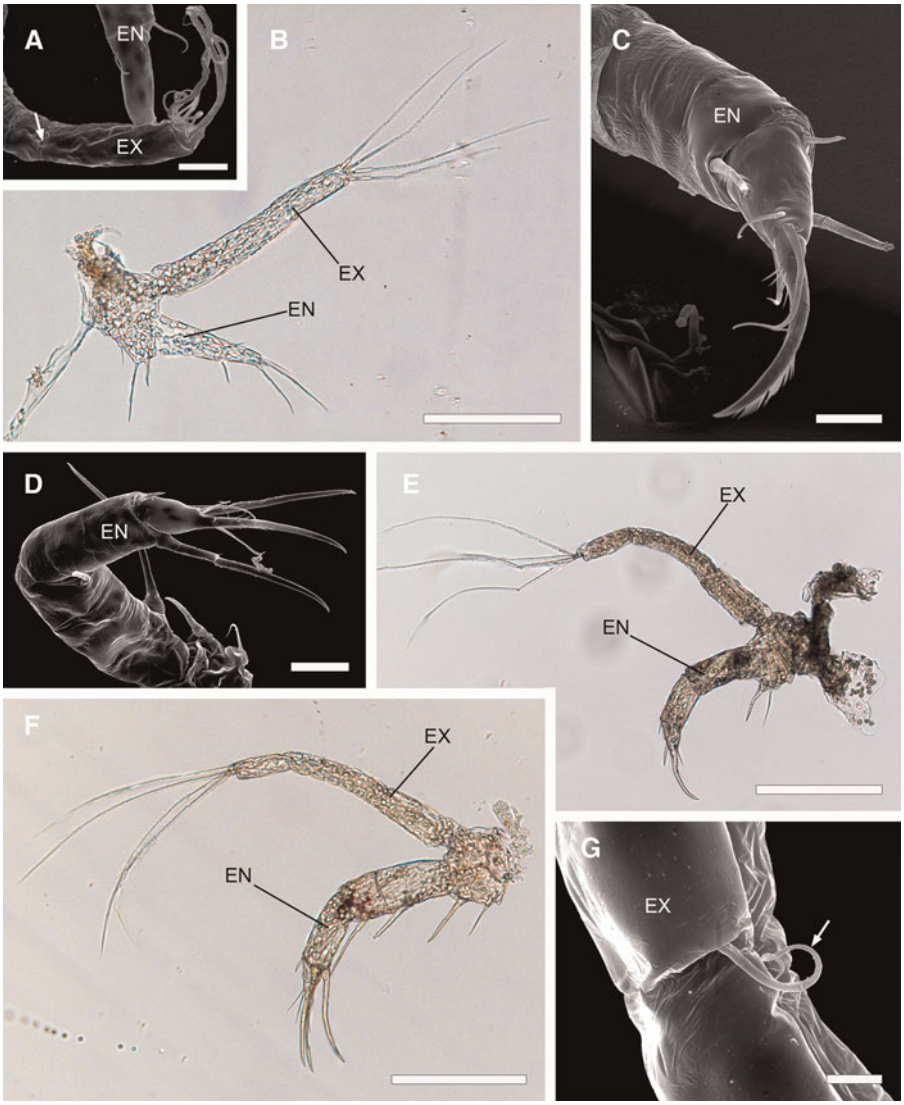


Fig. 3. *Gnathophyllum elegans*, first zoea, SEM (A, C, D, G) and LM (B, E, F) images showing maxillipeds: (A) endopod and exopod of first maxilliped, arrow = seta; (B) first maxilliped; (C) endopod of second maxilliped; (D) endopod of third maxilliped; (E) second maxilliped; (F) third maxilliped; (G) exopod of third maxilliped, arrow = seta. Abbreviations: EN, endopod; EX, exopod. Scale bars: A, 20 μm; B, 150 μm; C, 20 μm; D, 40 μm; E, 200 μm; F, 200 μm; G, 6 μm.

3-segmented; proximal segment with 1 + 1 simple setae; intermediate segment with two strong serrate distal setae, distal segment armed with proximolateral simple and three simple and strong serrate distally located setae. Exopod partially crossed by eight incisions, setae arranged as in first maxilliped.

First and second pereiopods (Figure 4C): present as biramous rudiments.

Third to fifth pereiopods: not differented.

Abdomen (Figures 1A, B & 4B): 6-segmented; strongly flexed between third and fourth segments; small simple seta on the posterior-lateral margin of the third and fourth somite present.

Pleopods and uropods: absent.

Telson (Figures 4A & 5B): fused with sixth abdominal segment; broadly triangular; 7 + 7 plumodenticulate setae on the posterior margin; the inner- and outermost smaller, the two outermost on each side with setules medially; minute spines only between four inner setae; setal bases armed with minute spines except inner and outermost.

DISCUSSION

The present description of the first zoea in *Gnathophyllum elegans* is based on a combination of SEM and LM techniques applied to fixed larvae and dissected appendages. Using the high resolution power of the SEM even minute structures such as setules, spines or the anterior sensory dorsal organ can be located and described. We also could analyse the gnathal edge of the mandibles in great detail, a feature often omitted in larval descriptions. Ingle (1992) mentioned that left and right mandibles in zoea-larvae are usually slightly dissimilar and that details are not easy to resolve by LM due to their gross three-dimensional structure. In the case of *G. elegans* this difference is marginal and can only be observed in the position of the submarginal spine on the incisor process.

The family Gnathophyllidae (superfamily Palaemonoidea Rafinesque, 1815) consists of five genera: *Gnathophylleptum* d'Udekem d'Acoz, 2001, *Gnathophylloides* Schmitt, 1933, *Gnathophyllum* Latreille, 1819, *Levicaris* Bruce, 1973, and *Pycnocaris* Bruce, 1972 (Martin & Davis, 2001; De Grave &

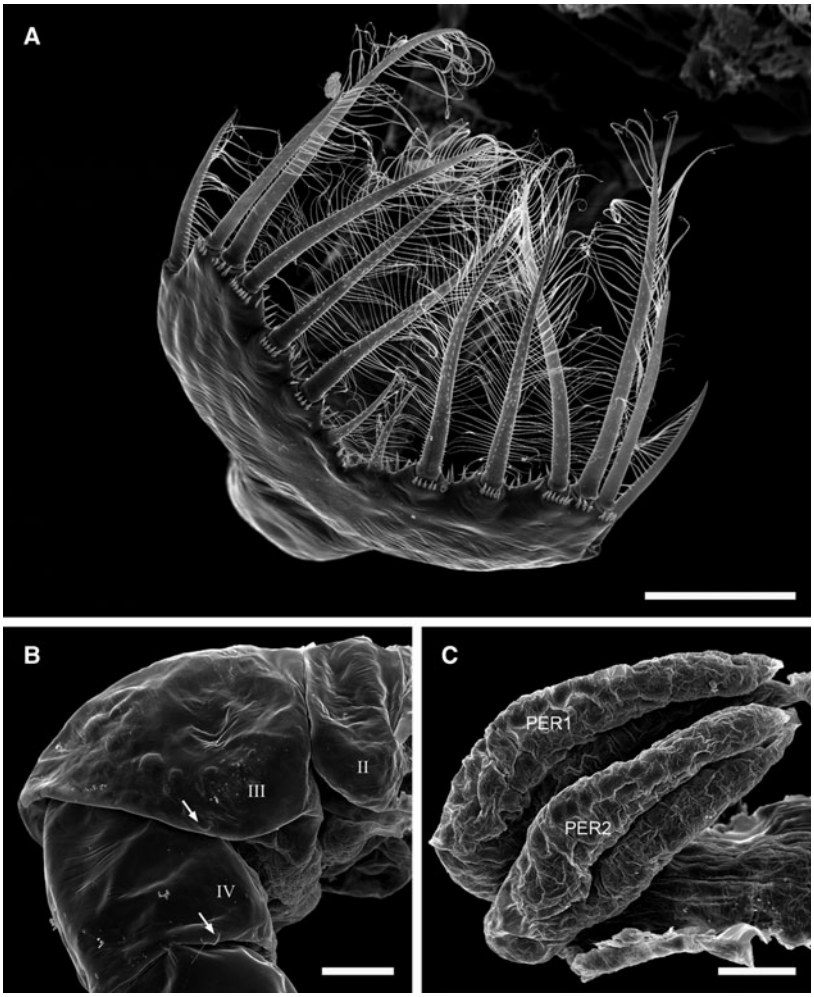


Fig. 4. *Gnathophyllum elegans*, first zoea, SEM images: (A) telson; (B) detail of abdomen segments II–IV, arrows = setae; (C) pereiopods 1 and 2. Abbreviations: II–IV, abdominal segments; PER1&2, pereiopods 1 and 2. Scale bars: A, 100 μm; B, 60 μm; C, 30 μm.

Fransen, 2011). Until now the description of the first zoeal stage in *Gnathophyllum americanum* was the only one present for a representative of this family (Bruce, 1986).

Differential diagnosis between the larval characters of the first zoea in *G. elegans* and *G. americanum* shows morphological differences in the carapace shape and the arrangement and number of setae on different appendages, e.g. the antennule, antenna, maxillule, maxilla, first to third maxillipeds and the telson (Table 1). The two *Gnathophyllum*-species can thus be readily distinguished from each other. However, some of the differences like the presence or absence of the pterygostomial spine and the segmentation of the endopod of the first maxilliped may also be a result from the different analysing techniques used in both examinations, since the SEM's resolution power is much higher than that of the LM.

The absence of the distinct basal lobe on the endopod of the maxilla and the branchiostegal spines as observed in the *G. elegans*-zoea are morphological characteristics thought to be specific for larvae of the palaemonid subfamily Pontoniinae as well as the Gnathophyllidae and the Hymenoceridae (Mitsuhashi *et al.*, 2007).

Thus, with respect to species specific features and above-family systematics our results fit well with what could be expected. However, the search for features that allow us to

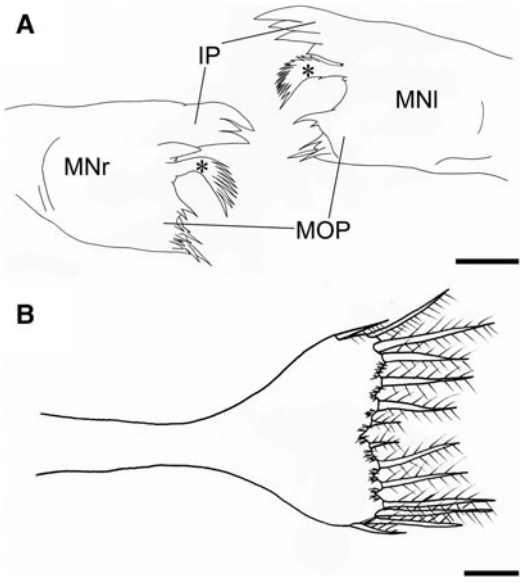


Fig. 5. *Gnathophyllum elegans*, first zoea, drawing: (A) posterio-ventral view of mandibles; (B) dorsal view of telson. Abbreviations: asterisk, 'lacinia mobilis'; IP, incisor process; MNl, left mandible; MNr, right mandible; MOP, molar process; Scale bars: A, 20 μm; B, 100 μm.

Table 1. Comparison of morphological features of the first zoeas Zoea-I larvae of *Gnathophyllum americanum, G. elegans, Periclimenes amethysteus* and *Hymenocera picta*. Data on *P. amethysteus* are taken from Geiselbrecht & Melzer (2010), *Gnathophyllum americanum* and *Hymenocera picta* from Bruce 1986, 1988. Bold marked features show similarity between *G. elegans* and *P. amethysteus*.

Appendage	*G. americanum*	*G. elegans*	*P. amethysteus*	*H. picta*
Carapace pterygostomial spine	Absent	Present	Present	Absent
Sensory dorsal organ	Not described	Present	Not described	Not described
Flagellum of antennule	3 aesthetascs + 2 sparsely setose setae	4 aesthetascs + 1 plumose seta	4 aesthetascs + 1 plumose seta	2 aesthetascs + 1 plumose seta
Exopod of antenna	9 plumose distomedial setae; 1st and last reduced	10 plumose distomedial setae and 1 simple seta; 1st and last reduced	10 plumose distomedial setae, 1st and last reduced	10 plumose distomedial setae; 1st and 2nd short, last reduced
Coxial endite of maxillule	3 short setae	4 simple setae + 1 proximal seta	4 simple setae + 1 proximal seta	3 simple setae + 1 proximal ventral seta
Basial endite of maxillule	2 stout + 1 slender setulose spines + 2 simple setae	2 stout + 1 slender plumose setae + 2 simple setae	3 strong plumose setae + 2 simple setae	3 stout setulose spines + 2 simple setae
Maxilla: distal lobe of basial endite	1 simple seta	2 simple setae	2 simple setae	1 simple seta
Maxilla: proximal lobe of basial endite	1 simple seta	1 simple seta	2 simple setae	1 simple seta
Maxilla: endopod	1 simple seta	1 simple seta	1 simple seta	1 simple seta
1st maxilliped	Endopod unsegmented 3 distal setae; exopod: 4 plumose setae	Endopod 3-segmented 0,1,1 + 3 setae; exopod: 4 plumose setae + 1 simple seta subterminal	Endopod weakly 3-segmented 0,1,1 + 3 setae; exopod: 4 plumose setae + 1 simple seta subterminal	Endopod 3-segmented 0,1,1 + 3 setae; exopod: 4 plumose setae
2nd maxilliped	Intermediate segment of endopod: distomedial seta and distolateral spine. Distal with strong laterally dentate terminal spine + 3 short simple setae	Intermediate segment of endopod: 1 distomedial serrate and 1 distolateral simple seta. Distal with strong serrate seta + 3 simple setae + 1 proximal located seta	Intermediate segment of endopod: 2 serrate distal setae. Distal with strong serrate seta + 3 simple setae + 1 proximal located seta	Intermediate segment of endopod: distomedial seta and distolateral spine. Distal with strong laterally dentate terminal spine + 2 short preterminal setae
3rd maxilliped	Distal segment of endopod: 1 strong spine + 2 setae distally, 1 proximolateral seta	Distal segment of endopod: 1 strong, serrated seta + 3 simple setae distally, 1 proximolateral seta	Distal segment of endopod: 1 strong serrate seta + 3 simple setae	Distal segment of endopod: 1 terminal spine + 3 simple preterminal setae
Abdomen	No setae described	1 simple seta on posterior-lateral margins on 3rd and 4th somite present	1 simple seta on posterior-lateral margins of 1st, 3rd and 4th somite present	No setae described
Telson	7 + 7 plumose setae, lateral pair setulose on medial margin	7 + 7 plumodenticulate setae, lateral pair setulose on medial margin	7 + 7 plumodenticulate setae, lateral pair setulose on medial margin	7 + 7 plumose setae, except lateral pair simple

distinguish the studied gnathophyllid zoeae from pontoniids and hymenocerids proved to be more difficult. In Table 1 we summarize characters of selected representatives of these taxa for detailed comparison. *Periclimenes amethysteus* was selected as a Pontoniine 'model' because the larval description is also based on a combined technique using LM and SEM and thus is most suitable for comparison with our results (Geiselbrecht & Melzer, 2009). A comparison of 14 zoeal characteristics in *G. elegans* and *P. amethysteus* shows a high degree of correspondence, though they are placed in different families. Seven of these features are identical, and only six differences are shown, i.e. setation of exopod of antenna, basal endite of maxillule, proximal lobe of basal endite of maxilla, second maxilliped, third maxilliped and the abdomen. It was not possible to compare the structure of the sensory dorsal organ, because an adequate description of this feature was lacking in *P. amethysteus*. This is surprising, since the two studied gnathophyllids, *G. elegans* and *G. americanum*, belonging to the same genus, show differences in eight conspicuous features. Moreover, *G. elegans* and *Hymenocera picta*, a representative of Hymenoceridae, the putative sister taxon of the Gnathophyllidae, show 11 differences (Table 1).

What could be explanations for these partly contradictory results? As mentioned above, our SEM technique used for the analysis of *G. elegans* and *P. amethysteus* shows minute details that are probably not detected with LM alone, viz., the technique used for description of *G. americanum* and *H. picta*. Differences in meticulousness could thus hinder comparison of zoeae and suggest false similarities. For example the structure of the sensory dorsal organ or the mandibles can only be described by use of the SEM. Nonetheless our study clearly supports a notion already put forward in several other differential diagnoses of pontoniine and gnathophyllid zoeas, i.e. their high degree of correspondence (Bruce, 1986, Bruce, 1988; Yang & Ko, 2002; Yang & Ko, 2004; Mitsuhashi *et al.*, 2007).

Another explanation for the character distribution shown here might lie in the not-well-understood relationships between the Gnathophyllidae and the Pontoniinae. Holthuis (1955) erected the family Gnathophyllidae based on adult mouthpart morphology (shape of the third maxilliped) by excision of genera *Gnathophylloides*, *Gnathophyllum*, *Levicaris* and *Pycnocaris* from the Pontoniinae, and thereby producing probably paraphyletic Pontoniinae as a 'leftover' group. With the first description of the larval features of *G. americanum* and their high degree of similarity to Pontoniinae zoeae, Bruce (1986) synonymized the Gnathophyllidae with the former, but Chace & Bruce (1993) concluded tentatively that the unique mouthparts of the Gnathophyllidae discard the possibility of synonymy. Moreover, Williamson & Rice (1996) and Williamson (2001) proposed that highly similar larvae do not necessarily signify related adults and, therefore, the Pontoniinae and the Gnathophyllidae may be distinct. This would suggest that some of the zoeal features presented in the present study might be plesiomorphies and thus not useful for phylogenetic considerations.

Recent molecular systematic analyses based on nuclear rDNA and mitochondrial sequences (Mitsuhashi *et al.*, 2007; Bracken *et al.*, 2009; Li *et al.*, 2011) show that the Gnathophyllidae are placed in a cluster of lineages including the Hymenoceridae as the sister group of Gnathophyllidae, plus several lineages belonging to Palaemoninae Rafinesque, 1815, Pontoniinae and Anchistioididae Borradaile, 1915 supporting the idea of pontoniine paraphyly.

It seems that the above described unclear character distribution between gnathophyllid and related zoeae is caused by a combination of still valid traditional taxonomic acts, lack of consequent cladistic analyses including molecular trees and insufficient knowledge of larval morphology. Further investigations combining molecular methods and classical morphological analyses (larval and adult) involving more gnathophyllid genera are therefore required.

ACKNOWLEDGEMENTS

We thank Stefan Friedrich (Zoologische Staatssammlung München, Germany) for expert technical assistance and collection management. Furthermore we would like to thank the captain Bernd Meyer and the crew of the SY 'Janu' for their support.

FINANCIAL SUPPORT

This study was partly supported by the Sea Life Center München, Germany.

REFERENCES

Bracken H.D., De Grave S. and Felder D.L. (2009) Phylogeny of the infraorder Caridea based on mitchondrial and nuclear genes (Crustacea: Decapoda). In Martin J.W., Crandall K.A. and Felder D.L. (eds) *Decapod crustacean phylogenetics*. Vol. 18. Boca Raton, FL CRC Press, pp. 281–305.

Bruce A.J. (1986) Observations of the family Gnathophyllidae Dana, 1852 (Crustacea: Decapoda). *Journal of Crustacean Biology* 6, 463–470.

Bruce A.J. (1988) A note on the first zoeal stage larva of *Hymenocera picta* Dana (Crustacea: Decapoda: Palaemonidae). *The Beagle, Records of the Museums and Art Galleries of the Northern Territory* 5, 119–124.

Chace F.A. Jr, and Bruce A.J. (1993) The caridean shrimps (Crustacea: Decapoda) of the *Albatross* Philippine Expedition, 1907–1910, part 6: Superfamily Palaemonoidea. *Smithsonian Contributions to Zoology* 543, 1–152.

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. *Patrimonies Naturels (M.N.H.N./S.P.N.)* 40, 1–383.

De Grave S. and Fransen C.H.J.M. (2011) Carideorum catalogus: the recent species of the dendrobranchiate, stenopodidean, procarididean and caridean shrimps (Crustacea: Decapoda). *Zoologische Mededelingen, Leiden* 89, 195–589.

Denitto F., Moscatello S. and Belmonte G. (2009) Occurrence and distribution pattern of *Palaemon* spp. shrimps in a shallow submarine cave environment: a study case in south-eastern Italy. *Marine Ecology* 30, 416–424.

Geiselbrecht H. and Melzer R.R. (2009) Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* (Risso, 1827) (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the scanning EM. *Zootaxa* 2140, 45–55.

Holthuis L.B. (1955) The recent genera of the caridean and stenopodidean shrimps (class Crustacea, order Decapoda, supersection

Natantia) with keys for their determination. *Zoologische Verhandelingen, Leiden* 26, 1–157.

Ingle R. (1992) *Larval stages of Northeastern Atlantic crabs. An illustrated key*. London: Chapman & Hall.

Li C.P., De Grave S., Chan T.-Y., Lei H.C. and Chu K.H. (2011) Molecular systematics of caridean shrimps based on five nuclear genes: implications for superfamily classification. *Zoologischer Anzeiger* 250, 270–279.

Martin J.W. and Davis G.E. (2001) An updated classification of the Recent Crustacea. *Natural History Museum of Los Angeles County, Science Series* 39, 1–124.

Meyer R. and Melzer R.R. (2004) Scanning EM diagnosis of marine decapoda larvae: a comparison of preparation techniques. *Crustaceana* 77, 883–886.

Mitsuhashi M., Sin Y.W., Lei H.C., Chan T.Y. and Chu K.H. (2007) Systematic status of the caridean families Gnathophyllidae Dana and Hymenoceridae Ortmann (Crustacea : Decapoda): a preliminary examination based on nuclear rDNA sequences. *Invertebrate Systematics* 21, 613–622.

Pipitone C. and Vaccaro M. (2011) Crustacea Decapoda from *Ustica* (southern Tyrrhenian Sea): species distribution in different habitats and sampling approach. In Pessani D., Tirelli T. and Froglia C. (eds) *IX Colloquium Crustacea Mediterranea Torino, 2–6 September 2008*. Torino, Italy: Museo Regionale di Scienze Naturali, pp. 413–434.

Türkay M. (2001) Decapoda. In Costello M.J., Emblow C.S. and White R.J. (eds) *European Register of Marine Species. A check-list of marine species in Europe and a bibliography of guides to their identification. Patrimoines Naturels* 50, 463 pp.

Williamson D.I. (2001) Larval transfer and the origins of larvae. *Zoological Journal of the Linnean Society* 131, 111–122.

Williamson D.I. and Rice A.L. (1996) Larval evolution in the crustacea. *Crustaceana* 69, 267–287.

Wirtz P. (1997) Crustacean symbionts of the sea anemone *Telmatactis cricoides* at Madeira and the Canary Islands. *Journal of Zoology* 242, 799–811.

Yang H.J. and Ko H.S. (2002) First zoea of *Palaemon ortmanni* (Decapoda, Caridea, Palaemonidae) hatched in the laboratory, with notes on the larval morphology of the Palaemonidae. *Korean Journal of Systematic Zoology* 18, 181–189.

Yang H.J. and Ko H.S. (2004) Zoeal stages of *Conchodytes nipponensis* (Deapoda: Palaemonidae) reared in the laboratory. *Journal Of Crustacean Biology* 24, 110–120.

Zariquiey Álvarez R. (1955) Crustáceos decápodos de la región de Cadaqués (España). *Vie et Milieu* 6, 397–409.

and

Zariquiey Álvarez R. (1968) Crustáceos Decápodos Ibéricos. *Investigacién Pesquera* 32, 1–510.

Correspondence should be addressed to:
R. Meyer
Zoologische Staatssammlung, Münchhausenstr. 21, D-81247 München, Germany
email: rolandmeyer@hotmail.de

5. Article III

Lerosey-Aubril R and **Meyer R** (2013) The sensory dorsal organs of crustaceans. Biological Reviews 88:406-426.

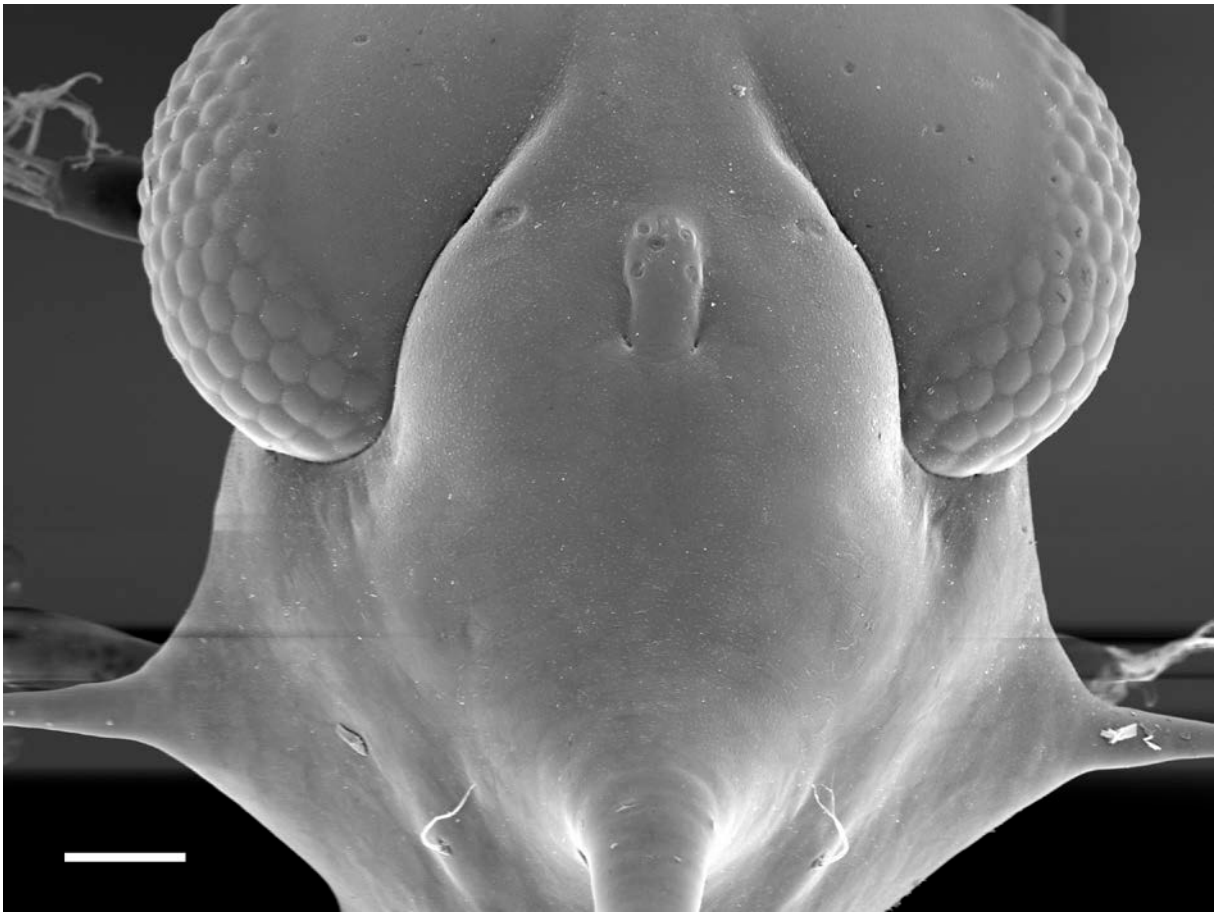


Figure 8: SEM-picture of the first zoea stage of *Portunus acuminatus* in dorsal view with the sensory dorsal organ present in the anterior region (bar 40 µm). Photo by the author.

BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2013), **88**, pp. 406–426.
doi: 10.1111/brv.12011

The sensory dorsal organs of crustaceans

Rudy Lerosey-Aubril[1,2,*] and Roland Meyer[3]

[1]*Laboratoire de Géologie de Lyon: Terre, Planètes, Environnement, Université Claude Bernard Lyon I, 2 rue Raphaël Dubois, 69622 Villeurbanne, France*
[2]*Senckenberg Research Institute, Senckenberganlage 25, 60325 Frankfurt-am-Main, Germany*
[3]*Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 Munich, Germany*

ABSTRACT

The cuticle of crustaceans bears numerous organs, of which the functions of many are unknown. One of these, the sensory dorsal organ (SDO), is present in a wide diversity of taxa. Here we critically review the variability, ultrastructure, distribution, and possible function of this enigmatic cuticular organ. Previous data are complemented by new observations on larvae and adults of various malacostracans. The SDO is composed of four sensors arranged as the corners of a square, the centre of which is occupied by a gland. Pores or pegs surrounding this central complex may also form part of the organ. The arrangement and the external aspect of the five main elements varies greatly, but this apparently has little impact on their ultrastructural organisation. The sensors and the gland are associated with a particularly thin cuticle. Each sensor contains four outer dendritic segments and the central gland is made of a single large cell. It is not yet known what this large cell secretes. The SDO is innervated from the tritocerebrum and therefore belongs to the third cephalic segment. A similar organ, here called the posterior SDO, has been repeatedly observed more posteriorly on the carapace. It resembles the SDO but has a greater number of sensors (usually six, but up to ten) apparently associated with only two outer dendritic segments. The SDO and the posterior SDO are known in the Eumalacostraca, the Hoplocarida, and the Phyllocarida. Some branchiopods also possess a 'dorsal organ' resembling both the SDO and the ion-transporting organ more typical of this group. This may indicate a common origin for these two functionally distinct groups of organs. New observations on the posterior SDO support the hypothesis that the SDO and the posterior SDO are homologous to the lattice organ complexes of thecostracans. However, the relationship between the SDO and the dorsal cephalic hump of calanoid copepods remains unclear. No correlation can be demonstrated between the presence of a SDO and a particular ecological or biological trait. In fossils, the most convincing examples of SDO-like organs are found in some Late Cambrian arthropods from the Alum Shale of southern Sweden. They suggest that related organs might have been present in non-crustacean Cambrian arthropods. The distribution of the SDO and posterior SDO in extant and fossil crustaceans strongly suggests that these organs originated early in the history of the group, and are crucial to the functioning of these organisms. However, except for knowing that the sensors are chemoreceptors and that in a given organ a functional relationship probably exists between them and the gland, little is known about this function. The description of a SDO in freshwater carideans, which can be easily reared in a laboratory, opens the way for behavioural and physiological experiments to be undertaken that could prove crucial for the determination of this function.

Key words: Crustacea, sensory dorsal organ, lattice organ, cephalic dorsal hump, anatomy.

CONTENTS

* Address for correspondence (Tel: +33 (0)4 72 44 81 44; Fax: +33 (0)4 72 43 15 26; E-mail: leroseyaubril@gmail.com).

I. INTRODUCTION

Arthropods are the most diverse group of metazoans, their abundance and diversity of morphology, size, and habitats attesting to their incomparable evolutionary success. Spanning some 520 million years (My), the fossil record of this phylum documents particularly well how the basic construction of the arthropod body has been modified to permit the evolution of a great variety of body plans. This plasticity in body patterning is not the sole explanation for their evolutionary success, for many other aspects of their biology are very significant (e.g. variety of developmental strategies, physiological tolerance). However, it seems difficult to conceive that arthropods could have acquired such a preponderant position in modern ecosystems without developing efficient sensorial abilities. Obvious sensory structures, such as eyes or antennae, have been extensively studied in both modern (e.g. crustaceans, Meyer-Rochow, 2001) and fossil arthropods (e.g. trilobites; Clarkson, Levi-Setti & Horváth, 2006), but as revealed by electronic microscopy, their cuticle also bears minute organs that apparently complement these major sensory structures (e.g. Laverack & Barrientos, 1985). One of these is the sensory dorsal organ (SDO), which has been observed in many crustaceans, situated along the sagittal line of the cephalic shield. Despite the many studies carried out by the late Professor Laverack and various collaborators from the mid-1980s to the late 1990s, this minute organ remains enigmatic.

Introduced by Laverack *et al*. (1996), the term 'sensory dorsal organ' refers to a small complex of structures (four sensors and a gland) often present in malacostracans. Previously, these structures were usually known as the 'dorsal organ', but great confusion existed about this term, for it had been used to refer to a variety of crustacean organs (e.g. Fioroni, 1980; Martin & Laverack, 1992). On the basis of ultrastructural features, Elofsson & Hessler (2008) proposed that most of these 'dorsal organs' could be regrouped into two functional categories: sensory organs ('dorsal sensory pit organs'; DSPOs) and ion-transporting organs ('dorsal ion-transporting complexes'; DITCs). In the present review, only the DSPOs are considered, with a special emphasis on the SDO, although the distinction between DSPOs and DITCs may sometimes be tenuous (e.g. in some branchiopods). The SDO was the first DSPO to be intensively studied, particularly its distribution and variability (Laverack & Macmillan, 1999), ultrastructure (Laverack *et al*., 1996), and innervation (Laverack & Sinclair, 1994). By the end of the 20th century it was clear that the SDO was extremely common in crustaceans and that it performed an essential function in these organisms. Surprisingly, however, not a single study has been devoted to this organ during the last decade, and its role and the exact nature of its relationships with similar organs of modern (e.g. the other DSPOs) or fossil (e.g. the cephalic median organ of trilobites) arthropods remains unknown. In the meantime, though, there has been a growing interest in another DSPO, the lattice organ (LO), which is found in clusters on the cephalic shield of the settling larval stages of thecostracans. Two clusters, one set anteriorly and comprising two pairs of LOs and one posterior comprising three pairs of LOs, have been described in representatives of the three subclasses of the Thecostraca (Høeg & Kolbasov, 2002). Each of these clusters is associated with a central gland to constitute a sensory glandular complex. The distribution and variability of these structures is fairly well known (e.g. Høeg & Kolbasov, 2002) and their ultrastructure has been described in a few taxa (Høeg, Hosfeld & Jensen, 1998; Høeg & Kolbasov, 2002). Their innervation, however, has not been investigated and, as with the SDO, their precise function remains unknown. A series of works dedicated to the LOs during the last 15 years has resulted in the accumulation of important data, which can now be used for a critical evaluation of the nature of their relationship with the SDO.

The present contribution originated from a simple statement of fact – despite an ever-growing accumulation of evidence, scientists have not fully appreciated the significance of the DSPO to crustaceans. Confusion about the term 'dorsal organ' may be a partial explanation for this situation.

The dispersal of data on the DSPO may be another. Herein we present a thorough review of the variability, ultrastructure, distribution, and possible function of the SDO. We complement these data with the results of investigations on adults of various freshwater carideans and on some malacostracan larvae, which include the first detailed description of a similar, but more posteriorly located organ, that we name the posterior SDO. A critical reappraisal of the relationships between the different types of DSPO identified by Elofsson & Hessler (2008) in living crustaceans is presented and we discuss the possible occurrences of related organs in fossil crustaceans. The main objective of this contribution is to stimulate renewed interest in these crustacean organs, which hopefully will enable their function to be determined.

II. MATERIALS AND METHODS

The following freshwater caridean species were examined: *Atya gabonensis* Giebel, 1875, *Atyaephyra desmaresti* (Millet, 1831), *Atyopsis moluccensis* (De Haan, 1849), *Caridina cantonensis* Yu, 1938 (variety 'tiger'), *C. balbauti* (Bouvier, 1918), *C. multidentata* Stimpson, 1860 ('Amano shrimps'), *C. spinata* Woltereck, 1937, *C. zeylanica* Arudpragasam & Costa, 1962, *Neocaridina heteropoda* Liang, 2002 (varieties 'red cherry' and 'sp. green'), *N. palmata* (Shen, 1948), and *Troglocaris* (*Troglocaris*) *planinensis* Birštejn, 1948. Apart from *A. desmaresti* and *T.* (*T.*) *planinensis*, individuals were bought in pet shops and reared in separate tanks in the laboratory. Depending on the taxa, individuals from one to many generations could be studied (e.g. descendants of a group of a dozen individuals over 4 years in the case of *N. heteropoda*). Some 30 specimens of *A. desmaresti* were collected in the Hérault River, at the 'Pont du diable' near Saint-Guilhem-le-Désert (Hérault) in southern France. These specimens and their offspring were reared in the laboratory for about 6 months. Six specimens of *T. (T.) planinensis* were collected in the Logarček cave (vicinity of Rakek, Western Slovenia), fixed in 70% ethanol, and kindly provided for this study by B. Sket and J. Jugovic (University of Ljubljana, Slovenia).

Investigations of the SDO and posterior SDO of these carideans were mainly conducted on exuviae, since their morphology is the same in dead specimens or on moults. Moreover, exuviae better support preparation for scanning electron microscopy, and their use permits, for a given individual, several attempts to study its cuticular organs; this was particularly useful for species that could not be maintained for more than one generation. The exuviae were collected as soon as possible after moulting, to minimise microbial decay, air-dried for 24–48 h, and stored in plastic boxes. They were then gold coated and studied at the Senckenberg Research Institute of Frankfurt am Main using a scanning electron microscope (SEM JEOL 310 JSM-6490LV) in high vacuum mode.

We also investigated the SDO and posterior SDO of larvae of 10 species of marine malacostracans, which represent four infra orders of Pleocyemata (i.e. Achelata, Anomura, Brachyura, Caridea). Ovigerous females were collected (see Table 1 for details), identified and held in separate aquaria. Recently hatched larvae were removed from the vials and fixed in a graded ethanol series as described in Meyer & Melzer (2004). For SEM preparation, fixed specimens were dehydrated in a graded acetone series (70, 80, 90%, 2 × 100%, 10 min each) and then critical-point-dried either in a Baltec CPD 030 or in hexamethyldisilazane (Nation, 1983; Laforsch & Tollrian, 2000). After coating with gold, they were studied with a LEO 1430VP SEM.

The size of the SDO and its different elements was calculated using the scale bars provided by the SEM or associated with the published illustrations thereof. The acronyms of the different organs discussed in this work are listed in Table 2. Abbreviations used herein are: exs., exsagittally, sag., sagittally, and tr., transversally.

III. THE SENSORY DORSAL ORGAN

(1) Morphology and variability

(a) *Description of a typical SDO*

As described by Laverack *et al*. (1996), the SDO is typically composed of a flexible, central area of cuticle exhibiting one or more pores and four peripheral sensory plates (Fig. 1A). The arrangement of these five elements usually forms a quincunx, like the fifth side of a die. Mean values for the area they occupy are about 33 μm × 36 μm [maximum length (exs.) X width (tr.)]. The mean size of the sensory plates is approximately 9 and 6.5 μm in maximum length and width, respectively, while that of the central element(s) is about 9 and 8.5 μm in maximum length and width, respectively. The SDO is located along the midline of the anterior half of the carapace (Figs 2A, 3A).

(b) *Variability*

The SDO is associated with a thin and flexible cuticle restricted to the central area or extending to the whole organ. This flexible cuticle frequently collapses during specimen preparation for electron microscopy, resulting in artificial differences in the external aspect of the organ. This problem must be kept in mind when describing the variability of the SDO, especially since this organ in many taxa has been described from only a few specimens.

Virtually every aspect of the SDO is subject to variation. For instance, the central area commonly exhibits one (e.g. *Eualus cranchii*, *Pandalus montagni*, Laverack & Crombie, 1988; Fig. 2B, C, F, H, K, L) to a few large pits (e.g. *Porcellana platycheles*, Barrientos & Laverack, 1986; Figs 2D, 3S), but these pits are sometimes replaced by many minute perforations (e.g. *Macrobrachium intermedium*, Laverack & MacMillan, 1999) or by a large slit (e.g. *Jasus edwardsii*, Laverack & MacMillan, 1999). In some carideans, it is composed of a smooth and large depressed area or a hole

Table 1. Taxonomic and collection details of the larval specimens investigated

Specimen	Origin
Infraorder Achelata	
Scyllarus sp. Sach. Kat. 34/10	19°38′N, 18°03′W off Cap Blanc, Mauritania (1 February 1970)
Infraorder Anomura	
Galathea squamifera Leach, 1814 ZSMA20035574	Saline bay, Rovinj Croatia (29 March 2003)
Infraorder Brachyura	
Eurypanopeus planus (Smith, 1869) ZSMA20080030	Cangrejal, Playa Samara Costa Rica (7 May 2004)
Goniopsis pulchra (Lockington, 1876) ZSMA20080030	Punta Morales, Playa Blanca Costa Rica (26 July 2004)
Panopeus chilensis Milne-Edwards & Lucas, 1843 ZSMA20080032	Punta Morales, Playa Blanca Costa Rica (25 July 2004)
Lophozozymus incisus (Milne-Edwards, 1834) ZSMA20071645	Roscoff, Bretagne France (1 June 2005)
Portunus acuminatus (Stimpson, 1871) ZSMA20050130	09°48.9′N, 84°40.5′W Golfo de Nicoya, Costa Rica (21 April 2004)
Xantho pilipes Milne-Edwards, 1867 ZSMA20035565	Saline bay, Rovinj Croatia (11 April 2003)
Xantho poressa (Olivi, 1792) ZSMA20035549	Saline bay, Rovinj Croatia (12 April 2003)
Infraorder Caridea	
Palaemon adspersus Rathke, 1837 ZSMA20035515	Saline bay, Rovinj Croatia (29 March 2003)

Repository: Bavarian State Collection of Zoology (ZSMA).

Table 2. Acronyms and descriptions of the different organs discussed in this review

Acronym	Name	Description/location/distribution	References
SDO	Sensory dorsal organ	Sensory glandular complex composed of four peripheral sensors and one central gland. In the anterior region of the carapace. Malacostraca, possibly Branchiopoda	Laverack *et al*. (1996)
Posterior SDO	Posterior sensory dorsal organ	Sensory glandular complex composed of six (exceptionally eight or ten) peripheral sensors and one central gland. Near posterior margin of the carapace. Malacostraca	Herein
LO	Lattice organ	Sensory organ. See LOC. Thecostraca	Elfimov (1986)
LOC (anterior or posterior)	Lattice organ complex (anterior or posterior)	Sensory glandular complex composed of four (anterior) or six (posterior) peripheral sensors (LO) and one central gland. Respectively in the anterior and posterior regions of the cephalic shield of settling larval stages. Thecostraca	Herein
CDH	Cephalic dorsal hump	Sensory glandular complex composed of one sensor and two glands. In the anterior of the cephalic shield of adult males. Copepoda (Calanoidea)	Nishida (1989)
DSPOs	Dorsal sensory pitted organs	Category regrouping the 'dorsal organs' with a sensory function. This contains the SDO and posterior SDO, the anterior and posterior LOC, and the CDH. All of them occur on the cephalic shield. Malacostraca, possibly Branchiopoda, Thecostraca, Copepoda (Calanoidea)	Elofsson & Hessler (2008)
DITCs	Dorsal ion-transporting complexes	Category regrouping the 'dorsal organs' with an ion-transporting function. This contains a great variety of (sometimes embryonic) organs. Their location is not restricted to the cephalic shield. In the Crustacea: Branchiopoda, Copepoda (Harpacticoidea), Malacostraca (Syncarida), Peracarida (Isopoda)	Elofsson & Hessler (2008)

surrounded by tiny pits (Fig. 3C, F, G, L). The central element can also take the form of a small knob (e.g. *Palaemon serenus*, Laverack & MacMillan, 1999) or a slightly raised area (e.g. *Lynceus brachyurus*, Olesen, 1996). More rarely, the central area is devoid of any perforations (e.g. *Scyllarus* sp., Fig. 2J; *Upogebia* sp., Laverack & MacMillan, 1999) or even of any features indicating the presence of a central element (e.g. *Callianassa australiensis*, Laverack & MacMillan, 1999; *Eurycercus glacialis*, Olesen, 1996). Lastly, this central area, which is associated with a flexible cuticle, was too strongly wrinkled in preparations of many taxa to permit description of its morphology (e.g. Fig. 3P, R).

The four sensory elements are less variable in external morphology, usually taking the form of ovoid plate-like areas (e.g. *Limnadia* sp., Laverack & MacMillan, 1999; *Eualus cranchi*, *Pandalus montagni*, Laverack & Crombie, 1988,

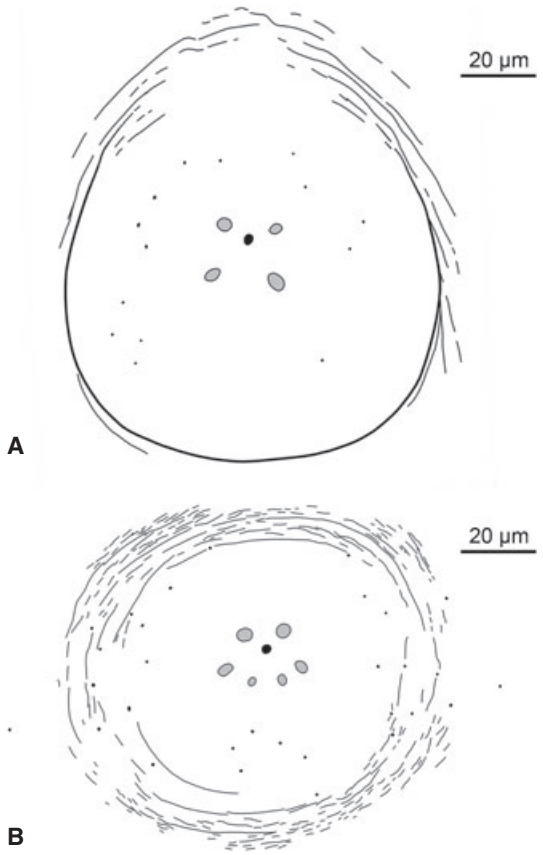


Fig. 1. Drawings of the anterior and posterior sensory dorsal organs (SDOs) of *Caridina multidentata*. (A) The anterior SDO is composed of a central complex made of four sensory plates and a large central pit, which is surrounded laterally and anteriorly by numerous tiny pores/pegs. (B) The posterior SDO comprises a central complex made of six sensory plates and a large central pit, which is surrounded laterally and posteriorly by numerous tiny pores/pegs.

Figs 2B–G, J–L, 3B, C, E, F, H, K–M, P–R, T–W). These areas are usually slightly depressed, more rarely forming true pits (e.g. *Homarus gammarus*, Barrientos & Laverack, 1986). As observed in *Atya gabonensis*, this difference may depend on the degree of collapse during the SEM preparation of the whole organ. Elevated bumps/papillae can replace the sensory plates in some taxa (e.g. *Anaspides tasmaniae*, *Crangon crangon*, Laverack *et al*., 1996), or in some specimens in species which otherwise exhibit sensory plates (e.g. *Neocaridina heteropoda*, compare Fig. 3R, S). Structures sometimes occur on these plates, the most common being four pores or pegs probably associated with four underlying dendrites (see Section III.2; Figs 2L, 3H, M, T, U). A more variable number (3–5) of pegs was observed in larval stages of *Homarus gammarus* by Barrientos & Laverack (1986), who also noted that they were not visible in the earliest stages. More rarely, each sensory plate can bear a nipple-like structure (e.g. *Dissodactylus crinitichelis*, Pohle & Telford, 1981; *Hyas cornatus*, Laverack & Barrientos, 1985; *Scyllarus* sp., Fig. 2J). The greatest departure from the usual morphology of the SDO is known in *Euphausia superba*, where the organ comprises six sensory plates (Laverack & MacMillan, 1999). This may indicate a closer affinity with a second, more posteriorly located organ we have observed in several species (see Section III.3), rather than an extreme evolution of the SDO in euphausiids.

Another source of variation is the positioning of the five elements. Most of the time, it forms a quincunx, with the sensory plates being at the corners of an imaginary square, the centre of which is occupied by the fifth element, the central element (e.g. Fig. 2B, D, F, J). In *Crangon crangon*, however, the five elements are aligned transversally, probably due to the position of the SDO at the base of a forwardly projecting spine (Laverack & Crombie, 1988). Rather than a square, the arrangement of the sensors forms a trapezoid in *Caridina zeylanica* (Fig. 3P) or *Neocaridina heteropoda* (Fig. 3Q–S) and an inverted trapezoid in several malacostracan larvae (Fig. 2C, E, K, L). The position of the fifth element is also frequently shifted forwards. In *N. heteropoda*, for example, the wrinkled area representing this fifth element is located between the two anterior sensory plates (Fig. 3R). The quincunx pattern is found in an overwhelming majority of cases, which suggests strong constraints on the relative position of the main elements of the organ, possibly due to functional requirements.

Our investigations revealed that this organ may sometimes be composed of additional elements in malacostracans. For instance, the complex of five elements is surrounded laterally and anteriorly by a constellation of minute pits/pegs in several carideans (e.g. *Atya gabonensis*, *Atyopsis moluccensis*, *Caridina multidentata*; Fig. 3B, D, E, F, I, J, K, N). These pits are not symmetrically arranged relative to the sagittal line and their numbers differ from one side to another. In adults of the other carideans investigated, the organ was too wrinkled to enable the detection of such tiny structures (e.g. Fig. 3O, V, W), but in *Neocaridina heteropoda* at least they are apparently absent. In the larvae of grapsoid and xanthoid crabs, the SDO is associated with a pair of pores located posteriorly at a variable distance from it (Fig. 2B–F, I). Again, this suggests that the SDO may comprise more than the five elements of the central complex. However, because no attention has been paid to such structures in the past (e.g. in Laverack & MacMillan, 1999), it is not possible to assess their frequency in the Crustacea, nor to make assumptions about their function.

Lastly, the presence/absence of a clear delimitation is another source of variation of the external aspect of the SDO. This is best exemplified in carideans, where a strong fold of the cuticle may isolate the organ from the rest of the carapace in some taxa (e.g. *Atya gabonensis*, *Atyopsis moluccensis*, *Caridina multidentata*; Fig. 3B, E, K), but not in others (e.g. *Caridina zeylanica*, *Neocaridina heteropoda*; Fig. 3P–S). The presence of a surrounding rim has only been observed in the putative SDO of some branchiopods (e.g. Olesen, 1996; see Section III.4a). This character suggests possible affinities of these SDO-like organs with DITC-type dorsal organs (Elofsson & Hessler, 2008) usually encountered in the Branchiopoda, supporting the assumption of Walossek (1993) that they might in fact represent both DSPO and DITC (see Section VI). All other

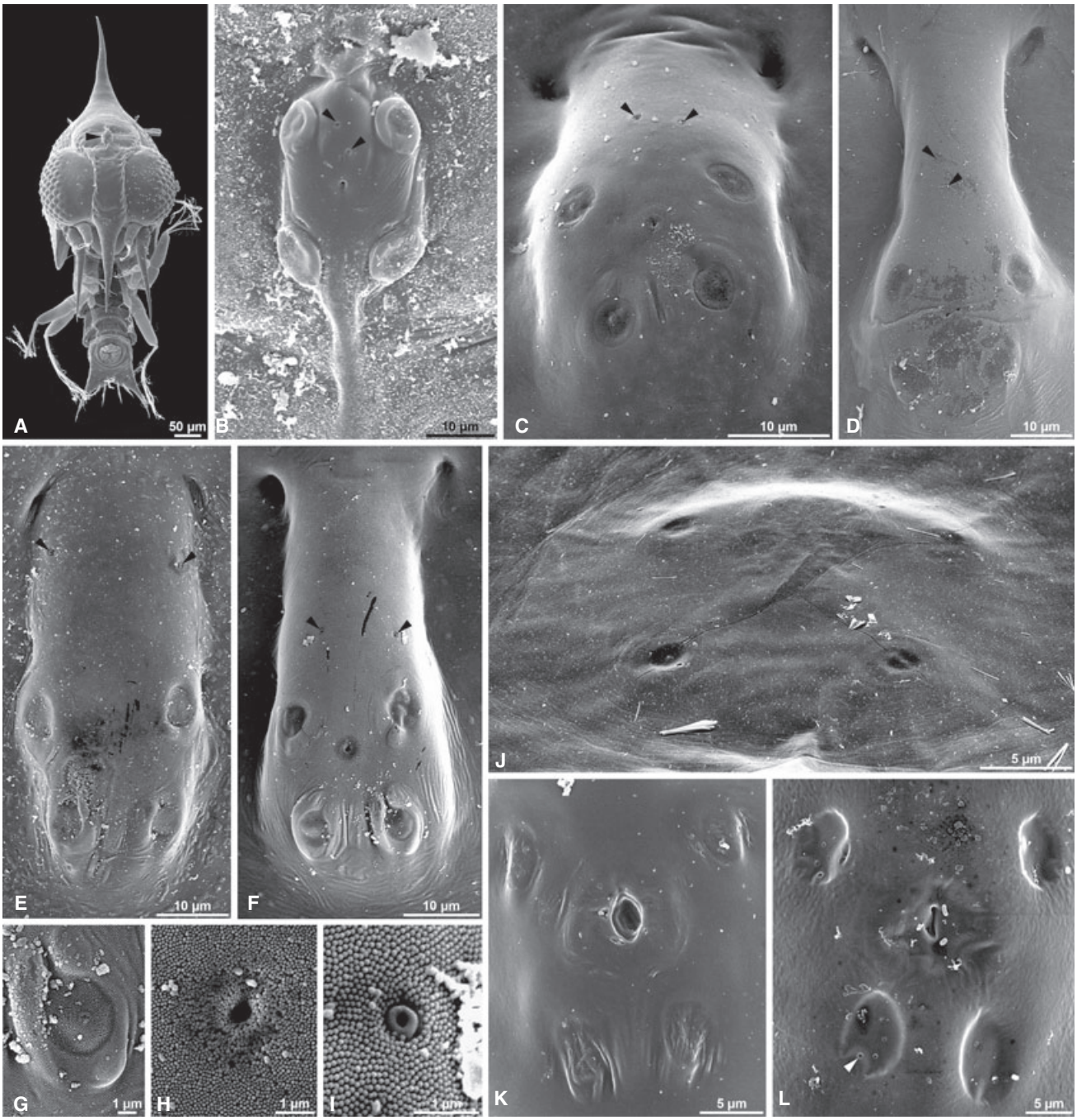


Fig. 2. The sensory dorsal organ (SDO) in larvae of diverse decapods. All figures are scanning electron micrographs of specimens coated with gold; anterior is to the bottom for all except J where anterior is to the top. (A, B) *Goniopsis pulchra* (Brachyura, Grapsoidea). (A) Entire specimen in frontal view showing the position of the SDO (arrow head). (B) General view of the SDO; note the pair of extra pores (arrow heads) behind the central pore. (C) *Eurypanopeus planus* (Brachyura, Xanthoidea), general view of the SDO; a pair of additional pores (arrow heads) is located a short distance behind the main central complex. (D) *Xantho poressa* (Brachyura, Xanthoidea), general view of the SDO; a pair of additional pores (arrow heads) is located a short distance behind the main central complex. (E) *Lophozozymus incisus* (Brachyura, Xanthoidea), general view of the SDO; a pair of additional pores (arrow heads) is located a short distance behind the main central complex. (F–I) *Panopeus chilensis* (Brachyura, Xanthoidea). (F) General view of the SDO; a pair of additional pores (arrow heads) is located a short distance behind the main central complex. (G) Detail of one of the four sensory plates. (H) Detail of the central pore. (I) Detail of one of the two extra pores located behind the main central complex. (J) *Scyllarus* sp. (Achelata), general view of the SDO; note that the central area is devoid of any perforations. (K) *Galathea squamifera* (Anomura), general view of the SDO. (L) *Palaemon adspersus* (Caridea), general view of the SDO; note that each sensory plate displays four tiny pores (arrow head).

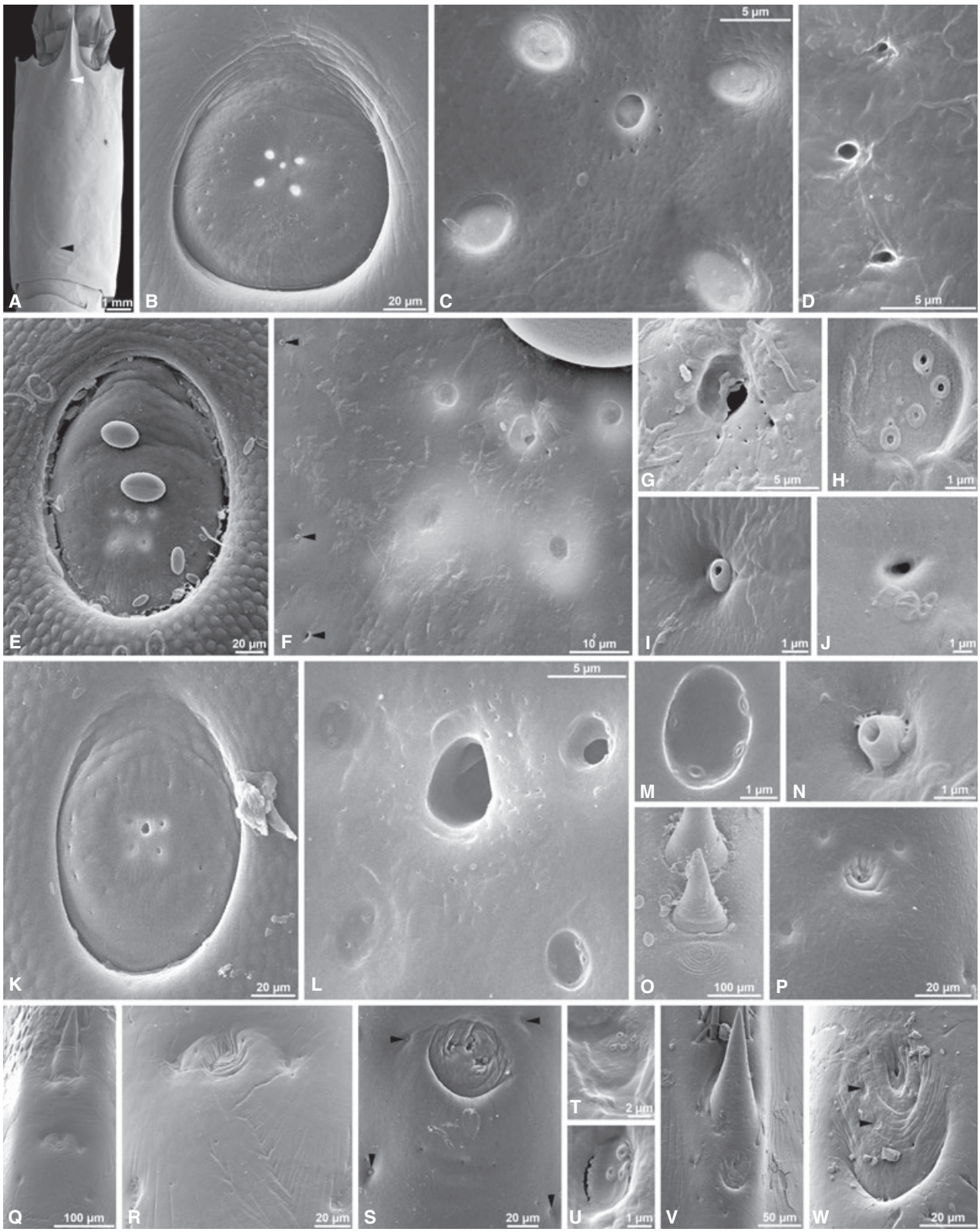


Fig. 3. Legend on next page.

known variations affecting these branchiopod SDOs can also be observed in malacostracan SDOs.

In most species, the central complex (sensors + central element) of the SDO is slightly less than 20 μm in length (sag.) and slightly more than 20 μm in width (tr.). It occupies a greater area in *Caridina zeylanica* [about 45 μm in length (sag.) and 75 μm in width (tr.)] and *Neocaridina heteropoda* [about 85 μm in length (sag.) and 105 μm in width (tr.)], but the size of the sensors in these species ranges from 3 to 7 μm in length and slightly less in width, as in most species. These greater dimensions of the central complex are not related to a greater body size, which is rather modest (2–3 cm) in these species. By contrast, in the two thalassanideans investigated by Laverack & MacMillan (1999), the central complex was not only notably larger [about 115 μm in length (sag.) and 145 μm in width (tr.) in *Callianassa australiensis*, and 100 μm in length (sag.) and 70 μm in width (tr.) in *Upogebia* sp.], but was associated with sensors about twice (*Upogebia* sp.) and four times (*C. australiensis*) larger than the sensors of other species. How this difference in the dimensions of the sensory plates affects the way they function is unknown, but it is noteworthy that these two taxa are endobenthic.

(2) Ultrastructure and innervation

Detailed investigations of the ultrastructure of the SDO have only been undertaken in two species: the syncarid *Anaspides tasmaniae* and the eucarid *Crangon crangon* (Laverack *et al*., 1996). Interestingly, while the SDO of these two taxa differ notably in external aspects, their ultrastructures are strikingly similar, suggesting that the internal organization of the SDO is conservative in malacostracans.

The whole complex is essentially an island of thin cuticle (Laverack *et al*., 1996, Fig. 4). The central area is associated with an invagination of an extremely thin epicuticle, which forms a blind-ending tube surrounded by a single large cell. This cell is not innervated and its strongly folded membrane and numerous vacuoles/vesicles are suggestive of a secretory function. In the two species investigated, the sensors take the form of slightly elevated bumps externally (i.e. papillae). In *Anaspides tasmaniae*, each bump contains a blind pocket, while it corresponds to a blind tube in *Crangon crangon*. The floor of the pocket/tube is made of an extremely thin layer of cuticle (0.05–0.1 μm). Immediately below is a thin layer of electron-dense, extracellular material of unknown nature. In both taxa, four outer dendritic segments, separated from one another by sheath cells, are found associated with each sensory area. Only in *C. crangon*, however, do the extremities of these dendrites pass through the electron-dense material and protrude into the thin epicuticle. One dendrite under each papilla lies alongside the pocket/tube, instead of beneath it, as in the three others. These dendrites correspond to four distinct monociliary nerve cells per papilla in both taxa.

The presence of a single, large secretory cell under the central area and four outer dendritic segments below each papilla has previously been described in larvae of the brachyuran *Hyas cornatus* by Laverack & Barrientos (1985). However, the outer dendritic segments correspond to only two biciliary nerve cells in these larvae. Four pegs, apparently associated with the four dendrites, are visible at the surface of each sensory plate in *Crangon crangon* (Laverack *et al*., 1996), but also in the adults of *Atya gabonensis* (Fig. 3H), *Atyopsis moluccensis* (Fig. 3M), *Caridina cantonensis* (Fig. 3U), *C. multidentata*, *Eualus cranchii* (Laverack & Crombie, 1988), *Macrobrachium intermedium*, *Neocaridina heteropoda* (Fig. 3T), *Palaemon serenus* [Laverack & MacMillan, 1999; also in larvae of *P. adspersus* (Fig. 2L)], and the larvae of *Homarus gammarus* (Barrientos & Laverack, 1986), *Jasus edwardsii* (Nishida & Kittaka, 1992), and an unidentified stomatopod (Laverack & MacMillan, 1999).

Lastly, it has been shown in *Macrobrachium intermedium* that the SDO is innervated from the tritocerebrum through the tortuous route of a particular branch of a large nerve (Laverack & Sinclair, 1994). This confirms a statement of Hanstrøm (1947) concerning the innervation of the SDO in

Fig. 3. The sensory dorsal organ (SDO) in adults of diverse freshwater caridean shrimps. All figures are scanning electron micrographs of exuviae coated with gold; anterior is to the top for all. (A–D) *Caridina multidentata*. (A) Carapace in dorsal view, with the locations of the SDO (white arrow head) and posterior SDO (black arrow head). (B) General view of the SDO; note the tiny pores surrounding the central complex laterally and anteriorly. (C) Detail of the central complex with four sensory plates and a large central pit associated with tiny perforations. (D) Three of the numerous pores surrounding the central complex. (E–J) *Atya gabonensis*. (E) General view of the SDO; the ovoid objects are pennate diatoms stuck to the cuticle. (F) Detail of the central complex; four of the pits/pegs surrounding the central complex are visible on the left (arrow heads). (G) Detail of the central area of the central complex made of a large depression (partially broken) and tiny perforations. (H) Sensory plate exhibiting four pegs. (I, J) Two of the numerous pegs (I) or pores (J) surrounding the central complex. (K–N) *Atyopsis moluccensis*. (K) General view of the SDO; note the tiny pores surrounding the central complex laterally and anteriorly. (L) Detail of the central complex made of four sensory plates and a large central pit associated with tiny perforations. (M) Sensory plate with four pegs. (N) One of the numerous pegs surrounding the central complex. (O) *Atyaephyra desmaresti*, detail of the carapace showing the location of the SDO a short distance posterior to the last rostral spine. (P) *Caridina zeylanica*, general view of the SDO; note that the organ is not associated with an island of thin cuticle as in B, E, or K. (Q–T) *Neocaridina heteropoda*. (Q) Detail of the carapace showing the location of the SDO a short distance posterior to the last rostral spine; as in *C. zeylanica*, the organ is not associated with an island of thin cuticle. (R) General view of the SDO; note that the glandular element is located between the two anterior sensory plates. (S) General view of the organ; note that each sensory plate is covered by a bump in this specimen (arrow heads). (T) Sensory plates with four pegs. (U–W) *Caridina cantonensis*. (U) Sensory plates bearing four pegs. (V) Detail of the carapace showing the location of the SDO a short distance posterior to the last rostral spine. (W) General view of the SDO; only two of the four sensory plates are visible (arrow heads).

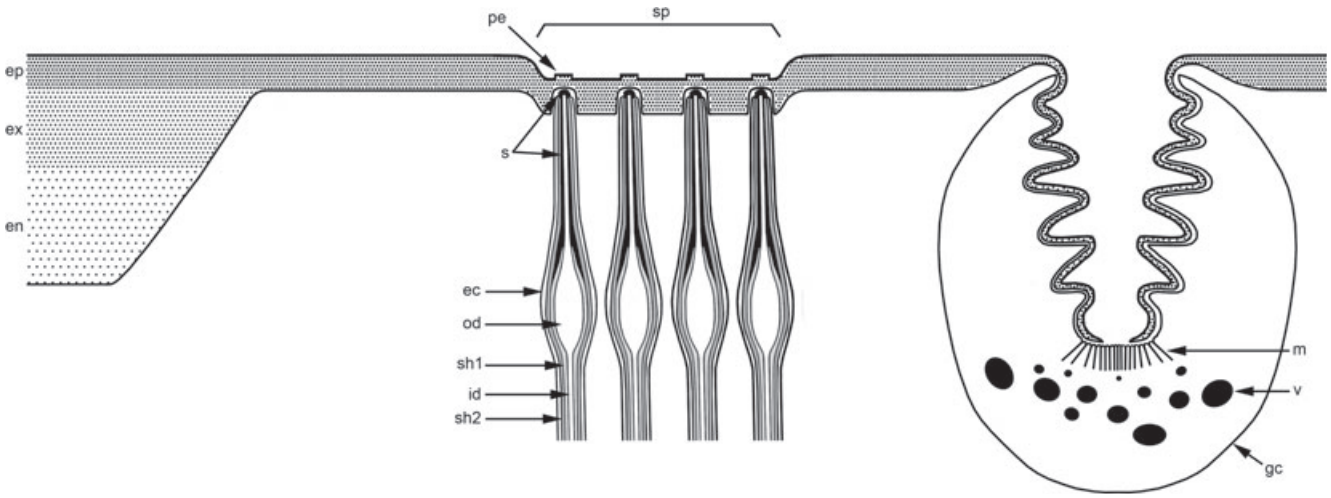


Fig. 4. Schematic representation of the ultrastructure of the sensory dorsal organ. Only one of the four sensors is represented. Each sensor is composed of four sensory cells, which are each surrounded by two sheath cells and an envelope cell. The central gland is made of a single large glandular cell exhibiting numerous microvilli. ec, envelope cell; ep, epicuticle; en, endocuticle; ex, exocuticle; gc, glandular cell; id, inner dendritic segment; m, microvilli; od, outer dendritic segment; pe, peg; s, sheath of electron-dense material; sh1, inner sheath cell; sh2, outer sheath cell; sp, sensory plate; v, vacuole. Modified after Laverack *et al*. (1996, fig. 5).

Anaspides tasmaniae. Unfortunately, nothing is known about the innervation of the SDO in other taxa. Such investigations would provide critical arguments for testing the homology of this organ both within and outside the Eumalacostraca.

(3) The posterior SDO

Laverack & MacMillan (1999) reported the presence in larvae or adults of several crustaceans of a second, posteriorly located organ. They suggested that this posterior organ may be related to the SDO, but neither their succinct descriptions nor their illustrations (Laverack & MacMillan, 1999, figs. 1.5, 2.4, 3.4) provide evidence in support of this relationship. Nishida & Kittaka (1992) described with greater precision the posterior organ of some phyllosoma larvae of *Jasus edwardsii* (Hutton, 1875), but unfortunately their only illustration of it is a schematic drawing (their fig. 3B). They clearly mentioned that this organ and the SDO were virtually identical, except for a greater number of sensory plates (10) in the former. They also mentioned the presence of two to four pegs on the sensory plates, but did not note whether the SDO and the posterior organ differ in this feature. Another posterior organ was illustrated by Meyer, Wehrtmann & Melzer (2006, fig. 4A; Fig. 5C herein) in a larva of the brachyuran *Portunus acuminatus*, which also displayed a SDO anteriorly. Our investigations have revealed that similar organs occur in larvae of xanthoid (*Eurypanopeus planus*, *Lophozozymus incisus*, *Panopeus chilensis*) and grapsoid (*Goniopsis pulchra*) brachyurans (Fig. 5A, B, D–H). They are composed of three pairs of sensory plates often located on a swollen area. Two pairs are arranged at the corners of a square. The sensory plates composing the third pair are more medially positioned in the anterior part of this square, or slightly in front of it, flanking a large pore (Fig. 5B–E). A second, smaller pore was repeatedly observed a short distance posteriorly from this large pore (Fig. 5B–E). A pair of pores also occurs behind the organ in the larvae of *P. acuminatus* (Fig. 5C) and *P. chilensis* (Fig. 5E, H). No posterior organs were detected in the larvae of *Galathea squamifera* (Anomura), *Palaemon adspersus* (Caridea), and *Scyllarus* sp. This latter observation suggests that within the Achelata the families Palinuridae and Scyllaridae may differ with regard to this character.

A specific search for a posterior organ in adult freshwater carideans revealed its occurrence in at least four species: *Atya gabonensis*, *Atyopsis moluccensis*, *Caridina multidentata*, and *Caridina spinata* (Fig. 5I–O). This organ occurs along the sagittal line of the carapace near its posterior margin (Fig. 3A). Its organisation is reminiscent of the SDO, but it appears more complex. In *C. multidentata*, it consists of a large, discoid island of flexible cuticle, with six plates surrounding a central area (Figs 1B, 5I, J). As in the sensory plates of the SDO, pegs occur at the surface, but only two instead of four for each plate (Fig. 5J). The central area bears one or a few large pores, sometimes surrounded by minute perforations. As in the SDO, the central complex (sensors + gland) is associated with numerous, minute pits, but these are located posteriorly (instead of anteriorly) and laterally (Figs 1B, 5I). Their arrangement is not symmetrical relative to the sagittal axis and they are seldom replaced by pegs. The organ is vaguely delimited by concentric wrinkles, but a few of the peripheral pits can occur beyond this limit (Fig. 1B). A similar organ has been observed on one individual (two exuviae) of *A. moluccensis* (Fig. 5K–M). As in *C. multidentata*, each sensory plate bears two pegs (Fig. 5L) and minute, peripheral pits occur on both sides (but not posteriorly) of the central complex. However, two additional sensory plates, each bearing two pegs, are visible posterior to the organ (Fig. 5K, M). The distance between these two additional sensors exceeds 30 μm.

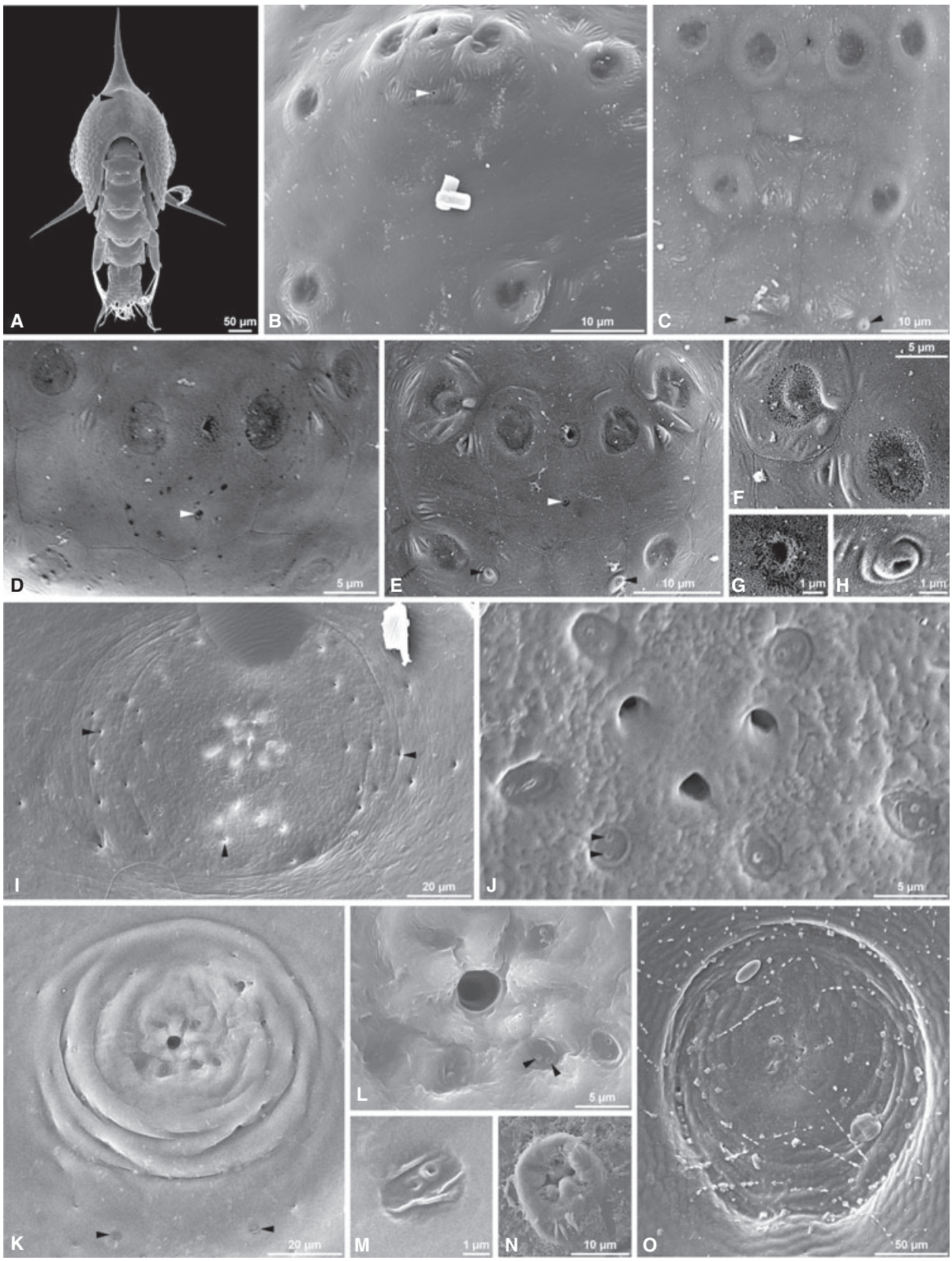


Fig. 5. Legend on next page.

The posterior organ observed in adult carideans and brachyuran larvae strongly resembles the SDO in the organisation of its central elements and the presence of pegs on the plates, suggesting that they are sensory plates. In adult carideans, additional similarities between the two organs are the presence of numerous, minute pits surrounding the central complex, their association with large islands of flexible cuticle, and their location along the sagittal line of the carapace. Accordingly, we consider that this posterior organ is related to the SDO and probably undertakes a similar function. We propose to name it the posterior SDO. In most cases, it comprises six sensors. The extra fourth pair observed in *Atyopsis moluccensis* is rather distant from the organ (Fig. 5K) and at present it is difficult to assess whether it is in fact part of this organ. In the posterior SDO of phyllosoma larvae of *Jasus edwardsii*, however, five pairs of sensors seem to be associated with the central swollen area, suggesting that variability may occur in the number of sensors composing this organ.

IV. DISTRIBUTION

(1) Distribution within the Crustacea

The distribution of the SDO within the Crustacea was surveyed by Laverack & Macmillan (1999). We complement their data with our own observations in various marine or freshwater malacostracans and with reports overlooked by these authors or published more recently. This updated distribution of the SDO in the Crustacea is presented in Table 3. This table only includes occurrences of an organ that we can reasonably assume to be a SDO. Several structures described by Hansen (1921) or Mauchline (1977) for example, were not included due to the absence of adequate illustrations.

The great majority of the 'dorsal organs' described in branchiopods are DITCs (Elofsson & Hessler, 2008). A few species, however, exhibit a more complex organ composed of five elements arranged in a quincunx, as in the SDO of malacostracans. Laverack & MacMillan (1999) mentioned the spinicaudatan *Limnadia* sp., and other examples have been identified in the Diplostraca (Table 3). Rieder *et al*. (1984) described the ultrastructure of such an organ in *Limnadia lenticularis* and suggested a possible role in ion regulation. However, its internal organization shows some similarities with that of the SDO, such as the presence of a central cell with microvilli and four nerve fibres (see Section III.2). On the other hand, this organ comprised more cell types than in the SDO; until further investigations are carried out on the ultrastructure and innervation of both organs, their homology remains uncertain.

The presence of a SDO in many malacostracans is less equivocal (Table 3). Mostly known in eumalacostracans, the organ has also been observed in the Phyllocarida and the Hoplocarida (Laverack & MacMillan, 1999, Table 3). A few remarks are necessary about its distribution within the Eumalacostraca. Firstly, the 'SDO' described by Laverack & MacMillan (1999) in the euphausiacean *Euphausia superba* comprised seven elements suggesting that it may in fact be a posterior SDO (Section III.1b). Secondly, the SDO has only been observed in larval stages in many decapods (Table 3). As illustrated by *Jasus edwardsii* (Laverack & MacMillan, 1999), the organ progressively disappears during ontogeny in these taxa, possibly due to the development of a thick and mineralized cuticle. Indeed, in the taxa where the SDO persists until adulthood, the cuticle remains rather thin and weakly mineralized. This is the case for all the carideans we investigated, which are rather small species (rarely exceeding 4 cm in length), and for the two thalassanideans described by Laverack & MacMillan (1999), which are burrowing species (Ruppert & Barnes, 1994, p. 703). Lastly, Laverack & MacMillan (1999) searched for the presence of a SDO in various adult peracarids without success. However, no younger developmental stages were investigated. Hansen (1921) described several dorsal structures in some Isopoda and Mysida, but none seems to be typical of a SDO. If confirmed, this absence of SDOs in the Peracarida could be regarded as an autapomorphy for this group. The posterior SDO has been observed in representatives of the Eumalacostraca, the Phyllocarida, and the Hoplocarida.

Fig. 5. Posterior sensory dorsal organs (posterior SDOs) in larvae and adults of diverse decapods. All figures are scanning electron micrographs of specimens coated with gold; anterior is to the top for B–O. (A, B) *Goniopsis pulchra* (Brachyura, Grapsoidea). (A) Entire specimen in posterior view showing the position of the posterior SDO (arrow head). (B) General view of the posterior SDO; note the tiny pore (arrow head) located behind the larger one which is framed laterally by sensory plates. (C) *Portunus acuminatus* (Brachyura, Portunoidea), general view of the posterior SDO; note the small pore (white arrow head) in the centre of the main central complex and the pair of pores located behind this complex (black arrow heads). (D) *Eurypanopeus planus* (Brachyura, Xanthoidea; larva), general view of the posterior SDO; note the small pore (arrow head) located in the centre of the main central complex. (E–H) *Panopeus chilensis* (Brachyura, Xanthoidea; larva). (E) General view of the posterior SDO; note the small pore (white arrow head) located behind the large pore and the pair of additional pores (black arrow heads) located a short distance behind the main central complex. (F) Detail of two sensory plates. (G) Detail of the large pore. (H) Detail of one of the two pores present behind the main central complex. (I, J) *Caridina multidentata* (Caridea; adult). (I) General view of the posterior SDO; the main central complex is surrounded laterally and posteriorly by numerous, tiny pits (arrow heads). (J) Detail of the main central complex; note that each sensory plate bears two pegs (arrow heads). (K–M) *Atyopsis moluccensis* (Caridea; adult). (K) General view of the posterior SDO; note the extra pair of sensory plates behind the organ (arrow heads). (L) Detail of the main central complex; the large central pit is surrounded by tiny perforations and each sensory plate bears two pegs (arrow heads). (M) Detail of one of the two extra sensory plates located behind the organ; note the two pegs. (N) *Caridina spinata* (Caridea; adult), general view of a possible posterior SDO. (O) *Atya gabonensis* (Caridea; adult), general view of the posterior SDO.

Table 3. Distribution of the sensory dorsal organ (SDO) and posterior SDO in the Crustacea

Class (subclass)	**Superorder** order (suborder or *infraorder*)	Species	Remarks	References
Branchiopoda (Phyllopoda)	Spinicaudata	*Limnadia* sp.?		Laverack & Macmillan (1999)
	Diplostraca (Cladocera)	*Eurycercus glacialis?*	-Quincunx organisation	Olesen (1996)
		E. lamellatus?	-Quincunx organisation	Olesen (1996)
	(Cyclestherida)	*Cyclestheria hislopi?*	-Quincunx organisation	Olesen (1996)
	(Laevicaudata)	*Lynceus brachyurus?*	-Quincunx organisation -In larvae and adults	Olesen (1996, 2005) and
		L. gracilicornis?	-Quincunx organisation	Martin & Belk (1988)
		Paralimnetis mapini?	-Quincunx organisation	Martin & Belk (1988)
Malacostraca (Phyllocarida)	Leptostraca	*Nebalia longicornis*	-Second organ posteriorly	Laverack & Macmillan (1999)
Malacostraca (Hoplocarida)	Stomatopoda	*Neogonodactylus oerstedii*	-Second organ posteriorly	Laverack & Macmillan (1999)
		Unidentified larvae	-Second organ posteriorly	Laverack & Macmillan (1999)
Malacostraca (Eumalacostraca)	**Syncarida** Anaspidacea	*Anaspides tasmaniae*		Laverack *et al*. (1996) Laverack & Macmillan (1999)
	Eucarida Euphausiacea	*Euphausia superba*	-Posterior organ	Laverack & Macmillan (1999)
	Eucarida Decapoda (Dendrobranchiata)	*Acetes sibogae*		Laverack & Macmillan (1999)
		Sergestes sp.	-Second organ posteriorly	Laverack & Macmillan (1999)
	(Pleocyemata) (*Achelata*)	*Jasus edwardsii*	-Larvae only; progressively disappear during ontogeny -Second organ posteriorly in earliest larval stages	Nishida & Kittaka (1992) and Laverack & Macmillan (1999)
		Scyllarus sp.	-In larvae -No second organ posteriorly	Herein
	(*Astacidea*)	*Homarus gammarus*	-In larvae only	Laverack & Barrientos (1985), Barrientos & Laverack (1986) and Laverack & Macmillan (1999)
		Nephrops sp.	-In larvae only	Laverack & Barrientos (1985)
	(*Anomura*)	*Galathea squamifera*	-In larvae	Herein
		Porcellana sp.	-In larvae only	Laverack (1988), Barrientos & Laverack (1986), and Laverack & Macmillan (1999)
		P. platycheles	-In larvae only	
	(*Brachyura*)	*Carcinus maenas*	-In larvae only	Laverack & Barrientos (1985) and Barrientos & Laverack (1986)
		Dissodactylus crinitichelis	-In larvae	Pohle & Telford (1981)
		Ebalia tuberosa	-In larvae only	Laverack & Macmillan (1999)
		Eurypanopeus planus	-In larvae -Second organ posteriorly	Herein
		Goniopsis pulchra	-In larvae -Second organ posteriorly	Herein
		Hyas cornatus	-In larvae only	Laverack & Barrientos (1985) and Barrientos & Laverack (1986)
		Leurocyclus tuberculosus	-In larvae -Second organ posteriorly	Santana & Marques (2009)
		Lophozozymus incisus	-In larvae -Second organ posteriorly	Herein

Table 3. (Cont.)

Class (subclass)	**Superorder** order (suborder or *infraorder*)	Species	Remarks	References
		Panopeus chilensis	-In larvae -Second organ posteriorly	Herein
		Portunus acuminatus	-In larvae -Second organ posteriorly	Meyer *et al*. (2006) Herein
		Sesarma elegans	-In larvae only	Laverack (1988)
		Xantho pilipes	-In larvae	Herein
		X. poressa	-In larvae	Herein
	(*Caridea*)	*Alpheus* sp.	-Second organ posteriorly	Laverack & Macmillan (1999)
		Atya gabonensis	-Second organ posteriorly	Herein
		Atyaephyra desmaresti		Herein
		Atyopsis moluccensis	-Second organ posteriorly	Herein
		Caridina cantonensis		Herein
		C. balbauti		Herein
		C. multidentata	-Second organ posteriorly	Herein
		C. spinata	-Second organ posteriorly	Herein
		C. zeylanica		Herein
		Crangon crangon		Laverack & Crombie (1988) and Laverack *et al*. (1996)
		Eualus cranchii		Laverack & Crombie (1988)
		Macrobrachium intermedium		Laverack & Sinclair (1994) and Laverack & Macmillan (1999)
		Neocaridina heteropoda		Herein
		N. palmata		Herein
		Palaemon adspersus	-In larvae	Herein
		P. serenus		Laverack & Macmillan (1999)
		Pandalus montagni		Laverack & Crombie (1988)
		Rhynchocinetes rugulosus		Laverack & Macmillan (1999)
		Troglocaris (T.) planinensis		Herein
		Unidentified larvae		Laverack & Macmillan (1999)
	(*Thalassanidea*)	*Callianassa australiensis*		Laverack & Macmillan (1999)
		Upogebia sp.	-In larvae and adults	Laverack & Macmillan (1999)

The presence of a question mark after a species name indicates that the organ present in this taxon may not be a typical SDO.

Within the Maxillopoda, the cephalic dorsal hump (CDH) of some copepods and especially the lattice organ complex (LOC) of thecostracan larvae may represent homologues to the SDO (see Section IV). If confirmed, this would mean that a DSPO might have been inherited from at least the common ancestor of the Branchiopoda, the Malacostraca, and the Maxillopoda. No DSPO has ever been described in the Cephalocarida, the Ostracoda, or the Remipedia (Elofsson & Hessler, 2008), but none of these groups has been specifically surveyed for the presence of such organs (Laverack & MacMillan, 1999). Such investigations will be essential for determining whether a DSPO has been acquired in specific groups of crustaceans, or if it was present in the common ancestor of all crustaceans and then lost secondarily in some clades.

(2) Distribution and ecological niche

Determining the function of the SDO will require physiological and behavioural experiments to be undertaken. In the meantime, analysing the distribution of this organ with regard to ecological and biological characteristics may help to elucidate its possible roles. SDO-bearing crustaceans occur in fresh water (e.g. most carideans discussed herein; *Anaspides tasmaniae*), brackish water (e.g. *Macrobrachium intermedium*), and purely marine environments [e.g. *Neogonodactylus oerstedii* (Hansen, 1895)]. Some are inhabitants of tide pools and are therefore able to survive significant variations in salinity [e.g. *Eualus cranchii* (Leach, 1817); Laverack & Crombie, 1988]. Others live in habitats where environmental parameters (including salinity) are remarkably stable [e.g. the cave shrimp *Troglocaris* (*T*.) *planinensis*]. It can therefore be concluded that the presence of a SDO is unlikely to be related to life in a particular environment. Likewise, it is not restricted to organisms with one particular feeding mode or diet. Indeed, most freshwater carideans investigated herein are detritivores, feeding mainly on algae and other organic remains they find on the substratum. A few of them (*Atya gabonensis*, *Atyopsis moluccensis*), however, are filter feeders, exposing fan-like chelae to water currents to trap organic particles and microorganisms. Some SDO-bearing crustaceans are predators, such as *Alpheus* sp., *N. oerstedii*,

and *M. intermedium*. A relationship between the presence of a SDO and a pelagic life style was suggested by Laverack (1988) based on the occurrence of the SDO only in planktonic larval stages in several decapods. However, this was rejected following the description of SDOs in some epibenthic (e.g. *N. oerstedii*) and endobenthic (e.g. *Callianassa australiensis*, *Upogebia* sp.) crustaceans (Laverack & MacMillan, 1999). The occurrence of a SDO and a posterior SDO only in the early developmental stages of many crustaceans (Table 3) is of interest, however, since it suggests that these organs may be primarily larval features. It also raises the question of the influence of developmental strategies on the persistence of these organs in adults. Freshwater carideans offer an opportunity to address this issue, since hatching in these shrimps is frequently delayed compared with marine forms as an adaptation to life in freshwater environments. Some of the taxa investigated (e.g. *Caridina multidentata*) still hatch as planktonic larvae and undergo partial development in downstream brackish environments, but others (e.g. *Neocaridina heteropoda*) hatch directly as minute adult-like individuals that immediately adopt a (semi-)benthic life style. The presence of the organ in adults of all these forms implies that this variation in developmental strategy does not affect the development of the SDO or its persistence into adulthood. Likewise, when we investigated the sex of individuals (in *Atyaephyra desmaresti*, *C. multidentata*, *N. heteropoda*), we found no difference in external aspect or location of the SDO between the sexes.

In summary, the SDO occurs in crustaceans with various ecological or biological characteristics and apparently in both sexes. Accordingly, a specific function cannot be inferred from the analysis of its distribution pattern. On the other hand, this significant ecological/biological diversity of SDO-bearing crustaceans suggests that this organ carries out an essential function in these organisms in a wide variety of ecological niches.

V. THE OTHER DORSAL SENSORY PIT ORGANS

(1) The lattice organ complex (Thecostraca)

(*a*) *Morphology, variability, internal structure, and ontogeny*

Settlement larval stages of thecostracans (i.e. cypris or homologous larvae) usually possess five pairs of cuticular structures along the dorsal midline of the head shield. These paired sensory structures, named lattice organs (LO) by Elfimov (1986), are arranged in two clusters (Fig. 6A). The first two pairs of LO (LO1 and LO2) are located in the anterior region of the head shield (Fig. 6A, B). The second cluster, comprising the remaining three pairs (LO3–LO5), occurs in the posterior-third of the head shield, with LO5 being frequently positioned at a greater distance from LO4 than LO4 is from LO3 (e.g. Celis *et al*., 2008, Fig. 6A, C). In the centre of the anterior cluster and of the area circumscribed by LO3 and LO4 sit one or more large pores. For a given pair, the LO are positioned symmetrically relative to the midline of the head shield. This distribution into two clusters of LO, each associated with a central gland, is fixed in the Thecostraca, which suggests that they represent two distinct sensory-glandular organs, only differing in the number of sensors involved. It is therefore unfortunate that the term 'organ' has been employed to refer to the sensory elements (LO) only. However, as 'lattice organ' has been repeatedly used in this sense in the past, we propose here the term of lattice organ complex (LOC) for the association of two or three pairs of LO with one central gland.

Two general types of LO can be recognized from their external morphology, the 'keel in a trough' and the 'pore field' types (Jensen *et al*., 1994*b*). As described by Rybakov *et al*. (2003, p. 16), the 'keel in a trough' type resembles 'an open-ended seta lying prostrate in an oblong depression and partially fused with the head shield' (e.g. Høeg & Kolbasov, 2002, fig. 5B). It has been observed in larvae of the Ascothoracida, some Facetotecta, and some acrothoracican Cirripedia (Jensen *et al*., 1994*b*; Høeg & Kolbasov, 2002). In the remaining Cirripedia, LO are represented by an elongate, plate-like area perforated by numerous pores ('pore field' type; e.g. Høeg & Kolbasov, 2002, fig. 5A). In both types, a large terminal pore is present at one or the other end of the area (except in the Rhizocephala Akentrogonida, Jensen *et al*., 1994*a*), depending on the specific LO or taxon concerned (Høeg & Kolbasov, 2002). The 'pore field' type occurs only within the Cirripedia and the existence of an intermediate type in this group suggests that it evolved from the 'keel in a trough' type (Jensen *et al*., 1994*b*; Høeg & Kolbasov, 2002).

The internal structure of the LO has been investigated in representatives of the Ascothoracida, Cirripedia, and Facetotecta (Høeg *et al*., 1998; Høeg & Kolbasov, 2002). These investigations revealed striking similarities in the internal organisation of LOs of the 'keel in a trough' and the 'pore field' types. Each LO is composed of a chamber situated in the exocuticle, which is, as a consequence, thickened locally (Fig. 6D). In 'pore field' LOs, numerous canals associated with the minute pores seen on the surface run through the roof of this chamber, but remain separated from the inside by an extremely thin layer of epicuticle and a thin layer of highly electron-lucent exocuticle (Høeg *et al*., 1998). It is not clear whether the canal associated with the large terminal pore opens into the chamber (Høeg *et al*., 1998; Høeg & Kolbasov, 2002). The chamber communicates with the interior of the larva by a large channel through the cuticle. Each organ is innervated by two sensory cells, whose inner dendritic segments continue into two outer dendritic segments (Høeg *et al*., 1998). The resulting four outer dendritic segments enter the cuticular chamber and run through it up to the vicinity of the terminal pore (Fig. 6D). Sheath cells devoid of scolopale envelop these dendrites, except at their most distal regions. In the chamber, the outer dendritic segments are also more or less enveloped by an electron-dense, extracellular sheath of unknown nature. This sheath extends to the terminal pore of the organ and is associated with balls of similarly electron-dense material adhering to the roof of the chamber (Høeg *et al*., 1998, Fig. 6D).

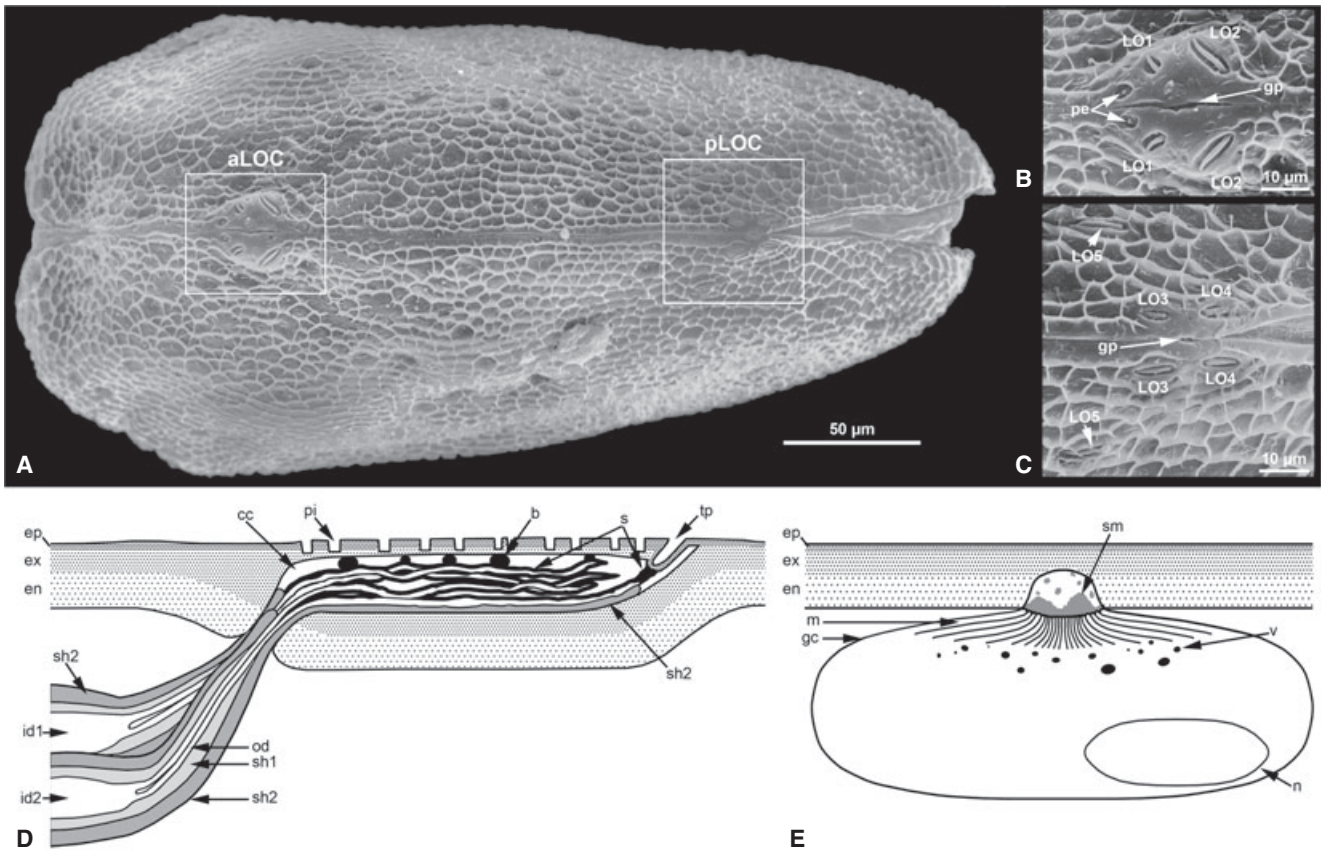


Fig. 6. External morphology and ultrastructure of the lattice organ complexes (LOC) of thecostracans. (A–C) *Capitulum mitella* (Cirripedia; cyprid larva), scanning electron micrographs (courtesy of J. Høeg); anterior is to the left. (A) Entire specimen in dorsal view showing the positions of the anterior and posterior lattice organ complexes (aLOC and pLOC, respectively). (B) Anterior LOC; note a pair of pegs anteriorly (*pe*), apparently associated with the anterior LOC in this species. (C) Posterior LOC; note that LO5 occupy unusual antero-lateral positions in this species. (D, E) Schematic representations of the ultrastructure of one lattice organ (LO) (D) and the central gland (E) of a generalised cirripede LOC. D is modified after Høeg *et al*. (1998, fig. 6). b, ball of electron-dense material; cc, cuticular chamber; ep, epicuticle; en, endocuticle; ex, exocuticle; gc, glandular cell; gp, gland pore; id1 and id2, inner dendritic segments 1 and 2; m, microvilli; n, nucleus; od, outer dendritic segment; pe, peg; pi, pit; s, sheath of electron-dense material; sh1, inner sheath cell; sh2, outer sheath cell; sm, secreted material; tp, terminal pore; v, vacuole.

The internal organisation of the central element of the LOC was also investigated by Høeg *et al*. (1998; their 'lattice organ glands'). In *Trypetesa lampas* (Acrothoracica), the putative central 'pore' represents a small cuticular chamber covered by the epicuticle and a thin layer of electron-lucent exocuticle (Fig. 6E). An electron-dense material is released into the chamber by a large secretory cell located beneath. In *Peltogaster paguri* (Rhizocephala), the ultrastructure of the central area is similar, but in the posterior cluster at least the gland is composed of two secretory cells.

Lastly, it has been shown that precursors of the LOs of cyprids (or cyprid-like larvae) exist in nauplii in the form of pore-bearing setae (Walossek, Høeg & Shirley, 1996; Rybakov *et al*., 2003; Høeg *et al*., 2009).

(*b*) *Comparison with the SDO*

It has been repeatedly mentioned that the LO exhibits interesting similarities with the sensors of the SDO (e.g. Høeg *et al*., 1998; Laverack & MacMillan, 1999; Elofsson & Hessler, 2008). The description of the posterior SDO in adult carideans and brachyuran larvae provides additional support for a possible homology between the LOC of thecostracans and the SDO of (mostly) malacostracans. Indeed, the posterior sensory-glandular complex differs from the anterior one in having an extra pair of sensors in both groups (for exceptions, see Section III.3). Thus, it is not only the number (two) and position (anterior and posterior) of the sensory-glandular complexes that are the same in thecostracans and many malacostracans, but also the number (five or seven) and gross disposition (four or six peripheral sensors, one central gland) of the elements of each of these complexes.

The most notable difference between the SDO and the LOC is to be found in the external aspect of their sensors. To date, there is no report of a SDO with sensory plates having a 'keel in a trough' or a 'pore field' morphology. These external characteristics of the sensors have apparently

evolved only in the Thecostraca, probably in relation to the development of a cuticular chamber housing the outer dendritic segments of the nerve cells. However, the external morphology of the sensors of the SDO is known to vary (see Section III.1b) and a comparable differentiation of the cuticle associated with the sensors might be described in the SDO of a malacostracan in the future. Moreover, this difference in external morphology should not mask the striking similarities in internal organisation between the SDO and the LOC. The sensors (sensory plates/LO), for example, are associated with four outer dendritic segments in both organs (Figs 4, 6D). These correspond to four nerve cells in adults of *Atyaephyra tasmaniae* and *Crangon crangon*, but in the zoea of *Hyas* they are associated with two biciliary sensory cells as in the LO of thecostracan larvae. Moreover, an electron-dense material has been observed in close association with these outer dendritic segments in both organs, and no scolopales occur within the enveloping sheath cells (Høeg *et al*., 1998). The central gland is also similar in the SDO and the LOC, being composed of a restricted number of secretory cells (one in the SDO, Laverack *et al*., 1996; one, more rarely two in the LOC, Høeg *et al*., 1998) with similar ultrastructural characteristics.

In summary, several lines of evidence (number, position, composition, and internal organisation) support the hypothesis that the thecostracan LOC and the SDO are homologous organs, a view shared with Høeg *et al*. (1998). A definitive confirmation of this could come from the demonstration that the anterior and posterior organs in the two groups are similarly innervated. Exploring the innervation of the posterior SDO and the two LOCs would therefore be of the utmost importance to demonstrate an ancient origin of DSPOs within the Crustacea.

(2) The cephalic dorsal hump (Copepoda)

The third organ considered by Elofsson & Hessler (2008) as a DSPO is the cephalic dorsal hump (CDH) of calanoid copepods. Like the SDO and the LOC, the CDH is a sensory glandular organ located antero-medially on the dorsal surface of the cephalic shield. However, it differs significantly from the other two DSPOs in its external morphology and ultrastructure and unlike them, has only been observed in males.

Nishida (1989) described the CDH as a keel-shaped process with four surfaces. The anterior and dorsal surfaces typically bear one pore each (the anterior and apical pores, respectively), while the lateral surfaces are characterized by an extremely thin cuticle. The number of pores associated with this organ varies greatly. There can be one, two, or several minute anterior pores, between zero and two apical pores and in a few instances, one or two additional pores posteriorly. Strikingly, these variations are sometimes observed within a single species.

The CDH is composed of two distinct glands, made of one secretory cell each, and one sensor composed of two biciliary sensory cells and two pairs of sheath cells (Nishida, 1989). The anterior gland (connected to the anterior pore) differs from the apical gland (connected to the apical pore) in the presence of a canal cell and the absence of modification of the overlying cuticle. In this regard, it also differs strongly from the glands of the SDO and the LOC. The sensory cells are more similar to the sensory cells of these latter two organs, being biciliary and associated with two sheath cells devoid of scolopales. However, these sheath cells form cavities housing the outer dendritic segments of the nerve cell, a feature not observed in other DSPOs. Lastly, Nishida (1989) mentioned that the glands and the receptor are surrounded by muscle cells, the presence of which has not been reported in the SDO or the LOC.

In summary, the CDH possesses an apical gland and a sensor which have some ultrastructural characteristics in common with the gland and the sensors of the other DSPOs. But it also exhibits important differences: the presence of an additional gland of a different type, a possible association with muscle cells, and the absence of the typical configuration of four/six sensors surrounding one gland. This latter characteristic is strongly constrained in the SDO and the LOC (see Section V.3), probably due to their specific functions. Also, while the CDH may deserve to be grouped within the DSPOs with regards to its sensory glandular nature and some of its ultrastructural characteristics, any possible homology with the SDO and the LOC remains uncertain.

VI. FUNCTION

While the SDO and the LOC are composed of both sensory and glandular elements, the function of these organs remains enigmatic. Indeed, neither the nature of the stimuli monitored by the sensors nor the material secreted by the gland have been determined.

(1) The sensors (sensory plates/LO)

Laverack *et al*. (1996; see also Laverack, 1988) suggested that the sensors of the SDO are mechanoreceptors, with the sensory cells monitoring the movements of the thin, overlying cuticle in response to pressure changes in the external environment. The description of a SDO in epibenthic or even endobenthic (burrowing) animals led Laverack & MacMillan (1999) to reconsider this hypothesis.

Alternatively, the sensors of the SDO could be chemoreceptors (Barrientos & Laverack, 1986; Laverack, 1988; Elofsson & Hessler, 2008). The most convincing evidence for this comes from their ultrastructural characteristics, especially the lack of scolopales in the sheath cells. As pointed out by Høeg *et al*. (1998), these intracellular elements are typical of mechanoreceptors or bimodal receptors, and their absence in the LO and the sensors of the SDO suggests they more likely represent chemoreceptors. In the LO, the outer dendritic segments are enclosed in a cuticular chamber. However, the roof of this chamber is most likely permeable, as suggested by the presence of a terminal pore and, in the 'pore field' type,

of the numerous pits (Fig. 6D). Likewise, the sensors of the SDO are associated with an extremely thin cuticle, which probably permits chemicals from the outside to come into contact with the outer dendritic segments lying below (Fig. 4). The description in both organs of an electron-dense material of unknown nature in contact with, or close to the outer dendritic segments is particularly intriguing. In the LO, this material is abundant at the opening of the canal leading to the terminal pore and the outside environment (Høeg *et al*., 1998, Fig. 6D), possibly suggesting an external origin.

(2) The gland

The role of the central gland is even more enigmatic. Barrientos & Laverack (1986) hypothesized that it could secrete a chemical that reduces surface tension and therefore facilitates buoyancy and/or swimming. Laverack (1988) suggested that the secreted product could be a surfactant or some sort of mucus. Laverack *et al*. (1996) suggested a more intimate functional relationship between the gland and the sensors, the former producing a gaseous or non-gaseous material to aid the latter in the monitoring of pressure changes. Until this product has been isolated and analysed, assumptions about the function of this gland will remain highly speculative. However, the suggestion of a possible functional link between the gland and the sensors of a given organ through the secretions of the former deserves consideration.

(3) Functional interactions between the gland and the sensors

Ultrastructural studies of the sensors and the glands of the SDO and the LOC have demonstrated that there are no direct (cell to cell contacts) or indirect (*via* the central nervous system and an innervation of the gland) physical relationships between them. However, these different elements are always found associated in a particular configuration, which implies a functional relationship between them (Laverack, 1988) and more specifically, the question of the sensitivity of the sensors to the product of the gland. Indeed, the sensors appear to encircle the gland. This is well illustrated by the lozenge configuration of LO around the central pore in the anterior LOC of some cirripeds (Jensen *et al*., 1994*b*, fig. 2; Høeg & Kolbasov, 2002, fig. 7). Similarly tight surrounding of the gland by sensors is also observed in the posterior SDO of adult carideans (Fig. 3C, F, L). Moreover, the presence of numerous pores/pegs surrounding the sensors and the gland in the SDO and posterior SDO in adult carideans (see Sections III.1b and III.3) strengthens the view that these organs are functionally organized around the gland (e.g. Figs 1A, 3B, E, K). The proximity and the size of the different elements of the SDO/LOC also need to be considered. Indeed, the gland is composed of one, or more rarely two, cells in these organs and therefore its production must be limited. This does not imply that the secreted substance could not be detected by other organs on the same individual or on another individual, but the proximity of the surrounding sensors make them the best candidates for its detection. This proximity might also explain the limited number of sensors and their simplicity (only 2–4 sensory cells per sensor). In this regard, it is noteworthy that the material secreted by the glandular cell and the material occurring in the cuticular chambers of the sensors both appear as electron-dense in the LOC.

However, one might wonder why such an indirect relationship exists between the sensors and the gland. This apparent complexity might be the result of the evolution of the organ. The different elements might have had separate functions, which later became integrated during their evolution, resolving into a more complex organ that performed a new function (exaptation). This indirect relationship may also be a way for the animal to detect a parameter of the environment that cannot be monitored by sensory cells alone. In this scenario, the secretion of the gland could be stimulated by a change in an environmental parameter or the contact of its apical membrane with an unknown chemical. The secreted material would then be detected by the sensors, which transmit the signal to the central nervous system (CNS). Lastly, the indirect relationship between the sensors and the gland could be a means of monitoring a physical parameter of the environment, such as water movement. In this hypothesis, the positions of the sensors surrounding the gland is crucial, since it would enable, after integration of the signals transmitted to the CNS by each of the sensors, the movement of the secreted substance from its central origin to the periphery to be monitored.

Another question is the possible interaction between the anterior and posterior organs within an individual. Theoretically, their positions at the two extremities of the cephalic shield should permit more accurate monitoring in space of changes in the external parameter they are meant to detect. However, cooperation between the two organs may also be more direct than the simple integration by the CNS of the information they provide. Indeed, both organs apparently function as effectors (gland) and as receptors (sensors) and if the sensors are sensitive to the chemical secreted by the gland, it can be imagined that the substance released by the gland of the anterior organ could also be detected by the sensors of the posterior organ and *vice versa*. In this regard, the absence of extra pores/pegs posterior to the central complex in the SDO and anterior to it in the posterior SDO in *Caridina multidentata* (Fig. 1) is particularly interesting.

We are well aware of the highly speculative nature of these suggestions considering our limited knowledge of the SDO and the LOC. However, they highlight some aspects of the morphology of these organs that are, in our opinion, crucial for understanding their function. The position of these organs on the carapace and the strongly constrained arrangement of their elements are obviously not fortuitous and as such they deserve greater attention, especially in the light of a possible functional relationship between the gland and the sensors. We also concur with others (e.g. Laverack *et al*., 1996; Laverack & MacMillan, 1999) that physiological experiments, possibly coupled with behavioural

observations, would be decisive in determining the function of the SDO/LOC. Perhaps the suggestions developed above will help to define the best way to conduct such investigations.

VII. POSSIBLE OCCURRENCE IN FOSSIL CRUSTACEANS AND RELATIVES

Various cuticular structures exhibited by fossil crustaceans, or forms phylogenetically close to the stem lineage of crustaceans, have been compared, if not homologized with the DSPOs of living taxa. However, several of these structures differ from DSPOs by at least one, and usually several, of the following criteria: external form, number, size, quincunx organisation or location (Table 4). We consider that there is no real justification for their comparison with the DSPOs of living crustaceans and accordingly, these organs are not discussed further herein.

By contrast, the dorsal structures possessed by three species of arthropods from the Late Cambrian of Orsten in Sweden may represent examples of DSPOs in fossils. This is particularly the case for an organ described in the eucrustacean *Bredocaris admirabilis*, which consists of four pores located on a slightly swollen area on the top of the cephalic shield ('neck organ'; Müller & Walossek, 1988, p. 8, fig. 4, pl. 3, fig. 2, pl. 9, fig. 6). A similar organ is present in a similar position in another eucrustacean, *Rehbachiella kinnekullensis*, but its pores are positioned at the margin of a plate-like, somewhat folded area ('neck organ'; Walossek, 1993, pp. 108–110, fig. 6, pl. 1, figs 1, 3, 6, pl. 2, figs 7, 8, pl. 3, fig. 5, pl. 5, fig. 5). This organ progressively disappears during the ontogeny of this species, while it is still visible in the largest specimens of *B. admirabilis*.

Walossek (1993) compared the anterior organ of *Rehbachiella kinnekullensis* with the 'dorsal organs' of modern branchiopods and found support for the attribution of this fossil taxon to the Branchiopoda. However, the location and the composition of this organ in both *R. kinnekullensis* and *Bredocaris admirabilis* are consistent with it being a DSPO and within the Branchiopoda, it is actually best compared to the SDO-like organ exhibited by some (e.g. in *Paralimnetis mapini*; Table 3). Consequently, it can be reasonably assumed that its function was in part, if not exclusively, sensory. The similarities between these fossil organs and the malacostracan SDO were noted by Walossek (1993) leading him to suggest that the fossil organs and the SDO-like organs of some modern branchiopods might represent composite organs assuming the functions of both a DSPO and a DITC. In this scenario, a role in ion transport would have been acquired secondarily, and the organ having this function would therefore constitute a synapomorphy of the Branchiopoda [and the Maxillopoda, but all the examples cited by Walossek (1993) in this group have proved to be DSPOs]. We have no new arguments in favour of or against this assumption, but we agree with Walossek (1993) that the organs of these two fossil species and the SDO-like organs of some branchiopods suggest a particularly ancient origin of DSPOs in crustaceans (at least in the common ancestor of the Branchiopoda, the Maxillopoda, and the Malacostraca).

Table 4. Cuticular structures in fossil crustaceans for which a supposed relationship with dorsal sensory pit organs (DSPOs) are rejected herein

Taxon (age)	Cuticular structure	Main differences compared with DSPO of living crustaceans	Age/References
Archaeostraca (Ordovician-Carboniferous)	(1) Anterolateral sensory structures (2) Dorsal sensory structures (3) Posterodorsal sensory structure	(1) No morphological similarities; not located along carapace midline (2) No morphological similarities; asymmetrically disposed (one valve only) (3) No morphological similarities; single structure	Crasquin *et al*. (2009)
Bradoriida (Cambrian)	'Dorsal organ'	No morphological similarities (tubercle bearing a pore at its apex); only one pair of structures	Zhang (2007)
Eucrustacean metanauplius (Cambrian)	'Dorsal organ'	'Plate-like suboval area' devoid of pits or pores	Zhang *et al*. (2010)
Thylacocephala (Cretaceous)	Organs supposedly homologous to keel-in-a-trough LO	Lack obvious keel (and terminal pore); not arranged as clusters with central pores; size about three times that of LO; more than 20 pairs (instead of five)	Lang & Schram (2002)

The organ of the Cambrian eucrustacean metanauplius described by Zhang *et al*. (2010) is more similar to a dorsal ion-transporting complex (DITC) than to a DSPO, as correctly mentioned by these authors. This review of the sensory dorsal organ (SDO) does not support the assumption of Lang & Schram (2002) that adult (larger) crustaceans may have a greater number of sensors and that these may be larger. Consequently, this is not an explanation for the differences observed between thecostracan lattice organs (LOs) and the structures described in thylacocephalans.

Interestingly, *Rehbachiella kinnekullensis* displays a second organ made of three pairs of pores, located near the posterior margin of the cephalic shield (Walossek, 1993, pl. 11, figs 6, 7). A similar organ was observed in another arthropod from the Orsten fauna, *Agnostus* (*Agnostus*) *pisiformis* (Müller & Walossek, 1987). It is composed of a cluster of pits on and around the glabellar node (Müller & Walossek, 1987, pl. 8, figs 5–7). This node is located in the posterior region of the cephalic shield at its highest point. At its apex, six large pores and a central smaller one are visible, the large pores being paired and symmetrically disposed as in *R. kinnekullensis*. Unlike this species, however, they are associated with a central pore and eight paired, smaller pores that surround the glabellar node posterolaterally. The precise arrangement of these different pores suggests that they might have constituted a single organ. The number (seven) and the disposition (six peripheral, one central, somewhat different) of the main pores are strongly suggestive of the morphology of the posterior SDO described herein (see Section III.3). The smaller peripheral pores and their posterolateral location relative to the main pores also recall the small pits/pegs which surround posterolaterally the central complex of the posterior SDO in *Caridina multidentata* (Fig. 5I). The agnostids (suborder Agnostina) have been traditionally recognized as trilobites (e.g. Cotton & Fortey, 2005), but strong arguments against this have been formulated (e.g. Walossek & Müller, 1990; Bergström & Hou, 2005), placing them instead close to the stem lineage of Crustaceans. Following the traditional view, Lerosey-Aubril & McNamara (2008) hypothesized that the organ of *A. (A.) pisiformis* might have evolved from the cephalic median organ of other trilobites. However, considering the new data on the posterior SDO of crustaceans presented herein, it seems now more sensible to suggest that the organ of *A. (A.) pisiformis*, the posterior organ of *R. kinnekullensis*, and the posterior SDO/LOC may represent homologous organs. If confirmed, this hypothesis would provide support to the claim that agnostids are more closely related to stem-group crustaceans than to trilobites. However, the number of SDO-like structures that might have been present on the cephalic shield of the earliest crustaceans remains ambiguous. Indeed, *A. (A.) pisiformis* does not possess a second, more anteriorly located organ, whereas this is the only SDO-like structure observed in the eucrustacean *B. admirabilis*. Moreover, *A. (A.) pisiformis* also exhibits clusters of regularly and symmetrically arranged pores on the axial and terminal nodes (i.e. on the pygidial axis). No SDO-like structure has ever been described posterior to the cephalic shield in living crustaceans. It remains likely that *A. (A.) pisiformis* provides a strong case for the presence of putative DSPO in fossil arthropods outside the Eucrustacea.

VIII. CONCLUSIONS

(1) The SDO is a sensory glandular complex made of four peripheral sensors and a central gland. These elements vary greatly in external appearance, but this has little impact on their ultrastructural organisation. This organ is associated with a thinning of the cuticle, particularly above the sensors and the central gland. Each sensor typically contains four outer dentritic segments that can be derived from either four monociliary or two biciliary nerve cells. The SDO is connected to the tritocerebrum, which indicates that it belongs to the third cephalic segment, but further investigations would be required to confirm this observation from a single species. Likewise, minute pits/pegs frequently surround the sensory glandular complex in adult carideans, suggesting that the SDO may be composed of a greater number of elements in some species. Determining whether these structures only occur in the Caridea and how they interact with the central sensory glandular complex would be of particular interest.

(2) Many malacostracans possess a second, more posteriorly located organ. It differs from the SDO by having six (occasionally more) sensory elements, possibly associated with only two dendritic extremities each. This number of sensors may vary, but this organ probably functions in a similar way to the SDO. How common its co-occurrence with the SDO is needs to be ascertained. The description of its innervation would also allow a determination to be made of the cephalic segment to which it belongs. This potentially might provide critical information about the nature of the carapace in malacostracans.

(3) The presence of the SDO is best documented in the Eumalacostraca, but it is also known in the Hoplocarida and the Phyllocarida. In branchiopods, most 'dorsal organs' are ion-transporting complexes (DITCs). However, in a few cases, the organ appears more complex and shows similarities with the SDO. This suggests that the two types of organ (DSPO and DITC), although having different functions, might have a common origin. In this scenario, the DSPOs would have evolved particularly early in the history of crustaceans. As for the SDO, the presence of the posterior SDO has been reported in the Eumalacostraca, the Hoplocarida, and the Phyllocarida. No correlation could be demonstrated between the presence of a SDO and a particular ecological (feeding and life habits, habitats) or biological (developmental strategies) trait. However, the organ and its sensors apparently display greater sizes in the Thalassanidea, which are endobenthic crustaceans.

(4) The homology of the two LOCs of thecostracans and the SDO and posterior SDO of malacostracans is supported by their similarities in location (along the sagittal line of the cephalic shield; one anterior, one posterior), composition (four sensors + one gland, usually six sensors + one gland), organisation (peripheral sensors, central glands), and ultrastructure. A more definitive demonstration of this might come from the description of the innervation of the LOC and of the posterior SDO. The CDH of calanoid copepods exhibits notable differences with the other DSPOs, which questions possible homology with the latter organs. However, some of its characteristics may indicate similarity in function. Again, determining the relationship of the CDH with the CNS would provide critical information.

(5) The SDO, the posterior SDO, and the LOC are sensory glandular complexes, but their function remains unknown. The ultrastructure of the sensors suggests that they are chemoreceptors, but the nature of the chemical they detect is unidentified. Likewise, the strongly constrained relative positions of the gland and sensors of a given organ suggests a functional relationship between them, but how these different elements interact is unknown. A possible interaction between the anterior and posterior organs (SDO/LOC) of an individual remains to be tested. Considering the wide distribution of these organs in crustaceans and the fact they have probably been conserved for a great part of the history of the group, it is of the utmost importance to determine their function. The description of the presence of such organs in freshwater carideans, which can be easily reared in a laboratory, opens the way for behavioural and physiological experiments that could prove crucial for achievement of this goal.

(6) The most convincing examples of the presence of DSPOs in fossil arthropods are found in two eucrustaceans and one arthropod close to the stem lineage of crustaceans from the Cambrian of Sweden. They suggest that DSPOs probably evolved very early in the history of the Crustacea and therefore their possible occurrence in non-crustacean Cambrian arthropods should not be excluded.

IX. ACKNOWLEDGEMENTS

R. L.-A. is particularly grateful to J.-B. Eymery (University of Picardie, France) for his help at an early phase of this project with SEM investigations of caridean shrimps. R. M. thanks Prof. R. Melzer (Zoologische Staatssammlung München) for his helpful comments and the Sea Life Center Munich, which supported him with rearing facilities. C. Franz (Senckenberg Research Institute) and A. Böttcher and A. Batel are thanked for their assistance with SEM. Specimens of *Troglocaris (Troglocaris) planinensis* were kindly provided by B. Sket and J. Jugovic (University of Ljubljana, Slovenia). We are also indebted to K. J. McNamara (University of Cambridge), who patiently corrected earlier drafts of this manuscript, to J. Høeg, who provided us with the images of the LOC presented in Fig. 6A–C, and to the two anonymous referees for their helpful suggestions and comments. This contribution greatly benefited from the comments of the editor W. Foster and the assistant editor A. Cooper. This is a contribution of the Laboratoire de Géologie de Lyon: Terre, Planètes, Environnement (UMR 5276, CNRS), Université Claude Bernard Lyon I and the Senckenberg Research Institute, Frankfurt am Main.

X. REFERENCES

Barrientos, Y. & Laverack, M. S. (1986). The larval crustacean dorsal organ and its relationship to the trilobite median tubercle. *Lethaia* **19**, 309–313.

Bergström, J. & Hou, X.-G. (2005). Early Palaeozoic non-lamellipedian arthropods. In *Crustacea and Arthropod Relationships*, *Crustacean Issues*, Volume 16 (eds S. Koenemann and R. Jenner), pp. 73–93. CRC Press, Boca Raton.

Celis, A., Alvarez, F., Newman, W. A. & Sumida, P. Y. G. (2008). Descriptions and phylogenetic significance of the fronto-lateral gland pores and dorsal lattice organs of cyprid larvae of seven species of barnacles (Cirripedia: Thoracica: Pedunculata). *Journal of Crustacean Biology* **28**, 203–215.

Clarkson, E., Levi-Setti, R. & Horváth, G. (2006). The eyes of trilobites: the oldest preserved visual system. *Arthropod Structure & Development* **35**, 247–259.

Cotton, T. J. & Fortey, R. A. (2005). Comparative morphology and relationships of the Agnostida. In *Crustacea and Arthropod Relationships*, *Crustacean Issues*, Volume 16 (eds S. Koenemann and R. Jenner), pp. 95–136. CRC Press, Boca Raton.

Crasquin, S., Racheboeuf, P. R. & Bergström, J. (2009). Sensory structures of Archaeostraca (Phyllocaridida, Crustacea). *Evolution and Development* **11**, 225–232.

Elfimov, A. S. (1986). Morphology of the carapace of cypris larva of the barnacle *Heteralepas mystacophora*. *Soviet Journal of Marine Biology* **12**, 152–156.

Elofsson, R. & Hessler, R. R. (2008). Two microvillar organs, new to Crustacea, in the Mystacocarida. *Arthropod Structure & Development* **37**, 522–534.

Fioroni, P. (1980). Das Dorsalorgan der Arthropodenunter besonderer Berucksichtigung der malacostraken Krebse. *Zoologische Jahrbücher, Abteilung Anatomie* **104**, 425–465.

Hansen, H. J. (1921). On the postembryonic occurrence of the median 'dorsal organ' in Crustacea Malacostraca. *Studies on Arthropoda I*, pp. 66–80. Bianco Luno, Copenhagen.

Hanstrøm, B. (1947). The brain, the sense organs and the incretory organs of the head in the Crustacea Malacostraca. *Acta Universitatis Lundensis, N.F. 2* **43**, 1–44.

Høeg, J., Achituv, Y., Chan, B. K. K., Chan, K., Jensen, P. G. & Pérez-Losada, M. (2009). Cypris morphology in the barnacles *Ibla* and *Paralepas* (Crustacea: Cirripedia Thoracica). Implications for cirripede evolution. *Journal of Morphology* **270**, 241–255.

Høeg, J., Hosfeld, B. & Jensen, P. G. (1998). TEM studies on the lattice organs of cirripede cypris larvae (Crustacea, Thecostraca, Cirripedia). *Zoomorphology* **118**, 195–205.

Høeg, J. T. & Kolbasov, G. A. (2002). Lattice organs in y-cyprids of Facetotecta and their significance in the phylogeny of the Crustacea Thecostraca. *Acta Zoologica (Stockholm)* **83**, 67–69.

Jensen, P. G., Høeg, J. T., Bower, S. & Rybakov, A. V. (1994*a*). Scanning electron microscopy of lattice organs in cyprids of the Rhizocephala Akentrogonida (Crustacea: Cirripedia). *Canadian Journal of Zoology* **72**, 1018–1026.

Jensen, P. G., Moyse, J., Høeg, J. T. & Al-Yahya, H. (1994*b*). Comparative SEM studies of lattice organs: putative sensory structures on the carapace of larvae from Ascothoracida and Cirripedia (Crustacea Maxillopoda Thecostraca). *Acta Zoologica (Stockholm)* **75**, 125–142.

Laforsch, C. & Tollrian, R. (2000). A new preparation technique of daphnids for Scanning Electron Microscopy using hexamethyldisilazane. *Archiv für Hydrobiologie* **149**, 587–596.

Lang, S. & Schram, F. R. (2002). Possible lattice organs in Cretaceous Thylacocephala. *Contributions to Zoology* **71**, 159–169.

Laverack, M. S. (1988). Larval locomotion, sensors, growth and their implication for the nervous system. *Symposia of the Zoological Society of London* **59**, 103–122.

Laverack, M. S. & Barrientos, Y. (1985). Sensory and other superficial structures in living marine Crustacea. *Transactions of the Royal Society of Edinburgh, Earth Sciences* **76**, 123–136.

Laverack, M. S. & Crombie, B. (1988). The dorsal organ of the natantian crustaceans *Crangon crangon*, *Thoralus cranchii* and *Pandalus montagui*: an SEM study. *Zoologischer Anzeiger* **220**, 17–24.

Laverack, M. S. & Macmillan, D. L. (1999). The sensory dorsal organ of crustaceans: theme and variations. *Marine and Freshwater Behaviour and Physiology* **32**, 75–86.

Laverack, M. S., Macmillan, D. L., Ritchie, G. & Sandow, S. L. (1996). The ultrastructure of the sensory dorsal organ of Crustacea. *Crustaceana* **69**, 636–651.

Laverack, M. S. & Sinclair, A. (1994). Innervation of the dorsal organ of the shrimp *Macrobrachium intermedium* (Decapoda: Natantia). *Journal of Crustacean Biology* **14**, 1–5.

Lerosey-Aubril, R. & McNamara, K. J. (2008). The cephalic median organ of trilobites. *Cuadernos del Museo Geominero* **9**, 229–235.

Martin, J. W. & Belk, D. (1988). Review of the clam shrimp family Lynceidae Stebbing, 1902 (Branchiopoda: Conchostraca), in the Americas. *Journal of Crustacean Biology* **8**, 451–482.

Martin, J. W. & Laverack, M. S. (1992). On the distribution of the crustacean dorsal organ. *Acta Zoologica* **73**, 357–368.

Mauchline, J. (1977). The integumental sensilla and glands of pelagic Crustacea. *Journal of the Marine Biological Association of the United Kingdom* **57**, 973–994.

Meyer, R. & Melzer, R. R. (2004). Scanning EM diagnosis of marine Decapoda larvae: a comparison of preparation techniques. *Crustaceana* **77**, 883–886.

Meyer, R., Wehrtmann, I. S. & Melzer, R. R. (2006). Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. *Scientia Marina* **70**, 261–270.

Meyer-Rochow, V. B. (2001). The crustacean eye: dark/light adaptation, polarization sensitivity, flicker fusion frequency, and photoreceptor damage. *Zoological Science* **18**, 1175–1197.

Müller, K. J. & Walossek, D. (1987). Morphology, ontogeny, and life habit of *Agnostus pisiformis* from the Upper Cambrian of Sweden. *Fossils and Strata* **19**, 1–124.

Müller, K. J. & Walossek, D. (1988). External morphology and larval development of the Upper Cambrian maxillopod *Bredocaris admirabilis*. *Fossils and Strata* **23**, 1–70.

Nation, J. L. (1983). A new preparation method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technology* **58**, 347–351.

Nishida, S. (1989). Distribution, structure and importance of the cephalic dorsal hump; a new sensory organ in calanoid copepods. *Marine Biology* **101**, 173–185.

Nishida, S. & Kittaka, J. (1992). Integumental organs of the phyllosoma larva of the rock lobster *Jasus edwardsii* (Hutton). *Journal of Plankton Research* **14**, 563–573.

Olesen, J. (1996). External morphology and phylogenetic significance of the dorsal/neck organ in the Conchostraca and the head pores of the cladoceran family Chydoridae (Crustacea, Branchiopoda). *Hydrobiologia* **330**, 213–226.

Olesen, J. (2005). Larval development of *Lynceus brachyurus* (Crustacea, Branchiopoda, Laevicaudata): redescription of unusual crustacean nauplii, with special attention to the molt between last nauplius and first juvenile. *Journal of Morphology* **264**, 131–148.

Pohle, G. & Telford, M. (1981). Morphology and classification of decapod crustacean larval setae: a scanning electron microscope study of *Dissodactylus crinitichelis* Moreira, 1901 (Brachyura: Pinnotheridae). *Bulletin of Marine Science* **31**, 736–752.

Rieder, N., Abaffi, P., Hauf, A., Lindel, M. & Weishäupl, H. (1984). Funkstionmorphologie Untersuchungen an den Conchostracen *Leptestheria dahalacensis* und *Limnadia lenticularis* (Crustacea, Phyllopoda, Conchostraca). *Zoologische Beiträge, Neue Folge* **28**, 417–444.

Ruppert, E. E. & Barnes, R. D. (1994). *Invertebrate Zoology*. Harcourt Brace & Company, Orlando.

Rybakov, A. V., Høeg, J. T., Jensen, P. G. & Kolbasov, G. A. (2003). The chemoreceptive lattice organs in Cypris larvae develop from naupliar setae (Thecostraca: Cirripedia, Ascothoracida and Facetotecta). *Zoologischer Anzeiger* **242**, 1–20.

Santana, W. & Marques, F. (2009). Larval morphology of the spider crab *Leurocyclus tuberculosus* (Decapoda: Majoidea: Inachoididae). *Nauplius* **17**, 49–58.

Walossek, D. (1993). The Upper Cambrian *Rehbachiella* and the phylogeny of Branchiopoda and Crustacea. *Fossils and Strata* **32**, 1–202.

Walossek, D., Høeg, J. T. & Shirley, T. C. (1996). Larval development of the rhizocephalan cirripede *Briarosaccus tenellus* (Maxillopoda: Thecostraca) reared in the laboratory: a scanning electron microscopy study. *Hydrobiologia* **328**, 9–47.

Walossek, D. & Müller, K. J. (1990). Upper Cambrian stem-lineage crustaceans and their bearing upon the monophyletic origin of Crustacea and the position of *Agnostus*. *Lethaia* **23**, 409–427.

Zhang, X.-G. (2007). Phosphatized bradoriids (Arthropoda) from the Cambrian of China. *Palaeontographica Abteilung A* **281**, 93–173.

Zhang, X.-G., Maas, A., Haug, J., Siveter, D. J. & Waloszek, D. (2010). A eucrustacean metanauplius from the Lower Cambrian. *Current Biology* **20**, 1075–1079.

(*Received 8 January 2012; revised 14 November 2012; accepted 20 November 2012; published online 24 December 2012*)

6. Article IV

Meyer R, Martin J, Melzer RR (2010) Nucleus patterns of zoea larvae (Crustacea: Decapoda) in the context of taxonomy. Zootaxa 2422: 31-42.

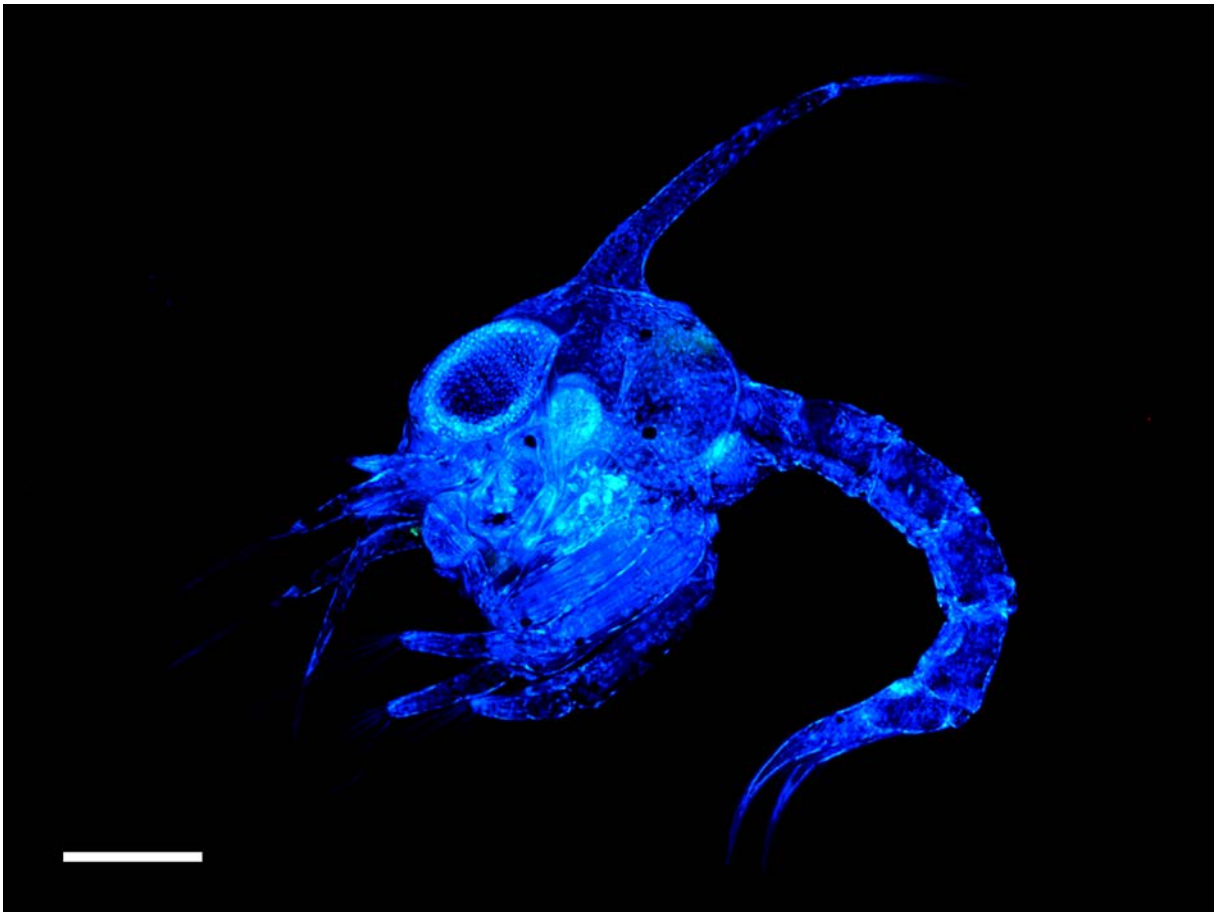


Figure 10: DAPI stained first zoea stage of *Lophozozymus incisus* (H. Milne Edwards, 1834) in lateral view (bar 250 µm). Photo by J. Martin.

Zootaxa 2422: 31–42 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Nucleus patterns of zoea I larvae (Crustacea: Decapoda) in the context of taxonomy

ROLAND MEYER, JANA MARTIN, & ROLAND R. MELZER[1]
Zoologische Staatssammlung, Münchhausenstr. 21, D-81247 München, Germany
[1]Corresponding author. E-mail: melzer@zsm.mwn.de

Abstract

Using DAPI as a nucleus marker, we studied zoeas of 6 decapods (*Palaemon adspersus* Rathke, 1837; *Palaemon elegans* Rathke, 1837; *Porcellana platycheles* (Pennant, 1777); *Pisidia longicornis* (Linnaeus, 1767); *Xantho hydrophilus* (Herbst, 1780) *Xantho pilipes* A. Milne Edwards, 1867) representing one species pair of Palaemonidae (Caridea), Porcellanidae (Anomura) and Xanthidae (Brachyura) each, with special reference to the telson, and correlated our observations with the general morphological features of the zoeas.

The different taxa exhibit specific features with respect to the distribution of nuclei, the patterns they exhibit, their size, density and numbers, thus being sets of characters potentially useful for taxonomic descriptions and diagnoses especially on the "above-species"-level. We discuss how nuclear patterns and classical morphological and/or morphogenetical features normally examined in zoeal larvae are related, and give some ideas on how "nucleus"-characters can contribute to taxonomic descriptions.

Key words: Zoea, DAPI, pattern, taxonomy, description (Decapoda)

Introduction

DAPI, a universal nucleic acid fluorescence dye (Kubista *et al.* 1987), is a popular marker for nuclei in a wide set of applications, e.g. mapping nuclei or giving evidence of the cellular composition of samples. This does not only account for organisms with well permeable body walls, but also for small arthropods, where DAPI can even be used as a vital marker in single dye preparations as well as a background stain for neuronal markers (Wohlfrom & Melzer 2001). Our preliminary tests showed that this is also possible in zoeal larvae of decapod crustaceans, and that mapping of nuclear features may reveal taxon-specific differences. In order to check the relevance of this method for comparison and diagnosis of different taxa, we therefore analysed the telsons of DAPI stained zoeal larvae of six different decapods with respect to nucleus distribution, patterns, size, density and numbers.

The studied zoeas represent three species pairs with the representatives of each pair belonging to the same or closely related genus, and one pair each represents the three infraorders Caridea (Palaemonidae: *Palaemon adspersus* and *Palaemon elegans*), Anomura (Porcellanidae: *Porcellana platycheles* and *Pisidia longicornis*) and Brachyura (Xanthidae: *Xantho hydrophilus* and *Xantho pilipes*). Detailed descriptions of the external morphology of the zoeas of these species are available: The *Palaemon* zoeas were described by Fincham (1977, 1985, 1986), those of *Porcellana* and *Pisidia* by Barnich (1995) and Gonzales-Gordillo *et al.* (1996), and those of the *Xantho* by Ingle (1983), Paula and Dos Santos (2000), and Meyer *et al.* (2004).

The combination of both very closely related species and representatives of different infraorders should allow to infer on which taxonomic level the observed features might be relevant. Apart from this, we seek to reveal in which way the commonly studied characters of zoeas are correlated with nucleus pattern, and what might be the potential use of these features in the context of taxonomy, hence adding this approach to the stock of species description techniques available for zoeas or small arthropods in general, such as scanning

Accepted by G. Karaman: 1 Mar. 2010; published: 9 Apr. 2010

EM diagnoses of zoeas that have been introduced as an additional source of data in zoeal descriptions (Meyer *et al*. 2004, 2006, Geiselbrecht & Melzer 2009). Comparatively analysing *all* the nuclei of zoeal larvae is, however, a very wide field for a first approach to this technique. Therefore, we study the telson here as a model organ, since its flattened form makes it relatively easy to visualize the nuclei completely.

Material and methods

Animals and fixation. Egg bearing females of *Palaemon adspersus* Rathke, 1837, *Palaemon elegans* Rathke, 1837, *Porcellana platycheles* (Pennant, 1777), *Pisidia longicornis* (Linnaeus, 1767), *Xantho hydrophilus* Herbst, 1780 and *Xantho pilipes* A. Milne Edwards, 1867 were caught in Roscoff (France) and Rovinj (Croatia) during courses in marine biology and kept in the aquarium in enclosures at a salinity of 3.7% and 22°C. The females were fed with Sera San and Krill Pacifica from local aquarium shops. After hatching, the juveniles were either kept alive for some hours for vital stains or either fixed in 4% formaldehyde in sea water, in 75% ethanol, or in a graded ethanol series as described in Meyer and Melzer (2004). Hence all our specimens represent very early zoea-I-stages. Prior to inspection or fixation the zoeas were anaesthetised in 7.14% magnesium chloride.

The studied animals are deposited at the Zoologische Staatssammlung München (Sektion Arthropoda varia) under the following collection numbers: *Palaemon adspersus* Rathke, 1837: female A20035514, larvae A20035516, SEM specimen A20071658. *Palaemon elegans* Rathke, 1837: female A20071637, larvae A20071636, A20071638, SEM specimens A20071628, A20071629. *Porcellana platycheles* (Pennant, 1777): female A20071639, larvae A20071640, SEM specimens A20071641-A20071643. *Pisidia longicornis* (Linnaeus, 1767): female A20071633, larvae A20071635, SEM specimens A20071630-A20072632. *Xantho hydrophilus* Herbst, 1780: larvae A20071646, SEM specimen A20071648. *Xantho pilipes* A. Milne Edwards, 1867: female A20071654, larvae A20071656, SEM specimen A20071657. Larvae were determined according to the above-cited larval descriptions, female adults from which the zoeae were obtained after Zariquiey Alvarez (1968).

DAPI staining. 1mg DAPI (4'6-Diamidino-2-Phenyindol-Dihydrochlorid; Sigma-Aldrich) was dissolved in 10ml distilled water (stock solution). 7 drops of the DAPI solution were either put into vials with 5-10 vital zoeas in 3ml sea water or with 5-10 zoeas in the respective fixans. The incubation time was 20-30 minutes in darkness for all stains (for details see Wohlfrom & Melzer 2001).

Fluorescence and conventional light microscopy. After staining zoeae, wholemounts or dissected telsons were studied with a Leica DMRBE at a wavelength of 365nm. The same and/or other specimens were also studied under conventional illumination or under both combined in order to get information on several parallel channels which enables us to correlate the fluorescence pictures with the conventional information on zoea morphology (Figs. 1, 3–5).

Focus series and normal pictures were made with a Visitron Spot Insight Color digital camera. 3D stacks were processed with Auto-Montage (Syncroscopy). Countings and measurements of nuclei were made on prints of the respective pictures. Partly, inverted enhanced contrast pictures were used. Depending on the shape and size of the telsons, the values for the number of nuclei per area were either counted from 100µm or 25µm squares.

Confocal microscopy. Some of the specimens were also studied under a Leica SP5 AOBS confocal microscope using a 405 nm diode laser and a Leica HCX Apo L UVI 40x NA 0.8 water dipping objective. Picture stacks were processed with ImageJ (Fig. 2).

Scanning EM. Specimens were dehydrated in Acetone, critical-point-dried in a Bal-Tec CPD 030 in carbon dioxide, mounted on stubs with self-adhesive carbon plates, sputtered with gold in a Polaron Sputter Coater and studied in a Leo 1430VP scanning EM at ca. 15kV (Figs. 3–5).

Results

General observations

In our zoeae all the nuclei are stained, those of the epidermis as well as those of the inner organs (Fig. 1). The nuclei located close to the cuticle are somewhat equally distributed all over the body while underlying clusters of nuclei mark inner organs, e.g. nervous system and gut. In addition regions with high fluorescence signal caused by densely arranged nuclei are recognisable. These are found at the base of the antennules, around the eyes, at the base of the mouthparts and maxillipeds, the ventral side of the thorax and at the ventral side of each pleon segment (Fig. 1). The latter are of varying shape or in the form of a continuous stripe. In addition, between the last pleon segment and the telson, in all the studied species a dense arrangement of nuclei is found (see below).

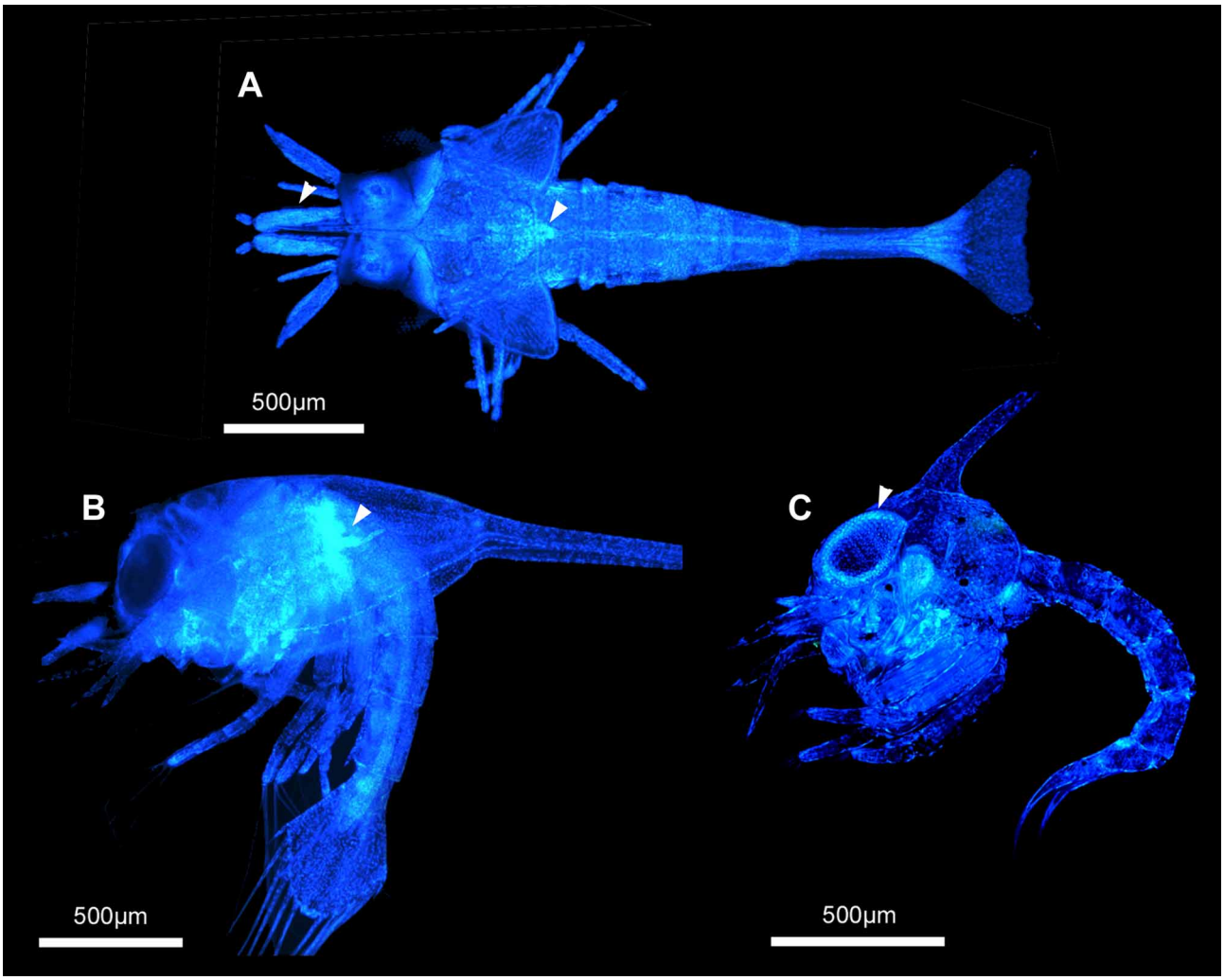


FIGURE 1. Survey of DAPI stained zoeas. **a.** *Palaemon elegans*, viewed from dorsally. b. *Porcellana platycheles*, viewed from laterally. **c.** *Xantho incisus*, viewed from laterally. *Arrowheads* regions with intensely stained nuclei.

Nuclear features of the telson

In the following, we will describe both the nuclear features as well as other morphological characteristics revealed by the light microscope and the scanning EM that are relevant for the understanding of the nuclear patterns.

In the zoea I of all the studied species, both pleopods and uropods are as yet not developed, and therefore the last pleon segments are of longitudinal shape and bear no other appendages than posteriolateral processes and setae. The last segment is confluent with the telson, which in all species is somewhat flattened and equipped with telson setae at the posterior edge (Figs. 2–5).

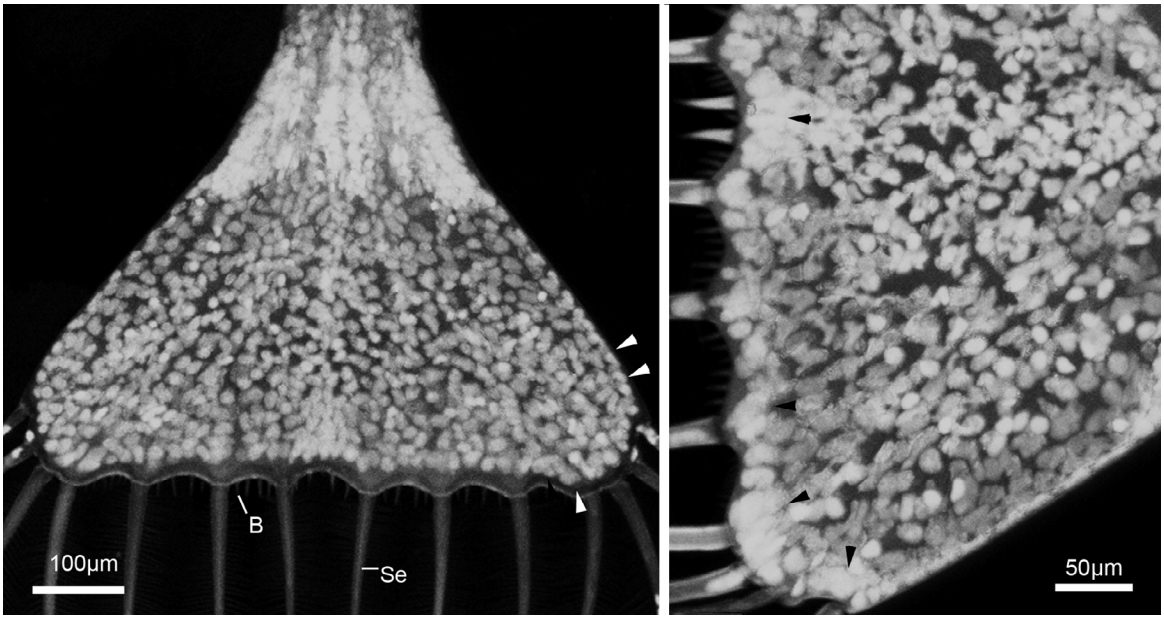


FIGURE 2. Confocal microscopy of telson of *Palaemon elegans* viewed from dorsally. **a.,** survey of telson, **b.,** detail. *Arrowehads*, dense arrangement of nuclei at the telson edge (**a.**) and nucleus clusters at the base of setae (**b.**); *B*, bristles; *Se*, setae.

Regarding the nucleus arrangement, two regions characterised by different arrangement of nuclei can be distinguished, the basal part, anterior to the anus, and the distal part, posterior to the anus. Anteriorly, one finds distinct organs, e.g. the gut, and muscles, hence densely arranged clusters of nuclei are visible here in addition to the epidermal nuclei (Figs. 2–5).

Posteriorly, i.e. in the flattened area of the telson, only the epidermis including sensory units forms the cellular part. The nuclei are not equally distributed here, but are arranged in distinct patterns. Two different regions have to be considered in this area as well: Along the edges of the telson, the nuclei are of elliptical appearance and – due to the angle under which they are viewed and the confluence of the dorsal and ventral epidermal layers – seem to exhibit a higher density than on the paddle surface (Fig. 2, 3B). In addition, at the base of setae, mostly on the posterior telsonal edge, small clusters of nuclei are seen, those of the seta forming cells and corresponding sensory cells (Fig. 2, 4A). In addition, in some preparations the sensory axons originating at the base of the sensory cells are labelled, too (Fig. 4A). Surprisingly, strong DAPI signal indicating presence of nuclei is also found inside the setal shafts in many preparations (see below).

Along the flat surface of the telson, our pictures show the nuclei of the epidermal cells located here, on the dorsal as well as on the ventral side. The arrangement of nuclei within the species pairs is very similar in this area (Figs. 3–5A,B). However, when the different genera studied are compared, one sees differences in nuclear pattern as well as other nuclear features that allow to unequivocally characterize them. Our countings and measurements are summarized in Table 1.

Palaemon adspersus Rathke, 1837 and *Palaemon elegans* Rathke, 1837

In the two *Palaemon* species studied, the telson is of an inverted triangular, flattened paddle like shape (Figs. 1A, 2, 3). Its posterior edge is of a wavelike form and armed with 14 plumodenticulate setae inserted on the wave crests (Fig. 2). Except for the two outer setae, they are of the same length and bear numerous small lateral setules giving them a feathered appearance (Figs. 2, 3E,F). The two outer setae are shorter and are equipped with only a small amount of setules. Between the setae, 4 to 6 tiny bristles are inserted on the paddle's edge (Fig. 2). At the base of the setae, densely arranged nuclei of the cells associated with the setae (sensory, trichogen and tormogen cells) are seen. A few strongly stained spots (4–7) indicating nuclei located within the setae are also labelled (Figs. 2, 3A,B; see also table 1). Around the anus, i.e. in the thicker basal area of the telson, muscle strands and the gut containing densely arranged nuclei are strongly labelled by DAPI. This area with densely arranged nuclei is cup-shaped in *P. adspersus* and heart-shaped in *P. elegans*. In addition, in *P. adspersus* two strands of nuclei project from the anus in a latero-posterior direction. In *P.*

elegans, these strands are shorter (Fig. 3A,B).

The light microscopes and the SEM show that posterior to this area, the telson is very flat and contains mostly the dorsal and ventral epidermal layers (Figs. 2, 3C–E). Here, the nuclei are not randomly distributed. They rather show a marmorated or mottled pattern, i.e. there are small groups of nuclei in a relatively dense arrangement, and areas of low nucleus density in between. This can be observed in ventral as well as dorsal views (Fig. 3A,B).

As Table 1 shows, in *Palaemon adspersus* we counted ca. 1000 nuclei within the telson from dorsal as well as from ventral side (average value dorsal = 1103 (n = 5; p = 1.242E-8; t = 148.2; one-sided t-test)), the nuclei having an average diameter of 6.59 µm (n = 15; dorsal: p = 1.445E-13, t = 27.41; ventral: p = 7.669E-12, t = 20.5; one-sided t-test). We counted ca. 190 nuclei per 100 µm^2. Remarkably, 4–5 nuclei were seen inside setae. The values for *Palaemon elegans* are very similar. The total amount of nuclei was between 998 (ventral) and 1007 (dorsal), and their average diameter 7.56 µm (n = 15; dorsal: p = 2.181E-2, t = 2.58; ventral: p = 7.199E-14, t = 28.84; one-sided t-test). We counted 170–180 nuclei per 100 µm^2. 4–7 nuclei were located inside the shaft of setae.

TABLE 1. Census and rating of nuclei in the 6 studied species.

	Palaemonidae				Porcellanidae				Xanthidae			
	Palaemon adspersus		*Palaemon elegans*		*Porcellana platycheles*		*Pisidia longicornis*		*Xantho hydrophilus*		*Xantho pilipes*	
	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral
Number of nuclei in telson	1103 (n=5)	1101	1007	998	1688	1200	1492	1511	679	690	766	777
Number of nuclei in setae	5	4	4	7	153	181	113	117	0	0	61	59
Mean number of nuclei per 100 µm^2	192 (n=3)	190 (n=3)	168 (n=3)	184 (n=3)	353 (n=3)	351 (n=3)	319 (n=3)	310 (n=3)	358 (n=5)	349 (n=5)	343 (n=5)	294 (n=5)
Mean nucleus diameter in µm (n = 30 for each species; SD = Standard deviation)	6,59 (SD = 1,196)		7,56 (SD = 1,266)		3,91 (SD = 0,536)		3,46 (SD = 0,687)		3,92 (SD = 0,588)		3,94 (SD = 0,744)	

Porcellana platycheles (Pennant, 1777) and *Pisidia longicornis* (Linnaeus, 1767)

In these two studied species (Figs. 1B, 4), pleon segments 4 and 5, though deprived of externally visible leg buds as other zoea I, are armed with appendages, i.e. a pair of posteriolateral protrusions each (Fig. 4E). Posterior to these segments, the telson is of an oval shape and dorsoventrally flattened (Fig. 4). The lateral edges posteriorly form a single spine each oriented along the telson's edges (Fig. 4 E,F). In between these spines, inserted on the parabola-shaped rear edge, 10 plumodenticulate setae are seen. In contrast to the 2 spines, they possess well-recognisable basal rings (Fig. 4E,F). Between the innermost of these large setae, two fine ones are found near the telson's midline. Furthermore, a similar pair of setae is located on the telson's dorsal side, at about 2/3 of its length (Fig. 4E).

As in the other studied zoeas, three telson regions can be distinguished using the nuclear patterns (Fig. 4A,B). (1) At the base of the telson, there is a dense arrangement of nuclei of a somewhat rectangular shape in *P. platycheles* and having the form of an "Y" in *P. longicornis*, confluent with the tissue of the 5th pleon segment, hence labelling the gut and muscle strands near the anus. (2) At the posterior edge of the telson, at the insertion of the setae, clusters of nuclei are found, those belonging to the setal cells (Fig. 4A). Small fibers originating from these cells indicate that sensory cell axons are labelled with DAPI (Fig. 4A). Remarkably, also in the 2 spines and the 10 setae numerous nuclei are visible. (3) On both the dorsal and the ventral sides of

the telson's surface, nuclei are arranged in a very distinct pattern, i.e. in somewhat parallel rows oriented anteroposteriorly (Fig. 4A,B).

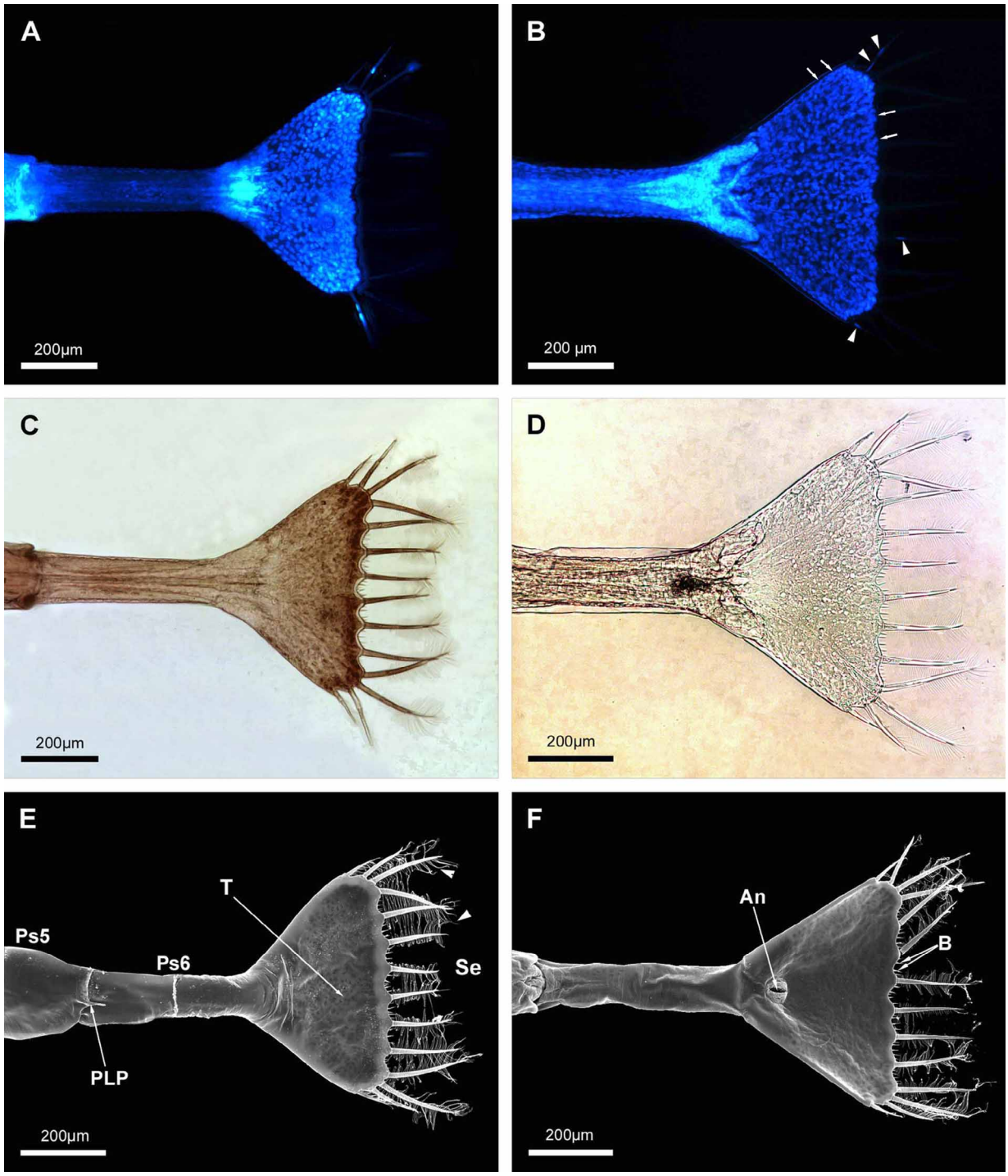


FIGURE 3. Telson of *Palaemon elegans* viewed from dorsally (**a., c., e.)** and *Palaemon adspersus* viewed from ventrally (**b., d., f.**), corresponding views made with different techniques. **a., b.** DAPI stained, **c., d.** conventional light microscopy, **e., f.** Scanning EM. *An*, anus; *B*, bristles; *PLP*, posteriolateral process; *Ps5* and *Ps6*, pleon segments 5 and 6; *Se*, setae; *arrowheads*, nuclei in setae (**b.**) and feather-like lateral branches on setae (**e.**), *arrows*, dense arrangement of nuclei at the telson edge.

In *Porcellana platycheles*, we counted 1688 and 1200 telsonal nuclei from dorsal and ventral, respectively. The nucleus diameter averages 3.91µm (n = 15; dorsal: p = 5.344E-12, t = 21.06; ventral: p = 2.876E-14, t = 30.82; one-sided t-test). We counted ca. 350 nuclei per 100 µm^2, and in the setae, more than

altogether 150 nuclei were observed. In *Pisidia longicornis*, the corresponding counts added to 1492 nuclei viewed from the dorsal side, and 1511 from the ventral side. The average nucleus diameter was 3.46µm (n = 15; dorsal: p = 1.397E-12, t = 23.23; ventral: p = 3.833E-13, t = 25.54; one-sided t-test). Per 100 µm^2, 310–319 nuclei were found, and in the setae 113 and 117 (Table 1).

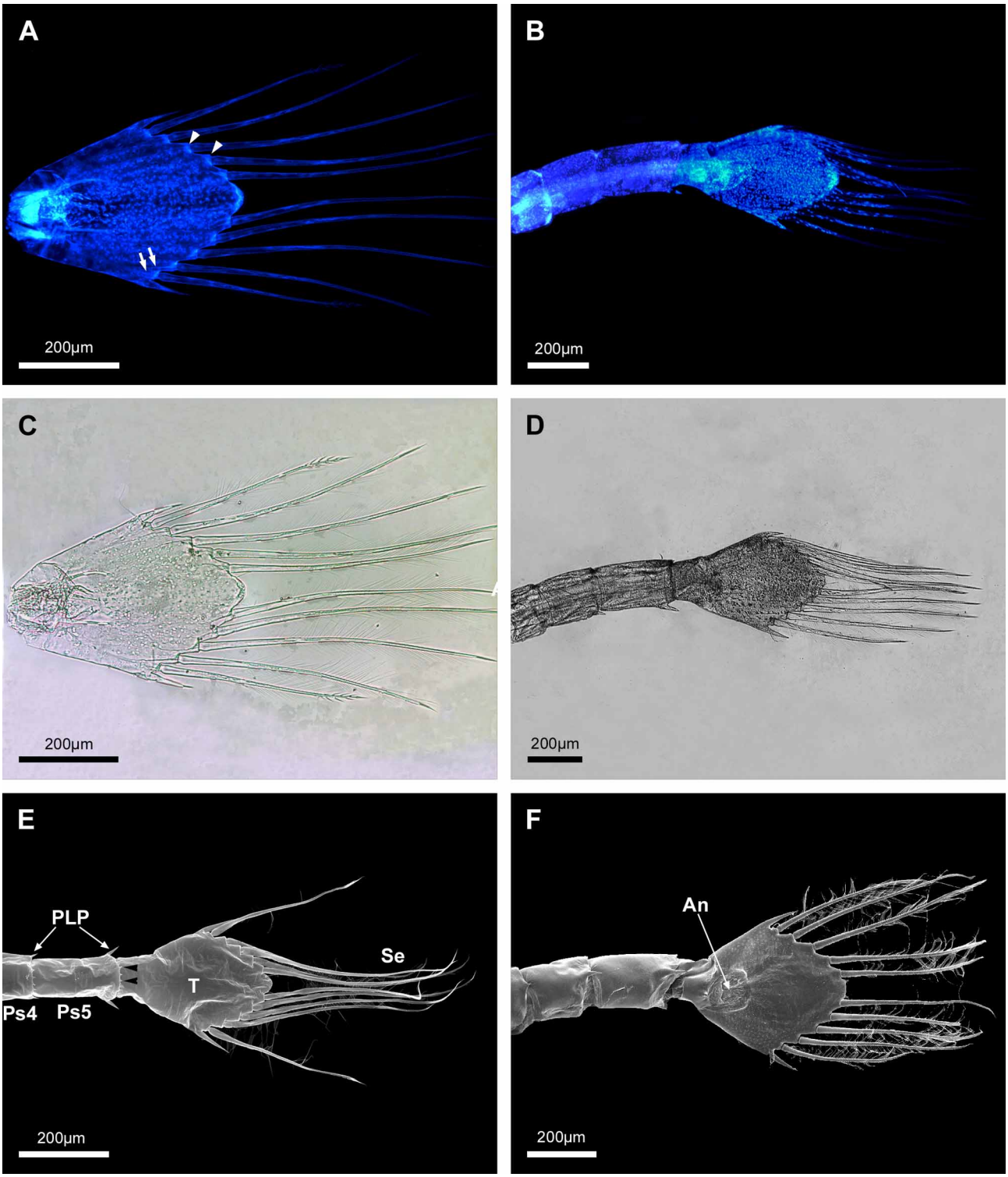


FIGURE 4. Telson of *Pisidia longicornis* viewed from dorsally (**a.**, **c.**, **e**.) and *Porcellana platycheles* viewed from ventrally (**b.**, **d.**, **f.**), corresponding views made with different techniques. (**a.**,**b**.) DAPI stained, (**c.**, **d.**) conventional light microscopy, (**e.** ,**f.**) Scanning EM. *An*, anus, *PLP*, posteriolateral process; *Ps4* and *Ps5*, pleon segments 4 and 5; *Se*, setae; *T*, telson .

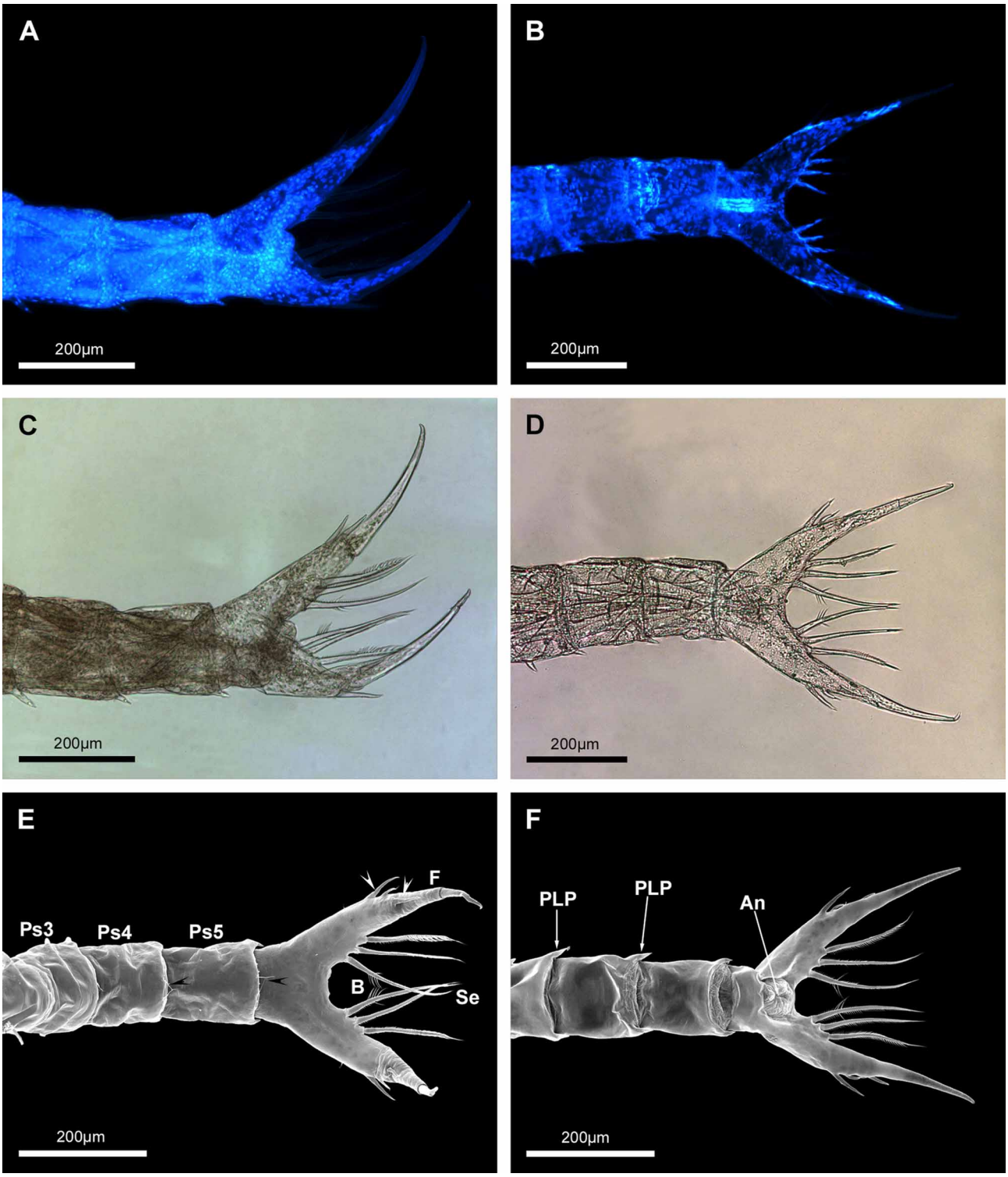


FIGURE 5. Telson of *Xantho hydrophilus* viewed from dorsally (**a., c., e.**) and *Xantho pilipes* viewed from ventrally (**b., d., f.**), corresponding views made with different techniques. **a., b.** DAPI stained, **c., d.** conventional light microscopy, **e., f.** Scanning EM. *An*, anus; *B*, bristles; *F*, furca branch; *PLP*, posteriolateral process; *Ps3-5*, pleon segments 3-5; *Se*, setae; *arrowheads*, small lateral processes on setae.

Xantho hydrophilus Herbst, 1780 and *Xantho pilipes* A. Milne Edwards, 1867

In the two species of *Xantho*, the telson is of a different shape than in the other studied taxa: A furca, composed of two lateroposteriorly oriented branches originating from the v-shaped base of the telson (Figs. 1C, 5) is well developed. Each branch narrows posteriorly, and has a pointed tip. At about 2/3 of the furca's

length, 2 lateral and 1 dorsal protrusion are inserted, and on the inner edge of the branches one finds 3 denticulate setae with numerous short setules. However, the most proximal one is equipped with 4–6 long setules (Fig. 5C–F).

As in the other studied taxa, a distinct accumulation of nuclei is found around the anus. In *Xantho*, it is of a rectangular shape. The other nuclei of the telson are irregularly arranged. From both the dorsal and the ventral sides, areas with higher and lower nucleus density can be distinguished (Fig. 5A,B).

Our counts (Table 1) yielded 679 telsonal nuclei from dorsal, and 690 from ventral in *Xantho hydrophilus*. The average nucleus diameter was 3.92µm (n = 15; dorsal: p = 2.995E-12, t = 21.97; ventral: p = 3.192E-12, t = 21.87; one-sided t-test). The average number of nuclei per 100 μm^2 was 358 from dorsal, and 349 from ventral. In the setae, no nuclei were detected. In *Xantho pilipes*, we counted from dorsally 766 nuclei, and ventrally 777. The diameter of the nuclei was 3.94µm (n = 15; dorsal: p = 1.797E-11, t = 19.26; ventral: p = 3.971E-12, t = 21.52; one-sided t-test), and per 100μm^2, we counted 343 nuclei from dorsal, and 294 nuclei from ventral. 61 and 59 nuclei were found inside the telson's appendages viewed from dorsally and ventrally, respectively.

Discussion

The epidermis of crustacean larvae has been studied in detail including the different cell types and their development (reviews, e.g. in Freeman 1993, Anger 2001). Furthermore, cellular and nuclear patterns have been analysed in an "evo-devo" context in various ways in various organisms, non-crustaceans (e.g., Höfer *et al.* 1995, Brook *et al.* 1996) as well as crustaceans (e.g., Scholtz and Dohle 1996, Scholtz 2000). The aim of the present study differs from these approaches by omitting the analysis of developmental processes and/or the underlying mechanisms, and focussing on the comparison of the patterns between taxa, hence a "horizontal" comparison as a test for taxonomic significance.

In general one can say that the closely related species forming the three studied species pairs are very similar with respect to the observed characteristics, and therefore our descriptions of the qualitative features could be made in common for both species of each pair. Differences, however, become obvious when comparisons between the pairs representing different decapod families (and infraorders) are made.

Here, the main difference is the pattern in which epidermal nuclei are arranged: in rows in the studied porcellanid zoeas, irregular or marmorated in palaemonids and xanthids. General traits seem to be the dense arrangement of non-epidermal nuclei around the anus, probably including nuclei of the intestine and of muscles terminating in this area, and the absence of nuclei other than those of the epidermis and sensilla in the telson's posterior part, which seems to be a passively maneuvered organ in which muscles are found only at its base.

Looking at our counts and measurements, more differences become apparent. The total amount of telsonal nuclei increases from the xanthids (ca. 700) to the palaemonids (ca. 1000) to the porcellanids (1200–1700). This seems to be correlated with the different shape and size of the telson in the three taxa: the porcellanids and palaemonids with their fin-shaped or roundish telsons (Gonzales-Gordillo *et al.* 1996, Fincham 1977, 1985, 1986) have more nuclei than the xanthids with their branched furca (Ingle 1983, Paula & Dos Santos 2000, Meyer at al. 2004) having the smallest surface area. However, also the number of nuclei per 100μm^2 differs between the taxa, with the palaemonids having a much lower number than the porcellanids and xanthids studied. Hence, the differences are not caused by the size of the telson alone, and there must be other immanent reasons. Conversely, the palaemonids have bigger nuclei than the other two taxa, a fact that is at the moment also hard to explain. One possibility is that the nucleus size might be correlated with the amount of DNA. It has been shown that polyploid nuclei are bigger than normal ones (Frankhauser 1945, Mundkur 1953), but in this respect data are missing for our studied zoeae.

Remarkable is the fact that in most of the studied species in addition to the seta-related cells at the setal base, we found spots strongly labelled with DAPI, and thus indicative of the presence of nuclei inside the telsonal setae, a few in the palaemonids, many in the porcellanids, and also a considerable amount in *Xantho*

pilipes, but not in *Xantho hydrophilus*. Especially the stains of *Porcellana* show so many of these structures labelled in such a strong way that their nuclear origin is quite clear. But, provided that these setae would be somewhat normal setae as found throughout the arthropods, the cell bodies of the cells assigned to them (and their nuclei), i.e. tormogen, thecogen, trichogen and sensory cells should be located at the base of the setae near the basal ring, and not inside their shafts (e.g., Ball and Cowen 1976, Altner 1977, Eguchi & Tominaga 1999). Do the large setae inserted at the hind edge of the telson therefore not represent setae s.str., but protrusions of another type containing their own epidermis sections? It is known that around molts, seta secreting cells move into the setal shafts (Berg & Schmidt 1996). However, all our zoeas were newly hatched animals, and it is highly improbable that they were in a close-to-molt stage. Furthermore, why should there be 180 nuclei, more than 10 per seta, in the setae, as found in *Porcellana platycheles*? Considering the high amount of lateral branches these setae possess, is it therefore possible that the telson is equipped with a previously unknown setal type, more complex than the normal ones? This point needs further investigation.

An interesting point is the question *why* there are such differences in the nucleus features of the studied species. It is obvious that we cannot expect any selection that directly brings about kind of an "evolution" of nucleus positions. Hence, the observed differences must be a secondary effect. In our stains every nucleus represents one cell, and the distance between nuclei gives some ideas about the dimensions of the cells they belong to. Form, size and function of cells thus determine the position of their nuclei. Specific structures, e.g. setae or protrusions, have a characteristic set of cells associated with them exhibiting a distinct arrangement. Hence there are morphological *constraints* that force cells – and secondarily also their nuclei – in certain positions. This is obvious for, e.g. the setal cell nuclei located at the base of the setae in all the studied species (review in Eguchi & Tominaga 1999), or the cells around the anus which have a well defined, unchangeable position. This idea is well supported by the light microscopical and the scanning EM observations we made parallel with the DAPI stains.

What might be the constraints producing the different nucleus patterns along the telsonal surface, i.e. within the "normal" epidermal layer? Principally, the epidermis nuclei – and the cells they are part of – could be evenly distributed and of equal size in all taxa studied, but, as we saw, they are not. One could think of inconsistencies in cuticle thickness or the arrangement of nerve bundles projecting from the setae anteriorly across the telson that bring about inhomogenous cell arrangement and thus specific nucleus pattern, but we could not find indications for such mechanisms.

Since a zoea I larva is a developing organism that will undergo thorough changes until it reaches the adult stage, ontogenetical constraints should be considered, too. Mitotic waves that increase the number of cells and alter the arrangement of nuclei by forming kind of embryonic cell nests have been analysed in detail in embryos of crustaceans (Scholtz & Dohle 1996, Scholtz 2000), and one can expect similar processes during larval development, e.g. in pre-molt zoeae that should differentiate the cellular material needed for morphogenetical changes and growth to the next instar. In our specimens, however, all of them being early rather than late zoeas in a pre-molt-stage, there was no indication of such changes, e.g. cleavage stages. One can conclude that the differences we observed are genuine taxon specific features and not morphogenetical effects caused by analysing different zoea I substages.

Whatever reason the differences of the nucleus features between the studied taxa might have, they seem to allow to distinguish taxa, and hence are potentially useful features in the context of taxonomy, and could be added to lists of diagnostic features, not on the species level – since we found no relevant differences here – but on a higher taxonomic level, e.g. genus or families or suborders. If we hypothesize the nucleus features seen in the two studied porcellanids to be characteristic of Porcellanidae, this diagnostic list would read as follows: "telson with big amount of nuclei (> 1000) arranged in rows with a density of 300–350 nuclei per 100µm^2 and a diameter of 3,5 to 4 µm". Our intention of course is not to introduce these features as a prerequisite for zoea I descriptions. What we want to show is that on many levels one can find features that may contribute to species diagnosis and apply them if they are helpful.

Acknowledgements

We thank Stefan Friedrich for excellent technical support. This study was partly supported by grant given by “Sea Life Centre München” and “Freunde der Zoologischen Staatssammlung e.V.”.

References

Altner, H. (1977) Insektensensillen: Bau- und Funktionsprinzipien. *Verhandlungen der Deutschen Zoologischen Gesellschaft 1977*, 139–153.

Anger, K. (2001) *The biology of decapod crustacean larvae*. A.A. Balkema Publishers, Lisse, Abington, Exton (PA), Tokyo, 420pp.

Ball, E.E. & Cowan, A.N. (1976) Ultrastructure of the Antennal Sensilla of *Acetes* (Crustacea, Decapoda, Natantia, Sergestidae). *Philosophical Transactions of the Royal Society of London (Ser. B, Biological Sciences)*, 277, 429–457.

Barnich, R. (1995) *The Larvae of the Crustacea Decapoda (excl. Brachyura) in the Plankton of the French Mediterranean Coast (Identification Keys and Systematic Review)*. Cuvillier Verlag, Göttingen, 189 pp.

Berg, J. & Schmidt, K. (1996) Moulting of mechanoreceptive hair sensilla of *Lepisma saccharina* (Zygentoma) and *Machilis* spec. (Archeognatha). *Tissue and Cell*, 28, 9–16.

Brook, W.J., Diaz-Benjumea, F.J., & Cohen, S.M. (1996) Organizing spatial pattern in limb development. *Annual Review of Cell and Developmental Biology*, 12, 161–180.

Eguchi, E. & Tominaga, Y. (Eds., 1999). *Atlas of Arthropod Sensory Receptors, Dynamic Morphology in Relation to Function*. Springer Verlag, Tokyo, 220 pp.

Fincham, A.A. (1977) Larval development of British Prawns and Shrimps (Crustacea: Decapoda: Natantia): 1. Laboratory methods and a review of *Palaemon* (*Paleander*) *elegans* Rathke, 1837. *Bulletin of the British Museum (Natural History), Zoology Series*, 31, 1–28.

Fincham, A.A. (1985) Larval development of British Prawns and Shrimps (Crustacea: Decapoda: Natantia): 4. *Palaemon* (*Palaemon*) *adspersus* Rathke, 1837. *Bulletin of the British Museum (Natural History), Zoology Series*, 48, 213–229.

Fincham, A.A. (1986) Larval keys and diagnoses for the subfamily Palaemoninae (Crustacea: Decapoda: Palaemonidae) in the north-east Atlantic and aspects of functional morphology. *Journal of Natural History*, 20, 203–224.

Frankhauser, G. (1945). The Effects of changes in chromosome number on amphibian development. *The Quarterly Review of Biology*, 20, 20–78.

Freeman, J.A. (1993) The crustacean epidermis during larval development. *In*: Horst, M.N. & Freeman, J.A. (Eds.), *The Crustacean Integument: Morphology and Biochemistry*. CRC Press, Boca Raton, pp. 193–221.

Geiselbrecht, H. & Melzer, R.R. (2009) Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* Risso, 1827 (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the Scanning EM. *Zootaxa*, 2140, 45–55.

González-Gordillo, J.I., Cuesta, J.A. & Rodriguez, A. (1996) Studies on the larval development of northeastern Atlantic and Mediterranean Porcellanidae (Decapoda, Anomura). I – Redescription of the larval stages of *Porcellana platycheles* (Pennant, 1777) reared under laboratory conditions. *Helgoländer Meeresuntersuchungen*, 50, 517–531.

Höfer, T., Sherrat, J.A. & Maini, P.K. (1995) Cellular pattern formation during Ditycostelium aggregation. *Physica D*, 85, 425–444.

Ingle, R.W. (1983) A comparative study of the larval development of *Monodaeus couchi* (Couch), *Xantho hydrophilus* Herbst and *Pilumnus hirtellus* (Linnaeus) (Crustacea: Brachyura: Xanthidae). *Journal of Natural History*, 17, 951–978.

Kubista, M., Aakerman, B. & Norden, B. (1987) Characterization of interaction between DNA and 4',6–diamidino-2-phenylindole by optical spectroscopy. *Biochemistry*, 26, 4545–4553.

Meyer, R. & Melzer, R.R. (2004) Scanning EM diagnosis of marine Decapoda larvae: A comparison of preparation techniques. *Crustaceana*, 77: 883–886.

Meyer, R., Friedrich, S. & Melzer, R.R. (2004). *Xantho poressa* (Olivi, 1792) and *Xantho pilipes* A. Milne-Edwards, 1867 Larvae (Brachyura, Xanthidae): Scanning EM Diagnosis of Zoea I from the Adriatic Sea. *Crustaceana*, 77, 997–1005.

Meyer, R., Wehrtmann, I.S. & Melzer, R.R. (2006) Morphology of the first zoeal stage of *Portunus acuminatus*, Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. *Scientia Marina*, 70: 261–270.

Mundkur, B.D. (1953) Interphase nuclei and cell sizes in a polyploid series of saccharomyces. *Cellular and Molecular Life Sciences*, 9, 373–374.

Paula, J. & Dos Santos, A. (2000) Larval and early post-larval stages of the crab, *Xantho pilipes* A. Milne-Edwards, 1867

(Crustacea, Decapoda, Xanthidae), reared under laboratory conditions. *Invertebrate Reproduction and Development*, 38, 253–264.
Scholtz, G. & Dohle, W. (1996) Cell lineage and cell fate in crustacean embryos – a comparative approach. *International Journal of Developmental Biology*, 40, 211–220.
Scholtz, G. (2000) Evolution of the nauplius stage in malacostracan crustaceans. *Journal of Zoological Systematics and Evolution Research*, 38, 175–187.
Wohlfrom, H. & Melzer, R.R. (2001) Development of the sensory system in larvae and pupae of *Chaborus crystallinus* (DeGeer, 1776; Diptera, Chaboridae): sensory cells, nerves and ganglia of the tail region. *Development, Genes and Evolution*, 211, 124–137.
Zariquiey Alvarez, R. (1968) Crustaceos Decapodos Ibericos. *Investigacion Pesquera, Barcelona*, 32, 1–510.

7. Article V

Meyer R, Weis A, Melzer RR (2013) Decapoda of southern Chile: DNA barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* and *Eurypodius*. Systematics and Biodiversity 11(3): 389-404.

The supplementary table of this article is available from the CD (Appendix) or the article's Taylor & Francis Online page http://dx.doi.org/10.1080/14772000.2013.833143.

Figure 12: The brachyuran crab *Eurypodius latreillii* Guérin, 1825 climbing on macroalgae. Photo by the author.

Systematics and Biodiversity (2013), **11(3)**: 389–404

Taylor & Francis
Taylor & Francis Group

Research Article

Decapoda of southern Chile: DNA barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* and *Eurypodius*

ROLAND MEYER, ANDREA WEIS & ROLAND R. MELZER

Zoologische Staatssammlung München, Münchhausenstrasse 21, 81247 München, Germany

(Received 22 October 2012; accepted 5 August 2013)

A combination of DNA sequence and morphological data is used to assess the taxonomy of Chilean decapods. The *c*. 657-base-pair long mitochondrial protein-coding gene COI (cytochrome c oxidase subunit 1) of 154 decapod specimens (41 species in 31 genera and 21 families) of the southern Chilean area (36°33′S–54°56′S) is analysed for the first time. The resulting phylogenetic consensus tree displays 41 distinct branches corresponding to the morphological determination of the studied species. These results confirm that standard DNA barcoding sequences are a suitable tool in addition to morphology for taxonomic analyses in Decapoda of the region. Genetic results are compared with morphological data to check and confirm species delimitations in morphologically closely related species, i.e. the representatives of the genera *Eurypodius* Guérin, 1825 (*E. latreillii* Guérin, 1825 and *E. longirostris* Miers, 1886) and *Acanthocyclus* Lucas, in H. Milne Edwards & Lucas, 1844 (*A. albatrossis* Rathbun, 1898, *A. hassleri* Rathbun, 1898 and *A. gayi* Lucas, in H. Milne Edwards & Lucas, 1844). Available morphological descriptions of these species are in many aspects contradictory, confusing and not always clear. The status of the different species is confirmed using the morphology-independent barcoding feature in combination with classic morphological features, clarifying species-specific morphological features for further species determination. An identification key of *Acanthocyclus* species based on constant features is given. Biogeographical aspects and distribution range for the selected species are discussed.

Key words: *Acanthocyclus*, Chile, COI, Decapoda, DNA barcoding, *Eurypodius*, integrative taxonomy, southern fjords

Introduction

The coast of Chilean Patagonia extends more than 2000 km, from Puerto Montt (41°S) in the north to Cape Horn (55°S) in the south, with a poorly explored coastline of approximately 84 000 km (Bustamente, 2009). This unique coast was covered and created by glaciers of the Northern and Southern Patagonian Ice Shield during the last ice age 15 000 years ago and was subsequently recolonized by benthic communities (Clapperton, 1993; Försterra, 2009). Steep slopes in coastal zones of the fjords and shallow water areas at SCUBA-accessible depths are nearly unexplored. Recent examinations show that these particular locations include hitherto undiscovered biodiversity hotspots awaiting exploration (Försterra, 2009). To close this gap of knowledge several Huinay Fjordos (HF) expeditions have been carried out in the southern Chilean region since 2005, organized by the Huinay Scientific Field Station (for details see: http://www.fundacionhuinay.cl/). The purpose of the expeditions is a complete faunistic inventory of the Chilean antiboreal region with a special sampling concept: species are documented *in situ* by underwater photography and then collected for further investigations during scuba diving (Häussermann & Försterra, 2009). Scuba diving allows collection activities in depths between 0 and 40 m and in all types of environment, e.g. steep walls, which is a great advantage in comparison to dredge-based sampling. During these expeditions about 600 decapod specimens were collected in the southern Chilean region between Dichato (36°33′S) and Islas Holger (54°56′S) as the basis for the present work, this extending over the southern Chilean fjord region and further northwards to the Región del Bío-Bío (Fig. 1).

In the present paper we report the first COI barcoding results for decapods of the southern Chilean region. For Crustacea COI barcoding has been found to be a useful tool for specimen identification (Bucklin *et al.*, 2007; Costa *et al.*, 2007; Miguel Pardo *et al.*, 2009), species delineation and the resolution of taxonomic problems of closely related species (Gusmao *et al.*, 2000; Daniels *et al.*, 2003; Machordom & MacPherson, 2004; Lefebure *et al.*, 2006; Pérez-Barros *et al.*, 2008).

Correspondence to: Roland Meyer. E-mail: rolandmeyer@hotmail.de

ISSN 1477-2000 print / 1478-0933 online

http://dx.doi.org/10.1080/14772000.2013.833143

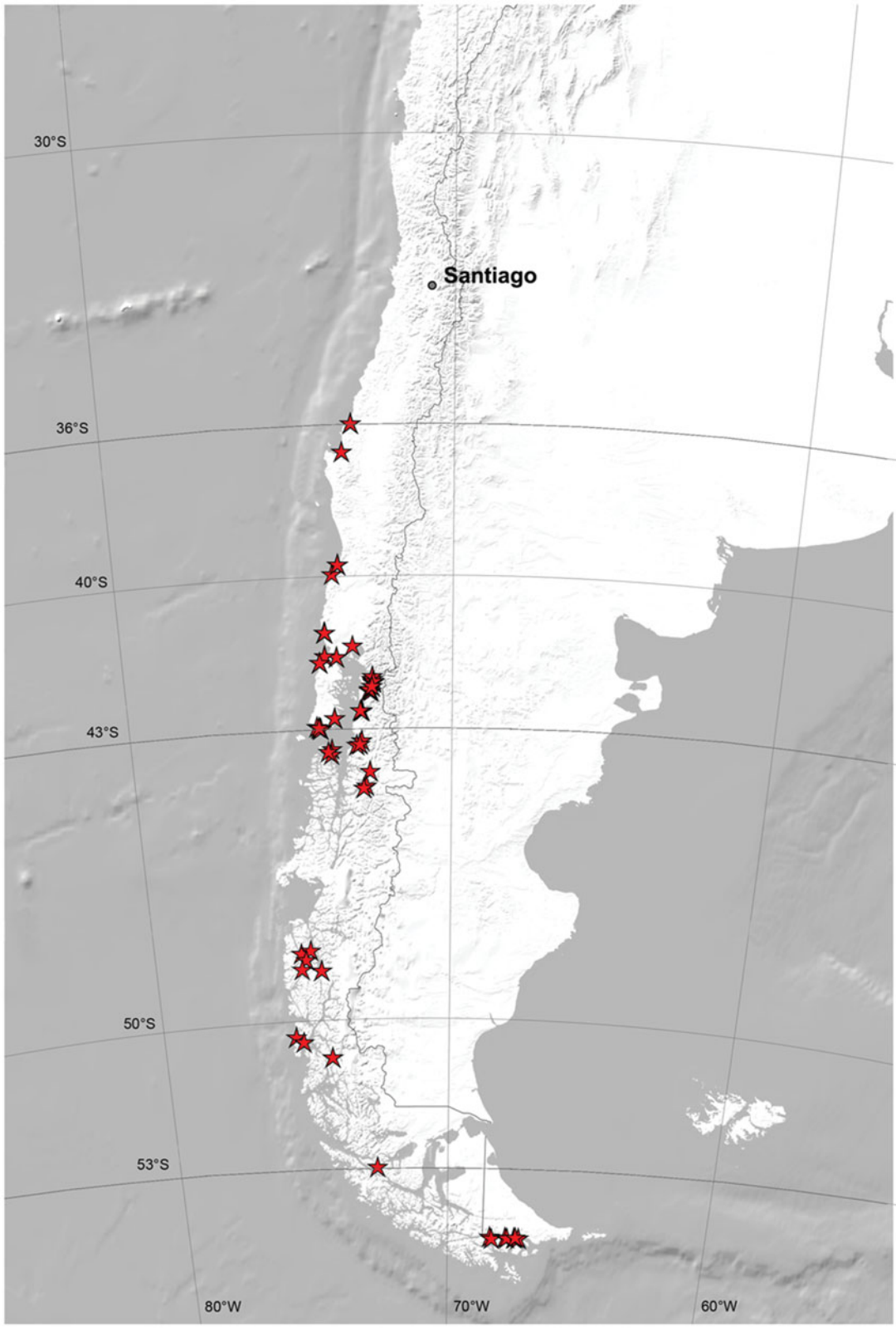


Fig. 1. Overview of collection sites of southern chilean decapods analysed in this study. Sampling area extends from Dichato (36°32′S) to Islas Holger (54°56′S). Red stars indicate sampling stations. Latitudes for species boundaries of *Acanthocyclus* and *Eurypodius* species are given.

The genus *Acanthocyclus* consists of 3 morphologically very closely related species found in southern Chile: *A. gayi* Lucas, in H. Milne Edwards & Lucas, 1844, *A. hassleri* Rathbun, 1898, and *A. albatrossis* Rathbun, 1898. Two morphologically very closely related species of the genus *Eurypodius* occur in southern Pacific waters: *Eurypodius longirostris* Miers, 1886 and *Eurypodius latreillii* Guérin, 1825 (Rathbun, 1925, 1930; Garth, 1957; Boschi & Gavio, 2005). These two genera raise difficulties for morphology based identification, since available descriptions are in many aspects contradictory and not always clear. We took the opportunity to recheck species delimitations and delineations using integrative taxonomy (Dayrat, 2005; Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010). We reanalysed the morphological characteristics, adjusted these data with available type-material of the species and corroborated them with the DNA sequences that represent a second character set independent from morphology.

Materials and methods

Samples

For DNA sequencing we used 190 specimens collected in southern Chile in the years between 2005 and 2011 during expeditions organized by the Huinay Scientific Field Station, Huinay, Chile (Huinay Fjordos 3-10). The collection area is displayed in Fig. 1. Samples were taken at depths between 0 and 30 m by scuba diving, snorkelling and hand collection in the intertidal zone. Muscle tissue of the ambulatory legs was preserved in 96% ethanol to ensure high-quality DNA for genetic analysis. All barcoded voucher specimens are stored in 75% ethanol and deposited at the Bavarian State Collection of Zoology and their respective DNA extract aliquots at the CCDB (Canadian Center for DNA Barcoding: www.dnabarcoding.ca) and the ZSM's DNA bank facility Munich (www.zsm.mwn.de).

All details regarding taxonomy, collection sites (including the geographical coordinates), BOLD and GenBank accession numbers are listed in Table S1 (see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2013.833143), and can also be accessed on the Barcode of Life Data System website (BOLD) (Ratnasingham & Hebert, 2007) under the project CFAD (Chile Fjord Arthropods Decapoda) as part of the campaign 'Marine Life (MarBOL)'.

In addition to these samples, type material of *Eurypodius longirostris* Miers, 1886 (1884.31 Natural History Museum, UK), *E. latreillii* Guérin, 1825 (RMNH D 42178, Nationaal Natuurhistorisch Museum, Leiden), *Acanthocyclus hassleri* Rathbun, 1898 (MCZ CRU-4889, Museum of Comparative Zoology, Harvard), *A. albatrossis* Rathbun, 1898 (USNM 1086178, Smithsonian Institution National Museum of Natural History) and *A. gayi* Lucas in H. Milne Edwards & Lucas, 1844 (RMNH D 43615, Nationaal Natuurhistorisch Museum, Leiden) was studied.

Species determination based on external morphological features used various sources (Rathbun, 1918, 1925, 1930; Haig, 1955; Garth, 1957; Retamal, 1981; Meyer *et al.*, 2009) including original descriptions of the *Eurypodius* and *Acanthocyclus* species.

DNA extraction, amplification and sequencing

Laboratory operations were carried out at the Canadian Centre for DNA Barcoding (CCDB), University of Guelph, Canada following the standard protocols of IBOL (http://dnabarcoding.ca/pa/ge/research/protocols). Depending on the size of the individuals, either whole legs (small specimens) or a sample of muscle tissue from each specimen was taken for DNA extraction and further sequencing. For the PCR a 1 : 1 ratio mix of LepF1/LepR1-primer (Hebert *et al.*, 2004*a*) and LCO1490/HCO2198 primer (Folmer *et al.*, 1994) was used. Prior to routine sequencing of samples at the barcoding facility in Guelph, a pilot study was conducted by kmbs (www.kmbioservices.com) to evaluate tissue quality and primer sequences.

Phylogenetic methods

In total 184 decapod sequences were used for phylogenetic analysis (154 sequences from this study, 21 additional sequences of species already represented in this study mined from GenBank and nine outgroup sequences of *Oratosquillina interrupta* (Kemp, 1911) (Squillidae, Stomatopoda) (Accession no. FJ229788-FJ229796)) (for details see Table 1) (Hultgren & Stachowicz, 2008; Pérez-Barros *et al.*, 2008; Miguel Pardo *et al.*, 2009; Tang *et al.*, 2010; Haye *et al.*, 2012). COI sequences were blasted with GENEIOUS Pro version 5.5.4 (Drummond *et al.*, 2011) using Megablast. The alignment was performed with GENEIOUS Pro version 5.5.4, using MUSCLE Alignment (Edgar, 2004). Aligned COI nucleotide sequences were manually checked for ambiguities and translated to amino acids to maintain the integrity of codon triplets and the alignment of amino acids.

The alignment was statistically tested for substitution saturation with the DAMBE 5.2.69 software package (Xia *et al.*, 2003; Xia & Lemey, 2009). MEGA 5.05 was used to find the best fitting substitution model. According to these results, we used the GTR (general time reversible) model with proportion of invariable sites (I) = 0.475 and the gamma shape parameter (G) = 1.169 (GTR+I+G), (Rodriguez *et al.*, 1990).

We used RAxML 7.0.4 (Stamatakis, 2006) to calculate the Maximum likelihood (ML) analyses with 1000 bootstraps; neighbour joining (NJ) trees based on Kimura

Table 1. Overview of decapods and outgroup specimens mined from GenBank.

GenBank ID	Species	Reference
FJ229788	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229789	*Oratosquillina interruptal*	Tang *et al*. 2010
FJ229790	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229791	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229792	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229793	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229794	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229795	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ229796	*Oratosquillina interruptal*	Tang *et al.* 2010
FJ155383	*Homalaspis plana*	Miguel Pardo *et al.* 2009
JN315643	*Homalaspis plana*	Haye *et al.* 2012
JN315644	*Homalaspis plana*	Haye *et al.* 2012
FJ155372	*Metacarcinus edwardsii*	Miguel Pardo *et al.* 2009
FJ155373	*Metacarcinus edwardsii*	Miguel Pardo *et al.* 2009
FJ155374	*Metacarcinus edwardsii*	Miguel Pardo *et al.* 2009
JN315645	*Metacarcinus edwardsii*	Haye *et al.* 2012
JN315646	*Metacarcinus edwardsii*	Haye *et al.* 2012
AY700163	*Munida gregaria*	Peréz-Barros *et al.* 2008
AY700164	*Munida gregaria*	Peréz-Barros *et al.* 2009
AY700165	*Munida gregaria*	Peréz-Barros *et al.* 2010
FJ155378	*Romaleon polydon*	Miguel Pardo *et al.* 2009
FJ155379	*Romaleon polydon*	Miguel Pardo *et al.* 2009
FJ155380	*Romaleon polydon*	Miguel Pardo *et al.* 2009
FJ155381	*Romaleon polydon*	Miguel Pardo *et al.* 2009
FJ155382	*Romaleon polydon*	Miguel Pardo *et al.* 2009
JN315651	*Romaleon polydon*	Haye *et al.* 2012
JN315652	*Romaleon polydon*	Haye *et al.* 2012
EU682872	*Taliepus dentatus*	Hultgren & Stachowicz 2008
JN315653	*Taliepus dentatus*	Haye *et al.* 2012
JN315654	*Taliepus dentatus*	Haye *et al.* 2012

2-parameter (K2p) model (Kimura, 1980; Saitou & Nei, 1987) and maximum parsimony (MP) (all 1000 bootstraps) analysis were performed using MEGA 5.05 software (Tamura *et al.*, 2011).

Bayesian inference (BI) MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was performed with 5.5 million Metropolis-coupled MCMC generations; every 200th tree was saved with a burn-in of 6875. The consensus tree was calculated under the 50% majority rule consensus. Graphic editing of the tree was done with FigTree 1.3.1 and MEGA 5.0.

Bootstrap values of the character-based trees (MP, ML, Bayes) and distance-based trees (NJ) were combined and given in the consensus tree of MrBayes (Fig. 2). Intra- and interspecific distances were calculated (excluding sequences mined from GenBank) using the K2P distance model in BOLD. The search for barcoding gaps was performed by the software Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012).

Results

Overall molecular results

The selected specimens represent 41 species in 31 genera and 21 families of the order Decapoda. Extraction and sequencing of the COI fragment was successful in 154 out of 190 specimens, i.e. 81.6%. We observed low success in barcoding anomuran species: for example the barcoding process for *Petrolisthes tuberculatus* (Guérin, 1835) failed completely and the total sequencing rate within this infraorder was 60.7%.

The morphological determinations of species accord very well with the results of the molecular analysis: 41 morphologically determined species correlate with 41 branches supported by high bootstrap (>90%) and posterior probability values (>93%) (Fig. 2). For species details see Fig. 2 and Table S1 (see supplemental material online).

For resolving relationships at a higher taxonomic level the COI gene appears to be inadequate: all representatives of the Porcellanidae Haworth, 1825 in this study (genera *Petrolisthes* Stimpson, 1858, *Allopetrolisthes* Haig, 1960, and *Pachycheles* Stimpson, 1858) cluster in one clade though only the species-level branches are well-supported by high values. Other representatives of the Anomura are not well separated at higher taxonomic levels: different families (Paguridae Latreille, 1802 with genera *Pagurus* Fabricius, 1775 and *Propagurus* McLaughlin & de Saint Laurent, 1998; Munididae Ahyong, Baba, Macpherson & Poore, 2010, with genera *Munida* Leach, 1820 and Lithodidae Samouelle, 1819, with genera *Lithodes* Latreille, 1806

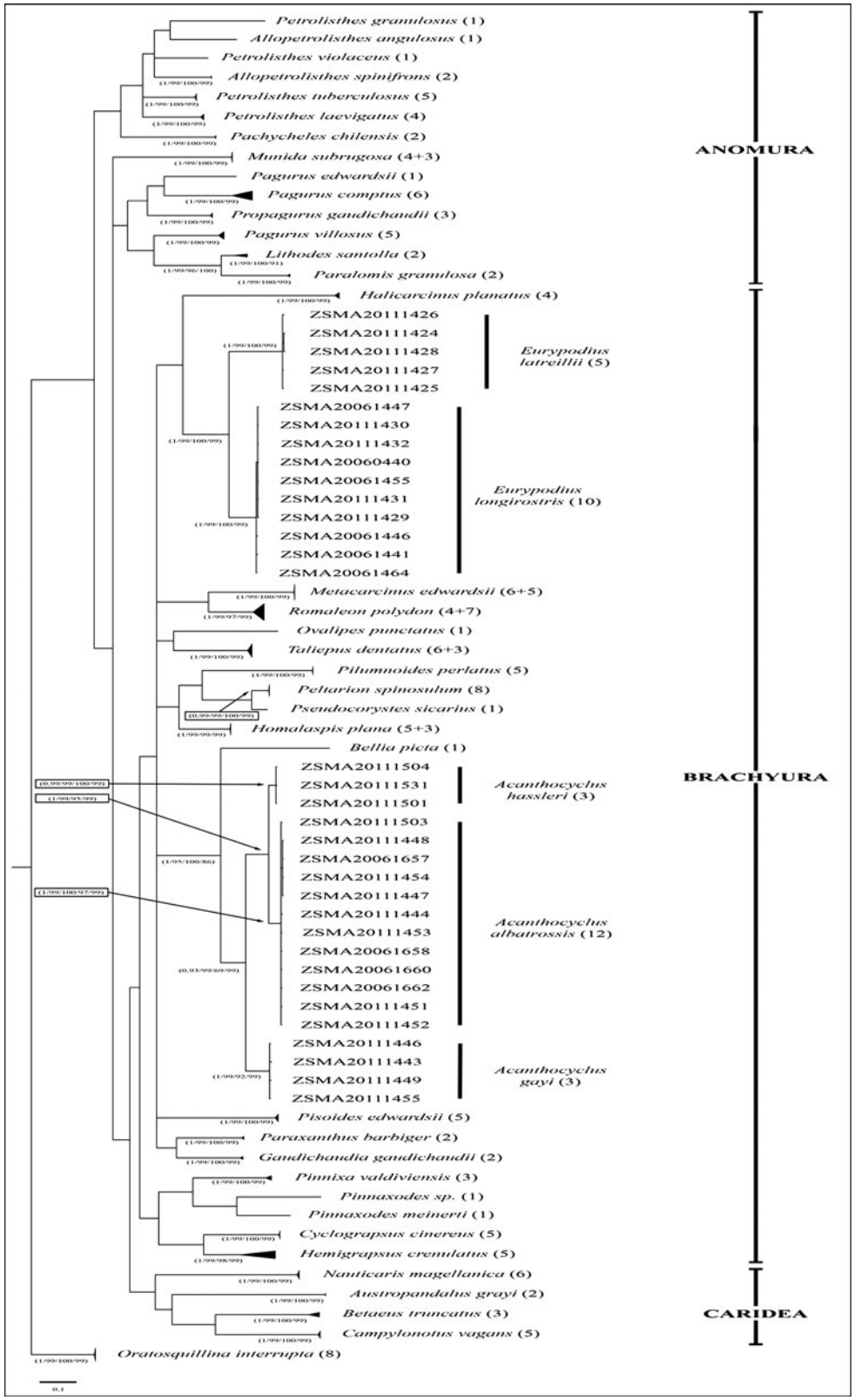


Fig. 2. Bayesian inference tree of cytochrome c oxydase I (COI) sequences, showing the placement of 154 decapods, plus eight outgroup specimens retrieved from GenBank. Numbers in parentheses indicate the number of analysed individuals (+ number of sequences of the respective species mined from GenBank). Numbers above and below branches show posterior probability of BI and bootstrap values (>90%) of NJ, MP and ML analysis branch length indicates substitutions per site.

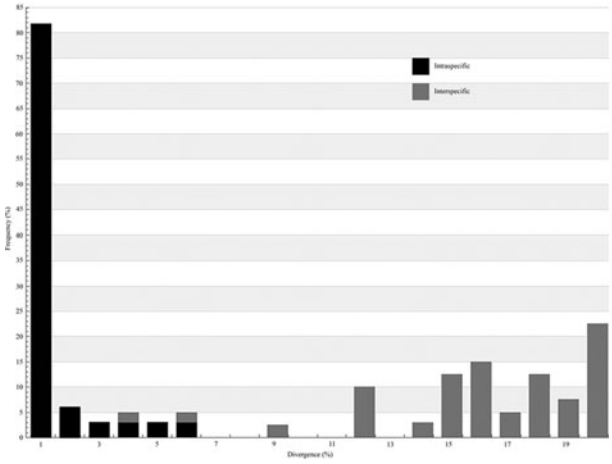


Fig. 3. Intraspecific and interspecific distance distribution among 154 south Chilean decapods for cytochrome c oxidase I sequences.

and *Paralomis* White, 1856) cluster in one clade. As in the Porcellanidae cluster, only the species-level branches are well supported.

In the Brachyura the Inachidae MacLeay, 1838, represented by the genus *Eurypodius* Guérin, 1825 (of which only two species are known, both in the study area: *E. latreillii* and *E. longirostris*), species-level discrimination is supported with high values. Furthermore the Bellidae Dana, 1852 cluster is remarkable; this cluster consists of two genera: *Bellia* H. Milne Edwards, 1848 (one species known) and *Acanthocyclus* Lucas in H. Milne Edwards & Lucas, 1844 (three species known; only occurring in South America). Both genera are well separated through high bootstrap support and species branches are well defined (Fig. 2).

Blasted in GenBank, only 15.38% of our sequences had matches since most species in our study had not been COI-sequenced before. The COI alignment had 331 variable sites, 329 conserved sites, while 316 sites were parsimony-informative. All sequences were longer than 500-bp and thus fulfil the requirements for barcoding (Ratnasingham & Hebert, 2007). Sequence compositions show a bias towards adenosine and thymine (average values: A 26.4%, C 19.4%, G 17.9%, T 36.3%) which is typical for arthropods. When sequences were translated into protein sequences the dataset showed no frame shift mutations or stop codons. According to the results of the substitution saturation test, the index of substitution saturation (Iss) was significantly lower than the critical value of the index of substitution saturation (Iss.c).

Results of the analysis of the intra- and interspecific distances are shown in Fig. 3. Among the studied specimens these values are as follows: the mean interspecific distance is 15.54% with a range of min. values of 3.46% (*Acanthocyclus albatrossis*/*Acanthocyclus hassleri*) and 5.61% (*Peltarion spinosulum*/*Pseudocorystes sicarius*) to a max. value of 25.11% (*Nauticaris magellanica*/*Propagurus gaudichaudii*). Intraspecific distances were calculated with an average value of 0.77% and with a min. value of 0.0% (*Peltarion spinosulum* n = 8, *Acanthocyclus hassleri* n = 3, *Austropandalus grayi* n = 2, *Pilumnoides perlatus* n = 5, *Allopetrolisthes spinifrons* n = 2) and a max. value of 5.04% (*Hemigrapsus crenulatus* n = 5). The high intraspecific distance in the *Hemigrapsus crenulatus* clade is based on a single specimen with a somewhat aberrant COI sequence (ZSMA 20111369). The clade's intraspecific distance value excluding this sample has an average of 0.535% (max. 1.075%); including this sample values go up to a mean of 5.04% (max. 12.522%).

The topology of the three constructed rooted phylograms (Bayesian inference, Maximum likelihood, Neighbour joining) and one cladogram (Maximum parsimony) calculated for the south Chilean decapods are similar and thus all values are integrated in the consensus tree of MrBayes indicating the bootstrap values of the other calculated trees (Fig. 2). Clades represented by a single specimen lack values. The chosen outgroup *Oratosquillina interrupta* (Kemp, 1911) clusters with high bootstrap values (>99%) and a high posterior probability value of 1 against all decapods studied in this paper.

Eurypodius Guérin, 1825

Molecular results. Specimens of the genus *Eurypodius* cluster in two clades in the COI analysis. These are supported by high bootstrap values (>99) and posterior probability values (1). Morphological comparisons of these two lineages with the type material of *E. latreilli* (Holotype RMNH D 42178, Nationaal Natuurhistorisch Museum, Leiden) and *E. longirostris* (Holotype, ZOO2012-247T, Natural History Museum, UK) show that these two lineages represent the two valid species.

The *Eurypodius latreillii* clade consists of five specimens (ZSMA20111424-428) while the *Eurypodius longirostris* clade is composed of 10 specimens (ZSMA20060440, ZSMA20061441, ZSMA20061446-447, ZSMA20061455, ZSMA20061464, ZSMA20111429-432). Intraspecific distances in these clades are low (*E. latreillii* mean 0.15%, max. 0.3% and *E. longirostris* mean 0.13%, max. 0.31%) while the interspecific distance in between these two clades is high (11.83%). To crosscheck the data and illustrate the barcode gap between these two clades we used the software ABGD. Based on the calculated different distance values (min. 0.01 and max. 0.18) this analysis confirms the two distinct clades (Fig. 4).

The two *Eurypodius* species show a distinct geographical distribution pattern: while *Eurypodius latreillii* was found in the northern part of the region investigated (range in this study: Dichato (36°S), Región del Bío-Bío and Inio 2 (43°S), Región de los Lagos) *Eurypodius longirostris* was found in the southern part between Inio 5 (43°S) and Los Gemelos (53°S), Región de Magallanes y de la Antártica Chilena.

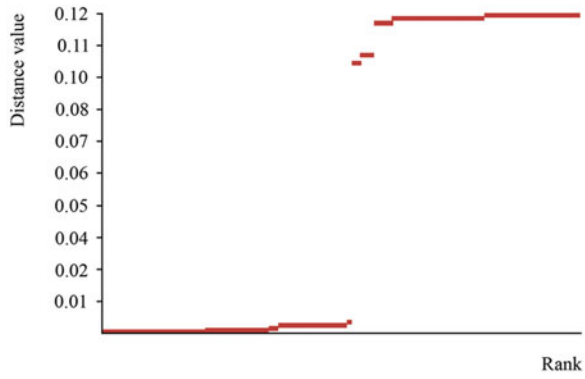


Fig. 4. Automatic Barcode Gap Discovery (ABGD) analysis for the *Eurypodius* specimens used in this study showing barcoding gaps.

Morphological aspects. Morphological features such as the rostral horns of *E. latreillii* are extremely variable. In small specimens (ZSMA20111603) they are divergent; in larger specimens they are contiguous (ZSMA20111425). The orientation of the rostrum also changes with size: horizontal in small specimens (ZSMA20061480) and with its distal portion slightly bent downwards in larger specimens (ZSMA20111427). All specimens collected during Huinay fjordos expeditions were characterized by the absence of a supraorbital spine (ZSMA20111427, RMNH D 42178).

Eurypodius longirostris is morphologically very similar to *E. latreillii*. The rostral horns can be divergent (ZOO2012-247T) or contiguous (ZSMA20061440). The orientation of the rostrum is more variable than in *E. latreillii* and can be orientated upwards (ZSMA20061447) or horizontal (ZSMA20061463) with its distal portion slightly bent downwards. The variability of these features was further shown through morphological analysis of the 44 specimens housed at the Bavarian State Collection of Zoology. Only the presence of the supraorbital spine was constant (ZSMA20061440, ZOO2012-247T) (Fig. 5–16).

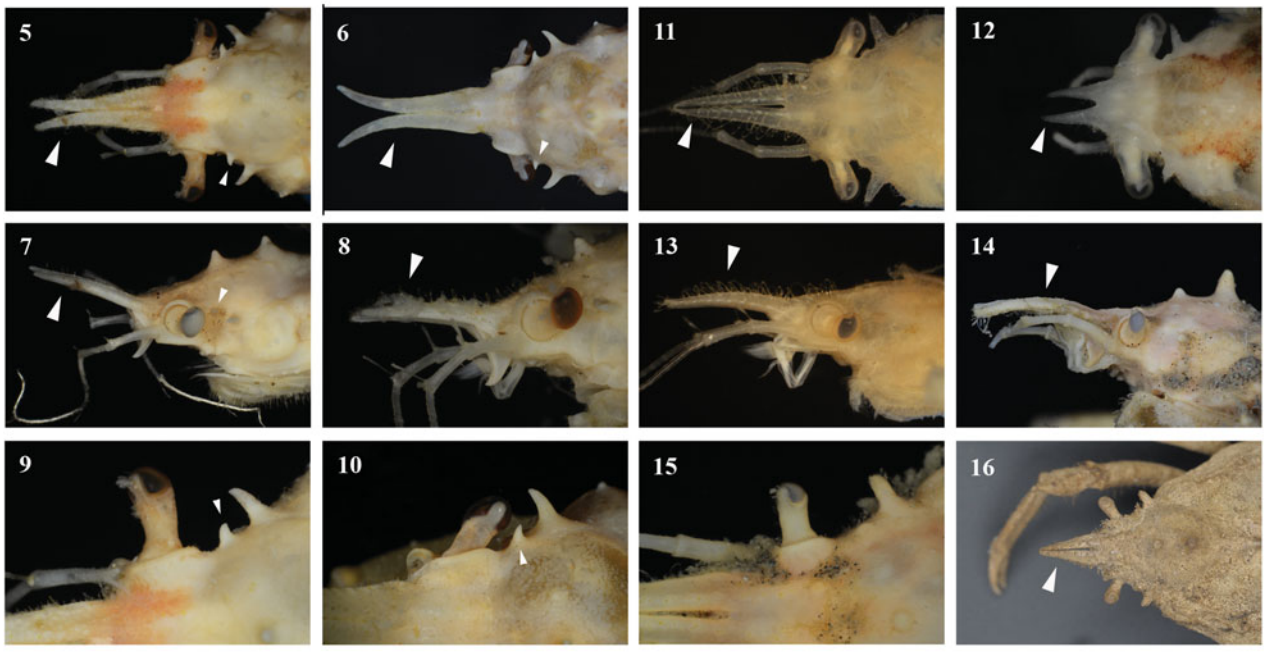
Morphological comparisons of representatives of the two lineages of the *Eurypodius* clade with the type material of *Eurypodius latreillii* and *E. longirostris* confirm this constant morphological feature.

Acanthocyclus Lucas, in H. Milne Edwards & Lucas, 1844

Molecular results. The *Acanthocyclus* clade consists of three lineages, all of which are supported by high bootstrap values (>92%) and high posterior probability values (>0.99). The *A. hassleri* clade is composed of three specimens from the same collection site (ZSMA20111501, ZSMA20111504, ZSMA20111531), the *A. albatrossis* clade of 12 specimens (ZSMA 20111503, ZSMA 20111447, ZSMA20111448, ZSMA20061657, ZSMA20061658, ZSMA20111444, ZSMA20061660, ZSMA20061662, ZSMA20111451, ZSMA20111452, ZSMA20111453, ZSMA20111454), and the *A. gayi* clade of four specimens (ZSMA20111446, ZSMA20111443, ZSMA20111449, ZSMA20111455). Intraspecific distances in the clades are low (*A. hassleri* 0%, *A. albatrossis* mean 0.1%, max. 0.3% and *A. gayi* mean 0.23%, max. 0.46%). Interspecific distances for the clades are as follows: *A. gayi*/*A. hassleri*: 8.39%, *A. albatrossis*/*A. hassleri*: 3.46%, *A. gayi*/*A. albatrossis*: 8.97%. To crosscheck the data and illustrate the barcode gap between these three clades we used ABGD. Based on the calculated different distance values (min. 0.01 and max. 0.18) this analysis confirms the three distinct clades (Fig. 17).

Analysis of the distribution of these three species in our sampling area (based on all available samples, those barcoded and others also collected by Huinay expeditions) show that all specimens of the *A. hassleri* clade were collected at the same location: Playa Chica (39°43′S), Región de los Ríos. *Acanthocyclus gayi* is distributed, according to our data, from Playa Chica (39°43′S) to Playa Corrales (41°15′S), Región de los Lagos. Specimens of *A. albatrossis* have a wide range: our northernmost sample locality is Dichato (36°32′S), Región del Bío-Bío and the southernmost Canal Messier (49°51′S), Región de Magallanes y de la Antártica Chilena.

Morphological aspects. The three lineages of *Acanthocyclus* correspond with the known morphological species. Morphological comparisons of the three clades with each other, with type material and with species descriptions in the literature show that these species can only be reliably distinguished from each other by a few features that were seen to be constant in our analysis. Conversely, many of the morphological characteristics given in the literature, e.g. the differential diagnosis of Rathbun, 1930 and Garth, 1957, using the width to length ratio of the carapace, the shape and form of the carapace lateral teeth, the carapace structure, the orientation and shape of ischium and merus joints of the 3rd maxilliped, the width–depth ratio of the orbit and the shape of the dactylus of the ambulatory legs of *Acanthocyclus* are, with analysis of a larger number of individuals, not consistent (Tables 2–4 and Figs 18–53). However, we observed several consistent features through our examinations: the shape and orientation of the front (entire or bilobed, directed forward or not) and the presence of hairs on the carapace and ambulatory legs. Our differential diagnosis between the three species shows a higher similarity of morphological features between *A. hassleri* and *A. albatrossis* (structure of male abdomen and 1st pleopod) than *A. hassleri* and *A. albatrossis* against *A. gayi*. On the basis of our data we can suggest an identification key for the



Figs 5–16. Selected morphological features and their variation of *Eurypodius longirostris* (6–11) and *E. latreillii* (12–17). **6**, **7**: dorsal view on rostrum (ZSMA20061440, Holotype ZOO2012-247T); **8**, **9**: lateral view of rostrum (ZSMA20061447, ZSMA20061463); **10**, **11**: detail of orbit with supraorbital spine (ZSMA20061440, Holotype ZOO2012-247T); **12**, **13**: dorsal view on rostrum (ZSMA20061480, 20111603); **14**, **15**: lateral view of rostrum (ZSMA20061480, ZSMA20111427); **16**, **17**: detail of orbit without supraorbital spine (ZSMA20111427, Holotype RMNH.CRUS42178). Small arrowheads pointing at supraorbital spine; large arrowheads at rostral horns.

three species of the genus *Acanthocyclus* of the southern Chilean region:

1. Front entire → (2)
   Front at least faintly bilobed and directed forward → *A. albatrossis*
2. Carapace and ambulatory legs covered with hair → *A. gayi*
   Carapace not covered with hair, front bent downwards → *A. hassleri*

Discussion

This study confirms that the barcode region of COI delivers species-level resolution for Decapoda lineages as suggested in Costa *et al.* (2007) and Lefebure *et al.* (2006) at least for the southern Chilean area.

Mean interspecific sequence divergence in southern Chilean decapods is high with respect to other groups of animals (15.54% with a max. of 25.11%), but fits well with the results of Costa *et al.* (2007) who observed a mean interspecific divergence for crustaceans of 17.16%. By comparison, variation of lepidopterans analysed worldwide is 6.1% (Hebert *et al.*, 2003), birds of North America 7.93% (Hebert *et al.*, 2004*b*), pycnogonids of southern Chile 18.83% (Weis & Melzer, 2012) and fishes of Australia 9.93% (Ward *et al.*, 2005). Hebert *et al*. (2004*a*) proposed that species can be resolved when their sequence divergence is 10× larger than the average intraspecific variation for the group. If applied to the decapods examined in this study (7.7% threshold), the 10× threshold would recognize 90.25% of the examined species. Species not identified at this threshold are

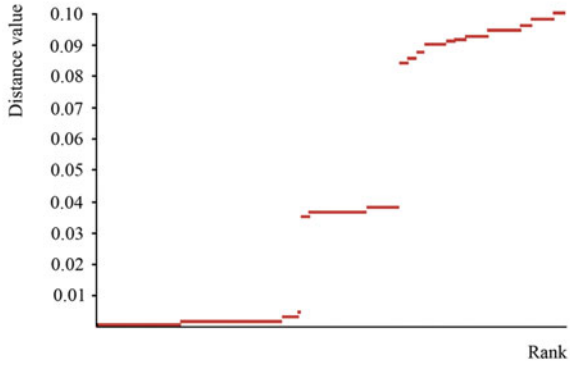


Fig 17. Automatic Barcode Gap Discovery (ABGD) analysis for the *Acanthocyclus* specimens used in this study showing barcoding gaps.

Table 2. Morphological features of *A. gayi* compared with features given in literature.

Feature	Literature (Rathbun, 1930)	This study	Reference*
carapace width length ratio	1.05-1.08	1.00-1.10	ZSMA20111443, ZSMA20111446
carapace lateral teeth	intermediate	appressed or intermediate	ZSMA20111605, ZSMA2011606
front	entire	entire	all specimens this study
pubescence of carapce and legs	carapace and legs	carapace and legs	all specimens this study
carapace structure	almost smooth	almost smooth	all specimens this study
orientation of ischium of 3rd maxilliped	ischium joints with inner margin subparalell, but leaving a wide hiatus	ischium joints with inner margins in contact or with hiatus	ZSMA20111443
orbit width depth ratio (viewed from above)	less than twice as wide as deep	more than twice as wide as deep	ZSMA20111443
structure of dactyli of legs	short, much curved from base	much or less curved from base	ZSMA20111605

*Specimen registration number.

P. sicarius and *P. spinosulum* with 5.61% and *A. albatrossis* and *A. hassleri* with 3.46% interspecific variation. In the first case, morphological differences are clear and define the two species; in the second case *A. albatrossis* and *A. hassleri* are closely related, but our examinations show significant morphological differences. The sequence divergence for the *Eurypodius* species, with a value of 11.83%, clearly is in accord with a 10× threshold.

Other recent publications suggest that divergences greater than 3% suggest either the presence of cryptic species (Radulovici *et al.*, 2009) or can be seen as the threshold for species delineation (Hebert *et al.*, 2003). In decapods, distances among haplotype clades varied from 2.78% to 9.6% (Oliveira-Biener *et al.*, 2010); in our study variation between 3.46% and 25.11% was found, supporting a 3% threshold for species level discrimination. Detailed analyses with the ABGD software on the focus genera *Acanthocyclus* and *Eurypodius* show obvious 'barcode gaps' between the individual species (Figs 4 and 17). Furthermore, the mean level of intraspecific variation of 0.77% in the studied decapods is slightly higher than the 0.46% reported in previous studies of crustacea (Costa *et al.*, 2007). The high intraspecific variation appearing in the *Hemigrapsus* clade might indicate presence of a cryptic species, or at least the beginning of genetic divergence between *Hemigrapsus* subgroups. Since this observation is based on a single specimen only and since this specimen does not show any morphological difference, we left this species tentatively in the *H. crenulatus* clade.

The values of interspecific variation and the methods applied for sequence-based delineation of taxa support the validity of DNA barcoding for species identification in decapod crustaceans of the studied region. However, these clear results are accepted primarily for the relatively small geographical area studied. Currently decapod species' barcode coverage worldwide is about 14.3% (2147 species with barcodes in BOLD by August 2012 compared with *c*. 15 000 described Decapoda species) and thus still at

Table 3. Morphological features of *A. albatrossis* compared with features given in literature.

Feature	Literature (Rathbun, 1930)	This study	Reference*
carapace width length ratio	1.08–1.13	1.07–1.21	ZSMA20111455, ZSMA20061657
carapace lateral teeth	prominent, acute	intermediate	all specimens this study
front	faintly bilobed	faintly bilobed	all specimens this study
pubescence of carapce and legs	less hairy than *A.gayi*	less hairy than *A.gayi*	all specimens this study
carapace structure	tuberculate or granulate	almost smooth, front part granular	all specimens this study
orientation of ischium of 3rd maxilliped	ischium joints with inner margins in contact	ischium joints with inner margins in contact or leaving a wide hiatus	ZSMA20111455, ZSMA20061657, ZSMA20111610, ZSMA20111447, ZSMA20061658, ZSMA20111607
orbit width depth ratio (viewed from above)	less than twice as wide as deep	more than twice as wide as deep	Sach.Kat.Nr 792/1
structure of dactyli of legs	long, little curved	in comparison to *A. gayi* longer and little curved	ZSMA20061662, ZSMA20061657

*Specimen registration number.

Table 4. Morphological features of *A. hassleri* compared with features given in literature.

Feature	Literature (Rathbun, 1930)	This study	Reference*
carapace width length ratio	1.16	1.18–1.29	ZSMA20111531, ZSMA20111504
carapace lateral teeth	teeth appressed	appressed or intermediate	all specimens this study
front	front entire	entire	all specimens this study
pubescence of carapce and legs	less hairy	carapace not hairy, legs little hairy	all specimens this study
carapace structure	carapce tuberculate	almost smooth, front part granular	all specimens this study
orientation of ischium of 3rd maxilliped	ischium joints with inner margins diverging anteriorly, gape less than in *A.gayi*	small gape, sometimes diverging anteriorly	ZSMA20111501, ZSMA20111531
orbit width depth ratio (viewed from above)	more than twice as wide as deep	twice or more than twice as wide as deep	ZSMA20111501
structure of dactyli of legs	dactyli short, much curved	much or less curved from base	ZSMA20111531

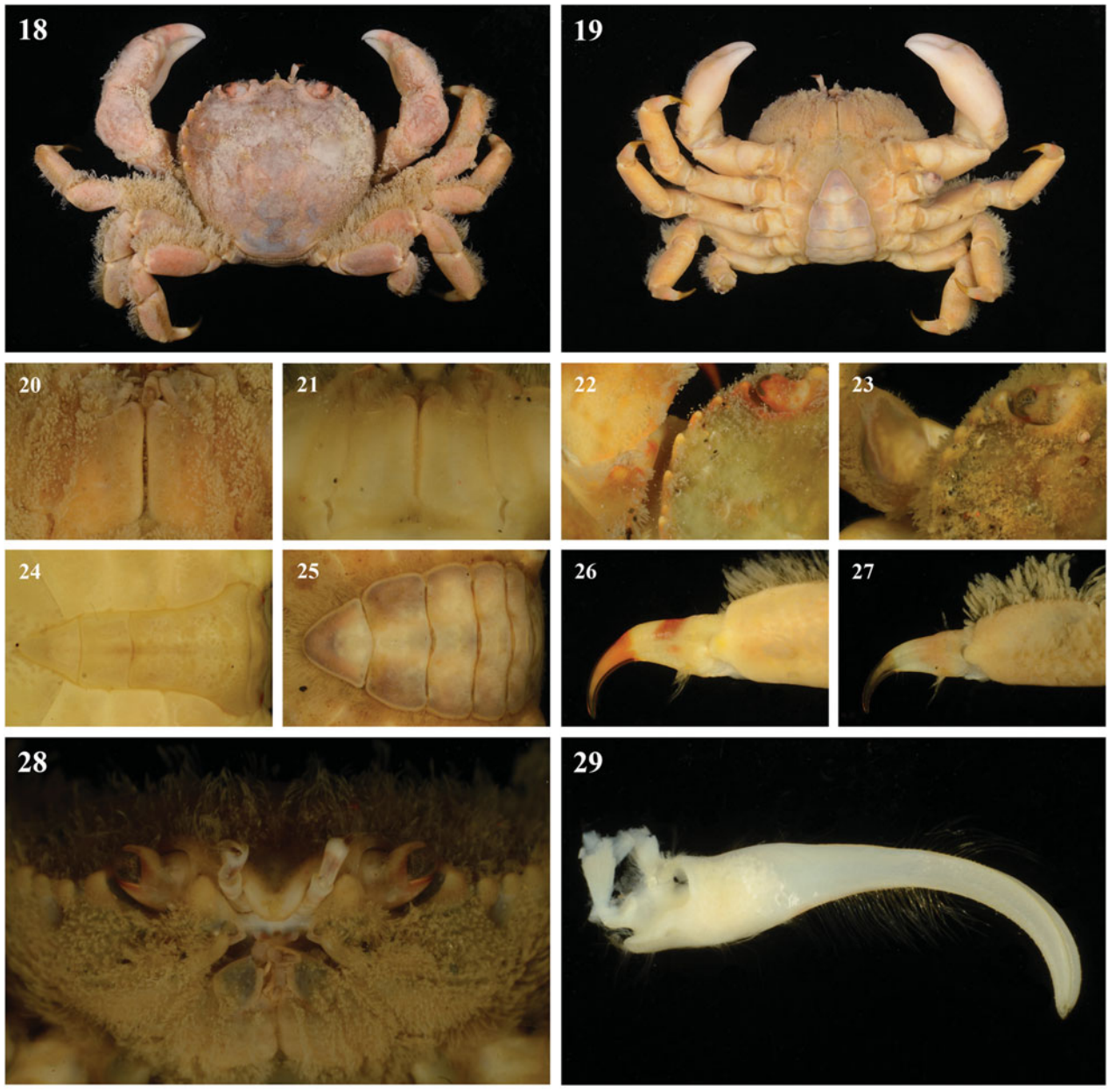
*Specimen registration number.

the beginning of the barcoding process. Within this study species of the genera *Acanthocyclus* and *Eurypodius* are the only representatives exclusively occurring in the study area but nowhere else; all other genera have a much wider distribution area and are not completely barcoded. Closing this information gap could reduce apparent interspecific variation once there is a global taxonomic view. However, in our two focus genera *Eurypodius* and *Acanthocyclus* we observed valid values.

The interspecific divergence value between the two branches of the genus *Eurypodius*, and the distinct morphological features, clearly define and confirm the two species *E. latreillii* and *E. longirostris*. We compared morphological species descriptions in the literature with all 44 specimens available from the Huinay expeditions and observed an extremely high morphological variation. However, the two species could be separated by the morphological feature of the absence or presence of the postorbital spine. This consistent feature was mentioned in Rathbun's examinations (Rathbun, 1925) and is confirmed by our study. Conversely, other features used historically for determination and separation of these two species, such as the orientation and shape of the rostral horns (Lagerberg, 1905; Stebbing, 1914; Garth, 1957, 1958), were shown not to be consistent throughout our samples and thus do not serve for species distinction. Putting the molecular results in context with the morphological analyses we were able to find and present a valid feature for further species discrimination, and clarify historical descriptions that were based on only a few specimens. In these cases there could be misinterpretation of features that seem distinctive between species if only a few specimens are analysed, but these disappear when a large sample of specimens is examined, as in this study.

Interspecific divergence of the three clades of the studied *Acanthocyclus* species complex is high in two cases (*A. gayi*/*A. albatrossis* 8.97%, *A. gayi*/*A. hassleri* 8.39%) and shows a well-defined threshold between these three species, confirming the specific status of *A. gayi*. The low distance value of 3.46% (*A. hassleri*/*A. albatrossis*) and the narrow differences in morphologically constant features between *A. hassleri* and *A. albatrossis* illustrates the closer relationship between these two species. Hence, we cannot exclude with certainty the possibility that *A. hassleri* is a subspecies of *A. albatrossis* (Garth, 1957). Interspecific values in this study for other decapods of this region range between 5.61% (*Peltarion spinosulum*/*Pseudocorystes sicarius*) and 25.11% (*Nauticaris magellanica*/*Propagurus gaudichaudii*).

Holthuis, 1952 and Ekman 1953 identified Chiloé Island (41°30′S) in the Pacific Ocean as the northern boundary of the South American antiboreal region. The geographical range and the distribution pattern of the *Eurypodius* species depicted in this study are very clear (we included all 44 available *Eurypodius* specimens (barcoding and collection) in the biogeographical analysis): *Eurypodius* samples north of Las Hermanas (43°46′S) were determined as *E. latreillii* (ZSMA20111589) and south of Inio 3, Chiloé (43°23′S) as *E. longirostris* (ZSMA20111597). However, distribution ranges for these species given in the literature (e.g. Rathbun, 1930; Garth, 1957) differ from our observations; the range of *E. latreillii* is stated to be 'from Peru south to Strait of Magellan' and *E. longirostris* is only known from its type locality (east coast of Madre de Diós Island, 50°08′S). Samples of *E. latreillii* and *E. longirostris* obtained in the Strait of Magellan and its southern channel system including the southern islands (52°57′S–55°47′S) document the occurrence of both species in this southern region (Arntz *et al*., 1999). Other expeditions (CIMAR-Fjordo III and CIMAR-Fiordo VII) sampled the South Patagonian Ice shield and the Strait of Magellan and collected *E. latreillii* at 24% of their stations (29 stations in total) (50°29′S–53°33′S) (Rios *et al.*, 2005). Notably, among the 1895 collected invertebrate samples collected by these expeditions not even one

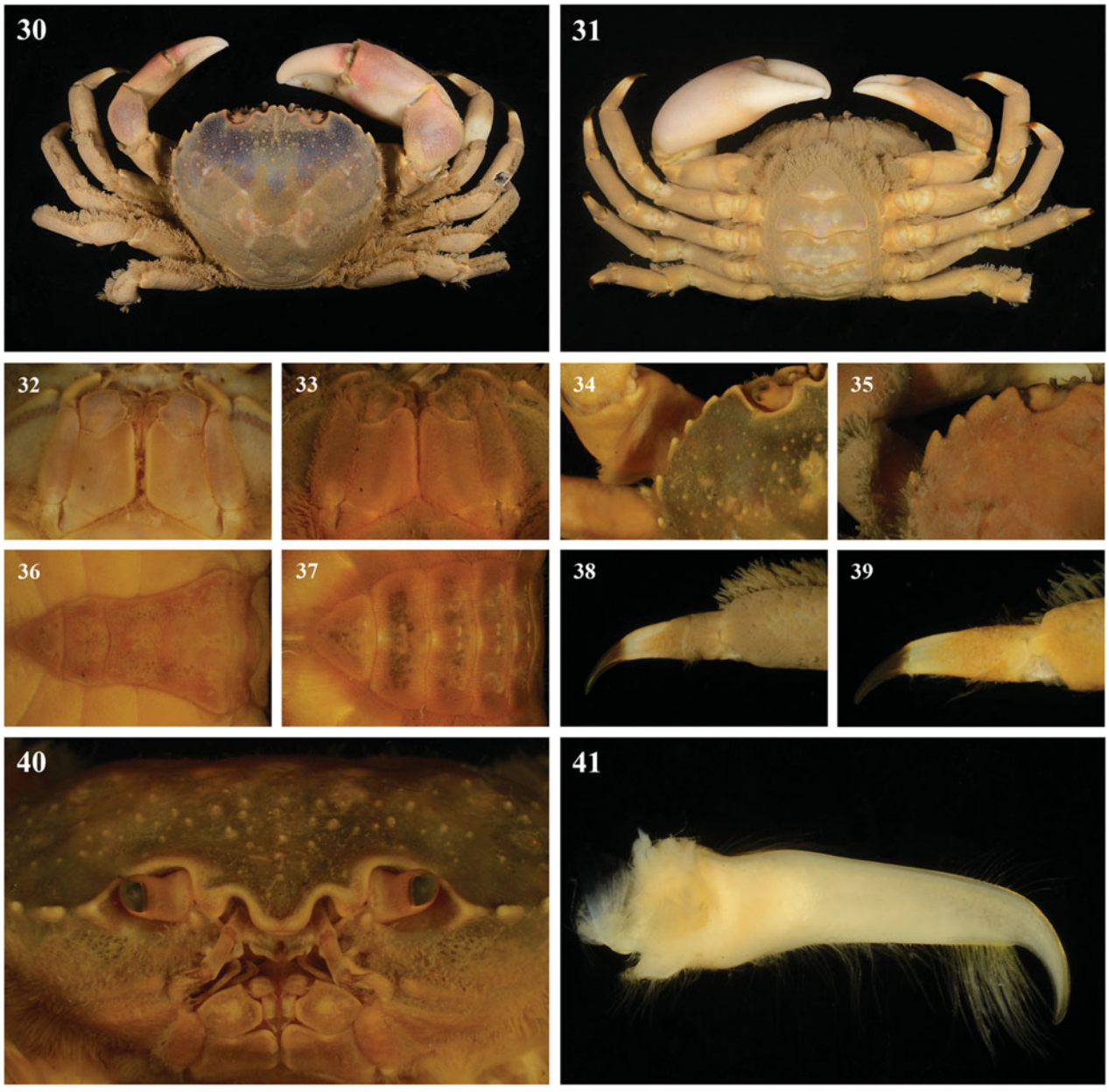


Figs 18–29. Selected morphological features of *Acanthocyclus gayi*. **18**: dorsal view, **19**: ventral view (both ZSMA20111449); **20**, **21**: variation of orientation of 3rd maxilliped (ZSMA20111449, ZSMA20111443); **22**, **23**: variation of lateral carapace teeth (ZSMA20111443, ZSMA2011605); **24**: male abdomen (ZSMA20111443); **25**: female abdomen (ZSMA20111449); **26**, **27**: variation of dactylus (ZSMA20111443, ZSMA20111605); **28**: frontal view (ZSMA20111605); **29**: 1st male pleopod (ZSMA20111443).

specimen of *E. longirostris* was found. Due to the similarity of the two species, it is hard to state to what extent specimens of *E. longirostris* were identified as *E. latreillii* and vice versa in older literature. Boschi & Gavio (2005) present a species checklist including the geographical distribution of decapods in the South American antiboreal region. According to these authors, both *Eurypodius* species occur in our sampling area, thus supporting our results.

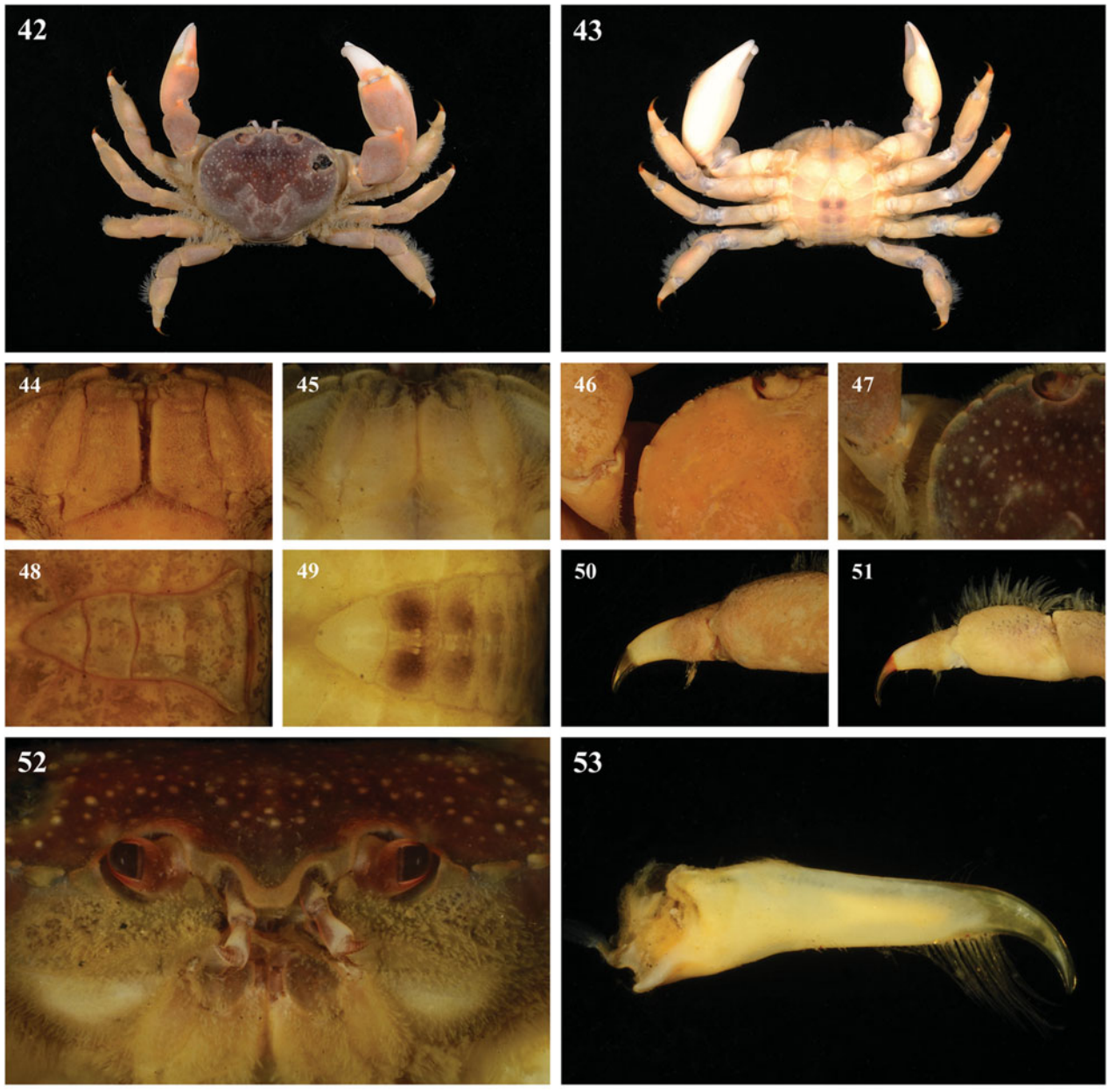
The different *Acanthocyclus* species have, according to Garth, 1957 and Rathbun, 1930, overlapping distribution areas: *A. gayi* from Salaverry, Peru (8°13′S) to Lota (37°05′S), *A. hassleri* from Alacrán Island (18°27′S) to Valparaiso (33°02′S) and *A. albatrossis* from Talcahuano (36°43′S) to the Strait of Magellan (53°31′S).

From our data we are able to clarify the southern distribution boundary of *A. gayi* and *A. hassleri*. *Acanthocyclus gayi*



Figs 30–41. Selected morphological features of *Acanthocyclus albatrossis*. **30**: dorsal view, **31**: ventral view (both ZSMA20061662); **32**, **33**: variation of orientation of 3rd maxilliped (ZSMA20111447, ZSMA20111453); **34**, **35**: variation of lateral carapace teeth (ZSMA20111451, ZSMA20061658); **36**: male abdomen (ZSMA20111451); **37**: female abdomen (ZSMA20111453); **38**, **39**: variation of dactylus (ZSMA20061662, ZSMA20061657); **40**: frontal view (ZSMA20111451); **41**: 1st male pleopod (ZSMA20061657).

is distributed to Playa Corrales (41°15′S) – about 500 km south of the previously proposed boundary. All specimens of *A. hassleri* were collected at one location: Playa Chica (39°43′S), which is located about 800 km south of the southern species boundary given in the literature. All specimens of *A. albatrossis* collected for this study were found in the range of distributions given for this species in the literature and thus confirm at least the northern boundary. Further information of the species ranges is hard to obtain: the *Acanthocyclus* species are shallow water inhabitants and thus not collected during large expeditions which mainly sampled at greater depths (Arntz *et al*., 1999; Mutschke and Gorny, 1999; Rios *et al.*, 2003). The occurrence of *A. albatrossis* in the antiboreal region is confirmed by Boschi & Gavio (2005), Campodonico & Guzman (1973) and Gorny (1999).



Figs 42–53. Selected morphological features of *Acanthocyclus hassleri*. **42**: dorsal view, **43**: ventral view (both ZSMA20111531); **44**, **45**: variation of morphology of 3rd maxilliped (MCZ CRU-4889, ZSMA20111531); **46**, **47**: variation of lateral carapace teeth (Holotype MCZ CRU-4889, ZSMA20111531); **48**: male abdomen (Holotype MCZ CRU-4889); **49**: female abdomen (ZSMA20111531); **50**, **51**: variation of dactylus (Holotype MCZ CRU-4889, ZSMA20111531); **52**: frontal view (ZSMA20111531); **53**: 1st male pleopod (Holotype MCZ CRU-4889).

At the moment the only comparable DNA barcoding study on Chilean arthropods is that on Pycnogonida by Weis & Melzer (2012), who observe a 'patchy' distribution pattern of different clades all attributed to *Achelia assimilis* (Haswell, 1885), and relate this phenomenon to post-glacial recolonization. In the present study no such effect has been detected. In comparison to the holobenthic life cycle of Pycnogonids, Decapods' pelago-benthic life cycle enables species to spread over wide geographical distances in a relatively short period of time, and minimize genetic divergences, i.e. intraspecific variation (Thatje *et al.*, 2005).

The use of a dataset independent of morphology (COI sequences) in addition to the classical morphological data of the two species complexes studied here in detail confirms the species status and delimitations in both cases. In addition the methodology allows clarification of the

diagnostic features and unequivocal diagnoses of these 'difficult species' in the future. By the support of the phylogenetic analysis and the high number of samples we were able to rank previously used morphological features according to their utility for species determination. Furthermore we expand the DNA barcode database involving these species of this hitherto unexplored region and reconfirm the usefulness of DNA barcoding for the identification of marine decapods.

In many DNA barcoding studies cryptic species are found that have not been detected using morphology alone (Malay *et al.*, 2012). This situation is more common that the opposite, i.e. the number of DNA branches is less than the number of already described valid species. In the present study, the molecular and morphological species are perfectly consistent. On the one hand, this contradicts the notion that DNA-based taxonomy is generally superior to the traditional one (Janzen, 2004; Hajibabaei *et al.*, 2006). On other hand it supports the excellent work done by the 'old' morphological taxonomists such as Mary J. Rathbun and John S. Garth who obviously 'knew their animals'.

Acknowledgements

We want to thank Günter Försterra and Dr Vreni Häussermann (Huinay Scientific Field Station, Chile) for the organization of the Huinay Fjordos expeditions and supporting us with samples. Special thanks go to all participants of the expeditions for their help with collecting, Dr Dirk Steinke from the Canadian Centre for barcoding at the Department of Zoology University of Guelph, Ontario, Canada for processing, coordinating and providing the sequence data, and Isabella Stöger (Zoologische Staatssammlung München) for the excellent support for the molecular analyses. For expert technical assistance and collection management we thank Stefan Friedrich (ZSM). Furthermore we would like to thank Dr Charles Fransen (National Natuurhistorisch Museum, Leiden), Ms Miranda Lowe (Natural History Museum, UK) and Mr Adam Baldinger (Museum of Comparative Zoology, Harvard) for making the type specimens available to us. This study was partly supported by the GeoBioCenter of the Ludwig-Maximilians-Universität (LMU) München, a 'Reisekostenstipendium of the LMU Auslandsamt für Internationale Angelegenheiten' a 'LMU Abschlusstipendium' and the SEA LIFE Center München. Publication # 77 of the Huinay Scientific Field Station.

Supplemental data

Supplemental data for this article can be accessed here.

References

Arntz, W.E., Gorny, M., Soto, R., Lardies, M.A., Retamal, M. & Wehrtmann, I.S. 1999. Species composition and distribution of decapod crustaceans in the waters off Patagonia and Tierra del Fuego, South America. *Scientia Marina* **63**, 303–314.

Boschi, E.E. & Gavio, M.A. 2005. On the distribution of decapod crustaceans from the Magellan Biogeographic Province and the Antarctic region. *Scientia Marina* **69**, 195–200.

Bucklin, A., Wiebe, P.H., Smolenack, S.B., Copley, N.J., Beaudet, J.G., Bonner, K.G., Farber-Lorda, J. & Pierson, J.J. 2007. DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *Journal of Plankton Research* **29**, 483–493.

Bustamente, M. 2009. The southern Chilean fjord region: oceanographic aspects. In: Häussermann, V. & Försterra, G., Eds., *Marine Benthic Fauna of Chilean Patagonia: Illustrated Identification Guide.* Nature in Focus, Puerto Montt, Chile, pp. 53–60.

Campodonico, G.I. & Guzman, M.L. 1973. Contribución a la biología de *Acanthocyclus albatrossis* Rathbun 1898. *Anales del Instituto de la Patagonia* **4**, 374–416.

Clapperton, C.M. 1993. Nature of environmental changes in south America at the last glacial maximum. *Palaeogeography Palaeoclimatology Palaeoecology* **101**, 189–208.

Costa, F.O., DeWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M. & Hebert, P.D.N. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272–295.

Daniels, S.R., Gouws, G., Stewart, B.A. & Coke, M. 2003. Molecular and morphometric data demonstrate the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae: *Potamonautes*) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnean Society* **78**, 129–147.

Dayrat, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**, 407–415.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrocks, S., Thierer, T. & Wilson, A. 2011. *Geneious v5.4* [available at http://www.geneious.com, accessed 5 August 2013].

Edgar, R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797.

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.

Försterra, G. 2009. Ecological and biogeographical aspects of the Chilean fjord region. In: Häussermann, V. & Försterra, G., Eds., *Marine Benthic Fauna of Chilean Patagonia: Illustrated Identification Guide.* Nature in Focus, Puerto Montt, Chile, pp. 61–76.

Garth, J.S. 1957. Reports of the Lund University Chile Expedition 1948–49, No. 29. The Crustacea Decapoda Brachyura of Chile. *Lunds Universitets Årsskrift. N.F. Avd. 2*. **53**, 1–129.

Garth, J.S. 1958. Brachyura of the Pacific coast of America: Oxyrhyncha. Text. *Allan Hancock Pacific Expeditions* **21**, 1–499.

Gorny, M. 1999. On the biogeography and ecology of the Southern Ocean decapod fauna. *Scientia Marina* **63**, 367–382.

Guérin, F.-E. 1825. *Histoire Naturelle. Entomologie, ou Histoire naturelle des Crustacés, des Arachnides et des Insectes*. In: Latreille, Ed., *Encyclopédie Méthodique*. Paris, France. 832 pp.

Gusmao, J., Lazoski, C. & Sole-Cava, A.M. 2000. A new species of *Penaeus* (Crustacea: Penaeidae) revealed by allozyme

and cytochrome oxidase I analyses. *Marine Biology* **137**, 435–446.

Haig, J. 1955. Reports of the Lund University Chile Expedition 1948–49, No. 20. The Crustacea Anomura of Chile. *Lunds Universtitets Arsskrift N.F. Avd. 2* **51**, 1–68, figs 61–12.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences USA* **103**, 968–971.

Häussermann, V. & Försterra, G. 2009. *Marine Benthic Fauna of Chilean Patagonia. Illustrated Identification Guide.* Nature in Focus, Santiago, Chile.

Haye, P.A., Segovia, N.I., Vera, R., de Los Angeles Gallardo, M. & Gallardo-Escarate, C. 2012. Authentication of commercialized crab-meat in Chile using DNA barcoding. *Food Control* **25**, 239–244.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B–Biological Sciences* **270**, 313–321.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. 2004*a*. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences USA* **101**, 14812–14817.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. 2004*b*. Identification of birds through DNA barcodes. *Public Library of Science Biology* **2**, 1657–1663.

Holthuis, L.B. 1952. Reports of the Lund University Chile Expedition 1948–1949, No. 4. On two species of Crustacea Decapoda Macrura from the N.W. coast of South America. *Lunds Universitets Årsskrift Ny Följd avdelningen 2* **47**, 1–11.

Hultgren, K.M. & Stachowicz, J.J. 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.

Janzen, D.H. 2004. Now is the time. *Philosophical Transactions of the Royal Society of London Series B–Biological Sciences* **359**, 731–732.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Lagerberg, T. 1905. Anomura und Brachyura der Schwedischen Südpolar Expedition. *Wissenschafteliche Ergebnisse der Schwedischen Südpolar.-Expedition 1901–1903* **5**, 1–39, pl. 31.

Lefebure, T., Douady, C.J., 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.

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.

Malay, M.C.D., Komai, T. & Chan, T.-Y. 2012. A new cryptic species in the "*Calcinus anani* Poupin & McLaughlin, 1998" species complex (Decapoda: Anomura: Diogenidae): evidence from colouration and molecular genetics. *Zootaxa* **3367**, 165–175.

Meyer, R., Lochner, S. & Melzer, R. 2009. Decapoda – crabs, shrimps & lobsters. In: Häussermann, V. & Försterra, G., Eds., *Marine Benthic Fauna of Chilean Patagonia*. Nature in Focus, Santiago, Chile, pp. 623–670.

Miers, E.J. 1886. Report on the Brachyura collected by H.M.S. Challenger during the years 1873–1876. In: Murray, J., Ed., *Zoology*. Neill and Company, Edinburgh, UK. 1–362 pp.

Miguel Pardo, L., Ampuero, D. & Veliz, D. 2009. Using morphological and molecular tools to identify megalopae larvae collected in the field: the case of sympatric *Cancer* crabs. *Journal of the Marine Biological Association of the United Kingdom* **89**, 481–490.

Mutschke, E. & Gorny, M. 1999. The benthic decapod fauna in the channels and fjords along the South Patagonian Icefield, Southern Chile. *Scientia Marina* **63**, 315–319.

Oliveira-Biener, U., Melzer, R.R. & Miller, M.A. 2010. *Pilumnus* Leach (Decapoda: Pilumnidae Samouelle) from Mediterranean and adjacent Atlantic waters: a COI analysis. *Invertebrate Systematics* **24**, 182–193.

Padial, J.M., Miralles, A., De La Riva, I. & Vences, M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7**, 16.

Pérez-Barros, P., D'amato, M.E., Guzman, N.V. & Lovrich, G.A. 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.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21**, 1864–1877.

Radulovici, A.E., 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.

Rathbun, M.J. 1898. The Brachyura collected by the U.S. Fish Commission steamer Albatross on the voyage from Norfolk, Virginia, to San Francisco, California, 1887–1888. *Proceedings of the United States National Museum* **21**, 567–616.

Rathbun, M.J. 1918. The grapsoid crabs of America. *Bulletin of the United States National Museum* **97**, 1–461.

Rathbun, M.J. 1925. The spider crabs of America. *Bulletin of the United States National Museum* **129**, 1–613.

Rathbun, M.J. 1930. The cancroid crabs of America of the families Euryalidae, Portunidae, Atelecyclidae, Cancridae, and Xanthidae. *Bulletin of the United States National Museum* **152**, 1–609.

Ratnasingham, S. & Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.

Retamal, M.A. 1981. Catalogo ilustrado de los crustáceos decapodos de Chile. *Gayana (Instituto Central de Biologia, Universidad de Conceptión) Zoologia*, 1–110.

Rios, C., Mutschke, E. & Morrison, E. 2003. Biodiversidad bentónica sublitoral en el estrecho de Magallanes, Chile. *Revista de Biologica Marina y Oceanografia* **38**, 1–12.

Rios, C., Mutschke, E., Montiel, A., Gerdes, D. & Arntz, W.E. 2005. Soft-bottom macrobenthic faunal associations in the southern Chilean glacial fjord complex. *Scientia Marina* **69**, 225–236.

Rodriguez, F., Oliver, J.F., Marlin, A. & Medina, J.R. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**, 485–501.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Saitou, N. & Nei, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* **5**, 421–438.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Stebbing, T.R.R. 1914. Crustacea from the Falkland Islands collected by Mr Rupert Vallentin, FLS. Part II. *Proceedings of the General Meetings for Scientific Business of the Zoological Society of London*, pp. 341–378.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Tang, R.W.K., 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.

Thatje, S., Hillenbrand, C.D. & Larter, R. 2005. On the origin of Antarctic marine benthic community structure. *Trends in Ecology and Evolution* **20**, 534–540.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B–Biological Sciences* **360**, 1847–1857.

Weis, A. & Melzer, R.R. 2012. How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity* **10**, 1–14.

Xia, X. & Lemey, P. 2009. Assessing substitution saturation with DAMBE. In: Lemey, P., Salemi, M. & Vandamme, A., Eds., *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny.* Cambridge University Press, Cambridge, UK, pp. 615–630.

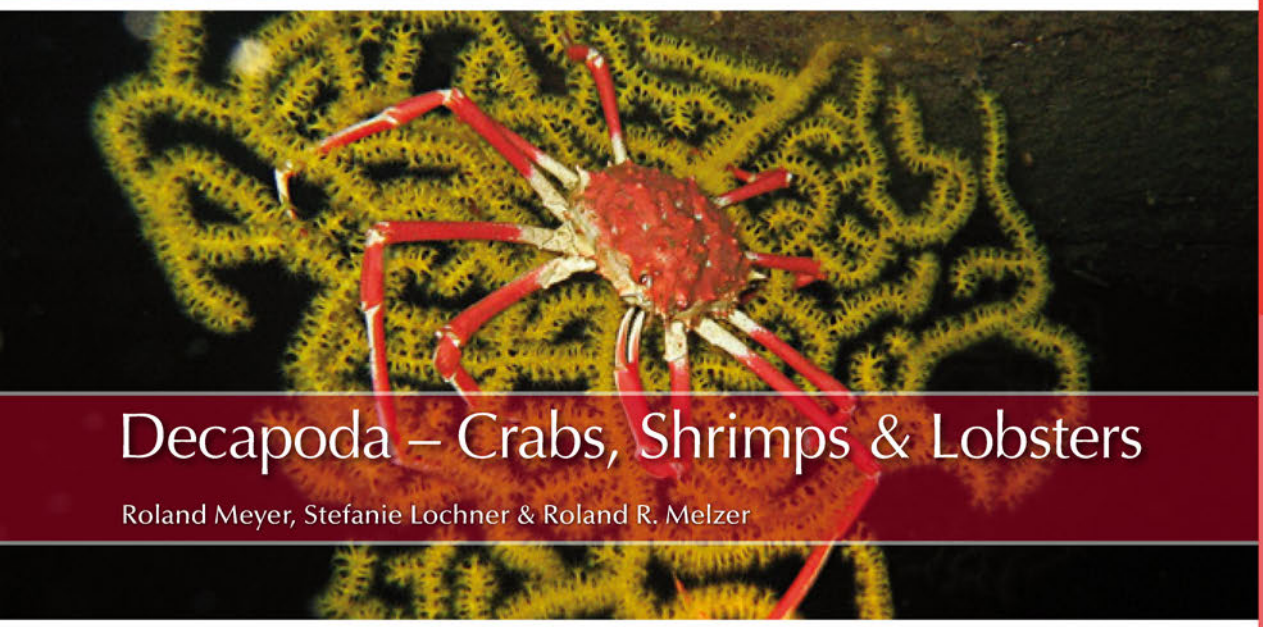
Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26**, 1–7.

Associate Editor: Chris Lyal

8. Article VI

Meyer R, Lochner S, Melzer RR (2009) Decapoda - crabs, shrimps & lobsters. In: Häussermann, V. & Försterra, G. (eds.) 2009. Marine Benthic Fauna of Chilean Patagonia, Nature in Focus, Santiago de Chile, 1000 pp. Spanish version: Häussermann, V. & Försterra, G. (eds.) 2009. Fauna Marina Bentonica de la Patagonia Chilena, Nature in Focus, Santiago de Chile, 1000 pp. ISBN 978-956-332-243-9 spanish; 978-956-332-244-6 english.

Figure 12: Close up of *Lithodes santolla* (Molina, 1782), an anomuran crab of the southern Chilean fjord area. Photo by the author.



Decapoda – Crabs, Shrimps & Lobsters

Roland Meyer, Stefanie Lochner & Roland R. Melzer

Decapoda – Crabs, Shrimps & Lobsters

Roland Meyer, Stefanie Lochner & Roland R. Melzer

General Introduction

Decapod crustaceans are among the most dominant and striking groups found in the benthic communities of the Chilean Fjord Region. Diversity is diminishing from the exposed coast towards the inner fjords. During the day intertidal species, like *Hemigrapsus crenulatus* and *Petrolisthes laevigatus*, can be found hidden under stones along the coast and in estuaries. Robust crabs, like *Acanthocyclus albatrossis*, are able to tolerate low salinities and can be observed in the low salinity layer of the fjords. In the dark and cold depths between seven and 40 m (the depths typical for a SCUBA dive) numerous and diverse decapods are found. Shrimps, such as the beautifully coloured *Campylonotus vagans* and *Nauticaris magellanica*, peer out of their holes or run around busily on sandy patches. Crab species, including *Pilumnoides perlatus*, *Peltarion spinosulum* and the impressively large *Cancer edwardsi*, are frequently seen. Hermit crabs, like the brightly coloured *Propagurus gaudichaudi*, can also be observed. The inachid, *Eurypodius latreillei*, sits on hydrozoans and other benthic animals with its chelipeds and first pereopods stretched out for food in a farcical way. More elusive decapods, such as the pinnotherids, are found hidden inside other organisms such as sea urchins and polychaete tubes. Large subantarctic deep water anomuran species, like *Lithodes santolla* and *Paralomis granulosa*, fill the diver with awe when seen at comparatively low depths in the fjords. Another anomuran, *Munida subrugosa*, sits between stones and rocks and swims away rapidly to a new hiding place if a diver approaches.

Systematics

The order Decapoda is divided into two suborders: the ancestral group, Dendrobranchiata (prawns), and the Pleocyemata (shrimps, true crabs, lobsters etc.). The two suborders are distinguished by their gill structure, which is branched in Dendrobranchiata (dendro: tree; branchia: gill) and has a lamellar structure in Pleocyemata. All taxa of Pleocyemata share a number of synapomorphic features, the most important of which is that the fertilised eggs are incubated by the females and remain stuck to the pleopods (Fig. 1) until the zoea larvae are ready to hatch. This character gave the group its name. The Pleocyemata are subdivided into seven infraorders: the **Stenopodidea** (Cleaner Shrimps), the **Caridea** (Shrimps, Coral Shrimps, Snapping Shrimps) (Fig. 1), the **Astacidea** (Freshwater Crayfish, True Lobsters, Reef Lobsters, Scampi), the **Thalassinidea** (Ghost Shrimps, Mud Shrimps, Sponge Shrimps), the **Palinura** (Flat Lobsters, Langoustines, Spiny Lobsters, Rock Lobsters, Lobsterettes, Slipper Lobsters), the **Anomura** (Hermit Crabs, King Crabs, Squat Lobsters, Porcelain Crabs, Mole Crabs) (Figs. 2a,b) and the **Brachyura** (True Crabs) (Figs.

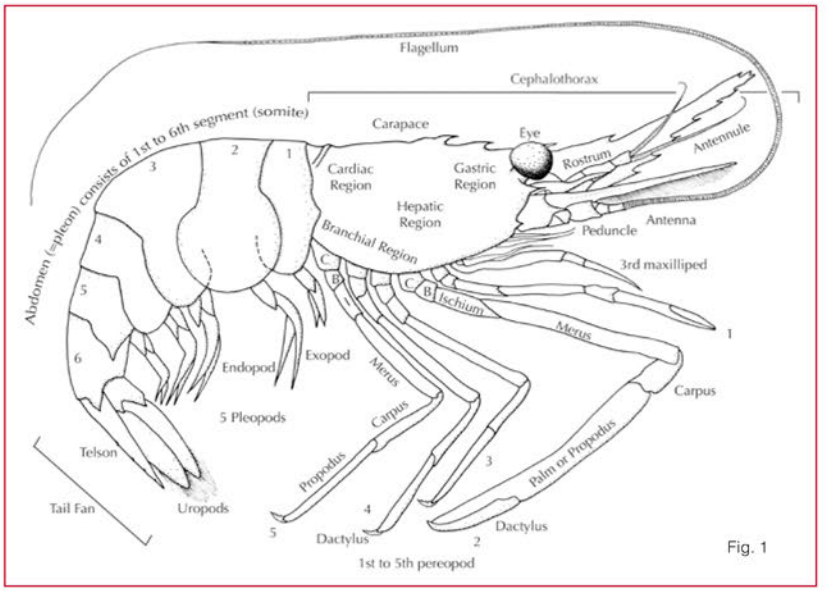


Fig. 1

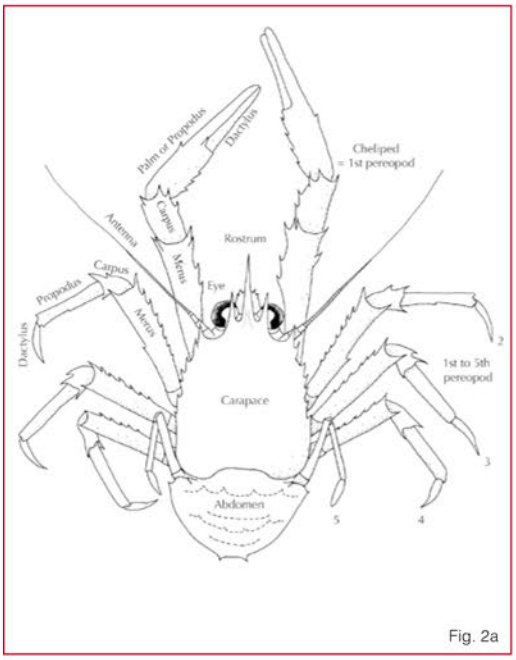


Fig. 2a

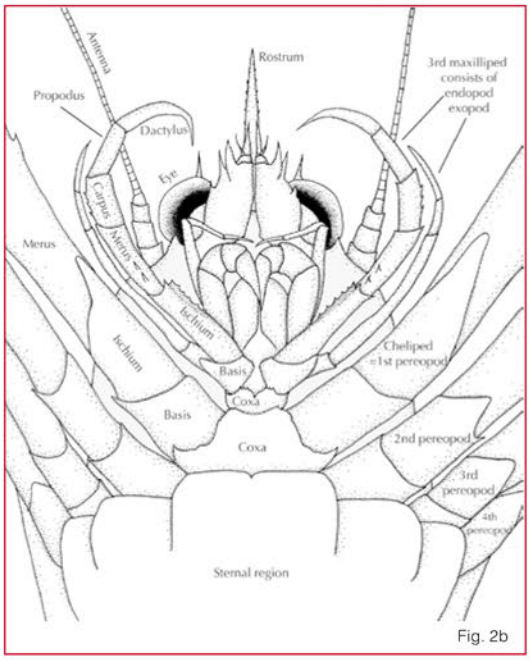


Fig. 2b

3a,b). For further information about Decapoda systematics and taxonomy, consult the "updated classification of the recent Crustacea" and "Systema Brachyurorum: Part 1. An annotated checklist of extant brachyuran crabs of the world" (Martin & Davis 2001; Ng et al. 2008).

Morphology

The decapod body is differentiated in two main sections (Fig. 1): (1) the cephalothorax, consisting of the fused head (cephalon) and trunk (thorax), and (2) the abdomen (pleon) (Fig. 2a). Appendages of the cephalothorax are the first and second antennae (antennule and antenna), all mouthparts (mandible, first and second maxilla, maxilliped one to three), and the thoracic appendages (five pairs of pereopods) (Fig. 2b). In many decapods the first pereopods have enlarged pincers (chelae) and are therefore called chelipeds (Brachyura). The cephalothorax is covered by a protective carapace, which is divided in the frontal, hepatic, gastric, cardiac, branchial and intestinal regions; further appendages are found on the seven-segmented pleon, with only six visible due to fusion of the last two segments. (Figs. 3a,3b). Each somite carries a pair of biramous pleopods except the sixth. The first pair of pleopods can be modified in the male as gonopods (e.g. the petasma, only in Dendrobranchiata). The last pleopods are called uropods and together with the telson form the tail fan.

Development

The Dendrobranchiata have a holo-pelagic life cycle—all the developmental stages are free-living in the water column. The eggs are released into the water column, and the juveniles hatch as nauplius larva. After six molts and anamorphic growth (the development of new segments at the posterior part of the larva) the nauplius develops into the zoea larva. The zoea larvae of the Dendrobranchiata develop into the adult pelagically-living prawn, through several molts.

The Pleocyemata undergo indirect development and have a pelago-benthic life cycle—the larvae are planktonic and the adults live on the ground. The fertilised eggs are carried on the female's pleopods. Unlike in the Dendrobranchiata, the development of the nauplius occurs inside the egg, and the larvae do not hatch until they reach the zoea stage. The zoea larvae (Fig. 4) pass through several morphologically-different zoea stages. After a series of molts they develop into the first benthic


Fig. 4

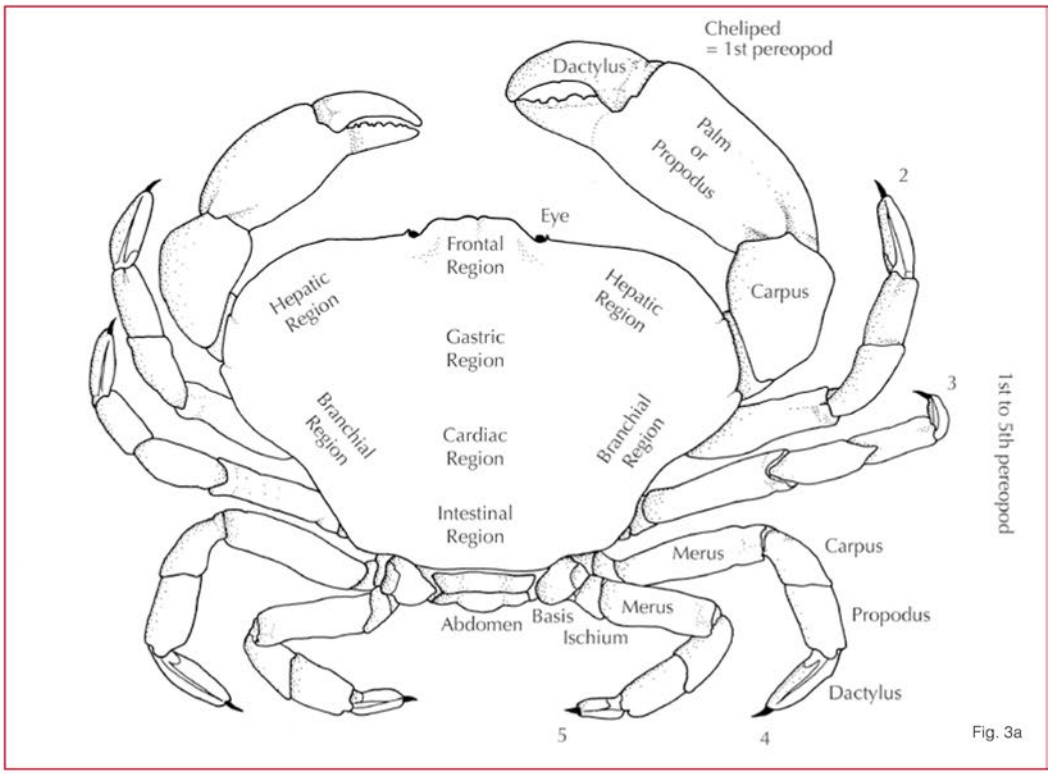

Cheliped
= 1st pereopod
Dactylus
Palm
or
Propodus
Eye
Frontal
Region
Hepatic
Region
Hepatic
Region
Carpus
Gastric
Region
Branchial
Region
Cardiac
Region
Branchial
Region
Intestinal
Region
Abdomen
Basis
Ischium
Merus
Merus
Carpus
Propodus
Dactylus
2
3
5
4
1st to 5th pereopod

Fig. 3a

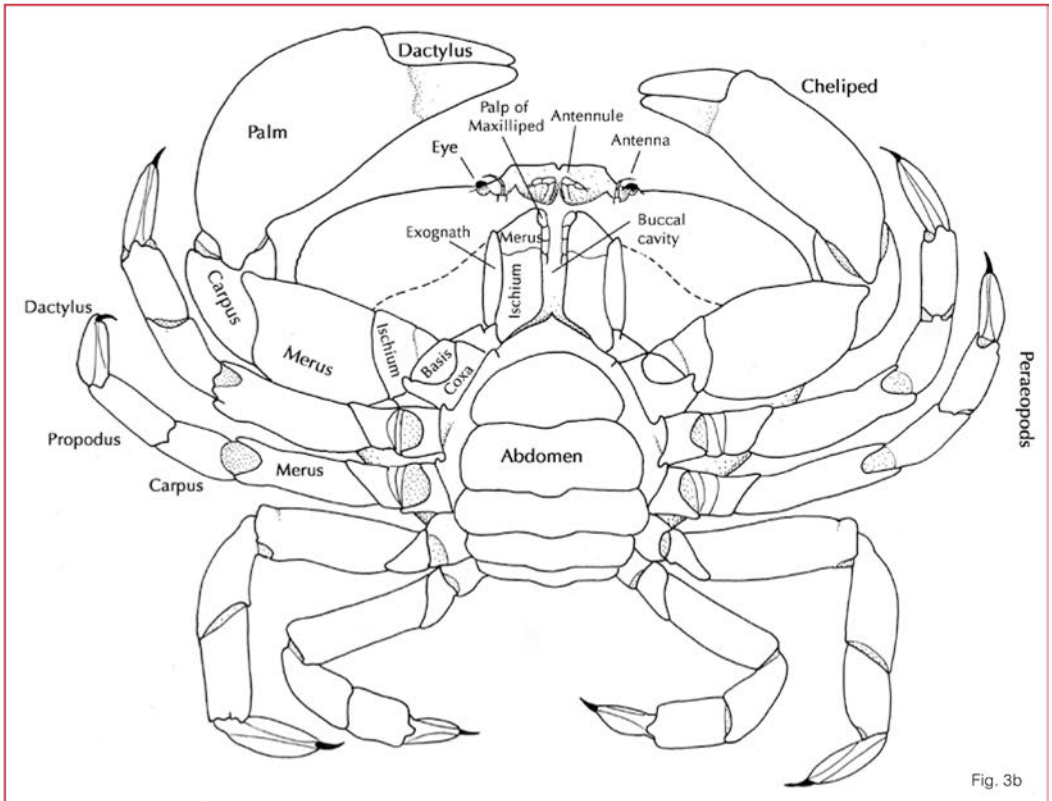

Dactylus
Palm
Palp of
Maxilliped
Antennule
Eye
Antenna
Cheliped
Exognath
Merus
Buccal
cavity
Ischium
Carpus
Dactylus
Merus
Ischium
Basis
Coxa
Propodus
Merus
Carpus
Abdomen
Pereopods

Fig. 3b

stage (the megalopa (Fig. 5)) and after another molt they develop into the adult-shaped pleocyemate decapod.
Both the indirect development and the ecological habitat separation of juvenile and adult forms (i.e. pelagic/benthic) allow Decapoda larva to be distributed over vast distances by the ocean currents and to colonize new areas as adults.

Collection and Preservation

Various methods are used for collecting decapod crustaceans including hand, trap and dredge collection. All the species presented in this chapter were sampled from various habitats on SCUBA trips to depths between 0 and 40 m. In order to collect the pelagic larval stages of decapod crustaceans (Zoea-larvae) a plankton-net, or the more selective light trap, were used. Determination of the adult specimens can be accomplished using eidonomical, i.e. external features such as the number of carapace spines or the shape of the pereopods and other appendages. Dissection is not necessary. The samples should be fixed at least overnight with a 4% formalin-seawater mixture to preserve the tissue and the thin cuticle at the articulations of appendages, and then transferred via a graded series of ethyl alcohol (i.e. 30%, 50% to 70%). The time for each step depends on the sample size; at least overnight duration is suggested. For DNA-samples non-denatured ethyl alcohol is used.


Fig. 5

The colours of the ethanol fixed species will bleach within a few days. Hence, a photo of the freshly fixed or living animal is needed for a colour description of the sample. The species that are described in this chapter and could not be photographed in their natural habitat were photographed after fixing with ethanol.

Crabs, Shrimps and Lobsters of the Chilean Fjord Region

Excavations made in the last decades show that around 10,200 years ago crustaceans had already been utilized by the native people of the fjord region as a food source (e.g. *Homalaspis plana*) and for religious rites (e.g. *Pagurus villosus*). But, the scientific description of the Chilean carcinofauna (or decapod fauna) only began about 200 years ago. The Chilean naturalist, Juan I. Molina (1740–1829), was the first to scientifically name the local crustacean species. Many international scientists of the last century (e.g. Charles R. Darwin (1809–1882), Alcide D'Orbigny (1802–1857) and Eduard Poeppig (1798–1868) also contributed their work to our knowledge of Chilean decapods. Notable amongst the numerous scientific vessel-based expeditions that were undertaken in the Chilean Fjord Region are the expeditions of the *H.M.S."Challenger"* (1872–76), the Swedish Lund University (1948–1949) and the *B/I "Victor Hensen"* (1994) to the Strait of Magellan.

Presently, the main inventory of the Chilean carcinofauna can be considered as complete. However, very little is known about the biology and ecology of most of the 51 decapod species described from the fjord region (for the complete list see App. 2). The researchers at the Huinay Scientific Field Station have recently begun to fill this information gap. They have lead numerous SCUBA excursions, between Puerto Montt and the Magellan Strait, since 2001 that have provided most of the observations, specimens and other information included in this chapter. In this chapter, 22 species of the infraorders Anomura, Brachyura and Caridea are described; several more were collected and will be included in the next edition. We present diagnoses on the family, generic and species levels and include pictures of the most relevant diagnostic features. We review the basic information on the biology of the species from both the literature and our own observations.

Classification

Infraorder Caridea
- Family Campylonotidae Sollaud, 1913
  - *Campylonotus vagans* Bate, 1888
- Family Hippolytidae Dana, 1852
  - *Nauticaris magellanica* (Milne Edwards, 1891)

Infraorder Anomura
- Family Galatheidae Samouelle, 1819
  - *Munida subrugosa* (White, 1847)
- Family Porcellanidae Haworth, 1825
  - *Petrolisthes laevigatus* (Guérin, 1835)
- Family Paguridae Latreille, 1802
  - *Pagurus villosus* Nicolet, 1849
  - *Pagurus comptus* White, 1847
  - *Pagurus edwardsi* (Dana, 1852)
  - *Propagurus gaudichaudi* H.Milne Edwards, 1836
- Family Lithodidae Samouelle, 1819
  - *Lithodes santolla* (Molina, 1782)
  - *Paralomis granulosa* (Jacquinot, 1847)

Infraorder Brachyura
- Family Inachidae Macleay, 1838
  - *Eurypodius latreillei* Guérin, 1825
- Family Majidae Samouelle, 1819
  - *Pisoides edwardsii* (Bell, 1835)
- Family Hymenosomatidae MacLeay, 1838
  - *Halicarcinus planatus* (Fabricius 1775)
- Family Atelecyclidae Ortmann, 1893
  - *Peltarion spinosulum* (White, 1843)
- Family Cancridae Latreille, 1583
  - *Cancer edwardsi* Bell, 1835
- Family Platyxanthidae Guinot, 1977
  - *Homalaspis plana* (H. Milne Edwards, 1834)
- Family Pilumnoididae Guinot & MacPherson, 1987
  - *Pilumnoides perlatus* (Poeppig, 1836)
- Family Belliidae Dana 1852
  - *Acanthocyclus albatrossis* Rathbun, 1898
- Family Pinnotheridae De Haan, 1833
  - *Pinnixa bahamondei* Garth, 1957
  - *Pinnaxodes chilensis* (Milne-Edwards, 1937)
- Family Varunidae H. Milne Edwards, 1853
  - *Cyclograpsus cinereus* Dana, 1851
  - *Hemigrapsus crenulatus* (H. Milne-Edwards, 1837)

Taxonomic Key to Infraorder Level (after Guzmán, 2003)

1) Shrimp-like body, epimeron of somite 2 not overlapping epimera of somites 1 and 3.
   a) Branching gill structure ..... Dendrobranchiata
   b) Other gill structure ..... Pleocyemata 2
2) Shrimp-like body, epimeron of somite 2 overlaps epimera of somites 1 and 3 ..... Caridea
3) Cephalothorax cylindrical, epimeron of somite 2 not overlapping epimera of somites 1 and 3 ..... Astacidea and Palinura
4) Crab-like body, pereopod 5 reduced (not visible) ..... Anomura
5) Crab-like body, 5 pairs of pereopods, 1st always chelate ..... Brachyura

Infraorder Caridea Dana, 1852

Infraorder Caridea includes worldwide 16 superfamilies with 36 families, 200 genera and ~2,000 species. Chile: 11 families with 44 genera and ≥73 species. Representatives of the Caridea can be found in a variety of habitats (e.g. in marine, brackish and freshwater habitats); can be semi-terrestrial, benthic or pelagic; and are free-living, parasitic or commensalic.The Caridea are characterised by the typical shrimp body plan (Fig. 1): carapace laterally compressed, subcylindrical to cylindrical or dorsoventrally flattened. 5 pereopods present, usually pereopod 1 and 2 chelate or subchelate. Abdomen consisting of 6 somites. Epimeron of somite 2 overlaps epimera of somite 1 and 3. Pleopods in 5 pairs. In males pleopods 1 or 2 can be modified to form a gonopod (e.g. the petasma). Tail fan composed of 1 pair of well-developed, ventrolaterally-positioned uropods and a median telson.

Family Campylonotidae Sollaud, 1913

Represented worldwide by 2 genera with 8 species. Chile: only 1 genus (*Campylonotus*). Dorsal antennular flagella without accessory branches. Pereopods 1–4 with arthrobranchs at bases; pereopod 1 and 2 subequal and chelate; pereopod 1 usually more slender than pereopod 2; pereopod 2 with undivided carpus.

Genus *Campylonotus* Bate, 1888

Chile: 4 species. Basal part of rostrum armed with a maximum of 4 teeth; 1st tooth behind middle of carapace. Upper antennal flagellum simple. Pereopods without exopods; pereopods 1 and 2 chelate; pereopod 2 equal; pereopods 1–4 with arthrobranchs and epipods at bases.

Taxonomic Key to the South American *Campylonotus* Species

(from Thatje, 2003)

1) a) Rostrum with 3–4 ventral teeth 2
   b) Rostrum with >4, normally 6–10, ventral teeth ..... *C. vagans,* (p. 635)
2) a) 1 subdorsal rostral spine 3
   b) No subdorsal rostral spine ..... *C. semistriatus*
3) a) Rostrum slightly curved, projecting, with short bristles at basis of ventral teeth; carapace without posterior tubercle; somite 4 with pleural tooth ..... *C. arntzianus*
   b) Rostrum strongly curved, without bristles; carapace with posterior tubercle; somite 4 without pleural tooth.. ..... *C. capensis*

Family Hippolytidae Dana, 1852

Represented worldwide by 30 genera and ~225 species. Chile: 9 genera with 10 species. Pereopod 1 always chelate, with 1 or more well-developed chela. Carpus of pereopod 2 multiarticulate in more or less than 5 joints.

Genus *Nauticaris* Bate, 1888

Characterised by presence of arthrobranchs at bases of pereopods and by movable pleura of somite 6.

Infraorder Anomura MacLeay, 1838

Anomura (Gr. anomos = uneven, asymmetrical & gr. oura = abdomen) (Fig. 2a,b)

Infraorder Anomura includes a wide variety of different forms such as the Hermit Crabs, King Crabs, Squat Lobsters, Porcelain Crabs and Mole Crabs. Most representatives of this Infraorder occur in marine habitats, but also semi-terrestrial or freshwater forms (e.g. in Chile the family Aeglidae) are known. The Anomura are distributed worldwide with ~1,800 species of 14 families arranged in 4 superfamilies: Paguroidea Latreille, 1802 (Coenobitidae, Diogenidae, Lithodidae, Paguridae, Parapaguridae, Pylochelidae), Galatheoidea Samouelle, 1819 (Aeglidae, Chirostylidae, Galatheidae, Porcellanidae), Hippoidea Latreille, 1825 (Albuneidae, Hippidae) and Lomisoidea Bouvier, 1895 (Lomisidae). Chile: 11 families with ~93 species. The Anomura are characterized by reduction of pereopod 5, which also differs in orientation from the other pereopods—it is folded under the thorax or hidden in the carapace.

Thorax and abdomen differentiated. Abdomen 6-segmented and well-developed (e.g. Squat Lobsters), reduced in size and folded under thorax (e.g. Porcelain Crabs), or asymmetric, soft and hidden in snail shell (e.g. Hermit Crabs). Uropods developed, positioned ventrolaterally.

Family Galatheidae Samouelle, 1819 (Squat lobsters)

Represented worldwide by 7 genera and ~258 species. Chile: 5 genera with 20 species. Carapace oblong, dorsoventrally flattened; surface covered with transversal lines (linea anomurica); rostrum triangular, sharp, overreaching distal corneal margin. Antennular flagellum biramous. Bases of maxilliped 3 close together. Chelipeds equal or subequal. Pereopods 2–4 similar in form; pereopod 5 reduced. Abdomen well-developed, consisting of distinct somites carried under thorax. Male with pleopods 1 and 2 modified as gonopods; females without sexual modifications on pereopods. Uropods positioned ventrolaterally, form a tail fan.

Genus *Munida* Leach, 1820

Chile: 5 species. Main character: shape of carapace: its latero-inferior regions form side walls of carapace and is only visible in ventral or lateral, but not in dorsal view; rostrum not keeled, slender, rounded, spine-like, flanked by supraorbital spines, eyes darkly pigmented, faceted.

Family Porcellanidae Haworth, 1825

Chile: 5 genera with 16 species. Carapace dorsoventrally flattened; rostrum reduced or absent, without outer orbital spines. Antennal flagellum reduced, vestigial or absent; basal segment of antennules broad, frequently touching midline. Pereopods 2–4 of similar form; basis and ischium fused; pereopod 5 reduced and folded under carapace. Chelipeds equal or subequal, usually broad and depressed, with short merus and elongated carpus. Abdomen adjacent to 7-segmented telson, divided into (5 or 7) multiple plates with uropods.

Genus *Petrolisthes* Stimpson, 1858

Carapace rounded or subquadrate, as broad as long; frontal region triangular or trilobate, often rather prominent and produced beyond eyes; eyes on short and stout eyestalks. Basal article of antenna not produced

to meet anterior margin of carapace, basal article of antennule large and broad. Chelipeds large, subequal; palms broad, flattened with dorsal surface somewhat swollen. Pereopods similar, of flattened shape except for reduced 5th carried on carapace.

Family Paguridae Latreille, 1802 (Right-handed hermit crabs)

Carapace cylindrical, subcylindrical or dorsoventrally flattened; lateral margins without projections; rostrum reduced or completely absent; without outer orbital spines. Flagellum of antennule biramous. Flagellum also on maxilliped 1. Bases of maxilliped 3 widely separated. Chelipeds equal or subequal (largest right). Pereopod 5 reduced. Pleon well-developed with distinct or indistinct somites; uropods present, positioned ventrolaterally. Telson entirely or partially divided longitudinally.

Taxonomic Key to Family Paguridae

(modified after Guzmán, 2004)

1) a) Bases of maxilliped 3 widely separate, maxilliped 1 each bearing a flagellum, 1 pleurobranch present above pereopod 3, carapace without projections, crista dentate equipped with strong spine, chelipeds dimorphic . ..... genus *Pagurus* 2
   b) Bases of the maxilliped 3 widely separate, maxilliped 1 each bearing a flagellum, carapace without projections, 3 rudimentary pleurobranchs present above pereopod 3, chelipeds markedly unequal .. genus *Propagurus* 5
2) a) Palm of chelipeds spare pilose without spines (except on dorsal margin) and granulated 3
   b) Palm of chelipeds pilose with irregular rows of spines on outer side, superior margin of pereopods unarmed, very small ..... *Pagurus villosus,* (p. 641)
3) a) Palm of right (large) cheliped with superior and inferior crest, carpus convex with its superior margin serrate ..... *Pagurus comptus,* (p. 641)
   b) Palm of right (large) cheliped without crest, superior margin of carpus not serrate 4
4) Palm of right (large) cheliped covered with blunt rounded white granules on red background. Eyestalks long and slender ..... *Pagurus edwardsi,* (p. 644)
5) Superior margins of pereopods with row of spines, chelipeds with several longitudinal series of black tipped spines ..... *Propagurus gaudichaudi,* (p. 644)

Genus *Pagurus* Fabricius, 1789

1 pleurobranch above pereopod 3. Rostrum of variable length and form, bases of maxilliped 3 widely separated. Flagellum on maxilliped 1. Chelipeds generally dimorphic, right one usually largest.

Family Lithodidae Samouelle, 1819 (Stone crab, King crab)

Distributed worldwide, with 16 genera and ~53 species. Chile: 5 genera with 15 species. Carapace as long as or wider than long, dorsoventrally flattened; dorsal regions usually well-defined, bearing granules and spines variable in number and size; rostrum always present. Chelipeds dimorphic, right more robust than left. Pereopods of similar shape (except 5 that is subchelate), reduced, folded underneath carapace. Plates distinct, calcified, variable in size and numbers, cover soft abdomen carried under thorax. Rudimentary pleopods in females; absent in males.

Genus *Lithodes* Latreille, 1806

Carapace well calcified. Chelipeds and pereopods armed with spines and granules variable in size and number; spines proportionally larger in juveniles than adults. Carapace regions well defined; gastric region prominent. Rostrum can be formed by a usually bifid long anterior projection with 1 or 2 pairs of located dorsally spines. 2nd article of antennular peduncle, with dorsal groove, thicker than others and as long as last article. Pereopods elongate; pereopod 2 longest. Chelipeds unequal; palms with numerous tufts of setae; right cheliped robust; palm with several thick, rounded teeth; left cheliped, less robust, with smaller, more numerous teeth.

Genus *Paralomis* White, 1856

Comprises ~46 species worldwide. Chile: 8 species: *P. papillata* (Benedict, 1895), *P. aspera* (Faxon, 1893), *P. longipes* (Faxon, 1893), *P. chilensis* (Andrade, 1980), *P. tuberipes* (Macpherson, 1988), *P. otsuae* (Wilson, 1990), *P. birsteini* (Macpherson, 1988) and *P. granulosa* (Jacquinot, 1847). A key to the eastern Pacific species is given. Carapace well-calcified, almost pentagonal

or pyriform; carapace regions well defined, convex; rostrum formed by basal spine and ≥1 pair of divergent, upwardly inclined, dorsal spines. Somites well-calcified, without membranous areas. Sternal region, located between pereopod 1, without longitudinal, medial groove. Chelipeds subequal; right stouter. Pereopods more or less elongate; pereopod 2 somewhat longer than pereopods 1 and 3.

Taxonomic Key to Eastern Pacific *Paralomis* Species

(from Macpherson, 1992)

1) a) Entire dorsal surface covered with spines or spiniform tubercles 2
   b) Dorsal carapace surface not entirely covered with spines, instead bearing numerous granules and, at most, a few scattered spines 3
2) a) Entire dorsal carapace surface without spines, covered with acute tubercles of uniform size ..... *P. chilensis*
   b) Entire dorsal carapace surface covered with spines; gastric region with 1 central spine more developed than rest and 1 median spine in centre of each branchial region ..... *P. phrixa*
3) a) Gastric region with central spine 4
   b) Gastric region without spines 5
4) a) Entire dorsal carapace surface verrucose, covered with prominent granules of approximately equal size ..... *P. verrilli*
   b) Dorsal carapace surface smooth, covered with small granules of different size ..... *P. otsuae*
5) a) External surface of pereopods without spines, having granules or tubercles 6
   b) External surface of pereopods with row of spines 8
6) a) External surface of pereopods covered with tubercles ..... *P. tuberipes*
   b) External surface of pereopods covered with small granules 7
7) a) Dorsal carapace surface covered by clustered granules ..... *P. granulosa*, (p. 647)
   b) Dorsal carapace surface covered by simple granules ..... *P. diomedeae*
8) a) Branchial regions much more protuberant than cardiac region 9
   b) Branchial regions as protuberant as cardiac region 10
9) a) Ventral surface of basal spine of rostrum strongly spinulated ..... *P. inca*
   b) Ventral surface of basal spine of rostrum unarmed or minutely spinulated ..... *P. papillata*
10) a) Carapace contour pyriform ..... *P. longipes*
    b) Carapace contour pentagonal ..... *P. aspera*

Infraorder Brachyura Latreille, 1802 (True Crabs, jaibas or pancoras cangrejos)

Infraorder Brachyura with worldwide ~5,000 species is the largest and the most diverse group within the Decapoda found in marine, freshwater and semi-terrestrial habitats. Chile: ~141 species in ~27 families. They are highly derived and well armoured decapods in that the body and the appendages are strongly modified compared to the ancestral shrimp-like decapod basic form (Fig. 3a, b). Carapace completely enclosing head and thorax, covering sternum; relatively small abdomen folded ventrally under thorax. Pereopod 1 forms chelipeds; antennae and antennules strongly reduced and short; uropods completely reduced. Form of abdomen usually revealing gender of crab; males with narrow abdomen; females with much wider one under which eggs are carried.

Familia Inachidae MacLeay, 1838

Carapace of subtriangular, subpyriform or subcircular shape, deprived of orbits. Eyestalks generally long, either non- retractile or if retractile then to sides of carapace or the present acute postorbital spine. Eyestalks not concealed. Basal antennal article usually long, slender and subcylindrical throughout its extent flattened or channelled ventrally, usually free distally. Pereiopods long and slender.

Genus *Eurypodius* Guérin, 1825

Carapace pyriform, moderately convex; dorsal side spinous or tuberculate; rostrum divided into 2 narrow pointed protrusions, antennae visible in dorsal view at sides of rostrum. With distinct postorbital spine, without preorbital spine. Eyestalks stout. Males with well-developed chelipeds; palms compressed or turgid. Females with smaller chelipeds. Pereopods long, prehensile. Abdomen 7-segmented.

Family Majidae Samouelle, 1819

Represented worldwide by 145 genera with 750 species. Chile: 12 genera and ~15 species. Carapace usually of pyriform shape, equipped with hooked hairs. Mouth field almost square, normal orientation of pereopod 5. Orbits incomplete, rostrum present. Basal article of antenna fused with epistome and frontal region. Supraocular spine at insertion of eye, with large, cupped postocular process; eyes on short eyestalks can be retracted into postocular process. Chelipeds shorter than pereopods.

Genus *Pisoides* Milne-Edwards & Lucas, 1843

Carapace longer than broad, slightly swollen, triangular; gastric and cardiac regions apparent and well separated from each other by deep furrows; rostrum elongate, usually armed with 2 moderately diverging spines. Upper margin of orbital cavity with cut, armed with sharp tooth at outer angle. Basal articles of antennae nearly as broad as long, peduncles reaching or overreaching end of rostrum.

Family Hymenosomatidae MacLeay, 1838

Carapace leather-like and soft, chelae of pereopod 1 without special features; with rostrum. Almost square mouth field, pereopod 5 in normal orientation. Rostrum present, orbits incomplete. 2nd segment of antenna long, fused with epistome. Genital opening of males in characteristic sternal position.

Genus *Halicarcinus* White, 1846

Epistome pronounced, antennules clearly visible, without septum between them. Merus and ischium of maxilliped 3 of almost same size.

Family Atelecyclidae Ortmann, 1893

Worldwide represented with 13 genera and 30 species. Chile: 3 genera (*Peltarion* Jacquinot, 1847, *Trachycarinus* Faxon, 1893, *Trichopeltarion* A. Milne-Edwards, 1880). Carapace suboval, broader than long; lateral margins and frontal region dentate. Antennules and antennae longitudinally directed; antennal flagellum normally pilose and robust, occasionally rudimentary or missing. Pereopods strong, pilose. Chelipeds in males stout, subequal.

Genus *Peltarion* Jacquinot, 1847

Carapace broad, oval-shaped, convex; anterior half broader than the posterior one; surface granulated; anterior and lateral margins dentate; rostrum horizontally directed, triangulate and tridentate, median tooth more advanced than lateral teeth. Basal article of antenna shorter than adjacent article. Chelipeds stout.

Family Cancridae Latreille, 1583

Carapace robust, broadly oval; frontal region with several teeth; one tooth median. Antennules folded lengthwise. Antennal flagellum present, short, hairy. Maxilliped 3 usually overlapping endostome.

Genus *Cancer* Linnaeus, 1758

Carapace transversely subelliptical, often indistinctly areolated; frontal region narrow, with 5 teeth or lobes inserted. Eyestalks short, orbits small, with 2 fissures in both upper and lower margin. Basal article of antenna usually enlarged and fused with frontal region.

Familia Platyxanthidae Guinot, 1977

Carapace transversely elliptical, with indistinct dorsal regions. Frontal margin four-lobed, with median notch. Antennular and antennal sinus distinct. Supraorbital margin of carapace bifissured. Antennule folded obliquely. Basal segment of antenna not in contact with front. Thoracic sternum relatively narrow to large.

Genus *Homalaspis* A. Milne-Edwards, 1863

Carapace broad and longitudinally convex; regions are indistinctly marked; frontal region advanced, deflexed, narrow; anterolateral borders obscurely lobed; posterior margins strongly convergent. Chelipeds unequal. Pereopods strong, robust.

Familia Pilumnoididae Guinot & MacPherson, 1987

Carapace, roundish, with well marked regions. Frontal margin bilobed. Sinus(es) of antennule and antenna rather distinct. Antennulae oblique, nearly transversely folded. Basal antennal segment not touching frontal margin. Endostomial ridge present. Chelipeds tightly pressed against carapace, dactyli slanting. Sternum narrow, elongate, sternal sutures 4/5 – 7/8 entire. Abdomen narrow in male, with freely articulated segments.

Genus *Pilumnoides* Milne-Edwards & Lucas, 1844

Carapace thick, swollen; posterior narrow; frontal region bilobed, deflexed; anterolateral margins slightly oblique. Orbits laterally deep, almost circular. Basal article and flagellum of antenna short; basal article of antennule wide, raised. Maxilliped 3 wide; merus rounded at external angle, slightly notched at insertion point of palp.

Chelipeds subequal stout; merus entirely concealed under carapace; palm with 3 large lobes or tubercles on upper margin.

Family Belliidae Dana, 1852

Contains worldwide 4 genera with ~7 species. Chile: 3 genera (*Acanthocyclus* Milne-Edwards & Lucas, 1844, *Bellia* Milne-Edwards, 1848 and *Corystoides,* Milne-Edwards & Lucas, 1844) with 5 species. Carapace subcircular or suboblong; frontal region terminating in subtriangular point. Antennae strongly reduced. Antennules well-developed, retractable within basal cavity.

Genus *Acanthocyclus* Milne-Edwards & Lucas, 1844

Represented in South American waters by 3 closely related species: *A. albatrossis* Rathbun, 1898, *A. gayi* Milne-Edwards, 1848 and *A. hassleri* Rathbun, 1898. Carapace rotund, slightly broader than long, with lateral teeth extending slightly along posterolateral margin; frontal region with large median tooth and 2 smaller lateral teeth. Orbits small with short eyes on stout peduncle. Antennae strongly reduced, terminate with basal article. Chelipeds stout, very unequal. Pereopods with curved, acuminate dactyli.

Taxonomic Key to the Genus *Acanthocyclus*

1) a) Carapace and pereopods very pilose ..... *A. gayi*
   b) Carapace and pereopods less pilose 2
2) a) Orbit viewed from above more than twice as wide as deep ..... *A. hassleri*
   b) Orbit viewed from above less than twice as wide as deep ..... *A. albatrossis*, (p. 660)

Family Pinnotheridae De Haan, 1833

Represented worldwide by 26 genera within 222 species. Chile: 5 genera with ~9 species. Carapace membranous or slightly calcified; regions not clearly visible. Orbits ovate, very small. Buccal cavity broad. Merus of maxilliped 3 large, palpus always on anterior external angle.

Genus *Pinnixa* White, 1846

Most species are commensals or parasites living in various organisms such as bivalve molluscs, in tubes or holes of polychete worms, in worm-like holothurians and sea urchins. Carapace much wider than long, with narrow frontal region and median groove. Orbits broadly ovate with wide inner hiatus; antennules transversely or obliquely folded. Eyestalks very short. Ischium of maxilliped 3 small, merus large, distal portion of outer margin convex. Chelipeds of moderate size, with triangular merus, smooth carpus, large and compressed palm. Pereopod 3 largest. Abdomen 7-jointed in both genders.

Genus *Pinnaxodes* Heller, 1865

Carapace slightly wider than long, thin, either soft or firm and parchment-like. Merus of maxilliped 3 almost fused with ischium, slightly oblique. Palpus of good size, sometimes as large as merus-ischium. Chelipeds much stouter than pereopods; 2 and 3 nearly equal in length.

Familia Varunidae H. Milne Edwards, 1853

Carapace of subquadrate to nearly subcircular shape, slightly wider than long and depressed. Anterolateral margin with 2 or more epibranchial lobes more or less crested, sometimes with submarginal row of setae. Antennule folding obliquely. Antennae well developped, diverging. Antennal flagellum relatively long. Buccal cavity of square shape. Dactyli of ambulatory legs deprived of spines.

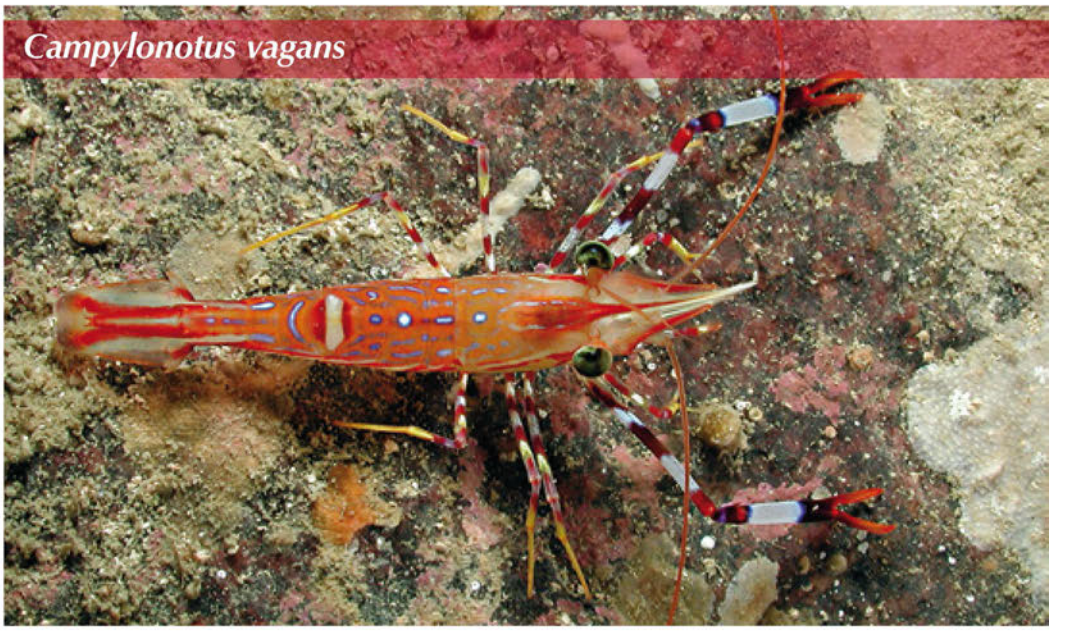
Genus *Cyclograpsus* Milne-Edwards, 1837

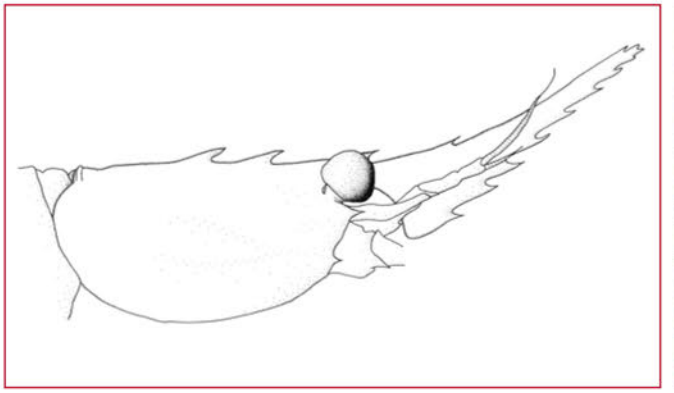
Carapace surface flat, smooth, except of deflexed anterior ⅓; frontal region and antero-lateral margins a regular curve. Lateral teeth not present. Front's width between ⅓ and ½ of width of carapace, orbits transversely oval, completely filled by eyes. Antennule folded transversely, antennas short. Buccal cavity narrowing anteriorly. External maxillipeds gaping widely at base. Chelipeds subequal, nearly smooth, with swollen palm. Pereopods narrow, of moderate length, 2nd pair longest.

Genus *Hemigrapsus* Dana, 1851

Carapace broader than long, rectangular. Antero-lateral margins rounded, dentate; frontal region extends less than ½ of carapace width. Antennule folding obliquely, antenna generally filling orbital hiatus. Inner angle of orbit with well-developed tooth. Chelipeds stout, palms and carpus of male with pilose patches inside.

Campylonotus vagans





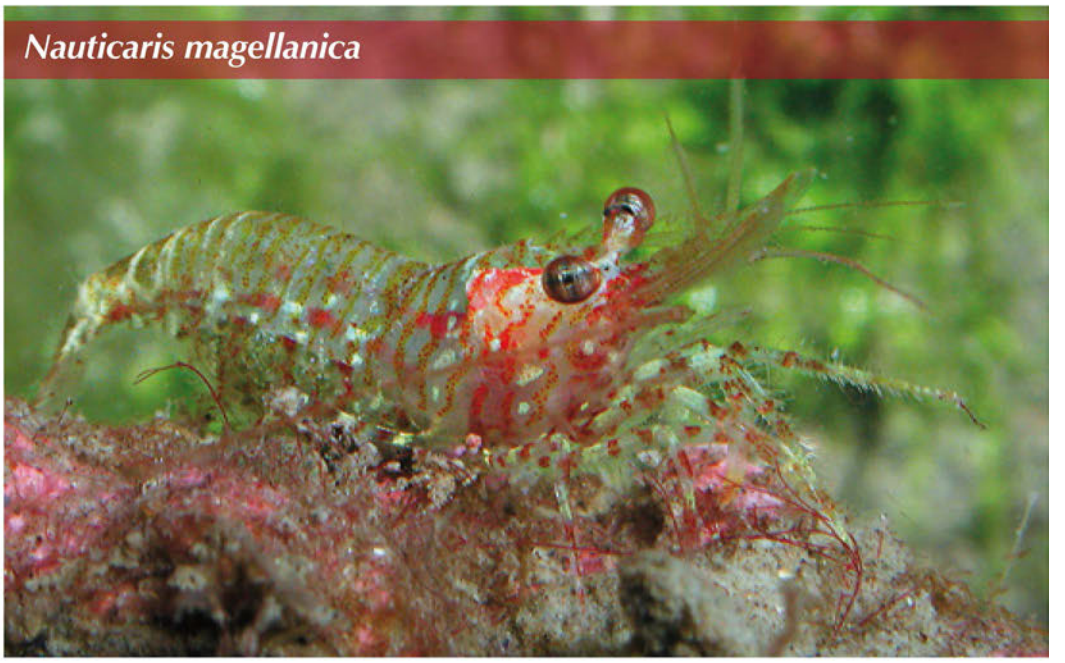
Campylonotus vagans Bate, 1888

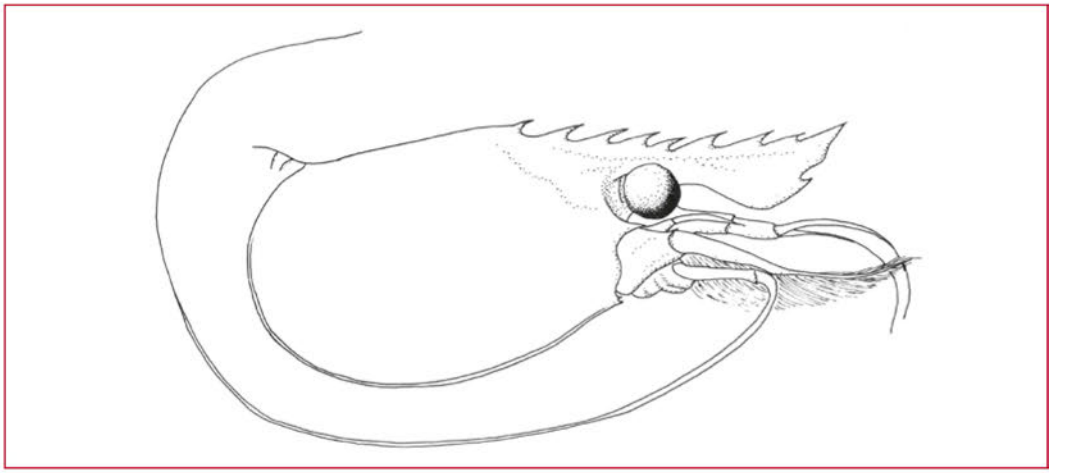
Common name: Painted shrimp; Camarón pintado
Synonymy: *Anchistiella hyadesi* Milne Edwards, 1891; *A. seneuili* Milne Edwards, 1891; *Campylonotus seneuili* Sollaud, 1910.
Description: Medium; maximum size 96 mm; carapace length 13–23 mm. Brightly coloured, base colour rather pale reddish brown; carapace with red longitudinal bands; abdomen with yellow and violet transverse bands. Carapace not compressed, bulged, smooth; rostrum obliquely, upwardly-directed, slightly longer than carapace, upper margin armed with 4 forwardly-directed spines, lower margin with 6–10 spines. Antennular stylocerite long, extending to median part of 2nd article of peduncle; antennular flagellum short, does not reach tip of rostrum. Antennal scaphocerite narrow, tapering, ends in sharp spine. Pereopods 1 and 2 chelate; 2 very long; dactyli short, with characteristic set of spines on lower margins. Abdomen with 5th abdominal pleura sharply angulated at posterior end. Telson with 4 pairs of spines on upper surface. **Possibility for confusion:** Can be differentiated from other *Campylonotus* species based on their distribution (see also key p. 628): *C. semistriatus* seems to be exclusively restricted to the channel and fjord system of the Strait of Magellan and Tierra del Fuego at depths of 150–500 m; and *C. capensis* occurs in the deep-sea along the continental platform of the Argentine Atlantic shelf at depths of 140–1,300 m.
Habitat: Intertidal rock pools to crevices at higher depths. **Depth:** Intertidal–320 m. **Abundance:** Common. **Distribution:** SW Atlantic (Argentina; Falkland Islands); SE Pacific (PP–SPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 41°S–56°S. **Biology:** Nocturnal. Larval development through 2 zoea stages and 1 decapodit stage. All Campylonotidae species are protandric hermaphrodites.
Main references: Torti & Boschi (1973); Boschi et al. (1992); Thatje et al. (2001); Thatje (2003).

Nauticaris magellanica (Milne Edwards, 1891)

Common name: Magellan shrimp; Camaroncito
Synonymy: *H. consobrinus* Milne Edwards, 1891; *Nauticaris marionis chilensis* Doflein & Balss, 1912.
Description: Small; maximum size 40 mm. Transparent; carapace with characteristic red longitudinal bands; abdomen with transversal bands. Carapace smooth; anterior margin with well-developed antennal spine; rostrum straight, dorsal margin with 7–8 regularly spaced spines (first 2 or 3 posterior to orbit), ventral margin with 1 or 2 teeth. Scaphocerite very slender, reaching far beyond rostrum. Pereopods 1 and 2 with chelae at tips. Pereopod 2 with carpus divided into 14–16 joints. Pleon smooth, composed of 6 somites; somites 1–3 with broadly rounded epimera; somites 4 and 5 with epimera terminating in sharp point. Telson with 2 pairs of spines on dorsal surface. **Possibility for confusion:** None. *N. magellanica* is the only species of the genus inhabiting the southern part of South America.
Habitat: Mostly hard substrates like gravel and rocks; also sandy bottoms and holdfasts of kelp (e.g. *Macrocystis* sp.). **Depth:** 5–100 m. **Abundance:** Common. **Distribution:** SW Atlantic (Falkland Islands); SE Pacific (PP–CPZ); Strait of Magellan. *Chile:* 20°S–54°S. **Biology:** Ovigerous females were observed in the months October to July. The eggs are numerous and reach 0.35–0.5 mm Ø. Larval development through 9 zoea stages and 1 decapodit stage.
Main references: Holthuis (1957); Wehrtmann & Albornoz (1998).

Nauticaris magellanica





Munida subrugosa

97

Munida subrugosa

(White, 1847)

Common name: Channel squat lobster; Langostino de los canales
Synonymy: *Munida gregaria* Miers, 1881 not *M. gregaria* (Fabricius, 1793).
Description: Medium size; total length to 69 mm. Carapace dorsally brown with transversal rims of whitish colour; ventrally reddish to brown. Carapace oblong, with several transversal rims, rim in anterior 3rd arcuated, anterior edges with small spines; rostrum straight, forwardly directed, flanked by supraocular spines. Merus of maxilliped 3 with sharp spine on outer margin. Chelipeds and pereopods with rows of strong spines on anterior margins especially on merus and carpus. Abdomen well-developed, folded under thorax. **Possibility for confusion:** Easily confused with *M. gregaria*, of which the merus of maxilliped 3 is unarmed (spine present in *M. subrugosa*) and the rostrum is much shorter and wider at its base.
Habitat: Sand, mud and hard substrates, less common in fjords and along exposed coast **Depth:** 5–137 m. **Abundance:** Common; sometimes in large aggregations. **Distribution:** Subantarctic Islands; SW Pacific (Australia; New Zealand); SW Atlantic (Uruguay; Argentina; Falkland Islands); SE Pacific (NPZ–CPZ); Strait of Magellan. *Chile:* 42°S–54°S. **Biology:** Feeds on crustaceans, algae, and polychaetes or consumes particulate organic matter and organisms associated with the superficial layer of the sediment (deposit feeder). Reproductive cycle starts in April. Ovigerous females carry eggs for 8–9 months, larval hatching occurs between October and January. Development series consists of 5 zoea stages and 1 megalopa stage. **Comments:** Galatheid species are used by humans in multiple ways: as cocktail shrimp, as a source of astaxanthins for pigmentation of chicken eggs and cultured salmon, as a source of lipids and proteins for balanced animal food and as a source of digestive enzymes for cheese manufacturing.
Main references: Roberts (1973); Rodríguez & Bahamonde (1986).

Petrolisthes laevigatus (Guérin, 1835)

Common name: Purple footed porcellan crab; Tijereta, Cangrejo porcelana de patas violetas
Synonymy: *P. granulosa* Guérin, 1835; *P. striata* Milne Edwards, 1837; *P. valida* Dana, 1852; *Petrolisthes validus* Stimpson, 1858; *P. granulosus* Ortmann, 1892; *P. granulosa* Boone, 1938.
Description: Small; carapace length to 23.9 mm. Carapace and appendages coloured above in dark purple-brown, interrupted by lighter, whitish dots. Joints of pereopods and chelipeds of bright red colour. Beneath, lighter brown. Carapace strongly convex from front to back; surface almost smooth, anteriorly covered with very fine granules; no epibranchial spine; frontal region triangular, strongly produced with shallow median sulcus. Orbits slightly concave, with outer orbital angle produced into narrow, distinct spine. Chelipeds broad, flattened; margins of carpus converging distally from highest point; anterior margin of carpus bearing strong proximal lobe; outer half of dorsal surface of palm, with smooth crest with short, thick pubescence. Pereopods nearly smooth to lightly granular, manus unarmed, carpus, propodus and dactylus scattered with short tufts of setae. **Possibility for confusion:** In the Chilean Fjord Region 2 more representatives can be found: *P. violaceus* and *P. tuberculosus*. Other Chilean *Petrolisthes* species can be identified by the key to species levels given in Haig (1960).
Habitat: On shores and under stones. **Depth:** Intertidal. **Abundance:** Locally common. **Distribution:** SE Pacific (Peru; PP–CPZ). *Chile:* 30°S–48°S. **Biology:** Ovigerous females were found in January, April, June and November and can produce up to 1,140 eggs. Larval development through 1 prezoea stage, 2 zoea stages and a megalopa stage. It feeds by filtering zooplankton.
Main references: Haig (1955; 1960).

Petrolisthes laevigatus

Pagurus villosus

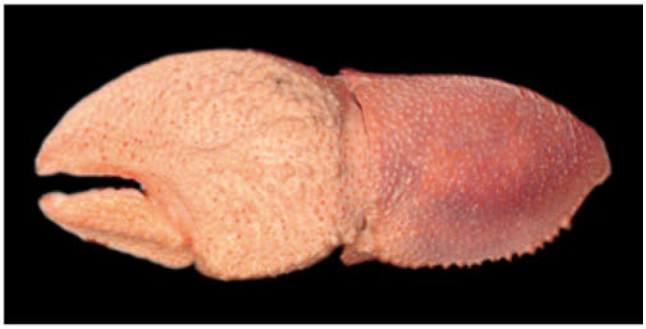
Pagurus villosus Nicolet, 1849

Common name: Small villous hermit crab;
Ermitaño chico velludo
Synonymy: *Pagurus benedicti* Rathbun, 1910 not *P. benedicti* Bouvier, 1898.
Description: Small; carapace length 5.3–8.9 mm. Carapace whitish; chelipeds light brown; pereopods and antennae with white and reddish-brown bands. Carapace without lateral projections; rostrum reduced. Chelipeds subequal (right cheliped larger), slender, hairy, with spiniform tubercles in longitudinal rows. Pereopods without spines on superior margin; pereopods 1 and 2 long, hairy. **Possibility for confusion:** None (see also key on p. 629).
Habitat: Hard substrates; algae, empty snail shells of *Nassarius gayi* and *N. dentifer*. **Depth:** 5–76 m. **Abundance:** Common, sometimes in large aggregations. **Distribution:** SE Pacific (Peru; PP–NPZ). *Chile:* 18°S–42°S. **Biology:** Nocturnal species. Feeds in large aggregations on algae. Ovigerous females were observed in the months of December through July. The larval development is as yet not described. **Comments:** Smallest of the Chilean pagurid species.
Main references: Haig (1955); Guzmán (2003; 2004).

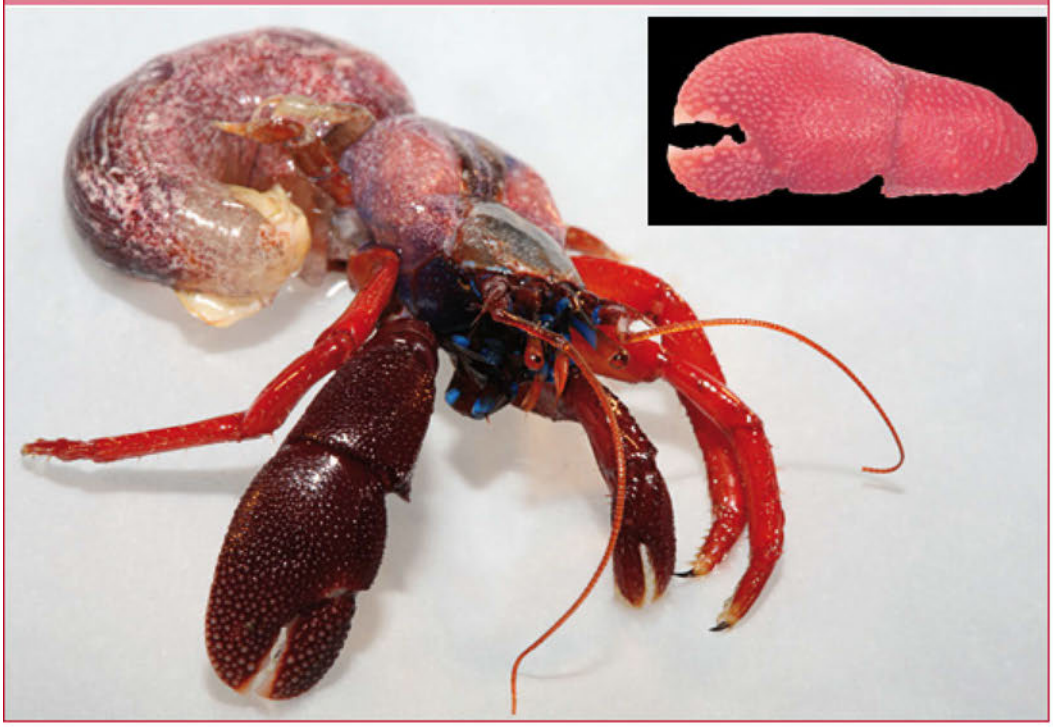
Pagurus comptus White, 1847

Common name: Common hermit crab;
Ermitaño comun
Description: Medium size; carapace length 0.7–10 mm. Carapace whitish; chelipeds beige; pereopods beige with wide reddish-brown stripes. Carapace with apical rostrum; rostrum dentiform, without lateral spines. Eyestalks nearly as long as base of antenna. Chelipeds very subequal, granulated; right cheliped glabrous with superior and inferior crest, carpus convex, superior margin serrate. **Possibility for confusion:** None (see also key on p. 629).
Habitat: Sediment, primary and secondary hard substrate (gorgonians etc.). **Depth:** Low intertidal–400 m. **Abundance:** Common. **Distribution:** SW Atlantic (Uruguay; Argentina; Falkland Islands); SE Pacific (PP–SPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 30°S–56°S. **Biology:** Ovigerous females have been observed in April and May. Larval development through 4 zoea stages and 1 megalopa. This hermit crab was mainly observed in shells of *Tegula atra, Fusitron cancelatus, Calliostoma* sp., *Parentheria* sp., *Faegotrophon pallidus, Nassarius dentifer* and *Drillia* sp.
Main reference: Retamal (2000).

Pagurus comptus



Pagurus edwardsi



103

Pagurus edwardsi (Dana, 1852)

Common name: Blood-red hermit crab;
Ermitaño rojo encendido
Synonymy: *Bernardus perlatus* Kinahan, 1857.
Description: Medium size; carapace length to 16.7 mm. Maxillipeds and antennae crimson with light blue patches, pereopods red, chelipeds crimson, with white blunt, rounded granules. Carapace dorsoventrally flattened, margins rounded, without lateral projections; rostrum reduced. Eyestalks long, slender. Chelipeds dimorphic, glabrous, covered with blunt rounded granules. Propodus of chelipeds, and margins of propodus and carpus of pereopods without spines. **Possibility for confusion:** None (see also key on p. 629).
Habitat: Rocks and other hard substrate. **Depth:** 5–15 m. **Abundance:** Locally common; less abundant in the inner fjords. **Distribution:** SE Pacific (Peru; PP–NPZ). *Chile:* 18°S–43°S. **Biology:** Ovigerous females were observed in March, April, May, July and October. Larval development has not yet been completely described. It inhabits shells of *Tegula atra*, *T. luctuosa*, *T. tridentata*, *Ocinebra crassilabrum*, *Acanthina calcar* and *Turbo niger*.
Main references: Haig (1955); Guzmán (2004).

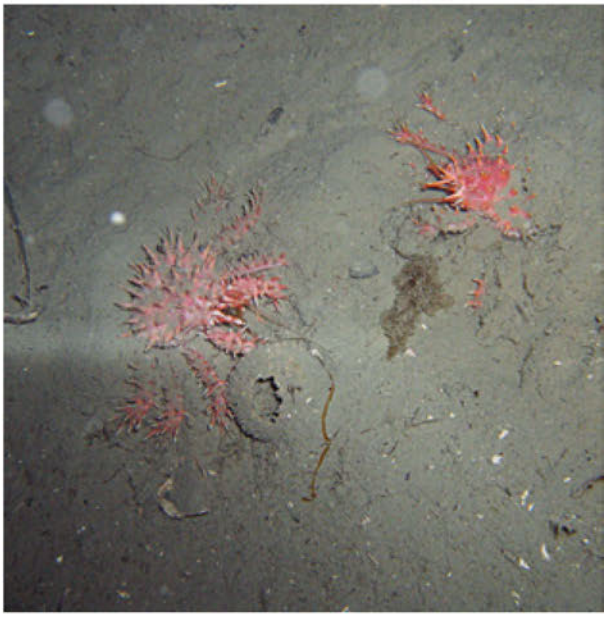
Propagurus gaudichaudi
H. Milne Edwards, 1836

Common name: Colourful hermit crab,
Gaudichaudi's hermit crab;
Ermitaño colorido
Synonymy: *Pagurus gaudichaudii* Milne Edwards, 1836; *Bernhardus barbiger* Milne Edwards, 1891; *Eupagurus patagoniensis* Benedict, 1892.
Description: Large; carapace length to 43.3 mm. Colourful; base colour of chelipeds and pereopods reddish-brown, superior margins purple-blue; chelipeds with several longitudinal series of black tipped spines. Carapace with triangular rostrum. Chelipeds unequal in size, with several longitudinal series of black tipped spines; right cheliped longer and stronger. Pereopods compressed, pilose; superior margins with row of spines. **Possibility for confusion:** None (see also key on p. 629).
Habitat: Rocks and other hard substrate. **Depth:** 5–150 m. **Abundance:** Infrequent. **Distribution:** SW Atlantic (Argentina); SE Pacific (PP–CPZ); Strait of Magellan. *Chile:* 31°S–54°S. **Biology:** Ovigerous females were found in the months January, April and July. Larval development has not yet been described. It inhabits empty snail-shells of *Voluta ancilla*, *V. magellanica*, *Fusus* sp., *Argobuccinum magellanicum*, *Trophon geversiaunus*, *Natica* sp. **Comments:** This is the largest of all Chilean hermit crabs.
Main references: Haig (1955); McLaughlin (2003).

Propagurus gaudichaudi

105

Lithodes santolla



Lithodes santolla

(Molina, 1782)

Common name: Southern king crab; Centolla del sur
Synonymy: *Lithodes antarctica* Jacquinot, 1844; *Pseudolithodes zenkevitchi* Birstein & Vinogradov, 1972.
Description: Large; carapace length to 19.8 cm, width to 25 cm. Carapace spiny; pereopods red, sometimes with violet tinge or white tipped spines; dorsal side whitish. Young densely covered with long acute spines, adults with less pronounced spines. Carapace almost pentagonal; gastric and cardiac regions well-developed, separated from other carapace regions by deep depressions, equipped with 4 spines of similar size arranged in square shaped pattern; antero-lateral edge with orbital cavity replaced by long, thick external orbital spine; rostrum with bifid, upwardly directed short anterior projection revealing the basal spine; basal spine with pair of dorsal spines located at base. Abdomen soft, covered with calcified plates, segment 2 composed of 3 plates. Chelipeds unequal; right cheliped larger than left; palms covered with tufts of setae. Pereopods elongate; 2 longer than others. Chelipeds and pereopods (except for antero-lateral portions) densely covered with spines. **Possibility for confusion:** *L. turkayi*, which differs in rostrum details: its anterior projection is long and concealing the basal spine.
Habitat: Adults: soft and hard bottoms; juveniles: holdfasts of *Macrocystis* sp. **Depth:** Intertidal–700 m. **Abundance:** Frequent. **Distribution:** SW Atlantic (Uruguay; Argentina); SE Pacific (PP–SPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 39°S–54°S. **Biology:** Ovigerous females carry 5,000–32,000 eggs; observed September–November. Hatching periods extended and vary in length (few weeks to months, see also *Paralomis granulosa*). Larval development through 3 zoea and 1 megalopa stage. **Population status:** Due to heavy over-fishing, populations dramatically declined in recent years. **Comments:** Deep-water emerging (eurybathic); reaching shallow depths in fjords.
Main references: Haig (1955); MacPherson (1988).

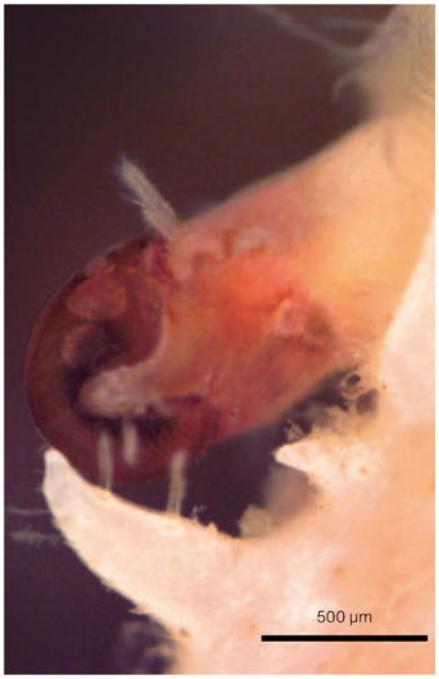
Paralomis granulosa

(Jacquinot, 1847)

Common name: False King crab; Centollón
Synonymy: *Lithodes granulata* Jacquinot, 1853; *L. verrucosa* Dana, 1852.
Description: Medium size; carapace length to 11 cm. Carapace red; dorsal surface covered with darker-red tubercles; ventral surface whitish. Carapace more or less pentagonal, somewhat broader than long; dorsal surface densely covered with clustered granules; gastric, cardiac and branchial regions pronounced, covered with clusters of granules variable in size, granules more widely spaced in adults than in juveniles; antero-lateral margins armed with 12–14 spines; rostrum slightly overtopping eyes, upwardly curved, armed with 2 forwardly directed, lateral spines. Chelipeds covered with granules, except on inner surface; anterior slightly pilose; carpus with 5–6 stout spines on high, medial crest. Pereopods short; external surface covered by small granules; dactyli slightly curved, with 5–6 spines on anterior margin. **Possibility for confusion:** None (see also key on p. 630).
Habitat: Adults: soft and hard bottoms in sheltered inshore waters; juveniles: holdfasts of *Macrocystis* sp. which provide them with mechanical shelter. **Depth:** 10–100 m. **Abundance:** Common, sometimes in large aggregations. **Distribution:** SW Atlantic (Argentina, Falkland Islands); SE Pacific (NPZ–CPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 41°S–54°S. **Biology:** In April, ovigerous females are observed carrying 1,540–8,200 eggs. Larval hatching occurs in low daily numbers, but over an extended period of time—up to several weeks. Larval development through 2 zoea and 1 megalopa stage. **Population Status:** Unknown.
Main references: Haig (1955); MacPherson (1988).

Paralomis granulosa

Eurypodius latreillei



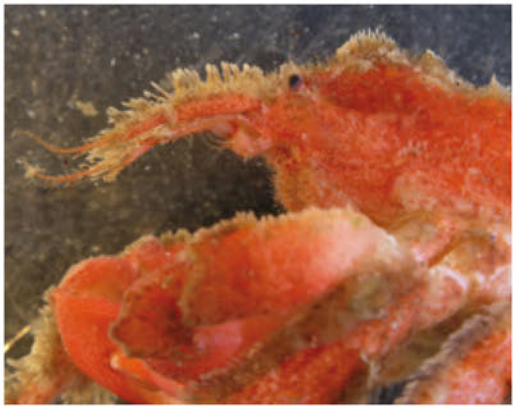
Eurypodius latreillei Guérin, 1825

Common name: Camouflaged spider crab;
Cangrejo arena
Synonymy: *Eurypodius cuviere* Andouin, in De Haan, 1838; *Eurypode tuberculateux* Eydoux & Souleyet, 1842; *E. tuberculatus* Eydoux & Souleyet, 1842; *Eurypodius andoninii* Milne Edwards & Lucas, 1842; *E. septentrionalis* Dana, 1851; *E. brevipes* Dana, 1851; *E. danae* Targioni-Tozzetti, 1877; *E. quiriquinensis* Yanez, 1948; *E. longirostris* Miers, 1886.
Description: Medium size; carapace lengths to 6.8 cm in males and 5.3 cm in females. Base colour greenish brown; lateral margins of carapace and pereopods beneath greyish. Carapace rough, equipped with tubercles and short spines (5 median, 2 gastric, 1 genital, 1 cardiac, and 1 at posterior margin); rostrum with horns stout, almost horizontally orientated, tapering distally, sometimes curving ventrally towards the tip. Supraorbital margin unarmed, interantennular tooth present and formed by a strong pointed spine. Pereopods with dilated, compressed propodi. Morphology extremely variable, especially direction and length of rostral horns, and prominence and sharpness of tubercles and spines on carapace. **Possibility for confusion:** None.
Habitat: Adults: in deeper waters on muddy or sandy substrates; juveniles: in sublitoral and shallow waters on hard substrates. **Depth:** An archibenthal species. 17–130 m. **Abundance:** Common. **Distribution:** SW Atlantic (Brazil–Argentina; Falkland Islands); SE Pacific (Peru; PP–CPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 18°S–54°S. **Biology:** Ovigerous females are observed in the months December through May. Larval development through 2 zoea stages and 1 megalopa stage. Specimens found in shallow waters (usually juveniles) were covered with algae, sponges and bryozoans. Adult individuals reported from deeper habitats were not camouflaged.
Main references: Rathbun (1918b); Garth (1957).

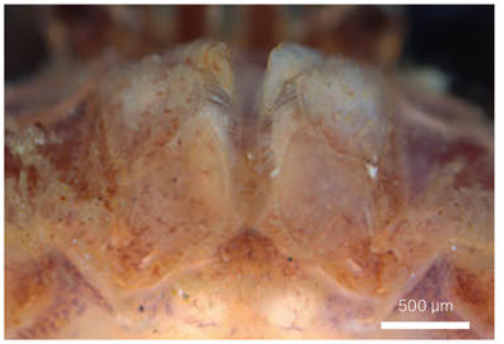
Pisoides edwardsii (Bell, 1835)

Common name: Edwards spider crab;
Cangrejo decorador, Cangrejo araña
Synonymy: *Pisoides tuberculosus* Milne Edwards & Lucas, 1843.
Description: Medium size; carapace length to 30 mm. Carapace and pereopods reddish, chelipeds red; ventral surface beige. Carapace triangular; branchial, gastric and genital regions with ornamentation of prominent tubercles; rostrum bifurcate; rostral horns of equal size, slightly divergent, pilose, downwardly directed. Preocular spine absent, postocular spine large and sharp. Antennae with subquadrate basal article; other articles flat and ciliate on outer side, overreaching rostral horns. Chelipeds short, robust; palm glabrous. Pereopods, and merus and carpus of chelipeds, covered with short dense velvet. **Possibility for confusion:** None. It is the only representative of this genus in Chile.
Habitat: Rocky and sandy bottoms and macroalgae (*Lessonia nigrescens, L. trabeculata, Macrocystes integrifolia*). **Depth:** 10–70 m. **Abundance:** Unknown. **Distribution:** SE Pacific (Panama; Galapagos Islands; Peru; PP–CPZ); Strait of Magellan. *Chile:* 18°S–54°S. **Biology:** Ovigerous females observed in February. Larval series consists of 2 zoea stages and 1 megalopa stage. As other representatives of the family Majidae, *P. edwardsii* covers its carapace with various benthic organisms for camouflage (e.g. sponges, hydroids and algae).
Main references: Rathbun (1918b); Garth (1957).

Pisoides edwardsii



Halicarcinus planatus



Halicarcinus planatus (Fabricius, 1775)

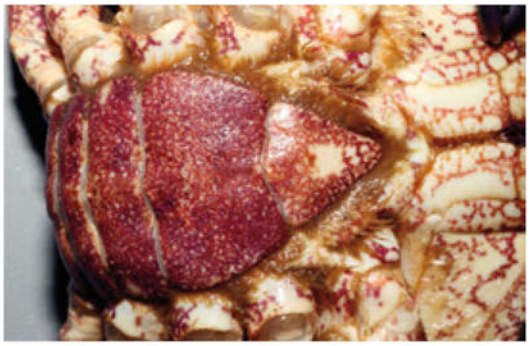
Common name: Flattened crab; Cangrejito aplanado
Synonymy: *Cancer orbiculus* Fabricius, 1775; *Hymenosoma leachii* Guérin-Méneville, 1838; *Liriopea lucasii* Nicolet, 1849; *Halicarcinus pubescens* Dana, 1851; *Hymenosoma tridentate* Hombron & Jacquinot, 1846.
Description: Small; carapace length to 8 mm, width to 13 mm. Colour brown to light brown or greyish, often with reddish areas. Pereopods sometimes banded. Carapace soft, oval, flat or depressed, with distinct rim; below with lateral teeth 3 frontal teeth, the medium one smaller and less extended than the outer ones; and 2 lateral teeth, the anterior one being small and obtuse, the posterior one well-developed and pointed. Pereopods successively shorter; dactyli flattened, slightly curved; pereopod 2 longer than others. **Possibility for confusion:** None. *H. planatus* is the only representative of the family Hymenosomatidae in American seas.
Habitat: Under stones, on various benthos organisms (e.g. algae, hydrozoan and bivalve colonies) and in rock pools. **Depth:** Low intertidal–270 m. **Abundance:** Frequent. **Distribution:** Subantarctic Islands; SW Pacific (New Zealand); SW Atlantic; SE Pacific (PP–CPZ); Strait of Magellan. *Chile:* 35°S–54°S. **Biology:** Ovigerous females were found in the months November to May. Larval development through 2 zoea and 1 megalopa stage. Moving slowly on substrate. **Comments:** *H. planatus* is the only brachyuran crab with a circum-Antarctic distribution.
Main references: Rathbun (1925); Garth (1957).

Peltarion spinosulum (White, 1843)

Common name: Tractor crab; Cangrejo peludo
Synonymy: *Peltarion magellanicus Jacquinot*, 1847; *Atelecyclus chilensis* Nicolet, 1849.
Description: Medium size; carapace length to 55 mm. Dorsal surface red with white spots; ventral surface white. Carapace as long as broad, with granular surface; anterior and lateral margins dentate; front tridentate; lateral teeth slender, about half as long as rostrum; rostrum narrow, biacuminate. Antennules and antennae longitudinally directed; basal article of antenna shorter than the following, less advanced than suborbital tooth. Chelipeds equal; upper margins spinous; surface rough with stout spinules in 5 longitudinal rows on outer surface of palm; dactyli subtriangular. Upper margin of chelae, pereopods, and lower part of carapace fringed with long silky hair. **Possibility for confusion:** None. *P. spinosulum* is the only representative of the genus *Peltarion* in South American waters.
Habitat: Soft bottoms: sand or mud. **Depth:** Intertidal–300 m. **Abundance:** Common. **Distribution:** SW Atlantic (Uruguay; Argentina; Falkland Islands); SE Pacific (PP–CPZ); Tierra del Fuego. *Chile:* 20°S–54°S. **Biology:** Lives hidden in burrows. Ovigerous females were found in March. Larval development through 4 zoea stages and 1 megalopa stage.
Main references: Rathbun (1930); Garth (1957).

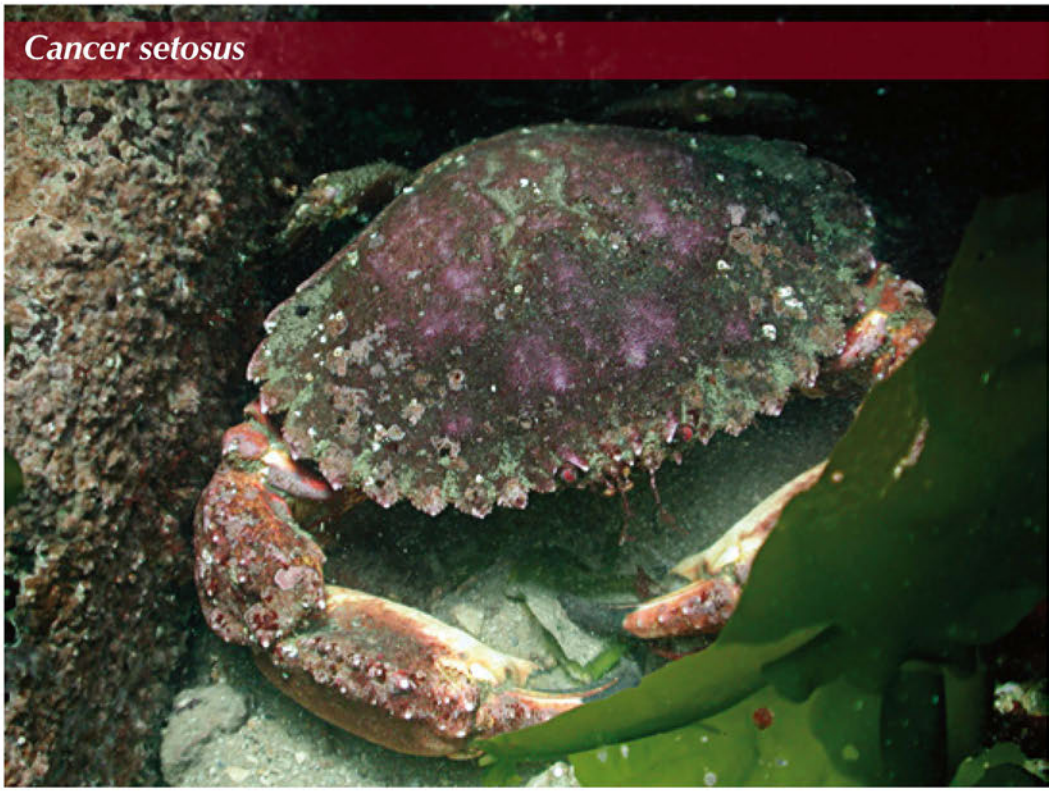
Peltarion spinosulum

Cancer edwardsi



Cancer coronatus

Cancer setosus



Cancer edwardsi Bell, 1835

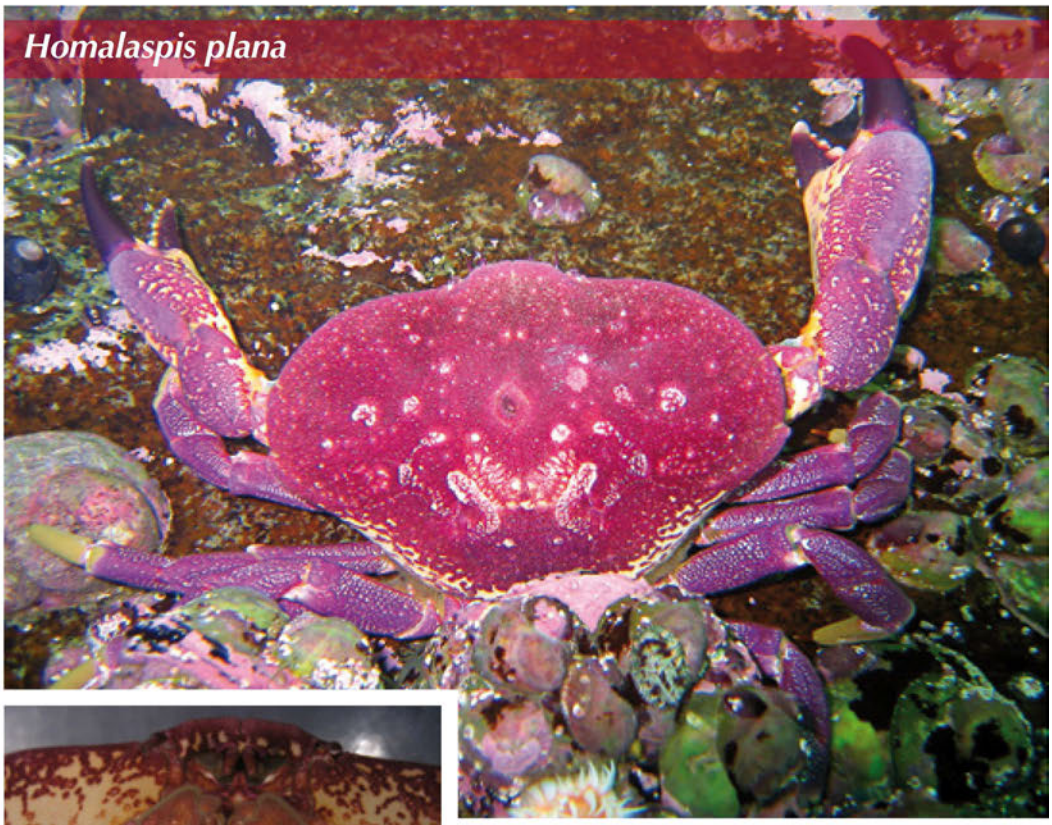
Common name: Rock crab; Jaiba marmola
Description: Large; carapace widths to 22 cm. Upper side reddish brown, sometimes variegated with orange, lower side yellow mottled with reddish. Carapace of juveniles with various colours; ventral side mostly light yellow, marbled or spotted in dark purple; joints of pereopods regularly annulated with broad bands of same colour. Carapace strongly convex; marginal rim slightly granulated; lateral margin with teeth alternating with 1 or 2 emarginations. 3 short, thick lobiform frontal teeth between antennae; median one smallest and only slightly overreaching adjacent pair. No teeth on outer orbital angle. Antenna with basal article 2x as long as broad. Cheliped with 5 outer and 2 upper carinae on palms; dactyli with black colour reaching ⅔ of length from tips. **Possibility for confusion:** May be confused with 3 other species from the same genus, 2 of which can be found in the fjord region; distinguishing characteristics are summarised in the table below.
Habitat: Sand, algae and rocks. **Depth:** 2–45 m. **Abundance:** Common. The most abundant of the Chilean *Cancer* species. **Distribution:** SE Pacific (Ecuador; Peru; PP–CPZ); Strait of Magellan. *Chile:* 18°S–54°S. **Biology:** Nocturnal species; during the day it hides in cavities and under stones. Ovigerous females were found between July and August. Larval development through 1 praezoea, 5 zoea and the megalopa stages. Like most decapod crustaceans, *C. edwardsi* is omnivorous and has a preference for polychaetes, bivalves and dead fish. It is well adapted for catching shell-protected prey, which they crush with their powerful chelipeds. **Population Status:** Unknown. **Comments:** *C. edwardsi* is fished all along the Chilean coast. Local fishermen collect it while diving; traps are also used.
Main references: Rathbun (1930); Garth (1957).

Feature	*C. edwardsi*	*C. coronatus*	*C. porteri*	*C. setosus*
Common names	Jaiba mamola	Jaiba reina	Jaiba limón	Jaiba peluda
Carapace	granulate	depressed granulated	densely granulated	pilose and finely granulated
Basal article of antenna	2x as long as broad	⅔ as broad as long	nearly as broad as long	2x as long as broad
Median frontal tooth	slightly over-reaching the adjacent pair	narrower, more projecting	broadly triangular	slender, more pronounced than outer pair
Distribution	SE Pacific (Ecuador; Peru; PP–CPZ); Strait of Magellan *Chile:* 18°S–54°S	SE Pacific (Peru, S of 12°S: PP–CPZ); Strait of Magellan *Chile:* 18°S–54°S	N Pacific (Panama) to SE Pacific (PP) *Chile:* 18°S–33°S	SE Pacific (Peru, S of 11°S; PP–NPZ) *Chile:* 18°S–47°S

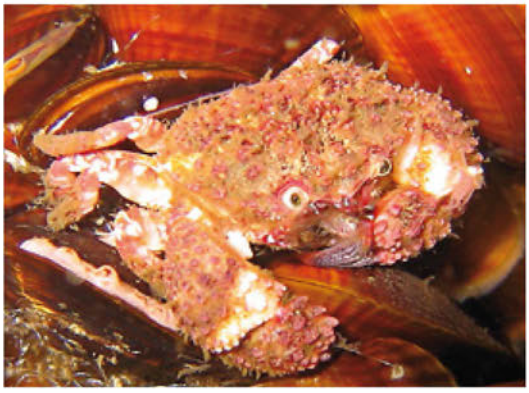
Homalaspis plana (H. Milne Edwards, 1834)

Common name: Blackberry crab; Jaiba mora
Synonymy: *Gecarcinus regius* Poeppig, 1836.
Description: Large; carapace diameter 1.5–10.5 cm. Dorsal side purple with white, outlined mesogastric region; ventral side (beneath lateral margins) whitish with purple spots; abdomen purple. Carapace robust, broad, longitudinally convex; covered with coarse, depressed granules; regions indistinct except for faintly outlined mesogastric region. Margins obscurely lobed: frontal region narrow, fused with orbital margin, anterior margin bilobed. Maxilliped 3 coarsely granulate, merus widest in middle, bearing 2 deep depressions. Chelipeds massive, merus almost entirely covered by carapace. Pereopods also strong; dactyli densely pilose. **Possibility for confusion:** None. *H. plana* is the only *Homalaspis* species in America.
Habitat: Rocks and other hard substrate. **Depth:** 2–18 m. **Abundance:** Probably rare. **Distribution:** SE Pacific (Ecuador; Peru; PP–CPZ); Juan Fernandez Islands; Strait of Magellan. *Chile:* 18°S–54°S. **Biology:** Ovigerous females were observed in the months July to December. The larval development through 4 zoea and 1 megalopa stage. Carrion, molluscs and sea urchins are their main nutrition. **Comments:** In Chile the "Jaiba mora" is commercial exploited.
Main references: Rathbun (1930); Garth (1957).

Homalaspis plana



Pilumnoides perlatus



Pilumnoides perlatus (Poeppig, 1836)

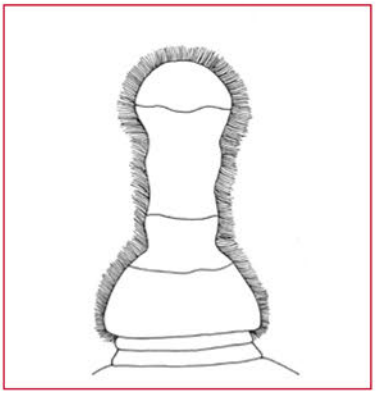
Common name: Garnet crab; Cangrejo granate
Synonymy: *Pilumnoides danai* Kinahan, 1857.
Description: Medium size; carapace length to 25 mm. Dorsal side reddish-brown with whitish patches. (juveniles with various colours between dark pink and reddish-brown); dactyli of chelipeds dark brown, usually white tipped. Carapace broader than long; anterior ²/₃ tuberculate; tubercles partly forming short transverse stripes; posterior ¹/₃ almost smooth; anterior margin with 5–6 irregular teeth. Cheliped with lower half of palm with tubercles arranged in rows; upper edge of palm trilobate. **Possibility for confusion:** None. *P. perlatus* is the only representive of this genus in Chile.
Habitat: On hard substrates; on kelp-holdfasts, on bivalve colonies and among ascidians (*Pyura chilensis*). **Depth:** Low intertidal–40 m. **Abundance:** Frequent. **Distribution:** NE Atlantic (Ireland; English Channel, England); Caribbean Sea (Tobago Islands; Panama); SE Pacific (Peru; PP–CPZ); Strait of Magellan. *Chile:* 18°S–54°S. **Biology:** Ovigerous females were found from June to November. Larval development through 5 zoea and 1 megalopa stage.
Main references: Rathbun (1930); Garth (1957).

Acanthocyclus albatrossis Rathbun, 1898

Common name: Fringy arm wrestler crab; Cangrejo a franjas pulseando
Description: Medium size; carapace length to 26.2 mm. Carapace surface dark brown; pereopods brighter; propodi whitish; dactyli of cheliped whitish with light purple areas. Carapace slightly broader than long, rotund; surfaces granulate or tuberculate; lateral margins armed with prominent, acute teeth. Median front tooth variable in shape, either entire or faintly bi-lobed, overreaching adjacent pair of lateral teeth in length. Orbit <2x as wide as deep in dorsal view. Chelipeds stout, very unequal. Pereopods with pilose upper margins; dactyli long, slightly curved. **Possibility for confusion:** None. From Chile 2 other Acanthocyclus species are described, which occur further north (*A. gayi*: 18°S–37°S and *A. hassleri*: 18°S–39°S) (see also key on p. 632).
Habitat: Rocks and other hard substrate. **Depth:** Intertidal–15 m. **Abundance:** Abundant. **Distribution:** SW Atlantic (Falkland Islands); SE Pacific (PP–SPZ); Strait of Magellan; Tierra del Fuego. *Chile:* 36°S–56°S. **Biology:** Nocturnal species. During daytime hides under stones and other sheltered places; at night feeds on sessile invertebrates (e.g. mussels, barnacles). Ovigerous females were observed in the months November to April. Larval development through 4 zoea stages and 1 megalopa stage.
Main references: Rathbun (1930); Campodónico & Guzmán (1973).

Acanthocyclus albatrossis

Pinnixa bahamondei



Pinnixa bahamondei Garth, 1957

Common name: Commensal parchment worm crab; Cangrejo comensal

Description: Small; carapace width to 10 mm. Males smaller than females. Carapace with brown cardiac and branchial regions; lateral margins densely punctuated with red spots; pereopods with pattern of light orange and red areas; joints red. Carapace sublong, without cardiac ridge; surface smooth; antero-lateral margins marked by crest; shoulders with dorsal, vertical side walls; posterior margin straight. Maxilliped 3 with large palpus, small, narrowly rectangular merus. Chelipeds slender, hairy; manus with superior and inferior row of granules. Pereopods 1 and 2 slender; last 2 pairs somewhat stout. Abdomen with widest part opposite segment 3 and narrowest part opposite segment 4; segments 4–6 constricted, with some degree of fusion. Subtidal specimens tend to have a more ornate spinulation than intertidal specimens. **Possibility for confusion:** None.

Habitat: Commensal crab found in tubes of the tube worm *Chaetopterus* sp. **Depth:** Intertidal–25 m. **Abundance:** Unknown. **Distribution:** SE Pacific (PP–NPZ). *Chile:* 29°S–43°S. **Biology:** Ovigerous females were observed in July. Larval development has not as yet been completely described.

Main references: Rathbun (1918a); Garth (1957).

Pinnaxodes chilensis

(Milne-Edwards, 1937)

Common name: Commensal sea urchin crab; Jaiba comensal del erizo

Synonymy: *Pinnaxodes hirtipes* Heller, 1865.

Description: Small; with pronounced sexual dimorphism; carapace length of females much larger (to 19.7 mm) than males (to 6.6 mm). *Females:* Whitish to beige. Carapace with hepatic regions and abdomen dotted with red patches. Body very soft. Carapace subquadrate, parchment-like, smooth; anterior margin straight; lateral margins rounded and posterior margin concave. Last of maxilliped 3 overreaches penultimate segment. Eyes segment partially visible in dorsal view. Ventral body surface (including margins of front, the chelipeds and pereopods) covered with long soft hairs. Chelipeds equal, stout, elongated. Pereopods of similar shape, nearly equal in length. Abdomen covering sternum. *Males:* With firmer body than females. Carapace wider than long, convex, narrows posteriorly, widest at level of pereopod 3; antero-lateral margins arcuate; lateral margins sloping steeply. Chelipeds equal, with concave, hirsute carpus on inner side. Pereopods long, slender; pereopod 2 longest; 4 shortest. Abdomen widest at middle of somite 3; somites 4 and 5 straight, somite 6 constricted in the middle, somite 7 alate at base, rounded at tip. **Possibility for confusion:** None. *P. chilensis* is the only representative of this genus in Chile and the female has a characteristic parasitic life.

Habitat: Hard substrates. **Depth:** 5–12 m. **Abundance:** Probably rare. **Distribution:** SE Pacific (Ecuador; Galapagos Islands; Peru; PP–CPZ). *Chile:* 18°S–53°S. **Biology:** The female of this parasitic crab is mainly found in the sea urchin species *Caenocentrotus gibbosus* and *Loxechinus albus*. It is eaten alive with lemon juice as a delicacy. The free-living male is seldom observed. **Comments:** Specimens shown were purchased at the "Mercado de mariscos Angelmo", Puerto Montt.

Main references: Rathbun (1918a); Garth (1957).

Pinnaxodes chilensis

Cyclograpsus cinereus

Cyclograpsus cinereus Dana, 1851

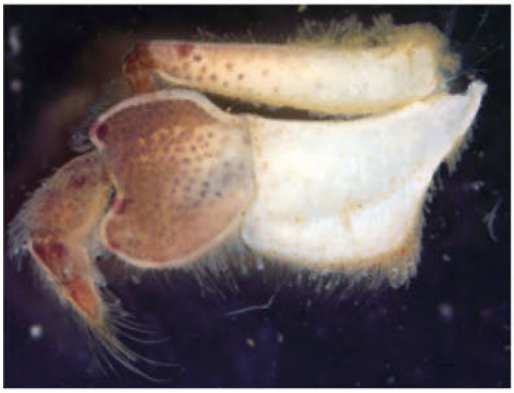
Common name: Rock crab; Jaiba de roca
Synonymy: *Cyclograpsus minutus* Hombron & Jacquinot, 1846.
Description: Small; carapace length increases with geographic latitude (20°S: 4.0–8.0 mm; 41°S: 5.7–14 mm). Carapace and pereopods bay-coloured on upper side, beneath lighter. Carapace with frontal surface deflexed, smooth; antero-lateral margins granulated. Deep furrow extending backwards from below orbit. Pereopods without projections; pereopod 2 longest, <2x as long as carapace; propodi with sparsely-pilose distal ½ of lower margin. **Possibility for confusion:** In Chile 2 more *Cyclograpsus* species are described: *C. punctatus* H. Milne Edwards, 1837 (Chile: 31°S–33°S), which has an obtuse tooth on the merus joints of the pereopods, and *C. longipes* Stimpson, 1858 (Chile: Easter Island), which is a small species (carapace range: 4.6–7.8 mm) and is characterized by having straight, posteriorly diverging, lateral carapace margins.
Habitat: Hides under stones and other hard substrate. **Depth:** Intertidal. **Abundance:** Locally common. **Distribution:** SE Pacific (Peru; PP–NPZ). *Chile:* 18°S–43°S. **Biology:** Ovigerous females can be found in northern Chile (ca. 20°S) in July and in southern Chile (ca. 42°S) in December. Larval development through 5 zoea and 1 megalopa stage.
Main references: Rathbun (1918a); Garth (1957).

Hemigrapsus crenulatus

(H. Milne Edwards, 1837)

Common name: Hairy-handed shore crab; Huillancha, Pancora, Yasca
Synonymy: *Trichodactylus granarius* Nicolet, 1849; *T. granulatus* Milne Edwards, 1853; *Heterograpsus barbigerus* Heller, 1862; *H. barbimanus* Heller, 1865; *H. sanguineus* Lenz, 1902.
Description: Medium size; males with carapace width of up to 40 mm; females smaller (up to 25 mm). Carapace with variable colours (grey brown, blue grey, olive-brown); ventral side and chelipeds whitish. Carapace broader than long, with 2 lateral teeth; postero-lateral margins convergent. Carapace covered with coarse, closely set granules; except for posterior region; posterior region punctate connected by fine impressed lines; front slightly arched. Antenna not filling orbital hiatus. Chelipeds granulate, carpus and palm of males with pilose patches inside, lacking in females. Pereopods of medium width, slightly pilose; dactylus of pereopod 5 relatively broader than those of other pairs.
Possibility for confusion: Easily distinguished from other shore crabs by the pilose patches on the inner side of the carpus and palm. The only species of the genus *Hemigrapsus* that occurs in Chile.
Habitat: Shallow water and estuaries. Lives in a wide variety of habitats: under stones, burrowing in sand and mud. **Depth:** Intertidal–10 m. **Abundance:** Common. **Distribution:** SW Pacific (New Zealand); SE Pacific (PP–CPZ); Strait of Magellan. *Chile:* 20°S–54°S. **Biology:** Ovigerous females were found in November. Larval development has not yet been completely described. Nocturnal; during the day hidden under stones, in sand holes and other sheltered places. They are very effective scavengers. **Comments:** The pilose patches inside the carpus and palms probably allow the crabs to wipe over the surfaces of various objects to capture food particles (diatoms).
Main references: Rathbun (1918a); Garth (1957).

Hemigrapsus crenulatus



127

Glossary

Abdomen	*Sensu lato* body part or tagma of an arthropod between thorax and telson, sometimes also called tail; an abdomen *sensu stricto* is deprived of legs, if there are legs it should be named pleon.
Accessory branches	Lateral branches originating from the main branch.
Acuminate	Pointed.
Acute	Pointed, sharp.
Alate	Wing-like.
Antenna	2nd pair of antennae situated between antennules and orbits.
Antennula	1st, anteriormost pair of antennae.
Arcuate	Bow-shaped.
Areolate	Around a central structure, an area of specific colour or form.
Arthrobranch	Gills located on joint membrane of coxopodit (basal leg segment).
Article	Segment, serially arranged cuticula ring.
Basis (pl. bases)	Basipodit, 2nd leg segment.
Biacuminate	Branched hair with 2 pointed tips.
Bifid, bifurcate	Forked.
Bilobed	Made of 2 lobes.
Biramous	Made of 2 branches.
Buccal cavity	Buccal cavern; cavity on ventral side of body containing mouthparts, posterior to epistome.
Carina (pl. carinae)	Keel.
Carpus (pl. carpi)	5th leg or maxilliped segment.
Cornea; corneal	Cuticle of compound eyes.
Crest	Comb-like structure.
Crista (pl. cristae)	Comb-like structure.
Dactylus (pl. dactyli)	7th or terminal (ultimate) leg or maxilliped segment.
Decapodit stage	Megalopa, late developmental stage with setose natatory pleopods on some or all of abdominal somites 1–5.
Deflexed	Not bent.
Dentate	Provided with teeth-like structures.
Dentiform	Tooth-shaped.
Dorsal groove	Notch having a dorsal position.
Emarginations	Protrusions.
Endopod(ite)	The leg of a decapod has an outer and an inner branch, the former called exopodite, the latter endopodite.
Epibranchial spine	Spine located on branchial region of carapax, i.e. its lateral parts.
Epimeron (pl. epimera)	Lateral sclerite of a segment, sometimes also called bladebone.
Epipod(ite)	Exit, appendix of a leg, e.g. gills.
Epistome	Cuticula plate forming anterior border of buccal cavity.
Exopod(ite)	The leg of a decapod has an outer and an inner branch, the former called exopodite, the latter endopodite.
Eyestalk	Movable protrusion on which compound eyes are inserted.
Flagellum (pl. flagella)	Distal, long section of antennules and antennae composed of numerous articles.
Glabrous	Deprived of hair.
Hiatus (pl. hiatuses)	Break or gap.
Inferior crest	Inner comb.
Interantennular tooth	Tooth located between the antennules.
Ischium (pl. ischia)	3rd leg segment.
Latero-inferior region	Outer-inner region.

Manus	Palm; proximal part of a cheliped's propodus.
Merus	4th leg segment.
Mesogastric lobe	Median section of gastric region.
Multiarticulate	Having many articles.
Nauplius	Early larva of many crustacea.
Orbit	Insertion of the compound eyes.
Ovigerous	Egg-carrying.
Palm	Manus; proximal part of cheliped's propodus.
Palp, palpus (pl. palpi)	Distal segments of maxilliped.
Peduncle	Proximal section of antennules and antennae.
Pilose	Hairy.
Pleopod	Leg located on one of the pleon segments.
Pleural tooth	Tooth-like structure having a lateral position.
Pleurobranch	Gill inserted on pleura or lateral sclerites of segment.
Pleuron (pl. pleura)	Lateral sclerite(s) of an arthropod leg.
Posterior tubercle	Nodule in hindmost position.
Postorbital spine	Spine behind orbits.
Preorbital spine	Spine in front of orbits.
Prezoea stage	Larval instar before zoea stage.
Propodus	6th (penultimate) leg segment.
Protandric	Animal passing male stage prior to becoming female.
Proximal lobe	Lobe close to observer or symmetry plane of body.
Pubescence	Structure having a fluffy shape.
Pyriform	Pear-shaped.
Rostrum (pl. rostra)	Front; anteriormost part of carapax projecting anteriorly from between eyestalks.
Scaphocerite	Stylocerite; process at base of antennules.
Septum (pl. septa)	Wall.
Seta (pl. setae)	Hair.
Somite	Body segment.
Spiniform tubercles	Protrusions with pointed tips.
Spinulation	Pattern and shape of spines.
Sternal region	Ventral part of body.
Sternum (pl. sterna)	Ventral, segmented wall of thorax.
Stylocerite	Scaphocerite; process at base of antennules.
Subdorsal	Dorsal, but not extremely dorsal.
Subelliptical	Not perfectly elliptical, but close to it.
Subequal	Almost equal.
Suborbital tooth	Tooth located under orbits.
Suboval	Almost oval.
Subquadrate	Almost of a square shape.
Subtriangular	Almost of a triangular shape.
Sulcus (pl. sulci)	Notch, groove.
Superior crest	Upper comb.
Supraocular spine	Spine located above eyes.
Supraorbital	Above the orbits.
Trident, tridentate	Having 3 pointed tips.
Trilobate	Terminating in 3 lobes.
Turgid	Swollen.
Zoea	Larval instars of Decapoda, between prezoea and Mysis, Megalopa or Decapodit stages.

Bibliography

Boschi, E.E., Fischbach, C.E. & Iorio, M.I. (1992) Catálogo ilustrado de los crustáceos estomatópodos y decápodos marinos de Argentina. *Frente Marítimo*, 10: 94 pp.

Campodónico, I. & Guzmán, L. (1973) Contribución a la biología de *Acanthocyclus albatrossis* Rathbun, 1898. (Crustacea, Decapoda, Brachyura, Atelecyclidae). *Anales del Instituto de la Patagonia, Punta Arenas (Chile)*, 4 (1-3): 373-416.

Garth, J.S. (1957) The Crustacea Decapoda Brachyura of Chile, Report No. 53 of the Lund University Chile Expedition 1948-1949. *Lunds Universitets Arsskrift*, 2: 1-130.

Guzmán, G. (2003) *Crustáceos Chilenos: Orden Decapoda. Guías de Identificación y biodiversidad Fauna Chilena*. Iquique, Universidad Arturo Prat.

Guzmán, G. (2004) *Isocheles aequimanus* (Dana, 1852) (Decapoda, Anomura, Paguroidea): revalidación para la carcinofauna chilena. *Investigaciones Marinas, Valparaíso*, 32 (2): 129-132.

Haig, J. (1955) The Crustacean Anomura of Chile, Report No. 51 of the Lund University Chile Expedition 1948-1949. *Lunds Universitets Arsskrift*, 2: 1-68.

Haig, J. (1960) *The Porcellanidae (Crustacea, Anomura) of the Eastern Pacific*, Allan Hancock Pacific Expedition.

Holthuis, L.B. (1957) The Crustacea Decapoda Macrura of Chile, Report No. 47 of the Lund University Chile Expedition 1948-1949. *Lunds Universitets Arsskrift*, 2 (10): 1-110.

Jara, C.G. (1997) Antecedentes sobre el desarrollo de la carcinología en Chile. *Investigaciones Marinas, Valparaíso*, 25: 245-254.

MacPherson, E. (1988) Revision of the Family Lithodidae Samouelle, 1819 (Crustacea, Decapoda, Anomura) in the Atlantic Ocean. *Monografías de Zoologia Marina*, 2: 9-153.

MacPherson, E. (1992) *Paralomis phrixa* (Decapoda, Anomura, Lithodidae), a new species from northern Peru, and a key to the Eastern Pacific species of the genus. *Crustaceana*, 63: 313-317.

Martin, J.W. & Davis, G.E. (2001) *An updated classification of the recent Crustacea*. Natural History Museum of Los Angeles County, 132 pp.

McLaughlin, P.A. (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 (1): 111-144.

Ng, P.K.L., Guinot, D. & Davie, P.J.F. (2008) Systema Brachyurorum: Part 1. An annotated checklist of extant brachyuran crabs of the world, *Raffles Bulletin of Zoology. Supplement Series* (17), 286 p.

Rathbun, M.J. (1918a) The grapsoid crabs of America. *Bulletin of the United States National Museum*, 97: 1-461.

Rathbun, M.J. (1918b) The spider crabs of America. *Bulletin of the United States National Museum*, 129: 1-611.

Rathbun, M.J. (1925) The spider crabs of America. *Bulletin of the United States National Museum*, 129: 1-613.

Rathbun, M.J. (1930) The cancroid crabs of America of the Families Euryalidae, Portunidae, Atelecyclidae, Cancridae and Xanthidae. *Bulletin of the United States National Museum*, 152: 1-609.

Retamal, M.A. (2000) Decápodos de Chile. *World Biodiversity Database*. Berlin, Springer Verlag.

Roberts, P.E. (1973) Larvae of *Munida subrugosa* (White), 1847, from Preseverance Harbour, Campbell Island. *Journal of the Royal Society of New Zealand. Zoology*, 3 (3): 393-408.

Rodríguez, L. & Bahamonde, R. (1986) Contribución al conocimiento de *Munida subrugosa* (White, 1847) en la XII Región, Chile. *In:* Arena, P. (ed.) *La pesca en Chile*. Universidad Católica de Valparaíso, Escuela de Ciencias del Mar, pp. 283-296.

Thatje, S. (2003) *Campylonotus arntzianthus*, a new species of the Campylonotidae (Crustacea: Decapoda: Caridea) from the Scotia Sea (Antarctica). *Polar Biology*, 26: 242-248.

Thatje, S., Bacardit, R., Romero, M.C., Tapella, F. & Lovrich, G.A. (2001) Description and key to the zoeal stages of the Campylonotidae (Decapoda, Caridea) from the Magallan region. *Journal of Crustacean Biology*, 21: 492-505.

Torti, M.R. & Boschi, E.E. (1973) *Nuevos aportes al conocimiento de los crustáceos decápodos Caridea del género Campylonotus Bate, 1888*. Physis, Buenos Aires, 65 pp.

Viviani, C. (1969) Los Porcellanidae (Crustacea, Anomura) chilenos. *Beitrage zur Neotropischen Fauna*, 4 (1): 40-56.

Wehrtmann, I.S. & Albornoz, L. (1998) Larval development of *Nauticaris magellanica* (A. Milne Edwards, 1891) (Decapoda: Caridea: Hippolytidae), reared under laboratory conditions. *Bulletin of Marine Science*, 62 (1): 45-72.

9. General discussion and results

The present thesis provides a variety of modern methods used to analyse the morphology of different life stages of decapods. The aim was to establish and characterize new morphological features in larval stages with the use of scanning electron, light and confocal microscopy for taxonomic purpose. Furthermore an inventory of the decapod fauna of the southern Chilean fjord area was carried out and results are analysed with both classical morphological methods and with the use of COI - barcoding in an integrative approach for species delimitation of southern Chilean Decapoda.

9.1. Larval morphology and SEM

The descriptions of the thitherto undescribed first zoea stage of *Gnathophyllum elegans* (article II) and *Portunus acuminatus* (article I) are mainly based on SEM data. With the use of the resolving power of the SEM it was possible to identify and describe several characters of zoea larvae not generally given in standard descriptions, e.g. the mandible structure and sensory dorsal organ (SDO). We furthermore were able to display tiny structures and describe their steric arrangement i.e. the composition of the carapace structure of *P. acuminatus* and the reliable description of the different types of setae and their classification according to Watling (1989) and Garm (2004).

Both larval descriptions included in this thesis, enable us to display details of the gross three-dimensional structure of the mandibles of the zoea larvae. This feature often is omitted in larval descriptions due to its complex structure and hidden position under other mouthparts, which is not easy to resolve with the predominantly used description technique of light microscopy (Ingle, 1992). Geiselbrecht and Melzer (2010) discussed the significance of mandible morphology as a set of characters for phylogenetic examinations and indicated that a significant phylogenetic signal is present on the features of the mandibles. Our results of the mandible structures of *G. elegans* and *P. acuminatus* support this conclusion on the basis of available data. Thus, structural analyses of this organ could give access to a relevant and yet poorly studied set of characters for larval diagnosis and further phylogenetic studies. Since the number of species for which detailed larval descriptions are available is still quite limited, this feature needs to be analyzed in more detail in future larval descriptions of larval stages.

The sensory dorsal organ (SDO) is a sensory glandular complex which is located along the sagittal line at the anterior part of the cephalic shield (see article III). It is documented in the Eumalacostraca but is also known in the Hoplocarida and the Phyllocarida (Laverack &

Macmillan, 1999). We observed this seldom described organ in various malacostracan larvae (articles I, II, III) and observed that the arrangement of the elements (4 sensors and 1 central gland) varies greatly in external appearance throughout different species. Presumably due to its small dimensions it is not described in classical descriptions based on light microscopy. Within the studied species the external morphology is a constant species-specific feature and therefore could be used as a potential character for species description and determination. A homologous structure can be found on the cephalic shield of cyprid larvae and is named the "lattice organ (LO)". Examinations of the LO show that the variations in the arrangement are phylogenetically informative (Celis et al., 2008). Due to the lack of described SODs in larval descriptions of decapod crustaceans it was not possible to carry out further comparative studies, but on the base of our examinations we expect this feature as phylogenetically informative.

9.2. Zoea larvae and confocal microscopy

Confocal microscopy was used to investigate the density and number of nuclei in zoea larvae as a diagnostic feature. As a model organ the telson was used since its flattened form makes it relatively easy to visualize the nuclei completely and evaluate its density.

This potential "taxonomically-relevant-feature" was checked within zoea larvae of representatives of all major infraorders of the Decapoda i.e. Caridea (*Palaemon adspersus* Rathke, 1837, *Palaemon elegans* Rathke, 1837), Anomura (*Porcellana platycheles* (Pennant, 1777), *Pisidia longicornis* (Linnaeus, 1767)) and Brachyura (*Xantho hydrophilus* Herbst, 1780, *Xantho pilipes* A. Milne Edwards, 1867). Results indicate that the arrangement as well as the density of nuclei varies at least on higher taxonomic level e.g. genus or families or suborders between the selected species. Comparisons of results between the nearly related species *Palaemon adspersus* / *Palaemon elegans* respectively *Xantho hydrophilus* / *Xantho pilipes* show no significant variations. Therefore on species level no relevant differences were recorded. Hence it can be stated that the detected taxonomic signal is exclusively valid at higher taxonomic level.

This approach based on confocal microscopy data as a modern method in taxonomy, shows that diagnostic features can be found on many different levels using various sets of characters. In the history of taxonomy, several of these "levels" have been checked for their taxonomic value i.e. biochemical characters such as protein, enzyme and hemoglobin chemistry, DNA hybridizations and immunochemistry (Baker, 1965, Manwell & Kerst, 1966, Throckmo,

1968, Goodman & Moore, 1971). However these biochemical characters seem to be most informative at higher taxonomic level (Sibley, 1960). Manwell and Baker (1963) studied biochemical factors in blood sera of marine arthropods and were able to observe qualitative and considerable qualitative variations of blood sera of different decapod-species (*Emerita talpoida* (Say, 1817) and *Uca (Leptuca) pugilator* (Bosc, 1802). However closely related species were not studied and the authors quoted that it is possible that under such circumstances the individual variations observed in these crustaceans could obscure existing species specificity. Furthermore, possible protein variations during physiological effects such as the moulting process were not considered in the study. Consequently these methods could serve as tools for higher taxonomy but have no resolution on species level. Further morphological independent approach was established by Moore and Goodman (1968) as the authors introduced immunodiffusion comparisons in taxonomy. In this approach, distances between species were calculated on the base of Ouchterlony data.

In our work, we studied the taxonomic potential of nuclei-pattern and sizes of decapod zoea larvae as a further, "exotic" character and the obtained results may contribute to species diagnosis as a new feature in taxonomy. Other relevant species features, including morphological and molecular characters can be supported by these results and form a substantiated set of taxonomic data for species identification and thus for taxonomic purpose.

9.3. Inventory and barcoding of Chilean decapods

The objectives of this project were (1) to get an overview of species richness through a systematic inventory of this nearly unexplored region as reference data for further investigations, (2) to check on this basis the systematic state of different species with integrative taxonomy (barcoding and classical morphology), and (3) to analyse these selected species complexes for the presence of cryptic species.

The systematic inventory of the southern Chilean fjord region is carried out since 2005 in organisation of the Huinay Scientific Field Station (www.fundacionhuinay.cl/projects.html). Since then over 650 lots with decapod samples have been analysed for this thesis at the Bavarian State Collection of Zoology and results have been published in the field guide "Marine Benthic Fauna of Chilean Patagonia" (article VI). The identification and determination of species was based on eidonomic, morphological features. In addition to the original descriptions, available identification literature was used (Rathbun, 1918, Rathbun, 1925, Garth, 1957, Retamal, 1981, Retamal & Gorny, 2001).

The taxonomic state of the species in the genera *Acanthocyclus* (*A. gayi*, *A. hassleri*, *A. albatrossis*) and *Eurypodius* (*E. longirostris* and *E. latreillii*) was not clear though species descriptions in literature are confusing. To check species boundaries of these "problematic cases" integrative taxonomy was used. In cooperation with Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007) selected decapod specimens of all collected species were barcoded for the first time for this region and analysed with the focus on theses 2 genera (article V). Data sets including collection data, specimen photos and the COI-sequence can be accessed on the BOLD website under the project CFAD (Chile Fjord Arthropods Decapoda) as part of the campaign 'Marine Life (MarBOL)'.

Species definition and identification based on eidonomic features only can sometimes be unclear because interpretation of character sets by different taxonomists can have a subjective component (Padial et al., 2010). On the other hand molecular data can be misinterpreted: false data could occur in the way of sampling specimen tissue, its preservation and other effects like the occurrence of mitochondrial pseudogenes (Song et al., 2008). To minimize and compensate these effects both methods have to be combined and furthermore new sets of characters must be added (like the SOD and setae morphology in larvae descriptions) to the given morphological data.

In the Chilean case study it was possible to identify constant morphological features in the species of the genera *Acanthocyclus* and *Eurypodius* (article V) by the the use of integrative taxonomy. Furthermore, on the basis of the collection data of the systematic inventory we were able to give new biogeographic information of distribution ranges of the collected species. Collection data including exact geographic coordinates are available at the BOLD website or on the sampling voucher of each specimen at the Bavarian State Collection of Zoology.

9.4. Conservation aspects

In the second half of the 20th century the Chilean Patagonia was discovered as one of the last natural marine and terrestrial exploitable areas on the planet. Infrastructure like forestry roads and the Carretera Austral offered better access into the remoter parts of the region. Industrial fisheries harvested large portions of fish with unknown consequences for food webs and artisanal fishermen not only satisfied own consumption and local markets but were integrated in the national and international markets. But the activity with the most dramatic impact on the Chilean Patagonian benthos and the marine life in the fjords is the development of

aquaculture. (Gowen & Bradbury, 1987, Johannessen et al., 1994, Häussermann et al., 2013). This alarming progress is now broadly recognized as a critical element of ecosystem change and a major threat to local diversity. Only by bringing these unique biocoenoses to the attention of the (scientific) public, the fish-farming community and the enforcement agencies will hopefully have a better understanding and acceptance for the need of protection of these scientifically little known resources (Reed, 2002).

With our efforts in supporting and participating at the "Huinay Fjordos" expeditions since 2006, and with publishing our results not only in scientific journals (article V, article VI) but also in popular scientific journals (Meyer & Melzer, 2012) and presentation on various conferences we hope to place this problem more and more in the public focus.

10. Conclusions and outlook

The future of taxonomy should be based in the combination of different methods (e.g. molecular, morphological, geographical, biochemical, data) to create a more and more perfect set of characters for each species. Taxonomy is a dynamic issue and methods applied are developing with the technological change. From the use of basic microscope technology and drawings for species description to high tech microscopy and next generation sequencing producing a high amount of specific data it took centuries. But in newer times, methods are changing faster and faster and will influence this scientific discipline rapidly.

In this thesis, we established new morphological data for zoea-larvae using modern methods. Molecular data for decapods of the unique southern Chilean fjord region are made available for colleagues around the world. We showed that different approaches can lead to well based taxonomic results and that features for species characterizations are available on several different levels. The combination of these results with the reinvestigation of type and other historical material create a significant species specific data set. Thus further research using integrative taxonomy can help to check species boundaries, identify cryptic species and finally characterize these properly.

But these results are just small pieces of a giant puzzle. To understand and characterize our environment, future projects have to pursue an interdisciplinary approach in order to identify new and cryptic species and define their species boundaries. The proper identification of species, their characterization and taxonomic evaluation forms an essential basis for a caring relationship with nature. To achieve these aims ongoing studies, especially in remote areas

like the southern Chilean fjord region are essential to create a platform for regimentations and conservation activities.

11. Relevant posters

Parts of this thesis by publication have been presented in poster format at different congresses. As a final summary and overview the relevant posters have been added.

11.1. Morphology of the first zoeal stage of the spotted bumblebee shrimp *Gnathophyllum elegans* (Risso,1816) studied with light and scanning electron microscopy. Presented at the Jahrestagung der DZG (Deutsche Zoologische Gesellschaft), 21.-24.09.2012, Konstanz, Germany.

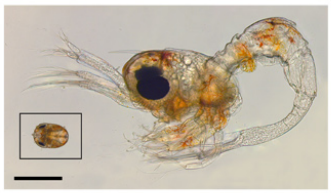
Morphology of the first zoeal stage of the spotted bumblebee shrimp *Gnathophyllum elegans* (Risso, 1816) (Decapoda: Caridea: Gnathophyllidae) studied with light microscopy and scanning EM

Roland Meyer, Tobias Lehmann, Roland R. Melzer, Hannes Geiselbrecht

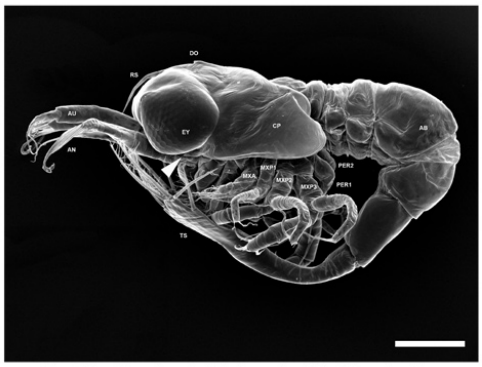
Zoologische Staatssammlung, Münchhausenstr. 21, D-81247 München, Germany; T: +49898107140, email: rolandmeyer@hotmail.de

INTRODUCTION

The morphology of the first stage zoea of *Gnathophyllum elegans* raised in the laboratory is illustrated and described for the first time. Selected features are presented here. Larvae were obtained from an ovigerous female, caught in shallow waters west of the isle of Šolta (43°23´00´´N,16°13´47´´O), Croatia.



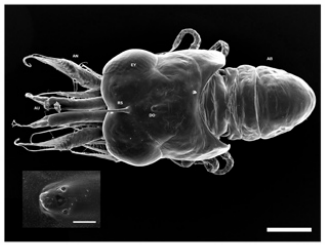
Above left: Adult specimen of *G. elegans* (size 1.7cm). Above right: Lateral view of zoea-I of *G. elegans*. Unfixed specimen showing natural coloration. (bar=250µm). Insert showing dorsal view of larvae in egg integument (egg size about 700µm).

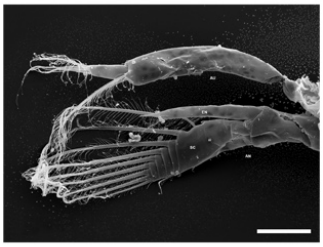


Above: Lateral view of zoea-I of *G. elegans* (bar=200µm). Arrowhead shows pterygostomial spine.

Carapace: Short, slender unarmed rostral process; epigastric tubercle with dorsal organ present; carapace armed with pterygostomial spine, otherwise absence of spines.
Abdomen: 6-segmented; strongly flexed between third and fourth segments; 1 small simple seta on the posterior-lateral margin of the 3rd and 4th somite present.

Figure: Dorsal view of zoea-I (bar=200µm); insert shows detail of dorsal organ (bar=20µm).



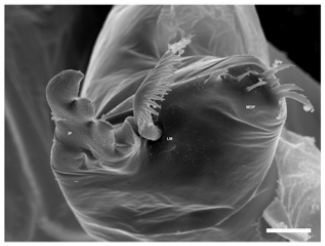


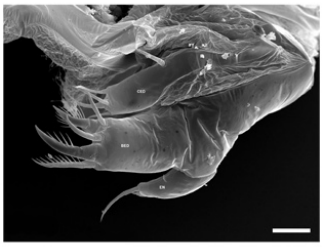
Antennule: Subcylindrical, unsegmented peduncle with 1 terminal plumose seta; flagellum with 4 aesthetascs and 1 plumose seta.
Antenna: Biramous, protopod unarticulated, with medio-terminal small spine; endopod apically with 1 plumose seta and 1 short spine; scaphocerite with 4 segmentations distally and 9 plumose setae distomedially, first and last reduced, with small tubercle proximally on medial border. 1 small seta on the outer side of the proximal article and 1 small terminal plumose seta, 1 small simple seta on the apex.

Figure: Dorsal view of left anntenule and antenna (bar=100µm).

Mandible: Right and left mandible almost identical; mandibular palp absent. Incisor process a marginal protrusion bearing 3 acute spines, 1 submarginal spine and a serrated `lacinia mobilis´. Molar process slender, bearing a group of small spines.

Figure: Inner view of left mandible (bar=10µm).



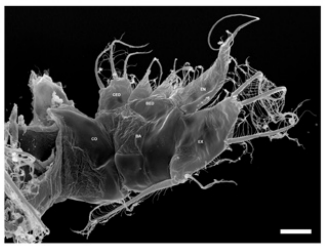


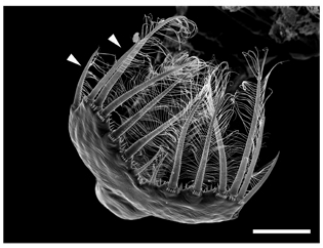
Maxillule: Endopod compressed, stout, terminally acute with 1 preterminal simple seta; coxal endite with 4 simple terminal setae and 1 slightly serrated proximal located seta; basal endite with 2 stout and 1 slender plumose setae and 2 simple setae distally; exopod absent.

Figure: Posterio-ventral view of right maxillule (bar=20µm).

Maxilla: Coxal endite with 3 simple setae, basal endite bilobed, proximal lobe with 1 simple seta, and distal lobe with 2 simple seta, endopod unsegmented with 1 long simple seta and microtrichia on inner margin. Exopod with 5 marginal plumose setae.

Figure: Posterio-ventral view of left maxilla (bar=20µm).





Telson: Fused with 6th abdominal segment; broadly triangular; 7+7 plumodenticulate setae on the posterior margin; the inner- and outermost smaller, the 2 outermost on each side with setules medially; minute spines only between 4 inner setae; setal bases armed with minute spines except inner and outermost.

Figure: Distal part of telson, arrowheads mark 2 outermost setae medially equipped with setules (bar=100µm).

DISCUSSION

Differential diagnosis between *G. elegans* and *G. americanum* Guérin-Méneville,1855 the only other *Gnathophyllum* species with available zoea description (Bruce, 1986) shows distinct differences in seta patterns on the antennule, maxilla, all maxillipeds and the telson. Hence both species can be clearly distinguished by larval morphology. The systematic position of the genus *Gnathophyllum* is under question since Bruce (1986) discovered strong similarities between the larvae of *G. americanum* and different representatives of the subfamily of the Pontoniinae. Comparison of our results with the partner shrimp *Periclimenes amethysteus* Risso, 1827, also studied with the scanning EM (Geiselbrecht & Melzer, 2009), shows several common features, hence supporting the need for further examinations on this systematic background.

Abbreviations
AB: Abdomen; AN: Antenna; AU: Antennule; BA: Basis; BED: basal endit;CED: coxal endit; CO: Coxa; CP: Carapace; DO: Dorsal organ; EN: Endopod; EX: Exopod; EY: Compound eye; IP: incisor process; LM: `lacinia mobilis´; MOP: molar process; MXA: Maxilla; MXP1-3: Maxilliped 1-3; PER1+2: Pereiopod 1+2; RS: Rostral spine; SC: Scaphocerite; TS: Telson.

References
Bruce, A.J. (1986) Obseravtions on the family Gnathophyllidae Dana, 1852 (Crustacea: Decapoda). Journal of Crustacean Biology 6(3): 463-470.
Geiselbrecht, H. & Melzer, R. R. (2009) Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* Risso, 1827 (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the scanning EM. Zootaxa 2140: 45-55.

Abstract

The morphology of the first stage zoea of *Gnathophyllum elegans* raised in the laboratory is illustrated and described for the first time. Larvae were obtained from an ovigerous female, caught in shallow waters west of the isle of Šolta (43°23´00´´N,16°13´47´´O), Croatia.

Differential diagnosis between *G. elegans* and *G. americanum* Guérin-Méneville, 1855 the only other *Gnathophyllum* species with available zoea description Bruce (1986) shows distinct differences in seta patterns on the antennule, maxilla, all maxillipeds and the telson. Hence both species can be clearly distinguished by larval morphology. The systematic position of the genus *Gnathophyllum* is under question since Bruce (1986) discovered strong similarities between the larvae of *G. americanum* and different representatives of the subfamily of the Pontoniinae. Comparison of our results with the partner shrimp *Periclimenes amethysteus* Risso, 1827, also studied with the scanning EM (Geiselbrecht & Melzer, 2009), shows several common features, hence supporting the need for further examinations on this systematic background.

11.2. Decapoda of the Chilean fjords: taxonomy and biogeography. Presented at the 104th Jahrestagung der DZG (Deutsche Zoologische Gesellschaft), 09.-12.09.2011, Saarbrücken, Germany.

Abstract

The decapod fauna of the southern Chilean fjord region (41°- 55° South) is partially well investigated by several large expeditions, e.g. the Lund university Chile expedition 1948-1949. However only easily accessible areas were hitherto studied and sampled. The aims of this work are to extend the sampling area to the benthos communities of scuba-accessible depths of the inner fjords, to include relatively unexplored subantarctic regions with its unique environmental conditions, e.g. eurybathy, and to establish a taxonomic and biogeographic survey of Decapoda fauna from the Chilean Fjords.

About 600 decapod samples representing 16 decapod families were collected predominantly by several expeditions carried out by the Huinay Scientific Field Station from 2005 to now. In addition to determination, and taxonomic revision, distribution patterns of different species are given and set in a biogeographic context. Selected morphological features of several species are reinvestigated and combined with molecular data in order to check species state and delimitations.

11.3. Decapoda of the Chilean Fjords: DNA Barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* H.Milne Edwards & Lucas, 1844 and *Eurypodius* Guérin, 1825. Presented at the Crustacean Society (TCS) summer meeting 03.-07.07.2012, Athens, Greece.

DECAPODA OF THE CHILEAN FJORDS: DNA Barcoding and integrative taxonomy with focus on the brachyuran genera *Acanthocyclus* (Belliidae) and *Eurypodius* (Inachidae)

Meyer, R. & Melzer, R. R.

Zoologische Staatssammlung München, Münchhausenstrasse 21, 81247 München, Germany;
Tel.: +49898107140, Fax: +49898107300, email: rolandmeyer@hotmail.de

About 600 decapod samples representing 41 species out of 21 decapod families were collected predominantly by several expeditions in the southern Chilean fjord region (41°- 55° South) organised by the Huinay Scientific Field Station, Chile from 2005 to now. COI sequence data of 154 specimens were analysed (project CFAD of MarBol., Marine Barcode of Life). Specimens for barcoding were chosen under geographic aspects to make sure selected samples cover the entire sampling area. The data set was extended with sequences of GenBank (see fig. 1,"+" numbers in brackets). Molecular analyses were performed using RAxML for maximum likelihood (ML), Mega5 for neighbour joining (NJ) and maximum parsimony (MP) (all 1000 bootstraps) and results are displayed in the consensus tree (fig.1) performed by MrBayes. Test for "barcoding gaps" was made with ABGD (Puillandre et al. 2011).

The species-level taxa are supported by high bootstrap and posterior probability values. This shows that DNA barcoding is a useful tool for specimen identification and resolving relationships in the fjord decapods at species level. The sequenced specimens grouped into clusters corresponding to known morphological species.
From this body of sequenced taxa we choose the genera *Eurypodius* Guérin, 1825 and *Acanthocyclus* Lucas, in H.Milne Edwards & Lucas, 1844 for detailed analysis. Both genera consist of 2 respectively 3 morphologically very closely related species. Species description and character diagnosis in literature is sometimes confusing and not always clear. To check species state and delimitations we combined molecular with morphological data.

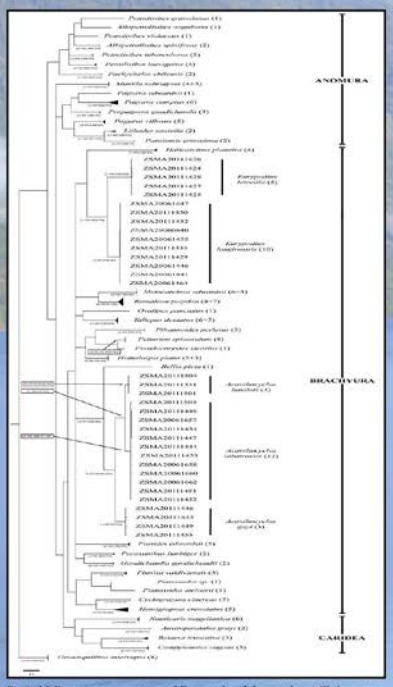


Fig.1: MrBayes consensus tree of Decapoda of the southern Chilean area. Posterior probabilities and bootstrap values (>90%) are given for MrBayes/ NJ/ML/MP; specimen number in brackets (Huinay samples + mined sequences of GenBank).

Fig.2: Ranked distances of *Eurypodius latreillii* and *E. longirostris* representing 2 taxa.

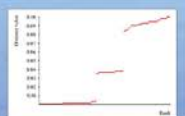


Fig.3: Ranked distances of *Acanthocyclus gayi*, *A. hassleri* and *A. albatrossis* representing 3 taxa.

***Eurypodius* Guérin, 1825**

This genus consists of 2 morphologically very close species: *Eurypodius latreillii* Guérin, 1825 and *E. longirostris* Miers, 1886 (Ng et al. 2008).

Eurypodius latreillii Guérin, 1825
Synonymy: *Eurypodius audouini* H. Milne Edwards & Lucas, 1842, *Eurypodius brevipes* Dana, 1851, *Eurypodius cuvieri* Audouin, in De Haan, 1838, *Eurypodius danae* Targioni-Tozzetti, 1877, *Eurypodius quirliquinensis* Yanez, 1948, *Eurypodius septentrionalis* Dana, 1851, *Eurypodius tuberculatus* Eydoux & Souleyet, 1842. The high number of synonyms displays the morphological variety of this species.

Eurypodius longirostris Miers,1886
Synonymy: none. The holotype of this species described by Miers was dredged off the coast of Chiloe and is "in a much broken condition" (Miers 1886).

Our molecular analyses of this genus show 2 strongly supported clades (posterior probability values 1, bootstrap values > 90%) with a mean distance value within the genus of 12, 1 % (fig.2). Morphological comparisons of these 2 clades with the type material of *E. latreilli* (Holotype RMNH D 42178, Nationaal Natuurhistorisch Museum, Leiden) and *E. longirostris* (Holotype, ZOO2012-247T, British Museum of National History) show that these 2 lineages correlate with the 2 valid species. Furthermore we compared in literature given morphological species diagnoses with all 54 specimens available from Huinay expeditions. We observed an extremely high morphological variety, e.g. orientation and direction of rostral horns (fig.4). However a single constant and therefore distinct morphological feature was found to distinguish these 2 species: the absence or presence of the supraorbital spine (fig.4).
Our examinations on the molecular level support the species state of *Eurypodius longirostris* and *E. latreillii*.

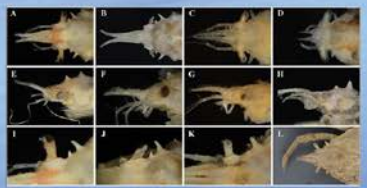


Fig.4: Variety of selected morphological features (A,B,E,F,I,J *E. longirostris*; C,D,G,H,K,L *E. latreillii*): A-D: direction and shape of rostral horns; E-H: orientation of rostrum in lateral view; I-L: presence or absence of the supraorbital spine; J,L: Type material.

***Acanthocyclus* Lucas, in H. Milne Edwards & Lucas, 1844**

Only 3 species are in this genus: *Acanthocyclus gayi* Lucas, in H. Milne Edwards & Lucas, 1844, *Acanthocyclus albatrossis* Rathbun, 1898 and *Acanthocyclus hassleri* Rathbun, 1898 (Ng et al. 2008). All species inhabit the Chilean coast and are so closely related that they may easily be mistaken for one another (Rathbun 1930).

Acanthocyclus gayi Lucas, in H. Milne Edwards & Lucas, 1844
Synonymy: *Acanthocyclus villosus* Strahl, 1862, *Plagusetes elatus* Heller, 1862

Acanthocyclus hassleri Rathbun, 1898 and *Acanthocyclus albatrossis* Rathbun, 1898 without synonym.

Results of the molecular analysis show that the *Acanthocyclus*-complex clusters in 3 clades. The clades are supported by high bootstrap values (posterior probability values >0,98, bootstrap values >90%) with a mean distance within the genus of 3, 5 % and 9, 6 % (fig.3). The 3 lineages correspond more or less with the known morphological species. However morphological comparisons of the 3 clades in-between and with (till this moment available) type material (Holotype *A. hassleri*, MCZ CRU-4889, Museum of Coomperative Zoology, Harvard and *A. gayi* RMNH D 43615, Nationaal Natuurhistorisch Museum, Leiden) and given species descriptions in literature show that these species can only be distinguished from each other by a set of morphological characters. Most of the morphological characteristics e.g. form of carapace lateral teeth, shape of front, existence of hairs on carapace and ambulatory legs, orientation and shape of ischium and merus joints of *Acanthocyclus* given in Rathbun, 1930 are due to their high variety not constant (fig.5). However on the base of our morphological and molecular data we can suggest an identification key for the 3 species of *Acanthocyclus* of the southern Chilean Fjord Region:

(1) Front entire (2)
Front bilobed.....*A. albatrossis*
(2) Carapace and ambulatory legs covered with hair.....*A. gayi*
Carapace not covered with hair.....*A. hassleri*

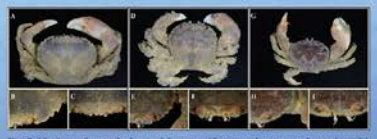


Fig.5: Variety of morphological features (front and carapace lateral teeth) in the *Acanthocyclus*-species-complex: A-C: *A. albatrossis*; D-F *A. gayi*; G-I: *A. hassleri*.

Conclusions

The use of a morphology independent data set (COI sequences) in addition to the classical morphological data of the species-complexes confirms in these 2 cases the species state and delimitations, but allows to clarify the diagnostic features that allow unequivocal diagnoses in the "difficult species" in the future. By the support of the phylogenetic analysis and the high amount of samples we were able to rank given morphological features to their utility for species determination. Furthermore we expand the DNA barcode database involving these species of this hitherto unexplored region and reconfirm the usefulness of DNA barcoding for the identification of marine decapods.

Acknowledgements

We want to thank Günter Försterra and Dr. Vreni Häussermann (Huinay Scientific Field Station, Chile) for the organisation of the Huinay Fjordos expeditions and supporting us with samples. Special thanks got to all participants of the expeditions for collecting and Dr. Dirk Steinke from the Canadian Centre for barcoding at the department of Zoology University of Guelph, Ontario, Canada for processing, coordinating and providing the sequence data. Furthermore we would like to thank Dr. Charles Fransen (National Naturhistorisch Museum, Leiden), Ms Miranda Lowe (British Museum of National History) and Mr. Adam Baldinger (Museum of Comparative Zoology, Harvard) for making us the type species available. This study was supported by the Sea Life Centre Munich, Germany.

References

Meyer R, Lochner S, Melzer RR (2009) Decapoda - crabs, shrimps & lobsters. In: Häussermann, V. & Försterra, G. (eds.) 2009. Marine Benthic Fauna of Chilean Patagonia, Nature in Focus, Santiago de Chile, 1000 pp. Spanish version: Häussermann, V. & Försterra, G. (eds.) 2009. Fauna Marina Bentonica de la Patagonia Chilena, Nature in Focus, Santiago de Chile, 1000 pp, ISBN 978-956-332-243-9 spanish; 978-956-332-244-6 english.
Miers, E.J. (1886) Report on the Brachyura collected by H.M.S. Challenger during the years 1873-1876. In: Murray, J. (ed.) Zoology. Report on the Scientific Results of the Voyage of H.M.S. Challenger During the Years 1873-76 Under the Command of Captain George S. Nares, R.N., F.R.S. and the Late Captain Frank Tourle Thomson, R.N. Wyville Thomson, C. and J. Murray (series eds.) Vol. 17. Edinburgh: Neil and Company. Pp. I-362, Plates 1-29.
Ng, P.K.L. Guinot, D. Davie, P.J.F. (2008) Systema Brachyurorum: Part I. An annotated checklist of the extant Brachyuran crabs of the world. The Raffles Bulletin of Zoology 17: 1-286.
Puillandre, N., Lambert, A., Brouillet, S., Achaz, G. (2011) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology, 21: 1864-1877.
Rathbun, M.J. (1930) The cancroid crabs of America of the families Euryalidae, Portunidae, Atelecyclidae, Cancridae, and Xanthidae. Bulletin of the United States National Museum 152: 1-609.

Abstract

The decapod fauna of the southern Chilean fjord region (41°- 55° South) is partially well investigated by several large expeditions, e.g. the Lund university Chile expedition 1948-1949. However only easily accessible areas were hitherto studied and sampled. The aims of this work are to extend the sampling area to the benthos communities of scuba-accessible depths of the inner fjords, to include relatively unexplored subantarctic regions with its unique environmental conditions, e.g. eurybathy, and to establish a taxonomic and biogeographic survey of the Decapoda fauna from the Chilean Fjords.

About 600 decapod samples representing 31 species out of 16 decapod families were collected predominantly by several expeditions carried out by the Huinay Scientific Field Station, Huinay, Chile from 2005 to now. In addition to determination, and taxonomic revision, distribution patterns of different species are given and set in a biogeographic context. Selected morphological features of several species are reinvestigated and combined with molecular data in order to check species state and delimitations. COI (cytochrome c oxidase subunit 1) sequence data of 190 specimens are analysed and set in context with morphological results. 93,5 % of the sequenced specimens grouped into clusters corresponding to known morphological species. For 2 species complexes (Acanthocyclus sp. and Eurypodius sp.) the occurrence of cryptic lineages is suggested by our data: specimens cluster in 3 and 2 groups respectively. Furthermore we expand the DNA barcode database involving these species of this hitherto unexplored region and reconfirm the usefulness of DNA barcoding for the identification of marine decapods.

11.4. Preliminary results of a marine expedition to the end of the world: status and perspectives. Presented at the 3rd International Marine Protected Areas Congress (IMPAC3), Marseille, France, 21.-27.10.2013.

Preliminary results of a marine expedition to the end of the world: Status and perspectives

Daniela Henkel[1], Roland Meyer[2], Roland R. Melzer[2], Vreni Häussermann[3], Günter Försterra[3], Carlos Spano[3], Kaitlin Mae McConnell[4], Ulrich Pörschmann[4] & David Bellhoff[4]

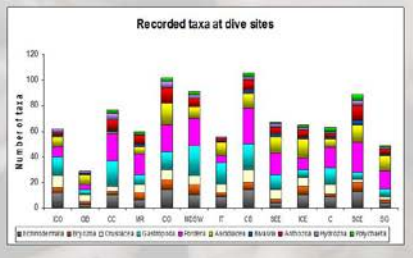
[1] University of Kiel, Leibnizstraße 3, 24118 Kiel, Germany;
[2] Zoologische Staatssammlung München, Münchhausenstraße 21, 81247 München, Germany;
[3] Pontificia Universidad Católica de Valparaíso, Avda. Brazil 2950 Valparaíso, Chile;
[4] Fundación Huinay, Casilla 462, Puerto Montt, Chile

Chile's economy is one of the fastest growing worldwide. As a consequence its environment faces increasing levels of anthropogenic pressure. With a coastline of more than 80.000 km, the Chilean fjord region is both the most extensive fjord region and one of the biggest estuarine systems in the world, hosting a unique diversity of marine life including a high level of endemic species. However, this region is exposed to various anthropogenic impacts such as global warming, intensive aquaculture and fishing. Especially salmon farming and fisheries, significant industries for Southern Chile, are negatively impacting marine life.

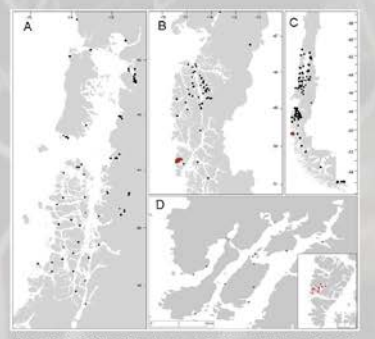
The research of the *Huinay Scientific Field Station* dedicates to the investigation of the biodiversity of the fjords of Southern Chile extending from Puerto Montt to Cape Horn. Since 2005 15 "*Huinay fiordos*" (HF) expeditions established marine species inventories of least studied regions.

In April 2013 the destination of the "HF 16" expedition was Guarello Island (50°21'54.15"S, 75°20'29.66"W) located in the remote archipelago Madre de Dios, XII Región de Magallanes y de la Antártica Chilena.

Species were obtained by scuba diving and hand collection in depths between 0 and 30m. The sampling concept followed the approved method of former expeditions: *in situ* documentation by underwater photography before preservation to ensure the documentation of habitat and coloration. In total 647 samples from 28 taxa were collected with a considerable number of species new to science (e.g. ~ 15 new poriferan species). Presence/absence lists for selected species were generated from scuba diving surveys and hand collection. Furthermore different biocoenoses were investigated and their status recorded. This recent study confirms the results of preliminary studies (HF 3) that the Madre de Dios Archipelago is a hotspot of diversity with unique communities such as hydrocoral reefs. Our data contribute to a long term biodiversity monitor program in this area, and will be compared to results of models that aim at filling data gaps to improve results from spatial planning tools.



Graph showing recorded taxa at each location of survey sites: *ICO* Isla Corbeta; *GB* Guarello Base; *CC* Canal Copihue; *MR* Muro Roberto; *CG* Conu Guarello; *MDSW* Madre de Dios South West; *IT* Islote Traro; *CB* Cross Bascuñan; *SEE* Seno Euleuterio E; *ICE* Isla Cerda; *C* Cueva; *SCE* Seno Contreras East; *SG* Seno Guarello.



Maps of the Chilean Fjord area: A-C show all "Huinay Fiordos" sampling locations since 2005 between Puerto Montt and Cape Horn (black dots); sampling locations of the "Huinay Fjordos 16" region are marked with red dots. Map D with insert show "HF 16" sampling region.

Members of the HF 16 expedition (from left to right): Ulrich Pörschmann, Daniela Henkel, Kaitlin McConnell, Roland Meyer, Luis Hernández Maldonado , David Bellhoff, Günter Försterra, Roland Melzer, Vreni Häussermann, Carlos Spano, Fiona and Fabian.

Further reading:

Häussermann V, Försterra G (2007) Large assemblages of cold-water corals in Chile: a summary of recent findings and potential impacts. In George R. Y. and S. D. Cairns, (eds): Conservation and adaptive management of seamount and deep-sea coral ecosystems. Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, 324 p:195-207.

Häussermann V, Försterra G (2007) Extraordinary abundance of hydrocorals (Cnidaria, Hydrozoa, Stylasteridae) in shallow water of the Patagonian fjord region. Polar Biology 30(4): 487-492 (online first: http://dx.doi.org/10.1007/s00300-006-0207-5).

Häussermann V, Försterra G (in review) Mass die-off of a reef-like accumulation of the hydrocoral Erina antarctica (Cnidaria, Hydrozoa) in Central Patagonia. Coral Reefs.

Häussermann, V. & Förstera, G. (eds) 2009. Marine Benthic Fauna of Chilean Patagonia, Nature in Focus, Santiago de Chile, 1000 pp.

Inarte JL, Gonzalez HE, Nahuelhual L (2010) Patagonian Fjord Ecosystems in Southern Chile as a Highly Vulnerable Region: Problems and Needs. A journal of the Human Environment 39: 463–466.

Weis A, Melzer RR (2012) How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity* 10: 361-374.

Meyer R, Weis A, Melzer RR (2013) Decapoda of the Chilean fjords: DNA Barcoding and integrative taxonomy with focus on the genera *Acanthocyclus* H. Milne Edwards & Lucas, 1844 and *Eurypodius* Guérin, 1825. *Systematics and Biodiversity*, in press.

Abstract

Chile's economy is one of the fastest growing worldwide. As a consequence its environment is faced to increasing level of anthropogenic pressure. With a coastline of more than 80.000 km, the Chilean fjord region is both the most extensive fjord region and one of the biggest estuarine systems in the world hosting a unique diversity of marine life including a high level of endemic species. However, this region is exposed to various anthropogenic impacts such as global warming, intensive aquaculture projects and illegal fishing. Especially salmon farming and cultured mussel production, significant industries for Southern Chile, are negatively impacting marine life.

The *Huinay Scientific Field Station* research is strongly focused on the investigation of the biodiversity of the fjords of Southern Chile extending from Puerto Montt to Cape Horn. Since 2005 15 "*Huinay fiordos*" (HF) expeditions established marine species inventories of least studied regions.

In April 2013 the destination of the "HF 16" expedition was Guarello Island (50°21'54.15"S, 75°20'29.66"W) located in the remote archipelago Madre de Dios, XII Región de Magallanes y de la Antártica Chilena.

Species were obtained by scuba diving and hand collection in depths between 0 and 30m. The sampling concept followed the approved method of former expeditions: *in situ* documentation by underwater photography before preservation to ensure the documentation of habitat and coloration. In total 647 samples consisting of 28 taxa were collected with a considerable number of species new to science (e.g. ~ 15 new poriferan species). The presence or absence of species was examined by scuba diving and hand collection. Furthermore different biocoenoses were investigated and their status recorded. This recent study confirms the results of preliminary studies (HF 3) as it is a hotspot of diversity with unique communities such as hydrocoral reefs. Our data are foreseen as a basis for comparisons with data obtained in further investigations in the context of a long term biodiversity monitor program in this area by the approach for detecting key data gaps by using MARXAN "reversely" though stimulation of data set improvements.

12. References

Abele L.G. (1974) Species diversity of decapod crustaceans in marine habitats. *Ecology*, **55**, 156-161.

Aikawa H. (1937) Further Notes on Brachyuran Larva. *Records of Oceanographic Works in Japan*, **9**(1), 87-162.

Anger K. (2001) *The Biology of Decapod Crustacean Larvae*, Rotterdam, Netherlands: A.A. Balkema.

Arabi J., Cruaud C., Couloux A. and Hassanin A. (2010) Studying sources of incongruence in arthropod molecular phylogenies: Sea spiders (Pycnogonida) as a case study. *Comptes Rendus Biologies*, **333**(5), 438-453.

Baker C.M.A. (1965) Molecular genetics of avian proteins. 4. Egg-white proteins of Golden Pheasant *Chrysolophus pictus* (Linnaeus, 1758) and Lady Amherst's Pheasant *Chrysolophus amherstiae* Leadbreater, 1829 and their possible evolutionary significance. *Comparative Biochemistry and Physiology*, **16**(1).

Bassler R.S., Resser C.E., Schmitt W.L. and Bartsch P. (1931) *Shelled Invertebrates of the Past and Present with Chapters on Geological History*, New York: Smithsonian Institution Series, Inc.

Bate C.S. (1888) Report on the Crustacea Macrura collected by H.M.S. Challenger during the Years 1873-76. In Murray J. (ed) *Zoology.* vol. 24, Edinburgh: Neill and Company, pp i–xc, 1-942. [Report on the Scientific Results of the Voyage of H.M.S. Challenger During the Years 1873-76 Under the Command of Captain George S. Nares, R.N., F.R.S. and the Late Captain Frank Tourle Thomson, R.N.

Borradaile L.A. (1907) On the classification of the decapod crustaceans. *The Annals and Magazine of Natural History, series 7*, **19**, 457-486.

Bribiesca-Contreras G., Solis-Marin F.A., Laguarda-Figueras A. and Zaldivar-Riveron A. (2013) Identification of echinoderms (Echinodermata) from an anchialine cave in Cozumel Island, Mexico, using DNA barcodes. *Molecular Ecology Resources*, **13**(6), 1137-1145.

Brown I.W., Fielder D.R. and Research A.C.f.I.A. (1991) *The Coconut crab: aspects of the biology and ecology of Birgus latro in the Republic of Vanuatu*: Australian Centre for International Agricultural Research.

Bruce A.J. (1986) Observations of the family Gnathophyllidae Dana, 1852 (Crustacea: Decapoda). *Journal Of Crustacean Biology*, **6**(3), 463-470.

Burkenroad M.D. (1963) The evolution of the Eucarida, (Crustacea, Eumalacostraca), in relation to the fossil record. *Tulane Studies in Geology*, **2**(1), 3-17.

Cain A.J. (1954) *Animal Species and their evolution*, New York: Harper & Row.

Celis A., Alvarez F., Newman W.A. and Sumida P.Y.G. (2008) Descriptions and Phylogenetic Significance of the Fronto-lateral Gland Pores and Dorsal Lattice Organs of Cyprid Larvae of Seven Species of Barnacles (Cirripedia: Thoracica: Pedunculata). *Journal Of Crustacean Biology*, **28**(2), 203-215.

Costa F.O., et al. (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences*, **64**(2), 272-295.

Dayrat B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, **85**(3), 407-415.

De Grave S., Cai Y. and Anker A. (2008) Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. *Hydrobiologia*, **595**, 287-293.

De Grave S., et al. (2009) A classification of living and fossil genera of decapod crustaceans. *The Raffles Bulletin of Zoology*, **Supplement No. 21**, 1-109.

Dietz L., Mayer C., Arango C.P. and Leese F. (2011) The mitochondrial genome of *Colossendeis megalonyx* supports a basal position of Colossendeidae within the Pycnogonida. *Molecular Phylogenetics and Evolution*, **58**(3), 553-558.

Drew M.M., Harzsch S., Stensmyr M., Erland S. and Hansson B.S. (2010) A review of the biology and ecology of the Robber Crab, *Birgus latro* (Linnaeus, 1767) (Anomura: Coenobitidae). *Zoologischer Anzeiger - A Journal of Comparative Zoology*, **249**(1), 45-67.

Garm A. (2004) Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. *Zoological Journal of the Linnean Society*, **142**(2), 233-252.

Garth J.S. (1957) Reports of the Lund University Chile Expedition 1948-49 29. The Crustacea Decapoda Brachyura of Chile. *Lunds Universitets Årsskrift. N.F. Avd. 2.*, **53**(7), 1-129.

Geiselbrecht H. and Melzer R.R. (2009) Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* (Risso, 1827) (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the Scanning EM. *Zootaxa*, (2140), 45-55.

Geiselbrecht H. and Melzer R.R. (2010) Mandible of zoea I larvae of nine decapod species: a scanning EM analysis. *Spixiana*, **33**(1), 27-47.

Geiselbrecht H. and Melzer R.R. (2013) How do mandibles sense? - The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (Decapoda, Palaemonidae). *Arthropod Structure & Development*, **42**(1), 1-16.

Goodman M. and Moore G.W. (1971) Immunodiffusion Systematics of the Primates I. The Catarrhini. *Systematic Zoology*, **20**(1), 19-62.

Gowen R.J. and Bradbury N.B. (1987) The ecological impact of salmonid farming in costal waters - a review. *Oceanography and Marine Biology*, **25**, 563-575.

Gurney R. (1938) The larvae of the decapod Crustacea. Palaemonidae and Alpheidae. *Great Barrier Reef Expedition 1928–29 Scientific Reports*, **6**(1), 1-60.

Gurney R. (1942) *Larvae of decapod Crustacea*, London: Ray Society.

Haig J. (1955) Reports of the Lund University Chile Expedition 1948–49. 20. The Crustacea Anomura of Chile. *Lunds Universtitets Arsskrift N.F. Avd. 2*, **51**(12), 1-68, figs 61-12.

Haig J. (1960) The Porcellanidae (Crustacea Anomura) of the eastern Pacific. *Allan Hancock Pacific Expeditions*, **24**, 1-440.

Häussermann V., Försterra G., Melzer R.R. and Meyer R. (2013) Gradual changes of benthic biodiversity in Comau Fjord, Chilean Patagonia - lateral observations over a decade of taxonomic research. *Spixiana*, **36**(2), 161-171.

Hebert P.D.N., Cywinska A., Ball S.L. and DeWaard J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**(1512), 313-321.

Heimeier D., Lavery S. and Sewell M.A. (2010) Using DNA barcoding and phylogenetics to identify Antarctic invertebrate larvae: Lessons from a large scale study. *Marine Genomics*, **3**(3-4), 165-177.

Ingle R. (1992) *Larval Stages of Northeastern Atlantic Crabs. An Illustrated Key*, London: Chapman and Hall.

Joerger K.M., Stoeger I., Kano Y., Fukuda H., Knebelsberger T. and Schroedl M. (2010) On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia. *BMC Evolutionary Biology*, **10**.

Johannessen P.J., Botnen H.B. and Tvedten O.F. (1994) Macrobenthos: before, during and after a fish farm. *Aquaculture Research*, **25**(1), 55-66.

Knowlton N. (1986) Cryptic and sibling species among the decapod Crustacea. *Journal Of Crustacean Biology*, **6**(3), 356-363.

Knowlton N. (1993) Sibling species in the sea. In Fautin D.G. (ed) *Annual Review of Ecology and Systematics.* vol. 24, pp 189-216. [Annual Review of Ecology and Systematics.

Krabbe K., Leese F., Mayer C., Tollrian R. and Held C. (2010) Cryptic mitochondrial lineages in the widespread pycnogonid *Colossendeis megalonyx* Hoek, 1881 from Antarctic and Subantarctic waters. *Polar Biology*, **33**(3), 281-292.

Laverack M.S. and Macmillan D.L. (1999) The sensory dorsal organ of crustaceans: Theme and variations. *Marine and Freshwater Behaviour and Physiology*, **32**(2-3), 75-86.

Lebour M.V. (1930) The larvae of the Plymouth Galatheidae. I. *Munida banffica, Galathea strigosa* and *Galathea dispersa*. *Journal of the Marine Biological Association of the United Kingdom*, **17**(1), 175-187.

Lefebure T., Douady C.J., Gouy M. and 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**(2), 435-447.

Manwell C. and Baker C.M. (1963) Starch gel electrophoresis of sera from some marine arthropods: studies on the heterogeneity of hemocyanin and on a "ceruloplasmin-like protein". *Comparative Biochemistry and Physiology*, **16**, 193-208.

Manwell C. and Kerst K.V. (1966) Possibilities of biochemical taxonomy of bats using hemoglobin lactate dehydrogenase esterases and other proteins. *Comparative Biochemistry and Physiology*, **17**(3).

Martin J.W. and Davis G.E. (2001) An updated classification of the Recent Crustacea. *Natural History Museum of Los Angeles County, Science Series*, **39**, 1-124.

Masta S.E., McCall A. and Longhorn S.J. (2010) Rare genomic changes and mitochondrial sequences provide independent support for congruent relationships among the sea spiders (Arthropoda, Pycnogonida). *Molecular Phylogenetics and Evolution*, **57**(1), 59-70.

Meyer R., Friedrich S. and Melzer R.R. (2004) *Xantho poressa* (Olivi, 1792) and *Xantho pilipes* A. Milne-Edwards, 1867 Larvae (Brachyura, Xanthidae): Scanning Em Diagnosis of Zoea I from the Adriatic Sea. *Crustaceana.* vol. 77, pp 997-1005.

Meyer R., Lehmann T., Melzer R.R. and Geiselbrecht H. (2013) Morphology of the first zoeal stage of the Mediterranean bumblebee shrimp *Gnathophyllum elegans* studied with both light microscopy and scanning EM. *The Journal of the Marine Biological Association of the United Kingdom*.

Meyer R., Lochner S. and Melzer R. (2009) Decapoda - crabs, shrimps & lobsters. In:. In Häussermann V. and Försterra G. (eds) *Marine Benthic Fauna of Chilean Patagonia,*. Santiago de Chile: Nature in Focus, pp 1000.

Meyer R. and Melzer R.R. (2004) Scanning EM diagnosis of marine Decapoda larvae: a comparison of preparation techniques. *Crustaceana*, **77**(7), 883-886.

Meyer R. and Melzer R.R. (2012) Bedrohtes Neuland - Zoologische Expeditionen in die südchilenische Fjordregion. *Die Aquarien- und Terrarienzeitschrift*, **02**, 36-41.

Meyer R., Wehrtmann I.S. and Melzer R.R. (2006) Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda : Portunidae : Portuninae) reared in the laboratory. *Scientia Marina*, **70**(2), 261-270.

Miguel Pardo L., Ampuero D. and Veliz D. (2009) Using morphological and molecular tools to identify megalopae larvae collected in the field: the case of sympatric *Cancer* crabs. *Journal of the Marine Biological Association of the United Kingdom*, **89**(3), 481-490.

Moore G.W. and Goodman M. (1968) A set theory approach to immunotaxonomy: Analysis of species comparisons in modified Ouchterlony plates. *U S Air Force Tech Doc Rep Amrl Tdr*, **68**((2)), 1-20.

Nielsen J.F., Lavery S. and Lorz A.-N. (2009) Synopsis of a new collection of sea spiders (Arthropoda: Pycnogonida) from the Ross Sea, Antarctica. *Polar Biology*, **32**(8), 1147-1155.

Padial J.M., Miralles A., De la Riva I. and Vences M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, **7**.

Pérez-Barros P., D'Amato M.E., Guzman N.V. and Lovrich G.A. (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.

Rathbun M.J. (1918) The grapsoid crabs of America. *Bulletin of the United States National Museum*, **97**, 1-461.

Rathbun M.J. (1925) The spider crabs of America. *Bulletin of the United States National Museum*, **129**, 1-613.

Rathbun M.J. (1937) The oxystomatous and allied crabs of America. *Bulletin of the United States National Museum*, **166**, 1-278.

Ratnasingham S. and Hebert P.D.N. (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, **7**(3), 355-364.

Reed D. (2002) Resource extraction industries in developing countries. *Journal of Business Ethics*, **39**(3), 199-226.

Retamal M.A. (1981) Catalogo ilustrado de los crustáceos decapodos de Chile. *Gayana (Instituto Central de Biologia, Universidad de Conceptión) Zoologia*, (44), 1-110.

Retamal M.A. and Gorny M. (2001) Decápodos de los fiordos de Chile (CIMAR-FIORDO 3). *Cienc.Tecnol.Mar.*, **24**, 91-97.

Rice A.L. (1980) Crab zoeal morphology and its bearing on the classification of the Brachyura. *Transactions of the Zoological Society of London*, **35**, 271-424.

Rice A.L. (1981) Crab zoeae and brachyuran classification: a re-appraisal. *Bulletin of the British Museum of Natural History (Zoology)*, **40**, 287-296.

Riedl R. (1983) *Fauna und Flora des Mittelmeeres*, Hamburg: Paul Parey.

Schander C. and Willassen E. (2005) What can biological barcoding do for marine biology? *Marine Biology Research*, **1**(1), 79-83.

Schram F.R., Feldmann R.M. and Copeland M.J. (1978) The late Devonian Palaeopalaemonidae and the earliest decapod crustaceans. *Journal of Paleontology*, **52**, 1375-1387.

Sibley C.G. (1960) The electrophoretic patterns of avian egg-white protein as taxonomic characters *Ibis*, **102**(2), 215-284.

Song H., Buhay J.E., Whiting M.F. and Crandall K.A. (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(36), 13486-13491.

Tang R.W.K., Yau C. and Ng W.-C. (2010) Identification of stomatopod larvae (Crustacea: Stomatopoda) from Hong Kong waters using DNA barcodes. *Molecular Ecology Resources*, **10**(3), 439-448.

Throckmo L. (1968) Biochemestry and taxonomy. *Annual Review of Entomology*, **13**.

Vardaro M.F. (2010) Genetic and anatomic relationships among three morphotypes of the echinoid *Echinocrepis rostrata*. *Invertebrate Biology*, **129**(4), 368-375.

Watling L. (1989) A classification system for crustacean setae based on the homology concept. In Felgenhauer B.E., Watling L. and Thistle A.B. (eds) *Functional Morphology of Feeding and Grooming in Crustacea.* vol. 6, Rotterdam: A.A. Balkema, pp 15-26.

Weis A. and Melzer R.R. (2012) How did sea spiders recolonize the Chilean fjords after glaciation? DNA barcoding of Pycnogonida, with remarks on phylogeography of *Achelia assimilis* (Haswell, 1885). *Systematics and Biodiversity*, **10**(1), 1-14.

Wilson N.G., Schroedl M. and Halanych K.M. (2009) Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology*, **18**(5), 965-984.

Zariquiey Álvarez R. (1968) Crustáceos Decápodos Ibéricos. *Investigacion Pesquera*, **32**, 1-510.

13. Acknowledgements / Dank

Keine Dissertation ohne Betreuung - mein größter Dank gilt meinen Doktorvater **Prof. Dr. Roland Melzer**. Dank seiner hervorragender Betreuung und Unterstützung - nicht nur während der Doktorandenzeit - konnte ich diese Arbeit vollenden. Unvergessen sind die zahlreichen Sammelexpeditionen an die Küsten verschiedenster Meere und natürlich die spannende und abenteuerlichen Tauchgänge die wir zusammen erlebten (und hoffentlich noch werden).

Natürlich will ich mich auch bei der gesamten Arbeitsgruppe „Arthropoda varia“ für das gute Arbeitsklima bedanken. Neben konstruktiven Gesprächen (vor allem im Kreise der Mittagspause - vielen Dank **Marc Ritzerfeld** für die vielen lustigen Momente) hatten auch alle zu jederzeit ein offenes Ohr für Probleme in allen Lebenslagen - besonderer Dank gilt hier meinen Mitdoktoranden **Tobias Lehmann, Hannes Geiselbrecht Ulla Biener-Miller, Andrea Weiss** und **Isabella Stöger** aber natürlich auch **Stefan Friedrich** „for the expert technical assistance“ in allen Lebenslagen sowie **Enrico Schwabe** und **Michael Schrödl** für die nette ZSM-Atmosphäre.

Ein herzliches Dankeschön möchte ich in den Süden Chiles schicken: vielen Dank **Dr. Verena Häussermann** alias „Vreni“ und **Günther Försterra** alias „Fossi“ dass ihr mir ermöglicht habt an eurem Fjord-Projekt mitzuarbeiten. Jeder der Expeditionen bleibt für mich unvergessen. Muchas gracias natürlich auch an die vielen Helfer (Sole und Reinhard, David, Kathi, Ulo, Boris, Liz, Emma…) auf der Station. Desweitern möchte ich **Dr. Ingo S. Wehrtmann** an der Universidad de Costa Rica für die gute Zusammenarbeit während meiner Zeit in San José danken.

Natürlich gab es während der Zeit nicht nur Rückhalt im Kreise der Arbeitskollegen (viele sind auch zu guten Freunden geworden) sondern auch von meiner Familie und Freunden. Vielen Dank für das Verständnis und die gute Zeit.

Ich möchte diese Arbeit meinem Vater Walter Meyer widmen der uns leider viel zu früh und unerwartet am 04.12.2010 verlassen hat. Vielen Dank für Alles.