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Decapod Crustacean Phylogenetics

edited by

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A Proposal for a New Classification of Portunoidea and Cancroidea (Brachyura: Heterotremata) Based on Two Independent Molecular Phylogenies

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ABSTRACT

Molecular methods are playing an increasingly important role in reconstructing phylogenetic relationships. Regardless of what source of DNA is used, the simple idea behind it is that the genetic distance (distinctness of DNA sequences) between any two taxa should be proportional to the time of their separation. Genetic markers with different degrees of variability appear appropriate for different taxonomic levels. The mitochondrial ribosomal RNA genes 12S and 16S have proven to be useful at the interspecific up to the interfamilial level in brachyuran crabs. Recent criticism has questioned the credibility of phylogenies based solely on mitochondrial DNA (mtDNA) as well as the specific value of commonly used mitochondrial markers such as 16S or Cox1. In this study, we present a molecular phylogeny of cancroid and portunoid crabs based on 1200 basepairs of mtDNA, which partly confirms and partly contradicts current morphology-based taxonomy. In order to test the reliability of mtDNA, we constructed a second phylogeny based on a nuclear gene corresponding to the histone H3. This phylogeny absolutely confirmed our initial results. Based on this independent evidence, we argue that mitochondrial DNA should still be considered a tool with high resolution power in decapod molecular phylogenies up to the interfamilial level. In view of the relatively unstable taxonomic classification of the two studied superfamilies, which are in the process of being revised (three new systems over the past three years), we propose a new taxonomy for the Cancroidea and Portunoidea that is based on significant evidence from two molecular markers and in part finds further support in larval morphology.

1 INTRODUCTION

The taxonomy of crabs included in the superfamilies Portunoidea and Cancroidea has been historically quite unstable (see Rathbun 1930; Karasawa et al. 2008). The swimming crabs of the genus *Portunus* and crabs of the genus *Cancer*, on which the superfamily names are based, clearly are different and easily separabale brachyuran heterotreme lineages. However, the establishment of higher taxonomic units in the form of subfamilies, families, and superfamilies, and the placement of different genera into those units based on sometimes convergent characters, has created a taxonomic system that is not necessarily composed of monophyletic units; it also has raised suspicions that members of the superfamilies Portunoidea and Cancroidea (as currently defined) would be better placed in the "other" superfamily or elsewhere (Schubart et al. 2000a; Flores & Paula 2000; Schubart & Reuschel 2005; Ng et al. 2008; Karasawa et al. 2008). Alternatively, genera or families classified elsewhere have been suggested to belong within the Portunoidea (Števčić 2005; Karasawa & Schweitzer 2006).

In order to obtain a stable and monophyletic taxonomic classification, corrections are often necessary at the superfamily, family, subfamily, and even genus level (e.g., Schubart et al. 2000b,

Table 1. Different arrangements of family (and subfamily) subdivisions of Portunoidea and Cancroidea, including extinct (†) and extant taxa.

Martin & Davis (2001)	Ng et al. (2008)	Karasawa et al. (2008)
PORTUNOIDEA		
Portunidae	**Portunidae**	**Portunidae**
(no subfamilies specified)		Atoportuninae
	Caphyrinae	Caphyrinae
	Carupinae	Carupinae
		Lupocyclinae
		Necronectinae
	Podophthalminae	Podophtalminae
	Portuninae	Portuninae
	Thalamitinae	Thalamitinae
		Carcinidae
	Carcininae	Carcininae
	Polybiinae	Polybiinae
		Macropipidae
		Catoptridae
		Mathildellidae
		Carcineretidae †
		Lithophylacidae †
		Longusorbiidae †
Geryonidae	**Geryonidae**	**Geryonidae**
Trichodactylidae	excluded	excluded
CANCROIDEA		
Cancridae	**Cancridae**	
Atelecyclidae	**Atelecyclidae**	
Pirimelidae	**Pirimelidae**	
Thiidae	excluded	
Corystidae	excluded	
Cheiragonidae	excluded	

2002, 2006 for the Grapsoidea). Therefore, it is necessary to understand the current taxonomy of Portunoidea and Cancroidea at different levels before contrasting it with our results based on two molecular phylogenies. Here, and in Table 1, we summarize the most important taxonomic revisions and conclusions at the family level for both superfamilies and at the subfamily level within the family Portunidae.

Portunoid and cancroid families. The composition of portunoid and cancroid crabs as used at the end of the 20th century was established by Bowman & Abele (1982). The history of classification of the Portunoidea previous to that has been summarized in detail by Karasawa et al. (2008: 83). Martin & Davis (2001) included the freshwater crab family Trichodactylidae within the Portunoidea based on findings by Rodríguez (1992), von Sternberg et al. (1999) and von Sternberg & Cumberlidge (2001). Števčić (2005) proposed his own explanation-free classification, in which he erected the Melybiidae as a portunoid family, moved the Geryonidae to the Goneplacoidea, and moved the Trichodactylidae to their own superfamily Trichodactyloidea. Ng et al. (2008) kept the Trichodactylidae removed from the Portunoidea (as also suggested by Schubart & Reuschel 2005), but left the Geryonidae within this superfamily. They also synonymized Števčić's (2005)

Melybiidae and kept the genus *Melybia* within the Xanthidae. That same year, Karasawa et al. (2008) published a taxonomic revision of the Portunoidea that emphasized fossil lineages and was based on a cladistic analysis of adult morphological characters. Their conclusion was that "the superfamily is much more diverse at the family level than has been previously recognized" (Karasawa et al. 2008: 82). Consequently, three subfamilies were elevated to family status (see below) and one new family, Longusorbiidae, and two new genera, exclusively composed of fossils, were described in their revision. According to Karasawa et al. (2008), and with inclusion of three additional fossil families (Carcineretidae, Lithophyllacidae, Longusorbiidae) and the extant Mathildellidae (which are Goneplacoidea according to Castro 2007 and Ng et al. 2008), the Portunoidea would consist of nine families (see Table 1; Karasawa et al. 2008: figs. 6-7).

Martin & Davis (2001) included six families within the superfamily Cancroidea (Table 1). In comparison to Bowman & Abele (1982), this meant the addition of the family Cheiragonidae Ortmann, 1893, with the two genera *Cheiragonus* and *Telmessus*, previously included within the Atelecyclidae. Ng et al. (2008) restricted the Cancroidea to the families Cancridae, Atelecyclidae, and Pirimelidae, separating the Cheiragonidae, Corystidae, and Thiidae into their own superfamilies: Cheiragonoidea, Corystoidea, and Thioidea (Table 1). Schweitzer & Feldmann (2000) redefined the family Cancridae with the inclusion of fossil taxa.

Subfamilies of the Portunidae. Ortmann (1893) included in his section Portuninea seven families, which later became subfamilies of the family Portunidae: Carupidae, Lissocarcinidae, Platyonychidae, Podophthalmidae, Polybiidae, Portunidae, and Thalamitidae. According to Davie (2002) and Ng et al. (2008), the Portunidae contains seven subfamilies: Caphyrinae Paul'son, 1875; Carcininae MacLeay, 1838; Carupinae Paul'son, 1875; Podophthalminae Dana, 1851; Polybiinae Ortmann, 1893; Portuninae Rafinesque, 1815; and Thalamitinae Paul'son, 1875. Števčić's (2005) system with eight subfamilies and 15 tribes will not be further discussed here, because it lacks supporting arguments and was not adopted in the more comprehensive revision by Ng et al. (2008). Most recently, previous taxonomies were challenged by the fossil work put forward by Karasawa et al. (2008). In addition to the inclusion of fossil taxa, Karasawa et al. (2008) elevated three subfamilies of the Portunidae, i.e., Catroptrinae, Carcininae, and Macropipinae, to full family level. Their results and conclusions will be discussed with our own later in this chapter.

The present study was initiated (Reuschel 2004; Schubart & Reuschel 2005) before the results of more recent revisions became available. Therefore, our taxon sampling was based on the classification by Martin & Davis (2001), with the goal to include taxa of all the portunoid and cancroid families listed in this monograph plus representatives of the seven subfamilies of the Portunidae as listed by Davie (2002). In this sense, our analysis is an independent revision to the ones by Ng et al. (2008) and Karasawa et al. (2008), which may also be said in terms of the methods used: adult morphology (Ng et al. 2008) and adult morphology plus fossils (Karasawa et al. 2008) versus DNA (present study). The goal of this study is to construct a phylogeny of cancroid and portunoid crabs (without claiming that these two superfamilies must represent sister taxa) and to propose a new taxonomy in which the taxa are classified according to their phylogenetic relationships based on two independent sources of DNA sequences. Based on these results, we propose a new taxonomic system, derived from two concordant phylogenetic hypotheses, that can be tested and ameliorated with additional morphological and molecular markers.

2 MATERIALS & METHODS

Samples for this study were obtained between 2000 and 2006, mostly from museum specimens and from colleagues (Table 2, Acknowledgements). All molecular studies were carried out at the University of Regensburg. DNA extractions and selective amplification of the mitochondrial complex, consisting of part of the large ribosomal subunit 16S rRNA, the $tRNA_{Leu}$, part of the NDH1

Table 2. List of crab species used for phylogenetic analyses with taxonomic classification following Martin & Davis (2001), locality of collection, museum catalogue number of voucher (if available), and genetic database accession numbers.

Species	Taxonomy	Collection Locality	Voucher	mtDNA	nDNA
PORTUNOIDEA					
Arenaeus cribrarius	Portunidae: Portuninae	USA: North Carolina	SMF-32753	FM208749	FM208799
Callinectes sapidus	Portunidae: Portuninae	GenBank: USA / USA: Lousiana	unknown/ULLZ3895	AY363392	FM208798
Laleonectes nipponensis	Portunidae: Portuninae	French Polynesia	MNHN-B31434	FM208753	FM208792
Portunus hastatus	Portunidae: Portuninae	Turkey: Beldibi	SMF-31989	FM208780	FM208796
Portunus inaequalis	Portunidae: Portuninae	Ghana: Cape Coast	SMF-32754	FM208752	FM208795
Portunus ordwayi	Portunidae: Portuninae	Jamaica: Priory	SMF-31988	FM208751	FM208794
Portunus pelagicus	Portunidae: Portuninae	Australia	CSIRO uncatalogued	FM208750	FM208797
Portunus trituberculatus	Portunidae: Portuninae	GenBank: Japan	unknown	AB093006	n.a.
Scylla serrata	Portunidae: Portuninae	Kenya: Lamu	MZUF 3657	FM208779	FM208793
Podophthalmus vigil	Portunidae: Podophthalminae	Malaysia: Pontian	ZRC Y4821	FM208760	FM208787
Thalamita crenata	Portunidae: Thalamitinae	Hawaii: Oahu	ULLZ 8664	FM208754	FM208800
Carupa ohashii	Portunidae: Carupinae	Japan: Okinawa Island	SMF-32756	FM208759	FM208790
Carupa tenuipes	Portunidae: Carupinae	New Caledonia	MNHN-B31436	FM208758	FM208789
Catoptrus nitidus	Portunidae: Carupinae	New Caledonia	MNHN-B31435	FM208755	n.a.
Libystes edwardsii	Portunidae: Carupinae	New Caledonia	MNHN-B31437	FM208761	n.a.
Libystes nitidus	Portunidae: Carupinae	New Caledonia	MNHN-B31438	FM208762	n.a.
Richerellus moosai	Portunidae: Carupinae	New Caledonia (paratype)	MNHN-B22838	FM208756	FM208788
Lissocarcinus orbicularis	Portunidae: Caphyrinae	Singapore: Southern Islands	no voucher, id. PKL Ng	FM208757	FM208791
Carcinus maenas	Portunidae: Carcininae	France: Le Havre	SMF-32757	FM208763	FM208811
Portumnus latipes	Portunidae: Carcininae	UK: Hastings	SMF-32758	FM208764	FM208812
Polybius henslowii	Portunidae: Polybiinae	Portugal	SMF-32759	FM208765	FM208816
Liocarcinus corrugatus	Portunidae: Polybiinae	Spain: Ibiza	SMF-32760	n.a.	FM208820
Liocarcinus depurator	Portunidae: Polybiinae	Alborn Sea	MNHN-B31439	FM208767	FM208819
Liocarcinus holsatus	Portunidae: Polybiinae	Germany: Helgoland	SMF-32750	FM208766	FM208817
Liocarcinus navigator	Portunidae: Polybiinae	France: Normandie	SMF-32775	n.a.	FM208821
Liocarcinus vernalis	Portunidae: Polybiinae	Italy: Naples: Fusaro	SMF-32761	FM208768	FM208818
Necora puber	Portunidae: Polybiinae	UK: Hastings	SMF-32749	FM208771	FM208813
Macropipus tuberculatus	Portunidae: Polybiinae	Alborn Sea	MNHN-B31440	FM208769	FM208815
Bathynectes maravigna	Portunidae: Polybiinae	Alborn Sea	MNHN-B31441	FM208770	FM208814
Benthochascon hemingi	Portunidae: Polybiinae	New Caledonia	ZRC 2000.102	FM208772	FM208826

Table 2. continued.

Species	Taxonomy	Collection Locality	Voucher	mtDNA	nDNA
Ovalipes trimaculatus	Portunidae: Polybiinae	Campagne MD50/Jasus	MNHN-B19785	FM208773	FM208823
Ovalipes iridescens	Portunidae: Polybiinae	Taiwan: NE coast	ZRC 1995.855	FM208774	FM208825
Ovalipes punctatus	Portunidae: Polybiinae	Taiwan	MNHN-B31442	n.a.	FM208824
Ovalipes australiensis	Portunidae: Polybiinae	Australia	CSIRO uncatalogued	n.a.	FM208822
Geryon longipes	Geryonidae	Spain: Ibiza, fish market	SMF-32747	FM208776	FM208828
Chaceon granulatus	Geryonidae	Japan	SMF-32762	FM208775	FM208827
Trichodactylus dentatus	Trichodactylidae	Brazil: Bahia	SMF-32763	FM208777	FM208785
CANCROIDEA					
Cancer pagurus	Cancridae	France: Le Havre	SMF-32764	FM207653	FM208806
Cancer irroratus	Cancridae	USA: Maine	ULLZ 3843	FM207654	FM208807
Atelecyclus rotundatus	Atelecyclidae	France: Bretagne	SMF-32765	FM207652	FM208804
Atelecyclus undecimdentatus	Atelecyclidae	Portugal: Algarve	SMF-32766	FM207651	FM208805
Pirimela denticulata	Pirimelidae	France: Guthary	SMF-32767	FM208783	FM208808
Sirpus zariquieyi	Pirimelidae	Greece: Parga	SMF-32768	FM208784	FM208809
Thia scutellata	Thiidae	France: Bretagne	SMF-32769	FM208782	FM208810
Corystes cassivelaunus	Corystidae	France: Bretagne	SMF-32770	FM208781	FM208801
Telmessus cheiragonus	Cheiragonidae	Japan: Hokkaido: Ozuchi	SMF-22475	FM207656	FM208802
Erimacrus isenbeckii	Cheiragonidae	Japan	SMF-32752	FM207657	FM208803
PSEUDOTHELPHUSOIDEA					
Epilobocera sinuatifrons	Pseudothelphusidae	Puerto Rico: Guilarte	SMF-32774	FM208778	FM208830
POTAMOIDEA					
Geothelphusa dehaani/sp.	Potamidae	GenBank: Japan	unknown	NC007379	DQ079677
CARPILIOIDEA					
Carpilius sp.	Carpiliidae	French Polynesia	SMF-32771	FM208748	FM208786

CSIRO Marine Research, Invertebrate Museum, Hobart; MNHN: Muséum National d'Histoire Naturelle, Paris; MZUF: Museo Zoologico Universitá di Firenze 'La Specola', Florence; SMF: Senckenberg Museum, Frankfurt a.M.; ULLZ: University of Louisiana at Lafayette Zoological Collection, Lafayette.

Table 3. Primers used for amplification of approximately 1200 basepairs mtDNA (consisting of 16S rRNA, $tRNA_{Leu}$, NDH1) and exactly 328 basepairs nDNA corresponding to histone H3.

16S towards NDH1:
16L2: 5'–TGCCTGTTTATCAAAAACAT–3' (Schubart et al. 2002)
16L6: 5'–TTGCGACCTCGATGTTGAAT–3' (Schubart this volume)
16L11: 5'–AGCCAGGTYGGTTTCTATCT–3' (Schubart this volume)
16LLeu: 5'–CTATTTTGKCAGATDATATG–3' (Schubart this volume)
NDL8: 5'– TTA GTD GSR GTW GCY TTT GT–3' (new)
NDH1 towards 16S:
16H37: 5'–CCGGTYTGAACTCAAATCATGT–3' (Klaus et al. 2006)
16H11: 5'–AGATAGAAACCRACCTGG–3' (Schubart this volume)
16H10: 5'–AATCCTTTCGTACTAAA–3' (Schubart this volume)
16HLeu: 5'–CATATTATCTGCCAAAATAG–3' (Schubart this volume)
NDH1: 5'–TCCCTTACGAATTTGAATATATCC–3' (Schubart this volume)
NDH5: 5'–GCYAAYCTWACTTCATAWGAAAT–3' (Schubart this volume)
H3 forward and reverse:
H3af: 5'–ATGGCTCGTACCAAGCAGACVGC–3' (Colgan et al. 1998)
H3ar: 5'–ATATCCTTRGGCATRATRGTGAC–3' (Colgan et al. 1998)
H3H2: 5'–GGCATRATGGTGACRCGCTT–3' (new)

(16S-NDH1), in addition to amplification of part of the nuclear histone H3, were performed as reported in Schubart et al. (2006). The primers used to amplify an approximately 1200-bp unit of mtDNA (16S-NDH1 complex) and 328 bp of the nuclear histone H3 are listed in Table 3. PCR-amplifications were carried out with four minutes of denaturation at 94°C, 40 cycles with 45 s at 94°C, 1 min at 48°C, 1 min at 72°C, and 10 min final denaturation at 72°C. PCR products were purified with Microcon 100 filters (Microcon), ExoSAP-IT (Amersham Biosciences), or Quick-Clean (Bioline) and then sequenced with the ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). Forward and reverse strands were obtained as well as overlapping regions for larger DNA fragments. New sequence data were submitted to the European molecular database EMBL (see Table 2 for accession numbers). In addition, the following sequences archived in molecular databases were included in our analyses: mtDNA of *Portunus trituberculatus* (AB093006), *Callinectes sapidus* (AY363392), and *Geothelphusa dehaani* (NC007379), and nuclear DNA (nDNA) of *Geothelphusa* sp. (DQ079677).

Sequences were aligned with CLUSTAL W (Thompson et al. 1994) as implemented in the software BioEdit version 7.5.0.3 (Hall 1999) and corrected manually with BioEdit or xESEE version 3.2 (Cabot and Beckenbach 1989). The data for 16S-NDH1 and H3 were always analyzed as separate datasets for subsequent independent phylogenetic analyses. DNA sequence of *Carpilius* sp. (Carpiliidae) was included as an outgroup.

Phylogenetic congruence among mtDNA partitions was performed using the incongruence length difference (ILD) test (Farris et al. 1995) implemented in PAUP as the partition-homogeneity test (Swofford 1998). For this test, we used random taxon addition, TBR branch swapping, and heuristic searches with 1000 randomizations of the data. The model of DNA substitution that fit our data best was determined using the software MODELTEST 3.6 (Posada and Crandall 1998). This approach consists of successive pairwise comparisons of alternative substitution models using the hLRT and Akaike tests. Model selections were done separately for the mtDNA and nDNA. Two methods of

phylogenetic inference were applied to our dataset: maximum parsimony (MP) using the software package PAUP (Swofford 1998) and Bayesian analysis (BI) as implemented in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001).

MP trees were obtained by a heuristic search with 100 replicates of random sequences addition and tree-bisection-reconnection as branch swapping options keeping multiple trees (MulTrees). Analyses were carried out by weighing transversions twice as much as transitions; gaps were always treated as missing. Subsequently, confidence values for the proposed groups within the inferred trees were calculated with the nonparametric bootstrap method (2000 pseudoreplicates, 10 replicates of sequence addition). Only minimal trees were retained and zero-length branches were collapsed. The BI trees were calculated using the suggested model of evolution. The Bayesian analysis was run with four MCMC (Markov chain Monte Carlo) chains for 2,000,000 generations, saving a tree every 500 generations (with a corresponding output of 4000 trees). The –lnL converged on a stable value between 20,000 and 60,000 generations ("burn-in phase"). The first 100,000 generations were thus excluded from the analysis to optimize the fit of the remaining trees. The posterior probabilities of the phylogeny were determined by constructing a 50% majority rule consensus of the remaining trees. Consensus trees were obtained using the "sumpt" option in MrBayes.

3 RESULTS

The total alignment of the sequenced portions of the 16S-NDH1 region consisted of 1497 bp, whereas the length of the sequenced region of the histone 3 gene consisted of 328 bp after removal of the primer regions. From the 1497-bp mtDNA, 671 were variable and 565 were parsimony-informative. The 328-bp nDNA had 111 variable positions and 100 parsimony-informative positions. The mtDNA fragment for most analyzed species was not longer than 1200 bp, but the sequence of the cancroid crab *Atelecyclus undecimdentatus* had an additional fragment of 284 bp inserted between the 16S rRNA and the $tRNA_{Leu}$ (explaining the high number of apparently constant characters). Comparing this fragment with sequences from the genetic database revealed that part of this DNA consists of a sequence corresponding to the $tRNA_{Val}$, whereas the rest of the sequence appears to be non-informative. Thus, we report a unique case of gene rearrangement, which appears to also occur in a similar fashion in other crabs of the genera *Cancer* and *Atelecylus*, based on the fact that we needed to amplify the apparently unconnected 16S rRNA and $tRNA_{Leu}$-NDH1 in separate PCRs (Schubart in preparation). Excluding this insertion in the DNA of *A. undecimdentatus*, we calculated a relatively high proportion of 46.6% parsimony-informative positions in the mtDNA as opposed to 30.5% parsimony-informative positions in the more conserved nDNA of histone 3.

The selected model of DNA substitution by hLRT and Akaike was the GTR + I + G model (Rodríguez et al. 1990) for the mitochondrial 16S-NDH1 as well as for the nuclear H3. This model was consequently used for the BI method. Character congruence between the 16S, $tRNA_{Leu}$, and the NDH1 gene fragments was not rejected according to the ILD test. We did not combine the mitochondrial and nuclear dataset, because one of the goals of this study was to compare results from the mitochondrial phylogeny with those from a nuclear dataset to address criticism concerning the credibility of phylogenies based on mtDNA (e.g., Mahon & Neigel 2008).

Both phylogenetic inference methods (BI and MP) resulted in trees that were surprisingly congruent in their overall topology for both sources of DNA, with most clusters showing consistently high confidence values. The results of the two methods are therefore shown together based on the topology of the BI tree, with all confidence values ≥ 50 plotted on the corresponding branches (figs. 1, 2). Posterior probabilities are expressed in a range from 0 to 100 (instead of from 0 to 1). In the case of H3, we also present the topology of the heuristic MP tree (Fig. 3), because the consensus tree of this relatively short gene fragment does not allow recognition of all branching patterns (without statistic support) at the base of the tree. The mtDNA MP heuristic search yielded

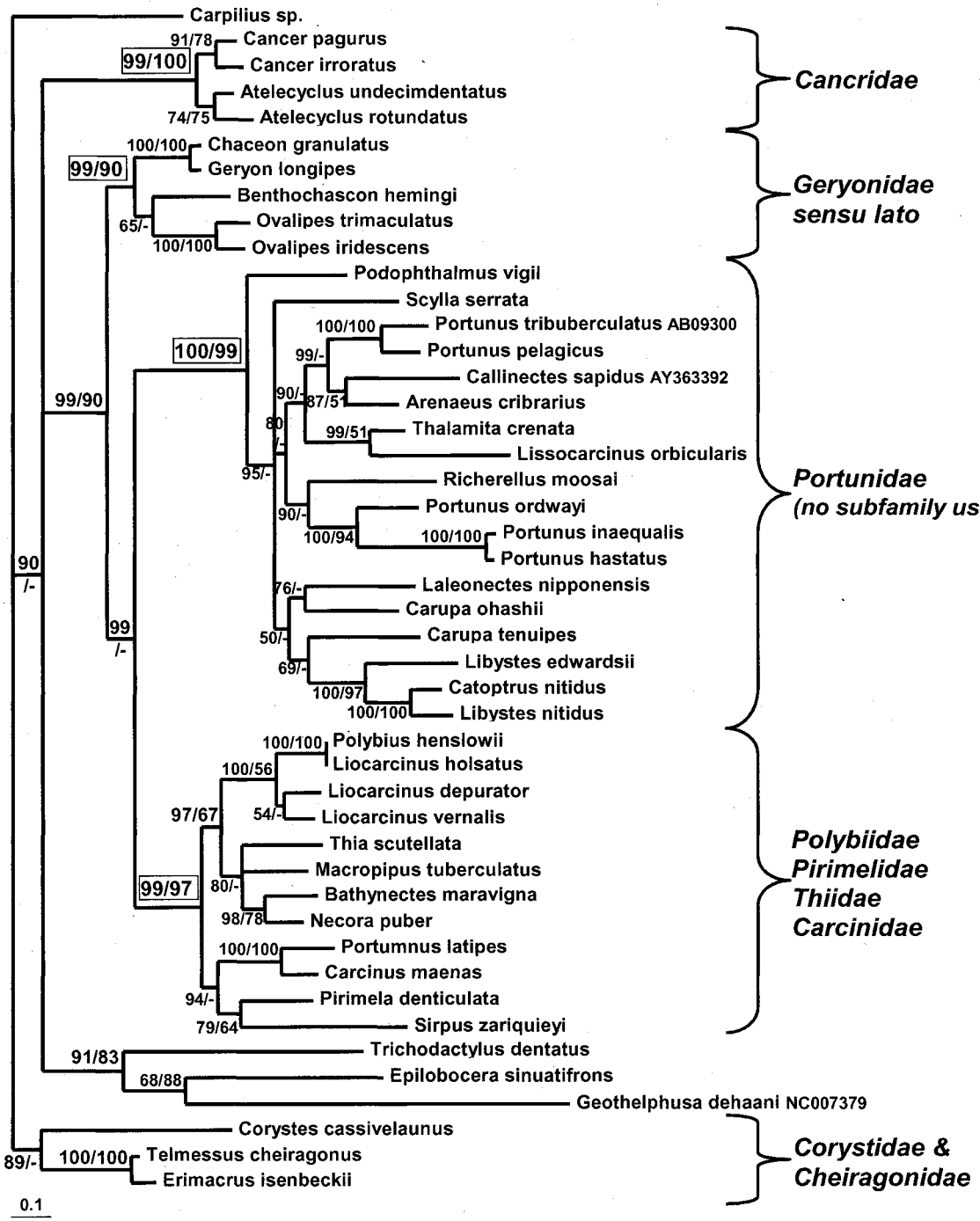

Figure 1. Phylogenetic consensus tree of 46 cancroid and portunoid crabs according to the classification of Martin & Davis (2001) based on 1497 basepairs of mtDNA (16S rRNA-NDH1); topology of a Bayesian Inference analysis with confidence values (only ≥ 50) corresponding to Bayesian posterior probabilities/maximum parsimony bootstrap values. *Carpilius* sp. was used as outgroup. The proposed taxonomic classification is given to the right.

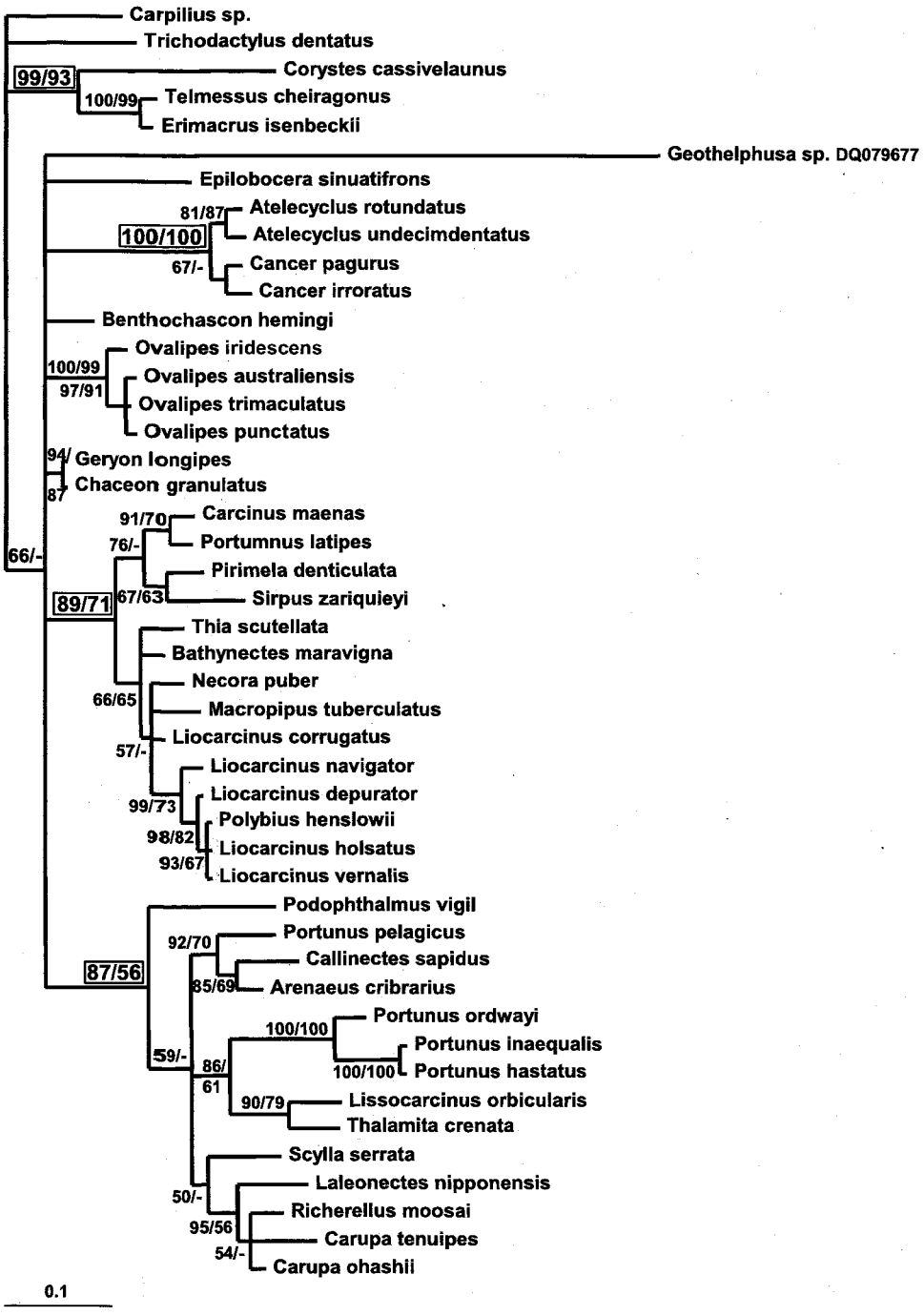

Figure 2. Phylogenetic consensus tree of 46 cancroid and portunoid crabs according to the classification of Martin & Davis (2001) based on 328 basepairs of nDNA (histone H3); topology of a Bayesian Inference analysis with confidence values (only ≥ 50) corresponding to Bayesian posterior probabilities/maximum parsimony bootstrap values. *Carpilius* sp. was used as outgroup.

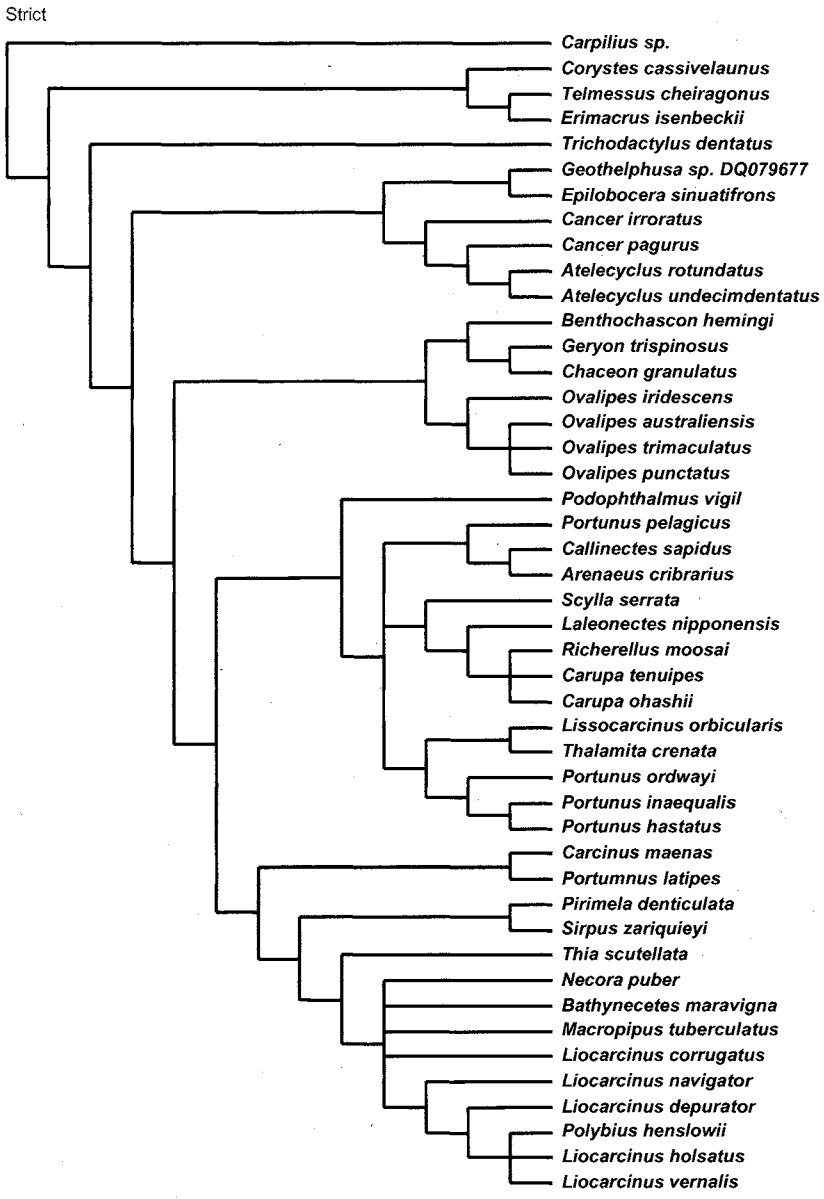

Figure 3. Strict consensus of 45 shortest trees of maximum parsimony heuristic search of 46 cancroid and portunoid crabs; 328 basepairs of nDNA (histone H3). *Carpilius*, sp. was used as outgroup.

one shortest tree of length 6751 with tree scores CI = 0.30, RI = 0.51. The topology of this search was congruent with the consensus topology obtained after bootstrapping, with resulting bootstrap values shown in Figure 1. The nDNA MP heuristic search yielded 45 shortest trees of length 696 with tree scores CI = 0.42, RI = 0.69. The strict consensus topology of these 45 shortest trees is shown in Figure 3, whereas MP bootstrap values after 2000 bootstrap reiterations are included in Figure 2 for comparison with BI posterior probabilities.

Comparison of the phylogenetic results derived from the mtDNA dataset (Fig. 1) with the current classifications (Table 1) reveals striking differences. Most evident is that both superfamilies (Portunoidea and Cancroidea) cannot be recognized as monophyletic clades in the tree, regardless of which of the taxonomic systems of Table 1 is followed. Crabs that have been considered Portunoidea fall into three to four major subgroups, depending on whether freshwater crabs of the family Trichodactylidae are included. Without the trichodactylids, which cluster with freshwater crabs from two other families (Pseudothelphusidae and Potamidae), three strongly supported (confidence always $\geq$ 90) groups including portunoid crabs remain: 1) With a support of 100/99 (BI/MP), there is a clade that contains the core of the Portunidae, including the type genus *Portunus* and the type species *Portunus pelagicus*, and all included members of the subfamilies Portuninae, Thalamitinae, Carupinae, Caphyrinae, and Podophthalminae. However, whenever more than one species of the subfamilies (Portuninae and Carupinae) were available, they did not cluster together, casting some doubt on the validity of these taxonomic units. Additionally, the genera *Portunus*, *Carupa*, and *Libystes* do not appear as monophyletic units on this tree. 2) The second group of portunoid crabs clusters with a support of 99/97. This group includes the European representatives of the other two units previously treated as subfamilies (Polybiinae and Carcininae), but also three other European species that were considered to belong elsewhere: *Pirimela denticulata* and *Sirpus zariquieyi* (both Pirimelidae) and *Thia scutellata* (Thiidae). Interestingly, the genus *Liocarcinus* is not monophyletic, and its type species, *L. holsatus*, is genetically almost identical to the type species of the genus *Polybius, P. henslowii*. Two non-European genera that are commonly classified as Polybiinae, *Benthochascon* and *Ovalipes*, are not found in this group, but in 3) a cluster where they are united, with a support of 99/90, to the two deep water representatives of the family Geryonidae.

The allocation of the different members of the Cancroidea *sensu* Martin & Davis (2001) on the phylogenetic tree is equally fragmented. The core of the Cancroidea, with the type genus *Cancer* and type species *C. pagurus*, is found in a well-defined clade (88/100) together with members of the genus *Atelecyclus* (type genus of the family Atelecyclidae). However, the remaining "Cancroidea" have little phylogenetic affinity to these crabs. As mentioned above, the two families Pirimelidae and Thiidae are now embedded among the European Carcininae and Polybiinae. The Corystidae and Cheiragonidae cluster together, but without absolute support (89/-). Both families appear to hold a basal and unrelated position to all other crabs analyzed in this study. However, this study was not designed to discern (and the tree does not resolve) phylogenetic relationships at the root of the Heterotremata.

All of these groups could also be recovered with the much shorter and more conserved nuclear marker. The only exception is the cluster consisting of Geryonidae-*Benthochascon*-*Ovalipes*, which is unresolved at the level above 50% confidence (see Fig. 2). However, the heuristic search (Fig. 3) and additional analyses based on neighbor joining distances (not shown) also grouped these taxa together. Additional taxa and longer DNA fragments may be necessary to provide strong enough support from nuclear DNA to this potential clade. We did find support from nDNA for 1) the portunid group consisting of the subfamilies Portuninae, Thalamitinae, Carupinae, Caphyrinae, and Podophthalminae (87/56); 2) the second "portunid" group consisting of the European representatives of Carcininae and Polybiinae together with the "cancroid" families Pirimelidae and Thiidae (89/71); 3) the core group of Cancroidea restricted to the families Cancridae and Atelecyclidae (100/100); and 4) a clade uniting Corystidae and Cheiragonidae (99/93) in a potentially monophyletic assemblage.

According to this phylogenetic congruence of the two datasets, and with the goal to establish a taxonomic system that is in agreement with phylogenetic relationships, we propose a taxonomic classification as depicted in Figure 1 and Table 4.

Table 4. Proposed taxonomy of extant Portunoidea and Cancroidea, as well as taxa excluded from those superfamilies, based on the current molecular phylogenies and supporting evidence.

Superfamily Portunoidea Rafinesque, 1815
Family Carcinidae MacLeay, 1838
Family Geryonidae Colosi, 1923
Family Pirimelidae Alcock, 1893
Family Polybiidae Ortmann, 1893
Family Portunidae Rafinesque, 1815
Family Thiidae Dana, 1852
Superfamily Cancroidea Latreille, 1802
Family Atelecyclidae Ortmann, 1892
Family Cancridae Latreille, 1802
Superfamily Corystoidea Samouelle, 1819
Family Corystidae Samouelle, 1819
Family Cheiragonidae Ortmann, 1893
Superfamily Trichodactyloidea H. Milne Edwards, 1853
Family Trichodactylidae H. Milne Edwards, 1853

4 DISCUSSION

The portunoid and cancroid taxonomic classifications as commonly used and summarized by Martin & Davis (2001) have been challenged by alternative classification schemes (Števčić 2005; Karasawa et al. 2008) and recently also by Ng et al. (2008, with the recognition of additional superfamilies). While Števčić's (2005) taxonomy was presented without further explanations, and evidently was based on subjective grouping according to adult morphology, Karasawa et al. (2008) used and listed adult morphological characters applied to extinct and extant portunoid crabs to support their classification. Adult morphology, especially carapace and chelar characters, is known to be influenced by convergent evolution. Therefore, we provide results from two molecular phylogenies (one mtDNA-based, the other nDNA-based) and use these to propose a new possible classification of portunoid and cancroid crabs. We do this realizing that all available classifications are still unsettled: "The composition of the superfamily Cancroidea has varied with different authors. The Portunoidea are sometimes included, and while there does appear(s) to be a link, we prefer to keep them apart until more compelling evidence surfaces" (Ng et al. 2008: 51). Nevertheless, we also propose a new taxonomy, because we are convinced that these molecular phylogenies correctly reflect the evolution of these groups and because we find independent confirmation of some of our conclusions in results from larval morphology (see below).

Our proposed taxonomy is summarized in Table 4 and with the labels of Figure 1. Most important is the recognition of six extant families within the superfamily Portunoidea instead of three (as in Martin & Davis 2001, Ng et al. 2008) or of a different six (Karasawa et al. 2008). In addition to the Geryonidae and the Portunidae *sensu novo*—which is now limited to members of the former subfamilies Carupinae, Caphyrinae, Podophthalminae, Portuninae, and Thalamitinae—we recognize the Carcinidae and Polybiidae as full families. We do not agree with Karasawa et al. (2008) in recognizing Mathildellidae Karasawa & Kato, 2003, as a portunoid family, based on preliminary DNA evidence that became available during revision of this manuscript (Schubart, in progress). This agrees with Ng & Manuel-Santos (2007), Castro (2007) and Ng et al. (2008), who

also do not consider Mathildellidae to belong to Portunoidea. The Thiidae and Pirimelidae, which had been recognized as full families within the Cancroidea (according to Martin & Davis 2001) or placed in their own superfamily (Thioidea in Ng et al. 2008), are herewith moved into the vicinity of Polybiidae and Carcinidae (and into the Portunoidea, if superfamilies continue to be used). The close relationship of Thiidae and Pirimelidae to the European Polybiidae and Carcinidae (Figures 1, 2) not only justifies the removal of these two families and three genera from the Cancroidea and their inclusion into the Portunoidea, but also requires elevation of Polybiinae and Carcininae to family level, if Pirimelidae and Thiidae continue to be regarded as full families. Alternatively, Carcinidae, Pirimelidae, Polybiidae *sensu stricto*, and Thiidae would all need to be included within the family Carcinidae MacLeay, 1838.

Bourdillon-Casanova (1956) and Flores & Paula (2000) described the larval development of *Pirimela denticulata* and noticed a close morphological similarity to larvae of European Portunidae, especially Polybiinae and Carcininae. Based on larval morphology, Bourdillon-Casanova (1960) suggested a continuous evolutionary line from *Macropipus* to *Portumnus*, with *Pirimela* and *Sirpus* as intermediate forms. Flores & Paula (2000) concurred with Bourdillon-Casanova's opinion and pointed out that the latter two genera share most morphological characters with those of larvae of the European Carcininae, *Carcinus* and *Portumnus*. This is exactly where the molecular results would place these two genera, and it is an important confirmation that larval morphology is often congruent with molecular results, even if contrary to results from adult morphology (see Schubart et al. 2000b, 2002). Consequently, Flores & Paula (2000: 2139) concluded: "pirimelids could be regarded as non-swimming portunids between portunines and carcinines."

Karasawa et al. (2008) independently reached the conclusion that the Carcinidae and Macropipidae should be regarded as full families. That means that they also recognized differences important enough in the former Polybiinae and Carcininae to separate them from the remaining Portunidae at a family level. However, more drastically than in our classification, they modified the composition of these two families with respect to the composition of the subfamilies. According to their results, the European Carcinidae and Polybiidae are not monophyletic but consist of two lineages, with some genera falling into Karasawa et al.'s (2008) redefined Carcinidae (*Liocarcinus*, *Polybius*, *Portumnus*, *Xaiva*, *Carcinus*) and some into the redefined Macropipidae (*Bathynectes*, *Necora*, *Macropipus*), both of which are considered full families. Based on our results, we disagree with this classification. All our European Polybiidae and Carcinidae appear closely related. This includes the European representative of the genus *Macropipus*, *M. tuberculatus* Prestandrea, 1833. Our separation into two families (Carcinidae and Polybiidae) is justified by the fact that the morphologically derived Pirimelidae and Thiidae cluster among these crabs and by the fact that *Carcinus* and *Portumnus* cluster together as sister genera, whereas the Polybiidae form a second branch together with Thiidae. Karasawa et al. (2008) used only *Macropipus australis* Guinot, 1961, for material of that genus. If this species turns out to belong to a different lineage than the European *Macropipus tuberculatus*, it would have to be reclassified. However, the subfamily name Macropipinae Stephenson & Campbell (or the derived family name Macropipidae) remains with *M. tuberculatus*, and this species clearly belongs to the European Polybiidae Ortmann, 1893, which is the older family name and thus has preference (see also Holthuis 1968).

It is certainly true that our definition of the new Polybiidae and Carcinidae cannot be satisfactorily completed without including all members (at least all genera) of the former subfamilies in our analysis (currently in progress). The genera *Brusinia*, *Coenophthalmus*, *Echinolatus*, *Nectocarcinus*, *Parathranites*, *Raymanninus*, and *Xaiva* may belong to different evolutionary lineages and thus might require the definition of new taxa. The Polybiidae, however, is defined by the position of *Polybius henslowii* Leach, 1820, and for the moment includes the genera *Polybius*, *Liocarcinus* (for which a revision of all species is in progress), *Necora*, *Bathynectes*, and *Macropipus*. We realize, however, that according to our mtDNA tree, even the Polybiidae *sensu stricto* may be paraphyletic if the Thiidae keep their family status.

The heterogeneous character of the former Polybiidae is discernible the phylogenetic position of the genera *Ovalipes* and *Benthochascon* in our trees. They are clearly more closely related to Geryonidae than to Polybiidae. We therefore exclude them from the Polybiidae and place them provisionally in the Geryonidae *sensu lato* (Fig. 1). Morphologically, they are clearly distinct from *Geryon* and *Chaceon*, and they may deserve their own family. We anticipate placing these two genera in a new family, but we await further results on the phylogenetic position of the American members of *Ovalipes* and of *Raymanninus schmitti* (for long considered to be a member of the genus *Benthochascon*; see Ng 2000) and more conclusive confirmation from nuclear DNA (work in progress).

All representatives of the other former subfamilies of the Portunidae (Portuninae, Caphyrinae, Carupinae, Thalamitinae, and Podophtahlminae) appear in the same cluster and are not segregated by their subfamily status. This is also shown by Mantelatto et al. (this volume) for the subfamilies Portuninae, Thalamitinae, and Podophtalminae, a result that again differs from Karasawa et al. (2008), who considered the Catoptridae, consisting of the genera *Catoptrus* and *Libystes*, a separate family. The possible paraphyly of these subfamilies can be confirmed only if additional representatives of the Thalamitinae, Caphyrinae, and Podophthalminae are included. For the moment we can say that the subfamilies Portuninae and Carupinae, and also the genera *Portunus*, with the type species *P. pelagicus* Linnaeus, 1758 (see also Mantelatto et al. 2007), and *Carupa*, with the type species *Carupa tenuipes* Dana, 1852, are paraphyletic, and we suggest refraining from using these subfamilies before a redefinition at the genus level has been carried out.

The Cancroidea as a superfamily should now be limited to the families Cancridae and Atelecyclidae, the latter maybe in its restriction to the genus *Atelecyclus* (see Guinot et al. 2008). A similar conclusion was reached by Ng et al. (2008) when removing Thiidae, Corystidae, and Cheiragonidae from the Cancroidea and placing them in their own independent superfamilies; Ng et al. (2008) noted that these single-family taxa may be preliminary groupings. Upgrading families into monofamilial superfamilies, however, underscores that the phylogenetic position of the included species is unknown and only changes the taxonomic level of uncertainty. Based on our results, we now place the Pirimelidae and Thiidae within the Portunoidea in close relationship to Carcinidae and Polybiidae and confirm the separate status of Corystidae and Cheiragonidae. These last two families cluster together in the mtDNA as well as in the nDNA phylogenies and should constitute sister families in the same superfamily. In that case, the name Corystoidea Samouelle, 1819, has preference. However, also in this case, additional genera of both families and clarification of the phylogenetic relationships of some of the current Atelecyclidae will be necessary before confirming this taxonomic change.

Overall, we feel that this study serves as an example that molecular phylogenies based on mitochondrial DNA can provide new insights into evolutionary relationships among decapod Crustacea (and other animals), insights that then can be used to implement a more phylogenetically based taxonomic system. The obvious congruence with a second tree based on the independent nuclear marker H3 gives confidence that results from previously published phylogenies of brachyuran crabs based on mitochondrial DNA alone (e.g., Schubart et al. 2000b, 2006 and others) do not necessarily have to be questioned. However, it also remains true that only the combination of a maximum number of approaches will lead to the best possible understanding of often-unexpected phylogenetic relationships in the natural world.

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