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Mitogenomic analysis of decapod crustacean phylogeny corroborates traditional views on their relationships

Hong Shen, Anke Braband, Gerhard Scholtz *

Humboldt-Universität zu Berlin, Institut für Biologie/Vergleichende Zoologie, Philippstr. 13, 10115 Berlin, Germany

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ABSTRACT

Phylogenetic relationships within decapod crustaceans are highly controversial. Even recent analyses based on molecular datasets have shown largely contradictory results. Previous studies using mitochondrial genomes are promising but suffer from a poor and unbalanced taxon sampling. To fill these gaps we sequenced the (nearly) complete mitochondrial genomes of 13 decapod species: *Stenopus hispidus*, *Polycheles typhlops*, *Panulirus versicolor*, *Scyllarides latus*, *Enoplometopus occidentalis*, *Homarus gammarus*, *Procambarus fallax* f. *virginalis*, *Upogebia major*, *Neaxius acanthus*, *Calocaris macandreae*, *Corallianassa coutierei*, *Cryptolithodes sitchensis*, *Neopetrolisthes maculatus*, and add that of *Dromia personata*. Our new data allow for comprehensive analyses of decapod phylogeny using the mitochondrial genomes of 50 species covering all major taxa of the Decapoda. Five species of Stomatopoda and one species of Euphausiacea serve as outgroups. Most of our analyses using Maximum Likelihood (ML) and Bayesian inference (BI) of nucleotide and amino acid datasets revealed congruent topologies for higher level decapod relationships: (((((((Anomala, Brachyura), Thalassinida: Gebiidea), Thalassinida: Axiidea), (Astacidea, Polychelida), Achelata), Stenopodidea), Caridea), Dendrobranchiata). This result corroborates several traditional morphological views and adds new perspectives. In particular, the position of Polychelida is surprising. Nevertheless, some problems can be identified. In a minority of analyses the basal branching of Reptantia is not fully resolved, Thalassinida are monophyletic; Polychelida are the sister group to Achelata, and Stenopodidea are resolved as sister group to Caridea. Despite this and although some nodal supports are low in our phylogenetic trees, we think that the largely stable topology of the trees regardless of different types of analyses suggests that mitochondrial genomes show good potential to resolve the relationship within Decapoda.

1. Introduction

Decapoda is the most species rich, diverse, visible, popular, and economically important group of all crustaceans. Currently, the Decapoda contains an estimated 18,000 living and extinct species (De Grave et al., 2009), among them popular animals such as shrimps, lobsters, freshwater crayfish, hermit crabs, or "true" crabs, some of which support seafood and marine industries worth billions of dollars each year to the world's economy. Not least because of the popularity of decapods, there has been a long-standing interest in their relationships. Beginning with Linnaeus and Lamarck, different hypotheses of decapod relationships have been put forward over the decades relying on sources of information such as adult and larval morphology or behavior. However, irrespective of the usage of just a few morphological characters in early investigations (e.g. tail length, gill type and number of chelae) or of a wider variety of characters in combination with comprehensive morphological cladistic analyses (Martin and Abele, 1986; Scholtz and Richter, 1995; Schram, 2001; Dixon et al., 2003), several questions are still controversial regarding the internal relationships of the major decapod taxa (Fig. 1).

Since the early 1990s, numerous comprehensive analyses of Decapoda have been published based on molecular data sets (Abele, 1991; Kim and Bae, 1992; Ahyong and O'Meally, 2004; Porter et al., 2005; Tsang et al., 2008b; Bracken et al., 2009; Chu et al., 2009; Toon et al., 2009; Bybee et al., 2011). However, the initial hope of the early single gene studies, that conflicts and open questions of decapod phylogeny and evolution could be easily and satisfactorily resolved by using molecular data has so far not been fulfilled. The topologies of the molecular trees published for the last 20 years are as different and contradictory concerning decapod phylogenetic relationships as are the morphological analyses (see Fig. 1). One promise of the new era of phylogenomics is that with increase in the number of genes, including whole genomes, molecular phylogenetic analyses gain greater robustness and reliability (Madsen et al., 2001; Rokas et al., 2003; Brinkmann

* Corresponding author. Tel.: +49 0 30 2093 6005; fax: +49 0 30 2093 6002.
E-mail addresses: hongshenks@hotmail.com (H. Shen), abraband@web.de (A. Braband), gerhard.scholtz@rz.hu-berlin.de (G. Scholtz).

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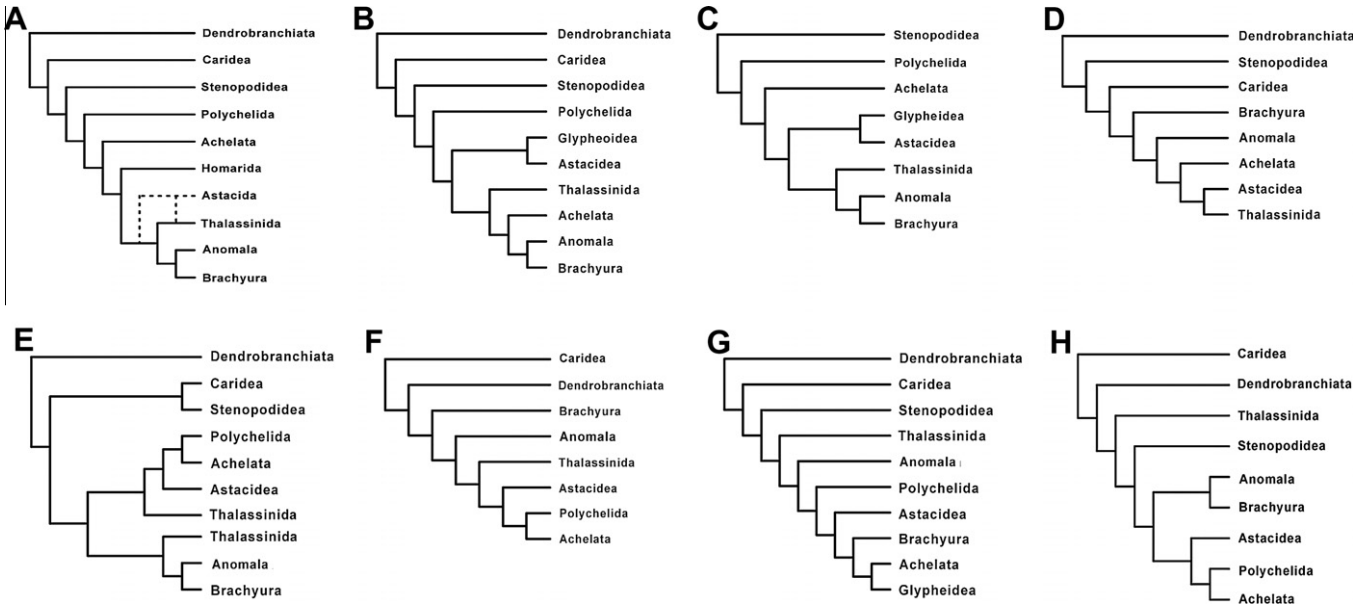

Fig. 1. Various hypotheses of phylogenetic relationships of Decapoda based on morphological cladistic analyses (A and B) and molecular phylogenetic analyses (C–H). A: Scholtz and Richter (1995); B: Dixon et al. (2003); C: Ahyong and O'Meally (2004); D: Porter et al. (2005); E: Tsang et al. (2008b); F: Toon et al. (2009); G: Bracken et al. (2009); H: Bybee et al. (2011).

and Philippe, 2008). In addition, it is evident that an increased taxon sampling is necessary to improve the quality of the analyses (Bergsten, 2005; Wägele and Mayer, 2007; Brinkmann and Philippe, 2008). Subsequently, more mitochondrial and nuclear genes have been concatenated together to build up large datasets, with the hope of reconstructing more reliable and robustly resolved trees compared to analyses based on few genes. However, the combination of different kinds of genes such as protein coding genes, *rRNA* genes, and non-coding nuclear genes made the alignment and the model selection quite difficult (Foster, 2004; Cox et al., 2008). Also, concatenated alignment may have lost important information in the missing parts (Philippe et al., 2004; Baurain et al., 2007), which together may result in unreliable trees. Therefore, large and reliable datasets are required for resolving the phylogenetic relationships within Decapoda based on molecular data.

Despite some problematic aspects such as strand bias and saturation (Hassanin et al., 2005), mitochondrial genomes (mt) might provide this kind of dataset. Accordingly, mt-data have been widely used in phylogenetic analyses of various metazoan groups including arthropods at different levels (e.g. Boore et al., 2005; Hassanin, 2006; Cameron et al., 2009; Masta et al., 2009; Braband et al., 2010; Rota-Stabelli et al., 2010; Liu and Cui, 2011).

Previous mt-genomic analyses of decapod crustaceans have been hampered by the relatively poor und unbalanced taxon sampling, despite its steady improvement over recent years (e.g. Miller and Austin, 2006; Liu and Cui, 2011). In contrast to the wealth of decapod species, so far the complete mt-genome has been sequenced from 36 species covering six major taxa, though often from only one species (Dendrobranchiata, Caridea, Achelata, Astacidea, Anomala and Brachyura). To fill these gaps, we add the (nearly) complete mt-genomes of 14 species. Hence, in our present study, we reconstruct the phylogeny of Decapoda based on the complete mt-genomes from 50 decapods. Our aim was to study interrelationships of decapods and test the monophyly of several controversial taxa (Palinura, Thalassinida, Astacidea and Meiura). This should provide important insights into the origin and evolution of the extraordinarily diverse Decapoda.

2. Materials and methods

2.1. Mitochondrial genome sequencing

We sequenced 13 species[1] covering most major decapod taxa (Table 1). The complete mitochondrial genomes were obtained using a combination of conventional PCR and long PCR to amplify overlapping fragments spanning the whole mitochondrial genome. Mitochondrial DNA was obtained from muscle tissue using DNeasy Tissue Kit (Qiagen), and amplified using the Repli-G Mini Kit (Qiagen). These steps all followed the manufacturer's protocols. Initially, *cox1* and *rrnS* were amplified by conventional PCR using universal primers: *cox1* primers, (Folmer et al., 1994), *rrnS* primers (Braband et al., 2006); *cox3*, *cob* and *nad5* were amplified using scorpion based primers which were designed in our lab. PCR conditions for *cox1* followed the protocol of Folmer et al. (1994), and others followed a standard three step protocol with an initial denaturing step at 96 °C for 3 min, then 40 cycles comprising denaturation at 94 °C for 30 s, annealing at a recommended temperature for different primers for 30 s, elongation at 72 °C for 45 s, then ended with a final extension at 72 °C for 5 min. The PCR products were sequenced commercially (Agowa Berlin, Germany) and the data were used to design species-specific primers to amplify large overlapping regions of the mitochondrial genomes using long range PCR kit (Long Range PCR kits, Qiagen). All fragments were sequenced by LGC (Berlin, Germany) using primer walking single/double strands methods. Primer sequences and fragment sizes can be found in Suppl. material 1 and Suppl. material 2, respectively.

2.2. Sequence annotation and analysis

Sequence annotations were done using the software Artemis Release 8 (Rutherford et al., 2000). Protein-coding and ribosomal RNAs were identified by BLAST search and gene boundaries were determined in comparison with alignments of other decapods.

[1] After submission of our manuscript the article of HuaFeng et al., 2012 on the mitochondrial genome of *Stenopus hispidus* appeared.

Table 1
List of species used in the analysis. The newly sequenced species are indicated by a grey background. Families and higher ranks of decapods follow mainly the suggestions of De Grave et al. (2009).

	Family	Species	Sampling location	GeneBank
Outgroup				
Stomatopoda				
	Gonodactylidae	Gonodactylus chiragra		NC_007442
	Lysiosquillidae	Lysiosquillina maculata		NC_007443
	Squillidae	Squilla empusa		NC_007444
		Harpiosquilla harpax		NC_006916
		Squilla mantis		NC_006081
Euphausiacea				
–	Euphausiidae	Euphausia pacifica		NC_016184.1
Ingroup				
Dendrobranchiata				
	Penaeidae	Marsupenaeus japonicus		NC_007010
		Penaeus monodon		NC_002184
		Farfantepenaeus californiensis		NC_012738
		Litopenaeus stylirostris		NC_012060
		Fenneropenaeus chinensis		NC_009679
		Litopenaeus vannamei		NC_009626
Pleocyemata				
Caridea				
	Atyidae	Halocaridina rubra		NC_008413
	Alpheidae	Alpheus distinguendus		NC_014883
	Palaemonidae	Macrobrachium rosenbergii		NC_006880
		Exopalaemon carinicauda		NC_012566
		Macrobrachium lanchesteri		NC_012217
		Macrobrachium nipponense		NC_015073
Stenopodidea				
	Stenopodidae	*Stenopus hispidus*	Aquarium shop, Berlin, Germany	KC107811
Reptantia				
Polychelida				
	Polychelidae	Polycheles typhlops	Alborán Island, Spain	KC107818
Achelata				
	Palinuridae	Panulirus japonicus		NC_004251
		Panulirus ornatus		NC_014854
		Panulirus stimpsoni		NC_014339
		Panulirus versicolor	Aquarium shop, Berlin, Germany	KC107808
	Scyllaridae	Scyllarides latus	Deutsches Meeresmuseum, Stralsund, Germany	KC107814
Astacidea				
	Parastacidae	Cherax destructor		NC_011243
	Cambaridae	Procambarus fallax f. virginalis	Own culture, Berlin, Germany	KC107813
		Procambarus clarkii		NC_016926.1
		Cambaroides similis		NC_016925.1
	Nephropidae	Homarus gammarus	Helgoland, Germany	KC107810
		Homarus americanus		NC_015607.1
	Enoplometopidae	Enoplometopus occidentalis	Aquarium shop, Berlin, Germany	KC107819
Axiidea				
	Calocarididae	Calocaris macandreae	Alborán Island, Spain	KC107812
	Strahlaxiidae	Neaxius acanthus	Bone Batang island, south Sulawesi, Indonesia	KC107821
	Callianassidae	Corallianassa coutierei	Bone Batang island, south Sulawesi, Indonesia	KC107817
Gebiidea				
	Upogebiidae	Upogebia pusilla	Adriatic sea, Italy	KC107815
		Upogebia major		JF793665.1
Anomala				
	Galatheidae	Shinkaia crosnieri		NC_011013
	Porcellanidae	Neopetrolisthes maculatus	Aquarium shop, Berlin, Germany	KC107816
	Paguridae	Pagurus longicarpus		NC_003058
	Lithodidae	Cryptolithodes sitchensis	San Juan island, USA	KC107809
				KC107820
Brachyura				
	Dromiidae	*Dromia personata*	Adriatic coast, Croatia	L. Podsiadlowski (pers. comm.)
	Bythograeidae	Gandalfus yunohana		NC_013713
	Portunidae	Callinectes sapidus		NC_006281
		Portunus trituberculatus		NC_005037
		Charybdis japonica		NC_013246
		Scylla paramamosain		NC_012572
		Scylla olivacea		NC_012569
		Scylla tranquebarica		NC_012567
		Scylla serrata		NC_012565

Table 1 (*continued*)

Family	Species	Sampling location	GeneBank
Potamidae	Geothelphusa dehaani		NC_007379
Menippidae	Pseudocarcinus gigas		NC_006891
Xenograpsidae	Xenograpsus testudinatus		NC_013480
Varunidae	Eriocheir sinensis		NC_006992
	Eriocheir hepuensis		NC_011598
	Eriocheir japonica		NC_011597

Structures and positions of most transfer RNAs were identified using the web-server of tRNA-scan SE (Lowe and Eddy, 1997), other parts were identified by their potential secondary structures and anticodons. Sequence data were deposited at NCBI database and access numbers were given in Table 1. GC content (Suppl. material 3) and skewness of protein-coding genes (Suppl. material 4) were calculated using DAMBE 5.2.57 (Xia and Xie, 2001).

2.3. Phylogenetic analysis

For the phylogenetic analyses, two concatened datasets: amino acid alignments (AA dataset) and nucleotide alignments (NT dataset) from 12 mt protein coding genes were combined. In addition, some calculations were done with a NT dataset supplemented with two mitochondrial *rRNAs*. The gene *nad2* was excluded from the analyses, because it is lost from two clawed lobsters (*Homarus gammarus* and *Enoplometopus occidentalis*) and because it shows a wide range of GC content (Suppl. material 3). Complete mt genomes of 36 decapods were retrieved from GenBank, and the mt genome of *Dromia personata* was provided by Lars Podsiadlowski and Nicola Dolgner (Universität Bonn). In total 50 decapods covering all major decapod groups were used in the analyses. Five stomatopod and one euphausiacean species were chosen as outgroups (Table 1). Euphausiacea have traditionally been considered as sister group to Decapoda, but some recent studies put them higher up in the malacostracan tree (Jarman et al., 2000; Richter and Scholtz, 2001). Stomatopoda are likely to be the sister group to all other Eumalacostraca (Richter and Scholtz, 2001). All alignments were done with ClustalW (Thompson et al., 1994) as implemented in Bioedit 7.0.9 (Hall, 1999) under default settings. Ambiguously aligned regions were removed by Aliscore v_1.0 (Misof and Misof, 2009; Kück et al., 2010), with the default parameter settings.

In attempt to decrease the effect of strand bias (see Hassanin et al., 2005; Hassanin, 2006; Braband et al., 2010; Rota-Stabelli et al., 2010; Wei et al., 2010) without excluding entire characters in the NT dataset, characters on the first and third codon positions, which could possibly undergo synonymous changes based on the invertebrate genetic code (Masta et al., 2009), were fully degenerated, using standard IUPAC codenames. In this way, fourfold degenerate sites were recoded as 'N', threefold as 'H' and twofold as 'Y' or 'R' (Regier et al., 2010). Due to the lack of effective recoding methods for the AA dataset, three cambarid crayfishes and one homarid lobster, which showed reversed strand bias compared with the other available decapods (Suppl. material 4), were excluded from the AA dataset in some analyses.

The RAxML Web Server (http://phylobench.vital-it.ch/raxml-bb/index.php) (Stamatakis et al., 2008) was used to run a maximum likelihood analyses for all datasets. A partitioned model optimization was done in that the datasets were partitioned according to the different genes. The GTRCAT + G + I model was used for the NT datasets, and MtRev + G + I was used for the AA datasets according to the results of ProtTest version 1.4 (Abascal et al., 2005). In all likelihood analyses, models were the same for each partition but optimized in an unlinked manner between the partitions, and branch statistical supports were obtained after 100 bootstrap replicates. Bayesian analyses of both datasets were carried out using PhyloBayes3.2e (Lartillot and Philippe, 2004), with the CAT-MtRev model for AA datasets and CAT-GTR model for NT datasets. Four independent chains were run in PhyloBayes3.2e, and the calculation was stopped once the largest discrepancy observed across all bipartitions being lower than 0.3. Bayesian posterior probability values were estimated after discarding the first 100 trees as burn-in.

3. Results

3.1. Mitochondrial genome content

Ten complete decapod mitochondrial genomes and three almost complete decapod mitochondrial genomes, coming from six major taxa (Stenopodidea, Polychelida, Achelata, Astacidea, Thalassinida, and Anomala), were sequenced in our lab (Table 1). We could not sequence the fragment including the *nad2*, control region and partial 12 s of *Neaxius acanthus*, while for *Calocaris macandreae* and *Cryptolithodes sitchensis* we failed to obtain the complete control region. Two astacids, *Homarus gammarus* and *Enoplometopus occidentalis* most likely lost the entire *nad2* and partial *nad2* in their mt genomes, respectively. In several taxa we recognized a derivation of the gene order compared to the decapod ground pattern (see Suppl. material 5). However, these changes are mostly either autapomorphic, convergent, or so variable that the phylogenetic information content is apparently very low. There are notable exceptions: for instance, the gene order of the three Axiidea species (*Neaxius acanthus, Calocaris macandreae, Corallianassa coutierei*) shows some apparently apomorphic characteristics which support axiid monophyly (see Suppl. material 5). Nevertheless, we refrained from including the gene order data in our analysis. A more detailed treatment of decapod mitochondrial gene order is in preparation.

3.2. Phylogenetic analyses

Neither the exclusion of the four astacidean species with inverted strand bias from the analysis nor the degenerate data set lead to largely divergent topologies when compared with the full AA- und NT-datasets. The Maximum likelihood analysis of the AA-data set without *Homarus gammarus*, *Procambarus clarkii, Procambarus fallax f. virginalis, and Cambaroides similis* is different to the analysis of the complete set with respect to the position of the *Upogebia* species (Figs. 2A and 3C). The Bayesian AA-datasets differ in the position of *Polycheles typhlops* and the incomplete resolution of the basal branching of the reptants (Figs. 2A and 3A). The Maximum Likelihood analyses of the degenerate NT-datasets (Fig. 2B) show the same topology as the Maximum Likelihood analyses of regularly coded NT-datasets (Fig. 3A). Hence, we conclude that strand bias has a negligible effect on the results.

Most of our analyses resulted in a largely congruent pattern of the higher level relationships among Decapoda. All analyses resolve Dendrobranchiata, Caridea, and Reptantia as monophyletic.

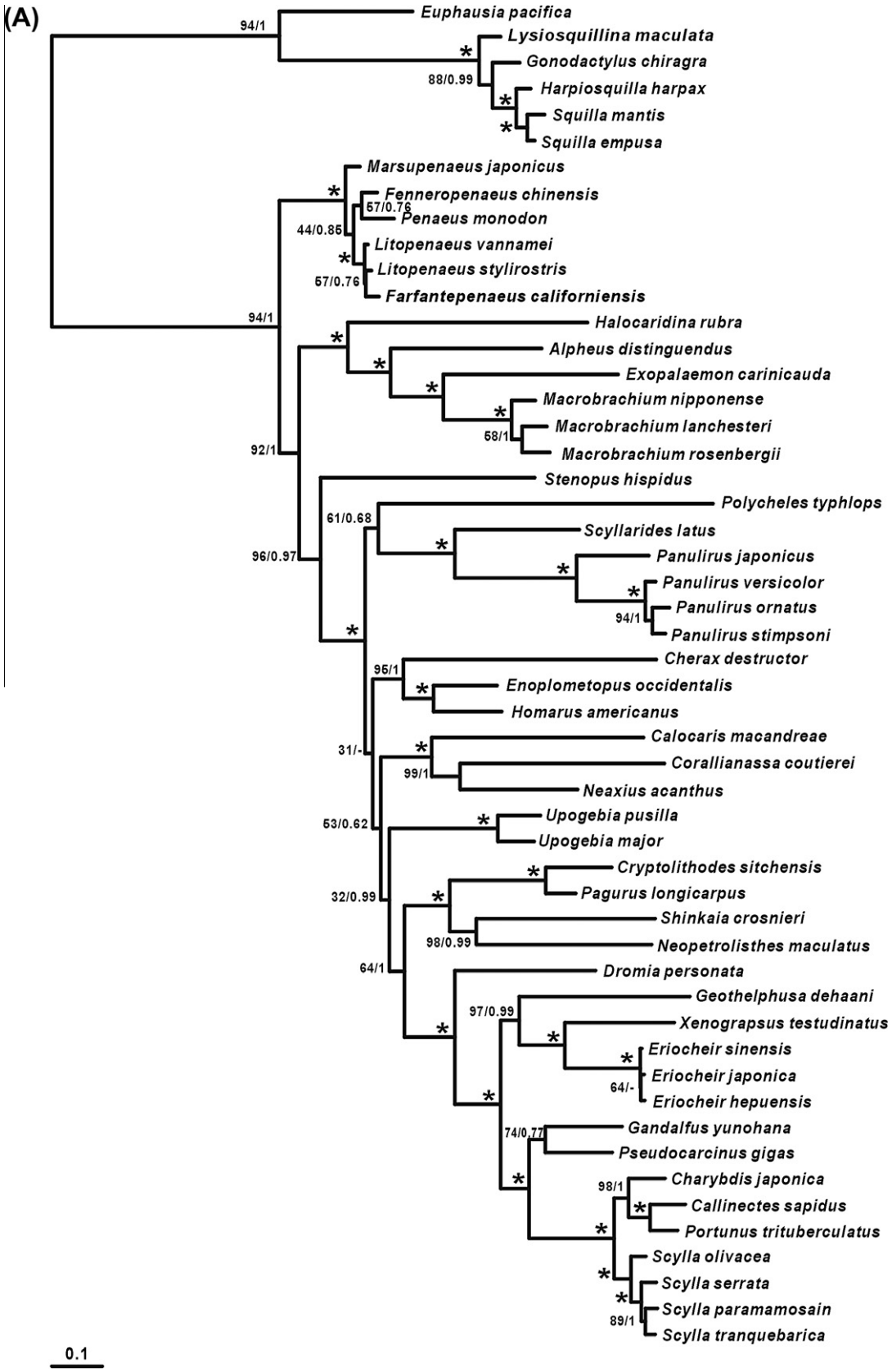

Fig. 2. Strand bias. (A) analysis of decapod relationships based on Maximum Likelihood (RaxML) and Bayesian (PhyloBayes) analyses of the amino acid dataset. The amino acids of 12 mitochondrial protein coding genes (except *nad2*) are concatenated in the dataset. To decrease the impacts of the strand bias, four astacids are excluded from AA dataset. Stars indicate bootstrap value is 100 ML and Posterior Probabilities BAY is 1. (B) Analysis of decapod relationships based on Maximum Likelihood analysis (RaxML) of a degenerated nucleotide dataset. 12 mitochondrial protein coding genes. Five stomatopods and *Euphausia pacifica* are used as outgroups.

Fig. 2. (*continued*)

Since we included only one species of stenopodids, we cannot make any statement about the monophyly of this shrimp group. As a general pattern, the sister group relationship between Dendrobranchiata and Pleocyemata is confirmed in all topologies. Within Pleocyemata, Caridea is the sister group to a clade formed by Stenopodidea and Reptantia. Only the Bayesian analyses of

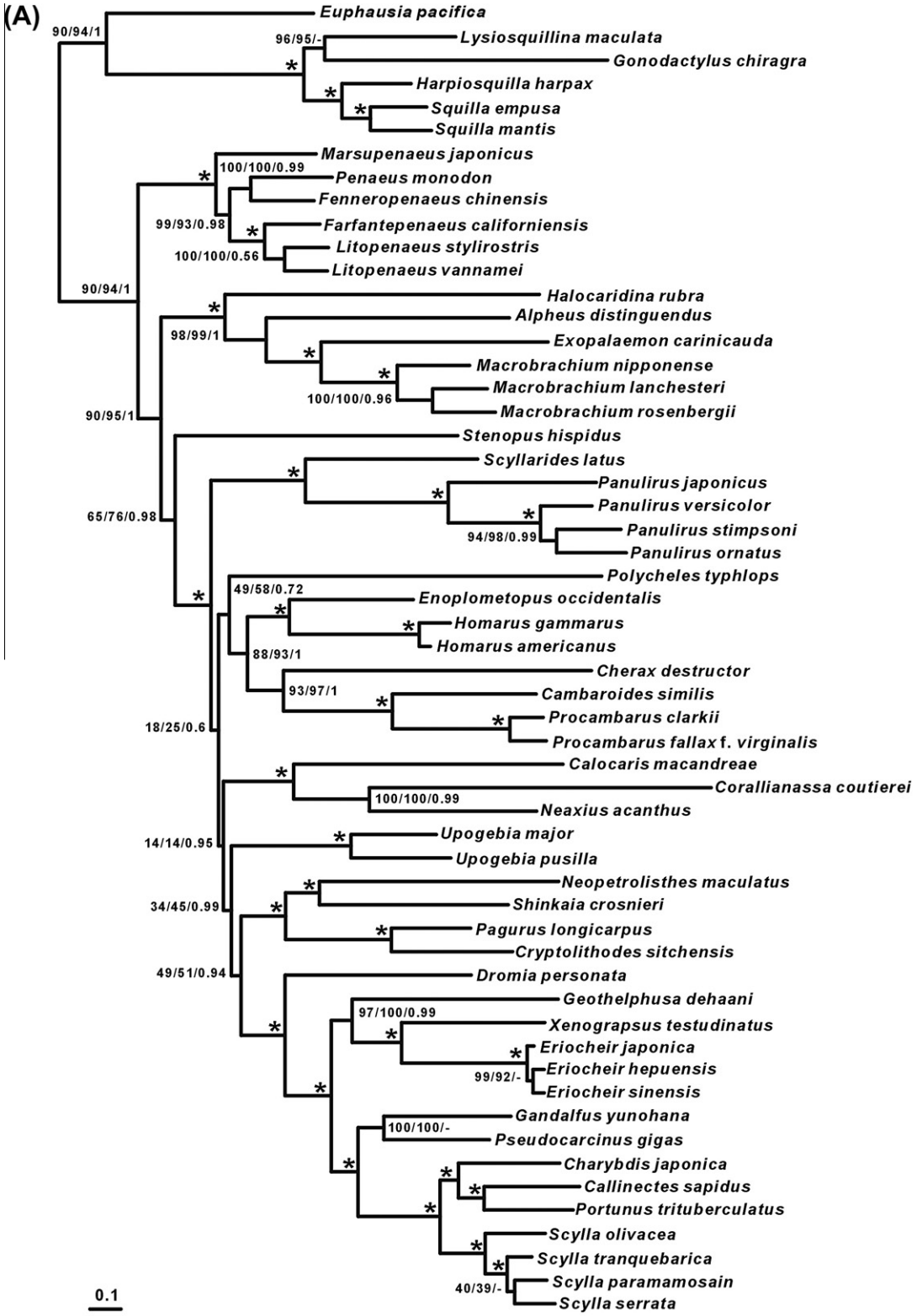

Fig. 3. Analyses of decapod relationships with different datasets. (A) Analysis based on maximum likelihood analysis (RaxML) of two different nucleotide datasets (12 mitochondrial protein coding genes (except *nad2*), and 12 protein coding genes plus 12s *rRNA* and 16s *rRNA*) and Bayesian analysis (PhyloBayes 3.2e) of amino acid dataset. The only topological differences between the two ML and the Bayesian analyses concern the internal relationships of stomatopods and those of the Brachyura *Eriocheir*, *Scylla* and *Gandalfus*/*Pseudocarcinus* as indicated by a minus. Stars indicate the two ML bootstrap values being 100, and BAY Posterior Probabilities being 1. (B). Bayesian analysis (PhyloBayes) of the two different nucleotide datasets (12 mitochondrial protein coding genes (except *nad2*), and 12 protein coding genes plus 12s *rRNA* and 16s *rRNA*). BAY Posterior Probabilities are above or below the branches. Star indicates both BAY Posterior Probabilities are 1. The only major differences to the topology in A are the position of *Stenopus* as sister to Caridea and the unresolved basal branching of the Reptantia. (C) Maximum likelihood analysis of the amino acid data set as in A. Bootstrap values above or under the branches. This is the only analysis showing monophyletic Thalassinida, although with low support. Furthermore, *Polycheles* is placed as sister group to Achelata.

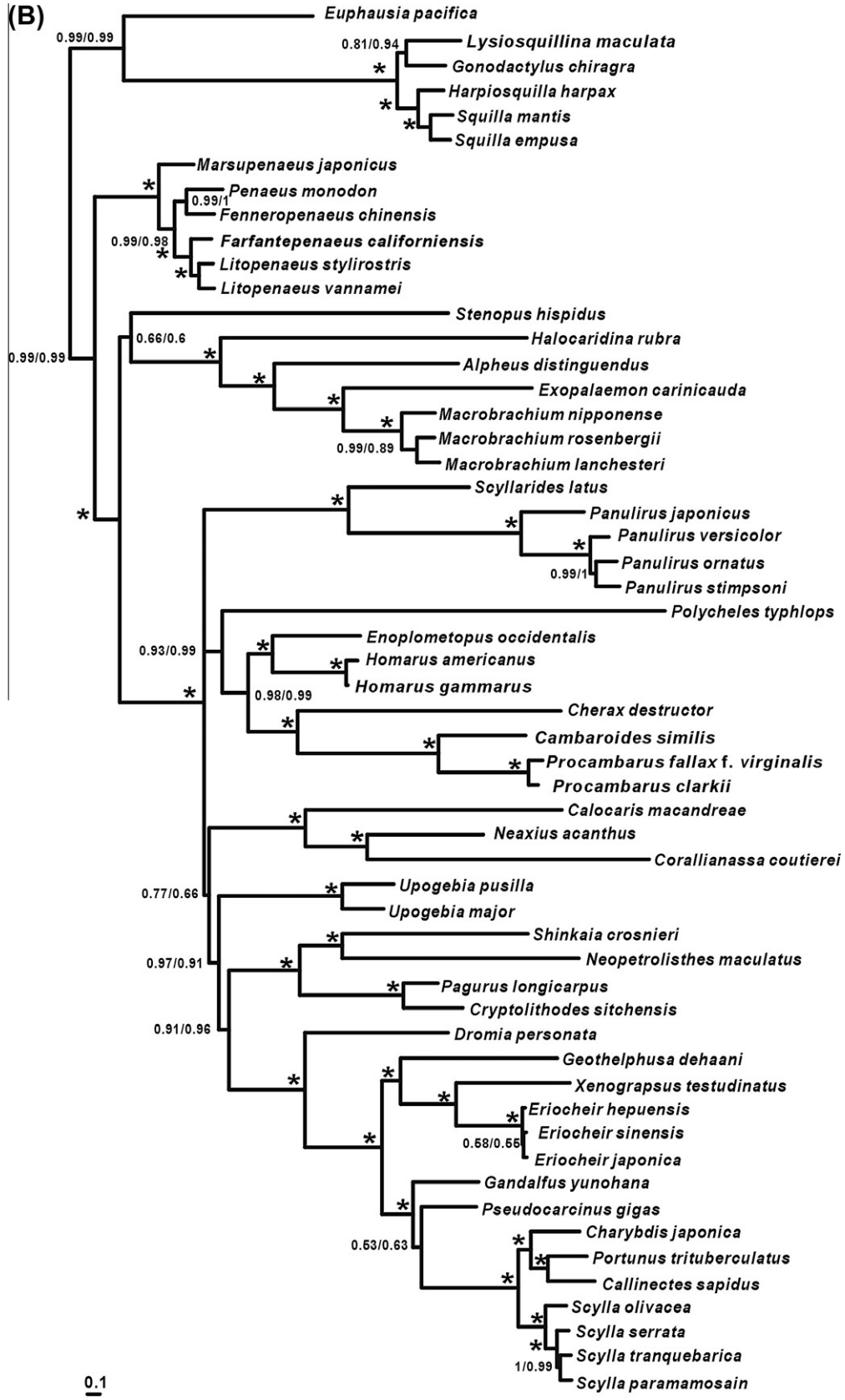

Fig. 3. (*continued*)

the NT-datasets resolve Stenopodidea as sister to Caridea but with low support (Fig. 3B). Among the Reptantia, Achelata, Astacidea, Anomala, and Brachyura are monophyletic. *Polycheles typhlops* is the sister group of Astacidea in the Maximum Likelihood and Bayesian analyses of the NT-datasets and the Bayesian AA-analysis (Fig. 3A and B). In the Maximum Likelihood AA tree (Fig. 3C), it is

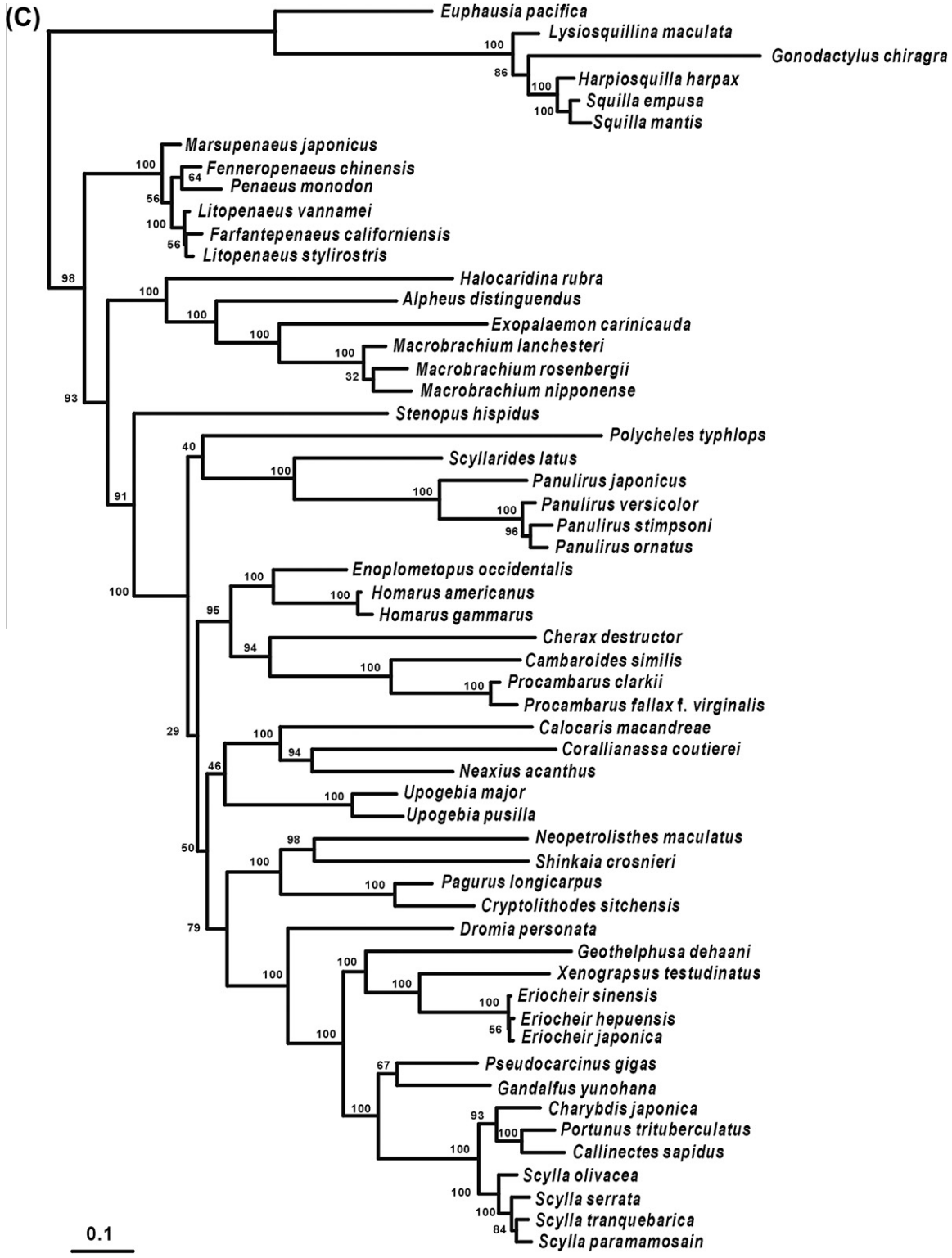

Fig. 3. (*continued*)

the sister group of Achelata. Of the traditional groupings, only Thalassinida is paraphyletic, in this case with respect to Meiura. Only the Maximum Likelihood analysis of AA-data shows monophyletic Thalassinida but with low statistical support (Fig. 3C). The Meiura, comprising Anomala and Brachyura, is consistently shown by all analyses. In general, all analyses corroborate this overall tree topology (Fig. 3). Nevertheless, bootstrap supports and posterior probabilities are low concerning some of the relationships within Reptantia. Furthermore, the Bayesian NT-analyses do not resolve the relative positions of Achelata and Astacidea to the remaining Reptantia (Fig. 3B).

At a lower level, Dendrobranchiata shows two different topologies in our trees (Fig. 3A–C). In the Caridea, the relationships of the three *Macrobrachium* species in the Maximum Likelihood AA tree

are different from those in the other trees ((Fig. 3A,C). Likewise, the three *Eriocheir* and three of the four *Scylla* species as well as the respective positions of *Pseudocarcinus gigas* and *Gandalfus yunohana* among the Brachyura show different sister-group relationships depending on the analysis (Fig. 3A–C).

4. Discussion

4.1. Phylogenetic relationships within shrimp-like decapods

Early classifications dividing the decapods into swimming (Natantia) and walking (Reptantia) lineages (e.g. Boas, 1880) have been abandoned for a long time. Burkenroad (1963, 1981) separated the Decapoda into Dendrobranchiata and Pleocyemata, largely based on gill morphology and reproductive biology, a view that is also consistent with decapod brain anatomy (Sandeman et al., 1993). This approach resulted in paraphyletic Natantia. Now there is little controversy about the monophyly of Dendrobranchiata and Pleocyemata (with the notable exception of Toon et al., 2009, who show a sister group relationship between Dendrobranchiata and Reptantia). Nevertheless, within Pleocyemata the relationships of two natant lineages (Caridea and Stenopodidea) relative to the Reptantia have been disputed. Morphological studies and some molecular analyses resolve Stenopodidea as sister group to Reptantia (Abele and Felgenhauer, 1986; Abele, 1991; Scholtz and Richter, 1995; Schram, 2001; Dixon et al., 2003; Schram and Dixon, 2004; Bracken et al., 2009). However, two alternative hypotheses have been proposed for this issue: (1) the Caridea and Stenopodidea together form a clade which is the sister group to Reptantia (Burkenroad, 1981; Tsang et al., 2008b; Chu et al., 2009) and (2) the Caridea is the sister group to the Reptantia (Christoffersen, 1988; Porter et al., 2005).

Our analyses support the Dendrobranchiata being the sister group of the remaining Decapoda, i.e. the Pleocyemata. Within Pleocyemata, Caridea is more basal than Stenopodidea, and Stenopodidea is likely the sister group to the Reptantia (with one exception resolving a stenopodid – caridean sister group relationship), which agrees with most recent morphological analyses (Scholtz and Richter, 1995; Schram, 2001; Dixon et al., 2003).

4.2. Relationships within Reptantia

The internal relationships of the highly diverse Reptantia are even more contentious. Based on morphological and molecular data, almost every possible combination of relationships between the reptant lineages has been suggested.

According to morphological cladistic analyses, a taxon Fractosternalia was proposed by Scholtz and Richter (1995) to describe a large monophyletic group including Astacida, Thalassinida, and Meiura (Anomala+Brachyura). Homarida is the sister group to this clade (Scholtz and Richter, 1995). However, the relationships of Astacida and Thalassinida and Meiura remained unresolved. An alternative grouping, Sterropoda was erected by Dixon et al. (2003) to describe a sister group relationship between Thalassinida and Eurysternalia (Achelata + Meiura). Astacidea is the sister group to this clade, with the Polychelida as sister group to the remaining reptants (Eureptantia, see also Scholtz and Richter, 1995). This topology was also supported when fossils were included (Schram and Dixon, 2004). Two recent largely contrasting topologies come from different molecular data: the topologies of Tsang et al. (2008b) based on two nuclear protein-coding genes, show two separate thalassinidean groups: a paraphyletic assemblage is closer to the Meiura, and the other group is more closely related to a clade formed by Palinura (Polychelida + Achelata) plus Astacidea. The analyses of Bracken et al. (2009), based on three ribosomal genes and one nuclear gene, show Brachyura as sister group to Achelata, Astacidea is sister group to both, Polychelida is sister group to this clade, and all of them are the sister group to Anomala, with Thalassinida as the basal branch within the Reptantia (Fig. 1).

Most analyses of our various datasets result in the same pattern of relationship among Reptantia. Anomala and Brachyura together constitute the Meiura. Thalassinida are paraphyletic with respect to Meiura. Astacidea is the sister group to the clade of "Thalassinida" and Meiura. Achelata are resolved as sister group of the remaining Reptantia. Polychelida is the sister group to the Astacidea (Fig. 4).

On a general level, our new trees are quite similar to the morphological cladistic analysis of Scholtz and Richter (1995) and the molecular study of Ahyong and O'Meally (2004). Two major clades of Scholtz and Richter (1995) are corroborated by our analysis, namely the Macrochelata (Astacidea, "Thalassinida", Anomala, Brachyura), however with the inclusion of Polychelida, and the Meiura (Anomala, Brachyura). In contrast, the clades Eureptantia (all reptants except Polychelida) and Fractosternalia (comprising freshwater crayfish, thalassinids, and meiurans) are not supported. Freshwater crayfish and homarids together are always resolved as monophyletic Astacidea (see also Dixon et al., 2003; Ahyong and O'Meally, 2004; Tsang et al., 2008b; Bracken et al., 2009; Chu et al., 2009; Toon et al., 2009; Bybee et al., 2011). This result implies that the movable last thoracic sternite, shared by freshwater crayfish, thalassinids, and anomalans might be either a convergent feature, or lost in the clawed lobsters and brachyurans. The latter view would extend the fractostern concept to a deeper reptant node (see Dixon et al., 2003 for discussion).

Ahyong and O'Meally (2004) proposed the name Lineata for a clade comprising thalassinids and Meiura. All our analyses reveal Lineata as monophyletic (compare also Boisselier-Dubayle et al., 2010) irrespective of whether thalassinids are resolved as monophyletic or paraphyletic (see below). The "Macrura Reptantia" of Tsang et al. (2008b) containing Polychelida, Achelata, Astacidea, and part of Thalassinida (see also Bybee et al., 2011) is not supported by our analysis.

4.3. Palinura (Achelata and Polychelida)

Achelata and Polychelida were first included in a monophyletic taxon "Palinura" (Borradaile, 1907) based on some morphological characters such as the reduction of the inner lobes of the 2nd maxillae and 1st maxillipeds. Nevertheless, this taxon was doubted by Scholtz and Richter (1995), who suggested that Polychelida is the sister group to the remaining Reptantia, which they called Eureptantia. They listed at least six morphological apomorphies shared by Eureptantia to the exclusion of Polychelida. According to this view, the latter show plesiomorphic character states, such as a pointed telson, the absence of a crista dentata from the ischium of the third maxilliped, or a sharp-tipped dactyl of the third maxilliped among others (see Scholtz and Richter, 1995). Furthermore, Scholtz and Richter (1995) suggested that scyllarids plus palinurids formed the monophyletic taxon Achelata. Additional morphological and molecular evidence supported these views (Schram, 2001; Dixon et al., 2003; Ahyong and O'Meally, 2004). However, recently there were some claims for a reunion of Palinura based on various molecular datasets (Crandall et al., 2000; Tsang et al., 2008b; Toon et al., 2009; Boisselier-Dubayle et al., 2010; Bybee et al., 2011). In our study only the RaxML analysis of the AA dataset shows this reunion but with low statistical support. All other analyses instead retrieved a polyphyletic Palinura. In these cases, Polychelida is the sister group to Astacidea. This topology differs from all previous phylogenetic studies. Interestingly, the omission of four Astacidea species shifts *Polycheles* from Astacidea to Achelata in the Bayesian analysis of the AA-dataset (Figs. 2 and 3).

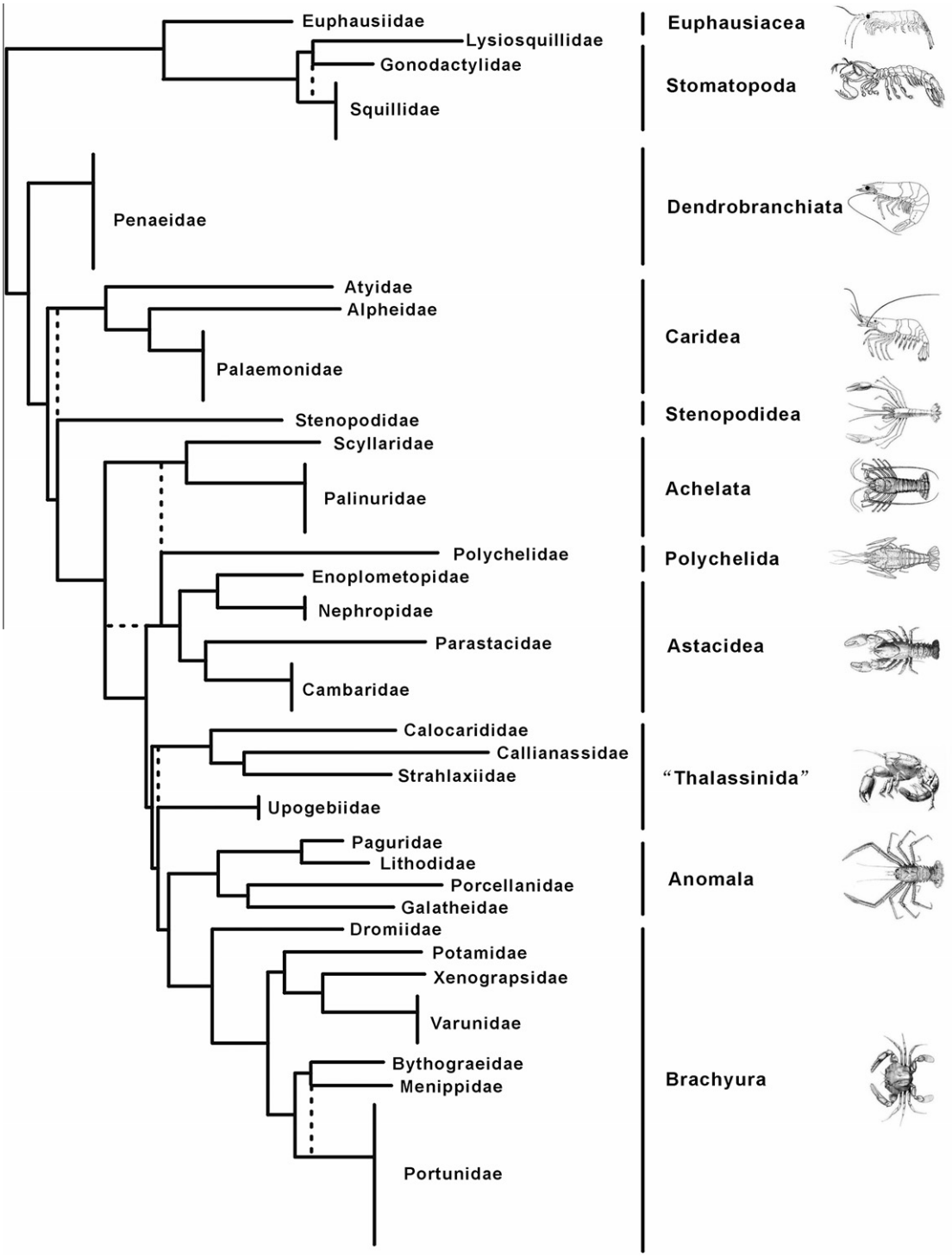

Fig. 4. Tree of Decapoda based on the analyses of all datasets. The branches are collapsed to show decapod families. Vertical bars indicate classical infraorders/superfamilies within Decapoda. Alternative or unresolved relationships which occurred in the minority of our analyses are indicated by broken lines.

A sister-group relationship between Polychelida and Astacidea or a monophyletic Palinura clearly conflicts with the mentioned morphological characters. Given the low level of support for the position of *Polycheles*, it is premature to come to a conclusive answer to the question of polychelid affinities.

4.4. Astacidea

The Astacidea include the freshwater crayfish and the marine clawed lobsters. The freshwater crayfish (Astacida) are nowadays considered to be a monophyletic taxon, with Astacoidea and Parastacoidea as major sister taxa (Scholtz, 1993, 1995; Crandall et al., 2000). The same is true for the marine clawed lobsters (e.g. Tshudy and Babcock, 1997; Ahyong, 2006; Karasawa et al., 2013). According to the analysis of Scholtz and Richter (1995), Astacida and Homarida (marine clawed lobsters) were paraphyletic. However, recent morphological and molecular analyses all supported the monophyly of the Astacidea (Dixon et al., 2003; Ahyong and O'Meally, 2004; Tsang et al., 2008b; Chu et al., 2009; Bracken et al., 2009; Toon et al., 2009).

Our trees agree with these recent results and clearly support the monophyly of the Astacidea (Fig. 3) with two subclades, the freshwater crayfish (Astacoidea and Parastacoidea) and the marine clawed lobsters (Nephropoidea and Enoplometopoidea). Thus, this recent outcome corroborates the traditional views of decapod systematists such as Boas (1880) and Borradaile (1907) (Astacura).

4.5. Thalassinida

There has been much debate about the interrelationships of the Thalassinida, and more specifically about the monophyly of this group. Paraphyletic or polyphyletic thalassinids with different affinities of the subgroups have been suggested based on larval, sperm, gastric, and general morphology (Gurney, 1938; de Saint Laurent, 1973; Tudge, 1995; Sakai, 2004). In contrast, the morphological phylogenetic analyses of Poore (1994), Scholtz and Richter (1995), Schram (2001), Dixon et al. (2003) proposed monophyletic Thalassinida. With the exception of Ahyong and O'Meally (2004), and Tsang et al. (2008a) most molecular studies do not resolve thalassinids as a clade (Morrison et al., 2002; Tudge and Cunningham, 2002; Tsang et al., 2008b; Chu et al., 2009; Bracken et al., 2009; Robles et al., 2009). Despite these differences, however, most analyses suggest two major monophyletic taxa within thalassinids, namely Gebiidea and Axiidea (see de Saint Laurent, 1973; Robles et al., 2009).

The monophyly of Axiidea and Gebiidea is also the outcome of our analyses. In addition, we find evidence for the idea of paraphyletic thalassinids. Apart from the topology of the Maximum Likelihood AA-dataset which resolves monophyletic Thalassinida with low support, in all our analyses Gebiidea is the sister group of Meiura, and Axiidea is the sister group to Gebiidea and Meiura. This result is different from all previous molecular analyses but it is not far removed from the ideas of Gurney (1938) and de Saint Laurent (1973) based on larval and morphological characters.

4.6. Meiura (Anomala and Brachyura)

The Meiura concept was first proposed by Scholtz and Richter (1995) with reference to a sister relationship between Anomala and Brachyura according to several morphological characters. This concept gained support from other morphological and molecular analyses (Schram, 2001; Dixon et al., 2003; Ahyong and O'Meally, 2004; Miller and Austin, 2006; Tsang et al., 2008b; Boisselier-Dubayle et al., 2010; Bybee et al., 2011). However, recently, the monophyletic Meiura was questioned by some molecular studies (Morrison et al., 2002; Porter et al., 2005; Bracken et al., 2009; Toon et al., 2009) (Fig. 1). Without exception our trees show Meiura as monophyletic (Figs. 2 and 3).

Our data, furthermore, indicate that Anomala and Brachyura are both monophyletic. This view that is held by almost all recent morphological or molecular studies (e.g. Scholtz and Richter, 1995; Schram, 2001; Morrison et al., 2002; Dixon et al., 2003; Ahyong and O'Meally, 2004; Ahyong et al., 2007, 2009; Brösing et al., 2007; Tsang et al., 2008b, 2011; Scholtz and McLay, 2009; Bybee et al., 2011; Reimann et al., 2011; Karasawa et al., 2011) with the notable exception of Spears et al. (1992), who resolved polyphyletic Brachyura.

The brachyuran crabs are traditionally divided into two major groups, the Podotremata and Eubrachyura, the latter comprising Heterotremata and Thoracotremata (e.g. Guinot, 1978; de Saint Laurent, 1980; Jamieson et al., 1995). There is significant doubt as to whether the Podotremata and the Heterotremata are actually monophyletic groups (see von Sternberg and Cumberlidge, 2001; Ahyong et al., 2007; Brösing et al., 2007; Scholtz and McLay, 2009). Unfortunately, our analysis includes only one podotrematan representative (*D. personata*), all other species being eubrachyurans. Hence, we cannot say anything about the status of the podotrematous crabs. However, one aspect in eubrachyurans is noteworthy. The Heterotremata, a group that has sometimes been interpreted as monophyletic (e.g. Guinot, 1978; Jamieson et al., 1995; Chu et al., 2009), is here resolved as paraphyletic (see also von Sternberg and Cumberlidge, 2001; Brösing et al., 2007). The heterotreme Potamidae is strongly supported in all our analyses as the sister group to the Thoracotremata.

Fig. 4 summarizes the results of our analyses. There is a stable gross topology of decapod relationships among all our approaches. Despite the sometimes low statistical support, the stability of most of the major sister group relationships irrespective of the analytical tools used is encouraging. It is plausible that the cases, in which certain relationships could not unambiguously be resolved, relate to long branches and poor taxon sampling. Hence, there is a realistic chance that future studies using a denser taxon sampling could improve the results.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.11.002.

References

Abascal, F., Zardoya, R., Posada, D., 2005. ProtTest: selection of best-fit models of protein evolution. Bioinformatics 21, 2104–2105.
Abele, L.G., 1991. Comparison of morphological and molecular phylogeny of the Decapoda. Mem. Queensl. Mus. 31, 101–108.
Abele, L.G., Felgenhauer, B.E., 1986. Phylogenetic and phenetic relationships among the lower Decapoda. J. Crust. Biol. 6, 385–400.
Ahyong, S.T., 2006. Phylogeny of the clawed lobsters (Decapoda Homarida). Zootaxa 1109, 1–14.
Ahyong, S.T., O'Meally, D., 2004. Phylogeny of the *Decapoda Reptantia*: resolution using three molecular loci and morphology. Raffles Bull. Zool. 52, 673–693.
Ahyong, S.T., Lai, J.C.Y., Sharkey, D., Colgan, D.J., Ng, P.K.L., 2007. Phylogenetics of the brachyuran crabs (Crustacea: Decapoda): the status of Podotremata based on small subunit nuclear ribosomal RNA. Mol. Phylogenet. Evol. 45, 576–586.
Ahyong, S.T., Schnabel, K.E., Maas, E.W., 2009. Anomuran phylogeny: new insights from molecular data. In: Martin, J.W., Felder, D.L., Crandall, K.A. (Eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). CRC Press, Boca Raton, pp. 399–414.
Baurain, D., Brinkmann, H., Philippe, H., 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? Mol. Biol. Evol. 24, 6–9.
Bergsten, J., 2005. A review of long-branch attraction. Cladistics 21, 163–193.
Boas, J.E.V., 1880. Studier over Decapodernes Slaegtskabsforhold. Vidensk. Selsk. Skr. 6. Raekke, naturvid. math. Afd. I, 2, 25–210.
Boisselier-Dubayle, M.-C., Bonillo, C., Cruaud, C., Couloux, A., Richer de Forges, B., Vidal, N., 2010. The phylogenetic position of the 'living fossils' *Neoglyphea* and *Laurentaeglyphea* (Decapoda: Glypheidea). C.R. Biologies 333, 755–759.
Boore, J.L., Macey, J.R., Medina, M., 2005. Sequencing and comparing whole mitochondrial genomes of animals. Meth. Enzymol. 395, 311–348.
Borradaile, L.A., 1907. On the classification of the decapod crustaceans. Ann. Mag. Nat. Hist. 19, 457–486.

Braband, A., Kawai, T., Scholtz, G., 2006. The phylogenetic position of the East Asian freshwater crayfish *Cambaroides* within the Northern Hemisphere Astacoidea (Crustacea, Decapoda, Astacida) based on molecular data. J. Zool. Syst. Evol. Res. 44, 17–24.

Braband, A., Cameron, S.L., Podsiadlowski, L., Daniels, S.R., Mayer, G., 2010. The mitochondrial genome of the onychophoran *Opisthopatus cinctipes* (Peripatopsidae) reflects the ancestral mitochondrial gene arrangement of Panarthropoda and Ecdysozoa. Mol. Phylogenet. Evol. 57, 285–292.

Bracken, H.D., Toon, A., Felder, D.L., Martin, J.W., Finley, M., Rasmussen, J., Palero, F., Crandall, K.A., 2009. The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. Arthr. Syst. Phylog. 67, 99–116.

Brinkmann, H., Philippe, H., 2008. Animal phylogeny and large-scale sequencing: progress and pitfalls. J. Syst. Evol. 46, 274–286.

Brösing, A., Richter, S., Scholtz, G., 2007. Phylogenetic analysis of the Brachyura (Crustacea, Decapoda) based on characters of the foregut with establishment of a new taxon. J. Zool. Syst. Evol. Res. 45, 20–32.

Burkenroad, M.D., 1963. The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record. Tulane Stud. Geol. 2, 1–17.

Burkenroad, M.D., 1981. The higher taxonomy and evolution of Decapoda (Crustacea). Trans. San Diego Soc. Nat. Hist. 19, 251–268.

Bybee, S.M., Bracken-Grissom, H.D., Hermansen, R.A., Clement, M.J., Crandall, K.A., Felder, D.L., 2011. Directed next generation sequencing for phylogenetics: an example using Decapoda. Zool. Anz. 250, 497–506.

Cameron, S.L., Sullivan, J., Song, H., Miller, K.B., Whiting, M.F., 2009. A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. Zool. Script. 38, 575–590.

Christoffersen, M.L., 1988. Phylogenetic systematics of the Eucarida (Crustacea, Malacostraca). Revist. Brasil. Zool. 5, 325–351.

Chu, K.H., Tsang, L.M., Ma, K.Y., Chan, T.Y., Ng, P.K.L., 2009. Decapod phylogeny: what can protein-coding genes tell us? In: Martin, J.W., Felder, D.L., Crandall, K.A. (Eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). CRC Press, Boca Raton, pp. 88–99.

Cox, C., Foster, P., Hirt, R., Harris, S.R., Embley, T.M., 2008. The archaebacterial origin of eukaryotes. Proc. Natl. Acad. Sci. USA 105, 20356–20361.

Crandall, K.A., Harris, D.J., Fetzner Jr., J.W., 2000. The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. Proc. R. Soc. Lond. B 267, 1679–1686.

De Grave, S.D., Pentcheff, N.D., Ahyong, S.T., Chan, T.Y., Crandall, K.A., Dworschak, P.C., Felder, D.L., Feldmann, R.M., Fransen, C.H.J.M., Goulding, L.Y.D., Lemaitre, R., Low, M.E.Y., Martin, J.W., Ng, P.K.L., Schweitzer, C.E., Tan, S.H., Tshudy, D., Wetzer, R., 2009. A classification of living and fossil genera of decapod crustaceans. Raffles Bull. Zool. Suppl. 21, 1–109.

De Saint Laurent, M., 1973. Sur la systématique et la phylogénie des Thalassinidea: definitions des familles des Callianassidae et des Upogebiidae et diagnose de cinq genres nouveaux (Crustacea Decapoda). Compt. Rend. Acad. Sci. (D) 277, 513–516.

De Saint Laurent, M., 1980. Sur la classification et la phylogénie des Crustacés Décapodes Brachyoures. Podotremata Guinot, 1977, et Eubrachyura sect. nov. Compt. Rend. Acad. Sci. (D) 290, 1265–1268.

Dixon, C.J., Ahyong, S.T., Schram, F.R., 2003. A new hypothesis of decapod phylogeny. Crustaceana 76, 935–975.

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. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Foster, P., 2004. Modeling compositional heterogeneity. Syst. Biol. 53, 485–495.

Guinot, D., 1978. Principles d'une classification èvolutive des crustacès dècapodes brachyoures (Principles of a new classification of the Crustacea Decapoda Brachyura). Bull. Biol. Fr. Belg., N. S. 112, 211–293.

Gurney, R., 1938. Larvae of decapod Crustacea. Part 5. Nephropsidae and Thalassinidae. Discov. Rep. 17, 291–344.

Hall, T.A., 1999. BioIEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. <http: // www. mbio. ncsu. Edu/ BioEdit/bioedit. html>.

Hassanin, A., 2006. Phylogeny of Arthropoda inferred from mitochondrial sequences: strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. Mol. Phylogenet. Evol. 38, 100–116.

Hassanin, A., Leger, N., Deutsch, J., 2005. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. Syst. Biol. 54, 277–298.

HuaFeng, S., RuiYu, L., ZhongLi, S., JiPing, M., 2012. Complete mitochondrial DNA sequence of *Stenopus hispidus* (Crustacea: Decapoda: Stenopodidea) and a novel tRNA gene cluster. Mar. Gen. 6, 7–15.

Jamieson, B.G.M., Guinot, D., Richer de Forges, B., 1995. Phylogeny of the Brachyura (Crustacea, Decapoda): evidence from spermatozoal ultrastructure. Mém. Mus. Nat. Hist. Nat. 166, 265–283.

Jarman, S.N., Nicol, S., Elliott, N.G., McMinn, A., 2000. 28S rDNA evolution in the Eumalacostraca and the phylogenetic position of krill. Mol. Phylogenet. Evol. 17, 26–36.

Karasawa, H., Schweitzer, C.E., Feldmann, R.M., 2011. Phylogenetic analysis and revised classification of podotrematous Brachyura (Decapoda) including extinct and extant families. J. Crust. Biol. 31, 523–565.

Karasawa, H., Schweitzer, C.E., Feldmann, R.M., 2013. Phylogeny and systematics of extant and extinct lobsters. J. Crust. Biol. 33, 78–123.

Kim, W., Bae, G.J., 1992. Nucleotide analysis of 18S rRNA and molecular phylogeny of the Korean decapods. Korean J. Zool. 35, 30–86.

Kück, P., Garcia, F.H., Misof, B., Meusemann, K., 2010. Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. PLoS ONE 6 (6), e21031.

Lartillot, N., Philippe, H., 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Mol. Biol. Evol. 21, 1095–1109.

Liu, Y., Cui, Z., 2011. Complete mitochondrial genome of the Chinese spiny lobster *Panulirus stimpsoni* (Crustacea: Decapoda): genome characterization and phylogenetic considerations. Mol. Biol. Rep. 38, 403–410.

Lowe, T.M., Eddy, S.R., 1997. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucl. Acids Res. 25, 955–964.

Madsen, O., Scally, M., Douady, C.J., Kao, D.J., Debry, R.W., Adkins, R., Amrine, H.M., Stanhope, M.J., Jong, W.W., Springer, M.S., 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409, 610–614.

Martin, J.W., Abele, L.G., 1986. Phylogenetic relationships of the genus *Aegla* (Decapoda: Anomura: Aeglidae), with comments on anomuran phylogeny. J. Crust. Biol. 6, 576–616.

Masta, S.E., Longhorn, S.J., Boore, J.L., 2009. Arachnid relationships based on mitochondrial genomes: Asymmetric nucleotide and amino acid bias affects phylogenetic analyses. Mol. Phylogenet. Evol. 50, 117–128.

Miller, A.D., Austin, C.M., 2006. The complete mitochondrial genome of the mantis shrimp *Harpiosquilla harpax*, and a phylogenetic investigation of the Decapoda using mitochondrial sequences. Mol. Phylogenet. Evol. 38, 565–574.

Misof, B., Misof, K., 2009. A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. Syst. Biol. 58, 21–34.

Morrison, C.L., Harvey, A.W., Lavery, S., Tieu, K., Huang, Y., Cunningham, C.W., 2002. Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like body form. Proc. Roy. Soc. Lond. B 269, 345–350.

Philippe, H., Snell, E.A., Bapteste, E., Lopez, P., Holland, P.W., Casane, D., 2004. Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol. Biol. Evol. 21, 1740–1752.

Poore, G.C.B., 1994. A phylogeny of the families of Thalassinidea (Crustacea: Decapoda) with keys to families and genera. Mem. Qld. Mus. 54, 79–120.

Porter, M.L., Pérez-Losada, M., Crandall, K.A., 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. Mol. Phylogenet. Evol. 37, 355–369.

Regier, J.C., Shultz, J.W., Zwick, A., Hussey, A., Ball, B., Wetzer, R., Martin, J.W., Cunningham, C.W., 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. Nature 463, 1079–1083.

Reimann, A., Richter, S., Scholtz, G., 2011. Phylogeny of the anomala (Crustacea, Decapoda, Reptantia) based on the ossicles of the foregut. Zool. Anz. 250, 316–342.

Richter, S., Scholtz, G., 2001. Phylogenetic analysis of the *Malacostraca* (Crustacea) J. Zool. Syst. Evol. Res. 39, 113–136.

Robles, R., Tudge, C.C., Dworschak, P.C., Poore, G.C.B., Felder, D.L., 2009. Molecular phylogeny of the Thalassinidea based on nuclear and mitochondrial genes. In: Martin, J.W., Felder, D.L., Crandall, K.A. (Eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). CRC Press, Boca Raton, pp. 309–326.

Rokas, A., Williams, B.L., King, N., Carroll, S.B., 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425, 798–804.

Rota-Stabelli, O., Kayal, E., Gleeson, D., Daub, J., Boore, J.L., Telford, M.J., Pisani, D., Blaxter, M., Lavrov, D.V., 2010. Ecdysozoan mitogenomics: evidence for a common origin of the legged invertebrates, the Panarthropoda. Genome Biol. Evol. 2, 425–440.

Rutherford, K., Parkhill, J., Crook, J., Horsnell, T., Rice, P., Rajandream, M.A., Barrell, B., 2000. Artemis: sequence visualization and annotation. Bioinformatics 16, 944–945.

Sakai, K., 2004. The diphyletic nature of the infraorder Thalassinidea (Decapoda, Pleocyemata) as derived from the morphology of the gastric mill. Crustaceana 77, 1117–1130.

Sandeman, D., Scholtz, G., Sandeman, R., 1993. Brain evolution in decapod Crustacea. J. Exp. Zool. 295, 112–133.

Scholtz, G., 1993. Teloblasts in decapod embryos: an embryonic character reveals the monophyletic origin of freshwater crayfishes (Crustacea, Decapoda). Zool. Anz. 230, 45–54.

Scholtz, G., 1995. Ursprung und Evolution der Flußkrebse (Crustacea, Astacida). Sitzber. naturforsch. Freunde Berlin (N.F.) 34, 93–115.

Scholtz, G., McLay, C.L., 2009. Is the Brachyura Podotremata a monophyletic group? In: Martin, J.W., Felder, D.L., Crandall, K.A. (Eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). CRC Press, Boca Raton, pp. 417–435.

Scholtz, G., Richter, S., 1995. Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). Zool. J. Lin. Soc. 113, 289–328.

Schram, F.R., 2001. Phylogeny of decapods: moving towards a consensus. Hydrobiologia 449, 1–20.

Schram, F.R., Dixon, C.J., 2004. Decapod phylogeny: addition of fossil evidence to a robust morphological cladistic data set. Bull. Mizu. Fos. Mus. 31, 1–19.

Spears, T., Abele, L.G., Kim, W., 1992. The monophyly of brachyuran crabs: a phylogenetic study based on 18S rRNA. Syst. Biol. 41, 446–461.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 57, 758–771.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal-W improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weigh matrix choice. Nucl. Acids Res. 22, 4673–4680.

Toon, A., Finley, M., Staples, J., Crandall, K.A., 2009. Decapod phylogenetics and molecular evolution. In: Martin, J.W., Felder, D.L., Crandall, K.A. (Eds.), Decapod Crustacean Phylogenetics (Crustacean Issues 18). CRC Press, Boca Raton, pp. 15–30.

Tsang, L.M., Lin, F.J., Chu, K.H., Chan, T.Y., 2008a. Phylogeny of Thalassinidea (Crustacea, Decapoda) inferred from three rDNA sequences: implications for morphological evolution and superfamily classification. J. Zool. Syst. Evol. Res. 46, 216–223.

Tsang, L.M., Ma, K.Y., Ahyong, S.T., Chan, T.Y., Chu, K.H., 2008b. Phylogeny of Decapoda using two nuclear protein-coding genes: origin and evolution of the Reptantia. Mol. Phylogenet. Evol. 48, 359–368.

Tsang, L.M., Chan, T.-Y., Ahyong, S.T., Chu, K.H., 2011. Hermit to king, or hermit to all: multiple transitions to crab-like forms from hermit crab ancestors. Syst. Biol. 60, 616–629.

Tshudy, D., Babcock, L.E., 1997. Morphology-based phylogenetic analysis of the clawed lobsters (family Nephopidae and the new family Chilenophoberidae). J. Crust. Biol. 17, 253–263.

Tudge, C.C., 1995. Ultrastructure and Phylogeny of the spermatozoa of the infraorders Thalassinidea and Anomura (Decapoda, Crustacea). In: Jamieson, B.G.M., Ausio, J., Justine, J.-L. (Eds.), Advances in Spermatozoal Phylogeny and Taxonomy. Mém. Mus. Nat. Hist. nat. (Paris), 166, 251–263.

Tudge, C.C., Cunningham, C.W., 2002. Molecular phylogeny of the mud lobsters and mud shrimps (Crustacea: Decapoda: Thalassinidea) using nuclear 18s rDNA and mitochondrial 16s rDNA. Invert. Syst. 16, 839–847.

von Sternberg, R., Cumberlidge, N., 2001. On the heterotreme–thoracotreme distinction in the Eubrachyura De Saint Laurent, 1980 (Decapoda: Brachyura). Crustaceana 74, 321–338.

Wägele, J.W., Mayer, C., 2007. Visualizing differences in phylogenetic information content of alignments and distinction of three classes of long-branch effects. BMC Evol. Biol. 7, 1–24.

Wei, S.-J., Shi, M., Chen, X.-X., Sharkey, M.J., van Achterberg, C., Ye, G.-Y., He, J.-H., 2010. New views on strand asymmetry in insect mitochondrial genomes. PloS One 5, 9.

Xia, X., Xie, Z., 2001. DAMBE: software package for data analysis in molecular biology and evolution. J. Hered. 92, 371–373.