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Phylogenetic relationships within the Mysidae (Crustacea, Peracarida, Mysida) based on nuclear 18S ribosomal RNA sequences

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Abstract

Species of the order Mysida (Crustacea, Peracarida) are shrimp-like animals that occur in vast numbers in coastal regions of the world. The order Mysida comprises 1053 species and 165 genera. The present study covers 25 species of the well-defined Mysidae, the most speciose family within the order Mysida. 18S rRNA sequence analysis confirms that the subfamily Siriellinae is monophyletic. On the other hand the subfamily Gastrosaccinae is paraphyletic and the subfamily Mysinae, represented in this study by the tribes Mysini and Leptomysini, consistently resolves into three independent clades, and hence is clearly not monophyletic. The tribe Mysini is not monophyletic either, and forms two clades of which one appears to be closely related to the Leptomysini. Our results are concordant with a number of morphological differences urging a taxonomic revision of the Mysidae.

Keywords: Mysida; Mysidae; Molecular phylogeny; 18S rRNA

1. Introduction

Mysid phylogeny is poorly understood and few attempts were made over the last decades to revise the earlier established systematic relationships between higher taxonomic levels within the Mysida. These attempts dealt with the status of orders and suborders within the superorder Peracarida (Casanova et al., 2002; De Jong and Casanova, 1997; De Jong-Moreau and Casanova, 2001; Jarman et al., 2000; Martin and Davis, 2001; Richter and Scholtz, 2001; Spears and Abele, 1997). These studies gave more insight in the evolutionary link between the formerly accepted suborders Lophogastrida and Mysida within the order Mysidacea, which now can be considered different orders while the "old" Mysidacea disappears. However, this ongoing debate does not discuss the status of lower taxonomic levels within the order Mysida (families, subfamilies, tribes, and genera). The latest systematic overviews, not based on a phylogenetic approach, date back to 1977 and 1993 (Mauchline and Murano, 1977; Müller, 1993), indicating the lack of novel morphological evidence since the early years of mysid systematics. Some recent efforts to study mysid phylogenetics were based on the foregut morphology (Kobusch, 1998), and statolith composition (Ariani et al., 1993; Wittmann et al., 1993). The development of molecular techniques and their application in recent phylogenetic research provides

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a useful tool to verify if the current morphology-based accepted systematic knowledge is supported by genetic evidence. DNA sequencing indeed could offer complementary information on phylogenetic relations in order to identify evolutionary relationships among morphologically similar taxa within the Mysida, as done for many other invertebrate and particularly crustacean taxa (e.g., Abele, 1991, 1992; Braga et al., 1999; Giribet et al., 2001; Palumbi and Benzie, 1991; Spears and Abele, 1997). To our knowledge no phylogenetic study of the order Mysida has been published so far using both molecular and morphological data.

In the present study, 25 species from 18 genera of the largest family within the Mysida, the Mysidae, were analysed based on 18S rRNA sequence data. The selected species represent a worldwide coverage of the three most important subfamilies in terms of numbers of species and/or genera i.e., the Siriellinae, the Gastrosaccinae, and the Mysinae. This is particularly true for the large subfamily Mysinae (sensu Müller, 1993) that comprises 91% of the genera and 80% of all species classified within the Mysidae. No members of the subfamilies Boreomysinae (1 genus), Rhopalophtalminae (1 genus), or Mysidellinae (3 genera) were included. However, the selected species should already provide a basis for beginning to infer the molecular phylogeny of the family Mysidae. Indeed, the present data analysis provides a tool to test the morphology-based classification of the Mysidae. The large subfamily Mysinae, which contains many genera and species compared to other subfamilies, can be questioned as a natural group. A molecular approach can supply additional evidence for, or reject the monophyletic character of the Mysinae, which are represented here by five genera of the tribe Leptomysini, and nine genera belonging to the Mysini. It is of particular interest to test the relationships between these tribes, in order to validate their phylogenetic strength. We show that both molecular and morphological evidence urges a taxonomic revision of the family Mysidae.

2. Materials and methods

A total of 25 mysid species were analysed (Table 1) in addition to four outgroup species from other crustacean taxa. All samples were stored in ethanol (70–95%) at 4 °C. Genomic DNA was extracted using a modified CTAB protocol (Kocher et al., 1989). Mysid tissue was crushed using a beadbeater and afterwards incubated for a minimum of 3 h at 60 °C in 500 μl CTAB buffer with 6 μl proteinase K (1 mg of 100 μl^{-1}). After an overnight incubation at 37 °C the DNA was extracted with phenol/chloroform/isoamylalcohol (25:24:1 pH 8) and chloroform:isoamylalcohol (24:1). Finally, DNA was precipitated with isopropanol and rehydrated in 25 μl water. Small aliquots of extracted nucleic acids were used as template for polymerase chain reaction amplification (PCR). The 18S ribosomal gene (1990 bp) was amplified using the 5′-EM (5′-TYC CTG GTT GAT YYT GCC AG-3′) and 3′-EM (5′-TGA TCC TTC CGC AGG TTC ACC T-3′) primers (Weekers et al., 1994). Cycle conditions were 95 °C for 1 min, 55 °C for 1.5 min, and 72 °C for 2 min for 35 cycles. PCR amplification products were sequenced using a Perkin–Elmer ABI Prism 377 automated DNA sequencer. PCR product was treated with shrimp alkaline phosphatase (1 U/μl, Amersham E70092Y) and exonuclease I (20 U/μl, Epicentre Technologies X40505K) for 15 min at 37 °C, followed by 15 min at 80 °C to inactivate enzymes. This material was then used for cycle sequencing without any further purification, using the ABI Prism BigDye Terminator Cycle Sequencing kit. The sequencing conditions were 30 s at 96 °C, 15 s at 50 °C and 4 min at 60 °C for 27 cycles. Cycle sequence products were precipitated by adding 25 μl of 95% ethanol and 1 μl 3 M sodium acetate, pH 4.6 to each cycle sequencing reaction (10 μl). The samples were placed at −20 °C for 15 min and centrifuged at 14,000 rpm for 15 min. After precipitation, an additional wash of the pellet was performed with 125 μl of 70% ethanol and centrifuged at 14,000 rpm for 5 min. The pellet was dried in a Speedvac concentrator, redissolved in loading buffer and run on a 48 cm 4.25% acrylamide:bisacrylamide (29:1) gel. All sequences have been submitted to EMBL (Accession Nos: AJ566084–AJ566109).

Four 18S ribosomal RNA sequences of the more or less closely related crustaceans *Diastylis* sp. (Peracarida, Cumacea), *Euphausia pacifica* (Eucarida, Euphausiacea), *Squilla empusa* (Hoplocarida, Stomatopoda) and *Nebalia* sp. (Leptostraca, Nebaliida) were obtained from GenBank, and used as outgroups in the analysis. All sequences were aligned with ClustalX (Version 1.74, Thompson et al., 1997) using the default settings (pairwise alignment parameters: slow-accurate pairwise alignment method, Gap opening penalty = 15.00, Gap extension penalty = 6.66, IUB DNA weight matrix; and multiple alignment parameters: Gap opening penalty = 15.00, Gap extension penalty = 6.66, Delay divergent sequences = 30%, DNA transition weight = 0.50), followed by limited manual editing to improve inferences of positional homology. Parsimony analysis was performed using PAUP 4.0b10 (Swofford, 2001) with the following heuristic search settings: 100,000 random taxon addition replicates followed by tree-bisection-reconnection (TBR) branch swapping. Nodal support was assessed by calculating bootstrap values (Felsenstein, 1985) from 1000 bootstrap replicates obtained by heuristic search with 10 random sequence addition replicates each. In addition, taxon jackknifing

Table 1
List of the different species used in this study with indication of the systematic position, geographic origin and GenBank sequence accession numbers

Order	Family	Subfamily	Tribe	Species	Geographic origin	EMBL Accession No.
Mysida	Mysidae	Siriellinae		*Siriella armata* (Milne-Edwards, 1837)	Coast of Apulia, Adriatic Sea, Italy	AJ566105
				Siriella clausii (G.O. Sars, 1877)	Coast of Apulia, Adriatic Sea, Italy	AJ566107
				Siriella jaltensis (Czerniavsky, 1868)	Coast of Apulia, Adriatic Sea, Italy	AJ566106
		Gastrosaccinae		*Anchialina agilis* (G.O. Sars, 1877)	Belgian continental shelf, Belgium	AJ566089
				Archaeomysis japonica (Hanamura, Jo and Murano,1996)	Otsuchi bay, Japan	AJ566084
				Archaeomysis kokuboi II, 1964	Otsuchi bay, Japan	AJ566085
				Bowmaniella sp.	Valdivia beach, Guayas province, Ecuador	AJ566086
				Gastrosaccus psammodytes (Tattersall, 1958)	Algoa bay, South Africa	AJ566087
				Gastrosaccus spinifer (Goës, 1863)	Westerschelde, The Netherlands	AJ566088
		Mysinae	Leptomysini	*Americamysis bahia* (Molenock, 1969)	West Coast USA	AJ566095
				Leptomysis lingvura adriatica (G.O. Sars, 1866)	Pilone estuary, Adriatic Sea, Italy	AJ566098
				Leptomysis lingvura lingvura (G.O.Sars, 1866)	Belgian continental shelf, Belgium	AJ566099
				Metamysidopsis sp.	Valdivia beach, Guayas province, Ecuador	AJ566096
				Mysidopsis sp.	Valdivia beach, Guayas province, Ecuador	AJ566094
				Mysidopsis gibbosa (G.O. Sars, 1864)	Belgian continental shelf, Belgium	AJ566097
			Mysini	*Acanthomysis longicornis* (Milne-Edwards, 1837)	Westerschelde, The Netherlands	AJ566093
				Diamysis mesohalobia mesohalobia (Ariani & Wittmann, 2000)	Coast of Apulia, Adriatic Sea, Italy	AJ566100
				Hemimysis anomala (Sars, 1907)	Danube river, Austria (orig. Caspian Lake)	AJ566104
				Holmesimysis costata (Holmes, 1910)	West coast USA	AJ566090
				Limnomysis benedeni (Czerniavsky, 1882)	Danube River, Austria	AJ566101
				Neomysis integer (Leach, 1814)	Westerschelde, The Netherlands	AJ566091
				Paramesopodopsis rufa (Fenton, 1985)	Taroona beach, Tasmania	AJ566108
				Praunus flexuosus (Müller, 1776)	Westerschelde, The Netherlands	AJ566102
				Schistomysis kervillei (Sars, 1885)	Belgian continental shelf, Belgium	AJ566103
				Schistomysis spiritus (Norman, 1860)	Voordelta, The Netherlands	AJ566109
Euphausiacea	Euphausiidae			*Euphausia pacifica* (Hansen, 1911)	N.A./from EMBL database	AY141010
Cumacea	Diastylidae			*Diastylis* sp.	N.A./from EMBL database	Z22519
Leptostraca	Nebaliidae			*Nebalia* sp.	N.A./from EMBL database	L81945
Stomatopoda	Squillidae			*Squilla empusa* (Smith, 1958)	N.A./from EMBL database	L81946

was performed to assess the effects of taxon sampling on the tree resolution (Lanyon, 1985). In this analysis, individual taxa were sequentially removed and the resulting data set of $n-1$ taxa was analyzed using parsimony with 1000 random addition replicates. All Jackknife generated trees were evaluated manually by comparing the nodes in each consensus tree with those in the bootstrapped parsimony tree generated by the full data set.

The likelihood ratio test in MODELTEST 3.06 (Posada and Crandall, 1998) was used to determine the model of DNA evolution that best fitted the dataset. Based on this test, the general time-reversible substitution model with a discrete γ correction for among site variation, and corrected for invariable sites (GTR + G + I model) (Rodriguez et al., 1990) was chosen for maximum likelihood analysis. ML was performed using the heuristic search option with TBR branch swapping, MulTrees option in effect, no steepest descent, rearrangements limited to 10,000 and with 50 random sequence addition replicates. Bootstrap values were determined from 100 bootstrap replicates obtained by heuristic search with 10 random sequence addition replicates each.

3. Results

3.1. Sequence data and alignment

A total of 25 different mysid species were sequenced, the length of the mysid 18S rRNA gene varies between 1788 bp (*Schistomysis spiritus*) and 1811 bp (*Archaeomysis japonica*). GC content varies between 46.6% (*Acanthomysis longicornis*) and 49.8% (*Anchialina agilis*), and has an average of 48.6%. The block of aligned 18S rRNA sequences contains 1889 positions: 1175 (62.2%) characters are constant, 439 (23.2%) are parsimony non-informative, and 275 (14.6%) are parsimony informative. No obvious large expansion segments are observed within the aligned 18S sequences.

3.2. Parsimony analysis

The parsimony (MP) analysis with heuristic search generated three most parsimonious trees of 2192 steps (consistency index = 0.5132, retention index = 0.5266, and rescaled consistency index = 0.2703) that had some topological changes. The strict consensus MP tree is shown in Fig. 1. The subfamily Gastrosaccinae is

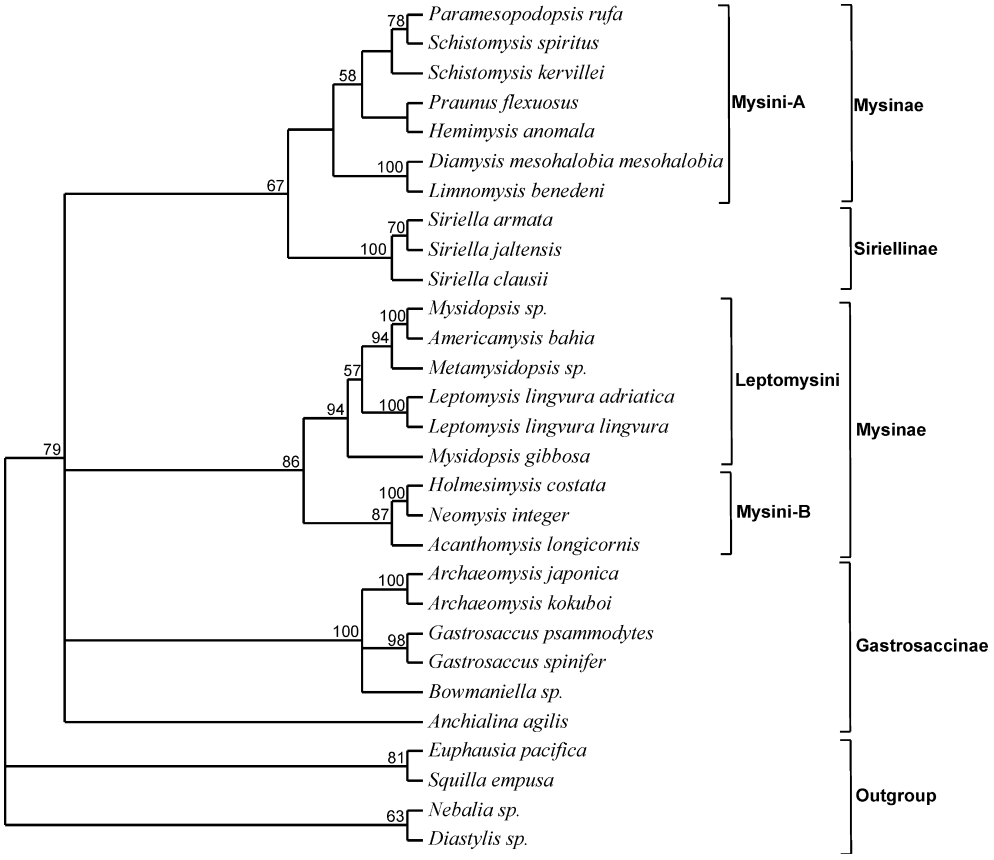

Fig. 1. Strict consensus maximum parsimony tree of 2192 steps obtained after 100,000 replicates (CI = 0.5132, RI = 0.5266, and RC = 0.2703). The numbers along the branches indicate MP bootstrap support, only bootstrap values higher than 50% are shown.

resolved as a paraphyletic group, while the Siriellinae are resolved as a well-defined monophyletic clade supported by high bootstrap values (100%) (Fig. 1). The relationships within the subfamily Gastrosaccinae are less clear, two most parsimonious trees suggests that *Bowmaniella* sp. is more closely related to the genus *Archaeomysis* than to *Gastrosaccus*, while the other tree suggest the opposite (trees not shown). The analysis also shows that the subfamily Mysinae, represented by the tribes Mysini and Leptomysini, is polyphyletic. One group of species belonging to the tribe Mysini (Mysini-A-group) forms a monophyletic clade that is closely related to the subfamily Siriellinae (Fig. 1). The MP analysis fails to resolve the two species of the genus *Schistomysis* as sister taxa. The three other species of this tribe (*Neomysis integer*, *Holmesimysis costata*, and *A. longicornis*) form a clade (Mysini-B-group) closely related to the species of the tribe Leptomysini (Fig. 1). It should also be noted that the genus *Mysidopsis* is resolved as a paraphyletic taxon by the MP analysis. Few trees obtained from the parsimony analysis with taxon jackknifing displayed deviations from the strict consensus MP tree. In particular the exclusion of the ingroup species *Gastrosaccus psammodytes*, *Bowmaniella* sp. and *A. agilis*, and the outgroup species *S. empusa* caused changes in the position of Gastrosaccinae and Siriellinae, and the relationships within the Mysini-A clade.

3.3. Maximum likelihood analysis

Maximum likelihood (ML) analysis was performed using the GTR + G + I model of molecular evolution with following values: substitution rates R = (1.1617, 2.2699, 1.4924, 0.646, and 4.569), proportion of invariable sites = 0.3798 and γ shape parameter, $\alpha = 0.4756$. The most likely tree had a $-\ln L = 12,677.09$ and is shown in Fig. 2. The subfamilies Siriellinae and Gastrosaccinae are each monophyletic, the latter only with 68% bootstrap support (Fig. 2). Interestingly, the Gastrosaccinae are now shown as a sister group to all other subfamilies. Also the ML tree confirms the morphology-based grouping of the genera within the subfamily Gastrosaccinae: *Bowmaniella* sp. is more closely related to the genus *Archaeomysis* than to *Gastrosaccus*. The polyphyly of the tribe Mysini within the subfamily Mysinae is indicated by the ML tree, with the split of the

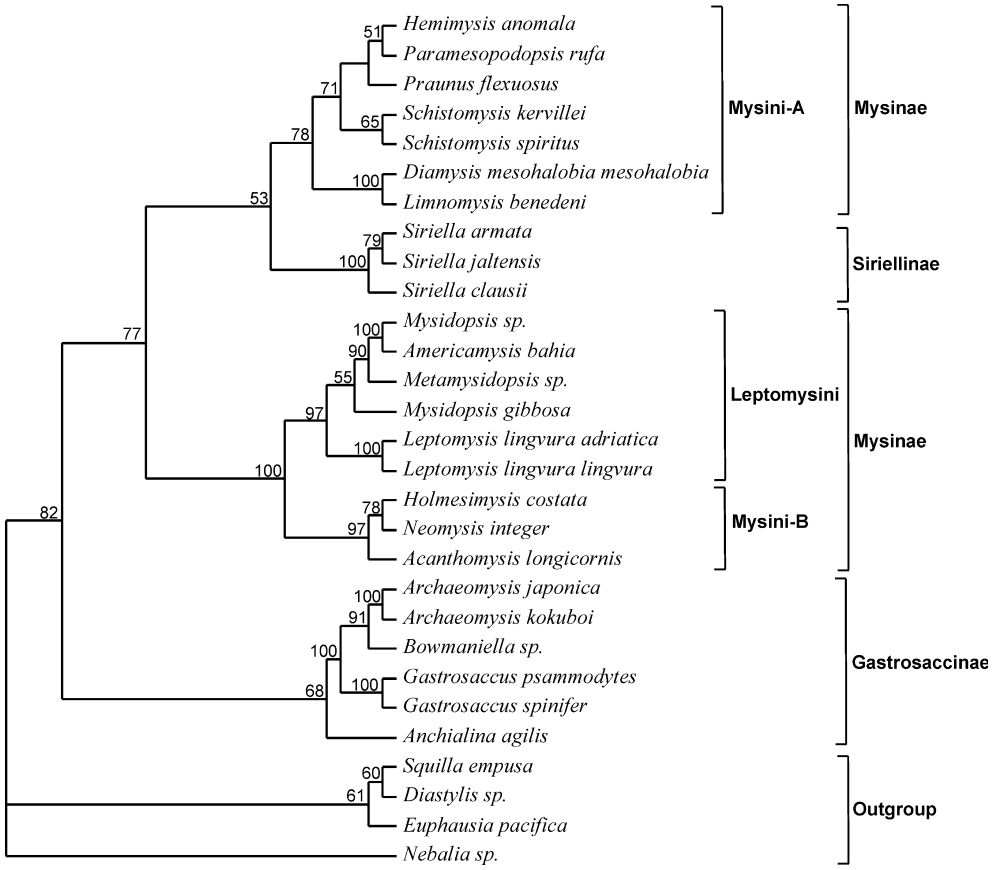

Fig. 2. Heuristic maximum likelihood tree based on the GTR + G + I model of sequence evolution and with $-\ln L = 12,677.09$. The parameters were: nucleotide frequencies: $A = 0.2488$, $C = 0.2171$, $G = 0.2701$, $T = 0.264$; substitution rates R = (1.1617, 2.2699, 1.4924, 0.646, and 4.569); proportion of invariable sites = 0.3798 and gamma shape parameter, $\alpha = 0.4756$. The numbers along the branches indicate ML bootstrap support, only bootstrap values higher than 50% are shown.

tribe Mysini in two different clades (Mysini-A and Mysini-B) as proposed by the MP analysis being confirmed by ML. The tribe Leptomysini is also resolved by ML as a monophyletic clade, and again the genus *Mysidopsis* is shown as a paraphyletic taxon. ML, unlike MP, supports the monophyly of the genus *Schistomysis.*

4. Discussion

The family Mysidae is divided into six subfamilies of which only three were represented in this study: Siriellinae, Gastrosaccinae, and Mysinae. In terms of numbers of species and genera these three subfamilies can be considered as the most important groups of the family, although the omission of the other three subfamilies (Boreomysinae, Rhopalophthalmidae, and Mysidellinae) lowers the value of the analysis in terms of general conclusions on phylogenetic relationships within the whole family.

According to the different methods (MP and ML) applied here to reconstruct phylogenetic relationships, the subfamily Siriellinae can be considered as a monophyletic clade. Some typical morphological characteristics support the monophyly of this group: the exopod of the uropod is divided into two segments, the mandibular molar process is reduced, the marsupium consists of three oostegites and males of almost every species have the typically spirally coiled pseudobranchiae at the pleopods (morphological data was taken from the NeMys database, http://intramar.ugent.be/nemys, see also Deprez et al., 2004).

The paraphyly of the Gastrosaccinae is caused by the deviant placement of *A. agilis*. The group formed by members of *Bowmaniella*, *Archaeomysis*, and *Gastrosaccus* can be considered as a well-defined monophyletic group. Morphologically this group of species (the "*Gastrosaccus*-group") indeed displays several differences with members of the genus *Anchialina*. Common characteristics for the whole subfamily are the presence of a spine on the antennal scale (which is setose all around), the typical shape of the telson (with a cleft, armed with spines, without setae), and the presence of spine on the labrum (absent in all other Mysinae species). Considering the combination of these characteristics taxonomists grouped the *Anchialina* species within the Gastrosaccinae subfamily although there are morphological differences, mainly in pleopod structures. Within the genus *Anchialina* the first pair of thoracopods bears a strongly developed claw on the dactylus, uniramous female pleopods are present, and the third pair of the male pleopods has an only slightly elongated exopod. In the "*Gastrosaccus*-group" at least the first pair of female pleopods are uniramous and in members of *Archaeomysis* and *Bowmaniella* also the second to the fifth pair are biramous. This may be an argument why in two of the three most parsimonious trees (Fig. 1, MP tree #2 and 3), and in the ML analysis (Fig. 4) *Bowmaniella* sp. is closer related to *Archaeomysis* than to *Gastrosaccus*. Members of *Anchialina* posses an uniramous first male pleopod while all male pleopods are biramous in the "*Gastrosaccus*-group."

Morphological evidence strongly suggests that the genus *Gastrosaccus* is the sister group to the genera *Bowmaniella* and *Archaeomysis*, which is partly supported by our molecular analysis (ML analysis). Biramy is considered to be more ancestral then uniramy (e.g., Wilson, 1989). By this criterion *Bowmaniella* and *Archaeomysis* are assumed to be more closely related to the ancestral form, while members of *Gastrosaccus* are more derived. Based on these morphological characteristics we can also classify the members of the subfamily Gastrosaccinae not included in this study either in the "*Anchialina*-group" (e.g., *Pseudanchialina* Hansen, 1910 and *Paranchialina* Hansen, 1910 species) or in the "*Gastrosaccus*-group" (e.g., *Haplostylus* Kossmann, 1880 and *Iiella* Bacescu, 1968 species). Already in 1882 Czerniavsky erroneously created the "divisio Anchialidae" (= tribe Anchialini in current terminology; this taxon was rejected by subsequent authors) based on the morphological characteristics that diverge the *Anchialina* species from the "true" Gastrosaccinae. A more profound study that would include more species might provide additional evidence for the creation of two monophyletic subfamilies as also indicated by our molecular analysis.

The subfamily Mysinae, represented in this study by the tribes Mysini and Leptomysini, consistently resolves into three clades (Leptomysini: 1 clade; Mysini: 2 clades), and hence is clearly not monophyletic. This subfamily was originally split into different tribes based on morphological characteristics (Bacescu and Iliffe, 1986; Hansen, 1910; Ii, 1964; Tattersall, 1955). Only two of the six tribes (Leptomysini with 31 genera and Mysini with 52 genera) are represented in our analysis. The subfamily Mysinae comprises the largest number of species (806) and genera (143) of the entire family Mysidae (157 genera, 1004 species) and even of the order Mysida (165 genera and 1053 species). The division into different tribes permitted structuring of this large subfamily, but the taxonomic value is doubtful—as reflected in our analysis.

Relationships within the Mysini are much less straightforward, since two clades are resolved in the analyses. One group includes *Praunus flexuosus*, *Hemimysis anomala*, *Schistomysis kervillei*, *S. spiritus*, *Limnomysis benedeni*, *Diamysis mesohalobia mesohalobia*, and *Paramesopodopsis rufa* (Mysini-A-group). The other group includes the species *N. integer*, *H. costata*, and *A. longicornis* (Mysini-B-group) and appears to be more closely related to the Leptomysini than to the Mysini-A-group. This is confirmed by the topology of

all tree construction methods. The Mysini are usually differentiated based on the following morphological characteristics: the second male pleopod is rudimentary and uniramous, and the fourth male pleopod is elongated, and mostly modified. The uniramous character of the second male pleopod constitutes the most important difference between the tribes Mysini and Leptomysini. Morphological indications for the splitting of the Mysini in two separate clades can be found in the exopod on the third male pleopod which is reduced in the Mysini-B-group whereas in the Mysini-A-group this structure is either slightly or well developed, and a cleft in telson is present. The Mysini-B-group seems to correspond to the definition of the tribe Mysini by Hansen (1910): the exopod of the male third pleopod has one or two segments, and mostly an entire telson. The genera *Acanthomysis*, *Neomysis*, and *Holmesimysis* display a very similar appearance, causing their pooling under a single generic name, *Neomysis* (Zimmer, 1915) in the past.

The Mysini-A-group comprises three species (*H. anomala*, *D. mesohalobia mesohalobia*, *L. benedeni*) that have calcareous (as the mineral vaterite) statoliths. The remaining four species of this group (*S. kervillei*, *S. spiritus*, *P. flexuosus*, and *P. rufa*) precipitate fluorite, as do the great majority of Mysidae. Although weakened by the absence of some essential taxa (e.g., *Mysis*, *Paramysis*) the present molecular analysis is in keeping with the conclusion of Ariani et al. (1993) that within the Mysini both calcareous and fluorite statoliths originate from common ancestors. These ancestors had the ability or predisposition to form calcareous statoliths, favouring a phylogenetically rapid shift of statolith mineral composition from fluorite to vaterite. The actual distribution of the mineral types (vaterite versus fluorite) seems to be paraphyletic with respect to the true phylogeny (i.e., mineral type represents analogy, not homology). The grouping of the closely related genera of Mysini in a '*Diamysis* group' (*Diamysis*, *Limnomysis* and *Antromysis* Creaser, 1936) and the '*Paramysis* group' (*Paramysis* Czerniavsky, 1882; *Katamysis*, Sars, 1877 and *Schistomysis*) based on features of antennal scale and male pleopods as suggested by Ariani et al. (1993) is also confirmed by our molecular analysis. However, more detailed molecular and morphological analyses covering members of the other tribes are needed to reach a more detailed and correct view of the genealogy of the different clades within the Mysidae.

The tree topology for the Leptomysini is nearly identical in all analyses. Morphological evidence suggests that *Mysidopsis* sensu Sars (1864) is more closely related to *Leptomysis*. The genera *Metamysidopsis*, *Brasilomysis* Bacescu, 1968 and *Americamysis* were more recently created and in many cases are synonymous with *Mysidopsis* species (e.g., *Americamysis almyra* was formerly known as *Mysidopsis almyra* Bowman, 1964; *Metamysidopsis munda* was formerly known as *Mysidopsis munda* Zimmer, 1918). However, even after later revisions the genus *Mysidopsis* sensu Price seems to remain a paraphyletic mixture of species (Price et al., 1994). This is consistent with our results and indicates that this genus is taxonomically not well defined and needs to be profoundly revised.

Based on molecular and morphological arguments we can conclude that the subfamily Siriellinae is a well-defined taxonomic unit. On the other hand the subfamily Gastrosaccinae is found to be paraphyletic and a split in two monophyletic subfamilies (the "*Gastrosaccus*-group" and the "*Anchialina*-group") should be considered. The third subfamily present in this study, Mysinae, represented here by the tribes Mysini, and Leptomysini, is clearly not monophyletic. A revision of the Mysini is suggested in order to tune taxonomy to phylogenetic relationships based on morphological and molecular data. On the other hand the tribe Leptomysini appears to be a well-defined taxonomical unit, although a revision of the genus *Mysidopsis*, and its related genera (e.g. *Metamysidopsis*, *Americamysis*) is needed. Obviously, future research should include more genes and more species, since the selection of taxa has a large and unpredictable effect on phylogeny (Lecointre et al., 1993). First, a sufficient number of representatives of the subfamilies Boreomysinae, Rhopalophthalmidae, and Mysidellinae, not included here, should be analysed to evaluate the taxonomic rigidity of the Mysidae. Second, species belonging to all existing tribes within the subfamily Mysinae must be included to assess the value of these taxonomical units as well as their relations.

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