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Phylogenetic relationships of the family Sphaeromatidae Latreille, 1825 (Crustacea: Peracarida: Isopoda) within Sphaeromatidea based on 18S-rDNA molecular data

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Abstract

Based on 18S-rDNA sequences of 97 isopods including 18 Sphaeromatidea, we show Sphaeromatidae, Valvifera, Serolidae, and Ancinidae is a well supported clade. The within clade relationships of these taxa are not as definitively demonstrated because taxon sampling for some groups is still limited. In our analyses the Sphaeromatidae are shown to be unequivocally monophyletic. This is contrary to the morphology-based analysis by A. Brandt and G. Poore in 2003, which included only five Sphaeromatidae and found the family to be paraphyletic. The Ancinidae are also upheld, and the Valvifera is the sister taxon to Serolidae. Surprisingly *Plakarthrium* (Plakarthiidae) is nested within the Sphaeromatidae in most analyses. We point out short-comings in our sampling and suggest areas which would benefit from better sampling. We also review the long and convoluted nomenclatural history of the Sphaeromatidea, Sphaeromatoidea, and Sphaeromatidae.

Key words: Isopoda, Sphaeromatidea, Sphaeromatoidea, Sphaeromatidae, Ancinidae, Tecticipitidae, 18S-rDNA, phylogeny

Introduction

This contribution assesses the proposed monophyly of the Sphaeromatoidea Latreille, 1825 (Brandt & Poore 2003) and the paraphyly of the Sphaeromatidae and their relationship to the other suborders and superfamilies within the Isopoda with 18S-rDNA sequence data. We summarize the long history of the group's defining characteristics and also provide a chronological summary of nomenclature for the Sphaeromatidae, Sphaeromatoidea, and Sphaeromatidea.

The Sphaeromatidae Latreille, 1825 is the largest family of free-living marine Isopoda with 100 genera (and many more undescribed) and more than 690 species (Schotte *et al.* 2008 onwards). Sphaeromatids are mostly small (3–10 mm, very few achieve 2 cm, e.g. *Ceratocephalus* Woodward, 1877, *Calcipila* Harrison & Holdich, 1984 and some *Exosphaeroma* Stebbing, 1900), often cryptic isopods. They are among the most frequently encountered of marine isopods on intertidal shores and shallow depths, reaching their greatest diversity in the southwestern Pacific (e.g. Australia and New Zealand with more than 263 species, or 37% of all named species; see Poore, Lew Ton & Bruce 2002; Poore 2005; Poore & Bruce 2009). Few genera and species extend beyond 100 m depth (Bruce 1994). Their morphology is hugely diverse (Fig. 1), ranging from the simple smooth-bodied ‘pill-bug’ forms to those with

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conspicuous cuticular sculpting, while others may be strongly dorso-ventrally flattened appearing scale-like. Numerous species have 'dorsal processes' and variously perforate pleotelson margins, the presence or absence of characters that, in the past, were used to define genera. Additionally, some genera exhibit extreme sexual dimorphism while other genera have virtually none at all. Female species-specific characters are often not available and hence diagnostic characters are almost exclusively based on males. This contributes to the difficulties of identifying females and juveniles when not associated with males from the same collecting event.

Over time the number of genera has increased, and defining generic characters have narrowed, a notable change being the rejection of the principle that the presence or absence of dorsal processes are axiomatically of generic merit. Equally, genera have in many cases been separated from larger genera on the basis of perceived differences, and yet other species simply could not be placed into existing genera. Consequently the family contains a disproportionally large number (60%) of 'small' genera (three species or less) as well as a high number of *incertae sedis* species (17%). Based on morphology, many of the larger genera (e.g. *Cilicaea* Leach, 1818, *Cymodoce* Leach, 1814, *Cymodocella* Pfeffer, 1887, *Dynamenella* Hansen, 1905, *Exosphaeroma* Stebbing, 1900, *Paracilicaea* Stebbing, 1910) are considered to be not monophyletic. The families Ancinidae (14 species) and Tecticipitidae (12 species) together with the Sphaeromatidae form the Sphaeromatoidea. Relationships of taxa such as *Paravireia* (3 species) and Plakarthriidae (3 species) remain ambiguous.

The composition and monophyly of the superfamily Sphaeromatoidea has been discussed based on morphological characters by Wägele (1989) and Brandt & Poore (2003) and is considered to be upheld by the coxal plates being fused to the tergites, pleonite 5 being fused to the pleotelson and the uropodal endopod fused to the peduncle [complete diagnosis provided in Brandt & Poore (2003)]. *Paravireia* Chilton, 1925 is placed within the Sphaeromatoidea, but is still regarded as *incertae sedis*, critically lacking any trace of uropods and the maxilla being of the same form as in the Cymothoidae (Brökeland *et al.* 2001). The families Sphaeromatidae, Ancinidae and Tecticipitidae all have scale patches on the endopod of pleopod 5. The sister group to the Sphaeromatoidea according to Brandt & Poore (2003) is the Seroloidea. These two superfamilies together constituting the Sphaeromatidea Wägele, 1989, are defined by ventral coxal plates of pereonite 7 not meeting in the middle, pleonite 1 narrower than pleonite 2 and the right lacinia mobilis is reduced and fused to the spine row.

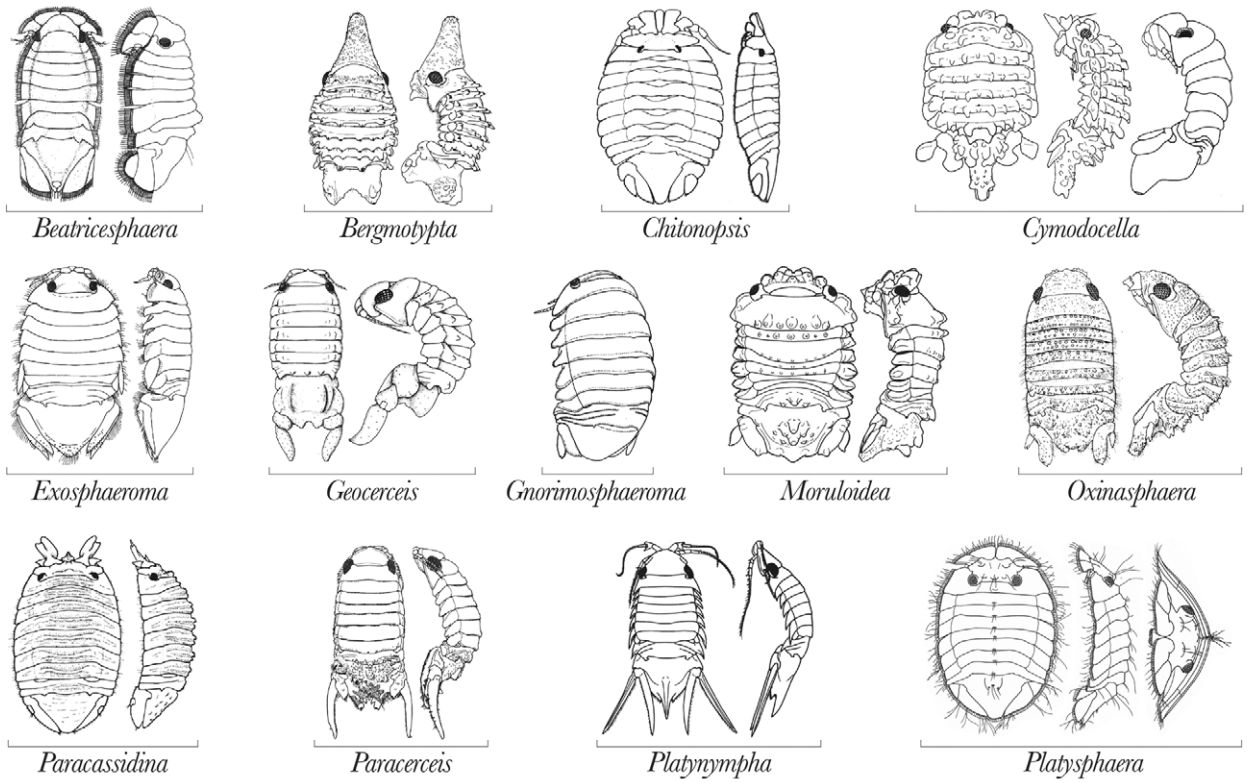

FIGURE 1. Examples of Sphaeromatidae diversity.

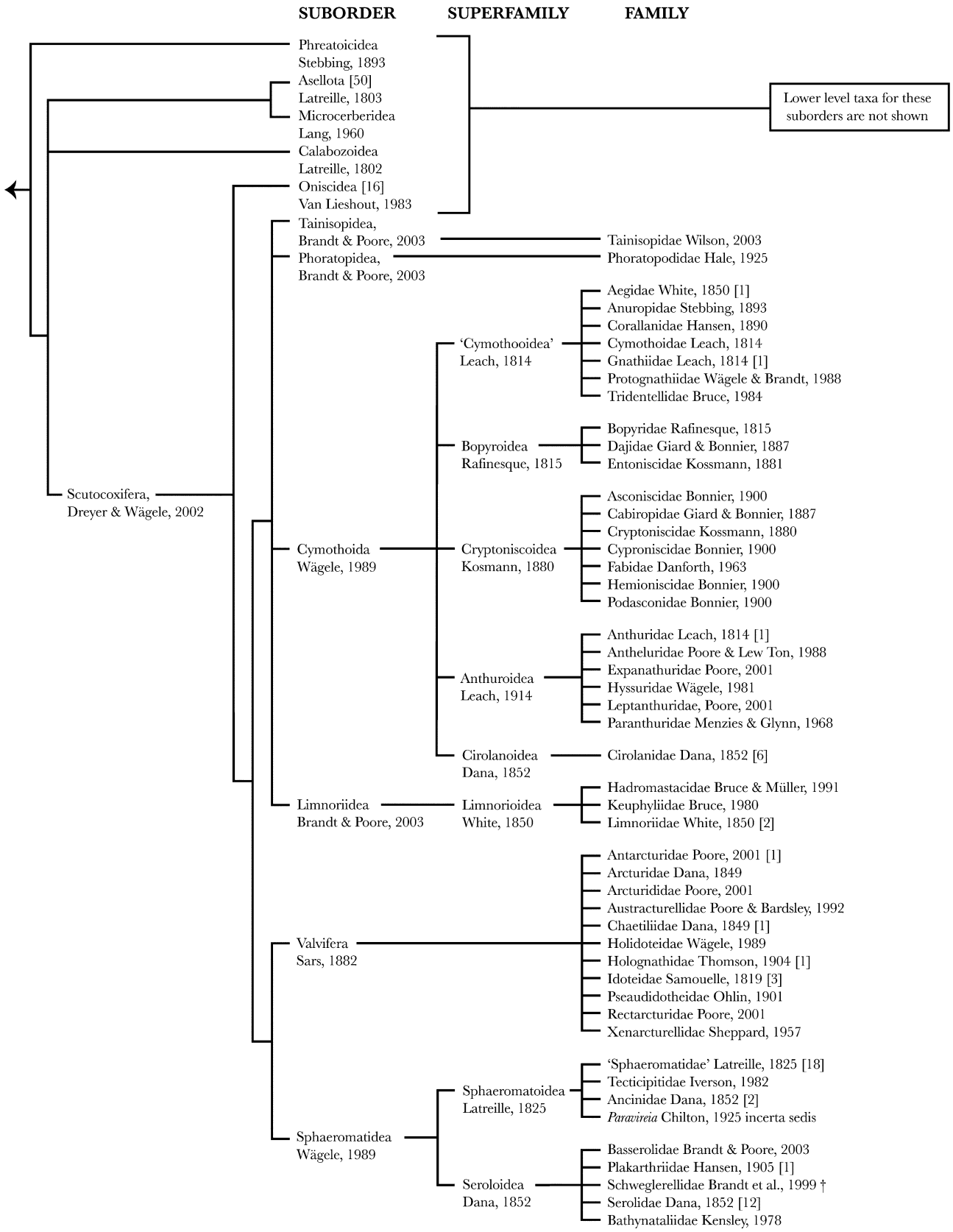

FIGURE 2. Reproduction of Brandt & Poore (2003) Table 3 presented as a phylogeny based on their analysis. Number of taxa used in this analysis are indicated in the brackets following the taxon names and are identified in Tables 2 and 3. Note spelling of Scutocoxifera was designated by Dreyer and Wägele 2002.

The monophyly of the Sphaeromatidae itself has been questioned, apparently lacking defining apomorphies (Brandt & Poore 2003). The Sphaeromatidae has a complex history of family-level nomenclature with eight available family-group names (see Appendix 1). Major divisions within the family were recognised first by Dana (1852), and then by Hansen (1905), based on pleopod morphology (among other characters), these later being given formal nomenclatural status (incorrectly by Hurley & Jansen 1977; later corrected by Bowman 1981 and Iverson 1982). Following Iverson's work, Bruce (1993) recognised the Ancinidae Dana, 1852, the Tecticipitidae Iverson, 1982 and the Sphaeromatidae Latreille, 1825, the latter with three subfamilies, Cassidininae Hansen, 1905, Dynameninae Bowman, 1981 and Sphaeromatinae Latreille, 1825. The core of the classification of within family relationships rested with the presence or absence of transverse thickened ridges on pleopods 4 and 5 (e.g. see schema presented by Harrison & Ellis 1991; Iverson 1982; Kensley & Schotte 1989). Another criterion, that of flattened body shape, was also influential in defining the subfamily Cassidininae, though this again was demonstrated to be homoplasious. Later works by Bruce (e.g. 1995, 1997, 2003) and Kussakin & Malyutina (1993) all argued that the principal defining pleopod characters for the subfamilies were homoplasious, overwhelmingly so for the supposed Cassidininae, and the use of these subfamilies was abandoned, as was the use of dorsal processes to define genera (Bruce 1997; Li 2000; Bruce & Holdich 2002). Nonetheless, Wägele (1989) and Bruce (1995, 1997, 2003) perceived that there were recognizable and potentially definable groups of genera within the family. These groups of genera, such as the informal '*Ischyromene* group' (Bruce 1995) and tribe 'Monolistrini' (Racovitza 1910; Sket 1964, 1986) have in some cases been used.

This complex history has resulted in some uncertainty over the monophyly of the Sphaeromatidae, the family being equivalent to the Sphaeromatoidea of Brandt & Poore (2003). The first assessment of the family and generic relationships of the Sphaeromatidae (*sensu latu* = Sphaeromatoidea) was that of Wägele (1989), part of a much wider 'all Isopoda' analysis, using the then traditional defining characters for the families and genera, and *a priori* assumptions of ancestry, but nonetheless demonstrating several groupings. Brusca & Wilson's (1991) analysis did not attempt to resolve the monophyletic status of the Sphaeromatidae (*sensu latu*). Brandt & Poore (2003), in their morphological analysis of the non-Asellota, included only five of the ~100 sphaeromatid genera, and stated that "we must conclude that the Sphaeromatidae are paraphyletic", this statement being justified on the basis of the apparent lack of supporting apomorphies. They defined the superfamily Sphaeromatoidea by several autapomorphies (p. 918) including: "pleonite 1 free, 2–4 fused, 5 fused to pleotelson, or more pleonites fused', 'pleopod 5 exopod with scale patches" and "Uropod ... endopod fused to peduncle". The Ancinidae and Tecticipitidae both have defining morphological apomorphies. These three families form a polytomy that is the sister group to *Paravireia*. In the present analysis the five Sphaeromatidae genera used in the Brandt & Poore (2003) analysis are a clade within the superfamily. The relationships of the Sphaeromatoidea are not fully resolved in Brandt & Poore's (2003: fig. 6) analysis (Fig. 2).

We provide new 18S-rDNA sequence data and combined with already available GenBank sequences, we address the status of Sphaeromatoidea and the monophyly of Sphaeromatidae.

Material and methods

Data analysis

We sequenced the 18S-rDNA nuclear gene for 18 Sphaeromatidae taxa (11 genera) previously not available in GenBank. Specimens of Tecticipitidae and of *Paravireia* were not available. We also sequenced one species each of Cirolanidae and Plakarthriidae, as well as, one species of Ancinidae (Table 1).

A pereopod, pleopod, anterior or posterior half of the body, or the entire animal was used in the extraction of DNA depending on the size of the specimen. Most material was fixed and preserved in 95% ethanol and stored at 4°C whenever possible. A few species were fixed in RNAlater (Applied Biosystems, Ambion, Austin, TX) and yielded the highest quality DNA; others had been collected in the mid-1980s and fixed and preserved in 70% ethanol. The latter had not been stored at reduced temperatures and yielded the lowest quantity and quality DNA, but nonetheless produced useful sequences. All were extracted with a QIAGEN DNeasy Kit (Qiagen, Valencia, CA) and the manufacturer's protocol was followed. Polymerase chain reaction (PCR, Sakai *et al.* 1988) was carried out with standard PCR conditions [2.5 µl of 10x PCR buffer, 1.5 µl of 50 mM $MgCl_2$, 4 µl of 10 mM dNTPs, 2.5 µl each of two 10 pmol primers, 0.15 Platinum Taq (5 units/µl), 9.6 µl double-distilled water, and 1 µl template] and

thermal cycled as follows: an initial denaturation at 96°C for 3 minutes followed by 40 cycles of 95°C for 1 minute, followed by 46°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. Primers are summarized in Table 2. A minimum of four 18S-rDNA primer pairs were needed to amplify the gene. In some instances, five or even six pairs were used. In all instances both directions of the gene were sequenced. The long insertions especially in the V4 and V7 regions (see Nelles *et al.* 1984; Wägele *et al.* 2003; Spears *et al.* 2005) were frequently difficult to sequence through and even though alternate overlapping primers were used, a few sequences have missing data. Total gene length sequenced is summarized in Table 1. PCR products were visualized by agarose (1.2%) gel electrophoresis with Sybr Gold (Invitrogen, Carlsbad, CA). PCR product was purified with Sephadex (Sigma Chemical, St. Louis, MO) on millipore multiscreen filter plates, and DNA was cycle sequenced with ABI Big-dye ready-reaction kit and following the standard cycle sequencing protocol with one quarter of the suggested reaction volume. Specimens and DNA are deposited in the Natural History Museum of Los Angeles County collections and can be retrieved by Genbank number.

TABLE 1. Nineteen new 18S-rDNA sequences with taxonomy, Genbank number, sequence length, and locality information. All specimens and DNA are deposited in the collectons at the Natural History Museum of Los Angeles County.

Family	*Genus/species*	Genbank No.	bp	Locality
Cymothoida				
Cirolanidae	*Cirolana kokoru*	JF699513	2493	New Zealand, Wellington, Island Bay, rocky intertidal, coralline red algae, fixed and preserved in 95% ethanol. 15 May 2004. Coll. K. Merrin. RW05.312.1482.
Sphaeromatidea				
Ancinidae	*Ancinus*	JF699514	2543	Pacific Panama, Naos Island, Flamenco Beach, beach sand, fixed and preserved in 95% ethanol. 23 Mar 2005. Coll. P. Glynn, I. Bethancourt, G. Hockensmith, T. Smith, A. Romanski. RW05.010.1475.
	Ancinus	JF699515	2330	Pacific Panama, Naos Island, Flamenco Beach, beach sand, fixed and preserved in 95% ethanol. 23 Mar 2005. Coll. P. Glynn, I. Bethancourt, G. Hockensmith, T. Smith, A. Romanski. RW05.010.1476.
Plakarthriidae	*Plakarthrium typicum*	JF699516	1748	New Zealand, North Island, Cape Palliser, 41.612°S 175.274°E, intertidal, mixed algae and under rocks, fixed and preserved in 95% ethanol. 24 Nov 2003. Coll. N.L. Bruce and J. Olesen. RW04.343.1441.
	Plakarthrium typicum	JF699517	2473	New Zealand, North Island, Cape Palliser, 41.612°S 175.274°E, intertidal, mixed algae and under rocks, fixed and preserved in 95% ethanol. 24 Nov 2003. Coll. N.L. Bruce and J. Olesen. RW04.343.1523.
Sphaeromatidae	*Campecopea hirsuta*	JF699520	2340	Atlantic, Canary Islands, Lanzarote, ~29°N ~13.38°W, empty barnacle shells from the upper shore, fixed in 95%, preserved in 95% ethanol. Received from D.M. Holdich, June 2002. RW02.038.1170.
	Dynamenella scaptocephala	JF699534	2637	Indian Ocean, Kenya, Mombasa, Ras Iwatine, 4.018°S 39.731°E, intertidal *Ulva*, hand, fixed in 100%, preserved in 95% ethanol. 15 Jul 2004. #121. Coll. R. Wetzer. RW04.177.1145
	Exosphaeroma	JF699544	2568	New Zealand, South Island, Kaikoura, Shark's Tooth, Atia Point, 42.24°S 173.41°E, intertidal, 2.5 m. Fixed in 100%, preserved in 95% ethanol. 19 Apr 2003. Coll. K. Merrin, rcvd. from N.L. Bruce. RW03.196.1504.
	Exosphaeroma	JF699547	2546	Atlantic, Namibia, south of Lüderitz, near Grossebucht (Big Bay), northern point, 0–5 m depth, ~26.38°S ~10.15°E, fixed and preserved in 95% ethanol. 29 Nov 1995. Coll. R. Wetzer (PharmaMar Expedition). RW95.030.1521.

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TABLE 1. (Continued)

Family	*Genus/species*	Genbank No.	bp	Locality
	Exosphaeroma obtusum	JF699548	1599	New Zealand, Wellington, Island Bay, rocky intertidal, coralline red algae, fixed and preserved in 95% ethanol. 15 May 2004. Coll. K. Merrin. RW05.311.1486.
	Exosphaeroma varicolor	JF699552	2523	Pacific, Chile, Coquimbo, ~29.57°S ~71.25°W, intertidal, under boulder, shell gravel, fixed and preserved in 95% ethanol. 17 Jun 2004. Coll. M. Thiel (Universidad Católica del Norte, Chile). RW04.206.1510.
	Gnorimosphaeroma oregonensis	JF699555	2048	Pacific, USA, Washington, westside of San Juan Island, Deadman Bay, 48.513°N 123.008°W, cobble/sand beach washes, hand, fixed and preserved in 95% ethanol. 8 Apr 2004. #5. Coll. R. Wetzer and N. D. Pentcheff. RW04.038.1151.
	Gnorimosphaeroma oregonensis	JF699556	2038	Pacific, USA, Washington, north end of Whidbey Island, Deception Pass, ~48.2°N ~122.4°W, rocky intertidal among mussels, fixed in 95%, preserved in 95% ethanol. 25 Jun 1998. Coll. T.J. Hilbish. RW98.031.1477.
	Ischyromene cordiforaminalis	JF699563	2341	New Zealand, North Island, Cape Palliser, 41.612°S 175.274°E, intertidal, mixed algae, encrusting algae, vertical rock face, fixed and preserved in 95% ethanol. 24 Nov 2003. Coll. N.L. Bruce and J. Olesen. RW04.335.1128.
	Neonaesa rugosa	JF699573	2376	Pacific, Australia, Queensland, Heron Island, between "Canyons" and "Lost Mooring", 23.458°S 151.925°E, dead *Acropora* from base of bommie, SCUBA, 13.5 m. Fixed in 100%, preserved in 95% ethanol. 12 Apr 2003. Sample #19. Coll. R. Wetzer, N.L. Bruce, N.D. Pentcheff. RW03.128.1550.
	Neosphaeroma laticaudum	JF699574	1661	Australia, New South Wales, Diamond Reef, south east of Hallidays Point, 32.091°S 152.552°E, orange sponge, hand collected on SCUBA, 17 m. Preserved in 95% ethanol. 21 Mar 2003. P 66313. Coll. RV Baragula, NSW 2216. RW04.291.1500.
	Oxinasphaera lobivia	JF699576	1490	Australia, Queensland, Amity Point, fixed and preserved in 95% ethanol. 1 Feb 2004. A804. Coll. A.N. Lörz. RW05.310.1490.
	Paradella garsonorum	JF699583	2540	Mexico, Sea of Cortez, Baja California Norte, Campo Linares, south of Campo Christina, north of Puertocitos, 30.471°N 114.634°W, intertidal barnacles, hand, fixed and preserved in 95% ethanol. 2 Aug 2003. Coll. R. Wetzer. RW03.223.1542.
	Pseudosphaeroma campbellensis	JF699589	2415	New Zealand, North Island, Wellington Evans Bay, 41.303°S 174.805°E, algae on barnacles, fixed and preserved in 95% ethanol. 30 Apr 2004. Coll. N. L. Bruce. RW04.336.1127.

Sequences were edited and assembled in Sequencher (Gene Codes Corporation), and all were BLAST searched. Table 3 lists the 97 previously published Genbank sequences (47 Asellota, 9 Cymothoida, 2 Limnoriidea, 16 Oniscidea, 12 Serolidae, 5 Sphaeromatidae, and 6 Valvifera) included in the dataset. 18S-rDNA was aligned using MAFFT (Multiple Alignment Program for amino acid or nucleotide sequences, Katoh *et al*. 2002, 2005) and manually adjusted where mismatches were made. All three LINS, EINS, and GINS alignment protocols were reviewed. Datasets containing all 116 taxa were reviewed. To determine what the effect of the considerably shorter Serolidae (671–700 bp in length) sequences might be, analyses excluding these sequences were considered. The datasets differed in how the taxa were combined during alignment, how profile constraints were implemented, and whether eliminating poorly aligned and divergent regions from the alignment with GBlocks (Castresana 2000; Talavera & Castresana 2007) had any effect. GBlocks was used to remove hypervariable regions.

TABLE 2. 18S-rDNA primer sequences used.

Primer Name	Primer Sequence (5'-3')
forward	
1F	TACCTGGTTGATCCTGCCAGTAG
1.2F	TGCTTGTCTCAAAGATTAAGC[2]
ai	CCTGAGAAACGGCTACCACATC[1]
a0.79	TTAGAGTGCTYAAAGC[1]
a2.0	ATGGTTGCAAAGCTGAAAC[1]
a3.5	TGGTGCATGGCCGYTCTTAGT
reverse	
b7.0	ATTTRCGYGCCTGCTGCCTTCCT
b5.0	TAACCGCAACAACTTTAAT
b3.0	GACGGTCCAACAATTTCACC[1]
bi	GAGTCTCGTTCGTTATCGGA[1]
7R	GCATCACAGACCTGTTATTGC[2]
9R	GATCCTTCCGCAGGTTCACCTAC[2]

[1] Whiting, M. F. *et al.* 1997.
[2] Whiting, M. F. 2002.

TABLE 3. Ninety-seven previously published 18S-rDNA sequences from Genbank with taxonomy, accession number, and sequence length and included in this dataset.

	genus/species	GenBank No.	bp
Asellota			
Acanthaspidiidae	*Acanthaspidia*	AY461455	2132
	Acanthaspidia bifurcatoides	AY461457	2137
	Acanthaspidia drygalskii	AY461458	2181
	Acanthaspidia pleuronotus	AY461459	2134
	Acanthaspidia rostratus	AY461456	2179
Asellidae	*Asellus aquaticus*	AJ287055	2123
	Asellus aquaticus	AF255701	2129
	Lirceus fontinalis	AF255702	2138
	Proasellus slavus	AF496662	2115
	Stenasellus racovitzai	AF496663	2216
	Stenasellus racovitzai	AF453248	597
Dendrotionidae	*Dendromunna*	AY461464	2092
Desmosomatidae	*Chelator*	AY461460	2088
	Eugerda	AY461463	2221
	Eugerdella natator	AY461462	2105
	Mirabilicoxa	AY461461	2124
Haplomunnidae	*Thylakogaster*	AY461470	2214
Haploniscidae	*Antennuloniscus armatus*	AY461468	2131
	Haploniscus	AY461467	2194
	Haploniscus	AY461466	2135
	Haploniscus	AY461465	2191
	Mastigoniscus	AY461469	2124
Ischnomesidae	*Haplomesus*	AY461474	2141
	Haplomesus	AY461473	2146
	Ischnomesus	AY461472	2154
	Stylomesus	AY461471	2080
Janirellidae	*Janirella*	AY461475	2098

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TABLE 3. (Continued)

	genus/species	GenBank No.	bp
Janiridae	*Carpias nereus*	AF496657	2162
	Iathrippa trilobatus	AF279606	2248
	Jaera albifrons	AF279609	2135
	Jaera nordmanni	AF279610	2137
	Janira maculosa	AF255700	2098
	Neojaera antarctica	AY461454	2179
Joeropsidae	*Joeropsis coralicola*	AF279608	2189
Macrostylidae	*Macrostylis*	AY461477	2160
	Macrostylis	AY461476	2174
Mesosignidae	*Mesosignum*	AY461478	2127
Munnopsidae	*Acanthocope galathea*	AF496656	2303
	Echinozone	AY461480	2117
	Echinozone spinosa	AF496658	2170
	Eurycope inermis	AF279607	2169
	Eurycope sarsi	AY461479	2117
	Ilyarachna antarctica	AY461481	2191
	Munnopsis typica	AF496661	2223
	Storthyngura falcata	AF498908	2165
	Storthyngurella triplospinosa	AY461482	2119
Stenetriidae		AY461453	2079
Cymothoida			
Aegidae	*Aega antarctica*	AF255689	2910
Anthuridae	*Cyathura carinata*	AF332146	2659
Cirolanidae	*Eurydice pulchra*	AF255690	2993
	Natatolana	AF255691	3269
	Natatolana albinota	AF255691	3269
	Typhlocirolana haouzensis	AF453249	619
	Typhlocirolana moraguesi	AF255692	2950
Corallanidae	*Excorallana quadricornis*	AF255688	2607
Gnathiidae	*Paragnathia formica*	AF255687	2116
Limnoriidea			
Limnoriidae	*Limnoria*	AY743943	1609
	Limnoria quadripunctata	AF279599	2686
Oniscidea			
Armadillidiidae	*Armadillidium vulgare*	AJ267293	3214
	Armadillidium vulgare	AJ287061	3232
	Cubaris murina	AJ287064	3537
Cylisticidae	*Cylisticus convexus*	AJ287059	3018
Ligiidae	*Ligia oceanica*	AF255698	2505
	Ligidium hypnorum	AJ287056	2414
Oniscidae	*Oniscus asellus*	AF255699	2924
	Oniscus asellus	AJ287057	2933
Philosciidae	*Philoscia muscorum*	AJ287058	2926
Platyarthridae	*Platyarthrus schoebli*	AJ287060	2969
Porcellionidae	*Porcellio scaber*	AJ287062	3192
Trachelipodidae	*Trachelipus rathkei*	AF279605	3402
	Trachelipus ratzeburgii	AJ287063	3291
Trichoniscidae	*Haplophthalmus danicus*	AJ287066	2574
	Hyloniscus riparius	AJ287065	2434
	Trichoniscus pusillus	AJ287067	2458

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TABLE 3. (Continued)

	genus/species	GenBank No.	bp
Sphaeromatidea			
Serolidae	*Acutiserolis bromleyana*	ABR269818	699
	Ceratoserolis meridionalis	CME269825	670
	Ceratoserolis pasternaki	CPA269826	671
	Ceratoserolis trilobitoides	CTR269824	671
	Cristaserolis gaudichaudii	CGA269828	690
	Cuspidoserolis johnstoni	CJO269817	700
	Cuspidoserolis luethjei	CLU269819	699
	Frontoserolis waegelei	FWA269822	699
	Paraserolis polita	PPO269823	701
	Serolella bouvieri	SBO269820	698
	Serolis glacialis	SGL269821	700
	Serolis paradoxa	SPA269827	673
Sphaeromatidae	*Campecopea hirsuta*	AF279601	2477
	Campecopea lusitanica	AF279602	2515
	Cassidinidea	AF255693	2743
	Lekanesphaera hookeri	AF279600	2461
	Sphaeroma serratum	AF255694	2413
Valvifera			
Antarcturidae	*Antarcturus spinacoronatus*	AF279604	2367
Chaetiliidae	*Glyptonotus antarcticus*	AF255696	2469
Holognathidae	*Cleantis prismatica*	AF255697	2646
Idoteidae	*Erichsonella attenuata*	AY743948	1671
	Idotea balthica	AF279603	2658
	Idotea balthica	IBAJ11390	2831

Phylogenetic trees were estimated with maximum likelihood (GARLI, Genetic Algorithm for Rapid Likelihood Inference, Zwickl 2006). GARLI phylogenetic searches on aligned nucleotide datasets begin with an assumed model of nucleotide substitutions (GTR), with gamma distributed rate heterogeneity and an estimated proportion of invariable sites. The implementation of this model is exactly equivalent to that in PAUP*, making the log likelihood (lnL) scores obtained directly comparable. All model parameters were estimated, including the equilibrium base frequencies. The gamma model of rate heterogeneity assumes four rate categories. GARLI uses a genetic algorithm approach to simultaneously find the topology, branch lengths, and model parameters that maximize the lnL (Zwickl 2006).

Phylogeny was also estimated with Mr. Bayes 3.0b4 (Ronquist & Huelsenbeck 2003) using Bayesian inferences coupled with Markov chain Monte Carlo techniques. Four Markov-Monte-Carlo chains were run for ten million generations, and a sample tree was saved every 1000 generations. Model parameters were treated as unknown variables with uniform default priors and estimated as part of the analysis. Convergence and mixing were monitored using Tracer v1.4 (Rambaut & Drummond 2009). All sample points prior to reaching stationary (one million generations) were discarded as burn-in. The posterior probabilities (p*P*) for individual clades obtained from separate analyses were compared for congruence and then combined and summarized on a 50% majority-rule consensus tree (Huelsenbeck & Imennov 2002; Huelsenbeck *et al.* 2002) in PAUP* (Swofford 2002). Clade support under the ML approach was assessed using the nonparametric bootstrap procedure (Felsenstein 1985) with 5,000 bootstrap replicates and one random addition per replicate.

In all analyses Asellota was used to root the phylogeny. Phylogenies were manipulated for presentation with Dendroscope 2.4 (Huson *et al.* 2007).

Results and Discussion

Sequence length variability

The 18S-rDNA sequences varied in length from 1807–2746 bp (aligned lengths ranged from 4878 bp to 5821 bp depending on alignment strategy and profile alignment constraints applied). Some sequences were difficult to obtain, despite multiple sequencing attempts with different primer pair combinations. Sequencing through the hypervariable regions and especially through the long insertions was difficult and not always successful. As a result some sequences, e.g. *Plakarthrium* with its long insertions, are not complete. Implementing GBlocks to eliminate questionably aligned regions reduced sequence length ~42% of total aligned sequence (from 2129 bp to 2143 bp removed). These iterations however produced outcomes with long branch length attraction and loss of phylogenetic signal. When using GBlocks the two *Plakarthrium* specimens of the same species collected from the same locality did not result in a sister relationship and make unlikely pairings with other taxa. All topologies were rooted in Asellota.

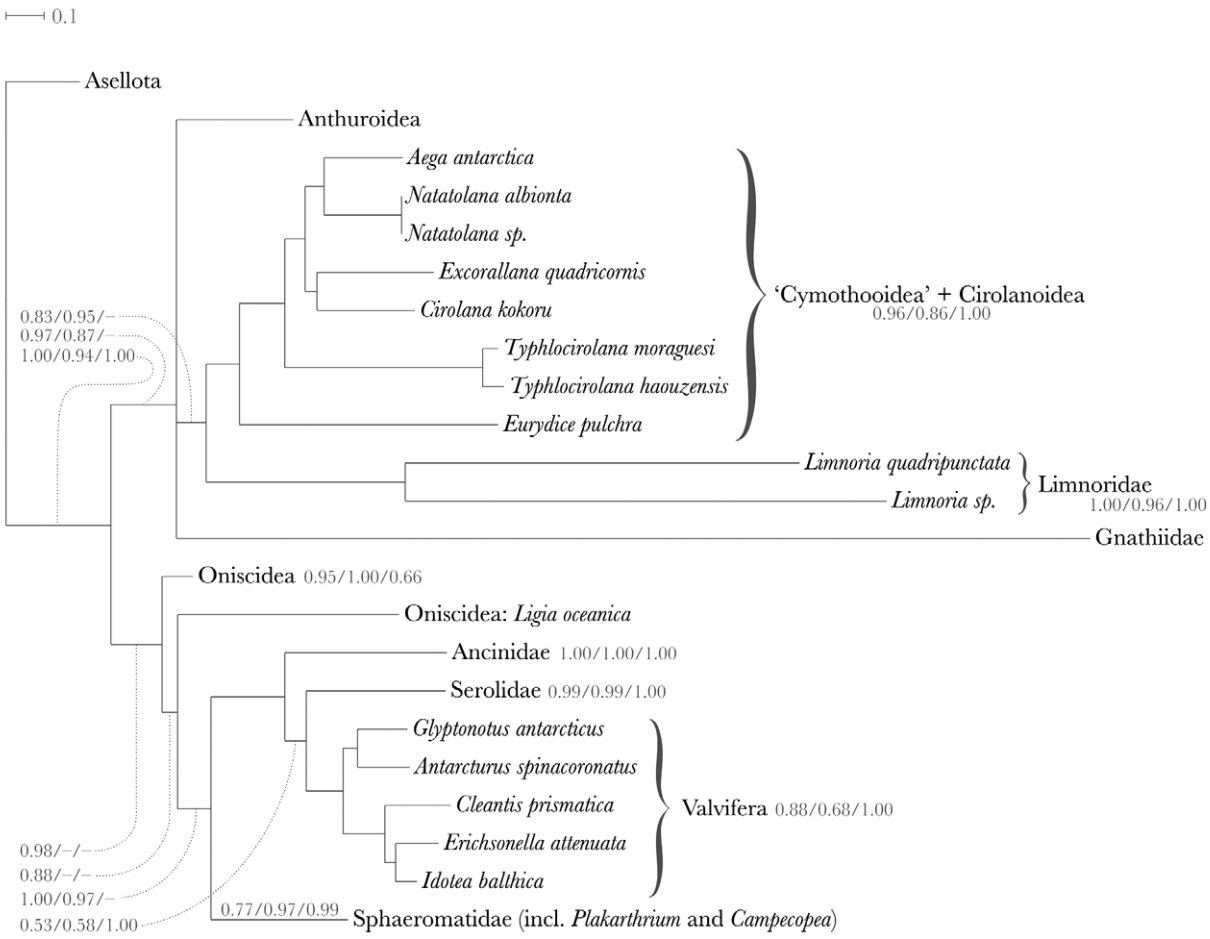

FIGURE 3. GARLI Best Tree, 116 taxa, aligned 4925 bp. Anthuridea represented by *Cyathura carinata* (GenBank AF332146), Gnathiidae represented by *Paragnathia formica* (GenBank AF255687), *Ligia oceanica* (GenBank AF255698). Posterior probabilities from three separate Bayesian analyses are indicated near nodes. The analyses differed in how alignments were created and whether GBlocks was employed or not. The first value is based on the identical alignment used in the Garli tree depicted here. The second set of posterior probabilities resulted from a dataset in which GBlocks retained only 2129 bp (43%) of the alignment and removed ambiguously aligned sections. Hyphens indicate the topology shown here was not recovered. The third posterior probability value resulted from an analysis in which serolids were added last to the alignment in an attempt to assess possible bias resulting from the short serolid sequences. As before, hypen indicates that this topology was not supported. All branches without posterior probabilities indicated were supported by values greater than 95%.

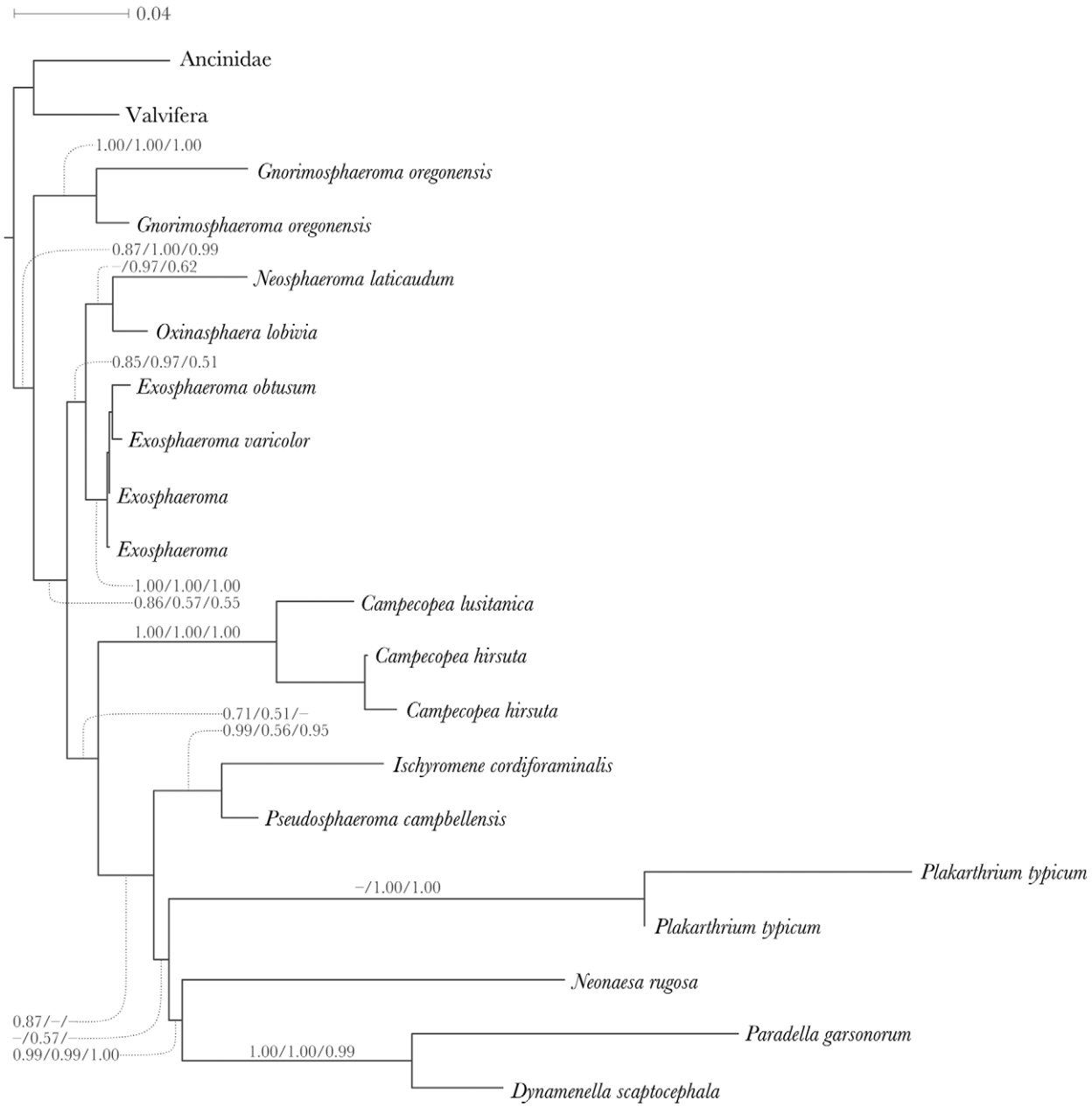

FIGURE 4. GARLI Best Tree, aligned 4925 bp. *Campecopea hirsuta* is GenBank AF279601 and *Campecopea lusitanica* AF279602. Analyses and posterior probabilities as described in Figure 3.

Phylogenetic analyses

The GARLI Best tree (Fig. 3) was selected as best representing all of the different analyses performed. Tree selection was based on internal relationships being upheld most often regardless of the analytical method used or data permutations performed. Terminal taxa are collapsed to suborder, superfamily, or family level except where clades commonly considered on morphological grounds as monophyletic do not reveal themselves in the molecular analyses (e.g. *Ligia*, never included within the Oniscidea). Bootstrap values and posterior probabilities are indicated for maximum likelihood and Bayesian analyses as described in the figure legend.

The Sphaeromatidae are always monophyletic. The proposed paraphyly of the Sphaeromatidae (Brandt & Poore 2003) is unambiguously refuted, no matter what permutation of the alignment (with or without profile alignments, application of GBlocks or not), and regardless of the analytical method (GARLI or MrBayes). The Scutocoxifera is upheld. In our analyses, the Oniscidea branch off before the Ancinidae, Serolidae, Valvifera and

Sphaeromatidae. Scutocoxifera Dreyer & Wägele 2002 had the Oniscidea sister to Valvifera, and their analytical methods found no support for Sphaeromatidea or Sphaeromatidae.

Within the Valvifera, Chaetiliidae (represented by *Glyptonotus*) and Antarcturidae (*Antarcturus*) are sister taxa and together they are sister to the Idoteidae. Within the Idoteidae, *Idotea* is sister to *Erichsonella* and together they have a sister relationship with *Cleantis* (Hologna thidae). Our analyses place Valvifera within the Sphaeromatidea as sister to the Serolidae, and the Valvifera + Serolidae as sister to Ancinidae, contrary to the Brandt & Poore (2003) phylogeny, which placed the Valvifera as sister to Sphaeromatidea. *Campecopea* is nested in the Sphaeromatidae, but its placement is not strongly supported. This result could possibly be attributed to the fact that only a single species of Ancinidae was included. Ancinidae contains two genera, *Ancinus* and *Bathycopea*, the latter known only from deep water. Future work should include Tecticipitidae with its single genus *Tecticeps*, Bathynataliidae, Basserolidae, and *Paravireia*. This improved taxon sampling and complete Serolidae sequences will go far to minimize long branch attraction (Whiting *et al.* 1997) and bring improved resolution.

Plakarthrium (Plakarthriidae), contained in the Seroloidea based on morphological characteristics (Brandt and Poore 2003), is nested deep within the Sphaeromatidae in our analyses. Two specimens were sequenced. Sequence data for this taxon is incomplete and the hypervariable region of a second sequence is not of the highest quality. The placement of *Plakarthrium* can not be definitively assessed. The short serolid sequences do have an effect on the analysis. Removing the Serolidae from the original alignment and adding them last, places Serolidae as sister to Valvifera. In other dataset combinations, Valvifera are the sister taxon to Sphaeromatidae (tree not shown).

Aega (Aegidae) always nests with the representative species of Cirolanidae (*Natatolana*, *Cirolana*). *Excorallana* (Corallanidae) is always the sister taxon of *Cirolana kokuru* (Cirolanidae). Placement of Cirolanidae in a superfamily separate from other cymothoidans appears not to be justified. Our analyses suggest that Cymothoida could be monophyletic. *Paragnathia* (Gnathiidae), *Cyathura* (Anthuridae), and *Ligia* (Ligiidae) have large divergences which result in very long branches. Too few related taxa were included in this analysis to bring any resolution to their position. Datasets in which GBlocks was implemented greatly increased the uncertainty of the placement of these taxa. We refrain from further speculation about Cymothoida as our analyses do not include representatives of Bopyroidea, Cryptoniscoidea, and we only have a single representative each of Anthuroidea (*Cyathura carinata*) and Gnathiidae (*Paragnathia formica*).

The Oniscidea are a monophyletic clade, excluding *Ligia*. *Ligia oceanica* (Ligiidae) (Genbank AF255687) consistently falls outside the Oniscidea. The sister taxon relationship of Ligiidae to the clade containing Ancinidae, Serolidae, Valvifera, and Sphaeromatidae is consistently and strongly supported. The Oniscidea branches off before the Sphaeromatoidea in most analyses and is congruent with previous morphological (Wägele 1989) and molecular (Michel-Salzat & Bouchon 2000; Wilson 2009) findings. This result was attributed to either there being too few closely related sequences in the analyses or their being a problem with this sequence. Wilson (2009) had problems with inconsistent placement of *Ligia* in his molecular analyses. However, it is noteworthy that Michel-Salzat & Bouchon 2000 had used 16S-rDNA and COI sequences, whereas Wilson 2009 had used 18S-rDNA, but both had similar results. Schmidt (2008) in his morphological review and analyses of onisc ideans, considers Ligiidae the most primitive Oniscidea. What is worth noting and exploring in the future is our finding of *Ligia* as sister to the clade Sphaeromatidea + Valvifera with Onsicidea ancestral to this clade in our analyses.

The alternative classification proposed by Wilson (2003: pp. 5, 6) based on morphology, reduces the number of major clades to four (Phreatoicidea, Asellota, Oniscidea, and Flabellifera), with the Flabellifera including the suborders Valvifera, Cymothoida, Limnoriidea and Sphaeromatidea. Wilson's classification is not supported by our analyses, differing notably in having the Oniscidea at the base of the clade containing the Sphaeromatidea and Valvifera and not a clade separating the 'Flabellifera' as in Wilson (2003). Contrary to Dreyer & Wägele (2002) *Limnoria* is not a Sphaeromatidae, but more closely allied with Cymothoida—as found by Brandt & Poore (2003). In our analysis Limnoriidea is the sister group to Cymothoida.

A limitation of this analysis specifically in relation to Sphaeromatidea is the lack of sequences from Bathynataliidae and Basserolidae. Future analyses should include complete Plakarthriidae sequences and full-length sequences of Serolidae. At present it is not known if the Serolidae also have long expansion segments. If they do, this might affect the placement of Plakarthriidae which in these analyses is placed within the Sphaeromatidae. Lemmon *et al*. (2009) demonstrated that 4-taxon simulations with missing characters or gaps can produce misleading estimates of topology and branch lengths. So not only are complete sequences desired, but future studies should include representatives of Tecticipitidae, Keuphyliidae and specimens of *Paravireia* Chilton,

1925 the latter currently considered *incertae sedis*. Within the Valvifera *Antarcturus* (Antacturidae) and *Glyptonotus* (Chaetiliidae) are always sister taxa. *Idotea* is always sister to *Erichsonella* (Idoteidae) and this pair is always sister to *Cleantis* (Hologнathidae), which is sister to the arcturids. Inclusion of many more taxa will be needed for finer scale resolution of the phylogenetic relationships of the valviferan families.

Crustacean 18S-rRNA sequence length variation was first remarked on by Spears *et al.* (1992, 2005), who noted the large variation of the V4 and V7 regions within the Peracarida (1807–2746 bp). Dreyer & Wägele (2002) found sequence length variation between 2098 and 3402 bp among the Isopoda used in their study. More recently Osborn (2009) did not find excessive sequence length variation in Asellota because there 'were many highly conserved regions and few highly variable regions'. The phylogenetic relevance of the 18S-rRNA hypervariable regions has been recognized for insects (Hwang *et al.* 2000; Xie *et al.* 2009) with some length-variable regions serving as synapomorphies for some groups. In this study we found that within the Sphaeromatidea sequence length varied between 1967 and 2865 bp), a length variation greater than found by Xie *et al.* (2009) for the insects (>600 bp). For the two clades (Asellota and Sphaeromatidea) for which multiple taxa have now been sequenced, it appears that length variation is comparably small for the Asellota compared to the length variation observed within the Sphaeromatidea. The latter has the largest length variation so far recognized within the Pancrustacea. We also found that removing these hypervariable regions which appear dubiously aligned seriously diminishes phylogenetic resolution of the deeper phylogeny. We agree with Dreyer & Wägele (2002) and Raupach *et al.* (2009) that alignment of these regions is problematic. These hyper-variable regions clearly have good phylogenetic signal, as cutting them out with G-Blocks (described earlier) contribute to phylogenetic instability.

Primary sequence and secondary structure have been worked out for several crustaceans including the isopod *Armadillidium vulgare* (Choe *et al.* 1999), the mysid *Boremysis megalops* (Meland 2007), and used in higher-level arthropod phylogeny by Koenemann *et al.* (2009). In addition to consideration of secondary structure further sampling is required to address the composition of Sphaeromatidea as proposed by Brandt & Poore (2003).

Although the 18S-rRNA gene is very useful for the questions being asked here, we concur with Osborn (2009) that sequencing single copy protein coding genes should be considered as we move forward. Wild & Maddison (2008) review nuclear protein-coding genes for phylogenetic utility in beetles, and Regier *et al.* (2009, 2010) offer nuclear protein-coding genes that are accessible across the Pancrustacea.

Conclusion

The Sphaeromatidae are always monophyletic. The sister group to the Sphaeromatidae is the clade Ancinidae, Valvifera + Serolidae (Fig 3). The Sphaeromatoidea is therefore not upheld, though critical data from Tecticipitidae were not available and the short sequence length of the Serolidae may affect their consistent placement. Our present taxonomic sampling is considered inadequate to warrant nomenclatural changes at this time. However, now that we have confirmed the validity of the taxon Sphaeromatidae, our focus will be directed to elucidating the relationships within the family.

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APPENDIX 1.
Chronological summary of the nomenclature of the Sphaeromatidae, Sphaeromatoidea and Sphaeromatidea

Sphaeromatidae LATREILLE 1825
1. HANSEN (1905)
The first family revision, the family at that time with about 30 genera.
Hemibranchiatae: with folds on the endopods of pleopods 4 and 5; included Sphaeromini and Cymodocini.
Eubranchiatae: with folds on both rami of pleopods 4 and 5; no family-group names were introduced within this division.
Platybranchiatae: without folds on both rami of pleopods 4 and 5; included Monolistrini, Cassidinini and Campecopeini. Although Monolistrini is a tribe name, therefore also a family-group name, it has been used by B. Sket as a 'group' name within the Cassidininae Hansen. 1905: the names Cassidinini, Campecopeini and Monolistrini were originally all established at equal rank, the Cassidininae later being given subfamily rank by IVERSON (1982).
2. RICHARDSON (1909)
Colobranchiatae, which also lack pleopodal folds and additionally possess prehensile first pereopods.
3. MILLER (1975)
Added the Pentabranchiatae, whose first pair of pereopods are gnathopods similar to Colobranchiatae, but which possess unique folding of both pleopodal 5 rami.
4. HURLEY & JANSEN (1977)
Elevated Hansen's group names to the subfamilies Eubranchiatinae, Hemibranchiatinae, and Platybranchiatinae. These subfamily names were not based on existing genera (i.e. did not conform to the International Code of Zoological Nomenclature).
5. BOWMAN (1981)
Replaced the Eubranchiatinae with the subfamily Dynameninae BOWMAN (1981).
6. IVERSON (1982)
Replaced the Hemibranchiatae with the subfamily Sphaeromatinae Latreille, 1825 and replaced Platybranchiatae with the subfamily Cassidininae Hansen, 1905 (incorrectly as new), reinstated Ancininae Dana, 1852 (replacing the name Colobranchiatae) and established a new subfamily Tecticeptinae Iverson, 1982 (replacing the Pentabrachiatae).
7. WÄGELE (1989) (pg. 170)
Established the Sphaeromatidea Wägele, 1989, which included the Plakarthriidae, Serolidae, Bathynatallidae, Sphaeromatidae, Keuphyliidae, Lynseiidae, Hadromastacidae, and Limnoriidae.
8. BRUCE (1993)
Raised the subfamilies Ancininae and Tecticepitinae to family rank.
9. BRANDT & POORE (2003)
Sphaeromatoidea restricted to Sphaeromatidae, Tecticipitidae, Ancinidae and *Paravireia* Chilton, 1925 (*incerta sedis*).