Relationships of the Sphaeromatidae genera (Peracarida: Isopoda) inferred from 18S rDNA and 16S rDNA genes

REGINA WETZER * , 1, NIEL L. BRUCE 2 & MARCOS PÉREZ-LOSADA 3, 4, 5

1 Research and Collections, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, California 90007 USA; Regina Wetzer * [rwetzer@nhm.org] — 2 Museum of Tropical Queensland, 70–102 Flinders Street, Townsville, 4810 Australia; Water Research Group, Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa; Niel L. Bruce [niel.bruc@qm.qld.gov.au] — 3 Computation Biology Institute, Milken Institute School of Public Health, The George Washington University, Ashburn, VA 20148, USA; Marcos Pérez-Losada [mlosada@gwu.edu] — 4 CIBIO-InBio, Centro de Investigación em Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal — 5 Department of Invertebrate Zoology, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA — * Corresponding author

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Abstract. The Sphaeromatidae has 100 genera and close to 700 species with a worldwide distribution. Most are abundant primarily in shallow (< 200 m) marine communities, but extend to 1.400 m, and are occasionally present in permanent freshwater habitats. They play an important role as prey for epibenthic fishes and are commensals and scavengers. Sphaeromatids’ impressive exploitation of diverse habitats, in combination with diversity in female life history strategies and elaborate male combat structures, has resulted in extraordinary levels of homoplasy. We sequenced specimens from 39 genera for nuclear 18S rDNA and mitochondrial 16S rDNA genes, comprehensively reviewed the effects of alignments on tree topology, and performed Garli and MrBayes analyses. These data consistently retrieved clades (genus groups), Sphaeroma, Exosphaeroma, Cymodoce, Ischyromene, Cerceis, and Dynamenella and the monogeneric clade of Gnorimosphaeroma. We define the major clades using morphological characters, attribute sampled taxa to consistently and strongly supported ones and suggest placement of unsampled genera based on their morphological characteristics. Within each clade, we also highlight unresolved and poorly sampled genera. We point out taxonomic problems in hopes of encouraging further phylogenetic exploration. Although we identify clades containing consistent generic groups and are confident that some groups will prove stable and reliable, we feel our sampling is insufficient to propose nomenclatural changes at this time.

Key words. Sphaeromatidae, 18S rDNA, 16S rDNA, Gnorimosphaeroma, Sphaeroma, Exosphaeroma, Cymodoce, Ischyromene, Cerceis, Dynamenella, phylogeny.

1. Introduction

The Sphaeromatidae Latreille, 1825 is an isopod family whose species are readily recognised and widely encountered in shallow-water marine environments, and as such came to the attention of the taxonomists early in the history of carcinology (e.g., Leach 1814, 1818; Say 1818; Milne Edwards 1840; Dana 1852). In the early 1900s to roughly the 1930s large numbers of species and genera were described, notably from southern Australia by Baker (literature can be sourced from Poore (2002) and Keppel H. Barnard from South Africa (see Kelsley 1978). The next era of description can be taken to be 1980 with the prolific work over a short period (1980–1984) of the English duo Keith Harrison and David Holdich followed on by Bruce (1992–2009), bringing the total to 100 accepted sphaeromatid genera and close to 700 species (Bruce & Schotte 2010).

The family received its first revision by the eminent Danish carcinologist Hans Jacob Hansen in 1905. The classification that Hansen (1905) proposed identified three large groups within the family, and within these groups he identified a further five groups for which he gave family-group names (as tribes). This classification was used largely unchanged until the late 20th century, although by the year 2000 the number of genera and spe-
cies had more than doubled. Later, other group names, not using accepted formal nomenclature, were also presented: Colobanchiatae Richardson, 1909 and the Pentabanchiatae Miller, 1975. The three major divisions were eventually formalized by Bowman (1981) and Iverson (1982), with all groups named as subfamilies and, other than the Cassidininae Hansen, 1905, no status given to the other family-group names proposed by Hansen. Of these other names only the Monolistini Hansen, 1905 (tribe) was used (e.g., Racovitza 1910), often informally as a group name within the Cassidininae, for the cave-dwelling sphaeromatids from the Balkans, notably by Sket (e.g., 1964, 1986) and a few others (Sbordoni et al. 1980; Stoch 1984). The Ancinidae Dana, 1852 and Tecticipitidae Iverson, 1982 were elevated to family level by Bruce (1993). These two families, together with the monophyletic Sphaeromatidae (Wetzer et al. 2013) and the unplaced genus Paravireia Chilton, 1925, constitute the superfamily Sphaeromatoidea Wägele, 1989 of Brandt & Poore (2003). Hansen’s (1905) divisions of the family was perceptive and were eventually given formal nomenclatural status in the 1980s (see Wetzer et al. 2013; El. Suppl. 1) and all genera known to date were placed into their five respective subfamilies in the key and generic listing of Harrison & Ellis (1991). This scheme was last formally presented by Roman & Dalens (1999).

Wägele (1989), as part of an overall phylogenetic reappraisal of the Isopoda and the only attempt to establish and test for groups within the Sphaeromatidae, presented in a brief “Hennigian analysis” of a dataset of 30 morphological characters which included overall body shape, cephalothorax, mandible, pereopod, pleopod, uropod, pleon, and brood pouch characters for the family; an unspecified number of genera (in some instances reference was to groups, e.g., “Gruppe Cassidina”) and genera were not coded into a matrix. Many of the characters used in that phylogeny have since been shown not to be of phylogenetic significance, notably flat body shape, uropods forming part of the body outline, presence or absence of dorsal processes, loss of the thickened folds (fleshy transverse ridges) on pleopods 4 and 5, presence or absence and form of pleotelson sinuses. At the generic level it also became apparent that dorsal processes, once considered to be axiomatically of generic significance (despite Hansen’s 1905 cautions) were inappropriate in terms of generic unity (e.g., see Bruce 1997; Bruce & Holdich 2002; Li 2000). Some ‘groups,’ such as the subfamily Cassidininae, are clearly not monophyletic, as recognized by Wägele (1989) himself, while some other groups are confirmed monophyletic by our analysis.

In the 1990s and later the generic revisions of Bruce (e.g., 1994a,b, 1995, 1997, 2003; Bruce & Holdich 2002) increasingly demonstrated that the critical purported subfamily characters – fleshy folds on pleopods 4 and 5 – were repeatedly lost within genera in the family and divisions based on those characters alone could no longer be upheld. Descriptions of new genera and generic revisions (e.g., Bruce 1993, 1994a,b, 1995, 1997, 2003, 2005; Poore 1994) did not correspond with the existing infra-family concepts. With 100 genera and roughly 700 species no alternative arrangement was offered, though definable generic groups were recognized by Bruce (1994, 1995). Infra-family groups were not used by Poore et al. (2002).

While several works dealing with the phylogeny of the Isopoda and former Flabellifera have been published (e.g., Wägele 1989; Brusca & Wilson 1991; Wilson 2003, 2009; Brandt & Poore 2003; Wetzer 2001, 2002) only Brandt & Poore (2003) questioned the integrity of the Sphaeromatidae itself, concluding that the family was paraphyletic. Wetzer et al. (2013) using 18S rDNA data demonstrated that the Sphaeromatidae is unequivocally monophyletic. The Sphaeromatidae, previously split into as many as six subfamilies, with the three largest divisions being based on pleopod morphology, is here revisited using DNA sequences from two genes (complete nomenclature summarized in Wetzer et al. 2013, Table 1).

We examine the viability of supra-generic groupings and the phylogenetic implications of these groups on classification within the family using combined 18S rDNA and 16S rDNA datasets. Our work further investigates within-clade relationships, mostly based on more extensive 16S rDNA sampling, and discusses morphological characters in the context of our genetic findings.

2. Methods

Taxon sampling. Ideally the type species of each of the Sphaeromatidae genera would be sequenced, as many of the large genera are not monophyletic or may have become a “catch-all genus” (e.g., Cymodoce Leach, 1814). In the perfect world, specimens from the type species would also come from the type locality. Prior to data acquisition and analysis, we divided the family Sphaeromatidae into perceived and plausible morphological groups of genera. Some of these groups had long been recognized, e.g., those genera related to Cercis. Some groups had been previously defined, e.g., the ‘Ischyromene-group’ (Bruce 1995). The basis for the present division lays in a DELTA (Dallwitz 1980; Dallwitz et al. 2006) phylogenetic generic morphological data set developed and in progress by NLB. These perceived divisions were then effectively assessed by the molecular analysis, and where upheld those data were used to present the morphological characterization of the major clades. Not all of the original groups held up as initially perceived (e.g. Gnorimosphaeroma separated from Exosphaeroma-like genera into a mono-generic clade). Other groups lacked sequence data.

Most specimens reported here were collected during expeditions to Australia (Great Barrier Reef, southeastern Queensland), East Africa (Kenya, Mombasa; Tanzania, Zanzibar), Singapore, Samoa and Palau. NLB collected specimens from around Australia and New Zealand, and RW contributed specimens from eastern Pacific shores.
(Chile, USA). Colleagues from all around the world (see Acknowledgements) sent many carefully collected specimens. All identifications were done by or verified by NLB. Currently there are 100 genera recognized in Sphaeromatidae. We were successful in sequencing specimens from 39 genera of the 52 genera collected and obtained, and in many instances several species and multiple individuals (El. Suppl. 1). In most instances multiple individuals were extracted, amplified, and sequenced for 18S rDNA and 16S rDNA genes. When type species were sequenced, these are indicated in El. Suppl. 1. Only in a few instances were 18S rDNA sequences incomplete (e.g., Plakarthrium Chilton, 1883) or not of the highest quality. This is reported in the ‘Results’ when unusual and unlikely placements could not be explained.

Our 18S rDNA dataset has 122 Sphaeromatidae sequences: 44 species in 33 genera. Fifty-seven of these sequences were generated for this project. This dataset contains one species of Ancinus Milne Edwards, 1840 (Acanthinae), five Valvifera species representing four families and twelve species of Serolidae (outgroup). The outgroup is as previously used in Wetzer et al. (2013).

Our 16S rDNA dataset has 201 Sphaeromatidae sequences: 94 species, in 46 genera, representing 179 sequences which are new for this project. The dataset includes two new Ancinus sequences and 45 Valvifera and Serolidae taxa (outgroup). The total aligned dataset was 634 bp long.

The concatenated 18S rDNA + 16S rDNA dataset (98 sequences) is based on 37 genera and 56 species, plus two Ancinidae, three Valvifera and six Serolidae, the latter three treated as outgroup. For 114 specimens both the 18S rDNA and 16S rDNA sequences came from the same individual (El. Suppl. 1). The combined dataset is smaller in terms of number of taxa compared to the separate 18S rDNA and 16S rDNA analyses, but still it is by far the most extensive sampling and sequencing of the family to date.

Specimen and sequence numbering scheme. All sequences used in the analyses are included with complete collection data in El. Suppl. 1. Unfortunately, the present Genbank (Benson et al. 2008) numbering scheme does not readily allow one to identify multiple gene fragments as coming from a single specimen. “RW numbers” (e.g., RW99.999) are collecting event identifiers. During DNA extraction from a single specimen, a unique 3 or 4-digit numeric identifier is appended to the locality identifier. This numeric tag readily allows association of the DNA in the spin tube, coming from a specific specimen, the collecting event, the locality, taxon name, and generated sequences (regardless of gene fragment). If a sequence used in our analyses came from Genbank, it too is assigned a 3–4 digit identifier for consistency. These unique identifiers are used here to assist the reader in identifying specimens from specific localities and collecting events and are helpful when nomenclature or taxonomic identification are troublesome. Identifiers either precede the taxon name or are reported in brackets following the taxon identification. Only in a few instances did we combine sequences from conspecifics in the combined 18S rDNA and 16S rDNA analyses. In these cases, the 3–4 digit identifier is separated by an underscore and are identified in Figs. 1 and 2. Nexus data has been submitted to TreeBASE (submission ID 21399) and will be added to Open Tree of Life upon publication. Specimens and DNA are deposited in the Natural History Museum of Los Angeles County (LACM) Collections and can be retrieved by GenBank lot, or specimen number indicated in El Suppl. 1.

Clade names used. Here we refer to clades based on the taxon that could be most extensively sampled. For example, we were able to include multiple specimens and species for the genera Exosphaeroma Stebbing, 1900, Cymodoce Leach, 1814, Ischyromene Racovitza, 1908, Dynamenella Hansen, 1905 in our analyses. As a result, these best characterize the species in the clade. The present use of these names does not imply any nomenclatural status nor their future applicability, as we are fully aware as additional taxa are included, some relationships are likely to change.

From tissue to analysis. Specimen preservation, tissue extraction, 18S rDNA primers, amplification, sequence editing, sequence assembly as well as alignment protocols are detailed in Wetzer et al. (2013). Isopod collecting and preservation methods are described in Wetzer 2015. Most material was fixed and preserved in 95% ethanol and stored in 4°C whenever possible. Specimens 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 10 × PCR buffer, 1.5 μl of 50 mM MgCl2, 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. A minimum of four 18S rDNA primer pairs were needed to amplify the gene. In some instances, five or even six pairs were used. Primer sequences are listed in Wetzer et al. (2013). 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, Waage 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. Sequence length for the 18S rDNA gene varied from 1,748–2,746 bp. 16S rDNA was amplified with universal 16Sar and 16Sbr primers (Palumbi et al. 1991; Wetzer 2001) resulting in ~550 bp fragments. 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.

As in the Wetzer et al. (2013) analyses which included only 19 Sphaeromatidae species, here we similarly explored all three MAFFT (Multiple Alignment Program for amino acid or nucleotide sequences, Katoh et al. (2002, 2005) alignment algorithms. Separate datasets were created using LINS, EINS, or GINS alignment protocols for 18S rDNA and 16S rDNA sequences. Separate analyses were run eliminating poorly aligned and divergent regions with GBLOCKS (Casteresana 2000; Tavaré & Casteresana 2007). We used default settings for all GBLOCKS parameters except for allowed gap positions, which we toggled to “with half” (i.e., only positions where 50% or more of the “sequences have a gap are treated as a gap position).

Phylogenetic congruence among mitochondrial 16S rDNA and nuclear 18S rDNA genes was assessed using Wüns (1998) protocol when genes were combined. No areas of strongly supported incongruence were observed among gene trees. Seventeen different datasets were assembled and analyzed. jModelTest v1.0.1 (Posada 2009; Darriba et al. 2012) was used to select the appropriate model of evolution for each gene partition under the Akaike Information Criterion AIC (Posada & Buckley 2004). The general time reversible model of evolution (Tavaré 1986), with proportion of invariable sites and gamma distribution, was selected for each gene (GTR + G + I). Both maximum likelihood (ML) and Bayesian methods of phylogenetic inference were applied. ML analysis was performed in GARLI under default settings for the genetic algorithm, except that searchreps = 10. Clade support was assessed using the non-parametric bootstrap procedure (Felsenstein 1985) with 1000 bootstrap replicates. Bayesian analysis coupled with Markov chain Monte Carlo (BMCMC) inference was performed in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003; Ronquist et al. 2012). Four independent BMCMC analyses were run in the CIPRES Science Gateway portal (Miller et al. 2010), each consisting of four chains. Each Markov chain was started from a random tree and run for $2 \times 10^7$ cycles, with sampling every $1000^\text{th}$ generation. Sequence evolution model parameters were estimated independently for each data partition starting as unknown variables with uniform default priors. Convergence and mixing were monitored using Tracer v1.5 (Rambaut & Drummond 2009). All sample points prior to reaching stationary levels were discarded as burn-in. The posterior probabilities for individual clades obtained from separate analyses were compared for congruence and then combined and summarized on a 50% majority-rule consensus tree.

Trees presented were selected as best representing all of the different datasets and analyses performed. Tree selection was based on internal relationships being upheld most often regardless of the analytical method used or data permutations performed. Parameters for the phylogenetic trees presented are as follows: Fig. 1 is based on 98 taxa, 5174 characters in total, 2089 constant characters, 2866 parsimony-informative characters, 219 autapomorphic characters. Fig. 2 contains the same 98 taxa as Fig. 1 and the same 5174 characters and is a 50%-majority-rule consensus of 18,002 trees. Figs. 3A, 4A, 5A, 6A, 7A, 8A, and 9A are 18S rDNA Garli BestTrees with MrBayes support values indicated on branches (110 taxa, 1841 bp characters, 854 constant characters, 873 parsimony-informative characters, 114 autapomorphic characters). Figs. 3B, 4B, 4C, 5B, 6B, 7B, 8B, 9B, 10 are 16S rDNA Garli BestTrees with MrBayes support values indicated on branches data matrix (246 taxa, 633 bp, 167 constant characters, 428 parsimony-informative characters, 39 autapomorphic characters).

MrBayes support values are indicated on all phylogenetic trees except Fig. 1. Nodes are considered strongly supported if $p_P > 0.95$. No support values are indicated in instances where maximum likelihood and Bayesian phylogenies are not congruent. Where readily available, dorsal and lateral line drawings from the primary literature have been added to terminal branches identified to the level of species. Sources are identified in the Acknowledgments.

3. Results and discussion: relationships within Sphaeromatidae

This paper infers a Sphaeromatidae phylogeny based molecular data. Key morphological features, i.e., existing morphological knowledge accumulated in the DELTA database (see Methods), is for the first time attributed to genetically derived clades. We present new molecular data, draw on morphological characters that support molecular findings, and discuss taxonomic problems and anomalies that need further review. Hence each section offers new insights and suggests new research opportunities.

Figs. 1 and 2 show the entire Sphaeromatidae and are based on the 18S rDNA + 16S rDNA combined datasets. Figs. 3A–9A show the 18S rDNA datasets, and Figs. 3B–9B, 10 are based on the 16S rDNA data; all show specific clades. The GARLI best tree (Fig. 1) and the MrBayes tree (Fig. 2) both based on the combined dataset (18S rDNA + 16S rDNA) most consistently captured deep nodes and internal generic relationships. Both of these analyses included the serolids, Plakarthrium, and did not apply GBLOCKS or profile alignments. Tree selection was based on internal relationships being upheld most often regardless of the analytical method used or data permutation performed. Branch lengths and posterior probabilities are indicated on the figures. Despite the long hypervariable regions and subsequent alignment difficulties, removing these regions with GBLOCKS produced trees we rejected as they no longer retained deep node support and the backbone of the Sphaeromatidae collapsed. Deep nodes are based primarily on combined 18S rDNA + 16S rDNA and 18S rDNA data. 16S rDNA data most consistently and robustly provides within clade relationships. We had also generated more 16S rDNA se-
quences than 18S rDNA sequences with 16S rDNA sequences increasing within clade resolution.

The phylogeny presented herein is based on the results of the molecular analyses depicted in Figs. 1 and 2. Morphological characters defining clades are presented with the relevant molecular results such that together these data will contribute to our future understanding and research of the family. Genera for which there was no genetic representation and lacking clear morphological affinities, remain as incertae sedis. All Sphaeromatidae genera are summarized in section 7. Appendix (Sphaeromatidae genera list) and organized according to our findings. A small number of genera (approximately 10% of all genera) are regarded as incertae sedis due to lack of descriptive data or simply a lack of clear morphological clues as to their phylogenetic affinities. Examples of the former are Botryias Richardson, 1910 and Hemisphaeroma Hansen, 1905. Examples of the latter are Xynosphaera Bruce, 1994b, a commensal of Alcyonacea (soft corals), with reduced morphology, and the genera Artropoles Barnard, 1920 (see Bruce 2001) and Cassidinella Whitelegen, 1901 (see Bruce 1994a).

The remaining genera form three basal clades – clade 1 (Gnorimosphaeroma) is always basal and the sister taxon to clade 2 and clade 3 (Figs. 1 and 2). Morphologically this clade is defined by pleopod and epistome morphology. The remaining clades are diagnosed, and the characters used are present in most taxa. Again, while some characters are secondarily lost or inconsistent, genera are placed on the overall balance of characters, with penial and pleopodal morphology, which show high consistency within genera, proving critical.

The hypothesis of relationships presented here is likely to undergo further refinement. Clades 2 and 3 equate to the subfamilies Sphaeromatinae and Dynameninae and while we are confident that they will remain stable, the generic composition and resolution of the relationships within the individual major clades is likely to change with the addition of taxa. In large part this is because many of the larger genera are not monophyletic, such as the large genus Cymodocea. This is evident on a morphological basis, but has been further demonstrated in the sequence data presented here, with species within such apparently classic ‘Sphaeroma-like’ genera, such as Gnorimosphaeroma Menzies, 1954, Sphaeromopsis Holdich & Jones, 1973 and Exosphaeroma, splitting into separate clades. Furthermore, the second author (NLB) is aware that there are numerous de novo genera in museum collections that remain to be described, and that exploration of deep-water hard-bottom habitats (< 1000 m of depth) will yield yet more new genera. There are many genera and species that remain inadequately described (notably species described by W.H. Baker from southern Australia, Keppel H. Barnard from South Africa and by Harriet Richardson from the USA), and consequently the relationships of these genera cannot be assessed on morphological criteria. Revision of such genera and description of new genera will inevitably change our understanding about the relationships between and within these clades.

3.1. Sphaeromatidae Latreille, 1825

Molecular results. The monophyly of the Sphaeromatidae was confirmed in Wetzer et al. (2013) and is not further discussed.

Diagnosis. The diagnosis presents the distinguishing characters that define the monophyletic Sphaeromatidae from the other families of both the superfAMILY Sphaeromatidea and the suborder Sphaeromatidea. Characters in bold italics are diagnostic.

Cephalon not fused with pereonite 1; pereonites 2–7 with coxal plates fused or with weak sutures; pleonite 1 tergite usually discrete, pleonites 2–5 fused bearing partial sutures, pleonite 5 indivisibly fused to pleonite 4; lateral suture lines variously indicated. Pleotelson entire, separate or partly fused with pleonite 5. Frontal lamina and clypeus fused, forming epistome; labrum present. Mandible stout, usually with multi-cusped incisor; lacinia mobilis short, multicusped, usually present on left mandible; spine row present; molar process forming flat nodulose, grinding or smooth crushing surface, or chitinised lobe. Maxillule mesial lobe with 3 or 4 long pectinate and 1 robust seta; lateral lobe gnathal surface with 9–13 stout, simple and/or serrate spines. Maxilliped endite elongate, bearing terminal plumose robust setae, usually with variously ornamented robust setae, usually with single coupling hook; palp articles 2–4 usually expanded to form lobes. Pereopods ambulatory, usually robust; pereopod 1 not chelate, not expanded, may be lobed (e.g., Moruloidea Harrison, 1984b; Monolistra Racovitza, 1910); dactylius usually with distinct secondary unguis. Pleopods contained within chamber formed by the strongly vaulted (dome) pleotelson, rami biramous, pleopods 1–3 usually lamellar, occasionally pleopod 1 indurate, occasionally operculate; pleopods 1–3 with plumose marginal setae; pleopods 4 and 5 with or without thickened ridges, exopod of pleopod 5 with distal scaled patches. Uropods anterolateral in position on pleotelson. Endopod fused to peduncle, may be reduced to a stub; exopod articulating, may be reduced, set laterally into endopod when present, often absent.

Remarks. Although the family has proved a challenge to define, in particular because of the high level of homoplasy that is present, most sphaeromatids are readily recognized. In part this is because many species have the ability to roll into a ball or fold themselves ‘closed’ clam shell-like. Most species appear calcified and have a rugose appearance when compared to families such as the smooth-bodied Cirolanidae and few genera have the discoidal shape of the Serolidae.

In almost all the Sphaeromatidae genera antennular articles are as follows: article 1 longest and widest; article 2 shortest but almost as broad as article 1; article 3 somewhat longer, however much narrower than the preceding articles. Expanded or broad antennular articles is an apomorphic character. All Sphaeromatidae

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Fig. 1. Garli BestTree, 98 taxa from Sphaeromatidae and outgroup based on 18S rDNA and 16S rDNA.
have the pleonites at least partly fused to each other, and all sphaeromatids have the uropodal endopod fused to the peduncle or variously reduced to absent. Similarly, the exopod can be large, and variously reduced to absent.

Characters that distinguish the Sphaeromatidae from the related families Ancinidae, Tecticipitidae and also the Serolidae are summarized in Table 1.

3.2. Clade 1: *Gnorimosphaeroma* clade

Fig. 3A,B

**Molecular results.** In all of our 18S rDNA and 18S rDNA + 16S rDNA phylogenies *Gnorimosphaeroma* is the most basal lineage within the Sphaeromatidae. With 25 species currently described (Schotte 2015) the genus is restricted to the western shores of North America and the eastern
Table 1. Sphaeromatidea Wägele, 1989: Morphological characters that distinguish the Sphaeromatidae from the related families Ancinidae, Tecticipitidae and Serolidae. Characters indicated in **bold** are synapomorphies.

<table>
<thead>
<tr>
<th>Character / Taxon</th>
<th>Serolidea</th>
<th>Tecticipitidae</th>
<th>Ancinidae</th>
<th>Sphaeromatidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>partly fused to pereonite 1</td>
<td>not fused</td>
<td>partly fused to pereonite 1</td>
<td>not fused</td>
</tr>
<tr>
<td>Mandible incisor</td>
<td>clypeate, without cups</td>
<td>clypeate, without cups</td>
<td>clypeate, with or without cups</td>
<td>gnathal, multicusp</td>
</tr>
<tr>
<td>Maxilliped endite</td>
<td>quadrate</td>
<td>quadrate</td>
<td>quadrate</td>
<td>elongate, distally rounded or acute</td>
</tr>
<tr>
<td>Maxilliped endite distal margin</td>
<td>without robust and slender setae</td>
<td>with slender setae</td>
<td>without or few slender setae</td>
<td>with many robust and simple setae</td>
</tr>
<tr>
<td>Pleopod 1 propodus</td>
<td>swollen, dactylus prehensile</td>
<td>swollen, dactylus prehensile</td>
<td>swollen, dactylus prehensile</td>
<td>not swollen, dactylus not prehensile</td>
</tr>
<tr>
<td>Pleonites</td>
<td>3 (1, 2 free; 3–5 fused)</td>
<td>4–fused</td>
<td>1 or 2</td>
<td>4 usually (many reductions to 0)</td>
</tr>
<tr>
<td>Uropods</td>
<td>biramous, articulated</td>
<td>biramous, endopod fused</td>
<td>uniramous, fused endopod absent</td>
<td>various, endopod fused when present</td>
</tr>
</tbody>
</table>

shores of Asia. The genus is unusual among sphaeromatids as it contains fresh-, brackish-, and salt water species (see Menzies 1954). Only few sphaeromatid genera have a broad salinity range. Our study has exemplars of two East Pacific species: marine *G. oregonensis* (Dana, 1853) and brackish/freshwater species *G. noblei* Menzies, 1954 both from the west coast of North America.

18Sr DNA + 16Sr DNA analyses (Figs. 1, 2): In the combined analyses the freshwater *G. noblei* and *G. oregonensis* are sister clades.

18Sr DNA analyses (Fig. 3A): In these analyses 1151 + 1496 + 1477 *G. oregonensis* cluster San Juan and Whidbey Island (Washington) specimens together and are derived with respect to the two freshwater specimens (1541 [Tomales Bay, Marine County, California, freshwater] and 1174 [San Gregorio Creek, San Mateo County, California, freshwater]) which are basal to 1151 + 1496 + 1477.

16Sr DNA analyses (Fig. 3B): A total of 7 sequences were available. Sequences 1174 + 1541 are *G. noblei* from San Gregorio Creek (salinity not measured) and Tomales Bay, head of bay were salinity was 20 ppt, respectively. The other five sequences are fully marine *G. oregonensis* collected in the intertidal of British Columbia and Washington State, San Juan and Whidbey Islands. Marine specimens clade together and are sister group to the *G. noblei* clade.

Morphological characters. The genus and clade is characterized by lamellar uropodal rami, the exopod being shorter than the endopod; the pleon lateral sutures run from the free lateral margins of the pleon, pleotelson posterior margin arculate, entire, not thickened; pleopods 4 and 5 are without folds, but otherwise similar to those of *Sphaeroma* Bosc, 1801 (now the accepted authority for the genus – see Low 2012) and *Exosphaeroma*. Generally, there are few distinguishing characters, in essence *Gnorimosphaeroma* superficially differs little from those species of *Exosphaeroma* with an arcuate pleotelson. *Gnorimosphaeroma* is distinguished by the shorter uropodal endopod and pleonal sutures running to the free lateral margin of the pleon (vs posterior pleon margin).


Remarks. Menzies (1954) erected *Gnorimosphaeroma* for *Exosphaeroma oregonensis* Dana, 1853. Although his diagnosis and accompanying figures for the type species, are reasonably detailed, until at least the type species, *Exosphaeroma oregonensis* is fully redescribed and the genus itself re-diagnosed uncertainty will remain over the systematic position of the genus. It should be noted that all of Dana’s isopod specimens were lost when the sloop Peacock sank at the bar of the Columbia River (see Bruce 2009: p. 211), so there is no type material for *Exosphaeroma oregonensis*. Type locality is Puget Sound, Washington State.

Similar genera are *Bilistra* Sket & Bruce, 2004 and *Neosphaeroma* Baker, 1926 (see Harrison & Holdich 1984). However, in our molecular analyses *Neosphaeroma* is basal to the *Cymodoce* clade (see below). We had no *Bilistra* sequences, and thus morphological relationships between these genera and the genera *Sphaeroma* and *Exosphaeroma* are unclear, only *Gnorimosphaeroma* can be attributed to this clade.

3.3. Clade 2 (equivalent to Sphaeromatinae Latreille, 1825)

Molecular results. Clade 2 is supported in all of our analyses (Figs. 1, 2). The bootstrap support (≈ bs) for Clade 2 is 72%. In the Bayesian analyses *Neosphaeroma* is included within *Cymodoce*. In the Garli analyses *Neosphaeroma* is the sister taxon to *Cymodoce*. Within Clade 2 the genus *Sphaeroma* is the sister taxon to the *Cymodoce – Oxinasphaera* Bruce, 1997 clade + the *Exosphaeroma* clade. The *Sphaeroma*, *Cymodoce* and *Exosphaeroma* clades each have 100% bs.

Morphological characters. Epistome long, anteriorly extended between antennula bases. Pleon of four visible pleonites. Pleopod 1 exopod truncate or sub-truncate (not rounded); endopod triangular to sub-triangular. Pleopods 1 and 2 lamellar. Pleopods 4 and 5 with transverse thickened ridges (when present). Pleopods 1 – 3 rami subequal in size.
Remarks. There are three clades within the Clade 2 sensu stricto: Sphaeroma, Cymodoce and Exosphaeroma (Figs. 1, 2). The Sphaeroma and Exosphaeroma clades, are characterised by biramous, lamellar uropods, maxillipeds without distinct lobes, pleotelson posterior margin entire (or with shallow, open, ventral exit channel), separate penial processes.

The Cymodoce clade is distinctive, distinguished by numerous derived morphological characters, such as excised pleotelson posterior margin, maxillipedal palps with 'finger-like' lobes, uropodal exopod reduced (e.g., Oxi-nasphaera) or uropodal endopod reduced (e.g., Cilicaea Leach, 1818, Paracilicaea Stebbing, 1910b and females with metamorphosed mouthparts [where known; Dynamenis] Richardson, 1905 not metamorphosed]. Species within the Cymodoce clade have the inferior margin of the merus, carpus and propodus of pereopod 1 with a pattern of large, evenly spaced robust setae that does not occur in any of the other groups of genera.

3.3.1. Sphaeroma clade
Fig. 4A,B,C

Molecular results. 18S rDNA + 16S rDNA analyses (Figs. 1, 2): Sphaeroma Bosc, 1801 is a large genus that today has 41 species. Most species of the genus can roll up tightly into a sphere. Over time some species formerly placed in Sphaeroma have been recognized as belonging to other genera such as Lekanesphaera Verhoeff, 1943, Isocladus Miers, 1876, Exosphaeroma and Gnorimosphaera, and have been removed from Sphaeroma. Our combined 18S rDNA and 16S rDNA analyses all resulted in a strongly supported clade regardless of the alignment or analysis method.

18S rDNA analyses (Fig. 4A): These included five sequences which in all analyses resulted in two distinct clades. All members of the genus Sphaeroma are the sister taxon to the clade containing exemplars of Lekanesphaera (100% bs). The two specimens of S. serratum (Fabricius, 1787) [1135 + 973] from Portugal and Spain, respectively, form the sister taxon to 1473 Sphaeroma sp. collected on the opposite side of the Atlantic (South Carolina, USA). They notably form a long branch, but have 100% bs.

GenBank AF279600 Lekanesphaera hookeri (Leach, 1814) (989 on tree) sequenced by Dreyer & Wägele (2002) is the sister taxon to 1529 L. hookeri from Greece. These three taxa form a well-supported clade and the species identifications are likely valid. 1529 L. hookeri was collected from a “spring in brackish lake”. This finding is interesting as the implication is another freshwater invasion – once in Gnorimosphaera, then again in the Sphaeroma clade with Lekanesphaera and again separately in the Dynamenella clade in Thermosphaeroma Cole & Bane, 1978 which is discussed later.

16S rDNA analyses (Fig. 4B,C): For these analyses we generated ten sequences for this project. Eleven sequences were previously published in GenBank mostly by Baratti et al. (2011). In most analyses Sphaeroma breaks up into two distinct clades with the Baratti et al. (2011) 16S rDNA S. terebrans Bate, 1866 sequences forming a clade that is distinct from a second clade containing Sphaeroma quoyanum Milne Edwards, 1840, S. walkerii Stebbing, 1905, S. quadridentatum Say, 1818 and Lekanesphaera hookeri.

Clade A: Baratti et al. (2011) extensively sampled Sphaeroma terebrans from the Seychelles, East Africa, Brazil, and Florida with 16S rDNA, COI and histone 3 genes. Their combined Bayesian analysis retrieves a clade containing Florida + Brazil sequences which together form the sister taxon to an African clade. Additionally, their sequences identified only as ‘Sphaeroma’ are an undescribed species [1601, 1609, 1608]. Adding our 812 S. terebrans sequence from South Carolina to the Baratti sequences retrieves a sister taxon relationship with 1603 S. terebrans from Florida, and together these form the sister taxon to the Brazilian specimen [1602]. The Baratti S. terebrans are all mangrove borers (Baratti et al. 2011; Baratti et al. 2005; Messana 2004). They acknowledge large genetic distances between populations.
that could suggest that these may be a species complex whose taxonomic status needs further evaluation. Within clade A bs is 100% for all specimens identified as *S. terebrans*.

**Clade B:** Based on 16S rDNA data, *Sphaeroma* is not monophyletic. The *S. terebrans* clade is distinct from a second clade containing *Sphaeroma quoyanum, S. walkeri, S. quadridentatum, and Lekanesphaera hookeri*. We do not have 18S rDNA *S. terebrans* sequences in our dataset, which quite possibly could change tree topology.

*Sphaeroma walkeri* [807 and 808] are both from Singapore. 408 *S. quadridentatum* and 409 *S. quoyanum* sequences are from specimens without locality data (donated by S. Shuster). *Sphaeroma* sp. [1473] is from South Carolina, 788 *Sphaeroma* (Florida), 1135 *S. serratum* (Portugal), and 1529 *L. hookeri* (Greece). 1042 *S. serratum* and 1043 *Sphaeroma* sp. are from the coast of France (Genbank, MICHEL-SALZAT et al. 2000). 1529 *Lekanesphaera* may be misidentified, or the identification is correct and this is additional evidence that the genus *Sphaeroma* is not monophyletic. *S. quadridentatum* is the sister taxon to 788 + 1473 *Sphaeroma* (100% bs). Together this clade is the sister taxon to 1135 *S. serratum* + 1043 *Sphaeroma* (100% bs). These in turn together form the sister taxon to 1042 *Sphaeroma serratum* + 1529 *L. hookeri* (100% bs). The sister clade to all these is 409 *S. quoyanum* (100% bs). Basalmost in the clade 808 + 807 *S. walkeri* (100% bs), with 100% bs to its sister group.

**Morphological characters.** Typically, smooth bodied, weakly or not sexually dimorphic; body can conglobate. Pereopods with superior margin with few to many long setae (shared with *Exosphaeroma*). Uropodal rami lamellar, usually subequal (shared with *Exosphaeroma*); exopod lateral margin usually smooth (*Benthosphaera Bruce, 1994, Bilistra Sket & Bruce, 2004*) or weakly to distinctly serrate (*Sphaeroma, Lekanesphaera*). Pleon of four visible somites (shared widely). Pleotelson posterior margin rounded or arcuate (never with exit channel, notches or foramen) – shared with *Exosphaeroma* and *Gnorimosphaeroma*; but not *Cymodoce* clade.

Remarks. Bruce (1994c: p. 400) and Sket & Bruce (2004) discussed a group of genera morphologically similar to Sphaeroma, primarily based on characters that appear to be plesiomorphic. These genera were: Apernosthaera Bruce, 1994b, Benthosphaera, Bilistra, Exosphaeroma, Exosphaeroidees Harrison & Holdich, 1983, Lekanesphaera, Neosphaeroma and Sphaeroma. The present analysis shows that this clade is restricted to the genera given above, Exosphaeroma forming a separate clade, and Neosphaeroma (a poorly characterized genus of doubtful monophyly) nesting within the Cymodoce clade. Note: According to Low (2012) the correct authority for Sphaeroma is Bosc, 1801 and predates the long accepted Latreille (1802).

3.3.2. Cymodoce clade

Fig. 5A,B

Molecular results. 18S rDNA + 16S rDNA analyses (Figs. 1, 2): The Cymodoce clade is strongly supported and is the sister clade to the well supported Exosphaeroma clade. In the MrBayes analyses the sister relationship of Cymodoce + Exosphaeroma lacks strong support and is possibly the result of inadequate taxon sampling. In the GARLI analysis Neosphaeroma is basal to the Cymodoce clade.

18S rDNA analyses (Fig. 5A): Ten sequences were available representing seven genera and eight species. Relationships are all strongly supported. 1489 + 1490 Oxinasphaera lobivia Bruce, 1997 from Queensland form the sister taxon to 1142 O. tetradon Schotte & Kenseley, 2005 (Tanzania). 1196 Cilicaea crassicaudata Haswell, 1881(Singapore) is the sister taxon of 1500 Neosphaeroma laticaudum (Whitelegge, 1901) (New South Wales). 1500 N. laticaudum has a long branch length and although strongly supported as included in the Cymodoce clade in the 18S rDNA GARLI analyses and the combined 18S rDNA + 16S rDNA Bayesian analyses (Fig. 2), it comes off basal to the Cymodoce clade in the 18S rDNA + 16S rDNA GARLI analyses (Fig. 1). There are three described species of Neosphaeroma. Two species are valid, and the third, N. pentaspinis Baker, 1926, is incertae sedis, probably or possibly a Gnorimosphaeroma. Genetic sampling both species might resolve their placement.

1143 Paracilicaea mossambica Barnard, 1914 (Kenya) is the sister taxon to 1180 Harrieta faxoni (Richardson, 1905) (Florida) (100% bs). Together they form the sister taxon to 1141 ? aff. Cymodopsis (Kenya) which is recognized to be at a minimum a new species or possibly a new genus (100% bs). Basalmost in the clade are sister taxa 1144 Cilicaeopsis whiteleggei (Stebbing, 1905) (Tanzania) and 1481 C. whiteleggei (Fiji) (100% bs).

16S rDNA analyses (Fig. 5B): The 16S rDNA gene fragment alone does not consistently reveal the deeper backbone of this otherwise strongly supported clade, but regardless of the analyses performed the following relationships are always supported. Taxa identified as Oxinasphaera have 100% bs. All of the Zanzibar specimens together form the sister taxon to the Mombasa specimens, and this entire group is the sister taxon to specimens from Queensland. At the species level, morphological determinations are more challenging between O. tetradon and O. penteumbonata Benvenuti, Messana & Schotte, 2000 and these are interspersed with “Oxinasphaera sp.” that could only be confidently identified to the level of genus. The sister clade to Oxinasphaera contains Neosphaeroma, Paracassidinopsis Nobili, 1906 and Platynympha Harrison, 1984. Notably this group has a long branch which may be the result of our poor sampling (see below “Genera Included” for proposed genera belonging to this clade), poor sequence quality, or misidentification/undescribed species. 1515 Platynympha longicaudata (Baker, 1908) (South Australia) should be regarded with caution as is not the best quality sequence. Four individual specimens from two localities (South Australia and Victoria) had been extracted/amplified and only 1515 yielded a useable sequence. 1519 Paracassidinopsis perlata (Roman, 1974) (Tanzania) is a high-quality sequence from a small whole individual. Annotations in the collecting notes indicate that the same lot contained immature ‘Cymodoce’ and Oxinasphaera. Based on its position within the clade our identification appears correct, but based on the specimen’s small size, the “Paracassidinopsis perlata” taxon label should be used cautiously. All Neosphaeroma laticaudum (1131, 1500, and 1513) are from the same New South Wales collecting event.

The sister taxon to this clade is 1128 Ischyromene cordinforcinalis (Chilton, 1883b) (New Zealand) with a long branch and no branch support. It is suspected that this is a long branch problem and the 16S rDNA gene fragments’ inability to resolve the phylogeny at this level. This is a high-quality sequence, but its placement is absurd. The combined 18S rDNA + 16S rDNA phylogenies (Figs. 1, 2), as well as the 18S rDNA phylogeny (Fig. 7A) firmly places 1128 Ischyromene cordinforcinalis in the Ischyromene clade.

The genera Cymodoce, Cilicaea, Paracilicaea and Cilicaeopsis together are composed of more than 118 described species, many of which are incertae sedis and do not belong to the respective genera sensu stricto. Sequences for only a few species were available here. As is evident from the groupings in Fig. 5A, species descriptions are difficult to apply and consistent identification was difficult. Together they are supported with 89% bs. All specimens in the clade containing 734, 750, 1143 Paracilicaea mossambica Barnard, 1914 and 728, 736, 755 Cymodoce are from Kenya. 830 Cymodoce tribulalis Harrison & Holdich, 1984 (Queensland) with a long branch is the sister taxon to the clade containing 742, 749, and 758 Cymodoce (Mombasa and Zanzibar) with the latter having 90% bs. These two clades together are the sister taxon to 1180 Harrieta faxoni (Florida).

Specimens 764 + 1144 Cilicaeopsis whiteleggei are from Zanzibar, and 1481 C. whiteleggei from Fiji. (Note: Cilicaeopsis whiteleggei is a group of cryptic species with at least six species or more.) Bootstrap value for Cilicaeopsis sequences is 100%. 1196 Cilicaea crassicau-
data, 809 C. latreillei Leach, 1818 are both from Singapore, and 739 Paracilicaea from Mombasa and 1130 Cymodoce aculeata Haswell, 1881 from New South Wales. 1130 was identified as Cymodoce aculeata (New South Wales). 1132 Cilicaea is also from New South Wales.

Some clades are strongly supported, others not. As already noted above, too few taxa were sequenced to reassign identifications based solely on the molecular analyses and some rearrangements would be expected as more genera and more sequence data are added.

**Morphological characters.** Body often setose, pleon and pleotelson variously with processes, nodules or spikes; pleotelson posterior margin variously excavate. Males and females strongly dimorphic; males often with, sometimes without prominent pleonal process; females with ‘metamorphosed’ mouthparts. Maxilliped endite articles with moderate to long finger-like lobes. Pereopods 1–3 inferior margin (merus, carpus and propodus) with series of prominent, close-set and straight serrate (bi-serrate) robust setae. Penial processes mutually adjacent, elongate;

![Fig. 5. Cymodoce. A: 18S rDNA Garli BestTree with MrBayes support values indicated on branches. B: 16S rDNA Garli BestTree with MrBayes support values indicated on branches.](image-url)
appendix masculina elongate (reflexed in Cilicaea; or ‘very long’). The uropodal rami are usually unequal, often with endopod largely or entirely reduced, and the exopod round in section (not lamellar). Some undescribed ‘Paracilicaea’ have biramous uropods (NLB pers. obs).


Remarks. Fifteen genera are included in the group, showing a diverse range of body appearances. The relationships between the genera within this group remain unclear. The larger genus such as Cymodoce, Cilicaea and Paracilicaea all include species that need to be housed in other mostly new genera. Pleopods are generally similar to Sphaeroma clade; penial processes are mutually adjacent (i.e., basally in contact, but separate) and long, extending beyond pleopod peduncle (vs. narrowly separated and short).

Bregmotypta Bruce, 1994, Kranosphaera Bruce, 1992 and Ceratocephalus Woodward, 1877 are included on the basis of maxilliped, pereopod, penial and pleopod morphology (Ceratocephalus female with metamorphosed mouthparts). No specimens of these genera were available for molecular analysis.

3.3.3. Exosphaeroma clade

Fig. 6A,B

Molecular results. 18S rDNA + 16S rDNA analyses (Figs. 1, 2): The Exosphaeroma clade is monophyletic for the taxa presently included, well supported (100%) and is the sister taxon to the Cymodoce clade.

18S rDNA analyses (Fig. 6A): Of all of the 18S rDNA clades, the Exosphaeroma clade maintains the least internal consistent structure. Internal structure of this clade is also not well supported and with different alignments and analysis permutations does not always return the same relationships. This is contrary to the 16S rDNA findings (see below). 1166 Sphaeramene polytylotos Barnard, 1914 and 1471 Parisocladus perforatus (Milne Edwards, 1840) are sister taxa (100% bs). 1474 and 1177 Exosphaeroma truncatellison Barnard, 1940 are both from Namibia and always are sister taxa, although not strongly supported (52% bs). For 1486 Exosphaeroma obtusum (Dana, 1853) (New Zealand) and 1522 Exosphaeroma (Namibia) a sister relationship is recovered only rarely. In this analysis it was recovered with 100% bs. Sequencing through the hypervariable region was problematic for both of these sequences, and they are not of the highest quality, although BLAST searches for each sequence was reasonable. 1197 Zuzara Leach, 1818 (South Australia), 1507 + 1164 Exosphaeroma (Victoria) is always recovered as a clade. The implication is that 1197 may actually be Exosphaeroma. The lot specimen 1197 came from contained what appeared to be single sphaeromatid genus, but specimen 1197 was a small individual not an adult male, but still large compared to most sphaeromatids, hence this may be an identification issue.

16S rDNA analyses (Fig. 6B): In all analyses the Exosphaeroma clade is always monophyletic for the 34 sequences generated. “Exosphaeroma” may appear morphologically simple, smooth bodied, and able to conglobate. At closer examination their dorsums can be highly diverse (many are smooth, others ornate and covered in tubercles, and there are two forms of pleotelson morphology – those with a simple arcuate rim, others with a ventrally thickened rim some with a produced apex; similarly, uropods can be simple, with sub-parallel margins and rounded apex, or expanded as in the Exosphaeroma ‘amplicauda group’ of species (see Wall et al. 2015). It is therefore to be expected that they appear genetically diverse, some with long branches and others not yet named.

Beginning with the most derived clade A, 1499 Exosphaeroma obtusum and 815 + 816 + 1504 Parisocladus (all New Zealand) form the sister taxon to 714, 1510 E. varicolor Barnard, 1914 (Chile). 663 + 1126 Isoscladus armatus (Milne Edwards, 1840) (New Zealand) together forms the sister taxon to 1486 E. obtusum (New Zealand). E. obtusum as presently defined needs to be revisited. Together this group is the sister taxon to 1195 and 829 Zuzara digitata Harrison & Holdich, 1984 (Queensland). Clade A has 100% bs.

In clade B, 1164, 1507, and 1511 Exosphaeroma are all from the same collecting event (Pt. Addis, Victoria). They form the sister clade to material identified as 818 Zuzara (Melbourne, Victoria). Basalmost in the clade is 1197 Zuzara (Ceduna, S. Australia). Clade B is well supported (100% bs).

All specimens contributing to clade C are from Namibia. 1166, 1472, 1552, and 1838 Sphaeramene polytylotos together form the sister taxon to 1471 Parisocladus perforatus. 1177, 1474 E. truncatellison as presently defined needs to be revisited. Clade C has 100% bs.

Specimens in clade D are all from Southern California, except 780 Exosphaeroma which is from La Paz,
Baja California Sur, Mexico and is the southernmost exemplar in the clade. 1134 and 777 *E. inornata* form the sister taxon to 1469 *E. aphrodita* Boone, 1923 (San Diego). 1470 *E. pentcheffi* Wall, Bruce & Wetzer, 2015 (Los Angeles, and dorsally ornately ornamented) is the sister taxon to 780 *Exosphaeroma* sp., possibly *E. bruscail* Espinosa-Pérez & Hendrickx, 2001 (La Paz, Baja California Sur, dorsum smooth). Clade D is strongly sup-

Fig. 6. *Exosphaeroma*. A: 18S rDNA Garli BestTree with MrBayes support values indicated on branches. B: 16S rDNA Garli BestTree with MrBayes support values indicated on branches.
ported (100% bs). 1121 Exosphaeroma is from the Pacific coast of Baja California Norte and in dorsal appearance would readily be recognized as E. inornata Dow, 1958, but genetically it is clearly not, and hence potentially an undescribed species. Most basal in the clade is 782 Exosphaeroma from the Gulf of California, Baja California Sur, also an undescribed species.

Morphological characters. Penial processes are narrowly separated, but longer than Sphaeroma clade. Mouthparts are not metamorphosed in females. Posterior margin of pleotelson entire, with or without shallow exit channel (except Zazara has complex pleotelson posterior margin). Pleopods are generally similar to Sphaeroma clade, though loss of transverse ridges on pleopods 4 and 5 is common. Uropods lamellar, usually subequal in size, occasionally with large, expanded rami [e.g., Ptyosphaera; Exosphaeroma amplicauda (Stimpson, 1857)].


Remarks. The monophyly of Exosphaeroma is far from assured. Subjectively three groups can be perceived; (1) those related to the type species E. gigas (Leach, 1818), which have an exclusively Southern Hemisphere distributions, (2) those species that with broad uropods and produced pleotelson apex (typified by Exosphaeroma amplicauda, see Wall et al. 2015), and (3) species similar to Exosphaeroma inornata, possibly restricted to the Northern Hemisphere. A particular problem with this genus is the large number of minimally described species, and, therefore, it is not possible to provide a more detailed morphological characterisation of this clade; furthermore, it is probable that some species will prove to be ‘species complexes’ (see Bruce 2003; Wall et al. 2015).

Distinguishing of the genera Zazara Leach, 1818 and Isocladus Miers, 1876 from Exosphaeroma Stebbing, 1900 is equally unclear. Exosphaeroma differs from Isocladus in lacking a dorsal process on pereonite 7 of males; females of the two genera are effectively indistinguishable using generic criteria. Zazara males also have the dorsal process but also have a short process and notch on the median point of the pleotelson posterior margin. Both these characters are absent from females, leaving them again indistinguishable at the generic level from Exosphaeroma. Exosphaeroma remains a paraphyletic taxon defined at present by the absence of these derived characters. This is further supported by both Zazara and Isocladus being embedded in clades that also have Exosphaeroma (Fig. 6B).

3.4. Clade 3 (equivalent to Dyameninae

Molecular results. Clade 3 is strongly supported (bs 97%) and in turn contains the strongly supported Ischyromene, Cerceis Milne Edwards, 1840 and Dyamenella clades (Figs. 1, 2). Both Campecopea Leach, 1814 and Plakarthrium (Plakarthriidae Hansen, 1905) are included here with Campecopea as the sister taxon to the other clades. Recall as noted earlier, the Plakarthrium sequence is incomplete and the placement of the taxon in our phylogeny is dubious.

Morphological characters. (Dyamenella and Cerceis are sister clades and together form the sister taxon to Ischyromene. Exceptions to the common clade state are noted in [parentheses].) Pleotelson complex with sinuses, excisions, upturned; or secondarily simple (as in Sphaeromopsis and Thermosthoma); pleonal sutures short, extend from the posterior margin [long in Cerceis, extend from lateral margin]. Epistome usually without mesial constriction [rarely with]. Maxilliped palp articles weakly lobate. Pleopods 4 and 5 with transverse ridges on both rami (when present); pleopod 2 appendix masculina basal [medial to distal in Cerceis clade]. Penial processes close set (but otherwise variable). In Cerceis and related genera the appendix masculina is even distally placed; also rami of pleopod 1 or 2 may be deeply serrate.

Remarks. Clade 3 includes three large clades, each rich in defining and characterizing derived characters, but have few shared characters. The Dyamenella + Cerceis – Ischyromene clades share a single character – both rami of pleopods 4 and 5 have transverse ridges when present.

This is the former “Eubranchiatinae”. Bowman (1981) designated a type genus and established the name Dynameninae, 1981, but with no diagnosis. Harrison & Holdich (1982a) and Bruce (1993) equally did not offer a diagnosis to the subfamily. Type genus is Dynamene Leach, 1814, type species Oniscus bidentata Adams, 1800 [= Dynamene bidentata (Adams, 1800)]. Dynamene is an atypical genus for this clade in being strongly sexually dimorphic.

3.4.1. Ischyromene clade

Fig. 7A,B

Molecular results. 18S rDNA + 16S rDNA analyses (Figs. 1, 2): Ischyromene, Scutaloidea, Pseudosphaeroma, Dynamenopsis and Amphoroidea Milne Edwards, 1840 representing 7 species were available and consistently produced a strongly supported Ischyromene clade (100% bs) with Campecopea being its sister group.

18S rDNA analyses (Fig. 7A): 1517+1518 Ischyromene huttoni (Thomson, 1879) (Chile) is the sister taxon to 1492 I. huttoni (New Zealand) (100% bs). Together they form the sister taxon to 1543 Amphoroidea typa Milne Edwards, 1840 (Chile) (79% bs). 1484+1516
Dynamenopsis varicolor (New Zealand) is the sister taxon to the aforementioned clade (94% bs). 1127 *Pseudosphaeroma cambellensis* Chilton, 1909 (New Zealand) is the sister taxon to 1410 + 1506 *P. lundae* (Menzies, 1962) (Chile) (100% bs). There is not strong support for the *Pseudosphaeroma + I. huttoni / D. varicolor / A. typa* clade. 1128 *Ischyromene cordiforminalis* (New Zealand) is basal and with a long branch. 1190 Scutuloides is basal to all.

**16S rDNA analyses (Fig. 7B):** 715 + 1543 *Amphiroidea typa* (Chile) together form the sister taxon of 1129 *A. media* (New Zealand) (100% bs). Together they are the sister group to 1517 + 1518 *Ischyromene huttoni* (Canal Darwin, Chile) (100% bs). 811 + 1178 *Cassidinidea ovalis* (Say, 1818) (South Carolina, USA) is the sister taxon to the *I. huttoni – Amphiroidea* clade in this particular analysis (100% bs). In most 16S rDNA analyses *Cassidinidea* is basal within *Ischyromene*, which contradicts its placement in the combined 18S rDNA + 16S rDNA analyses in the *Dynamenella* clade (Figs. 1, 2) and see discussion under *Dynamenella* clade below. 1485 + 1532 *Cymodocella foveolata* (Coquimbo, Chile), 1517 + 1518 *I. huttoni* (Canal Darwin, Chile), and 1128 *I. cordiforminalis* (New Zealand). In all combined gene 18S rDNA + 16S rDNA analyses and in all 18S rDNA analyses, the *C. foveolata* are members of the *Dynamenella* clade. The contradiction of these data is attributed to the influence of the extremely variable 18S rDNA V4 and V7 regions. The Chilean *C. foveolata* and *I. huttoni* are separated by nearly 1,900 km. All of the sequences are complete and of good quality. Based on morphology, *Cymodocella* would be expected to be within the *Ischyromene* clade.

**Morphological features.** Antennula peduncle article 2 is always relatively long (> 40% length of article 1); article 3 is short (equal in length or shorter than article 2) [compared to most other genera; e.g., the *Cymodocella, Cerceis* and *Cilicaeopsis* genus groups]. Pereopods secondary unguis with 2 accessory cusps. Pleopod 1 endopod medial margin is indurate (exopod may also be indurate and operculate). Pleopods 2 and 3 endopod distinctly longer than exopod. Pleopods 3 and 4 exopods always lacking a

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**Fig. 7. Ischyromene. A:** 18S rDNA Garli BestTree with MrBayes support values indicated on branches. **B:** 16S rDNA Garli BestTree with MrBayes support values indicated on branches.
transverse suture. Short pleonal sternite present. Appendix masculina basally attached, extends beyond the pleopodal rami. Maxilliped endite distal margin with clubbed robust setae. Brood pouch of ovigerous females with a posterior pocket. Sexual dimorphism weak or absent.

This group of genera was recognised by Bruce (1995), and has several consistent and distinctive characters. The antennula morphology is unique (the only similar arrangement is in some genera with strongly flattened antennula peduncles), as is the morphology of pleopods 1–3, with pleopod 1 endopod with a thickened medial margin, occasionally pleopod 1 exopod operculate or thickened and operculate, and pleopods 1–3 with the endopod distinctly longer than the exopod; all included genera have a characteristic secondary unguis (robust seta) on the dactylus, which has two secondary cusps and is described as being bifid.

Only in this clade some genera show a high degree of pleonite fusion – *Austrasphaera* entirely lacks any indication of pleonal sutures, in *Margueritta* the pleon and pleotelson join is indicated laterally only, and in *Juletta* the pleon and pleotelson is medially fused.

The only exception to these morphological characters is *Pseudosphaeroma*, which shows none of the *Ischyromene*-clade characters.


**Remarks.** This clade has a predominantly Southern Hemisphere distribution, and most species are small in size (< 5 mm) with the exception of some cold-water taxa such as *Amphoroidea*.

### 3.4.2. *Cerceis* clade

Fig. 8A,B

**Molecular results.** *18S rDNA + 16S rDNA analyses (Figs. 1, 2):* This is a consistent grouping. We were able to sample 10 genera and 14 species out of the 16 genera we hypothesize to be contained in this clade. In all analyses the *Cerceis* clade is always strongly supported (100% bs) and *Dynamene* and *Dynamenoides* are always basal to all other *Cerceis* genera (99% bs).

**18S rDNA analyses (Fig. 8A):** Our analyses contained representatives of 10 genera. 1169 *Paracerceis sculpta* (Holmes, 1904) (Gulf of California, Sonora, Mexico) and 1176 *Paracerceis* Hansen, 1905 (Gulf of California, Baja California Sur) form the sister clade to 1505 *Cassidias* Richardson, 1906 (Singapore) (100% bs). The former taxa together form the sister taxon to 1536 *Paracassidinopsis perlata* (Kenya) (99% bs). In our analyses 1539 *Cerceis* (Queensland) is the sister taxon to 1414 *Pseudocerceis* Harrison & Holdich, 1982b (Queensland) (76% bs). Together they are the sister taxon to 1193 *Haswellia* Miers, 1884 (New South Wales) (100% bs). 1192 + 1550 *Neonaesa rugosa* Harrison & Holdich, 1982b (Queensland) forms a clade with 1483 *N. rugosa* (Fiji), then with 1187 *N. rugosa* (Tanzania) (100% bs). Basal to *Neonaesa* is 1173 *Acrocercis kenynis* Müller, 1995 (Kenya) (99% bs). Basalmost in the *Cerceis* clade is the subclade comprising 1186 + 1544 *Dynamene curaliti* Holdich & Harrison, 1980 (Queensland) and 1530 + 1531 *Dynamenoides decima* Hurley & Jansen, 1977 (New Zealand) as sister taxa (100% bs).

**16S rDNA analyses (Fig. 8B):** As in the *18S* rDNA analyses *Neonaesa* is the sister taxon to *Acrocercis*, however for this gene they are the most derived with *Haswellia* basal to the pair (78% bs). 754 *Cerceis* (Kenya) is the sister taxon to 831 *Cerceis* (Queensland) (100% bs). 1179 *Cerceis pravidolus* is from Singapore and 1539 *Cerceis* from Queensland (100% bs). The genus *Cerceis* is well supported (100%). 1530 + 1531 *Dynamenoides decima* and 1124 *D. vulcanta* Hurley & Jansen, 1977 (New Zealand) form the sister taxon to 1536 *Paracassidinopsis perlata* (Kenya) (75% bs). 810 *Cilicaea latreilli* (Singapore) is a near match to 1133 *P. sculpta* from a marina in Los Angeles, California. 1169 *P. sculpta* is from Sonora, Mexico. 1505 *Cassidias* (Singapore) is the sister taxon to 1534 an undescribed species of *Cas­sidias* (Palau). *Cassidias* is the strongly supported sister taxon of *Paracerceis* (100% bs). Both 1190 *Scutuloidea maculata* Chilton, 1883 and 1533 *Cassidinopsis admirabilis* Hurley & Jansen, 1977 are from New Zealand, and 745 is an undescribed genus from Kenya. Their relationship is not strongly supported and placement of these within the *Cerceis* clade is not well understood.

**Morphological characters.** Pleopod 1 exopod longitudinal axis distinctly oblique to longitudinal axis of endopod, rami not collinear. Pleopod 2 with appendix masculina medial to distal in position. Pleopods 1 and 2 exopod distal margin usually deeply serrate (*) or not serrate (‘). Pleonal sternite usually prominent (i.e., long). Pleopod 1 inferior margin usually with prominent stout robust setae. Female with metamorphosed mouthparts (shared with *Cymodoce* clade). In some genera the anterior margin of the head over-rides the antennula and antenna peduncles.


Remarks. This clade includes a group of fifteen distinctive genera such as Cerceis, Afrocerceis and Haswellia, typified by a greater or lesser degree by its marginal serration on pleopods 1 and 2; the axis of pleopod 1 exopod is strongly oblique to both endopod and peduncle, and the appendix masculina has a medial to distal point of attachment. The epistome of several genera has a medial constriction, in some instances appearing the same as that of the Sphaeroma clade. Females of all genera have metamorphosed mouthparts, a character shared with the Cymodoce clade.

Dynamene Leach, 1814 and Dynamenoides Hurley & Jansen, 1977 clade as sister clade to Cerceis clade – so here they are included in the clade on the basis of pleopod morphology and female mouthparts. Dynamene has metamorphosed mouthparts – characteristic also of Cymodoce and Oxinasphaera.

In the molecular analysis Paracassidinopsis is included in the Cerceis clade, but Paracassidinopsis has few Cerceis clade morphological characters and appears more like an aberrant Dynamenella. Its epistome is not medially constricted, penial process is like the *Dynamenella clade* form, but pleopods are collinear and are otherwise not similar.

Fig. 8. Cerceis. A: 18S rDNA Garli BestTree with MrBayes support values indicated on branches. B: 16S rDNA Garli BestTree with MrBayes support values indicated on branches.
3.4.3. *Dynamenella* clade

Figs. 9A,B, 10

**Molecular results.** 18S rDNA + 16S rDNA analyses (Figs. 1, 2): In all analyses, except a single MrBayes analysis, *Dynoides* is always basalmost in the *Dynamenella* clade, and *Dynamenella* is the most derived and the clade has 95% bs. For the combined analysis we had representatives of the nine genera. Striking is that *Sphaeromopsis* falls into two distinct clades. The genera *Paradella* and *Dynamenella* need to be revisited with regional taxonomy overriding phylogenetic relationships. Specimens 1140 + 1532 identified as *Cymodocella foveolata* from Coquimbo, Chile are always included in this clade. Using GBLOCKS to remove 1841 bp (37%) of the hypervariable regions does not change the outcome.

**18S rDNA analyses** (Fig. 9A): 1145 + 1526 *Dynamenella scaptocephala* Messana, 1990 (Kenya) forms the sister taxon to 1182 + 1509 *Sphaeromopsis amathitis* Holdich & Jones, 1973 (Kenya) (100% bs). Together they form the sister taxon to 1137 *Dynamenella* (Washington State, USA) (63% bs). The three 1148, 1524, and 1525 *Striella* (Baja California Sur, Mexico) specimens form the sister taxon to the long branched 1512 *Sphaeromopsis serriguberna* Holdich & Harrison, 1981 (Queensland) (70% bs). 1147 *Pistorius bidens* Harrison & Holdich, 1982b (Kenya) and 1527 *P. bidens* (Queensland) together form sister clade relationship by a long branch to 1120 + 1542 *Paradella garsonorum* Wetzer & Bruce, 2007 (Baja California Norte and Baja California Sur, respectively) (72% bs). The strongly supported (100% bs) clade 1139 + 1548 + 1549 “? aff. *Heterodina* sp.” (Kenya) forms the sister taxon to 1538 *Paraimene* Javed & Ahmed, 1988 (Kenya), but with low support (58% bs). Our “? aff. *Heterodina* sp.” is an undescribed genus that differs from *Heterodina* by having a small, anteriorly acute epistome that does not extend anteriorly, not visible dorsally, and with large flattened antenna peduncle. Specimens are small and there are no males in this sample. 1181 and 1189 *Dynamenella ptychura* (Queensland) are always sister specimens, but their exact position in the clade is not definitive as in different analyses they have a tendency to move within the *Dynamenella* clade (not shown). Specimens 1185, 1497, and 1498 *Sphaeromopsis* (Ecuador) always form a clade together and never with the specimens identified as *Sphaeromopsis* from Queensland or Kenya.

1198 *Beatricephaera ruthae* Wetzer & Bruce, 1999 (Atlantic, Costa Rica) in this analysis is the sister taxon to 1140 + 1532 *Cymodocella foveolata* (Coquimbo, Chile). As already noted under the 18S rDNA and 16S rDNA combined analyses above, these animals never group with *I. huttoni* from Darwin Channel, Chile or *I. cordiforaminalis* (New Zealand), yet in all of the 16S rDNA analyses (discussed in *Ischyromene*) they always belong to the *Ischyromene* clade. *B. ruthae* collected in 1986 and preserved in 70% ethanol are by far the oldest specimens in our dataset. Multiple extractions and sequencing attempts finally did yield a complete, high-quality sequence for both genes, but with long branches and tendency to move around in the clade. 972 *Cassidinidea* (Dreyer & Wägele Genbank AF255693, collecting locality not indicated) and 1178 *C. ovalis* (South Carolina, USA) form a sister taxon to 1172 *Thermosphaeroma subequaleum* Cole & Bane, 1978 (Texas, USA).

In all 18S rDNA analyses 1149 + 1150 *Dynoides* (California, USA) (not shown) have a long branch, are basal to *Dynamenella*, but in some analyses appear in unlikely pairings with other long-branched taxa, e.g., *Plakarthrium typicum*.

16S rDNA analyses (Figs. 9B, 10): *Dynamenella* and *Paradella* are the most derived genera in the clade and nodes are mostly well supported. 411 *Paradella* (specimen gift from S. Shuster, locality unknown) is the sister taxon to 797 *Dynamenella* (Singapore) (95% bs). Together these are the sister taxon to 1542 *P. garsonorum* (Baja California Norte, Mexico) (100% bs). The sister clade 783 + 1120 *P. garsonorum* (Baja California Sur) is well supported (100% bs). 410 *P. dianae* (Menzies, 1962) (S. Shuster, locality unknown) forms the sister clade (90% bs). 733, 738, and 746 *P. harrisoni* Müller, 1995 are all from Kenya. 1181 + 1189 + 1540 *D. ptychura* Harrison & Holdich, 1982 (Queensland) together form the sister taxon to 1148 + 1524 + 1525 *Striella* (Baja California Sur) (79% bs).

1185 + 1497 + 1498 *Sphaeromopsis* (Ecuador) form the sister taxon to the *Dynamenella*, *Paradella*, *Striella*. As in the 18S rDNA alone and the 18S rDNA + 16S DNA combined analyses, the Ecuadoran *Sphaeromopsis* are more closely related to *Dynamenella ptychura* (Queensland) than *Sphaeromopsis* from Kenya and Tanzania. The African *Sphaeromopsis* [729, 741, 748, 768, 1182, 1509] are more closely allied to *D. scaptocephala* [753, 1145, 1526] also from Kenya (94% bs). As presently identified *Sphaeromopsis* is not monophyletic. These are likely two distinct and separate genera in need of reassignment. The sister taxon to the African *Sphaero­mopsis* and *D. scaptocephala* is 1538 *Paraimene*, also from Kenya (100% bs).

*Thermosphaeroma* with eight species occurs in freshwater springs in the southwest U.S. and northern and central Mexico. It is endemic to thermal springs in Texas and New Mexico, as well as in Chihuahua, Durango, Coahuila and Aguascalientes, Mexico. The genus has interesting North American post-Cretaceous biogeographic implications with diversification during receding ocean levels. The genus is supported with 100% bs.

775 *Paraimene tumulus* (Atlantic, Costa Rica) is not closely related to 1538 *Paraimene* from Africa.

In our 16S rDNA analyses *Cassidinidea* ovalis pairs with *I. huttoni* (Canal Darwin, Chile) in the *Ischyromene* clade rather than as expected within the *Dynamenella* clade. Here again we believe the resolution of the 16S rDNA marker may be misleading and inaccurate at this taxonomic level. Based on morphology, 18S rDNA and the combined 18S rDNA + 16S rDNA markers *Cassidinidea* clearly belongs with members of the *Dynamenella* clade.
Also in the 16S rDNA analyses the species level structure of *Dynoides* (Fig. 10) is always strongly supported, but deeper node resolution is lost (not shown). 1150 + 1149 *Dynoides elegans* (Southern California, USA) is the sister taxon to 1171 *Dynoides* (Baja California Sur, Mexico). 1508 *D. daguilarensis* (Hong Kong) is the sister taxon to 1514 *D. sheareri* – specimens identified as *Dynamenella sheareri* (San Juan Island, Washington, USA). The later with a long branch. Based on these analyses 1514 D. sheareri would be attributed to *Dynoides* rather than *Dynamenella*.

*Beatricesphaera* is always in or near the *Dynamenella* clade in all 18S rDNA and some 16S rDNA analyses. In 16S rDNA analyses 741 ? aff. *Heterodina* is not included in the *Dynamenella* clade, but rather is sometimes the sister taxon to *Amphoridae* (*Ischyromene* clade). Trees not shown.

**Morphological characters.** Pleopod 1 endopod has a characteristic shape, endopod oblique, apex acute, disstolateral margin concave, posteromesial angle projecting (‘heel’) *Beatricesphaera, Cassidinidea, Dynoides, Thermosphaeroma*. Pleopod 1 peduncle mesial margin forming narrowly produced lobe in some genera [* = present]. Epistome long but lacking medial constriction. Penial processes basally adjacent (i.e., not fused) or basally, partially or entirely fused. Pleonal sutures to posterior margin (pleonites occasionally wholly fused).


**Remarks.** In contrast to the *Ischyromene* and *Cercois* clades, few characters are consistently present throughout the included genera. *Dynamenella* and related genera have a typical pleopod 1 and pleopod 2 morphology, and the penial processes are always mutually adjacent, and often fused to various degrees from basally to entirely fused including the fused vasa deferentia. Many genera have a perforate pleotelson. The genus *Sphaeromopsis* is superficially similar to the Sphaeromatinae but the morphology of the penial processes and pleopods 1 and 2 shows clear *Dynamenella* clade characteristics. *Thermosphaeroma* appears morphologically more closely related to genera such as *Bilistra*.

### 3.4.4. *Campecopea*

**Molecular results.** 18S rDNA + 16S rDNA analyses (Figs. 1, 2): In our analyses *Campecopea* is the sister taxon to the remaining clade 3 taxa. *Campecopea* was represented by a single species [*C. hirsuta* (Montagu, 1804)]. 18S rDNA of 1044 *C. hirsuta* had been previously generated by D. Bouchon (GenBank AJ388076) and was paired with 16S rDNA 990 *C. hirsuta* (GenBank AF279601). 1170 C. hirsuta sequences are from a Canary Island specimen. This strongly supported pair in these combined analyses is always basal to the *Dynamenella* clade. The genus presently contains five species and *C. hirsuta* is the type species of genus.

18S rDNA analyses (not shown): In these analyses *Campecopea* is still basal but with much weaker support. 990 + 1170 C. hirsuta is the sister taxon to 991 *C. lusitanica* (GenBank AF279602, previously generated by H. Dreyer).

16S rDNA analyses (not shown): Here *Campecopea* clades with other long-branch taxa in biologically inexplicable relationships, but not unexpected based on characters of this genetic marker.


**Remarks.** *BRUCE & HOLDICHI* (2002) in their revision of *Campecopea* concluded that the genus was incerta sedis. The present analyses place the genus as the sister clade to the *Dynamenella* clade, supporting its position apart from other sphaeromatid genera.

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**Fig. 9. Dynamenella.** A: 18S rDNA Garli BestTree with MrBayes support values indicated on branches. B: 16S rDNA Garli BestTree with MrBayes support values indicated on branches.
3.5. Sphaeromatidae clades incertae sedis

A further four “groups” of genera are based only on morphological characters. These groups lacked any representation in the molecular analysis, but the morphological characters that unite each group are unlikely to be homoplasious (e.g., reflexed and robust antennula articles in Moruloidea group; maxillipeds article 2 and lateral head margins expanded in Cassidina group). The phylogenetic validity of these “groups” remains to be tested by either morphological or molecular analyses.

3.5.1. Cassidina group

Molecular results. Our 18S rDNA 1488 Paracassidina dama Bruce, 1994 sequence is basal to the Gnorimosphaeroma clade in all analyses (not shown). This is not unexpected since we had no other representatives for the group and including other genera will most likely result in a different topology. There was no 16S rDNA sequence available and hence the genus was not included in the combined analyses.

Morphological characters. Body margins expressed laterally (not ventrally) giving an overall flat appearance. Head lateral margins expanded, not set into pereonite 1. Epistome visible in dorsal view. Pereonites with coxal extensions. Pleonal sternite prominent. Pleon of 3 segments, laterally unfused. Antennula peduncle articles 1 and 2 flattened. Maxillipeds 2 expanded, articles 3 and 4 finger-like medial lobes, article 5 elongate. Pereopods modified, with 1 or more elongate articles and elongate dactylus; accessory unguis slender. Pleopods 1 modified, with 1 or more elongate articles and elongate dactylus; accessory unguis slender. Pereopod 1 rami subequal in width, distally rounded, usually collinear (from BRUCE 1994).

Genera included. Agostodina Bruce, 1994; Cassidina Milne Edwards, 1840; Chitonopsis Whitelegge, 1902; Paracassidina Baker, 1911.

Remarks. These genera, together with the next “group” are at the core of the former “Cassidinae”.

3.5.2. Leptosphaeroma group

Molecular results. Only 16S rDNA for Platysphaera was available.


Genera included. Chitonosphaera Kussakin & Malyshtina, 1993; Discidina Bruce, 1994; Leptosphaeroma Hilgendorf, 1885; Paraleptosphaeroma Buss & Iverson, 1981; Platysphaera Holdich & Harrison, 1981.

Remarks. All are strongly flattened genera, sharing few uniting characters with the “Cassidina-group” (see above).

3.5.3. Moruloidea group

Molecular results. No species were sequenced for this group.

Morphological characters. Antenna with peduncular articles 4 and 5 robust, reflexed. Male pereopod 1 propodus with inferoproximal lobe (= ‘heel’). Uropodal exopod reduced. Rugose and heavily calcified dorsum. Fold ‘purse-like’ at pereonite 5, with coxae 5 overlapping both anteriorly and posteriorly.


Remarks. Pleopods are of the Sphaeroma clade form as is the setation of the pleopods. Apart from Moruloidea and Caecocassidias Kussakin, 1967 (see BRANDT 1998) all genera can be satisfactorily placed in the Sphaeroma clade genera on morphological criteria. At least one species, Cymodopsis beageli Brandt, 1998 may belong to this group though it lacks the reflexed antenna peduncle. It is plausible that Kranosphaera Bruce, 1992 also belongs with this group. Characters for Waiteolana Baker, 1926 are unclear; maxilliped palp not as produced as in Cymodoce clade s.str, but pleopod morphology is as in clade 2 (Sphaeromatinae).

3.5.4. Monolistra group

Molecular Results. No species were sequenced for this group.

Morphological characters. This is probably a monophyletic clade, as all have similar pleopod, antennule, and antenna morphology.

Genera included. Caecospheroma Dollfus, 1896; Monolistra Gerstaecker, 1856; Merozoon Sket, 2012.

Remarks. This group comprises three aquatic cave-dwelling genera, Caecospheroma in France, Monolistra and Merozoon in central Europe (SKET 1986). Monolistra, a large genus of about 20 species and many subspecies, has been split into five subgenera (see SKET 1986). The affinities of these genera in relation to marine genera remain unapprised, though it is likely that the genera form a monophyletic group.
3.6. Sphaeromatidae genera incertae sedis

The genera listed below cannot reliably be placed in the proposed groupings and are considered incertae sedis. Genera in normal type face are morphologically reasonably well described and their placement is hypothesized. Bold-faced genera lack unambiguous morphological characters to place them. For all these genera no specimens were available for molecular analysis. Genera are listed alphabetically.

*Artopoles* Barnard, 1920: the only ‘clue’ is that pleopod 1 is generally similar to those of *Dynamenella* clade (Bruce 2001). Pereopod morphology is unique.

*Botryias* Richardson, 1910: No data – minimally described; remains unplaced.

*Cassidinella* Whitelegge, 1901: Good data (see Bruce 1994a) but still not clear to which genus this genus belongs; probably in the *Sphaeroma* clade s.l.

*Cœcosphaera* Dollfus, 1896: Closely related to *Monolistra*.

*Cercosphaera* Bruce, 1994c: Has metamorphosed females, but shares few other characteristics; pereopod setation also fits with *Cymodoce*.

*Cymodetta* Bowman & Kühne, 1974: There are no obvious character links; likeliest and most similar is the *Dynamenella* clade.

*Cymodopsis* Baker, 1926: The status of the poorly known *Cymodopsis* Baker, 1926 is unclear, and the genus is not monophyletic. The type species remains effectively undescribed, and several species are inappropriate placed in the genus.

*Hemisphaeroma* Hansen, 1905: Genus needs detailed redescription. At present its affinities are entirely uncertain.

*Monolistra* Gerstaecker, 1886: Probably monophyletic, all with similar pleopod and antennula and antenna morphology; all are European cave taxa

*Naesicopea* Stebbing, 1893: Few data; probably in with the *Cymodoce* group.

*Syncassidina* Baker, 1928: Possibly belongs in with the *Dynamenella* clade.

*Xynosphaera* Bruce, 1994c: A commensal or parasite of soft corals; no real morphological clues to where it belongs except *Ischyromene* and *Cereis* groups can be excluded – i.e., *Sphaeroma* clade in the broadest sense.

4. Conclusions

Sphaeromatid isopods are ideal for addressing life history, ecology, biogeography, and phylogenetic hypotheses, because: they exhibit extreme morphological diversity; they are readily collectable in many habitats; some genera are speciose, while others are monotypic; and described genera include biogeographically widespread as well as regionally endemic species. However, we demonstrate here that many (perhaps most) of the large genera are not monophyletic.


Variation in brood pouch morphology among females (Harrison 194b) and extreme sexual dimorphism among males (wide variation in uropod shape, size and ornamentation; cephalic shape, size and ornamentation; and variation in body size and growth rate; Hurley & Jansen 1977; Shuster 1987, 1992) make sphaeromatids excellent organisms for studying mating system evolution. However, this research direction (and similar comparative questions) can only be pursued with a robust phylogeny in hand.

With the exception of *Gnorimosphaeroma* clade 1) the family divides into two large clades, these corresponding to the Sphaeromatinae (clade 2) and the Dynameninae (clade 3). The morphological support for these two clades is the presence of thickened transverse ridges on both rami of pleopods 4 and 5 (Dynameninae) or the endopod only (Sphaeromatinae). These characters are consistent, but also repeatedly lost within genera and also in those taxa occupying freshwater, and to a lesser degree, estuarine habitats.

Our major clades supported by molecular data are recognizable morphologically. The concept of the subfamily Cassidininae (flat bodied, ‘simple’ pleopods, flat uropods with reduced exopod and whatever else has been used over the years) is not upheld, but unfortunately we have few sequences for these taxa (the genera and species are mostly sub-tidal benthic, and some didn’t yield molecular sequences). Evaluation, description and redescription of morphological characters including brood pouch characters will go a long way in aiding taxonomists in identifying this beautifully diverse group of crustaceans.

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6. References


BROUCE N.L. 1993. Two new genera of marine isopod crustaceans (Flabellifera: Sphaeromatidae) from southern Australia, with a reappraisal of the Sphaeromatidae. – Invertebrate Taxonomy 7: 151 – 171.


HARRISON K. 1994a. Some sphaeromatid isopods (Crustacea) from southern and south-western Australia, with description of a new
genus and two new species. – Records of the Western Australian Museum 11: 259 – 286.


7. Appendix: Sphaeromatidae genera list

Currently recognized Sphaeromatidae genera list containing recognizable clades. The World Register of Marine Species (http://www.marinespecies.org on 2017-05-05) currently records 100 genera in the family. Genera marked with * denote that at a minimum of one species and one individual is represented in our dataset. In most instances multiple species and multiple individuals were sequenced, and whenever possible for both genes. Based on our molecular phylogeny, genera are organized into monophyletic clades and these are numbered (see Figs. 1, 2). At present it is possible to place 76 of the 100 (76%) genera. Obvious morphological characters and other annotations augment the list. Groups missing from our analysis include representatives of the Moruloidea group, Cassidina group, Leptosphaeroma group (and the randoms) about another 13 species.

Clade 1 – Gnorimosphaeroma

1 *Gnorimosphaeroma Menzies, 1954

Clade 2 – Sphaeromatinae

Sphaeroma clade
2 *Benthosphera Bruce, 1994
3 *Bilistra Sket & Bruce, 2004
4 *Lekanesphaera Verhoeff, 1943
5 *Sphaeroma Bosc, 1801

Neosphaeroma clade
6 *Neosphaeroma Baker, 1926

Cymodoce clade
7 Bregmotrema Bruce, 1994
8 Calicippia Harrison & Holdich, 1984
9 Ceratocephalus Woodward, 1877
10 Cercosphaera Bruce, 1994
11 *Ciliaeae Leach, 1818
12 *Cilicaeopsis Hansen, 1905
13 *Cymodoce Leach, 1814
14 *aff. Cymodopsis (Kenya; an undescribed species, possible new genus. See Cymodopsis under incertae sedis genera (intentionally not numbered).
15 *Harrieta Kelsney, 1987
16 Korenasphaera Bruce, 2003
17 Kranaosphaera Bruce, 1992
18 *Oxinasphaera Bruce, 1997
19 *Paracilicaeae Stebbing 1910b
20 Parasphaeroma Stebbing 1902
21 Porosreode Bruce, 2009

Exosphaeroma clade
22 *Apemosphera Bruce, 1994
23 *Exosphaeroides Holdich & Harrison, 1983
24 *Exosphaeroma Stebbing, 1900
25 *Isocladus Miers, 1876
26 *Parisoiscladus Barnard, 1914
27 Pysphaeroma Holdich & Harrison, 1983
28 *Sphaeramene Barnard, 1914
29 Stathmos Barnard, 1940
30 *Zaza Leach, 1818

Clade 3 – Dynameninae

Campecopea clade
31 *Campecopea Leach, 1814 – sister clade to all Dynameninae.

Ischyromene clade
32 *Amphoroidea H. Milne Edwards, 1840
33 Amorpho Milea Baker, 1908
34 Austrasphaera Bruce, 2003
35 Cassidinopsis Hansen, 1905
36 *Cymocella Pfeffer, 1887
37 Dicilicella Bruce, 1995
38 *Dynamenopsis Baker, 1908
39 *Ischyromene Racevitra, 1908
40 Juleto Bruce, 1993
41 Marguerita Bruce, 1993
42 Maricoccus Poore, 1994
43 Pedinura Bruce, 2003
44 *Pseudosphaeroma Chilton, 1909
45 *Scutuloida Chilton, 1883

Cerceis clade – comprising two clades: Dynamene + Cerceis
46 *Afrocerceis Muller, 1995
47 *Cassidias Richardson 1906
48 *Cerceis Milne Edwards, 1840
49 Discerceis Richardson, 1905
50 *Dynamene Leach, 1814
51 *Dynamenoides Hurley & Jansen, 1977
52 Eterocercaes Messana, 1990
53 Exocercaes Baker, 1926
54 Geoceceis Menzies & Glynn, 1968
55 *Haswelia Miers, 1884
56 Holotelson Richardson, 1909
57 *Neonaesa Harrison & Holdich, 1982
58 *Paracassidinopsis Nobili, 1906
59 *Pararceceis Hansen, 1905
60 Platycercaes Baker, 1926
61 Platynympha Harrison, 1984
62 Pseudocercaes Harrison & Holdich, 1982

Dynamenella clade – comprising two clades: Dynoides + Dynamenella
63 *Beatricesphaera Wetzer & Bruce, 1999
64 *Cassidinedia Hansen, 1905
65 Chiamenella Kussakin & Malyutina, 1987
66 *Dynamenella Hansen, 1905
67 *Dynoides Barnard, 1914
68 *Heterodina Scholle & Kelsney, 2005
69 Makarasphaera Bruce, 2005
Incertae sedis genera

Three morphologically defined groups lacked molecular representation and at present cannot be associated with any of our clades. The remaining genera lack descriptive data or do not show any clear morphological indication as to where they belong in the schema presented here. The Cassidina group and the Leptosphaeroma group were defined by Bruce (1994).

Cassidina group
77 Agostodina Bruce, 1994
78 Cassidina H. Milne Edwards, 1840
79 Chitonopsis Whitelegge, 1902
80 Paracassidina Baker, 1911

Leptosphaeroma group
81 Chitonosphaera Kussakin & Malyutina, 1993
82 Discidina Bruce, 1994
83 Leptosphaeroma Hilgendorf, 1885
84 Para leptosphaeroma Buss & Iverson, 1981
85 Platysphaera Holdich & Harrison, 1981

Moruloidea group
86 Caecocassidias Kussakin, 1967
87 Moruloidea Baker, 1908
88 Waiteolana Baker, 1926

Monilistra group
89 Caecosphaeroma Dollfus, 1896
90 Monilistra Gerstaecker, 1856
91 Merozoon Sket, 2012. The description of the genus (and species) is based on a fragment so affinities cannot be assessed other than one in this group of genera.

The following genera cannot be reliably placed in the proposed groupings and are considered incertae sedis. Genera in normal type face are morphologically reasonably well described and their placement is hypothesized. Bold-faced genera lack unambiguous morphological characters to place them. For the genera below no specimens were available for molecular analysis.

92 Artopoles Barnard, 1920 – the only ‘clue’ is that pleopod 1 is generally similar to those of Dynamenella clade. Pereopod morphology is unique.
93 Botryias Richardson, 1910 – minimal morphological data; remains unplaced.
94 Cassadinella Whitelegge, 1901 – good data, but still not clear what its phylogenetic affiliation is; probably in the Sphaeromatinae sensu latu, but not confident.
95 Cymodetta Bowman & Kuhne, 1974 – descriptions are fine, just no obvious character links; likeliest and most similar is the Dynamenella clade.
96 Cymodopsis Baker, 1926 – the status of this poorly known genus (with eight species) is unclear. As presently constituted the genus is not monophyletic. The type species remains effectively undescribed, and several species are inappropriately placed in the genus.
97 Hemisphaeroma Hansen, 1905 – lack of descriptive data for this monotypic freshwater genus precludes assessment of its affinities.
98 Naesicopea Stebbing, 1893 – few data; some specimens held at Muséum national d’Histoire naturelle (Paris, NLB personal observation); probably in with the Cymodoce group.
99 Syn cassidina Baker, 1928 – to Dynamenella clade?
100 Xynosphaera Bruce, 1994c – commensal or parasite of soft corals; no real morphological clues to where it belongs except Ischyromene and Cerceis groups can be excluded – i.e., excluded from the ‘Sphaeromatinae’ in the broad sense.

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics

File 1: wetzer&al-sphaeromatidaephylogeny-asp2018-electronic supplement-1.xls — Sequences from GenBank and new sequences generated by this project and used in analyses.