Phylogenetic assessment and systematic revision of the acoel family Isodiametridae

SARAH ATHERTON1 and ULF JONDELIUS FLs1,2,*

1Department of Zoology, Naturhistoriska riksmuseet, Stockholm, Sweden
2Department of Zoology, Systematics and Evolution, Stockholm Universitet, Stockholm, Sweden

Received 26 February 2021; revised 7 June 2021; accepted for publication 28 June 2021

Isodiametridae is a large family of Acoela with 22 nominal genera and nearly 100 species. Unfortunately, systematics of Isodiametridae, as it stands, is highly problematic. Genera frequently have been proposed without reference to an explicit phylogenetic hypothesis, such that the current classification system holds little or no predictive power. Many taxa do not fit with the family diagnosis, and it is increasingly difficult to determine in which taxon a new species should be described. Herein, we reconstruct the phylogenetic relationships of Acoela with a focus on Isodiametridae using both previously published and new ribosomal and mitochondrial sequence data. Our dataset comprises sequences from 45 species representing 16 of the 22 isodiametrid genera. Our results recovered a well-supported Isodiametridae, but provided further evidence that the family and several genera within require revision. We have updated the classification system of Isodiametridae to be consistent with its phylogeny, including the transference of Otocelis to Otocelididae, Postaphanostoma and Faerlea to Mecynostomidae and Alluna to Actinoposthiidae. Six other genera are placed in synonymy. We review the morphological taxonomy and provide an identification key of the genera in the revised family.


INTRODUCTION

Acoela comprises approximately 450 nominal species of marine worms found primarily in sediments or among algae all over the world. Through the 20th century, acoels were placed in Platyhelminthes (e.g. Ehlers, 1985), but more recent phylogenetic analyses of nucleotide sequences reject this position (e.g. Cannon et al., 2016; Rouse et al., 2016). Currently, Acoela together with Nemertodermatida and Xenoturbellida form the group Xenacoelomorpha, separate from Platyhelminthes. The phylogenetic position of Xenacoelomorpha is contentious, but the current mainstream view is that they form the sister-group of the Nephrozoa (Ruiz-Trillo et al., 1999; Egger et al., 2004; Cannon et al., 2016; Rouse et al., 2016), although they have also been considered closely related to ambulacrarians (Philippe et al., 2011).

Acoels can be difficult to study since they are small (~0.2–5.0 mm), often fragile and have comparatively few morphological characters that, nevertheless, can be extremely variable (Jondelius et al., 2011). Early classification systems reflect the challenges in working with such animals, and numerous, sometimes conflicting, taxonomical changes were frequently proposed without reference to a phylogenetic hypothesis (e.g. Graff, 1912; Luther, 1912; Westblad, 1948; Faubel, 1974; Kostenko, 1989). Dörjes (1968) revised acoel classification based in large part on characters of the relatively complex hermaphroditic reproductive system and proposed a large number of new taxa at the generic, family and species levels. Modifications to Dörjes’s system were based on the structure of the nervous system (e.g. Raikova et al., 1998, 2004), sperm ultrastructure (Hendelberg, 1977; Raikova et al., 2001), body wall musculature (Hooge, 2001, Tekle et al., 2005) and on the musculature of the copulatory organs (Hooge & Tyler, 2005).

Analyses of nucleotide sequences have generated testable phylogenetic hypotheses that necessitated revisions of acoel classification (e.g. Hooge et al., 2002; Raikova et al., 2004; Hooge & Tyler, 2005). The hitherto largest study of acoel phylogeny combined data from two nuclear genes and one mitochondrial

*Corresponding author: E-mail: ulf.jondelius@nrm.se
[Version of record, published online 1 October 2021; http://zoobank.org/urn:lsid:zoobank.org:pub:9D60E284-31D3-4FBB-B6F1-88AB5AC91EAE]
gene with 37 multistate morphological characters to assess the relationships and morphological evolution of 126 different acoel species, approximately one-third of the species known at the time, and to generate a classification consistent with the phylogenetic hypothesis (Jondelius et al., 2011). The resulting classification system included a total of 21 families, with several (e.g. Convolutidae Graff, 1905; Hofsteniidae Bock, 1923; Paratomellidae Dörjes, 1966; and Solenofilomorphidae Dörjes, 1968) considered well-resolved and with clear morphological diagnoses, although the positions, interrelationships and morphology of others (e.g. Actinoposthiidae Hooge, 2001 and Otocelididae Westblad, 1948) were still ambiguous.

Isodiametridae Hooge & Tyler, 2005 was proposed by Hooge & Tyler (2005) to accommodate ‘small convolutids’: acoel species with 9 + 2 sperm axonemes and lacking symbiotic algae that were previously classified in Convolutidae. This was in response to findings by Hooge et al. (2002) and Petrov et al. (2004) that nine species of ‘large convolutids’ with 9 + 0 axonemes and symbiotic algae form a monophyletic group separate from another nine species of ‘small convolutids’ from three nominal genera in their analysis of 185 rDNA sequences. In addition, Hooge & Tyler (2005) studied the musculature of the male copulatory organ of 17 species of ‘small-bodied convolutids’ from seven nominal genera and determined that these specimens all possessed a seminal vesicle that encloses a tubular isodiametric penis with inner circular muscle fibres and outer non-anastomosing longitudinal fibres.

Hooge & Tyler (2005) decided to reclassify all ‘small convolutids’ in the new family Isodiametridae, including species that had not been included in the 18S rDNA gene tree or studied in detail with regards to the male copulatory organ. As a consequence, Isodiametridae is currently a large family comprising 22 nominal genera and nearly 100 species, but many taxa do not fit the family diagnosis provided by Hooge & Tyler (2005). For instance, a muscular seminal vesicle is absent from species of Alluna Faubel & Rieger, 1983, Proconvoluta Dörjes, 1968 and Pseudoposthia Westblad, 1946, while the male copulatory organ is a simple inpocketing of the epidermis in the genera Aragina Leiper, 1902 and Prophanostoma Dörjes, 1972.

While results from the molecular analyses of Jondelius et al. (2011) corroborated the monophyly of Isodiametridae with high support, relationships within the family remained largely unresolved. Generic classification within Isodiametridae is problematic, because genera have been introduced without reference to an explicit phylogenetic hypothesis, often on the basis of a single morphological character (e.g. Ancylocirrus Kozloff, 2000; Raphidophallus Kozloff, 1965; Rimicola Bohmig, 1908), without actually assessing the prevalence of that character in other acoel species (e.g. Bohmig, 1908; Kozloff, 1965, 2000; Dörjes, 1968). The result is a confused classification system rife with monotypic genera, with little or no predictive power (Jondelius et al., 2011) and with which it is difficult to determine into which taxon a new species should be referred.

Herein, we reconstruct the phylogenetic relationships of Isodiametridae and closely related groups within Acoela using both previously published and new ribosomal and mitochondrial sequence data. Our dataset comprises sequences from 45 species representing 16 of the 22 isodiametrid genera. We aim to provide a robust classificatory backbone for future work. Thus, our dataset did not include any unidentified or undescribed species. We update the classification of the family to be consistent with our phylogenetic hypothesis, review the morphological taxonomy of the genera and provide an identification key to these genera. Our goal is to clarify and simplify the taxonomy to facilitate future research into Isodiametridae diversity.

MATERIAL AND METHODS

New specimens were collected over a period of 21 years from a variety of marine sediments and aquatic vegetation (Supporting Information, Table S1). Specimens were collected through multiple methods, including by hand from the beach at low tide, via SCUBA or snorkelling or by dredging at lower depths. Samples were transported back to laboratories and processed according to type. Animals were extracted from marine sandy or mixed sediments following the anesthetization-decantation protocols of Martens (1984) and from mud or finer/silt sediments via the siphoning method detailed in Holovachov et al. (2017). Vegetation was simply washed through a fine mesh sieve.

Following extraction, animals were manually isolated and identified using a compound microscope equipped with DIC (differential interference contrast). When possible, images and digital video were captured and uploaded to the Acoela Scratchpad project (https://acoela.myspecies.info/en). Measurements were taken with an ocular micrometre. Following documentation, specimens were directly fixed in 96% ethanol, RNASHield (Zymo Research) or Histochoice (Sigma-Aldrich) for later processing.

For histological sections, specimens fixed in Histochoice were washed in PBS, dehydrated in an ethanol series, embedded in paraffin and finally serially sectioned in 4–6 µm slices using an LKB
Bromma 2219 Historange microtome. Sections were stained with haematoxylin and eosin as counter stain following the protocols of Aescht et al. (2010).

For molecular analyses, DNA was extracted from whole animals using the DNeasy Blood & Tissue kit (Qiagen) following the instructions of the manufacturer. Polymerase chain reaction (PCR) amplification was performed using 0.2 mL PuReTaq Ready-To-Go PCR beads (GE Healthcare) with 2 µL DNA and 5 pmol each of forward and reverse primers. Products were viewed on 1% agarose gel and then purified with ExoSAP-IT enzymes (Exonuclease and Shrimp Alkaline Phosphatase; GE Healthcare). Sequencing was performed commercially at Macrogen and Shrimp Alkaline Phosphatase; GE Healthcare).

Products were viewed on 1% agarose gel and then DNA and 5 pmol each of forward and reverse primers.

The complete nuclear 18S rRNA and 28S rRNA genes and the ~600 bp ‘Folmer’ segment (Folmer Europe (Netherlands). Sequencing was performed commercially at Macrogen and Shrimp Alkaline Phosphatase; GE Healthcare).

The complete nuclear 18S rRNA and 28S rRNA genes and the ~600 bp ‘Folmer’ segment (Folmer et al., 1994) of the mitochondrial cytochrome c oxidase subunit I (COI) gene were selected as molecular markers for phylogenetic analyses following the protocols of Jondelius et al. (2011). These three gene regions are the most widely available molecular markers for acoels in GenBank and have been shown to be useful in resolving the phylogenetic relationships for Acoela (Hooge et al., 2002; Jondelius & Raikova, 2002; Hooge & Tyler, 2005; Jondelius et al., 2011) and numerous other meiofaunal groups (e.g. Kieneke & Nikoukar, 2017; Zhao et al., 2018; Atherton & Jondelius, 2019).

New DNA sequences were combined with the dataset of Jondelius et al. (2011) and acoel sequences available from GenBank. To ensure accuracy, sequences representing undescribed or uncertain species of acoels were excluded from the database. Further, analyses were run both including and excluding sequences from six species of Isodiametridae and Actinoposthidae (Supporting Information, Table S1) that were represented only by 18S sequences downloaded from GenBank and that grouped together in a clade with a highly unstable position in the analyses of Jondelius et al. (2011). Overall, the final analyses included sequences from a total of 128 different acoel species, with 47 species from 16 of the 22 genera of Isodiametridae represented. Table S1 in the Supporting Information lists the collection information and GenBank accession numbers of all specimens used in this study. Because Diopisthiporidae Westblad, 1940 is the sister-taxon to all other acoels (Jondelius et al., 2011), sequences from two specimens, Diopisthopus longitubus Westblad, 1940 and Diopisthuspsammophilus Dörjes, 1968, were defined as outgroups.

Sequences were aligned with Multiple Alignment using Fast Fourier Transformation (MAFFT; Katoh & Toh, 2008). COI sequences were translated to amino acids using the standard invertebrate mitochondrial genetic code, manually checked for stop codons and reading frame shifts, aligned and reverted back to the original nucleotides. As acoels are known to have unusually variable ribosomal sequences compared with most metazoans (Ruiz-Trillo et al., 1999), 18S and 28S alignments were filtered with GBLOCKS, available online through the CSIC-UPF webserver (http://molevol.cmima.es/castresana/Gblocks_server.html), allowing for gap positions within the final blocks, although all analyses were performed using both non-filtered and filtered alignments. Maximum likelihood (ML) analysis was performed on each marker individually and with concatenated datasets in IQTree v.1.6.12 (Trifinopoulos et al., 2016; https://www.iqtrees.cibiv.univie.ac.at) with 1000 ultrafast bootstrap replicates. The best-fitting substitution models were determined using the ModelFinder Algorithm via BIC, with the GTR+G+I+G4 model selected for all gene datasets, excepting the 18S dataset filtered with GBLOCKS, for which the GTR+I+F+R4 model was selected.

In order to examine the pertinence of morphological characters typically utilized to delimit genera, 21 morphological characters considered in the past (e.g. Hooge & Tyler, 2005) to be taxonomically important were coded for the 32 species positioned within the Isodiametridae clade of our molecular phylogeny, as well as nine additional outgroup species, and mapped onto the concatenated tree (Supporting Information, Table S1). The coding method followed that of Hooge & Tyler (2005). The morphological characters primarily comprised details of the reproductive system, although some other general morphology, such as body size and presence of pigmented eyespots, were also considered. Characters were coded following original descriptions, illustrations, photos and live observations of each species, whenever possible. The outgroup species were selected from diverse positions across the closest sister-clade of Isodiametridae (see Results; Fig. 1).

RESULTS

Results from the phylogenetic analyses can be found in Figure 1 and Figures S1–S8 in the Supporting Information. Tree topologies were consistent across individual genes and concatenated analyses regardless of whether or not datasets were filtered with GBLOCKS, and the deeper nodes were generally recovered with high support in the 18S and 28S gene trees and in the concatenated analyses. Much lower support occurred for the deep nodes of the COI gene tree, although this was expected given that the COI gene is known to be highly variable and thus less suited to assessing distant relationships (Sanna et al., 2009; Tang et al., 2012).

Figure 1 summarizes the concatenated phylogeny. Results were overall consistent with the previous
findings of Jondelius et al. (2011). Mecynostomidae Dörjes, 1968 and Convolutidae form a clade with high support. Dakuidae Hooge, 2003 is recovered, although it does not include Notocelis Dörjes, 1968, and indeed the relationships between Dakuidae, Notocelis gullmarensis (Westblad, 1946), Mecynostomidae + Convolutidae and

Figure 1. Concatenated 28S, 18S and COI gene tree summary. Percent bootstrap values are given at each node. Sequences from six species of Isodiametridae and Actinoposthiidae represented only by 18S sequences downloaded from GenBank (Table S1) were excluded.
a clade consisting of three species of *Otocelis* Diesing, 1862 (*Otocelidiae*) are all unresolved. Outside this polytomy, Proporidae Graff, 1882 and Isodiametridae are both resolved; *Hallangia proporoides* Westblad, 1946 (*Hallangiidae Westblad*, 1946) is sister to a Solenofilomorphidae clade + Hofsteniidae clade; and Paratomellidae is sister to the remaining Bitesticulata.

Isodiametridae is recovered in all analyses, although the support varied somewhat depending on whether six species that were difficult to place in Isodiametridae/Actinoposthiidae (Supporting Information, Table S1) are included in the analyses. Each of these species is represented only by 18S sequences downloaded from GenBank. Jondelius et al. (2011) found that the six species formed part of a clade with a highly unstable position that grouped either within or outside Isodiametridae. When included in our analyses, they form a clade within Isodiametridae, but the overall support for Isodiametridae is low. Without these six species, Isodiametridae is highly supported. We chose not to reclassify the species of this group until more sequence data can be attained and their position inside or outside Isodiametridae can be determined with more certainty. The remaining results and discussion concerns only the analyses with these species excluded unless otherwise stated. Results from the three-gene concatenated and 18S datasets that include all species are available as Figures S1 and S3 in the Supporting Information.

There are several highly supported groups in the larger Isodiametridae clade: (1) six species of *Archaphanostoma* Dörjes, 1968 grouped together; (2) eight species of *Isodiametra* Hooge & Tyler, 2005, *Praeconvoluta* Dörjes, 1968 and *Aphanostoma* Ørsted, 1845 formed the sister-group to the *Archaphanostoma* clade. *Prophanostoma tenuissima* (Westblad, 1946) was sister to (3) a diverse clade of ten species, including *Otocelis erinae* Hooge & Rocha, 2006, *Raphidophallus actuosus* Kozloff, 1965, *Aphanostoma virescens* Ørsted, 1845 and seven species of *Isodiametra*; and (4) a clade containing *Pseudaphanostoma hyalinorhabdoida* Kånneby & Jondelius, 2013, *Pseudaphanostoma smithrii* Hooge & Tyler, 2003, *Haplogonaria schillingi* Hooge & Tyler, 2015, *Praeaphanostoma rubrum* Dörjes, 1968 and *Praeaphanostoma longum* Dörjes, 1968, which is sister to the previous three clades. Finally, *Haplocelis dichona* Dörjes, 1968 followed by *Pharyngia furva* Nilsson et al., 2011 are sister to the remaining species of Isodiametridae.

Several species previously considered isodiametrids did not group within Isodiametridae. As previously stated, three species of *Otocelis* grouped together outside any represented acoel family. In addition, *Proconvoluta primitiva* Dörjes, 1968 nests in a clade of four species of *Faerlea* Westblad, 1945 with high support, which in turn is nested in Mecynostomidae, and two species of *Postphanostoma* Dörjes, 1968 form a clade that is sister to all other species of Mecynostomidae. Thus, results from our molecular analyses provide further evidence that Isodiametridae require revision.

**TAXONOMIC NOTES AND NOMENCLURAL ACTS**

**Otocelidiae Westblad, 1948**

Westblad (1948) recognized the monotypic family *Otocelidiae* for acoels with a vagina opening posterior to the male copulatory organ. Only two species, *Otocelis rubropunctata* (Schmidt, 1852) and *O. gullmarensis* Westblad, 1946, were known at the time. Dörjes (1968) transferred the latter to a new genus, *Notocelis*, on account of its lack of a tubular penis and at the same time introduced the genera *Archocelis* Dörjes, 1968, *Haplocelis* Dörjes, 1968, *Haplotestis* Dörjes, 1968 and *Philocelis* Dörjes, 1968. Following this, Ehlers & Dörjes (1979) erected the genus *Exocelis* Ehlers & Dörjes, 1979; Kozloff (2000) recognized *Posticopora* Kozloff, 2000; and Hooge (2003) introduced *Stomatricha* Hooge, 2003. However, the presence of symbiotic algae within *Stomatricha hochbergi* Hooge, 2003 caused Hooge & Tyler (2005) to subsequently transfer the genus to Convolutidae and question the taxonomic importance of the position of the vagina and hence the validity of *Otocelidiae* as a monophyletic family. Based on this and the morphology of the male copulatory organ, Hooge & Rocha (2006) proposed the transfer of the genera *Haplocelis* and *Otocelis* to Isodiametridae. Building on a phylogenetic hypothesis derived from a combination of nucleotide sequences and morphological characters, Jondelius et al. (2011) placed *Notocelis* and *Philocelis* to Dakuidae, while refraining from reclassifying the type genus *Otocelis*, due to ambiguities in its phylogenetic position. *Archocelis, Exocelis, Haplotestis* and *Posticopora* were not represented in the study by Jondelius et al. (2011). The results of our analyses (Fig. 1) support the inclusion of *Stomatricha* in Convolutidae and *Philocelis* in Dakuidae, but show that *Otocelis* forms a clade outside Isodiametridae, herein interpreted to represent the family *Otocelidiae*.

The position outside Isodiametridae is somewhat problematic, since at least three species of *Otocelis* (*O. westbladi* Ax, 1959, *O. rubropunctata* and *O. erinae*) are all known to possess the diagnostic character of Isodiametridae – penis musculature with inner circular and outer non-anastomosing longitudinal fibres (Hooge & Rocha, 2006). Notably, this morphology was not found in *O. sandara* Hooge & Tyler, 2003, leading Hooge & Rocha (2006) to question the relationship between *O. sandara* and the remaining species of *Otocelis*, although they ultimately chose not to reclassify the species until further data could be attained. Results from our DNA analyses clearly
confirm the position of *O. sandara* within *Otocelis* and Otocelididae, but surprisingly do not include *O. erinae* in the family. The latter species is positioned within a diverse Isodiametridae clade containing several species of *Isodiametra*, *Raphidophallus actuosus* and *Aphanostoma virescens*. There are some morphological characters that support such a grouping, such as a vagina with a well-developed muscular sphincter, which is found in *Otocelis erinae* and the remaining species of the clade, but is lacking in all other species of *Otocelis* and Otocelididae, and the presence of a bursa with a well-developed nozzle. The position of *O. erinae* – as well as *Haplocelis dichona*, *Stomatricha hochbergi*, *Philocelis bruggemannii* *Hooge & Tyler, 2003* and *P. rochbrochai* *Hooge & Rocha, 2006* – outside of Otocelididae further supports *Hooge & Tyler's* assertion that the positioning of the vagina is taxonomically of low importance. These ambiguities perfectly illustrate the shortcomings of basing a taxonomically of low importance. These ambiguities perfectly illustrate the shortcomings of basing a classification on perceived morphological apomorphies without reference to an explicit, testable phylogenetic hypothesis. Inclusion of the remaining Otocelididae genera and species in a comprehensive phylogenetic analysis is clearly needed to establish their positions and to uncover any synapomorphic characters that potentially exist for the family.

**REASSIGNMENT OF THE GENUS FAERLEA WESTBLAD, 1945 TO THE FAMILY MECYNSOTOMIDAE DÖRJES, 1968**

Results from our phylogenetic analyses show that the species of *Faerlea*, as well as *Proconvoluta primitive*, form a clade with high support nested within the larger Mecynostomidae clade, and indeed, the general structure of the male copulatory organ of *Faerlea* does share some similarities to those typical of species of Mecynostomidae. For instance, the copulatory organ is often circular and appears as two concentric circles with the gonopore (e.g. Fig. 2A), and numerous glands surround the distal part of the penis, particularly in, for example, *F. antora* *Marcus, 1952* and *F. fragilis* *Westblad, 1945*. Additionally, species of *Faerlea* lack rhabdoids, a condition much more common to species of Mecynostomidae than Isodiametridae. *Hooge & Tyler* (2005) assigned *Faerlea* to Isodiametridae without specifying any reason beyond that the genus was previously within Convolutidae and lacked symbionts. Yet to the best of our knowledge, there has been no detailed investigation into the morphology of the musculature, male copulatory organ or sperms for any species of *Faerlea*, and so it is not certain if any, or all, of the species possess morphology consistent with Isodiametridae (= penis musculature with inner circular and outer non-anastomosing longitudinal fibres; *Hooge & Tyler, 2005*) or Mecynostomidae (= sperm with 9 + 1 axonemes and distal microtubules only and four layers of dorsal body-wall musculature; *Jondelius et al., 2011*). Regardless, the position of the four species of *Faerlea*, in particular the type species *F. fragilis*, justify the reassignment of the genus into Mecynostomidae (Fig. 1).

**SYNONYMIZATION OF FAERLEA WESTBLAD, 1945 AND PROCONVOLUTA DÖRJES, 1968**

Results from our phylogenetic analyses found *Proconvoluta primitiva* clearly nested in the *Faerlea* clade as sister to the new species *Faerlea assembli* (Fig. 1). *Dörjes (1968)* separated *Proconvoluta* from *Faerlea* based on the seminal bursa, which is present in *Proconvoluta*, although small and difficult to distinguish in live specimens, and absent in species of *Faerlea*. Otherwise, the morphologies of the two genera generally overlap, in particular with the absence of rhabdoid glands and the heavily vacuolated parenchyma. *Proconvoluta primitiva* is the only species currently recognized for the genus, and so, based on the results of the phylogenetic analyses, *Proconvoluta* becomes a junior synonym of *Faerlea*.

**ORDER ACOELA ULIJANIN, 1870**

**FAMILY MECYNSOTOMIDAE DÖRJES, 1968**

**GENUS FAERLEA WESTBLAD, 1945**

Unpigmented and often glassy in appearance. Parenchyma heavily vacuolated. Frontal glands well developed. Rhabdoids absent. Testes and ovaries paired. Male system includes a round seminal vesicle, a small muscular penis and a short antrum masculinum. Copulatory bursa, if present, is small, difficult to discern in live animals and includes a ventrofrontally directed cellular appendage. Gonopore(s) separate or male only. Type species: *Faerlea fragilis Westblad, 1945*.

**FAERLEA ASSEMBLI SP. NOV.**

*Fig 2–3*

*Zoobank registration: urn:lsid:zoobank.org:act:C46A5FAF-F9E0-4C44-8B5C-D69CD8CD46EC*

© 2021 The Linnean Society of London, Zoological Journal of the Linnean Society, 2021, **XX**, 1–25
Material examined: Holotype (SMNH-Type-9334) and paratypes (SMNH-Type-9335): serially sectioned specimens. Digital video and photographs of original specimens.

Type locality: SPAIN. Mutriku, Siete Playas. 43°19’41.5”N, 2°22’46.7”W

Habitat: Marine sediments. Subtidal, medium sand.

Diagnosis: Species of Faerlea without pigmentation, glassy. Body 0.8 mm long, vermiform with rounded anterior and posterior. Large vacuoles in posterior. Smaller vacuoles present laterally and in the anterior. Frontal glands large. Rhabdoids absent. Testes and ovaries paired. Male system with round seminal vesicle, small antrum. Penis a simple inpocketing of the epidermis. Female system with bursa with a single small, ventrofrontally directed appendage. Gonopores separate.

Etymology: This species is named after the Assemble Plus programme, funded by the European Community, which has supported a variety of marine field studies.

Description
Living specimens approximately 0.8 mm long. Width ~50 µm at position of stylet and slightly increasing toward posterior; ~75 µm at position of largest egg. Body shape vermiform with rounded anterior and posterior...
ends. Without body pigmentation or eyespots. Glassy in appearance. Statocyst 10–12 μm in diameter located ~50 μm from anterior end. Frontal organ present and large. Two large vacuoles present at the posterior end; smaller vacuoles present laterally and in the anterior, though more numerous in the posterior body. Epidermis 4 μm thick, uniformly covered with cilia. Cilia ~4 μm long. Rhabdoids absent. Mouth located at the second-quarter of the body, 15 μm long in fixed specimens. Ovaries paired. Female pore clearly separate from male pore, ciliated, opening ventral, ~9 μm long. Female atrium present, ~11 μm deep in fixed specimens leading to a short vagina and bursa. Bursa present 75 μm from posterior end, 28 μm long and 34 μm wide, with a single ventrofrontally directed cellular appendage. Sperm was not observed in the bursa of any specimen examined. Testes paired, located laterally approximately one-quarter of the way from the anterior end of the body. Vas deferens large and clearly evident toward male copulatory organ, with evident sperm. Male copulatory organ present ~45 μm from posterior end, spheroid, ~35 μm long and ~30 μm wide in living specimens. Penis present as a simple inpocketing of the epidermis, 17 μm in fixed specimens, with a shallow male atrium. Male pore large, 15 μm long, ciliated.

Remarks

There are seven species of Faerlea, following the synonymization of Proconvoluta and Faerlea. Faerlea assembli is most similar to Faerlea primitiva in that they both possess a bursa with a ventrofrontally directed cellular appendage. The two species can be distinguished by the size of the bursa and male copulatory organ, which are more clearly evident and larger in Faerlea assembli, and through the small, brownish-black pigments that are present in Faerlea primitiva and absent in the new species.

**Faerlea tyleri sp. nov.**

(Fig. 4)

Zoobank registration: urn:lsid:zoobank.org:act:D7ACB50A-70AB-40E3-ADB7-86310945FB0F

Material examined: Holotype (SMNH-Type-9336) and paratypes (SMNH-Type-9337): serially sectioned specimens. Digital video and photographs of original specimens.

Type locality: USA, Hawaii, Waimanolo Beach. 21°19'8.23"N, 157°40'11.92"W.
Habitat: Marine sediments; mixed fine and medium sand with some organic content; 1.5 m deep.

Diagnosis: Species of Faerlea without pigmentation, glassy. Body up to 1 mm long, vermiform with rounded anterior and posterior. Large vacuoles in posterior and laterally. Smaller vacuoles in the anterior. Frontal glands well developed. Rhabdoids absent. Testes and ovaries paired. Male copulatory organ located close the posterior end. Small, round seminal vesicle present with a penis as a simple inpocketing of the epidermis. Bursa or female accessory organs absent.

Etymology: This species is named after Seth Tyler in recognition of his contributions to the taxonomy of Acoela.

Description
Living specimens up to 1 mm long. Glassy and without body pigmentation or eyespots. Body width ~67 μm at position of stylet and increasing toward posterior; ~100 μm at position of largest egg. Body shape vermiform with rounded anterior and posterior ends. Statocyst to 8 μm in diameter located 67 μm from anterior end. Frontal organ well developed. Large vacuoles present at the posterior end and extending laterally, decreasing in size toward the anterior; smaller vacuoles present in the anterior. Epidermis 4–5 μm thick, uniformly covered with cilia. Cilia 3–5 μm long. Rhabdoids absent. Mouth located at the second-quarter of the body. Ovaries paired, located in third-quarter of the body. Bursa or female accessory organs absent. Testes paired, located laterally approximately ~300 μm from the anterior end the body. Vas deferens more evident toward male copulatory organ, with clear sperm. Male copulatory organ small, ~20 μm long and ~25 μm deep in fixed specimens, spheroid. Present 55 μm from posterior end in living specimens. Penis present as a simple inpocketing of the epidermis, 9 μm long and projecting outward in fixed specimens. Male pore small, ~5 μm long, ciliated.
Remarks

**Faerlea tyleri** is the first species of its genus to be documented from Hawaii or the Pacific. In general, the species fits well with the other known species of **Faerlea**: **Faerlea tyleri** is fragile due to its heavily vacuolated parenchyma; the position of the testes is far toward the anterior; and the position and composition of the male copulatory organ is consistent with the other species. The species is most similar to **Fragilis fragilis** and **F. glomerata** in that it is non-parasitic and does not possess female accessory organs. However, **Faerlea tyleri** differs from either species in its general body shape, which is more strap-shaped with rounded anterior and posterior ends than either **Fragilis fragilis** or **F. glomerata**, and the small size of the male copulatory organ. Additionally, **Faerlea tyleri** can be distinguished from **F. glomerata** by the size of the frontal organ, which, although well developed, is smaller in comparison, the more distinctly paired ovaries and wide separation between the ovaries and the male copulatory organ. **Faerlea tyleri** can be distinguished from **F. fragilis** by the relatively few vacuoles and the position of the ovaries, which begin further toward the posterior end.

Reassignment of **Postaphanostoma Dörjes, 1968** to the family **Mecynostomidae Dörjes, 1968**

Both species of Postaphanostoma included in our analyses, **P. glandulosum Dörjes 1968** and **P. nilssoni Känneby & Jondelius, 2013**, grouped together as the highly supported sister-group of Mecynostomidae, separate from Isodiametridae. As with Faerlea, once again there has not been any detailed investigation into the body and reproductive musculature or sperm ultrastructure for any species of Postaphanostoma, and so it cannot be definitively stated if the animals of this group possess any of the morphological characteristics of Isodiametridae, Mecynostomidae or any other group. Indeed, the position of **P. glandulosum** and **P. nilssoni** as sister of Mecynostomidae in our phylogenetic analyses suggests such detailed morphological investigations should be of high priority for future studies. Unfortunately, no gene sequences of the type species of Postaphanostoma, **P. atriomagnum Dörjes, 1968**, currently exist. However, there are also no significant morphological characters that distinguish **P. glandulosum** and **P. nilssoni** from the remainder of the genus with which to justify a separation, and so, rather than create confusion by forming a new genus in which to transfer the two species, we choose to reassign Postaphanostoma in its entirety to Mecynostomidae until such time as sequences or more detailed morphology can be assessed for the type.

Reassignment of **Alluna Faubel & Regier, 1983** to the family **Actinoposthiidae Hooge, 2001**

There were several inconsistencies in Faubel & Regier’s (1983) original description of Alluna. First, they stated in the genus diagnosis that **A. sublittoralis** Faubel & Regier, 1983 was the type and only species, but entitled Alluna vulgaris as the caption of their illustrations. Since no other species description occurred apart from that of **A. sublittoralis**, and no illustration of **A. sublittoralis** was provided, Alluna vulgaris can be considered a synonym of Alluna sublittoralis. More importantly, Faubel & Regier (1983) stated in the diagnosis of Alluna that a true seminal vesicle is absent, but they described paired false seminal vesicles attached to a (true) seminal vesicle in the subsequent description of **A. sublittoralis**, a feature that was also clearly present in the following illustrations.

If the genus diagnosis is thus emended to fit its type and only species, its morphology becomes more consistent with species of Actinoposthiidae than with Isodiametridae. Following the family diagnosis of Hooge (2001), Actinoposthiidae includes those species of Acoela with a penis built of either sclerotized or muscular elements that is never invaginated into a seminal vesicle. Alternately and according to the diagnosis of Hooge & Tyler (2005), Isodiametridae includes those species of Acoela with a muscular, not isodiametric (following the original illustrations, the proximal part of the penis swells to form a bulb), and – different to all other species of Isodiametridae – lies entirely outside of the seminal vesicle. Unfortunately, sequences of Alluna sublittoralis are currently not available for analysis, so the position of the species could not be tested using molecular techniques. However, in consideration of its morphology, the species and genus must be transferred to Actinoposthiidae.

Synonymization of **Baltalimania Ax, 1959 and Archaphanostoma Dörjes, 1968**

Dörjes (1968) placed the three species of Baltalimania of the time into different genera, based solely on the presence or absence of a female bursa. Archaphanostoma was created to encompass two species [A. agile (Jensen, 1878) and A. macrosipferum (Westblad, 1946)] with bursal tissue, leaving the one species that lacked bursal tissue (B. kosswigi Dörjes, 1968) to comprise its own monotypic genus. Subsequently and without mention of Baltalimania, Känneby et al. (2014) amended the
diagnosis of *Archaphanostoma* to include both species with and without bursal tissue, following phylogenetic analysis of nucleotide sequences from three genes that demonstrated that four new species without bursa (*A. fontaneti* Kånneby et al., 2015; *A. occulta* Kånneby et al., 2015; *A. sublitorialis* Kånneby et al., 2015 and *A. ylvae* Kånneby et al., 2015) nested within the *Archaphanostoma* clade. Unfortunately, the amended diagnosis caused *Baltalimania* and *Archaphanostoma* to be morphologically indistinguishable. Phylogenetic analyses oppose Dörjes’ (1968) argument that bursal tissue is a good character to differentiate these genera (Figs 1, 5). Although no DNA sequences currently exist for *B. kosswigi*, the identical generic diagnoses and similarities in the morphologies of *B. kosswigi* and species of *Archaphanostoma* without bursal tissue strongly suggest that the two genera are synonymous. Therefore, *Archaphanostoma* is a junior synonym for *Baltalimania*.

**SYNONYMIZATION OF *BURSOSAPHIA* DÖRJES, 1968 AND *PRAEAAPHANOSTOMA* DÖRJES, 1968**

Dörjes (1968) placed great emphasis on the presence and structure of the bursa in his classification system, and numerous genera were created or separated based on small differences of this organ. When Dörjes (1968) described the species *Bursosaphia baltalimaniaformis* Dörjes, 1968, he justified the creation of a new genus based on what he interpreted as bursal tissue ('*bursales Gewebe*') in which the true bursa was embedded. However, Dörjes (1968) was somewhat unclear in his description of the bursal tissue that surrounds the female system, stating only that it is a tissue that stores foreign sperm, that it is separate from the parenchyma of the rest of the body and that it similarly is present in species of *Archaphanostoma* (= *Baltalimania*), which otherwise lack a true bursa. Dörjes (1968) also stated that bursal tissue may occur in other genera, but will typically disappear after a

---

**Figure 5.** Bursal morphology of *Isodiametridae* mapped onto the concatenated tree. The bursal morphology of each species was coded following original descriptions and illustrations, as well as photos and live observation of species where possible.
true bursa has formed. Unfortunately, this character has not been assessed for any species with a true bursa in any of the literature outside Dörjes’ (1968) description of Bursosaphia and, therefore, it is unclear how prevalent it is within Isodiametridae.

Unfortunately, no sequences of B. baltalimaniaformis are currently available, and so the relationship between this species and other acoels cannot be directly tested at this time. Regardless, results from our phylogenetic analyses suggest that bursal tissue is not a good character for distinguishing genera, since the only species other than B. baltalimaniaformis known to possess it – Baltalimania macrospiriferum and B. agile – do not on their own form a clade, but instead group with species of Baltalimania without bursal tissue (Fig. 1). Given these results, and that B. baltalimaniaformis is not a good character for distinguishing genera, since the species differ only in the size of the rhabdoids (small in P. longum, large in H. schillingi), possession of a genital atrium (absent in P. longum, present in H. schillingi) and, of course, the penis (present in P. longum, absent in H. schillingi). Given such similarities, the position of H. schillingi is conceivable, and the absence of a penis could potentially represent a secondary loss. Praeaphanostoma includes several species – including those in our analysis that were the closest to H. schillingi – where the penis is a simple inpocketing of the epidermis, a condition that in Isodiametridae is only otherwise present in Proaphanostoma.

Thus, based on the positioning of the species in our phylogenetic analyses and the morphological similarity, we formally reassign Haplogonaria schillingi to Praeaphanostoma in Isodiametridae.

REASSIGNMENT OF HAPLOGONARIA SCHILLINGI
HOoge & TYLER, 2015 TO THE GENUS
Praeaphanostoma Dörjes, 1968

Our results show Haplogonaria schillingi to be nested in Praeaphanostoma and closely related to Praeaphanostoma rubrum. Sequences of the 18S, 28S and COI genes were published for H. schillingi and used in several phylogenetic analyses before the species was formally described. Jondelius et al. (2011) found the species (denoted there as Haplogonaria ‘schillingi’) positioned closely to Pseudaphanostoma smithrii and transferred the species to Pseudaphanostoma on this basis. Later, Nilsson et al. (2011) and Kånneby & Jondelius (2013) used the sequences in their phylogenetic analyses with results congruent to Jondelius et al. (2011), but still listing the species as Haplogonaria schillingi. Of note, at the time of all three publications, no sequences of any species of Praeaphanostoma were available and, indeed, results from our analysis show that P. smithrii is the closest sister to all other species of Praeaphanostoma. Hooge & Tyler (2015) eventually described the species, but found that the male copulatory organ lacks a muscular, tubular penis. Because such morphology is incongruous with the diagnostic character of Isodiametridae, Hooge & Tyler (2015) dubbed the species Haplogonaria schillingi and assumed the molecular data resulted from an error in handling specimens.

Our results, with the additional sequence data of Praeaphanostoma longum and especially P. rubrum, contradict the assertion that the position of H. schillingi in the phylogenetic analyses was due to some error. The morphology of H. schillingi is generally consistent with species of Praeaphanostoma. Praeaphanostoma is in part characterized by a moderate to small frontal organ, a muscular seminal vesicle, a seminal bursa with well-defined walls but without nozzles and a well-defined vagina with a sphincter, all characters that H. schillingi also possesses. Praeaphanostoma longum is additionally morphologically similar to H. schillingi in the shared possession of an unpaired ovary, common gonopore, diagonal muscles restricted to the anterior end and a characteristic bright-red pigmentation. The two species differ only in the size of the rhabdoids (small in P. longum, large in H. schillingi), possession of a genital atrium (absent in P. longum, present in H. schillingi) and, of course, the penis (present in P. longum, absent in H. schillingi). Following a re-examination of the original collection notes and materials, it is clear from both photographs of live animals and from fixed, sectioned animals that Kånneby & Jondelius (2013) incorrectly interpreted the morphology of the reproductive system of Pseudaphanostoma hyalinorhabdoida in their original description. The female bursa was described as the seminal vesicle; the actual seminal vesicle and connecting vas deferens were dismissed as patches of free sperm outside the male copulatory organ, and the penis was not identified. Kånneby & Jondelius (2013) based the inclusion of the species in Pseudaphanostoma on the mistaken lack of female accessory organs and the relationship between this species and Pseudaphanostoma smithrii in their phylogenetic tree.

It is clear that this species does possess a female bursa, ciliated vagina and gonopore common to both the male and female system (Figs 6–7). The presence of an unadorned female bursa is a characteristic of species of Praeaphanostoma, while species of Pseudaphanostoma lack all female accessory organs. Further, results from our DNA analyses confirm a close relationship between this species with Praeaphanostoma longum and P. rubrum (Fig. 1), neither of which had sequences available when
Kånneby & Jondeilius (2013) performed their analysis. The morphological inaccuracies of the original description require that the species be redescribed, and the updated morphology and phylogenetic relationships support the transference of the species from *Pseudaphanostoma* to *Praeaphanostoma*.

**Family IsodiameTridae Hooge & Tyler, 2005**

**Genus Praeaphanostoma Dörjes, 1968**

*Praeaphanostoma hyalinorhabdoida* (Kånneby & Jondeilius, 2013), comb. nov.

Figs 6–7

**Material examined:** Holotype (SMNH Type-8468) and paratypes (SMNH Type-8469–8471): serially sectioned specimens. Digital video and photographs of original living specimens.

**Type locality:** CHILE. El Quisco, Marina, 33°23′35.664″S, 71°41′53.088″W.

**Habitat:** Marine sediments; fine sand at 10 m depth.

**Diagnosis:** Species of *Praeaphostoma* without body pigmentation or eyespots. Body 0.5 mm long, vermiform with rounded anterior and more pointed posterior end. A single, large vacuole present in the posterior end with additional smaller vacuoles present laterally and in the anterior. Rhabdoid glands distinct in longitudinal rows, more numerous towards body ends. Paired ovaries and testes. Female system includes muscular seminal bursa without adornments and a short, ciliated vagina with muscular sphincter. Male system includes short, cylindrical penis invaginated in seminal vesicle. Penis with two distal, fingerlike lobes that reach outward toward genital pore. Gonopore common.

**Description**

Living specimens approximately 500 μm long and 150–200 μm wide. Fixed specimens up to 300 μm in body length. Body shape vermiform with a tapering,
conically rounded anterior end and a somewhat more sloped and pointed posterior end. Without body pigmentation or pigmented eyespots. Central parenchyma may appear yellow-orange from food. Statocyst and statolith 15 µm and 9 µm in diameter, respectively, located 75 µm from anterior end. Frontal organ present, extending from the frontal pore to the level of the mouth. Mouth 25 µm long in fixed specimens, located ventrally approximately one-quarter of the way from the anterior of the body. A single, large vacuole present in the anterior end; other smaller vacuoles may also be present laterally and in the anterior. Epidermis 3–4 µm thick, uniformly covered with cilia, and penetrated by numerous well-developed rhabdoids. Rhabdoid glands present in distinct longitudinal rows, more numerous dorsally and towards the anterior and especially posterior ends. Ovaries paired, ventral. Thick-walled, muscular seminal bursa clearly present, usually filled with allosperm. Bursa shape an irregular prolate spheroid, ~20 µm long and ~60 µm wide in living specimen (Fig. 6D). A short, ciliated vagina connects the bursa ventrally to a ciliated common antrum and gonopore. Muscular sphincter present at the junction between the vagina and bursa. Testes paired, diffuse, located laterally just anterior to the midpoint of the body and extending to the seminal vesicle. Male copulatory organ present closely between the bursa and the posterior vacuole, consisting of a glandular, short penis invaginated into a seminal vesicle. Penis cylindrical with two distal, fingerlike lobes. The twin lobes of the penis reach outwards into the common antrum and are clearly visible in living specimens (Fig. 6C, D).

Remarks

*Praeaphanostoma hyalinorhabdoida* can be easily distinguished from all known species of *Praeaphanostoma* by its larger, distinct rhabdoids and small penis with distal, fingerlike lobes that are clearly visible in live specimens (Fig. 6C, D). Apart from these characters, *P. hyalinorhabdoida* is perhaps most similar to *P. foramivora* Hooge & Tyler, 2008 and *P. thalasophilum* Ehlers & Dürjes, 1979 in general body shape and size, and in that they all possesses a single, large, posterior vacuole and a common gonopore. However, *P. foramivora* has a much larger seminal vesicle and smaller bursa, and *P. thalasophilum* has a longer vagina and unpaired ovary.
Synonymization of Ancylocirrus Kozloff, 2000 and Praeaphanostoma Dörjes, 1968

Kozloff (2000) proposed the genus Ancylocirrus after finding secretory granules in the lumen of the cirrus and in the vagina of its one species, A. ornatus Kozloff, 2000. The only details presented about these granules were that they were colourless, ~3–5 µm in diameter and likely secreted by the male system, since they were associated with sperm masses and present in the female system only after insemination. Kozloff (2000) stated that such secreted granules had not been reported in any other genera at the time and was thus sufficient for the establishment of a new genus.

Sequence data is not available for Ancylocirrus ornatus at this time. However, while secretory granules such as those described by Kozloff (2000) may not be a character that has typically been assessed in the past (although, see e.g. Aphanostoma album Dörjes, 1968; Baltalimania histobursalium; Diatomovora amoena Kozloff, 1965), we can confirm that it is present across multiple species of Acoela (Fig. 8), including in at least one species of Praeaphanostoma (P. rubrum; Fig. 8D). Further, based on our own personal observations, we hypothesize that far from being unique to one species, such secretions are probably common, especially in species with larger and more glandular male copulatory organs, such as can be found in species of, for example, Baltalimania (Fig. 8B, C).

Ancylocirrus is further characterized by the morphology of the reproductive system: a long, curved cirrus invaginated into a seminal vesicle; a small, unciliated antrum masculinum; a long, muscular vagina; and, finally, a conspicuous seminal bursa without adornment. This morphology, along with the general body shape and pigmentation, fits in its entirety with species of Praeaphanostoma. While Ancylocirrus ornatus does differ in that it has pigmented eyespots, genera with species both with and without eyespots are widespread throughout Acoela (e.g. Amphiscolopis Graff, 1904; Isodiametra; Nadina Ulajnin, 1870; Otocelis; Proporus Schmidt, 1848) and the character is unlikely to be of much taxonomic importance. Thereby, Ancylocirrus is designated a junior synonym of Praeaphanostoma.

Aphanostoma Ørsted 1845, Isodiametra Hooge & Tyler, 2005 and Praeconvoluta Dörjes, 1968

Species of Aphanostoma, Isodiametra and Praeconvoluta are united by the possession of a single bursa with some type of bursal appendage, the morphology of which, following the classification systems of Dörjes (1968), Faubel (1974) and Hooge & Tyler (2005), in large part distinguishes each genus (for a list of bursa terminology, see also: Petrov et al., 2006). Isodiametra encompassed those species of Isodiametridae with a single sclerotized bursal nozzle, and Aphanostoma comprises species with a cellular bursal cap, which may also include small actin-sclerotized bodies (Petrov et al., 2006; Hooge & Tyler, 2008). For Praeconvoluta, Dörjes (1968) and later Faubel (1974) specified a ‘simple bursa’ in the genus diagnosis. However, all species, except P. tigrina Hooge & Tyler, 2003, were described with a bursal cap, and one species, P. bocasensis Hooge & Tyler, 2008, further possesses a bursal cap with several spots of concentrated actin, and Hooge & Tyler (2005) listed Praeconvoluta and Aphanostoma as morphologically indistinguishable in their identification table. There was some discussion of combining Aphanostoma and Praeconvoluta (Nilsson et al., 2011; Zauchner et al., 2015), since the general morphologies of the species of the two genera are the same, although no formal synonymization ever actually occurred.

All species of Praeconvoluta in our analyses form a clade with Aphanostoma bruscai Dörjes, 1968 and
A. collinae Hooge & Tyler, 2008, a result that is not surprising, given that the genera are morphologically indistinguishable from each other (Hooge & Tyler, 2005; Zauchner et al., 2015). This clade also included Isodiametra norvegica (Westblad, 1946), the type species of Isodiametra, and I. hortulous (Hooge & Tyler, 2003) (Fig. 1). Thus, this clade includes species with all forms of bursa morphology (Fig. 5): simple without adornments (P. tigrina), with a cellular cap reported with (A. bruscai, A. collinae and P. bocasensis) or without (P. castinea Hooge & Tyler, 2003 and P. tornuva Hooge & Tyler, 1999) spots of actin and with a fully sclerotized nozzle (I. norvegica and I. hortulous). The type species of Aphanostoma, A. virescens, was not included in this group, but formed a clade with Otocelis erinae, Raphidophallus actuosus and seven other species of Isodiametra (Fig. 1). The bursal appendages in the species of this clade are more similar to each other, since all species, except for A. virescens, possess a bursa with a sclerotized nozzle (Fig. 5), and the morphology of the bursal cap of the latter is somewhat unique. Aphanostoma virescens has a large and highly muscular bursal cap with numerous (six to eight according to Steinböck, 1931, although Graff, 1905, recorded as many as 14) sclerotic spines. Both clades were maximally supported.

Our results indicate that, while the presence or absence of a walled/true bursa may be useful to distinguish genera of Isodiametridae, the details and composition of the bursal appendage is not (Fig. 5). This is perhaps not so surprising given the similarities in the composition of the different bursal appendages. Each sclerotized bursal nozzle in Acoela is composed of a sperm duct with actin-reinforced inner edges, while the bursal cap of at least some species includes disjunct spots of intracellular actin localized along a sperm duct (Petrov et al., 2006). Further, because intracellular actin is not always visible in squeeze preparations and living specimens, it can easily be missed if the bursal cap of a species has not been properly examined with phallloidin-labelled probes and fluorescence microscopy (Hooge & Tyler, 2008). Petrov et al. (2006) warned that actin-sclerotised sections of bursal caps could be confused with muscle fibres following conventional staining by iron haematoxylin and, in fact, they were highly skeptical that muscles were a component of any bursal appendage in Acoela at all. Thus, the analysis of bursal morphology may be incomplete or incorrect in some species considered to have only muscular or cellular caps, especially for species with older descriptions.

Below, we attempt to update the current classification system to reflect as best as possible their phylogenetic relationships as represented by the results of our molecular analyses (Fig. 1), and we reassign species to, and emend, the genera accordingly. Information gathered from live observations and original descriptions of each species included in the analyses form the basis for the morphological summary of each genus, while those species for which sequence data are currently unavailable, were classified based on morphology. Specifically, our results supported the existence of two clades representing two genera with walled bursas (Figs 1, 5), which appeared to be best distinguished morphologically based on the presence or absence of one or more distinct muscular vaginal sphincters (Fig. 9). This result notably concurs with the findings of Jondelius et al. (2011) that details of the musculature have higher potential to be taxonomically informative at the genus level for Isodiametridae. Nevertheless, we must underscore the need for further detailed morphological study using modern methodology to clarify any potential discrepancies that may exist in the reproductive anatomy and musculature, as well as the importance of obtaining DNA sequences of the remaining species of the genera.

EMENDED DIAGNOSIS OF APHANOSTOMA ØRSTED, 1845 AND REASSIGNMENT OF SPECIES; SYNONYMIZATION OF RAPHIDOPHALLUS KOZLOFF, 1965 AND APHANOSTOMA ØRSTED, 1845

Aphanostoma is hereby emended to include the species that form a clade with its type species, A. virescens. Isodiametra bajaensis Hooge & Eppinger, 2005, I. cuernos Hooge & Tyler, 2008, I. divae (Marcus, 1950), I. finkei Kâneby & Jondelius, 2013, I. nicki Hooge & Tyler, 2008, I. pulchra (Smith & Bush, 1991), Otocelis erinae and Raphidophallus actuosus are all transferred to Aphanostoma.

Raphidophallus was distinguished from other genera solely based on the delicate cuticularized rods that occurred in the lumen of the penis. Otherwise, Kozloff (1965) noted that its morphology was highly similar to other species of Isodiametra, particularly with reference to the bursa with nozzle, which the majority of the species in the clade do also possess (although see notes above). Raphidophallus actuosus is the type and only species of its genus and, therefore, Raphidophallus is considered a junior synonym of Aphanostoma.

All species in this clade are united by the possession of a muscular bursa with evident actin in the form of a nozzle or spines, a distinct male or common atrium and one or more well-developed vaginal sphincters. Of the species for which DNA is currently unavailable, Isodiametra earnhardtii (Hooge & Smith, 2004), I. helgolandica (Dörjes, 1968), I. karpredi (Hooge & Tyler, 2003), I. vexillaria (Marcus, 1948) and I. westbladi (Marcus, 1949) all also possess these characters and are, therefore, transferred to Aphanostoma. In addition, Aphanostoma album and A. rhomboides Jensen, 1878 both possess strong vaginal sphincters that are characteristic of the
The morphology of each species was coded following original descriptions and illustrations, as well as photos and live observation of species where possible. *?* indicates that the presence or absence of a vaginal sphincter was not examined/could not be determined.

Figure 9. Vaginal sphincter presence or absence in species of Isodiametridae mapped on to the concatenated tree. The morphology of each species was coded following original descriptions and illustrations, as well as photos and live observation of species where possible. *?* indicates that the presence or absence of a vaginal sphincter was not examined/could not be determined.

Synonymization of Praeconvoluta Dörjes, 1968 and Isodiametra Hooge & Tyler, 2005; Emended Diagnosis of Praeconvoluta Dörjes, 1968 and Reassignment of Species

All four species of Praeconvoluta group with Aphanostoma bruscae Hooge & Tyler, 2003, A. collinae Hooge & Tyler, 2008, Isodiametra norvegica and I. hortulous in our analyses to form a second clade of species of Isodiametridae with true/walled bursas (Fig. 5). The species of this clade are united together and distinguished from Aphanostoma through a lack of well-defined vaginal sphincter muscles (Fig. 9). Nine of the 16 species with true bursas without DNA sequences currently available all lack well-defined vaginal sphincters, including all remaining four species of Praeconvoluta, Aphanostoma piscæ Zauchner et al., 2015, Isodiametra colorata (Ehlers & Dörjes, 1979), I. marginalis (Ivanov, 1952), I. urua (Marcus, 1954) and I. variomorpha (Dörjes, 1968).

Isodiametra norvegica is the type species of Isodiametra, which was proposed by Hooge & Tyler (2005) for species with a single bursa nozzle. According to our phylogenetic hypothesis, this genus is polyphyletic with the majority of the species grouping separately from the type species. Isodiametra norvegica groups with the four species of Praeconvoluta included in the analyses. Although DNA sequences are not available for the type species Praeconvoluta karinae, it and other species of the genus lack well-defined vaginal sphincters. Based on our phylogenetic hypothesis, Praeconvoluta and Isodiametra are synonymized such that Isodiametra is a junior synonym of Praeconvoluta.
Isodiametra was the type genus of Isodiametridae. Nevertheless, no name replacement for the family is necessary in this instance since replacing Isodiametridae with another name would be exclusively due to the type genus being considered a junior synonym (see ICZN section 40.1). Hence Isodiametridae is retained and Praeconvoluta becomes the type genus.

CONCLUSIONS

The classification system of Isodiametridae has been updated to be consistent with its phylogeny following the results of molecular analyses that included sequence data from 128 species of Acoela and 16 of the 22 genera of Isodiametridae. Following the transfer of Otocelis to Otocelididae, Postphanostoma and Faerlea to Mecynostomidae and Alluna to Actinoposthiidae, as well as the synonymisation of six other genera, Isodiametridae now includes 12 genera and 78 nominal species. Below we provide a morphological summary of the genera of Isodiametridae and a family key.

TAXONOMIC SUMMARY OF ISODIAMETRIDAE

APHANOSTOMA ØRSTED, 1845

With or without orange pigmented eyespots in the anterior. Rhabdoids present in rows. Frontal glands often prominent. Testes paired. Ovaries paired or unpaired. Male system includes curved, muscular penis invaginated into a muscular seminal vesicle and a distinct male or common atrium. Penis glandular, with one or more well-developed sphincter muscles in lumen. Female system includes distinct vagina of one or more well-developed sphincter muscles and walled bursa with cap or sclerotized single nozzle. Bursal cap may have multiple spines. Gonopore common or separate; if separate, female pore may be either anterior or posterior to male pore. Type species: Aphanostoma virescens Ørsted, 1845.

Marine, free-living, 16 species:

- Aphanostoma virescens Ørsted, 1845
- Aphanostoma actuosus (Kozloff, 1965), comb. nov.
- Aphanostoma album Dörjes, 1968
- Aphanostoma bafiaensis (Hooge & Eppinger, 2005), comb. nov.
- Aphanostoma cuernos (Hooge & Tyler, 2006), comb. nov.
- Aphanostoma divae (Marcus, 1950), comb. nov.
- Aphanostoma earnhardti (Hooge & Smith, 2004), comb. nov.
- Aphanostoma eriniae (Hooge & Rocha, 2006), comb. nov.
- Aphanostoma finkei (Kanneby & Jondelius, 2013), comb. nov.
- Aphanostoma helgolandica (Dörjes, 1968), comb. nov.
- Aphanostoma kapredi (Hooge & Tyler, 2003), comb. nov.
- Aphanostoma nicki (Hooge & Tyler, 2008), comb. nov.
- Aphanostoma pulchra (Smith & Bush, 1991), comb. nov.
- Aphanostoma rhomboides Jensen, 1878
- Aphanostoma vexillaria (Marcus, 1948), comb. nov.
- Aphanostoma westbladi (Marcus, 1949), comb. nov.

AVAGINA LEIPER, 1902

Frontal glands weakly developed. Rhabdoids absent. Paired testes and paired or unpaired ovaries. Male system includes a muscular seminal vesicle, an eversible muscular penis and a ventral or subterminal gonopore. Antrum masculinum and female reproductive accessory organs absent. Type species: Avagina incola Leiper, 1902.

Marine, free-living or parasitic; six species:

- Avagina incola Leiper, 1902
- Avagina glandulifera Westblad, 1953
- Avagina marci Dorjes & Karling, 1975
- Avagina polyvacuola Ehlers & Dörjes, 1979
- Avagina sublitoralis Faubel, 1976
- Avagina vivipara Hickman, 1956

Notes

Avagina incola was first described by Leiper in 1902. Graff subsequently moved it to Haplodiscus in 1905 arguing that the description was not thorough enough to warrant another genus. Westblad then moved it back to Avagina in 1948 based on the paired testes and parasitic lifestyle, where it has remained since. At the time of writing, Avagina incola is incorrectly listed as a synonym of Haplodiscus incola in WoRMS (Tyler et al., 2006–21).

Three genera of Isodiametridae include species that lack a seminal bursa yet have a muscular seminal vesicle (Baltalimania, Pharyngia and Pseudaphanostoma). Avagina differs from all three genera through the absence of rhabdoids and a distinct antrum masculinum. Otherwise, Avagina is similar to Alluna, a genus of Actinoposthiidae, and Faerlea, a genus that molecular analyses suggests belongs in Mecynostomidae. The former is distinguishable by the morphology of the seminal vesicle, which is muscular in
Avagina and non-muscular with paired, false seminal vesicles in Alluna, while the sole defining morphological difference for the latter is the small antrum masculinum in species of Faerlea that is absent in species of Avagina. Further investigation, including obtaining DNA sequences for, especially, Avagina incola, is needed to assess the validity of the genus and the position within Isodiametridae and Acoela.

BALTALIMANIA AX, 1959
Frontal organ well developed. Rhabdoid glands present in longitudinal rows. Yellow-orange lipid globules often present. Testes paired; ovaries paired or unpaired. Male system includes a muscular seminal vesicle, a muscular penis, a distinct antrum masculinum and a gonopore. Penis distinctly curved, typically relatively large, completely or incompletely invaginated into the seminal vesicle. Antrum masculinum ciliated or unciliated. Gonopore terminal or subterminal. Female bursa absent or, if present, indistinct without walls and without any adornments. Type species: Baltalimania kosswigi Ax, 1959.

Marine, free-living; nine species:
- Baltalimania kosswigi Ax, 1959
- Baltalimania agile Jensen, 1978
- Baltalimania fontaneti (Kånneby et al., 2015), comb. nov.
- Baltalimania histobursalium (Dörjes, 1968), comb. nov.
- Baltalimania macrospiriferum Westblad, 1946
- Baltalimania marcsi (Hooge & Rocha, 2006), comb. nov.
- Baltalimania occulta (Kånneby et al., 2015), comb. nov.
- Baltalimania sublittoralis (Kånneby et al., 2015), comb. nov.
- Baltalimania ylvae (Kånneby et al., 2015), comb. nov.

DIATOMOVORA KOZLOFF, 1965
Rhabdoids present in distinct longitudinal rows or scattered. Paired or unpaired ovary and paired testes. Male system includes a highly muscular, curved penis partially invaginated into a seminal vesicle. Female system includes a heavily muscularized vagina and one or two muscular seminal bursae with two to six cuticularized nozzles each. Common gonopore ventral. Common, ciliated genital atrium may be present. Type species: Diatomovora amoena Kozloff, 1965.

Marine, free-living; two species:
- Diatomovora amoena Kozloff, 1965
- Diatomovora jacki Hooge & Tyler, 2008

Notes
Species of Diatomovora have been reported with a wide amount of morphological variability in the female anatomy. Diatomovora amoena, as originally described by Kozloff (1965), was characterized by a single bursa with two bursal nozzles, a highly muscular vagina and a common ciliated antrum. Dörjes & Karling (1975) re-examined materials of the same species deposited in SMNH and found specimens with one or two bursae and three nozzles per bursa. Hooge & Tyler (2008) described Diatomovora jacki with a single bursa with ‘~6 bursal nozzles’ that were not visible in squeeze preparations or live materials. The nozzles were visible in the actin-stained whole mounts viewed with fluorescence microscopy.

The only other species of Isodiametridae that possesses a bursa with multiple nozzles is Haplocelis dichona. Species of Diatomovora and Haplocelis are easily distinguished through the positioning of the gonopore (ventral in Diatomovora, terminal in Haplocelis) and the positioning of the vagina (anterior to the male copulatory organ in Diatomovora, dorsal to the male copulatory organ in Haplocelis).

HAPLOCELIS DÖRJES, 1968
Without body pigmentation. Rhabdoids present, scattered across body. Paired testes and ovaries. Vagina dorsal to the male copulatory organ. Male system includes a muscular, glandular penis invaginated into a muscular seminal vesicle. Female system includes ciliated, muscular vagina and a bursa with one or two coiled bursal nozzles. Common gonopore terminal. Type species: Haplocelis dichona (Marcus, 1954).

Marine, free-living; one species:
- Haplocelis dichona (Marcus, 1954), Dörjes 1968

PHARYNGIA NILLSON ET AL., 2011
Pharynx present. With dark-brown body pigmentation. Testes paired; ovary unpaired. Male system includes small penis invaginated into muscular seminal vesicle, well-developed ciliated antrum masculinum and ventral gonopore. Seminal bursa or any female accessory organs absent. Type species: Pharyngia furva Nillson et al., 2011.

Marine, free-living; one species:
- Pharyngia furva Nillson et al., 2011

Notes
Two other species of Isodiametridae have a pharynx (Isodiametra helgolandica and Praeaphanosta
Species of *Pharyngia* can be separated from both based on the absence of a seminal bursa.

**Praeaphanostoma** Dörjes, 1968

With or without body pigmentation. With small frontal organ. Rhabdoids small, often inconspicuous, in rows. Testes paired. Ovaries paired or unpaired. Male system includes a straight, tubular penis partially or completely invaginated into a muscular seminal vesicle. Female bursa present without appendage or cellular cap. Vagina with sphincter typically present. Gonopore(s) separate or common, ventral. Type species: *Praeaphanostoma chaetocaudatum* Dörjes, 1968.

- *Praeaphanostoma chaetocaudatum* Dörjes, 1968
- *Praeaphanostoma baltalimaniaformis* (Dörjes, 1968), comb. nov.
- *Praeaphanostoma bremifrons* Dörjes, 1968
- *Praeaphanostoma foramivora* Hooge & Tyler, 2008
- *Praeaphanostoma gusana* Hooge & Eppinger, 2005
- *Praeaphanostoma hyalinorhabdoida* (Kånneby & Jondelius, 2013), comb. nov.
- *Praeaphanostoma longum* Dörjes, 1968
- *Praeaphanostoma musculosum* Ehlers & Dörjes, 1979
- *Praeaphanostoma ornatus* (Kozloff, 2000), comb. nov.
- *Praeaphanostoma parvum* Rieger & Ott, 1971
- *Praeaphanostoma rubrum* Dörjes, 1968
- *Praeaphanostoma schillingi* (Hooge & Tyler, 2015), comb. nov.
- *Praeaphanostoma thalasophilum* Ehlers & Dörjes, 1979
- *Praeaphanostoma vitreum* Ehlers & Dörjes, 1979
- *Praeaphanostoma wadsworthi* Hooge & Tyler, 2003

**Praeconvoluta** Dörjes, 1968

Rhabdoids absent or sparsely present and small, scattered or in longitudinal rows. Frontal organ present. Testes paired. Ovary single or paired. Male system includes a muscular penis capped with a prostatic vesicle and invaginated into a muscular seminal vesicle. Female system includes a bursa with distinctly thick walls. Bursal appendage may be absent or present; if present may be a distinct nozzle or a cellular cap with or without one or more spots of concentrated actin. Vagina typically present without a well-developed sphincter. Sphincter always absent or weak. Gonopore common or male only, ventral. Type species: *Praeconvoluta karinae* Dörjes, 1968.

- *Praeconvoluta karinae* Dörjes, 1968
- *Praeconvoluta bocasensis* Hooge & Tyler, 2008
- *Praeconvoluta bruscai* (Hooge & Tyler, 2003), comb. nov.
- *Praeconvoluta castinea* Hooge & Tyler, 2003
- *Praeconvoluta collinae* (Hooge & Tyler, 2008), comb. nov.
- *Praeconvoluta colorata* (Ehlers & Dörjes, 1979), comb. nov.
- *Praeconvoluta hortalous* (Hooge & Tyler, 2003), comb. nov.
- *Praeconvoluta marginalis* (Ivanov, 1952), comb. nov.
- *Praeconvoluta minor* Faubel, 1974
- *Praeconvoluta norvegica* (Westl. 1946), comb. nov.
- *Praeconvoluta piscae* (Zauchner et al., 2015), comb. nov.
- *Praeconvoluta schmidtii* Faubel, 1977
- *Praeconvoluta stepphanii* Faubel & Regier, 1983
- *Praeconvoluta tigrina* Hooge & Tyler, 2003
- *Praeconvoluta tornuva* Hooge & Tyler, 1999
- *Praeconvoluta urua* (Marcus, 1954), comb. nov.
- *Praeconvoluta variomorpha* (Dörjes, 1968), comb. nov.

**Proaphanostoma** Dörjes, 1972


- *Proaphanostoma tenuissima* Dörjes, 1972

**Pseudaphanostoma** Westblad, 1946

Frontal organ present. Rhabdoids small and in longitudinal rows. Ovaries and testes paired or unpaired. Male system consists of a straight, eversible penis fully invaginated into a seminal vesicle and a distinct, ciliated antrum masculinum. Female system includes penis invaginated into a seminal vesicle and a distinct nozzle or cellular cap with or without one or more spots of concentrated actin. Vagina typically present without a well-developed sphincter. Sphincter always absent or weak. Gonopore common or male only, ventral. Type species: *Pseudaphanostoma variabilis* Westblad, 1946.

- *Pseudaphanostoma variabilis* Westblad, 1946
- *Pseudaphanostoma brevicaudatum* Dörjes, 1968
- *Pseudaphanostoma divae* Marcus, 1952
• *Pseudaphanostoma herringi* Hooge & Rocha, 2006
• *Pseudaphanostoma murmanicus* (Mamkaev, 1967) Dörjes, 1968
• *Pseudaphanostoma pelophilum* Dörjes, 1968
• *Pseudaphanostoma psmmophilum* Dörjes, 1968
• *Pseudaphanostoma smithri* Hooge & Tyler, 2003

**PSEUDOPOSTHIA WESTBLAD, 1946**

Without body pigmentation. With frontal organ and numerous small rhabdoid glands. Tests and ovaries paired. Male system includes a muscular penis associated with a separate glandular organ, a false seminal vesicle and a gonopore. False seminal vesicle not connected to the penis. True seminal vesicle absent. Glandular organ opens together with the penis at the gonopore. Male gonopore ventral, located at mid-body. Female accessory organs absent. Type species: *Pseudoposthia macrogonopora* Westblad, 1946.

Marine, free-living; one species:
• *Pseudoposthia macrogonopora* Westblad, 1946

**RIMICOLA BÖHMIG, 1908**

Without body pigmentation. Frontal organ present; rhabdoid glands absent. With paired testes and ovaries. Male system includes a well-developed penis, paired false seminal vesicles, a short antrum masculinum and ventral gonopore. True seminal vesicle absent. Female accessory organs absent. Type species: *Rimicola glacilis* Böhmig, 1908.

Marine, free-lining; one species:
• *Rimicola glacilis* Böhmig, 1908

**Notes**

*Rimicola* can be distinguished from most of the other genera of Isodiametridae by the absence of rhabdoid glands, a muscular seminal vesicle and female accessory organs. It is closest in morphology to *Pseudoposthia*, which also lacks a true seminal vesicle and bursa, but *Pseudoposthia* can be differentiated through the absence of an antrum masculinum and the presence of rhabdoids.

### Key to Isodiametridae

1a. Seminal bursa absent ................................................................. 2
1b. Seminal bursa present ................................................................. 7
2a. Pharynx absent .................................................................................. 3
2b. Pharynx present ................................................................................... Pharyngia
3a. Penis distinct. True seminal vesicle absent ........................................ 4
3b. Penis at least partially invaginated into muscular seminal vesicle ............ 5
4a. Rhabdoid glands absent. Male system includes false seminal vesicle, short antrum masculinum and muscular penis ...................................................................................................................... RIMICOLA
4b. Rhabdoid glands present. Male system includes muscular penis associated with a separate glandular organ and a separate false seminal vesicle ................................................................. PSEUDOPOSTHIA
5a. Rhabdoids absent ................................................................................... Avagina
5b. Rhabdoids present, usually in longitudinal rows .................................... 6
6a. Penis well developed and distinctly curved, only partially invaginated into seminal vesicle ...................................................................................................................... Baltalimania (B. fontaneti, B. occulta, B. sublitoralis, B. ylvae)
6b. Penis straight or nearly so, fully invaginated into seminal vesicle ............. PSEUDAPHANOSTOMA
7a. Bursa with distinct muscular walls .......................................................... 8
7b. Bursa tissue only ...................................................................................... Haploclisis
8a. Common gonopore terminal; vagina dorsal to male copulatory organ; bursa with one or two large, coiled nozzles ............................................................................................................................... Aphanostoma
8b. Gonopore usually ventral or subterminal. If terminal, gonopores separate, with female pore anterior (ventral) to male pore (terminal). Vagina never dorsal to male copulatory organ ....................................................... Diatomovora
9a. One or two bursas present, each with multiple sclerotized nozzles .............................................................. Praeaphanostoma
9b. Only a single bursa present, always without multiple sclerotized nozzles ........................................................... Praeaphanostoma
10a. Rhabdoids absent. Antrum masculinum absent. Penis an inpocketing of the epidermis ... Praeaphanostoma
10b. Rhabdoids present, scattered or in longitudinal rows ................................... 11
11a. Vaginal sphincter weak or absent .......................................................... Praeconvoluta
11b. Vaginal sphincter muscles well developed ............................................ Praeaphanostoma
12a. Frontal glands prominent. Penis curved. Bursal appendage present as nozzle or cap .......... Aphanostoma
12b. Frontal glands weak. Penis straight. Bursa always simple without appendage ....... Praeaphanostoma
and a glandular organ associated with the male copulatory organ.

*Rimicola* is also similar in morphology to other species of *Faerlea* (Mecynostomiidae). Species of both lack rhabdoids and a seminal bursa and have a small antrum masculinum. Unlike *Rimicola*, species of *Faerlea* are characterized, among other things, by a short penis with associated glands and a muscular seminal vesicle. Further investigation, including obtaining DNA sequences, is needed to assess the position of the genus within Isodiametridae and Acoela.

ACKNOWLEDGEMENTS

Material for this study was collected over many years and in multiple locations. We are grateful to the staff at the Tjärnö and Kristineberg Marine Labs, Biologische Station Helgoland, CCMAR Faro, Hawaii Institute of Marine Biology at Coconut Island and Plentzia Marine Station for providing opportunities for fieldwork. Our colleagues, Marco Curini Galletti, Francesca Leasi and Jon Norenburg, organized meiofauna workshops where we also collected material. Funding was received from the Swedish Taxonomy Initiative, the Swedish Species Information Centre (ArtDatabanken) grant number SLU.dha.2019.4.3-11 to UJ and access to the Plentzia Information Centre (ArtDatabanken) grant number 2018.4.3-11 to 22 S. ATHERTON and U. JONDELIUS.

ETHICS STATEMENTS

All necessary permits for field study and sampling have been obtained by the authors from the competent authorities. The study is compliant with the United Nations Convention of Biological Diversity (CBD) and Nagoya protocols.

DATA AVAILABILITY

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with accession numbers MZ518796-816, MZ519760-776, and MZ520441-463.

REFERENCES


Hooge MD, Rocha CEF. 2006. Acoela (Acoelomorpha) from the northern beaches of the state of São Paulo, Brazil, and a systematic revision of the family Otocelididae. Zootaxa 1335: 1–50.


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Table S1. List of species used in this study along with the corresponding collection country and GenBank accession number, where available. New genetic sequences are highlighted in bold. *** five species of Isodiametridae and Actinopodistidae with unstable positions that were excluded from analyses.

Table S2. Morphological character matrix. The morphology of each species was coded following original descriptions and illustrations, as well as photos and live observations where possible.? indicates the presence or absence of the character could not be determined or was not examined.

Figure S1. Concat tree full. Concatenated 28S, 18S and COI gene tree summary. Percent bootstrap values are given at each node. Sequences from all species in the study were included.
Figure S2. 18S tree full. 18S gene tree summary. Percent bootstrap values are given at each node. Sequences from all species in the study were included.

Figure S3. 18S tree full GBlocks. 18S gene tree summary. The alignment was filtered with GBlocks allowing for gap positions within the final blocks. Percent bootstrap values are given at each node. Sequences from all species in the study were included.

Figure S4. 18S tree part. 18S gene tree summary. Percent bootstrap values are given at each node. Sequences from six species of Isodiamentridae and Actinoposthiidae represented only by 18S sequences downloaded from GenBank (Table 1) were excluded.

Figure S5. 18S tree part GBlocks. 18S gene tree summary. The alignment was filtered with GBlocks allowing for gap positions within the final blocks. Percent bootstrap values are given at each node. Sequences from six species of Isodiamentridae and Actinoposthiidae represented only by 18S sequences downloaded from GenBank (Table 1) were excluded.

Figure S6. COI tree. Mitochondrial Cytochrome C Oxidase (COI) gene tree summary. Percent bootstrap values are given at each node.

Figure S7. 28S tree. 28S gene tree summary. Percent bootstrap values are given at each node.

Figure S8. 28S tree GBlocks. 28S gene tree summary. The alignment was filtered with GBlocks allowing for gap positions within the final blocks. Percent bootstrap values are given at each node.