International Journal for Parasitology 39 (2009) 497–511

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Proposal for a new tapeworm order, Rhinebothriidea

Claire J. Healy [a], Janine N. Caira [b,*], Kirsten Jensen [c], Bonnie L. Webster [d], D. Timothy J. Littlewood [d]

[a] Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, Ont., Canada M5S 2C6
[b] Department of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Road, Storrs, CT 06269-3043, USA
[c] Department of Ecology and Evolutionary Biology and the Natural History Museum and Biodiversity Research Center, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045, USA
[d] Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 4 September 2008
Accepted 5 September 2008

Keywords:
Cestoda
New order
Rhinebothriidea
Rhinebothriinae
Tetraphyllidea
Batoids
Elasmobranchs
ssrDNA
lsrDNA
Phylogeny
Stalked bothridia

ABSTRACT

The polyphyletic nature of the tapeworm order Tetraphyllidea Carus, 1863 is addressed in part with the establishment of the new order Rhinebothriidea for a subset of the taxa formerly comprising the phyllobothriid subfamily Rhinebothriinae (Platyhelminthes: Eucestoda). Support for the order comes from Bayesian, maximum likelihood, and parsimony analyses of complete ssrDNA and partial (D1–D3) lsrDNA sequence data for 58 cestode species. These data consisted of novel data generated for 40 species in 15 genera of candidate rhinebothriines and the cathetocephalidean species *Sanguilevator yearsleyi* as well as comparable data taken from GenBank for an additional 18 cestode species in 17 genera. In total, the species analyzed consisted of two Cathetocephalidea, two Litobothriidea, two Lecanicephalidea, three Proteocephalidea, and 49 Tetraphyllidea. The tetraphyllideans consisted of three Onchobothriidae, three Serendipidae, and 43 Phyllobothriidae (one Thysanocephalinae, one Echeneibothriinae, five Phyllobothriinae, 35 candidate Rhinebothriinae and the poorly known *Spongiobothrium*). This work suggests that some elements of current membership in the group are in need of revision. For example, while inclusion of the echeneibothriine genus *Echeneibothrium* and the phyllobothriine genera *Rhodobothrium* and *Anthocephalum*, and also *Spongiobothrium*, in the Rhinebothriidea is supported, inclusion of *Duplicibothrium* and *Caulobothrium* in the new order is not. Histological sections and scanning electron microscopy of selected members of the study group suggest that the presence of bothridial stalks may serve as an effective morphological feature to characterise the order. The group is restricted to elasmobranchs, and appears to have a particular affinity for Myliobatiformes. The new order includes at least 13 genera. Intraordinal relationships were determined to be insufficiently stable to justify the formal reorganization of rhinebothriidean families at this time.

1. Introduction

Recent interest in the reassessment of ordinal level relationships of the Class Cestoda has been fueled by preliminary phylogenetic analyses that resolve some of the traditionally recognized orders (see Khalil et al., 1994) as para- or even polyphyletic (e.g., Brooks et al., 1991; Hoberg et al., 1997, 1999a; Mariaux, 1998; Olson and Caira, 1999; Kodedová et al., 2000; Olson et al., 2001). In a number of cases, these results have been confirmed by analyses targeting broader taxon sampling of the orders in question (e.g., Hoberg et al., 1997, 1999b; Mariaux, 1998; Olson et al., 1999; Caira et al., 1999, 2001, 2005; Rego et al., 1998; de Chambrier et al., 2004; Brabec et al., 2006; Kuchta et al., 2007) and also by those emphasising greater amounts of data (e.g., Waeschenbach et al., 2007).

Reorganization of the ordinal level classification of the cestodes to reflect new views of their phylogenetic relationships has also now begun in earnest. For example, the paraphyly of the pseudophyllideans, which emerged from early phylogenetic work based on limited sampling of taxa (e.g., Hoberg et al., 1997; Mariaux, 1998; Kodedová et al., 2000; Olson et al., 2001), has subsequently been confirmed by studies based on denser taxon sampling (Brabec et al., 2006; Kuchta et al., 2007). In 2007, Kuchta and his collaborators formally brought the classification of these cestodes into line with their phylogenetic relationships by subdividing the Pseudophyllidea into two new orders, the Diphyllobothriidea and Bothriocephalidea (see Kuchta et al., 2007).

Another order that has emerged as uncontestably non-monophyletic is the Tetraphyllidea. Essentially all previous phylogenetic analyses, regardless of whether taxon sampling has been sparse (e.g., Hoberg et al., 1997; Waeschenbach et al., 2007) or relatively dense (Caira et al., 1999, 2001; Olson et al., 1999), have supported the polyphyly of the tetraphyllideans. Conspicuous among the groups contributing to this polyphyly is the most diverse of the

* Corresponding author. Tel.: +1 860 486 4060; fax: +1 860 486 6364.
E-mail address: janine.caira@uconn.edu (J.N. Caira).

0020-7519/$34.00
doi:10.1016/j.ijpara.2008.09.002

tetraphyllidean families, the Phyllobothriidae Braun, 1900, the artificial nature of which has been previously recognized by many previous workers (e.g., Wardle and McLeod, 1952; Williams, 1968; Brooks et al., 1981b; Ruhnke, 1993, 1994). The present paper is aimed at initiating the process of formally dismantling the Tetraphyllidea and reconfiguring its included taxa into monophyletic assemblages. Unfortunately, a complete reorganization of the order is beyond the scope of this study. Instead, we will focus here on a group for which there is mounting evidence to support both its monophyly and independence from the remaining Tetraphyllidea, specifically the phyllobothriid subfamily Rhinebothriinae Euzet, 1956.

The Rhinebothriinae was established in 1956 by Euzet for tetraphyllideans lacking a myzorhynchus, but bearing subdivided unarmed bothridia (Euzet, 1956). At that time Euzet considered the subfamily to include only the genus *Rhinebothrium* Linton, 1890. However, in his more recent, fuller treatment of the Tetraphyllidea, based on the same features, Euzet (1994) considered the Rhinebothriinae to include the five additional genera, *Rhinebothroides* Mayes, Brooks and Thorson, 1981, *Rhabdotobothrium* Euzet, 1953, *Duplicibothrium* Williams and Campbell, 1978, *Glyphobothrium* Williams and Campbell, 1977 and *Caulobothrium* Baer, 1948. More recently, Ball et al. (2003) added *Scalithrium* Ball, Neifar and Euzet, 2003 to the Rhinebothriinae. However, constituency of this subfamily, and the Phyllobothriidae in general, was viewed somewhat differently by Brooks and Barriga, who, in 1995, erected the family Serendipidae Brooks and Barriga, 1995 with *Serendip* Brooks and Barriga, 1995 as its type genus. These authors also transferred *Duplicibothrium* and *Glyphobothrium* from the Rhinebothriinae into the new family. Furthermore, they recognized a "*Rhinebothrium* group" comprised of *Caulobothrium*, *Rhabdotobothrium*, *Rhinebothrium*, *Tritaphros* Lönnberg, 1889, *Rhinebothroides* and the "*Phyllobothrium centrurum*" species group, the latter a collection of species for which Ruhnke (1994) had resurrected *Anthocephalum* Linton, 1890.

Molecular work conducted to date has resulted in the generation of sequence data for only eight species of Rhinebothriinae (sensu Euzet, 1994). These include *Rhinebothrium maccallumi* Linton, 1924 (see Olson and Caira, 1999: complete ssrDNA and partial elongation factor 1-alpha [Ef1-α]; Olson et al., 2001: partial lsrDNA) and *Rhabdotobothrium anterophallum* Campbell, 1975 (see Olson et al., 2001: ssrDNA and partial (D1-D3) lsrDNA). In addition, Olson et al. (1999) provided ssrDNA data for *Caulobothrium* sp., *Duplicibothrium minutum* Williams and Campbell, 1978, *Duplicibothrium* n. sp. 1 and n. sp. 2, *Rhinebothrium* sp. and *Rhinebothrium* n. sp. Subsets of these data have subsequently been used in molecular studies aimed at exploring the relationships of other cestode orders (e.g., Kodedová et al., 2000; de Chambrier et al., 2004; Caira et al., 2005; Brabec et al., 2006; Waeschenbach et al., 2007). However, in only three cases were more than a single rhinebothriine species included in any analysis. Two of these studies (Olson et al., 2001 and Caira et al., 2005) included only *R. maccallumi* and *R. anterophallum*. In both cases, these rhinebothriine species grouped as sister taxa. The study of Olson et al. (1999) included seven rhinebothriines (sensu Euzet, 1994) and thus represents the most dense sampling of the subfamily to date. The results of Olson et al. (1999) are intriguing both with respect to the monophyly of the Rhinebothriinae as well as the relationships among other tetraphyllidean taxa. In no case did the *Duplicibothrium* species group with the rhinebothriine species, a result consistent with Brooks and Barriga's (1995) concept of the subfamily, nor did the *Caulobothrium* species. However, in the tree resulting from parsimony analysis, the echeneibothriine *Echeneibothrium vernetae* Euzet, 1956 and the phyllobothriines *Anthocephalum* (three species) and *Rhodobothrium* Linton, 1889 (one species) grouped among the rhinebothriines. Also consistently grouping among the rhinebothriines was *Spongiobothrium* Linton, 1889, a taxon considered a *genus inquirendum* by Euzet (1994). The close affinity between species of *Rhodobothrium* and *Rhinebothrium* was also seen by Waeschenbach et al. (2007).

Several morphological studies investigating the relationships among select rhinebothriine taxa have also been conducted. The relationships of *Rhinebothrium* and *Rhinebothroides* species were explored in a series of papers by Brooks and his colleagues (e.g., Brooks et al., 1981a,b; Brooks and Deardorff, 1988). However, the paucity of information provided on characters and methods employed to analyze the character data make the results of these studies difficult to assess. Caira et al. (1999, 2001) examined tetraphyllidean relationships in a much broader context. Caira et al. (1999) found *Rhodobothrium* sp., *Rhinebothroides moralarai* (Brooks and Thorson, 1976) Mayes, Brooks and Thorson, 1981, *Caulobothrium* n. sp. and *Rhinebothrium* sp. to comprise a clade, only distantly related to a clade that included *D. minutum* and *Glyphobothrium zwerneri* Williams and Campbell, 1977. The latter result was also seen by Caira et al. (2001). However, in the latter more expanded analysis, relationships among the former genera were much less well resolved.

With respect to the taxonomic independence of the Rhinebothriinae from the other cestode orders and, in particular, from the remaining Tetraphyllidea, the limited taxon sampling in studies conducted to date has not allowed this idea to be fully explored. However, assuming the constituency of the group may need to be adjusted somewhat, at least preliminary support for the independence of the Rhinebothriinae from other tetraphyllidean taxa is provided in the minimum evolution trees of Olson and Caira (1999), and also in the analyses of Kodedová et al. (2000), Olson et al. (2001), Caira et al. (2005) and Waeschenbach et al. (2007).

The present study was undertaken with a substantially expanded sampling of Rhinebothriinae to address three specific issues: (i) the constituency and monophyly of the Rhinebothriinae, (ii) the relationships of the Rhinebothriinae to the remaining Tetraphyllidea, and (iii) the relationships of the Rhinebothriinae to the other cestode orders. Partial (D1–D3) lsrDNA and complete ssrDNA sequence data were generated de novo for 15 genera and 39 species of candidate rhinebothriines. These particular genes were chosen because previous studies have shown data from these genes to be of utility in resolving intra- and intergeneric relationships, as well as higher level relationships, among cestode orders (e.g., Olson et al., 1999, 2001). Data for each gene were analyzed individually and in combination, using maximum likelihood (ML), Bayesian inference (BI) and maximum parsimony (MP) methods. The ultimate goal of this study was to evaluate elevation of the Rhinebothriinae to the ordinal level and, if supported by analyses of this expanded dataset, to take the appropriate taxonomic action.

2. Materials and methods

2.1. Study taxa

In selecting study taxa, we strove to achieve as broad a taxon sampling of the Rhinebothriinae as possible. The only rhinebothriine genus recognized by Euzet (1994) or by Ball et al. (2003) for which material was unavailable was *Glyphobothrium*. Also taken into account was the fact that Healy (2006. A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium*, *Rhabdotobothrium*, *Rhinebothrium*, *Scalithrium* and *Spongiobothrium*. Doctoral dissertation, University of Connecticut, Storrs, CT, USA) showed the Rhinebothriinae to be much more diverse than previously thought, including a wide array of novel genera and species. Although many of these novel taxa have not yet been formally described, the availability of ethanol preserved material of representatives of many,

made their inclusion in this study possible, which greatly enhanced the power of the analyses presented here. The identities of these taxa are supported by the deposition of voucher specimens and by inclusion of scanning electron micrographs of a representative of each genus. A diversity of other tetraphyllideans, and also representatives of other cestode orders, with particular emphasis on taxa considered, like the rhinebothriines, to be acetabulate (sensu Caira et al., 1999) were also included (i.e., Lecanicephalidea and Proteocephalidea). Figs. 1–16 provide illustrations of 15 of the focus (potentially rhinebothriine) genera, as well as one of the non-rhinebothriine genera, *Acanthobothrium* Van Beneden, 1850.

The 58 species analyzed in this study are listed in Table 1 along with their hosts and collection localities. For the purposes of this study, new ssrDNA and partial lsrDNA data were generated for 40 of these species; ssrDNA and partial lsrDNA data for the remaining 18 species were obtained from published sequences available in GenBank; GenBank accession numbers for the ssrDNA and partial lsrDNA data are provided in Table 1. The ordinal level placement of each species, based on current cestode classification (sensu Khalil et al., 1994 as modified by Brooks and Barriga, 1995 for the Rhinebothriinae), and family or subfamily placement in the case of the tetraphyllideans are also provided. Based on these classification schemes, the 58 species included here consisted of two cathetocephalideans (two genera), two litobothriideans (one genus), two lecanicephalideans (two genera), three proteocephalideans (two genera), and 49 tetraphyllideans. The tetraphyllideans included three Onchobothriidae Braun, 1900 (three genera) and three Serendipidae (two genera); representation of phyllobothriid subfamilies was as follows: one Thysanocephalinae Euzet, 1953, one Echeneibothriinae de Beauchamp, 1905, five Phyllobothriinae de Beauchamp, 1905 (five genera), 35 Rhinebothriinae (10 genera) and one species of *Spongiobothrium*, a *genus inquirendum* of uncertain sub familial placement according to Euzet (1994). Among the tetraphyllideans, only *Ceratobothrium* and *Phyllobothrium* were represented by their type species.

A single specimen of each of the 40 species for which new data were generated was sequenced. For all but one of these, the tissue sequenced consisted of only a portion of the specimen; the remaining portion was prepared for light microscopy as a permanent whole mount to serve as a voucher for the identification of the sequenced specimen. These vouchers have been deposited in the Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, Storrs, CT, USA. Accession numbers are provided in Table 1. The single species for which a specimen voucher was not retained was the currently undetermined rhinebothriine from *Pristis clavata*. The small size of this specimen precluded division of the specimen; thus, the voucher of this specimen consists of a photograph taken of a wet mount of the specimen sequenced. This too has been deposited in LRP.

The litobothriidean *Litobothrium janovyi* Olson and Caira, 2001 was employed as the outgroup in the BI and ML analyses of all three data partitions (partial lsrDNA, ssrDNA and combined lsrDNA + ssrDNA). Both *L. janovyi* and *L. amplifica* (Kurochkin and Slankis, 1973) Euzet, 1994 were employed as outgroups in the MP analyses of all three data partitions. Litobothriideans were chosen as outgroups because they have consistently been placed as sister to a clade comprised of the acetabulate cestode lineages and their relatives in phylogenetic studies with broad taxonomic coverage of cestodes (e.g., Olson et al., 2001; Caira et al., 2005; Waeschenbach et al., 2007).

2.2. Scanning electron microscopy

Scolices of exemplars of 15 of the study genera were prepared for scanning electron microscopy (SEM). These specimens were hydrated in a graded ethanol series, transferred to 1.5% osmium tetroxide overnight, dehydrated in a graded ethanol series and placed in hexamethyldisilizane (HMDS, Ted Pella Inc., Redding, CA) for 15 min. They were subsequently allowed to air dry and were then mounted on carbon tape on aluminum stubs. Specimens were sputter-coated with approximately 40 nm of gold/palladium and examined with a LEO/Zeiss DSM 982 Gemini field emission scanning electron microscope (FESEM) or with a Zeiss LEO 1550 FESEM. The image of *Anthocephalum* was retaken from material prepared by Caira et al. (2001).

2.3. Histology

For the investigation of stalks, sections through the scolices of representatives of five genera (*Acanthobothrium*, *Duplicibothrium*, *Caulobothrium*, *Rhodobothrium* and *Rhinebothrium*) were prepared as follows. Specimens were dehydrated, cleared, embedded in paraffin and sectioned at 8 μm intervals using an Olympus CUT4060 retracting rotary microtome. They were subsequently placed on glass slides flooded with 3% sodium silicate, and allowed to dry on a slide warmer for 4–8 h. Sections were stained with Gill's or Delafield's hematoxylin, counterstained with eosin, cleared in xylene and mounted on glass slides under coverslips in Canada balsam. In the cases of *Anthocephalum* and *Clistobothrium* Dailey and Vogelbein, 1990 the scolex sections examined were borrowed from LRP (Nos. 2290 and 2468, respectively).

2.4. DNA extraction, gene amplification and sequencing

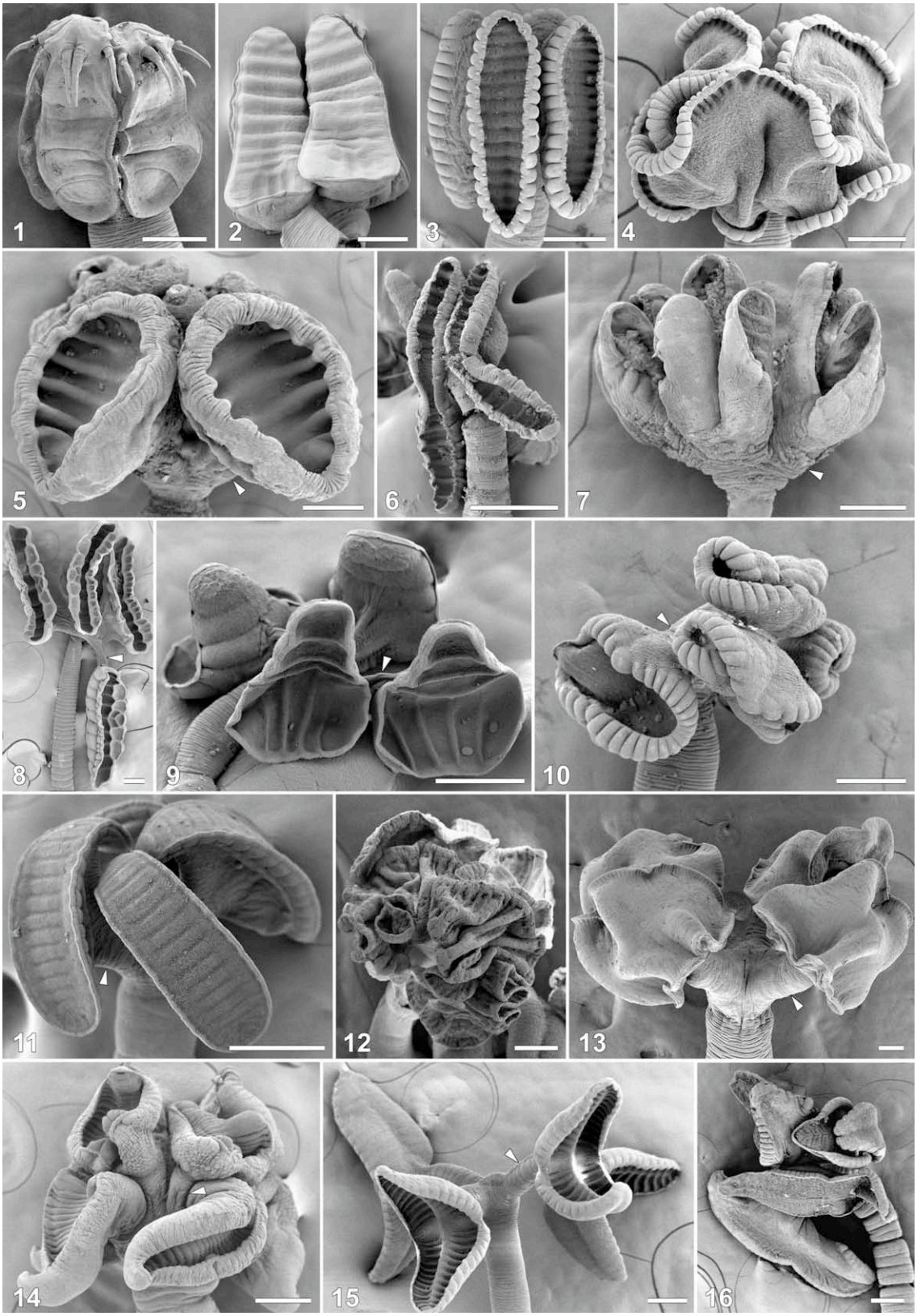
Total genomic DNA was extracted from ethanol preserved specimens using the DNeasy Tissue kit (Qiagen) according to the manufacturer's instructions. Partial lsrDNA (D1–D3) was amplified using the primer combination LSU5 and 1500R and complete ssrDNA was amplified using the primers WormA and WormB (see Table 2).

PCR amplifications were performed in a total reaction volume of 25 μl using Ready-to-go PCR Beads (Amersham Pharmacia Biotech) each containing 1.5 units DNA Taq Polymerase, 10 mM Tris–HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and stabilizers including BSA, 0.4 μM of each primer and 2 μl of DNA (~10 ng). Thermal cycling was performed in a Perkin Elmer 9600 Thermal Cycler and the PCR cycling conditions for both genes were: 2 min denaturing at 94 °C: 40 cycles of 1 min at 94 °C, 30 s at 52 °C, 2 min at 72 °C; followed by final extension period of 7 min at 72 °C.

PCR products were purified using QIAquick Gel Extraction Kit or QIAquick PCR purification Kit (QIAGEN). Sequencing of both strands was carried out on an Applied Biosystems 3730 DNA Analyser, using Big Dye version 1.1, using the original PCR primers together with a variety of internal primers to obtain the full sequence of each fragment on both strands (see Table 2). Sequence identity was checked using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nih.gov/BLAST/). Contigs were assembled using Sequencher 4.5 (GeneCodes Corporation).

2.5. Sequence alignment and phylogenetic analyses

Sequences were aligned initially with ClustalX (Thompson et al., 1997), with default settings and penalties as follows: gap opening 10, gap extension 0.20, delay divergent sequences 30%, DNA transition weight 0.5. The alignment was improved by eye in MacClade (Maddison, D.R., Maddison, W.P., 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4.08. Sinauer Associates, Sunderland, Massachusetts, USA) and with reference to secondary structure models (European Ribosomal RNA database; http://www.psb.ugent.be/rRNA/index.html). Regions that could not be unambiguously aligned were excluded from the analysis.



Figs. 1–16. Scanning electron micrographs of species representing select genera included in this study; scale bars: 100 μm. Fig. 1. *Acanthobothrium* sp.; Fig. 2. *Duplicibothrium minutum* Williams and Campbell, 1978; Fig. 3. *Caulobothrium* n. sp. 1; Fig. 4. *Anthocephalum alicae* Ruhnke, 1994; Fig. 5. *Echeneibothrium* sp.; Fig. 6. Rhinebothriinae n. sp. (ex *Pristis clavata*); Fig. 7. New genus 1 n. sp.; Fig. 8. New genus 2 *shipleyi*; Fig. 9. New genus 3 *cadenati*; Fig. 10. New genus 4 *kinabatanganensis*; Fig. 11. *Scalithrium* n. sp.; Fig. 12. *Spongiobothrium* sp.; Fig. 13. *Rhodobothrium paucitesticulare* Mayes & Brooks, 1981; Fig. 14. *Rhabdotobothrium anterophallum*; Fig. 15. *Rhinebothrium* sp.; Fig. 16. *Rhinebothroides* sp. Note, arrowheads in Figs. 5, 7–11, and 13–15 indicate stalks.

Table 1
Specimens included in molecular analyses.

Subfamily	Taxon	GenBank SSU	GenBank LSU	LRP voucher	Host	Locality
Order Cathetocephalidea						
	Cathetocephalus thatcheri Dailey & Overstreet, 1973	AY961398	AY961397			
	Sanguilevator yearsleyi Caira, Mega & Ruhnke, 2007[a]	FJ177057	FJ177097	LRP 4218 (TE-114)	*Lamiopsis temmincki* (BO-488)	South China Sea, off Mukah (2°53′52.16″N, 112°5′44.12″E), Sarawak, Malaysia
Order Lecanicephalidea						
	Cephalobothrium aetobatidis Shipley & Hornell, 1906[b]	AF124466	AF296927		*Aetobatus narinari*	Thailand
	Eniochobothrium gracilis Shipley & Hornell, 1906[c]	AF124465	AF286928		*Rhinoptera* sp.	Australia
Order Litobothriidea						
	Litobothrium amplifica (Kurochkin & Slankis, 1973) Euzet, 1994	AF124467	AF286931		*Alopias pelagicus*	Mexico
	Litobothrium janovyi Olson & Caira, 2001	AF124468	AF286930		*Alopias superciliosus*	Mexico
Order Proteocephalidea						
	Gangesia parasiluri Yamaguti, 1934	AJ287515	AF286935		*Silurus asotus*	Japan
	Proteocephalus macrocephalus (Creplin, 1825) Nufer, 1905	EF095247	EF095261		*Anguilla anguilla*	River Thames, Windsor, United Kingdom
	Proteocephalus perplexus LaRue, 1911	AF124472	AF286940		*Amia calva*	Canada
Order Tetraphyllidea family Onchobothriidae Braun, 1900						
	Acanthobothrium sp. 1	AF286993	AF286953		*Dasyatis longus*	Mexico
	Phoreibothrium sp.	AF286994	AF286954		*Sphyrna mokarran*	Gulf of Mexico, Mexico
	Platybothrium auriculatum Yamaguti, 1952	AF124470	AF286955		*Prionace glauca*	USA
Order Tetraphyllidea family Phyllobothriidae Braun, 1900						
Echeneibothriinae	*Echeneibothrium* sp.[a]	FJ177058	FJ177098	LRP 4217 (TE-94)	*Raja velezi* (BJ-243)	Gulf of California, off Santa Rosalia (27°20′44.15″N, 112°16′8.96″W), Baja California Sur, Mexico
Phyllobothriinae	*Anthocephalum* cf. *centrurum*[a]	FJ177059	FJ177099	LRP 4219 (TE-141)	*Dasyatis centroura* (SE-222)	Eastern Atlantic Ocean, off Mbour (14°24′21.75″N, 16°58′5.85″W), Senegal
Phyllobothriinae	*Ceratobothrium xanthocephalum* Monticelli, 1892	AF126085	AF382089		*Isurus oxyrinchus*	USA
Phyllobothriinae	*Clistobothrium montaukensis* Ruhnke, 1993	AF286996	AF286957		*Isurus oxyrinchus*	USA
Phyllobothriinae	*Crossobothrium longicolle* (Molin, 1958) Euzet, 1959[b]	AF286997	AF286958		*Scyliorhinus canicula*	United Kingdom
Phyllobothriinae	*Phyllobothrium lactuca* van Beneden, 1849	AF286999	AF286960		*Mustelus asterias*	United Kingdom
Phyllobothriinae	*Rhodobothrium paucitesticulare* Mayes & Brooks, 1981[a]	FJ177060	FJ177100	LRP 4216 (TE-61)	*Rhinoptera bonasus* (BNC-22)	Core Sound, Western Atlantic Ocean, off Davis (34°47′8.60″N, 76°26′24.71″W), North Carolina, USA
Rhinebothriinae	*Caulobothrium* n. sp. 1[a]	FJ177061	FJ177101	LRP 3912 (CH-23)	*Myliobatis californicus* (BJ-626)	Gulf of California, off Bahia de Los Angeles (28°59′8.71″N, 113°32′53.43″W), Baja California, Mexico
Rhinebothriinae	*Caulobothrium* n. sp. 2[a]	FJ177062	FJ177102	LRP 3916 (CH-28)	*Pteromylaeus bovinus* (SE-257)	Eastern Atlantic Ocean, off Diogué (12°34′29.57″N, 16°45′2.41″W), Senegal
Rhinebothriinae	*Caulobothrium* n. sp. 3[a]	FJ177063	FJ177103	LRP 3915 (CH-27)	*Pteromylaeus bovinus* (SE-143)	Eastern Atlantic Ocean, off Saint-Louis (16°1′28.04″N, 16°30′33.45″W), Senegal
Rhinebothriinae	*Caulobothrium* n. sp. 4[a]	FJ177064	FJ177104	LRP 3913 (CH-24)	*Pastinachus* cf. *sephen* (BO-164)	South China Sea, off Sematan (1°48′15.45″N, 109°46′47.17″E), Sarawak, Malaysia
Rhinebothriinae	*Caulobothrium* n. sp. 5[a]	FJ177065	FJ177105	LRP 3914 (CH-25)	*Pastinachus sephen* (NT-25)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Rhinebothriinae	*Caulobothrium opisthorchis* Riser, 1955[a]	FJ177066	FJ177106	LRP 3910 (CH-21)	*Myliobatis californicus* (BJ-626)	Gulf of California, off Bahia de Los Angeles (28°59′8.71″N, 113°32′53.43″W), Baja California, Mexico
Rhinebothriinae	New genus 1 n. sp.[a]	FJ177067	FJ177107	LRP 3902 (CH-11)	*Himantura* cf. *gerrardi* (BO-466)	South China Sea, off Mukah (2°53′52.16″N, 112°5′44.12″E), Sarawak, Malaysia

(*continued on next page*)

Table 1 (*continued*)

Subfamily	Taxon	GenBank SSU	GenBank LSU	LRP voucher	Host	Locality
Rhinebothriinae	New genus 2 cf. *sexorchidum*[a]	FJ177068	FJ177108	LRP 3922 (CH-35)	*Taeniura lymma* (BO-86)	Celebes Sea, off Semporna (4°35′56.83″N, 118°39′44.42″E), Sabah, Malaysia
Rhinebothriinae	New genus 2 *shipleyi*[a]	FJ177069	FJ177109	LRP 3894 (CH-3)	*Dasyatis kuhlii* (BO-336)	South China Sea north of Kuching (2°30′7.34″N, 110°40′16.82″E), Sarawak, Malaysia
Rhinebothriinae	New genus 3 *cadenati*[a]	FJ177070	FJ177110	LRP 3924 (CH-37)	*Zanobatus schoenleinii* (SE-201)	Eastern Atlantic Ocean, off Soumbédioune (14°40′42.00″N, 17°27′42.00″W), Senegal
Rhinebothriinae	New genus 3 n. sp. 1[a]	FJ177071	FJ177111	LRP 3898 (CH-7)	*Dasyatis* cf. *margaritella* (SE-125)	Eastern Atlantic Ocean, off Mbour (14°24′21.75″N, 16°58′5.85″W), Senegal
Rhinebothriinae	New genus 3 n. sp. 2[a]	FJ177072	FJ177112	LRP 3899 (CH-8)	*Dasyatis* cf. *margaritella* (SE-125)	Eastern Atlantic Ocean, off Mbour (14°24′21.75″N, 16°58′5.85″W), Senegal
Rhinebothriinae	New genus 3 n. sp. 3[a]	FJ177073	FJ177113	LRP 3905 (CH-14)	*Himantura toshi* (NT-26)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Rhinebothriinae	New genus 3 n. sp. 4[a]	FJ177074	FJ177114	LRP 3906 (CH-15)	*Himantura toshi* (NT-26)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Rhinebothriinae	New genus 3 n. sp. 5[a]	FJ177075	FJ177115	LRP 3909 (CH-20)	*Himantura undulata* (NT-117)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Rhinebothriinae	New genus 3 n. sp. 6[a]	FJ177076	FJ177116	LRP 3926 (CH-45)	*Himantura walga* (BO-237)	South China Sea, off Mukah (2°53′52.16″N, 112°5′44.12″E), Sarawak, Malaysia
Rhinebothriinae	New genus 3 n. sp. 7[a]	FJ177077	FJ177117	LRP 3917 (CH-30)	*Rhinobatos typus* (AU-56)	Timor Sea, Fog Bay, off Dundee Beach (12°45′32.56″S, 130°21′6.90″E), Northern Territory, Australia
Rhinebothriinae	New genus 4 *kinabatanganensis* (Healy, 2006)[a]	FJ177078	FJ177118	LRP 3900 (CH-9)	*Himantura chaophraya* (BO-108)	Kinabatangan River, off Kampung Abai (5°41′10.81″N, 118°23′8.35″E), Sabah, Malaysia
Rhinebothriinae	*Rhabdotobothrium anterophallum* Campbell, 1975	AF287000	AF286961		*Mobula hypostoma*	USA.
Rhinebothriinae	Rhinebothriinae n. sp.[a]	FJ177079	FJ177119	LRP 4220 (CH-26)	*Pristis clavata* (AU-36)	Timor Sea, near Buffalo Creek (12°20′11.49″S, 130°54′39.01″E), Lee Point, Northern Territory, Australia
Rhinebothriinae	*Rhinebothrium maccallumi* Linton, 1924[b]	AF124476	AF286962		*Dasyatis americana*	Mexico
Rhinebothriinae	*Rhinebothrium megacanthophallus* Healy, 2006[a]	FJ177080	FJ177120	LRP 3901 (CH-10)	*Himantura chaophraya* (BO-108)	Kinabatangan River, off Kampung Abai (5°41′10.81″N, 118°23′8.35″E), Sabah, Malaysia
Rhinebothriinae	*Rhinebothrium* sp. 1[a]	FJ177081	FJ177121	LRP 3903 (CH-12)	*Himantura pastinacoides* (B0-76)	Sulu Sea, off Kampung Tetabuan (6°1′6.89″N, 117°42′19.67″E), Sabah, Malaysia
Rhinebothriinae	*Rhinebothrium* sp. 10[a]	FJ177082	FJ177122	LRP 3920 (CH-33)	*Taeniura lymma* (BO-86)	Celebes Sea, off Semporna (4°35′56.83″N, 118°39′44.42″E), Sabah, Malaysia
Rhinebothriinae	*Rhinebothrium* sp. 11[a]	FJ177083	FJ177123	LRP 3923 (CH-36)	*Taeniura lymma* (BO-131)	Celebes Sea, off Pulau Mabul (4°14′44.02″N, 118°37′53.32″E), Sabah, Malaysia

Table 1 (*continued*)

Subfamily	Taxon	GenBank SSU	GenBank LSU	LRP voucher	Host	Locality
Rhinebothriinae	*Rhinebothrium* sp. 2[a]	FJ177084	FJ177124	LRP 3907 (CH-16)	*Himantura uarnacoides* (BO-91)	Sulu Sea, off Beluran (5°53′52.04″N, 117°33′21.29″E), Sabah, Malaysia
Rhinebothriinae	*Rhinebothrium* sp. 3[a]	FJ177085	FJ177125	LRP 3908 (CH-19)	*Himantura undulata* (NT-117)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Rhinebothriinae	*Rhinebothrium* sp. 4[a]	FJ177086	FJ177126	LRP 3892 (CH-1)	*Dasyatis akajei* (JN-1)	Western Pacific Ocean, Suruga Bay, off Shimizu (35°01′00″N, 138°29′00″E), Shizuoka, Central Honshu, Japan
Rhinebothriinae	*Rhinebothrium* sp. 5[a]	FJ177087	FJ177127	LRP 3893 (CH-2)	*Dasyatis brevis* (BJ-51)	Gulf of California, off Puertecitos (30°20′58.15″N, 114°38′21.61″W), Baja California, Mexico
Rhinebothriinae	*Rhinebothrium* sp. 6[a]	FJ177088	FJ177128	LRP 3896 (CH-5)	*Dasyatis longus* (BJ-423)	Gulf of California, off San José del Cabo (23°2′45.22″N, 109°41′33.19″W), Baja California Sur Mexico
Rhinebothriinae	*Rhinebothrium* sp. 7[a]	FJ177089	FJ177129	LRP 3897 (CH-6)	*Dasyatis* cf. *margaritella* (SE-123)	Eastern Atlantic Ocean, off Mbour (14°24′21.75″N, 16°58′5.85″W), Senegal
Rhinebothriinae	*Rhinebothrium* sp. 8[a]	FJ177090	FJ177130	LRP 3930 (CH-55)	*Paratrygon* cf. *aiereba* (PU-10m)	Blanquillo (12°23′26.75″S, 70°42′32.06″W), Madre de Dios, Peru
Rhinebothriinae	*Rhinebothrium* sp. 9[a]	FJ177091	FJ177131	LRP 3921 (CH-34)	*Taeniura lymma* (BO-86)	Celebes Sea, off Semporna (4°35′56.83″N, 118°39′44.42″E), Sabah, Malaysia
Rhinebothriinae	*Rhinebothroides* cf. *freitasi*[a]	FJ177092	FJ177132	LRP 3929 (CH-54)	*Potamotrygon* cf. *castexi* (PU-25 b)	Boca Manu (12°16′16.27″S, 70°55′6.84″W), Madre de Dios, Peru
Rhinebothriinae	*Scalithrium* n. sp.[a]	FJ177093	FJ177133	LRP 3895 (CH-4)	*Dasyatis longus* (BJ-423)	Gulf of California, off San José del Cabo (23°2′45.22″N, 109°41′33.19″W), Baja California Sur Mexico
Rhinebothriinae	*Spongiobothrium* sp.[a]	FJ177094	FJ177134	LRP 3919 (CH-32)	*Rhynchobatus* cf. *australiae* (NT-66)	Gulf of Carpentaria, Arafura Sea, east of Wessel Islands (11°17′43.63″S, 136°59′48.26″E), Northern Territory, Australia
Thysanocephalinae	*Thysanocephalum* sp.	AF287001	AF286963		*Galeocerdo cuvier*	U.S.A.
Order Tetraphyllidea family Serendipidae Brooks and Barriga, 1995						
	Duplicibothrium n. sp.[a]	FJ177095	FJ177135	LRP 3918 (CH-31)	*Rhinoptera* sp. (SE-84)	Eastern Atlantic Ocean, off Saint-Louis (16°1′28.04″N, 16°30′33.45″W), Senegal
	Duplicibothrium cf. minutum[a]	FJ177096	FJ177136	LRP 3928 (CH-49)	*Rhinoptera bonasus* (SE-254)	Eastern Atlantic Ocean, off Diogué (12°34′29.57″N, 16°45′2.41″W), Senegal

[a] Species for which new data were generated for this study (FJ177057–FJ177136).
[b] Species identification requires verification.
[c] *Eniochobothrium euaxos* Jensen, 2005 (see Jensen, 2005).

The full alignments for ssrDNA and partial lsrDNA gene partitions are available in Supplementary Table S1, which also provides an indication of exclusion sets. Modeltest version 3.7macX (Posada and Crandall, 1998) was used to select a model of evolution using the Akaike Information Criterion. In all BI, ML, MP analyses, gaps were treated as missing data (i.e., "?").

The data were partitioned into three character sets: (1) partial lsrDNA, (2) complete ssrDNA, (3) combined lsrDNA + ssrDNA. Phylogenetic trees were constructed using Bayesian Inference with MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003), Maximum Likelihood with PAUP* version 4.0b10 (Swofford, D.L. 2002. PAUP: Phylogenetic analysis using parsimony. Version PAUP* 4.0b10. Sinauer Associates, Sunderland, Massachusetts, USA) and Maximum Parsimony with PAUP* version 4.0b10 (Swofford, D.L. 2002. PAUP: Phylogenetic analysis using parsimony. Version PAUP* 4.0b10. Sinauer Assocaites, Sunderland, Massachusetts, USA) and Tree analysis using New Technology (TNT) version 1.1 (Goloboff, Farris and Nixon, 2003, http://www.zmuc.dk/public/phylogeny/TNT/). The BI and ML analyses were run on a four dual-core Opteron-based Unix cluster (http://pug.nhm.ac.uk); MP analyses were run on an Intel Pentium D (3.4 GHz) processor.

For BI, likelihood settings were set to nst = 6, rates = invgamma, ngammacat = 4 (equivalent to the GTR+I+G model of evolution, as suggested by MrModeltest 2.2; Nylander, J. A. A. 2004. MrModelTest, a program distributed by the author. Evolutionary Biology Center, Upsala University, Sweden). In the 'lsrDNA + ssrDNA' analyses, parameters were estimated separately for each gene. Four chains (temp = 0.2) were run for 5,000,000 generations and

Table 2
Primers used for the amplification and sequencing of partial (D1–D3) lsrDNA and complete ssrDNA.

Primers	Sequence (5′–3′)
lsrDNA	
Amplification & sequencing	
LSU5	TAGGTCGACCCGCTGAAYTTAAGCA
L1500R	CGAAGTTTCCCTCAGGATAGCAAC
Internal sequencing	
L300R	GTTCATGGCACTCCCTTTCAAC
ECD2	CTTGGTCCGTGTTTCAAGACGGG
L1200F	CCCGAAAGATGGTGAACTATGC
L1200R	CCGAAAGATGGTGAACTATGC
ssrDNA	
Amplification & sequencing	
WormA	GCGAATGGCTCATTAAATCAG
WormB	CTTGTTACGACTTTTACTTCC
Internal sequencing	
300F	AGGGTTCGATTCCGGAG
300R	TCAGGCTCCCTCTCCGGA
930F	GCATGGAATAATGGAATAGG
1200F	CAGGTCTGTGATGCCC
1200R	GGGCATCACAGACCTG

sampled every 1,000 generations. Three million generations were discarded as 'burn-in', after checking that log likelihood values had 'plateaued' (usually after 500,000 generations). Tracer v1.4 (Rambaut and Drummond, University of Edinburgh) was used to determine this burn-in value and assess whether each of the Bayesian analyses had reached stationarity within the burn-in sample. Nodal support is indicated with posterior probabilities. Clades with BI posterior probabilities of $\geqslant$95% were considered to have high support.

ML analyses were performed using successive approximation: model parameters were estimated based on a starting tree determined by neighbor-joining (NJ). A heuristic search was performed implementing the estimated model parameters using nearest-neighbor-interchange (NNI) branch swapping. Model parameters were estimated on the best tree and a heuristic search performed using subtree-pruning-regrafting (SPR) branch swapping. After estimating model parameters, heuristic searches using tree-bisection-reconnection (TBR) branch swapping were performed until the topology remained unchanged. ML bootstrap values for 100 replicates were obtained using Genetic Algorithm for Rapid Likelihood Inference (GARLI) version 0.951 (Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, USA) using default settings, except setting 'Genthreshfortopoterm' to 10,000 generations. Clades with ML bootstrap resampling values of $\geqslant$70% were considered to have high support.

For parsimony analyses, heuristic searches were conducted using 100,000 random-addition sequence replicates and TBR branch swapping was employed. Starting trees were obtained through stepwise addition. Characters were treated as unweighted and unordered, gaps were treated as missing ("?"), and ACCTRAN character optimisation was in effect. Branches were collapsed if their minimum length equaled zero. The results of the MP analysis of each data partition were summarised as strict consensus trees. MP bootstrap values for 10,000 replicates were obtained using TNT. Clades with MP bootstrap resampling values of $\geqslant$80% were considered to have high nodal support.

3. Results

The combined aligned dataset of ssrDNA and partial lsrDNA consisted of 3,978 characters. Of these, 1,212 characters could not be unambiguously aligned, leaving 2,766 alignable characters, of which 1,743 were from ssrDNA and 1,023 from lsrDNA. The number of variable positions was 355 (20.37%) for ssrDNA and 469 (45.85%) for lsrDNA; under the principles of parsimony there were 253 (14.52%) and 380 (37.16%) informative positions for ssrDNA and lsrDNA, respectively. Per unit length of gene sequenced, lsrDNA provided more than twice as many variable positions and phylogenetically informative characters (under the principles of parsimony) as ssrDNA. The results of incongruence length difference (ILD) tests, as implemented in PAUP*, indicated that the ssrDNA and lsrDNA datasets were not congruent ($P = 0.01$). Nonetheless, these datasets were analyzed separately and in combination in order to examine the effects of combining these data.

3.1. Molecular phylogenies

3.1.1. Combined ssrDNA + lsrDNA analyses

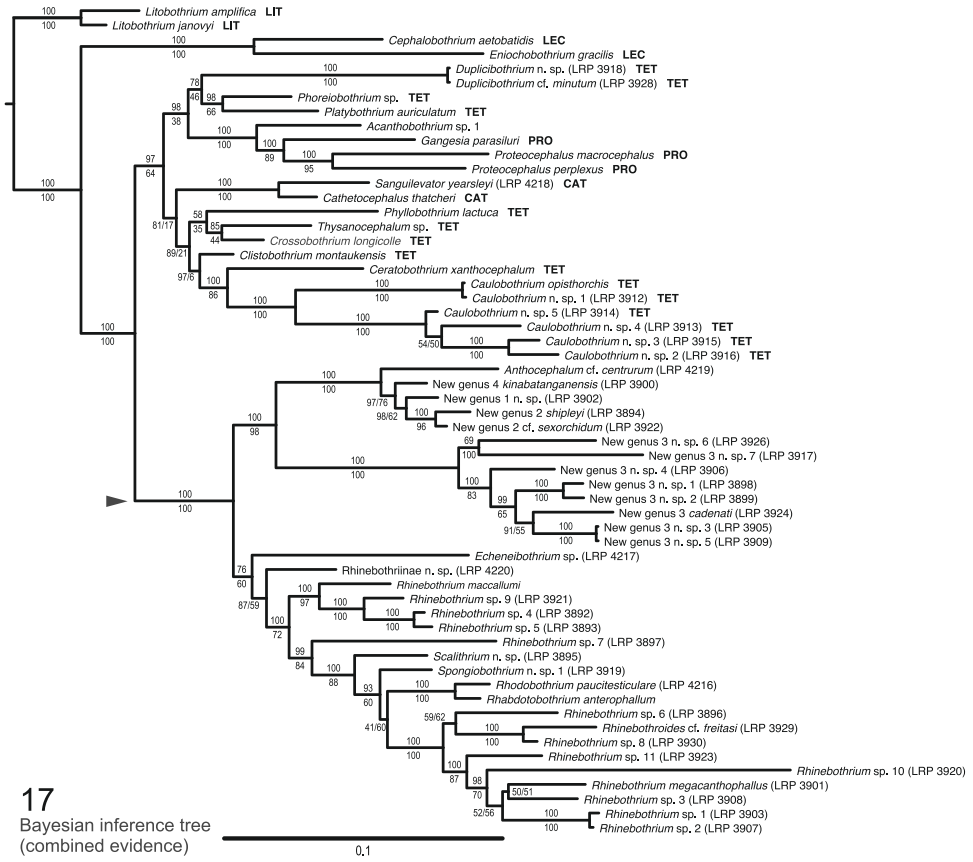
The combined evidence (ssrDNA + lsrDNA) BI tree was inferred using the GTR+I+G model for each partition and is shown in Fig. 17 with nodal support from posterior probabilities given above the lines. Because the tree resulting from ML analysis differed only slightly from the BI tree, also indicated in Fig. 17, below the lines, are the ML bootstrap resampling percentages from ML ($n = 100$) for the topology shown. Differences in topology between the ML and BI trees were as follows: (i) *Phyllobothrium lactuca* Van Beneden, 1850 and *Clistobothrium montaukensis* Ruhnke, 1993 were sister taxa in the former (as in the topology shown in Fig. 20), rather than *P. lactuca* appearing as sister taxon to *Thysanocephalum* sp. + *Crossobothrium longicolle* (Molin, 1858) Euzet, 1959 and the latter sister taxon to the group comprised of *Ceratobothrium xanthocephalum* Monticelli, 1892 and the *Caulobothrium* spp., (ii) *Caulobothrium* n. sp. 4 was the sister taxon to *Caulobothrium* n. sp. 5 (as in the topology shown in Fig. 20), rather than to the group comprised of *Caulobothrium* n. sp. 2 and n. sp. 3, (iii) *Spongiobothrium* n. sp. 1 was the sister taxon to *Rhodobothrium paucitesticulare* Mayes and Brooks, 1981 + *R. anterophallum* (as in the topology shown in Fig. 22), rather than sister taxon to a group comprised of the latter two taxa plus *Rhinebothrium* species 1, 2, 3, 6, 8, 10, 11, and *Rhinebothrium megacanthophallus* Healy, 2006 and *Rhinebothroides* cf. *freitasi*.

The combined evidence (ssrDNA + lsrDNA) MP tree is the strict consensus of the four most parsimonious trees and is shown in Fig. 18, with nodal support, as indicated by bootstrap values >50%, shown above the lines. As is evident by comparison of Figs. 17 and 18, differences between the BI and ML, and MP tree topologies were seen within, but not among major clades. Conspicuous differences between the trees, outside of the rhinebothriines were seen in the positions of *Duplicibothrium* spp., *C. montaukensis*, the two cathetocephalidean species, and *P. lactuca*. Within the rhinebothriines, the greatest differences were seen in the positions of *Echeneibothrium* sp., the Rhinebothriinae n. sp. and *Rhinebothrium* sp. 10.

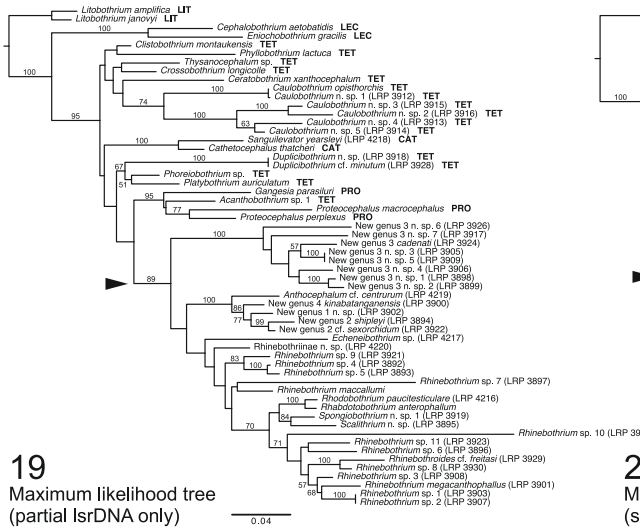
All analyses of the combined data fully supported monophyly of the rhinebothriines if the group is considered to include *Anthocephalum*, New genera 1–4, *Echeneibothrium*, the new rhinebothriine from *P. clavata*, *Rhinebothrium*, *Rhodobothrium*, *Rhabdotobothrium*, *Rhinebothroides*, *Scalithrium* and *Spongiobothrium*. All analyses were also unanimous in their lack of support for *Duplicibothrium* and *Caulobothrium* species as members of the Rhinebothriinae.

3.1.2. Analyses of lsrDNA

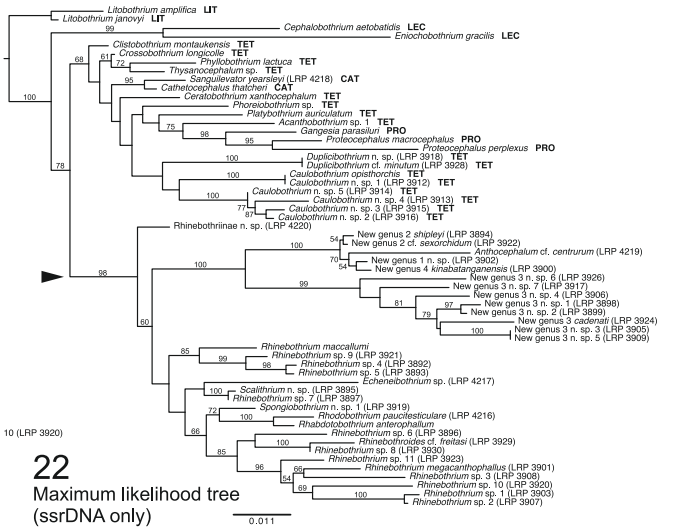
With lsrDNA, branching patterns from the estimates provided by ML (Fig. 19) and BI (Fig. 20) were identical with the single exception that *R. maccallumi* was resolved as the sister taxon to *Rhinebothrium* sp. 7 in ML but paraphyletic relative to this species



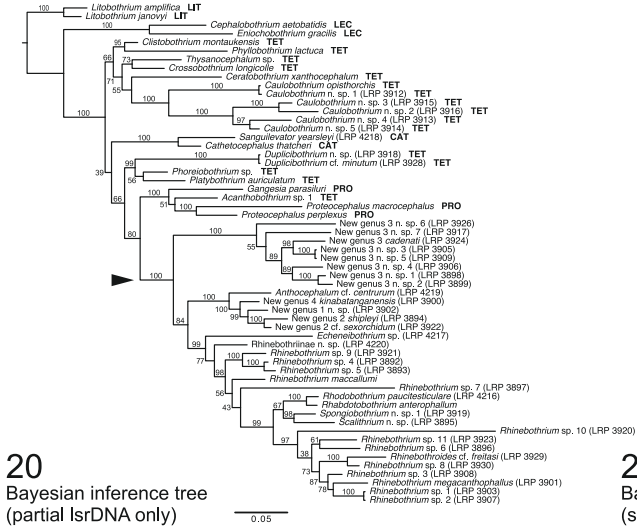
Figs. 17 and 18. Fig. 17. Combined evidence (ssrDNA and partial lsrDNA) Bayesian inference tree. Nodal support is given as posterior probabilities (above the line) and bootstrap values (below the line); arrowhead indicates the root of the rhinebothriidean clade; −ln likelihood = 20097.95, scale: number of substitutions per site; Fig. 18. Combined evidence (ssrDNA and partial lsrDNA) maximum parsimony tree (strict consensus of four most parsimonious trees; 3,189 steps, consistency index = 0.334, retention index = 0.645)); arrowhead indicates the root of the rhinebothriidean clade. Nodal support is given as bootstrap values >50% above the line. Abbreviations: CAT, Cathetocephalidea; LEC, Lecanicephalidea; LIT, Litobothriidea; PRO, Proteocephalidea; TET, Tetraphyllidea.



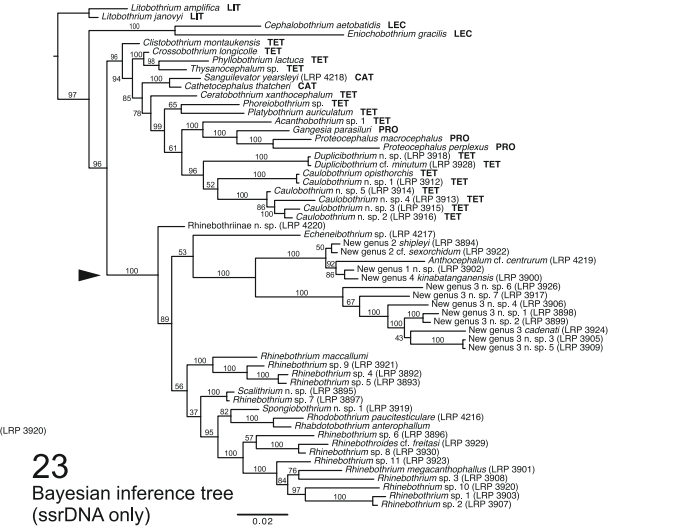
19
Maximum likelihood tree
(partial lsrDNA only)



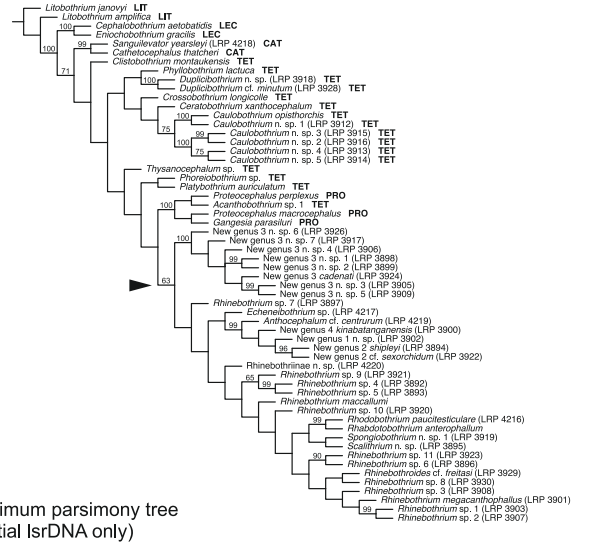
22
Maximum likelihood tree
(ssrDNA only)



20
Bayesian inference tree
(partial lsrDNA only)



23
Bayesian inference tree
(ssrDNA only)



21
Maximum parsimony tree
(partial lsrDNA only)

24
Maximum parsimony tree
(ssrDNA only)

Figs. 19–24. Fig. 19. Maximum likelihood tree (partial lsrDNA only). Nodal support is given as bootstrap values >50% above the line; Fig. 20. Bayesian inference tree (partial lsrDNA only); −ln likelihood = 11,057.59. Nodal support is given as posterior probabilities above the line; Fig. 21. Maximum parsimony tree (partial lsrDNA only) (strict consensus of two most parsimonious trees; 2,012 steps, consistency index = 0.316, retention index = 0.617). Nodal support is given as bootstrap values >50% above the line; Fig. 22. Maximum likelihood tree (ssrDNA only). Nodal support is given as bootstrap values >50% above the line; Fig. 23. Bayesian inference tree (ssrDNA only); −ln likelihood = 8,798.86. Nodal support is given as posterior probabilities above the line; Fig. 24. Maximum parsimony tree (ssrDNA only) (strict consensus of three most parsimonious trees; 1,127 steps, consistency index = 0.386, retention index = 0.713). Nodal support is given as bootstrap values >50% above the line. Note, arrowheads in Figs. 19–24 indicate the root of the rhinebothriidean clade. Abbreviations: CAT, Cathetocephalidea; LEC, Lecanicephalidea; LIT, Litobothriidea; PRO, Proteocephalidea; TET, Tetraphyllidea.

in BI. However, the estimate provided by MP (Fig. 21, strict consensus of two most parsimonious trees) differed in a number of respects from the BI and ML estimates. With respect to potential rhinebothriine taxa, the *Duplicibothrium* spp. were sister taxa to *P. lactuca* (MP), rather than sister to *Phoreiobothrium* sp. and *Platybothrium auriculatum* Yamaguti, 1952 (BI and ML), but in all three cases grouped outside the rhinebothriine clade. New genus 3 n. sp. 4 was part of a polytomy with the remaining two lineages of New genus 3 (MP), rather than the sister taxon to New genus 3 n. sp. 1 and 2 (BI and ML). *Rhinebothrium* sp. 7 was basal to a clade comprised of all rhinebothriines except for New genus 3 (MP), rather than sister to a clade comprised of a much more restricted subset of rhinebothriines (BI and ML). *Echeneibothrium* sp. was sister to a clade comprised of *Anthocephalum* cf. *centrurum* and New genera 1, 2 and 4 (MP), rather than the sister taxon to a clade comprised of *Rhinebothrium*, *Rhinebothroides*, *Rhabdotobothrium*, *Spongiobothrium* and *Scalithrium* spp. (BI and ML). *Rhinebothrium* sp. 10 was the sister taxon to a clade comprised of *Rhodobothrium*, *Rhabdotobothrium*, *Spongiobothrium*, *Scalithrium* spp. + *Rhinebothrium* and *Rhinebothroides* species (MP), rather than the sister taxon to the clade of *Rhinebothrium* and *Rhinebothroides* species (BI and ML).

3.1.3. Analyses of ssrDNA

The ML (Fig. 22) and BI (Fig. 23) estimates of ssrDNA trees differed only in two places. First, in the relationship between *Phoreibothrium* sp. and *P. auriculatum*; with BI these were estimated as being sister taxa, but with ML, they were paraphyletic to the clade including *Acanthobothrium* sp. 1 and the Proteocephalidea. *Echeneibothrium* sp. 1 was the only other taxon differing in position between the BI and ML estimates of the ssrDNA phylogeny, falling either as sister to the clade comprised of New genera 1–4 + *Anthocephalum* cf. *centrurum* (BI), or as the sister group to a clade comprised of *Scalithrium* n. sp. + *Rhinebothrium* sp. 7 (ML). The MP estimate of ssrDNA (Fig. 24, strict consensus of three most parsimonious trees) was most similar to the BI estimate, differing only in that *Caulobothrium* sp. 5 was part of a polytomy with *Caulobothrium* spp. 4 and 2 + 3, rather than sister to a clade comprised of these taxa, and in that *Anthocephalum* cf. *centrurum* was sister to a clade comprised of New genera 1, 2 and 4, rather than sister to New genus 1 and 4, and finally in that New genus 3 *cadenati* was sister to a clade containing four of its congeners, rather than sister to New genus 3 n. spp. 3 and 5.

3.1.4. Comparison of ssrDNA and lsrDNA trees

Comparison of the trees resulting from analysis of each gene individually using ML (Fig. 19 lsrDNA and Fig. 22 ssrDNA), BI (Fig. 20 lsrDNA and Fig. 23 ssrDNA) and MP (Fig. 21 lsrDNA and Fig. 24 ssrDNA) revealed considerable differences between trees estimated for the individual genes, regardless of the type of analysis performed. These differences are presented in the light of varying levels of nodal support arising from the differences in information content provided by these two ribosomal genes. Topological differences arose only amongst taxa whose positions were supported by low nodal support. Bearing in mind that our criteria for high nodal support was BI posterior probability with $\geqslant$95%, ML bootstrap resampling values of $\geqslant$70%, and MP bootstrap values of $\geqslant$80%.

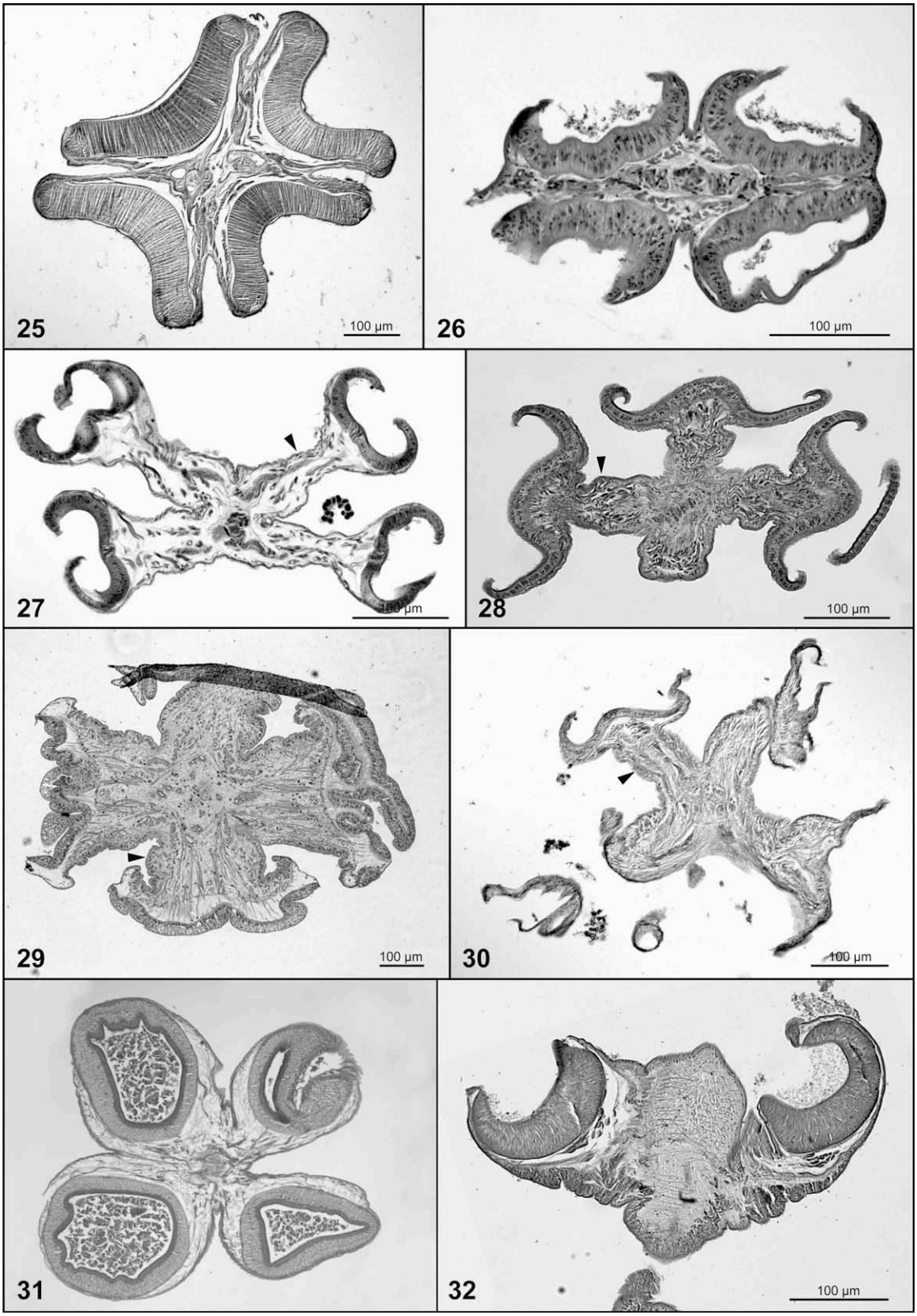
However, most relevant to the primary objective here is that in the trees resulting from all analyses of all data partitions, support was high for a clade comprised of species of the rhinebothriine genera *Rhinebothrium*, *Rhinebothroides*, *Rhabdotobothrium*, *Scalithrium*, *Spongiobothrium*, New genus 1, New genus 2, New genus 3 and New genus 4, as well as the included species of *Anthocephalum*, *Echeneibothrium* and *Rhodobothrium*. In the combined analyses this clade had a posterior probability of 100% (BI), a bootstrap resampling value of 100% (ML), and a bootstrap resampling value of 99% (MP). In the analysis of partial lsrDNA alone, the clade had a bootstrap resampling value of 89% (ML), a posterior probability of 100% (BI), and a bootstrap resampling value of 63% (MP). In the analysis of ssrDNA alone, the clade had a bootstrap resampling value of 98% (ML), a posterior probability of 100% (BI), and a bootstrap resampling value of 98% (MP). All of the analyses were also consistent in the placement of the six species of *Caulobothrium* and two species of *Duplicibothrium* outside the rhinebothriines.

4. Discussion

Molecular phylogenetic analysis has confirmed previous hypotheses concerning the need to recognise rhinobothriine cestodes as a monophyletic clade independent of the remaining Tetraphyllidea. We consider this group to be worthy of ordinal status within the Cestoda. While the constituency of the clade requires some revision with respect to specific phyllobothriine and echeneibothriine taxa, it is generally consistent with Euzet's (1994) concept of the group as revised by Brooks and Barriga (1995).

The monophyly of the rhinebothriines relative to the other acetabulate cestode taxa was strongly supported by all three types of phylogenetic analyses of all three data partitions. However, all analyses also supported the suggestion of Brooks and Barriga (1995), and the work of Olson et al. (1999), that *Duplicibothrium* and *Caulobothrium* are not members of the Rhinebothriinae. This placement of the former genus is also consistent with the morphological work of Caira et al. (1999, 2001), but placement of the latter genus is inconsistent with their results. Results from all analyses of all data partitions conducted here were also consistent with those of Olson et al. (1999) in that the species of the echeneibothriine genus *Echeneibothrium* and of each of the phyllobothriine genera *Anthocephalum* and *Rhodobothrium* grouped among the rhinebothriines, as did *Spongiobothrium*. The close affinity between *Rhodobothrium* and *Rhinebothrium* was also recognised by Waeschenbach et al. (2007). As a consequence, we propose that only the following genera should be considered for inclusion in the Rhinebothriinae: *Rhabdotobothrium*, *Rhinebothrium*, *Rhinebothroides*, *Scalithrium*, *Spongiobothrium*, New genera 1–4, the undescribed rhinebothriine from *P. clavata*, *Anthocephalum*, *Echeneibothrium* and *Rhodobothrium*. However, much more extensive sampling of the last three genera is clearly in order to confirm placement of these echeneibothriine and phyllobothriine taxa in the Rhinebothriinae.

The taxon sampling employed here was designed to evaluate the relationships of rhinebothriines relative to other acetabulate cestode lineages, and also relative to Litobothriidea and Cathetocephalidea whose members also parasitise elasmobranchs but are considered to lack bothria and acetabula. With the exception of the orders Tetrabothriidea, Nippotaeniidea and Cyclophyllidea, which have collectively been shown to comprise a clade (e.g., Mariaux, 1998; Olson et al., 2001), and also to be particularly divergent relative to the other acetabulate orders (e.g., Mariaux, 1998; Olson et al., 2001), exemplars of all acetabulate cestode orders were included. These acetabulate lineages consisted of representatives of the orders Proteocephalidea, Lecanicephalidea, and a relatively disparate suite of species in 10 non-rhinebothriine genera of tetraphyllideans. In all analyses of all data partitions, the Rhinebothriinae grouped outside these taxa and support for this result was strong. Given that representation of these taxa was extensive, this result is likely to be robust relative to rhinebothriine taxa in general. With the exception of *Glyphobothrium*, a genus transferred from the Phyllobothriidae to the Serendipidae by Brooks and Barriga (1995), all genera considered as rhinebothriines by one or more previous authors were represented in our analyses. Similarly, we believe the tetraphyllidean representation was sufficiently broad so as to capture much of tetraphyllidean diversity and also exemplify the polyphyly of this group. In most trees, the observed



Figs. 25-32. Cross sections through the scolices of genera representing those included in this study. Fig. 25. *Acanthobothrium* sp.; Fig. 26. *Duplicibothrium minutum*; Fig. 27. *Caulobothrium* sp.; Fig. 28. *Anthocephalum alicae*; Fig. 29. *Rhodobothrium paucitesticulare*; Fig. 30. *Rhinebothrium* sp.; Fig. 31. *Marsupiobothrium* sp.; Fig. 32. Frontal section through the scolex of *Clistobothrium carcharodoni* Dailey and Vogelbein, 1990. Note, arrowheads in Figs. 27–30 indicate stalks.

polyphyly of the tetraphyllideans was completely independent of the rhinebothriines. The single exception was in the strict consensus tree resulting from the MP of partial lsrDNA data alone (Fig. 21), but even in this instance the rhinebothriines were monophyletic with respect to a basal suite of tetraphyllideans and proteocephalideans.

We believe there is considerable evidence to warrant elevation of the Rhinebothriinae, as strictly circumscribed above, to the ordinal level, as the Rhinebothriidea. However, of key importance to the utility and validity of this action is the issue of morphological criteria that might be employed to diagnose this new order. The fact that the bothridia (i.e, acetabula) of the Rhinebothriidea are born on stalks is the most conspicuous candidate feature.

In stalked taxa, the bulk of the scolex is comprised of four parallel-sided stalks, each of which attaches to the proximal surface of a bothridium. The region considered to represent the scolex proper in these taxa is restricted to the site of confluence of the four stalks. Stalks are most clearly seen in histological sections of the scolex (e.g. Figs. 27–30). However, they are also readily visible with both SEM (e.g. Figs. 9, 13 and 15) and light microscopy. Although several tetraphyllidan taxa are also considered to possess features called stalks, it is clear from comparative examinations of these stalks that, in some cases, they are not homologous with the stalks of rhinebothriideans. In contrast, the scolex condition seen in the majority of the remaining tetraphyllideans is one in which the scolex proper is relatively substantial (sensu Caira et al., 1999, 2001). In such cases (e.g. Figs. 25 and 26), the bothridia are fused directly to the scolex proper, their ability to move independent of the scolex proper being restricted to their lateral margins. We note that both of these conditions differ from that seen in *Yorkeria* and *Spiniloculus*, in which the left and right bothridia are borne in back-to-back pairs on pedicels which are considered to be a divided cephalic peduncle (see Caira et al., 1999, 2001).

We believe elevation of the subfamily Rhinebothriinae, as circumscribed here, to the ordinal level is justified at this time. However, removal of the Rhinebothriidea from the Tetraphyllidea does not fully resolve the polyphyly of the latter group, because the tetraphyllidean taxa that remain do not form a monophyletic assemblage relative to the other major cestode lineages. In addition, although we have described the relationships among the Rhinebothriidea and the remaining Tetraphyllidea found here, we have not dwelt on them because we feel the differences seen among methods of analysis and, in particular, among data partitions, attest to the fact that additional data and taxa must be explored before these relationships are fully understood.

4.1. Proposal of new order Rhinebothriidea

Synonyms: Tetraphyllidea Carus, 1863 *in part*

4.1.1. Diagnosis

Cestoda: Eucestoda. Small tapeworms. Strobila polyzoic and proglottized. Proglottids hermaphroditic, craspedote or acraspedote, generally euapolytic, but occasionally apolytic or hyperapolytic. One set of male and female reproductive organs per segment. Two pairs of lateral osmoregulatory canals; ventral canals usually wider than dorsal canals. Neck absent. Scolex with four muscular unarmed bothridia. Bothridia stalked, usually lacking distinct apical suckers, with or without marginal and/or facial septa. Myzorhynchus present or absent. Testes usually numerous, rarely 2; post-poral testes usually lacking. Vas deferens convoluted. External seminal vesicle present or absent. Cirrus-sac without internal seminal vesicle; cirrus with spiniform microtriches. Genital pores lateral, irregularly alternating. Vagina opening anterior to cirrus sac into common genital atrium. Ovary posterior, bilobed or tetralobed in cross section. Vitellarium follicular; follicles arranged in lateral fields, occasionally encroaching on median line of proglottid. Uterus tubular, with or without lateral diverticula; pre-formed uterine pores lacking. Parasites in spiral intestines of batoids.

List of genera recognised in the new order Rhinebothriidea

Type-genus: Rhinebothrium Linton, 1890.

Other valid genera: Anthocephalum Linton, 1890; *Echeneibothrium* van Beneden, 1850; *Rhabdotobothrium* Euzet, 1953; *Rhinebothroides* Mayes, Brooks and Thorson, 1981; *Rhodobothrium* Linton, 1889; *Scalithrium* Ball, Neifar, and Euzet, 2003; *Spongiobothrium* Linton, 1889.

Undescribed genera: New genus 1 (Fig. 7); New genus 2 (Fig. 8); New genus 3 (Fig. 9); New genus 4 (Fig. 10).

4.1.2. Differential diagnosis

The Rhinebothriidea is readily distinguished from all other valid cestode orders (sensu Khalil et al., 1994; Olson et al., 2001; Caira et al., 2005; Kuchta et al., 2007) as follows. It differs from the Amphilinidea and Gyrocotylidea in its possession of a distinct scolex, and also in that it is polyzoic. It differs from the Bothriocephalidea, Caryophyllidea, Diphyllidea, Diphyllobothriidea, Haplobothriidea, Spathebothriidea and Trypanorhyncha in its possession of a scolex bearing four acetabula (which are generally in the form of bothridia), rather than bothria. It similarly differs from the Cathetocephalidea, Litobothriidea and Nippotaeniidea in scolex form, given that unlike the Rhinebothriidea, the Cathetocephalidea possesses a scolex that is essentially undivided and laterally expanded, and the Litobothriidea and Nippotaeniidea possess scolices that bear essentially only a single apical sucker. Among the acetabulate orders Lecanicephalidea, Tetraphyllidea, Proteocephalidea, Cyclophyllidea and Tetrabothriidea, the Rhinebothriidea differs most conspicuously in its possession of bothridia (i.e., acetabula) that are stalked. However, it further differs from each of these taxa as follows: whereas the Cyclophyllidea and Tetrabothriidea each exhibit a compact vitellarium, the vitellarium in the Rhinebothriidea is follicular. The scolex of the Lecanicephalidea is generally considered to possess an apical organ, with a few exceptions, whereas an apical organ is absent in most Rhinebothriidea. Whereas the parenchyma in the Proteocephalidea is divided by its conspicuous longitudinal musculature into cortical and medullary regions, no such distinction is seen in the Rhinebothriidea. Clearly the rhinebothriideans most closely resemble the tetraphyllideans. The distinction between the features considered to be stalks in tetraphyllideans and the stalks seen in the Rhinebothriidea is discussed below.

4.1.3. Remarks

With respect to membership of the Rhinebothriidea, we list only genera included in the formal analyses conducted here. However, it is likely that molecular and histological work will reveal that at least the other echeneibothriine genera, specifically *Pseudanthobothrium* Baer, 1946, *Clydonobothrium* Euzet, 1959, *Phormobothrium* Alexander, 1963 and *Tritaphros*, will be found to also belong in the new order. Given its initial inclusion in the Rhinebothriinae, *Glyphobothrium* would also be worthy of further investigation, as would other members of the Serendipidae such as *Serendip*, and of the Triloculariidae such as *Escherbothrium* Berman and Brooks, 1994. We have refrained from formally designating families within the Rhinebothriidea until a more robust and stable hypothesis of the interrelationships of taxa within the order is available and the New genera have been formally described.

If the presence of bothridial stalks is sufficient to unite all members of the Rhinebothriidea, the inclusion of *Anthocephalum*, *Echeneibothrium* and *Rhodobothrium* among the Rhinebothriidea on the basis of molecular data (despite their current placement in subfamilies outside of the Rhinebothriinae) would suggest that these taxa too should be found to possess bothridial stalks. In fact, cross-sections and/or SEM of the scolices of species in these three genera confirm their possession of bothridial stalks (e.g. Figs. 28, 5 and 29, respectively). The morphologies of these three genera are entirely consistent with the diagnosis of the proposed new order.

The suggestion of Brooks and Barriga (1995) that *Duplicibothrium* does not belong within this group is supported by cross-sections made through the scolex (Fig. 26), which reveal that bothridial stalks are lacking from members of this genus. However, it is of note that cross-sections through the scolex of *Caulobothrium* (e.g. Fig. 27), a genus determined to lie within the Rhinebothriinae based on the morphological work conducted by Caira et al. (1999, 2001), but also considered to lie outside of the Rhinebothriinae by Brooks and Barriga (1995), a position supported by the molecular analyses performed here, reveals the existence of bothridial stalks. Despite its possession of stalks, Healy (2006. A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium*, *Rhabdotobothrium*, *Rhinebothrium*, *Scalithrium* and *Spongiobothrium*. Doctoral Dissertation, University of Connecticut, Storrs, CT, USA) provided several morphological features to support the placement of *Caulobothrium* outside of the Rhinebothriinae. She noted that *Caulobothrium* spp. differed from rhinebothriine species in their lack of spiniform microtriches on the cirrus, and also in that the cirrus sac is joined by the vas deferens at its proximal, rather than anterior, margin. In addition, unlike most rhinebothriideans, *Caulobothrium* spp. possess post-poral testes. Thus, at this point, we consider the presence of bothridial stalks in *Caulobothrium* species to represent a homoplasious feature, rather than evidence that *Caulobothrium* belongs in the Rhinebothriidea.

The existence of structures that potentially constitute bothridial stalks in other tetraphyllidean taxa is also of relevance here. In the most recent revision of the order, Euzet (1994) recognised eight families of tetraphyllideans. Three of these families (the Disculicipitidae, Cathetocephalidae and Litobothriidae), lack bothridia, and in fact, the latter two have since been accepted as taxa deserving ordinal level status, reducing the number of bothridiate tetraphyllidean families to six. Brooks and Barriga's (1995) subsequent erection of the Serendipidae brings the number to seven. Among these seven bothridiate families, only the Phyllobothriidae and Serendipidae include any taxa that possess structures even remotely resembling bothridial stalks as defined by Caira et al. (1999, 2001). Among the 32 genera of phyllobothriids and serendipids included in the analyses of Caira et al. (2001), only five genera beyond those discussed here were considered to possess stalks. In addition, an examination of the generic diagnoses for all other valid tetraphyllidean genera indicated that an additional nine tetraphyllidean genera appear to possess stalks. All 14 genera are phyllobothriids; they consist of the echeneibothriines *Clydonobothrium* Euzet, 1959, *Notomegarhynchus* Ivanov and Campbell, 2002, *Phormobothrium* Alexander, 1963, *Pseudanthobothrium* and *Tritaphros*; the phyllobothriines *Anthobothrium* van Beneden, 1850, *Carpobothrium* Shipley and Hornell, 1906, *Clistobothrium* and *Marsupiobothrium* Yamaguti, 1952; the thysanocephalines *Rhoptrobothrium* Shipley and Hornell, 1906, *Myzocephalus* Shipley and Hornell, 1906 and *Myzophyllobothrium* Shipley and Hornell, 1906; and two phyllobothriid genera with no current sub familial membership, *Anindobothrium* Marques, Brooks and Lasso, 2001 and *Pararhinebothroides* Zamparo, Brooks and Barriga, 1999. Given the level of support for inclusion of the echeneibothriine genus *Echeneibothrium* within the Rhinebothriidea seen here, it seems likely that among these, at least the other five echeneibothriine genera, which appear to bear structures very much like the bothridial stalks seen in *Echeneibothrium* (e.g. see *Pseudanthobothrium* in fig. 41 in Caira et al., 2001), will also be confirmed to possess stalks and to belong within the Rhinebothriidea once comparable morphological and molecular data are available. However, this is not necessarily the case for the phyllobothriine, thysanocephaline, or the remaining phyllobothriid genera that appear to bear stalks, but that did not group among the rhinebothriideans in any of the analyses performed here. In fact, sections through the scolices of *Marsupiobothrium* and *Clistobothrium* (Figs. 31 and 32, respectively) reveal that, rather than consisting of rectangular structures (i.e., with parallel sides) attaching to the centre of the backs of the bothridia, the "stalks" are essentially triangular in shape and completely surround the bothridia. This construction leads one to believe that the structures seen in the latter genera may not be homologous to those seen in the rhinebothriidean taxa. Additional work is required to test this hypothesis. Closer examinations of the "stalks" of *Anindobothrium*, *Anthobothrium*, *Carpobothrium* and *Pararhinebothroides* are also clearly in order. The stalks seen in the three thysanocephaline genera are considered to be extensions of the cephalic peduncle (see Jensen and Caira, 2006 for a detailed description), rather than the scolex proper. These stalks should be viewed as non-homologous with the stalks of the rhinebothriideans.

Host records for the Rhinebothriidea (see Healy, 2006. A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium*, *Rhabdotobothrium*, *Rhinebothrium*, *Scalithrium* and *Spongiobothrium*. Doctoral Dissertation, University of Connecticut, Storrs, CT, USA), indicate that its species are restricted to batoids; there appear to be no valid records of members of the order from sharks. Among batoids, the Rhinebothriidea have been reported from all orders recognised by Compagno (1999), including the Pristiformes, Rhiniformes, Rhinobatiformes, Rajiformes and Torpediniformes. However, the majority of existing records come from rays of the order Myliobatiformes (i.e., the stingrays).

Acknowledgements

We thank Julia Llewellyn-Hughes and Claire Griffin for providing expert technical assistance with the sequencer, and Andrea Waeschenbach for sequencing genes for four taxa. Florian Reyda kindly provided the specimens of *Rhinebothrium* sp. 8 and *Rhinebothroides* cf. *freitasi* for molecular analysis, the SEM image of *Rhinebothroides* in Fig. 16, as well as helpful comments on an earlier version of this manuscript. This work was supported by a grant to C.J.H. from The Systematics Research Fund. C.J.H., J.N.C., and K.J. gratefully acknowledge funding from NSF PEET award No. DEB 0118882, and NSF BS & I award Nos. 0542846 and 0542941. B.L.W. and D.T.J.L. were funded in part by a Wellcome Trust Senior Research Fellowship (043965) to D.T.J.L. and NERC (NER/A/S/2003/00313).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2008.09.002.

References

Ball, D., Neifar, L., Euzet, L., 2003. Proposition de *Scalithrium* n. gen. (Cestoda, Tetraphyllidea) avec comme espèce-type *Scalithrium minimum* (van Beneden, 1850) n. comb. parasite de *Dasyatis pastinaca* (Elasmobranchii, Dasyatidae). Parasite 10, 31–37.

Berman, R., Brooks, D.R., 1994. *Escherbothrium molinae* n. gen. et n. sp. (Eucestoda: Tetraphyllidea: Triloculariidae) in *Urotrygon chilensis* (Chondrichthyes: Myliobatiformes: Urolophidae) from the Gulf of Nicoya, Costa Rica. Journal of Parasitology 80, 775–780.

Brabec, J., Kuchta, R., Scholz, T., 2006. Paraphyly of the Pseudophyllidea (Platyhelminthes: Cestoda): Circumscription of monophyletic clades based on phylogenetic analysis of ribosomal RNA. International Journal for Parasitology 36, 1535–1541.

Brooks, D.R., Mayes, M.A., Thorson, T.B., 1981a. Systematic review of cestodes infecting freshwater stingrays (Chondrichthyes: Potamotrydonidae) including four new species from Venezuela. Proceedings of the Helminthological Society of Washington 48, 43–64.

Brooks, D.R., Mayes, M.A., Thorson, T.B., 1981b. Cestode parasites in *Myliobatis goodei* Garman (Myliobatiformes: Myliobatidae) from Rio de la Plata, Uruguay, with a summary of cestodes collected from South American elasmobranchs during 1975–1979. Proceedings of the Biological Society of Washington 93, 1239–1252.

Brooks, D.R., Barriga, R., 1995. *Serendip deborahae* n. gen., n. sp. (Eucestoda: Tetraphyllidea: Serendipidae n. fam.) in *Rhinoptera steindachneri* Evermann, Jenkins, 1891 (Chondrichthyes: Myliobatiformes: Myliobatidae) from southeastern Ecuador. Journal of Parasitology 81, 80–84.

Brooks, D.R., Deardorff, T.L., 1988. *Rhinebothrium devaneyi* n.s p. (Eucestoda: Tetraphyllidea), *Echinocephalus overstreeti* Deardorff, Ko, 1983 (Nematoda: Gnathostomatidae) in a thorny back ray, *Urogymnus asperrimus*, from Enewetak Atoll, with phylogenetic analysis of both species groups. Journal of Parasitology 74, 459–465.

Brooks, D.R., Hoberg, E.P., Weekes, P.J., 1991. Preliminary phylogenetic systematic analysis of the major lineages of the Eucestoda (Platyhelminthes: Cercomaria). Proceedings of the Biological Society of Washington 104, 651–668.

Caira, J.N., Jensen, K., Healy, C.J., 1999. On the phylogenetic relationships among tetraphyllidean, lecanicephalidean and diphyllidean tapeworm genera. Systematic Parasitology 42, 77–151.

Caira, J.N., Jensen, K., Healy, C.J., 2001. Interrelationships among tetraphyllidean and lecanicephalidean cestodes. In: Littlewood, D.T.J., Bray, R.A. (Eds.), Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp. 135–158.

Caira, J.N., Mega, J., Ruhnke, T.R., 2005. An unusual blood sequestering tapeworm (*Sanguilevator yearsleyi* n. gen., n. sp.) from Borneo with description of *Cathetocephalus resendezi* n. sp. from Mexico and molecular support for the recognition of the order Cathetocephalidea (Platyhelminthes: Eucestoda). International Journal for Parasitology 35, 1135–1152.

Compagno, L.J.V., 1999. Checklist of living elasmobranchs. In: Hamlett, W.C. (Ed.), Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes. Johns Hopkins University Press, Baltimore, pp. 471–498.

de Chambrier, A., Zehnder, M., Vaucher, C., Mariaux, J., 2004. The evolution of the Proteocephalidea (Platyhelminthes, Eucestoda) based on an enlarged molecular phylogeny, with comments on their uterine development. Systematic Parasitology 57, 159–171.

Euzet, L., 1956. Suggestions pour une nouvelle classification des cestodes tétraphyllides. In: Proceedings XIV International Congress of Zoology, pp. 347–349.

Euzet, L., 1994. Order Lecanicephalidea Wardle & McLeod, 1952. In: Khalil, L.F., Jones, A., Bray, R.A. (Eds.), Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, England, pp. 195–204.

Hoberg, E.P., Mariaux, J., Justine, J.-L., Brooks, D.R., Weekes, P.J., 1997. Phylogeny of the orders of the Eucestoda (Cercomeromorphae) based on comparative morphology: Historical perspectives and a new working hypothesis. Journal of Parasitology 83, 1128–1147.

Hoberg, E.P., Gardner, S.L., Campbell, R.A., 1999a. Systematics of the Eucestoda: Advances towards a new phylogenetic paradigm, and observations on the early diversification of tapeworms and vertebrates. Systematic Parasitology 42, 1–12.

Hoberg, E.P., Jones, A., Bray, R.A., 1999b. Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. Systematic Parasitology 42, 51–73.

Jensen, K.J., 2005. A monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). Bulletin of the University of Nebraska State Museum vol. 18, 249 pp.

Jensen, K., Caira, J.N., 2006. The status of Rhoptrobothrium Shipley et Hornell, 1906 (Cestoda: Tetraphyllidea), with redescription of the type species, *R. myliobatidis*, and description of three new species from eagle rays in the genus *Aetomylaeus* from Malaysian Borneo. Folia Parasitologia 53, 189–207.

Khalil, L.F., Jones, A., Bray, R.A., 1994. Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, U.K.

Kodedová, I., Dolezel, D., Broucková, M., Jirku, M., Hypsa, V., Lukes, J., Scholz, T., 2000. On the phylogenetic positions of the Caryophyllidea, Pseudophyllidea and Proteocephalidea (Eucestoda) inferred from 18S rRNA. International Journal for Parasitology 30, 1109–1113.

Kuchta, R., Scholz, T., Brabec, J., Bray, R.A., 2007. Suppression of the tapeworms order Pseudophyllidea (Platyhelminthes: Eucestoda) and the proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. International Journal for Parasitology 38, 49–55.

Mariaux, J., 1998. A molecular phylogeny of the Eucestoda. Journal of Parasitology 84, 114–124.

Olson, P.D., Ruhnke, T.R., Sanney, J., Hudson, T., 1999. Evidence for host-specific clades of tetraphyllidean tapeworms (Platyhelminthes: Eucestoda) revealed by analysis of 18S ssrDNA. International Journal for Parasitology 29, 1465–1476.

Olson, P.D., Caira, J.N., 1999. Evolution of the major lineages of tapeworms (Platyhelminthes: Castoidea) inferred from 18S ribosomal DNA and *elongation factor-1α*. Journal of Parasitology 85, 1134–1159.

Olson, P.D., Littlewood, D.T.J., Bray, R.A., Mariaux, J., 2001. Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Molecular Phylogenetics and Evolution 19, 443–467.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rego, A.A., de Chambrier, A., Hanzelová, V., Hoberg, E., Scholz, T., Weekes, P., Zehnder, M., 1998. Preliminary phylogenetic analysis of subfamilies of the Proteocephalidea (Eucestoda). Systematic Parasitology 40, 1–19.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ruhnke, T.R., 1993. A new species of *Clistobothrium* (Cestoda: Tetraphyllidea), with an evaluation of the systematic status of the genus. Journal of Parasitology 79, 37–43.

Ruhnke, T.R., 1994. Resurrection of *Anthocephalum* Linton, 1890 (Cestoda: Tetraphyllidea) and taxonomic information on five proposed members. Systematic Parasitology 29, 159–176.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Research 25, 4876–4882.

Waeschenbach, A., Webster, B.L., Bray, R.A., Littlewood, D.T.J., 2007. Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. Molecular Phylogenetics and Evolution 45, 311–325.

Wardle, R.A., McLeod, J.A., 1952. The Zoology of Tapeworms. University of Minnesota Press, Minneapolis.

Williams, H.H., 1968. The taxonomy, ecology and host-specificity of some Phyllobothriidae (Cestoda: Tetraphyllidea), a critical revision of *Phyllobothrium* Beneden, 1849 and comments on some allied genera. Philosophical Transactions of the Royal Society of London 253, 231–307.