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Orders out of chaos – molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships



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ABSTRACT

Novel molecular data are presented to resolve the long-standing issue of the non-monophyly of the elasmobranch-hosted tapeworm order Tetraphyllidea relative to the other acetabulate eucestode orders. Bayesian inference analyses of various combinations of full ssrDNA, and full or partial IsrDNA (D1–D3), sequence data, which included 134 species representing 97 genera across the 15 eucestode orders, were conducted. New ssrDNA data were generated for 82 species, partial IsrDNA data for 53 species, and full IsrDNA data for 29 species. The monophyly of each of the elasmobranch-hosted orders Cathetocephalidea, Litobothriidea, Lecanicephalidea and Rhinebothriidea was confirmed, as was the non-monophyly of the Tetraphyllidea. Two relatively stable groups of tetraphyllidean taxa emerged and are hereby designated as new orders. The Onchoproteocephalidea n. ord. is established to recognise the integrated nature of one undescribed and 10 described genera of hook-bearing tetraphyllideans, previously placed in the family Onchobothriidae, with the members of the order Proteocephalidea. The Phyllobothriidea n. ord. is established for a subset of 12 non-hooked genera characterised by scoleces bearing four bothridia each with an anterior accessory sucker; most parasitise sharks and have been assigned to the Phyllobothriidae at one time or another. Tentative ordinal placements are suggested for eight additional genera; placements for the remaining tetraphyllidean genera have not yet emerged. We propose that these 17 genera remain in the “Tetraphyllidea”. Among these, particularly labile across analyses were *Anthobothrium*, *Megalonchos*, *Carpobothrium*, *Calliobothrium* and *Caulobothrium*. The unique association of *Chimaerocestus* with holocephalans, rather than with elasmobranchs, appears to represent a host-switching event. Both of the non-elasmobranch hosted clades of acetabulate cestodes (i.e. Proteocephalidea and Cyclophyllidea and their kin) appear to have had their origins with elasmobranch cestodes. Across analyses, the sister group to the clade of “terrestrial” cestode orders was found to be an elasmobranch-hosted genus, as was the sister to the freshwater fish- and tetrapod-hosted Proteocephalidea. Whilst further data are required to resolve outstanding nomenclatural and phylogenetic issues, the present analyses contribute significantly to an understanding of the evolutionary radiation of the entire Cestoda. Clearly, elasmobranch tapeworms comprise the backbone of cestode phylogeny.

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1. Introduction

Despite global efforts to bring cestode classification into line with phylogeny, one of the most speciose of orders parasitising elasmobranchs (i.e. the Tetraphyllidea) remains the most problematic. All phylogenetic work that has included representatives of this order, whether morphological (Euzet et al., 1981; Brooks et al., 1991; Hoberg et al., 1997; Caira et al., 1999, 2001) or molecular (Mariaux, 1998; Olson and Caira, 1999; Kodedová et al., 2000; Olson et al., 2001; Waeschenbach et al., 2007, 2012), has shown the group to be paraphyletic. As a consequence, the pivotal position of

the tetraphyllideans in the larger context of tapeworm evolution, and thus the importance of establishing their phylogenetic relationships, is widely recognised.

The formal dismantling of the Tetraphyllidea, as traditionally circumscribed, consisting of the speciose Onchobothriidae Braun, 1900 and Phyllobothriidae Braun, 1900, the morphologically unique Chimaerocestidae Williams and Bray, 1989 and Dioecotaeniidae Schmidt, 1969, as well as the Triloculariidae Yamaguti, 1959 (e.g., see Schmidt, 1986; Euzet, 1994), and the Serendipidae Brooks and Barriga, 2005 (see Brooks and Barriga, 2005), began almost a decade ago largely as a result of the application of molecular methods. Three suites of taxa that exhibit proglottid morphology similar to that of the Tetraphyllidea but bear scoleces that lack acetabula characteristic of Tetraphyllidea and their derived relatives, were

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removed from the order. Olson and Caira (2001) resurrected the order Litobothriidea of Dailey (1969) for the members of the genus *Litobothrium* Dailey, 1969. Caira et al. (2005) resurrected the order Cathetocephalidea (of Schmidt and Beveridge, 1990) for several genera exhibiting cushion-like scoleces. In addition, most recently, Healy et al. (2009) erected the Rhinebothriidea to house the tetraphyllideans bearing stalked acetabula.

However, even with these modifications the Tetraphyllidea persists as a paraphyletic assemblage (e.g. see Waeschenbach et al., 2012). This paraphyly has particularly important implications for our understanding of the evolutionary history of the other acetabulate cestode orders (i.e., Proteocephalidea, Tetrabothriidea, Cyclophyllidea and Mesocoeloides), and the non-acetabulate Nippotaeniidea, all of which parasitise vertebrate groups other than elasmobranchs. Tetraphyllidean paraphyly has manifested itself somewhat differently among molecular analyses. For example, Mariaux (1998) found tetraphyllideans placed in a ladder-like arrangement basal to a clade comprised of diphyllidean, proteocephalidean, nippotaeniidean, tetrabothriidean and cyclophyllidean exemplars. Of the four tetraphyllideans included by Olson and Caira (1999), three comprised a clade that also included a proteocephalidean exemplar, while the fourth grouped as sister to a clade comprised of the former clade and nippotaeniidean, tetrabothriidean and cyclophyllidean exemplars. Kodedová et al. (2000) found a similar result but with less resolution within both clades. Olson et al. (2001) reported a diversity of results depending on the data partition and method of analysis, but in general, found one of their tetraphyllidean exemplars (*Acanthobothrium* van Beneden, 1850) to group with proteocephalidean exemplars, and their remaining eight tetraphyllidean exemplars placed in a ladder-like arrangement basal to a clade comprised of the proteocephalideans and *Acanthobothrium* as well as the cyclophyllidean, tetrabothriidean, nippotaeniidean exemplars. The analyses of Waeschenbach et al. (2007, 2012) yielded similar results. Caira et al. (2005) found the nine tetraphyllidean exemplars included in their analyses to comprise a clade together with six proteocephalidean exemplars; this clade was sister to a clade comprised of the cyclophyllidean, tetrabothriidean and nippotaeniidean exemplars. Because these studies were all largely based on nuclear ribosomal (RNA) gene data, the differences in topologies could be ascribed to taxon sampling.

By sampling densely across the Tetraphyllidea, the present molecular study was undertaken (i) to examine the relationships among tetraphyllidean taxa to establish a foundation for systematic revision of the order, and (ii) to investigate tetraphyllidean relationships with respect to the monophyly and origins of the other acetabulate cestode lineages, many of which parasitise vertebrates other than elasmobranchs. Efforts were made to include representatives of as many tetraphyllidean genera as possible so as to capture their extensive morphological variation, host associations, and the multitude of positions they appear to occupy across the cestode phylogeny. Also included, at least in some analyses, were one or more representatives of all other cestode orders, with the exception of the Amphilinidea. We build on previous studies by concentrating on large and small subunits of nuclear ribosomal RNA genes, lsrDNA (=28S rDNA) and ssrDNA (=18S rDNA), respectively.

2. Materials and methods

2.1. Sampling and study taxa

Specimens sequenced de novo were obtained from hosts collected around the globe over the last two decades. Sampling of elasmobranch hosts was conducted as follows: off Australia using

gill nets, fish traps, and in conjunction with the commercial trawling vessels including the FV Ocean Harvest; in Canada off New Brunswick with a local trawling vessel; off Chile in conjunction with local trawling vessels; in Malaysian Borneo from fish markets, in conjunction with trawling vessels and with gill nets; in Mexico in the Gulf of California using gill nets; off Horta in the Azores, Portugal, in conjunction with Azores Fisheries; off New Zealand in conjunction with the research vessel NZ Tangaroa; in Peru with hand spears and fish nets; off Senegal in conjunction with local net fishermen; off Thailand in conjunction with commercial trawlers. Within the United States: in the Gulf of Mexico using hand lines; in North Carolina with gill nets; off New York, Connecticut and Rhode Island in conjunction with shark tournaments; off South Carolina in conjunction with the RV Oregon II; in the Florida Keys with hand lines. The two species of *Chimaerocoelus* Williams and Bray, 1984 were collected from holocephalans in conjunction with the RV Tangaroa. Specimens of *Proteocephalus perplexus* La Rue, 1911 were collected from Hay Bay, Ontario, Canada. All specimens were preserved in 95% ethanol. An effort was made to preserve vouchers of all specimens sequenced in this study. In most instances, whole mounts of hologenophores consisting of either the scolex and terminal proglottids, or in the cases of smaller specimens, the scolex only, were prepared following standard methods (see Pickering and Caira, 2008). In the cases of extremely tiny species, specimens were photographed and the entire specimen was then used for sequencing; in such cases photographic vouchers were deposited. Elasmobranch identities follow Naylor et al. (2012).

In total, 134 cestode species were analysed in this study. These are listed in Table 1 together with their hosts and collection localities. For the purposes of this study, new ssrDNA data were generated for 82 of these species; partial lsrDNA data were generated for 53 and full lsrDNA data for 29 species. Accession numbers for hologenophores and photographic vouchers for 82 species deposited in the Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, Storrs, Connecticut, USA are provided in Table 1. In the cases of 34 of the remaining 57 species, partial lsrDNA and ssrDNA data were obtained from GenBank; for 23 of the 34 species full lsrDNA and ssrDNA were obtained from GenBank. Sequences for which vouchers are available were preferred. GenBank accession numbers and sources for all species are provided in Table 1 as well as in Fig. 2 and Supplementary Fig. S5. Also indicated in Table 1 is the ordinal level placement of each species based on current cestode classification (sensu Khalil et al., 1994 as modified by Caira and Olson, 2001; Caira et al., 2005; Healy et al., 2009), as well as its revised ordinal placement as a result of this study.

Two data sets were generated. In both cases, all acetabulate taxa were considered as members of the ingroup and, with the exception of the nippotaeniids and cathetocephalideans, all non-acetabulate taxa were considered as members of the outgroup. Inclusion of the nippotaeniids as members of the ingroup, despite their non-acetabulate nature, followed previous work (e.g., Hoberg et al., 2001; Waeschenbach et al., 2012) as did treatment of the cathetocephalideans (e.g. Caira et al., 2005). Dataset_I consisted of partial (i.e. D1–D3 region) lsrDNA and complete ssrDNA for 134 eucestode taxa. Informed by Dataset_I and to deeper explore the phylogenetic relationships, 47 of these 134 taxa were chosen for which sequence data were generated for the remaining domains of lsrDNA and included in Dataset_II (i.e., complete ssrDNA and complete lsrDNA). Outgroup taxa in Dataset_I consisted of exemplars of: Bothriocephalidea, Diphyllidea, Diphyllbothriidea, Litobothriidea and Trypanorhyncha. Outgroup taxa in Dataset_II consisted of exemplars of the cestode orders Bothriocephalidea, Caryophyllidea, Diphyllidea, Diphyllbothriidea, Gyrocotylidea, Litobothriidea, Spathebothriidea and Trypanorhyncha, as well as

Table 1
List of Ingroup (IG) and outgroup (OG) taxa included in the analyses with their ordinal placement, specimen numbers, and host and collection data. GenBank numbers in bold indicate sequences generated as part of this study.

Ingroup (IG)/ Outgroup (OG)	Taxa in analyses 1, 3 and 5	Taxa in analyses 2, 4 and 6	Taxon	Specimen ID	Current Order	Revised Order	Voucher Acc. No.	GenBank Acc. No. (1stDNA)	GenBank Acc. No. (1stDNA [D1–D3])	GenBank Acc. No. (ssrDNA)	Host code	Host species (host order: host family)	Locality	Source
IG	Yes	Yes	<i>Disculiceps</i> sp. 1	TE-130	Tet	Cat	LRP 8328	KF685761	KF685839	MS05-24	<i>Carcharhinus limbatus</i> (Carcharhiniformes: Carcharhinidae)	Horn Island, off Ocean Springs, Mississippi, USA		
IG		Yes	<i>Cathetocephalus thatcheri</i>	TE-28	Cat	Cat	LRP 8281		KF685884	KF685838		<i>Carcharhinus leucas</i> (Carcharhiniformes: Carcharhinidae)	Gulf of Mexico, USA	
IG	Yes	Yes	<i>Sanguilevator yearsleyi</i>	TE-114	Cat	Cat	LRP 4218	KF685762		FJ177057	BO-488	<i>Lamiopsis tephrodes</i> (Carcharhiniformes: Carcharhinidae)	Mukah, Sarawak, Borneo	Healy et al. (2009)
IG	Yes	Yes	<i>Dilepis undula</i>	Dun	Cyc	Cyc		AF286915		AF286981	REF 97/47	<i>Turdus merula</i> (Passeriformes: Turdidae)	Nova Cherna, Silistra, Bulgaria	Olson et al. (2001)
IG	Yes	Yes	<i>Hymenolepis diminuta</i>	Hymd	Cyc	Cyc		AY157181		AF286983		<i>Rattus norvegicus</i> (Rodentia: Muridae)	Lab strain, University of Copenhagen, Denmark	Lockyer et al. (2003) and Olson and Cairn (1999)
IG	Yes	Yes	<i>Mesocoestoides</i> sp.	Mesc	Cyc	Cyc		EF095263		EF095248	BG2002.08.20.02.1	<i>Apodemus agrarius</i> (Rodentia: Muridae)	Nova Cherna, Silistra, Bulgaria	Waeschenbach et al. (2007)
IG	Yes	Yes	<i>Adelobothrium aetobatidis</i>	TE-16B	Lec	Lec	LRP 8272	EF095257		EF095249	AU-57	<i>Aetobatus ocellatus</i> (Myliobatiformes: Myliobatidae)	Darwin, Northern Territory, Australia	Waeschenbach et al. (2007)
IG		Yes	<i>Anteropora joanna</i>	TE-122	Lec	Lec	LRP 8326		KF685864	KF685789	BO-86	<i>Taeniura lymna</i> 1 (Myliobatiformes: Dasypatiidae)	Mukah, Sarawak, Borneo	
IG		Yes	<i>Anteropora leelongi</i>	TE-23A	Lec	Lec	LRP 8278		KF685857	KF685787	JO-12	<i>Hemiscyllium ocellatum</i> (Orectolobiformes: Hemiscylliidae)	Cairns, Queensland, Australia	
IG		Yes	<i>Anteropora patulobothridium</i>	TE-90	Lec	Lec	LRP 8307		KF685863	KF685788	BO-86	<i>Taeniura lymna</i> 1 (Myliobatiformes: Dasypatiidae)	Semporna, Sabah, Borneo	
IG		Yes	<i>Cephalobothrium</i> n. sp. 1	TE-21	Lec	Lec	LRP 8275		KF685858	KF685782	TH-19	<i>Aetobatus ocellatus</i> (Myliobatiformes: Myliobatidae)	Bangsaray, Thailand	
IG		Yes	<i>Eniochobothrium euaxos</i>	TE-18A	Lec	Lec	LRP 8274		KF685859	KF685784	AU-85	<i>Rhinoptera neglecta</i> (Rhinoptera: Rhinopteroidea)	Dundee Beach, Northern Territory, Australia	
IG		Yes	<i>Eniochobothrium</i> n. sp. 1	TE-91	Lec	Lec	LRP 8308		KF685860	KF685785	MS05-49	<i>Rhinoptera</i> cf. <i>steindachneri</i> (Myliobatiformes: Rhinopteroidea)	Ship Island, off Ocean Springs, Mississippi, USA	
IG		Yes	<i>Flapocephalus</i> n. sp. 1	TE-88	Lec	Lec	LRP 8302		KF685861	KF685795	CM03-79	<i>Pastinachus atrus</i> (Myliobatiformes: Dasypatiidae)	Weipa, Queensland, Australia	
IG		Yes	<i>Flapocephalus</i> n. sp. 2	TE-30	Lec	Lec	LRP 8283		KF685862	KF685794	AU-61	<i>Pastinachus atrus</i> (Myliobatiformes: Dasypatiidae)	Dundee Beach, Northern Territory, Australia	
IG		Yes	<i>Hornellobothrium</i> n. sp. 1	TE-32B	Lec	Lec	LRP 8285		KF685865	KF685793	NT-76	<i>Aetobatus ocellatus</i> (Myliobatiformes: Myliobatidae)	Wessel Islands, Northern Territory, Australia	
IG		Yes	New genus 5 n. sp. 1	TE-87	Lec	Lec	LRP 8305		KF685866	KF685783	CM03-75	<i>Glaucoctegus typus</i> (Rhinopteroidea: "Rhinopteroidea")	Weipa, Queensland, Australia	
IG		Yes	New genus 6 n. sp. 1	TE-134	Lec	Lec	LRP 8331		KF685867	KF685792	CM03-24	<i>Himantura uarnak</i> 2 (Myliobatiformes: Dasypatiidae)	Weipa, Queensland, Australia	
IG		Yes	<i>Paraberapex manifestus</i>	TE-142	Lec	Lec			KF685868	KF685781	BJ-298	<i>Squatina californica</i> (Squatinoidea: Squatinidae)	Santa Rosalia, Baja California Sur, México	

(continued on next page)

Table 1 (continued)

Ingroup (IG)/ Outgroup (OG)	Taxa in analyses 1, 3 and 5	Taxa in analyses 2, 4 and 6	Taxon	Specimen ID	Current Order	Revised Order	Voucher Acc. No.	GenBank Acc. No. (IstDNA)	GenBank Acc. No. (IstDNA [D1–D3])	GenBank Acc. No. (ssrDNA)	Host code	Host species (host order: host family)	Locality	Source
IG	Yes	Yes	<i>Polypocephalus helmuti</i>	TE-17A	Lec	Lec	LRP 8273	KF685869	KF685786	AU-85		Dundee Beach, Northern Territory, Australia	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Polypocephalus</i> sp. 1	TE-47A	Lec	Lec	LRP 8292	KF685870	KF685790	NT-44		Wessel Islands, Northern Territory, Australia	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Polypocephalus</i> sp. 2	TE-133	Lec	Lec	LRP 8330	KF685748	KF685791	CM03-74		Wessel Islands, Northern Territory, Australia	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Terragonocephalum passeyi</i>	TE-19A	Lec	Lec	LRP 7276	KF685871	KF685856	NT-32		Wessel Islands, Northern Territory, Australia	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Terragonocephalum</i> sp. 1	TE-89	Lec	Lec	LRP 8306	KF685872	KF685796	CM03-53		Weipa, Queensland, Australia	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Amurotaenia deciduas</i>	Ada	Nip	Nip		AF286932	AF124474			Lake Taupo, New Zealand	Olson et al. (2001); Olson and Caira (1999)	
IG	Yes	Yes	<i>Nippotaenia chaenogobii</i>	Ncha	Nip	Nip	BMNH 2000.3.7.11-12	AF286933	AF286987			Lake Suwa, Nagano Prefecture, Japan	Olson et al. (2001)	
IG	Yes	Yes	<i>Nippotaenia mogurnidae</i>	Nip	Nip	Nip	BMNH 2000.3.7.13	AF286934	AJ287545			Nukui River at Babadai, Higashihiroshima, Hiroshima, Japan	Olson et al. (2001) and Littlewood and Olson (2001)	
IG	Yes	Yes	<i>Gangesia parasituri</i>	Gpar	Pro	Onc	INVE-22436	AF286935	AJ287515			Lake Suwa, Nagano Prefecture, Japan	Olson et al. (2001) and Littlewood and Olson (2001)	
IG	Yes	Yes	<i>Pelidocotyle rugosa</i>	Pelt	Pro	Onc	INVE-22374	AF286937	AF286989			Rio Paraguay, San Antonio, Central Province, Paraguay	Olson et al. (2001) and Littlewood and Olson (2001)	
IG	Yes	Yes	<i>Proteocephalus macrocephalus</i>	Prom	Pro	Onc	EF095261	EF095247	EF095247			River Thames, Windsor, United Kingdom	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Proteocephalus perplexus</i>	TE-58	Pro	Onc	LRP 8299	KF685873	KF685833	LO-8		Hay Bay, Lake Ontario, Canada	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Acanthobothrium parvuncinatum</i>	TE-44	Tet	Onc	LRP 8291	EF095264	EF095250	BJ-95		Bahia de Los Angeles, Baja California Sur, México	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Acanthobothrium santarosaliense</i>	TE-136	Tet	Onc	LRP 8300	KF685751	KF685834	BJ-300		Santa Rosalia, Baja California Sur, México	Waeschenbach et al. (2007)	
IG	Yes	Yes	New genus 8 n. sp. 1	TE-92	Tet	Onc	LRP 8309	KF685765	KF685836	AU-36		Darwin, Northern Territory, Australia	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Phoreiobothrium lewintense</i>	TE-53	Tet	Onc	LRP 8295	KF685896	KF685830	DEL-1		Straits of Florida, Florida, USA	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Platybothrium auriculatum</i>	TE-38	Tet	Onc	LRP 8289	KF685898	KF685837	EH-1		Montauk, New York, USA	Waeschenbach et al. (2007)	
IG	Yes	Yes	<i>Platybothrium jondorum</i>	TE-60A	Tet	Onc	LRP 8301	KF685772	KF685829	AU-30		Darwin, Northern Territory, Australia	Waeschenbach et al. (2007)	

IG	Yes	Yes	<i>Potamotrygonocoestus</i> cf. <i>fitzgeraldi</i>	TE-37B (1stDNA); TE-37A (ssrDNA)	Tet	Onc	LRP 8288; LRP 8287	KF685773	KF685832	PU-23	<i>Potamotrygon castexi</i> (Myliobatiformes: Potamotrygonidae)	Madre de Dios River, Madre de Dios, Peru
IG	Yes	Yes	<i>Prosobothrium armigerum</i>	TE-31A	Tet	Onc	LRP 8284	KF685899	KF685828	OJ-2	(Carcharhiniformes: Carcharhinidae)	Montauk, New York, USA
IG	Yes	Yes	<i>Triloculatum andersonorum</i>	TE-54	Tet	Onc	LRP 8296	KF685895	KF685831	CM03-34	(Carcharhinidae)	Weipa, Queensland, Australia
IG	Yes	Yes	<i>Uncibilocularis okei</i>	TE-93	Tet	Onc	LRP 8310	KF685777	KF685835	AU-61	(Myliobatiformes: Dasyatidae)	Dundee Beach, Northern Territory, Australia
IG	Yes	Yes	<i>Megalanchos shawae</i>	TE-153	Tet	Onc?	LRP 8339	KF685764	KF685807	NT-39	(Hemipristis elongata)	Wessel Islands, Northern Territory, Australia
IG	Yes	Yes	<i>Calyptrobothrium</i> sp. 1	TE-163	Tet	Phy	LRP 8342	KF685754	KF685848	TN-118	(Hemigaleidae)	Rhode Island, USA
IG	Yes	Yes	<i>Chimaerocoestus</i> n. sp. 1	TE-85	Tet	Phy	LRP 8503	KF685758	KF685827	CR-46	(Torpediniformes: Torpedinidae)	Chatham Rise, New Zealand
IG	Yes	Yes	<i>Chimaerocoestus</i> n. sp. 2	TE-172	Tet	Phy	LRP 8548	KF685882	KF685850	CR-165	(Chimaeriformes: Rhinochimaeridae)	Chatham Rise, New Zealand
IG	Yes	Yes	<i>Marsupiobothrium</i> sp. 1	TE-145 (1stDNA); TE-144 (ssrDNA)	Tet	Phy	LRP 8337; LRP 8336	KF685771	KF685821	BJ-719	(Rhinochimaeridae)	Chatham Rise, New Zealand
IG	Yes	Yes	<i>Nandoceustus guariticus</i>	TE-34A	Tet	Phy	LRP 8286	KF685888	KF685817	PU-15	(Alopiidae)	Santa Rosalia, Baja California Sur, México
IG	Yes	Yes	New genus 10 n. sp. 1	TE-86	Tet	Phy	LRP 8304	KF685889	KF685814	DEL-6	(Pararhynchiformes: Myliobatiformes: Potamotrygonidae)	Mouth of Rio Shilve, Madre de Dios, Peru
IG	Yes	Yes	<i>Orectolobiceustus randyi</i>	TE-105	Tet	Phy	LRP 8318	KF685767	KF685820	BO-493	(Sphyrnidae)	Straits of Florida, Florida, USA
IG	Yes	Yes	<i>Orectolobiceustus tyleri</i>	TE-101	Tet	Phy	LRP 8315	KF685890	KF685819	BO-472	(Chiloscyllium hasseltii)	Mukah, Sarawak, Borneo
IG	Yes	Yes	<i>Orygmatobothrium</i> cf. <i>musteli</i> 1	TE-52 (1stDNA); TE-111 (ssrDNA)	Tet	Phy	LRP 8294	KF685768	KF685815	SE-99; SE-316	(Orectolobiformes: Hemiscylliidae)	Mukah, Sarawak, Borneo
IG	Yes	Yes	<i>Orygmatobothrium</i> cf. <i>musteli</i> 2	TE-112	Tet	Phy	LRP 8311	KF685891	KF685816	SE-187	(Mustelus mustelus)	Soumbédioune, Senegal
IG	Yes	Yes	<i>Paraorygmatobothrium exiguum</i>	TE-98	Tet	Phy	LRP 8313	KF685769	KF685822	TK-1	(Triakidae)	Senegal
IG	Yes	Yes	<i>Paraorygmatobothrium prionacis</i>	TE-49	Tet	Phy	LRP 8293	KF685892	KF685818	IF-7	(Alopiidae)	Quakam, Senegal
IG	Yes	Yes	<i>Phyllobothrium</i> cf. <i>lactuca</i>	TE-181	Tet	Phy	LRP 8313	KF685770	KF685845	PEET 136	(Carcharhiniformes: Carcharhinidae)	Montauk, New York, USA
IG	Yes	Yes	<i>Phyllobothrium squali</i>	TE-180	Tet	Phy	LRP 8345	KF685897	KF685846	RDM-189	(Carcharhiniformes: Mustelus mento)	Puerto Montt, Chile
IG	Yes	Yes	<i>Ruhnkeceustus latipi</i>	TE-167	Tet	Phy	LRP 8345	KF685900	KF685853	BO-265	(Triakidae)	Rhode Island, USA
IG	Yes	Yes	<i>Scyphophyllitidium</i> cf. <i>giganteum</i>	TE-170	Tet	Phy	LRP 8346	KF685901	KF685854	CR-140	(Squalidae)	Mukah, Sarawak, Borneo

(continued on next page)

Table 1 (continued)

Ingroup (IG)/ Outgroup (OG)	Taxa in analyses 1, 3 and 5	Taxa in analyses 2, 4 and 6	Taxon	Specimen ID	Current Order	Revised Order	Voucher Acc. No.	GenBank Acc. No. (lsrDNA)	GenBank Acc. No. (ssrDNA) [D1–D3]	GenBank Acc. No. (ssrDNA)	Host code	Host species (host order: host family)	Locality	Source
IG	Yes	Yes	<i>Thysanocephalum crispum</i>	TE-43B	Tet	Phy	LRP 8290	KF685902	KF685823	KC-13	Galeocerdo curvier (Carcharhiniformes: Carcharhinidae)	South Carolina, USA		
IG	Yes	Yes	<i>Glyptocephalus montaukensis</i>	TE-22A (lsrDNA); Clis (ssrDNA)	Tet	Phy?	LRP 8277	EF095259	AF286996	PO-1	<i>Isurus paucus</i> (Lamniformes: Lamnidae)	Montauk, New York, USA	Olson et al. (2001) and Waeschebach et al. (2007)	
IG	Yes	Yes	<i>Crossobothrium dolini</i>	TE-173	Tet	Phy?	LRP 8349	KF685759	KF685847	MS05-553	<i>Hepranchius perlo</i> (Hexanchiformes: Hexanchidae)	Gulf of Mexico		
IG	Yes	Yes	<i>Crossobothrium laciniatum</i>	TE-127	Tet	Phy?		KF685883	KF685824	Cl-2	<i>Hexanchus griseus</i> (Hexanchiformes: Hexanchidae)	Pacific Ocean, Puerto Montt, Chile		
IG	Yes	Yes	<i>Antiocephalum cf. centrurum</i>	TE-141	Rhi	Rhi	LRP 4219	FJ177099	FJ177059	SE-222	<i>Dasyatis</i> sp. (Myliobatiformes: Dasyatidae)	Mbour, Senegal	Healy et al. (2009)	
IG	Yes	Yes	<i>Echenebothrium sp. 1^a</i>	TE-94	Rhi	Rhi	LRP 4217	FJ177098	FJ177058	BJ-243	<i>Raja velezi</i> (Rajiformes: Rajidae)	Santa Rosalia, Baja California Sur, Mexico	Healy et al. (2009)	
IG	Yes	Yes	<i>Echenebothrium sp. 2</i>	TE-95	Rhi	Rhi	LRP 8312	KF685876	KF685842	SE-188	<i>Raja miraletus</i> (Rajiformes: Rajidae)	Quakam, Senegal		
IG	Yes	Yes	New genus 1 n. sp.	CH-11	Rhi	Rhi	LRP 3902	FJ177107	FJ177067	BO-412	<i>Himantura cf. gerrardi</i> 1 (Myliobatiformes: Dasyatidae)	Tanjung Manis, Sarawak, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	New genus 2 cf. <i>sexortidum</i>	CH-35	Rhi	Rhi	LRP 3922	FJ177108	FJ177068	BO-86	<i>Taeniura lymna</i> 1 (Myliobatiformes: Dasyatidae)	Semporna, Sabah, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	New genus 3 <i>cadernati</i>	CH-3	Rhi	Rhi	LRP 3894	FJ177109	FJ177069	BO-336	<i>Neotrygon kuhlii</i> 1 (Myliobatiformes: Dasyatidae)	Sarawak, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	New genus 3 n. sp. 6	CH-45	Rhi	Rhi	LRP 3926	FJ177116	FJ177076	BO-237	<i>Zanobatus schoenleinii</i> (Rhinoipristiformes: Zanobatidae)	Mukah, Sarawak, Borneo	Healy et al. (2009)	
IG	Yes	Yes	New genus 3 n. sp. 7	CH-30	Rhi	Rhi	LRP 3917	FJ177117	FJ177077	AU-56	<i>Himantura walga</i> (Myliobatiformes: Dasyatidae)	Dundee Beach, Northern Territory, Australia	Healy et al. (2009)	
IG	Yes	Yes	New genus 4 <i>kinabatanganensis</i>	CH-9	Rhi	Rhi	LRP 3900	FJ177118	FJ177078	BO-108	<i>Glaucoctegus typus</i> (Rhinoipristiformes: "rhinobatidae")	Kinabatangan River, Sabah, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	<i>Pseudanthobothrium sp. 1</i>	TE-117	Tet	Rhi	LRP 8324	KF685750	KF685841	HM-7	<i>Leucoraja erinacea</i> (Rajiformes: Rajidae)	St. Andrews, New Brunswick, Canada		
IG	Yes	Yes	<i>Rhabdothoracium anterophallum</i>	Rhab	Rhi	Rhi	BMNH-2001.1.31.3-4	AF286961	AF287000	M-99-2442	<i>Mobula hypostoma</i> (Myliobatiformes: Mobulidae)	Gulf of Mexico, Mississippi, USA	Olson et al. (2001)	
IG	Yes	Yes	<i>Rhinebothrium sp. 1</i>	CH-12	Rhi	Rhi	LRP 3903	FJ177121	FJ177081	BO-76	<i>Himantura pastinacoides</i> (Myliobatiformes: Dasyatidae)	Kampung Tetabuan, Sabah, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	<i>Rhinebothrium cf. maccallumi</i>	Rnac	Rhi	Rhi	LRP 2108	AF286962	AF124476		<i>Dasyatis americana</i> (Myliobatiformes: Dasyatidae)	Mississippi, USA	Olson et al. (2001)	
IG	Yes	Yes	<i>Rhinebothrium megacanthophallus</i>	CH-10	Rhi	Rhi	LRP 3901	FJ177120	FJ177080	BO-108	<i>Himantura polyplepis</i> (Myliobatiformes: Dasyatidae)	Kinabatangan River, Sabah, Malaysia	Healy et al. (2009)	
IG	Yes	Yes	<i>Rhinebothrium sp. 7</i>	CH-6	Rhi	Rhi	LRP 3897	FJ177129	FJ177089	SE-123	Rhinopteridae (Myliobatiformes: Dasyatidae)	Mbour, Senegal	Healy et al. (2009)	
IG	Yes	Yes	<i>Rhinebothrium sp. 8</i>	CH-55	Rhi	Rhi	LRP 3930	FJ177130	FJ177090	PU-10	<i>Paratrygon aieba</i> (Myliobatiformes: Potamotrygonidae)	Madre de Dios River, Madre de Dios, Peru	Healy et al. (2009)	

IG	Yes	<i>Rhineboothrium</i> sp. 9	CH-34	Rhi	Rhi	LRP 3921	FJ177131	FJ177091	BO-86	<i>Taeniura lymnae</i> 1 (Myliobatiformes: Dasyatidae)	Semporna, Sabah, Malaysia	Healy et al. (2009)
IG	Yes	<i>Rhineboothroides</i> cf. <i>fretasi</i>	CH-54	Rhi	Rhi	LRP 3929	FJ177132^b	FJ177092	PU-25b	<i>Potamotrygon</i> cf. <i>castrei</i>	Madre de Dios River, Boca Manu, Peru	Healy et al. (2009)
IG	Yes	<i>Rhodobothrium paucitesticulare</i>	TE-61	Rhi	Rhi	LRP 4216	FJ177100	FJ177060	BNC-22	(Myliobatiformes: Potamotrygonidae)	Davis, North Carolina, USA	Healy et al. (2009)
IG	Yes	<i>Scalithrium</i> n. sp.	CH-4	Rhi	Rhi	LRP 3895	FJ177133	FJ177093	BJ-423	(Myliobatiformes: Rhinopteridae)	San Jose del Cabo, Baja California Sur, México	Healy et al. (2009)
IG	Yes	<i>Scalithrium</i> sp. 1	TE-140	Rhi	Rhi	LRP 8333	KF685878	KF685840	BJ-423	<i>Dasyatis longa</i> (Myliobatiformes: Dasyatidae)	San Jose del Cabo, Baja California Sur, México	Healy et al. (2009)
IG	Yes	<i>Spongiobothrium</i> sp.	CH-32	Rhi	Rhi	LRP 3919	FJ177134	FJ177094	NT-66	<i>Dasyatis</i> (Myliobatiformes: Dasyatidae)	Wessel Islands, Northern Territory, Australia	Healy et al. (2009)
IG	Yes	New genus 7 n. sp. 1	TE-166	Tet	Rhi?	LRP 8344	KF685749	KF685851	KJG-17	(Rhinopristiformes: Rhynchobatidae)	Sydney, New South Wales, Australia	
IG	Yes	<i>Pentaloculum</i> n. sp. 1	TE-171	Tet	Rhi?	LRP 8347	KF685877	KF685852	CR-136	<i>Parascyllium collare</i> (Orectolobiformes: Parascylliidae)	Chatham Rise, New Zealand	
IG	Yes	<i>Terrabothrium erosiris</i>	Tero	Teb	Teb	AF286950	AF286950	AJ287581		<i>Typhlonarke tarakea</i> (Torpediniformes: Narkidae)	Danube Delta, Romania	Olson et al. (2001) and Littlewood and Olson (2001)
IG	Yes	<i>Terrabothrium</i> sp.	Tsp	Teb	Teb	AF286952	AF286952	AJ287582		<i>Larus argentatus</i> (Charadriiformes: Laridae)	St. Kilda, Victoria Australia	Olson et al. (2001) and Littlewood and Olson (2001)
IG	Yes	<i>Anthobothrium caseyi</i>	TE-100	Tet	"Tet"	LRP 8314	KF685879	KF685805	EH-1	<i>Puffinus tenuirostris</i> (Procellariiformes: Procellariidae)	Montauk, New York, USA	Olson (2001)
IG	Yes	<i>Anthobothrium</i> n. sp. 1	TE-119	Tet	"Tet"	LRP 8325	KF685752	KF685806	NT-55	(Carcharhinidae)	Wessel Islands, Northern Territory, Australia	
IG	Yes	<i>Balanobothrium</i> sp.	TE-108	Tet	"Tet"		KF685880	KF685802	BO-450	<i>Carcharias risoni</i> (Carcharhiniformes: Carcharhinidae)	Mukah, Sarawak, Borneo	
IG	Yes	<i>Calliobothrium violae</i>	TE-182	Tet	"Tet"	LRP 8350	KF685881	KF685813	EM-4	<i>Stegostoma fasciatum</i> (Orectolobiformes: Stegostomatidae)	Old Lyme, Connecticut, USA	
IG	Yes	<i>Calliobothrium</i> cf. <i>verticillatum</i>	TE-155	Tet	"Tet"	LRP 8341	KF685753	KF685812	EM-3	<i>Mustelus canis</i> (Carcharhiniformes: Triakidae)	Old Lyme, Connecticut, USA	
IG	Yes	<i>Carpobothrium</i> n. sp. 1	TE-103	Tet	"Tet"	LRP 8317	KF685755	KF685804	BO-270	<i>Chiloscyllium hasseltii</i> (Orectolobiformes: Hemiscylliidae)	Mukah, Sarawak, Borneo	
IG	Yes	<i>Caulobothrium opisthorchis</i>	CH-21	Tet	"Tet"	LRP 3910	FJ177106^b	FJ177066	BJ-626	<i>Myliobatis californica</i> (Myliobatiformes: Myliobatidae)	Bahia de Los Angeles, Baja California Sur, México	Healy et al. (2009)
IG	Yes	<i>Caulobothrium</i> n. sp. 5	CH-25	Tet	"Tet"	LRP 3914	FJ177105	FJ177065	NT-105	<i>Pseudocarcharias kamoharui</i> (Myliobatiformes: Dasyatidae)	Wessel Islands, Northern Territory, Australia	Healy et al. (2009)
IG	Yes	<i>Ceratobothrium xanthocephalum</i>	TE-7 (srDNA [D1-D3]); TE-124 (srDNA [D3-12]; sstfDNA)	Tet	"Tet"	LRP 8351; LRP 8327	KF685756; KF685757	KF685849	PO-3	<i>Isurus paucus</i> (Lamniformes: Lamnidae)	Montauk, New York, USA	
IG	Yes	<i>Dinobothrium planum</i>	TE-29B	Tet	"Tet"	LRP 8282	KF685886	KF685797	QU-1	<i>Cetorhinus maximus</i> (Lamnidae: Cetorhinidae)	Long Island Sound, New York, USA	

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Table 1 (continued)

Ingroup (IG)/ Outgroup (OG)	Taxa in analyses 1, 3 and 5	Taxa in analyses 2, 4 and 6	Taxon	Specimen ID	Current Order	Revised Order	Voucher Acc. No.	GenBank Acc. No. (IstDNA) [D1–D3]	GenBank Acc. No. (ssrDNA)	Host code	Host species (host order: host family)	Locality	Source
IG	Yes	Yes	<i>Dioecotaenia cancellata</i>	TE-132	Tet	"Tet"		KF685760	KF685810	MS05-156	<i>Rhinoptera</i> cf. <i>streindachneri</i> (Myliobatiformes: Rhinopteridae)	East Ship Island, off Ocean Springs, Mississippi, USA	
IG	Yes	Yes	<i>Duplicobothrium minutum</i>	TE-135	Tet	"Tet"	LRP 8332	KF685885	KF685809	MS05-49	<i>Rhinoptera</i> cf. <i>streindachneri</i> (Myliobatiformes: Rhinopteridae)	East Ship Island, off Ocean Springs, Mississippi, USA	
IG	Yes	Yes	<i>Duplicobothrium</i> n. sp. 1	TE-131	Tet	"Tet"	LRP 8329	KF685763	KF685808	MS05-49	<i>Rhinoptera</i> cf. <i>streindachneri</i> (Myliobatiformes: Rhinopteridae)	Ship Island, off Ocean Springs, Mississippi, USA	
IG	Yes	Yes	<i>Myzocephalus</i> sp. 1	TE-27A	Tet	"Tet"	LRP 8280	KF685887	KF685826	AU-41	<i>Aerobatus ocellatus</i> (Myliobatiformes: Myliobatidae)	Darwin Northern Territory, Australia	
IG	Yes	Yes	New genus 9 n. sp. 1	TE-102	Tet	"Tet"	LRP 8316	KF685766	KF685811	BO-47	<i>Himantura uarnak</i> 3 (Myliobatiformes: Dasypatidae)	Mukah, Sarawak, Borneo	
IG	Yes	Yes	<i>Pachybothrium hutsomi</i>	PED-11	Tet	"Tet"	LRP 8322	EF095260	EF095246	CM03-16	<i>Nebrius ferrugineus</i> (Orectolobiformes: Ginglymostomatidae)	Weipa, Queensland, Australia	Waeschenbach et al. (2007)
IG	Yes	Yes	<i>Pedibothrium mouseyi</i>	TE-116	Tet	"Tet"	LRP 8323	KF685893	KF685803	CM03-16	<i>Nebrius ferrugineus</i> (Orectolobiformes: Ginglymostomatidae)	Weipa, Queensland, Australia	
IG	Yes	Yes	<i>Pedibothrium veravalensis</i>	TE-107	Tet	"Tet"	LRP 8320	KF685894	KF685801	BO-450	<i>Stegostoma fasciatum</i> (Orectolobiformes: Stegostomatidae)	Mukah, Sarawak, Borneo	
IG	Yes	Yes	<i>Rhoptrorhynchium cf. gambangi</i>	TE-146	Tet	"Tet"	LRP 8338	KF685774	KF685825	BO-492	<i>Stegostoma fasciatum</i> (Orectolobiformes: Stegostomatidae)	Mukah, Sarawak, Borneo	
IG	Yes	Yes	<i>Spiniloculus</i> n. sp. 1	TE-57	Tet	"Tet"	LRP 8298	KF685775	KF685800	TH-3	<i>Chiloscyllium punctatum</i> (Orectolobiformes: Hemiscylliidae)	Bangsaray, Thailand	
IG	Yes	Yes	<i>Trilocularia gracilis</i>	TE-165	Tet	"Tet"	LRP 8343	KF685776	KF685855	SQ-2	<i>Squalus acanthias</i> (Squaliformes: Squalidae)	Rhode Island, USA	
IG	Yes	Yes	<i>Yorkeria hilli</i>	TE-56	Tet	"Tet"	LRP 8297	KF685903	KF685798	TH-2	<i>Chiloscyllium punctatum</i> (Orectolobiformes: Hemiscylliidae)	Bangsaray, Thailand	
IG	Yes	Yes	<i>Yorkeria izardi</i>	TE-106	Tet	"Tet"	LRP 8319	KF685904	KF685799	CM02-2	<i>Chiloscyllium cf. punctatum</i> (Orectolobiformes: Hemiscylliidae)	Cairns, Queensland, Australia	
OG	Yes	Yes	<i>Abotrium gadi</i>	Agad	Bot	Bot		AF286945	AJ287773		<i>Gadus morhua</i> (Gadiformes: Gadidae)	South of Shetland Isles, Scotland	Olson et al. (2001) and Littlewood and Olson (2001)
OG	Yes	Yes	<i>Ananterum tortum</i>	Anat	Bot	Bot	BMNH 2001.2.1.1	AF286941	AF286992	M-99-2478	<i>Synodus foetens</i> (Aulopiformes: Synodontidae)	Horn Island, off Ocean Springs, Mississippi, USA	Olson et al. (2001)
OG	Yes	Yes	<i>Bothriocephalus scorpii</i>	Bsco	Bot	Bot		AF286942	AJ287776		<i>Myoxocephalus scorpius</i> (Scorpaeniformes: Cottidae)	Off St. Abbs Head, United Kingdom	Waeschenbach et al. (2007)
OG	Yes	Yes	<i>Balanotaenia bancrofti</i>	Ban	Car	Car		AF286909	AF286977	THC6321	<i>Tandanus tandanus</i> (Siluriformes: Plotosidae)	Brisbane, Australia	Waeschenbach et al. (2007)
OG	Yes	Yes	<i>Caryophyllaeus laticeps</i>	Ciat (IstDNA): CAR-Cal (ssrDNA)	Car	Car		AY157180	AJ287488		<i>Abramis brama</i> (Rutilus rutilus) (Cypriniformes: Cyprinidae)	Neuchatel Lake, Neuchatel, Switzerland	Waeschenbach et al. (2007)

OG	Yes	<i>Ditrachyobothrium</i> cf. <i>macrocephalum</i>	Dibm2	Dip	Dip	BMNH 2004.1.6.1-5	AY584864	DQ642903	AF1584864	BMNH 2004.1.6.1-5	Dip	Dip	Goban Spur, off Ireland	Bray and Olson (2004) and Olson et al. (2001)
OG	Yes	<i>Echinobothrium chisholmae</i>	Eho	Dip	Dip	BMNH 2000.8.3.4-7	AF286922	AF286986	AF286922	BMNH 2000.8.3.4-7	Dip	Dip	Heron Island, Queensland, Australia	Olson et al. (2010)
OG	Yes	<i>Echinobothrium</i> sp. 1	TE-143	Dip	Dip	LRP 8335	KF685905	KF685778	SE-192	LRP 8335	Dip	Dip	Ouakam, Senegal	
OG	Yes	<i>Diphyllobothrium stemmacephalum</i>	Dstm	Dib	Dib	USNPC 86992	AF286943	AF124459	AF124459	USNPC 86992	Dib	Dib	Cape Cod, Massachusetts, USA	Olson and Caira (1999) and Olson et al. (2001)
OG	Yes	<i>Schistocephalus solidus</i>	Ssol	Dib	Dib		AF286944	AF124460	AF124460		Dib	Dib	Hidden Lake, Matanuska-Susitna Valley, Alaska, USA	Olson and Caira (1999) and Olson et al. (2001)
OG	Yes	<i>Gyrocotyle urna</i>	Gyro	Gyr	Gyr	AV157178	AV157178	AJ228782	AJ228782	AV157178	Gyr	Gyr	Bergen, Norway	Waeschenbach et al. (2007)
OG	Yes	<i>Litobothrium amplifica</i>	TE-26	Lit	Lit	LRP 8279	KF685906	KF685843	BJ-713	LRP 8279	Lit	Lit	Santa Rosalia, Baja California Sur, México	Waeschenbach et al. (2007)
OG	Yes	<i>Litobothrium jamoyvi</i>	Lalo	Lit	Lit	BMNH 2000.3.7.3-5	AF286930	AF124468	BJ-716	BMNH 2000.3.7.3-5	Lit	Lit	Santa Maria, Baja California, México	
OG	Yes	<i>Litobothrium nickoli</i>	TE-113	Lit	Lit	LRP 8321	KF685907	KF685844	BJ-713	LRP 8321	Lit	Lit	Santa Rosalia, Baja California Sur, México	
OG	Yes	<i>Didymobothrium rudolphi</i>	Didb3	Spa	Spa	BMNH 2006.10.4.9	EF095255	EF095245	EF095245	BMNH 2006.10.4.9	Spa	Spa	Northern Portuguese coast	Waeschenbach et al. (2007)
OG	Yes	<i>Spathobothrium simplex</i>	Sps	Spa	Spa		AF286949	AF124456	AF124456		Spa	Spa	Rye Beach, New Hampshire, USA	Waeschenbach et al. (2007)
OG	Yes	<i>Aporhynchus mesenezi</i>	TE-154	Try	Try	LRP 8340	KF685908	KF685780	AZ-1	LRP 8340	Try	Try	Horta, Faial Island, Portugal	
OG	Yes	<i>Diesingium lomentaceum</i>	Dies	Try	Try	LRP 3713	DQ642760	DQ642922	SE-99	LRP 3713	Try	Try	Sombédioune, Senegal	Olson et al. (2010)
OG	Yes	<i>Doliffusiella geraschmidti</i>	Dfisp	Try	Try	BMNH 2001.1.25.6-7	DQ642793	DQ642955	DQ642955	BMNH 2001.1.25.6-7	Try	Try	Queenscliff, Victoria, Australia	Olson et al. (2010)
OG	Yes	<i>Doliffusiella martini</i>	Dolm	Try	Try	BMNH 2001.1.25.2-4	DQ642802	DQ642964	TF1	BMNH 2001.1.25.2-4	Try	Try	Queenscliff, Victoria, Australia	Olson et al. (2010)
OG	Yes	<i>Grilloria erinaceus</i>	Geri	Try	Try	AF286967	AF286967	AJ228781	123/B	AF286967	Try	Try	South of Fair Isle, United Kingdom	Waeschenbach et al. (2007)
OG	Yes	<i>Grilloria pristiophori</i>	GripA	Try	Try	SAMA 28386	DQ642763	DQ642925	DQ642925	SAMA 28386	Try	Try	San Remo, Victoria, Australia	Olson et al. (2010)
OG	Yes	<i>Paragrilloria similis</i>	TE-51	Try	Try	LRP 4280	KF685909	KF685779	GC-1	LRP 4280	Try	Try	Florida Bay, Florida, USA	
OG	Yes	<i>Molicola uncinatus</i>	Moli	Try	Try	BMNH 2004.3.18.102	DQ642746	DQ642908	DQ642908	BMNH 2004.3.18.102	Try	Try	Apollo Bay, Victoria, Australia	Olson et al. (2010)
OG	Yes	<i>Nybelinia africana</i>	Nyb5	Try	Try		DQ642786	DQ642948	BO-74-01		Try	Try	Mukah, Sarawak, Borneo	Olson et al. (2010)
OG	Yes	<i>Oncomegas australensis</i>	Onco1	Try	Try	LRP 3678	DQ642795	DQ642957	NT-76	LRP 3678	Try	Try	Wessel Islands, Northern Territory, Australia	Olson et al. (2010)
OG	Yes	<i>Parachristianella indonensis</i>	Pbav5	Try	Try	LRP3673	DQ642777	DQ642939	NT-49	LRP3673	Try	Try	Wessel Islands, Northern Territory, Australia	Olson et al. (2010)

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Table 1 (continued)

Ingroup (IG)/ Outgroup (OG)	Taxa in analyses 1, 3 and 5	Taxa in analyses 2, 4 and 6	Taxon	Specimen ID	Current Order	Revised Order	Voucher Acc. No.	GenBank Acc. No. (lsrDNA)	GenBank Acc. No. (lsrDNA ID1–D31)	GenBank Acc. No. (ssrDNA)	Host code	Host species (host order: host family)	Locality	Source
OG	Yes		<i>Lepidophyllum steenstrupi</i>	Lste	DIG	DIG	AY157175	AJ287530				(Rhinopristiformes: Rhynchobatidae) <i>Anarchichas lupus</i> (Perciformes: Anarhichadidae) <i>Callorhynchus milii</i> (Chimaeriformes: Callorhynchidae) <i>Micromesistius pourtaussou/pollachius virens</i> (Gadiformes: Gadidae)	Australia North Sea, United Kingdom	Waeschenbach et al. (2007)
OG	Yes		<i>Rugogaster hydrologi</i>	Rug	ASP	ASP	AY157176	AJ287573				(Chimaeriformes: Callorhynchidae)	Hobart, Tasmania, Australia	Waeschenbach et al. (2007)
OG	Yes		<i>Diclidophora minor</i> ; <i>Diclidophora denticulata</i>	Dicm (lsrDNA); Dden (ssrDNA)	MON	MON	AY157169	AJ287779				(Microsporidia: Puccinelliales)	North Sea, United Kingdom	Waeschenbach et al. (2007)

ASP, Aspidogastrea; Bot, Bothriocephalidae; Car, Caryophyllidae; Cat, Catherocephalidae; Cyc, Cyclophyllidae; Dib, Diphyllididae; Dig, Digenea; Dip, Diphyllididae; Lec, Lecanicephalidae; Lit, Litobothriidae; MON, Monogenea; Nip, Nippotaeniidae; Onc, Onchoprotocephalidae; Phy, Phyllobothriidae; Pro, Proteocephalidae; Rhi, Rhinebothriidae; Spa, Spathebothriidae; Tet, Tetrabothriidae; Try, Trypanorhyncha.

^a As *Echeneibothrium* sp. in Healy et al. (2009).

^b lsrDNA (D4–D12) generated as part of this study.

one digenean, one aspidogastrea and one monogenean taxon (represented by chimaeric sequences).

In order to maximise the diversity of morphological forms, representatives of 10 undescribed genera were included in one or both data sets. These consisted of the four novel genera of rhinebothriids from Healy et al. (2009) (i.e., N. gen. 1–N. gen. 4) and six novel genera introduced here (i.e., N. gen. 5–N. gen. 10). The identities of these taxa are formally anchored by voucher specimens deposited in LRP, as indicated in Table 1. Scanning electron micrographs (SEMs) of the scoleces of N. gen. 1–N. gen. 4 can be found in Healy et al. (2009; figs. 7–10). SEMs of the scoleces of the six remaining new genera, prepared for SEM following Healy et al. (2009), are provided in Fig. 1.

2.2. DNA extraction, gene amplification and sequencing

Total genomic DNA was extracted from ethanol-preserved specimens using the DNeasy Blood and Tissue kit or Genomic-tip 20/G (QIAGEN, UK) following the manufacturer's instructions. PCR and sequencing primers are listed in Supplementary Table S1. Partial lsrDNA (1,172–1,597 bp) was amplified in one fragment using LSU5 or ZX-1 + 1500R; in the case of poor amplification, semi-nested PCRs on primary amplicons using the same forward primer and reverse primer 1200R were carried out. Complete lsrDNA (4,132–4,438 bp) was amplified in a number of overlapping fragments, depending on amplification success: LSU5 or ZX-1 + either L2230, LSUD6-3' or L1642; and U1846 or 1600F + OR-2. Complete ssrDNA (1,912–2,260 bp) was amplified in either one fragment using WormA + WormB primers or by a number of overlapping fragments, depending on amplification success: 18S-8 + WormB or 1200R; WormA + A27'; Tet460F + Tet1420R; Tet1100F + 1200R or WormB. PCRs were carried out in 25 µl reaction volumes using Illustra PuRe Taq Ready-to-go PCR beads (GE Healthcare, UK) and 1 µl of 10 µM of each primer. Cycling conditions for lsrDNA were as follows: initial denaturation for 5 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 2 min at 72 °C and completed by 10 min at 72 °C. Cycling conditions for ssrDNA were as follows: initial denaturation for 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 54 °C, 2 min at 72 °C and completed by 10 min at 72 °C; in the case of nested PCRs, the annealing temperature was increased by two degrees. 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. Sequence identity was checked using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST/). Contigs were assembled using Sequencher 4.8 (GeneCodes Corporation, USA).

2.3. Sequence alignment and phylogenetic analyses

Sequences were aligned 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 and Maddison, 2005). Regions that could not be unambiguously aligned were excluded from the analysis; alignments are available from the authors on request. Modeltest version 3.7macX (Posada and Crandall, 1998) was used to select a model of evolution using the Akaike Information Criterion.

A total of six phylogenetic analyses were conducted as follows. Analysis 1: complete lsrDNA for the restricted set of 55 taxa (Supplementary Fig. S1). Analysis 2: partial lsrDNA for the suite of 134 taxa (Supplementary Fig. S2). Analysis 3: ssrDNA for the 55 taxa (Supplementary Fig. S3). Analysis 4: ssrDNA for the 134 taxa (Supplementary Fig. S4). Analysis 5: complete lsrDNA +

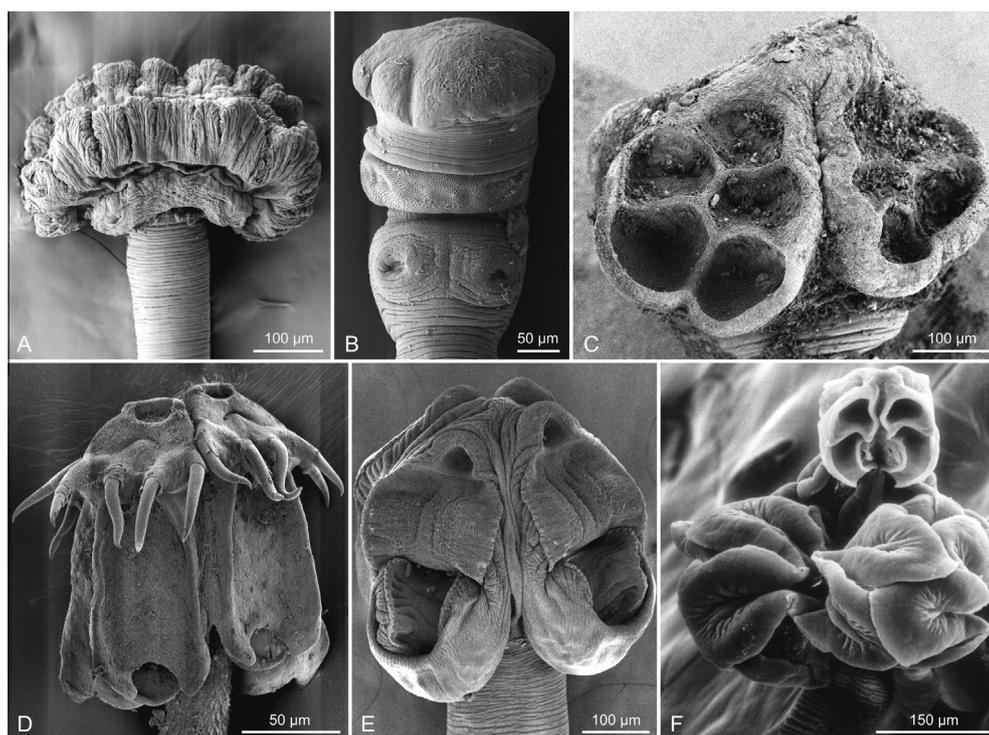


Fig. 1. Scanning electron micrographs of scoleces of undescribed genera included in molecular analyses. (A) New genus 5. (B) New genus 6. (C) New genus 7. (D) New genus 8. (E) New Genus 9. (F) New genus 10.

ssrDNA for the 55 taxa (Supplementary Figs. S5 and S6). Analysis 6: partial *l*srDNA + *ssr*DNA for the 134 taxa (Supplementary Figs. S5 and S6). Thus, Analyses 1–4 were based on data from single genes; Analyses 5 and 6 were based on data from both genes.

Phylogenetic trees were constructed using Bayesian inference (BI) with MrBayes, version 3.1 (Huelsenbeck and Ronquist, 2001); likelihood settings were set to $nst = 6$, $rates = invgamma$, $ngammat = 4$ (equivalent to the GTR + I + G model of evolution). In the analyses with multiple character partitions, parameters were estimated separately for each partition. Four chains ($temp = 0.2$) were run for 10,000,000 generations and sampled every 1,000 generations. ‘Burn-in’ was determined as the point at which average standard deviation of split frequencies (ASDOSF) was < 0.01 . Maximum likelihood (ML) bootstrap values for 100 replicates were obtained using Genetic Algorithm for Rapid Likelihood Inference (GARLI) Version 0.942 (Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Doctoral dissertation, University of Texas at Austin, TX, USA) using default settings, except setting ‘Genthreshfortopterm’ to 10,000 generations. Clades were considered to have high nodal support if BI posterior probability was $\geq 95\%$ and bootstrap values $\geq 75\%$.

3. Results

3.1. Tetracystidean relationships

Analyses of individual genes (i.e., Analyses 1–4) yielded tree topologies that differed conspicuously from one another, and from those resulting from the combined analyses (i.e., Analyses 5 and 6), signaling a good deal of instability in any of the topologies chosen for formal presentation here. Incongruences included not only the placement of certain taxa (e.g., *Anthobothrium* van Beneden, 1850, *Calliobothrium* van Beneden,

1850, *Carpobothrium* Shipley and Hornell, 1906, *Caulobothrium* Baer, 1948, *Megalonchos* Baer and Euzet, 1962), but also potential ordinal membership and interrelationships. As a consequence, the trees from the analyses of individual genes and of the smaller number of taxa are presented as supplementary documents only (see Supplementary Figs. S1–S6), and we have concentrated here on the tree generated from the greater taxon sampling and data from both genes (i.e. Analysis 6 Fig. 2). We have also provided a schematic diagram (Fig. 3) summarising the better supported nodes for the purposes of discussion. The tree illustrating the phylogenetic relationships among the 55 species included in the analysis of complete *l*srDNA and complete *ssr*DNA (i.e. Analysis 5), together with potential ordinal placements, is shown in Supplementary Fig. S5. The more robust nodes (i.e., with ≥ 0.95 Bayesian posterior probabilities [pp] and/or 75% ML bootstrap support) are summarised in the schematic topology in Supplementary Fig. S6. Owing to its less dense taxon sampling, the results of Analysis 5 are limited with respect to their implications for the interrelationships among genera. The tree illustrating the phylogenetic relationships among the 134 cestode species included in the analysis of partial *l*srDNA and complete *ssr*DNA data (i.e. Analysis 6), together with resulting potential ordinal placements, is shown in Fig. 2. The more robust nodes (with ≥ 0.95 Bayesian pp and/or 75% ML bootstrap support) are summarised in the schematic topology in Fig. 3, which has been expanded to include additional outgroups based on Waeschenbach et al. (2012). This figure also includes the major vertebrate groups hosting each cestode taxon.

Given the instability noted above, our goal of achieving breadth across tetracystidean genera rather than depth within tetracystidean genera, and the limited representation of genera of the other acetabulate orders, we have refrained from commenting on the implications of our results for the non-monophyly of individual genera (e.g., *Polypocephalus* Braun, 1878, *Echeneiobothrium* van Beneden, 1849, *Rhinebothrium* Linton, 1890, *Phyllobothrium* van Beneden, 1849, *Paraorygmatobothrium* Ruhnke,

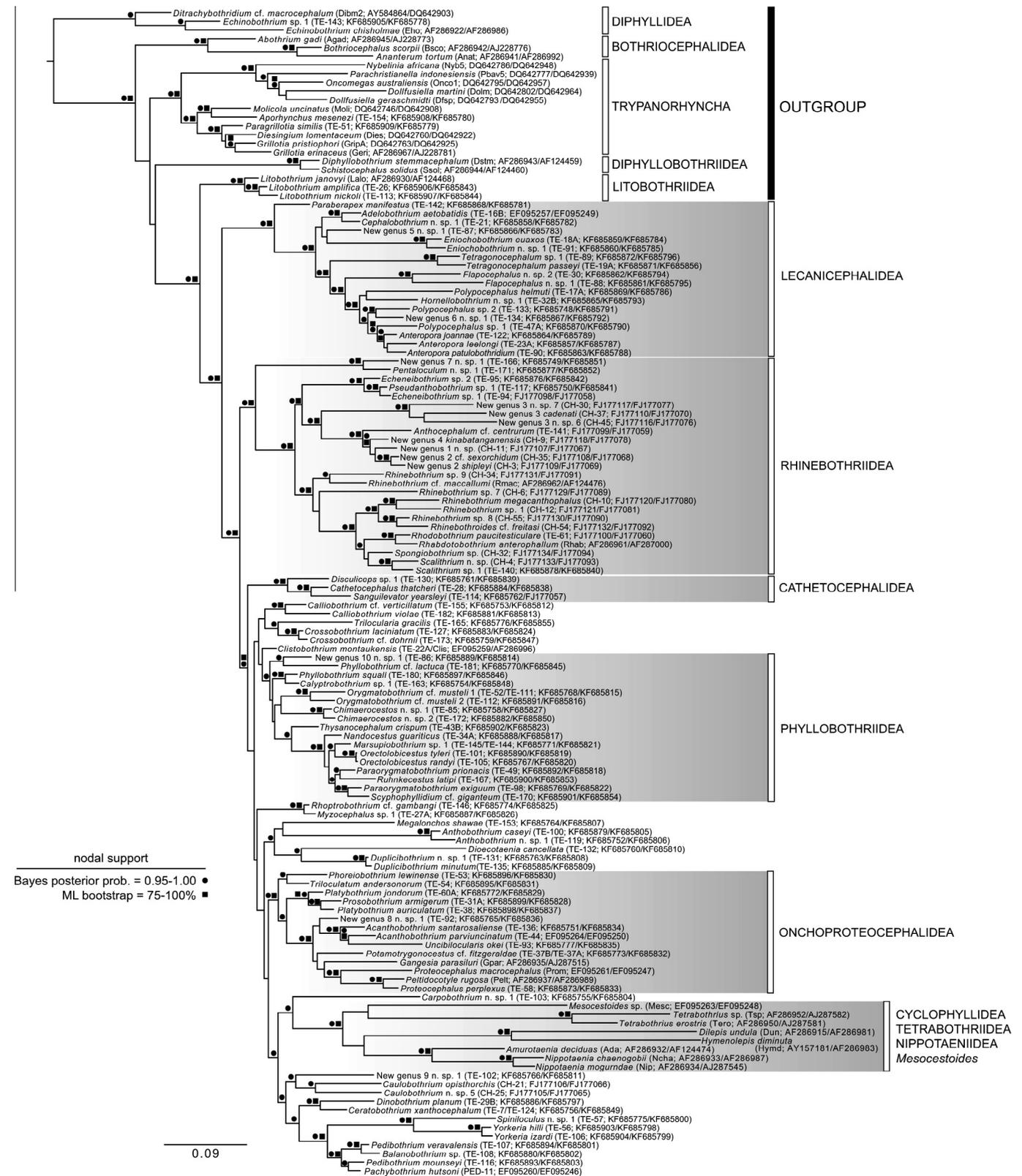


Fig. 2. Phylogenetic tree: Analysis 6. Bayesian analysis of the concatenated partial IsrDNA (D1–D3) + complete ssrDNA data of 134 taxa (Dataset_I). Constructed using MrBayes under the GTR+I+G model. Analysis was run for 10,000,000 generations, with 4,000,000 generations discarded as burn-in. Branch length scale bar indicates number of substitutions per site. ML, maximum likelihood.

1994, *Platybothrium* Lindon, 1890, *Acanthobothrium*, *Pedibothrium* Linton, 1908 and *Proteocephalus* Weinland, 1858). We feel strongly that these issues are best addressed in analyses that include much denser taxon sampling. Furthermore, it is important

to note that the outgroups employed in the analyses of Dataset_I (i.e., Analyses 2, 4, and 6) were much more limited than those employed in analyses of Dataset_II (i.e., Analyses 1, 3, and 5) and thus the interrelationships of the bothriate (i.e., Diphylloidea,

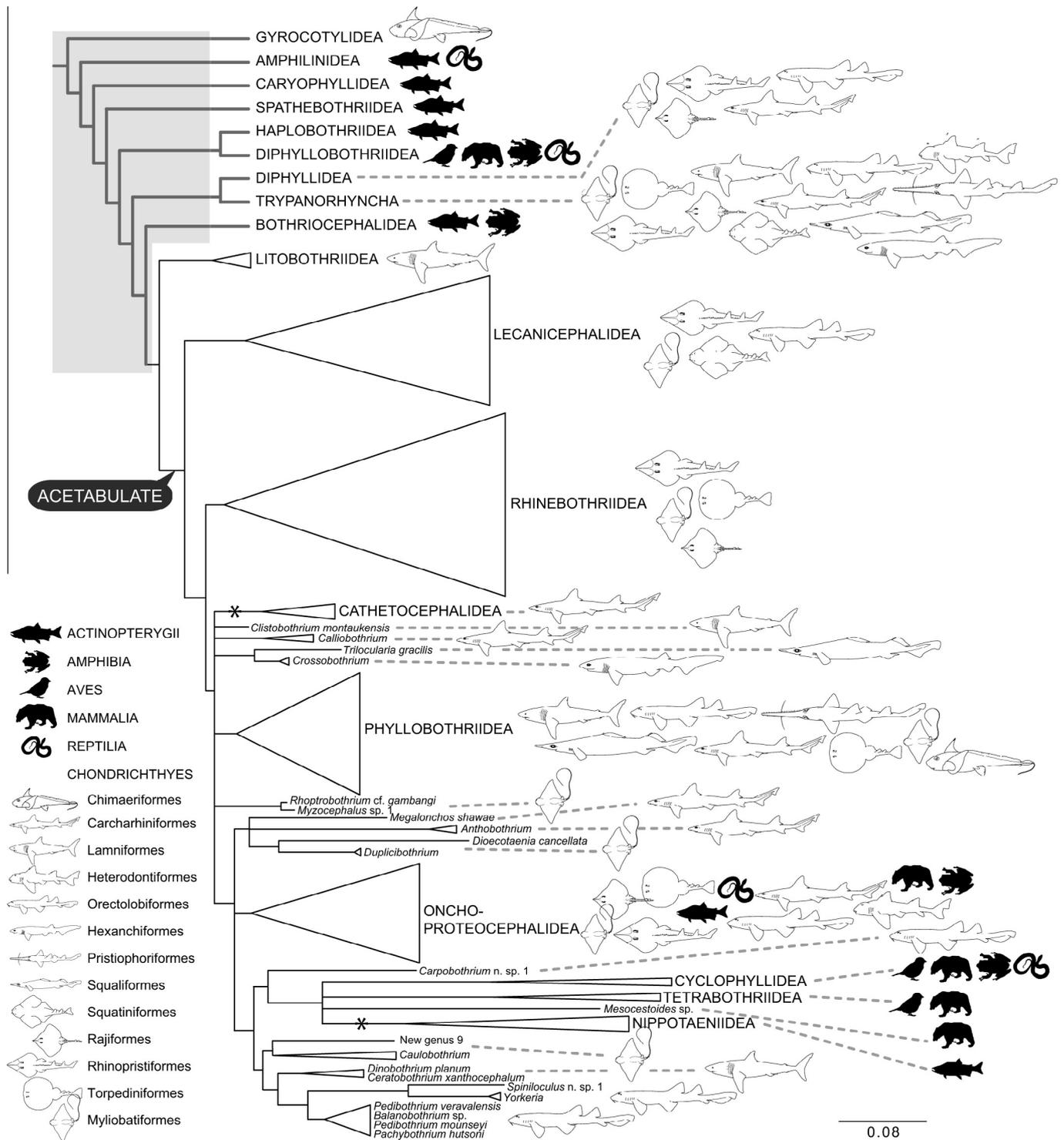


Fig. 3. Schematic diagram of phylogenetic tree from Analysis 6 (Fig. 2) expanded to include additional outgroups based on Waeschenbach et al. (2012). Major vertebrate host groups are indicated. Nodes which were supported by <0.95 Bayesian posterior probability and/or <75% Maximum Likelihood bootstrap support were collapsed. Asterisks indicate loss of acetabulae. Branch length scale bar indicates number of substitutions per site.

Bothriocephalidae, Trypanorhyncha, Diphyllbothriidea) and other non-acetabulate (i.e., Caryophyllidae, Spathebothriidea, etc.) cestode orders have not been addressed.

Not unexpectedly, the combined analysis with broadest taxon representation (Analysis 6) confirmed the monophyly, and thus ordinal status, of the elasmobranch-hosted Lecanicephalidae, Rhinebothriidea, Litobothriidea and Cathetocephalidae (Fig. 2). It also confirmed the monophyly of the acetabulate cestodes with one

exception. Despite their non-acetabulate nature (and thus their original inclusion among the outgroup taxa), the Cathetocephalidae grouped robustly among the acetabulate taxa in both Analysis 5 (Supplementary Figs. S5 and S6) and Analysis 6 (Figs. 2 and 3).

In contrast, the tetraphyllideans were distributed throughout the topology of the trees resulting from both combined analyses. Taxon-dense Analysis 6 yielded the following notable affinities. Although poorly supported, the Proteocephalidae (represented by

Gangesia Woodland, 1924, *Proteocephalus*, and *Peltidocotyle* Diesing, 1850) emerged as monophyletic, but nested with a high degree of support among a selection of mostly hooked tetraphyllidean genera (e.g., *Acanthobothrium*, *Uncibilocularis* Southwell, 1925, *Platybothrium*, *Prosobothrium* Cohn, 1902, *Phoreiobothrium* Linton, 1889, *Triloculatum* Caira and Jensen, 2009, New genus 8), with the freshwater stingray-hosted *Potamotrygonocestus* Brooks and Thorson, 1976 as its closest relative (Fig. 2). This result provides guidance for the reconfiguration of the ordinal classification of the proteocephalideans and their kin that follows below. Furthermore, a selection of hooked (i.e., *Spiniloculus* Southwell, 1925, *Yorkeria* Southwell, 1927, *Pedibothrium*, *Pachybothrium* Baer and Euzet, 1962, and *Balanobothrium* Hornell, 1911) and non-hooked (e.g., *Caulobothrium*, New genus 9, *Dinobothrium* van Beneden, 1889, *Ceratobothrium* Monticelli, 1892) tetraphyllidean genera grouped as sister to a clade consisting of *Mesocestoides* Vaillant, 1863 + Tetrabothriidea (i.e. *Tetrabothrius* Rudolphi, 1819) + Cyclophyllidea (i.e., *Dilepis* Weinland, 1858 and *Hymenolepis* Weinland, 1858) + Nippotaeniidea (i.e. *Nippotaenia* Yamaguti, 1939), with the tetraphyllidean *Carpobothrium* as the closest relative of the latter clade (Fig. 2).

A relatively large clade of tetraphyllideans that appears to have emerged, albeit with relatively weaker support, consisted of a diversity of non-hooked genera with acetabula in the form of relatively simple bothridia, many of which parasitise sharks (i.e., New genus 10, *Phyllobothrium*, *Orygmatobothrium* Diesing, 1863, *Thysanoccephalum* Linton, 1890, *Pithophorus* Southwell, 1925, *Orectolobicestus* Ruhnke, Caira and Carpenter, 2006, *Paraorygmatobothrium*, *Ruhnkecestus* Caira and Durkin, 2006, and *Scyphophyllidum* Woodland, 1927), but some of which parasitise rays (e.g., *Calyptrobthrium* Monticelli, 1893 and *Nandocestus* Reyda, 2008). *Chimaerocestos*, the only known genus of tetraphyllidean hosted by non-elasmobranch chondrichthyans (holocephalans), also nested within this clade. Within this larger clade, six genera (*Nandocestus*, *Paraorygmatobothrium*, *Pithophorus*, *Orectolobicestus*, *Ruhnkecestus* and *Scyphophyllidum*) comprised a subclade that was particularly well supported in the tree resulting from Analysis 6 (Fig. 2).

Three additional, albeit smaller, clades of tetraphyllidean genera (i.e., *Rhoptrobthrium* Shipley and Hornell, 1906 + *Myzocephalus* Shipley and Hornell, 1906; *Calliobothrium* + *Trilocularia* Olsson, 1867 + *Crossobothrium* Linton, 1889; *Megalonchos* Baer and Euzet, 1962 + *Anthobothrium* + *Dioecotaenia* Schmidt, 1969 + *Duplicibothrium* Williams and Campbell, 1978) were found intermingled among other cestode groups in the tree resulting from Analysis 6 (Fig. 2). These taxa were found to be by far the most labile in position across the six analyses. Morphologically, the latter two clades contain a particularly puzzling assemblage of genera and the relatively low support for each raises questions about their true affinities.

3.2. Interrelationships among currently recognised cestode orders

Both Analyses 5 and 6 (i.e. analyses of both datasets) strongly support the Lecanicephalidea as the earliest divergent order of acetabulate cestodes, followed by the Rhinebothriidea, which forms the sister to a large clade consisting of the remaining orders (Supplementary Figs. S5 and S6, and Figs. 2 and 3). Also emerging, albeit with strong support only in Analysis 5, is the Cathetocephalidea as the earliest diverging order within the latter, larger clade (Supplementary Figs. S5 and S6). Interrelationships among the other members of the larger clade are less well resolved and, in some cases, also less stable across analyses. A clade comprised of the orders Cyclophyllidea, Tetrabothriidea, Nippotaeniidea and *Mesocestoides* is robustly supported in all analyses of both datasets, however their interrelationships differed between analyses.

With respect to the Tetraphyllidea, Analyses 5 and 6 are consistent in the following elements. The non-monophyly of the order is

indisputable. A suite of tetraphyllidean genera are sister to the Proteocephalidea. Another suite of tetraphyllidean genera are sister to the clade comprised of the Cyclophyllidea, Tetrabothriidea, Nippotaeniidea and *Mesocestoides*. However, in both cases the closest tetraphyllidean relative is ambiguous.

4. Discussion

Phylogenetic analysis of *lsr*DNA and *ssr*DNA based on a comprehensive sampling of tetraphyllidean taxa in the context of other acetabulate cestode orders has provided support for a number of previously proposed systematic hypotheses, challenged others, and has suggested new phylogenetic scenarios. These require consideration with reference to morphology, and synapomorphies supported, challenged, or newly revealed. Such phylogenetic hypotheses are discussed separately below in the context of their implications for cestode orders and our understanding of cestode evolution overall.

The three litobothriidean species included in our analyses consistently formed a monophyletic group distinct from the remaining major lineages. This result supports Dailey (1969) and Olson and Caira's (2001) recognition of the Litobothriidea as an independent order. The litobothriideans were found to be sister to a clade comprised of the acetabulate cestode groups. This suggests that the unusual form of the scolex seen in the seven known litobothriidean species (i.e. an apical sucker followed by a series of pseudosegments) likely represents a uniquely derived condition, rather than a modification of the acetabulate scolex form characteristic of the members of its sister clade.

The monophyly of the Lecanicephalidea and its status as an independent order were both well supported by all of the analyses conducted here. Our results suggest that this lineage of apical organ-bearing cestodes represents the sister taxon of the remaining acetabulate cestode taxa. It is interesting that *Paraberapex manifestus* Jensen, 2001, the only lecanicephalidean species included that lacks an apical organ, grouped as the sister taxon to the clade comprised of the 17 apical organ-bearing species. However the analysis included representatives of only nine of the 21 described genera and thus much remains to be explored with respect to the interrelationships within the order in general.

The erection of the Rhinebothriidea by Healy et al. (2009), was fully supported by the results of our analyses. In addition, *Pseudanthobothrium* Baer, 1956, not treated by Healy et al. (2009), was found to group among species of *Echeneiobothrium* suggesting that within the Rhinebothriidea there exists a clade of taxa the adult form of which bears an apical modification of the scolex proper (in this case a myzorhynchus). Although lacking facial loculi, *Pseudanthobothrium* exhibits bothridial stalks and on this basis was predicted by Healy et al. (2009) to belong in the Rhinebothriidea. Our results also support inclusion of *Anthocephalum* Linton, 1890 in the Rhinebothriidea as was suggested by Healy et al. (2009) despite its lack of facial bothridial loculi. In addition, a novel genus (New genus 7; Fig. 1C) and the tetraphyllidean *Pentaloculum* Alexander, 1963, not treated by Healy et al. (2009), were found to group as close relatives of the Rhinebothriidea. Although both genera exhibit facial loculi, neither is well known and their morphology and status as members of the Rhinebothriidea require further investigation.

Although missing from our analyses and theirs, we support the suggestion of Healy et al. (2009) that the following five genera should be considered candidates for inclusion in the Rhinebothriidea based on their possession of facial loculi and possibly also bothridial stalks: *Clydonobothrium* Euzet, 1959, *Notomegarhynchus* Ivanov and Campbell, 2002, *Phormobothrium* Alexander, 1963, *Tri-taphros* Lönnberg, 1889 and *Escherbothrium* Berman and Brooks, 1994. We propose that on this morphological basis *Biotobothrium*

Tan, Zhou and Yang, 2009 and *Zyxiobothrium* Hayden and Campbell, 1981 be added to this list. However, the rhinebothriidean status of these seven genera remains to be confirmed with molecular data.

Our analyses consistently yielded a clade comprised of three genera, two of which (*Cathetocephalus* Dailey and Overstreet, 1973 and *Sanguilevator* Caira, Mega and Ruhnke, 2005) were formally recognised in the order Cathetocephalidea by Caira et al. (2005). The inclusion of a third genus, *Disculiceps* Joyeux and Baer, 1936, in this clade, and thus this order, is a novel result and indicates that *Disculiceps* should be transferred from the Tetracystida to the Cathetocephalidea. This action is fully consistent with the morphology of the scolex of *Disculiceps* which is bipartite consisting of an anterior cushion and posterior collar, and which, like the scoleces of *Cathetocephalus* and *Sanguilevator*, lacks all evidence of acetabula. Our results support recognition of the Cathetocephalidea as a distinct order at this time. However, its position relative to many of the tetracystid groupings was found to be ambiguous across analyses.

The non-monophyly of the Tetracystida was anticipated based on previous investigations, but among the most striking results of our analyses was the remarkably widespread intermingling of tetracystid taxa among and across the other cestode orders. Also somewhat unexpected was the determination that the hooked tetracystids (i.e. the Onchobothriidae) represent at least three independent lineages.

It is beyond the scope of this study to fully revise the classification of the Tetracystida so as to render it entirely monophyletic given that in some cases the relationships recovered were labile, differing across analyses and the solution for absolute monophyly is unclear. However, two new orders are erected below to accommodate the two clades of genera that were mostly stable across analyses. Although not ideal, we propose that the other genera remain in the non-monophyletic "Tetracystida" until such time as their relationships can be more fully and definitively resolved based on more dense taxon sampling and additional molecular data. Ultimately, the complete dismantling of the "Tetracystida" may be required to promote systematic clarity.

4.1. Onchoproteocephalidea n. ord.

Synonyms: Tetracystida Carus, 1863 *pro parte*; Proteocephalidea Mola, 1928 *in toto*.

Diagnosis

Cestoda: Eucestoda. Small to medium sized tapeworms. Strobila polyzoic, proglottised; simple gladiate spinitriches present posterior to scolex proper, at a minimum on cephalic peduncle, neck, and/or proliferation zone, on entire strobila in some. Proglottids hermaphroditic, generally euapolytic or apolytic, occasionally hyperapolytic. One set of reproductive organs per proglottid. Two pairs of lateral osmoregulatory canals; ventral canals usually wider than dorsal canals. Scolex with four muscular bothridia. Bothridia unarmed or with one pair of hooks, facially divided or not, lacking stalks. Apical organ present in some. Metascolex present in some. Testes numerous, post-poral field present. Vas deferens convoluted. External seminal vesicle present or absent. Cirrus armed with spinitriches. Genital pores lateral, irregularly alternating. Vagina opening anterior or posterior to cirrus sac. Vitellarium follicular; follicles generally in lateral fields. Uterus with or without lateral diverticula. Adults in freshwater fish, amphibians, reptiles occasionally mammals, and elasmobranchs. Taxa included: all proteocephalidean genera and the tetracystid genera *Acanthobothrium*, *Acanthobothroides*, *Onchobothrium*, *Pinguicollum*, *Platybothrium*, *Phoreiobothrium*, *Potamotrygonocestus*, *Prosobothrium*, *Triloculatum*, *Uncibilocularis*, New genus 8; likely also *Megalonchos*.

Within the Onchoproteocephalidea, the reciprocal monophyly of the Proteocephalidea sensu de Chambrier et al. (2004) and the genera traditionally assigned to the Tetracystida was either not supported (Analysis 2) or only weakly supported (Analyses 4 and 6) in all analyses with relevant representation. The establishment of this new order is thus necessary to preserve ordinal monophyly. However, it is somewhat radical in that it expands the concept of the Proteocephalidea, which heretofore has consisted solely of non-hooked taxa that primarily parasitise bony fishes and some terrestrial tetrapods, such as lizards, snakes and the occasional mammal (de Chambrier et al., 2004), to include a selection of hooked taxa that parasitise stingrays, a few skates and sharks. Nonetheless, this concept has gained substantial momentum over the past decade with *Acanthobothrium* and/or *Phoreiobothrium* being found to group with the proteocephalideans on the basis of molecular data (e.g., Olson et al., 2001; Caira et al., 2005; Waeschenbach et al., 2007, 2012; Healy et al., 2009). Although a morphological synapomorphy has not previously been identified to support this new order, the overall morphological resemblances between certain proteocephalideans and tetracystids has been noted previously by several authors (e.g., de Chambrier et al., 2009b; Scholz et al., 2013).

Our results provide definitive evidence of the integrated nature of the affinities between some members of the hook-bearing tetracystid family Onchobothriidae and the order Proteocephalidea. This result is consistent with all previous molecular analyses that have included relevant representation of both groups (Olson and Caira, 1999; Olson et al., 2001; Kodedová et al., 2000; Caira et al., 2005; Waeschenbach et al., 2007, 2012; Healy et al., 2009). The lack of representation across the full spectrum of onchobothriids in these previous works, in part, impeded the taxonomic action required to formally recognise these affinities so as to maintain monophyly at the ordinal level. Our analyses included 13 of the 18 described and one undescribed genus of onchobothriids, and thus revision of the classification in an informed manner is now possible. The name Onchoproteocephalidea n. ord. is proposed to reflect the hybrid nature of the order.

It is much easier to identify morphological differences, rather than similarities, between genera previously assigned to the Proteocephalidea and the subset of onchobothriid genera proposed here to belong to this new order. Indeed, despite the mounting molecular evidence, formal recognition of this as a cohesive group has also been hampered by the lack of one or more morphological features uniting its members. In searching for diagnostic features we observed that among onchobothriids, genera that exhibit gladiate spinitriches throughout the length of their strobila (i.e., *Phoreiobothrium*, *Platybothrium*, *Potamotrygonocestus*, *Prosobothrium*, *Triloculatum* and New genus 8) were included in the molecularly-defined Onchoproteocephalidea. This led us to explore this feature in proteocephalidean genera. Remarkably, in essentially all cases for which SEM data are available for body surfaces posterior to the scolex proper (i.e., proliferation zone, immature and/or mature proglottids), simple gladiate spinitriches are reported. This was true for multiple genera in both the Proteocephalidae (e.g., Scholz et al., 1999; de Chambrier, 2006; de Chambrier and de Chambrier, 2010; Ash et al., 2012; Rambeloson et al., 2012) and the Monticelliidae (e.g., Gil de Pertierra, 2002, 2005; de Chambrier et al., 2006; Scholz et al., 2008; Arredondo et al., 2013; Gil de Pertierra and de Chambrier, 2013). While promising, this feature requires more detailed study across genera in both families as SEM data are available only for more recently described taxa and a few exceptions may exist (e.g., possibly de Chambrier et al., 2009a; Gil de Pertierra, 2009). Also worth noting is the fact that species of *Acanthobothrium* and *Uncibilocularis* bear a dense covering of gladiate spinitriches restricted to the cephalic peduncle (sensu Caira et al., 1999), a region of the body immedi-

ately posterior to the scolex proper that may be homologous to the neck and/or proliferation zone.

Although not represented here, based on their scolex morphology, possession of gladiate spinitriches posterior to the bothridia and host associations, the onchobothriid genera *Acanthobothroides* Brooks, 1977, *Onchobothrium* de Blainville, 1828, and *Pinguicollum* Riser, 1955 should also be included in this order. Based on these same features and perhaps additional molecular data, we predict that *Megalonchos* may ultimately also be found to belong to this new order. Transfer of the type genus of Onchobothriidae (i.e. *Onchobothrium*) to the Onchoproteocephalidea makes it available to house at least a subset of genera of the Onchoproteocephalidea. Inclusion of a representative of *Onchobothrium* in future molecular analyses is required to confirm this action. Furthermore, the composition of the family, if retained, remains to be determined as the onchobothriids transferred to the new order do not represent a monophyletic group relative to proteocephalidean taxa.

4.2. Phyllobothriidea n. ord.

Synonyms: Tetraphyllidea Carus, 1863 *pro parte*.

Diagnosis

Cestoda: Eucestoda. Small to medium sized tapeworms. Strobila polyzoic, proglottised; spinitriches restricted to bothridial surfaces, often serrate or gongylate. Neck and strobilar surfaces with filitriches arranged in scutes, or leaf-like structures in some. Proglottids hermaphroditic, euapolytic or apolytic, craspedote or acraspedote. One set of reproductive organs per proglottid. Two pairs of lateral osmoregulatory canals; ventral canals usually wider than dorsal canals. Scolex with four muscular bothridia. Bothridia with anterior accessory sucker, unarmed, most without facial loculi, some with marginal loculi, extensively folded posteriorly in some, lacking stalks; accessory sucker without lateral muscular projections. Apical organ, metascolex and remi (sensu Jensen and Caira, 2006) absent. Testes numerous, post-poral field present. Vas deferens convoluted. External seminal vesicle present or absent. Cirrus armed with spinitriches. Genital pores lateral, irregularly alternating. Vagina opening anterior to cirrus sac. Vitellarium follicular; follicles generally in lateral fields, occasionally circumcortical. Uterus without lateral diverticula. Adults primarily in sharks, occasionally in batoids (*Nandocestus* and *Calyptrobothrium*) and ratfish (*Chimaerocestos*). Taxa included: *Calyptrobothrium*, *Chimaerocestos*, *Marsupiobothrium* Yamaguti, 1952, *Nandocestus*, *Orectolobicestus*, *Orygmatobothrium*, *Paraorygmatobothrium*, *Phyllobothrium*, *Ruhnkecestus*, *Scyphophyllidium*, *Thysanocephalum*, and New genus 10 (Fig. 1F); likely also to include *Alexandercestus* Ruhnke and Workman, 2013, *Bibursibothrium* McKenzie and Caira, 1998, *Cardiobothrium* McKenzie and Caira, 1998, *Clistobothrium*, *Crossobothrium*, *Flexibothrium* McKenzie and Caira, 1998 and *Pelichnibothrium* Monticelli, 1889.

The second order that has emerged from among the tetraphyllideans, but admittedly less definitively, is one comprised of a subset of the non-hooked tetraphyllidean taxa, most of which have at one time been assigned to the family Phyllobothriidae (see Ruhnke, 2011). With respect to morphological attributes supporting this order, all of the above genera exhibit bothridia that bear an anterior accessory sucker; most parasitise carcharhiniform or orectolobiform sharks.

We recognise that the order as circumscribed above was not consistently supported across analyses; monophyly of the representatives of these genera was not recovered in the trees resulting from Analyses 1, 2 and 5, whereas their monophyly was supported in the trees resulting from Analyses 3 and 4 and, perhaps most importantly, also in Analysis 6 which included data for both genera

for the full complement of taxa. Nonetheless, we believe that establishment of a distinct order to house these taxa is justified. The name Phyllobothriidea is proposed for this new order given this clade includes *Phyllobothrium*, the type genus of the family Phyllobothriidae and current home to the majority of these genera (see Ruhnke, 2011). In fact, Ruhnke (2011) considered 10 of the above 11 described genera as confirmed or at least provisional members of the family in his monograph revising the Phyllobothriidae. While recognising its affinities with the phyllobothriids, Ruhnke (2011) considered *Chimaerocestos*, which is unique among eucestodes in parasitising holocephalans, to belong to the monogeneric Chimaerocestidae. Our results have led us to include it here in the Phyllobothriidae.

With respect to other potential members of the order, *Crossobothrium* and *Clistobothrium* Dailey and Vogelbein, 1990 are candidates. They were treated as members of the Phyllobothriidae by Ruhnke (2011); both also bear an anterior accessory sucker and parasitise sharks. However, they were either labile in position across analyses and/or their association with the other members of the order was only weakly supported, thus we have refrained from formally including them in the order at this time. Although not represented here, three other confirmed or provisional members of the Phyllobothriidae sensu Ruhnke (2011) (i.e., *Bibursibothrium* McKenzie and Caira, 1998, *Cardiobothrium* McKenzie and Caira, 1998, *Flexibothrium* McKenzie and Caira, 1998) and one erected since (i.e. *Alexandercestus* Ruhnke and Workman, 2013) are worth consideration. Although still poorly known, *Pelichnibothrium* may also ultimately be found to belong in this order. These genera all bear anterior accessory suckers and parasitise sharks.

The presence of an anterior accessory sucker in the rhinebothriidean genera *Anthocephalum* and *Escherbothrium* Berman and Brooks, 1994 is likely a homoplasious occurrence of this feature. Among the other tetraphyllidean genera included in our analyses but not formally assigned to this order, only *Caulobothrium* and *Dinobothrium* appear to exhibit accessory suckers. At this time we have refrained from transferring either for there is little other evidence to support their inclusion in the Phyllobothriidea. In the former case, its placement relative to the other genera among analyses was particularly unstable (e.g. Fig. 2 versus Supplementary Fig. S5) and the presence of an anterior accessory sucker on its otherwise rhinebothriidean-like loculated bothridia remains to be confirmed. *Dinobothrium* failed to group with the above genera in any of the analyses and exhibits anterior lappets that support its affiliation with *Ceratobothrium*.

4.3. Tetraphyllidea

While adoption of the proposed two new orders will do much to reduce the polyphyletic nature of the Tetraphyllidea by providing new or provisional ordinal placements for 31 genera, the relationships and appropriate ordinal homes for the remaining described genera (see Table 1) are unresolved. The remaining genera clearly do not represent a monophyletic assemblage and in most cases their hypothesised affinities differed across analyses and support for their inclusion in any larger clade was generally low. We propose they remain in the order in its revised sense ("Tetraphyllidea") until such time as additional sampling and data can be generated to explore their phylogenetic affinities more fully. So, to guide future work, issues with each suite of taxa are treated below.

Among the cestodes remaining in the "Tetraphyllidea" are two independent clades of hooked taxa. The most diverse is the clade *Pachybothrium* + *Pedibothrium* + *Balanobothrium* + *Spiniloculus* + *Yorkeria*. These genera are united by their lack of post-poral testes and by their association with orectolobiform sharks. They differ from most other hooked taxa in that their single pair of hooks are

either bipronged with internal channels that open separately in each prong (rather than via a single pore), or are unipronged. It is puzzling that this clade consistently grouped with subsets of the following taxa: *Ceratobothrium* + *Dinobothrium*, *Caulobothrium*, New gen. 9, and in one instance (Analysis 2) *Carpobothrium*. This fact is difficult to reconcile with morphology (all four of the latter genera lack hooks) or host associations (the first two parasitise lamniform sharks, the second two batoid rays and the latter oreoichthiform sharks). Furthermore, the interrelationships between the various non-hooked taxa and the hooked clade are unstable across analyses.

The second clade of hooked taxa, consisting of two species of *Calliobothrium* that parasitise triakid sharks, was similarly problematic. This genus consistently grouped well away from all other hooked genera, a fact that is reconcilable with its possession of a unique armature in the form of two pairs of hooks per bothridium, rather than a single pair. However, its hypothesised affinities varied substantially across analyses and in all cases support for these affinities was weak. Although not included in the present study, we anticipate that *Biloculuncus* Nasin, Caira and Euzet, 1997 and *Erudituncus* Healy, Scholz and Caira, 2001 will be found to be closely allied with *Calliobothrium* based on their association with triakid sharks and possession of multiple pairs of hooks. Their inclusion in future analyses may serve to improve resolution of the affinities of what is likely a third lineage of hooked tetraphyllideans.

In terms of reconciling morphology, host associations and stability of their overall relationships, by far the most problematic of the non-hooked genera remaining in the “Tetraphyllidea” (apart from *Megalonchos* which was treated above with the Onchoproteocephalidea) are *Dioecotaenia*, *Duplicibothrium*, *Rhoptrobothrium*, *Myzocephalus*, *Ceratobothrium*, *Dinobothrium* and *Anthobothrium*. Among these genera three robust pairs of sister-taxa have emerged: (i) *Duplicibothrium* + *Dioecotaenia*, both of which parasitise cownose rays and bear facial bothridial loculi; this association was strongly supported in all six analyses. (ii) *Rhoptrobothrium* + *Myzocephalus*, both of which parasitise myliobatid stingrays and share their possession of a bipartite scolex bearing an elongate cephalic peduncle equipped with four flap-like remi: this association was strongly supported in all three analyses that included both genera (Analyses 2, 4 and 6). Based on morphology and host associations, we predict that *Myzophyllobothrium* Shipley and Hornell, 1906, if valid, will ultimately be found to belong to this clade. (iii) *Ceratobothrium* + *Dinobothrium*, both of which infect lamniform sharks and bear lateral muscular lappets on the apical region of each bothridium; this association was highly supported in all three analyses that included both genera (Analyses 2, 4 and 6). Based on its morphology and host associations we believe *Dinobothrium* will ultimately also be found to belong in this clade. Nonetheless, these three clades and *Anthobothrium* were by far the most labile of tetraphyllidean taxa in that their phylogenetic positions differed conspicuously across analyses and they generally grouped with taxa with which they bear no obvious morphological and/or host similarities. Curiously, *Anthobothrium* which is essentially unique among tetraphyllideans in lacking apical bothridial suckers, failed to group consistently with any genus or clade but was most commonly found allied, with only weak support, with the hooked genus *Megalonchos*.

4.4. Cyclophyllidea and their kin

An intriguing result of our analyses was the fact that the sister taxon to the clade composed of the primarily terrestrial, tetrapod-parasitising Cyclophyllidea + *Mesocestoides* + Tetrabothriidea + Nippotaeniidea was consistently found to be a member of the “Tetraphyllidea”. The tetraphyllideans comprising the sister group to this clade could not, however, be determined unambiguously because the specific tetraphyllidean taxon (or suite of taxa)

and the level of support differed across analyses. Candidates are: New genus 9 (Analyses 1 and 5), New genus 9 + *Caulobothrium* (Analysis 2), *Carpobothrium* (Analyses 3 and 6), and *Carpobothrium* + *Caulobothrium* (Analysis 4). This result is generally inconsistent with previous works (Olson and Caira, 1999; Olson et al., 2001; Hoberg et al., 2001; Waeschenbach et al., 2007, 2012), all of which have found candidate sister taxa to the tetrapod-parasitising cestodes to include tetraphyllidean genera we have referred to here as the Onchoproteocephalidea. We would attribute this to the fact that none of these previous studies included any of the tetraphyllidean candidate sister taxa identified here. Thus, these new results are of wider taxonomic and evolutionary significance.

4.5. Evolution and Host Associations of major Eucestode Lineages

Based on the new configuration of orders proposed here, the Eucestoda, or true tapeworms, should be considered to consist of the following 17 orders: Bothriocephalidea, Caryophyllidea, Cathetocephalidea, Cyclophyllidea, Diphyllidea, Diphyllbothriidea, Haplobothriidea, Lecanicephalidea, Litobothriidea, Nippotaeniidea, Onchoproteocephalidea n. ord., Phyllobothriidea n. ord., Rhinebothriidea, Spathebothriidea, Tetrabothriidea, Trypanorhyncha and “Tetraphyllidea”. This brings the total number of orders in the class Cestoda to 19 (i.e., including the Gyrocotylidea and Amphilinidea). It is interesting that nine of these orders (i.e. ~50%) are associated with elasmobranchs. However, truly remarkable is the key role that elasmobranch-hosted taxa appear to have played in the evolution of cestodes overall. Mapping of major vertebrate groups on the schematic diagram of the tree from Analysis 6 shown in Fig. 3 emphasises elasmobranch-hosted cestodes comprising the backbone of the cestode phylogeny overall. Our results suggest that both of the non-elasmobranch hosted clades of acetabulate cestodes had their origins in elasmobranch-hosted taxa. Depending on resolution of the relationships among bothriate taxa, this is possibly also true for the Bothriocephalidea relative to the Diphyllidea and Trypanorhyncha. Furthermore, several additional elasmobranch-hosted cestode lineages will likely need to be recognised among the “Tetraphyllidea” once these relationships are more fully understood.

With respect to the taxa previously assigned to the Proteocephalidea, the majority of which parasitise freshwater teleosts (see de Chambrier et al., 2004), their candidate elasmobranch-hosted sister taxa are illuminating, for all have some affinity with freshwater habitats. *Potamotrygonocestus* parasitises freshwater stingrays of the family Potamotrygonidae Garman, 1877; New genus 8 parasitises sawfish of the genus *Pristis* Linck, 1790 and *Uncibilocularis* parasitises stingrays of the genus *Pastinachus* Rüppell, 1829, both of which frequent coastal euryhaline habitats and in some instances even freshwater (Martin, 2005). This suggests that teleost-parasitising cestodes may have had their origin with freshwater, or at least euryhaline, elasmobranchs. The final determination awaits definitive resolution of the sister taxon to the clade.

The fact that marine elasmobranchs may also constitute the sister taxon to what is generally referred to as the “terrestrial” clade (i.e., Cyclophyllidea + Tetrabothriidea + Nippotaeniidea + *Mesocestoides*) clearly invites further investigation. Much denser taxon sampling of the thousands of species and hundreds of genera in this “terrestrial” clade (i.e. beyond the six to eight species employed here), of the Cyclophyllidea in particular, is required to resolve these interrelationships with confidence. Inclusion of more dense taxon sampling of the three elasmobranch-hosted candidate sister genera (i.e., *Carpobothrium*, New genus 9 and *Caulobothrium*) would also be informative. These three genera offer remarkably different evolutionary scenarios with respect to the potential ori-

gins of the “terrestrial” clade. *Carpobothrium* parasitises bamboo-sharks of the family Hemiscylliidae Gill, 1892 (order Orectolobiformes) and is currently restricted to Indo-Pacific waters; *Caulobothrium* parasitises stingrays of the family Dasyatidae Jordan, 1888 (order Myliobatiformes) and occurs in essentially a circumtropical band; New genus 9 parasitises stingrays of the family Dasyatidae and guitarfish of the family “Rhinobatidae” Müller and Henle, 1837 (order Rhinopristiformes) and also is restricted to Indo-Pacific waters.

Another surprising result of this work was the relatively derived phylogenetic position occupied by the two species of *Chimaerocestos*. Given their association with holocephalans, rather than elasmobranchs, we had anticipated they would occupy a much earlier divergent position on the tree relative to species parasitising elasmobranchs. Instead, both species consistently and robustly grouped among the members of the new order Phyllobothriidea. This suggests that the association of this genus with holocephalans represents a host-switching event from elasmobranchs (most likely sharks) to holocephalans, rather than vice versa.

4.6. Morphological Evolution

The spectacular diversity of scolex forms seen among the genera that have been assigned to the Tetraphyllidea over time (e.g., see Euzet, 1994; Caira et al., 2001) is essentially without parallel in any other cestode order. Scolex elaborations seen in these elasmobranch-hosted cestodes include, for example, hooks, suckers, bothridia, pedicels, facial loculi, marginal loculi both with or without external septa, anterior pads, fusion of portions resulting in pouch-like structures, circular muscle bands, extensions on lateral margins of bothridia, stalked remi with primary and secondary areolae, etc. Several preliminary attempts to place this diversity of forms into a phylogenetic context have been made. For example, Caira et al. (1999) circumscribed 120 morphological characters, nearly 80 of which involved scolex features; Caira et al. (2001) expanded this list to 157 characters, over 100 of which treated scolex features. Unfortunately, in both cases the phylogenetic trees resulting from analyses of these data were relatively unresolved, and the phylogenetic relationships implied were relatively incongruent. However, in both studies, the non-monophyly of the Tetraphyllidea relative to other cestode orders was apparent. Given the results presented here, it now seems clear that much of the remarkable morphological diversity is a result of multiple radiations that represent independent lineages. Our proposal of two new orders resolves issues with a subset of the genera. However, the phylogenetic relationships and thus proper ordinal placement of many genera remain to be determined. It is intriguing that in most cases these genera are among the most morphologically enigmatic tetraphyllidean taxa. Unlike all other elasmobranch-hosted cestodes, *Dioecotaenia* bears proglottids that are dioecious rather than monoecious. Whether this represents sequential or simultaneous dioecy across the strobila remains to be determined. Similarly, unlike essentially all other “tetraphyllidean” taxa, *Anthobothrium* bears bothridia that lack, rather than possess, an apical orientation and/or apical modification of the bothridia. *Rhoptrobothrium* and *Myzocephalus* are unique in their possession of a scolex with four stalked remi extending from their cephalic peduncle, each of which bears primary and secondary areoli.

An even further dismantling of the “Tetraphyllidea” is inevitable and likely desirable, perhaps even to its final destruction. Our results suggest this may ultimately result in the recognition of even a greater number of elasmobranch-hosted orders but if monophyly of cestode orders is to be maintained will likely be necessary. The application of data from other molecular markers to further explore cestode interrelationships is now indicated. Only

in this way can we achieve taxonomic clarity and reveal further the complex evolutionary histories of cestodes and their elasmobranch hosts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijpara.2013.10.004>.

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