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Interrelationships and Evolution of the Tapeworms (Platyhelminthes: Cestoda)

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Interrelationships of the tapeworms (Platyhelminthes: Cestoda) were examined by use of small (SSU) and large (LSU) subunit ribosomal DNA sequences and morphological characters. Fifty new complete SSU sequences were added to 21 sequences previously determined, and 71 new LSU (D1–D3) sequences were determined for the complementary set of taxa representing each of the major lineages of cestodes as currently understood. New sequences were determined for three amphilinidean taxa, but were removed from both alignments due to their excessively high degree of divergence from other cestode sequences. A morphological character matrix coded for supraspecific taxa was constructed by the modification of matrices from recently published studies. Maximum-parsimony (MP) analyses were performed on the LSU, SSU, LSU+SSU, and morphological data partitions, and minimum-evolution (ME) analyses utilizing a general time reversible model of nucleotide substitution including estimates of among-site rate heterogeneity were performed on the molecular data partitions. Resulting topologies were rooted at the node separating the Gyrocotylidea from the Eucestoda. The LSU data were found to be more informative than the SSU data and were more consistent with inferences from morphology, although nodal support was generally weak for most basal nodes. One class of transitions was found to be saturated for comparisons between the most distantly related taxa (gyrocotylideans vs cyclophyllideans and tetrabothriideans). Differences in the topologies resulting from MP and ME analyses were not statistically significant. Nonstrobilate orders formed the basal lineages of trees resulting from analysis of LSU data and morphology. Difossate orders were basal to tetrafossate orders, the latter of which formed a strongly supported clade. A clade including the orders Cyclophyllidea, Nippotaeniidea, and Tetrabothriidea was supported by all data partitions and methods of analysis. Paraphyly of the orders Pseudophyllidea, Tetraphyllidea, and Trypanorhyncha was consistent among the molecular data partitions. Inferences are made regarding a monozoic (nonsegmented) origin of the Eucestoda as represented by the Caryophyllidea and for the evolution of the strobilate and acetabulate/tetrafossate conditions having evolved in a stepwise pattern. © 2001 Academic Press

Key Words: **Cestoda; Eucestoda; rDNA; phylogeny; molecular systematics.**

INTRODUCTION

Tapeworms (Platyhelminthes: Cestoda) are obligate internal parasites of vertebrates that display a wide range of body forms, life histories, and host associations (Table 1). Existing evidence suggests that extant groups evolved as parasites of fishes and subsequently radiated to parasitize all major vertebrate groups (Hoberg *et al.,* 1999a) with their greatest diversification found among tetrapod hosts (i.e., the Cyclophyllidea; Khalil *et al.,* 1994). A few species (e.g., *Echinococcus* spp.) are etiological agents of major diseases in human beings and domesticated animals and have received considerable attention from biologists in a variety of fields. From a phylogenetic perspective, however, such species are recent evolutionary novelties and offer little insight into the evolution of the group as a whole.

Recent efforts based primarily on comparative analysis of morphology, and to a lesser extent on molecules, have advanced our understanding of tapeworm systematics and evolution considerably (see reviews by Hoberg *et al.,* 1997b, 1999a) and more generally of their position within the phylum Platyhelminthes (Littlewood and Olson, 2001; Littlewood *et al.,* 1999, Fig. 1). The use of molecular data for the study of relationships among tapeworms has been largely limited to those species of medical or economic importance (for reviews see Mariaux, 1996; Mariaux and Olson, 2001), although a few recent studies have begun to address the systematics of a wider diversity of tapeworms (Kodedová *et al.,* 2000; Olson *et al.,* 1999; von Nickisch-Rosenegk *et al.,* 1999; Zehnder and Mariaux, 1999). Two works provided the foundation for the present study of ordinal-level interrelationships: Mariaux (1998) used partial nuclear small subunit (SSU)

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TABLE 1

Recognition of the Major Cestode Lineages, Their Representation, and Their Characteristics

Taxon	No. of exemplar taxa: Mariaux (1998)	Olson and Caira (1999)	Present study	Scolex condition[a]	Definitive host group(s)[b]
Amphilinidea	1	1	3[c]	"Monofossate"	Chondrosteans, primitive freshwater, and advanced marine teleosts (and *Austramphilina elongata* in freshwater chelonians in Australia)
Gyrocotylidea	0	1	2	"Monofossate"	Holocephalans
Eucestoda					
Caryophyllidea	1	1	5	"Monofossate"	Siluriform and cypriniform freshwater fishes (and *Archigetes* spp. in tubificid annelids)
Spathebothriidea	1	1	2	"Monofossate"	Freshwater, euryhaline, and marine chondrosteans and teleosts in the Northern Hemisphere
Diphyllidea	1	2	3	Difossate/bothriate	Elasmobranchs (predominantly squaliform)
Haplobothriidea	0	1	1	"Bothriate"	Amiiformes (bowfins)
Pseudophyllidea	4	0	5	Difossate/bothriate	Freshwater and marine teleosts (and *Cephalochlamys namaquensis* in anurans and caudates)
Diphyllobothriidae	2	2	2	Difossate/bothriate	Birds, reptiles, and mammals
Trypanorhyncha	2	2	13	Bothriate	Elasmobranchs
Cyclophyllidea	21	1	6	Tetrafossate/acetabulate	Tetrapods
Mesocestoididae	1	0	1	Tetrafossate/acetabulate	Mammals (and rarely birds)
Lecanicephalidea	0	2	3	Tetrafossate/acetabulate	Elasmobranchs (predominately squaliform)
Litobothriidea	0	2	2	Acetabulate	Elasmobranchs (lamniform sharks)
Nippotaeniidea	1	1	3	Acetabulate	Freshwater teleosts in China, Japan, New Zealand, and Russia
Proteocephalidea	7	1	6	Tetrafossate/acetabulate	Freshwater teleosts, amphibians, reptiles (and *Thaumasioscolex didelphidis* in opossums[d])
Tetrabothriidea	1	1	3	Tetrafossate/acetabulate	Marine mammals and birds
Tetraphyllidea	3	4	11	Tetrafossate/acetabulate	Elasmobranchs
Total	46	23	71		

[a] See Appendix 2, character 12, for discussion on terminology and characterization of scolex forms.
[b] Information modified from Khalil *et al.* (1994).
[c] Reported herein but not included in the analyses (see text).
[d] The first report of a proteocephalidean from a homeotherm vertebrate (*Didelphis marsupialis*) was recently described by Cañeda-Guzmán *et al.* (2001); see Kodedová *et al.* (2000) for an analysis of SSU rDNA that includes this unusual proteocephalidean species.

ribosomal DNA (rDNA) sequences of 43 taxa representing 11 orders, and Olson and Caira (1999) used complete SSU and partial elongation factor-1α (Ef-1α) sequences of 23 taxa representing 14 orders. Although both studies resolved most of the internodes delineating the major lineages, a small number of exemplar taxa was utilized, including single species in some cases (see Table 1), and significant differences were found among the estimates of phylogeny. A better representation of eucestode diversity and additional sequence data were considered necessary to produce more robust results. The present study combines the majority of taxa used in previous studies with a large number of additional taxa and fully complements complete SSU sequences with partial sequences from the LSU gene (domains D1–D3). In addition, a modified morphological matrix derived from Hoberg *et al.* (2001) and Justine (2001) was constructed primarily to examine the congruence between morphological and molecular data.

Subsequent to the publications of Mariaux (1998) and Olson and Caira (1999) three sequences of cestodes were determined to be erroneous: partial SSU sequences of a diphyllidean (*Echinobothrium* sp.) (Mariaux, 1998; GenBank Nos. Z93841–43) and partial Ef-1α sequences of an amphilinidean (*Schizochoerus liguloideus*) and a spathebothriidean (*Spathebothrium simplex*) (Olson and Caira, 1999; GenBank Nos. AF124793 and AF124795, respectively). Inferences based on these sequences in the original and subsequent publications must therefore be considered unsubstantiated: specifically, that SSU supports a sister group relationship between the Diphyllidea and the Proteocephalidea (Hoberg *et al.*, 2001; Mariaux, 1998; Mariaux and Olson, 2001) and that Ef-1α supports a basal position of the Spathebothriidea (Mariaux and

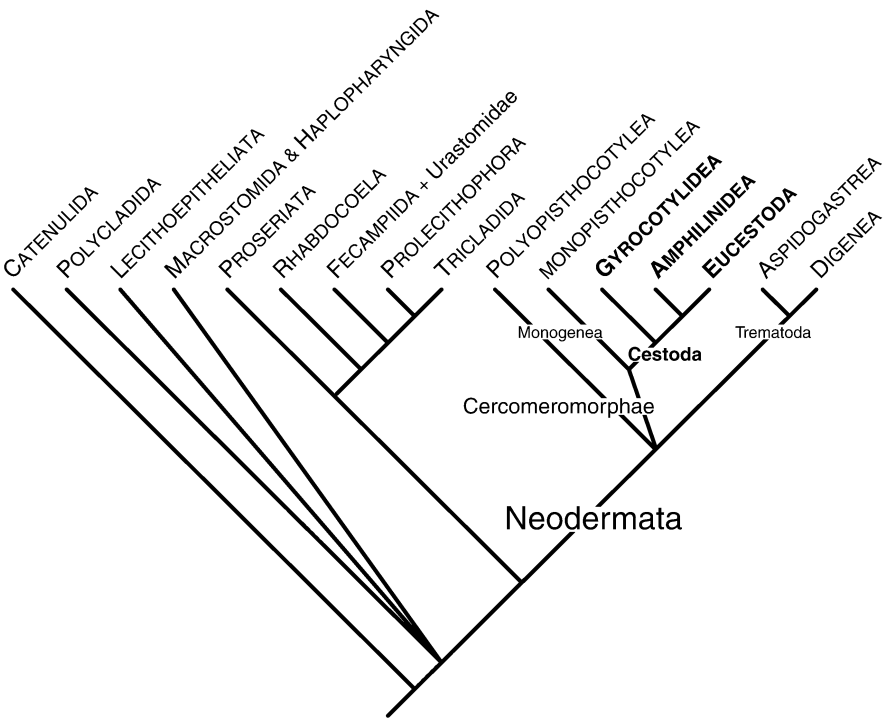

FIG. 1. Interrelationships of the Platyhelminthes showing the position of the Cestoda based on a phylum-level analysis of SSU rDNA of 270 taxa by Littlewood and Olson (2001). Note the positions of the orders Amphilinidea and Gyrocotylidea relative to the Eucestoda. Monophyly of the Cercomeromorphae and Monogenea was not resolved in these analyses.

Olson, 2001; Olson and Caira, 1999) within the Eucestoda. The authors (J.M. and P.D.O.) have removed these sequences from the GenBank/EMBL public databases.

Spelling of the terms proglottis/proglottides/proglottisation, scolex/scoleces, and strobila/strobilation follow the recommendations of Arme (1984). We use the taxon name Eucestoda to include all tapeworms exhibiting hexacanth larvae, whether monozoic or polyzoic (= Cestoidea *sensu* Ax, 1996; Ehlers, 1985), and Cestoda to include the Gyrocotylidea + Amphilinidea + Eucestoda (= Cestoidea *sensu* Brooks and McLennan, 1993).

MATERIALS AND METHODS

Collection of Taxa

New specimens were collected or obtained to supplement the previously collected taxa used in the studies of Mariaux (1998), Olson and Caira (1999), and Littlewood *et al.* (1999). Table 2 shows a complete taxonomic listing of the species analyzed, their hosts, collection localities, and sequence and voucher specimen accession numbers. Multiple exemplar species representing each of the 14 orders recognized in Khalil *et al.* (1994) and the putatively independent lineages Diphyllobothriidae (see Kodedová *et al.*, 2000; Mariaux, 1998), Litobothriidae (see Olson and Caira, 1999), and Mesocestoididae (see Mariaux, 1998) were included in the present data set. Specimens new to this study were fixed in 90–100% EtOH and stored at −20°C. Genomic DNA extracts were used to generate new data from taxa previously collected, although new extractions were performed in some cases.

DNA Isolation, PCR Amplification, and Gene Sequencing

Ethanol in the tissue samples was replaced with 1 M Tris–EDTA (pH 8) buffer (TE) via repeated washings. Genomic DNA was extracted by the grinding of the samples in TE containing 1% sodium dodecyl sulfate followed by 24 h incubation with the addition of proteinase K. Proteins were removed by phenol precipitation and the gDNA was concentrated with Millipore Microcon columns. Then 25-μl PCR amplifications were performed with Ready-To-Go (Amersham Pharmacia Biotech) PCR beads (each containing ~1.5 units *Taq* DNA polymerase, 10 mM Tris–HCl at pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, and stabilizers, including BSA), 1 μl of genomic extract, and 10 mM each PCR primer. The following thermocycling profile was used: 3 min denaturation hold at 96°C; 40 cycles of 1 min at 96°C, 1 min at 54°C, 2 min at 72°C; and 7 min extension hold at 72°C. The complete SSU gene was amplified in two overlapping fragments as described in Olson and Caira (1999) and the

TABLE 2

Taxonomic Listing of Species Analyzed with Hosts, Collection Localities, and Accession Numbers

Classification[a] *Taxon sequenced* (specimen voucher Accession No.[c]) [*host species* (common name), collection locality]	GenBank No.[b] SSU	LSU
Amphilinidea Poche, 1922		
Amphilinidae Claus, 1879		
Austramphilina elongata [*Chelodina longicollis* (Eastern long-necked turtle), Armidale, NSW, Australia]	AJ287480§	AF286907§
Gigantolina magna [*Diagramma labiosum* (Painted sweetlips), Coral Sea, Heron Lagoon, Heron Island, Queensland, Australia]	AJ243681	AF286908§
Schizochoeridae Poche, 1922		
Schizochoerus liguloideus [*Arapaima gigas* (Piraruchu), Itacoatiara, Provence Amazonas, Brazil]	AF124454	n/a
Caryophyllidea van Beneden in Carus, 1863		
Balanotaeniidae Mackiewicz & Blair, 1978		
Balanotaenia bancrofti [*Tandanus tandanus* (Dewfish), Pullen Pullen Creek, Brisbane River, Brisbane, Australia]	AF286977§	AF286909§
Capingentidae Hunter, 1930		
Breviscolex orientalis (BMNH-2001.1.30.1-4) [*Hemibarbus barbus* (Barbel steed), Hiroi River at Kotobuki, Iiyama City, Nagano Prefecture, Japan]	AF286978§	AF286910§
Caryophyllaeidae Leuckart, 1878		
Caryophyllaeus laticeps [*Rutilus rutilus* (Roach), Neuchâtel Lake, Neuchâtel, Switzerland]	AJ287488§	AF286911§*
Hunterella nodulosa (LRP-2123-28) [*Catostomus commersoni* (White sucker), Illinois, USA]	AF124457	AF286912§
Lytocestidae Hunter, 1927		
Caryophyllaeides ergensi (BMNH-2001.1.29.4-5) [*Tribolodon hakunensis* (*Ugui*), Hiroi River at Kotobuki, Iiyama City, Nagano Prefecture, Japan]	AF286979§	AF286913§
Cyclophyllidea van Beneden in Braun, 1900		
Davaineidae Braun, 1900		
Raillietina australis [*Dromaeus novaehollandiae* (Emu), Werribee Park, Victoria, Australia]	AF286980§	AF286914§*
Dilepididae Railliet & Henry, 1909		
Dilepis undula [*Turdus merula* (Blackbird), Wimbourne, Dorset, UK]	AF286981§	AF286915§
Hymenolepididae Ariola, 1899		
Fimbriaria sp. (BMNH-2000.9.19.2) [*Anas platyrhynchus* (Mallard duck), Lake Butte des Morts, Wisconsin, USA]	AF286982§	AF286916§
Hymenolepis diminuta [*Rattus norvegicus* (Rat), laboratory strain, University of Copenhagen, Denmark]	AF286983§	AF286917§
Hymenolepis microstoma [*Mus musculus* (Mouse), laboratory strain, University of Copenhagen, Denmark]	AJ287525§	AF286918§
Wardoides nyrocae [*Cygnus olor* (Mute swan), Scotland]	AJ287587§	AF286919§
Mesocestoididae Fuhrmann, 1907		
Mesocestoides corti[e] [laboratory strain, University of Zürich, Switzerland]	AF286984§	AF286920§
Diphyllidea van Beneden in Carus, 1863		
Echinobothriidae Perrier, 1897		
Echinobothrium chisholmae (BMNH-2000.8.3.4-7) [*Rhinobatos typus* (Giant shovelnose ray), Coral Sea, Heron Island, Queensland, Australia]	AF286986§	AF286922§
Echinobothrium harfordi (BMNH-2001.1.23.4-7) [*Raja naevus* (Cuckoo ray), North Sea, UK]	AF286985§	AF286921§
Macrobothridiidae Khalil & Abdul-Salam, 1989		
Macrobothridium sp. (LRP-2149) [*Rhinobatos typus* (Giant shovelnose ray), Shoal Bay, Darwin, NT, Australia]	AF124463	AF286923§
Gyrocotylidea Poche, 1926		
Gyrocotylidae Benham, 1901		
Gyrocotyle urna [*Chimaera monstrosa* (Rabbit fish), unknown fjord, Norway]	AJ228782	AF286924§
Gyrocotyle rugosa (LRP-2129-31) [*Hydrolagus colliei* (Spotted ratfish), Gulf of Alaska, Alaska, USA]	AF124455	AF286925§
Haplobothriidea Joyeux & Baer, 1961		
Haplobothriidae Cooper, 1917		
Haplobothrium globuliforme (LRP-2139-44) [*Amia calva* (Bowfin), Hay Bay, Lake Ontario, Ontario, Canada]	AF124458	AF286926§
Lecanicephalidea Wardle & McLeod, 1952		
Lecanicephalidae Braun, 1900		
Cephalobothrium cf. *aetobatidis* (LRP-2150) [*Aetobatus narinari* (Spotted eagle ray), Gulf of Thailand, Bangsaray, Thailand]	AF124466	AF286927§
Eniochobothrium gracile (LRP-2151) [*Rhinoptera* sp. (Cownose ray), Timor Sea, Fog Bay, NT, Australia]	AF124465	AF286928§
Tetragonocephalidae Yamaguti, 1959		
Tylocephalum sp. [*Dasyatis* sp. (Stingray), Noumea, New Caledonia]	AJ287586§	AF286929§*

TABLE 2—*Continued*

Classification[a] *Taxon sequenced* (specimen voucher Accession No.[c]) [*host species* (common name), collection locality]	GenBank No.[b] SSU	LSU
Litobothriidea Dailey, 1969		
Litobothriidae Dailey, 1969		
Litobothrium amplifica (BMNH-2000.3.7.8-10) [*Alopias pelagicus* (Pelagic thresher shark), Gulf of California, Santa Maria, Baja Mexico]	AF124467	AF286931§
Litobothrium janovyi[d] (BMNH-2000.3.7.3-5) [*Alopias superciliosus* (Bigeye thresher shark), Gulf of California, Santa Maria, Baja Mexico]	AF124468[d]	AF286930§
Nippotaeniidea Yamaguti, 1939		
Nippotaeniidae Yamaguti, 1939		
Amurotaenia decidua (LRP-2133-38) [*Gobiomorphus cotidanus* (Common bully), Mouth of Kuratan River, Lake Taupo, New Zealand]	AF124474	AF286932§*
Nippotaenia chaenogobii (BMNH-2000.3.7.11-12) [*Chaenogobius urotaenia* (*Ukigori*), Lake Suwa, Suwa, Nagano Prefecture, Japan]	AF286987§	AF286933§
Nippotaenia mogurndae (BMNH-2000.3.7.13) [*Odontobutis obscura* (*Donko*), Nukui River at Babadai, Higashihirosima, Hiroshima Prefecture, Japan]	AJ287545§	AF286934§*
Proteocephalidea Mola, 1928		
Monticelliidae La Rue, 1911		
Nomimoscolex piraeeba (INVE-22284) [*Brachyplatystoma filamentosum* (Lau-lau), Itacoatiara, Province Amazonas, Brazil]	AF286988§	AF286936§
Peltidocotyle rugosa (INVE-22374) [*Pseudoplatystoma fasciatum* (Tiger catfish), Rio Paraguay, San Antonio, Central Province, Paraguay]	AF286989§	AF286937§
Rudolphiella szidati (INVE-24668) [*Luciopimelodus pati* (Pati), Rio Parana, Corrientes, Corrientes Province, Argentina]	AF286990§	AF286938§
Zygobothrium megacephalum (INVE-21846) [*Phractocephalus hemioliopterus* (Redtail catfish), Itacoataria, Province Amazonas, Brazil]	AF286991§	AF286939§
Proteocephalidae La Rue, 1911		
Gangesia parasiluri (INVE-22436) [*Silurus asotus* (Japanese catfish), Lake Suwa, Suwa, Nagano Prefecture, Japan]	AJ287515§	AF286935§
Proteocephalus perplexus (LRP-2121-22) [*Amia calva* (Bowfin), Hay Bay, Lake Ontario, Ontario, Canada]	AF124472	AF286940§
Pseudophyllidea Carus, 1863		
Bothriocephalidae Blanchard, 1849		
Anantrum tortum (BMNH-2001.2.1.1) [*Synodus foetens* (Inshore lizardfish) Gulf of Mexico off Horn Island, Mississippi, USA]	AF286992§	AF286941§
Bothriocephalus scorpii [*Myoxocephalus scorpius* (Shorthorn sculpin), North Sea off St. Abbs Head, UK]	AJ228776	AF286942§
Diphyllobothriidae Lühe, 1910		
Diphyllobothrium stemmacephalum (USNPC-86992) [*Lagenorhynchus acutus* (Atlantic white-sided dolphin), Wellfleet Bay, Massachusetts, USA]	AF124459	AF286943§
Schistocephalus solidus[e] [*Gasterosteus aculatus* (3-spined stickleback), Hidden Lake, Matanuska-Sustina Valley, Alaska, USA]	AF124460	AF286944§
Triaenophoridae Lönnberg, 1889		
Abothrium gadi [*Gadus morhua* (Atlantic cod), North Sea south of Shetland Isles, UK]	AJ228773	AF286945§
Anchistrocephalus microcephalus [*Mola mola* (Ocean sunfish), North Sea, Lincolnshire, England]	AJ287473§	AF286946§
Eubothrium crassum (BMNH-1999.4.9.1) [*Salmo trutta* (Sea trout), North Ireland]	AJ287509§	AF286947§
Spathebothriidea Wardle & McLeod, 1952		
Acrobothriidae Olsson, 1872		
Cyathocephalus truncatus [*Salmo trutta fario* (Sea trout), Areuse River, Switzerland]	AJ287493§	AF286948§
Spathebothriidae Yamaguti, 1934		
Spathebothrium simplex (LRP-2132) [*Liparis atlanticus* (Atlantic seasnail), Atlantic Ocean, Rye Beach, New Hampshire, USA]	AF124456	AF286949§
Tetrabothriidea Baer, 1954		
Tetrabothriidae Linton, 1891		
Tetrabothrius erostris [*Larus argentatus* (Herring gull), Danube Delta, Ukraine]	AJ287581§	AF286950§
Tetrabothrius forsteri (USNPC-86991) [*Lagenorhynchus acutus* (Atlantic white-sided dolphin), Wellfleet Bay, Massachusetts, USA]	AF124473	AF286951§
Tetrabothrius sp. [*Puffinus tenuirostris* (Short-tailed shearwater), St. Kilda, Victoria, Australia]	AJ287582§	AF286952§
Tetraphyllidea Carus, 1863		
Onchobothriidae Braun, 1900		
Acanthobothrium sp. 1 (LRP-2112) [*Dasyatis longus* (Longtail stingray), Gulf of California, La Paz, Baja Mexico]	AF286993§	AF286953§
Phoreiobothrium sp. (LRP-2111) [*Sphyrna mokarran* (Great hammerhead), Gulf of Mexico]	AF286994§	AF286954§

TABLE 2—*Continued*

Classification[a] *Taxon sequenced* (specimen voucher Accession No.[c]) [*host species* (common name), collection locality]	GenBank No.[b] SSU	LSU
Platybothrium auriculatum (LRP-2145-48) [*Prionace glauca* (Blue shark), Atlantic Ocean, Montauk, New York, USA]	AF124470	AF286955§
Prosobothriidae Baer & Euzet, 1955		
Prosobothrium armigerum (LRP-2109) [*Prionace glauca* (Blue shark), Atlantic Ocean, Montauk, New York, USA]	AF286995§	AF286956§
Phyllobothriidae Braun, 1900		
Clistobothrium montaukensis (LRP-2114) [*Isurus oxyrinchus* (Shortfin mako), Atlantic Ocean, Montauk, New York, USA]	AF286996§	AF286957§
Crossobothrium longicolle (LRP-2113) [*Scyliorhinus canicula* (Small-spotted catshark), North Sea, UK]	AF286997§	AF286958§
Marsupiobothrium sp. (LRP-2110) [*Alopias pelagicus* (Pelagic thresher shark), Gulf of California, Baja Mexico]	AF286998§	AF286959§
Phyllobothrium lactuca (LRP-2115) [*Mustelus asterias* (Starry smooth-hound), North Sea south of Fair Isle, UK]	AF286999§	AF286960§
Rhabdotobothrium anterophallum (BMNH-2001.1.31.3-4) [*Mobula hypostoma* (Devil ray), Gulf of Mexico, Mississippi, USA]	AF287000§	AF286961§
Rhinebothrium maccallumi (LRP-2108) [*Dasyatis americana* (Southern stingray), Gulf of Mexico]	AF124476	AF286962§
Thysanocephalum sp. (LRP-2116) [*Galeocerdo cuvier* (Tiger shark), Atlantic Ocean, Montauk, New York, USA]	AF287001§	AF286963§
Trypanorhyncha Diesing, 1863		
Dasyrhynchidae Dollfus, 1935		
Dasyrhynchus pillersi[e] [*Lethrinus atkinsoni* (Pacific yellowtail emperor), Coral Sea, Heron Island, Queensland, Australia]	AJ287496§	AF286964§
Eutetrarhynchidae Guiart, 1927		
Dollfusiella sp. (BMNH-2001.1.26.1) [*Carcharhinus melanopterus* (Blacktip reef shark), Coral Sea, Heron Island, Queensland, Australia]	AF287002§	AF286965§
Gilquiniidae Dollfus, 1942		
Gilquinia squali [*Squalus acanthias* (Spiny dogfish), North Sea, UK]	AJ287516§	AF286966§
Grillotiidae Dollfus, 1969		
Grillotia erinaceus [*Raja radiata* (Starry skate), North Sea south of Fair Isle, UK]	AJ228781	AF286967§
Grillotia heronensis[e] [*Plectropomus leopardus* (Leopard coralgrouper), Heron Island, Queensland, Australia]	AJ287519§	AF286968§
Hepatoxylidae Dollfus, 1940		
Hepatoxylon sp.[e] [*Prionace glauca* (Blue shark), Atlantic Ocean, Montauk, New York, USA]	AF124462	AF286969§
Lacistorhynchidae Guiart, 1927		
Callitetrarhynchus gracilis [*Carcharhinus melanopterus* (Blacktip reef shark), Coral Sea, Heron Island, Queensland, Australia]	AJ287487§	AF286970§
Floriceps minacanthus[e] [*Euthynnus affinis* (Bonito), Coral Sea, Heron Island, Queensland, Australia]	AF287003§	AF286971§
Otobothriidae Dollfus, 1942		
Otobothrium dipsacum[e] [*Choerodon venustus* (Venus tuskfish), Coral Sea, Heron Island, Queensland, Australia]	AJ287552§	AF286972§*
Pterobothriidae Pintner, 1931		
Pterobothrium lintoni[e] [*Choerodon venustus* (Venus tuskfish), Coral Sea, Heron Island, Queensland, Australia]	AF287004§	AF286973§
Sphyriocephalidae Pintner, 1913		
Sphyriocephalus sp.[e] (BMNH-2000.1.18.4) [*Dalatias licha* (Kitefin shark), Goban Spur, off southwest Ireland]	AJ287576§	AF286974§
Tentaculariidae Poche, 1926		
Nybelinia queenslandensis [*Carcharhinus melanopterus* (Blacktip reef shark), Coral Sea, Heron Island, Queensland, Australia]	AF287005§	AF286975§*
Tentacularia sp.[e] [*Prionace glauca* (Blue shark), Atlantic Ocean, Montauk, New York, USA]	AF124461	AF286976§*

[a] Classification and authorities follow Khalil *et al.* (1994) except in the recognition herein of the order Litobothriidea Dailey, 1969.

[b] GenBank accession numbers followed by § represent sequences new to the present study. All SSU sequences are complete and all LSU sequences are of the D1–D3 regions except where noted (*) which span the D1–D6 regions (and *Pterobothrium lintoni* which spans the D2–D3 regions).

[c] Where noted, specimen vouchers have been deposited in public collections: INVE, Museum d'Histoire Naturelle, Genève, Switzerland; BMNH, The Natural History Museum, Department of Zoology, Parasitic Worms Division, London, UK; LRP, Larry R. Penner Collection, University of Connecticut, Storrs, USA; USNPC, U.S. National Parasite Collection, Beltsville, Maryland, USA. Note that many of these vouchers represent specimens from a population collected from the same host individual and not the actual specimens that were sequenced.

[d] *Litobothrium janovyi* was referred to as *L. alopias* in Olson and Caira (1999) and Olson *et al.* (1999); it was subsequently determined to represent a new species (see Olson and Caira, 2001).

[e] Identification and genetic analyses based on plerocercoid larvae (except *Mesocestoides corti*, based on tetrathyridea larvae); all other specimens were collected as adult worms.

TABLE 3

Summary of Character Statistics and Results of Maximum-Parsimony Analyses[a]

Data partition	Mean nucleotide[b] frequencies				Number of characters (%)				Parsimony analyses					
	A	C	G	T	Aligned	Included	Constant	Pars. Inf.	No. EPTs	Length (steps)	CI	RI	RC	HI
SSU rDNA	24	23	28	25	2554	1595 (62)	1183 (74)	266 (17)	2435	1208	0.47	0.68	0.32	0.53
LSU rDNA	24	23	32	21	1614	752 (47)	393 (52)	294 (39)	27	1533	0.35	0.67	0.23	0.65
SSU + LSU rDNA	24	23	30	23	4168	2347 (56)	1576 (67)	560 (24)	16	2770	0.39	0.67	0.26	0.60

[a] CI, consistency index; EPTs, equally parsimonious trees; HI, homoplasy index; Pars. Inf., informative under the criterion of parsimony; RC, rescaled consistency index; RI, retention index.
[b] χ^2 tests implemented in PAUP* (Swofford, 1998) do not support significant base frequency heterogeneity among taxa.

D1–D3 region of the LSU gene was amplified with primers LSU5 and 1200R as described by Littlewood *et al.* (2000).

PCR amplicons were purified with Qiagen Qiaquick columns, cycle-sequenced directly from both strands with ABI BigDye chemistry, alcohol-precipitated, and run on an ABI Prism 377 automated sequencer. A variety of internal sequencing primers was used for sequencing in addition to the primers used for PCR. A complete list of SSU primers designed or used for platyhelminth taxa is given in Littlewood and Olson (2001), and LSU primer definitions are given in Littlewood *et al.* (2000). Contiguous sequences were assembled and edited with Sequencher ver. 3.1.1 (GeneCodes Corp.) and submitted to GenBank/EMBL (accession numbers shown in Table 2).

Sequence Alignment

Alignments were handled initially with GDE (Smith *et al.*, 1994). Fifty new SSU sequences were combined with 21 sequences previously published and aligned by eye based on the alignment shown in Appendix A of Olson and Caira (1999), which includes reference to secondary structure. Seventy-one new LSU sequences were aligned by eye with reference to conserved sequence motifs. Both alignments were entirely complementary with regard to taxon representation and both excluded the amphilinidean sequences (see below). The SSU and LSU alignments were then imported to MacClade 4.0b28 (Maddison and Maddison, 2000) and combined, and character-exclusion sets were embedded into a NEXUS-formatted file (available on TreeBASE at www.herbaria.harvard.edu/treebase/, Accession No. SN556). Regions in which homology could not be determined unambiguously were excluded from the analyses. This resulted in the exclusion of ~40% of the aligned positions from both data partitions due to numerous insertion/deletions of varying length among the taxa (see Table 3).

Outgroup Selection

Although the Amphilinidea is well accepted to be the sister group to the Eucestoda based on both morphology (Ehlers, 1985; Rohde, 1990; Xylander, 2001) and molecules (e.g., Littlewood and Olson, 2001; Fig. 1), Olson and Caira (1999) showed the extreme divergence of the SSU sequence of the amphilinidean, *Schizochoerus liguloideus*, in comparison with other cestode taxa. It was hoped that the inclusion of additional amphilinidean species might help subdivide the long branch and show greater similarity to eucestode sequences; however, extremes in sequence divergence were also observed in *Austramphilina elongata* and *Gigantolina magna*. Although largely restricted to the V4 and V7 regions of the SSU gene, other regions, including a highly conserved stem region, were ambiguous or missing entirely from one or more of the amphilinidean sequences. Similarly, LSU sequences of these taxa showed a high degree of divergence. These sequences made confident alignment of both genes difficult and substantially increased the number of positions excluded due to the lack of apparent positional homology. We therefore excluded the amphilinidean taxa from both the alignments and the analyses and rooted the resulting topologies with the more distantly related, but more genetically similar, gyrocotylidean sequences. New SSU and LSU sequences of the amphilinidean taxa have been made publicly available despite the fact that they were not utilized herein (Table 2).

Phylogenetic Analyses

All phylogenetic analyses were performed with PAUP* ver. 4.0b4a (Swofford, 1998) and the resulting networks rooted with the outgroup (Gyrocotylidean) taxa. The SSU and LSU sequence data were analyzed both independently and combined by the methods of maximum-parsimony (MP) and minimum-evolution (ME). Analyses by MP were performed with a heuristic search strategy (1000 search replicates), random-addition sequence, and tree-bisection-reconnection (TBR) branch-swapping options. All characters were run unordered and equally weighted. Gaps were treated as missing data. Nodal support was assessed by bootstrap resampling (1000 bootstrap replicates with three heu-

TABLE 4

Maximum-Likelihood Parameter Estimates[a]

	ASRV			Ts		Tv			
Data partition	α	Inv-E	Inv-O	AG	CT	AC	AT	GC	GT
SSU rDNA	0.52	0.59	0.74	3.75	6.33	1.19	2.16	0.89	1
LSU rDNA	0.61	0.35	0.51	5.19	8.32	1.09	2.57	0.41	1
SSU + LSU rDNA	0.54	0.51	0.66	4.61	7.01	1.06	2.29	0.65	1

[a] All estimates based on a general time reversible model of nucleotide substitution incorporating estimates of among-site rate variation (ASRV) calculated over the strict consensus topology corresponding to the data partition (Figs. 2A–4A). Inv-E, estimated proportion of invariant sites; Inv-O, observed proportion of invariant sites; Ts, transition substitution rates; Tv, transversion substitution rates; α, alpha shape parameter of the gamma distribution.

ristic searches/replicate) and by decay analysis with AutoDecay ver. 4.01 (Eriksson, 1998) with three heuristic searches/constraint tree. A partition homogeneity test was performed on the SSU and LSU data partitions with the incongruence-length difference test (ILD) of Farris *et al.* (1995) as implemented in PAUP*.

Genetic distances used for ME analyses were estimated by maximum-likelihood with a general time reversible (GTR) model of nucleotide substitution including estimates of invariant sites (I) and among-site rate heterogeneity (G) as it was found through χ^2 analysis to provide a significantly higher likelihood score than less parameter-rich models for each of the three data partitions (SSU, LSU, and combined) when calculated over their corresponding strict consensus topologies [Figs. 2A–4A; see Page and Holmes (1998) and Posada and Crandall (1998) for discussions on use of χ^2 analysis to select among models of nucleotide substitution]. Table 4 shows the parameter estimates as calculated over the strict consensus topologies. In calculating genetic distances used for ME, values of I and G were set to those shown in Table 4; substitution rate parameters were free to vary and nucleotide frequencies used were empirical.

Comparisons of the SSU and LSU Data Partitions

The degree to which the two rDNA data partitions estimated the same relative distances among the taxa was examined by the plotting of the corresponding observed distances for all pairwise comparisons of the taxa ($N = 2279$) from the SSU and LSU data. MacClade (Maddison and Maddison, 2000) was used to examine the distribution of site variability in each data partition as a function of the percentage of positions in each change (step) class. Saturation of substitutions was examined by the plotting of the observed values of A-G and C-T substitutions for all pairwise comparisons against their corresponding patristic distances (i.e., distances based on the most parsimonious distribution of character states). The MUST package (Philippe, 1993) was used to generate pairwise patristic distances because PAUP* (Swofford, 1998) ignores all multistate characters in its calculations and thus underestimates the values. Distributions of site variability and patristic distances were based on 1 of the 16 equally parsimonious trees (EPTs) resulting from analysis of the combined data partition because (1) it provided an estimate of phylogeny influenced by both data partitions and (2) it allowed us to avoid making calculations that would be affected by the polytomies present in the strict consensus topology.

Morphological Characters

The publications of Hoberg *et al.* (2001) and Justine (2001) provided the foundation for the character matrix shown in Appendix 1, consisting of 33 characters (3 egg, 5 larval, 1 metacestode, 13 adult, 8 spermatozoan, and 3 spermiogenesis characters) coded at the taxonomic level of order (and family in some cases), of which 5 were multistate. Not including the spermatozoan/spermiogenesis characters [39–44 and 47–49 in Hoberg *et al.* (2001) that were updated according to revisions found in Justine (2001)] 19 characters used by Hoberg *et al.* (2001) were not included in our matrix for one or more of the following reasons: uncertain or questionable homology of character states among taxa (their characters 1, 2, 8, 10, 12, 13, 14, 15, 17, 27, 33, 38, 45, 46, 51), some characters representing a mixture of characters (their characters 8, 33), character or character states too poorly or incompletely defined to be evaluated or understood (their characters 1, 2, 11, 13, 14, 15, 17, 18, 38, 45, 46), and character states known from too few representative species to be generalized for higher taxa (their characters 18, 27, 37, 45, 50). In addition, 13 of the included characters were redefined and/or the character state(s) assigned to one or more taxa changed as discussed in Appendix 2.

Our ability to examine the evolution of the morphological traits in light of the results based on molecules was confounded by differences in the coding of terminal taxa; the molecular data represent species (or more precisely, individuals), whereas the morphological data represent supraspecific taxa (orders and families). Because of this, no attempt was made to analyze the molecular and morphological data partitions simultaneously. However, to evaluate the congruence between

the morphological and the molecular data partitions, we attempted to better match the terminal clades diagnosed by the "reduced" trees based on molecular data (Figs. 2–4) with the terminal taxa coded morphologically. The Monogenea and Amphilinidea were therefore removed from the matrices of Hoberg *et al.* (2001) as they were not represented in our molecular matrices. Character states for egg, larval, metacestode, and adult characters were coded for the Gyrocotylidea based on examination of specimens and the literature (Gibson, 1994a; Löser, 1965; Malmberg, 1974), and spermatozoan/spermiogenesis characters were taken from Justine (2001). Characters 9 and 15 were applicable only to eucestodes and were coded as "9" for the Gyrocotylidea (see Caira *et al.*, 1999). The Trypanorhyncha was divided into the "Trypanorhyncha*" (= *Dollfusiella* sp., *Nybelinia queenslandensis, Tentacularia* sp.; see Figs. 2–4) and the remaining members of the order ("Trypanorhyncha"), and the Tetraphyllidea were represented by the "Onchobothriidae" and "Phyllobothriidae" (as found in Hoberg *et al.*, 2001) and by the Rhinebothriinae and the tetraphyllidean genus *Acanthobothrium*. Egg, larval, metacestode, and adult characters were coded from the literature (Caira *et al.*, 1999, 2001; Campbell and Beveridge, 1994; Euzet, 1994b) and spermatozoan/spermiogenesis characters had to be simply replicated in the matrix to accommodate the new terminal taxa (e.g., states for *Acanthobothrium* sp. were assumed to be identical to those of the "Onchobothriidae"). The morphological character matrix shown in Appendix 1 is available as a NEXUS-formatted file on TreeBASE (Accession No. SN556).

The matrix was analyzed by MP with a heuristic search strategy and TBR branch-swapping (1000 replicates), and all characters were equally weighted and unordered. Multistate taxa were treated as polymorphic rather than as uncertainties, and tree statistics were calculated with the lowest possible values when polymorphic states were considered. Resulting topologies were rooted at the node separating the Gyrocotylidea from the Eucestoda. Nodal support was assessed by bootstrap (1000 replicates) and decay analyses as described above. Analyses were run both with and without the inclusion of the Litobothriidea to examine the effect that the high number of unknown character states for this taxon had on the resulting topologies. The fit of the hypotheses based on molecules to that based on morphology was compared with MacClade (Maddison and Maddison, 2000) by rearrangement of the 20 terminal taxa coded for morphology to match as closely as possible the "reduced" topologies based on molecular data (Figs. 2–4); in doing so, some groups found to be paraphyletic (or unresolved) by molecular data had to be represented as monophyletic taxa in the comparisons (notably the tetraphyllidean groups). In addition, the Litobothriidea was pruned from the molecular trees for comparisons with the morphological analysis that excluded this taxon.

RESULTS

SSU rDNA Analyses

Analysis of the SSU data partition by MP resulted in 2435 equally parsimonious trees (Fig. 2) and provided the least resolution and nodal support of the three data partitions but had the highest consistency, retention, and rescaled consistency indices (Table 3), illustrating the limited utility of these statistics. In ME analysis, the Haplobothriidea was the most basal order of eucestodes, whereas MP analysis resulted in a large polytomy of the monofossate and difossate lineages. Monophyly of a derived tetrafossate clade was refuted by the position of the Pseudophyllidea (not including the Diphyllobothriidae) in the MP analysis and by the position of the Diphyllidea in the ME analysis. The ME topology was 16 steps longer than the MP topology (1224 vs 1208) and was not found to be statistically different based on the Kishino–Hasegawa or Templeton tests implemented in PAUP*.

LSU rDNA Analyses

Analysis of the LSU data partition by MP resulted in 27 EPTs (Fig. 3) that showed greater resolution in their strict consensus than that of either the SSU or the combined data partitions. Both MP and ME analyses supported a basal position of the Caryophyllidea (albeit a paraphyletic assemblage composed of either two (MP) or three (ME) separate lineages), followed by the Spathebothriidea. A derived tetrafossate clade was strongly supported with the interrelationships of its members well resolved (except for the tetraphyllidean taxa). The ME topology was 48 steps longer than the MP topology (1581 vs 1533) and was not found to be statistically different based on the Kishino–Hasegawa or Templeton tests implemented in PAUP*.

Combined rDNA Analyses

The validity of combining the SSU and LSU data partitions, under the criteria of conditional combination (Cunningham, 1997; Huelsenbeck *et al.*, 1996), was supported by the ILD test implemented in PAUP* ($P = 0.48$). Analysis by MP resulted in the fewest EPTs (16) (Fig. 4) but provided less resolution than the LSU data partition. In ME analysis, the Haplobothriidea, followed by the Diphyllobothriidae, were the basal eucestode lineages, followed by a sister group relationship between the Caryophyllidea and the Spathebothriidea. In MP analysis the latter three groups formed a trichotomy, followed by a trichotomy of the major difossate groups. A derived tetrafossate clade was highly supported, as were most of the interrelationships within the clade. The ME topology was 20 steps longer than the MP topology (2790 vs 2770) and was not found to be statistically different based on the Kishino–Hasegawa or Templeton tests.

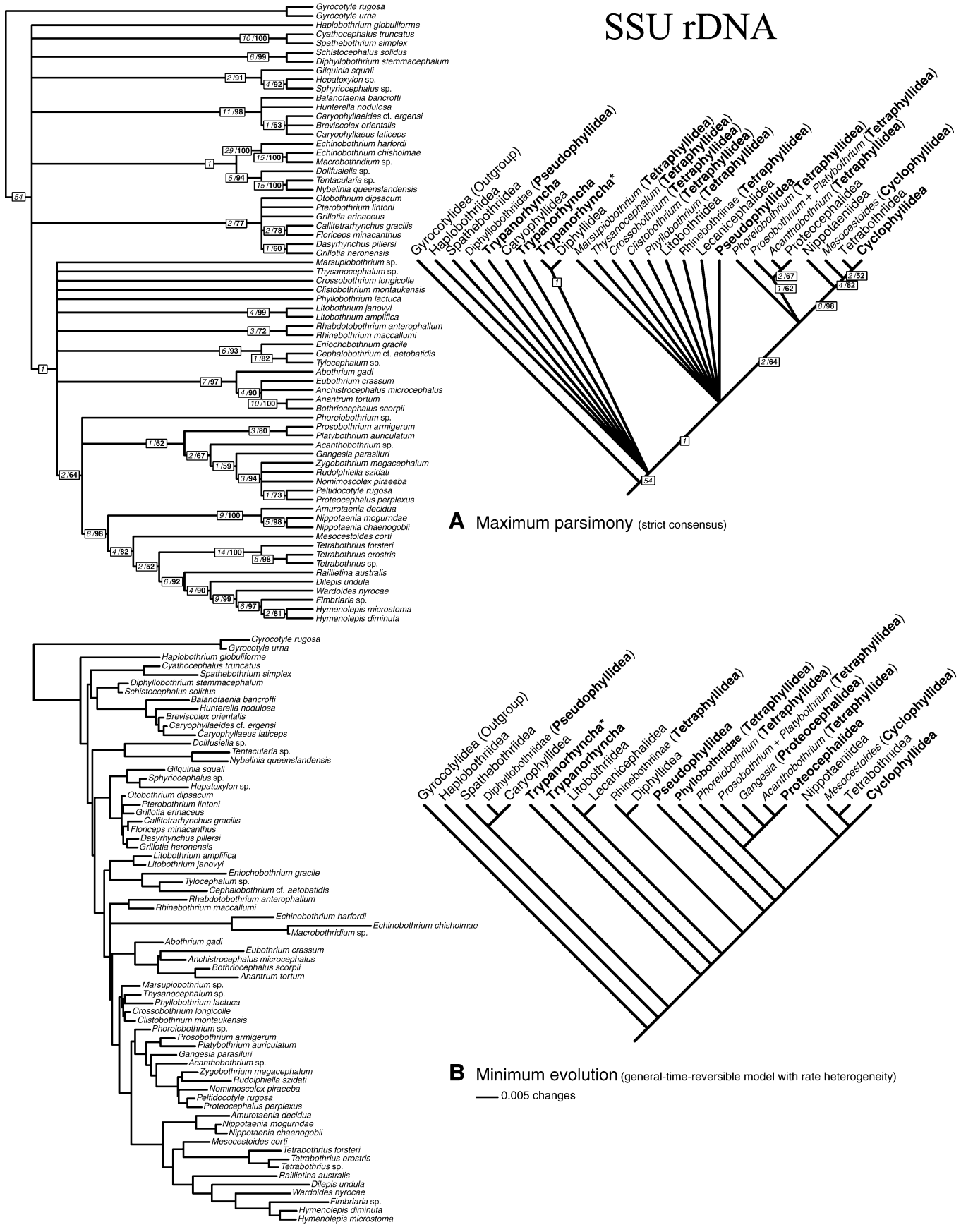

FIG. 2. Analyses of SSU rDNA. (A) Strict consensus of 2435 equally parsimonious trees (nodal support shows *decay indices*/bootstrap values ≥ 50%). (B) Results of analysis by minimum-evolution. Reduced trees on right show relationships among terminal clades representing higher taxa; those found to be paraphyletic are shown in boldface. Trypanorhyncha* = (*Dollfusiella* sp. (*Nybelinia queenslandensis*, *Tentacularia* sp.)).

FIG. 3. Analyses of LSU rDNA. (A) Strict consensus of 27 equally parsimonious trees (nodal support shows *decay indices*/bootstrap values ≥ 50%). (B) Results of analysis by minimum-evolution. Reduced trees on right show relationships among terminal clades representing higher taxa; those found to be paraphyletic are shown in boldface. Trypanorhyncha* = (*Dollfusiella* sp. (*Nybelinia queenslandensis, Tentacularia* sp.)).

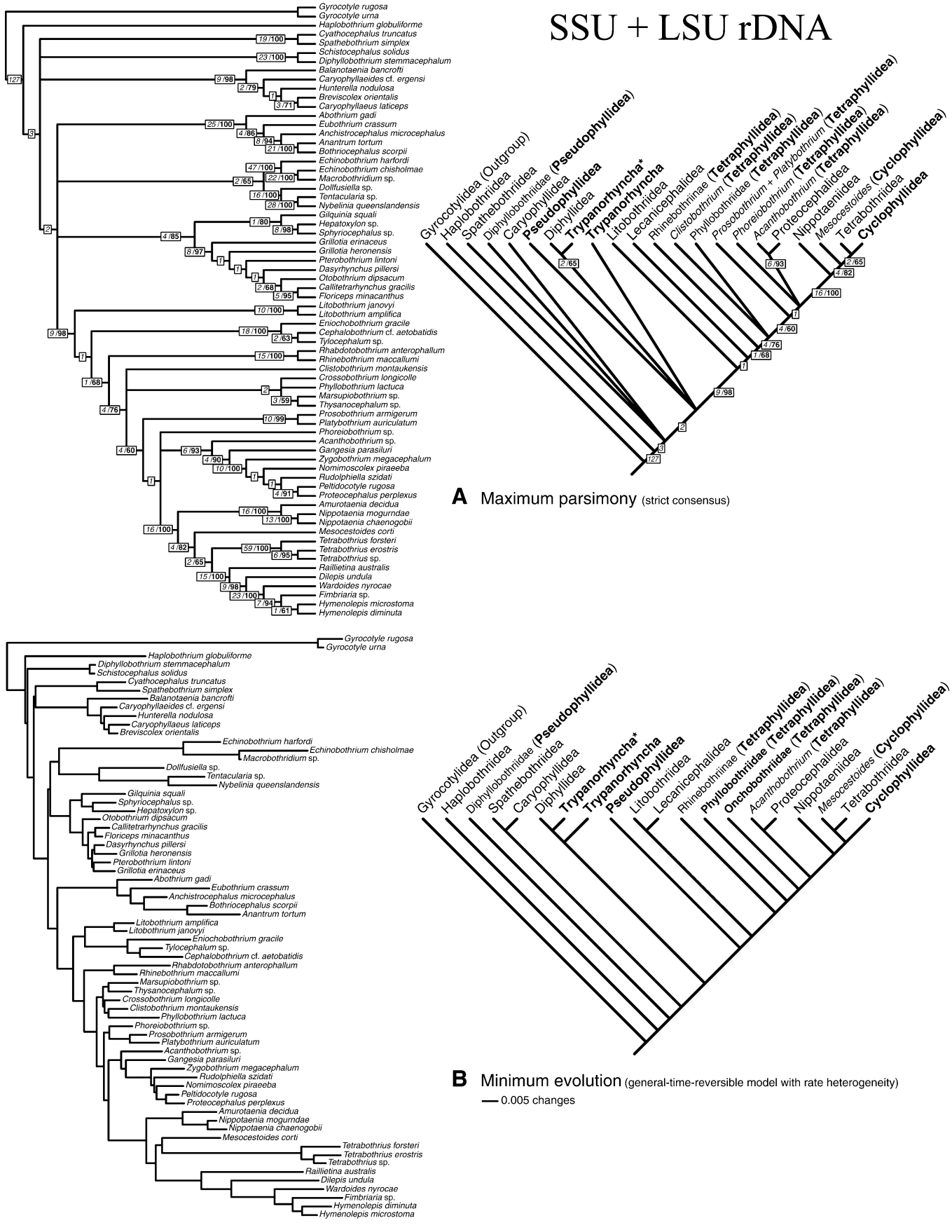

FIG. 4. Analyses of SSU and LSU rDNA combined. (A) Strict consensus of 16 equally parsimonious trees (nodal support shows *decay indices*/bootstrap values ≥ 50%). (B) Results of analysis by minimum-evolution. Reduced trees on right show relationships among terminal clades representing higher taxa; those found to be paraphyletic are shown in boldface. Trypanorhyncha* = (*Dollfusiella* sp. (*Nybelinia queenslandensis, Tentacularia* sp.)).

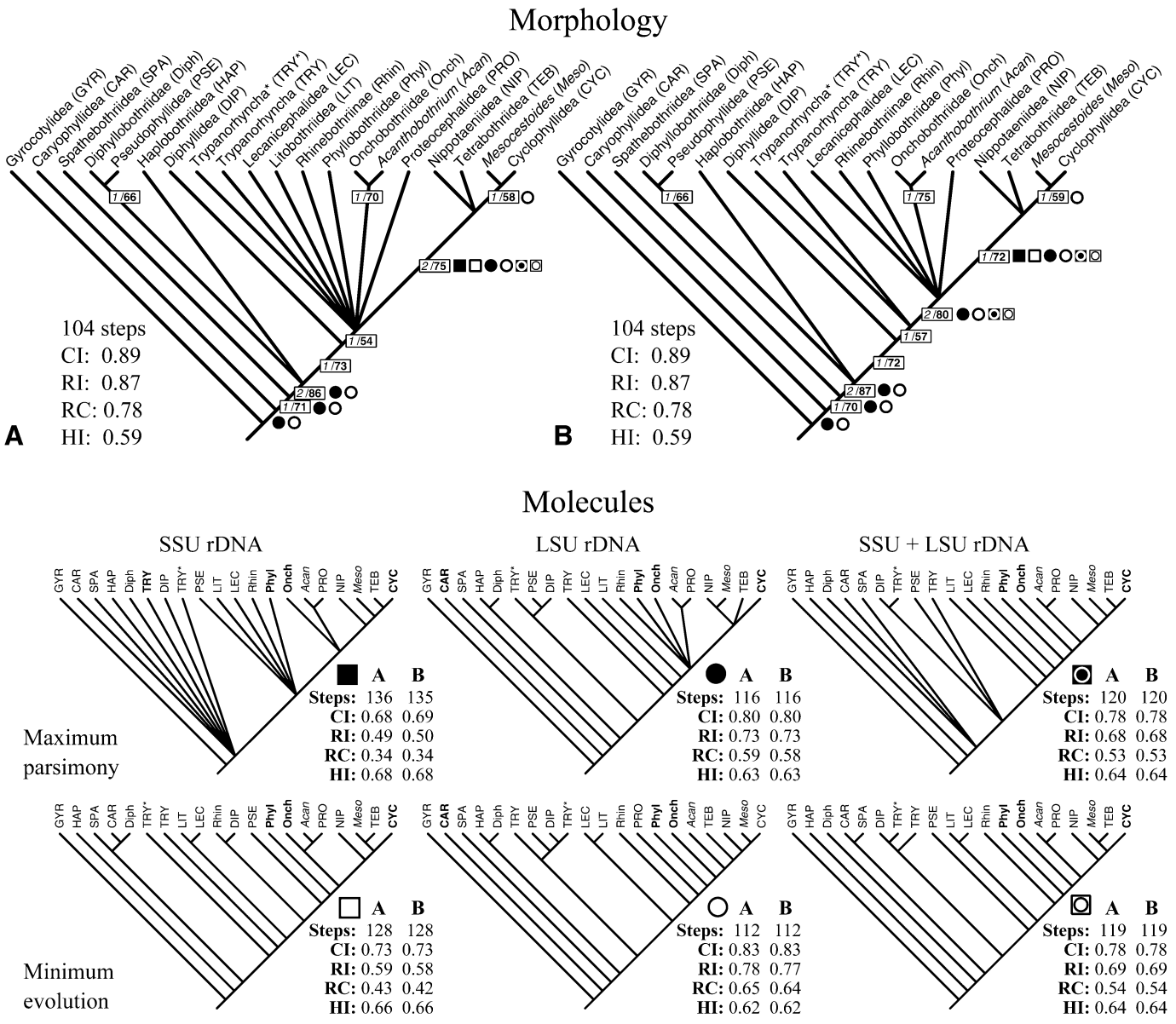

FIG. 5. Comparison of topologies and tree statistics based on analysis of morphology and the "reduced" trees based on the molecular data partitions (Figs. 2–4). (A) Srict consensus of 576 equally parsimonious trees (EPTs) from analysis including all supraspecific taxa; (B) strict consensus of 216 EPTs from analysis excluding the Litobothriidea for which numerous character states were unknown. Boxes show *decay*/bootstrap indices and symbols indicate clades present in the molecular-based topologies. Taxon names in boldface were found to be paraphyletic in the original analyses of the molecular data but were represented as being monophyletic for the purposes of the comparisons with morphology. Minimum-evolution analysis of the LSU data partition shows the greatest degree of congruence with morphology, and all molecular data partitions supported the clade including the Cyclophyllidea + *Mesocestoides* + Nippotaeniidea + Tetrabothriidea. CI, consistency index; HI, homoplasy index; RI, retention index; RC, rescaled consistency index.

Morphological Analysis

Of the 33 characters, 22 were parsimony informative. MP analysis resulted in 576 EPTs when all taxa were included and 216 EPTs when the Litobothriidea were excluded from analysis; strict consensus topologies are shown in Figs. 5A and B, respectively. Both analyses resulted in EPTs of 104 steps regardless of the inclusion/exclusion of the Litobothriidea, although its exclusion resulted in greater overall resolution. Comparisons with estimates based on the molecular data show that ME analysis of the LSU data alone was most consistent with the most parsimonious distribution of the morphological character states and resulted in a tree only 8 steps longer.

DISCUSSION

Interrelationships of the "Monofossate" and Difossate/Bothriate Orders

The monofossate and difossate/bothriate groups comprise the basal lineages of all recovered trees with few exceptions, although strong character support for the interrelationships of these lineages has been, and continues to be, elusive from a molecular standpoint. Consistent among all molecular analyses, however, is a separation between four groups at the base of the tree, Caryophyllidea, Diphyllobothriidae, Haplobothriidea, and Spathebothriidea, and the remaining orders that were more derived. Among these groups, a basal position of the Haplobothriidea as supported by ME analysis of the SSU data seems unlikely; in previous works that considered the position of the order, only Baer (1950) placed haplobothriids at the base of the Eucestoda, whereas a majority of authors have allied them with the Pseudophyllidea (Brooks and McLennan, 1993; Dubinina, 1980; Euzet, 1959, 1974; Euzet *et al.*, 1981; Hoberg *et al.*, 1997a; 2001; Olson and Caira, 1999; Wardle and McLeod, 1952). The LSU data support their position as the sister group to the diphyllobothriid pseudophyllideans, as do previous analyses based on SSU data (Hoberg *et al.*, 2001; Kodedová *et al.*, 2000; Olson and Caira, 1999).

If the Pseudophyllidea are indeed a paraphyletic assemblage with the Diphyllobothriidae forming a distinct clade, as all molecular evidence to date suggests (Kodedová *et al.*, 2000; Mariaux, 1998; Olson and Caira, 1999; this study), it is somewhat surprising that the diphyllobothriids are not found to be derived relative to the remaining "Pseudophyllidea," as the family is restricted to reptile, bird, and mammal definitive hosts unlike other pseudophyllidean families that are predominantly parasites of teleosts. Alternatively, if the group is monophyletic, as indicated by morphological analyses (Fig. 5), the divergence of the Diphyllobothriidae must have occurred very early during the evolution of the order. Previous morphological analysis of the internal structure of the Pseudophyllidea supported a separate diphyllobothriid clade, but did not test the potential paraphyly of the order (Bray *et al.*, 1999). Nevertheless, no unambiguous synapomorphy was found to support monophyly of the order (Bray *et al.*, 1999). In our molecular analyses, the nondiphyllobothriid species representing the families Bothriocephalidae and Triaenophoridae formed a well-supported clade that was unstable in its position. Based on morphological analysis, however, the presence of a median genital pore (although not universally observed in the group) places them basal to the tetrafossate orders.

Like the Pseudophyllidea, the Diphyllidea and Trypanorhyncha were also unstable in their placement, but showed a weak affinity in both the SSU and the LSU data. No analysis supported monophyly of the Trypanorhyncha as currently defined (Campbell and Beveridge, 1994), due in part to the strong support for a separate lineage composed of the species *Dollfusiella* sp., *Nybelinia queenslandensis*, and *Tentacularia* sp. (labeled Trypanorhyncha* in Figs. 2–4), all of which appear as long-branching taxa (see Figs. 2B–4B). Whereas the genera *Nybelinia* and *Tentacularia* are both members of the Homeacanthoidea, *Dollfusiella* is a member of the Heteracanthoidea (Campbell and Beveridge, 1994), and in a preliminary cladistic analysis based on morphology (Beveridge *et al.*, 1999), the latter genus is well separated from the former two. Thus, without morphological justification and with the possibility of error due to long-branch attraction (LBA; Felsenstein, 1978), a more comprehensive analysis of the trypanorhynchs and their kin is needed to help explain the existence of this clade and its separation from the other members of the order.

The exact placement of the Diphyllidea has long been problematic, although most workers have generally allied them with the Trypanorhyncha as both groups are bothriate and hosted by elasmobranchs (see reviews in Hoberg *et al.*, 1997a). Present analyses strongly support monophyly of the Diphyllidea, whereas the genus *Echinobothrium* was found to be paraphyletic, consistent with the morphological analysis of Ivanov and Hoberg (1999) but not that of Caira *et al.* (2001). In addition to the gyrocotylidean taxa, the diphyllidean species were among the longest branches of the trees (Figs. 2B–4B), and their affinity with the three-taxon Trypanorhyncha* clade may have been influenced by the similarly high divergence rates of the two clades (i.e., LBA). Nevertheless, a sister group relationship between the two orders is also supported by the morphological analyses of Caira *et al.* (2001).

Interrelationships of the Tetrafossate/Acetabulate Orders

Strong support was found for a derived clade of the acetabulate orders with the Lecanicephalidea and Litobothriidea forming the basal branch(es). The branch subtending this clade is supported by a large number of morphological characters including oligolecithal, quinone-tanned eggs with two embryonic membranes, medullary vitellaria, and the acetabulate scolex condition itself. The monophyly and interrelationships of the three lecanicephalidean species are consistent with analyses based on morphology (Caira *et al.*, 2001). Placement of the Litobothriidea outside of the Tetraphyllidea supports their recognition as a separate order (Dailey, 1969) rather than as a family within the Tetraphyllidea (Euzet, 1994b; see also discussions in Olson and Caira, 1999, 2001), although a sister group relationship between the Litobothriidea and the Lecanicephalidea is not suggested by morphological analyses (Caira *et al.*, 2001; Hoberg *et al.*, 2001). The

Tetraphyllidea, represented by 13 species in the analysis, never formed a clade, nor was monophyly of either of the two major families, Onchobothriidae and Phyllobothriidae, supported. Almost all analyses supported the two members of the Rhinebothriinae (as defined by Euzet, 1994b) as basal to the remaining tetraphyllidean taxa, and other members of the "Phyllobothriidae" were generally basal to those of the "Onchobothriidae." Paraphyly of the order is consistent with analyses based on morphology (Caira *et al.*, 1999, 2001; Hoberg *et al.*, 2001) and molecules (Mariaux, 1998; Olson and Caira, 1999) and will take far denser sampling of both genes and taxa to resolve.

With a single exception (Fig. 3B), all molecular analyses supported a position of the onchobothriid tetraphyllidean, *Acanthobothrium* sp., as the sister taxon to a monophyletic Proteocephalidea. This suggests not only the inclusion of the Proteocephalidea within the Tetraphyllidea (as is also supported by morphological analyses, Caira *et al.*, 2001), but also alludes to Brook's (1978) contentions regarding the origin of the Proteocephalidea in Gondwanan South America. Although the tetraphyllidean genus *Acanthobothrium* is large and cosmopolitan in distribution (Jensen, 1996; Schmidt, 1986), at least four members of the genus are found in *Potamotrygon* spp. (Brooks and Amato, 1992), a group of freshwater stingrays restricted to the Atlantic watersheds of South America (Nelson, 1994). Contrary to this hypothesis is the basal position of the genus *Gangesia* within the Proteocephalidea (also supported by the more comprehensive analysis of proteocephalidean relationships based on LSU data by Zehnder and Mariaux, 1999), a genus found only in the Northern Hemisphere. Nevertheless, molecular evidence that the Proteocephalidea is descended from a lineage of the elasmobranch-hosted Tetraphyllidea is strong, and the most likely route for host-switching, to, for example, siluriform fishes, would be expected to come from tetraphyllidean species that had found their way into freshwater. A direct ancestor–descendent relationship would also do much to explain the near identity of the proglottis morphology in many members of these two orders.

The only universally recovered clade from these and previous analyses based on molecules (Mariaux, 1998; Olson and Caira, 1999) is that including the Cyclophyllidea + Nippotaeniidea + Tetrabothriidea ("higher acetabulates"). This clade is supported by present and recent morphological analyses in which the three groups are united on the basis of their compact vitellarium (Hoberg *et al.*, 1997a, 2001), first postulated as evidence of their common origin by Galkin (1996). Although not universally recovered, a sister group relationship between the Cyclophyllidea and the Tetrabothriidea is supported by SSU and a few spermatozoan characters. This arrangement suggests that the progenitor of these two orders evolved via a primary colonization of tetrapods from a teleost-hosted ancestor. Although the family Mesocestoididae is unique among cyclophyllideans in a number of fundamental aspects (Rausch, 1994), its exclusion from the order should not be based on the unstable position of *Mesocestoides corti* which resulted from analyses of SSU and LSU without the elimination of the possibility of LBA and the obtaining of stronger support for an alternate position.

Morphological Matrix

Our modified morphological matrix was far more conservative with regard to assumptions of homology than that of Hoberg *et al.* (2001) and included a greater number of polymorphic character states that more accurately describe the conditions of the supraspecific taxa. Nevertheless, our results differ from those of Hoberg *et al.* (2001) only in lack of resolution and character support (compare our Fig. 5B with their Fig. 12.1). Ultrastructural information on spermatozoan morphology provided little evidence of interordinal relationships, but it is important to note that very few species have been examined in such detail and the states for a number of potentially informative characters are not yet known from some groups [see Justine (1998, 2001) for more detailed evaluations on the utility of these data]. Even with our more conservative approach, there is still considerable room for debate on the homology of a number of the characters and we encourage future workers to scrutinize our interpretations as we have done those of Hoberg *et al.* (2001).

Evolution of Strobilation within the Eucestoda

Like the digeneans, the eucestodes have evolved their own novel life history strategy for achieving enormous reproductive capability. Whereas digeneans increase their number of progeny through multiple asexual generations (rediae and sporocysts), the eucestodes have increased fecundity through serial repetition of their reproductive organs (proglottisation). The evolution of this key adaptation has remained of fundamental interest centered around the phylogenetic positions of two enigmatic groups: the Caryophyllidea, which exhibit neither proglottisation nor segmentation (external division of proglottides), and the Spathebothriidea, which exhibit proglottisation without external segmentation. The nonsegmented (monozoic) condition of the Caryophyllidea has been hypothesized as either evidence of their basal position among the eucestodes or as a secondary loss from a segmented, pseudophyllidean ancestor. The Spathebothriidea is a much smaller group (5 vs 42 recognized genera; Gibson, 1994b; Mackiewicz, 1994), rarer in nature, and consequently less understood than the Caryophyllidea. Only a partial life cycle is known from a spathebothriidean (Sandeman and Burt, 1972) and most authors have simply assumed that their condition represents a loss of external segmentation from, again, a pseudophyl-

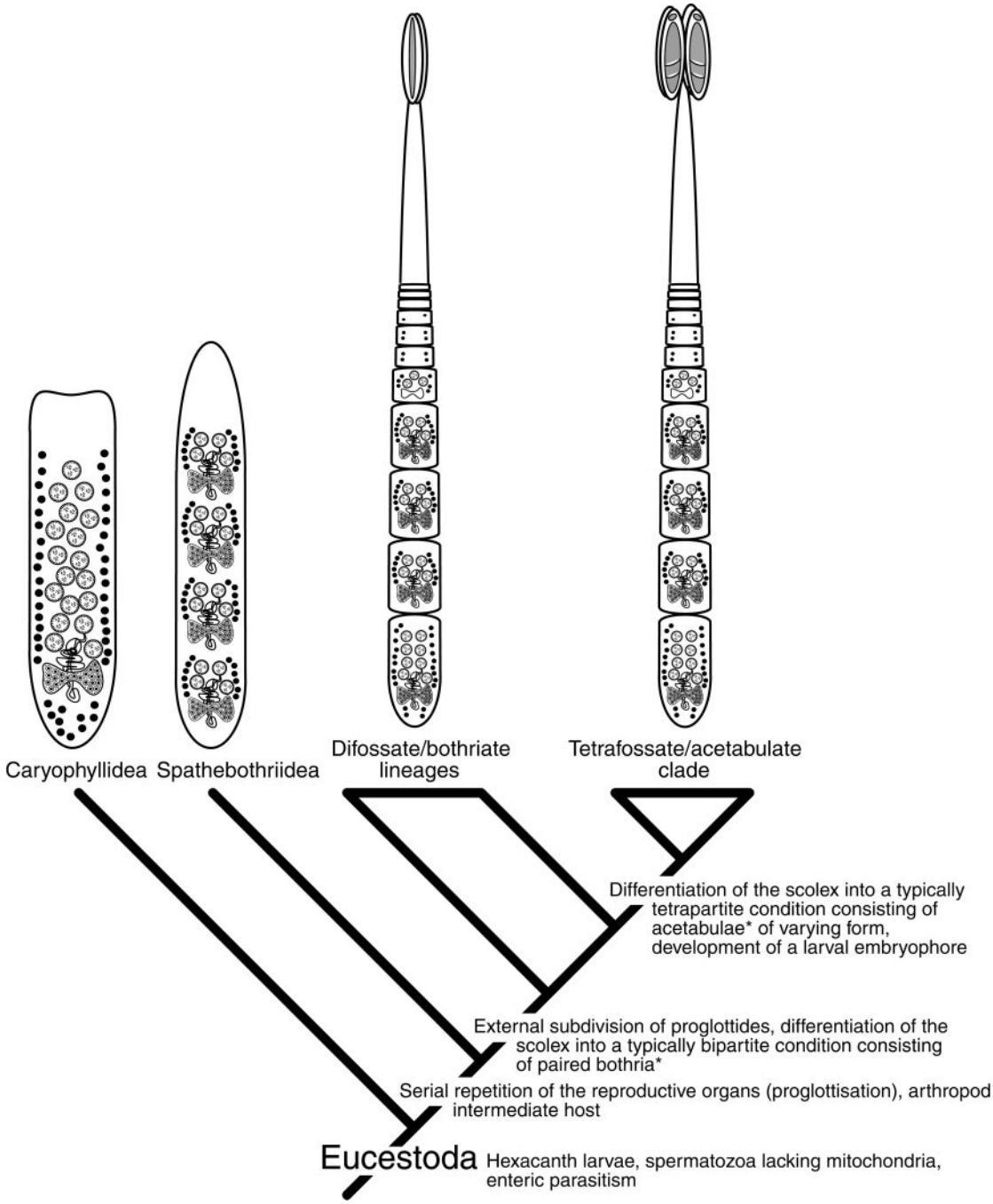

FIG. 6. Diagrammatic representation of a stepwise evolutionary pattern resulting in the strobilate, tetrafossate/acetabulate condition characteristic of higher eucestodes, consistent with analyses of LSU rDNA (Fig. 3), morphology (Fig. 5), and other recent works (e.g., Hoberg *et al.*, 1997a, 2001; Mariaux, 1998). See text for discussion. *See Caira *et al.* (1999) for definitions and discussion of the difossate/bothriate and tetrafossate/acetabulate scolex conditions.

lidean ancestor. Such ideas were implicit in previous classification schemes that considered the Caryophyllidea and Spathebothriidea to be members of the order Pseudophyllidea (e.g., Fuhrmann, 1931; Joyeux and Baer, 1961).

That the Spathebothriidea represented an intermediate form in the evolution of the strobilate condition that was to become the hallmark of the Eucestoda was not broadly envisioned by cestodologists, irrespective of their views on a basal position of the Caryophyllidea. Figure 6 illustrates the evolution of strobilation in the Eucestoda as stemming from the plesiomorphic "caryophyllidean" condition and culminating in the derived strobilate condition via a stepwise pattern in which pro-

glottisation and external segmentation were independent evolutionary events. The potential evolutionary advantages of these processes differ; proglottisation increases fecundity, whereas external segmentation can allow (in apolytic species) for development and fertilization to occur in niches other than that occupied by the parental worm, such as in a different region of the gut or in the external environment, and in this way promotes cross-fertilization. We may only speculate as to how much more advantageous it is to have compartmentalized proglottides and why some cyclophyllidean and pseudophyllidean species apparently lost this key adaptation (see below). Present day diversity suggests that the acquisition of the trait was indeed significant, with so few extant lineages of the Spathebothriidea in comparison to the nearly 600 described strobilate genera (Khalil *et al.*, 1994).

The scenario presented in Fig. 6 is not new among hypotheses of eucestode evolution (see, e.g., Mackiewicz, 1981; Rees, 1969), but perhaps requires evidence independent of morphology and life history to achieve broader acceptance. Cladistic support for this scenario was first published by Hoberg *et al.* (1997a) and was later supported by SSU data of Mariaux (1998). The SSU data of Olson and Caira (1999) supported a basal position of the Spathebothriidea with the Caryophyllidea placed in a more derived "difossate" clade, as did the recent study of Kodedová *et al.* (2000) which added 18 SSU sequences to those generated by Olson and Caira (1999). Ignoring the sequence of *Spathebothrium simplex* (see Introduction), analysis of Ef-1α amino acid data (Olson and Caira, 1999) supported a basal position of the Caryophyllidea. Although the present analyses based on SSU data are largely inconclusive with regard to the positions of the basal orders, results based on LSU data provide additional independent support for the hypothesis shown in Fig. 6.

Albeit rare, examples of higher eucestodes that exhibit no or incomplete external segmentation are known from the Cyclophyllidea (e.g., some members of the Anoplocephalidae, Hymenolepididae, and all Nematotaeniidae; Beveridge, 1994; Czaplinski and Vaucher, 1994; Jones, 1994b), the Pseudophyllidea (e.g., members of the genera *Anantrum, Baylisia, Digramma, Ligula,* and *Triaenophorus;* Bray *et al.*, 1994), and the Nippotaeniidea, which may be considered to be only weakly segmented. These examples support the notions that proglottisation and external segmentation are, or can be, decoupled genetically and that a number of higher eucestodes have lost the latter feature secondarily. One such example, *Anantrum tortum,* was described by Overstreet (1968) and placed in the pseudophyllidean family Bothriocephalidae despite its lack of external segmentation. The following year Rees (1969), unaware of the erection of *Anantrum,* erected the genus *Acompsocephalum* for the same type species and placed it in a new family of Pseudophyllidea, although she considered it to be neither pseudophyllidean nor spathebothriidean *sensu stricto.* Instead, she suggested that *A. tortum* represented a "grade" between the more basal cyathocephalid spathebothrideans and the more derived strobilate Pseudophyllidea (Rees, 1969). Molecular analyses in our study confirm the original designation by Overstreet (1968), which was also accepted by Schmidt (1986) and Bray *et al.* (1999), and thus supports secondary loss of external segmentation in the genus.

Patterns of Host Association

Hoberg *et al.* (1999a) discussed the pattern of host associations and radiation of the cestodes as inferred from a morphologically based hypothesis of ordinal-level interrelationships and suggest possible divergence dates based on the ages of specific host groups. Differences in the hypotheses presented herein do not change significantly the inferences that they made and readers are thus referred to their comments for a more detailed account. In short, it appears that cestodes were first and foremost parasites of fish and were present in both basal teleost and elasmobranch (holocephalan) fishes before the first eucestodes appeared. Early eucestodes are found in basal teleost groups, and there was perhaps a single primary colonization event of galeomorph and/or squalimorph elasmobranchs, with subsequent radiation(s) back to teleosts (e.g., in the Proteocephalidea). Host-switching appears common, and whereas the Cyclophyllidea and Tetrabothriidea are the only groups inferred to have evolved from a single primary radiation into tetrapods (leading to the evolution of terrestrial life cycles in the former group), the Proteocephalidea, Pseudophyllidea, and even Amphilinidea have also secondarily acquired tetrapod hosts. Stricter patterns of host coevolution are more likely to be found at lower taxonomic levels, especially in groups that exhibit high degrees of host specificity (see Adamson and Caira, 1994; Caira, 1990), but given the presumed antiquity of these lineages, it is not particularly surprising that patterns of host association appear to be complicated at all taxonomic levels (see, e.g., Olson *et al.*, 1999; Zehnder and Mariaux, 1999).

Effects of Additional Taxa

The present study utilized a much larger number of species and generally more equitable representation of the higher taxa than the studies of either Mariaux (1998) or Olson and Caira (1999) (Table 1); the effects of this depend somewhat upon the type of analyses compared. With regard to parsimony-based estimates, most of the internodes and/or clades that were strongly supported were also recovered by the previous studies. Likewise, the lack of support for monophyly of the orders Cyclophyllidea, Pseudophyllidea, and Tetraphyllidea were apparent in earlier studies (Mariaux, 1998; Olson

and Caira, 1999). Indeed, even the Trypanorhyncha was previously indicated to be paraphyletic despite the fact that the two separate clades recovered here were each represented by only a single species (Olson and Caira, 1999). Considering only the SSU data, the addition of taxa has resulted in even less resolution than that previously obtained with regard to estimates based on MP. Additional taxa certainly help to better define the lineages that they represent and may help subdivide long branches, but they do not necessarily provide any additional characters for resolution of the branching pattern of the separate lineages themselves. As Poe and Swofford (1999, p. 300) stated, "the best way to improve accuracy is to increase the chance of detecting the relatively few changes occurring on the short internal branch[es], which is better accomplished by adding characters [than by adding taxa]." To be clear, we do not argue against dense sampling of taxa (which necessarily provides more robust tests of monophyly), but we argue only that our results show that the addition of the LSU data was more informative than if we had simply increased the taxon sampling in the SSU data set. This is further illustrated by the analysis of Kodedová *et al.* (2000) in which new SSU sequences representing the orders Caryophyllidea, Pseudophyllidea, and Proteocephalidea added to the SSU sequences of Olson and Caira (1999) resulted in less resolution of the basal branching pattern than did the analyses of fewer taxa in the original study (Olson and Caira, 1999). It seems evident that continued sampling of the SSU gene will not improve this situation.

The addition of taxa may have a bigger influence on methods that rely on estimates of genetic distance such as ME. As with all statistical estimates, the larger the sample size (in this case the number of sequences representing a clade), the greater is the accuracy of the estimate (the genetic distance of the branch subtending the clade). Minimum-evolution analyses based on GTR+I+G "corrected" distances in the study of Olson and Caira (1999), in which many orders, and thus estimates of branch lengths representing major lineages, were represented by single taxa, generally resulted in phylogenetic estimates that differed greatly from those of MP or ME based on LogDet-transformed (Lockhart *et al.*, 1994) distances and were even less congruent with hypotheses based on morphology. In the present study, results from the ME analyses based on the GTR+I+G model did not differ statistically from those from the MP analyses and showed greater congruence to the morphological estimates (Fig. 5).

Effects of New Sequence Data

This work represents the first use of LSU rDNA for examining the higher-level relationships of cestodes. Previous works utilizing LSU data for cestodes (reviewed in Mariaux and Olson, 2001) are limited to studies on *Taenia* spp. (Bowles and McManus, 1994) and on members of the order Proteocephalidea (Zehnder and de Chambrier, 2000; Zehnder *et al.*, 2000; Zehnder and Mariaux, 1999). In comparison with previous works on SSU rDNA (Mariaux, 1998; Olson and Caira, 1999) and Ef-1α (Olson and Caira, 1999), the LSU gene appears to be the most informative of the three, especially when the sequencing effort is taken into account. In the present study, roughly half the number of nucleotide positions was determined for the LSU gene compared to that for the SSU gene, and yet a greater number of positions was phylogenetically informative via parsimony (294 vs 266; Table 3). Analysis of the LSU data also resulted in far fewer EPTs (27 vs 2435), indicating a greater degree of hierarchical structure in the data. The combination of both data sets resulted in higher nodal support in general, but conflicting signal resulted in less resolution among the basal orders (Fig. 4A).

The comparison of raw distances (Fig. 7A) resulted in a linear regression value of 0.71, indicating that, although the estimates of relative genetic distances between the two data partitions are similar, they are not exactly equivalent; deviation from the line of identity (dashed line) shows that estimated distances are larger for the LSU gene. The shallower left-skewed distribution of variable sites of the SSU data in comparison to LSU (Fig. 7B) is reflected in the lower alpha value for SSU (Table 4) and thus somewhat greater among-site rate variation (ASRV). This comparison is biased, however, in that the LSU data are based on only the D1–D3 regions of the gene in which variability is relatively high throughout. The D4–D6 regions were determined for eight species (indicated with asterisks in Table 2) representing five orders and were found to be too conserved to be informative for this level of inference. Had these invariant and more conserved positions been determined for all of the taxa, the level of ASRV in the LSU data would be more similar to that of the complete SSU gene. Presumably, the sites most heavily saturated in both genes were those for which positional homology was obscured in the first place and could not be included in the alignment.

Multiple substitutions are the greatest impediment to accurate phylogenetic reconstruction with sequence data because they obscure the historical record of genetic change. For most taxonomic groups, both substitution rate and divergence time must be estimated from data that are themselves subject to multiple substitutions, and thus it is difficult to accurately determine the presence or degree of saturation from a given data set. In the absence of a fossil record or other means of dating divergences, time must be represented by genetic distance, which in turn may be estimated in a number of different ways from the data (e.g., "corrected" vs uncorrected). Patristic distances by definition provide an estimate of the lower bound of evolutionary change (see Page and Holmes, 1998 and references therein) and are thus useful for comparisons

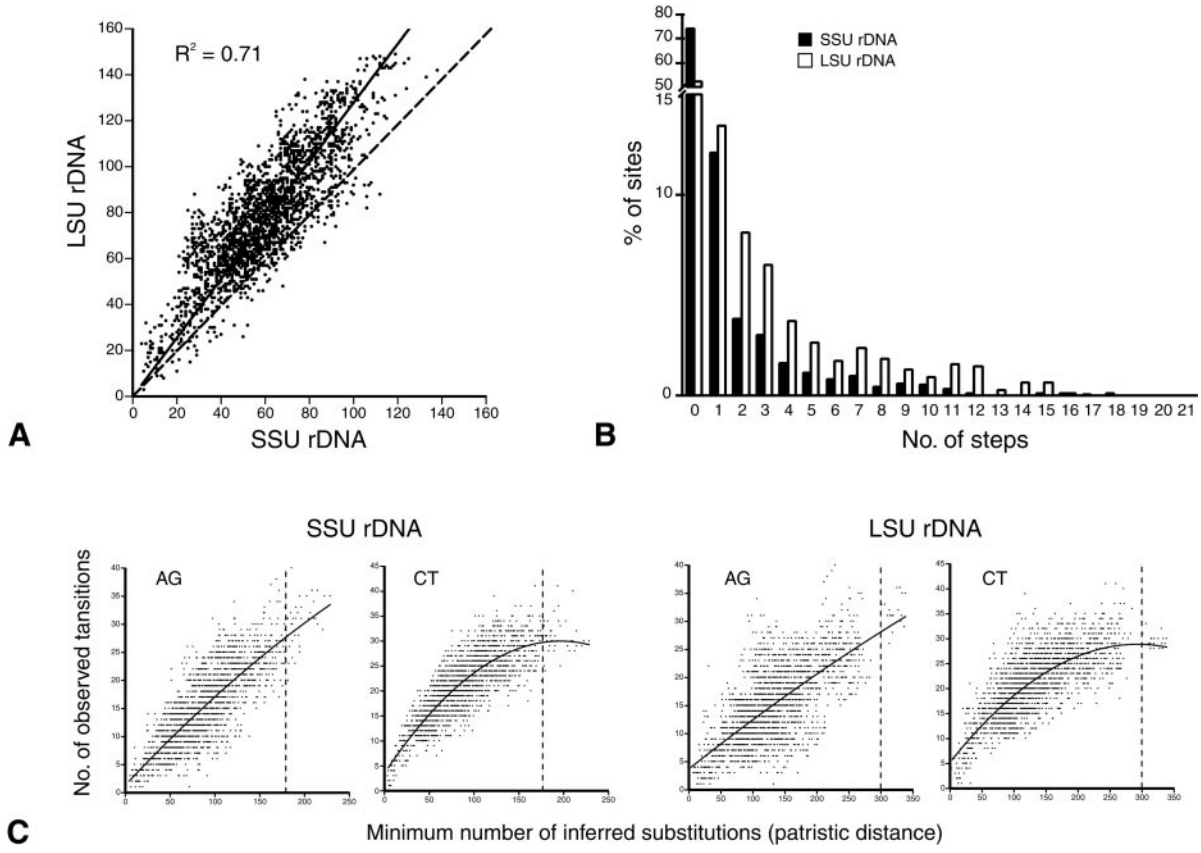

FIG. 7. Comparisons of the SSU and LSU rDNA data partitions. (A) Correspondence between the observed genetic distances of all pairwise comparisons of the taxa ($N = 2279$). Regression analysis shows that both data sets provide similar estimates of distance, although those based on LSU are greater in actual value (note deviation from dashed line). (B) Percentage of sites vs number of steps/site. SSU data show a shallower curve (and thus lower alpha value; Table 4) with over 70% of the sites observed to be constant. LSU data are biased in comparison because they are based on only the D1–D3 region of the molecule which contains the majority of variable positions. (C) Number of observed substitutions in each transition class vs patristic distance for all pairwise comparisons of the taxa. All curves were fitted using the same two-degree polynomial formula as implemented in DeltaGraph (ver. 4.0, SPSS Inc.). Points to the right of the dashed lines are comparisons between the highly derived cyclophyllidean and tetrabothriidean taxa and the gyrocotylidean outgroup taxa (and between the cyclophyllidean and the diphyllidean taxa in some instances of the SSU data) and appear to be saturated with regard to the C-T transition class. The higher rate of C-T than A-G transitions in both genes (see Table 4) is consistent with the fact that both pyrimidines form a strong bond with guanine and therefore such changes do not disrupt the secondary structure of rDNA. Number of steps/site and patristic distances were based on 1 of the 16 equally parsimonious trees resulting from analyses of the combined data (strict consensus shown in Fig. 4A).

with the amount of observable change (uncorrected distances). Figure 7C shows these comparisons for the two transition substitution classes in each gene. Both classes of transitions in both genes show a high degree of scatter as a result of wide differences in substitution rates among lineages. C-T transitions show higher rates of substitution than A-G transitions (see Table 4), consistent with the fact that there is little cost to C-T substitutions; the secondary structure of rDNA is maintained through both G-C and G-T bonding (Gutell, 1996; Gutell *et al.*, 1994; Simon *et al.*, 1994). These graphs also indicate that the gyrocotylideans are too divergent from the higher acetabulate taxa for such comparisons to be reliable. One way to remedy this would be to down-weight C-T substitutions, but unless it was possible to do this only for comparisons between the outgroup taxa and the cyclophyllidean and tetrabothriidean taxa, these changes would also be down-weighted for all other comparisons in which they do not appear to be saturated, and thus support for the interrelationships of more closely related taxa would be reduced or lost altogether. Perhaps a better method that should be considered for future studies is functional outgroup rooting (Watrous and Wheeler, 1981) with the Caryophyllidea, for example.

CONCLUSION

The multiple hypotheses presented in Figs. 2–5 belie the congruence, or at least the nonconflict that is found among them, and the significant degree of progress that has resulted from an intensive effort by an international group of cestodologists (see reviews by Hoberg *et al.*, 1997b; Olson, 2000), notably since the publica-

tion of the widely adopted taxonomic framework of Khalil *et al.* (1994). The rate at which new phylogenetic hypotheses have been published, the majority of which are based on morphological analyses, has never been greater and most major taxonomic groups now have a least a working hypothesis of their internal structure (e.g., Beveridge *et al.*, 1999; Bray *et al.*, 1999; Caira *et al.*, 1999, 2001; Hoberg *et al.*, 1999b; Ivanov and Hoberg, 1999; Olson *et al.*, 1999; Rego *et al.*, 1998; Zehnder and Mariaux, 1999). A few groups, however, such as the Caryophyllidea, have received little attention despite their unique biology, diversity, and importance with regard to the evolution of the class; still, the most recent and eloquent review of the group is by Mackiewicz (1982). Molecular studies have provided a wealth of new characters and have laid to rest at least some long-standing debates regarding the affinities of enigmatic taxa such as the Haplobothriidea, Litobothriidea, and Nippotaeniidea; debates that were unlikely to be resolved by comparative morphology alone.

Continued research in tapeworm systematics is particularly needed to circumscribe monophyletic groups within the orders shown to be paraphyletic (especially the Tetraphyllidea), to test the putative homology of many of the morphological character states used herein and elsewhere, and to fill in the gaps in our knowledge in groups that are still largely unknown (e.g., Litobothriidea, Spathebothriidea). In turn, such studies will likely result in the need to reevaluate higher-level relationships once again and to formally revise our classification schemes to better reflect this new knowledge.

APPENDIX 1

Morphological Character Matrix

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Gyrocotylidea	0	0	0	?	0	0	?	0	9	0	0	0	?	0	9	0	1
Caryophyllidea	0	0	0	0	1	0	0,1	0	0	0	0	0	0	0	0	0	1
Spathebothriidea	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1
Diphyllobothriidae	0	0	0	1	1	0	0	0	0	0,1	1	1	0	0	0	0	1
Pseudophyllidea	0	0,1	0	1	1	0	0,1	0	0	0,1	1	1	0	0	0	0	0,1
Haplobothriidea	0	0	0	0	1	0	0	0	0	1	1	?	0	0	0	1	1
Diphyllidea	0	1	?	0	1	?	1	?	1	1	1	1	0	0	0	3	1
Trypanorhyncha*	0	0,1	0	0,1	1	1	0,1	0	0,1	1	1	1	0	0	0	1,3	0
Trypanorhyncha	0	0,1	0	0,1	1	1	0,1	0	0,1	1	1	1	0	0	0	1,3	0
Rhinebothriinae	1	1	1	0	1	1	1	1	0	1	1	2	0	0,1	0	1	0
Phyllobothriidae	1	1	1	0	1	1	1	1	0	1	1	2	0	0	0	1	0
Onchobothriidae	1	1	1	0	1	1	1	1	0	1	1	2	1	0	0	1	0
Acanthobothrium	1	1	1	0	1	1	1	1	0	1	1	2	1	0	0	1	0
Litobothriidea	?	1	?	?	1	?	?	?	0	1	1	?	?	0	0	1	0
Lecanicephalidea	1	1	1	0	1	1	1	1	0	1	1	2	0	0,1	0	1	0
Proteocephalidea	1	1	1	0,1	1	1	1,2	1	0,1	1	1	2	0	0	0	1	0
Nippotaeniidea	1	1	1	0	1	1	1	1	0	1	1	2	0	0	0	3	0
Tetrabothriidea	1	1	1	0	1	1	1	1	0	1	1	2	1	0	1	2	0
Cyclophyllidea	1	1	1	0	1	1	2	1	1	0,1	1	2	0,1	0	0,1	3	0
Mesocestoididae	1	1	1	0	1	1	2	1	1	1	1	2	0	0	0	3	1

Taxon	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Gyrocotylidea	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Caryophyllidea	0,1	0	1	0,1	0,1	1	?	0	0	0	0	1	0	0	0	0
Spathebothriidea	0	0	1	0	0	1	?	?	?	0	?	0	?	?	?	?
Diphyllobothriidae	0,1	0	0,1	0,2	1	1	1	0	0	0	0	0	0	0	0	0
Pseudophyllidea	1	0,1	0,1,3	0,1,2	1	1	1	0	0	0	0	0	0	0	0	0
Haplobothriidea	1	0	3	1	1	1	?	0	0	0	0	0	0	0	0	0
Diphyllidea	1	0	0,1	1	1	1	1	0	0	0	0	0,1	0	0	0	0
Trypanorhyncha*	1	0	1	0,1	1	1	?	0	0	0	0	0	0	0	0	0
Trypanorhyncha	1	0	0,1	0,1	1	1	?	0	0	0	0	0	0	0	0	0
Rhinebothriinae	1	0	0,1	1	1	1	1	0	0	0	0	1	1	0	0	0
Phyllobothriidae	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0
Onchobothriidae	1	0	0,1	1	1	1	1	0,1	0	0	0	0	1	0	0	0
Acanthobothrium	1	0	0	1	1	1	1	0,1	0	0	0	0	1	0	0	0
Litobothriidea	1	0	1	1	1	?	?	?	?	?	?	?	?	?	?	?
Lecanicephalidea	1	0	0,1	1	1	1	1	1	0	0	0	1	0	0	0	0
Proteocephalidea	1	0	0	0,1,2	0,1	1	1	0,1	0	0,1	0	0,1	0	0	0	0
Nippotaeniidea	1	1	2	1	1	1	?	?	?	0	?	1	?	?	?	?
Tetrabothriidea	1	1	2	1	1	1	1	2	0	1	1	1	0	0	0	0
Cyclophyllidea	1	1	3	1	1	1	1	2	1	1	0,1	1	0	1	1	0,1
Mesocestoididae	1	1	3	1	1	1	1	1	0	0	0	1	0	0	0	0

APPENDIX 2

Morphological Characters and Their States

Morphological characters and their states are modified from those of Hoberg *et al.* (2001) (1–22) and Justine (2001) (23–33). Numbers below shown parenthetically refer to the original numbering scheme of Hoberg *et al.* (2001). Characters followed by an asterisk have been recoded and/or redefined from their original versions as described. See text for a list of the characters in Hoberg *et al.* (2001) not included herein and for additions and deletions to terminal taxa.

1. EGG, development* (19): 0, polylecithal; 1, oligolecithal.

Hoberg *et al.* (2001) coded the Litobothriidea as having oligolecithal eggs, but information on egg development is not found in the literature and we have changed the character state of this taxon to "?"

2. EGG, operculum (20): 0, present; 1, absent.
3. EGG, quinone tanning (21): 0, present; 1, absent.
4. LARVAE, embryogenesis* (25): 0, not delayed; 1, delayed.

Following Beveridge (2001), we have coded this character as polymorphic for the Trypanorhyncha.

5. LARVAE, form (26): 0, decacanth; 1, hexacanth.
6. LARVAE, protonephridium (23): 0, present; 1, absent.
7. LARVAE, embryophore* (24): 0, ciliated; 1, nucleated, nonrigid; 2, rigid.

Following Beveridge (2001), we have coded this character as polymorphic for the Trypanorhyncha.

8. LARVAE, No. of embryonic membranes (22): 0, single membrane formed by embryo; 1, two membranes with embryophore.
9. METACESTODE, cercoid structure* (35): 0, scolex not invaginated or retracted; 1, scolex invaginated or retracted.

Following Beveridge (2001), we have coded this character as invaginated or retracted for the Diphyllidea and as polymorphic for the Trypanorhyncha.

10. ADULT, external segmentation* (31): 0, absent; 1, present.

In contrast to Hoberg *et al.* (2001), we have coded this character as polymorphic for the Cyclophyllidea, Diphyllobothriidae, and "Pseudophyllidea" (see text for discussion).

11. ADULT, proglottisation (32): 0, absent; 1, present.
12. ADULT, scolex condition* (7): 0, monofossate; 1, bothriate; 2, acetabulate.

Few detailed studies have evaluated the underlying homology among the diverse array of scolex conditions (e.g., Caira *et al.*, 1999, 2001; Dubinina, 1980; Galkin, 1996; Hoberg, 1987), although the historical importance of this character in morphologically defining the major lineages of the class continues to the present day (e.g., Khalil *et al.*, 1994). Comprehensive studies dedicated solely to this topic have the potential to significantly enhance the foundation upon which stands current classification schemes.

We have replaced the character states "apical sucker, single," "bilateral, difossate," and "bilateral, tetrafossate" in Hoberg *et al.* (2001) with "monofossate," "bothriate" and "acetabulate," respectively; the latter two states better reflect the terminology used by Caira *et al.* (1999, 2001) whose works more precisely define the various scolex structures generally classified as either "difossate" or "tetrafossate." Although "acetabulate," for example, more accurately describes the state of the character (i.e., "membrane bound, muscular organs of attachment;" Caira *et al.*, 1999, p. 102), elsewhere in the text we generally refer to bothriate and acetabulate groups, using the more traditional terms.

Character state "0" (apical sucker, single) in Hoberg *et al.* (2001) is used to characterize the Monogenea, Amphilinidea, Litobothriidea, and Nippotaeniidea. We have changed the state to "monofossate," encompassing the various nonbothriate and nonacetabulate forms found in caryophyllideans, gyrocotylideans, and spathebothriideans. This state admittedly requires further study of its putative, and perhaps unlikely, homology, especially between the two eucestode groups and the gyrocotylideans. We have coded the Nippotaeniidea as acetabulate, following the definition of Caira *et al.* (1999) above. The scolex of the Litobothriidea was originally described as consisting only of an apical sucker followed by modified pseudosegments that are cruciform in transverse section (Dailey, 1969). Alternative views suggest that the scolex in this group is represented by more than just the apical sucker (Caira *et al.*, 1999; Olson and Caira, 2001), and Caira *et al.* (1999) code this character state for the Litobothriidea as "pseudosegmentation." We have chosen to code the group as an uncertainty (?), although it could be considered acetabulate based on the definition above.

Hoberg *et al.* (2001) coded the Haplobothriidea as bilateral, difossate, and possessing bothria (their characters 7 and 8). The primary scolex of the sole species (but see Premvati, 1969), *Haplobothrium globuliforme*, is undivided with four simple tentacles (lacking the more complex rhyncheal apparatus found in trypanorhynchs), and the secondary scolex is similarly undivided but depressed on four sides (Jones, 1994a). Thus, there are no physical features to support Hoberg *et al.*'s (2001) coding. Like the Litobothriidea, the enigmatic,

perhaps relictual form of the haplobothriidean scolex required us to code the group as an uncertainty.

13. ADULT, ontogeny of scolex (16): 0, adult form in intermediate host; 1, adult form in definitive host only.
14. ADULT, myzorhynchus* (9): 0, absent; 1, present.

Hoberg *et al.* (2001) coded both the phyllobothriid tetraphyllideans and the lecanicephallideans as having a myzorhynchus; however, this character, if indeed homologous, is polymorphic in both groups (see Euzet, 1994a,b).

15. ADULT, uterus position (4): 0, ventral; 1, dorsal.
16. ADULT, uterine pore position (3): 0, permanent, ventral; 1, dehiscent, ventral; 2, dehiscent, dorsal; 3, absent.
17. ADULT, genital pore position* (5): 0, marginal; 1, median.

Hoberg *et al.* (2001) had three states, splitting the marginal condition into two states: that with the male and female pores separate and that with them forming a single pore. We feel that these two latter states are redundant, already being covered by character 18 (their character 6) and have changed the character to be binary.

18. ADULT, genital pore fusion* (6): 0, separate; 1, fused.

Hoberg *et al.* (2001) differentiated between fused pores opening into a common atrium and simply "fused pores" (the latter condition not defined by the authors). We consider the genital pore to be the opening of the genital ducts on the body surface and recognize two states.

19. ADULT, vitellarium structure* (30): 0, follicular; 1, compact.

Character 30 of Hoberg *et al.* (2001) is more complex with three states encompassing two characters (structure of the vitellarium and condition of the vitelline ducts). We have retained only the conditions relating to structure.

20. ADULT, vitellarium arrangement* (29): 0, lateral, 1, circum-segmental; 2, median, preovarian; 3, median, postovarian.

Hoberg *et al.* (2001) include states of both distribution (lateral, median, pre/postovarian) and structure (follicular or globular). We have retained only the definitions related to arrangement as the latter condition is redundant of character 19 above. In addition, we have added the character state "circum-segmental" with codings based on information in Khalil *et al.* (1994).

21. ADULT, vitellarium position in transverse-section* (28): 0, cortical; 1, medullary; 2, paramuscular.

In contrast to Hoberg *et al.* (2001), we have included both cortical and paramuscular states for the Diphyllobothriidae (Bray *et al.*, 1994), all three states for the Pseudophyllidea (Bray *et al.*, 1994), and both cortical and medullary states for the Trypanorhyncha (Campbell and Beveridge, 1994) and changed the Diphyllidea from cortical to medullary (Caira *et al.*, 1999, 2001).

22. ADULT, testes position* (34): 0, cortical; 1, medullary.

Hoberg *et al.* (2001) coded the Caryophyllidea as having cortical testes, but this is characteristic only of the family Balanotaeniidae which houses two species (Mackiewicz, 1994). We have changed the state to be polymorphic.

23. SPERMATOZOA, mitochondria: 0, present; 1, absent.
24. SPERMATOZOA, crested body: 0, absent; 1, present.
25. SPERMATOZOA, intercentriolar body: 0, present; 1, single plate; 2, absent.
26. SPERMATOZOA, striated root: 0, present; 1, absent.
27. SPERMATOZOA, peripheral microtubules: 0, parallel; 1, twisted.
28. SPERMATOZOA, periaxonemal sheath: 0, absent; 1, present.
29. SPERMATOZOA, No. of axonemes in mature spermatozoa: 0, two; 1, one.
30. SPERMATOZOA, No. of types of cortical microtubules: 0, one; 1, two.
31. SPERMIOGENESIS, No. of axonemes in zone of differentiation: 0, two; 1, one.
32. SPERMIOGENESIS, flagellar rotation: 0, present; 1, absent.
33. SPERMIOGENESIS, proximodistal fusion: 0, present; 1, absent.

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