ON THE POSITION OF *ARCHIGETES* AND ITS BEARING ON THE EARLY EVOLUTION OF THE TAPEWORMS

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ABSTRACT: The tapeworm *Archigetes sieboldi* Leuckart, 1878 (Platyhelminthes: Cestoda: Caryophyllidea) has been cited as a likely representative of the "protocestode" condition, owing to its lack of segmentation and ability to attain sexual maturity in the invertebrate host (aquatic oligochaetes). The idea has been variously amplified or rejected in the literature, although the actual phylogenetic position of the species has not been investigated until now. New collections of *Archigetes* sp. from both its vertebrate and invertebrate hosts provided the opportunity to estimate its phylogenetic position with the use of molecular systematics, while prompting new analyses aimed at assessing the early diversification of the Cestoda. Additional collections representing the Amphilinidea, Caryophyllidea, and Gyrocotylidea were combined with published gene sequences to construct data sets of complete 18S (110 taxa) and partial (D1-D3) 28S (107 taxa) rDNA sequences, including 8 neodermatan outgroup taxa. Estimates resulting from Bayesian inference, maximum likelihood, and maximum parsimony analyses of the separate and combined data sets supported a derived position of the genus within the Caryophyllidea, and thus reject the idea that *Archigetes* sp. may exemplify a "primitive" condition. Topological constraint analyses rejected the hypothesis that *Archigetes* represents the most basal lineage of the Eucestoda, but did not rule out that it could represent the earliest branching taxon of the Caryophyllidea. In all analyses, the Eucestoda were monophyletic and supported basal positions of the nonsegmented Caryophyllidea and Spathebothriidea relative to other major lineages of the Eucestoda, implying that segmentation is a derived feature of the common ancestor of the di- and tetrafossate eucestodes. However, constraint analyses could not provide unequivocal evidence as to the precise branching patterns of the cestodarian, spathebothriidean, and caryophyllidean lineages. Phylogenetic analyses favor the interpretation that sexual maturity of *Archigetes* sp. in the invertebrate host, and similar examples in members of the Spathebothriidea, are the result of progenesis and have little if any bearing on understanding the protocestode condition.

During more than a century of speculation on the origin and evolution of the tapeworms, the significance of the nonsegmented Caryophyllidea, and particularly of the genus *Archigetes* Leuckart, 1878, has been an ongoing theme. Authors inclined to believe that the first hosts of cestodes were invertebrates pointed to *Archigetes* sp., members of which may self-fertilize and become gravid in the invertebrate (tubificid annelid) host (Calentine, 1962), as evidence of the "protocestode" condition. Implicit in this hypothesis is that the Caryophyllidea represents the most basal eucestode order, and that *Archigetes* sp. is either the most basal lineage within the order, or within the entire Eucestoda (a scenario in which the Caryophyllidea would be rendered paraphyletic). Alternative views held that the Caryophyllidea (and Spathebothriidea) were an offshoot of the "Pseudophyllidea" and had thus lost segmentation secondarily, forcing the interpretation that *Archigetes* sp. evolved through progenesis, i.e., the precocious development of the reproductive system leading to sexual maturity in an otherwise juvenile state. Still others accept a basal position of the Caryophyllidea, but suggest that their monozoic condition may be nevertheless derived, once again evolving by way of heterochrony, i.e., progenesis, as exemplified by *Archigetes* sp. The issues surrounding these opposing views have been discussed at greatest length by Mackiewicz (1972, 1981, 1982, 2003).

As alluded to by Olson et al. (2001) and Mackiewicz (2003), the identification of unique genes that control the processes of proglottization (the formation of serially repeated reproductive organs, or proglottids) and segmentation (the somatic compartmentalization of proglottids), if such specific genes exist, would provide the most direct means of inferring whether the lack of segmentation in the Caryophyllidea (as well as the Spathebothriidea) is a primary or secondary characteristic (see also Olson, 2008, for a discussion of this topic in relation to Hox genes). Should such genes be lacking in the Caryophyllidea, then this would support their primary lack of segmentation and corroborate a basal position of the group. Alternatively, should such genes occur in the Caryophyllidea, but not be expressed, then secondary loss of segmentation would be inferred. Commonly, it has been assumed that parasites with complex life cycles must have first entered the intermediate host before the definitive host, but this is not necessarily the most parsimonious interpretation; e.g., Littlewood et al. (1999) posit that the original hosts of all parasitic platyhelminths were vertebrates (see also Cribb et al., 2001, 2003). More recently, it has been argued that as the Trematoda and Cestoda appear to be sister taxa, the acquisition of complex life cycles involving invertebrate intermediate hosts was a single event after vertebrate parasitism was acquired by stem group neodermatans (Lockyer et al., 2003; Park et al., 2007).

Despite the significance that has been attached to *Archigetes* sp., its phylogenetic position has never been tested and only relatively recently has the application of cladistic methodology and molecular sequence data provided both a testable framework and a source of data independent of morphology. Previous molecular estimates of cestode interrelationships based predominantly on ribosomal DNA (i.e., Mariaux, 1998; Olson and Caira, 1999; Kodedová et al., 2000; Hoberg et al., 2001; Olson et al., 2001; Brabec et al., 2006; Waeschenbach et al., 2007) have not provided conclusive support of the early branching pattern of the Eucestoda, but have nevertheless generally supported either the Caryophyllidea (28S) or Spathebothriidea (18S), both groups of which lack segmentation, at the base of the Eucestoda (for a review see Olson and Tkach, 2005). Thus, although further corroboration is needed, molecular data point to the monozoic (unsegmented) condition of the Caryophyllidea as plesiomorphic, and not resulting from secondary loss.

Collection of *Archigetes* sp. from both its vertebrate (fish)

and invertebrate (oligochaete) hosts afforded the opportunity to examine its phylogenetic position with the use of molecular data for the first time and, in doing so, to revisit the question of the early diversification of the tapeworms. Starting with 71 complete 18S and partial (D1–D3) 28S rDNA sequences from Olson et al. (2001), including representatives of all major lineages recognized by Khalil et al. (1994), additional taxa representing early branching monofossate (Amphilinidea, Gyrocotylidea, Caryophyllidea, and Spathebothriidea) and difossate (Diphyllidea, Diphyllobothriidea, and Bothriocephalidea) lineages have been added based on both recently published (Bray and Olson, 2004; Brabec et al., 2006; Marques et al., 2007) and newly characterized sequences. The position of *Archigetes* sp. is thus analyzed within the context of the entire Cestoda with representatives of the Monopisthocotylea, Polyopisthocotylea, Aspidogastrea, and Digenea used as outgroups.

MATERIALS AND METHODS

New collections of adult *Archigetes sieboldi* were made from the fish host *Gnathopogon elongatus* in Japan (experimental research river A, Kasada, Kawashima-cho, Hashima-gun, Gifu Prefecture) and of ''progenetic'' forms from the coelom of the tubificid oligochaete *Limnodrilus hoffmeisteri* in Russia (Latka Stream, flowing into the Rybinsk Reservoir). In addition, new collections were made of 3 caryophyllideans (*Atractolytocestus huronensis*, ex *Cyprinus carpio*, Tisa River, East Slovakia; *Caryophyllaeides fennica*, ex *Rutilus rutilus*, Třeboň, Czech Republic; and *Monobothroides chalmersius*, ex *Clarias gariepinus* [=*C. lazera*], River Nile, Egypt), 1 amphilinidean (*Amphilina foliacea*, ex *Acipenser ruthenus*, lower Danube River, Bulgaria), and 1 gyrocotylidean (*Gyrocotyle* sp. ex *Callorhinchus milii*, Hobart, Tasmania, Australia).

Complete 18S and partial (D1-D3) 28S rDNA sequences were characterized from the taxa above as detailed in Olson et al. (2003), screened via BLASTn (McGinnis and Madden, 2004) to confirm their identities, and submitted to GenBank (accession numbers EU343734-49; see also Fig. 1). The sequences were added to the 18S and 28S alignments of 71 cestode taxa from Olson et al. (2001), together with recently published sequences representing the Spathebothriidea (Marques et al., 2007; Waeschenbach et al., 2007), Diphyllidea (Bray and Olson, 2004), and diphyllobothriid and bothriocephalid ''pseudophyllideans'' (Brabec et al., 2006). To provide an independent, i.e., noncestode, root to the tree, published sequences were added from 2 taxa representing each additional major parasitic flatworm lineage, i.e., Monopisthocotylea, Polyopisthocotylea, Aspidogastrea, and Digenea (see Fig. 1 for taxa and sequence accession numbers and Olson and Tkach, 2005, for an overview of the interrelationships of the parasitic flatworms). An alignment consisting of 110 (18S) or 107 (28S) sequences was constructed by eye with the use of MacClade 4.08 (Maddison and Maddison, 2005), and only positions that could be aligned confidently across the entire spectrum of taxa (including outgroups) were included in the analyses, resulting in 1,514 of 3,291 aligned (including gapped) positions for 18S (46%) and 554 of 2,244 for 28S (25%). The 18S and 28S data partitions were analyzed both individually and combined by Bayesian inference with the use of MrBayes (ver. 3.1.2, Ronquist and Huelsenbeck, 2003) and by parsimony-based bootstrapping (10,000 replicates evaluated with the use of a fast heuristic search strategy) using PAUP* (ver. 4.0b10, Swofford, 2001). Bayesian analyses employed a general time reversible (GTR) model of nucleotide substitution including estimates of both invariant sites and among-site rate variation, as this was found to provide the best fit to each partition independently by MrModelTest 1.1b (Nylander, 2004). Two MCMCMC runs with 4 chains each were run for 2 million generations (saving topologies every 100th generation) and used default starting prior probabilities. MrBayes was used to assess convergence of the 2 runs, and the ''burnin'' value (the number of generations completed before parameter estimates plateau) was estimated by plotting log-likelihood values against generation number. Consensus trees were constructed using the ''sumt'' command with the ''contype=allcompat'' option and ''burnin'' values of 2,000 for each partition analyzed individually and 1,500 for the combined analysis.

Maximum-likelihood (ML) analyses were performed with the use of successive approximation: model parameters were estimated based on a starting tree determined by neighbor joining (NJ). A heuristic search was performed implementing the estimated model parameters using nearest-neighbor-interchange (NNI) branch swapping. Model parameters were estimated on the best tree and a heuristic search performed using subtree-pruning-regrafting (SPR) branch swapping. After estimating model parameters, heuristic searches using tree-bisection-reconnection (TBR) branch swapping were performed until the topology remained unchanged.

Topological constraint analyses were run using ML analyses, as described, to test alternative positions of *Archigetes* sp. and of the basal branching cestode lineages. For each constraint analysis, a tree with the desired topology was loaded as a backbone constraint prior to ML analysis. Finally, the unconstrained ML tree was compared statistically with the results from the constraint analyses using the Shimodaira–Hasegawa test as implemented in PAUP*. Hypotheses tested included (see also Table I): (1) *Archigetes* sp. as the earliest branch of the Caryophyllidea; (2) *Archigetes* sp. as the earliest branch of the Eucestoda; and (3) the Caryophyllidea (including *Archigetes* sp.) as the earliest branch of the Eucestoda.

RESULTS

Both the 18S and 28S rDNA sequences of *A. sieboldi* from fishes in Japan and from tubificid worms in Russia were identical and all phylogenetic analyses placed the species in a derived position within the Caryophyllidea, nearest to *Hunterella* sp. (Figs. 1, 2). The Gyrocotylidea and Amphilinidea were each monophyletic and formed either a single lineage that was the sister group of the Eucestoda (28S) or 2 separate lineages (18S) in which the Amphilinidea was sister to the Eucestoda. Independent analyses of 18S and 28S (Fig. 1) showed other differences, particularly with respect to the relative positions of the basal mono- and difossate orders, which were poorly supported by the single-gene analyses. Similar to the findings of Olson et al. (2001) and other studies stemming from their data (e.g., Brabec et al., 2006; Waeschenbach et al., 2007), 28S data weakly support the Caryophyllidea as the most basal eucestode lineage, i.e., sister to all other eucestodes, whereas 18S places the Spathebothriidea at the root of the eucestode tree, grouping the Caryophyllidea in a slightly more derived position, typically as the sister group to the Diphyllobothriidea.

Combining the genes (Fig. 2) increases support and thus resolution across the tree, with the interrelationships of the early branching groups more similar to the results of 18S alone than to 28S. The combined analysis supports independent cestodarian lineages with the Amphilinidea as sister to the Eucestoda and the Spathebothriidea as the most basal branching lineage of the Eucestoda followed by a lineage in which the Caryophyllidea is the sister group to the Diphyllobothriidea (albeit without nodal support for the latter grouping). Within the Caryophyllidea, *Archigetes* sp. is again placed in a relatively derived position nearest to *Hunterella*.

Other aspects of the trees reflect previous findings based on subsets of the present data (Olson et al., 2001; Brabec et al. 2006), or the addition of complete 28S sequences for a subset of the present taxa (Waeschenbach et al., 2007). These include support of 2 independent lineages of the former Pseudophyllidea, i.e., Diphyllobothriidea and Bothriocephalidea (see also Brabec et al., 2006; Kuchta et al., 2008) and of the ''Trypanorhyncha.'' Similarly, present analyses support a derived clade of tetrafossate lineages and of a ''higher tetrafossate'' clade (Tetrabothriidea + Nippotaeniidea + Cyclophyllidea) within it.
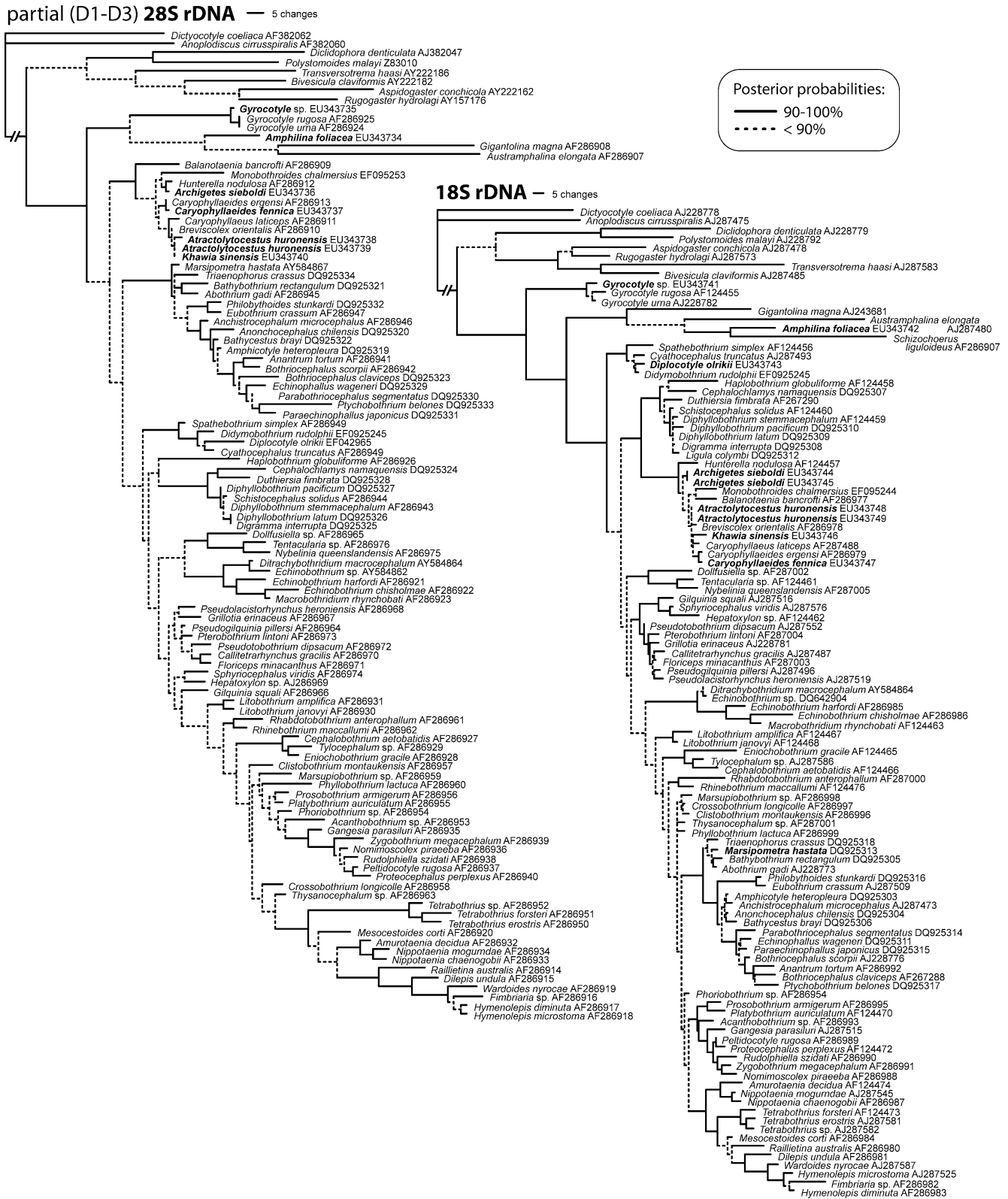
FIGURE 1.   Independent analyses of 28S and 18S rDNA based on Bayesian inference. Sequence accession numbers follow taxon names; new sequences shown in bold.

TABLE I. Results of topological constraint analyses.

Constraint topology	Shimodaira–Hasegawa	
	−ln likelihood	P
Unconstrained ML tree	21,904.22	Best
1. *Archigetes* sister to (or most basal lineage of) Caryophyllidea	21,924.70	0.321
2. *Archigetes* sister to (or most basal lineage of) Eucestoda	21,969.33	0.018*
3. *Archigetes* + Caryophyllidea as sister to other Eucestoda	21,912.20	0.680
4. Amphilinidea sister to all other Cestoda	21,926.79	0.287

* Asterisked P values indicate that the constraint topology is significantly worse than the consensus at $P < 0.05$ or less.

Within the tetrafossate clade, the polyphyletic Tetraphyllidea forms a number of independent lineages, including 1 comprising members the Onchobothriidae together with a monophyletic Proteocephalidea. In the combined analysis, the Litobothriidea and Lecanicephalidea are unusually found embedded among the various phyllobothriid lineages, whereas in the separate analyses and in previous studies they more frequently formed the earliest branches of the tetrafossate clade.

Results from constraint analyses on ML trees of the combined data set with the use of the Shimodaira and Hasegawa (1999) test are shown in Table I. Although all phylogenetic analyses resolved *Archigetes* sp. as a derived member of the Caryophyllidea, constraints showed that placing it as the sister group to the remaining Caryophyllidea was not significantly different from the unconstrained solution (Constraint 1). Forcing *Archigetes* to occupy the position as sister group to all Eucestoda was significantly different, and so the hypothesis that it may represent the earliest eucestode lineage is rejected (Constraint 2). However, support for the placement of Caryophyllidea + *Archigetes* as sister group to all remaining Eucestoda was not significant (Constraint 3), suggesting that insufficient signal exists to resolve the deeper cestode lineages; further evidence for this comes from relatively poor nodal support. Together, the constraint tests and the resolved phylogenies strongly indicate that *Archigetes* sp. is a derived member of the Caryophyllidea

DISCUSSION

In addition to testing the position of *Archigetes* sp. and the conspecificity of its forms maturing in invertebrate and vertebrate hosts, the analyses herein include a number of important taxa not found in previous studies. For example, amphilinideans are included for the first time in the context of an ordinal level analysis of the Cestoda and are herein represented by 3 (28S) or 4 (18S) different genera, including the type species for the order, *Amphilina foliacea*. Amphilinidean rDNA sequences are typified by being exceedingly divergent, including long stem-loop structures in the expansion regions, e.g., amphilinidean 18S sequences are 400–600 bps longer than those of other cestode groups (see Appendix A in Olson and Caira, 1999). Accommodating their rDNA sequences, therefore, significantly reduces the amount of alignable data, and previous analyses (Olson et al., 2001; Waeschenbach et al., 2007) have intentionally excluded these taxa in favor of analyzing a greater number of characters. Lower eucestode groups are also herein better represented with 4 of 5 known genera of the Spathebothriidea included and increased sampling of the Caryophyllidea, Diphyllidea, Diphyllobothriidea, and Bothriocephalidea.

Other than the large and small nuclear rDNA genes, only elongation factor 1-α has been used previously to estimate the interrelationships of cestodes and was judged to be neither more nor less informative than 18S rDNA (Olson and Caira, 1999). Indeed, the signal contained in rDNA seems reasonably robust given that similar results are achieved when analyzing various amounts and combinations of both data and taxa (Olson and Caira, 1999; Kodedová et al., 2000; Olson et al., 2001; Brabec et al., 2006; Waeschenbach et al., 2007). This similarity includes both support for the tetrafossate and higher tetrafossate clades and paraphyly of the Pseudophyllidea, etc., and lack of support. The latter has been particularly problematic with regard to resolving the interrelationships of the more early branching, nontetrafossate clades. Here, some differences are found comparing 18S and 28S, but neither individual gene nor their combination provides a single, robust picture of the early branching pattern. That the combined solution (Fig. 2) is more similar to the analysis of 18S than 28S most likely reflects a greater number of informative characters (400 for 18S vs. 251 for 28S); almost three-quarters of the 28S data were too variable to be aligned across the entire spectrum of taxa. Nevertheless, even with the inclusion of only highly conserved positions, additional taxa and a full range of neodermatan outgroups, the ''core'' signal in the genes differs little from previous analyses.

Hunterella sp. and the other caryophyllidean lineages all represent the standard 2-host cycle, supporting the inference that the monoxenous form of *Archigetes* sp. results from progenesis. Poddubnaya et al. (2003) recently examined the ultrastructural evidence for progenesis in *A. sieboldi*, concluding that ''in the final analysis, there is only one characteristic that basically distinguishes a progenetic procercoid from a normal one: maturity of the reproductive system.'' In other words, gravid *A. sieboldi* in the annelid host show all of the ultrastructural hallmarks of a larval worm, except in regard to the precocious development of the reproductive system. Such forms are thus accurately termed *progenetic*. What triggers such a change in the relative timing of development is unknown, but its evolutionary significance undoubtedly lies in its ability to ''perpetuate the parasite in those instances where the definitive host is absent'' (Poddubnaya et al., 2003).

As a phenomenon, progenesis in tapeworms is rare. Until recently, it had been documented only in *Archigetes* sp., and this fact has likely contributed to the special status of the genus. However, like *Archigetes* sp., there is also a tendency for a monoxenous life cycle in the amphipod host in 2 species belonging to the Spathebothriidea, i.e., *Diplocotyle olrikii* (Sandeman and Burt, 1972; Leontovich and Valovaya, 1989; Kuperman et al., 1995; Davydov et al., 1997) and *Cyathocephalus truncatus* (Kulkina, 1990; Protasova and Roytman, 1995; Okaka, 2000). Davydov et al. (1997) postulated that progenesis in tapeworms may appear as a result of a combination of 2 conditions, i.e., the availability of physical space in which to accommodate the growth and development of the parasite, and the presence of protective structures to provide immunological protection. Both of these conditions are observed in monoxe-
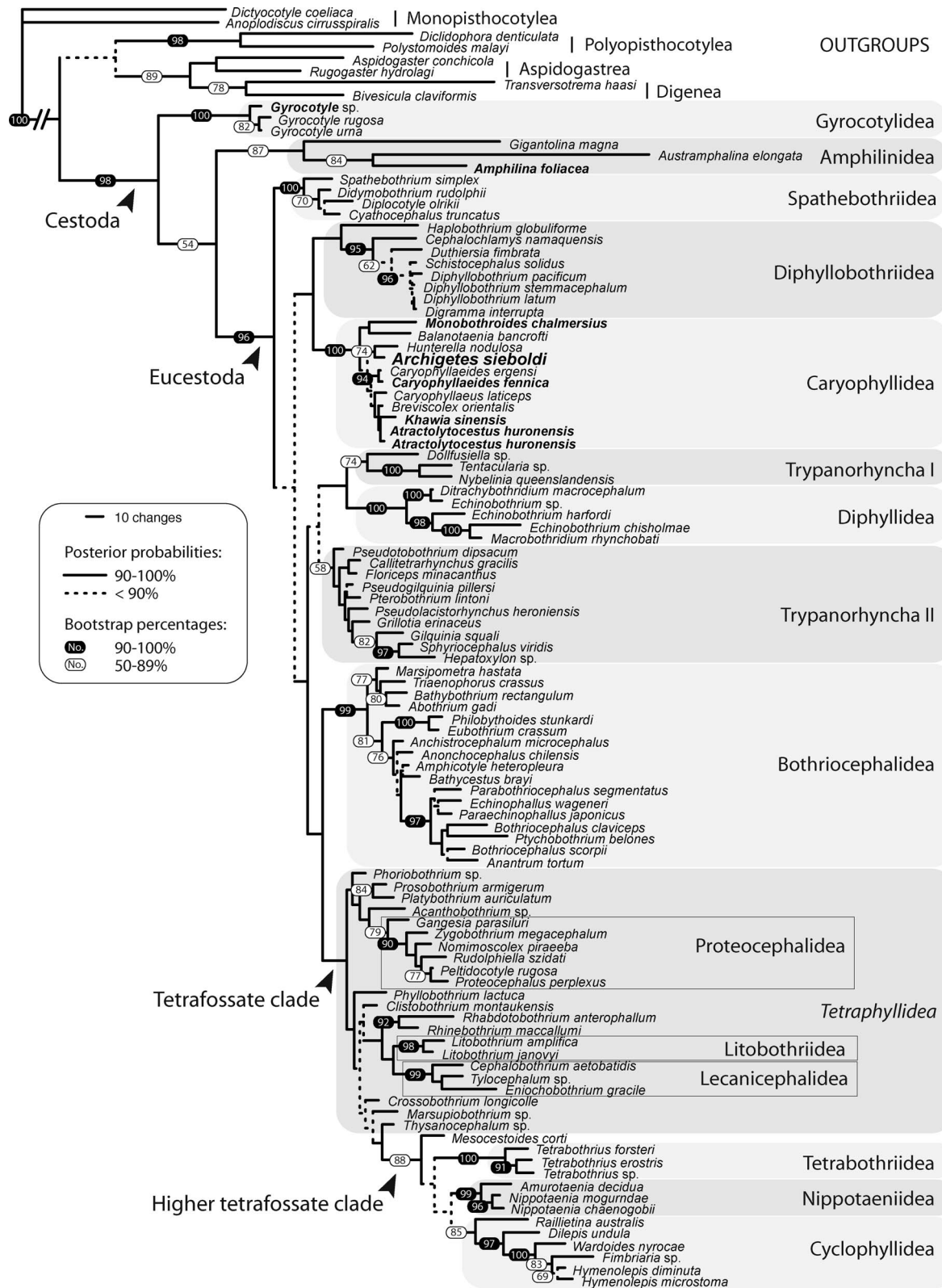
FIGURE 2.   Combined analysis of 28S and 18S rDNA based on Bayesian inference, with additional nodal support via parsimony bootstrapping (N.B. for greater clarity, relative branch lengths depict the number of character state changes and not mean Bayesian divergence estimates).

nous *A. sieboldi*. Thus, tiny larval worms attain sexual maturity in the comparatively large coelom of aquatic oligochaetes, and a thick, filamentous coating (dense glycocalyx layer) observed on the worm's surface presumably offers protection against host coelomocytes (Poddubnaya et al., 2003). A coating similar to the glycocalyx layer of *A. sieboldi* has also been shown in progenetic *D. olrikii* from the body cavity of gammarids (Davydov et al., 1997). It is important to appreciate, however, that although such conditions may allow for progenetic development in these worms, it does not necessarily follow that the conditions promote progenesis, and there are far more numerous examples of cyst-forming cestodes that do not exhibit precocious development. On the whole, life-cycle truncation among parasitic platyhelminths appears more frequently in the Digenea, where progenesis is just 1 method by which it is thought to have been achieved (Poulin and Cribb, 2002; Lefebvre and Poulin, 2005); heterochrony generally is hypothesized to have played a significant role in the evolution of many free-living flatworm groups (Tyler, 2001).

The conceptual link between progenesis as exemplified by *A. sieboldi* and the origin of the tapeworms is difficult to dispel. For example, even when postulating a basal phylogenetic position for the Caryophyllidea, Mackiewicz (2003) still favored their monozoic condition as being secondarily derived, originating (possibly) through progenetic development from a strobilate ancestor. Certainly paedomorphosis provides a plausible solution for the evolution of the Caryophyllidea. However, if the Caryophyllidea are both basal and evolved via progenesis, then one must invoke strobilate ancestors that are no longer extant. Whereas we agree with that such silent players may well tell a very different story from that which can be deduced today (Mackiewicz, 2003), such a scenario is not only nonparsimonious, but also begs the question as to why these lineages would have gone extinct when the strobilate bauplan has proven the most successful among contemporary cestode groups. In any case, although the monoxenic form of *A. sieboldi* appears to be a classic example of progenesis, a derived position of the genus within the Caryophyllidea negates the possibility that the lineage itself played an important role in the evolution of the order, let alone of the tapeworms generally.

ACKNOWLEDGMENTS

LITERATURE CITED

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

BRAY, R. A., AND P. D. OLSON. 2004. The plerocercus of *Ditrachybothridium macrocephalum* Rees, 1959 from two deep-sea elasmobranches, with a molecular analysis of its position within the order Diphyllidea and a checklist of the hosts of larval diphyllideans. Systematic Parasitology **59:** 159–167.

CALENTINE, R. L. 1962. *Archigetes iowensis* sp. n. (Cestoda: Caryophyl-
laeidae) from *Cyprinus carpio* L. and *Limnodrilus hoffmeisteri* Claparède. Journal of Parasitology **48:** 513–524.

CRIBB, T. H., R. A. BRAY, AND D. T. J. LITTLEWOOD. 2001. The nature and evolution of the association among digeneans, molluscs and fishes. International Journal for Parasitology **31:** 997–1011.

———, ———, P. D. OLSON, AND D. T. J. LITTLEWOOD. 2003. Life cycle evolution in the Digenea: A new perspective from phylogeny. Advances in Parasitology **54:** 197–254.

DAVYDOV, V. G., L. G. PODDUBNAYA, AND B. I. KUPERMAN. 1997. An ultrastructure of some systems of *Diplocotyle olrikii* (Cestoda: Cyathocephalata) in relation to peculiarities of its life cycle. Parazitologiya **31:** 132–141.

HOBERG, E. P., J. MARIAUX, AND D. R. BROOKS. 2001. Phylogeny among orders of the Eucestoda (Cercomeromorphae): Integrating morphology, molecules and total evidence. *In* Interrelationships of the Platyhelminthes, D. T. J. Littlewood and R. A. Bray (eds.). Taylor and Francis, London, U.K., p. 112–126.

KHALIL, L. F., A. JONES, AND R. A. BRAY. 1994. Keys to the cestode parasites of vertebrates. CAB International, Wallingford, U.K., 751 p.

KODEDOVÁ, I., D. DOLEŽEL, M. BROUČKOVÁ, M. JIRKŮ, V. HYPSA, J. LUKEŠ, AND T. SCHOLZ. 2000. On the phylogenetic positions of the Caryophyllidea, Pseudophyllidea and Proteocephalidea (Eucestoda) inferred from 18S rRNA. International Journal for Parasitology **30:** 1109–1113.

KUCHTA, R., T. SCHOLZ, J. BRABEC, AND R. A. BRAY. 2008. Suppression of the tapeworm order Pseudophyllidea (Platyhelminthes: Eucestoda) and the proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. International Journal for Parasitology **38:** 49–55.

KULKINA, L. V. 1990. Progenetic cestode *Cyathocephalus* in *Gammarus hirsutus* from waters of West Tien-shan. Parazitologiya **24:** 232–235.

KUPERMAN, B. I., L. G. PODDUBNAYA, AND M. A. VALOVAYA. 1995. Biology and ultrastructure of *Diplocotyle olrikii* (Cestoda, Spathebothriidea) from the White Sea. Bulletin of the Scandinavian Society for Parasitology **5:** 78.

LEFEVBRE, F., AND R. POULIN. 2005. Progenesis in digenean trematodes: A taxonomic and synthetic overview of species reproducing in their second intermediate host. Parasitology **130:** 1–19.

LEONTOVICH, O. N., AND M. A. VALOVAYA. 1989. Description of progenetic cestode genus *Diplocotyle olrikii* (Pseudophyllidea, Cyathocephalidae) from *Gammarus oceanicus*. Vestnik Moskovskogo Universiteta Seriya Biologiya **16:** 39–52.

LITTLEWOOD, D. T. J., K. ROHDE, R. A. BRAY, AND E. HERNIOU. 1999. Phylogeny of the Platyhelminthes and the evolution of parasitism. Biological Journal of the Linnean Society **68:** 257–287.

LOCKYER, A., P. D. OLSON, AND D. T. J. LITTLEWOOD. 2003. Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): Implications and a review of the cercomer theory. Biological Journal of Linnean Society **78:** 155–171.

MACKIEWICZ, J. S. 1972. Caryophyllidea (Cestoidea): A review. Experimental Parasitology **31:** 417–512.

———. 1981. Caryophyllidea (Cestoidea): Evolution and classification. Advances in Parasitology **19:** 139–206.

———. 1982. Caryophyllidea (Cestoidea): Perspectives. Parasitology **84:** 397–417.

———. 2003. Caryophyllidea (Cestoidea): Molecules, morphology and evolution. Acta Parasitologica **48:** 143–154.

MADDISON, W. P., AND D. R. MADDISON. 2005. MacClade: Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts.

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

MARQUES, J. F., M. J. SANTOS, D. I. GIBSON, H. N. CABRAL, AND P. D. OLSON. 2007. Cryptic speciation of *Didymobothrium rudolphii* (Cestoda: Spatheobothriidea) from the sand sole, *Solea lascaris*, off the Portuguese coast, with an analysis of their molecules, morphology, ultrastructure and phylogeny. Parasitology **134:** 1057–1072.

MCGINNIS, S., AND T. L. MADDEN. 2004. BLAST: At the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Research **32:** W20–W25.

NYLANDER, J. A. A. 2004. MrModelTest, a program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.

OKAKA, C. E. 2000. Maturity of the procercoid of *Cyathocephalus truncates* (Eucestoda: Spathebothriidae) in *Gammarus pulex* (Crustacea: Amphipoda) and the tapeworm life cycle using the amphipod as the sole host. Helminthologia **37:** 153–157.

OLSON, P. D. 2008. Hox genes and the parasitic Platyhelminthes: New opportunities, challenges and lessons from the free-living. Parasitology International **57:** 8–17.

———, AND J. N. CAIRA. 1999. Evolution of the major lineages of tapeworms (Platyhelminthes: Cestoidea) inferred from 18S ribosomal DNA and *elongation factor-1α*. Journal of Parasitology **85:** 1134–1159.

———, T. H. CRIBB, V. V. TKACH, R. A. BRAY, AND D. T. J. LITTLEWOOD. 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). International Journal for Parasitology **33:** 733–755.

———, D. T. J. LITTLEWOOD, R. A. BRAY, AND J. MARIAUX. 2001. Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Molecular Phylogenetics and Evolution **19:** 443–467.

———, AND V. V. TKACH. 2005. Advances and trends in the molecular systematics of the parasitic Platyhelminthes. Advances in Parasitology **60:** 165–243.

PARK, J. K., K. H. KIM, S. KANG, W. KIM, K. E. EOM, AND D. T. J. LITTLEWOOD. 2007. A common origin of complex life cycles in parasitic flatworms: Evidence from the complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes). BMC Evolutionary Biology **7:** 11.

PODDUBNAYA, L. G., J. S. MACKIEWICZ, AND B. I. KUPERMAN. 2003. Ultrastructure of *Archigetes sieboldi* (Cestoda: Caryophyllidea):

Relationship between progenesis, development and evolution. Folia Parasitologica **50:** 275–292.

POULIN, R., AND T. H. CRIBB. 2002. Trematode life cycles: Short is sweet? Trends in Parasitology **18:** 176–183.

PROTASOVA, E. N., AND V. A. ROYTMAN. 1995. Cyathocephalates, tapeworm helminths of marine and freshwater fish (Cestoda: Pseudophyllidea: Cyathocephalata). Vol. 12. Essentials of cestodology. Institute of Parasitology, RAS, Moskva, Russia, 134 p.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572–1574.

SANDEMAN, I. M., AND M. B. D. BURT. 1972. Biology of *Bothrimonus* (= *Diplocotyle*) (Pseudophyllidea: Cestoda): Ecology, life cycle, and evolution; a review and synthesis. Journal of Fisheries Research Board of Canada **29:** 1381–1395.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution **16:** 1114–1116.

SWOFFORD, D. L. 2001. PAUP*, phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TYLER, S. 2001. The early worm: Origins and relationships of the lower flatworms. *In* Interrelationships of the Platyhelminthes, D. T. J. Littlewood and R. A. Bray (eds.). Taylor and Francis, London, U.K., p. 3–12.

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