

# Phylogenetic Relationships of Platyhelminthes Based on 18S Ribosomal Gene Sequences

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**Nucleotide sequences of 18S ribosomal RNA from 71 species of Platyhelminthes, the flatworms, were analyzed using maximum likelihood, and the resulting phylogenetic trees were compared with previous phylogenetic hypotheses. Analyses including 15 outgroup species belonging to eight other phyla show that Platyhelminthes are monophyletic with the exception of a sequence putatively from Acoela sp., Lecithoepitheliata, Polycladida, Tricladida, Trematoda (Aspidobothrii + Digenea), Monogenea, and Cestoda (Gyrocotylidae + Amphilinidea + Eucestoda) are monophyletic groups. Catenulids form the sister group to the rest of platyhelminths, whereas a complex clade formed by Acoela, Tricladida, "Dalyellioida," and perhaps "Typhloplanoida" is sister to Neodermata. "Typhloplanoida" does not appear to be monophyletic; Fecampiida does not appear to belong within "Dalyellioida," nor Kalyptorhynchia within "Typhloplanoida." Trematoda is the sister group to the rest of Neodermata, and Monogenea is sister group to Cestoda. Within Trematoda, Aspidobothrii is the sister group of Digenea and Heronimidae is the most basal family in Digenea. Our trees support the hypothesis that parasitism evolved at least twice in Platyhelminthes, once in the ancestor to Neodermata and again in the ancestor of Fecampiida, independently to the ancestor of putatively parasitic "Dalyellioida."** © 1998 Academic Press

## INTRODUCTION

Platyhelminthes, the flatworms, are a diverse group of terrestrial and aquatic organisms that display a

range of life histories from free-living to parasitic. Platyhelminthes are among the most phylogenetically basal group of bilateral animals (Field *et al.*, 1988; Morris, 1993); however, their evolutionary history is still unclear because of the only fragmentary information on their morphology, physiology, life cycles, and fossil remains. With the increased use of light and electron microscopy there has been an explosive interest in the systematics of Platyhelminthes, as indicated by the large number of recent papers (listed in Rhode *et al.*, 1993). Molecular systematic methods have also been used to assess relationships within Platyhelminthes (Ohama *et al.*, 1993; Qu *et al.*, 1986; Field *et al.*, 1988; Hori *et al.*, 1988; Ali *et al.*, 1991; Baverstock *et al.*, 1991; Korbssrisate *et al.*, 1991; Turbeville *et al.*, 1992; Riutort *et al.*, 1993; Blair, 1993; Blair and Barker, 1993; Barker *et al.*, 1993; Johnston *et al.*, 1993; Katayama *et al.*, 1993; Rhode *et al.*, 1993, 1994; Kuznedelov and Timoshkin 1993, 1995; see also review by Blair *et al.*, 1996). Nevertheless, questions regarding relationships within Platyhelminthes remain, in part due to poor representation of some groups in previous studies. Numerous 18S ribosomal sequences are available from Platyhelminthes, and our aim was to bring these sequences together to perform a comprehensive study of the phylogenetic relationships within Platyhelminthes using molecular data. We have compiled whole and partial sequences of 18S ribosomal RNA from 65 species of platyhelminths available and include 6 new partial sequences.

The present state of Platyhelminthes systematics features both controversies and nomenclature complexities. For consistency, in this paper we use the relationships and names of the major groups provided in Ehlers (1986), including quotation marks on "Dalyellioida" and "Typhloplanoida," which are not thought to be monophyletic.

## MATERIALS AND METHODS

### *Specimens*

The following organisms were collected for DNA sequencing: *Taenia crassiceps* (Eucestoda) cysticerci

Sequence data from this article have been deposited with the GenBank Data Libraries under Accession Nos. U88071–U88076.

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(ORF strain) from peritoneal cavity of Balb/c mice after 6 to 8 weeks of infection; *Taenia solium* cysticerci from skeletal muscle of naturally infected pigs; *Octomacrum mexicanum* (Monogenea) (Lamothe-Argumedo, 1980) adult worms from the gills of the fish *Algansea lacustris* (Cyprinidae), from Pátzcuaro Lake, State of Michoacán, México; *Posthodiplostomum minimum* (Digenea) metacercariae from the liver of the fish *Chirostoma attenuatum*, from Pátzcuaro Lake, State of Michoacán, México (Pérez-Ponce de León *et al.*, 1994); *Geoplana mexicana* (Tricladida), collected on the campus of the Universidad Nacional Autónoma de México, México City.

#### DNA Preparation and Cloning

*T. solium* and *O. mexicanum* specimens were immediately frozen in dry ice and stored at  $-75^{\circ}\text{C}$  until use. *T. crassiceps* and *G. mexicana* specimens were immediately used for DNA isolation. *P. minimum* specimens were fixed in 70% alcohol. DNA was purified according to Sambrook *et al.* (1989) by overnight incubation at  $50^{\circ}\text{C}$  in 0.5 M EDTA, 0.5% Sarkosyl, and 0.1 mg/ml Proteinase K. All samples were extracted with phenol:chloroform (1:1). The genomic DNA from *Echinococcus granulosus* was kindly provided by A. Flisser.

Clones of 18S ribosomal genes of *T. crassiceps* were obtained from a genomic DNA library in EMBL4, using a 18S rRNA probe from *Trypanosoma cruzi* (kindly provided by R. Hernández), and subcloned into M13 using TG1 cells (Gibson, 1984).

#### PCR Amplification

Amplification of 18S ribosomal DNA fragments from all other species was carried out by PCR (Saiki *et al.*, 1988) using primers JLR24, 5'-CGGAATTCGCTAGAGGTGAAATTCTTGG-3' (1009–1027), and JLR25, 5'-CCGAATTCGCGAGGTTACCTACGG-3' (1964–1981), designed from the sequence of *T. crassiceps*. Numbers in parentheses correspond to positions of the 18S gene of *Schistosoma mansoni* (Ali *et al.*, 1991).

PCR reactions were performed in 100- $\mu\text{l}$  volumes of Tris buffer (70 mM, pH 8.8) containing 20 mM ammonium sulfate, 2 mM magnesium chloride, 100 mg/ml bovine serum albumin, 0.1% Triton X-100, 0.2 mM each dNTP, 50 pmol of each primer, 250 ng of template DNA, and *Taq* polymerase (2.5 U; Boehringer). PCR fragments were purified by agarose gel electrophoresis followed by adsorption to glass powder (Vogelstein and Gillespie, 1979).

#### Sequencing Strategy

Sequence of clones was obtained by the dideoxy chain termination method (Sanger *et al.*, 1977), using the Sequenase 2.0 Kit (United States Biochemical Corporation) and [ $\alpha$ - $^{35}\text{S}$ ]dATP (NEN Research Products). Sequencing primers were either M13 primers or oligonucleotides internal to the genomic DNA fragment.

PCR products were sequenced using the CircumVent Thermal Cycle Sequencing Kit (New England BioLabs) and [ $\alpha$ - $^{35}\text{S}$ ]dATP (NEN Research Products), following recommendations of the manufacturer. Sequencing primers were: JLR24, JLR25, or other internal oligonucleotides.

#### Data Analysis

In addition to the sequences generated for this study, sequences were obtained from GenBank or the literature as listed in the Appendix. All nucleotide sequences were initially aligned with the program CLUSTAL W (Thompson *et al.*, 1994) and then manually adjusted. Variable regions V4 and V7 of the ribosomal sequences were excluded from the analyses, because these regions are highly variable across taxa and difficult to align.

Three analyses were conducted with fastDNAm1 (Olsen *et al.*, 1994) using empirical base frequencies, global branch swapping, and random taxon addition. From these initial analyses several putatively monophyletic groups were identified; Cestoda, Digenea, Lecithoepitheliata, Monogenea, Polycladida, and Tricladida. Each putatively monophyletic group, along with an outgroup taxon chosen from within Platyhelminthes, was separately analyzed with PAUP\* 4.0 (Swofford, 1996) with a one-parameter maximum likelihood model with empirical base frequencies and either a branch-and-bound search (Cestoda, Lecithoepitheliata, Monogenea, Polycladida, and Tricladida) or 10 heuristic searches with random sequence taxon addition and tree bisection–reconnection branch swapping (Digenea). To obtain the best tree for the relationships between the putative monophyletic groups and the rest of the taxa, each putative monophyletic group was constrained with the best topology found and 10 analyses were performed with prerelease versions of PAUP\* 4.0 using a one-parameter model, empirical base frequencies, random sequence taxon addition, and subtree pruning branch swapping. The best tree obtained at this point was used as the starting tree in an analysis, again with PAUP\* 4.0, using no constraints, a one-parameter model, empirical base frequencies, and tree bisection–reconnection branch swapping. Alternative topologies were compared with the test of Kishino and Hasegawa (1989) using a prerelease version of PHYLIP 4.0 (Felsenstein, 1996), and reported likelihood values are those from PHYLIP. All other program options, unless noted, were default.

## RESULTS AND DISCUSSION

#### Platyhelminthes Are Monophyletic

The results of our analyses are consistent, if rooting is ignored, with other recent studies using 18S RNA gene sequences (Wainwright *et al.*, 1993; Smothers *et al.*, 1994) with respect to the relative relationships of Ctenophora, Porifera, and Cnidaria. Other consistent

associations in all global analysis are: Chaetognatha–Nematoda and Mollusca–Nemertina, in reasonable agreement with a traditional view of invertebrate phylogeny (Brusca and Brusca, 1990). Only Acanthocephala (represented by a single species) might seem misplaced, appearing as the sister group of a clade including Cnidaria, Ctenophora, and Porifera.

Using these outgroups, Platyhelminthes appear to be monophyletic with the exception of one of three species of Acoela (*Acoela* sp.), which was placed with Nematoda in all analyses, suggesting that it is a highly anomalous sequence (Fig. 1). Therefore, we favor the monophyly of Platyhelminthes, also supported by previous work (Ehlers, 1986; Ax, 1987), as well as by recent trees also based on 18S sequence analysis of a small number of taxa (Turbeville *et al.*, 1992; Riutort *et al.*, 1992). However, a more thorough analysis to check this point should include sequences of other groups (i.e., gnathostomulids) not yet available.

#### *Relationships among the Major Groups of Platyhelminthes*

The relationships among the major groups of Platyhelminthes as presented by Ehlers (1986) are shown in Fig. 2, compared with the relationships determined in our analyses as shown in Fig. 3.

Our analysis places Catenulida and Fecampiida (*Kroenborgia isopodicola*) as sister taxa that together comprise the most basal clade of Platyhelminthes. Fecampiids are usually placed within “Dalyellioida” Meixner 1924 (Crezée, 1984; Ehlers, 1985) and have been proposed to be the sister group of Neodermata. This Fecampiida–“Dalyellioida” association does not appear solid according to our results, as the rest of dalyellioid species included in our study (*Luridae* sp., *Syndisyrinx punicea*, *Temnocephala dendyi*, and *Pterastericola australis*) are grouped in a clade, well apart from *K. isopodicola* (see below). The uniqueness of fecampiids has been previously noticed by Rohde (1990), who also analyzed the relationships of *K. isopodicola* with other platyhelminths through comparison of 18S ribosomal sequences (Rohde *et al.*, 1994) and proposed the establishment of a separate class for them. Whatever the affinity of fecampiids, they do not appear closely related to Neodermata.

In contrast, the placement of Catenulida within the sister clade to the rest of the phylum is in agreement with several previous studies based on nonmolecular data (Hyman, 1951; Ehlers, 1985, 1986; Ax, 1987), as well as with some trees also based on 18S sequence data (Rohde *et al.*, 1993).

One group that is not positioned by Ehlers (1985, 1986), Lecithoepitheliata, has also been placed in our tree. Lecithoepitheliata (six species of *Geocentrophora* and *Prorhynchus* sp.) and Macrostromida (*Macrostromum* sp.) form a clade emerging after Catenulida as sister group of the rest of Platyhelminthes. This associa-

tion appears consistently in all global analysis we carried out and suggests that Lecithoepitheliata occupies a more basal position than generally believed.

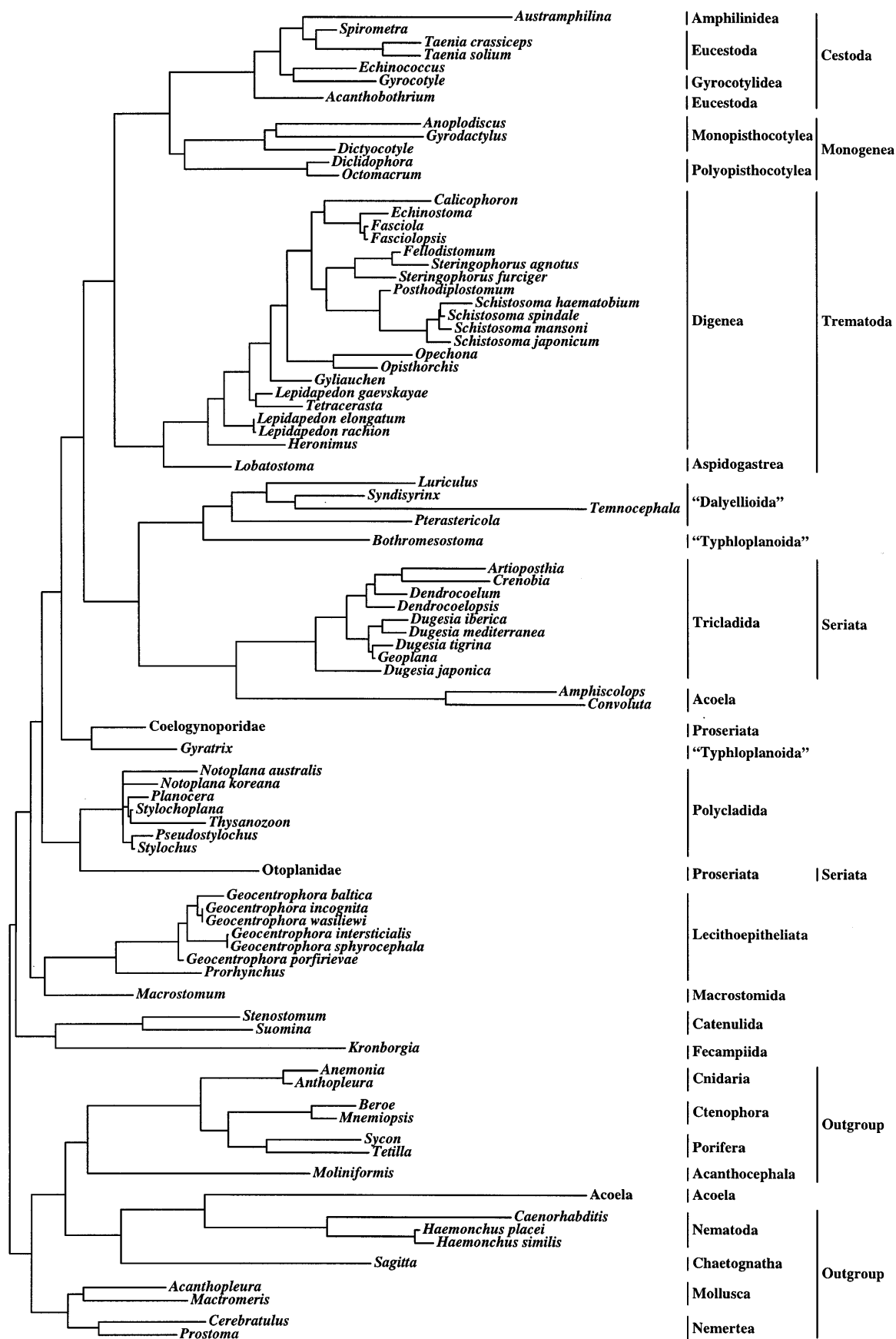
Polycladida appears associated with one of two species of Proseriata (Otoplanidae gen. sp.), whereas the second species (Coelogynoporidae gen. sp.) is related with one species of Typhloplanoida (*Gyratrix* sp.), suggesting that Proseriata is not monophyletic.

There is a striking similarity in the arrangement of Catenulida, Macrostromida (placed as a sister clade of Lecithoepitheliata), and Polycladida between our tree and a reconstruction (Ehlers 1985, 1986), based on nonmolecular data. The only exception is the placement of Acoela, which appears more basal in the reconstruction of Ehlers.

Finally, our tree places Acoela, Tricladida, “Dalyellioida,” and one of two species of “Typhloplanoida” in a monophyletic group that is sister to Neodermata (see below).

#### *Relationships of Acoela, Tricladida, “Dalyellioida,” and “Typhloplanoida”*

The sister group of Neodermata is a complex group divided in two major clades (Fig. 1). The first is formed by “Dalyellioida” (*Luriculus australiensis*, *S. punicea*, *T. dendyi*, and *P. australis*) and one of two species of “Typhloplanoida” (*Bothromesostoma personatum*) and the second includes Acoela (*Amphiscolops* sp. and *Convoluta naikaiensis*) and Tricladida (with nine species). “Dalyellioida” is a crucial group often involved in hypotheses regarding the origin of parasitism and of Neodermata (Ehlers, 1985, 1986; Brooks, 1989b; Brooks and McLennan, 1993). “Dalyellioida” show a variety of associations with other organisms from mutualism, ecto- and endo-comensalism, and parasitism. Ehlers (1986) acknowledged that “Dalyellioida” and “Typhloplanoida” might be paraphyletic and denoted these groups with quotation marks. A recent study based on morphological characters (Rohde, 1990, 1994) supports the idea that the similarities between Neodermata and “turbellarian” Rhabdocoelans (“Dalyellioida” and “Typhloplanoida”) are mostly due to morphological convergence rather than phylogenetic relationship. Our results are not consistent with “Dalyellioida” alone being sister group to Neodermata or with previously suggested monophyletic groups such as Rhabdocoela and Dolyopharyngiophora (Ehlers, 1985) and Cercomeria (Brooks and McLennan, 1993). However, all dalyellioid species included in this study appear to form a monophyletic group, with the exception of the parasite *K. isopodicola* (Fecampiidae) (see above), and support the inclusion of Temnocephalidea within part of “Dalyellioida.” Further studies are required on Udonellida before the relationships within the complete “Dalyellioida” (sensu Ehlers, 1985) are better understood. In contrast, in none of our analysis did the two species of “Typhloplanoida” (*Gyratrix* sp. and *B. personatum*)



**FIG. 1.** Phylogenetic tree of Platyhelminthes and other taxa determined by maximum likelihood, based on 18S RNA gene sequence data. The tree has a log likelihood value of  $-38376.707$  and is midpoint rooted for presentation purposes.

appear together in a single clade, suggesting that "Typhloplanoida" is not monophyletic. Indeed, the Kalyptorhynchid (*Gyratrix* sp.) always appears closely related to the proseriatan *Coelogyneporidae* sp.

Acoelans are anatomically relatively simple; they lack of digestive and body cavities and have been considered one of the earliest emerging groups of flatworms involved in the origin of all bilaterians (Becklemishev, 1963, 1969; Hanson, 1977; von Salvini-Plawen, 1978). On the other hand, their simplicity has also been interpreted as a secondary reduction from a coelomate bilaterian ancestor (Ax, 1963; Siewing, 1980; Smith and Tyler, 1985). Our phylogenetic reconstruction places Acoela, with the exception of an apparently anomalous *Acoela* sp. sequence, and Tricladida as sister group to "Dalyellioida"/Typhloplanoida, well apart from the common ancestor of all Platyhelminthes and metazoans. Recent analysis also based on 18S ribosomal sequences, placing Polycladida diverging later than Acoela and Tricladida (Katayama *et al.*, 1993), are difficult to compare to the present study because of differing taxa representation in the former study (i.e., absence of catenulids, macrostomids, lecitioepitheliata, etc.).

Tricladida in this study include two land (*Artioposthia* sp. and *Geoplana maxicana*) and seven freshwater (*Crenobia alpina*, *Dendrocoelopsis lactea*, *Dendrocoelum lacteum*, *Dugesia iberica*, *D. japonica*, *D. mediterranea*, and *D. tigrina*) planarian species. None of our analyses produced a monophyletic genus *Dugesia* nor monophyletic Terricola and Paludicola groupings.

#### Relationships within the Neodermata

Within Neodermata, our global analyses place Trematoda as the sister group of Monogenea and Cestoda, in agreement with schemes presented by several authors in recent work (Ehlers, 1985; Ax, 1987; Justine, 1991; Brooks and McLennan, 1993), and support the monophyly of Cercomeromorphae (Ehlers, 1985, 1986; Brooks, 1989a).

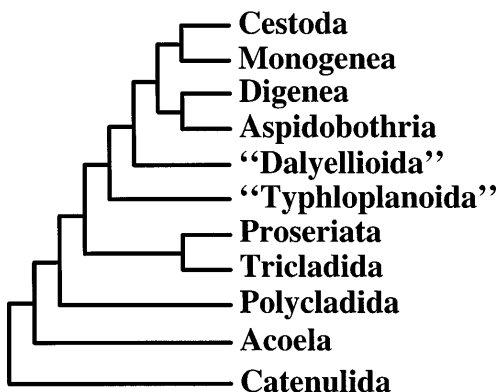


FIG. 2. Phylogenetic tree of the major groups of Platyhelminthes derived from Ehlers (1986).

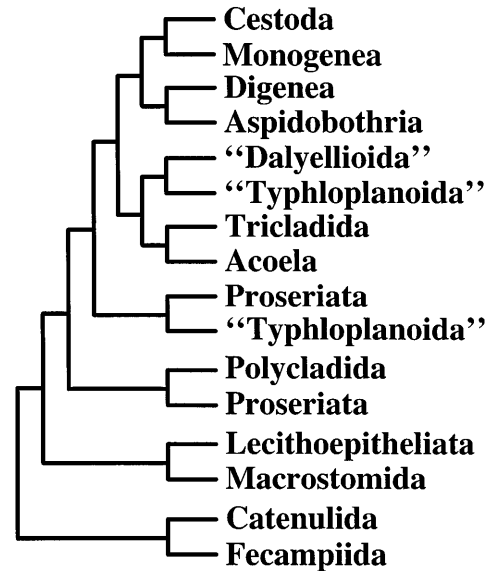


FIG. 3. Phylogenetic tree of the major groups Platyhelminthes summarized from Fig. 1.

#### Relationships within the Monogenea

Recent analyses of relationships within monogeneans based on partial 18S ribosomal sequences (Baverstock *et al.*, 1991; Blair, 1993; Rohde *et al.*, 1993) have led to the suggestion that the group might be paraphyletic. Our trees show that monogeneans are monophyletic and furthermore, that the traditionally recognized subgroups Monopisthocotylea and Polyopisthocotylea are also monophyletic (Fig. 1).

#### Relationships within the Trematoda

Trematoda is the flatworm group that has received the greatest attention in recent molecular systematic studies (Ali *et al.*, 1991; Rohde *et al.*, 1993; Blair, 1993; Blair and Barker, 1993; Barker *et al.*, 1993; Johnston *et al.*, 1993; Lumb *et al.*, 1993). Analysis of a small number of 18S sequences of Platyhelminthes (Blair, 1993) could not resolve whether Aspidobothrii (=Aspidobothrea, Aspidobothria) is the sister group of Digenaea alone or of the rest of Neodermata. The global analyses in the present study, carried out on a much greater number of sequences, show that Aspidobothrii is the sister group of the digeneans alone (Fig. 1). This is in agreement with most proposals based on morphological and life history characters (Rhode, 1990; Ehlers, 1985; Ax, 1987; Gibson, 1987; Brooks, 1989b; Brooks and McLennan, 1993).

The phylogenetic relationships among Digenea are still controversial. Among digenean families included in our study, at least three have been proposed as the sister group of all others: Fellodistomidae (Køie, 1985), Paramphistomidae (Gibson, 1987), and Heronimidae (Brooks *et al.*, 1985). Also, *Schistosoma mansoni* was

proposed to represent the sister group to seven families of digeneans including Paramphistomidae and Heronimidae (Barker *et al.*, 1993). Furthermore, some families of digeneans have been placed as *incerta sedis*: Paramphistomidae and Fellodistomidae (Brooks *et al.*, 1985) and Gyliachenidae (Schell, 1982).

Our results suggest that Heronimidae is the sister family to the rest of the digeneans (Fig. 4). One large clade in Digenea is formed by 12 species belonging to six families; Schistosomatidae, Diplostomidae, Fellodistomidae, Fasciolidae, Echinostomidae, and Paramphistomidae. This clade is divided in two major branches; one including Schistosomatidae, Diplostomidae, and Fellodistomidae as a monophyletic group as proposed previously (Brooks *et al.*, 1985). The second branch includes Fasciolidae, Echinostomidae, and Paramphistomidae as previously found in a study also based in 18S ribosomal genes (Barker *et al.*, 1993). Relationships within the group of schistosomes (Fig. 1) coincide exactly with those obtained by Johnston *et al.* (1993), who used the same sequence data. As for the taxa *incerta sedis* mentioned above, Paramphistomidae appears in our trees as the sister group of Fasciolidae and Echinostomidae. In turn, Fellodistomidae is the sister group to Schistosomatidae and Diplostomidae. Gyliachenidae is also clearly positioned. The organization of *Lepidapedon* species coincides with some results of Lumb *et al.* (1993), although *Tetracerasta blepta* appears within this clade. However, in our tree Lepocreadiidae is not monophyletic. One species within Lepocreadiidae, *Opechona bacillaris*, appears as sister group to *Opisthorchis viverrini* (Opisthorchiidae), and other Lepocreadiidae appear to have diverged separately between the divergence of Heronimidae and Gyliachenidae (Fig. 4). This might be due, in part, to the use of complete sequence data for *O. viverrini* and the inclusion of a larger number of taxa in our study.

#### Relationships within Cestoda

Several studies have suggested that Gyrocotyle is the sister group of the rest of Cestoda and that Amphilini-

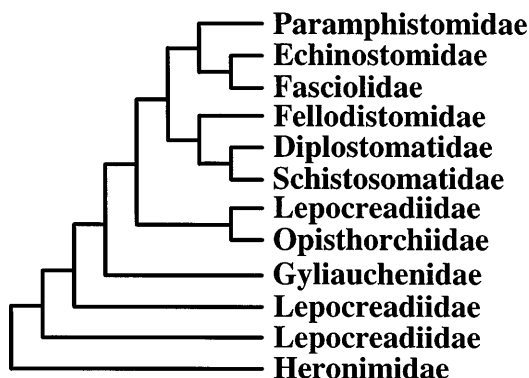


FIG. 4. Phylogenetic tree of familial relationships within Digenea.

dea is sister group to Eucestoda. However, our global tree (Fig. 1) shows that Eucestoda is not monophyletic: *Acanthobothrium heterodonti* appears as the sister group to the rest of Cestoda. *Spirometra erinacei* forms a group with *Taenia*, and *Gyrocotyle rugosa* (Gyrocotylidae) is placed with *Echinococcus granulosus*. Several different topologies within Cestoda were compared using the test of Kishino and Hasegawa (1989), but there were no significant differences among the alternative topologies examined. We conclude that the present data are insufficient to clearly resolve the relationships of these taxa. A more comprehensive study of the phylogenetic relationships within Cestoda is needed, with both a larger number of species and more DNA sequence data.

#### The Origin of Parasitism within Platyhelminthes

A number of hypotheses have been proposed to explain the origin of the major parasitic groups within Platyhelminthes. For example, Hyman (1951) proposed that trematodes (including monogeneans) and cestodes originated independently from parasitic "turbellarian" rhabdocoelan ancestors ("Dalyellioida" and "Typhloplanoida" sensu Ehlers). Stunkard (1983) stated that digenetic trematodes and cestodes originated from free-living turbellarian-like ancestors. Bychowsky (1937, 1957) and Llewellyn (1965, 1986) claimed a monogenean ancestry for cestodes. In this scheme monogeneans originated from a free-living "turbellarian" rhabdocoelan ancestor and had separate origins from digeneans. Another hypothesis is that the ancestor of trematodes and the common ancestor to cestodes and monogeneans both evolved from free-living "turbellarian" rhabdocoel-like ancestors, but at different times (Freeman, 1973). Similarly, Cannon (1986) suggested that monogeneans, digeneans, and cestodes arose from different families of "Dalyellioida" and had separate origins. Finally, Janicki (1921, cited by Hyman, 1951) argued that both digenetic trematodes and cestodes originated from a monogenean ancestor.

From these previous hypotheses, it can be inferred that parasitism may have originated one, two, or three times in the evolution of Neodermata and one or more times in the evolution of "Dalyellioida." Our analysis of sequence data suggests that Neodermata is monophyletic and supports the idea that parasitism originated once within the ancestor of this major parasitic group and separately from the origin of the ancestor to Fecampiidae (Fig. 1). It remains to be established if several "Dalyellioida" groups are truly parasitic (Rohde, 1994) and thus could represent other independent origins of parasitism.

In 1982 Mackiewicz stated, "Evolutionary biology of helminths has come of age." The large volume of literature in the last decade certifies the accuracy of his assertion. Part of this literature includes molecular systematic studies, which were carried out to analyze

relationships within small groups or within large groups but using a small number of species (reviewed in Blair *et al.*, 1996). The present paper represents an attempt at a comprehensive analysis of the phylogenetic relationships of flatworms using available sequence data. However, the number of species included is still low for such a diverse phylum, with some groups not represented or only poorly represented. In addition, many sequences are relatively short, consisting of only partial gene sequences, and it has been demonstrated that more sequence data are required to clearly resolve phylogenetic relationships (Cummings *et al.*, 1995). Extension of these molecular data, both in the number of taxa and the amount of sequence, should allow molecular systematics to continue to make a significant contribution to elucidate the evolutionary history of these bizarre and fascinating organisms.

#### APPENDIX<sup>4</sup>

##### Platyhelminthes

###### Catenulida

###### Catenulidae

*Suomina* sp. L41129 (Rohde *et al.*, 1993)

###### Stenostomatidae

*Stenostomum* sp. L41136 (Rohde *et al.*, 1993)

###### Acoela

Fam. gen. sp. L41123 (Rohde *et al.*, 1993)

###### Convolutidae

*Amphiscolops* sp. D17557 (Katayama *et al.*, 1993)

*Convoluta naikaiensis* D17558 (Katayama *et al.*, 1993)

###### Polycladida

###### Leptoplanidae

*Notoplana australis* (Baverstock *et al.*, 1991)

*N. koreana* D17561 (Katayama *et al.*, 1993)

*Stylochoplanea pusilla* D17565 (Katayama *et al.*, 1993)

###### Planoceridae

*Planocera multitentaculata* D17562 (Katayama *et al.*, 1993)

###### Callioplanidae

*Pseudostylochus obsuculus* D17563 (Katayama *et al.*, 1993)

###### Stylochidae

*Stylochus orientalis* D17564 (Katayama *et al.*, 1993)

###### Pseudoceridae

*Thysanozoon brocchii* D17566 (Katayama *et al.*, 1993)

###### Lecithoepitheliata

###### Prorhynchidae

*Geocentrophora baltica* X65073

*G. incognita* X65074

*G. interstitialis* X65071

*G. porfirievae* X65075

*G. sphyrocephala* X65072

*G. wasiliewi* X65076

*Prorhynchus* sp. L41134 (Rohde *et al.*, 1993)

###### Macrostomidae

*Macrostomum* sp. L41127 (Rohde *et al.*, 1994)

###### Seriata

###### Proseriata

###### Coelogyneporidae

gen. sp. L41132 (Rohde *et al.*, 1993)

###### Otoplanidae

gen. sp. L41128 (Rohde *et al.*, 1993)

###### Tricladida

###### Geoplanidae

*Artioposthia* sp. L41133 (Rohde *et al.*, 1993)

*Geoplana mexicana* U88072 (this paper);

###### Planariidae

*Crenobia alpina* M58345 (Riutort *et al.*, 1992, 1993)

###### Dendrocoelidae

*Dendrocoelopsis lactea* D17559 (Katayama *et al.*, 1993)

*Dendrocoelum lacteum* M58346 (Riutort *et al.*, 1992, 1993)

###### Dugesidae

*Dugesia iberica* M58343 (Riutort *et al.*, 1992, 1993)

*D. japonica* D17560 (Katayama *et al.*, 1993)

*D. mediterranea* M58344 (Riutort *et al.*, 1992, 1993)

*D. tigrina* M20068, M20069, M20070 (Field *et al.*, 1988; Riutort *et al.*, 1992)

###### "Typhloplanoida"

###### Polycystidae

*Gyratrix* sp. L41131 (Rohde *et al.*, 1993)

###### Typhloplanidae

*Bothrosostoma personatum* M58347 (Riutort *et al.*, 1992, 1993)

###### "Dalyellioida"

###### Luridae

*Liriculus australiensis* L41124 (Rohde *et al.*, 1993)

###### Umagillidae

*Pterastericola australis* L41135 (Rohde *et al.*, 1993)

*Syndisyrinx punicea* L41138 (Rohde *et al.*, 1993)

###### Temnocephalidae

*Temnocephala dendyi* L41137 (Baverstock *et al.*, 1991; Rohde *et al.*, 1993)

###### Fecampiida

*Kronborgia isopodicola* L41125 (Rohde *et al.*, 1994)

###### Trematoda

###### Aspidobothrii

###### Aspidogastridae

<sup>4</sup> Highest taxon names within Platyhelminthes based on Ehlers (1986). GenBank accession numbers and citations are given where known.

- Lobatostoma manteri* L16911 (Blair, 1993)
- Digenea
- Paramphistomidae
- Calicophoron calicophorum* L06566
- Echinostomidae
- Echinostoma caproni* L06567 (Blair and Barker, 1993)
- Fasciolidae
- Fasciola hepatica* X56041 (Riutort *et al.*, 1992, 1993)
- Fasciolopsis buski* L06668 (Blair and Barker, 1993)
- Fellodistomidae
- Fellodistomum fellis* Z12601 (Lumb *et al.*, 1993)
- Steringophorus agnotus* Z12598 Z12599 (Lumb *et al.*, 1993)
- S. furciger* Z25818
- Gyliauchenidae
- Gyliauchen* sp. L06669 (Blair and Barker, 1993)
- Heronimidae
- Heronimus mollis* L14486 (Barker *et al.*, 1993)
- Lepocreadiidae
- Lepidapedon elongatum* Z12600 (Lumb *et al.*, 1993)
- L. gaevskayae* Z12605 Z12606 (Lumb *et al.*, 1993)
- L. rachion* Z12607 (Lumb *et al.*, 1993)
- Opechona bacillaris* Z12596 Z12597 (Lumb *et al.*, 1993)
- Tetracerasta blepta* L06670 (Blair and Barker, 1993)
- Opisthorchiidae
- Opisthorchis viverrini* X55357 (Korbsrisate *et al.*, 1991)
- Diplostomatidae
- Posthodiplostomum minimum* U88074 (this paper)
- Schistosomatidae
- Schistosoma haematobium* Z11976 (Johnston *et al.*, 1993)
- S. japonicum* Z11590 (Johnston *et al.*, 1993)
- S. mansoni* X53047 S61067 (Ali *et al.*, 1991)
- S. spindale* Z11979 (Johnston *et al.*, 1993)
- Cercomeromorpha
- Monogenea
- Gyrodactylodea
- Gyrodactylodae
- Gyrodactylus salaris* Z26942
- Monopisthocotylea
- Anoplodiscidae
- Anoplodiscus cirrusspiralis* L41130 (Rohde *et al.*, 1993)
- Monocotylidae
- Dictyocotyle coeliaca* (Baverstock *et al.*, 1991)
- Polyopisthocotylidae
- Diclidophoridae
- Diclidophora merlangi* (Baverstock *et al.*, 1991)
- Discocotylidae
- Octomacrum mexicanum* U88073 (this paper);
- Cestoda
- Gyrocotylidae
- Gyrocotylidae
- Gyrocotyle rugosa* (Baverstock *et al.*, 1991)
- Amphilinidea
- Austramphilinidae
- Austramphilina elongata* (Baverstock *et al.*, 1991)
- Eucestoda
- Tetraphyllidae
- Oncobothriidae
- Acanthobothrium heterodonti* (Baverstock *et al.*, 1991)
- Cyclophyllidae
- Taeniidae
- Echinococcus granulosus* U88071 (this paper)
- Taenia crassiceps* U88075 (this paper)
- T. solium* U88076 (this paper);
- Pseudophyllidae
- Diphyllobothriidae
- Spirometra erinacei* (Baverstock *et al.*, 1991)
- Outgroup taxa
- Acanthocephala
- Moliniformis moliniformis* Z19562
- Chaetognatha
- Sagitta elegans* Z19551
- Cnidaria
- Anemonia sulcata* X53498
- Anthopleura kurogane* Z21671
- Ctenophora
- Beroe cucumis* D15068
- Mnemiopsis leidyi* L10826
- Mollusca
- Acanthopleura japonica* X70210
- Mactromeris polynyma* L11230
- Nematoda
- Caenorhabditis elegans* X03680
- Haemonchus placei* L04154
- Haemonchus similis* L04152
- Nemertea
- Cerebratulus lacteus* M90051/M81167, M90052/M81167, M90053/M81167
- Prostoma* sp. (Rohde *et al.*, 1993)
- Porifera
- Sycon calcaravis* D15066
- Tetilla japonica* D15067

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