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Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences[1]

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Abstract

Platyhelminth phylogeny is controversial. Phylogenetic analyses of the partial domain C1 and the full domains D1 and C2 (358 nucleotides) from the 28S ribosomal RNA gene for 21 species from the Monogenea, Digenea, Cestoda, and, as the outgroup, Tricladida reveal major departures from prevailing theory. The Digenea and not the Monogenea (Monopisthocotylea and Polyopisthocotylea) form the sister group of the cestodes; the Monopisthocotylea and Polyopisthocotylea are each monophyletic, but the Monogenea do not form a monophylum; the sister group of the Digenea + Cestoda is the Polyopisthocotylea; and Monopisthocotylea are the sister group of all other parasitic flatworms. © 1997 Elsevier Science B.V.

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1. Introduction

The Platyhelminthes (flatworms) are presently the center of a discussion on the origin of the Metazoa [1,2]. Among the Platyhelminthes, the parasitic flatworms (flukes and tapeworms), which

Abbreviations: PCR, polymerase chain reaction.

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are a major cause of disease in animals including humans, constitute a monophyletic group [2–7]. Monogenea (monogenetic flukes), which are the chief subject of this report, occupy a key position in hypotheses of phylogenetic relationships of parasitic Platyhelminthes. Since its appearance in 1985, the phylogenetic system of flatworm relationships of Ehlers [4,5] and of Brooks et al. [6,7] has been widely accepted [3]. More recently, the phylogenetic relationships of the major groups of the Platyhelminthes have been investigated using molecular methods, analysing 18S rDNA, albeit from a limited number of species [8–11]. Molecular phylogeny has also been inferred within the Digenea [12] and the Cestoda [13,14] using 18S rDNA and, within the Digenea [15,16], using 28S rDNA.

Disagreement still remains as to the monophyly of the Monogenea. In the systems based on non-molecular phylogenetic systematics [4,5,7,17], the Monogenea, composed of two groups (Monopisthocotylea and Polyopisthocotylea), are considered to be a clade (monophylum). This clade is based on a few synapomorphies concerning the eyes and the number of ciliated bands in the larvae [3]. However, in the analyses using 18S rDNA sequences [8–11] there was no support for monophyly of the Monogenea. A cladistic analysis of sperm ultrastructure [18,19] also failed to demonstrate any synapomorphies for the Monogenea, but monophyly of the Monopisthocotylea and the Polyopisthocotylea, respectively, was supported by distinctive synapomorphies for each group.

The 28S rDNA gene was used because of its variety of domains of variable evolutionary rate which contain a strong potential for reconstructing phylogenies [20–22]. We analyzed our data with neighbour-joining, parsimony and maximum likelihood methods in order to test the hypothesis of monophyly of the Monogenea, monophyly of the Monopisthocotylea and, separately, of the Polyopisthocotylea and to infer the phylogenetic relationships of the Monogenea with Digenea and Eucestoda. Two Tricladida were used as the outgroup. The three methods of analysis give a phylogeny which is fundamentally different from the prevailing system based on morphology [4–7]. Most notably, it will be shown that the Monogenea no longer appear monophyletic and are displaced from a sister group relationship to the cestodes.

2. Material and methods

The following species were included in this analysis: the digenean *Echinostoma caproni* from laboratory mice (France), the cestodes *Proteocephalus neglectus* from *Coregonus* sp. and *Caryophyllaeus* sp. from *Rutilus rutilus* (Switzerland), the triclads *Polycelis* sp. (France) and *Bipalium kewense* (Australia), and monogeneans collected from fishes (Heron Island, Queensland, Australia), *Zeuxaptera seriolae* from *Seriola lalandi*, *Gotocotyla secunda* from *Scomberomorus commerson*, *Pricea multae* from *Scomberomorus commerson* (polyopisthocotyleans); *Acleotrema* sp. from *Xyphosus vaigienis*, *Haliotrema chrysotaeniae* from *Lutjanus carponotatus*, *Tetrancistrum* sp. from *Siganus fuscescens*, *Merizocotyle icopae* from *Rhinobatos typus*, *Encotyllabe caballeroi* from *Scolopsis monogramma*, *Troglocephalus rhinobatidis* from *Rhinobatos typus*, *Entobdella australis* from *Taeniura lymna*, *Neoheterocotyle rhinobatidis* from *Rhinobatos typus*, *Benedenia lutjani* from *Lutjanus carponotatus* (monopisthocotyleans). Sequences from GenBank™ were added, for the digeneans *Heterobilharzia americana* (Z46506), *Lepidapedon sommervillae* (Z29502) and *Schistosoma mansoni* (X13836) and the cestode *Hymenolepis diminuta* (K03537, K03538).

The partial domains C1 and D2 and the full domains D1 and C2 of the 28S ribosomal RNA gene were sequenced. Genomic DNA was extracted [23]. A portion of the 28S rDNA gene was amplified by PCR (polymerase chain reaction), following the Gibco BRL (Australia) protocol with primer C1: ACCCGCTGAATTTAAGCAT (5′-3′ position 25) and primer D2: TGG TCC GTG TTT CAA GAC (5′-3′ position 1128) [24]. The amplified portion varied in length from 660 to 907 bp and contained the partial domains C1, D2 and the full domains D1 and C2. All PCR products were gel purified on 1.5% agarose. The sequencing reaction for the automated DNA sequencing was carried out using the ABI PRISM reagents from Perkin Elmer with both primers C1 and D2 and following

their protocol. The DNA was then precipitated using the ethanol precipitation method. The primers sequenced well enough for up to 500 bp of overlap to be confirmed on both strands, any remaining bases at either end have been read from one strand only.

The 21 nucleotide sequences used in this analysis have been aligned using both CLUSTAL W [25] and visual inspection using the ED program of the MUST package [26]. A saturation study of the molecule has been performed. Saturation plots were obtained using PAUP 3.1.1 (computer program distributed by D.L. Swofford and IL Natural History Survey, Champaign, Illinois, USA) and the NET, AF_PAUP3 and COMP_MAT programs of the MUST package. Relative saturation was evaluated by plotting the pairwise number of transversions against the pairwise number of transitions. The absolute saturation of both transitions and transversions has been evaluated by plotting the pairwise number of observed differences against the pairwise number of inferred substitutions obtained in a most parsimonious tree resulting from an heuristic search from PAUP 3.1.1. Trees were constructed using the Neighbour-Joining method [27] as implemented by the MUST package and the parsimony method implemented by PAUP 3.1.1 via the heuristic search command. For both methods, trees were constructed successively according weight of 1, 0.5 and 0 to transitions. For each tree construction method and each weighting scheme, bootstrapping was performed with 1000 iterations as implemented respectively by MUST with the NJboot command and PAUP 3.1.1. The maximum likelihood approach [28] was performed using the FastDNAml software [29]. The options, empirical frequencies and jumble were used in the analysis.

3. Results

The nucleotide sequences used in our analysis comprised 459 sites from the domains C1, D1 and C2, including alignment gaps. The 101 sites leading to ambiguous alignment were removed from the analysis. The domain D2 was found to be too variable to be aligned. Of the 358 remaining sites, 201 were variable and, of these, 156 were informative for phylogenetic analysis. Tests for saturation of nucleotide substitutions were performed. Transitions were not completely saturated and transversions were less saturated than transitions.

PAUP yielded a single most-parsimonious tree by heuristic search and equally weighting transitions and transversions, using the two triclads as the outgroup. This tree is shown as a phylogram in Fig. 1.

Other analyses were performed giving weights of 0 and 0.5 to transitions against transversions. When the transitions were down-weighted to 0.5, PAUP yielded two most-parsimonious trees. The strict consensus tree (736 steps, consistency index 0.556, rescaled consistency index 0.338, retention index 0.607) had the same topology as the unweighted tree except for the relative position of Digenea, Eucestoda and Polyopisthocotylea which form a polytomy and the position of the monopisthocotylean *Merizocotyle icopae* which was grouped with *Neoheterocotyle rhinobatidis* and *Troglocephalus rhinobatidis*. When transitions were excluded, PAUP again yielded two most-parsimonious trees. In the strict consensus tree (742 steps, consistency index 0.551, rescaled consistency index 0.331, retention index 0.600), the Polyopisthocotylea were the sister group of the group consisting of the Monopisthocotylea and the two sister groups Digenea and Eucestoda. In the Monopisthocotylea, the position of the group *Tetrancistrum* sp. and *Haliotrema chrysotaeniae* was unresolved.

The Monopisthocotylea (Fig. 1) constitute a monophyletic group (bootstrap value of 87). This group appears as the sister group of the combined Polyopisthocotylea, Eucestoda and Digenea (bootstrap value of 100). Monophyly of the Polyopisthocotylea is supported by a strong bootstrap value of 96. The Eucestoda appear as the sister group of the Digenea, though with a low bootstrap value (45). These two groups appear as the sister group of the Polyopisthocotylea (bootstrap value of 74). The paraphyly of the monogeneans is therefore supported by a bootstrap value of 74. Boostrap values above 70 are considered robust [30,31].

The neighbour-joining method led to one tree the topology of which differs from the most-parsimonious PAUP tree only in the position of the monopisthocotylean *Merizocotyle icopae* (Fig. 1).

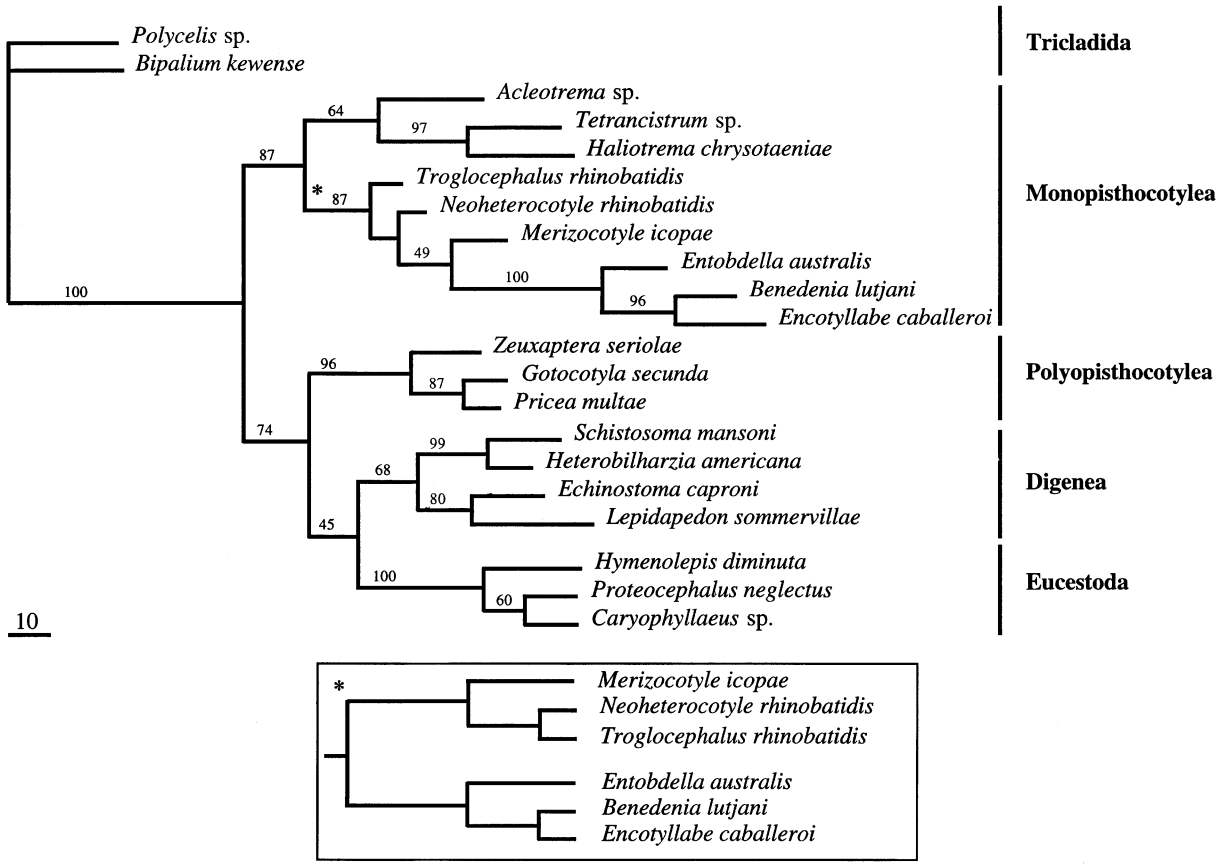

Fig. 1. The single most-parsimonious tree obtained for 21 species of Platyhelminthes by heuristic searching with equal weighting of transitions and transversions (729 steps, consistency index 0.556, rescaled consistency index 0.339, retention index 0.610). Bootstrap values are indicated on the branches. The asterisk node indicates the only difference in topology of this tree from the neighbour-joining and maximum likelihood trees. The inset shows the relevant node in the latter two trees.

This latter is grouped with *Neoheterocotyle rhinobatidis* and *Troglocephalus rhinobatidis*. The same topology was found with the neighbour-joining method for the three weightings of transitions (0, 0.5 and 1), with high bootstrap values.

The maximum likelihood analysis produced a tree (Ln likelihood = −4003.67148) with exactly the same topology as the neighbour-joining tree.

With both neighbour-joining and parsimony methods, although there was substantial saturation of the transitions, the bootstrap values were high. The homoplasy observed comes mainly from the transitions. Nevertheless, it is evident that the transitions also contain information. When we deleted the transitions or gave a smaller weight to them, some information was lost as indicated by the fact that the resulting analysis led to unresolved nodes.

Thus, all three methods used (neighbour-joining, parsimony and maximum likelihood) gave the same topology with a small difference at one node in the parsimony analysis. With regards to the paraphyly of the monogeneans, we consider the congruence of the three methods as an indication of the strength of the present analysis.

4. Discussion

In all analyses, the two groups of Monogenea, the Monopisthocotylea and the Polyopisthocotylea never grouped together. Thus, the Monogenea never appeared as a clade. However, the nine

Monopisthocotylea formed a monophylum as, separately, did the three Polyopisthocotylea. This confirmed the results of the molecular phylogenies for 18S RNA [8–11]. However, in each of those studies the number of monogenean taxa was much lower than in the present analysis. The non-monophyly of the Monogenea was also suggested in a cladistic analysis of sperm structure [18,19]. These results are in opposition to previous phylogenetic schemes [4,5,7,17,32,33] which showed the Monogenea as monophyletic. Monophyly of the Monopisthocotylea and of the Polyopisthocotylea is in accordance with phylogenies based on morphology [17] and sperm structure [18,19].

The present analysis was also of value in testing and confirming the monophyly of constituent families within the Monopisthocotylea. With regard to the relative positions of families in the phylogeny, the diplectanid *Acleotrema* sp. appears as the sister group of the dactylogyrids, *Tetrancistrum* sp. and *Haliotrema chrysotaeniae*. If we exclude the pseudomurraytrematids from Boeger and Kritsky's analysis [17] this result is in accordance with their phylogenetic scheme. In our analysis, the Capsalidae appear as a monophylum, whereas the Monocotylidae appear paraphyletic since *Merizocotyle icopae* appears as the sister group of the Capsalidae. The non-monophyly of the Monocotylidae could explain the disparate sperm structures found in this family [34].

This study indicates that the Digenea and Eucestoda are monophyletic sister-groups and that the Polyopisthocotylea form the sister group of this group (Fig. 2). As the Digenea have been shown to be the sister-group of the Aspidogastrea [9,10], the two groups comprising the Trematoda, a trematode–eucestode relationship is suggested. Our analysis (Fig. 2), is in opposition to the view that the Trematoda are the sister group of the Monogenea + Cestodaria (Fig. 3) [4–7]. However, bootstrap values supporting the grouping of Digenea and Eucestoda as monophyletic sister-groups are weak and addition of more taxa and ideally of longer sequences, is therefore required in order to test the trematode–eucestode relationships. What emerges as strongly supported in the present analysis is that the Monogenea do not form a monophylum, the Monopisthocotylea and Polyopisthocotylea are each and separately, monophyletic and that neither forms the sister group of the Eucestoda.

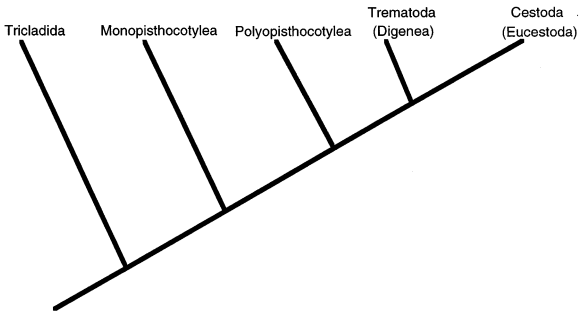

Fig. 2. Relationships of the major Platyhelminthes taxa inferred from the present analysis of 28S rDNA. The Monopisthocotylea and the Polyopisthocotylea are each monophyletic, but do not group as a monophylum Monogenea. The Monopisthocotylea are the sister-group for all other parasitic Platyhelminthes.

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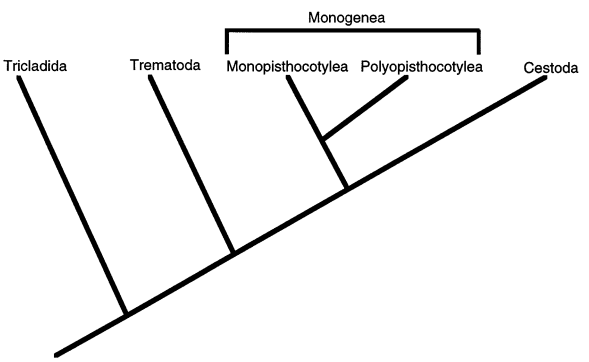

Fig. 3. Relationships of the major Platyhelminthes taxa as proposed from non-molecular data [5,6]. Contrary to the present analysis, the Monogenea were considered a monophyletic group and the sister-group of the Cestoda.

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