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Phylogeny of the Platyhelminthes and the evolution of parasitism

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Robust phylogenies provide the basis for interpreting biological variation in the light of evolution. Homologous features provide phylogenetically informative characters whereas homoplasious characters provide phylogenetic noise. Both provide evolutionary signal. We have constructed molecular and morphologically based phylogenies of the phylum Platyhelminthes using a recently revised morphological character matrix and complete 18S and two partial 28S rRNA gene sequences in order to evaluate the emergence and subsequent divergence of parasitic forms. In total we examine 65 morphological characters, 97 18S rDNA, 41 D1 domain 28S rDNA, and 49 D3–D6 domain 28S rDNA sequences. For the molecular data there were 748, 132 and 249 phylogenetically informative sites for the 18S, D1 and D3–D6 28S rDNA data sets respectively. Morphological and molecular phylogenetic solutions are incongruent but not incompatible, and using the principles of conditional combination (18S rDNA + morphology passing Templeton's test) they demonstrate: a single and relatively early origin for the parasitic Neodermata (including the cestodes, trematodes and monogeneans); sister-group status between the cestodes and monogeneans, and between these taxa and the trematodes (digeneans and aspidogastreans). The sister-group to the Neodermata is likely to be a large clade of neoophoran turbellarians, based on combined evidence, or a clade consisting of the Fecampiid + Urastomid turbellarians, based on morphological evidence alone. The combined evidence solution for the phylogeny of flatworms based on 18S rDNA and morphology is used to interpret morphological and life-history data and to support a model for the evolution and radiation of neodermatan parasites in the group.

ADDITIONAL KEYWORDS:—total evidence – 18S rDNA – 28S rDNA – phylogenetics – cladistics.

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INTRODUCTION

It is fashionable for studies involving comparative data to point out that systematics is the basis of biology, and where possible a phylogeny based on shared derived characteristics is the starting point when interpreting comparative data in an evolutionary framework (e.g. see Harvey & Pagel, 1991 and contributions in Martins, 1996). We concur with this stance and have taken such a phylogenetic approach in tracking the evolution of parasitism in the phylum Platyhelminthes. Flatworms include common helminth parasites such as tapeworms and flukes that have attracted a great deal of attention from biologists who quite often have had little reason to dwell on systematic issues. Nevertheless, with the wealth of comparative data available it is not surprising that workers are continually drawn to explain the origins and subsequent radiations of these highly successful parasitic taxa. Early studies tended to take a phenetic approach with the consequence that a wide variety of evolutionary scenarios were put forward or popularized, none of which could be tested in any meaningful or objective way. Although conjectural, these ideas remain widespread in the literature today (e.g. see examples in Kearn, 1998). Phylogenetic systematics provides a testable, if not fully objective, methodology. However, although early cladistic studies yielded strictly bifurcating phylogenies (e.g. Ax, 1984; Ehlers, 1985), the relatively few and often contentious morphologically based character sets still left us with a number of alternatives (e.g. Rohde, 1990) and no single robust solution. Elegant and persuasive analyses on the nature of parasitism in the group (e.g. Brooks, Thorson & Mayes, 1981) have too often been undermined by apparent flaws in the basic phylogeny (cf. Lovejoy, 1997). Phylogenetics aims to resolve interrelationships between taxa and ultimately, as data sets are published, tested, compared and argued over, the interpretations that they underpin may gain credence.

Here we present another 'state of the art' phylogeny of the Platyhelminthes drawing on a recent 'total evidence' approach (Littlewood, Rohde & Clough, 1999) and whilst there may still be flaws, lack of robustness and the need for consensus of opinion, we have gathered all available molecular data, generated new molecular data and have included a morphological data set with which collectively we can review some of the favoured hypotheses concerning the origins and evolution of parasitism in the group. Regardless of the topology, a phylogeny must make biological sense and here we investigate the consequences of accepting a combined morphological and molecular solution that is at least testable if not definitive.

Background and aims

We believe it is now well accepted that the Neodermata, comprising the obligate parasitic platyhelminth groups, the Monogenea (Monopisthocotylea and Polyopisthocotylea), Trematoda (Aspidogastrea and Digenea), and Cestoda (Amphilinidea, Gyrocotylidea and Eucestoda), is a monophyletic group, as first proposed by Ehlers (1984, 1985). This has been doubted in the past but both morphological and molecular evidence is overwhelming (by Brooks, O'Grady & Glen, 1985; Rohde, 1990 using mainly morphological data, by Baverstock *et al.*, 1991, Blair, 1993 and Rohde *et al.*, 1993 using partial 18SrDNA sequences, by Rohde *et al.*, 1995 using combined ultrastructural and DNA evidence). We have two basic aims in this study. The first is phylogenetic, where we wish to review and test the evidence for the sister-group relationship of the Neodermata and to resolve the interrelationships of the major neodermatan taxa using the available morphological and molecular data. Based on these analyses our second aim is to review the likely origins and radiation of parasitism, particularly with reference to the Neodermata.

We have brought together morphological data collated and scored by one of us (KR) and published in Littlewood, Rohde & Clough (1999), with three molecular data sets from ribosomal RNA/DNA. Two sections of the 28S rRNA gene provided separate data sets; the first is from the D1 variable domain, used largely by Mollaret *et al.* (1997) and the second is from the D3 to D6 domains (D3D6) used primarily by Litvaitis & Rohde (1999). Complete 18S rDNA sequences comprised the largest set and builds on a variety of earlier studies (e.g. Katayama, Nishioka & Yamamoto, 1996; Carranza, Riutort & Baguñà, 1997; Littlewood *et al.*, 1999). The molecular data sets draw heavily upon previously published material although we do add new sequence data to increase taxon sampling, particularly with a view to establishing a more acceptable position for the Nemertodermatida, which previously appeared amongst the Proseriata, and to place the Amphilinidea in the 18S rDNA analysis. The molecular data sets are all nuclear ribosomal genes or gene fragments from the small and large subunit coding regions. Only the D3D6 28S rDNA and 18S rDNA data sets have been used previously for a phylum-wide phylogenetic treatment. They have yielded markedly different results (Littlewood *et al.*, 1999; Litvaitis & Rohde, 1999). Only the D1 28S rDNA data set has not been used for a phylum-wide analysis and it reflects the lowest diversity of sampling across the various flatworm groups. The densest sampling of the D1 and D3D6 data sets is from the Monogenea and the Proseriata, respectively, which reflects the interests of previous molecular phylogenetic studies on these groups (Mollaret *et al.*, 1997; Litvaitis *et al.*, 1996).

Collectively, the data sets represent an enormous amount of information with which, when modern systematic techniques, algorithms, opinions and philosophies change daily, will keep us busy for a long time (e.g. see Cunningham, Omland & Oakley, 1998). However, we would prefer this study to be viewed as an initial foray into the evolutionary parasitology of flatworms from a combined morphological and molecular approach. At least, it takes a fresh look at the consequences of accepting one or other phylogeny that has been promoted in the literature and that is supported by the current phylogenetic evidence, and at best it hopes to provide a convincing framework with which to guide further studies. Clearly, the value of these interpretations is dependent on the veracity of the data and the analyses. We have

analysed each data set independently and have chosen conditional combination (Huelsenbeck, Bull & Cunningham, 1996; Cunningham, 1997) as the basis for a subsequent 'total evidence' approach.

MATERIAL AND METHODS

We used the morphological data from Littlewood *et al.* (1999), 89 published 18S rDNA/rRNA, 31 published D1 28S rDNA/rRNA, and 49 published D3D6 28S rDNA/rRNA nucleotide sequences available from EMBL/GenBank.

New molecular data

In addition to the published sequences we have added six D1 28S rDNA and seven complete 18S rDNA sequences to the data sets, of which one was kindly supplied by Ulf Jondelius; 18S rDNA sequence of the nemertodermatid *Meara stichopi* is unpublished and may be available from the author (e-mail: ulfj@nrm.se). The full list of published and new sequences is shown in the Appendix and is organized with reference to current taxonomy.

DNA extraction, gene amplification and sequencing

We used previously published techniques for extracting DNA, PCR amplifying and direct sequencing D1 28S and complete 18S rDNA molecules (Littlewood, Rohde & Clough, 1997, 1999). Fragments of D1 28S rDNA were larger than those needed to complement existing nucleotide sequences and in fact incorporate some of the D2 variable domain (homologous to sections used in Littlewood & Johnston, 1995). Full sequences are deposited in EMBL/GenBank with accession numbers shown in the Appendix.

Sequence alignment

The 18S rDNA alignment reported in Littlewood *et al.* (1999) was used as the starting point (profile alignment in ClustalW; Thompson, Higgins & Gibson, 1994) for adding new 18S rRNA gene sequences. The D1 and D3D6 28S rDNA sequence sets were each aligned *de novo* using ClustalW with default settings. Alignments were checked finally by eye and, following the previously reported protocol, regions of ambiguity were removed prior to analysis. The full sequence alignment used in these analyses has been deposited with EMBL under accession DS39182 and is available via anonymous FTP from FTP.EBI.AC.UK under directory pub/databases/embl/align.

Phylogenetic analysis and conditional combination

All analyses were conducted using PAUP* (PAUP 4.0.0d64; Swofford, 1999). For maximum parsimony analysis, only consistency indices (CI) excluding uninformative characters are presented. Wherever possible data sets were bootstrap resampled ($n=$ 1000) using the full heuristic search or the fast step-wise addition options, depending on the size of the data sets and the time required for each replicate. Molecular data sets were too large for branch-and-bound searches and for these only heuristic

searches were employed. Trees were rooted using selected outgroups and character states were optimized using the ACCTRAN option; i.e. reversals were preferred over parallelisms.

Each molecular data set was also analysed using a minimum evolution (ME) model, starting with a Logdet neighbour-joining tree (NJ_{logdet}) and subsequently optimizing the transition:transversion ratio, the gamma statistic and the proportion of invariable sites through repeated, iterative estimates until the estimates for each parameter variable (and the log likelihood) did not change. A final maximum-likelihood model using these statistics was then employed to estimate the molecular phylogeny under the neighbour-joining method; see Lake (1991) and Lockhart *et al.* (1994) for further details. Branch support was estimated with bootstrap resampling ($n = 1000$).

Data sets were analysed independently first. A full matrix was then assembled consisting of 160 taxa and 2309 characters with which we could test the compatibility of the individual data sets using Templeton's test (Larson, 1994) in order to determine which data sets could be added for a 'total evidence', or more realistically 'combined, compatible evidence' solution. Only taxa common between pairs of data sets were used when testing compatibility. For each pair of data sets to be tested, phylogenetic solutions were found initially with the individual reduced number of taxa using maximum parsimony (MP). The tree solutions from each analysis were saved and then the data sets from which they were each determined were mapped onto the two phylogenetic solutions. Templeton's (Wilcoxon's signed rank) test was employed to determine whether the data from one set could be mapped onto the topological solution yielded by the other data set to determine whether there was a difference between the two trees. Full reciprocal tests were made between all data sets and where no significant difference between data sets was found, they were combined. In the cases where there were multiple equally most parsimonious solutions for individual data sets, 50% majority-rule consensus trees were used for comparison and testing. In the cases of comparing molecular data sets with morphology there was the problem of no morphological data at lower taxonomic levels such that certain taxa (e.g. Monopisthocotylea) would be represented by a large polytomy in the morphological solution. In such instances polytomies were redrawn as 'ladderized' clades which simply represented one possible branching pattern, allowed the unambiguous mapping of character data and did not affect the tree length. Full details of the Templeton's test and rationale can be found in Cunningham (1997); Incongruence Length Difference and Rodrigo tests (*op cit.*) were considered but not undertaken due to the large data sets requiring excessive computer time. After conditionally combining data sets that were not arguing for statistically different solutions, we chose to run a MP analysis with the full data set; 160 taxa 2309 characters with missing data coded accordingly (N for molecular, ? for morphology) in what was an unconditional combination of data.

RESULTS

The data sets

(*a*) *Morphology*. The full data set formulated by one of us (KR) and published in Littlewood *et al.* (1999) was used, comprising 65 strictly binary characters from 25

ingroup and six outgroup taxa. Under the criteria of parsimony 60 characters were informative. (Note: it is the second version of the matrix used here, after modifications suggested by Kornakova & Joffe (1999); see original article).

(*b*) *18S rDNA.* A total of 109 sequences from 90 ingroup and 19 outgroup taxa yielded 1358 unambiguously aligned nucleotide positions, of which 268 were invariant and 778 parsimony-informative.

(*c*) *D1 28S rDNA.* Forty-one sequences from 37 ingroup and four outgroup taxa yielded 288 unambiguously aligned positions, of which 100 were invariant and 132 parsimony-informative.

(*d*) *D3D6 28S rDNA.* The data set published by Litvaitis & Rohde (1999), which used only one taxon as outgroup (the nemertine worm *Ototyphlonomertes pallida*) was supplemented with three additional outgroups (*Mus musculus*, *Caenorhabditis elegans* and *Drosophila melanogaster*). Those taxa with very short sequences reported by Litvaitis & Rohde (1999; D3 only) were not used and neither was the acoel *Neochildia fusca* as it could not easily be aligned with confidence. The final data set included 49 sequences from 45 ingroup and four outgroup taxa and yielded 596 unambiguously aligned positions, of which 145 were invariant and 249 parsimony-informative.

Phylogenetic analyses

Morphology. Full details of the morphological analyses may be found in Littlewood *et al.* (1999). The strict consensus of the 104 equally most parsimonious trees is shown in Figure 1 with bootstrap and Bremer support (Bremer, 1994) estimated for each branch. The 50% majority-rule consensus solution (not shown) also failed to completely resolve the sister-group to the Neodermata but placed *Urastoma* and the Fecampiida as sister taxa.

Molecules. Treated separately, only the 18S and D3D6 28S rDNA data sets were sampled sufficiently to address the aims of this work as independent data sets. However, the D1 data set was included to test for conditional combination (see below). Only major clades are highlighted as space limitations prevent us from detailing individual taxa on the figures.

D1 28S rDNA. Few turbellarian taxa were sampled for this gene region, and although major taxa appeared as monophyletic groups for the MP solution, the ME solution indicated long-branching taxa and polyphyletic or paraphyletic groups (Fig. 2a,b) and no clear sister-group to or interrelationships within the Neodermata. Interestingly, the amphilinid and gyrocotylid appeared as sister taxa within the Cestoda. The NJ_{logdet} solution (from which the ME solution was derived/optimised; tree not shown) was identical to that provided by MP.

D3D6 28S rDNA. We had hoped to increase the robustness of the analyses of Litvaitis & Rohde (1999) by adding three extra outgroup taxa; a single outgroup taxon was not judged as satisfactory (Smith, 1994). However, although the nucleotide alignment was conserved with unambiguous regions diligently avoided, the phylogenetic solutions with both MP and ME were disappointing. The ingroup was not monophyletic even though no acoels, which have never appeared as members of the Platyhelminthes *sensu stricto* in molecular systematic analyses, were sampled. Consequently we have chosen to highlight those groups appearing only as monophyletic assemblages (Fig. 2c, d) and to allow Templeton's test to detect whether

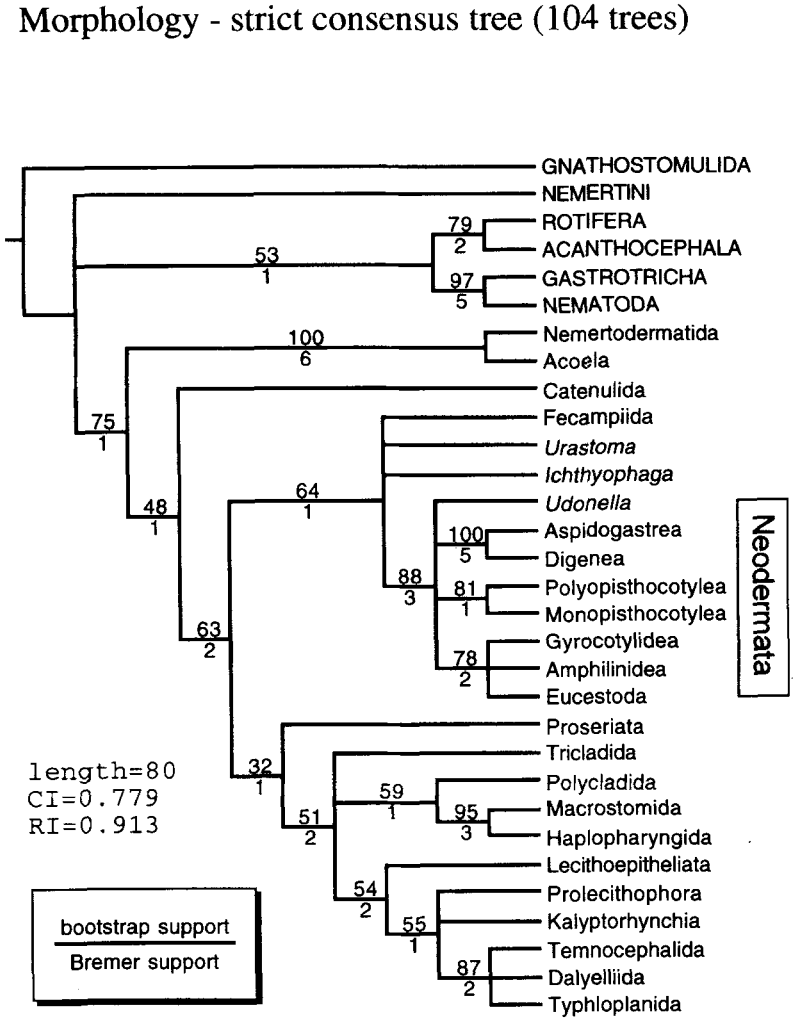
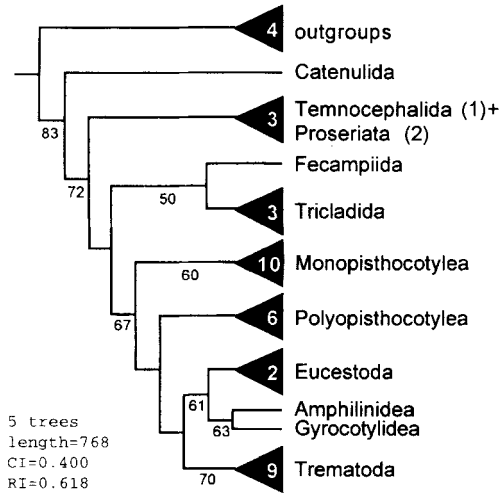


Figure 1. Maximum parsimony solution using the morphological matrix of Rohde in Littlewood *et al.* (1999; Fig. 7 including modifications by Kornakova & Joffe, 1999) with bootstrap ($n=1000$) and Bremer support; see original article for full details.

the phylogenetic signal in the data is congruent with that in the other data sets, so that we may use these data in a combined evidence solution. The utility of the D3D6 28S rDNA for phylum-wide phylogenetic analysis is questionable given the non-monophyly of clades that are considered to be robust entities on the basis of morphological and other molecular evidence, but as our analyses included additional outgroup taxa to the original reference we encourage readers to see the original treatment and discussion of these data (Litvaitis & Rohde, 1999).

18S rDNA. Maximum parsimony yielded 108 equally most parsimonious trees (Fig. 3a). With this solution the interrelationships of the major flatworm taxa do not differ greatly from the data set used in Littlewood *et al.* (1999), although the proseriates are here resolved as a monophyletic group and the amphilinids are

a. D1 domain 28S rDNA - MP



b. D1 domain 28S rDNA - ME

solution not shown

problems:

long branches to outgroups
low bootstrap support throughout

monophyletic	poly/paraphyletic
Cestoda	Tricladida
Trematoda	Proseriata
	Monopisthocotylea
	Polyopisthocotylea

c. D3D6 domain 28S rDNA - MP

solution not shown

32 trees
length=1497
CI=0.408
RI=0.532

problems:

ingroup not monophyletic
outgroup paraphyletic assemblage
including *Udonella, Provortex, Nemertinoides, Suomina* and *Catenula*

monophyletic	poly/paraphyletic
Eucestoda	Polyopisthocotylea
Macrostomida	Monopisthocotylea
Tricladida	Trematoda
Catenulida	Proseriata
	Neodermata

d. D3D6 domain 28S rDNA - ME

solution not shown

problems:

ingroup not monophyletic
outgroup paraphyletic assemblage
including *Udonella*, and *Nemertinoides*

monophyletic	poly/paraphyletic
Eucestoda	Polyopisthocotylea
Macrostomida	Monopisthocotylea
Tricladida	Trematoda
Catenulida	Proseriata
	Neodermata

Figure 2. Molecular solutions from analyses performed in this study for (a,b) D1 28S rDNA and (c,d) D3D6 28S rDNA gene data using maximum parsimony (MP) and minumum evolution (ME). Tree topologies for (b, c and d) are summarized rather than drawn to focus on the phylogenetic signal (monophyletic taxa with high bootstrap support) and highlight the noise (poly/paraphyletic taxa with very low bootstrap support).

represented. The sister-group to the neodermatans was again a large clade of neoophoran turbellarians, although this clade was poorly supported by bootstrap resampling. The minimum evolution solution (Fig. 3b) provided an alternative topology with some important differences. Albeit with poor support for each of the essential differences, this method placed the nemertine worms as sister-group to the catenulids, the polyclads, macrostomids and haplopharyngids as a clade, the two

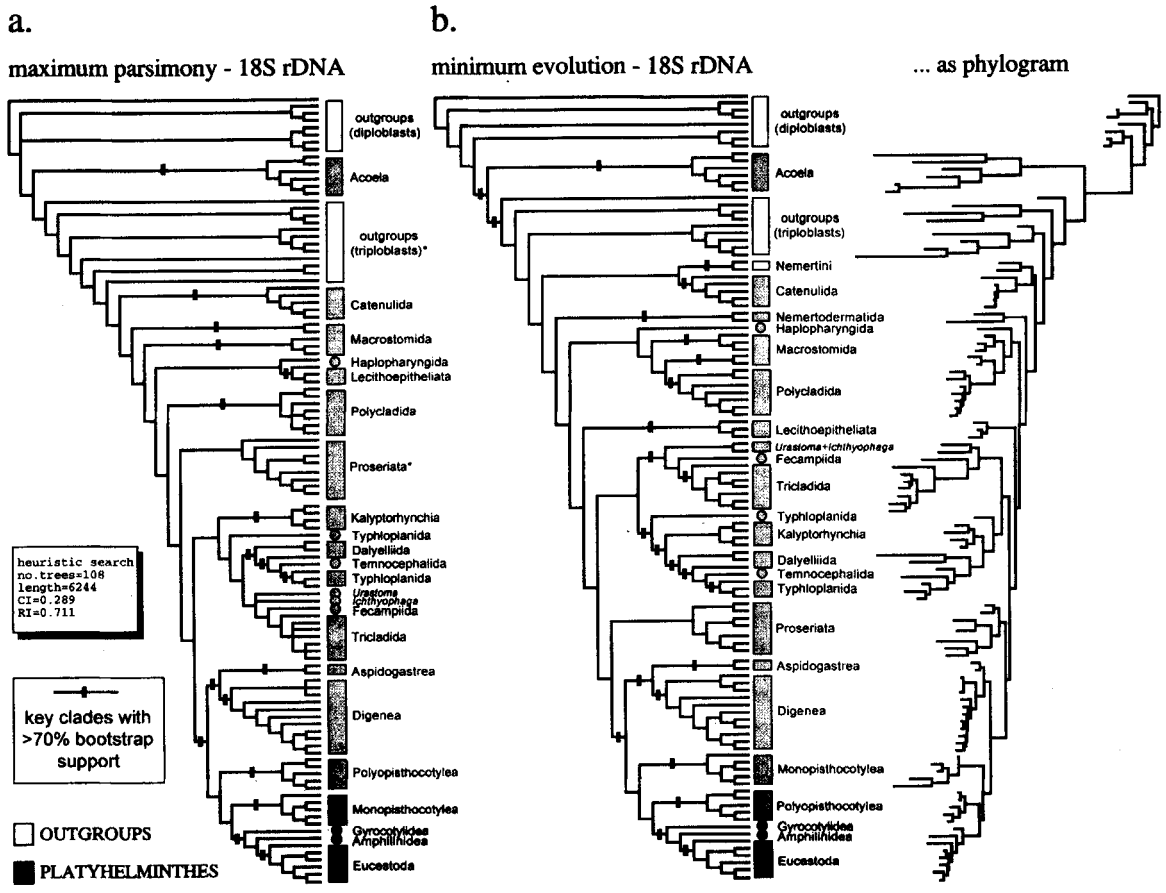


Figure 3. (a) maximum parsimony and (b) minimum evolution solutions for the 18S rDNA data set indicating major flatworm clades with high (>70%) bootstrap support. The phylogram indicates long-branching taxa; * the Nemertodermatida are polyphyletic with MP, *Meara* appearing in the outgroup and *Nemertinoides* amongst the Proseriata. CI = consistency index excluding uninformative sites; RI = retention index.

TABLE 1. Results of Templeton's (Wilcoxon signed-rank) tests testing the combinability of the molecular (d1, d3d6, and 18S) and morphological data sets with one another. Each data set was tested against each most parsimonious tree, or strict consensus of the most parsimonious trees, generated from successive data sets, where only the taxa common to each pair of data sets being compared were used. In the case of comparing morphological data/solutions against 18S rDNA data/the morphological tree was not found empirically as there were too few phylogenetically informative sites in the morphological data set; instead the morphological tree used was based on that found on a reduced data set with major clades represented as polytomies (see text for further details). Number of taxa (n), length of shortest tree (length; found empirically with a heuristic MP search), number of additional steps required to map alternative data set (Δ L) and probability (P) that there is no difference between the 2 trees being compared. P values in italics indicate a significant difference between the trees being compared with a particular data set. Boxed pairs indicate combinability of 18S rDNA and morphology data sets

n length Δ L P	Morphology tree	d1 tree	d3d6 tree	18S tree
	–	42	49	108
Morphology	–	69	71	138
data	–	22	49	1
	–	*0.0525*	*0.0001*	0.5847
	42	–	11	16
d1	973	–	317	400
data	110	–	62	31
	0.0001	–	*0.0001*	*0.0007*
	49	11	–	21
d3d6	1498	497	–	692
data	168	36	–	55
	0.0001	*0.0015*	–	*0.0001*
	108	16	21	–
18S	7354	1028	1388	–
data	343	61	119	–
	0.1604	*0.0001*	*0.0001*	–

nemertodermatids as a monophyletic group basal to the rhabditophoran turbellarians, the proseriates as sister-group to the Neodermata and the positions of the monopisthocotylean and polyopisthocotylean monogeneans was inverted. The amphilinid *Gigantolina*, a species not previously sampled and a taxonomic group not previously fully sequenced, fell consistently as the sister-group to the Eucestoda (i.e. supporting a cestodarian clade: Amphilinidea + Eucestoda). Importantly, the Neodermata, Aspidogastrea, Digenea, Trematoda (Aspidogastrea + Digenea), Polyopisthocotylea, Monopisthocotylea, Cestoda (Gyrocotylidea + Amphilinidea + Eucestoda) and the Eucestoda were all strongly monophyletic but in no case was there a strong candidate for the sister-group to the Neodermata. The NJ_{logdet} solution (tree not shown) gave the same tree topology as maximum parsimony with respect to all major taxa. Amongst the Neodermata the conservative solution from all methods of reconstruction would be (outgroup (Trematoda, (Polyopisthocotylea, Monopisthocotylea, (Cestoda)))).

Data compatibility: towards a working phylogeny

Templeton's test

The results of full reciprocal Templeton's tests between the four data sets are shown in Table 1. The number of taxa common to each pair of data sets compared

is indicated, although the results of the original heuristic searches (MP) are not. Only the 18S rDNA and morphological data sets are compatible with one another, suggesting the data from one set can account for the tree topology resulting from the analysis of the other data set within the limits of statistical significance. Therefore only these data sets can be combined under the principles of conditional combination (Huelsenbeck *et al.*, 1996; Cunningham, 1997; but see Miyamoto & Fitch, 1995 for arguments against combination). Does this mean that the D1 and D3D6 data are arguing for separate phylogenetic solutions? Rather than speculate on this we take the view that the sampling of both these genes is inadequate, particularly when compared with the 18S rDNA data. For the combined analysis (18S rDNA + morphology) we scored each morphological character for the diploblast outgroups as zero as they were simply to root the tree. The morphological matrix did not differentiate taxa within major clades (e.g. amongst polyopisthocotylean monon-ogeneans). Consequently, it was not surprising for large polytomies to appear amongst these major clades and the heuristic searches had to be stopped on what was believed to be its final 'island' (Swofford *et al.*, 1996); all analyses were run on a DEC-alpha UNIX machine for a minimum of 2 days. The majority-rule consensus solution of 2,579 equally most parsimonious trees combining 18S rDNA and morphology is shown in Figure 4. The solution is almost identical to that given by MP analysis of 18S rDNA alone (Fig. 3a), with the sister-group to the Neodermata represented by a large clade of neoophoran turbellarians. Again, only poor bootstrap support was given for this alternative.

Unconditional 'total evidence' solution

When combining all available data from each of the four data sets maximum parsimony was stopped when it had found 4150 equally most parsimonious trees (length = 8796, CI = 0.32, RI = 0.626). The consensus trees indicated a neodermatan sister-group of (Triclads (*Urastoma*, *Ichthyophaga*, *Kronborgia*)). Bootstrap support for this clade was, as expected, very low and a tree one step longer would result in the collapse of the clade.

DISCUSSION

Recent studies since Littlewood *et al.* (1999, and references therein) question the monophyly of the Platyhelminthes, particularly with respect to the position of the Acoela. Justine *et al.* (1998) made a comparative immunocytochemical study of acetylated tubulin in an acoel, temnocephalid, digenean and monogenean and found evidence that suggests that the sperm cortical microtubules of Acoela are not homologous with those of the other platyhelminths. Further studies by Reuter, Raikova & Gustaffson (1998) and Raikova *et al.* (1998) have demonstrated that the brain-like structure of the acoel is not homologous with other platyhelminths. These studies, along with earlier and the latest, most exhaustive molecular studies using 18S rDNA (Carranza *et al.*, 1997; Ruiz-Trillo *et al.*, 1999, respectively), clearly imply an independent evolution of the Acoela as does the molecular data presented herein.

In spite of a recent dismissal of the notion (Kearn, 1998), there is now convincing evidence that all the Neodermata are monophyletic, as first proposed by Ehlers (1984, 1985) on the basis of morphological (including ultrastructural) evidence, and

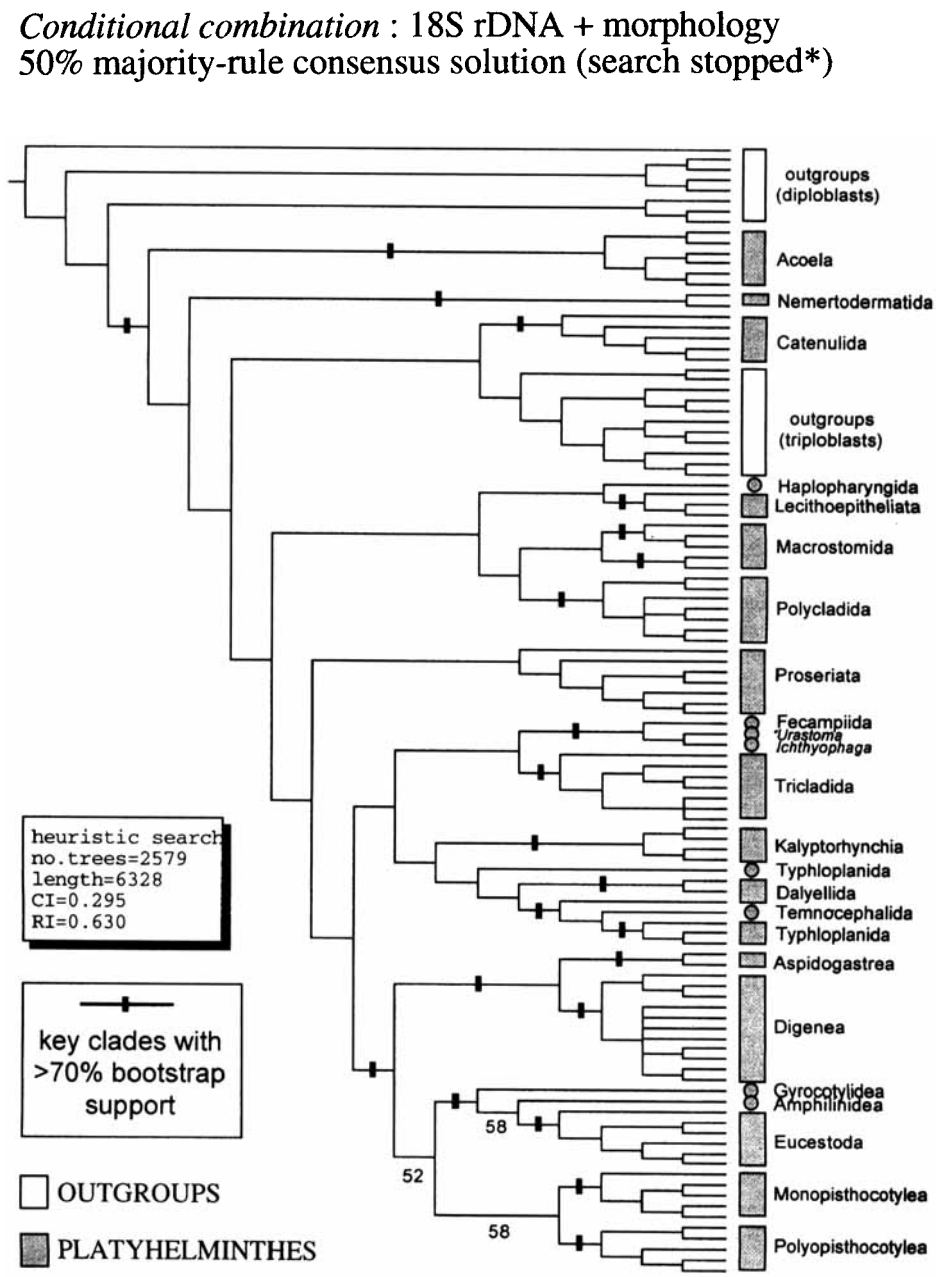


Figure 4. Majority-rule consensus of 2579 equally most parsimonious trees combining 18S rDNA and morphology; CI = consistency index excluding uninformative sites; RI = retention index. Percentage bootstrap support ($n = 1000$) indicated for values >70% with vertical bars and for clades under investigation (i.e. sister-group to Neodermata and interrelationships of major neodermatan clades) with values.

supported by complete 18S rDNA and partial 28S rDNA sequences (Figs 1–4). Synapomorphies of the group are replacement of the larval epidermis by a neodermis with 'insunk' nuclei (tegument), lack of vertical ciliary rootlets of epidermal cilia, presence of characteristic 'neodermatan-type' electron-dense collars of sensory receptors, axonemes of sperm incorporated in sperm body by proximo-distal fusion, protonephridial flame bulbs formed by two cells, and incorporation of vertebrate host in life cycle either as a single host (Monogenea) or as a facultative (some Aspidogastrea) or usually an obligate final host (all the others). The type of flame bulb is possibly plesiomorphic, since a similar type is also known from some turbellarians, e.g. the Proseriata.

We have no evidence for the adaptive value of the various morphological characters, but we suggest the following possibilities. A neodermis with perikarya below the surface may be useful in protecting the surface layer from host actions (abrasions or immune reactions), a vertical ciliary rootlet of epidermal cilia may be useful for anchoring cilia in the epidermis in free-living turbellarians which retain the epidermis throughout their life, but it may not be necessary in neodermatan larvae which shed the cilia when infecting a host. The neodermis also has an important role in nutrient acquisition with a much increased surface area from microvilli, microridges and pits, and a highly active glycocalyx involved with active nutrient intake and transport (Halton, 1997; Tyler & Tyler, 1997). Axonemes incorporated in the sperm body may bring about more effective locomotion of sperm towards the egg cells, essential in parasitic forms which generally are much larger than their free-living relatives, with corresponding longer distances for sperm to travel. Flame bulbs formed by two cells may permit a larger size of the bulbs, essential in large species.

Alternatively, possession of these synapomorphies may be coincidental, characteristic of ancestral forms that happened to adopt a parasitic way of life. Whilst one may hesitate to elect any single morphological 'key innovation' that has enabled the Neodermata to undergo their extensive adaptive radiation (approximately 40 000 species, see Rohde [1996] RAB has estimated that there may be as many 100 000 neodermatan species, based on the number of vertebrate hosts, so far unstudied), it is tempting to focus on the neodermis (Tyler & Tyler, 1997). However, the reason for the large numbers of species seems to be their ability to infect vertebrate hosts, which depends on a wide range of characters, probably gradually acquired during their long evolutionary history. In other words, adaptive radiation of the parasitic platyhelminths went hand in hand with that of their vertebrate hosts.

The Neodermata do not only show certain morphological characteristics, distinguishing them from the free-living turbellarians, they also have a number of functional adaptations to a parasitic way of life, many of them related to food intake and digestion (Halton, 1997). However, as for the morphological features, no single functional key adaptation for all the neodermatans can be identified. The monogeneans resemble free-living, predatory flatworms most closely. Thus, certain monopisthocotyleans possess a protusible pharynx and have extracorporeal digestion, followed by intracellular digestion in their intestine. Blood feeding polyopisthocotyleans have intracellular digestion, with intracellular accumulation of haematin. Many digeneans also feed on blood, but digestion is largely extracellular. The neodermis also contributes to feeding. Small organic solutes diffuse passively through it or are actively absorbed by it. In the cestodes, which lack an intestine, feeding is

entirely via the tegument (Halton, 1997). Glycogen storage predominates in the Neodermata (Jennings, 1997).

The review of Whittington (1997) shows that parasitic flatworms have a variety of behavioural adaptations for host location and settlement on a host. These include attachment of eggs to their host, egg hatching in response to host chemicals, endogenous hatching rhythms, special behaviour of larvae and host recognition. There is no single behavioural 'key innovation' that can be identified as being critical for the success of parasitic platyhelminths.

One way to identify key innovations would be to look for preadaptations, present in free-living forms and used (perhaps in a modified way) by the parasites. Production of many offspring may be such a key innovation (see above and Jennings, 1997; Rohde, 1997). Tyler and Tyler (1997) have proposed that epidermal replacement during embryogenesis in many turbellarians may be another preadaptation. Turbellarians generally have a surface epidermis, whereas in the Neodermata the epidermis is replaced by a syncytial tegument (neodermis) when the larva infects the host. Many turbellarians have two or three generations of epidermis, in some taxa possibly an adaptation to development of ectolecithal eggs, facilitating the use of yolk outside the blastomeres. Such replacement may have been used by the Neodermata in the formation of their neodermis. However, evidence for this assumption is circumstantial, and we do not know whether the neodermis is indeed the key innovation essential for the great success of the Neodermata.

By far the greatest number of parasitic species is found in the Neodermata, but parasitism (or other symbiotic associations) have evolved several times among the turbellarians. Cannon (1998) lists symbionts in nine rhabdocoel, three temnocephalan, one nemertodermatid, three polyclad, four prolecithophoran and one triclad turbellarian families. Jennings (1997) estimates that 200 turbellarians in total live in permanent association with hosts, most of them invertebrates. In general, such symbiotic forms resemble their free-living relatives more closely than the Neodermata. Thus, most species have retained a ciliated epidermis and types of epidermal cilia, sensory receptors, sperm and spermiogenesis, and protonephridia as also found in the free-living forms. Exceptions are the Fecampiida, *Urastoma*, *Ichthyophaga* and *Notentera*, which resemble the neodermatans at least in some characters, especially in the ultrastructure of the protonephridial flame bulb, sperm and spermiogenesis. Concerning functional adaptations, ectosymbiotic forms like the temnocephalids feed on prey similar to free-living turbellarians, but also show opportunistic commensalism (Jennings, 1997). Food reserves and digestive physiology are identical in these forms and their free-living relatives. In contrast, endosymbiotic forms differ distinctly. Some feed on symbiotic protozoans, supplemented by ingestion of the host's food, gut cells or coelomocytes, others feed mainly on intestinal cells. Some species lack digestive enzymes altogether and rely entirely on those ingested from the host tissues or host's ingesta. The Fecampiida and Acholadidae absorb food through the epidermis. There is a shift from lipid storage in the free-living and ectosymbiotic species to glycogen storage in most endosymbiotic turbellaria (and Neodermata, see above). Some endosymbiotic turbellarians have active haemoglobins, which allows them to absorb oxygen preferentially from their hosts (Jennings, 1997). High fecundity in Neodermata and endosymbiotic turbellarians may be consequence of this shift to glycogen storage, guaranteeing a rich and continuous food supply (Jennings, 1997). Alternatively, it may be a prerequisite (perhaps a preadaptation, see below) for a parasitic way of life. Trouve *et al.* (1998) examined

the relationships between life history traits of free-living and parasitic flatworms using phylogenetically-independent contrasts. They examined patterns of interspecific covariation in adult size, progeny volume, daily fecundity, total reproductive capacity, age at first reproduction and longevity. Progeny volume was defined as egg volume in parasitic forms, and as hatchling volume from free-living forms. Daily fecundity was defined as number of eggs (hatchlings) produced per day per individual. Total reproductive capacity was defined as the product of the number of eggs produced over the whole life span and the egg volume. Most of the data were obtained from experimental infections and no parasitic turbellarians were included. The conclusions that the total reproductive capacity is directly determined by the size of the worm, whether parasitic or not, and that the way of life "does not seem to influence the basic patterns of life history evolution" are not convincing, considering the fact that the parasitic turbellarian *Kronborgia* produces an enormous number of eggs 30 000 eggs over 4 days—>1 million eggs during its life (Christensen & Kanneworff, 1964; Kanneworff & Christensen, 1966)—much larger than for instance many larger polyclads, although experimentally determined data for them are not available. Furthermore, multiplication of offspring in digenean parthenitae is not considered, and each of these can increase the population size by many orders of magnitude through the production of high numbers of cercariae (Wright, 1971; Rohde, 1993).

Why are there so few species of Platyhelminthes that are parasites of invertebrates in their adult stage? Only the turbellarians have succeeded but then only infrequently (200 species; Jennings, 1997). The small number cannot be due to competition from the Neodermata which are parasites of vertebrates. Also, it has been shown repeatedly that many empty niches exist (references in Rohde, 1997). The likely reason for the small number of species parasitizing invertebrates appears to be that species are trapped in their niches and they cannot 'long-jump' to other 'peaks' in rugged fitness landscapes (Rohde, 1997), i.e. they have not succeeded in conquering other host taxa (peaks) because of their rigid adaptation to their original niches (peaks). Amongst the evidence is the case of the temnocephalans in New Zealand and Australia which are still very similar to those in South America, even after many millions of years separation.

The sister-group to the Neodermata and the origins of parasitism

There are essentially seven alternative phylogenies we need to consider based on suites of morphological and molecular characters (see Fig. 5). We believe we can reject some of these. There are fundamental differences in spermiogensis and ultrastructure of the protonephridia of 'Dalyellioida' and Neodermata, which make it highly unlikely that these two taxa are sister groups (Fig. 5a; for details of spermiogenesis see Watson & Rohde, 1995; for details of protonephridia see Rohde, 1990). Nowhere in the molecular analyses do temnocephalids appear anywhere near the Neodermata (Fig. 5b), except buried deeply as a derived member of the turbellarians. Although 15 characters apparently unite the temnocephalans and the Neodermata (in the Cercomeria; Brooks & McLennan, 1993; Fig. 5b) a critical examination of this hypothesis by one of us and published elsewhere clearly rejects the clade (Rohde, 1996). Ultrastructure of spermiogenesis and the protonephridia of temnocephalids indicated an affinity with 'dalyellioids' modified by a symbiotic way of life.

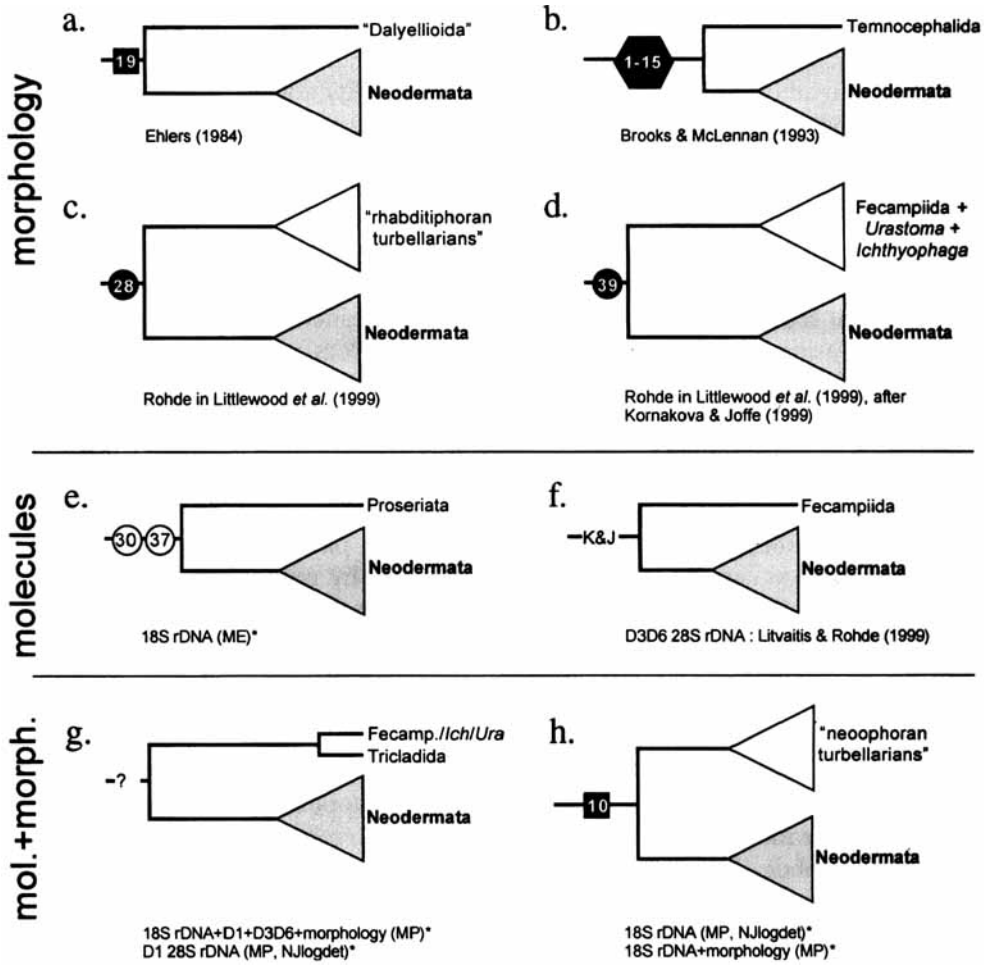


Figure 5. Alternative sister-groups to the Neodermata from (a,b,c,d) morphological, (e,f) molecular and (g,h) both data sets including combined analyses with sources cited. Synapomorphies for the groups are from the original sources or from mapping morphological characters found in (a) Ehlers (1984; square), (b) Brooks & McLennan (1993; hexagon) and (c) Rohde (in Littlewood *et al.*, 1999; circle, representing unambiguous change only) with relevant character numbers from original sources indicated. Character changes mapped with MacClade (Maddison & Maddison, 1992). Solutions (e) through (h) indicate individual or combined data set solutions using maximum parsimony (MP), minimum evolution (ME) or neighbour-joining using the Logdet model (NJ_{logdet}). Closed symbols indicate character acquisitions, open symbols indicate character losses, ? indicates no known synapomorphy, K&J refers to unpublished morphological observations by Kornakova and Joffe and coded in Littlewood *et al.* (1999); see text for full details.

A sister-group relationship of the Rhabditophora and Neodermata (Fig. 5c) is suggested by the morphological data matrix in Littlewood *et al.* (1999). A sister group relationship of the Neodermata and a large taxon comprising all or most turbellarians was earlier suggested by Rohde *et al.* (1993, 1995) on the basis of partial 18S rDNA sequences and protonephridial ultrastructure.

Proseriata and Neodermata (Fig. 5e) share a similar type of protonephridial flame bulb, formed by two cells. However, in the Neodermata the external ribs of the

filtration apparatus are outgrowths of the proximal canal cell, whereas in the Proseriata they are outgrowths of the terminal cell (Rohde, 1990), a difference which may not be of major importance. Similar flame bulbs are also found in some other turbellarian groups.

A possible sister group relationship of the Fecampiidae and Neodermata (Fig. 5d, f) was first suggested by Ehlers (1995, also Rohde, 1990). Subsequent electron-microscopic studies of the protonephridia, eyes and spermiogenesis of *Kronborgia isopodicola* have demonstrated remarkable similarities between this species and the Neodermata (Watson, Williams & Rohde, 1992; Watson, Rohde & Williams, 1992; Watson & Rohde, 1993). Assuming that these similarities are indeed due to common origin and not convergence, re-analysis of the morphological data matrix in Littlewood *et al.* (1999; see also Fig. 1 herein) showed a sister-group relationship of the Neodermata and the Fecampiida (as well as *Urastoma* and *Ichthyophaga*). Kornakova and Joffe (1999) have made an ultrastructural study of spermiogenesis of *Notentera ivanovi* and suggested the following scheme: Neodermata and Urastomidae (*Urastoma cyprinae*) are sister-groups comprising the taxon Mediofusata (axonemes fusing in a proximo-distal direction with a median cytoplasmic process); Mediofusata and Fecampiida (*Kronborgia* and *Notentera ivanova*) are sister-groups, comprising the Revertospermata (proximo-distal development of sperm, but no median cytoplasmic process).

There is strong ultrastructural and limited molecular evidence that fecampiids and certain related forms may indeed comprise the sister group of the Neodermata (Fig. 5d, f). Unpublished studies of the protonephridium of *Ichthyophaga*, as well as Figure 4, suggest that this species must be included in this sister-group as well. However, there is no evidence that the Tricladida are closely related to the Neodermata. Both the ultrastructure of their protonephridia (Rohde & Watson, 1995) and of spermiogensis (Watson & Rohde, 1995) is fundamentally different in the two groups.

The sister-group status between a clade comprising (*Kronborgia*, *Ichthyophaga*, *Urastoma*) and the Tricladida (Fig. 5g) is supported by D1 28S (Fig. 2a) and 18S rDNA (Fig. 3) but this clade's sister-group status with the Neodermata has practically no support from bootstrap resampling: 32% (ME) and 39% (MP) for the D1 data which failed to sample widely across the phylum and a mere 1% for the unconditionally combined solution using all data, a procedure which in itself is of dubious value (Lanyon, 1993). Perhaps more importantly we can find no morphological synapomorphy for this grouping and therefore discount this option as poorly supported and unlikely.

A sister group relationship of the Neodermata and Neoophora (Fig. 5h) is indicated by the total evidence solution using an extensive data matrix and complete 18SrDNA sequences in Littlewood *et al.* (1999). However, the morphological data matrix on its own suggests a sister group relationship of the Neodermata and all turbellarians (excluding the Acoela and Catenulida). A sister group relationship of the Neodermata and a large taxon comprising all or most turbellarians was earlier suggested by Rohde *et al.* (1993, 1995) on the basis of partial 18S rDNA sequences and protonephridial ultrastructure.

In summary, two alternative sister-groups come to the fore: strong ultrastructural evidence suggests a Fecampiida + Urastomidae candidate clade (Fig. 5d with modification), whilst the conditionally combined morphology + molecules solution suggests a neoophoran clade (Fig. 5h).

Future studies should concentrate on sequencing some Prolecithophora and *Notentera*. Prolecithophora have been claimed to form a monophylum (the Eulecithophora) with the turbellarian rhabdocoels and the Neodermata by Sopott-Ehlers (1997) on the basis of the ultrastructure of female gametes, and *Notentera* has been claimed to belong to the sister-group of the Neodermata by Kornakova & Joffe (1999).

The interrelationships of the major neodermatan taxa and the radiation of parasitic forms

With seven taxa there are 10 395 possible strictly bifurcating rooted trees (Felsenstein, 1978). Four hypotheses only reflect the morphological and molecular evidence (Fig. 6) and we can discount three of them. Critically, it is the phyletic status of the Monogenea that is the source of conflict. One solution places the Monogenea as polyphyletic (Fig. 6d, D3D6 data; Litvaitis & Rohde, 1999 and analyses conducted herein), two as paraphyletic and one as monophyletic; in all cases the Monopisthocotylea and Polyopisthocotylea are each strongly monophyletic. While the monophyletic status of the Monogenea is poorly supported by molecular data alone (Mollaret *et al.*, 1997; Littlewood *et al.*, 1998), the Monogenea as a whole have several synapomorphies: two pairs of eyes, three bands of ciliary patches, tapering epidermal cilia with a reduced number of microtubules in the apical parts, and a remarkable similarity in the gross morphology of the protonephridia at least in some species (Whittington, Chisholm & Rohde, in prep; see also Boeger & Kritsky [1993, 1997] but see Justine [1998] for putative support for paraphly from sperm morphology). Clearly, the monopisthocotylean and polyopisthocotylean monogeneans diverged rapidly, at least in terms of molecular evolution, but the strong morphological synapomorphies support the tree shown in Figure 6a derived from morphology and morphology + 18S rDNA. There are two main branches—the Trematoda and the Cercomeromorphae (cestodes and monogeneans)—that have split at the very base of the Neodermata, i.e. they are comparatively old. The Aspidogastrea are the sister-group of the Digenea. Among the Cercomeromorphae there are two branches, the cestodes and the monogeneans. The gyrocotylids are the most basal group of the cestodes, sharing the character 'anterior excretory pores' with the monogeneans. The following synapomorphies can be given in support of this tree:

Trematoda: posterior excretory pore(s), reproductive system with Laurer's canal; epidermal ciliated cells of larva with intraepithelial nuclei and separated from each other by neodermis; male copulatory organ a cirrus (also found in some other Neodermata, but probably not homologous); invertebrate (mollusc) and facultative or obligate vertebrate host; posterior or ventral sucker delimited from parenchyma by distinct capsule.

Monogenea: vertebrate host only, usually more than 10 hooks, anterior excretory pores; oncomiracidia with three bands of ciliary patches, epidermal cilia with tapering tips containing reduced number of microtubules, two pairs of eyes in oncomiracidium; copulatory organ a penis or penis stylet; well defined posterior attachment organ (haptor), but not separated from parenchyma by capsule.

Cestoda: posterior excretory pores; syncytial ciliated epidermis of larva with intraepithelial nuclei; neodermatan type of flame bulb and protonephridial capillary

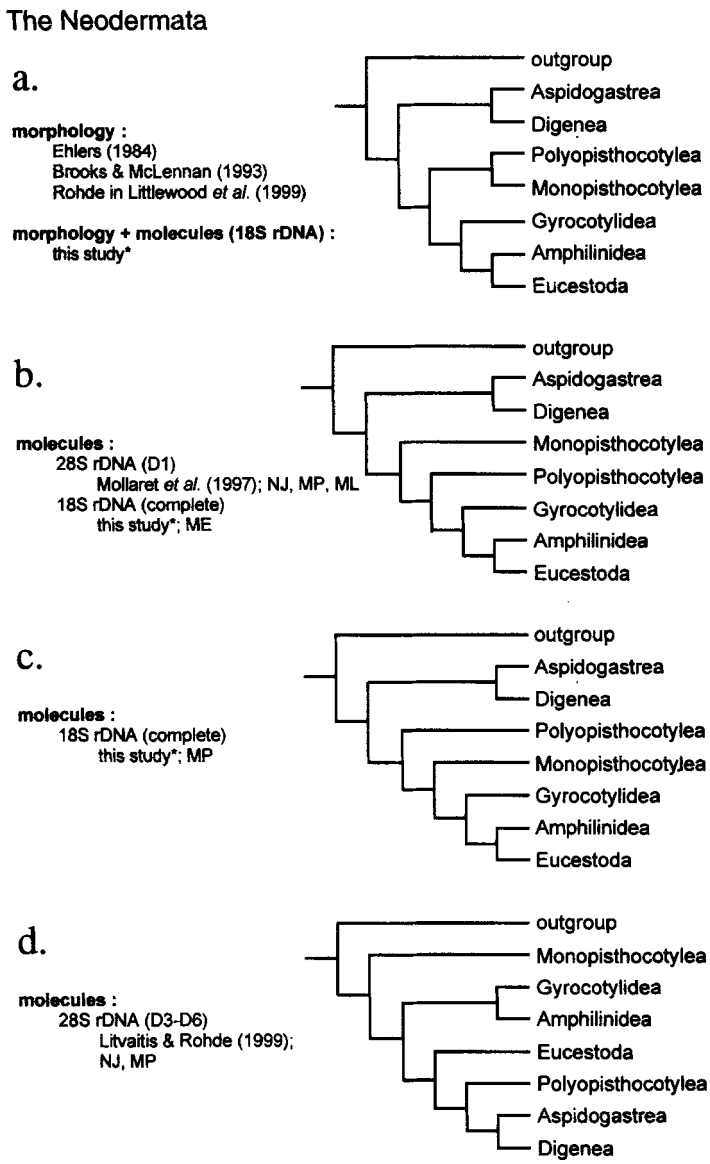


Figure 6. (a–d) Alternative interrelationships of the major neodermatan taxa with sources. Only sources sampling the majority of ingroup taxa are mentioned; other relevant sources with fewer taxa but congruent with these hypotheses are mentioned in the text.

without septate junction (also in two monopisthocotyleans including *Udonella*, but probably due to secondary loss and therefore not homologous); no intestine (probably convergently evolved in Fecampiida).

Cercomeromorphae: posteriorly located hooks for attachment.

Monogenea and Gyrocotylidea: paired anterior excretory pores in larva and adult.

Major events in the evolution of parasitism drawn from phylogenetics

We have used the principle of parsimony to interpret the events and life-history traits that are associated with the divergence of neodermatan taxa as arranged in the most likely phylogeny (i.e. Fig. 6a). In the absence of knowing the identity of the sister-group to the Neodermata, we are restricted to interpreting the evolutionary events which mark the adaptive radiation of the parasites alone or, with caution suggest scenarios based on our two favoured sister-group alternatives (a large clade of neoophoran turbellarians or a clade of the Fecampiids + *Ichthyophaga* + *Urastoma*). Some of the major events may be dated, albeit with added caution, by reference to the divergence dates of the hosts.

Brooks (1989) must be credited with the first serious cladistic interpretation of the neodermatan divergence, although many interpretations dependent on sister-group identity must be discounted. For example, Brooks (1989) reckoned that the plesiomorphic cercomerians were ectoparasitic on arthropods. Cercomeria was defined as the clade (Temnocephala ((Udonellida) (Cercomeridia))). Justine (1997) accepts a system largely agreeing with this scheme based on sperm morphology (Cercomeria and Cercomeridea). None of our results suggest that Temnocephala is sister to the Cercomeridia (= our Neodermata) and Littlewood *et al.* (1998) have shown that *Udonella* is a monopisthocotylean monogenean. It is apparent, therefore, that there is no reason to sustain this idea, nor Brooks' (1989) idea that the Cercomeridians (= our Neodermata) have a plesiomorphic two host vertebrate/arthropod life-cycle. Nevertheless, we have found that many of Brooks' interpretations (1989; Brooks & McLennan, 1993) still hold and readers are encouraged to read these interpretations in the light of the phylogenies presented here.

The vertebrate host

The common ancestor to the Neodermata not only acquired or had already acquired the morphological synapomorphies for the group (detailed above), but gained a vertebrate host and, we believe, was initially endoparasitic (Fig. 7). The vertebrate host is common to all Neodermata and accepting endoparasitism as the first mode of parasitism is more parsimonious as only the Monogenea move towards ectoparasitism whilst retaining a neodermis; i.e. a single gain of endoparasitism in the neodermatan stem-group followed by a single move to ectoparasitism in the stem-group Monogenea (suggested also by Brooks, 1989). The same rationale suggests a molluscan intermediate host was acquired by the common ancestor of the Trematoda, and subsequently confirmed to be lost in only one digenean, namely *Aporocotyle* which uses an annelid (Køie, 1982); also, no molluscan host is known for the aspidogastrean *Stichocotyle*. Meanwhile a crustacean host is most likely to have been acquired by the stem-group Cestoda (although the intermediate hosts of Gyrocotylidea are not known), or, perhaps more likely, the Amphilinidea + Eucestoda.

Gibson (1987) and Brooks (1989) suggested that the vertebrates which were first parasitized were placoderms prior to the divergence of the selachians and 'ray finned fishes' (actinopterygians). The phylogeny of fishes illustrated by Long (1995) has the placoderms as sister-group to the chondrichthyans, and not as an ancestral group to the osteichthyans. However, taking the 'protovertebrates' prior to this divergence as the plesiomorphic host, a scheme can be developed from the observations of

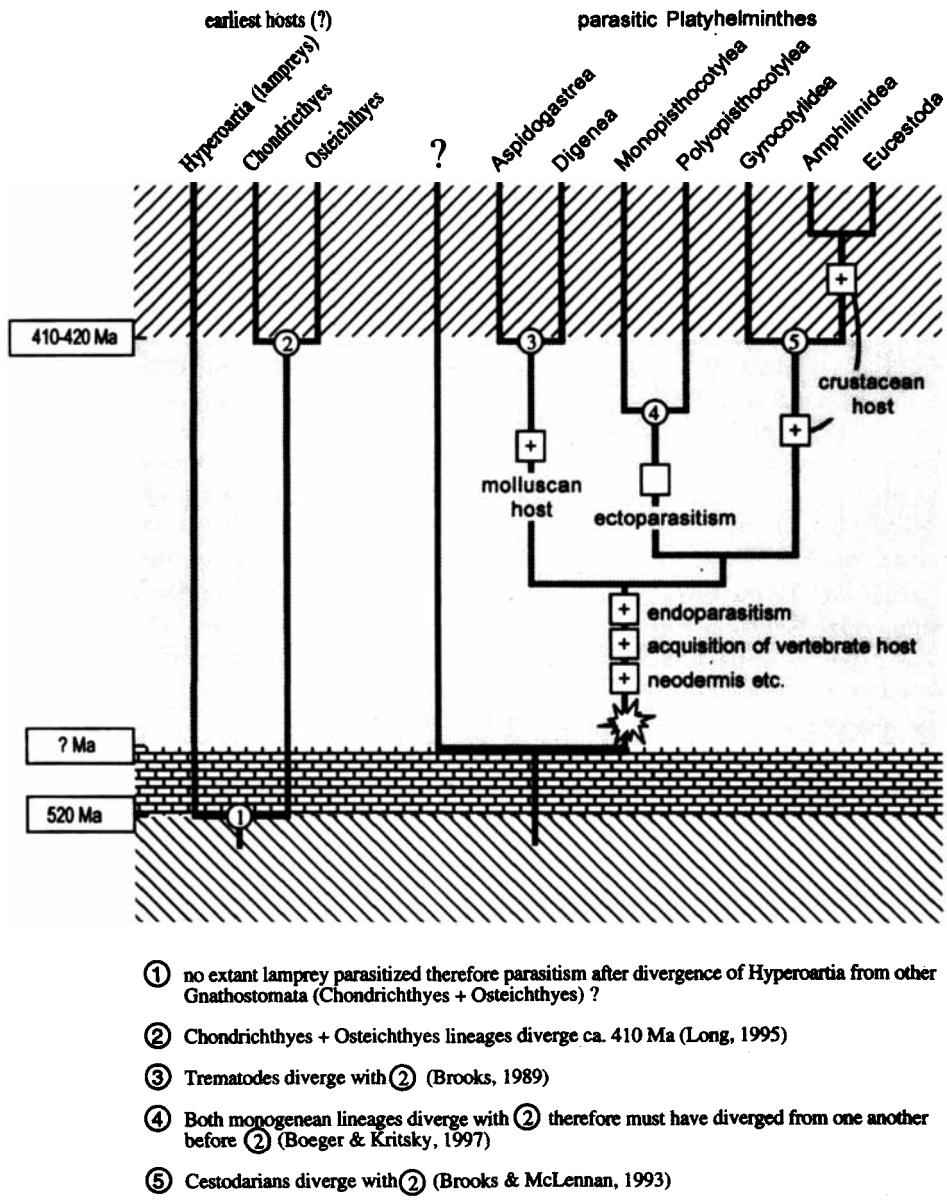


Figure 7. Interpretation of the major events associated with the divergence of the Neodermata. See text for rationale and also Brooks, 1989 for similar conclusions.

Brooks (1989) and Boeger & Kritsky (1997). Brooks (1989) suggested that the divergence of aspidogastreans from digeneans and that of the gyrocotylideans from the cestodarians (amphilideans + eucestodes) is associated with the divergence of chondrichthyeans and osteichthyeans. Similarly Boeger & Kritsky (1997) suggested that the Oligonchoinea and Polystomatoinea diverged with the divergence of the chondrichthyans and osteichthyans, and that at this same time a divergence within the Polyonchoinea (= Monopisthocotylea) occurred. This suggest that much diverging was going on during the early Ordovician, some 500 Ma (Long, 1995;

Janvier, 1996). The Neodermata must then have diverged from the platyhelminth stock earlier than that, in the Ordovician or Cambrian, as must the divergence of the Trematoda and Cercomeromorphae and the Cestodaria and the Monogenea. The strong association of the Neodermata to vertebrates suggests that this occurred in 'protovertebrates'.

The plesiomorphic host of trematodes

Kearn (1998: 56) suggested that molluscs were the first host of trematodes and further pointed out that digeneans [parthenitae-implied] only occurred in gastropods, bivalves and scaphopods, not in amphineurans or cephalopods. Using implications on mollusc phylogeny attributed to Wright (1971) he suggested that cephalopods and amphineurans [polyplacophora] were the first major groups to 'emerge' from the main molluscan lineage, leaving the ancestor of the gastropod/bivalve/scaphopod line to be colonized by ancestral digeneans. More recent views on molluscan phylogeny are summarized by Ponder (1998), showing that the polyplacophorans diverged early (Upper Vendian >570 Ma), and early in the Palaeozoic [<570 Ma] the main mollusc divergence occurred with three lines evolving, i.e. the Scaphopod/Bivalvia, the Monoplacophora and the Gastropoda/Cephalopoda. The scenario envisaged by Kearn (1998) cannot be reconciled with these more recent findings.

The early divergence of the Trematoda may indicate that they may have become associated with molluscs prior to the divergence of the Bivalvia/Scaphopoda and Gastropoda/Cephalopoda clades, nearly 570 Ma (Ponder, 1998). There is no evidence, however, that the digeneans using bivalves are monophyletic (Hall, Cribb & Barker, 1999) or that the Ptychogonimidae (using scaphopods) are related to those groups inhabiting bivalves (Blair, Bray & Barker, 1998).

The plesiomorphic host of the neodermatans

A quite different scenario can be developed if the frequency of the neodermatan/teleost association is considered along with the observations on the frequency of host-switching that has occurred, for example, in the digenean/mollusc association (Gibson & Bray, 1994). If teleosts or their ancestral actinopterygians are considered plesiomorphic hosts, then the divergences of various neodermatan groups becomes de-coupled from the divergence of host-groups. The divergence of the Aspidogastrea and Digenea, both of which inhabit teleosts, need not be associated with host-divergence. The gyrocotylideans presumably diverged into the holocephalans early, but there is no need to postulate that amphilinideans (found in actinopterygians and basal teleosts) and eucestodes (whose plesiomorphic hosts were probably 'ray-finned fishes', i.e. teleosts or actinopterygians; Hoberg *et al.*, 1999) co-diverged with the host-group. The 'historical relationships' of the Monogenea (=Monogenoidea – sic) as illustrated by Boeger & Kritsky (1997), show a miasma of crossing lines (horizontal transfer – Page & Charleston, 1998) with, for example, a group of large taxa transferring from the elasmobranchs to the teleosts (see their figure 6). Both the scenarios postulated here should be testable against the consensus phylogeny we present, but our argument may serve to illustrate the different hypotheses that can be put forward if co-evolution/extinction is considered as just one of several phylogenetic possibilities.

The alternatives suggested here indicate that the plesiomorphic condition of the Trematoda is a two host cycle involving vertebrates and molluscs. There seems no

reason to postulate the loss of a vertebrate host, the acquisition of a mollusc host and the re-acquisition of the vertebrate host. The best candidate for the most primitive digenean is probably the teleost parasite, the Bivesiculidae (Blair *et al.*, 1998), which has several primitive features (Pearson, 1992) although a three-host life-cycle has recently been described (Cribb *et al.*, 1998).

Some major open questions remain, both in establishing a 'definitive' system of the Platyhelminthes, and in defining key events that have led to the branching of platyhelminths and the relative success of various clades within them. It is encouraging that molecular systematics contributes as much as morphology to our phylogenetic knowledge of the group so far. It is clear we need both data sets for phylogenetic reconstruction and resolution, and as more genes and more taxa are sampled we can continue to interpret comparative data in the light of these phylogenies with greater confidence.

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REFERENCES

Ax P. 1984. *Das phylogenetische System. Systematisierung der lebenden Natur aufgrund ihrer Phylogenese.* Stuttgart: Gustav Fischer Verlag.

Baverstock PR, Fielke R, Johnson AM, Bray RA, Beveridge I. 1991. Conflicting phylogenetic hypotheses for the parasitic Platyhelminthes tested by partial sequencing of 18S ribosomal RNA. *International Journal for Parasitology* **21:** 329–339.

Blair D. 1993. The phylogenetic position of the Aspidobothrea within the parasitic flatworms inferred from ribosomal RNA sequence data. *International Journal for Parasitology* **23:** 169–178.

Blair D, Bray RA, Barker SC. 1998. Molecules and morphology in phylogenetic studies of the Hemiuroidea (Digenea: Trematoda: Platyhelminthes). *Molecular Phylogenetics and Evolution* **9:** 15–25.

Boeger WA, Kritsky DC. 1993. Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* **26:** 1–32.

Boeger WA, Kritsky DC. 1997. Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology* **27:** 1495–1511.

Bremer K. 1994. Branch support and tree stability. *Cladistics* **10:** 295–304.

Brooks DR. 1989. The phylogeny of the Cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. *Journal of Parasitology* **75:** 606–616.

Brooks DR, McLennan DA. 1993. *Parascript: parasites and the language of evolution.* Washington DC: Smithsonian Institution Press.

Brooks DR, Thorson TB, Mayes MA. 1981. Fresh-water stingrays (Potamotrygonidae) and their helminth parasites: testing hypotheses of evolution and coevolution. In: Funk VA, Brooks Dr, eds, *Advances in cladistics.* New York: New York Botanical Garden, 147–175.

Brooks DR, O'Grady RT, Glen DR. 1985. The phylogeny of the Cercomeria Brooks, 1982 (Platyhelminthes). *Proceedings of the Helminthological Society of Washington* **52:** 1–20.

Cannon LRG. 1998. *Turbellaria of the World. A guide to families and genera.* CD-ROM format version 1.0. Amsterdam: Expert Center for Taxonomic Identification, University of Amsterdam. Distributed by Springer-Verlag and UNESCO.

Carranza S, Baguñà J, Riutort M. 1997. Are the Platyhelminthes a monophyletic primitive group? An assessment using 18S rDNA sequences. *Molecular Biology and Evolution* **14:** 485–479.

Christensen AM, Kanneworff B. 1964. *Kronborgia amphipodicola* gen. et sp. nov., a dioecious turbellarian parasitizing ampeliscid amphipods. *Ophelia* **1:** 147–166.

Cribb TH, Adlard RD, Anderson GR, Bray RA. 1998. A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). *International Journal for Parasitology* **28:** 1791–1795.

Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* **14:** 733–740.

Cunningham CW, Omland KE, Oakley TH. 1998. Reconstructing ancestral character states: a critical reappraisal. *Trends in Ecology and Evolution* **13:** 361–366.

Ehlers U. 1984. Phylogenetisches System der Plathelminthes. *Verhandlungen des naturwissenschaftlichen Vereins Hamburg* **27:** 291 294.

Ehlers U. 1985. *Das Phylogenetische System der Plathelminthes.* Stuttgart: Gustav Fischer.

Ehlers U. 1995. The basic organization of the Plathelminthes. *Hydrobiologia* **305:** 21–26.

Felsenstein J. 1978. The number of evolutionary trees. *Systematic Zoology:* **27:** 27–33.

Gibson DI. 1987. Questions in digenean systematics and evolution. *Parasitology* **95:** 429–460.

Gibson DI, Bray RA. 1994. The evolutionary expansion and host-parasite relationship of the Digenea. *International Journal for Parasitology* **24:** 1213–1226.

Hall KA, Cribb TH, Barker SC. 1999. V4 region of small subunit rDNA indicates polyphyly of the Fellodistomidae (Digenea) which is supported by morphology and life-cycle data. *Systematic Parasitology* **43:** 81–92.

Halton DW. 1997. Nutritional adaptations to parasitism within the platyhelminthes. *International Journal for Parasitology* **27:** 693–704.

Harvey PH, Pagel MD. 1991. *The Comparative Method in Evolutionary Biology.* Oxford: Oxford University Press.

Hoberg EP, Gardner SL, Campbell RA. 1999. Systematics of the Eucestoda: advances toward a new phylogenetic paradigm, and observations on the early diversification of tapeworms and vertebrates. *Systematic Parasitology* **42:** 1–12.

Huelsenbeck JP, Bull JP, Cunningham CW. 1996. Combining data in phylogenetic analysis. *Trends in Ecology and Evolution* **11:** 152–158.

Janvier P. 1996. *Early Vertebrates.* Oxford: Clarendon Press.

Jennings JB. 1997. Nutritional and respiratory pathways to parasitism exemplified in the Turbellaria. *International Journal for Parasitology* **27:** 679–691.

Justine JL. 1997. The general classification of parasitic platyhelminthes – recent changes, and the use of ultrastructural characters, particularly those of spermatozoa. *Bulletin de la société Zoologique de France-Evolution et Zoologie* **122:** 269–277.

Justine JL. 1998. Non-monophyly of the monogeneans? *International Journal for Parasitology* **28:** 1653–1657.

Justine JL, Iomini C, Raikova OI, Mollaret I. 1998. The homology of cortical microtubules in platyhelminth spermatozoa – a comparative immunocytochemical study of acetylated tubulin. *Acta Zoologica* **79:** 235–241.

Kanneworff B, Christensen AM. 1966. *Kronborgia caridicola* sp. nov., an endoparasitic turbellarian from North American shrimps. *Ophelia* **3:** 65–80.

Katayama T, Nishioka M, Yamamoto M. 1996. Phylogenetic relationships among turbellarian orders inferred from 18S rDNA sequences. *Zoological Science* **13:** 747–756.

Kearn GC. 1998. *Parasitism and the Platyhelminths.* London: Chapman & Hall.

Køie M. 1982. The redia, cercaria and early stages of *Aporocotyle simplex* Odhner, 1900 (Sanguinicolidae) – A digenetic trematode which has a polychaete annelid as the only intermediate host. *Ophelia* **21:** 115–145.

Kornakova EE, Joffe BI. 1999. A new variant of the neodermatan-type spermiogenesis in a parasitic turbellarian, *Notentera ivanovi* (Plathelminthes) and the origin of the Neodermata. *Acta Zoologica (Stockholm)* **80:** 135–151.

Lake JA. 1991. Reconstructing evolutionary trees from DNA and protein sequences: paralinear distances. *Proceedings of the National Academy of Sciences, USA* **91:** 1455–1459.

Lanyon SM. 1993. Phylogenetic frameworks – towards a firmer foundation for the comparative approach. *Biological Journal of the Linnean Society* **49:** 45–61.

Larson A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. In: Schierwater B, Streit B, Wagner GP, DeSalle R, eds. *Molecular ecology and evolution: approaches and applications.* Berlin: Birkhauser Verlag.

Littlewood DTJ, Johnston DA. 1995. Molecular phylogenetics of the four *Schistosoma* species groups determined with partial 28S ribosomal RNA gene sequences. *Parasitology* **111:** 167–175.

Littlewood DTJ, Rohde K, Clough KA. 1997. Parasite speciation within or between host species? - phylogenetic evidence from site-specific polystome monogeneans. *International Journal for Parasitology* **27:** 1289–1297.

Littlewood DTJ, Rohde K, Clough KA. 1998. The phylogenetic position of *Udonella* (Platyhelminthes). *International Journal for Parasitology* **28:** 1241–1250.

Littlewood DTJ, Rohde K, Clough KA. 1999. Interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biological Journal of the Linnean Society* **66:** 75–114.

Litvaitis MK, Curini-Galletti MC, Martens PM, Kocher TD. 1996. A reappraisal of the systematics of the Monocelididae (Platyhelminthes, Proseriata): Inferences from rDNA sequences. *Molecular Phylogenetics and Evolution* **6:** 150–156.

Litvaitis MK, Rohde K. 1999. A molecular test of platyhelminth phylogeny: inferences from partial 28S rDNA sequences. *Invertebrate Biology* **118:** 42–56.

Lockhart PJ, Steel MA, Hendy MD, Penny D. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. *Molecular Biology and Evolution* **11:** 605–612.

Long JA. 1995. *The Rise of Fishes. 500 million years of evolution.* Sydney: University of New South Wales Press.

Lovejoy NR. 1997. Stingrays, parasites, and neotropical biogeography: a closer look at Brooks *et al.*'s hypotheses concerning the origins of neotropical freshwater rays (Potatotrygonidae). *Systematic Biology* **46:** 218–230.

Maddison WP, Maddison DR. 1992. *MacClade, version 3.06.* Massachusetts: Sinauer Associates.

Martins E. 1996 ed. *Phylogenies and the Comparative Method in Animal Behaviour.* Oxford: Oxford University Press.

Miyamoto MM, Fitch WM. 1995. Testing species phylogenies and phylogenetic methods with congruence. *Systematic Biology* **44:** 64–76.

Mollaret I, Jamieson BGM, Adlard RD, Hugall A, Lecointre G, Chombard C, Justine J-L. 1997. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Molecular and Biochemical Parasitology* **90:** 433–438.

Page RDM, Charleston MA. 1998. Trees within trees: phylogeny and historical associations. *Trends in Ecology and Evolution* **13:** 356–359.

Pearson JC. 1992. On the position of the digenean family Heronimidae: an inquiry into a cladistic classification of the Digenea. *Systematic Parasitology* **21:** 81–166.

Ponder W.F. 1998. Classification of Mollusca. In: Beesley PL, Ross GJB, Wells A, eds. *Mollusca: The southern synthesis. Fauna of Australia Vol.5.* Part A. Melbourne: CSIRO.

Raikova OI, Reuter M, Kotikova EA, Gustafsson MKS. 1998. A commissural brain! The pattern of 5-HT immunoreactivity in Acoela (Plathelminthes). *Zoomorphology* **118:** 69–77.

Reuter M, Raikova OI, Gustafsson MKS. 1998. An endocrine brain – the pattern of FMRF-amide immunoreactivity in Acoela (Plathelminthes). *Tissue & Cell* **30:** 57–63.

Rohde K. 1990. Phylogeny of Platyhelminthes, with special reference to parasitic groups. *International Journal for Parasitology* **20:** 979–1007.

Rohde K. 1993. *Ecology of marine parasites.* 2nd ed. Wallingford, UK: CAB International.

Rohde K. 1996. Robust phylogenies and adaptive radiations: a critical examination of methods used to identify key innovations. *American Naturalist* **148:** 481–500.

Rohde K. 1997. The origins of parasitism in the Platyhelminthes: a summary interpreted on the basis of recent literature. *International Journal for Parasitology* **27:** 739–746.

Rohde K, Watson NA. 1995. Comparative ultrastructural study of the posterior suckers of four species of symbiotic Platyhelminthes, *Temnocephala* sp. (Temnocephalida), *Udonella caligorum* (Udonellidea), *Anoplodiscus cirrusspiralis* (Monogenea, Monopisthocotylea), and *Philophthalmus* sp. (Trematoda, Digenea). *Folia Parasitologica* **42:** 11–28.

Rohde K, Hefford C, Ellis JT, Baverstock PR, Johnson AM, Watson NA, Dittmann S. 1993. Contributions to the phylogeny of Platyhelminthes based on partial sequencing of 18S ribosomal DNA. *International Journal for Parasitology* **23:** 705–724.

Rohde K, Johnson AM, Baverstock PR, Watson NA. 1995. Aspects of the phylogeny of Platyhelminthes based on 18S ribosomal DNA and protonephridial ultrastructure. *Hydrobiologia* **305:** 27–35.

Ruiz-Trillo I, Riutort M, Littlewood DTJ, Herniou EA, Baguñà J. 1999. Acoel flatworms: earliest extant bilaterian metazoans. *Science* **283:** 1919–1923.

Smith AB. 1994. Rooting molecular trees – problems and strategies. *Biological Journal of the Linnean Society* **51:** 279–292.

Sopott-Ehlers B. 1997. Fine-structural features of male and female gonads in *Jensenia angulata* (Plathelminthes, Rhabdocoela, 'Dalyellioida'). *Microfauna marina* **11:** 251–270.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM. 1996. Phylogenetic inference. In: Hillis DM, Moritz C, Mable BK eds. *Molecular Systematics* 2nd ed. Massachussetts: Sinauer Associates

Swofford DL. 1999. *PAUP*: Phylogenetic Analysis Using Parsimony (and other methods), version 4.0.* Massachussetts: Sinauer Associates.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL-W – improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Trouve S, Sasal P, Jourdane J, Renaud F, Morand S. 1998. The evolution of life history traits in parasitic and free-living platyhelminthes – a new perspective. *Oecologia* **115:** 370–378.

Tyler S, Tyler MS. 1997. Origin of the epidermis in parasitic platyhelminths. *International Journal for Parasitology* **27:** 715–738.

Watson NA, Rohde K. 1993. Ultrastructure of sperm and spermiogenesis of *Kronborgia isopodicola* (Platyhelminthes, Fecampiidae). *International Journal for Parasitology* **23:** 737–744.

Watson NA, Rohde K. 1995. Sperm and spermiogenesis of the 'Turbellaria' and implications for the phylogeny of the phylum Platyhelminthes. In: Jamieson BG, Ausio MJ, Justine J-L, eds. *Advances in spermatozoal taxonomy and phylogeny.* Paris: Mémoires du Muséum national d'Histoire naturelle, 37–54.

Watson NA, Rohde K, Williams JB. 1992. Ultrastructure of the protonephridial system of larval *Kronborgia isopodicola* (Platyhelminthes, Fecampiidae). *Journal of Submicroscopic Cytology and Pathology* **24:** 43–49.

Watson NA, Williams JB, Rohde K. 1992. Ultrastructure and development of the eyes of larval *Kronborgia isopodicola* (Platyhelminthes, Fecampiidae). *Acta Zoologica* **73:** 95–102.

Whittington ID. 1997. Reproduction and host-location among the parasitic platyhelminthes. *International Journal for Parasitology* **27:** 705–714.

Wright CA. 1971. *Flukes and snails.* London: George Allen and Unwin.

APPENDIX

Species used in the molecular analysis with classification and GenBank/EMBL accession numbers. Classification of turbellarians following Cannon (1998). § indicates new sequence for this study; UJ indicates unpublished sequence provided by Ulf Jondelius.

Classification	18S	D1	D3D6
Phylum			
outgroups			
Placozoa			
Trichoplax sp.	Z22783		
Ctenophora			
Mnemiopsis leidyi	L10826		
Beroe cucumis	D15068		
Porifera			
Scypha calcaravis	D15066		
Microciona prolifera	L10825		
Reniera fulva		AJ225829	
Cnidaria			
Tripedalia cystophora	L10829		
Anthopleura kurogane	Z21671		
Anemonia sulcata	X53498		
Cyanea capillata		UG5481	
Eunicella stricta		X57255	
Nematoda			
Haemonchus similis	L04152		
Meloidogyne arenaria	U42342		
Caenorhabditis elegans			X03680
Hexapoda			
Drosophila melanogaster			M21017
Acanthocephala			
Moniliformis moliniformis	Z19562		
Neoechinorhynchus pseudemydis	U41400		
Centrorhynchus conspectus	U41399		
Rotifera			
Brachionus plicatilis	U49911		
Philodina acuticornis	U41281		
Gnathostomulida			
Gnathostomula paradoxa	Z81325		
Nemertini			
Lineus sp.	X79878		
Prostoma eilhardi	U29494		
Ototyphlonemertes pallida			AF023124
Gastrotricha			
Lepidodermella squamata	U29198		
Vertebrata			
Mus musculus		X00525	
ingroup			
Platyhelminthes			
'Turbellaria'			
Order Catenulida			
Family Stenostomidae			
Stenostomum sp.	U95947		
Stenostomum leucops	U70084		
Stenostomum leucops	D85095		
Stenostomum leucops aquariorum	AJ012519	AJ228801§	
Suomina sp.	AJ012532§		AF021322
Catenula sp.			AF021320
Order Nemertodermatida			
Family Nemertodermatidae			
Nemertinoides elongatus	U70084		AF021326
Meara stichopi	UJ		

Continued

APPENDIX—*continued*

Classification	18S	D1	D3D6
Order Acoela			
Family Childiidae			
Actinoposthia beklemischevi	AJ012522		
Family Convolutidae			
Convoluta pulchra	U70086		
Convoluta naikaiensis	D83381		
Convoluta convoluta	AJ012524		
Amphiscolops sp.	D85099		
Amphiscolops sp.	AJ012523		
Order Macrostomida			
Family Dolichomacrostomidae			
Paromalostomum fusculum	AJ012531		AF021328
Family Macrostomidae			
Macrostomum tuba	U70082		
Macrostomum tuba	D85092		
Family Microstomidae			
Microstomum lineare	U70081		
Microstomum lineare	D85091		
Microstomum sp.			AF021332
Microstomum papillosum			AF021330
Order Haplopharyngida			
Family Haplopharyngidae			
Haplopharynx rostratus	AJ012511		AF022746
Of uncertain status (see text)			
Family Urastomidae			
Urastoma cyprinae	U70086		
Ichthyophaga sp.	AJ012512		
Order Lecithoepitheliata			
Family Prorhynchidae			
Geocentrophora sphyrocephala	D85089		
Geocentrophora sp.	U70080		
Geocentrophora wagini	AJ012509		
Order Proseriata			
Family Monocelididae			
Monocelis lineata	U45961		
Archiloa rivularis	U70077		
Family Otoplanidae			
Otoplana sp.	D85090		
Paratoplana renatae	AJ012517		
Archotoplana holotricha	AJ243676§		
Monostichoplana filum		AJ243683§	
Otoplanella schulzi			AF022750
Family Nematoplanidae			
Nematoplana coelogynoporoide	AJ012516		AF022748
Family Coelogynopridae			
Coelogynopora gynocotyla	AJ243679§	AJ243680§	U40207
Family Archimonocelidae			
Archimonocelis staresoi			U40209
Order Rhabdocoela			
Dalyellida			
Family Dalyellidae			
Microdalyellia rossi	AJ012515		
Family Graffilidae			
Graffila buccinicola	AJ012521		
Family Pterastericolidae			
Pterastericola australis	AJ012518		
Family Fecampiidae			
Kronborgia isopodicola	AJ012513	AJ228800§	AF022862
Family Provorticidae			
Provortex psammophilus			AF022754

Continued

APPENDIX—*continued*

Classification	18S	D1	D3D6
Temnocephalida			
Family Temnocephalidae			
Temnocephala sp.	AJ012520	AJ228802§	
Temnocephala minor			AF022864
Typholoplanida			
Family Trigonostomidae			
Mariplanella frisia	AJ012514		
Family Typhloplanidae			
Bothromesostoma sp.	D85098		
Mesocastrada foremani	U70082		
Mesostoma lingua	AJ243682§		
Kalyptorhynchia			
Family Polycystidae			
Gyratrix hermaphroditus	AJ012510		
Arrawaria n.gen.	AJ243677§		
Schizorhynchia			
Family Diascorhynchidae			
Diascorhynchus rubrus	AJ012508		AF022752
Family Karkinorhynchidae			
Cheliplana cf. *orthocirra*	AJ012507		
Order Tricladida			
Family Procerodidae			
Ectoplana limuli	D85088		
Family Bdellouridae			
Bdelloura candida		AJ228798§	
Family Dendrocoelidae			
Dendrocoelum lacteum	M58346		
Dendrocoelopsis lactea	D85087		
Family Dugesiidae			
Dugesia (Schmidtea) mediterranea	U31084		
Romankenkius lidinosus	Z99951		AF022766
Family Planariidae			
Crenobia alpina	M58345		
Polycelis tenuis		AF022762	
Polycelis sp.		AF026105	
Family Bipaliidae			
Bipalium sp.	X91402		
Bipalium adventitium			AF022758
Bipalium kewense		AF026119	AF022756
Order Polycladida			
Acotylea			
Family Leptoplanidae			
Notoplana koreana	D85097		
Notoplana australis	AJ228786		
Family Planoceridae			
Planocera multitentaculata	D83383		
	D17562		
Family Discocoelidae			
Discocelis tigrina	U70079		AF02274
Cotylea			
Family Pseudocerotidae			
Thysanozoon brocchii	D85096		
Pseudoceros tritriatus	AJ228794		
Neodermata			
Class Monogenea			
Monopisthocotylea			

Continued

APPENDIX—*continued*

Classification	18S	D1	D3D6
Order Monocotylidea			
Family Monocotylidae			
Calicotyle affinis	AJ228777		
Neoheterocotyle rhinobatidis		AF026107	
Troglocephalus rhinobatidis		AF026110	
Merizocotyle icopae		AF026113	
Order Gyrodactylidea			
Family Gyrodactylidae			
Gyrodactylus salaris	Z26942		
Order Dactylogyridea			
Family Pseudomurraytrematidae			
Pseudomurraytrema sp.	AJ228793		
Family Microbothriidae			
Leptocotyle minor	AJ228784		
Family Ancyrocephalidae			
Tetrancistrum sp.		AF026114	
Haliotrema chrysotaeniae		AF026115	
Family Dactyolgyridae			
Acleotrema sp.		AF026118	
Order Capsalidea			
Family Capsalidae			
Benedenia lutjani		AF026106	
Entobdella australis		AF026108	
Encotyllabe caballeroi		AF026112	
Order Udonellidea			
Family Udonellidae			
Udonella caligorum	AJ228796	AJ228803§	
Udonella sp.			AF022866
Polypisthocotylea			
Order Diclybothriidea			
Family Diclybothriidae			
Pseudohexabothrium taeniurae	AJ228791		
Order Polystomatidea			
Family Polystomatidae			
Neopolystoma spratti	AJ228788		AF023105
Polystomoides malayi	AJ228792		
Order Mazocraeidea			
Family Mazocraeidae			
Kuhnia scombri	AJ228783		
Family Diclidophoridae			
Diclidophora merlangi	AJ228779		
Family Microcotylidae			
Bivagina pagrosomi	AJ228775	AJ243678§	
Family Axinidae			
Zeuxapta seriolae		AF026103	
Family Gotocotylidae			
Gotocotyla secunda		AF026109	
Family Gastrocotylidae			
Pricea multae		AF026111	
Class Trematoda			
Subclass Aspidogastrea			
Order Aspidogastrida			
Family Aspidogastridae			
Lobatostoma manteri	L16911		
Multicotyle purvisi	AJ228785	AJ243684§	AF023115
Subclass Digenea			

Continued

APPENDIX—*continued*

Classification	18S	D1	D3D6
Order Strigeida			
Family Bucephalidae			
Prosorhynchoides gracilescens	AJ228789		
Family Schistosomatidae			
Schistosoma spindale	Z11979	Z46505	
Schistosoma haematobium	Z11976	Z46521	
Schistosoma mansoni	X53047	Z46503	
Schistosoma japonicum		Z46504	
Heterobilharzia americana		Z46506	
Order Echinostomida			
Family Echinostomatidae			
Echinostoma caproni	L06567	AF026104	
Family Fasciolidae			
Fasciolopsis buski	L06668		
Family Heronimidae			
Heronimus mollis	L14486		
Family Paramphistomidae			
Calicophoron calicophorum	L06566		AF023109
Order Plagiorchiida			
Family Gyliauchenidae			
Gyliauchen sp.	L06669		
Family Lepocreadiidae			
Tetracerasta blepta	L06670		
Lepidapedon sommervillae		Z29502	
Family Opithorchiidae			
Opithorchis viverrini	X55357		
Family Opecoelidae			
Peracreadium idoneum		AJ243685§	
Class Eucestoda			
Order Gyrocotylidea			
Family Gyrocotylidae			
Gyrocotyle urna	AJ228782	AJ228799§	
Order Amphilinidea			
Family Amphilinidae			
Gigantolina magna	AJ243681§		
Austramphilina elongata		Aj243675§	AF023123
Order Otobothrioidea			
Family Grillotidae			
Grillotia erinaceus	AJ228781		
Order Pseudophyliidea			
Family Bothriocephalidae			
Bothriocephalus scorpii	AJ228776		
Family Triaenophoridae			
Abothrium gadi	AJ228773		
Family Diphyllobothriidae			
Spirometra erinacei	D64072		
Order Proteocephalidea			
Family Proteocephalidae			
Proteocephalus exiguus	X99976		
Proteocephalus neglectus			AF026116
Order Cyclophyllidea			
Family Taeniidae			
Echinococcus granulosus	U27015		
Family Dipylididae			
Dipylidium caninum			AF023120
Family Hymenolepididae			
Hymenolepis diminuta			AF023118
Order Caryophyllidea			
Family Caryophyllaeidae			
Caryophyllaeus sp.	AF026117		