

Platyhelminth phylogenetics – a key to understanding parasitism ?

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ABSTRACT. The comparative method, the inference of biological processes from phylogenetic patterns, is founded on the reliability of the phylogenetic tree. In attempting to apply the comparative method to the understanding of the evolution of parasitism in the phylum Platyhelminthes, we have highlighted several points we consider to be of value along with many problems. We discuss four of these topics. Firstly, we view the group at a phylum level, in particular discussing the importance of establishing the sister taxon to the obligate parasite group, the Neodermata, for addressing such questions as the monophyly, parasitism or the endo or ectoparasitic nature of the early parasites. The variety of non-congruent phylogenetic trees presented so far, utilising either or both morphological and molecular data, gives rise to the suggestion that any evolutionary scenarios presented at this stage be treated as interesting hypotheses rather than well-supported theories. Our second point of discussion is the conflict between morphological and molecular estimates of monogenean evolution. The Monogenea presents several well-established morphological autapomorphies, such that morphology consistently estimates the group as monophyletic, whereas molecular sequence analyses indicate paraphyly, with different genes giving different topologies. We discuss the problem of reconciling gene and species trees. Thirdly, we use recent phylogenetic results on the tapeworms to interpret the evolution of strobilation, proglottization, segmentation and scolex structure. In relation to the latter, the results presented indicate that the higher cestodes are diphyletic, with one branch difossate and the other tetrafossate. Finally, we use a SSU rDNA phylogenetic tree of the Trematoda as a basis for the discussion of an aspect of the digenean life-cycle, namely the nature of the first intermediate host. Frequent episodes of host-switching, between gastropod and bivalve hosts or even into annelids, are indicated.

KEY WORDS: Platyhelminthes, parasitism, phylogenetics, Monogenea, Cestoda, Digenea, gene trees, life-history evolution

INTRODUCTION

Phylogenies aim to reveal patterns of inter-relatedness and the radiation of constituent taxa. Inferring evolutionary processes from phylogenies, the comparative method (HARVEY & PAGEL, 1991), is a well-established practice and one that has become increasingly more refined as our understanding and development of tree-building methods improves, and more popular as new sources of comparative data, particularly molecular, become available (e.g. PAGEL, 1998). In spite of the early seminal work on platyhelminth phylogenetics coming from turbellarian workers (e.g. EHLERS, 1985a,b) it has been the parasitologists who have embraced the technique wholeheartedly. The nature

of parasitism, its commercial and medical consequences and the wealth of comparative information concerning parasites no doubt allow greater scope. Regarding flatworms in this light, perhaps the most significant milestone has been the publication of *Parascript* (BROOKS & MCLENNAN, 1993), a book based on the phylogenetic assessment of numerous parasitic platyhelminths, in which the authors used trees as the basis for addressing and testing many long-standing “myths” associated with the study of evolutionary parasitology. The eloquent story-telling, unravelling of myths and ultimate enlightenment as each tree yields its secrets, herald a most compelling union between systematics and comparative flatworm biology. Alas, the book has not been without its critics as the foundations upon which it draws its strength, namely its trees and the characters that form them, have been denounced, reproached and debated sufficiently (e.g.

PEARSON, 1992; CAIRA, 1994; ROHDE, 1996) to erode them and to induce caution or suspicion. Indeed, whilst phylogenetics clearly holds the key to unravelling evolutionary mysteries, a review of the flatworm literature reveals few single trees that consistently and strictly bifurcate, satisfy all those scrutinising their foundations (i.e. homology assessment, character definition and coding), or provide topologies congruent with those trees derived from additional, independent sources of data.

Whilst congruence and consensus provide us with the necessary confidence to proceed with the comparative method, it is always compelling to take stock of the phylogenetic trees available and interpret the biological consequences of accepting them. Here we take four examples from our own interests and show how the evolution of parasitism can be interpreted from available phylogenies. The first example dwells on the variety of phylum-wide phylogenies and the consequences of accepting any one of them, highlighting the need for congruence and consensus. The second emphasises the need to treat any conflict between gene trees and species trees with care, reviewing the apparent paraphyletic status of the Monogenea based on molecular data. The third and final examples demonstrate the power and frustration of phylogenetics in interpreting the evolution and radiation of the most speciose of platyhelminth and parasitic groups, the digeneans and cestodes. All the examples pose more questions than they answer, hence the title of this contribution.

THE EXAMPLES

Example 1. Origins and evolution of parasitism in the Phylum Platyhelminthes

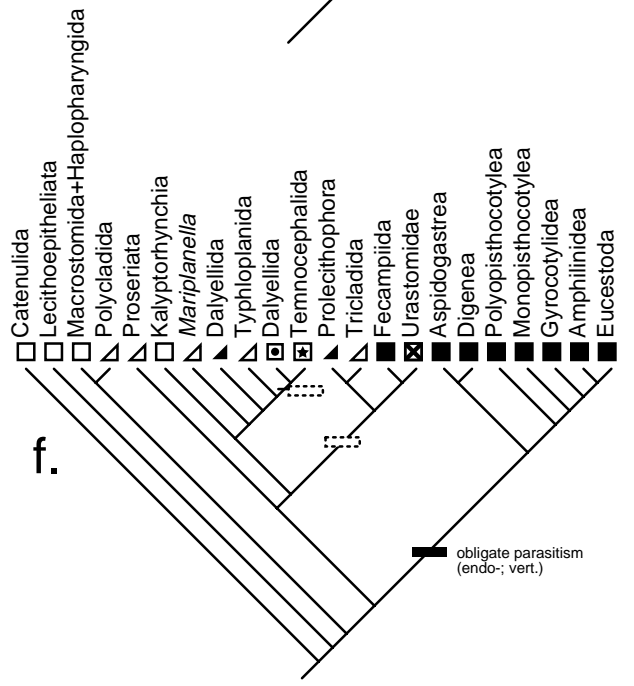
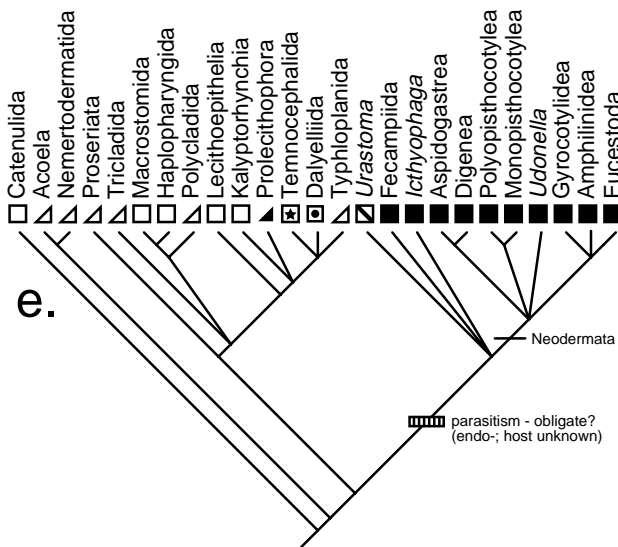
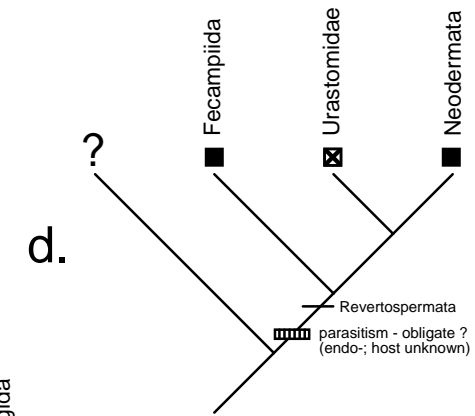
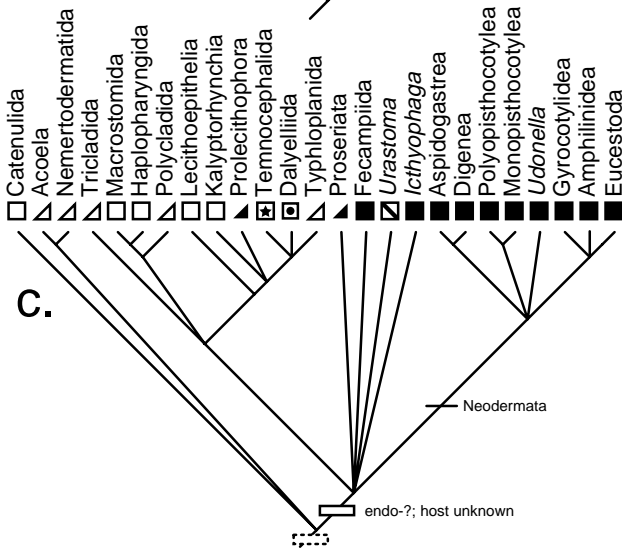
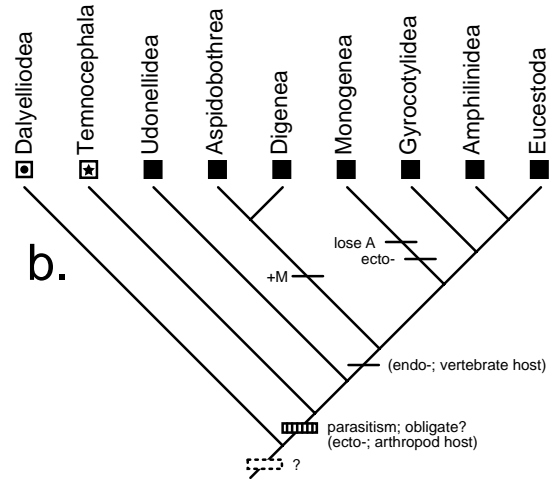
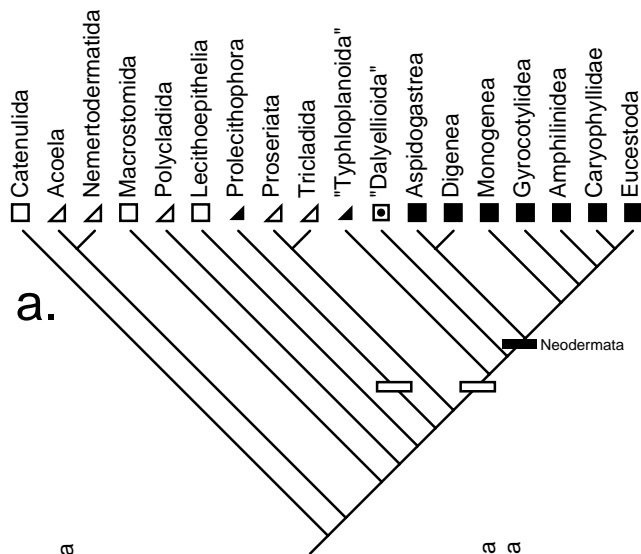
The term 'parasitism' means different things to different workers, yet it is generally accepted that an association between host and parasite is to the detriment of the host and the benefit of the parasite. Commensals live in close association with other organisms and, although their existence may appear inextricably linked with another organism, their survival is not thought to depend fully on the association. In contrast, obligate parasites require hosts for the completion of their life-cycle and generally derive some or all of their nutrients from their host during the parasitic phase. The Neodermata include the most familiar of obligate flatworm parasites, and the very presence of a neodermis appears to be inextricably linked at the physical, biochemical and immunological levels (TYLER & TYLER, 1997) with host-parasite interactions that protect and nurture the parasite. Arguably, the neodermis contributes most significantly to the success of the neodermatan taxa. Nevertheless, many 'turbellarians' are found in close association with other taxa; 200 species from 35 families live in permanent association with other animals according to JENNINGS (1971; 1974). A few groups within this paraphyletic assemblage include flatworms that are also exclusively parasitic: members of the

Urastomidae (ROHDE, 1994a), and the Fecampiida (WILLIAMS, 1988). Members of the Temnocephalida are almost exclusively ectocommensals on a variety of hosts, but predominantly freshwater crustaceans (CANNON & JOFFE, in press), and appear to be the most modified of turbellarians for this mode of life. However, among the remaining turbellarian taxa that include species living in close association with other animals, few demonstrate obvious morphological adaptations to a parasitic way of life, such as the development of attachment organs, although many show nutritional and respiratory adaptations to parasitism (JENNINGS, 1997). The incidence of commensalism is high and few higher order taxa of flatworms appear to include exclusively free-living species. One might argue that generally, the phylum demonstrates a propensity towards parasitism.

Fig. 1 illustrates a selection of six phylogenetic hypotheses that each has a bearing on our understanding of parasitism and commensalism in the phylum (see legend for full details). Figs 1a-e illustrate phylogenies that are based solely on morphological characters, and Fig. 1f represents a tree derived from small subunit (SSU) ribosomal DNA. EHLERS' pectinate scheme (EHLERS, 1985a,b) illustrates the major single evolutionary event that was the emergence of the Neodermata; Fig. 1a. His grouping of the "Dalyellioida" includes many taxa with molluscan, annelid and crustacean hosts, and its sister-group status to the Neodermata would indicate a common ancestry that, based on numbers, has a proclivity for parasitism. Identification of sister-groups is the basis of phylogenetics, and therefore also the comparative method. Brooks' scheme, shown in Fig. 1b (BROOKS et al., 1985; BROOKS & MCLENNAN, 1993), argues for ectoparasitism and an arthropod host as plesiomorphic conditions for the obligate parasites. Whilst from a strictly parasitological point of view the move from ecto- to endoparasitism seems compelling, the evidence uniting temnocephalans as sister-group to the Neodermata is contentious (ROHDE, 1994b; LITTLEWOOD et al., 1999a; CANNON & JOFFE, 2001). Additionally, both morphological (BOEGER & KRITSKY, 2001) and molecular data (LITTLEWOOD et al.,

Legend to Fig. 1 (see opposite page)

Phylogenetic trees from various sources illustrating the interrelationships of key parasitic platyhelminths, with an indication of the life-history strategy of constituent taxa and the possible origins of obligate parasitism; from a. EHLERS (1985a) based on morphology; b. BROOKS & MCLENNAN (1993) based on morphology, indicating intermediate host use and endo/ecto parasitism in/on final host; c. solution based on ROHDE's initial matrix argued in LITTLEWOOD et al. (1998); d. the interrelationships of the Revertospermata, argued by KORNAKOVA & JOFFE (1999); e. solution based on revised matrix of Rohde, argued in LITTLEWOOD et al. (1998); f. maximum parsimony solution of 270 complete SSU rDNA genes, excluding aceolomorphs in LITTLEWOOD & OLSON (2001). Symbols indicate the frequency of commensals and parasites within taxa – see key.



- free-living
- △ includes some commensals
- ▲ includes some parasites/commensals
- ▴ predominantly parasites/commensals
- ⊠ exclusively parasites/commensals
- ▣ facultative parasites
- ⊞ includes fac./obl. parasites
- obligate parasites

- +M — gain mollusc intermediate host (Trematoda)
- lose A — arthropod host lost
- ecto- — ectoparasitic adult
- endo- — endoparasitic adult
- ⋯ possible origin of parasitism?
- origin of parasitism
- ▣ possible origin of obligate parasitism?
- origin of obligate parasitism

1998) argue strongly for udonellids as monopisthocotylean monogeneans. Brooks' eloquent interpretations based on the phylogeny of the 'cercomerians' fails at the point where inferences are drawn on the basis of the positions of Temnocephalida and Udonellidea as critical polarising sister taxa, simply because their position is so poorly supported. Indeed, it is the identity of the sister-group to the Neodermata that is pivotal to the discussion of obligate parasitism.

ROHDE's explicit morphological character matrix, constructed for a phylum-wide estimation (in LITTLEWOOD et al., 1999a), failed to resolve a clear sister-group candidate to the Neodermata (Fig. 1c), in contrast to his earlier prediction based on a non-cladistic assessment, that the Fecampiida occupied this position (ROHDE, 1990). Considering this unresolved, highly polytomous tree, we may infer that the origin of parasitism is as likely to be at the radiation of the Rhabditophora as it is to be at the radiation of the Neodermata. Most recently, the examination of sperm morphology suggested that Urastomidae is the sister-group to the Neodermata with Fecampiida completing a clade of obligate parasites, the Revertospermata (KORNAKOVA & JOFFE, 1999); see Fig. 1d. Fecampiids are parasites of decapod and isopod crustaceans (JENNINGS, 1971), and urastomids are parasites of molluscs and teleosts. If members of the Urastomidae and Fecampiida are truly the closest living relatives of the neodermatans, then it is not possible to predict the plesiomorphic host phylum of the Revertospermata, although, as all revertospermata are found within their host's tissues, they would presumably have been endoparasitic. Given the very different nature of parasitism and host identity among members of Urastomidae and Fecampiida, these large-scale phylogenetic assessments suggest that finer phylogenetic resolution of these taxa would be well worth pursuing, particularly with a view to tracking the appearance of obligate parasitism. *Ichthyophaga*, unlike other urastomids, is an obligate parasite found embedded in the tissues of teleost fishes and thereby shares features with the predicted ancestor of the Neodermata (LITTLEWOOD et al., 1999b).

The inclusion of the 'revertospermata' sperm data into ROHDE's morphological matrix yielded a more resolved tree (LITTLEWOOD et al., 1999a) suggesting a Urastomidae+Fecampiida clade as sister-group to the Neodermata; Fig. 1e. The wide distribution of parasites and commensals within the remaining large neophoran turbellarian clade once again does not rule out the possibility that parasitism originated at the base of the Rhabditophora.

Finally, SSU rDNA sequences of 270 taxa (LITTLEWOOD & OLSON, 2001) maintain the accepted interrelationships of the Neodermata, albeit with the Monogenea paraphyletic (see Example 2 below), with a distinct but poorly supported sister-group of neophoran turbellarians, and notably including highly derived Fecampiida+Urastomidae. There are few unambiguous morphological characters that support the major group-

ings of the turbellarians in this molecular tree. Furthermore, obligate parasitic turbellarian groups are dispersed throughout the tree, suggesting no fewer than three distinct origins of obligate parasitism, although again, from a parsimony principle it is equally likely that obligate parasitism was the plesiomorphic condition for the Rhabditophora.

Which scenario is correct? Until the incongruence between the independently derived molecular and morphological trees can be reconciled, and a morphologically based matrix can be refined and improved, including additional information and refinements that reflect the problems associated with character coding (e.g. FOREY & KITCHING, 2000), we are left with few well-resolved clades that reflect the phylogenetic content of independent data sets. Notably, Brooks' interpretation of the radiation of the Neodermata (BROOKS & MCLENNAN, 1993; LITTLEWOOD et al., 1999b) holds true as the interrelationships of its constituent groups appear well resolved (but see Example 2 below). However, the origins of parasitism throughout the phylum cannot, as yet, be recovered. Indeed, if it is the case that a large clade of neophoran turbellarians is truly the sister-group to the Neodermata, we will neither be able to predict the plesiomorphic host nor have much confidence in whether the first parasites were ecto- or endoparasitic. Nevertheless, the divergence patterns of the neophoran turbellarians will still hold a key to understanding the prevalence and radiation of parasitic taxa.

It is generally accepted that once a lineage embarks upon parasitism as a way of life (certainly endoparasitism), there is no going back, and there appear to be few phylogenies of any taxonomic group that suggest the adoption of parasitism is a reversible process (see SIDDALL, 1993, and references therein). Even in the Nematoda, a group that includes many parasites and free-living species throughout its constituent taxa, interpretations of phylogenies based on evolutionary parsimony suggest multiple origins of parasitism rather than secondary loss of parasitism (DORRIS et al., 1999). At what point is the irreversibility set? Presumably, only when a species is truly an obligate (endo)parasite, of which there are only a few well-proven examples in the turbellarian flatworms. Thus, although many of the phylogenies in Fig. 1 suggest the appearance and disappearance of commensalism and association with a host on multiple occasions, until finer phylogenetic resolution of the obligate turbellarian parasites is established, we cannot fully resolve the number of times obligate parasitism has arisen in the phylum, nor test whether it may be a reversible process.

Example 2. Monogeneans, morphology, molecules and the question of monophyly

The interrelationships of the Neodermata are well argued from morphology (EHLERS, 1985b), and preliminary combined morphological and molecular evidence is wholly

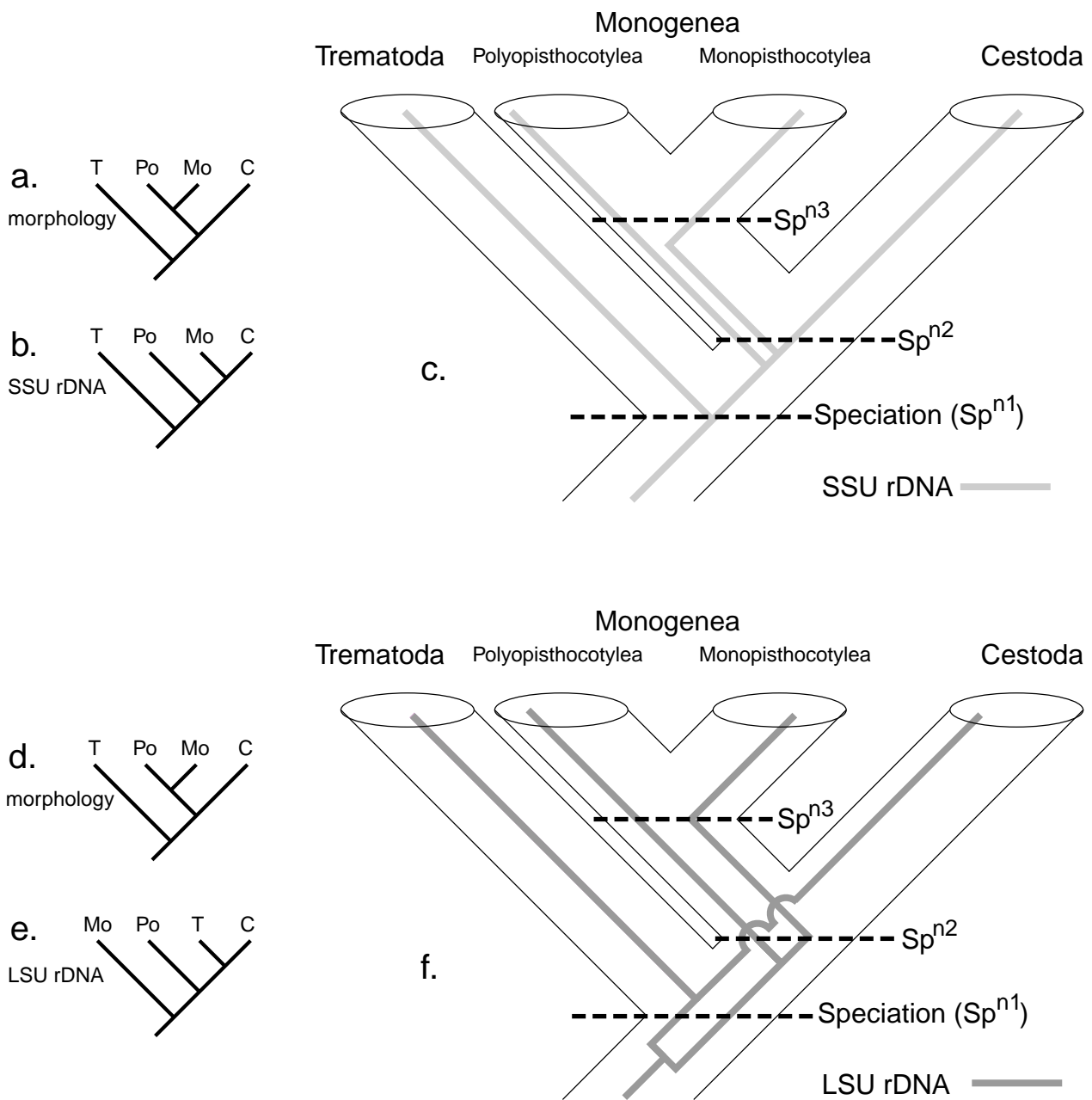


Fig. 2. – Species and gene trees for the Monogenea. Most morphologically based phylogenies argue for monophyly among the Monogenea (a, d). SSU rDNA consistently argues for a paraphyletic solution (LITTLEWOOD et al., 1999a; LITTLEWOOD & OLSON, 2001) with Polyopisthocotylea as basal monogeneans (b). When mapped on to the morphology tree, deep coalescence of the SSU gene prior to the divergence of monogeneans and cestodes, such that the genes had already split during the stem-lineage of the Monogenea, could account for incongruence (c). However, LSU rDNA (e) argues for a very different solution to morphology (e) and problems in lineage sorting are unlikely to account for the incongruence (f). Speciation events (Sp^{n1-3}) are discussed in the text.

congruent (LITTLEWOOD et al., 1999a) with the traditional view (Fig. 2a). Recent morphological data strongly support the contention that the Monogenea is a monophyletic group (BOEGER & KRITSKY, in press), but ribosomal DNA consistently supports paraphyly (MOLLARET et al., 1997; LITTLEWOOD et al., 1998). Disturbingly, the order of paraphyly depends on the gene utilised (SSU and LSU offer different results; Fig. 2b,e) and whilst a call for morphological reassessment is clearly justified (JUSTINE, 1998), in the absence of additional molecular or morphological evidence

to support paraphyly, the three possibilities (Fig. 2a, b, e) need to be addressed independently or reconciled. It is worth noting here that LITTLEWOOD et al. (1999b) incorrectly reported that the difference between SSU and LSU was simply the relative placement of the Monopisthocotylea and the Polyopisthocotylea (compare Fig. 2b and 2e); MOLLARET et al.'s (1997) interpretation of neodermatan interrelationships suggested that Trematoda and Cestoda were sister-groups.

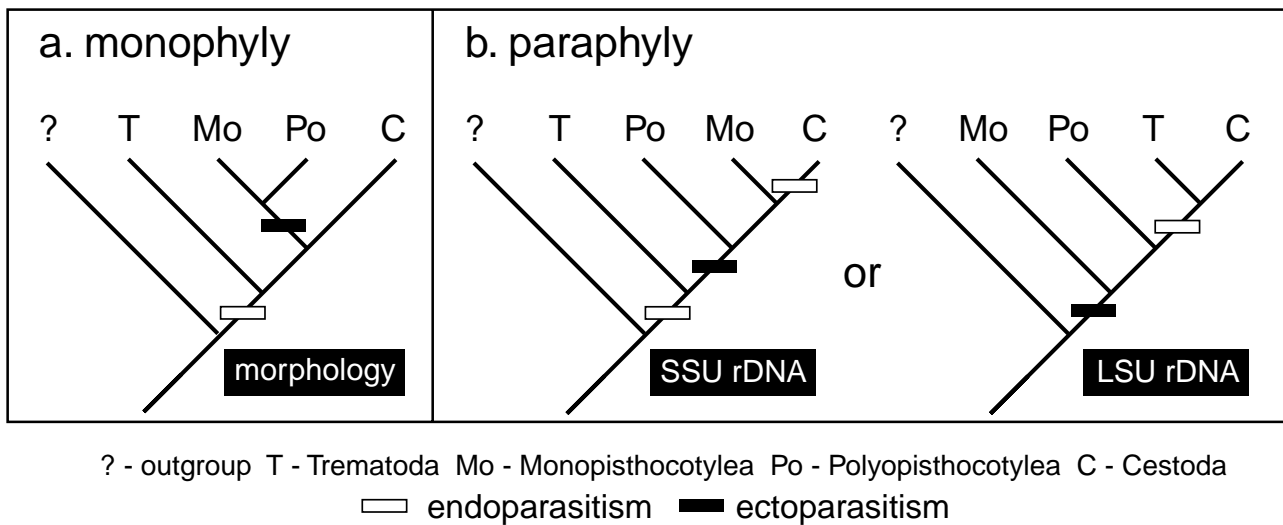


Fig. 3. – Possible origins and evolution of endo- and ectoparasitism in the Neodermata depending on the monophyly/paraphyly of the Monogenea and interrelationships estimated from a. morphology (e.g. EHLERS, 1985b), and b. molecular data; SSU rDNA (e.g. LITTLEWOOD et al., 1999a) and LSU rDNA (MOLLARET et al., 1997).

From an evolutionary parsimony perspective where the number of changes is minimised, a monophyletic Monogenea suggests that the shift toward ectoparasitism was a single event, even when we are uncertain of the sister-group to the Neodermata; Fig. 3a (LITTLEWOOD et al., 1999b). Paraphyly (Fig. 3b) requires that if endoparasitism was the plesiomorphic condition, suggested by morphology and SSU rDNA (LITTLEWOOD et al., 1999b), ectoparasitism arose at the base of the Cercomeromorphae (Cestoda+Monogenea), and along the cestode lineage endoparasitism was reacquired. However, if ectoparasitism was the plesiomorphic condition, suggested by LSU rDNA, then endoparasitism appeared just once, with the divergence of the Trematoda and Cestoda. Intuitively, in the case of paraphyly we might predict that ectoparasitism was the more likely plesiomorphic condition for the Neodermata, unless one considers the neodermis a likely adaptation to endoparasitism, but it seems highly unlikely that Trematoda and Cestoda are sister-groups (e.g. see BYCHOWSKY, 1937; ROHDE & WATSON, 1995; LITTLEWOOD et al., 1999b). Furthermore, the monophyly of the Monogenea remains the favoured solution as neither paraphyletic tree based on molecular data is particularly well supported at its base, there are a number of well-argued morphological synapomorphies for the Monogenea, and paraphyly requires a greater number of life-style switches, at least for the SSU data. If the gene trees are a correct estimation of the divergence of SSU and LSU rDNA, how do we reconcile the morphological solution? Figs 2c and 2f map each of the gene trees onto the morphologically based species tree. A number of possibilities exist for having a gene tree differing from a true species tree and include gene duplication, problems in lineage sorting, and horizontal gene transfer (PAGE & HOLMES, 1998). For the purposes of this discussion we will not discuss the last and, although ribosomal gene paralogy is known with SSU rDNA in triclads (CARRANZA et

al., 1996; CARRANZA et al., 1999), there is currently no evidence to suggest this has occurred within the Neodermata. Lineage sorting problems, detected by tracing gene phylogenies back in time, relate to the failure of gene alleles to coalesce before the time when species diverge. In the words of SLOWINSKI & PAGE (1999, p.815) “deep coalescence can produce conflict between a gene tree and the overlying species tree because there is a window of opportunity for a sequence from a less related species to coalesce with one of the descendant sequences of the deep coalescence”. However, invoking deep coalescence as the basis for gene and species tree discrepancies for the Monogenea is not wholly satisfactory. The scenario presented for SSU rDNA (Fig. 2c) suggests that between the second and third speciation events (Sp^{n2} and Sp^{n3}), the SSU genes that evolved within the stem lineage of the Monogenea have a more ancient coalescence time, which pre-dates the age of the lineage (see PAGE & HOLMES, 1998 for further examples and rationale), and whilst this single example remains plausible, the situation for LSU rDNA appears highly tenuous (Fig. 2f). The simpler, but incorrect, interpretation of LSU-based paraphyly (Fig. 6b in LITTLEWOOD et al., 1999b) could be explained by deep coalescence time in a way similar to that proposed in Fig. 2c (with the gene lineages of the Monopisthocotylea and Polyopisthocotylea transposed). However, whilst SSU and morphology based trees are compatible under lineage sorting problems, LSU is compatible with neither. LSU and SSU ribosomal genes are members of the same tandemly repeated chromosomal arrays and we would expect concerted evolution to at least provide congruent gene trees (HILLIS & DIXON, 1991).

Another, perhaps more powerful interpretation is that there must have been a relatively short period between the monogeneans diverging from the cestodes and when this stem lineage split into the Monopisthocotylea and

Polyopisthocotylea. Molecular changes accumulated within the stem lineage of the Monogenea have either been obliterated through base saturation, or were very few, and cannot be resolved satisfactorily with either nuclear ribosomal gene fragment. It remains that morphology suggests that a number of important morphological changes took place that unite the Monogenea (for examples see EHLERS, 1985a; LITTLEWOOD et al., 1999a; BOEGER & KRITSKY, 2001) and existing molecular data are incapable of resolving this stem lineage.

Example 3. Cestodes and the evolution of segmentation and attachment

Segmentation is a hallmark of the eucestodes and represents one of the evolutionarily novel means by which the parasitic neodermatans have achieved their enormous reproductive capabilities. It is not universally observed among cestodes, however, and thus the evolution of the trait within the group can be examined by means of a phylogenetic tree derived from other sources of data (e.g. molecules; Fig. 4). The strobilate (segmented) condition may be seen as the result of two separate processes: proglottization, the serial repetition of the gonads; and segmentation, the external division of the proglottids into self-contained compartments that may develop and become fertilized independently of the parental worm. The evolutionary advantages of these processes differ; proglottization increases fecundity, whereas segmentation can allow for development and fertilization to occur in niches other than that occupied by the parental worm (e.g. in the external

environment). Evidence for the recognition of strobilation being the result of two processes rather than one is found in the peculiar form of the members of the Spathebothriidea, which exhibit proglottization without external segmentation. Albeit rare, this condition is also found in higher eucestodes such as the pseudophyllidean *Anantrum tortum* Overstreet 1968, and to a lesser extent, in the nippotaeniids which show only weak external segmentation. As the closest relatives of the eucestodes (the cestodarian groups Amphilinidea and Gyrocotylidea) are themselves non-strobilate, it follows that the two non-strobilate eucestode groups, Caryophyllidea and Spathebothriidea, represent the ancestral condition and are placed most parsimoniously at the base of the eucestode tree. The utilisation of oligochaete, rather than arthropod, intermediate hosts has also been argued to provide support for a basal position of the Caryophyllidea (HOBERG et al., 1999); life-histories of spathebothriideans are as yet unknown (BEVERIDGE, 2001). Indeed, BURT & JARECKA (1982) went as far as to propose that the caryophyllidean genus *Archigetes*, species of which may obtain reproductive maturity in oligochaetes, represented the first true tapeworms; however, as the eucestodes are otherwise universally observed to have at least one intermediate host and a vertebrate definitive host, it seems more likely that the life-cycle of *Archigetes* spp. evolved through progenesis of a larval stage (MACKIEWICZ, 1982). Despite such observations, many authors have considered the lack of segmentation in caryophyllideans and spathebothriideans to be secondarily derived from a strobilate ancestor, namely the Pseudophyllidea (e.g. FUHRMANN,

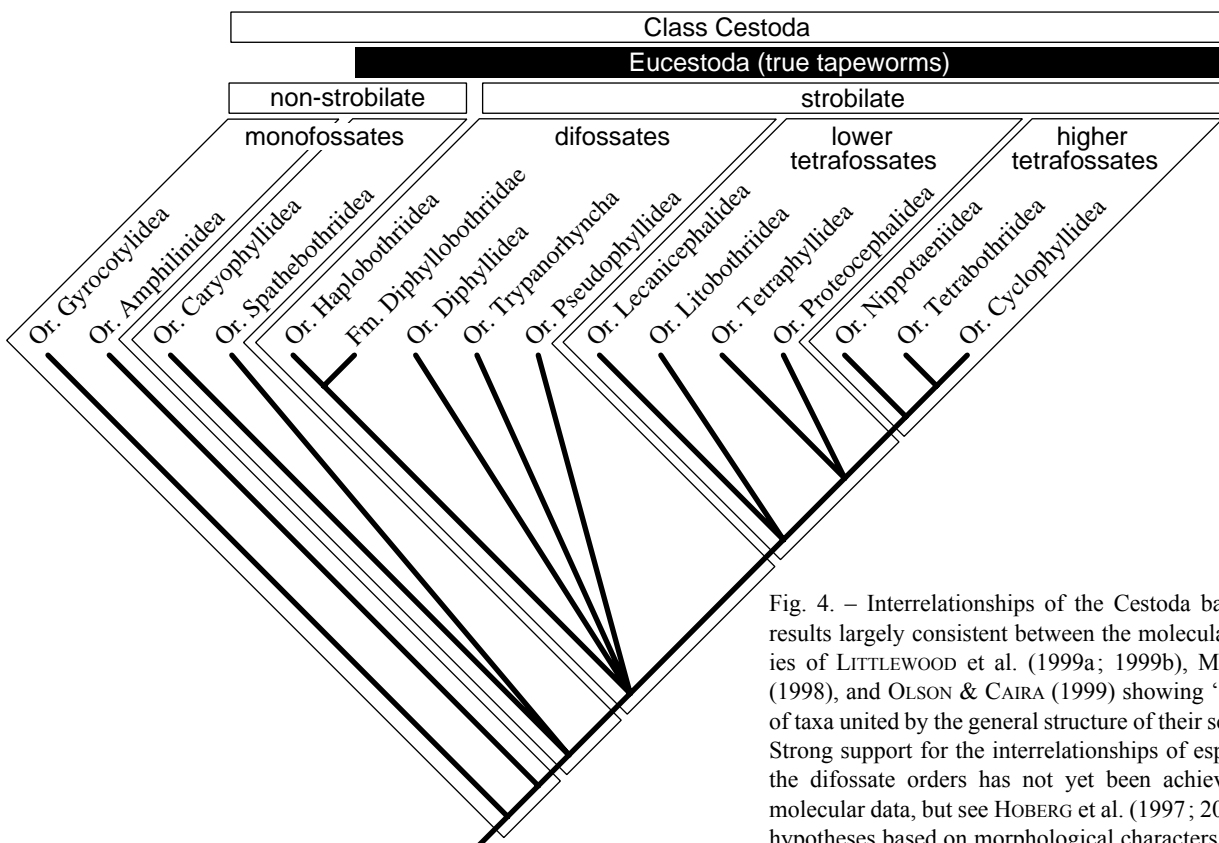


Fig. 4. – Interrelationships of the Cestoda based on results largely consistent between the molecular studies of LITTLEWOOD et al. (1999a; 1999b), MARIAUX (1998), and OLSON & CAIRA (1999) showing 'grades' of taxa united by the general structure of their scoleces. Strong support for the interrelationships of especially the difossate orders has not yet been achieved via molecular data, but see HOBERG et al. (1997; 2001) for hypotheses based on morphological characters.

1931; JOYEUX & BAER, 1961). Thus, only through an independently derived phylogeny can the evolution of segmentation be addressed objectively.

Molecular data support a basal position of the non-strobilate eucestodes (MARIAUX, 1998; OLSON & CAIRA, 1999; MARIAUX & OLSON, 2001), although the published results differ in regards to which group appeared first. MARIAUX's (1998) analysis supported the more commonly hypothesized arrangement inferring strobilation as a step-wise process evolving from non-proglottized, non-segmented worms (Caryophyllidea), to proglottized, non-segmented worms (Spathebothriidea), to the proglottized, segmented condition (higher eucestodes), consistent with previous hypotheses as well as recent analyses based on morphology (HOBERG et al., 1997; HOBERG et al., 2001). OLSON & CAIRA's (1999) work supported a basal position of the Spathebothriidea, whereas the Caryophyllidea was placed in a clade that implied the group to be secondarily non-strobilate. Newer analyses (OLSON et al., unpublished data) involving larger numbers of both exemplar taxa and sequence data are showing better support for the former hypothesis (Caryophyllidea (Spathebothriidea(strobilate eucestodes))).

Another classic example from the cestodes is the evolution of their holdfast structures. In their most general form, cestode scolices can be divided among three basic divisions: monofossate, having only a single part to the scolex; difossate, having a bipartite scolex; or tetrafossate, having four parts. Fig. 4 shows 'grades' based on these divisions. Most orders readily fit into one of the grades, whereas the scolex morphology of other groups is more enigmatic. For example, members of Haplobothriidea possess four tentacles and have occasionally been allied with the trypanorhynch on this basis (FUHRMANN, 1931). Trypanorhynch also have four tentacles, but their tentacular structures differ significantly and the scolex of a majority of species is otherwise typically difossate. Nippotaeniid scolices possess a single apical sucker thus making them monofossate. Unlike the other monofossate groups (e.g. Caryophyllidea, Spathebothriidea), however, they are strobilate worms. Litobothriids are characterized by a scolex that is cruciform in cross section and differs markedly from the scolex morphologies of other tetrafossate lineages. For groups such as these, little evidence of their phylogenetic affinities can be gained from comparison of scolex features.

Many authors have hypothesized a diphyletic evolution of the cestodes split between difossate and tetrafossate lineages (FUHRMANN, 1931; EUZET, 1959; EUZET, 1974), whereas others show a step-wise evolutionary pattern starting with the monofossate condition and culminating with the tetrafossate condition (HOBERG et al., 1997; HOBERG et al., 2001). Molecular-based results from OLSON & CAIRA (1999) supported a largely diphyletic pattern whereas MARIAUX's results (1998) supported a step-wise pattern; results of neither study were strictly diphyletic or strictly bifurcating. Both, however, supported a derived, monophyletic clade of tetrafossate groups that also included the

Litobothriidea and Nippotaeniidea (Fig. 4). Within the tetrafossate clade was another derived clade uniting the Cyclophyllidea, Nippotaeniidea, and Tetrabothriidea. Internal branch lengths and levels of support for the internodes subtending the tetrafossate clade and that of the higher tetrafossate clade within it were greater than for any other internode subtending major groupings, with the exception of that separating the Eucestoda from the cestodarian orders. From this we can infer that the monofossate condition seen in the Nippotaeniidea is a reversal to the plesiomorphic condition, and that the affinities of the Litobothriidea are with the Lecanicephalidea, not the Tetrephyllidea as suggested by the classification of EUZET (1994). Likewise, the affinities of the Haplobothriidea are among the difossate group Diphyllbothriidae (Fig. 4) despite their having four tentacles and an undivided scolex.

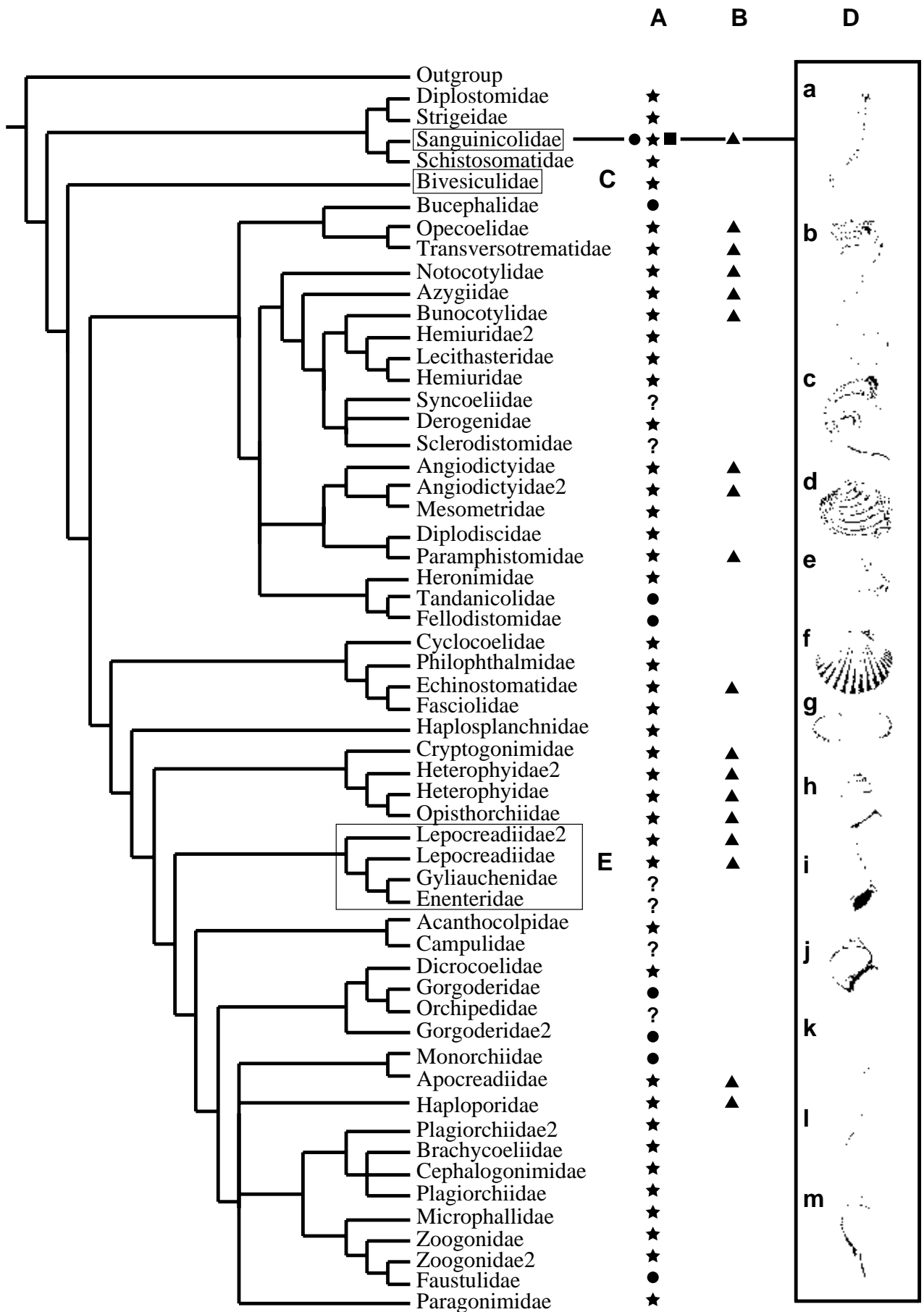
Example 4. Digenean phylogenetics and the evolution of life-history

Digenean trematodes are obligatorily parasitic in invertebrate intermediate and vertebrate definitive hosts. A key aspect of the evolution of parasitism within the group is the exploitation of different taxa as first intermediate hosts, namely gastropods, bivalves, scaphopods and a few polychaete annelids. Clearly, present-day associations are the result of the interaction between the twin processes of coevolution and host-switching but the balance between these two processes is not understood in any detail. Strict host-parasite coevolution could be expected to be reflected by distributions of parasite taxa restricted to closely related molluscs and, as a corollary, mollusc taxa infected with single parasite taxa. A credible phylogeny of the Digenea makes it possible to analyse the significance of present distributions.

To explore these issues we have constructed a database of life-cycle information for the Digenea. The data is derived from the compendium of YAMAGUTI (1975) and subsequent publications. Here we focus on the inferences that can be drawn by exploring how individual mollusc taxa are exploited by digeneans and how individual digenean taxa exploit the potential range of first intermediate hosts. The distributions are mapped on the maximum parsimony tree inferred from 18S rDNA sequence data (Fig. 5, for details see CRIBB et al., 2001);

Legend to Fig. 5 (see opposite page)

Fig. 5. – Phylogeny of the Digenea inferred from 18S rDNA showing distribution of life-cycle attributes for selected taxa. Where parasite taxa appear twice it is as an indication of putative paraphyly. **A.** The class of first intermediate hosts associated with each family: ★ Gastropoda, ● Bivalvia, ■ Polychaeta. **B.** Families that infect hydrobiid gastropods. **C.** The nearly basal taxon Bivesiculidae, which infects only cerithiid gastropods. **D.** Hosts of Sanguinicolidae: Polychaeta – a. Ampharetidae, b. Serpulidae, c. Terebellidae; Bivalvia – d. Veneridae, e. Donacidae, f. Pectenidae, g. Solemyidae; Gastropoda Prosobranchia – h. Bithyniidae, i. Hydrobiidae, j. Viviparidae; Gastropoda Pulmonata – k. Ancyliidae, l. Lymnaeidae, m. Planorbidae. **E.** – see text.



A. The distribution of trematodes that use bivalves as first intermediate hosts (Fig. 5A) shows six entirely separate occurrences (members of Allocreadiidae and Gymnophallidae also use bivalves but these taxa are not yet incorporated in the phylogenetic analysis). Because all the other taxa in the analysis use gastropods as first intermediate hosts, this distribution allows the parsimonious inference that parasitism of bivalves has arisen independently within the Digenea at least six times, instead of the other possibilities of being plesiomorphic or suggesting relationship between the taxa concerned. This observation also emphasises how frequent and dramatic host-switching has been in the evolution of the Digenea.

B. The Hydrobiidae is a group of cosmopolitan prosobranch gastropods found in fresh and brackish water. Populations of hydrobiids are frequently extraordinarily dense and heavily infected with trematodes and, as a result, have been studied extensively. Fifteen families of trematodes in the present analysis have been reported from hydrobiids. These are distributed very widely in the phylogeny of the Digenea (Fig. 5B). Such a distribution can be interpreted as the result of either extensive coevolution or of extensive host-switching (or of course as a mixture of both processes). The extent to which these families are also found in other groups of molluscs resolves this question. Of the 15 families reported from hydrobiids, 13 are also known from other gastropod families and 12 from other than the Rissooidea, the superfamily to which the Hydrobiidae belongs. We thus infer that, although some coevolution may be obscured, in general the hydrobiids have become infected through repeated cases of host-switching which relates in turn to their "attractiveness" as intermediate hosts.

C. There is extraordinary variation in the host-specificity shown by families of trematodes. At one extreme the Bivesiculidae is known only from the prosobranch gastropod family Cerithiidae. The implication of such a distribution is, presumably, that the evolutionary history of the parasite family is linked to that of the mollusc family. In the case of the Bivesiculidae this creates a still unresolved problem. The basal position of the family (Fig. 5C) suggests that it may be a relatively ancient taxon (perhaps consistent with elements of its morphology) but there is nothing particularly ancient about the Cerithiidae, known definitively from no earlier than the Upper Cretaceous (HEALY & WELLS, 1998). It seems certain that the Digenea had undergone its major radiations well before the Upper Cretaceous so that this host-parasite distribution is enigmatic.

D. At the other extreme the Sanguinicolidae, the fish blood flukes, are known from 16 families of first intermediate hosts including bivalves, prosobranch and pulmonate gastropods, and polychaete annelids (Fig. 5D). Members of Sanguinicolidae have a broad distribution within fishes (both teleosts and chondrichthyans). Because the Sanguinicolidae falls within the most basal group of Digenea, the host distribution of the Sanguinicolidae is consistent with an ancient coevolution-

ary radiation between these host groups. However, this hypothesis requires exploration by resolution of phylogenetic relationships within the Sanguinicolidae, information that is not yet available.

E. Finally we can attempt to use the relationships inferred from the phylogeny to predict the intermediate hosts of digeneans for which no first intermediate hosts are known. For example, the Gyliuchenidae and Eenteridae, parasites of marine herbivorous fishes, occur in the clade containing the Lepocreadiidae (Fig. 5E). Many lepecreadiid cercariae are known, but none are known for the Gyliuchenidae or Eenteridae. The first intermediate hosts of lepecreadiids are all gastropods, Subclass Orthogastropoda, Superorder Caenogastropoda, Order Sorbeoconcha and include representatives from four superfamilies (Conoidea, Muricoidea, Naticoidea and Rissooidea) and nine families. Because the parasite phylogeny suggests that the Gyliuchenidae and Eenteridae are families derived from within the Lepocreadiidae we might predict that the first intermediate hosts would be from the Sorbeoconcha. Unfortunately the Sorbeoconcha includes dozens of families so that the predictive power is limited.

For the understanding of the evolution of the digenean life-cycle, the phylogeny of the Digenea solves some questions satisfactorily, leaves others ambiguous, and identifies whole new areas of inquiry. Most importantly, the preliminary nature of the gene tree on which these hypotheses are based must be emphasised (see CRIBB et al., 2001), and our future efforts are set to address this issue as much as to test hypotheses of life-history evolution.

CONCLUSION

As with any evolutionary interpretation dependent upon an estimate of phylogeny, the utility of the approach relies almost exclusively on the validity of the tree and the underlying data that it is founded upon. We have shown that there remain some serious discrepancies between independent estimates of plathyhelminth phylogeny, at a multitude of taxonomic levels. Such incongruence needs to be reconciled, as might be possible in some instances of gene/species tree mismatching, or perhaps more likely, additional data needs to be gathered. Even in the absence of conflict we are ever mindful that relatively low nodal support values (estimates of the strength of the tree) limit the confidence in our subsequent interpretations. Nevertheless, with examples from the Cestoda and Digenea, we have shown that phylogenetics remains the most profitable key to understanding the evolution of parasites and parasitism.

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