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Phylogenetic Analysis of the Rhabdocoela (Platyhelminthes) with emphasis on the Neodermata and Relatives

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Phylogenetic systematic analysis of 24 taxa representing the rhabdocoel platyhelminths, based on a suite of 89 morphological characters, produced two equally parsimonious trees, 181 steps long, with a consistency index (CI) of 0.69 and a rescaled consistency index (RCI) of 0.56, differing only with respect to that portion of the tree containing Umagillidae, Acholadidae, Graffillinae, Pseudograffillinae, Pterastericolidae and Hypoblepharinidae. Our results accommodate all previously proposed sister taxa to the Neodermata in a single clade in which ((Dalyelliidae + Temnocephalida) Typhloplanidae) is the sister group of ((Fecampiidae + *Urastoma*) (*Udonella* ((Aspidogastrea + Digenea) (Monogenea (Gyrocotylidea (Amphilinidea + Eucestoda)))))). Bootstrap and jackknife analyses indicate that the groupings of ((Dalyelliidae + Temnocephalida) Typhloplanidae) and of ((Fecampiidae + *Urastoma*) (*Udonella* ((Aspidogastrea + Digenea) (Monogenea (Gyrocotylidea (Amphilinidea + Eucestoda)))))) are highly robust, with the latter clade having a CI of 90% and RCI of 82%. Disagreements among previous analyses of these taxa have been due to the influence of missing data for critical characters in key taxa and differences in the taxa analysed, rather than any inherent weakness in the morphological data. Non-phylogenetic systematic approaches to homology assessment and misconceptions regarding phylogenetic systematic methodology are discussed. Recent analyses combining sequence data with a subset of approximately 60% of the morphological characters should be re-assessed using the entire morphological database. Even if *Udonella* is a monogenean, it is most parsimonious to suggest that the common ancestor of the Neodermata had a vertebrate–arthropod two-host life cycle.

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Introduction

The phylogenetic relationships among members of the phylum Platyhelminthes have received extensive scrutiny for nearly 20 years. Ehlers (1984) published the first phylogenetic systematic treatment for the phylum at about the same time as parasitologists were turning their attention towards intensive phylogenetic analysis of the parasitic groups within that phylum (the Neodermata and relatives: Brooks 1982, 1989a,b; Brooks *et al*. 1985a,b). Although most of the studies since those initial attempts have produced remarkably congruent results, there have been some disagreements, especially about the identity of the sister group to the Neodermata (Brooks 1982, 1989a,b; Ehlers 1984, 1985a,b, 1986; Brooks *et al*. 1985a; Rohde 1990, 1991; Rohde *et al*. 1990; Brooks & McLennan 1993c; Jondelius & Tholleson 1993; Williams 1993; Watson 1997).

Congruence notwithstanding, some parasite taxonomists (e.g. Rohde 1990, 1994a, 1996) have objected to the hypothesized relationships among the parasitic groups, the choice of characters and the evolutionary implications of the phylogenetic systematic analyses, which call into question a number of long-standing myths about parasite evolution (Brooks & McLennan 1993a,b,c). More recently, the debate has shifted to assertions that molecular data are inherently superior to morphological data as markers of phylogeny (e.g. Justine 1998b; Littlewood *et al*. 1999a,b; Litvaitis & Rohde 1999). Recent molecular studies, for example, have either ignored (e.g. Baverstock *et al*. 1991; Blair 1993; Litvatis & Rohde

1999) or minimized (Rohde *et al*. 1995; Littlewood *et al*. 1999a,b) the extensive morphological database that has been collected for the parasitic platyhelminths over the last 200 years. The assertion that morphological data are not as reliable as molecular data is a curious one, given that: (1) morphological studies routinely produce fewer equally parsimonious trees with better goodness of fit values than their wholly molecular counterparts; and (2) molecular studies have often produced results virtually identical to those already published by morphologists (e.g. Hoberg *et al*. 1997, in press; Mariaux 1998). This same debate has been carried out by systematists working on many different taxa. The result of the debate has been widespread agreement that the goal of systematics should be the production of phylogenetic hypotheses based on the most parsimonious (i.e. most scientifically robust) arrangement of all available evidence (see Kluge 1989, 1997, 1998a,b, 1999).

Jondelius & Tholleson (1993) provided the first direct phylogenetic systematic link between intense analysis of the parasitic groups and extensive analysis of the Platyhelminthes as a whole with their pioneering analysis of the Rhabdocoela. In this study, we present an updated analysis of the Rhabdocoela incorporating new character information that has been collected since the study by Jondelius & Tholleson (1993). The emphasis of this study is on the Neodermata and their closest relatives. We are particularly interested in answering two questions. What is the sister group of the Neodermata? Do the new data support or refute previous hypotheses of phylogenetic relationships within the Neodermata? In doing so, we will discuss the rationale for *a priori* exclusion of many morphological characters from recent phylogenetic analyses of the taxa herein considered. In this regard, we hope to show that the database of suitable morphological characters is far larger than that used in recent 'total evidence' studies.

Materials and methods

Taxa

The following taxa were included in this study (see also Jondelius & Tholleson 1993): Umagillidae, Pseudograffillinae, Graffillinae, Acholadidae, Pterastericolidae, Fecampiidae, Hypoblepharinidae, Dalyelliidae, Provorticidae, Temnocephalida, Kytorhynchidae, Promesostomidae, Solenopharyngidae, Trigonostomidae, Typhloplanidae, Kalyptorhynchia, *Urastoma*, *Udonella*, Aspidogastrea, Digenea, Monogenea, Gyrocotylidea, Amphilinidea and the Eucestoda.

Character list

Characters were recorded based upon extensive descriptions in the literature: Aken'ova & Lester (1996); Bandoni & Brooks (1987a,b); Boeger & Kritsky (1993, 1997); Brooks (1982, 1989a,b); Brooks & McLennan (1993a,b,c); Brooks *et al*. (1985a,b, 1989, 1991); Bullock (1965); Cannon (1982, 1987); Ching & Leighton (1993); Christensen (1976); Christensen & Kanneworff (1965); DeClerk & Schockaert (1995); Ehlers (1984, 1985a,b, 1986, 1995); Ehlers & Sopott-Ehlers (1993); Fleming (1986); Fleming *et al*. (1981); Hoberg *et al*. (1997, in press); Hyman (1951); Ivanov (1952); Joffe & Kornakova (1998); Jondelius (1991, 1992); Jondelius & Tholleson (1993); Justine (1990, 1991, 1993, 1995, 1998a); Kanneworff & Christensen (1966); Kornakova & Joffe (1999); Koie & Bresciani (1973); Lee (1972); Littlewood *et al*. (1998, 1999a); Noury-Srairi *et al*. (1989a,b); Rohde (1986a,b, 1987, 1989, 1990, 1991, 1994b, 1998); Rohde & Watson (1993); Rohde *et al*. (1987a,b, 1989a,b, 1992, 1995, 1999); Shinn & Christensen (1985); Sopott-Ehlers (1991, 1996, 1998, 2000); Sopott-Ehlers & Ehlers (1995, 1997, 1998); Watson (1997, 1998a,b); Watson & Jondelius (1995); Watson & L'Hardy (1995); Watson & Rohde (1994a,b, 1995a,b,c); Watson & Schockaert (1996, 1997); Watson *et al*. (1992, 1995); Williams (1993); Wirth (1984); Xylander (1986, 1987a,b,c,d, 1988a,b, 1989, 1990). Characters were polarized using information on platyhelminth groups other than the Rhabdocoela summarized primarily in Ehlers (1984, 1985a,b, 1986, 1995), Jondelius & Tholleson (1993) and Littlewood *et al*. (1998, 1999a). '?' indicates that the state of the character is unknown in a particular taxon. Higher taxa that are polymorphic for a character were coded with the plesiomorphic state, as per Jondelius & Tholleson (1993) and standard phylogenetic systematic practice (Wiley 1981; Brooks & McLennan 1991; Wiley *et al*. 1991, in press; McLennan & Brooks, in press). Table 1 is the data matrix.

Spermatozoal ultrastructure

1 Number of sperm axonemes. Two (0); none (1).

2 Axonemes. Free (0); incorporated into sperm cell body by proximo-distal fusion (1); incorporated into sperm cell body by distal proximal fusion (2).

3 Dense bodies. Present (0); absent (1).

4 Reverting migration which leads to the nucleus occupying a more distal position relative to the basal bodies. Absent (0); present (1).

5 Reverting migration includes a backward movement of the basal bodies and their axonemes to a proximal position. Absent (0); present (1).

6 Basal bodies retain their proximal position. Absent (0); present (1).

7 Electron-dense granules. Absent (0); present (1).

8 Spermatogenesis. Mature spermatozoa lacking dense heel, rotation of flagella and spur (0); mature spermatozoa possessing dense heel, rotation of flagella and spur (1).

9 Intercentriolar body during spermatogenesis. Present, weakly developed (0); absent (1).

Table 1 Data matrix for phylogenetic analysis of the rhabdocoels. In this study, 91 morphological transformation series were considered. The most robust and inclusive results are based on 89 transformation series (17, 28 excluded) and with characters 16, 22, 24, 41, 60, 61, 78, 79 and 82 ordered. For identities of characters and states, refer to text. 0, plesiomorphic state; 1, 2, 3, 4, 5, apomorphic states; ?, unknown; OG, outgroup function (composite outgroup based on character argumentations for each transformation series).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Umagillidae	1	0	0	0	0	0	0	0	0	0	0	?	0	1	1	?	?	0	0	0	0	?	0	0	0	1	1	1	2	0
Pseudograffillinae	?	?	?	0	0	0	?	0	?	0	0	?	0	?	?	?	?	0	0	0	0	?	0	?	0	1	1	0	0	0
Graffillinae	1	0	0	0	0	0	1	0	0	0	0	?	0	?	?	?	?	0	0	0	0	0	0	0	0	1	1	?	?	0
Acholadidae	?	?	?	0	0	0	?	?	?	0	0	?	0	?	?	?	?	0	0	0	0	0	0	0	0	1	1	1	2	0
Pterastericolidae	1	0	0	0	0	0	0	0	0	0	0	2	0	1	1	?	?	0	0	0	0	0	0	0	0	1	1	1	2	0
Hypoblepharinidae	?	?	?	0	0	0	?	?	?	0	0	?	0	0	?	?	?	0	0	0	0	0	0	0	1	0	1	1	2	0
Provorticidae	1	?	0	0	0	0	0	0	0	0	0	?	0	1	?	?	?	0	0	0	0	0	0	1	0	1	1	?	1	0
Kytorhynchidae	?	?	?	0	0	0	?	?	?	0	0	?	0	?	?	?	?	0	0	0	0	0	0	1	0	0	?	?	?	0
Promesostomidae	1	0	?	0	0	0	0	0	?	0	0	?	0	0	?	?	?	0	0	0	0	0	0	0	0	0	?	?	?	0
Solenopharyngidae	1	0	0	0	0	0	1	0	?	0	0	?	0	0	?	?	?	0	0	0	0	0	0	0	0	1	?	?	?	0
Trigonostomidae	1	2	1	0	0	0	?	0	?	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Kalyptorhynchia	1	2	0	0	0	0	0	0	0	0	0	2	0	1	1	?	?	0	0	0	0	0	0	1	0	0	0	?	?	1
Dalyelliidae	1	2	0	0	0	0	1	1	0	1	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	?	1	0
Temnocephalida	1	0	0	0	0	0	1	1	0	1	0	2	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	?	?	0
Typhloplanidae	1	0	0	0	0	0	0	1	0	1	0	2	0	1	1	0	?	0	0	0	0	0	0	0	?	0	?	0	1	0
Urastoma	1	1	1	1	0	1	0	0	1	0	0	1	1	0	0	?	1	0	0	0	0	0	0	?	0	?	?	0	1	1
Fecampiidae	1	1	1	1	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1
Udonella	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	2	1	1	1	2	0
Aspidogastrea	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	2	1	1	0	1	0
Digenea	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0	1	2	1	1	0	1	0
Monogenea	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0	1	2	1	1	2	1	0
Gyrocotylidea	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	2	0	0	0	1	0	1	1	2	2	1	1	1	2	0
Amphilinidea	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	2	0	0	0	1	0	2	1	2	2	1	1	1	2	0
Eucestoda	1	1	1	1	0	1	0	0	1	0	1	1	0	0	0	2	0	0	0	1	1	2	1	2	2	1	1	1	2	0

Taxa	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Umagillidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1
Pseudograffillinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Graffillinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0
Acholadidae	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0
Pterastericolidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Hypoblepharinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0
Provorticidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kytorhynchidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Promesostomidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Solenopharyngidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Trigonostomidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Kalyptorhynchia	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1	0	0	0	1
Dalyelliidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1
Temnocephalida	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1
Typhloplanidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Urastoma	1	?	?	0	0	?	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
Fecampiidae	1	3	?	0	0	1	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	1	1
Udonella	0	2	1	1	1	1	1	1	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2	1	1	1
Aspidogastrea	0	2	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	2
Digenea	0	2	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	2
Monogenea	0	2	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0	3
Gyrocotylidea	0	2	1	1	1	1	1	1	0	0	2	1	0	1	1	1	1	1	1	1	0	0	0	2	1	2	0	2	4
Amphilinidea	0	2	1	1	1	1	1	1	0	0	2	1	0	1	1	1	1	0	0	0	1	1	1	0	2	1	3	0	2	4
Eucestoda	0	2	1	1	1	2	1	1	0	0	2	1	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	0	2	4

Table 1 *Continued*

Taxa	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Umagillidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudograffillinae	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Graffillinae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acholadidae	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pterastericolidae	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypoblepharinidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Provorticidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kytorhynchidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Promesostomidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Solenopharyngidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trigonostomidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kalyptorhynchia	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dalyelliidae	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temnocephalida	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Typhloplanidae	1	0	0	0	1	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urastoma	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fecampiidae	1	1	2	1	2	0	0	0	0	0	1	1	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Udonella	1	1	1	1	1	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Aspidogastrea	1	1	1	1	1	0	0	1	0	0	0	0	1	0	1	1	2	1	1	2	1	0	0	0	0	0	0	0	0	0	0
Digenea	1	1	1	1	1	0	0	1	0	0	0	0	1	1	1	1	2	1	1	2	0	0	0	0	1	1	1	0	0	0	0
Monogenea	1	1	1	2	1	0	0	2	0	0	0	0	1	0	1	1	1	2	1	1	0	1	0	0	0	0	0	0	0	0	0
Gyrocotylidea	2	1	1	1	0	0	1	2	0	0	0	0	1	0	2	0	1	2	2	1	0	2	1	1	0	0	0	0	0	0	0
Amphilinidea	2	1	1	1	0	0	0	2	1	1	0	0	1	0	2	0	1	2	2	1	0	3	1	0	0	0	0	0	0	0	0
Eucestoda	2	1	1	1	0	0	0	2	0	0	0	0	1	0	2	0	1	2	2	1	0	4	1	0	0	0	0	1	1	1	1

10 Peripheral layer of microtubules in spermatozoa. Not spirally arranged (0); spirally arranged (1).
11 Mitochondria in sperm. Present (0); absent (1).

Protonephridia ultrastructure

12 Longitudinal ribs (rods). Absent (0); present, in two rows, inner formed by terminal cell, outer formed by canal cell (1); present, in single row of longitudinal ribs formed by canal cell (2).
13 Interdigitating processes of weir. Absent (0); present (1).
14 Terminal perikaryon. Present (0); absent (not close to flame) (1).
15 Support structure of ribs (rods). Microtubules absent (0); microtubules present (1).
16 Pair of cytoplasmic cords from canal cell connected by a desmosome. Absent (0); present (1).
17 Surface of capillary. 'Saccate'/simple (0); lamellae of connected spaces (1); microvilli (2).

Osmoregulatory system microstructure

18 Secondary protonephridial system of canals and pores. Absent (0); present (1).
19 Giant paranephrocytes. Absent (0); present (1).
20 Osmoregulatory system. Never reticulate (0); becomes reticulate in late ontogeny (1).
21 Osmoregulatory system in early ontogeny. Not reticulate (0); reticulate (1).
22 Protonephridia in larvae. In anterior end of body (0); in anterior and posterior end of body (1); in posterior end of body (2).
23 Desmosomes in the passage of the first excretory canal cell. Present (0); absent (1).

Tegument

24 Tegument. Cellular (0); syncytial, protruding to surface between epidermal cells (1); syncytial, not protruding to surface between epidermal cells (2).
25 Adult body ciliation. Completely ciliated (0); at least some body ciliation lost (1); all ciliation lost (2). Some umagillids have lost body ciliation (Jondelius 1991); we con-sider this to be a derived trait within the group and con-sider the family to be plesiomorphically ciliated.
26 Rhabdites. Present (0); absent (1).
27 Duo gland organ. Present (0); absent (1).
28 Rhabdomeric eyes. Two (0); none (1); four (2).
29 Lensing. Non-mitochondrial (0); mitochondrial (1); no lenses (2).
30 Rhabdoids (large granular and vesicular bodies in epi-dermis). Absent (0); present (1).
31 Spur projecting from the basal body opposite the

horizontal rootlet of epidermal cilia. Absent (0); present (1).
32 Pharyngeal musculature. Circular muscle innermost (0); longitudinal muscle innermost; (1) circular muscle layer only (2); pharynx absent (3).
33 Dictyosomes and endoplasmic reticulum in larval/juvenile epidermis. Present (0); absent (1).
34 Larval epidermis. Not shed at end of larval stage (0); shed at end of larval stage (1).
35 Cilia of larval epidermis. With more than one rostrally directed rootlet (0); with one rostrally directed rootlet (1).
36 Specialized microvilli and microtubules in epithelium. Absent (0); present (1); modified into microtriches (2).
37 Epithelial sensory cells. Electron-dense collars absent (0); electron-dense collars present (1).
38 Post-larval epidermis. Not syncytial (0); syncytial [neodermis] (1).
39 Excretory vesicles. Lateral, paired (0); single, medial opening postero-dorsally (1).
40 Cephalic tentacles. Absent (0); present (1).
41 Vitelloducts. Absent (0); present, lining not syncytial (1); present, lining syncytial (2).
42 Anterior and posterior nervous system commissures. Single bilobed units (0); two bilobed units (1).
43 Ciliary bands on embryo. Absent (0); present, in three rows (1).
44 Larval epidermis. Not syncytial (0); syncytial (1).
45 Endoderm. Present in embryos (0); absent in embryos (1).
46 Vitellogenic cells. With more than one kind of electron-dense vesiculated inclusions (0); with one kind of electron-dense vesiculated inclusion (1).
47 Inner longitudinal muscle layer. Poorly developed (0); well developed (1).
48 Antero-lateral notch. Absent (0); present (1).
49 Nuclei in larval epidermis. Present (0); absent (1).
50 Multiciliary nervous receptors. Present (0); absent (1).
51 Epithelial lining of genital ducts. Not syncytial (0); syncytial (1).
52 Protonephridial ductules. Ciliated (0); not ciliated (1).
53 Medullary and cortical distinction. Not apparent (0); apparent (1).
54 Protein embedments in larval epidermis. Absent (0); present (1).

Reproductive system

55 Male intromittent organ. Simple stylet (0); cirrus [sometimes mistakenly called a penis] (1); copulatory papilla (2); complex stylet (3); absent (4). Monogeneans do not have a copulatory stylet (the accessory piece in some monogeneans is an independently evolved structure, and a cirrus is plesiomorphic for the group: Boeger & Kritsky 1993, 1997). The copulatory papillae of Gyrocotylidea and Amphilinidea may be vestigial/reduced cirri.
56 Openings of male and female gonopores. Common genital atrium (0); separate (1); separate sexes (2).
57 Position of genital atrium or genital pores. Posterior (0); caudal (1); anterior (2); lateral (3).
58 Muscular copulatory bulb. Present (0); absent (1).
59 Testes. Paired (0); single (1); multiple, in two lateral bands (2). A single testis occurs convergently within Aspidogastrea, Digenea and Monogenea, but phylogenetic analyses (Brooks *et al*. 1985b, 1989; Boeger & Kritsky 1993, 1997) have shown that paired testes are plesiomorphic in each case.
60 Female reproductive system. Simple oviduct (0); oviduct expanded to form antrum (functional uterus) without separate opening (1); oviduct coiled, with small secondary tube (Laurer's canal) opening to the surface (not opening to surface or absent in derived taxa), used to vent excess material from oviduct (2); oviduct relatively straight, with secondary tube forming separate tubular uterus with uterine pore opening to surface (3); oviduct relatively straight, uterus highly coiled (4). Previous phylogenetic analyses of the Cercomeria (Brooks *et al*. 1985a) and Rhabdocoela (Jondelius & Tholleson 1993) have treated various portions of the female reproductive system as a series of separate characters. These include the presence or absence of a vagina, presence or absence of a uterus, and their position(s) relative to the male gonopore and to the body in general. This has been complicated in part by the fact that most neodermatans possess two (or even three) openings of the female reproductive tract.

The majority of cercomerideans (Trematoda + Cercomeromorphae) exhibit a bifurcated oviduct, with each bifurcation forming a tube that opens to the exterior. These tubes have been functionally defined in the parasitic taxa, i.e. any egg-containing tube is called the uterus, and the alternative tube is called the vagina. Thus, in the trematodes, the male gonopore and uterine pore are said to be proximate, with the vagina separate. The vagina (called the Laurer's canal) is almost always short, narrow and relatively straight (in many cases it does not open to the exterior or is even lost) and the uterus is generally coiled. In the Monogenea, all three pores are separate plesiomorphically, with the apomorphic state 'uterine and male pores proximate' being displayed by some taxa. The uterus and vagina are relatively well developed, short and straight. Doubling the vagina (considered by Brooks *et al*. 1985a to be an autapomorphy for the Monogenea) appears to be an apomorphic trait within the Monogenea (Boeger & Kritsky 1993, 1997). In the Gyrocotylidea, all three pores are proximal and separate (the plesiomorphic condition for the Monogenea). In the Cestoidea (Amphilinidea + Eucestoda), the male pore and the vaginal pore

are proximal, with the uterine pore distantly situated. Finally, within the Cestodaria (Gyrocotylidea + Amphilinidea + Eucestoda), the uterus is plesiomorphically highly coiled (it is apomorphically saccate in the Eucestoda: Brooks *et al.* 1991; Hoberg *et al.* 1997, in press). Such coiling also occurs convergently within the Monogenea (Boeger & Kritsky 1993, 1997). Establishing homologies for these structures across taxa has been difficult, demanding complex evolutionary scenarios to explain the diversity of ducts, tubes, pores and their positions relative to each other. We suggest that these scenarios have been unnecessarily complex and propose the following alternative.

The basic unit of the platyhelminth female reproductive system is an ovary (paired plesiomorphically) connected to a tubular oviduct, a canal which originates from the ovary and terminates in a genital pore that communicates with the external environment. Plesiomorphically, this canal functions as both vagina (receiving sperm) and uterus (delivering eggs to the external environment) and is situated near the male genital pore, either sharing a common atrium with the male pore or not (Fig. 1). Within the rhabdocoels, including fecampiids, *Urastoma* and *Udonella*, the oviduct is expanded, producing a functional uterus, or antrum. The antrum may be symmetrical or asymmetrical, small, containing a single egg, or large, containing several eggs, and saccate or somewhat tubular.

We propose that, regardless of perceived function, the oviduct is the portion of the female reproductive system plesiomorphically proximal to the male genital pore, with which it may or may not share a common gonopore (genital atrium). The secondary duct may be proximal to (Monogenea, Gyrocotylidea) or distant from the openings of the oviduct and male genital pore (dorsal in the trematodes, ventral in the Amphilinidea and Eucestoda). The Laurer's canal is thus actually homologous with the uterus, not the vagina, of the Cercomeromorphae. The current function of the Laurer's canal, expulsion or digestion of sperm and other debris from the fertilization and egg-making process (e.g. Juel's organ in some hemiuriform digeneans), may well have been the original function of the duct. The widespread belief that the Laurer's canal is a vestigial vagina stems from discussions of the presumed degenerate evolutionary nature

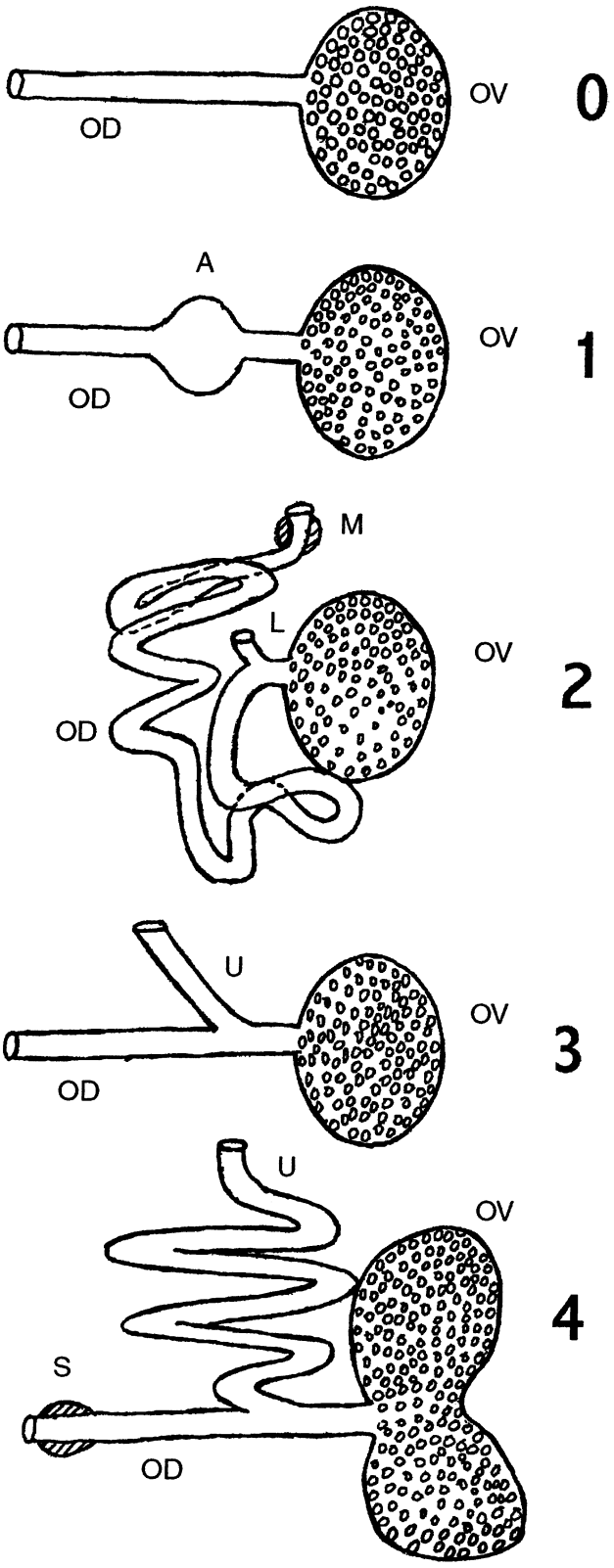

Fig. 1 Schematic representation of diversity in the female reproductive system of neodermatans. 0, 1, 2, 3, 4 refer to the character states used in this analysis. State 0 is the condition found among various rhabdocoels. State 1 occurs in *Urastoma*, Fecampiidae, *Udonella* and various rhabdocoels. State 2 is the condition found in trematodes. State 3 is the condition among the monogeneans. State 4 is the condition of the Cestodaria. A, antrum; L, Laurer's canal; M, metraterm; OD, oviduct; OV, ovary; S, sphincter; U, uterus.

of parasites beginning in the late 19th century. Actual evidence is rare. For example, without sectioning his material, Cohn (1902) stated that he had found one specimen of *Liolope copulans* extruding its cirrus into the Laurer's canal of another. Brooks & Overstreet (1978), however, noted that they never found any evidence of this behaviour in a close relative of *L. copulans*, *Dracovermis occidentalis* Brooks & Overstreet, 1978. They stated that '… based on the narrow Laurer's canal, wide cirrus, thick and large genital atrium, and uterus occasionally entirely packed with sperm in *Dracovermis occidentalis*, we doubt that Laurer's canal in that species serves for more than the elimination of excess products.' Increased egg-holding capacity in the trematodes is made possible by extensive coiling of the oviduct, while in the cercomeromorphs it is due to the elongation (Monogenea) and coiling (gyrocotylids, amphilinids and eucestodes) of the Laurer's canal, co-opted (an *exaptation*: Gould & Vrba 1982) as a functional uterus distinct from the oviduct.

The above proposal provides a succinct conception of the evolution of the number, nature and position of the ducts and pores of the female reproductive system in the Cercomeridea. Interestingly, it is also the scheme proposed by Looss (1893) but apparently forgotten until now. Our coding of characters associated with these structures reflects this new hypothesis. Finally, many trematodes have been described as exhibiting a glandular muscle surrounding the terminal end of the uterus called the 'metatherm' (Smyth 1994) or 'metraterm' (Noble *et al*. 1989). Many eucestodes have been described as having a muscular structure at the terminal end of the vagina called a 'vaginal sphincter'. If our hypothesis above is true, it is likely that these structures are homologous. At present, we lack sufficient information to use this as a character.

61 Ovary. Paired (0); single and spherical (1); single and bilobed (2).

62 Mehlis' gland. Absent (0); present (1).

63 Vitellaria. Paired, compact, medial (0); lateral and follicular (1); compact and medial vitellarium (2). Compact vitellaria occur convergently in a number of digenean and eucestode groups, but are apomorphic within these taxa (Brooks *et al*. 1985b, 1989, 1991; Hoberg *et al*. 1997, in press).

64 Cirrus. Absent (0); present, muscular and aspinose (1); present, muscular and spinose (2).

65 Testes. Preovarian (0); postovarian (1); dioecious (2). Dioecy appears convergently in some digenean (e.g. Schistosomatidae) and some eucestode (e.g. *Dioecotaenia*, *Dioecocestus*, *Shipleya*, *Gyrocoelia*) groups (Brooks *et al*. 1985b, 1989, 1991; Hoberg *et al*. 1997, in press). Because the Fecampiidae are dioecious, the character is inappropriate. We have the option of either coding the fecampiids as '9' — inappropriate, or as '2', as the condition is autapomorphic. The choice of coding in this instance does not affect the analysis.

66 Eggs. Round adhesive disc at the end of filament where the substance of the disc is secreted later when the worm attaches the egg to the body of the host. Absent (0); present (1).

67 Vitellaria. Not encircling entire body (0); encircling entire body, extending along entire body length (1). The apomorphic state appears convergently in some eucestode groups (Hoberg *et al*. 1997, in press).

68 Permanent uterine pore. Absent (0); present, dorsal (1); present, ventral (2).

69 Uterine pore. Not proximal to pharynx (0); proximal to pharynx (1).

70 Uterus. Coiled, not 'N'-shaped (0); 'N'-shaped (1).

Digestive system

71 Mouth and pharynx. Present (0); absent (1). The apharyngeate condition exhibited by some monogeneans and digeneans is convergently evolved within these groups (Brooks *et al*. 1985b, 1989; Boeger & Kritsky 1993, 1997).

72 Doliiform pharynx (pharynx bulbosus of Jondelius & Tholleson 1993). Present (0); absent (1).

73 Pharynx placement. In anterior half of worm (1); medial to posterior half of worm (2); absent (3). This is a difficult character to polarize because most outgroups are polymorphic. Jondelius & Tholleson (1993) proposed that anterior was plesiomorphic for the rhabdocoels, but their own argument can also be used to support the contention that a pharynx in the mid to posterior half of the body is plesiomorphic; therefore, we have coded the outgroup state as '?' and given each ingroup state a non-zero number.

74 Oral sucker. Lacking a capsule (0); with a capsule (1).

75 Gut shape. Saccate (0); bifurcate (1); lacking in adults (2). Convergent reversal to a saccate gut from a plesiomorphically bifurcate gut occurs within the aspidogastreans, digeneans and monogeneans (Brooks *et al*. 1985b, 1989; Boeger & Kritsky 1993, 1997).

76 Oral sucker. Absent (0); present (1).

Posterior adhesive organs

77 Posterior adhesive organ. Absent (0); present, not delimited by capsule (1); present, delimited by capsule (2).

78 Posterior adhesive organ. Absent (0); present, no hooks (1); present, with hooks (2).

79 Posterior adhesive organ. Absent (0); present throughout life (1); present only during early development, partially invaginated (2).

80 Posterior adhesive organ. Absent (0); present, terminal (1); present, ventral (2).

81 Posterior sucker. Without transverse septa (0); hypertrophy and linear subdivision of posterior sucker by transverse septa (1).

82 Hooks on posterior end of larva. Absent (0); sixteen equal-sized hooks (1); ten equal-sized hooks (2); six large and four small hooks (3); six hooks (4).
83 Posterior body invagination. Absent (0); present (1).
84 Rosette at posterior end of body. Absent (0); present (1).

Ontogeny
85 Miracidium. Absent (0); present (1).
86 Sporocyst. Absent (0); present (1).
87 Cercaria. Absent (0); present (1).
88 Procercoid. Absent (0); present (1).
89 Plerocercoid. Absent (0); present (1).
90 Cerebral development in larvae. Present (0); absent (1).
91 Extra-embryonic membrane. Not formed by embryo (0); formed by embryo (1).

Analyses performed
Data were analysed using standard Hennigian argumentation (see Hennig 1966; Wiley 1981; Brooks & McLennan 1991; Wiley *et al.* 1991, in press), and results were confirmed using the 'branch and bound option' on the computer program PAUP 4*, implemented on Macintosh G3/400, G4/450 and G4/500 computers. Acctran and Deltran character optimization produced the same results. Bootstrap and jackknife analyses

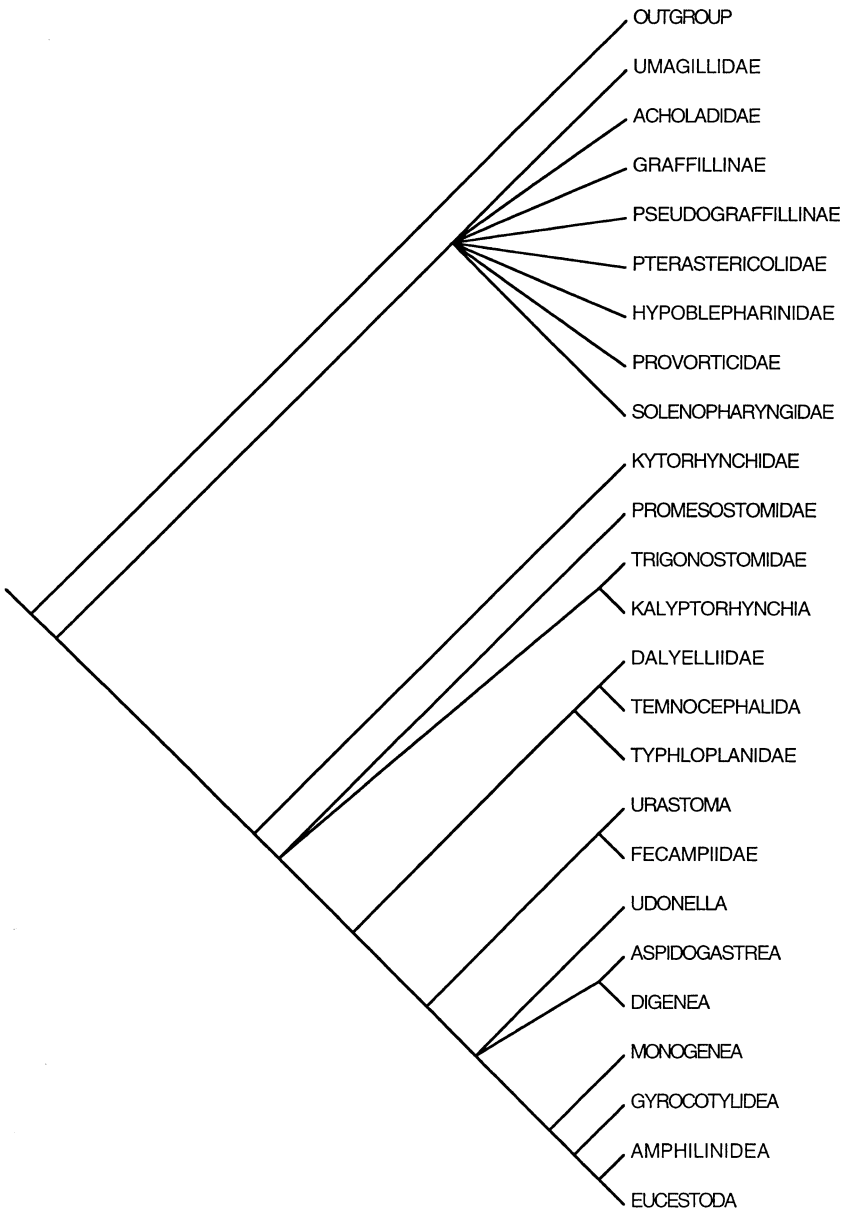

Fig. 2 Majority rule consensus tree for 24 rhabdocoel taxa based on 98 most parsimonious trees (MPTs) (tree length (TL = 190), consistency index (CI = 67%) and rescaled consistency index (RCI = 55%) produced by phylogenetic systematic analysis of 91 morphological characters.

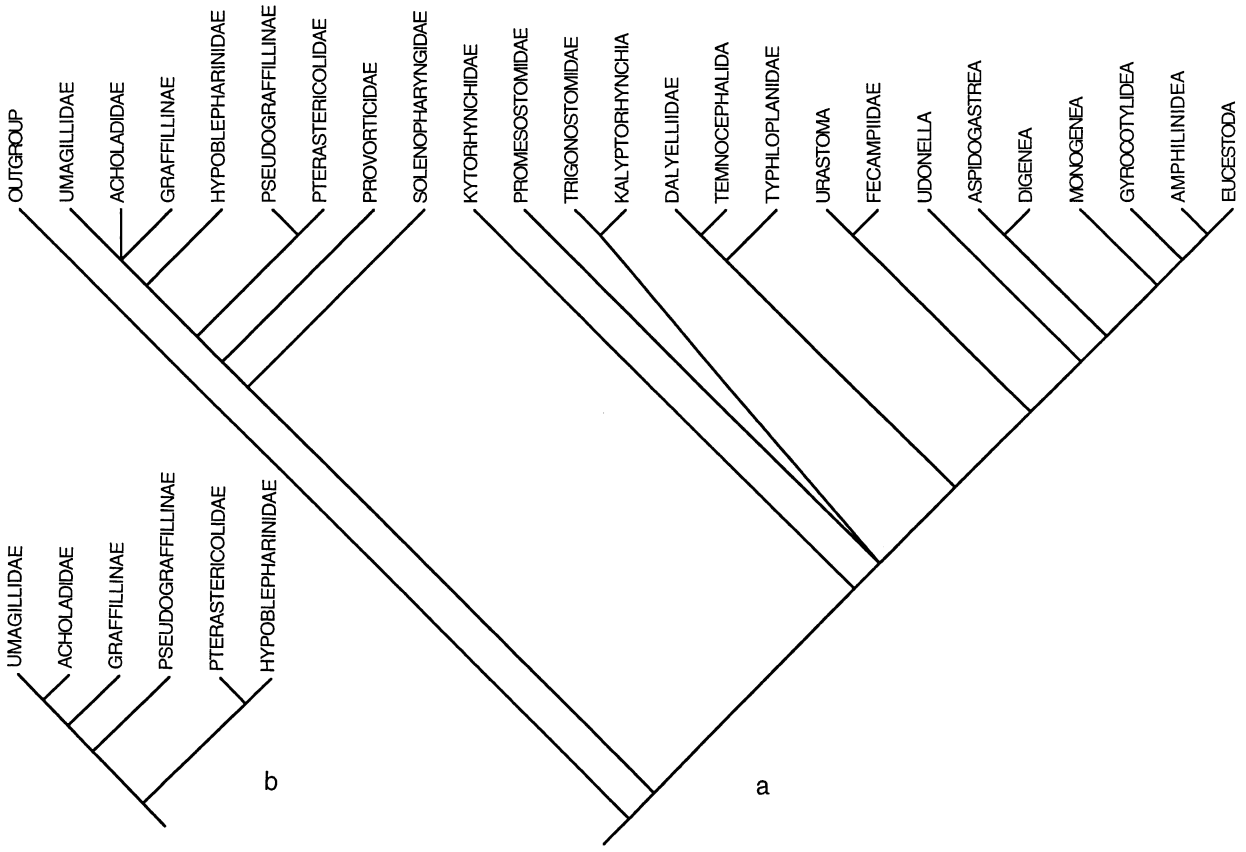

Fig. 3 Two most parsimonious trees (MPTs) (tree length (TL = 181), consistency index (CI = 69%) and rescaled consistency index (RCI = 56%)) for 24 rhabdocoel taxa produced by phylogenetic systematic analysis of 89 morphological characters. Ordering multistate characters 16, 22, 24, 41, 60, 61,78, 79 and 82 produced the same results.

were performed using 10 000 replicates, with the exception of the complete data set, for which only 100 replicates were performed due to computational constraints.

Results

The analysis of all 91 characters, unordered, produces 98 most parsimonious trees (MPTs), each 190 steps long, with a consistency index (CI) of 67% and rescaled consistency index (RCI) of 55%. Forty-one of these MPTs place the Kytorhynchidae, Promesostomidae, Trigonostomidae, Typhloplanidae, Dalyelliidae and Temnocephalida at the base of the tree, similar to results reported by Jondelius & Tholleson (1993) and Littlewood *et al.* (1999a,b). The remaining 57 MPTs suggest that these taxa are part of an inclusive clade also containing the Neodermata, a result more similar to the hypothesis proposed by Ehlers (1984, 1985a,b, 1986, 1995) and Brooks and coworkers (Brooks *et al.* 1985a; Brooks 1989a,b; Brooks & McLennan 1993a). Figure 2 is the 50% majority rule consensus tree for the 98 MPTs. We have discovered that this 'dichotomous' result in the placement of the Kytorhynchidae, Promesostomidae, Trigonostomidae, Typhloplanidae, Dalyelliidae and Temnocephalida is the product of missing data for key taxa in characters 17 and 28. In computer-assisted phylogenetic studies, some configurations of missing data can produce effects similar to long branch attraction effects in the analysis of nucleotide sequence data (see Nixon & Davis 1991; Platnick *et al.* 1991; Maddison 1993; Wilkinson 1995). Other characters show low character consistencies on the tree as well, but their inclusion does not affect the stability of the results. We believe that characters 17 and 28 are too poorly documented at present to be useful.

Removing characters 17 and 28 produces two MPTs (Fig. 3), 181 steps long, with a CI of 0.69 and an RCI of 0.56, differing only in the degree of resolution of that portion of the tree containing the Umagillidae, Acholadidae, Graffillinae, Pseudograffillinae, Pterastericolidae and Hypoblepharinidae. Characters 16, 22, 24, 41, 60, 61, 78, 79 and 82 are multistate transformation series produced by combining what were previously considered to be a series of binary characters

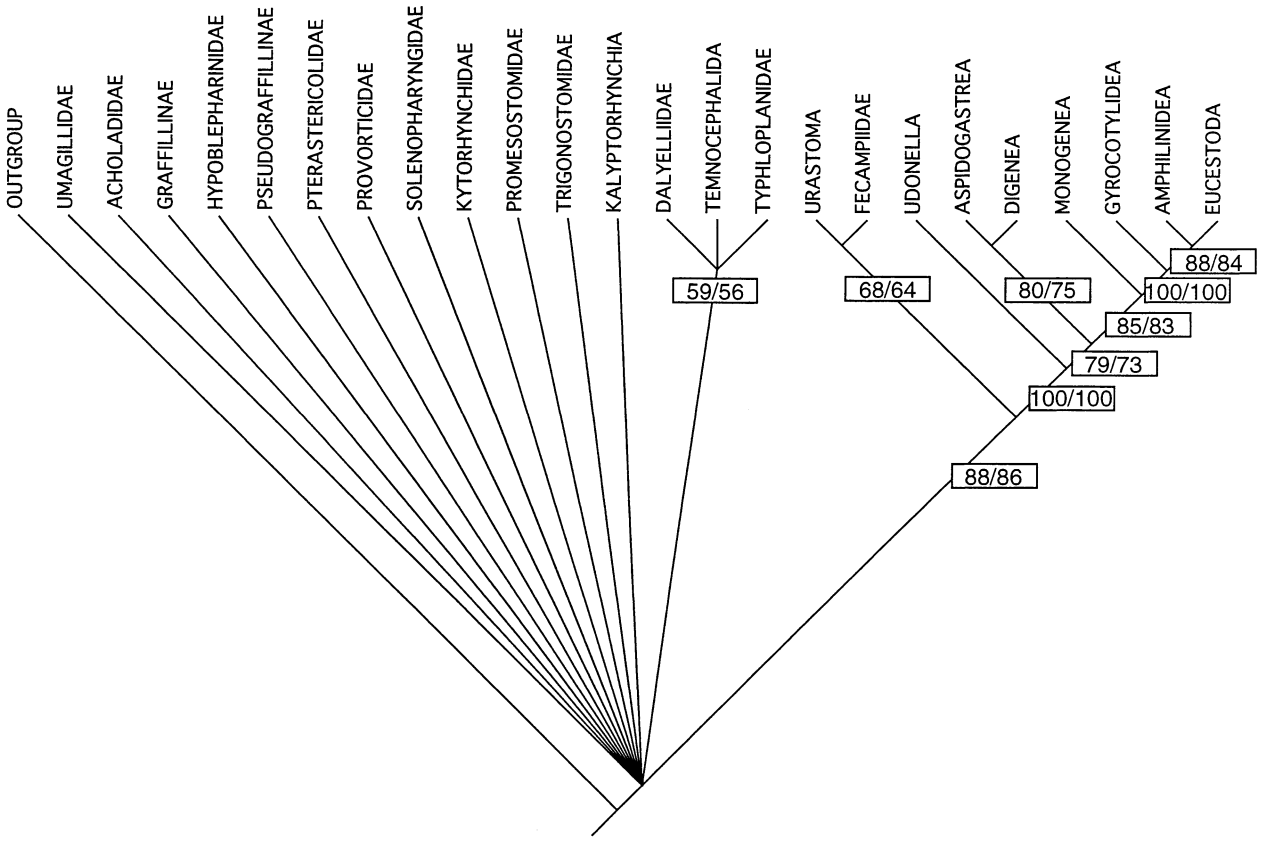

Fig. 4 Bootstrap and jackknife consensus tree for 24 rhabdocoel taxa based on 89 morphological characters, with multistate characters 16, 22, 24, 41, 60, 61, 78, 79 and 82 ordered. Bootstrap/jackknife values appear on appropriate branches.

(Brooks & McLennan 1993c). The relationships shown in Fig. 3 supported the ordering of these transformation series. Phylogenetic analysis with these nine characters ordered produced the same results as Fig. 3. Successive approximations with reweighting of the data produced the single tree shown in Fig. 3(A), which we consider to be the most robust hypothesis incorporating the maximum amount of information possible for this data set.

Six taxa in the present study, the Acholadidae, Pseudograffillinae, Hypoblepharinidae, Solenopharyngidae, Promesostomidae and Kytorhynchidae, have substantial missing data entries, and the portion of the tree containing the Umagillidae, Pseudograffillinae, Graffillinae, Acholadidae, Pterastericolidae and Hypoblepharinidae produces the two MPTs shown in Fig. 3. Not surprisingly, then, bootstrap and jackknife analyses indicate that only the groupings of ((Dalyelliidae + Temnocephalida) Typhloplanidae) and of ((Fecampiidae + *Urastoma*) (*Udonella* ((Aspidogastrea + Digenea) (Monogenea (Gyrocotylidea (Amphilinidea + Eucestoda)))))) are robust (Fig. 4). Given recent successes at finding many morphological traits for other platyhelminth groups (e.g. Lundin 2000), we feel confident that sufficient characters are there to be discovered, and a fully robust assessment of the Rhabdocoela is feasible. In the rest of this study, we will concentrate on the Neodermata and their closest relatives.

The placement of the Temnocephalida in our analysis precludes the interpretation that all posterior holdfast organs in this clade are homologous. The taxon Cercomeria Brooks, 1982 therefore cannot be maintained, as suggested by Ehlers & Sopott-Ehlers (1993) and Rohde & Watson (1995). The clade of Fecampiidae + *Urastoma* as the sister group of the Neodermata supports the monophyly of the Revertospermata Kornakova & Joffe, 1999, but not the Mediofusata Kornakova & Joffe, 1999.

Discussion

Discussions of the phylogeny of the Neodermata revolve around two questions: (1) What is the sister group of the Neodermata? (2) How does the choice of sister group affect

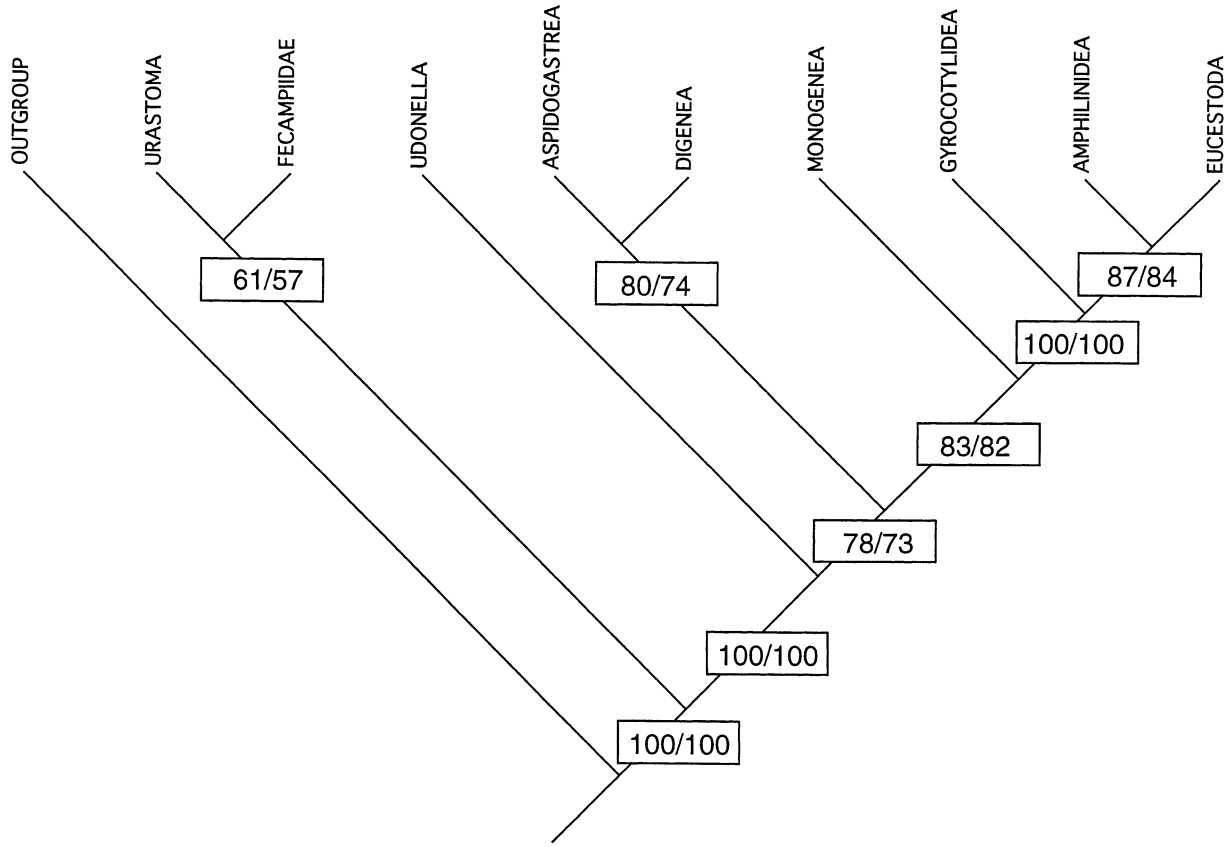

Fig. 5 Bootstrap and jackknife consensus trees for the Revertospermata Kornakova & Joffe (Neodermata (Fecampiidae + *Urastoma*)) based on 89 morphological characters, with multistate characters 16, 22, 24, 41, 60, 61, 78, 79 and 82 ordered. Bootstrap/jackknife values appear on appropriate branches.

the hypotheses of the relationships among taxa within the Neodermata? With regard to the first question, four taxa have been previously suggested as sister groups of the Neodermata: (1) the Dalyelliidae and Typhloplanidae (Ehlers 1984, 1985a,b, 1986, 1995; Ehlers & Sopott-Ehlers 1993); (2) the Temnocephalida (Brooks 1982, 1989a,b; Brooks *et al.* 1985a; Brooks & McLennan 1993c); (3) *Urastoma* (Rohde *et al.* 1990; Williams 1993; Watson 1997; Kornakova & Joffe 1999); and (4) the Fecampiidae (Rohde 1990, 1991; Litvaitis & Rohde 1999). This study included all four candidates in the same analysis, and the results indicate that they comprise the four closest relatives of the Neodermata (Fig. 3). With respect to the second question, the present analysis supports the monophyly of the Monogenea and the placement of *Udonella* as the basal member of the Neodermata as originally proposed by Brooks *et al.* (1985a). Re-analysing the present data set using any number and combination of the four putative sister groups as outgroup taxa produces the same result. This occurs because the data for relationships within the Neodermata are highly robust (CI = 94%, RCI = 87%), making any combination of the four candidates suitable outgroups. The portion of the tree comprising the (Fecampiidae + *Urastoma*) + Neodermata is slightly less robust (CI = 90%, RCI = 82%) because the Fecampiidae + *Urastoma* clade is not as well supported (see bootstrap and jackknife values in Fig. 5).

Brooks *et al.* (1985a) used a data set of 39 transformation series in their initial analysis of the Neodermata; this produced a single MPT, 41 steps long (CI = 95%), depicting the same relationships as shown in Figs 2–5. In that analysis, the authors used only attributes deemed informative by authors of numerous earlier studies in order to demonstrate that differences in results were due to differences in methods of analysis, not in choice of characters. Adding more morphological traits produced a data set of 127 binary characters (Brooks 1989a,b), corroborating the original phylogenetic hypothesis, producing a single MPT, 131 steps long (CI = 97%). Brooks & McLennan (1993c) produced

the same MPT, 161 steps long, for 153 apomorphic traits (CI = 95%). In the present study, some characters were modified according to new findings, some redundant characters listed by Brooks & McLennan (1993c) were combined, and 47 fewer autapomorphies were used, resulting in the same MPT, 107 steps long, for 100 apomorphic traits (CI = 93%); including the 47 autapomorphies produces a single MPT for the Neodermata, 154 steps long, for 147 apomorphic traits (CI = 95%).

Despite consistent robust support for this hypothesis during the past 15 years, some researchers have felt uncomfortable with the results (Rohde 1990, 1994a, 1996; Justine 1998b; Littlewood *et al.* 1998, 1999a,b). We believe that misunderstandings about phylogenetic systematics have been responsible for the differences of opinion. The most fundamental misunderstanding stems from the way in which phylogeneticists determine homologous character states. All systematists begin the search for homology by using a set of criteria, such as those proposed by Remane (1952), to determine whether two or more characters are 'similar' (see discussion in de Pinna 1991). These similarities apply to both identity (a finger is a finger) and also transformation (a bird's wing is a tetrapod forearm). Assessing similarity based upon such biological criteria, without recourse to knowledge of underlying genealogical relationships, eliminates any hint of circularity in the process (see Eldredge & Cracraft 1980; Wiley 1981; Brooks & McLennan 1991; Wiley *et al.* 1991, in press; McLennan & Brooks, in press). The difference among systematists begins with how these similarities are treated next. Phylogenetic systematists use assessments of similarity to construct *hypotheses* of homology 'If a and b look the same (e.g. are in the same position, develop from the same tissue), then they are homologous'. This is called Hennig's auxiliary principle (see Hennig 1966; Wiley 1981; Brooks & McLennan 1991; Wiley *et al.* 1991, in press; McLennan & Brooks, in press). These hypotheses are tested by using phylogenetic systematics and are ultimately corroborated or rejected. In the latter case, we conclude that the similarity is due to homoplasy.

Some taxonomists, on the other hand, believe that they can make *a priori* judgements about which similarities are due to homology, and which are due to homoplasy, and thus eliminate some characters (the putative homoplasies) from the data set before the analysis begins. Such *a priori* judgements are valid only if they are supported by evidence. For example, experimental research has demonstrated that characters such as the number of vertebrae or fin rays in stickleback fish are strongly influenced by the temperature under which the larvae develop (Lindsey 1962; Hagen 1967). Reporting the number of vertebrae or fin rays without adjusting for developmental temperature, an almost impossible feat in wild caught fish, thus introduces a known source of homoplasy into the data set. In this case, systematists are justified in eliminating these traits from their analysis *a priori*. Because such data are rare, however, it becomes important to ask: 'what supports the elimination of a particular character, or type of character, from an analysis?'.

With regard to the Neodermata, it has been asserted that complex characters are more likely to be homologous than simple characters (Rohde 1990, 1994a, 1996; Littlewood *et al.* 1999a). What evidence is there to support this assertion? There is a large body of evidence documenting simple genetic bases for many homologous behavioural and morphological characters in *Drosophila* species. That alone would seem to falsify the hypothesis that simple characters are not likely to be homologous. This assertion stems, in part, from a misunderstanding of levels of homology. The presence of bristles may be homologous across *Drosophila*, but the exact number of bristles may display some homoplasy. In other words, there is no evidence indicating that sweeping generalities can be made about the nature of homology vs. homoplasy based upon a vague notion of simple vs. complex character structure.

The hypothesis about the relative merits of simple vs. complex characters as markers of genealogical relationships could be examined by assigning a 'simple' vs. 'complex' status to characters *a priori*, running those characters through a phylogenetic systematic analysis, and then asking whether there is a significant difference in homoplasy among the two character classes. Once this process has been repeated for a substantial number of data sets from different groups of organisms, we could then begin to determine the validity of the hypothesis. In lieu of this evidence, we opted to use all available characters, presuming maximum homology and character independence *a priori*, and relying on phylogenetic congruence among all characters *a posteriori* as the final arbiter of homology (Wiley 1981; Kluge 1989, 1997, 1998a,b, 1999; de Pinna 1991).

While the primary function of phylogenetic analysis is to produce a robust hypothesis of phylogenetic relationships, it also provides a means to help us determine when our *a priori* presumptions are incorrect. Once we have a phylogenetic hypothesis based on as many characters as possible, we can move from homology presumptions to homology determinations. Hennig (1966) considered such 'reciprocal illumination', using the overall analysis to assess individual *a priori* presumptions of homology, to be a primary benefit of phylogenetic systematics. The homologies are the traits that are congruent with the phylogenetic tree, whether they are complex or simple in nature; homoplasies are those that are incongruent with the tree. For example, this study supports the proposal by Ehlers & Sopott-Ehlers (1993) and Rohde & Watson (1995) that the holdfast organ of the temnocephalids is not homologous with the holdfasts of neodermatans

(characters 77–84). Brooks *et al.* (1985a) hypothesized that the various holdfasts, while demonstrably different, were all part of a homologous transformation series. Within phylogenetic systematic methodology, this hypothesis could not be falsified by reiterating that the holdfasts were different (Rohde & Watson 1995), but could be falsified by including more taxa in the analysis, as we have done herein.

Additionally, Rohde and coworkers (Rohde 1990, 1994a, 1996; Littlewood *et al.* 1999a) suggested that protonephridial characters should be given high weight in phylogenetic analyses of the Platyhelminthes. We considered six protonephridial characters in this study. Three of them (12, 13, 16) have character consistencies of 100%, character 15 has a character consistency of 50%, 17 has a character consistency of 33% and character 14 has a character consistency of 25%. The combined character consistencies for these traits is 68%, and their exclusion from the analysis produces the same tree topology as shown in Fig. 3(A) and increases the CI slightly. In addition, character 17 is one of the characters producing marked instability due to missing data. Reciprocal illumination thus tells us that protonephridial characters are, at best, no better than any other character.

Phylogeneticists expect that the analysis of a data set comprising incorrect homology assessments will produce a distinctive result — many MPTs with low CIs. This is not the case with the Brooks *et al.* (1985a) (see also Brooks 1989a,b; Brooks & McLennan 1993c) data sets, nor is it the case with the present data set. In the current study, 90% of the characters support the relationships indicated for the Revertospermata, and these results strongly corroborate previous analyses. In the past, these results have been rejected because we are dealing with parasites (Neodermata) and symbiotic 'turbellaria', and adaptation to a common lifestyle is 'known' to produce high degrees of correlated homoplasy (Rohde 1990, 1994a, 1996; Littlewood *et al.* 1999a). To correct for this problem, characters 'known' to be adaptations to parasitism/symbiosis have been discounted (eliminated from the analysis *a priori*). For example, Rieger & Tyler (1985) suggested that similar structures in taxa sharing similar environments (e.g. exposed to similar selection pressures) should be coded *a priori* as homoplasious, or ambiguous as in Littlewood *et al.* (1999a,b).

Such suggestions ignore the basic Darwinian notion that homologies can be adaptations and that adaptation need not produce homoplasy. In the past decade, a substantial amount of evidence has accumulated indicating that most similarities in structure, function and preferred environment are due to common ancestry (Wanntorp *et al.* 1990; Brooks & McLennan 1991; Harvey & Pagel 1991). There is thus no reason to exclude, or manipulate, any 'adaptive' character from any analysis (McLennan *et al.* 1988; Brooks & McLennan 1991, 1993c, 1994; McLennan 1993). In addition, Ronquist's (1994) study on the evolution of inquilinism in cynipid hymenopterans, for example, showed that removal of characters associated with a parasitic lifestyle did not alter the phylogenetic assessment that inquilinism had arisen only a single time in the group. Finally, Trouvé *et al.* (1998) showed that a suite of life history traits for free-living and parasitic platyhelminths did not differ, suggesting that neodermatans do not have a 'parasitic mode of life' so much as a 'platyhelminth mode of life' in a parasitic context.

In recent years, some authors have disparaged the morphological data upon which previous analyses of the Neodermata and their relatives had been performed because the results were not compatible with molecular data (Rohde 1990, 1994a, 1996; Littlewood *et al.* 1999a; Litvatis & Rohde 1999; Mollaret *et al.* 2000). It has also been suggested that the phylogenies based upon morphological data have been highly variable and greatly differ among each other (Littlewood *et al.* 1999a). This has not actually been the case. First, the relationships among the neodermatan groups have been the same in multiple studies using phylogenetic systematic methods beginning in 1985, with CI values remaining between 95% and 97% despite an increase in the number of characters used from 39 to 147. Second, differences in hypotheses of the sister group of the Neodermata have been based on differences in the taxa analysed; our analysis herein accommodates all previously proposed sister groups in a manner that is congruent with all previous hypotheses.

In addition, Kornakova & Joffe (1999) pointed out that molecular results have failed to reproduce the monophyly of several firmly established taxa (based on morphology) and suggest that we consider sampling and long-branch attraction as serious effects in molecular analyses. For example, molecular studies suggest various combinations of para- or even polyphyly for the Monogenea, whereas morphological studies consistently suggest that the group is monophyletic. Some authors take this as an indication that we should question all morphological traits used in phylogenetic studies of monogeneans (Rohde 1990, 1994a, 1996; Justine 1998b; Litvatis & Rohde 1999; Mollaret *et al.* 2000). Littlewood *et al.* (1999b) showed that a combination of sequence data and only 50 of the 89 characters used herein supported a monophyletic Monogenea, and accepted that grouping. Since the molecular data alone did not support Monogenean monophyly, the study by Littlewood *et al.* (1999b) provides evidence of insufficiencies in the sequence data as suggested by Kornakova & Joffe (1999).

This thinking needs to be carried through consistently in all future total evidence studies. Littlewood *et al.* (1999a) coded nine characters shared uniquely by *Urastoma*, the Fecampiidae and the Neodermata as ambiguous for *Urastoma* and Fecampiidae, presumably based on Rohde's (1994a: 1104) assertion that 'comparison of DNA sequences … suggests that

the [fecampiids are] not a close relative of the Neodermata … thus the morphological similarities of the two groups appear indeed to be due to convergent evolution'. Likewise, Littlewood *et al*. (1999a,b) made a number of *ad hoc* assumptions concerning *Udonella*. For example, the absence of larval hooks was coded *a priori* as apomorphic secondary loss, when the same absence of larval hooks in aspidogastreans and digeneans was coded as plesiomorphic absence. These added assumptions clearly demonstrate an *a priori* coding 'preference' for regarding *Udonella* as a Monogenean. Finally, Littlewood *et al*. (1999a,b) utilized only slightly more than half of the available morphological characters that had been summarized in Brooks & McLennan (1993c). Many of these traits were characterized by Rohde (1990, 1994a, 1996) (also Litvaitis & Rohde 1999) as exhibiting a low probability of being homologous. Our study herein does not support that characterization. In fact, the total morphological database provides very strong support not only for the monophyly of the Monogenea, which Littlewood *et al*. (1999b) accepted, but also for the Fecampiids + *Urastoma* as the sister group of the Neodermata and *Udonella* as the sister group of the Cercomeridea Brooks, O'Grady & Glen 1985 (Trematoda + Cercomeromorphae).

Finally, our study corroborates the hypothesis that the ancestor of the Trematoda + Cercomeromorphae had a two-host life cycle involving the addition of a vertebrate host to the plesiomorphic arthropod host direct life cycle (Brooks *et al*. 1985a; Brooks 1989b; Brooks & McLennan 1993c), contrary to the proposal by Littlewood *et al*. (1999b) that the original life cycle was a single vertebrate host direct cycle. This is the most parsimonious explanation even if *Udonella* is a monogenean. It supports the notion that vertebrate endoparasitism in this group originated through the predation of vertebrates on arthropods. It may also be an example supporting the hypothesis that the alternation of hosts is an adaptive response to avoid the evolutionary costs of overspecialization (Moran 1988, 1994; see also Kuris & Norton 1985).

Conclusions

The morphological database for the Neodermata and close relatives is highly robust. This is partly due to the fact that the data themselves are numerous and unambiguous. More importantly, scientific hypotheses are more robust in proportion to the number of tests they have survived (Popper 1960, 1968a,b, 1972, 1976, 1992), and the current database reflects the efforts of a number of specialists to refute the hypothesis first proposed by Brooks *et al*. (1985a). The current study also shows that phylogenetic systematic analysis is capable of uncovering instances in which our *a priori* presumption of homology is not supported. Thus, the selective removal of characters *a priori* is not necessary and indeed is counterproductive if our aim is always to produce the most robust hypothesis of phylogenetic relationships possible given all available evidence. The parasitic platyhelminths represent one of the most extensively studied animal groups, with a database assembled over the past 200 years that will soon exceed 2500 morphological character states. This represents historical continuity in our studies of flatworms, which comprises a formidable assemblage of knowledge about structure and biology. The results of the current study indicate that comparative morphology remains viable, tractable and powerful. Phylogenetic analyses using morphological data provide an excellent framework for assessing our young but growing molecular database. We suggest that future total evidence studies should make full use of the large and robust morphological database documented herein.

This study also highlights two other benefits of a phylogenetic systematic approach: the ability, through reciprocal illumination, to falsify previous hypotheses of character evolution, and the ability to highlight areas where further research would be the most immediately beneficial. In this case, we clearly need more studies on the enigmatic groups such as the Acholadidae, Pseudograffillinae, Hypoblepharinidae, Solenopharyngidae, Promesostomidae, Trigonostomidae and Kytorhynchidae.

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