



Preliminary cladistic analysis of genera of the cestode order Trypanorhyncha Diesing, 1863*

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Abstract

A preliminary cladistic analysis was carried out on the 49 currently recognised genera of the order Trypanorhyncha. Forty-four characters were analysed; a functional outgroup was used for scolex and strobilar characters, while *Nybelinia* was utilised to polarise characters related to the rhyncheal system. Eight well-resolved clades were evident in the resultant cladogram, which is compared with existing phenetic classifications. An analysis of families resulted in a similar clustering of taxa to that observed in the case of the genera. The results suggest that two key characters used in existing classifications, namely the presence of sensory fossettes on the bothridia and the development of atypical heteroacanth and poeciloacanth armatures from typical heteroacanth armatures, have occurred on several occasions. Some clades provide support for the arrangements used in current classifications. Suggestions are made for future avenues of research which might provide more robust phylogenetic data for the Trypanorhyncha.

Introduction

There have been few attempts to investigate phylogenetic relationships within the cestode order Trypanorhyncha. Dollfus (1942) tentatively suggested a phylogenetic arrangement based on the number of bothridia and the tentacular armature. He considered that: (a) genera with two bothridia had evolved from those with four bothridia on several occasions; (b) hollow hooks had evolved from solid hooks; and (c) the evolution of patterns in the tentacular armature was from genera with hooks arranged in ascending spirals (the homeoacanth) to genera in which the hooks were arranged in ascending half-circles (the heteroacanth) and then genera with distinct files, extra rows or bands of hooks on the external surface of the tentacle (atypical heteroacanth and poeciloacanth). Dollfus (1942) did not however discuss the relationships of specific

families or genera, preferring to restrict himself to an overview of evolutionary pathways within the order.

Campbell & Beveridge (1994) revised Dollfus' (1942) classification by adding new genera and families, but retained its essential structure (Table I). They considered that a cladistic analysis of the order was probably premature, but presented their essentially phenetic classification within a cladistic framework as a working hypothesis (Figure 1), entering family names and potential synapomorphies onto branches where this was possible. While utilising the same morphological characters as Dollfus (1942), they also included the form of the uterus, the presence of sensory fossettes, whether or not bothridia were pedicellate and features of the genital system, namely paired or single genitalia, presence of an hermaphroditic duct and presence of an accessory seminal vesicle. The tree they produced therefore resembled that proposed by Dollfus (1942) but provided greater detail. Their earlier speculations (Beveridge & Campbell, 1988a) on the relationships between the families Tetrarhynchobothriidae and Eutetrarhynchidae and the relation-

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ships among genera bearing files of hooks termed "chainettes" (poeciloacanthus by their definition) (Beveridge & Campbell, 1989) were not included in their classification due to uncertainty of the validity of the hypotheses suggested in the earlier paper and because the primary goal was the production of a stable phenetic classification.

Most recently, Palm (1995, 1997) has proposed an alternative classification of the Trypanorhyncha (Figure 2) based on four primary characters: (a) presence or absence of a blastocyst; (b) presence of sensory fossettes; (c) presence of a prebulbar organ; and (d) the possession of two or four bothridia. Less importance was placed on a fifth character, the pattern of the tentacular armature. Palm (1995) argued that since this latter character was frequently difficult to interpret and was homoplasious, it could only be used at family level or below. The presence or absence of a blastocyst separates the same group of genera in the system of Palm (1995) as in the systems of Dollfus (1942) and Campbell & Beveridge (1994), while the use of sensory fossettes and prebulbar organs at the superfamily level is novel. Although developed initially from a phenetic point of view (1995), the characters utilised can be polarised and therefore a phylogenetic hypothesis can be developed from it (Palm, 1997), as is the case with the essentially phenetic classification of Campbell & Beveridge (1994).

The two different phenetic classifications of Campbell & Beveridge (1994) and Palm (1995, 1997) differ significantly in structure and in the weight placed on different morphological characteristics in constructing the respective classifications.

A formal phylogenetic analysis of the Trypanorhyncha could therefore provide further insights into the classification of the order and the way in which classifications currently in use might be altered or improved.

Critical to such an analysis is an understanding of the position of the Trypanorhyncha within the Eucestoda. A detailed comparative historical account of relationships (Hoberg et al., 1997) indicates that, broadly, the trypanorhynchs have been allied either with the Tetraphyllidea (see for example Baer, 1950) or with the Pseudophyllidea (see for example Euzet, 1959). Brooks et al. (1991) provided a series of arguments for an alliance between the Trypanorhyncha and the Tetraphyllidea, including as synapomorphies: (a) ovaries four-lobed in transverse section; (b) four bothridia with rigid margins (in some genera fusing to form two bothridia); and (c) the lack of flame-cells in the

hexacanth embryo. Their hypothesis is not invalidated by the fact that a few trypanorhynchs have bilobed ovaries (Campbell & Beveridge, 1994) and could be strengthened by the adoption of the hypothesis, first advanced by Pintner (1896), that the rhyncheal system peculiar to trypanorhynchs, with its four tentacles, is the homologue of the four suckers of tetraphyllideans. Ultrastructural features of the rhyncheal system are at least concordant with this hypothesis (Beveridge & Smith, 1988).

An additional morphological feature supporting an association with the tetraphyllideans is the presence of a linear, central uterus (except in the Homeacanthoidea) in contrast to the coiled uterus (plesiomorphic) present in most Pseudophyllidea as well as in the Amphilinidea, used by Brooks et al. (1991) as an outgroup for the Eucestoda. Synapomorphies supporting an association with the Pseudophyllidea are (a) the presence of ciliated embryos and (b) circumedullary vitelline distribution compared with lateral bands of vitelline follicles in the Tetraphyllidea and in the amphilinidean outgroup. Thus there is some evidence which supports both hypotheses.

In a subsequent cladistic analysis of the eucestode orders, Hoberg et al. (1997) concluded that the Trypanorhyncha was basal to the "higher cestodes", the Tetraphyllidea, Lecanicephalidea, Proteocephalidea, Nippotaeniidea, Tetrabothriidea and Cyclophyllidea, but arose after the Pseudophyllidea. The molecular data of Mariaux (1998), however, place the Trypanorhyncha as the most primitive group of polyzoic cestodes, derived from caryophyllidean and spathebothriidean ancestors but in turn ancestral to the Pseudophyllidea.

The current cladistic analysis of the Trypanorhyncha is considered preliminary in nature for a number of reasons. Character polarity is likely to be controversial because of the degree of uncertainty in the position of the Trypanorhyncha within the Eucestoda. In addition, for many genera which are incompletely described or known only from metacestodes, scoring of all characters is not possible. Furthermore, a number of potentially useful characters has been recognised for which insufficient information exists across genera to permit their utilisation. The current analysis is therefore presented as a tentative hypothesis in the hope that it will suggest further directions for study of this cestode order.

Table 1. Comparison of the classifications of the Trypanorhyncha Diesing, 1863 by Campbell & Beveridge (1994) and Palm (1997).

Campbell & Beveridge (1994)	Palm (1977)
Homeacanthoidea Dollfus, 1942	Tentacularioidea Palm, 1995
Tentaculariidae Poche, 1926	Tentaculariidae Poche, 1926
<i>Kotorella</i> Euzet & Radujkovic, 1989	<i>Kotorella</i> Euzet & Radujkovic, 1989
<i>Tentacularia</i> Bosc, 1797	<i>Tentacularia</i> Bosc, 1797
<i>Nybelinia</i> Poche, 1926	<i>Nybelinia</i> Poche, 1926
Paranybeliniidae Schmidt, 1970	
<i>Paranybelinia</i> Dollfus, 1966	
<i>Pseudonybelinia</i> Dollfus, 1966	
Hepatoxylidae Dollfus, 1940	Sphyriocephalidae Pintner, 1913 (= Hepatoxylidae of Palm (1995))
<i>Hepatoxylon</i> Bosc, 1811	<i>Hepatoxylon</i> Bosc, 1811
Sphyriocephalidae Pintner, 1913	<i>Sphyriocephalus</i> Pintner, 1913
<i>Sphyriocephalus</i> Pintner, 1913	
Tetrarhynchobothriidae Dollfus, 1969	Gilquiniidae Dollfus, 1942
<i>Tetrarhynchobothrium</i> Diesing, 1854	<i>Gilquinia</i> Guiart, 1927
<i>Didymorhynchus</i> Beveridge & Campbell, 1988	<i>Deanicola</i> Beveridge, 1990
<i>Zygorhynchus</i> Beveridge & Campbell, 1988	<i>Plesiorhynchus</i> Beveridge, 1990
Heteracanthoidea Dollfus, 1942	Aporhynchidae (Poche, 1926)
Eutetrarhynchidae Guiart, 1927	<i>Aporhynchus</i> Nybelin, 1918
<i>Eutetrarhynchus</i> Pintner, 1913	Pterobothriidae Pintner, 1931 (= Gymnorhynchidae of Palm (1995))
<i>Oncomegas</i> Dollfus, 1929	<i>Gymnorhynchus</i> Rudolphi, 1819
<i>Dollfusiella</i> Campbell & Beveridge, 1994	<i>Chimaerarhynchus</i> Beveridge & Campbell, 1989
<i>Mecistobothrium</i> Heinz & Dailey, 1974	<i>Molicola</i> Dollfus, 1935
<i>Trigonobium</i> Dollfus, 1929	<i>Pterobothrium</i> Diesing, 1850
<i>Parachristianella</i> Dollfus, 1946	<i>Rhinoptericola</i> Carvajal & Campbell, 1975
<i>Prochristianella</i> Dollfus, 1946	<i>Stragulorhynchus</i> Beveridge & Campbell, 1988
<i>Trimacracanthus</i> Beveridge & Campbell, 1987	Lacistorhynchidae Guiart, 1927
<i>Pseudochristianella</i> Campbell & Beveridge, 1990	<i>Lacistorhynchus</i> Pintner, 1913
	<i>Callitetrarhynchus</i> Pintner, 1931
	<i>Dasyrhynchus</i> Pintner, 1928
	<i>Diesingium</i> Pintner, 1929
	<i>Floriceps</i> Cuvier, 1817
	<i>Grillotia</i> Guiart, 1927
	<i>Hornelliella</i> Yamaguti, 1954
	<i>Patellobothrium</i> Beveridge & Campbell, 1989
	<i>Progrillotia</i> Dollfus, 1946
	<i>Pseudogrillotia</i> Dollfus, 1969
	<i>Pseudolacistorhynchus</i> Palm, 1995**
Gilquiniidae Dollfus, 1942	
<i>Gilquinia</i> Guiart, 1927	
<i>Aporhynchus</i> Nybelin, 1918	
<i>Deanicola</i> Beveridge, 1990	
<i>Plesiorhynchus</i> Beveridge, 1990	
Shirleyrhynchidae Campbell & Beveridge, 1994	
<i>Shirleyrhynchus</i> Beveridge & Campbell, 1988	
<i>Cetorhynchicola</i> Beveridge & Campbell, 1988	
Otobothrioidea Dollfus, 1942	Otobothrioidea Dollfus, 1942
Otobothriidae Dollfus, 1942	Otobothriidae Dollfus, 1942
<i>Otobothrium</i> Linton, 1890	<i>Otobothrium</i> Linton, 1890
(<i>Pseudotobothrium</i> Dollfus, 1942*)	<i>Pseudotobothrium</i> Dollfus, 1942
<i>Diplootobothrium</i> Chandler, 1942	<i>Diplootobothrium</i> Chandler, 1942
<i>Poecilancistrum</i> Dollfus, 1929	<i>Poecilancistrum</i> Dollfus, 1929
	<i>Poecilacanthum</i> Palm, 1995**

Table 1. Continued

Campbell & Beveridge (1994)	Palm (1997)
Rhinoptericolidae Carvajal & Campbell, 1975 <i>Rhinoptericola</i> Carvajal & Campbell, 1975	Paranybeliniidae Schmidt, 1970 <i>Paranybelinia</i> Dollfus, 1966 <i>Pseudonybelinia</i> Dollfus, 1966
Pterobothriidae Pintner, 1931 <i>Pterobothrium</i> Diesing, 1850	Pseudotobothriidae Palm, 1995 <i>Pseudotobothrium</i> Dollfus, 1942
Grillotiidae Dollfus, 1969 <i>Grillotia</i> Guiart, 1927 <i>Pseudogrillotia</i> Dollfus, 1969 <i>Progrillotia</i> Dollfus, 1946	
Molicolidae Beveridge & Campbell, 1989 <i>Molicola</i> Dollfus, 1935 <i>Stragulatorhynchus</i> Beveridge & Campbell, 1988	
Poecilacanthoidea Dollfus, 1942	Eutetrarhynchoidea Dollfus, 1969
Lacistorhynchidae Guiart, 1927 <i>Lacistorhynchus</i> Pintner, 1913 <i>Floriceps</i> Cuvier, 1817 <i>Callitetrarhynchus</i> Pintner, 1931	Eutetrarhynchidae Guiart, 1927 <i>Eutetrarhynchus</i> Pintner, 1913 <i>Dollfusiella</i> Campbell & Beveridge, 1994 <i>Mecistobothrium</i> Heinz & Dailey, 1974 <i>Oncomegas</i> Dollfus, 1929 <i>Parachristianella</i> Dollfus, 1946 <i>Prochristianella</i> Dollfus, 1946 <i>Pseudochristianella</i> Campbell & Beveridge, 1994 <i>Trigonolobium</i> Dollfus, 1929 <i>Trimacracanthus</i> Beveridge & Campbell, 1987 <i>Didymorhynchus</i> Beveridge & Campbell, 1988 <i>Tetrarhynchobothrium</i> Diesing, 1854 <i>Zygorhynchus</i> Beveridge & Campbell, 1988
Dasyrhynchidae Dollfus, 1935 <i>Dasyrhynchus</i> Pintner, 1928	
Hornelliellidae Yamaguti, 1954 <i>Hornelliella</i> Yamaguti, 1954	
Mustelicolidae Dollfus, 1969 <i>Patellobothrium</i> Beveridge & Campbell, 1989 <i>Diesingium</i> Pintner, 1929	
Gymnorhynchidae Dollfus, 1935 <i>Gymnorhynchus</i> Rudolphi, 1819 <i>Chimaerarhynchus</i> Beveridge & Campbell, 1989	
Mixodigmatidae Dailey & Vogelbein, 1982 <i>Mixodigma</i> Dailey & Vogelbein, 1982 <i>Halysiorhynchus</i> Pintner, 1913	Mixodigmatidae Dailey & Vogelbein, 1982 <i>Mixodigma</i> Dailey & Vogelbein, 1982 <i>Halysiorhynchus</i> Pintner, 1913
	Shirleyrhynchidae Campbell & Beveridge, 1994 <i>Shirleyrhynchus</i> Beveridge & Campbell, 1988 <i>Cetorhynchicola</i> Beveridge & Campbell, 1988

* Recognised as subgenus.

** New genera described by Palm (1995).

Materials and methods

General approach

The initial approach taken in the current analysis was to investigate the relationships of genera of the Trypanorhyncha. The obvious deficiencies of such a strategy are that the number of genera (49) exceeds the number of available characters (44), thereby leading to relatively poor resolution in the phylogeny. The alternative possibility of analysing families was also undertaken realising that, while genera are rel-

atively well defined (Campbell & Beveridge, 1994), recent discoveries have made higher levels of the classification, particularly the composition of families, controversial (Palm, 1995, 1997). Analysis of families has the advantage that the number of characters available exceeds the number of taxa and therefore is likely to produce a higher level of resolution.

Outgroups

The relatively basal position of the Trypanorhyncha within the Eucestoda provides significant difficulties

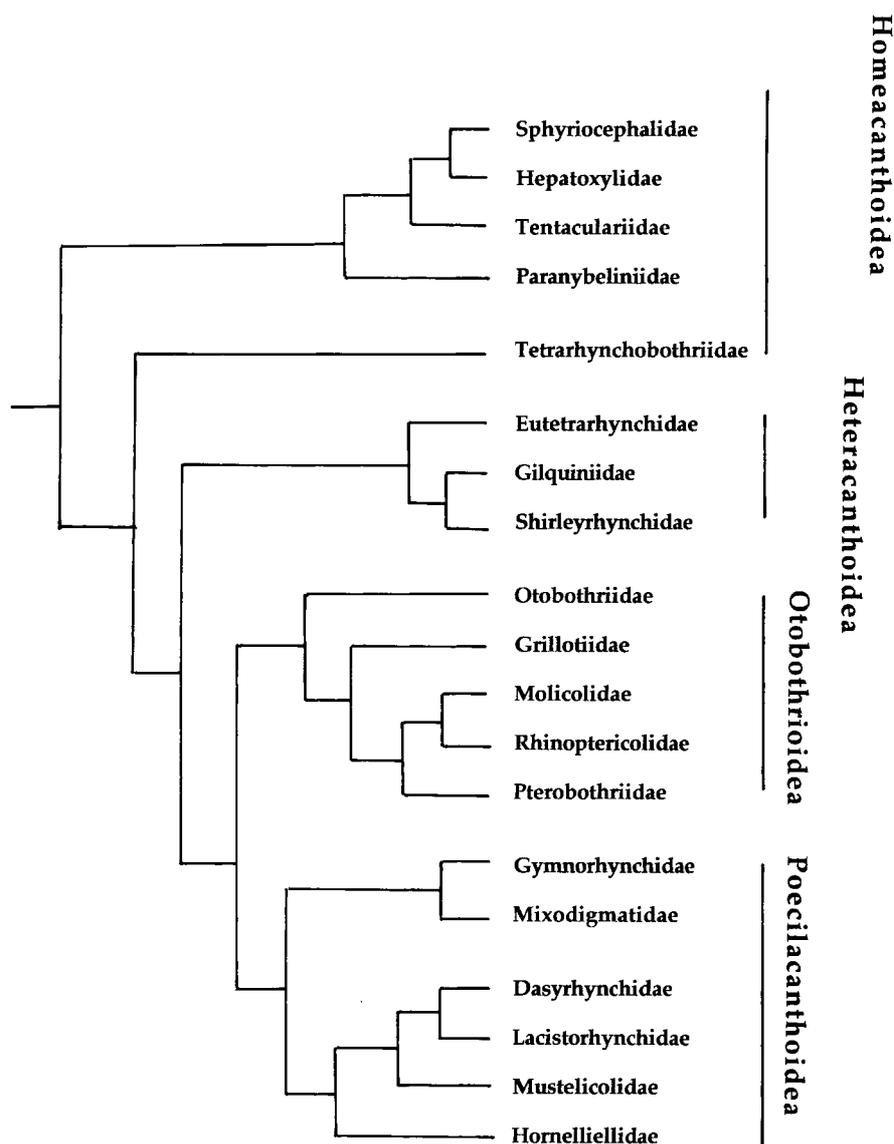


Figure 1. Classification of the Trypanorhyncha to family level, proposed by Campbell & Beveridge (1994).

in determining an appropriate outgroup. Both the Tetrephyllidea or the Pseudophyllidea might have been considered as potential outgroups based on earlier phylogenetic hypotheses (Hoberg et al., 1997), but both provide difficulties, not only because neither is probably a sister group to the Trypanorhyncha, but also because many of the taxonomic characters used within the Trypanorhyncha relate to their unique rhyneal system, which is absent in all potential outgroups. To overcome some of these difficulties, amphilinideans were used as the outgroup (Watrous & Wheeler, 1981) based on the cladistic analysis of

the Eucestoda by Hoberg et al. (1997). Wherever possible, characters used by Hoberg et al. (1997) for the cestode orders were also applied to the genera of the Trypanorhyncha. The arguments used in character polarisation are therefore presented in some detail as they are potentially controversial. Polarisation of rhyneal characters is also discussed below.

1. *Scolex*. The scolex of trypanorhynchs is considered to be essentially tetrafossate since, although there may be two or four bothridia, there are always four tentacles, sheaths and bulbs. The uniform presence of

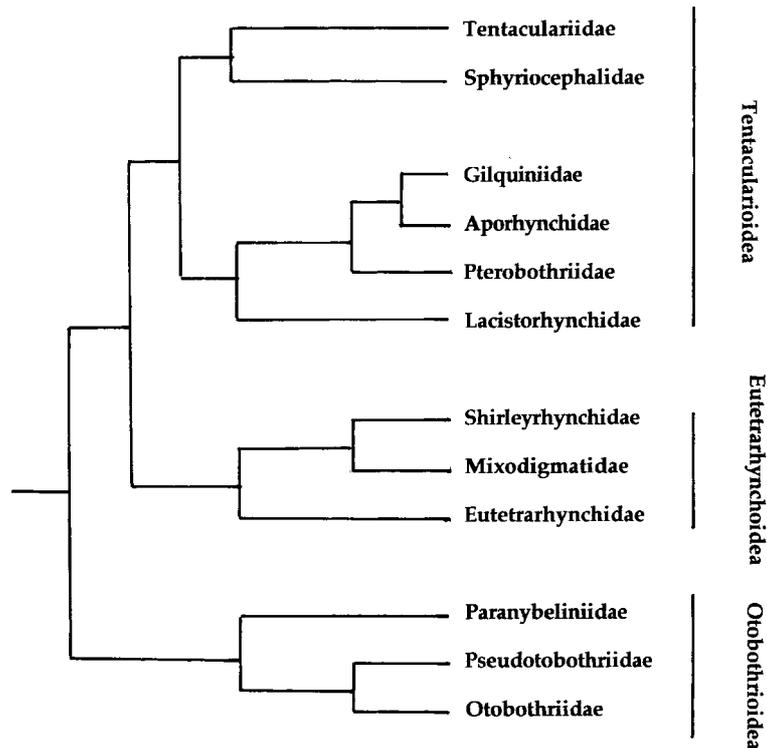


Figure 2. Classification of the Trypanorhyncha to family level, proposed by Palm (1995, 1997).

four elements in the rhyncheal system, whether the bothridia number four, two or are incompletely fused, appears to provide compelling evidence that four bothridia represents the plesiomorphic condition within the trypanorhynchs. An alternative polarity might have been obtained if the pseudophyllideans were adopted as the outgroup. Pseudophyllideans bear two bothria rather than four bothridia and the two organs are not necessarily homologues. Brooks et al. (1991) considered the difossate condition apomorphic among polyzoic cestodes, while Hoberg et al. (1997) considered the tetrafossate bothridia of the trypanorhynchs plesiomorphic with respect to the remaining tetrafossate orders. In spite of potentially conflicting views, the presence of four tentacles is used as evidence for treating the tetrafossate condition as plesiomorphic within the order.

The presence of sensory fossettes on the margins of the bothridia is considered apomorphic because they are absent in all other cestode orders. Similarly, pedicellate bothridia represent an apomorphic character both within the trypanorhynchs and the tetraphyllideans and do not occur in other cestodes. Elongation of the pars pedunculus scolecis with a scolex

clearly distinguished from the strobila is again considered apomorphic by comparison with all other cestode orders.

2. *Rhyncheal system.* The rhyncheal system is autapomorphic for the Trypanorhyncha (see Brooks et al., 1991), is presumably homoplasious with the tentacular system of the Haplobothriidea (see Hoberg et al., 1997) and may have been lost secondarily in a single gilquiniid genus *Aporhynchus*. Polarisation of characters associated with this organ system was based on *Nybelinia*, the genus which has the "simplest" armature pattern in which hooks are homeomorphous and exhibit a pattern of rotational symmetry (Campbell & Beveridge, 1994). All other patterns found in the order can be derived from that exhibited by *Nybelinia* (see Campbell & Beveridge, 1994). With no outgroup polarisation possible in this instance, the transition from a simple, symmetrical armature to complex and asymmetrical armatures has been assumed rather than demonstrated. The reverse polarisation would be extremely difficult to defend. This leads to the assumptions that homeomorphous armatures are plesiomorphic and that all specialised hook

forms (bill-hooks, bidentate hooks) are apomorphic. As well, hook patterns exhibiting rotational symmetry are plesiomorphic while those exhibiting glide reflection symmetry or complex arrangements of hooks (chainettes, bands of hooks) are apomorphic.

Other features of the rhyncheal system (bulbs, retractor muscles) are also polarised based on the condition present in *Nybelinia*.

3. *Genital pore and genital ducts.* Several characters associated with these structures warrant comment.

(a) Position of genital pores. In the Amphilinidea, the genital pores are ventral, in the Pseudophyllidea the pores can be ventral, marginal, sub-marginal or dorsal (Bray et al., 1994) while in the Tetraphyllidea genital pores are marginal or sub-marginal (Euzet, 1994). In the trypanorhynch genital pores are either marginal or ventro-submarginal; the ventro-submarginal character was considered plesiomorphic with respect to the marginal position by comparison with the amphilinideans, although clearly, using the same criterion with the amphilinideans as the outgroup, the ventro-submarginal position is also apomorphic.

(b) Hermaphroditic ducts occur commonly in trypanorhynchs (Campbell & Beveridge, 1994) but are not found in any other orders of cestodes. The occurrence of this character is therefore considered to be apomorphic.

(c) Seminal vesicles. Three types of seminal vesicles are present within the Trypanorhyncha: internal, external and accessory (see Dollfus, 1942; Campbell & Beveridge, 1994). Since these structures are absent in basal cestodes and accessory seminal vesicles occur exclusively in the trypanorhynchs, their occurrence has been treated as apomorphic.

(d) Testes. Testes are distributed in lateral pre-ovarian bands in cestodarians (Gibson, 1994a,b) and in most tetraphyllideans (Euzet, 1994). In Pseudophyllideans, testes are frequently in lateral bands but may be pre- or post-ovarian (Bray et al., 1994). Consequently, testes in the pre-ovarian position were considered plesiomorphic within the Trypanorhyncha, using the condition in cestodarians as the out-group state.

4. *Uterus.* Uterine pores are present both in the Amphilinidea and the Pseudophyllidea. Their presence was therefore considered plesiomorphic within the Trypanorhyncha in agreement with the coding of this same character for the cestode orders by Hoberg et al. (1997). Two types of uterine development are observed within the Trypanorhyncha, species in which

the uterus forms *in situ* and species in which the uterus develops gradually from a sac or anlage present at the end of the uterine duct. A preformed uterus is present in amphilinideans, pseudophyllideans and tetraphyllideans and is therefore considered plesiomorphic. A uterus which grows from the end of the uterine duct occurs in certain trypanorhynchans as well as in some cyclophyllidean families and is therefore considered apomorphic. A similar polarisation was used by Hoberg et al. (1997).

5. *The development of the metacystode.* Trypanorhynch plerocerci occur either within a blastocyst or as a post-larva in which the blastocyst is lacking. Following Hoberg et al. (1997) (character 36), the lack of a blastocyst is considered plesiomorphic.

Character analysis

A character matrix (Table II) was constructed based on the genera included in the revision of the order by Campbell & Beveridge (1994) as well as three additional genera, two of which, *Poeciloacanthum* and *Pseudolacistorhynchus*, were erected by Palm (1995) and a third, *Pseudotobothrium* Dollfus, 1942, was elevated from sub-generic rank by Palm (1995). Most of the morphological features utilised are taken from the works cited. In addition, features of the strobila of *Gymnorhynchus* were derived from the description of Caira & Bardos (1996).

Most of the characters utilised are illustrated in the key to genera of Campbell & Beveridge (1994) and the figure numbers cited refer to this work. Plesiomorphic states are indicated by 0, apomorphic states by 1 or 2. In instances where both character states existed within a genus (polymorphic characters), three approaches were used. The character was coded directly as a polymorphism or additive binary coding was utilised (Scotland, 1992) in which character states are coded as either 00, 01 or 11. As an alternative method of coding, states were scored as apomorphic if any species in the genus exhibited the apomorphic condition.

For analysis at the family level, the matrix was re-coded to include all families recognised both by Campbell & Beveridge (1994) and Palm (1995, 1997). Direct coding of polymorphisms and additive binary coding were used for instances in which both character states were present within a single family and were compared with analyses based on recognising only apomorphic states.

Table II. Character matrix for cladistic analysis of the Trypanorhyncha.

<i>Kotorella</i>	??00000010????1??00011000001000000000000000
<i>Tentacularia</i>	1000000011000110000111000100000000000000000
<i>Nybelinia</i>	100000001 $\frac{0}{1}$ 00011000010100000 $\frac{0}{1}$ 0000000000000000
<i>Pseudonybelinia</i>	??11?00?0?????1??0?01?0?000000000000000000
<i>Paranybelinia</i>	??11?00?0?????1??0?01?0?000000000000000000
<i>Hepatoxylon</i>	1001100200100000000010?00001000000000000000
<i>Sphyricephalus</i>	1001000200?00000000010?00001000000000000000
<i>Didymorhynchus</i>	0?0100101010000000101101?00?0000000000000000
<i>Zygorhynchus</i>	0?01001010100001001101011001 $\frac{0}{1}$ 0000000000000000
<i>Tetrarhynchobothrium</i>	01010010102000010011110110010000000000000000
<i>Oncomegas</i>	01010010100100010011100111111100000100000000
<i>Dollfusiella</i>	01010010101000010011010011111100000000000000
<i>Eutetrarhynchus</i>	01010010102000010011110110011100000000000000
<i>Mecistobothrium</i>	0?01001010110001000?110111111110000000000000
<i>Trigonolobium</i>	0?010010100000010011011011111100000000000000
<i>Parachristianella</i>	010100101000000100110100111111100000000000000
<i>Prochristianella</i>	010100101000000100110100111111100000000000000
<i>Trimacracanthus</i>	0?010010100000010011011011111110000000100000
<i>Pseudochristianella</i>	0?0100101000000?00110100111?111000000000000000
<i>Aporhynchus</i>	0?00000?101110001000?110???????????????????
<i>Deanicola</i>	0?0000001011101010 $\frac{0}{1}$ 0 $\frac{0}{1}$ 110011111100000000000000
<i>Gilquinia</i>	010000001011100110000110111111110000000000000
<i>Plesiorhynchus</i>	0?0000001011100 $\frac{0}{1}$ 100011 $\frac{0}{1}$ 0100111 $\frac{0}{1}$ 00000000000000
<i>Shirleyrhynchus</i>	0?000010100000010110010010101110000000100000
<i>Cetorhynchus</i>	??00?0101?????0??01101?110101110000000100001
<i>Diplootobothrium</i>	0?1110?010010011001?011010011110100000000000
<i>Otobothrium</i>	0111000 $\frac{0}{1}$ 1010001100 $\frac{0}{1}$ 001101 $\frac{00}{11}$ 11110200000001000
<i>Pseudotobothrium</i>	?111?0011?????0??1001?0100111102000000000000
<i>Peocilancistrum</i>	01110001101100010010011010011101200000000000
<i>Rhinoptericola</i>	0?000000100100010110010010101110100000100000
<i>Pterobothrium</i>	0100010 $\frac{0}{1}$ 10 $\frac{00}{11}$ 0 $\frac{0}{1}$ 010010011010 $\frac{0}{1}$ 111112000000000000
<i>Pseudogrillotia</i>	0?01001010010?100000011010 $\frac{0}{1}$ 111112000000000000
<i>Grillotia</i>	010100001011000100 $\frac{0}{1}$ 001101 $\frac{00}{11}$ 11111200000001000
<i>Progrillotia</i>	0?01000010?????0??010010010011111200000000000
<i>Stragulorhynchus</i>	0?000001100111011000001111111111200000000001
<i>Molicola</i>	01000001100110000000001011111110200000010001
<i>Floriceps</i>	01010001101001010000011010011110010001000010
<i>Lacistorhynchus</i>	01010001101001010000111111111110010001001000
<i>Callitetrarhynchus</i>	01010001101001000000011010011110010001000000
<i>Dasyrhynchus</i>	01010001101101110010011110 $\frac{0}{1}$ 11111011010010000
<i>Hornelliella</i>	0?010001101001000010000010111110001000010000
<i>Patellobothrium</i>	?101?0010?????0??00001?010011110000101000000
<i>Diesingium</i>	0?010001101001000000011110011110000101000000
<i>Gymnorhynchus</i>	01000000100100001000100010111110001010010101
<i>Chimaerarhynchus</i>	0?000000101110001000010010011110001000000110
<i>Mixodigma</i>	0?000010100000010010110010101110010000000010
<i>Halysiorhynchus</i>	0?000110101100010010010010001110010010000010
<i>Pseudolacistorhynchus</i>	?101?0011?????0??00?1?01111111010000001000
<i>Poecilacanthum</i>	?111?00?1?????1??0001?01110110010000000010

Characters

Cited figures relate to Campbell & Beveridge (1994).

1. Uterus: Uterus pre-formed in the midline of the segment (figure 7.7a) (0) or uterus growing from an anlage at the end of the uterine duct to form an inverted U-shaped gravid uterus. (figure 7.7d) (1).
2. Metacestode. Plerocercus without blastocyst (figure 7.9a, b) (0) or with blastocyst (figure 7.8a, b) (1).
3. Sensory fossettes. Absent (0) or present (figures 7.64, 7.243, 7.250) (1).
4. Bothridia. Four bothridia (0) or two bothridia (1). Some genera with two bothridia have partially subdivided bothridia. To qualify as four bothridia, complete separation of the bothridia was deemed necessary.
5. Genitalia. Genitalia single (0) or paired (1).
6. Bothridia. Sessile (0) or pedicellate (1).
7. Prebulbar organ. Absent (0) or present (figure 7.4) (1).
8. Retractor muscle. Origin of retractor muscle at base of bulb (figures 7.56, 7.103) (0), within bulb but not at base (1) or at entry to bulb (2). These characters were considered as unordered.
9. Relative length of pars bothridialis of scolex. Pars bothridialis equal to or greater than the pars vaginalis (figure 5.56) (0) or pars bothridialis much shorter than the pars vaginalis (figure 7.102) (1).
10. Position of genital pore. Genital pore ventrosubmarginal (0) or marginal (1).
11. Internal seminal vesicle. Vesicle absent (figure 7.63) (0), present and single (figure 7.11) (1) or present and subdivided into proximal and distal regions (figure 7.12) (2). These states were treated as unordered characters.
12. External seminal vesicle. Vesicle absent (figure 7.63) (0) or present (figure 7.13) (1).
13. Accessory seminal vesicle. Vesicle absent (figure 7.63) (0) or present (figure 7.15) (1).
14. Hermaphroditic duct. Duct absent (figure 7.11) (0) or present (figures 7.14, 7.16) (1).
15. Scolex. Scolex acraspedote (figure 7.1) (0) or craspedote (figure 7.250) (1).
16. Uterine pore. Pore present (figure 7.84) (0) or absent (figure 7.94) (1).
17. Shape of uterus. Uterus central, symmetrical (figures 7.62, 7.142) (0) or uterus deviating anteriorly towards genital pore (figures 7.194, 7.203) (1).
18. Uterine lobes. Uterus without paired posterior lobes or diverticula (figure 7.142) (0) or with two posterior diverticula (figures 7.223, 7.266) (1).
19. Bulbs. Bulbs short (length to width ratio less than 5 : 1) (0) or bulbs long (length to width ratio greater than 5 : 1) (1).
20. Gland cells. Gland cells absent within bulb (figure 7.332) (0) or present (figure 7.103) (1).
21. Pars post bulbosa. Pars post bulbosa small or absent (figures 7.344, 7.301) (0) or prominent (figure 7.102) (1).
22. Bothridial margins. Margins relatively thin (0) or prominently thickened (figures 7.68, 7.75) (1).
23. Distribution of testes. Testes exclusively pre-ovarian (figure 7.172) (0) or some testes postovarian (figure 7.348) (1).
24. Pintner's cells. Cells absent (0) or present (figures 7.102, 7.138) (1).
25. Tentacle length. Tentacles short (less than 20–25 principal rows of hooks) (figures 7.52, 7.69) (0) or tentacles elongate (more than 20–25 principal rows of hooks) (1).
26. Basal swelling on tentacle. Swelling absent (figures 7.52) (0) or present (figure 7.180) (1).
27. Basal armature. Distinctive basal armature absent (figure 7.60) (0) or present (figure 7.180) (1).
28. Hook type. Hooks solid (0) or hollow (1).
29. Type of metabasal armature. Metabasalar armature homeomorphous (figure 7.59) (0) or heteromorphous (figure 7.178) (1).
30. Symmetry of metabasal armature. Symmetry rotational (figure 7.25) (0) or of glide reflection type (figure 7.29) (1).
31. Hook files 1(1'). Hook files 1(1') on internal surface convergent (figure 7.40) (0) or divergent (figure 7.41) (1).
32. Intercalary hook rows. Rows absent (figure 7.25) (0) or present (figure 7.44) (1).
33. Hook arrangement. Hooks homeoacanthous or heteroacanthous typical (figures 7.36, 7.42) (0), atypical (figure 7.43) (1) or with a band of hooks (figure 7.44) (2). These states are treated as unordered.
34. Chainette. Chainette absent (0) or present (figure 7.45) (1).
35. Chainette. Chainette single (figure 7.74) (0) or double (figure 7.353) (1).
36. Chainette. Chainette single (0) or multiple (figure 7.46) (1).

37. Chainette elements. One chainette element per principal row of hooks (0) or multiple elements per principal row (figure 7.45) (1).
38. Satellite hooks. Satellite hooks absent (0) or present (figure 7.347, hooks 7,8) (1).
39. Macrohooks on basal swelling of tentacle. Macrohooks absent (0) or present (figure 7.114) (1).
40. Macrohooks at base of tentacle. Falcate macrohooks absent at very base of tentacle (0) or present (figure 7.319) (1).
41. Bill-hooks. Base of tentacle without bill-hooks (0) or with bill-hooks (figure 7.343) (1).
42. Winged chainette elements. Chainette elements without wings (figure 7.382) (0) or with single wings (figure 7.402) (1).
43. Double winged chainette elements. Chainette elements with single wing or no wings (0) or with double wings (figures 7.402, 7.408) (1).
44. External tentacle sheath. Basal region of tentacle without unarmed sheath (0) or with unarmed sheath (figures 7.235, 7.236) (1).

The following characters were excluded from analysis as they occurred in a single genus only, usually as autapomorphies: secondary loss of the rhyncheal system (*Aporhynchus*), the presence of an hermaphroditic vesicle (*Hornelliella*); uterus x-shaped in dorso-ventral view (*Didymorhynchus*); vitelline follicles in lateral fields only (*Didymorhynchus*) (a plesiomorphic character); principal hook rows interrupted (some species of *Grillotia*); presence of hooks with dentate tips (*Pterobothrium*).

Characters considered for use but rejected only on the basis of there being insufficient information for each genus were: coracidium present or absent, eggs operculate or not, eggs quinone tanned or not, presence of protonephridia in embryo, embryophore present or absent, egg oligolecithal or polyolecithal, number of ovarian lobes, presence of an ovarian isthmus, presence of muscular band encircling the opening of the bulb, presence of palmate microtriches on the scolex and axis of orientation of the tentacular armature.

The consistency indices of the characters used are given in Table IV.

Relationships with hosts

A preliminary investigation was undertaken of the relationships between genera of trypanorhynch and orders of hosts as defined by Shirai (1996). Presence or absence of each genus was recorded, based

on the published literature, for each trypanorhynch genus in the Heterodontiformes, Orectolobiformes, Lamniformes, Carcharhiniformes, Hexanchiformes, Squaliformes, Squatiniformes, Pristiophoriformes and Rajiformes (Table III). Because of the preliminary nature of the analysis, the data for each clade of trypanorhynchs (except for the Paranybeliniidae whose adults are unknown) were simply superimposed onto the cladogram of host phylogeny of Shirai (1996) (Figure 5).

Data analysis

Analysis of the character matrix was performed using PAUP 3.1.1. Settings for the heuristic search were with random addition of taxa, tree-bisection-reconnection (TBR) branch swapping, MULPARS option in effect and topological constraints not enforced. Analyses were also carried out using the subtree pruning regrafting (SPR) and nearest neighbour interchange (NNI) options.

The data matrix was also analysed excluding those genera known only from their metacestodes or as immature adults (*Cetorhynchicola*, *Paranybelinia*, *Patellobothrium*, *Poeciloacanthum*, *Pseudolacistorhynchus*, *Pseudonybelinia*, *Pseudotobothrium*). The 50% majority rule consensus tree was calculated for each set of data. The analysis of the families was performed with and without the families Aporhynchidae and Pseudotobothriidae, families recognised by Palm (1995, 1997) but not by Campbell & Beveridge (1994).

Results

Analysis of genera

Different methods of coding polymorphisms and different methods of analysis (use of TBR, SPR and NNI options) had relatively little overall effect on the topology of the resulting consensus trees. The NNI produced trees with a greater number of steps than those using the TBR and SPR options and the latter trees were therefore examined in greater detail. The trees derived from the data set in which genera known only from the metacestode or immature adult stage had been excluded did not differ significantly in topology from trees based on the entire data set. The former trees were not therefore considered in detail. There were significant differences in tree topology between analyses based on different coding methods. (a) The

Table III. Occurrence of trypanorhynch genera in different orders of elasmobranch hosts. Host classification follows Shirai (1996).

Host order	Heterodontiformes	Orectolobiformes	Lamniformes	Carchariformes	Hexanchiformes	Squaliformes	Squatiniiformes	Pristiophoriformes	Rajiformes
Cestode genus									
Clade 1									
<i>Sphyriocephalus</i>			+	+		+			
<i>Hepatoxylon</i>			+						
<i>Tentacularia</i>			+	+					
<i>Nybelinia</i>	+	+		+		+			+
†									
Clade 3									
<i>Zygorhynchus</i>									+
<i>Didymorhynchus</i>									+
<i>Tetrarhynchobothrium</i>									+
<i>Eutetrarhynchus</i>				(+)*		(+)			+
<i>Dollfusiella</i>		(+)		(+)					+
Clade 4									
<i>Oncomegas</i>									+
<i>Mecistobothrium</i>									+
<i>Trigonolobium</i>				(+)					+
<i>Trimaacracanthus</i>				(+)					+
<i>Parachristianella</i>									+
<i>Prochristianella</i>									+
Clade 5									
<i>Rhinoptericola</i>									+
<i>Shirleyrhynchus</i>									+
<i>Cetorhnicola</i>			+						
<i>Mixodigma</i>			+						
<i>Halysiorhynchus</i>									+
<i>Diplobothrium</i>				+					
<i>Pseudogrillotia</i>				+					
<i>Poecilancistrum</i>				+					
<i>Progrillotia</i>									+
<i>Otobothrium</i>			+	+					+
<i>Grillotia</i>		+		+	+	+			+
<i>Pterobothrium</i>									+
Clade 6									
<i>Gymmorhynchus</i>			+						
<i>Chimaerarhynchus</i>							+		
<i>Molicola</i>			+						
<i>Stragulorhynchus</i>		+							
<i>Aporhynchus</i>						+			
<i>Deanicola</i>							+		
<i>Gilquinia</i>						+			
<i>Plesiorhynchus</i>				+		+			
Clade 7									
<i>Diesingium</i>				+					
<i>Patellobothrium</i>				+					
<i>Callitetrarhynchus</i>				+					
<i>Floriceps</i>				+					
<i>Lacistorhynchus</i>				+	+				
<i>Dasyrhynchus</i>				+	+				
<i>Hornelliella</i>		+							

* (+) indicates identifiable case of host switching.

† No adults are known for *Pseudonybelinia* and *Paranybelinia* which constitute Clade 2.

Table IV. Consistency indices (CI) for individual characters used in analyses of the genera of the Trypanorhyncha.

Character No.	Character	CI
1.	Uterus – development	1.00
2.	Blastocyst – presence	1.00
3.	Sensory fossettes – presence	0.20
4.	Bothridia – number	0.20
5.	Genitalia – single or paired	0.50
6.	Bothridia – sessile or pedicellate	0.50
7.	Prebulbar organ – presence	0.33
8.	Retractor muscle – origin	0.50
9.	Scolex ratios	0.50
10.	Position of genital pore	1.00
11.	Internal seminal vesicle	0.24
12.	External seminal vesicle	0.17
13.	Accessory seminal vesicle	0.50
14.	Hermaphroditic duct	0.27
15.	Scolex-craspedote or acraspedote	0.09
16.	Uterine pore	0.25
17.	Uterine shape	0.50
18.	Uterine lobes	1.00
19.	Bulb length	0.19
20.	Gland-cells within bulb	0.25
21.	Pars post bulbosa – presence	0.14
22.	Thickness of bothridial margins	0.13
23.	Distribution of testes	0.15
24.	Pintner's cells – presence	0.17
25.	Tentacle length	0.50
26.	Basal tentacular swelling	0.21
27.	Distinctive basal armature	0.11
28.	Hooks solid or hollow	0.25
29.	Homeomorphous or heteromorphous armature	0.37
30.	Symmetry of tentacular hook patterns	0.25
31.	Hook files 1 (1') divergent	0.22
32.	Intercalary hook rows	0.14
33.	Heteroacanthous armature	0.40
34.	Chainette – presence	0.25
35.	Chainette – single or double	0.50
36.	Chainette – multiple	0.50
37.	Number of chainette elements per principal row	0.33
38.	Satellite hooks	0.50
39.	Macrohooks on basal swelling of tentacle	0.33
40.	Macrohooks at very base of tentacle	0.33
41.	Bill-hooks – presence	0.33
42.	Winged chainette elements	1.00
43.	Double-winged chainette elements	0.25
44.	External tentacle sheath	0.33

position of the genus *Progrillotia* was not resolved using the additive binary coding method, while direct coding of polymorphisms allied *Progrillotia* closely with *Grillotia*. (b) The otophryniids formed a distinct clade in analyses using additive binary coding (Figure 3, Clade 6), but were less well resolved following direct coding methods. (c) The clade *Dasyrhynchus* + *Hornelliella* was aligned with genera of the Lacistorhynchidae in analyses based on additive binary coding but was allied with the eutetrarhynchids (clades 3–5) in analyses based on direct coding, except for those using the NNI option which also aligned this clade with the lacistorhynchid genera. The analysis based on additive binary coding (Figure 3) is presented because it provided a greater degree of resolution and because it agreed more closely with the family analysis.

The consensus of 1,000 equally parsimonious trees was calculated. Using binary coding, the consistency index was 0.25, the homoplasy index 0.75, retention index 0.66 and rescaled consistency index 0.17. Coding based on the occurrence of apomorphic characters resulted in a consensus tree with a consistency index of 0.28, a retention index of 0.62 and a rescaled consistency index of 0.18. Using direct coding of polymorphisms, the consensus tree had a consistency index of 0.29, a retention index of 0.67 and a rescaled consistency index of 0.19. Branches occurring in fewer than 80% of trees were collapsed for simplicity.

The majority rule consensus tree was relatively well resolved at the higher level, with eight readily identifiable clades (Figure 3), 6 of which occurred in 100% of the trees examined and 2 in 82% of the trees. The analysis did not resolve the relationships of the genera *Kotorella* or *Progrillotia*. Consistency indices for individual characters varied widely (Table IV), values of 1.00 being obtained for characters 1, 2, 10, 18 and 42 only.

Analysis of families

Several morphological characters (10, 36 and 42) became autapomorphies in the family analysis and were removed. Using direct coding of polymorphisms, 336 equally parsimonious trees were obtained. The consistency index was 0.52, the retention index 0.66 and the rescaled consistency index 0.35. Using additive binary coding, the trees had a consistency index of 0.37, a homoplasy index of 0.63, a retention index of 0.56 and a rescaled consistency index of 0.21. Without binary coding, there were 81 equally parsimonious

trees. The consistency index was 0.35, the retention index 0.54 and the rescaled consistency index 0.19. As with the generic analysis, the method of coding had relatively little effect on tree topology but direct coding provided a greater consistency index and hence this tree only is presented (Figure 4). Removal of the families Aporhynchidae and Pseudotobothriidae had no significant effect on tree topology.

Relationships with hosts

Comparisons of the parasite distributions in host orders with the host cladogram revealed several clear patterns (Figure 5). The homeacanthoid trypanorhynchids (clade 1) occurred primarily in heterodontiform, orectolobiform, lamniform and carchariniform sharks. Individual species were also found in squaliform and rajiform elasmobranchs, but these appeared to be exceptional. Trypanorhynchids from clade 8, the gilquiniids, gymnorhynchids and molicolids occurred equally in Orectolobiformes, Lamniformes, Carchariniformes and Squaliformes. The Otophryniid genera (*sensu* Campbell & Beveridge, 1994) of clade 6 had the broadest host range, occurring in all major clades of elasmobranchs. The poeciloacanthoid genera (clade 7) occurred in the clades from Orectolobiformes to Squaliformes. Trypanorhynchids from clades 3 and 4 belonging to the families Eutetrarhynchidae and Tetrarhynchobothriidae were almost exclusively restricted to Rajiformes. In all instances in which members of this clade were reported from sharks (e.g. Beveridge, 1990), they were identifiable as accidental infections as they occurred at a very low prevalence and intensity and/or did not develop to maturity. Trypanorhynchids of the related clade 5, Shirleyrhynchidae + Mixodigmatidae + Rhinoptericolidae again occurred primarily in Rajiformes. The two genera which constitute an apparent exception, *Mixodigma* and *Cetorhynchicola*, occur in large planctivorous lamnid shark genera (*Cetorhinus* and *Megachasma*).

Discussion

Genera

Although the current cladistic analysis of the trypanorhynchid genera produced a moderate degree of resolution of the relationships, caution is needed in its interpretation. The tree presented is the consensus of 1,000 equally parsimonious trees and had a

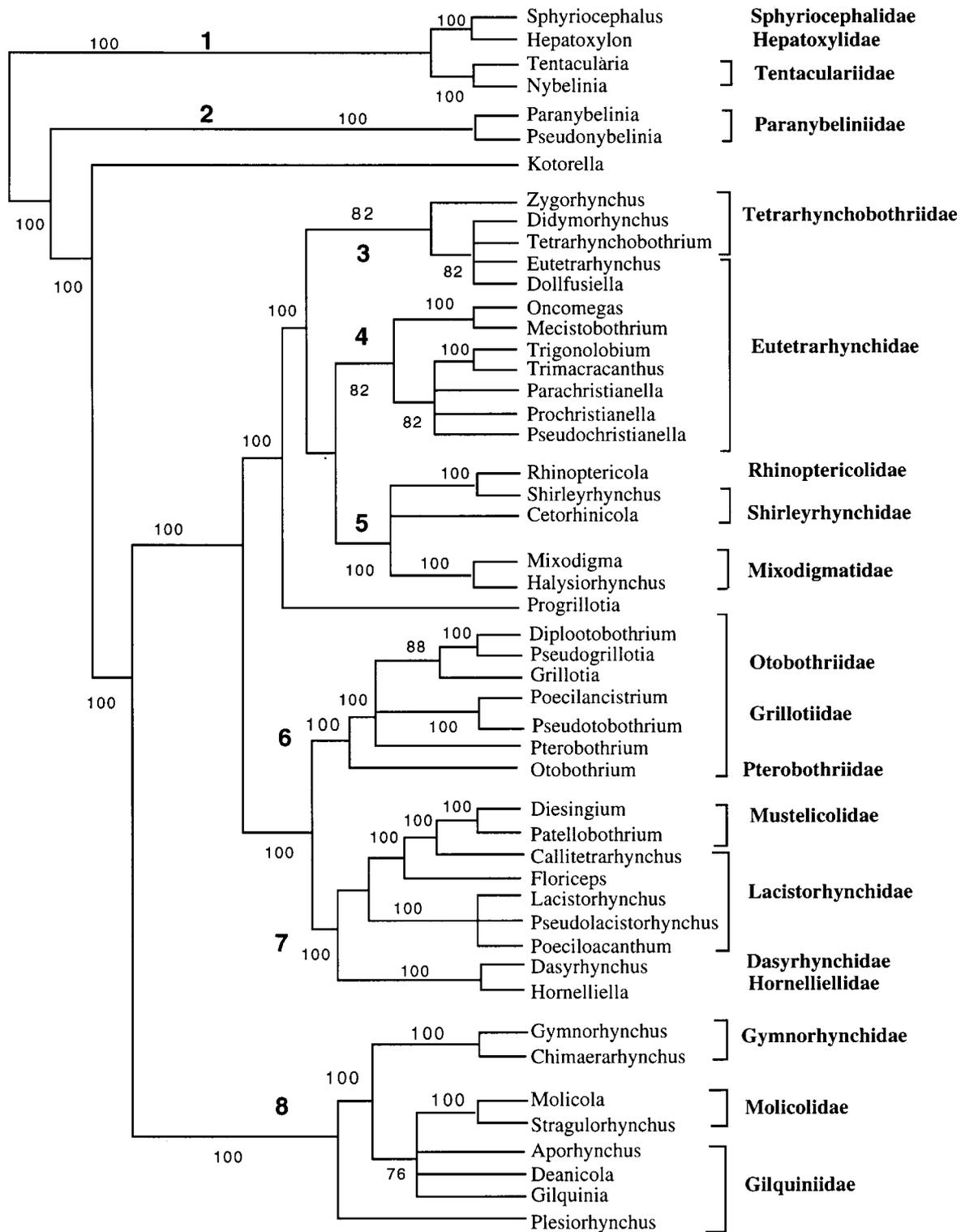


Figure 3. Majority rule consensus tree for cladistic analysis of 49 genera within the order Trypanorhyncha. Large numerals indicate major clades identified. Smaller numerals indicate the percentage occurrence of clades (>80%) in the individual trees from which the consensus tree was derived.

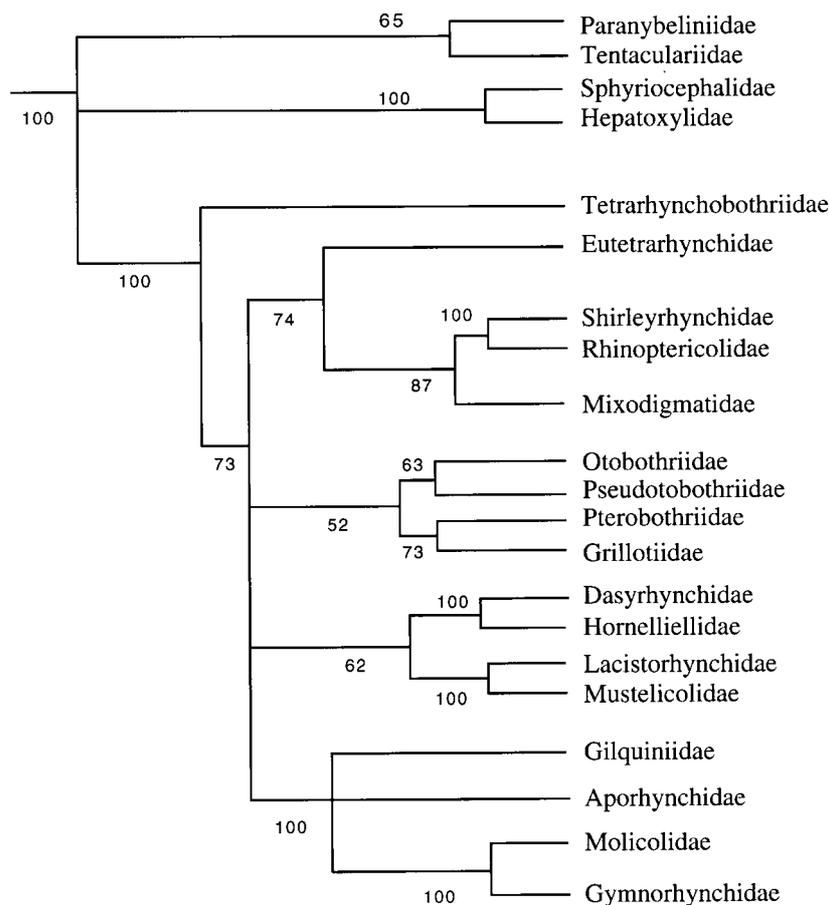


Figure 4. Majority rule consensus tree for cladistic analysis of 21 families within the order Trypanorhyncha. Numerals indicate the percentage occurrence of clades in the individual trees from which the consensus tree was derived.

consistency index of 0.25, indicating a high level of homoplasy. That most of the major branches occur in a high percentage of the individual trees suggests that the higher level clades are robust and that most of the homoplasy is in the terminal branches. The discussion is therefore restricted to the major clades, together with the reservation that the addition of new characters might produce a more robust tree or even a tree with a different topology. That the high level of homoplasy is not due to the inclusion of genera based on metacestodes and hence the significant numbers of characters which cannot be coded in the matrix was demonstrated by the fact that exclusion of these genera produced a tree that was no different topologically and did not have a higher consistency index. Similarly, different methods of coding polymorphisms did not significantly affect tree topology. Nevertheless, the large number of characters in the matrix which with our present level of knowledge cannot be coded is suf-

ficient cause for interpreting the current results with caution.

The position of the genus *Kotorella* was unresolved with the tree. On phenetic grounds, this genus is easily classified within an existing family using either existing system of classification (Campbell & Beveridge, 1994; Palm, 1995, 1997). *Kotorella* is in some respects a poorly characterised genus, with nine of its 44 characters being unscorable and with only five characters providing synapomorphies. This may be one reason for uncertainty over its position. Similarly, *Progrillotia* has nine apomorphic characters shared with the Eutetrarhynchidae and eleven with the Grillotiidae, which may account for the uncertainty of its position in the analysis. Using direct coding of polymorphisms, this genus was allied closely with *Grillotia*, suggesting that uncertainty over its position may be related primarily to methods of coding.

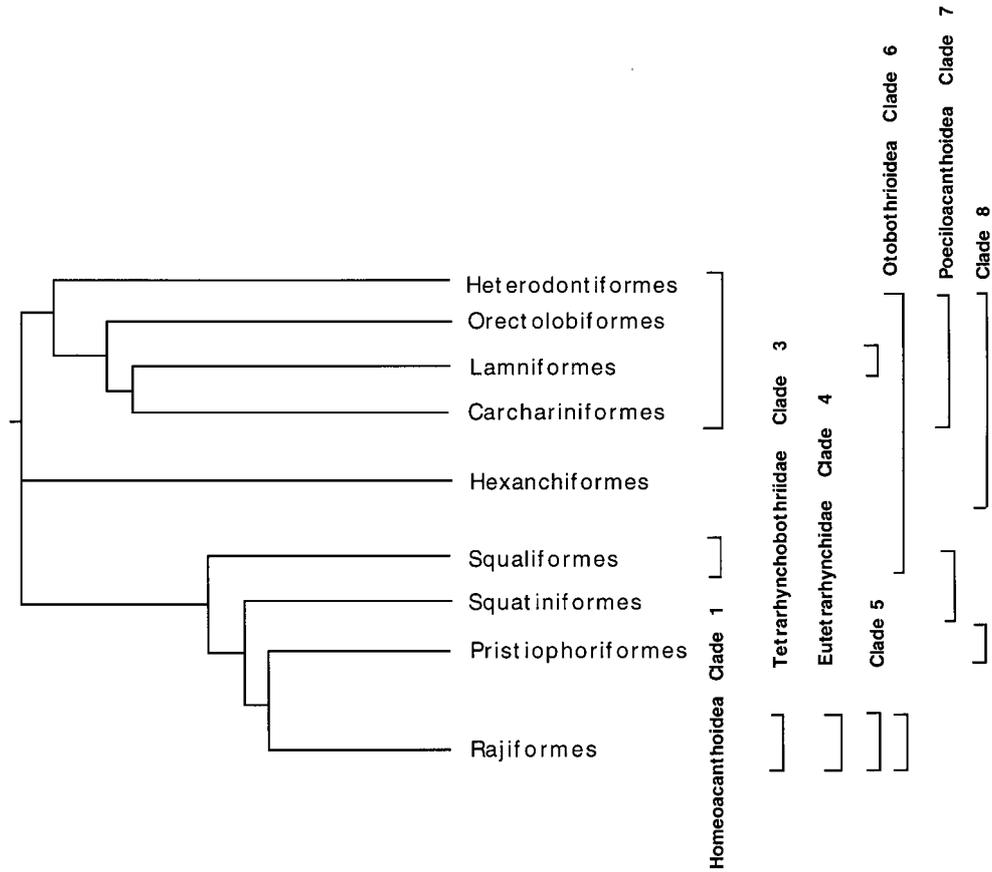


Figure 5. Phylogeny of the elasmobranchs after Shirai (1996) showing the distribution of different clades of trypanorhynch cestodes.

Comparisons can be made between the phylogeny present here and the classifications of Campbell & Beveridge (1994) and Palm (1995, 1997) given the caveat that the latter two classifications are essentially phenetic in origin, though both have also been presented in a phylogenetic fashion.

Comparison with the classification of Campbell & Beveridge (1994)

The present phylogenetic analysis demonstrated eight well-defined clades (Figure 3). These could represent up to eight superfamilies in an hierarchical classification. Campbell & Beveridge (1994) used four superfamilies to subdivide the trypanorhynchs, suggesting that their level of subdivision may have been conservative.

There is correspondence between some of the eight groupings resulting from the phylogenetic analysis and the superfamilies used by Campbell and Beveridge (1994). Their superfamily Homeacanthoidea

corresponds in part with clades 1 and 2, clade 6 corresponds broadly with their Otophthrioidea, clade 7 with their superfamily Poecilacanthoidea and clade 4 with their Heteracanthoidea (in part). Clades 5 and 8 represent novel groupings of genera.

Excluding the six families represented each by a single genus, seven of the remaining families utilised by Campbell & Beveridge (1994) (Table I) remain essentially intact in the cladistic analysis, although some change their position in the phylogenetic tree and the genera of two or more related families are mixed within the same clade in the five remaining families. Major changes are: (a) the genera of the Otophthriidae, Pterobothriidae and Grillotiidae are mixed together in clade 6 (Figure 3); and (b) the genera of the Gymnorhynchidae are nested with the genera of the Gilquiniidae and Molicolidae (clade 8).

Clade 1. This clade represents the “traditional” homeacanth families (Dollfus, 1942) within the su-

perfamily Homeacanthoidea. Campbell & Beveridge (1994) included the Tetrarhynchobothriidae in this superfamily, although it is grouped with the Eutetrarhynchidae in the cladistic analysis. Earlier, Beveridge & Campbell (1988a) aligned the Tetrarhynchobothriidae with the Eutetrarhynchidae. This divergence in opinion is discussed below. The apomorphic character defining the clade is the development of the uterus (character 1).

Clade 2. The Paranybeliniidae is an anomalous family known only from the metacestodes occurring in plankton or in teleosts. This family was placed together with other homeacanth families by Campbell & Beveridge (1994). It is suggested that the Paranybeliniidae is paraphyletic with the remaining homeacanthoid families, a suggestion which is not surprising since its members have armatures similar to the homeacanth yet possess sensory fossettes, a feature of genera of the Otobothriidae. This character is apomorphic for clade 2.

Clades 3 and 4. The Tetrarhynchobothriidae and Eutetrarhynchidae occur in these clades. The two families were synonymised by Schmidt (1986) but were maintained provisionally by Beveridge & Campbell (1988a), who emphasised the importance of the morphological characters exhibited in the Tetrarhynchobothriidae as being intermediate between homeacanth and eutetrarhynchids. Beveridge & Campbell (1988a) placed considerable weight on uterine structure which, together with the reported presence of a blastocyst, aligned the family Tetrarhynchobothriidae with the heteroacanth family Eutetrarhynchidae. To facilitate a phenetic classification, Campbell & Beveridge (1994) however included the Tetrarhynchobothriidae in the Homeacanthoidea. The current phylogenetic analysis supports an alignment with the Eutetrarhynchidae, but suggests that disagreement over the validity of the Tetrarhynchobothriidae could be resolved by the transfer of the genera *Eutetrarhynchus* and *Dollfusiella*, both possessing a relatively homeomorphous metabasal armature, from the eutetrarhynchid clade (4) to the tetrarhynchobothriid clade (3).

Clade 5. The families Mixodigmatidae, Shirleyrhynchidae and Rhinoptercolidae, families with many eutetrarhynchid features (Beveridge & Campbell, 1988b, 1989) but having four bothridia in contrast to the two bothridia of the eutetrarhynchids, are arranged

in this clade. Clade 5 is the sister group to clade 4 which contains most of the eutetrarhynchid families (Figure 3). Beveridge & Campbell (1989, p. 223) suggested that the "Mixodigmatidae may be derived from a common ancestor with *Shirleyrhynchus* and *Cetorhynchicola*, two genera (= Shirleyrhynchidae) with eutetrarhynchid characters, but four bothridia". In contrast to the arrangement used by Campbell & Beveridge (1994), the former view is supported by the current phylogenetic analysis and unites genera with typical, atypical and poeciloacanthous armatures, suggesting again the independent evolution of these armature types in both clade 5 and clade 8.

Clade 6. The genera included in this group in broad terms constitute the Otobothrioidea of Campbell & Beveridge (1994). The Rhinoptercolidae, with its single genus *Rhinoptercolia*, is removed in the phylogenetic analysis from the otobothrioid group where it was placed by Campbell & Beveridge (1994) and is aligned with the Shirleyrhynchidae in clade 5. The presence of an atypical heteroacanthous armature unites the genera of clade 6. This is the weakest clade in the analysis and was not consistently present when different tree building options were utilised. In the analysis of families, this clade is very weakly supported.

Clade 7. The superfamily Poecilacanthoidea, as defined by Campbell & Beveridge (1994), corresponds roughly with clade 7, with the exception that the families with four bothridia, Gymnorhynchidae and Mixodigmatidae, are removed from it to clades 8 and 5 respectively. This removal in fact facilitates the characterisation of the clade in morphological terms since all of its component genera possess two bothridia, chainettes and an hermaphroditic duct, all of which are synapomorphies. The genera *Dasyrhynchus* and *Hornelliella* invariably formed a clade and were usually aligned with the lacistorhynchids, although some analyses aligned them with the members of clades 3–5. In the analyses of families, the Dasyrhynchidae and Hornelliellidae were aligned with the Lacistorhynchidae.

Clade 8 The genera included in clade 8 belong to the families Gilquiniidae, Gymnorhynchidae and Molicolidae of Campbell & Beveridge (1994). Although not grouped together in their key, Beveridge & Campbell (1989, p. 223) speculated that the "Gymnorhynchidae may be derived from a common ances-

tor with the Gilquiniidae”, a guess consistent with the current findings. *Gymnorhynchus* and *Molicola* were placed in the same family and even the same genus (Dollfus, 1942) prior to their separation into separate families by Beveridge & Campbell (1989). Clade 8 groups together genera with four bothridia, uterine pores or accessory seminal vesicles, or with a uterus deviated towards the genital atrium, the last three characters being synapomorphies. It groups together genera with typical heteroacanth armatures (Gilquinidae), poecilacanth (*Gymnorhynchidae*) armatures and with bands of hooks (family *Molicolidae*), suggesting that these armature types can develop from a common ancestor and that both have developed independently in several clades (e.g. clade 5).

Comparison with the classification of Palm (1995, 1997)

Palm (1995, 1997) recognised three superfamilies, Tentacularioidea, Obothrioidea and Eutetrarhynchoidea compared with the eight groupings identifiable in the present phylogenetic analysis and therefore sub-divided the order on a conservative basis. Parts of Palm’s (1995) classification are supported by the cladistic analysis.

Palm’s (1997) superfamily Eutetrarhynchoidea, including the families Shirleyrhynchidae, Mixodigmidae and Eutetrarhynchidae, corresponds with the related clades 4 and 5 of the phylogenetic analysis. Palm (1997) characterised this superfamily as possessing a blastocyst and a prebulbar organ, both synapomorphies, but lacking sensory fossettes, a symplesiomorphy. A combination of characters rather than a single character unite this group: elongate bulbs, retractor originating at base of bulb, presence of gland-cells in the bulb and the presence of a prebulbar organ. Not every feature is present in each genus: a prebulbar organ is lacking in *Rhinoptericola*, the bulbs in *Mecistobothrium* are short, gland-cells are absent in *Shirleyrhynchus* and, according to Campbell & Beveridge (1993), a prebulbar organ is present in *Pseudogrillotia*. Prior to the addition of *Rhinoptericola* in the present analysis, the Eutetrarhynchoidea was united by the possession of a prebulbar organ. However, the combination of the three characters encompasses all genera, and the correlation between these related clades and the Eutetrarhynchoidea of Palm (1995, 1997) is apparently close. All are synapomorphies except for the origin of the retractor muscle.

The Obothrioidea as defined by Palm (1995, 1997) grouped together all genera with sensory fossettes. However, the separation of the family Paranybeliniidae in a different clade (2) to the remainder of the Obothriidae (clade 6) in the cladistic analysis fails to support this hypothesis, as does the mixing of genera of the Obothriidae (*Obothrium*, *Diplobothrium*, *Poecilancistrum*, *Pseudobothrium*) with *Pterobothrium* and genera of the Grillotiidae (*Grillotia*, *Pseudogrillotia*) as well as the occurrence of *Poeciloacanthum*, a genus bearing sensory fossettes, in clade 7. Palm (1997) placed the two families, Pterobothriidae and Grillotiidae in the Tentacularioidea as they lacked sensory fossettes. The use of sensory fossettes as a synapomorphic character is not supported by the current analysis and warrants more detailed investigation.

The Tentacularioidea as defined by Palm (1997) would be considered a polyphyletic assemblage based on the cladistic analysis. This may be due to the fact that the Tentacularioidea is defined on the basis of the absence of sensory fossettes and the absence of a prebulbar organ, both of which states are plesiomorphic. In examining the family arrangement within the Tentacularioidea, the phylogenetic analysis demonstrates a very close relationship between *Hepatoxylon* and *Sphyricephalus* which Palm (1997) considered belonged to the same family. Together with the genera of the Tentaculariidae (*Tentacularia* and *Nybelinia*), these genera are united by uteri developing from an anlage, here considered an apomorphic character. The arrangement of these families suggested by Palm (1995, 1997) thus corresponds with the cladistic analysis (clade 1). The remaining genera occurring in clades 7 and 8, except *Poeciloacanthum*, were also placed in the Tentacularioidea by Palm (1995, 1997). Clade 7 corresponds with the family Lacistorhynchidae as defined by Palm (1995, 1997), with the exception that the three genera of the Grillotiidae *sensu* Campbell & Beveridge (1994) were removed from it and the genus *Poeciloacanthum* was added.

Clade 8 includes the families Gilquiniidae, Aporhynchidae and Pterobothriidae (= *Gymnorhynchidae* of Palm, 1995) with the exception of two genera, *Rhinoptericola* and *Pterobothrium*. The analysis thus supports a close relationship between the *Gymnorhynchidae* and *Molicolidae* (*sensu* Beveridge & Campbell, 1994) as proposed by Beveridge & Campbell (1989) and Palm (1997). No significant support was demonstrated for resurrecting the Aporhynchidae. The latter is a matter of phenetic versus phylogenetic

judgement based on the absence or secondary loss of a rhynceal system in its sole genus *Aporhynchus*.

Families

The analysis based on families produced a consensus tree (Figure 4) with a slightly higher consistency index than that derived from genera, but, due to the high level of homoplasy, the same cautions apply in its interpretation. The topology of the tree is essentially similar to that derived from genera, with the exception that the Tetrarhynchobothriidae is ancestral to the remaining heteroacanth. The relationships between the Shirleyrhyndidae + Rhinoptericolidae + Mixodigmatidae and Gilquiniidae + Aporhynchidae + Molicolidae + Gymnorhynchidae are maintained in the family analysis and warrant no additional comment. Again, neither method of coding polymorphisms nor the exclusion of the families Aporhynchidae and Pseudogrillotiidae, recognised by Palm (1997) but not by Campbell & Beveridge (1994), significantly affected the topology of the trees. As indicated earlier, the composition of the families is disputed (Campbell & Beveridge, 1994, Palm, 1997) complicating any analysis of their relationships.

Relationships with hosts

Any analysis of host parasite relationships must be considered preliminary because of the obvious imperfections of published records. For these reasons a simple comparison only was made between parasite clades and their host distributions. Nevertheless, the preliminary observations presented here (Figure 5) provide working hypotheses which can be tested by additional studies. The data available suggest a complex relationship. The basal, homeoacanthoid trypanorhynch genera occur primarily in sharks, principally in Lamniformes and Carchariformes. The tetrarhynchobothriids and eutetrarhynchids constituting the next clade (3) are primarily parasitic in Rajiformes. The inner clades of the trypanorhynch cladogram include clades essentially restricted to sharks (the poeciloacanthoids – clade 7 and the gilquiniids + gymnorhynchids + molicolids – clade 8); others are restricted to rays (clades 4 and 5) (with the notable exception of two genera occurring in plankton feeding sharks); and a cluster of genera belonging to the Obothrioidea (*sensu* Campbell & Beveridge, 1994) (clade 6) has a very broad host range involving both sharks and rays. Thus there is not a simple co-evolutionary relationship between the trypanorhynchs

and their hosts. Brooks *et al.* (1991) suggested that the cestodes of elasmobranchs originated from ancestors in teleosts, but that the colonisation event may have occurred early in the evolution of fishes, allowing for diversification and some degree of co-evolution. Their hypothesis would explain observed instances of co-evolution on the one hand and associations based on the food preferences of the hosts on the other hand. The data available at present are too imprecise to draw any firm conclusions, but they do indicate that the issue warrants further investigation.

Conclusions

The preliminary cladistic analysis of the Trypanorhyncha presented above provides a number of insights into the phylogeny of the order when compared with recent phenetic classifications. While obviously providing support for some parts of the classifications used by Campbell & Beveridge (1994) and Palm (1995, 1997), it also questions some of the assumptions underlying each classification. For example, the current analysis suggests that the transition in armature types from homeoacanthous to heteroacanthous has occurred once, while transitions from typical heteroacanth to atypical heteroacanth or poeciloacanth types have occurred independently in several lineages, as suggested tentatively by Beveridge & Campbell (1989) and more strongly by Palm (1995, 1997). Likewise, the use of sensory fossettes as a synapomorphy, as proposed by Palm (1995, 1997), is unsupported by the current analysis. The results of the present study however suggest that the homeoacanth trypanorhynchs, lacking blastocysts, are basal, that the Shirleyrhyndidae, Rhinoptericolidae and Mixodigmatidae are related to the Eutetrarhynchidae (Beveridge & Campbell, 1989; Palm, 1995, 1997), the Gymnorhynchidae and Molicolidae are related to the Gilquiniidae (Beveridge & Campbell, 1989), the Grillotiidae are separate from the Lacistorhynchidae (Campbell & Beveridge, 1993; Palm, 1995, 1997) and are more closely related to the Obothriidae (Campbell & Beveridge, 1994), and that the Mustelicolidae, Lacistorhynchidae, Dasyrhyndidae and Hornelliellidae are closely related. How this phylogenetic information can be incorporated into a classification represents a future challenge. The taxonomy of the order is currently in a state of considerable flux, with new genera being added as a result of recent faunal surveys. A phylogenetic analysis is therefore timely in forcing a re-evaluation of the assumptions

involved in classifications constructed to date if a truly optimal taxonomy is desired. At the same time, the limitations of the current phylogenetic analysis need to be borne in mind: many characters used cannot be scored for every genus; a series of potential characters exist for the utilisation of which insufficient knowledge currently exists; and the consistency of the current phylogenetic hypothesis is low, a situation which might be remedied by the addition of new characters. Further research is clearly required to adequately characterise several genera morphologically. Many are known only from metacestodes, while others cannot be included in the analysis because they are too poorly known and are currently considered *inquirenda* (see Campbell & Beveridge, 1994). Some large genera (*Nybelinia*, *Grillotia*, *Otobothrium*) are currently in need of revision and our observations suggest that they may be more complex than hitherto suspected. In addition, the range of new genera awaiting description could provide considerable insight into our current understanding of morphological features and their homologies. The filling of these obvious gaps in knowledge and the inclusion of new morphological characters, as well as ultrastructural and molecular studies may well provide valuable new data on the evolution of the Trypanorhyncha.

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