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Preliminary phylogenetic analysis of subfamilies of the Proteocephalidea (Eucestoda)*

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Abstract

Cladistic analysis based on comparative morphology was used to examine the subfamily-level relationships within the cestode order Proteocephalidea. A single most parsimonious tree (70 steps, CI = 0.571; RC = 0.295; HI = 0.471) is consistent with monophyly for the Proteocephalidea and showed a relatively high consistency at the family level with the diagnosis of two major subclades. Unambiguous support for a Proteocephalidae subclade, including the Corallobothriinae, Proteocephalinae, Gangesiinae and Sandonellinae, and a Monticelliidae subclade, including the Marsypocephalinae, Zygobothriinae, Monticelliinae, Rudolphiellinae, Ephedrocephalinae and Othinoscolecinae was evident. Two subfamilies, the Acanthotaeniinae (historically in the Proteocephalidae) and Nupeliinae (historically in the Monticelliidae), were however, basal to all other subfamilies, indicating that neither family as currently conceived is monophyletic. Trees one or two steps longer, however, would be consistent with monophyly for the Proteocephalidae and Monticelliidae (excluding Acanthotaeniinae) or would result in the monophyly for both families, including all respective subfamilies congruent with current concepts for systematics of the order. Zoogeographical analysis demonstrated a strong Gondwanan association; proteocephalideans originated in Africa, with subsequent development linking Africa and South America. Colonisation of the Northern Hemisphere by proteocephalid subfamilies, the Proteocephalinae, Corallobothriinae and Gangesiinae, was secondary. Analysis of parasite-host relationships indicated that the basal hosts for the Proteocephalidea are equivocal; siluriform teleosts were, however, the basal hosts for the Nupeliinae + the Monticelliidae and Proteocephalidae subclades and an extensive co-evolutionary history with this host group is postulated. Independent colonisation events of reptilians by species of the Proteocephalinae and Acanthotaeniinae, non-siluriform teleosts associated with the Sandonellinae and some Proteocephalinae, and amphibians by some Proteocephalinae, are recognised. Some points which should be considered for further development of the systematics of the group are proposed, with special emphasis given to thorough morphological descriptions and investigations of life-cycles.

* A report of results of phylogenetic analyses conducted during the 2nd International Workshop for Tapeworm Systematics, Lin-

coln, Nebraska, 2–6 October 1996; E.P. Hoberg, S.L. Gardner and R.A. Campbell, organisers. Contributions edited by E.P. Hoberg.

Introduction

The tapeworms of the order Proteocephalida are parasites of freshwater fish, amphibians and reptiles (Freze, 1965; Rego, 1994). In excess of 300 species have been described in two families, the Proteocephalidae and Monticelliidae, that contain 12 subfamilies and 46 genera. More than one half of the genealogical diversity of the group is limited to species parasitising freshwater fishes, principally siluriforms, from South American (Schmidt, 1986; Rego, 1994).

Subsequent to the description of the first species of the Proteocephalida, *Taenia* (= *Proteocephalus*) *percae* by Müller (1780), the first complex account on the group was provided by La Rue (1911, 1914). He erected two families, the Proteocephalidae with the genera *Proteocephalus* Weinland, 1858, *Corallobothrium* Fritsch, 1886, *Crepidobothrium* Monticelli, 1899, *Acanthotaenia* von Linstow, 1903 and *Ophiotaenia* La Rue, 1911; and the Monticelliidae, to accommodate the species *Tetracotylus* (= *Monticellia*) *coryphicephala* Monticelli, 1891. Later, Woodland (1933a,b,c, 1934a,b,c, 1935a,b,c) described a number of species and genera from South American siluriform fishes, mainly those placed into the Monticelliidae.

Freze (1965) presented in his monograph of the Proteocephalida a new arrangement of the group. He recognised three families: (1) Proteocephalidae La Rue, 1911 with subfamilies Proteocephalinae, Corallobothriinae Freze, 1965, Gangesiinae Mola, 1929, Paraproteocephalinae Freze, 1963, Sandonellinae Khalil, 1960, and Zygobothriinae Woodland, 1933; (2) Ophiotaeniidae Freze, 1963 with the Ophiotaeniinae and Acanthotaeniinae Freze, 1963; and (3) Monticelliidae La Rue, 1911 with the Monticelliinae, Endorchiinae Woodland, 1934, Ephedrocephalinae Mola, 1929, Marsypocephalinae Woodland, 1933, Othinoscolecinae Woodland, 1933 (syn. Peltidocotyliinae Woodland, 1934) and Rudolphiellinae Woodland, 1935. Brooks (1978a), however, synonymised the Ophiotaeniidae with the Proteocephalidae; this synonymy has widely been accepted by other workers.

Twenty years later, Schmidt (1986) primarily mirrored the classification of Freze (1965), but did not retain the family Ophiotaeniidae and placed the Zygobothriinae in the Monticelliidae. Schmidt (1986) recognised two families, the Proteocephalidae and Monticelliidae, the former comprising the Gangesiinae, Prosobothriinae Yamaguti 1959 (now placed to the order Lecanicephalida Wardle & McLeod, 1952 – see Euzet, 1994), Sandonellinae, Coral-

lobothriinae, Acanthotaeniinae, Proteocephalinae and Marsypocephalinae; the latter including the Monticelliinae, Zygobothriinae, Endorchiinae, Ephedrocephalinae, Othinoscolecinae Woodland, 1934 (syn. Peltidocotyliinae) and Rudolphiellinae.

Rego (1994) presented a similar system in his review of the Proteocephalida. A new subfamily, Nupeliinae Pavanelli & Rego, 1991, was placed into the Monticelliidae. The same author (Rego 1995) proposed completely different classification of the group, retaining one family, the Proteocephalidae, and suppressing the Monticelliidae to subfamily level.

Phylogenetic analysis of the Proteocephalida based on cladistic assessment of morphological characters has been performed by Brooks (1978a,b, 1995), Brooks & Rasmussen (1984), Brooks et al. (1991) and Brooks & McLennan (1993). Brooks (1978b) conducted the first analysis of morphological, zoogeographical and host relationships among genera of the order. The Marsypocephalinae was suppressed, being grouped within the Corallobothriinae. This classification recognised the Proteocephalidae with the Corallobothriinae, Sandonellinae, Proteocephalinae, Acanthotaeniinae and Gangesiinae; and the Monticelliidae with the Zygobothriinae, Monticelliinae and Othinoscolecinae (syn. Peltidocotyliinae).

Brooks & Rasmussen (1984) revised the classification of the Monticelliidae on the basis of reinterpretation of some characters listed previously by Brooks (1978b). In contrast to earlier studies, some degree of parallel evolution was found in the majority of morphological characters. The authors pointed out the need to revise the taxonomy of the Monticelliidae in order to be more consistent with their phylogeny.

Brooks et al. (1991) postulated that proteocephalideans might be polyphyletic and that the Monticelliidae might not be monophyletic, because the most important diagnostic feature, the cortical vitellarium, is plesiomorphic. In this analysis, which concentrated on the relationships among the major lineages of the Eucestoda, the Proteocephalidae were divided into two branches. These were diagnosed by the presence/absence of a cysticercoid in the life-cycle, and the Gangesiinae were considered to be related and basal to Cyclophyllidea due to the presence of an armed rostellum.

Brooks (1995) presented a new classification of monticelliid genera based on the phylogenetic tree of this family. This classification resulted in synonymy of numerous genera, e.g. *Endorchis* Woodland, 1934, *Gibsoniela* Rego, 1985 and *Nupelia* Pavanelli & Rego,

1991 with *Nomimoscolex* Woodland, 1934; *Ephedrocephalus* Diesing, 1850 and *Othinoscolex* Woodland, 1933 with *Rudolphiella* Fuhrmann, 1916; *Peltidocotyle* Diesing, 1850 *Choanoscolex* La Rue, 1911, *Goezeella* Fuhrmann, 1916 and *Jauella* Rego & Pavanelli, 1985 with *Spatulifer* Woodland, 1934.

The analyses by Brooks set the foundation for detailed study of the phylogeny of the Proteocephalidea and constitute a set of testable phylogenetic hypotheses for relationships among the genera and subfamilies. Interpretations presented by Brooks and co-workers were limited, however, by the uneven quality of comparative morphological and biological data in the literature. A shortage of reliable and complete data for many taxa, mainly those from South American fishes described by Woodland between 1933 and 1935 (cf. de Chambrier & Vaucher, 1997) makes it difficult to perform a consistent phylogenetic analysis of the group at the generic-level or to assess the phylogenetic importance of individual characters. In addition, there is a considerable gap in data on the life-cycles of most proteocephalideans and no information has been provided on the life-cycle of any member of the family Monticelliidae.

In order to evaluate the current stage of knowledge of the systematics of the Proteocephalidea, a phylogenetic analysis of the group was performed by the present authors. The present study is based on critical observations on taxa, mainly those from South America (see numerous papers by de Chambrier, Rego and co-authors). It must be emphasised, however, that it reflects the present state of knowledge, which is still far from being satisfactorily complete. For this reason, the present analysis is limited to the study of relationships among subfamilies of the Proteocephalidea, taking a top down approach to assessment of genealogical diversity (see Hoberg et al., 1997). Consistent with all phylogenetic studies, this will be open to critical and continued evaluation with the advent of a more complete database for comparative morphology and biology.

The main part of the analysis was carried out by members of the Proteocephalidea group (chairman A.A. Rego) during the Workshop on Tapeworm Systematics in Lincoln, Nebraska, October 2–6, 1996. Corrections and re-interpretation of some characters, based mainly on the re-examination of new material, analysis of amended data and final editing of the manuscript were made subsequently and are the responsibility of A. de Chambrier, E. Hoberg and T. Scholz.

Materials and methods

Relationships among proteocephalidean subfamilies were analysed using phylogenetic systematics (Hennig, 1950, 1966; Wiley et al., 1991). Characters were analysed with the program “Phylogenetic Analysis Using Parsimony”, Version 3.05 (PAUP) (Swofford, 1993); further analyses of characters, host association and biogeography were conducted using MacClade Version 3.05 (Maddison & Maddison, 1993).

Specimens examined

Taxonomy and classification of subfamilies considered here is based on the system proposed by Rego (1994). The analysis was based on critical observations of features in most proteocephalidean genera studied by the present authors: A.A. Rego and A. de Chambrier in South America (see Rego et al., 1974; de Chambrier & Vaucher, 1984, 1992, 1994, 1997; Rego, 1987, 1991, 1994; de Chambrier, 1987, 1988, 1989a,b, 1990; Rego & Pavanelli, 1990, 1991, 1992; de Chambrier et al., 1992, 1996; de Chambrier & Paulino, 1997; de Chambrier & Rego, 1994, 1995; Rego & de Chambrier, 1995; Scholz et al., 1996) and V. Hanzelová & T. Scholz in the Holarctic (Scholz, 1989, 1991, 1993; Scholz & Ergens, 1990; Hanzelová & Spakulová, 1992; Hanzelová & Scholz, 1992, 1993; Scholz & Cappellaro, 1993; Scholz & Hanzelová, 1994; Hanzelová et al., 1995a,b; Scholz et al., 1995, 1997).

Results of the studies of extensive material of proteocephalideans from Amazonia (Brazil) and Paraguay, part of which has yet to be published (A. de Chambrier and A.A. Rego), were also used for analysis. In addition, some type and voucher specimens of Woodland’s material were examined by one of the authors (A. de Chambrier); vouchers of the following taxa recently have been examined: *Sandonella sandoni* (MHNG 18155 INVE), *Kapsulotænia* sp. (Harold W. Manter Laboratory, University of Nebraska Museum, Lincoln, Coll. No. 33942), *Silurotaenia siluri* and *Gangesia parasiluri* (both Institute of Parasitology, České Budějovice, Coll. Nos. C-52 and C-125, respectively).

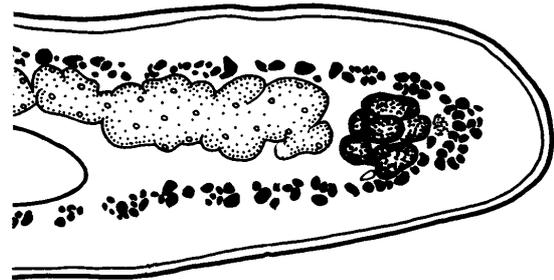
Character analysis

Characters used in the analysis were derived mainly from comparative morphological and taxonomic studies of the present authors (see above). In some groups, we relied on morphological data derived from the extensive literature; it is recognised, however, that some

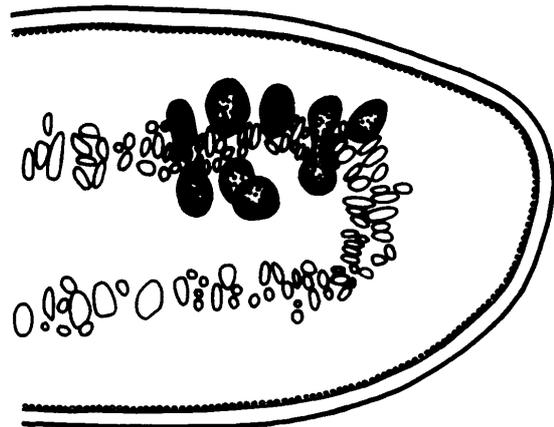
characters might have been described incorrectly in their original descriptions. Polarisation of characters was based on comparisons to a taxonomic outgroup (Maddison et al., 1984; Wiley et al., 1991). The Tetraphyllidea (see Euzet, 1994) was the primary outgroup, as it is a putative paraphyletic taxon basal to the ingroup as determined by results of analyses at the ordinal level among the Eucestoda (Hoberg et al., 1997). Characters of the outgroup were taken from data provided by Euzet (1994). In addition, some details regarding individual characters and putative plesiomorphy or apomorphy were discussed personally with L. Euzet during the Workshop.

In subfamilies containing multistate taxa (genera or species with both the primitive and derived state, mainly in Proteocephalinae and Zygobothriinae), we chose a conservative approach with respect to coding for polymorphism. We recognise that coding for polymorphism can result in ambiguity and potentially may lead to incorrect reconstructions (see Maddison & Maddison, 1993). Thus, few characters and taxa are regarded as polymorphic. In most cases, explicit decisions have been made relative to coding a specific attribute as plesiomorphic or apomorphic within a given taxon. As a generality, if the derived state occurred in the subfamily, then coding was considered apomorphic to recognise acquisition of a specific state in at least some genera (or species) within a particular subfamily (see Hoberg & Lichtenfels, 1994).

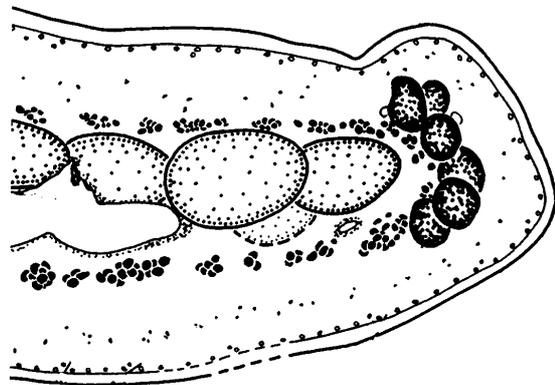
Some characters, previously used for phylogenetic analysis (e.g. Brooks, 1978b), were not used in the present study because they were considered to have minimal information or require more detailed evaluation; e.g. thickness of the longitudinal musculature (inconspicuous versus well developed – see Brooks & Rasmussen, 1984), formation of the uterus (performed versus not performed in mature proglottides – Freze, 1965); structure of the ovary (follicular versus non-follicular - Brooks, 1978b); orientation of uterine branches (lateral versus antero-posterior - Brooks, 1978b), etc. A summary of the 27 characters, representing 38 character states, is presented below and in a numerical matrix (Table I). Plesiomorphic states are coded as 0, apomorphic as 1, 2, or 3. Analyses with PAUP were conducted with Branch and Bound and all multistate characters were run as unordered; results are presented a phylogenetic tree with associated statistics including the Consistency Index (CI), Homoplasy Index (HI) and Rescaled Consistency Index (RC) as specified by Swofford (1993).



1



2



3

Figures 1–3. Position of vitelline follicles: 1. medullary, *Proteocephalus soniae* de Chambrier & Vaucher, 1994 (after de Chambrier & Vaucher, 1994); 2. paramuscular, *P. paraguayensis* (Rudin, 1917) (after de Chambrier, 1990); 3. cortical, *Nomimoscolex piraeeba* Woodland, 1934 (after de Chambrier & Vaucher, 1997).

Table 1. Character matrix for the subfamilies of the Protocephalidea and the Tetrphyllidea.

Subfamilies	Character																											
	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	
Corallobothriinae	0	0	0	0	2	0	1	0	0	0	1	0	0	3	0	1	0	0	0	1	0	0	1	1	0	0	0	0
Marsypocephalinae	0	0	0	2	0	0	1	0	?	1	1	0	0	3	1	0	0	0	0	1	0	0	0	1	0	0	1	0
Sandonellinae	0	2	0	0	0	0	1	0	?	1	1	0	1	1&2	0	0	0	0	0	0	0	1	1	1	1	0	0	0
Proteocephalinae	1	0	0	0	1	0	1	0&1	0	0	2	0	0	3	0	0	1	1	0	0	0	0	1	1	1	0	1	0
Gangesiinae	0	0	0	0	0	0	1	0	0	0	1	0	0	3	0	0	0	0	0	0	1	0	0	1	1	0	0	0
Acanthotaeniinae	0	0	0	0	2	0	2	0	?	1	2	0	0	0&1	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Zygobothriinae	2	0	1	0	2	2	1	0	1	0	2	1	1	3	1	1	0	1	1	1	0	0	1	0	1	0	1	0
Nupeliinae	2	0	1	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Rudolphiellinae	2	0	1	0	0	0	1	0	0	1	2	0	0	3	1	1	0	0	0	0	0	0	0	1	0	0	0	0
Monticelliinae	2	0	2	2	0	1	1	2	1	0	1	0	0	3	1	1	0	1	0	0	0	0	0	1	0	0	1	0
Ephedrocephalinae	2	1	0	2	0	0	1	0	0	1	0	0	0	2	1	1	0	0	0	0	0	0	1	1	0	0	1	0
Othinoscolecinae	2	0	0	2	0	1	1	0	0	1	0	0	0	2	1	1	0	1	0	1	0	0	1	1	0	0	1	0
Tetrphyllidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0

Results

Characters

(1) Position of vitelline follicles (Figures 1-3). Three states: 0 = medullar; 1 = paramuscular; 2 = cortical. Paramuscular position of vitelline follicles is defined according to de Chambrier (1990): follicles encircle bundles of the longitudinal musculature, with some follicles reaching to the cortex and some follicles to the medulla (Figure 2).

(2) Topography (distribution) of vitelline follicles (Figures 4-6). Three states: 0 = two lateral fields; 1 = scattered throughout proglottis forming one ventral field; 2 = compact, posterior to ovary. State 1 is present in *Ephedrocephalus microcephalus* Diesing, 1850 (Ephedrocephalinae) (Figure 5); state 2 in *Sandonella sandoni* (Lynsdale 1960) (Sandonellinae) (Figure 6).

(3) Position of ovary. Three states: 0 = medullar; 1 = originally medullary, developing cortically; 2 = cortical. State 1 is present in the genera *Endorchis* Woodland, 1934 and *Gibsoniela* Rego, 1984 and in *Nomimoscolex admonticellia* (Woodland, 1934) (all Zygobothriinae) (Figure 7).

(4) Position of testes. Three states: 0 = medullar; 1 = paramuscular; 2 = cortical. State 1 has been observed only in the Nupeliinae (Pavanelli & Rego, 1991; Figure 8).

(5) Position of testicular fields (Figures 9-11). Three states: 0 = one field; 1 = two fields connected anteriorly; 2 = two distinctly separated fields.

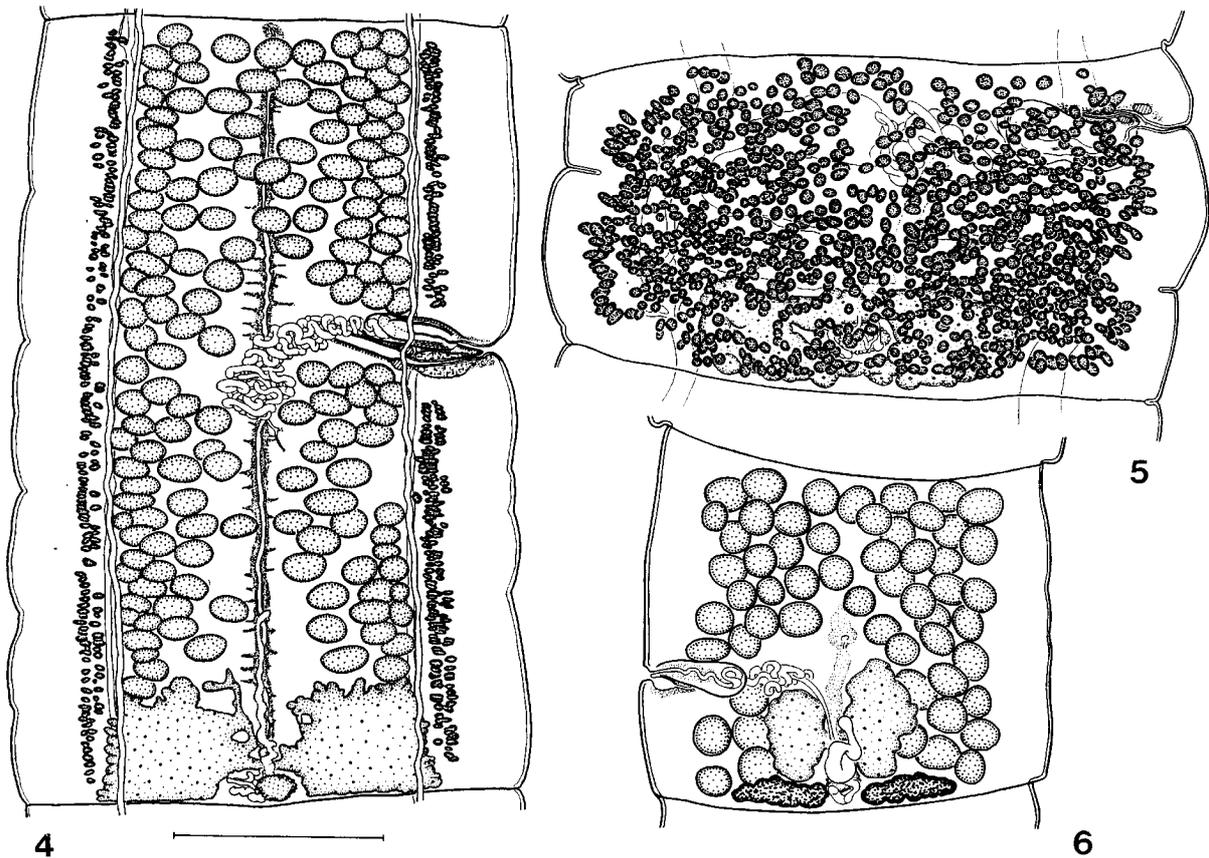
(6) Position of uterus. Three states: 0 = medullar; 1 = cortical; 2 = originally cortical, developing medullarly. State 0 is the most common; state 1 is present in the Monticelliinae and Othinoscolecinae; state 2 in some *Proteocephalus* (Proteocephalinae), *Zygobothrium* Diesing, 1850, *Endorchis* Woodland, 1934 and *Nomimoscolex* Woodland, 1934 (all Zygobothriinae).

(7) Original shape of uterus. Three states: 0 = saccate, without lateral expansions (diverticula); 1 = saccate, with lateral expansions (diverticula); 2 = forming capsules. Capsules, present in *Kapsulotaenia* Freze, 1965 (Acanthotaeniinae), are formed by eggs grouped together, covered with a thin-walled membrane; the capsules are within the lumen of the uterine diverticula connected with the uterine stem.

(8) Egg structure (Figures 12-14). Three states: 0 = egg spherical to oval, external hyaline membrane present; 1 = internal polar circle-like structures present; 2 = polar filaments present. State 1 is present in eggs of *Crepidobotrium eirasi* Rego & de Chambrier, 1995 (Proteocephalinae; Figure 13); state 2 in *Goezeella siluri* Fuhrmann, 1916 (Monticelliinae) and *Proteocephalus renaudi* de Chambrier & Vaucher, 1994 (Proteocephalinae; Figure 14).

(9) Embryonation of eggs. Two states: 0 = embryonated when laid (= ovoviviparus); 1 = unembryonated when laid (= oviparous; see Brooks et al., 1991).

(10) Vaginal sphincter. Two states: 0 = present; 1 = absent. The vaginal sphincter is considered to be the



Figures 4–6. Topography (distribution) of vitelline follicles: 4. two lateral fields, *Proteocephalus renaudi* de Chambrier & Vaucher, 1994 (after de Chambrier & Vaucher, 1994); 5. scattered throughout proglottis forming one ventral field, *Ephedrocephalus microcephalus* Diesing, 1850 (original); 6. compact, posterior to ovary, *Sandonella sandoni* (Lynsdale, 1963) (original). Scale-bars: 4, 5, 500 μm ; 6, 250 μm .

circular muscular sphincter surrounding the terminal (distal) part of the vagina (Figure 15).

(11) Position of vagina relative to cirrus-sac. Three states: 0 = anterior; 1 = posterior; 2 = alternating.

(12) Genital pore. Two states: 0 = alternating irregularly; 1 = unilateral. The unilateral position of the genital pore is represented by some *Amphoteromorphus* spp. (Zygobothriinae; see de Chambrier & Vaucher, 1997).

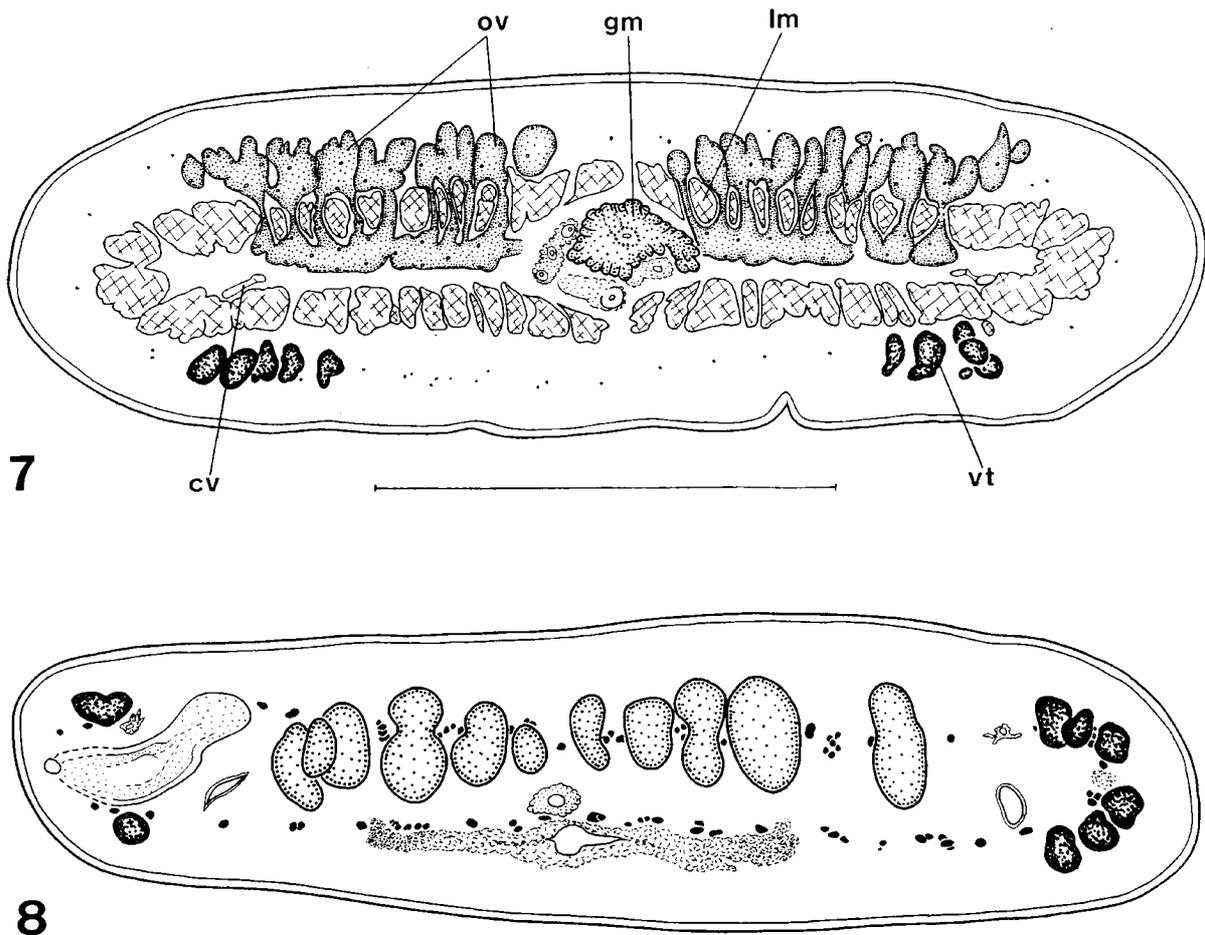
(13) Type of proglottides. Two states: 0 = acraspedote; 1 = craspedote.

(14) Shape of mature proglottides. Four states: 0 = longer than wide; 1 = quadrate; 2 = wider than long; 3 = variable.

(15) Transverse tegumental wrinkles. Two states: 0 = absent; 1 = present; (Figure 17). State 1 is observed in the Marsypocephalinae and in all subfamilies referred to the Monticelliidae. Tegumental wrinkles

which are not artifacts of inappropriate fixation (contraction of worms) should be considered.

(16) Metascolex. Two states: 0 = absent; 1 = present. Several types of the metascolex have been recognised by different authors (e.g. Brooks & Rasmussen, 1984; Rego, 1995; de Chambrier & Paulino, 1997). There is however a high degree of intraspecific variation of this feature. The shape of the metascolex may be dependent on fixation (see Rego & Pavanelli, 1985 for *Megathylacus brooksi* Rego & Pavanelli, 1985 and Scholz & Cappellaro, 1993 for *Corallobothrium parafimbriatum* Befus & Freeman, 1973 – both Corallobothriinae) and there may be forms that are intermediate to specific types of metascoleces (i.e. in *Choanoscolex abscisus* (Riggenbach, 1896) – Monticelliinae). Consequently, only two states (presence versus absence) are now considered until the various forms of the metascolex are clearly defined and distinguished.



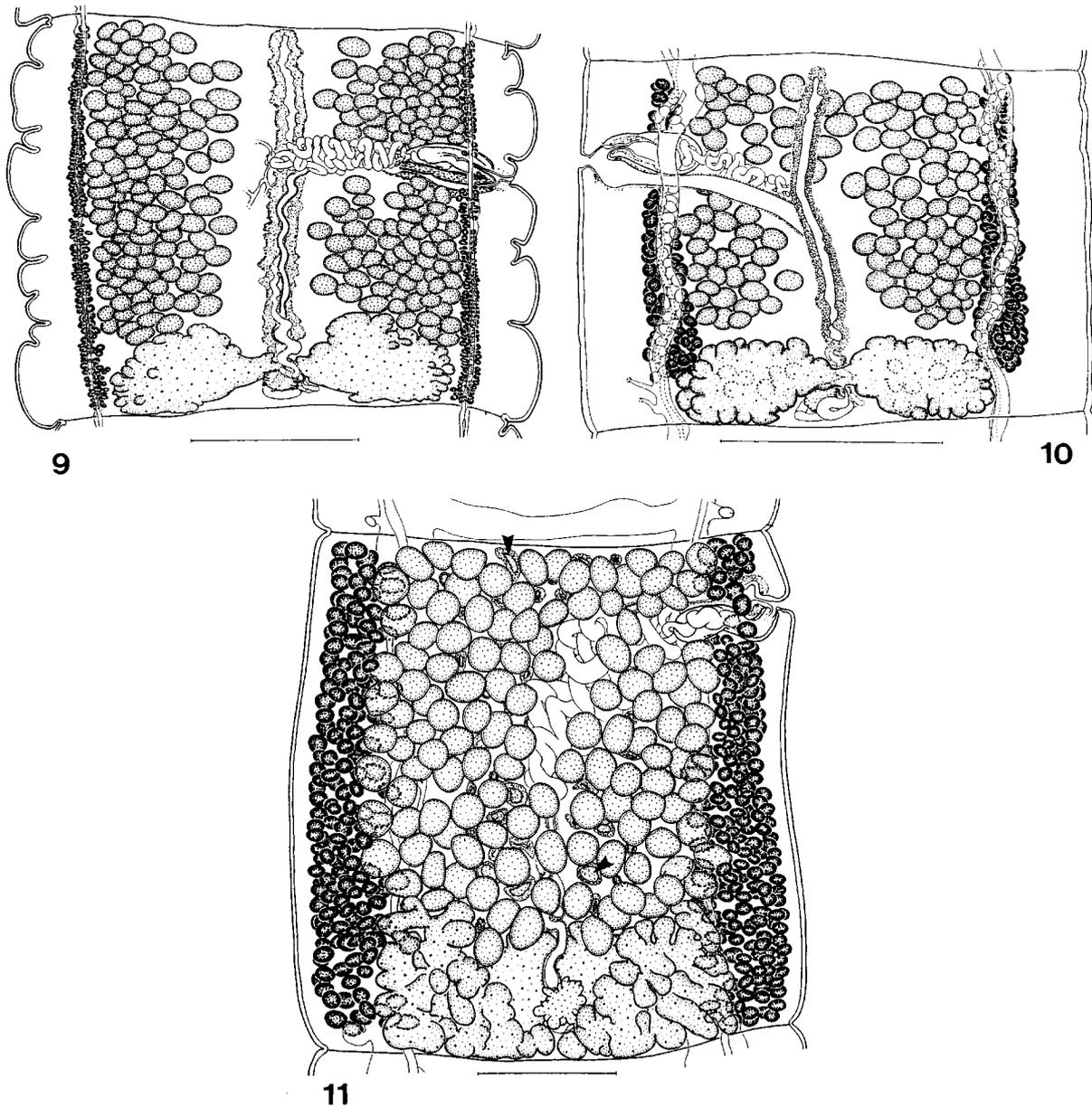
Figures 7–8. Position of ovary: originally medullary, developing cortically. *Endorchis piraeeba* Woodland, 1934 (after de Chambrier & Vaucher, 1997). 8 Paramuscular position of testes, *Nupelia portoricensis* Pavanelli & Rego, 1991 (original). Abbreviations: cv, ventral osmoregulatory canal; gm, Mehlis' gland; lm, internal longitudinal musculature; ov, ovary outgrowth; vt, vitelline follicles. Scale-bars: 7,8, 500 μm .

(17) Apical organ. Three states: 0 = absent; 1 = muscular; 2 = glandular. State 1 is present in some *Proteocephalus* spp.; state 2 in *Proteocephalus glandiger* (Janicki, 1928) and *P. joanae* de Chambrier & Paulino, 1997 (Proteocephalinae), *Jauella glandicephalus* Rego & Pavanelli, 1985 (Othinoscolecinae = Peltidocotylinae), *Nomimoscolex piraeeba* Woodland, 1934 (Zygobothriinae), etc. (Fuhrmann, 1933; Rego & Pavanelli, 1985; de Chambrier & Vaucher, 1997; de Chambrier & Paulino, 1997).

(18) Sucker structure. Two states: 0 = spherical; 1 = other forms. State 1 is present in the proteocephaline genera *Crepidobothrium* Monticelli, 1900 and *Deblocktaenia* Odening, 1963 in some Zygobothriinae, Othinoscolecinae (syn. Peltidocotylinae) and Monticelliinae.

(19) Auricular (papilla-like) projections on suckers. Two states: 0 = absent; 1 = present. State 1 is presented in the genera *Harriscolex* Rego, 1987 and *Houssayela* Rego, 1987 and the species *Nomimoscolex alovarius* Brooks & Deardorff, 1980 (all Zygobothriinae).

(20) Distal sphincter on suckers. We consider that this structure is not a true sphincter but merely acting as one (see de Chambrier & Rego, 1995). Two states: 0 = present; 1 = absent. State 0 is present in the genera *Marsypocephalus* Weld, 1861 (Marsypocephalinae), *Megathylacus* Woodland, 1934, *Megathylacoides* Jones, Kerley & Sneed, 1956 (Corallobothriinae), *Zygobothrium* Diesing, 1850 (Zygobothriinae), *Mariauxiella* de Chambrier & Rego, 1995 (Othinoscolecinae = Peltidocotylinae) (Figure 16).



Figures 9–11. Position of testicular fields: 9. two distinctly separated fields, *Proteocephalus soniae* de Chambrier & Vaucher, 1994 (after de Chambrier & Vaucher, 1994); 10. two fields connected anteriorly, *P. regoi* de Chambrier & al., 1996 (after Chambrier & al., 1996); 11. one field, *Mariauxiella pimelodi* de Chambrier & Rego, 1995 (after de Chambrier & Rego, 1995). Scale-bars: 9, 10, 500 μm ; 11, 250 μm .

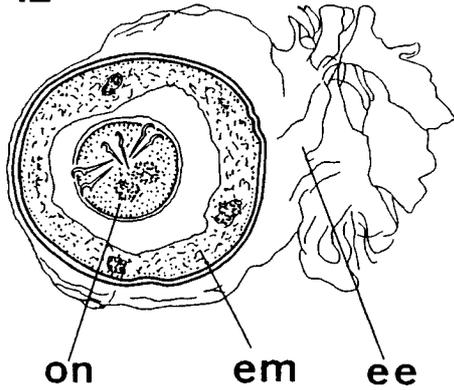
(21) Rostellum-like apical part of scolex armed with hooks. Two states: 0 = absent; 1 = present. State 1 is present in the Gangesiinae.

(22) Lappet structures on apical part of scolex. Two states: 0 = absent; 1 = present. State 1 is present only in *Sandonella sandoni* (Lynsdale, 1960) (Sandonellinae).

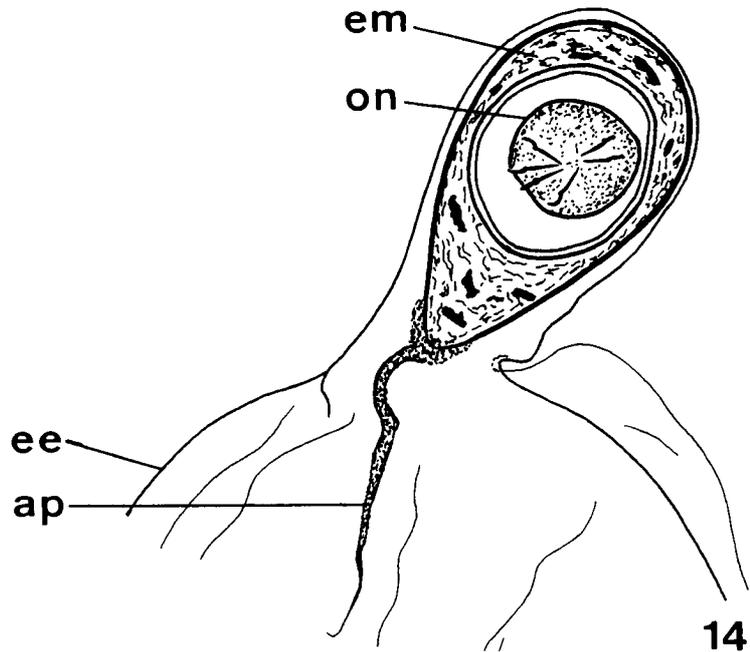
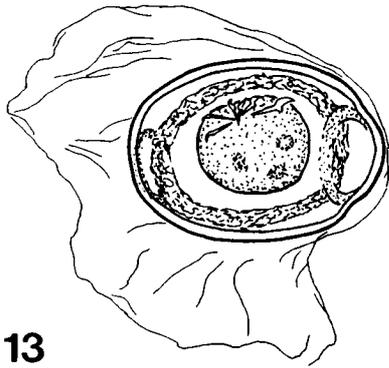
(23) Arrangement of longitudinal musculature. Two states: 0 = isolated fibres; 1 = fibres concentrated together, forming distinct bundles.

(24) Spination on cirrus. Two states: 0 = present; 1 = absent. State 0 is present in *Zygothrium megacephalum* Diesing, 1850 and in *Nomimoscolex pirarara* (Woodland, 1935) (both Zygothriinae) and in Acanthotaeniinae and Gangesiinae.

12



13



Figures 12–14. Egg structure: 12. egg spherical to oval, external hyaline membrane present, *Nomimoscolex piraeeba* Woodland, 1934 (after de Chambrier & Vaucher, 1997); 13. internal polar circle-like structure present, *Crepidobothrium eirasi* Rego & de Chambrier, 1995 (after Rego & de Chambrier, 1995); 14. polar filaments present, *Proteocephalus renaudi* de Chambrier & Vaucher, 1994 (de Chambrier & Vaucher, 1994). Abbreviations: ap, appendix; ee, outer envelope; em, embryophore; on, oncosphere.

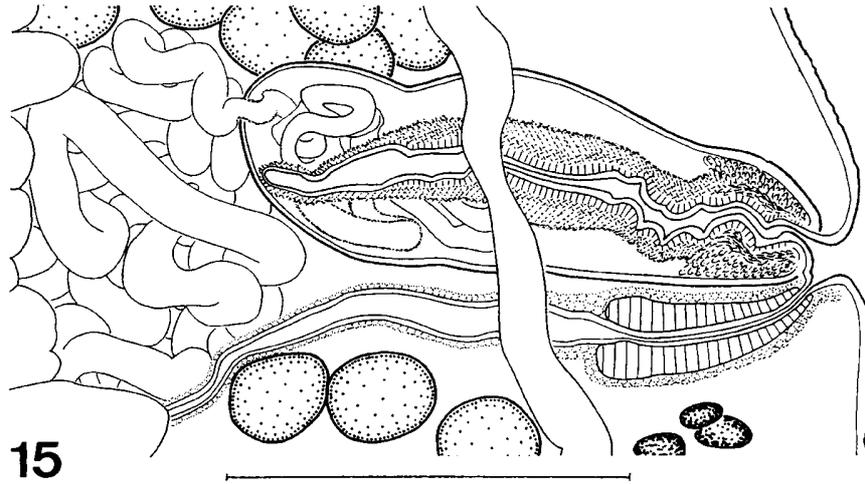
(25) “Spination” on suckers (presence of giant, spine-like microtriches on suckers). Two states: 0 = absent; 1 = present. State 1 is present in some *Proteocephalus* spp. from snakes (Proteocephalinae), in *Nomimoscolex piraeeba* Woodland, 1934 (Zygobothriinae), in two species of *Spasskyellina* Freze, 1965 (Monticelliinae), and in the Acanthotaeniinae and Gangesiinae (de Chambrier & Vaucher, 1997). “Spination” is considered here to be the presence of giant, spine-like microtriches forming a few rows on the margins and face of the suckers; microtriches are not considered homologous with true spines. Additionally, spination on the anterior region of the strobila as reported for the Acanthotaeniinae and Gangesiinae has been misinterpreted. The latter are identical to the microtriches present on the suckers, and similar to the very dense, filiform microtriches, previously observed (Thompson et al., 1980).

(26) Presence of piercing organ. Two states: 0 = absent; 1 = present. State 1 is present only in the Acanthotaeniinae.

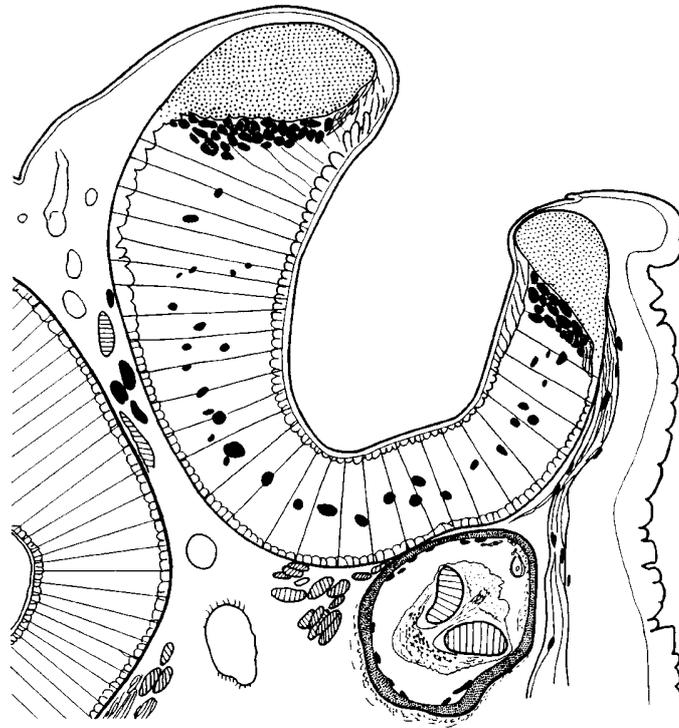
(27) Longitudinal tegumental wrinkles. Two states: 0 = absent; 1 = present; State 1 is observed in the Marsypocephalinae, Proteocephalinae and in all subfamilies of the Monticelliidae except Rudolphiellinae. Longitudinal wrinkles are independent to the transverse wrinkles defined by character 15.

Phylogeny of subfamilies of the Proteocephalidea

A single most parsimonious phylogenetic tree for relationships among the 12 proteocephalidean subfamilies (70 steps; CI = 0.571, excluding uninformative characters = 0.468; HI = 0.471 and 0.559; RC = 0.295), resulted from analysis of 27 morphological characters (Figure 18). Monophyly for the Proteocephalidea is



15



16

Figures 15–16. 15. Vaginal sphincter, *Proteocephalus sophiae* de Chambrier & Rego, 1994 (after de Chambrier & Rego, 1994). 16. Distal “sphincter” on suckers, *Mariauxiella pimelodi* de Chambrier & Rego, 1995 (after de Chambrier & Rego, 1995). Scale-bars: 15, 250 μm ; 16, 100 μm .

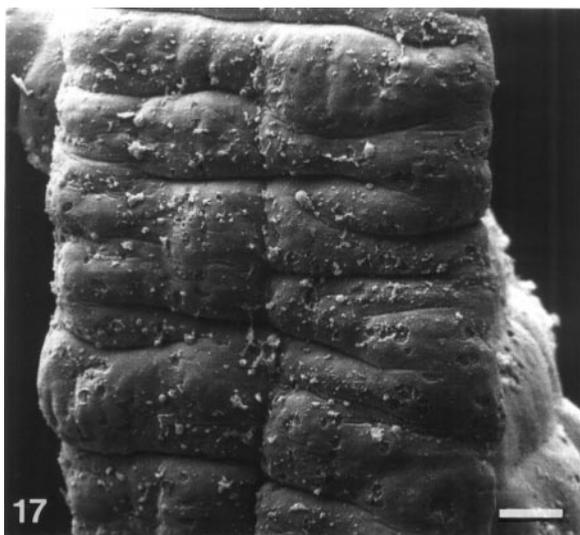


Figure 17. Transverse tegumental wrinkles, *Endorchis piraeeba* Woodland, 1934. Scale-bar: 100 μm .

diagnosed by the structure of the uterus (char. 7) and the presence of a vaginal sphincter (10). The latter character is ambiguous due to putative reversal in two subclades: (1) the Corallobothriinae + Proteocephalinae and Gangesiinae; and (2) the Zygobothriinae and Monticelliidae (Figure 18).

The Acanthotaeniinae are basal, followed by the Nupeliinae and two relatively diverse subclades: (1) the “Proteocephalidae” clade (Sandonellinae + Corallobothriinae + Proteocephalinae and Gangesiinae) and (2) the “Monticelliidae” clade (the Marsypocephalinae + Ephedrocephalinae and Othinoscolecinae + Rudolphiellinae + Monticelliinae and Zygobothriinae) (Figure 18). Support for the Proteocephalidae is based on the arrangement of the longitudinal musculature (char. 23), an attribute influenced by homoplasy. The Monticelliidae is diagnosed by the presence of transverse tegumental wrinkles (char. 15), an unambiguous attribute, and by the position of the testes (4) and longitudinal tegumental wrinkles (27). The latter character undergoes postulated reversal in the Rudolphiellinae.

It can be seen that neither Proteocephalidae nor Monticelliidae as currently conceived are monophyletic based on this analysis. For instance, the Nupeliinae are placed basally with respect to the remaining monticelliids. Additionally, two subfamilies regarded representatives of the Proteocephalidae, namely the Marsypocephalinae and Acanthotaeniinae, are not included within the family as defined by Rego (1994). The Acanthotaeniinae are placed as the basal

subfamily of the Proteocephalidae and are separated from the other nominal taxa of the family by four synapomorphies (chars. 4, 11, 23 and 24) that diagnose relationships for higher taxa in the order. The Marsypocephalinae is grouped as the basal member of the Monticelliidae clade based on the presence of transverse tegumental wrinkles (char. 15). However, a tree one step longer results in the monophyly of both the Proteocephalidae and Monticelliidae, if the Acanthotaeniinae are excluded from the Proteocephalidae. Similarly, a tree two steps longer results in the monophyly for both families and includes all respective subfamilies consistent with current systematics as proposed by Rego (1994).

Regardless of these differences, some groupings are stable in the most parsimonious tree and those one or two steps longer. The Proteocephalinae + Gangesiinae are the sister group of the Corallobothriinae. The Sandonellinae are consistently placed as the basal taxon of the Proteocephalidae clade and are further diagnosed by a number of autapomorphies including the form of the vitellarium in two massive bodies posterior to ovary (char. 2), and structure of the scolex with lappets (char. 22). Additionally, subfamilies of the Monticelliidae clade, including the Monticelliinae + Zygobothriinae, are placed as the sister group of the Rudolphiellinae diagnosed by three homoplasious characters (3, 4, and 11); these subfamilies are the sister group of the Ephedrocephalinae + Othinoscolecinae diagnosed by two ambiguous attributes (14 and 23) (Figure 18). Homoplasy is widespread within the Proteocephalidae, particularly parallelism which was associated with 14 characters of the 27 characters examined in this analysis (1, 3-6, 10, 13, 14, 16, 18, 20, 23, 25, and 27) (Table II).

Biogeography

The contemporary biogeographic distributions for subfamily level taxa are given in Table III and are mapped and optimised onto the parasite cladogram (Figure 19). Results of zoogeographical analysis appear to be compatible with a basal Gondwanan relationship (Figure 19, Table III). Extensive diversification occurred in Africa and South America, with the origin of the group probably in the former region. Subsequent distribution of some groups (proteocephalid subfamilies Proteocephalinae, Corallobothriinae and Gangesiinae) extended into the Northern hemisphere (Holarctic Region).

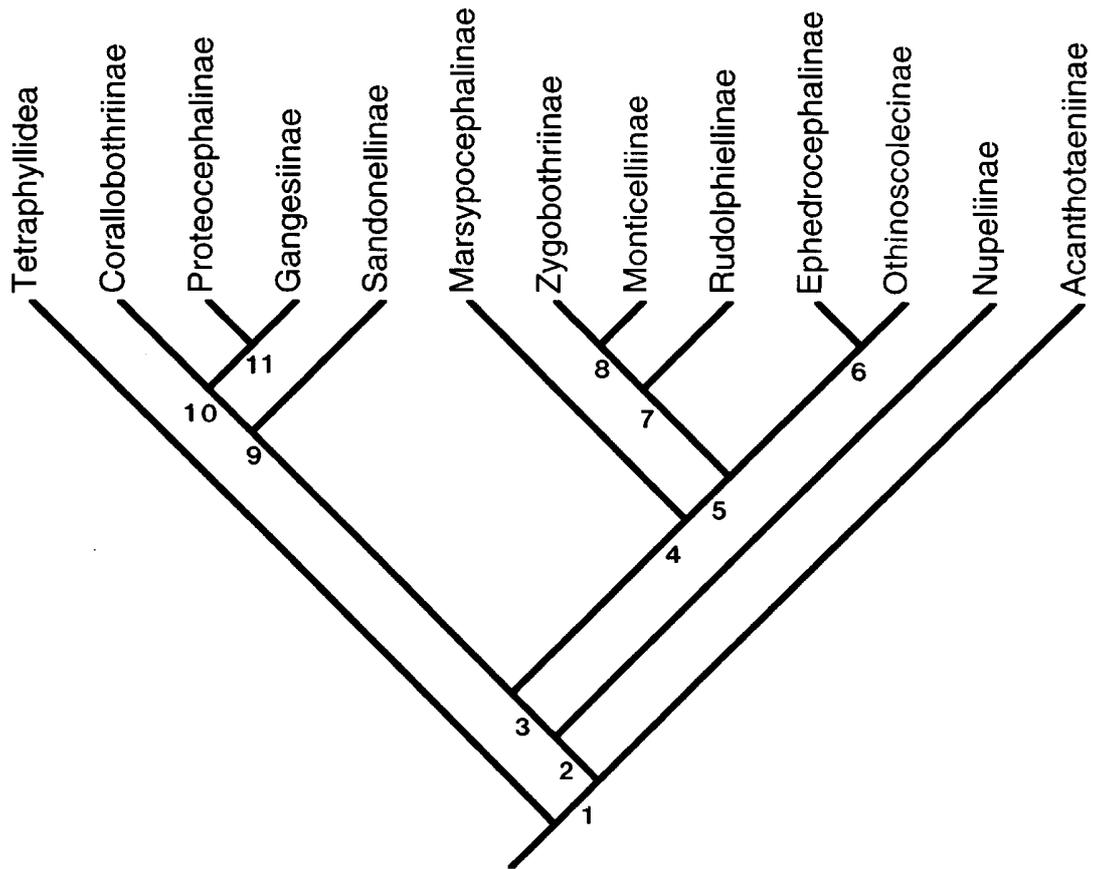


Figure 18. Phylogenetic hypothesis for the 12 nominal subfamilies of the Proteocephalida based on comparative morphology. Shown is the single most parsimonious tree (minimum 40 steps, 70 required; CI = 0.571; HI = 0.471). Character support for each taxon and internode (numbered) is defined below and includes apomorphy (A), homoplasy as parallelism and convergence (H), reversal (R), and polymorphism or change within terminal taxa (P). Terminal taxa are labelled accordingly: Acanthotaeniinae (A: 7, 26; H: 5, 11, 25; P: 14); Nupeliinae (A: 6, 4; H: 1, 3); Marsypocephalinae (H: 20); Rudolphiellinae (R: 27); Monticelliinae (A: 3, 8; H: 4; R: 11); Zygobothriinae (A: 6, 12, 17, 19; H: 5, 13, 20, 23, 25; R: 24); Othinoscolecinae (H: 16, 1, 20); Ephedrocephalinae (A: 2); Sandonellinae (A: 2, 22; H: 13, 14; P: 14); Corallobothriinae (H: 5, 16, 20); Gangesiinae (A: 21; R: 23); and Proteocephalinae (A: 1, 5, 11; H: 18, 27; P: 8). Internodes starting basally are designated as 1-11: 1: (A: 7, 10); 2: (A: 24); 3: (A: 11, 14); 4: (A: 15; H: 4, 27); 5: (H: 1, 16; R: 11); 6: (H: 14, 23); 7: H: 3, 11; R: 4); 8: (A: 9; H: 6, 18; R: 10); 9: (H: 23); 10: (R: 10); 11: (H: 25).

Host relationships

Analysis of parasite-host relationships indicates (Figure 20) that siluriform teleosts were the basal host group for the Nupeliinae + the proteocephalid and monticelliid subclades; basal hosts for the Proteocephalida are unresolved. Non-siluriform teleosts were secondarily colonised by some proteocephalines and sandonellines. It can also be assumed that two independent colonisation events of Reptilia by the Proteocephalinae and Acanthotaeniinae took place, together with one colonisation of amphibians by some other proteocephaline cestodes (Table III, Figure 20).

Discussion

Monophyly for the Proteocephalida is not disputed (see also Hoberg et al., 1997), but results indicate that concepts for the currently recognised families are not supported. Neither the Proteocephalidae nor the Monticelliidae constitute natural groups, based on the present analysis (see Figure 18; Table III). Two major and distinct subclades are consistently recognised: a Proteocephalidae group, including the Sandonellinae, Proteocephalinae, Corallobothriinae and Gangesiinae, and a Monticelliidae group, consisting of all monticelliid subfamilies, except for the Nupeliinae,

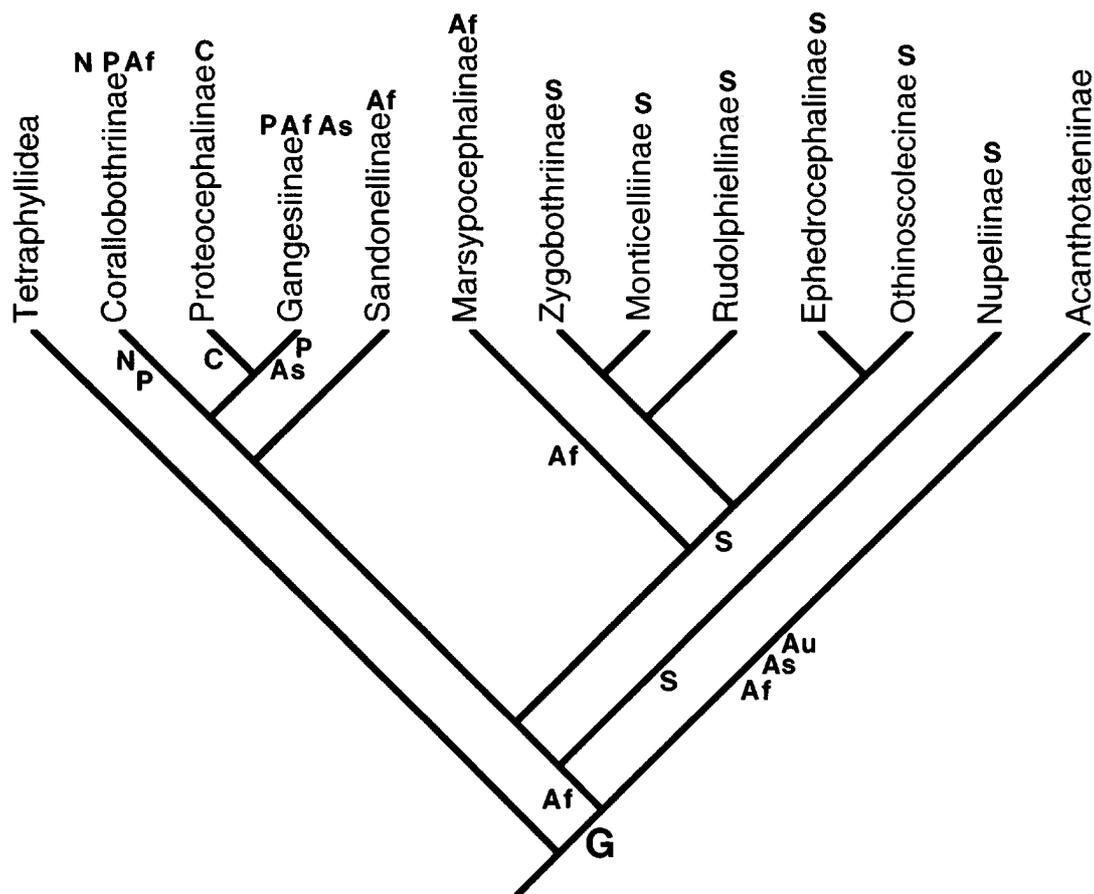


Figure 19. Phylogenetic hypothesis for the subfamilies of the Proteocephalidae with geographical distributions optimised onto the parasite cladogram. Labelling is consistent with Figure 18 and Table III. Geographical localities are indicated as follows: Af, Africa; As, Asia; Au, Australia; C, Cosmopolitan (some groups particularly the Proteocephalinae may have distributions which have been influenced secondarily by translocation and introduction of hosts and parasites across the Holarctic); G, Gondwanan; N, Nearctic; P, Palearctic; and S, South American. Terminal taxa are labelled and geographical distributions are optimised on the tree.

a subfamily established quite recently (Pavanelli & Rego, 1991). Brooks et al. (1991) also suggested the polyphyletic origin of the Monticelliidae. In general, however, the topology of the tree and the relationships specified, are largely congruent with the systematic structure and classification of the proteocephalideans at the higher-level as presented by Rego (1994). It appears that the division of the order into two families is justified with each reflecting phylogenetic relationships among individual subfamilies. No support for recent proposal to suppress the Monticelliidae as a subfamily of the Proteocephalidae (Rego, 1995) has been obtained.

Considering relationships within the monticelliids, consistent with Brooks (1978b), a close relationship between Ephedrocephalinae and Othinoscolecinae was found. On the contrary, the present analysis

did not support Brooks' (1978b) synonymy of the Rudolphiellinae as a member of the Monticelliinae. These subfamilies were placed in distinct subclades within the putative family, with the latter as the sister group of the Zygobothriinae (Figure 18).

The most significant departure from the current classification is in the placement of the Marsypocephalinae as the basal taxon of the monticelliid clade, and the basal placement of the Nupeliinae and the Acanthotaeniinae. As indicated, however, potential alternative trees, either one or two steps longer, would be entirely congruent with the currently proposed systematics for the order and relationships among the subfamilies. Consequently, it is premature to propose changes to the current taxonomy for the group.

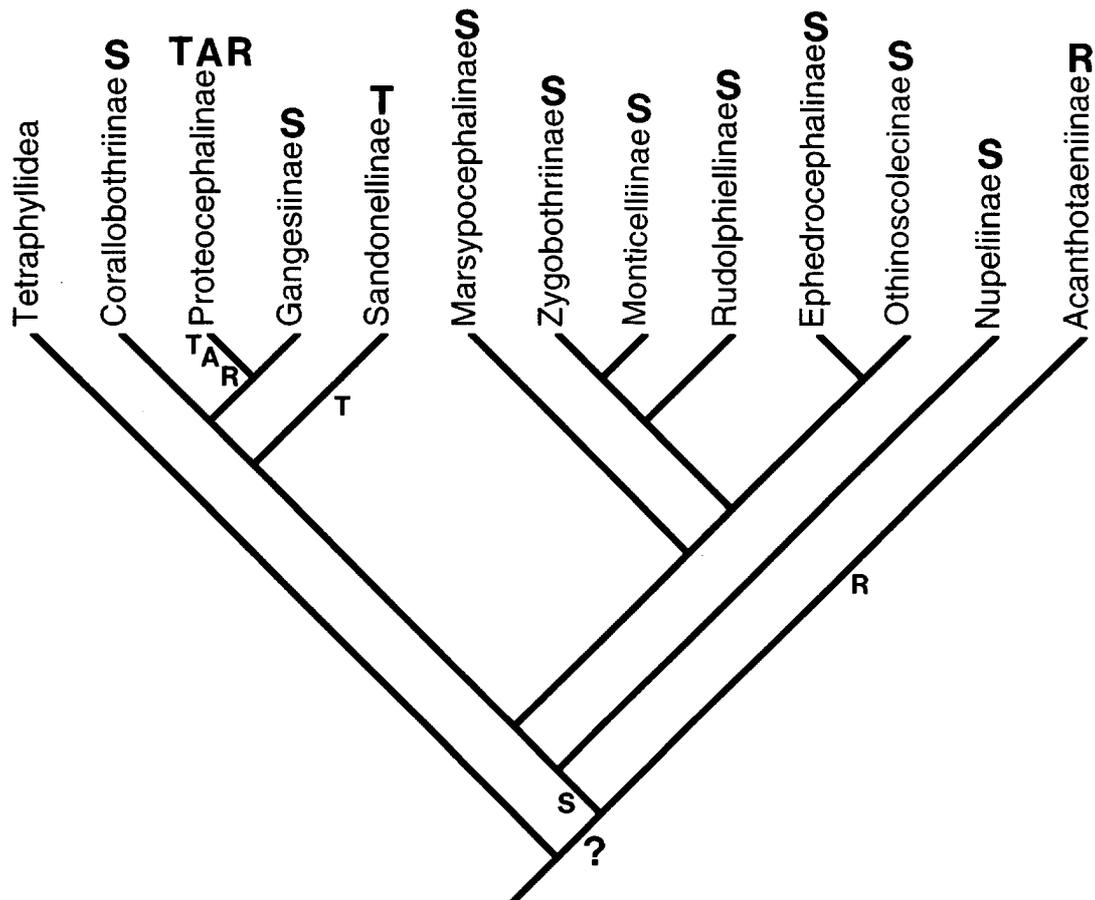


Figure 20. Phylogenetic hypothesis for the subfamilies of the Proteocephalidea with host occurrence optimised onto the parasite cladogram. Labelling is consistent with Figure 1 and Table III. Hosts are designated as follows: A, Amphibia; T, non-siluriform teleosts; R, Reptilia; S, siluriform fishes; ?, unresolved association. Terminal taxa are labelled and host associations are optimised on the tree.

Since the present study did not evaluate relationships at the generic level, it was not possible to evaluate groupings of many genera, particularly those within the Monticelliidae that were considered artificial by Brooks & Rasmussen (1984, figure 11). Results presented by these authors may reflect existing confusion in generic diagnoses of many taxa rather than a clear indication of the necessity to modify classifications of the group. Similarly, a new arrangement of the Monticelliidae, including numerous synonymies of genera proposed by Brooks (1995), requires critical evaluation coinciding with the broadening of our knowledge from comparative morphology. As an example, the recent revision of the monticelliid genus *Nomimoscolex*, which resulted in numerous synonymies and new combinations (de Chambrier & Vaucher, 1997), can be mentioned.

Brooks (1995) suggested that the current level of resolution in phylogenetic studies of the monticelliids (and this can be extended to the proteocephalideans in general) was limited by the availability of character information for many taxa. Homoplasy was considered to be relatively minimal in the characters evaluated (e.g. CI = 0.73). In contrast, the current analysis resulted in recognition of substantial levels of homoplasy, primarily definable as parallel development (e.g. CI = 0.571; with 14 of 27 characters exhibiting some level of homoplasy) (Table II). Thus, it is apparent that much more data must be evaluated in order to critically assess relationships within the order Proteocephalidea.

Historical biogeography for the proteocephalideans is complex and clearly associated with Gondwana (Figure 19). The current analysis is compatible with basal Gondwanan relationships, initial diversification in Africa, and with radiation in some taxa occurring

Table II. Consistency indices for individual characters used in analysis of the Proteocephalidea as an indication of the degree of homoplasy

Character number	Character	CI
1	vitelline position	0.667
2	vitelline topography	1.000
3	ovary position	0.667
4	testes position	0.500
5	testicular field	0.500
6	uterus position	0.500
7	uterus shape	1.000
8	egg structure	0.500
9	egg embryonation	1.000
10	vaginal sphincter	0.333
11	vagina position	0.333
12	genital pore	1.000
13	proglottis type	0.500
14	proglottis shape	0.800
15	teg. wrinkles, transverse	1.000
16	metascolex	0.333
17	apical organ	1.000
18	sucker structure	0.250
19	auricula	1.000
20	“sphincter” on suckers	0.250
21	rostellum	1.000
22	lappet structures	1.000
23	longitudinal muscles	0.250
24	“spination” of cirrus	0.500
25	“spination” of suckers	0.333
26	piercing organ	1.000
27	teg. wrinkles, longitudinal	0.333

prior to the breakup of the supercontinent. Fragmentation of Gondwana is reflected in the geographical distributions of the Marsypocephalinae + remaining monticelliids (Figure 19). Secondary expansion into the Northern Hemisphere is evident among the Coralbothriinae, Proteocephalinae and the Gangesiinae.

Brooks (1978b), however, considered Gondwanian South America to be the centre of origin for the Proteocephalidea. Irrespective of this difference, the present analysis suggests subsequent distributional history of proteocephalideans linking Africa and South America, with an extensive radiation in the latter continent after its separation from Africa. It can also be presumed that proteocephalid subfamilies (Proteocephalinae, Coralbothriinae and Gangesiinae) subsequently dispersed with their hosts into the Northern Hemisphere, with members of the former

Table III. Geographical distribution and host spectrum for the subfamilies of the Proteocephalidea

Subfamily	Family ¹	Hosts ²	Geographical distribution
Coralbothriinae	P	Pi-S	Nearctic, Palearctic & Africa
Gangesiinae	P	Pi-S	Africa, Asia, Palearctic
Proteocephalinae	P	Pi,A,R	cosmopolitan
Sandonellinae	P	Pi	Africa
Marsypocephalinae	P	Pi-S	Africa
Zygobothriinae	M	Pi-S	S. America ³
Monticelliinae	M	Pi-S	S. America
Rudolphiellinae	M	Pi-S	S. America
Ephedrocephalinae	M	Pi-S	S. America
Othinoscotecinae	M	Pi-S	S. America
Nupeliinae	M	Pi-S	S. America
Acanthotaeniinae	P	R	Africa, Asia, Australia ⁴

¹ P, Proteocephalidae; M, Monticelliidae.

² Pi, Pisces [teleosts] (mostly siluriform – S); A, Amphibia; R, Reptilia.

³ *Postgangesia parasiluri*, previously placed in the Zygobothriinae (see Schmidt, 1986; Rego, 1994), is in fact *Gangesia parasiluri*, a member of the Gangesiinae, as stated previously by Dubinina (1971, 1987).

⁴ One species, *Acanthotaenia overstreeti* Brooks & Schmidt, 1978, was described from *Cyclura stejnegeri* in Puerto Rico.

subfamily group becoming cosmopolitan in distribution.

Examination of host-parasite associations suggests that teleosts were basal hosts for the proteocephalideans, although the basal node linking the Acanthotaeniinae and other subfamily level taxa must be regarded as equivocal. Excluding the Acanthotaeniinae, which occur in reptilian hosts, optimisation of host occurrence on the phylogenetic tree would suggest siluriform fishes as basal hosts for the remaining proteocephalideans (Figure 20); an extensive history of co-evolution with catfishes of the Neotropics is postulated. In contrast, the host associations of proteocephaline and acanthotaeniine cestodes in the present phylogenetic tree may be consistent with two independent colonisation events of reptilians. Additionally associations of proteocephalines with amphibians and other teleosts, and sandonellines with teleosts, represent putative event of host-switching (Figure 20). This strongly corroborates the original hypothesis for host relationships among the proteocephalideans, as proposed by Brooks (1978b; see figure 8 of Brooks)

Summary and conclusions

Despite some differences between results of the present phylogenetic analysis and current classification of the Proteocephalidea, no changes in the taxonomy of this cestode group are proposed. This analysis, although the first one based on critical study of existing data, is considered to be preliminary due to the limited information about some taxa. Similarly, the taxonomic position of some subfamilies (Acanthotaeniinae, Nupeliinae, Marsypocephalinae and Sandonellinae) remains problematical. Although the current analysis supports recognition of the proteocephalideans as monophyletic, this hypothesis requires continued evaluation (see Brooks et al., 1991).

Regarding the future, any phylogenetic analysis at generic or lower levels within the Proteocephalidea should be preceded by a revision of the characters based on type, voucher and recently collected material. Particularly, consideration of the following points for the future progress in the taxonomy of the group and its phylogenetic relationships is strongly encouraged by the present authors:

(1) More detailed and complete redescriptions of inadequately characterised taxa are recommended. For example, members of some subfamilies like the Sandonellinae, Nupeliinae, Gangesiinae, Acanthotaeniinae, and Marsypocephalinae are poorly known.

(2) We suggest that further taxonomic work should be based only on adequately processed material. For example, good results were obtained with fixation of living worms with hot 4% formaldehyde and staining with Mayer's iron paracarmine. It is recommended to avoid excessive relaxation of worms in water, which often results in vacuolisation of tissues and damage of the tegument. The fixation process should attempt to eliminate excessive levels of contraction of the strobila; artificial flattening of the worms is to be discouraged. Technical information for fixation and preparation of specimens has been published and discussed in several papers, including Anonymous (1957), Pritchard & Kruse (1982), Gibson (1984), Georgiev et al. (1986) and de Chambrier (1987).

(3) In order to precisely observe the internal topography of genital organs and longitudinal musculature, morphological study must include transverse sections.

(4) The search for new characters should continue (see Mariaux, 1996; Hoberg et al., 1997). Of particular promise may be attributes derived from histology, ultrastructural analysis (SEM and TEM) and comparative evaluations of the surface morphology of the

scolex allowing observations of complex structures such as the apical organ, glandular cells and other attributes. It is vital to have synoptic descriptions of uterine ontogeny and structure, along with details of oncospheres and egg envelopes. Helpful observations of egg structure can be obtained with eggs released from the terminal proglottides and observed in water.

(5) Substantial progress also could be achieved with respect to studies of the life-cycles of most proteocephalidean groups. For example, it is vital that this information be obtained for species parasitising South American fishes and reptiles, including all members of the Monticelliidae. Studies published previously, limited primarily to members of the Proteocephalinae, have indicated that copepods are exclusive first intermediate hosts of proteocephalideans (Freze, 1965; Scholz, 1991, 1993). Variation in egg structure among some South American species however, suggests that several life-cycle patterns exist among Proteocephalidea.

The current analysis summarises the current body of data available from comparative morphology. Generalities are apparent with respect to conclusions of this analysis and previous concepts for the systematics of the Proteocephalidea. This can now promote a hierarchical and top down approach to the evaluation of genera and species (e.g. Hoberg et al., 1997) in the pursuit of a refined understanding of the systematics, host-associations and historical biogeography of these fascinating tapeworms.

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