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The evolution of the Proteocephalidea (Platyhelminthes, Eucestoda) based on an enlarged molecular phylogeny, with comments on their uterine development

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Abstract

We present a molecular phylogeny of the Proteocephalidea based on 28S rDNA sequence data that is a follow-up to the paper by Zehnder & Mariaux (1999). Twenty-three new sequences, including three outgroups are added in our new data-set. The Gangesiinae Mola, 1929 and the Acanthotaeniinae Freze, 1963 appear to be the most primitive clades. They are followed by a robust clade comprising the Palaearctic Proteocephalinae Mola, 1929 from freshwater fishes. The structure of the more derived clades, comprising most Neotropical and Nearctic species, is less resolved. At the nomenclatural level, we erect a new genus, *Glanitaenia* n. g. for *G. osculata* (Goeze, 1782) n. comb., previously *Proteocephalus osculatus*, and define an aggregate for the Palaearctic *Proteocephalus* Weinland, 1858. After a re-examination of all of the studied taxa, we identify two types of uterine development and show the importance of this character for the systematics of the order. Our phylogeny does not support the classical view of a Neotropical origin for the Proteocephalidea but rather favours an Old World origin of the group either in saurians or Palaearctic Siluriformes.

Introduction

This study represents a follow-up to the molecular phylogenetic analysis of the Proteocephalidea published by Zehnder & Mariaux (1999). We present here the results obtained after appending the sequences of 23 species (11 genera) to our previous 28S rDNA database. Hence, we examine the phylogenetic relationships of 71 proteocephalideans representing 10 subfamilies and 30 genera and using four tetraphyllideans, including three from Olson et al. (2001), as outgroup species. The main goals were to verify the strength of the basal nodes, especially in relation to the basal position of the monitor parasite *Acanthotaenia* Linstow, 1903 indicated in previous contributions (Kodedova et al., 2000; Rego et al., 1998; Skeríková et al., 2001), to base the analyses on a more representative sampling, and to eventually define new synapomorphies based on morphological characters.

Materials and methods

Twenty newly sequenced proteocephalidean species as well as three extra outgroups (obtained from GenBank) were added to the former database examined by Zehnder & Mariaux (1999). They are listed below with the host, geographical origin, references to mounted vouchers from the Geneva Natural History Museum (INVE) and EMBL/Genbank data library accession numbers. Analyses were thus performed on an enlarged data-set of 75 taxa:

Tetraphyllidea

Acanthobothrium sp. – Oncobothriidae ex *Dasyatis longus* Garman, Mexico, AF286953 (Olson et al., 2001).
Phyllobothrium lactuca Beneden, 1850 – Phyllobothriidae ex *Mustelus asterias* Cloquet, off the UK, AF286960 (Olson et al., 2001).
Rhinebothrium maccalumi (Linton, 1924) – Phyllobothriidae ex *Dasyatis americana* Hildebrand &

Schroeder, Mexico, AF286962 (Olson et al., 2001).
Tetraphyllidea gen. sp. ex *Squalus acanthias*, North Sea (Zehnder & Mariaux, 1999), AJ388591.

Proteocephalidea

Proteocephalidae

Acanthotaeniinae

Acanthotaenia cf. *shipleyi*, Linstow, 1903 ex *Varanus salvator* (Laurenti), Klang, Selangor, Malaysia, 05.x.2001, INVE 32837, AJ583453.
Kapsulotaenia sp. 1 ex *Varanus rosenbergi* Mertens, Rocky River, road to Snake Lagoon, Kangaroo Island, South Australia, Australia, 16.xi.2001, INVE 32842, AJ583452.
Kapsulotaenia sp. 2 ex *Varanus gouldi* (Gray), Carnarvon National Park, Mt Mofatt, Queensland, Australia, 07.xii.2000, INVE 32839, AJ583455.
Kapsulotaenia sp. 3 ex *Varanus gouldi* (Gray), Culgoa Floodplain National Park (CFNP), Queensland, Australia, 17.xii.2000, INVE 32840, AJ583451.
Kapsulotaenia sp. 4, ex *Varanus varius* (Shaw), Roma, Injune road, Taroom turnoff, Queensland, Australia, 30.iii.2000, INVE 32838, AJ583454.

Corallobothriinae

Corallobothrium solidum Fritsch, 1886, ex *Malapterurus electricus* (Gmelin), Luxor, River Nile, Egypt, 18.iv.2002, INVE 31553, AJ583450.
Corallotaenia intermedia (Fritts, 1959) ex *Ictalurus melas* (Rafinesque), Hay Bay, Lake Ontario, Canada, 18.vii.1995, INVE 25795, AJ275232.

Proteocephalinae

Proteocephalus chamelensis Pérez-Ponce de León, Brooks & Berman, 1995 ex *Gobiomorus maculatus* (Günther), Chamelá, Jalisco, Mexico, xii.1998, AJ275233.
Proteocephalus hobergi de Chambrier & Vaucher, 1999 ex *Oxydoras kneri* Bleeker, San Antonio, Central Province, Paraguay, 06.xi.1995, INVE 24643, AJ275062.
Proteocephalus perplexus La Rue, 1911 ex *Amia calva* (L.), Hay Bay, Lake Ontario, Canada, 17.vii.1995, INVE 25658, AJ275228.
Proteocephalus sp. (larva from liver) ex *Perca flavescens* (Mitchill), Lake Ontario, Canada, 18.vii.1995, AJ275230.
Pseudocrepidobothrium sp. ex *Phractocephalus hemioliopterus* (Schneider), Itacoatiara, Province Amazonas, Brazil, 01.x.1995, INVE 22108, AJ275063.
Testudotaenia cf. *testudo* (Magath, 1924) ex *Apalone spinifera hartwegi* (Conant & Goin), Nishnabotna River, Riverton, Iowa, USA, 31.viii.2000, INVE 30486, AJ583456.
Thaumasioscolex didelphidis Cañeda-Guzmán, de Chambrier & Scholz, 2001 ex *Didelphis marsupialis* L., Los Tuxtlas, Veracruz, Mexico, 21.v.1999, INVE 28993, AJ275065.

Monticelliidae

Monticelliinae

Choanoscolex cf. *abscisus* (Riggenbach, 1896) ex *Pseudoplatystoma fasciatum* (L.), Itacoatiara, Province Amazonas, Brazil, 11.x.1995, INVE 25102, AJ275064.

Zygobothriinae

Amphoteromorphus piriformis Carfora, de Chambrier & Vaucher, 2003 ex *Brachyplatystoma flavicans* (Castelnau), Itacoatiara, Province Amazonas, Brazil, 21.ix.1992, INVE 22211, AJ275231.
Amphoteromorphus ninoi Carfora, de Chambrier & Vaucher, 2003 ex *Brachyplatystoma vaillanti* (Cuvier & Valenciennes), Itacoatiara, Province Amazonas, Brazil, 02.x.1995, INVE 22205, AJ275066.
Amphoteromorphus ninoi Carfora, de Chambrier & Vaucher, 2003 ex *Brachyplatystoma filamentosum* (Lichtenstein), Itacoatiara, Province Amazonas, 07.x.1995, INVE 22239, AJ388624.
Brooksiella praeputialis (Rego, Dos Santos & Silva, 1974) ex *Cetopsis coecutiens* Spix & Agassiz, Itacoatiara, Province Amazonas, Brazil, 16.x.1995, INVE 21996, AJ275229.
Harriscolex kaparari (Woodland, 1935) ex *Pseudoplatystoma tigrinum* (Valenciennes), Itacoatiara, Province Amazonas, Brazil, 09.x.1995, INVE 22018, AJ275227.

Since the publication of Zehnder & Mariaux (1999), the names or the identification of some taxa used in their work needs to be updated:
Rostellotaenia sp. (INVE 25026, AJ388593) instead of *Acanthotaenia* sp.;
Amphoteromorphus ninoi Carfora, de Chambrier & Vaucher 2003 instead of *A. piraeeba* Woodland, 1934 (INVE 22239, AJ275066);
Pseudocrepidobothrium eirasi (Rego & de Chambrier, 1995) instead of *Crepidobothrium eirasi* (INVE 27431, AJ388623) (Rego & Ivanov, 2001);
Nomimoscolex suspectus Zehnder, de Chambrier,

Vaucher & Mariaux, 2000 (INVE 22298, AJ388602) instead of *Nomimoscolex* sp;

Peltidocotyle lenha (Woodland, 1933) Zehnder & de Chambrier, 2000 instead of *Peltidocotyle* sp. and *Othinoscolex lenha* (Woodland, 1933) (respectively INVE 21912, AJ238834; INVE 22373, AJ238837; INVE 22021, AJ238836);

Rudolphiella szidati Gil de Pertierra & de Chambrier, 2000 instead of *Rudolphiella* sp. (INVE 24668, AJ388617);

Spatulifer sp. instead of *Spatulifer maringaensis* Pavanelli & Rego, 1989 (INVE 21986, AJ388634);

'*Proteocephalus longicollis* 1' instead of *P. pollanicola* Gresson, 1952 (AJ388599);

'*Proteocephalus longicollis* 2' instead of *P. exiguus* La Rue, 1911 (AJ388626).

DNA extraction, PCR amplification of a 5′ portion of the 28S rDNA molecule and PCR product purification were performed as previously described (Zehnder & Mariaux, 1999). Sequencing was done on an ABI 377 sequencer. The alignment was performed with Sequencher v.4.1.2, refined by hand, and is available freely at the following URL: *http://www.treebase.org/treebase* with the accession numbers S968 and M1605.

A parsimony analysis was performed with PAUP* (V4.0b10) (Swofford, 2002) with the following parameters: heuristic search, uninformative characters removed, no maxtree, acctran, gaps considered as missing, and 2,500 replicates with random seed and input order. Furthermore, 200 full heuristic bootstraps were performed with 10 repeats each and a maxtree setting of 10000.

Results

The enlarged 28S rDNA data-set comprised 1,134 characters, 146 of which were excluded due to alignment difficulties. Regarding the remaining 988 positions, 480 were variable and 316 were phylogenetically informative. Parsimony analyses yielded 2,895 most parsimonious trees with a length of 1,555 steps, $CI = 0.319$, $RI = 0.664$, $RC = 0.212$. A strict consensus with bootstrap values is shown in Figure 1. It essentially confirms results previously obtained by Zehnder & Mariaux (1999), and adds some new information.

Within the Proteocephalidea, *Acanthotaenia* Linstow, 1903, *Rostellotaenia* Freze, 1963, *Kapsulotaenia* Freze, 1965, *Gangesia parasiluri* Yamaguti, 1934 and *Silurotaenia siluri* (Batsch, 1786) are the most basal taxa. Exact relationships between these taxa are difficult to determine as the basal position of the *Silurotaenia-Gangesia* clade relative to the *Rostellotaenia-Acanthotaenia-Kapsulotaenia* one is only weakly supported.

The next, and well-supported clade, comprises the European *Proteocephalus* together with *Paraproteocephalus parasiluri* (Yamaguti, 1934) and *Proteocephalus osculatus* (Goeze, 1782) (=*Glanitaenia osculata* – see below). These basal taxa are followed by a large clade with a weak internal structure. Within it *Proteocephalus hemioliopteri* de Chambrier & Vaucher, 1999, *Zygobothrium megacephalum* Diesing, 1850, *P. pirarara* (Woodland, 1935), *Corallotaenia intermedia* (Fritts, 1959) and *C. solidum* Fritsch, 1886 are basal, but their relative position is unclear. In the remaining crown clade only a few groups of two to four species are supported with high bootstrap values. Among them, we find well defined only *Peltidocotyle* Diesing, 1850 and some *Nomimoscolex* Woodland, 1934 (see Zehnder et al., 2000) clades (Figure 1).

Discussion

Systematics of the order

Woodland (1934, 1935) set the foundation of the present classification based on the arrangements of the genital organs relative to the internal longitudinal musculature. His system comprised one family and eight subfamilies. Freze (1965) followed the general scheme of Woodland, but introduced features or characteristics of the life-cycle and host relationships in his classification, with three families and 14 subfamilies. His family Ophiotaeniidae was, however, never really accepted, and later Schmidt (1986), like many others, only retained a two-family structure for the order. Based on morphology, ontogeny, life-cycle and distribution, Rego (1995) proposed the suppression of the family Monticelliidae Mola, 1929, keeping only one family, the Proteocephalidae Mola, 1929, and five subfamilies. Nevertheless, the two-family scheme is presently the most widely accepted (Rego, 1994; Scholz & de Chambrier, 2003), but it has been questioned by Zehnder & Mariaux (1999) among others.

The results presented here verify the conclusions of Zehnder & Mariaux (1999) that both the Proteo-

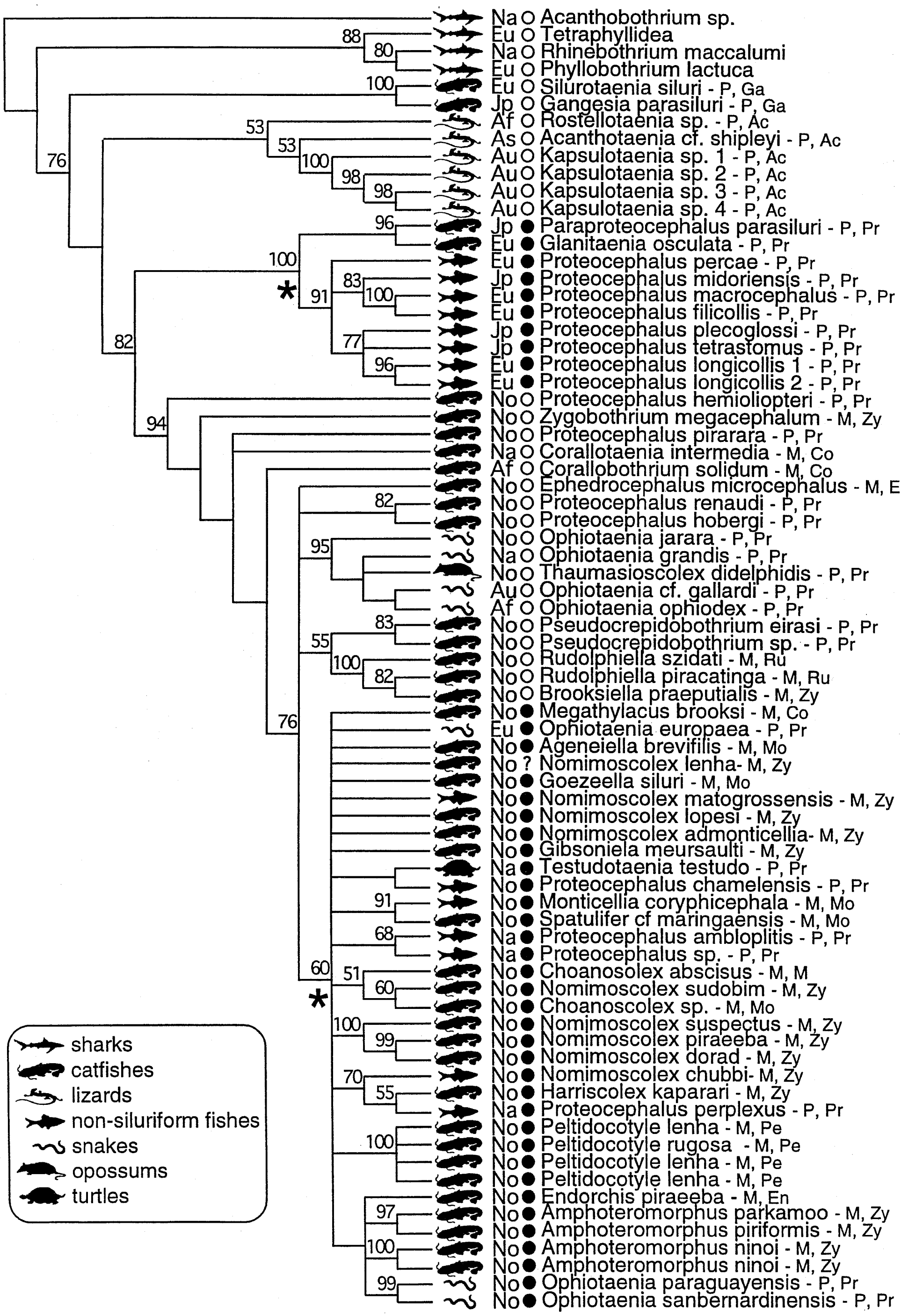

Figure 1. Strict consensus tree of 2,895 equally parsimonious trees for the Proteocephalidea obtained from a parsimony analysis of the nuclear 28S rRNA data-set, including four tetraphyllidean outgroups (heuristic, 2,500 repeats). Figures above the branches indicate the support values obtained from 200 × 10 full heuristic bootstrap replicates. *Abbreviations*: Af, Africa; Au, Australia; As, Asia; Eu, Europe; Ja, Japan; Na, Nearctic; No, Neotropical. Icons representing the hosts are explained on the figure. The present taxonomic position of each taxon within the order are indicated by a three-letters abbreviation after its name, the first one indicating the family: (P, Proteocephalidae; M, Monticelliidae), the next ones the subfamily (Pr, Proteocephalinae, Zy, Zygobothriinae; Co, Corallobothriinae; Ru, Rudolphiellinae; Mo, Monticelliinae; Ac, Acanthotaeniinae; Ga,. Gangesiinae; Pe, Peltidocotylinae; Ep, Ephedrocephalinae, En, Endorchiinae); ○, uterine type 1; ●, uterine type 2; ?, unclear. Stars (⋆) indicate the appearance of the type 2 uterus.
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cephalidae and Monticelliidae are paraphyletic. Figure 1 indicates that the most basal taxa belong to Proteocephalidae, from which Monticelliidae appear to be derived. The most derived clade in our tree is a mixture of members from both families. At the subfamilial level, our data do not support the monophyly of the Proteocephalinae Mola, 1929, Zygobothriinae Woodland, 1933, Corallobothriinae Freze, 1965, Rudolphiellinae Woodland, 1935 or Monticelliinae Mola, 1929. Conversely, the Acanthotaeniinae Freze, 1963, Gangesiinae Mola, 1929 and Peltidocotylinae Woodland, 1934 appear as natural groups, the latter two being strongly supported. Nevertheless, our results based on molecular data fail to confirm any previous classification scheme.

In Zehnder & Mariaux (1999), Rego et al. (1998) and Skeríková et al. (2001), *Acanthotaenia* was basal. With the addition of several additional parasites from monitors and some gangesiines to the database, this position is more uncertain, as a clade comprising *Silurotaenia* and *Gangesia* appears to be the most basal of the proteocephalideans. Nevertheless, the position of the parasites of monitors at, or very near the basal branch is confirmed in this work.

Proteocephalus Weinland, 1858, one of the largest genera with regard to the number of species, *Ophiotaenia* La Rue, 1911 and *Nomimoscolex* Woodland, 1934, plus several other genera and subfamilies, do not emerge as monophyletic, emphasising the need for investigating new characters in order to find natural groupings. Such attempts have been made for *Nomimoscolex* (see Zehnder et al., 2000) but, so far, no attempt has been made to define different groups for *Proteocephalus* subsequent to the suggestion of Brooks et al. (1991) that it may indeed represent a composite taxon. The present classification scheme is no longer satisfactory, and an alternative structure for the genus must be defined. Our data show that eight Palaearctic species (in addition to *Paraproteocephalus* Chen Yan-Hsin, 1962 and *Glanitaenia* n. g. – see below) form a robust, distinct and morphologically homogenous clade, and that the remaining eight Neotropical and Nearctic species from our sampling, the affinities of which are not satisfactorily resolved in this analysis, are scattered in as many as six clades. They will eventually be removed from the genus when their evolutionary relationships can be established.

This result persuaded us to reorganise *Proteocephalus*, but, as we are unable at this point to define natural taxa to accommodate all *Proteocephalus* species, we propose the establishment of an aggregate of species of *Proteocephalus*, complying with Article 6b of the International Code of Zoological Nomenclature (1999), for a well-defined clade consisting of the Palaearctic species only (see below).

As for the other species of the genus, *P. ambloplitis* (Leidy, 1887) clusters with the newly added Nearctic *Proteocephalus* specimen recovered from the liver of *Perca flavescens* (Mitchill). A morphological comparison between both taxa was not possible, since only a larval stage of the latter specimen was available. The parenteral location of plerocercoids in the second intermediate host represents a pattern differing from that usually found in Palaearctic *Proteocephalus* (see Scholz, 1999). Finally, we could find no morphological or life-cycle arguments to account for the relationships of the remaining species of '*Proteocephalus*' dispersed in our tree.

We observed a similar situation for *Ophiotaenia* La Rue, 1911, the members of which are dispersed in three clades. One robust clade is formed by four parasites of venomous ophidians (Viperidae and Elapidae): *O. ophiodex* Mettrick, 1960 (Africa), *O. gallardi* (Johnston, 1911) (Australia), *O. grandis* La Rue, 1911 (North America) and *O. jarara* Fuhrmann, 1927 (South America) with *Thaumasioscolex didelphidis* from a Mexican marsupial. These five species possess the type 1 uterine formation (see below for the definitions of uterine types).

Considering the strong association of *T. didelphidis* with four ophidian parasites, we can assume that the colonisation of the mammalian host is the result of a capture from an ancient ophidian host. Morphological observations (de Chambrier et al., 1991; Cañeda-Guzmán, 1997; Cañeda-Guzmán et al., 2001) indicate that in all parasites from this cluster the scolex is huge with powerful suckers, the testes are in two lateral fields, the internal longitudinal musculature is well developed and the uterine development is of type 1 with a medullary stem. However, a number of characters clearly differentiate *Thaumasioscolex* from *Ophiotaenia*: a lateral opening of non-circular suckers, inversely craspedote proglottides, a larger number of testes (> 500), a larger strobila size (> 1 m) and eggs in clusters of four to six (Cañeda-Guzmán et al., 2001).

Interestingly, the remaining Palaearctic and Neotropical *Ophiotaenia* analysed are found in colubrid snakes, possess the type 2 uterine formation and are dispersed in a large unresolved clade. However, we are unable to decide how to organise *Ophiotaenia* until more material from both venomous and non-venomous snakes is analysed.

The Rudolphiellinae, with *Rudolphiella szidati* Gil de Pertierra & de Chambrier, 2000 and *R. lobosa* (Riggenbach, 1895), were previously found to be monophyletic (Zehnder & Mariaux, 1999). This is no longer the case after adding *Brooksiella praeputialis* (Rego, Santos & Silva, 1974) (Zygobothriinae) to the database: this species groups strongly with the *Rudolphiella* taxa. This clade not only associates species from different genera, but from different subfamilies. The Rudolphiellinae and the Zygobothriinae are characterised by a different arrangement of gonads relative to the longitudinal musculature: in *Rudolphiella*, the vitelline follicles and testes are completely cortical, the ovary is largely cortical and the uterus is entirely medullary, whereas in *Brooksiella* Rego, Chubb & Pavanelli, 1999, the vitelline follicles alone lie in the cortex. One trait of the three species is the peculiar morphology of their eggs which bear two elongate polar processes on either side. This uniting feature is common to all species of *Rudolphiella* (see Gil de Pertierra & de Chambrier, 2000) and has been reported in no other proteocephalid genus except for the monotypic *Brooksiella*. This genus also shares with *Rudolphiella* a very folliculated ovary, ventral vitelline follicles, a metascolex with uniloculate suckers, type 1 uterine development and a short strobila with proglottides generally wider than long.

Other cases of (weaker) associations involving species from different subfamilies include *Harriscolex kaparari* (Woodland, 1935) and *Nomimoscolex chubbi* (Pavanelli & Takemoto, 1995) (both Zygobothriinae) with *Proteocephalus perplexus* La Rue, 1911 (Proteocephalinae). Moreover, two species of *Choanoscolex* La Rue, 1911 (Monticelliinae) cluster with *Nomimoscolex sudobim* Woodland, 1935 (Zygobothriinae). The latter two monticelliid genera differ substantially in gonad arrangement within the proglottis, thus rendering the associations difficult to interpret on the basis of morphological criteria.

All these observations once again confirm that the cortical/medullary criterion for the arrangement of the gonads is not correlated with natural taxa in previous classifications, i.e. since the work of La Rue (1911, 1914). In fact, the traditional concept of the Proteocephalidea split into two families based on this character has to be abandoned, even if the cortical/medullary position of the gonads still appears to be very useful at lower taxonomic levels (e.g. for the present subfamilies). Finally, at the generic level, and on the basis of the four taxa included in this analysis, the monophyly of *Amphoteromorphus* Diesing, 1850 is unclear (unresolved in the strict consensus tree, but supported by a 65% value in the bootstrap analysis) and may only be ultimately assessed by adding its last missing species, *A. peniculus* Diesing, 1850, to the analysis. Morphologically, the three analysed species are the unique representatives of the order Proteocephalidea in that they display unilateral genital pores (de Chambrier & Vaucher, 1997), a character not shared with *A. peniculus*. The composition of this genus is discussed in greater detail elsewhere (Carfora et al., 2003).

Pseudocrepidobothrium Rego & Ivanov, 2001 represents a natural taxon on the basis of the two piscine species from this study: *P. eirasi* (Rego & de Chambrier, 1995) and *Pseudocrepidobothrium* sp., which represents a new species.

Taxonomy

A number of nomenclatural acts are consequent upon the results summarised above and presented in Figure 1.

(a) The erection of Glanitaenia *n. g.*

Due to its particular morphological features (Goeze, 1782; Nybelin, 1942; Scholz & Hanzelová, 1998) and its basal position, *Proteocephalus osculatus* (Goeze, 1782) is clearly separated from the other Palaearctic species of *Proteocephalus*. This justifies the erection of a new genus.

***Glanitaenia* n. g.**

Diagnosis: Proteocephalidea, Proteocephalidae, Proteocephalinae. Large tapeworms with slightly craspedote proglottides. Unarmed scolex with four uniloculate suckers. True apical sucker well developed, strongly muscular, with deep cavity. Testes medullary, in one continuous field and in one layer. Ovary medullary, bilobed. Uterus medullary, ventral, with lateral outgrowths. Vitelline follicles lateral, occupying almost entire proglottis length. Vagina always anterior to cirrus-sac, without vaginal sphincter. Genital pore near median proglottis margin. Parasites of Palaearctic siluriform fishes. Type and only species: *G. osculata* (Goeze, 1782) n. comb. [syn. *Proteocephalus osculatus* (Goeze, 1782)].

Etymology: Named after the catfish host species *Silurus glanis*.

Differential diagnosis

Glanitaenia n. g. belongs to the subfamily Proteocephalinae on the basis of the presence of a medullary vitellarium, ovary, uterus and testes, and a scolex without a metascolex. Twelve genera belong to Proteocephalinae: *Brayela* Rego, 1984, *Cangatiella* Pavanelli & Machado dos Santos, 1991, *Crepidobothrium* Monticelli, 1900, *Deblocktaenia* Odening, 1963, *Euzetiella* de Chambrier, Rego & Vaucher, 1999, *Macrobothriotaenia* Freze, 1965, *Ophiotaenia, Proteocephalus, Pseudocrepidobothrium, Tejidotaenia* Freze, 1965, *Thaumasioscolex* and *Travassiella* Rego & Pavanelli, 1987, of which only seven possess uniloculate suckers, i.e. *Cangatiella, Euzetiella, Ophiotaenia, Proteocephalus, Tejidotaenia, Thaumasioscolex* and *Travassiella*. *Glanitaenia* differs from them all by the presence of a well-developed, strongly muscular apical sucker with a deep cavity (Scholz et al., 1998).

The presence of such an apical sucker is rare among the Proteocephalidea, *G. osculata* being one of the only three known species, all belonging to monotypic genera, with such an apical structure. The second one, *Paraproteocephalus parasiluri* (Yamaguti, 1934), is likewise a parasite of a Palaearctic silurid, *Parasilurus (= Silurus) asotus* (Linnaeus), and also strongly clusters with *G. osculata* in our tree. They differ however by a number of characters, including the presence in *P. parasiluri* of a metascolex, a transverse orientation of the uterus and by a wider than long shape of the proglottides, which in our opinion justifies their placement in different genera (Freze, 1965; Shimazu, 1993).

The third species is *Sciadocephalus megalodiscus* Diesing, 1850, a parasite of *Cichla monoculus* (Spix & Agassiz) from Brazil. It has recently been redescribed by Rego et al. (1999) and differs from *G. osculata* by an umbrella-shaped metascolex, a very short strobila (< 10 mm) and the presence of ovigerous capsules in the uterus.

(b) The splitting of Proteocephalus *Weinland, 1858*

The strongly supported Palaearctic *Proteocephalus* clade displays a particular development of the uterus described below as type 2. Furthermore, all these taxa possess lateral vitelline follicles which rarely reach the mid-level of the ovary and scarcely reach the posterior margin of the proglottides. They were also clustered in previous analyses using 5.8S, ITS-2 and 16S rDNA (Zehnder & Mariaux, 1999) and using 18S rDNA plus morphological data (Skeríková et al., 2001). Finally, their life-cycle pattern differs from that of the Nearctic *Proteocephalus ambloplitis* (Leidy, 1887), for which a parenteral localisation of the plerocercoid larvae has been demonstrated within the second intermediate host (Fischer & Freeman, 1969; Scholz, 1999). These characters allow us to isolate an aggregate of species defined as follows:

***Proteocephalus* aggregate**

Diagnosis (in part after Scholz & Hanzelová, 1998): Proteocephalidea, Proteocephalidae, Proteocephalinae. Testes, ovary, uterus and vitellarium medullary. Scolex simple with four suckers; apical organ present or absent. Suckers situated two by two, dorsoventrally. Segmentation well marked; proglottides anapolytic, acraspedote and exceptionally slightly craspedote. Longitudinal internal muscles bundles well developed. Testes oval to spherical, in one central field between vitelline follicles and ovary. Vas deferens coiled. Cirrus-sac thick-walled opening into small genital atrium. Genital pore lateral, irregularly alternating. Ovary bilobed, situated near posterior margin of proglottis. Vitelline follicles forming two bands on lateral sides of proglottis, not exceeding anterior or middle part of ovary, scarcely ever reaching anterior margin of proglottis, interrupted on poral side ventrally by cirrus-sac and vagina. Vagina tubular, opening anterior, antero-dorsal or dorsal to cirrus-sac. *Formation of uterus*. Presence in immature proglottides of undifferentiated longitudinal median concentration of cells, sometimes difficult to observe. In last immatures proglottides, presence on either side of uterine stem of lateral digitate diverticula as concentration of numerous intensely staining cells. Lumen present in uterine stem and at base of diverticula in mature proglottides. Apex of diverticula covered by numerous intensely staining cells. Uterine diverticula gradually develop from central stem to lateral margins of proglottis. Gravid uterus with diverticula on each side some of which occasionally reach vitellarium, occupies most of gravid proglottis width. Parasites of Holarctic freshwater fishes. Type-species: *Proteocephalus ambiguus* (Dujardin, 1845).

Note: The type-species, *P. ambiguus*, was not available for our molecular study. Its description, however, is compatible with our definition of the Holarctic *Proteocephalus* taxa (Willemse, 1968; Rødland, 1983, Scholz & Hanzelová, 1998).

The following species belong to the new *Proteocephalus* aggregate:
Proteocephalus ambiguus, P. cernuae (Gmelin, 1790), *P. filicollis** (Rudolphi, 1802), *P. gobiorum* Dogiel & Bychowsky, 1939, *P. longicollis** (Zeder, 1800) (=*P. exiguus***, *P. pollanicola***), *P. macrocephalus** (Creplin, 1825), *P. midoriensis** Shimazu, 1990, *P. percae** (Müller, 1780), *P. plecoglossi** Yamaguti, 1934, *P. sagittus* (Grimm, 1872), *P. tetrastomus** (Rudolphi, 1810), *P. thymalli* (Annenkova-Chlopina, 1923) and *P. torulosus* (Batsch, 1786).

A few more Palaearctic [*P. pronini* Ruzinek, 2001, *P. pamirensis* Dzhalilov & Ashurova, 1971] and Nearctic species [such as *P. fluviatilis* Bangham, 1925, *P. luciopercae* Wardle, 1932, *P. osburni* Bangham, 1925, *P. pearsei* La Rue, 1919, *P. pinguis* La Rue, 1911, etc.] may also belong to the *Proteocephalus* aggregate (Hoffman, 1999; Scholz & Hanzelová, 1999). However, we refrain from including them in it until new material is available, and either their morphology or their sequences can be assessed.

Uterine development

The structure and development of the uterus has long been recognised as an important source of information for cyclophyllidean systematics (see Hoberg et al., 1999). This has been re-emphasised by Beveridge (2003), who recently developed the coding proposed by Hoberg et al. (1999) for these characters. Although detailed observations are only available for a few families, such as the Dilepididae and the Anoplocephalidae (see Bona 1994; Beveridge 1994), they have proved highly useful for understanding the relationships within these groups. Within the Proteocephalidea, the ontogeny of uterus also appears to be an interesting character. A detailed morphological reanalysis of all taxa included in this work (apart from *P. filicollis*) allowed us to propose a new interpretation for the uterine structure and development in the Proteocephalidea.

Within the Proteocephalidea, the uterus, in its immature and mature stages, shows two distinct and fundamentally different types of development. Although this character is difficult to observe, we could assign each species we observed to one of these types. This new character may become important in proteocephalidean systematics, as previously suggested by de Chambrier (1990, p. 96).

Type 1 (Figure 2a–e)
In immature proglottides, the uterine stem is in the form of an elongate concentration of chromophilic cells. In mature proglottides, the uterus is tubular with a dense wall of chromophilic cells and a central lumen. Further development occurs as a progressive lateral formation of thin-walled diverticula with very few isolated chromophilic cells.

This type is found notably in the Acanthotaeniinae, the Gangesiinae and proteocephalideans from viperids and elapids.

Type 2 (Figure 2f–j)
In immature proglottides, the uterine stem is in the form of an undifferentiated longitudinal median concentration of chromophilic cells; this is sometimes difficult to see. In premature proglottides, the uterine stem develops dense elongate lateral digitations which may be ramified, or develops into a median field of undifferentiated chromophilic cells. In mature proglottides, there is a gradual appearance and extension of a lumen from the base to the apex into each digitate diverticula; the apex is composed of numerous chromophilic cells. It is not easy to see whether a continuous multiplication of cells occurs or whether a limited stock of cells is already in place in the premature uterine wall. In the gravid uterus, diverticula on each side comprise numerous chromophilic cells and occupy most of the proglottis width.

Type 2 is observed in members of the new *Proteocephalus* aggregate and most former 'monticelliids', although in some cases it was not possible to observe the entire process of uterine development, as that material of *P. ambiguus* was in a poor condition and that of *P. filicollis* was immature. For the latter two species, we could only presume that the complete development is of type 2.

For more detailed illustrations of type 1, see: de Chambrier (1988, Figures 1–2); de Chambrier & Vaucher (1994, Figure 8); and Rego & de Chambrier (1995, Figures 2–3). For type 2, see: de Chambrier & Vaucher (1999, Figures 31–32, 39–40, 51, 75–76, 91); de Chambrier & Vaucher (1997, Figures 4, 10, 13); and Zehnder & de Chambrier (2000, Figure 15).

In both types, the lumen of diverticula appears prior to (or simultaneously with) the appearance of the eggs. Following the opinion of La Rue (1914, pp. 31–

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*Species included in the present molecular analysis.

**According to Scholz & Hanzelová (1998), Hanzelová & Scholz (1999) and Hanzelová et al. (1995)

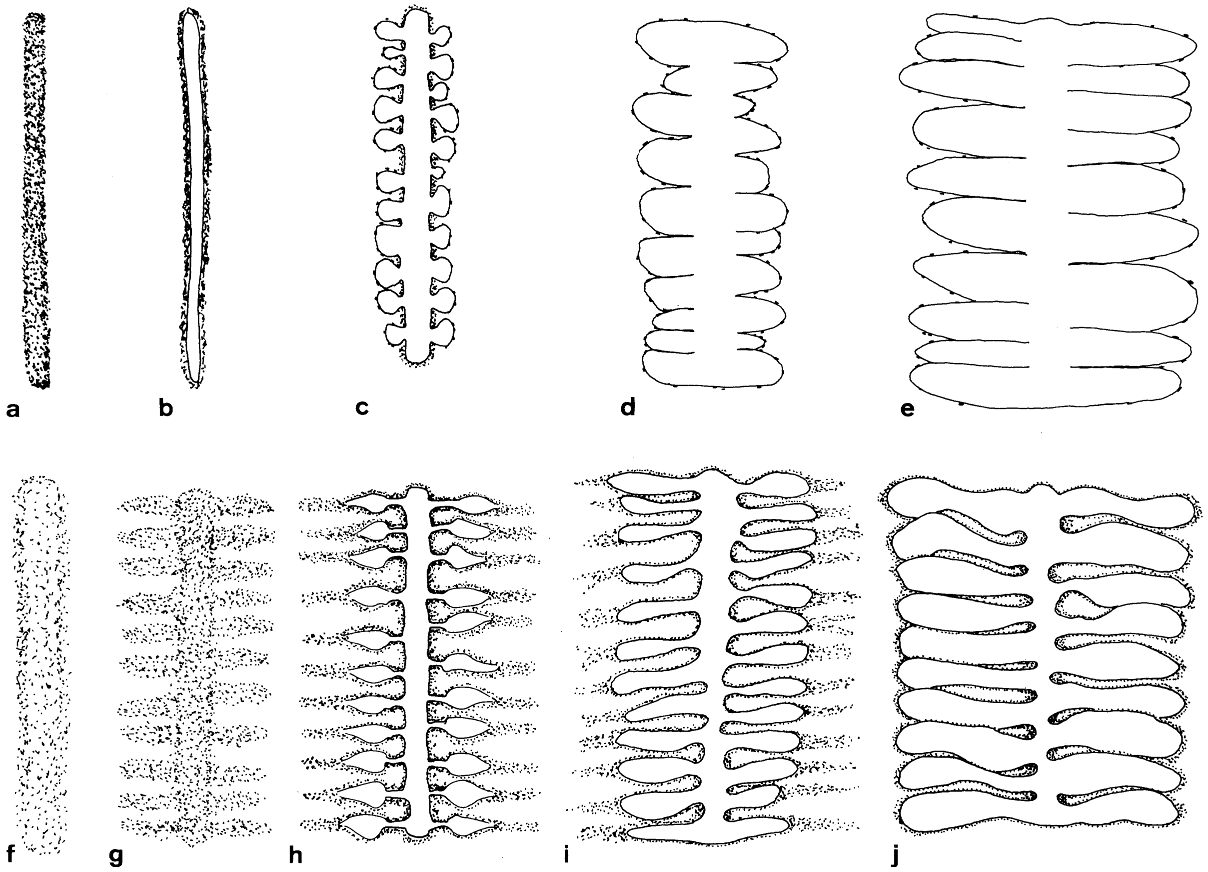

Figure 2. Diagrammatic view of the developmental stages of the uterus: a–e, type 1 uterine development; f–j, type 2 uterine development. a,b,f,g, immature proglottides; c,h, mature proglottides; d,i, pregravid proglottides; e,j, gravid proglottides.

32), we do not believe that the diverticula are formed by the pressure of the eggs.

The type 1 uterine development appears to be primitive as it is present in the Tetraphyllidea (Euzet *in lit.*) and in both gangesiine and acanthotaeniine species. Type 2 appears twice in our tree, firstly in the strongly supported clade of the Proteocephalinae from Palaearctic fishes, and secondly in the large clade comprising members of the Proteocephalinae, Zygobothriinae, Monticelliinae, Peltidocotylinae and Endorchiinae Woodland, 1934, with the exception of *Nomimoscolex lenha* (Woodland, 1933) (see asterisks, Figure 1).

In our tree, the uterine development character we define in this paper is less homoplastic than other characters previously used to define higher level groupings of the Proteocephalidea, such as the presence/absence of a metascolex (see Rego, 1995) or the relative position of the genital organs in relation to the internal longitudinal musculature (see the classification of Woodland 1934, 1935). When mapped onto our tree, these characters exhibit a much higher level of homoplasy.

Biogeography of the Proteocephalidea

Brooks (1978) placed the geographical origin of the Proteocephalidea in siluriform hosts from Gondwanian South America where the largest number of proteocephalid genera occurs. He suggested that proteocephalidean genera, *Proteocephalus* in particular, later colonised new geographical areas and other host groups. Brooks (1978) noted that these distribution tracks were supported by the early biogeography of ostariophysan fishes. Rego (1994) similarly situated the origin of the Proteocephalidea in primitive fishes '. . .in Gondwana, probably in South American rivers.' (p. 257). This hypothesis was later reinforced by Olson et al. (2001), who found the Proteocephalidea to be the sister-group of a tetraphyllidean (*Acanthobothrium* van Beneden, 1849) parasite of a (marine) stingray; this indicated the inclusion of the Proteocephalidea within the Tetraphyllidea. Euzet (1982), on the other hand, favoured an African origin with a subsequent expansion to other continents. In their cladistic analyses of proteocephalidean subfamilies based on morphological characters, Rego et al. (1998) favoured a Gondwanan African origin for these parasites, with an extensive diversification in Africa and South America. The Northern Hemisphere was thought to have been colonised subsequently.

In contrast to the views of Brooks (1978) and Rego (1994), our analyses do not support a South American origin for the Proteocephalidea (the same conclusion was reached by Zehnder & Mariaux, 1999): our phylogenetic reconstructions present two Palaearctic gangesiins, or six ccanthotaeniines (an African *Rostellotaenia*, a Malaysian *Acanthotaenia* and four Australian *Kapsulotaenia*) followed by a clade of Palaearctic *Proteocephalus* species as the most basal taxa. The remaining species mostly originate from the Nearctic and Neotropical areas but also include two African, one Australian and one Palaearctic specimen. The zoogeographical distribution pattern is more straightforward, however, when strictly piscine parasites are considered: the most basal taxa are all Palaearctic and the more derived species are Nearctic and Neotropical. Thus, we rather favour a Palaearctic origin for Proteocephalidea, with a subsequent radiation to the Nearctic and the Neotropics, where the great morphological diversification displayed by the Monticelliidae occurred, and ultimately to other regions. However, since members of the two strictly African subfamilies, the Marsypocephalinae Woodland, 1933 and Sandonellinae Khalil, 1960, are absent from our sampling, the likelihood of an African origin cannot be definitely ruled out. Both hypotheses are difficult to reconcile with the scenarii of Brooks (1978) or Olson et al. (2001); they are nevertheless the only ones supported by our data at this point.

Assuming that the primitive hosts of the Proteocephalidea are indeed monitors, we conclude that the colonisation of fishes in western Gondwana and the Palaearctic must have occurred after the Antarctic-Indian-Australian plates separated from the South America-African block (during the lower Cretaceous, i.e. 135–96 mya). The saurian to fish transfer did not occur in Australia, and Australian fishes were subsequently prevented from acquiring parasites from other freshwater fishes since the oceans represent a barrier for the dispersion of these hosts. However, several scenarios may explain a late colonisation of terrestrial hosts, even in Australia (e.g. in snakes or amphibians).

Host-parasite relationships

We found no indication that any co-evolutionary process at a higher level may have occurred between the proteocephalideans included in this work and their respective hosts. The most primitive host, the fish *Amia calva* L., is parasitised by *Proteocephalus perplexus*, which appears at a derived level on the tree. Conversely, the Acanthotaeniinae parasitic in varanoid lizards are among the most basal taxa.

Brooks (1978) and Rego (1994) proposed that piscine proteocephalideans originated as parasites of South American siluriform fishes. The present data suggest that their origin may indeed be associated with the Siluridae, one of the most primitive siluroid families according to Mo (1991), but of Palaearctic origin. On the other hand, the basal position of the Acanthotaeniinae suggests an origin for proteocephalideans in varanoid lizards, quite contrary to the hypotheses proposed to date. However, our data do not favour one scenario over the other. Subsequently, one lineage, the Palaearctic *Proteocephalus* species, appears to have colonised a variety of non-siluriform fishes, whereas another lineage colonised the Pimelodidae (one of the two most important siluriform fish groups found in South America) and, more specifically, the Pimelodinae. Thus, there is an extremely strong association of South American proteocephalideans with the Pimelodinae, from which they sporadically colonised other siluriform fishes, e.g. the Cetopsidae, Doradidae and Ageneiosidae from the Neotropics or the Ictaluridae from the Nearctic. On the other hand, a few species colonised a variety of non-siluriform fish hosts (de Chambrier & Vaucher, 1999; Rego et al., 1999). We additionally suggest, as was also proposed by Brooks (1978) and Rego et al. (1998), that the colonisation of terrestrial hosts (reptiles and mammals) occurred on several occasions during the history of Proteocephalidea. We found these parasites to be distributed in at least four separate clades mainly following switches from the Pimelodinae (Figure 1).

Brooks & Rassmussen (1984) suggested that, the bulk of hosts of the Monticelliidae were siluroid fishes of the family Pimelodidae and that host-switching was only responsible for a fraction of the host relationships postulated by their cladogram, all the rest being due to co-evolution. These authors thus concluded that the monticelliids may 'have co-evolved with a fairly small number of siluriforms, and may have speciated more often than their hosts (p. 758). We found however that six proteocephalidean species (*Proteocephalus hemioliopteri, P. pirarara, Zygobothrium megacephalum, Pseudocrepidobothrium eirasi, Pseudocrepidobothrium* sp. and *Ephedrocephalus macrocephalus* Diesing, 1850) harboured by the Amazon siluroid fish *Phractocephalus hemioliopterus* not only belong to different subfamilies but do not form a monophyletic group. Our conclusions thus converge with those of Euzet (1982), who warned against searching for a possible parallel evolution of the Monticelliidae and their siluriform hosts. Host-switching may be a more common phenomenon than previously anticipated (Brooks, 1978; Brooks & Rassmussen, 1984; Rego, 1994), especially among the Monticelliidae, which are generally known to have an oioxenic host-specificity (de Chambrier & Vaucher, 1999), and in the *Proteocephalus* aggregate, as previously shown by Skeríková et al., (2001).

Conclusions

The 5′ portion of the 28S rRNA gene used for phylogenetic relationship inference in this work performs variably in different sections of our tree. Although a number of deeper relationships are well supported, it cannot clearly decipher relationships resulting from more recent events, especially for several proteocephalideans of Nearctic and Neotropical origin.

The poor resolution of relationships for numerous North and South American species, of which a large proportion are monticelliids, may be explained by the emergence of these species being associated with a particular event, such as a rapid radiation of species, during the evolutionary history of these parasites (hard polytomy). Thus, when considering the association of New World Proteocephalidea with Neotropical fishes, it is of interest to note that Lundberg et al. (1998), in their work on Neotropical fish diversification, stressed that the 'long and complex history of South America's landscape and river systems', and in particular the formation of the Andes, the shifting courses of rivers and repeated incursions and regressions of marine waters, 'must have produced many vicariance events'. They noted in particular that during the late Miocene (*c*.22–*c*.6 mya), 'a great diversity of small clades ranked as genera, species groups and even some species of modern Neotropical fishes appear in the fossil record'. They suggested that the evolution of Neotropical landscape and drainage that occurred at that time 'could have been among the most important in influencing the diversification of fishes'. During that very interval, when marine incursions spread deep inland into the continent, 'salinity-intolerant fishes could have been allopatrically fragmented in peripheral river systems' (p. 43). Hence, it cannot be ruled out that a similar event of species diversification may have taken place simultaneously for Neotropical fishes and their proteocephalidean parasites. This may be confirmed by the extreme morphological diversity of the 'Monticelliidae' that has so far prevented a satisfactory system being developed for these parasites.

The use of molecular data for phylogenetic inference inclined us to examine some morphological characters more carefully. Indeed, the evolution of the two types of uterine development was found to be consistent with our tree (Figure 1). The type 1 uterine development is present in basal lineages (Tetraphyllidea, Gangesiinae and Acanthotaeniinae) and therefore appears to be primitive, as seems to be the case for characters such as the presence of hooklets (Gangesiinae), a rostellum-like apical organ (Acanthotaeniinae) and a medullary position of the vitellarium (Gangesiinae, Acanthotaeniinae, *Paraproteocephalus, Glanitaenia* and *Proteocephalus* aggregate).

Nevertheless, the phylogenetic relationships of many proteocephalideans are still not well resolved. In order to improve our understanding of the evolution of this group, a more extensive sampling is certainly needed. Combined analyses of sequences of several genes might also prove very useful, as recently demonstrated for parasitic flatworms by Olson et al. (2001, 2003); Olson & Littlewood, (2002); Lockyer et al. (2003) or Telford et al. (2003), although practical alternatives or complements to rDNA sequences are still difficult to find (Mariaux & Olson, 2001). Finally, more traditional but largely ignored characters, such as larval features, should also be studied, as suggested by Mariaux (1998).

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References

Beveridge, I. (1994) Family Anoplocephalidae Cholodkosky. 1902. *In*: Khalil L.F., Jones A. & Bray R.A. (Eds) *Keys to the cestode parasites of vertebrates*, Wallingford: CAB International, pp. 315–366.

Beveridge, I. (2003) Phylogenetic relationships of *Anoplotaenia dasyuri* (Cestoda) from dasyurid marsupials, with observations on uterine development in Cyclophyllideans. *In*: Combes, C. & Jourdane, J. (Eds). *Taxonomie, écologie et évolution des métazoaires parasites*. Livre-hommage à Louis Euzet. Perpignan: Presses Universitaires de Perpignan, Vol. 1, pp. 47–66.

Bona, F. (1994) Family Dilepididae Railliet & Henry, 1909. *In*: Khalil L.F., Jones A. & Bray R.A. (Eds) *Keys to the cestode parasites of vertebrates*, Wallingford: CAB International, pp. 443–554.

Brooks, D.R. (1978) Evolutionary history of the cestode order Proteocephalidea. *Systematic Zoology*, **27**, 312–323.

Brooks, D.R., Hoberg, E.P. & Weekes P.J. (1991) Preliminary phylogenetic systematic analysis of the major lineages of the Eucestoda (Platyhelminthes: Cercomeria). *Proceedings of the Biological Society of Washington*, **104**, 651–668.

Brooks, D.R. & Rasmussen, G. (1984) Proteocephalid cestodes from Venezuelan catfish, with a new classification of the Monticelliidae. *Proceedings of the Biological Society of Washington*, **97**, 748–760.

Cañeda-Guzmán, I.C. (1997) *Parásitos de tres especies de Marsupiales de la Estación 'Los Tuxtlas' y algunas zonas cercanas, Veracruz, México*. BSc Thesis. Facultad de Ciencias, Universidad Nacional Autonóma de México, 193 pp.

Cañeda-Guzmán, I.C., de Chambrier, A. & Scholz, T. (2001) *Thaumasioscolex didelphidis* n. gen. and n. sp. (Eucestoda: Proteocephalidae) from the black-eared opossum *Didelphis marsupialis* from Mexico, the first proteocephalidean tapeworm from a mammal. *Journal of Parasitology*, **87**, 639–646.

Carfora, M., de Chambrier, A. & Vaucher, C. (2003) Le genre *Amphoteromorphus* (Cestoda: Proteocephalidea), parasite de poissons-chats d'Amérique tropicale: étude morphologique et approche biosystématique par électrophorèse des protéines. *Revue Suisse de Zoologie*, **110**, 381–409.

de Chambrier, A. (1988) *Crepidobothrium garzonii* n. sp. (Cestoda: Proteocephalidae) parasite de *Bothrops alternatus* Dum. Bibr. & Dum.1854 (Serpentes: Viperidae) au Paraguay. *Revue Suisse de Zoologie*, **95**, 1163–1170.

de Chambrier, A. (1990) Redescription de *Proteocephalus paraguayensis* (Rudin, 1917) (Cestoda: Proteocephalidae) parasite de *Hydrodynastes gigas* (Dum., Bibr. & Dum., 1854) du Paraguay. *Systematic Parasitology*, **16**, 85–97.

de Chambrier, A., d'Alessio, M.L. & de Azevedo Corrêa, F.M. (1991) Redescription de *Proteocephalus jarara* (Fuhrmann, 1927) (Cestoda: Proteocephalidae) parasite de *Bothrops alternatus* (Viperidae) au Brésil. *Revue Suisse de Zoologie*, **98**, 15–32.

de Chambrier, A. & Vaucher, C. (1994) Etude morpho-anatomique et génétique de deux nouveaux *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae) parasites de *Platydoras costatus* (L.), poisson siluriforme du Paraguay. *Systematic Parasitology*, **27**, 173–185.

de Chambrier, A. & Vaucher, C. (1997) Révision des cestodes (Monticelliidae) décrits par Woodland (1934) chez *Brachyplatystoma filamentosum* avec redéfinition des genres *Endorchis* Woodland, 1934 et *Nomimoscolex* Woodland, 1934. *Systematic Parasitology*, **37**, 219–233.

de Chambrier, A. & Vaucher, C. (1999) Proteocephalidae et Monticelliidae (Eucestoda: Proteocephalidea) parasites de poissons d'eau douce au Paraguay, avec descriptions d'un genre nouveau et de dix espèces nouvelles. *Revue Suisse de Zoologie*, **106**, 165–240.

Euzet, L. (1982) Problèmes posés par la spécificité parasitaire des cestodes Proteocephalidea et Pseudophyllidea parasites de poissons. *Mémoires du Museum d'Histoire Naturelle, Série A, Zoologie*, **123**, 279–287.

Fischer, H. & Freeman, R.S. (1969) Penetration of parenteral plerocercoids of *Proteocephalus ambloplitis* (Leidy) into the gut of smallmouth bass. *Journal of Parasitology*, **55**, 766–774.

Freze, V.I. (1965) [*Essentials of Cestodology.*] Vol. V. [*Proteocephalata in fish, amphibians and reptiles.*] Moskva: Izdatel'stvo Nauka, 538 pp. (In Russian: English translation, Israel Program of Scientific Translation, 1969, Cat. No. 1853. v + 597 pp).

Gil de Pertierra, A.A. & de Chambrier, A. (2000) *Rudophiella szidati* sp. n. (Proteocephalidea: Monticelliidae, Rudolphiellinae) parasite of *Luciopimelodus pati* (Valenciennes, 1840) (Pisces: Pimelodidae) from Argentina with new observations on *Rudolphiella lobosa* (Riggenbach, 1895). *Revue Suisse de Zoologie*, **107**, 81–95.

Goeze, J.A.E. (1782) *Versuch einer Naturgeschichte der eingeweidewürmer tierischer Körper.* Blankenburg, 471 pp.

Hanzelová, V. & Scholz, T. (1999) Species of *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), parasites of coregonid and salmonid fishes from North America: taxonomic reappraisal. *Journal of Parasitology*, **85**, 94–101.

Hanzelová, V., Scholz, T. & Fagerholm, H.-P. (1995) Synonymy of *Proteocephalus neglectus* La Rue, 1911 with *P. exiguus* La Rue, 1911, two fish cestodes from the Holarctic Region. *Systematic Parasitology*, **30**, 173–185.

Hoberg, E.P., Jones, A. & Bray, R.A. (1999) Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. *Systematic Parasitology*, **42**, 51–73.

Hoffman, G.L. (1999) *Parasites of North American freshwater fishes*. Second Edition. Ithaca & London: Comstock Publishing Associates, Cornell University Press, 539 pp.

International Code of Zoological Nomenclature (1999) Fourth Edition. London: The International Trust for Zoological Nomenclature, xxix + 306 pp.

Kodedová, I., Dolezel, D., Broucková, M., Jirku, M., Hypsa, V., Lukes, J. & Scholz, T. (2000) On the phylogenetic positions of the Caryophyllidea, Pseudophyllidea and Proteocephalidea (Eucestoda) inferred from 18S rRNA. *International Journal for Parasitology*, **30**, 1109–1113.

La Rue, G.R. (1911) A revision of the cestode family Proteocephalidae. *Zoologischer Anzeiger*, **38**, 473–482.

La Rue, G.R. (1914) A revision of the cestode family Proteocephalidae. *Illinois Biological Monographs*, **1**, 1–349.

Lockyer, A.E., Olson, P.D. & Littlewood, D.T.J. (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, **78**, 155–171.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L. & Wesselingh, F. (1998) The stage for Neotropical fish diversification: A history of tropical south American rivers. *In*: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S. & Lucena, C.A.S. (Eds) *Phylogeny and classification of Neotropical fishes*. Porto Alegre: Edipucrs, pp. 13–48.

Mariaux, J. (1998) A molecular phylogeny of the Eucestoda. *Journal of Parasitology*, **84**, 114–124.

Mariaux, J. & Olson, P.D. (2001) Cestode systematics in the molecular era. *In*: Littlewood, D.T.J & Bray, R.A. (Eds). *Interrelationships of the Platyhelminthes*. London: Taylor & Francis, pp. 127–134.

Mo, T. (1991) *Anatomy and systematics of Bagridae (Teleostei), and siluroid phylogeny*. Königstein: Koeltz Scientific Books, 216 pp.

Nybelin, O. (1942) Zur Helminthen Fauna der Süsswasserfische Schwedens. II. Die Cestodes des Welses. *Göteborgs Kungl Vetenkaps Och Vitterhets-samhalles Handlingar Series* B1, 1–24.

Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A. & Littlewood, D.T.J. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, **33**, 733–755.

Olson, P.D. & Littlewood, D.T.J. (2002) Phylogenetics of the Monogenea – evidence from a medley of molecules. *International Journal for Parasitology*, **32**, 233–244.

Olson, P.D., Littlewood, T.J., Bray, R.A. & Mariaux, J. (2001) Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution*, **19**, 443–467.

Rego, A.A. (1994) Order Proteocephalidea Mola, 1928. *In*: Khalil L.F., Jones A. & Bray R.A. (Eds) *Keys to the cestode parasites of vertebrates*, Wallingford: CAB International, pp. 257–293.

Rego, A.A. (1995) A new classification of the cestode order Proteocephalidea Mola. *Revista Brasileira de Zoologia*, **12**, 791–814.

Rego, A.A. & de Chambrier, A. (1995) *Crepidobothrium eirasi* n. sp. (Cestoda: Proteocephalidae), a parasite of the siluroid fish *Phractocephalus hemioliopterus* (Schneider, 1801) (Pisces: Pimelodidae) from the Brazilian Amazon. *Revue Suisse de Zoologie*, **102**, 3–11.

Rego, A.A., de Chambrier, A., Hanzelová, V., Hoberg, E., Scholz, T., Weekes, P. & Zehnder, M. (1998) Preliminary phylogenetic analysis of subfamilies of the Proteocephalidea (Eucestoda). *Systematic Parasitology*, **40**, 1–19.

Rego, A.A., Chubb, J.C. & Pavanelli, G. C. (1999) Cestodes in South American freshwater teleost fishes: keys to genera and brief description of species. *Revista Brasileira de Zoologia*, **16**, 299–367.

Rego, A.A. & Ivanov, V. (2001) *Pseudocrepidobothrium eirasi* (Rego and de Chambrier, 1995) gen. n. and comb. nov. (Cestoda, Proteocephalidea), parasite of a South American freshwater fish, and comparative cladistic analysis with *Crepidobothrium* spp. *Acta Scientarum*, **23**, 363–367.

Rego, A.A., Machado, P.M. & Pavanelli, G.C. (1999) *Sciadocephalus megalodiscus* Diesing, 1850 (Cestoda: Corallobothriinae) a parasite of *Cichla monoculus* Spix, 1831 (Cichlidae), in the Paraná River, State of Paraná, Brazil. *Journal of the Helminthological Society of Washington*, **66**, 133–137.

Rødland, J.T. (1983) A redescription of the cestodes *Proteocephalus filicollis* (Rudolphi) from *Gasterosteus aculeatus* L., and *P. ambiguus* (Dujardin) from *Pungitius pungitius* (L.). *Zoologica Scripta*, **12**, 19–23.

Schmidt, G.D. (1986) CRC *Handbook of tapeworm identification*. Boca Raton: CRC Press, 675 pp.

Scholz, T. (1999) Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic Region: a review. *Journal of Helminthology*, **73**, 1–18.

Scholz, T. & de Chambrier, A. (2003) Taxonomy and biology of proteocephalidean cestodes: current state and perspectives. *Helminthologia*, **40**, 65–77.

Scholz, T., Drábek, R. & Hanzelová, V. (1998) Scolex morphology of *Proteocephalus* tapeworms (Cestoda: Proteocephalidae), parasites of freshwater fish in the Palaearctic Region. *Folia Parasitologica*, **45**, 27–43.

Scholz, T. & Hanzelová, V. (1998) Tapeworms of the genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), parasites of fishes in Europe. *Studie AV CR*. Prague: Academia, 119 pp.

Scholz, T. & Hanzelová, V. (1999) Species of *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae) from cyprinid fishes in North America. *Journal of Parasitology*, **85**, 150–154.

Shimazu, T. (1993) Redescription of *Paraproteocephalus parasiluri* (Yamaguti, 1934) n. comb. (Cestoidea: Proteocephalidae), with notes on four species of the genus *Proteocephalus*, from Japanese freshwater fishes. *Journal of the Nagano Prefectural College*, **48**, 1–9.

Skeríková, A., Hypsa, V. & Scholz, T. (2001) Phylogenetic analysis of European species of *Proteocephalus* (Cestoda: Proteocephalidea): compatibility of molecular and morphological data, and parasite-host coevolution. *International Journal for Parasitology*, **31**, 1121–1128.

Swofford, D.L. (2002) PAUP*. *Phylogenetic analysis using parsimony* (*and other methods). Version 4.0b10. Sunderland, Massachussets: Sinauer Associates.

Telford, M.J., Lockyer, A.E., Cartwright, C. & Littlewood, D.T.J. (2003) Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. *Proceedings of the Royal Society of London B*, **270**, 1077–1083.

Willemse, J.J. (1968) *Proteocephalus filicollis* (Rudolphi, 1802) and *Proteocephalus ambiguus* (Dujardin, 1845), two hitherto confused species of cestodes. *Journal of Helminthology*, **42**, 395–410.

Woodland, W.N.F. (1934) On some remarkable new cestodes from the Amazon siluroid fish, *Brachyplatystoma filamentosum* (Lichtenstein). *Parasitology*, **26**, 268–277.

Woodland, W.N.F. (1935) Additional cestodes from the Amazon siluroids Pirarará, Dorad and Sudobim. *Proceedings of the Zoological Society of London*, **104**, 851–862.

Zehnder, M.P. & Mariaux, J. (1999) Molecular systematic analysis of the order Proteocephalidea (Eucestoda) based on mitochondrial and nuclear rDNA sequences. *International Journal for Parasitology*, **29**, 1841–1852.

Zehnder, M.P. & de Chambrier, A. (2000) Morphological and molecular analyses of the genera *Peltidocotyle* Diesing, 1850, *Othinoscolex* and *Woodlandiella* (Eucestoda, Proteocephalidea), parasites of South American siluriform fishes (Pimelodidae). *Systematic Parasitology*, **46**, 33–43.

Zehnder, M.P., de Chambrier, A., Vaucher, C. & Mariaux, J. (2000) *Nomimoscolex suspectus* n. sp. (Eucestoda: Zygobothriinae) with morphological and molecular phylogenetic analysis of the genus. *Systematic Parasitology*, **47**, 157–172.