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The metascolex in proteocephalids

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ABSTRACT. The morphology of metascolex of Proteocephalid species, parasites of freshwater fishes from Brazil, is discussed by using scanning electron microscope. The importance of metascolex in the classification is evaluated. The following species were analyzed: *Corallobothrium* sp., *Paramonticellia itaipuensis, Spatulifer rugosa, Amphoteromorphus peniculus, Choanoscolex abscisus, Jauella glandicephalus* and *Rudolphiella* sp.

Key words: Proteocephalid cestodes, South American freshwater fish parasites, metascolex, scolex.

RESUMO. Metaescólex em proteocefalídeos. É avaliada a importância do metaescólex na taxonomia da ordem Proteocephalidea, especialmente no que se refere às espécies da América do Sul. É discutida a morfologia do metaescólex das seguintes espécies: *Corallobothrium* sp., *Paramonticellia itaipuensis, Spatulifer rugosa, Amphoteromorphus peniculus, Choanoscolex abscisus, Jauella glandicephalus* e *Rudolphiella* sp.

Palavras-chave: cestóides, proteocefalídeos, metaescólex, escólex, parasitas de peixes de água doce da América do Sul.

Members of the Cestoda Order Proteocephalidea parasite freshwater fishes (mostly Siluriform), amphibians and reptiles. Proteocephalids constitute an evolved link between the marine tapeworms and the terrestrial Cyclophyllidea; consequently the Proteocephalideans are of great interest for phylogenetic studies of the parasites and their hosts. The proteocephalids are also closely related to the Tetraphyllideans, a group found in marine elasmobranchs. According the Freeman (1973), the Proteocephalidea and Tetraphyllidea evolved from a common ancestor (Figure 1).

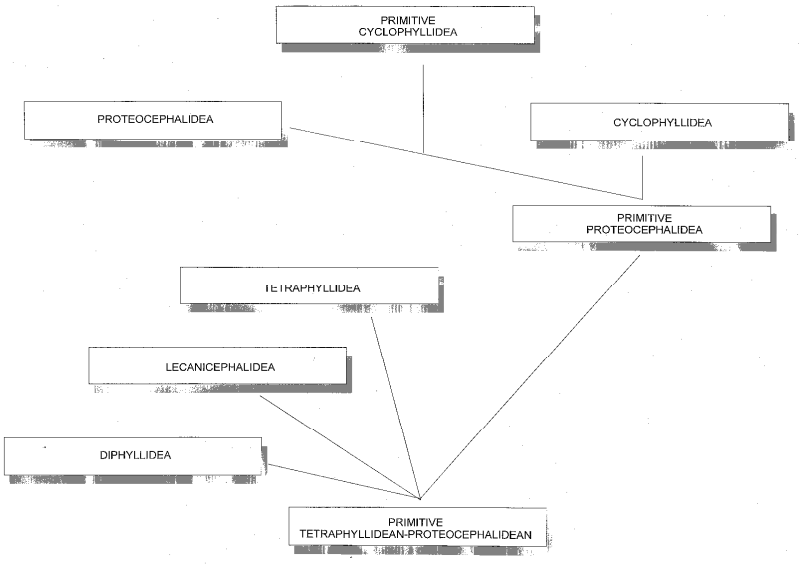

Figure 1. Evolution of proteocephalids and cyclophyllides. Adapted from Freeman (1973)

The metascolex was defined as "the posterior portion of a divided scolex. Consists of folds of tissue generally encircling or hiding the suckers. It either originates from the base of scolex, sometimes forming a sort of "collar", or from folds of tissue between the suckers (Rego, 1994).

The aim of this study is to discuss data and details of the metascolex, a structure common in Proteocephalids, but not well understood by the students of the group. As a matter of fact, to describe a proteocephalid species it is necessary to study the reproductive organs as seen in cross section, however it is equally important to careful study the scolex and the concerned structures, that include metascolex, spines of scolex and suckers, apical glands, rostellum with hooks, etc.

Woodland (1925, 1933, 1934, 1935) described most of the known proteocephalid species from Africa and South America, but unfortunately he didn't attribute great importance to scolex. In recent papers the authors have described better these structures and provided scanning electron micrographies (SEM) of scolex and metascolex as well.

Material and methods

Proteocephalids must be collected immediately from freshly killed fishes. Specimens to SEM must be examined in freshwater, with a binocular stereomicroscope and have cleaned the holdfast with a brush. Cestodes are fixed using boiling (100ºC) 4% formaldehyde solution. In the laboratory the specimens are transferred to alcohol 70%. Scolices are prepared by cutting the strobila, behind the neck region, to facilitate mounting and viewing from all sides in the specimen chamber. Cestodes in 7O% ethanol are taken through absolute ethanol, critical point dried, mounted on viewing stub, sputter-coated in 60% gold/palladium and viewed under a Phillips 501 SEM.

Remarks and discussion

Some authors believe that the tetraphyllids and the "collared" proteocephalids could be the most primitive tapeworms; the "collared" Proteocephalids seem to be closer to Tetraphyllidea than the "non collared" forms (Wardle and McLeod, 1952). It is interesting to note that a sort of "collar" can also be observed in genera of the Orders Caryophyllidea (Figure 2), Tetraphyllidea (Figure 3), Lecanicephalidea (Figure 4) and Tetrabothriidea (Figure 5). The types of "collar" presented by these forms could be compared to some found in Proteocephalids. To explain the origin of metascolex, Wardle and McLeod (1952) theorized: "In response to holdfast pressure against the host gut mucous membrane, many forms evolved a postacetabular fold or collar".

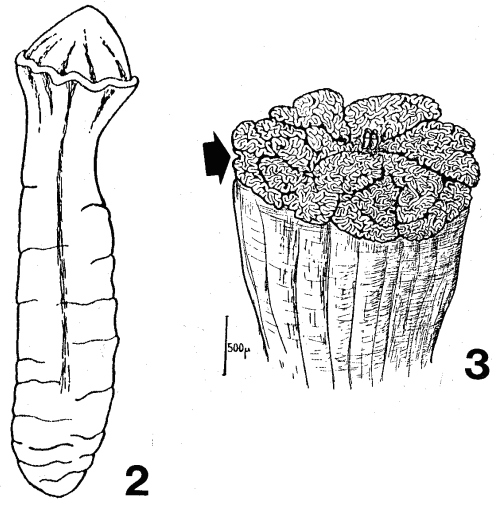

Figure 2. Caryophyllid, *Balanotaenia bancrofti;* entire worm. After Wardle and McLeod (1952)
Figure 3. Tetraphyllid, *Thysanocephalum thysanocephalum*; metascolex and scolex. Scolex (arrow). After Euzet (1959)

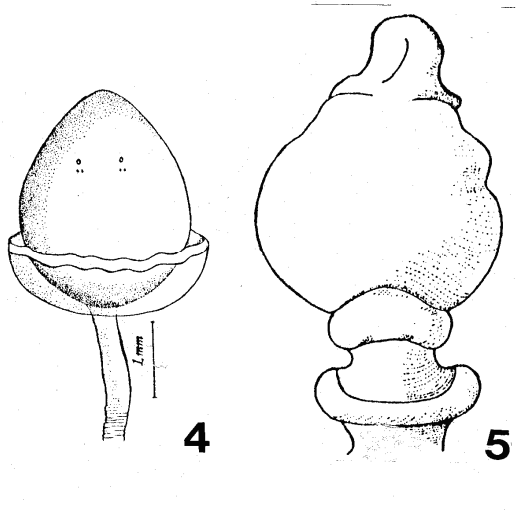

Figure 4. Lecanicephalid, *Balanobothrium;* holdfast. After Wardle and McLeod (1952)
Figure 5. Tetrabothriidea, *Priapocephalus*; holdfast. After Wardle and McLeod (1952)

Freze (1965) criticized Wardle and McLeod's (1952) statement about the primitiviness of metascolex; he noted that these assumptions cannot be evidenced. In Freze's opinion the metascolex is a secondary sign, as he said: "new structures that appear in proteocephalids after bifurcation of the ancestral stem found not even in all proteocephalid forms, as vaginal sphincter, L-shaped vitellaria and

metascolex". He added: "The evolution of subfamilies Corallobothriinae and Paraproteocephalinae also followed the path of holdfast function reinforcement, but from a morphological base other than Gangesinae, as shown by the metascolex development. This manner of holdfast reinforcement is less effective than of the rostellum and hooks formation".

Species with collared metascolex, as from the genera *Corallobothrium* and *Rudolphiella*, and from *Monticellia* a genera without metascolex, were classified in the same taxa by the former authors, but Fuhrmann (1916) in describing *Goezeella siluri*, showed the existence of fundamental internal differences between the reproductive organs and vitellaria of these forms, i.é, in spite of the similitude of metascolices (*Corallobothrium* has medullary gonads and the South American genera, *Rudolphiella, Goezeella* and others have cortical or partly cortical gonads). Later, Fuhrmann (1934), in revising the types of Diesing, redescribed some of these species, *Ephedrocephalus microscopicus, Amphoteromorphus peniculus* and *Peltidocotyle rugosa*, providing excellent descriptions of the metascolices of these species. Curiously, his good description of the species *Peltidocotyle rugosa* was ignored by the following authors; they referred this species only on the base of the scarce original description of Diesing (1855).

Since these species-groups differ in the relative situation of the reproductive organs with respect to the cortico-medullary boundary, it may be assumed that, this corolla character has evolved independently among them (Woodland, 1925; Wardle and McLeod, 1952); a clear example of convergent adaptation among Proteocephalids.

Discussing the form and function of metascolex, Freze (1965) stated: "The superficial attachment organs in Proteocephalids include the widely distributed metascolex, convergently formed in various groups of the suborder; it consists of a small number of large folds, situated behind the suckers. The cuticular and subcuticular layers and the cortical parenchyma tissue participate in the formation of the folds. Usually, however, the function of the metascolex and the suckers mutually supplement each others. In the species *Amphoteromorphus piraeeba* and *A. peniculus*, numerous minute digitate processes are presented on the anterior scolex surface". He added: "The significance of the metascolex for attachment is unquestionable. It is sufficient to mention that Proteocephalids contain examples of physiological substitution, when the suckers degenerate fully or their function is carried out by a highly developed metascolex (genera *Othinoscolex*).

The mode of attachment of the species *Amphoteromorphus peniculus* (and other species), shows an intimate correspondence between the microvilli and folds of the metascolex-scolex to the villi of the gut host (Figure 8).

Metascolex is a common structure in the Proteocephalidea, from the existing forty-five Proteocephalidea genera, seventeen have some kind of metascolex (Rego, Chubb and Pavanelli, 1999).

A typical metascolex is "collar-like", term utilized in the papers of La Rue (1914), Riggenbach (1896), Fuhrmann (1916, 1934), Woodland (1925, 1933, 1934, 1935) and Freze (1965). There are several metascolex forms, however, in some of them, the metascolex is not well defined or even weakly developed. Frequently it is hard to decide if the wrinkles and the creases in the scolex could be of a true metascolex, as observed in *Zygobothrium megacephalum* Diesing, 1850 (Figure 9).

Recently, Chambrier and Paulino (1997) described *Proteocephalus joanae* from a South American snake; a species with a peculiar metascolex (Figure 6). The holdfast is peculiar because it crosses the gut wall until the peritoneum. On judging by the figure, it is not a true metascolex, but a post scolex, or a pseudometascolex. The species *Marsypocephalus tanganicae* from a siluriform fish from Africa is also interesting; the scolex has a certain amount of loose tissue, that reminds a metascolex, but it is insufficient for the formation of a true folded metascolex (Figure 7).

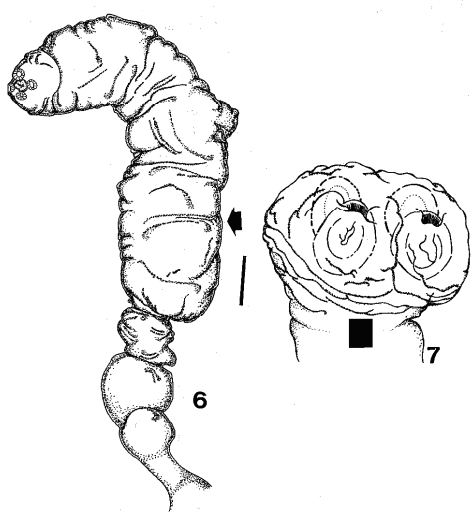

Figure 6. *Proteocephalus joanae*; anterior extremity; note the peculiar metascolex. After Chambrier and Paulino (1997)
Figure 7. *Marsypocephalus tanganicae*; scolex. After Wardle and McLeod (1952)

Figure 8. *Amphoteromorphus peniculus*; metascolex in intimate contact with gut villi of host, *Brachyplatystoma* sp. (original). p = parasite, h = host. Scale bar 0,500 mm. Original

Unfortunately to the taxonomy of the Proteocephalidea, some authors, specially Woodland, have not attributed taxonomic importance to the external characters, metascolex included.

They prefer to give more importance to internal characters, as position of gonads and vitellaria in relation to longitudinal (cortical, medular) musculatures. Woodland (1925) stated that any small modification of the scolex, however conspicuous, cannot be regarded as a character of more than specific value (...) all of which genera are similarly founded on trivial scolex characters (...) while Fuhrmann's *Goezeella siluri* becomes *Monticellia siluri*". In a following paper, Woodland (1933a) recognized the internal organs arrangement as the top character in the classification of Proteocephalidea.

Figure 9. *Zygobothrium megacephalum*; scolex. Note the wrinkles on entire surface of scolex and suckers. SEM. Scale bar 0,300 mm. Original

The following authors, Wardle and McLeod (1952), Yamaguti (1959), Freze (1965) and Schmidt (1986) accepted in general terms the scheme of Woodland's classification of the Order Proteocephalidea.

It is interesting to note their emphasis on the the internal disposition of gonads relative to the longitudinal muscles in Proteocephalids, if compared with the other great taxa, as Tetraphyllidea, Trypanorhyncha, and Cyclophyllidea, in which, on the contrary, the external scolex characteristics, as bothridial apparatus, tentacles, rostellum with hooks etc. are emphasized. Even in other Proteocephalids than Monticelliids, the emphasis on reliable taxonomic characters was given mostly to characteristics of scolex, as in Gangesiinae and Sandonelliinae.

As mentioned, there are several types of metascolex: inconspicuous, *Paramonticellia* (Figure 10), weakly developed as in *Corallotaenia*, and very conspicuous as in *Amphoteromorphus* (Figure 14). In general terms, the conspicuous ones are the "collared types', found in the group species of *Corallobothrium*, *Paraproteocephalus, Goezeela, Amphoteromorphus, Spatulifer, Rudolphiella and Ephedrocephalus.*

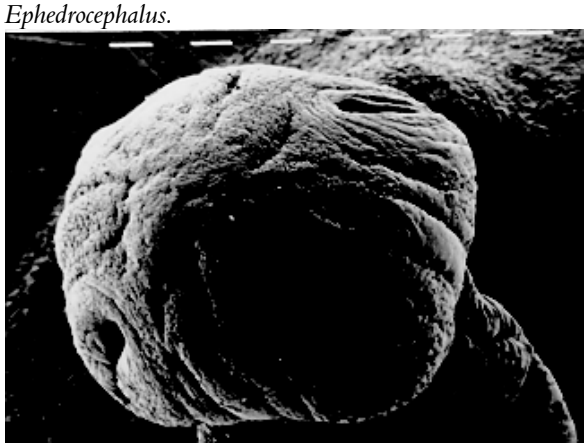

Figure 10. *Paramonticellia itaipuensis*; atypical metascolex; only wrinkles on entire scolex; the suckers appearing as slits on scolex. SEM. Scale bar 100. Original

The following genera were evaluated concerning the morphology of metascolex:

Corallobothrium: genera not referred in South America; the metascolex is highly developed; it has the form of a symmetrical folded collar surrounding the scolex and protruding anteriorly to the scolex apex. The suckers are better seen when the apical part of scolex is protruded.

Spatulifer: metascolex well-developed, "collar'like", folded posterior to suckers; folds mostly longitudinal; large, uniloculated suckers, directed laterally (Figure 12).

Rudolphiella: massive metascolex, with many furrows or wrinkles in the anterior part of scolex;

"collar-like", but less folded in posterior part of scolex.; uniloculate suckers (Figure 13).

Figure 11. *Corallobothrium parafimbriatum*; holdfast, apical view of scolex. SEM. Scale bar 0,100mm. After Scholz and Capellaro (1993)

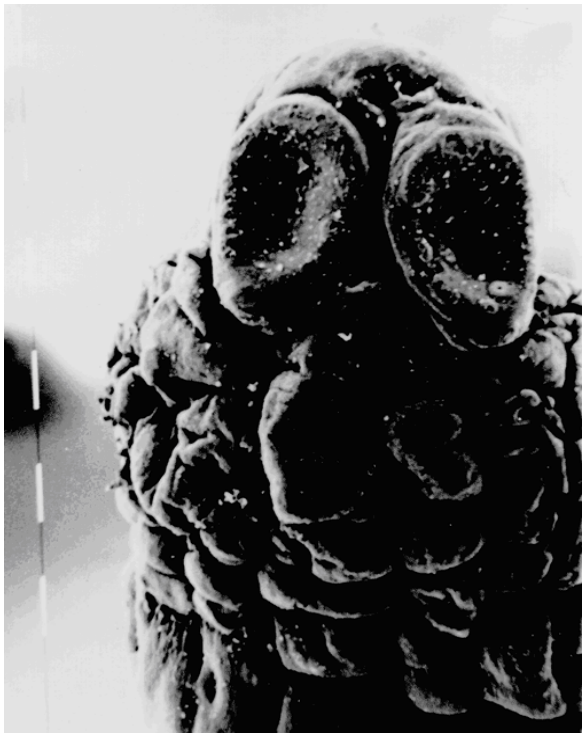

Figure 12. *Spatulifer rugosa* ; metascolex "collar-like", visible scolex; large suckers. SEM. Scale bar 0,200mm. Original

Choanoscolex: Conical scolex; the metascolex is formed by developed folds at the base of scolex wich partially envelop the base of the uniloculate suckers (Figure 15).

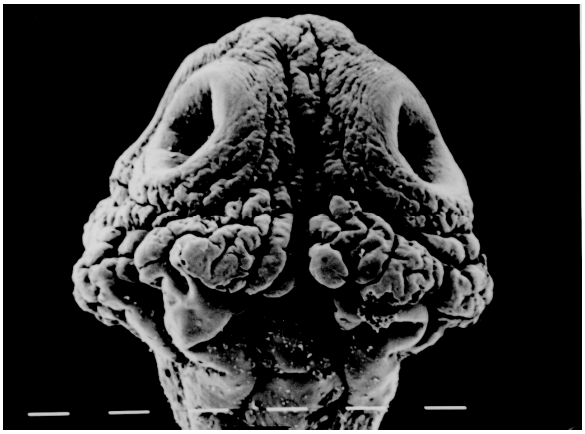

Figure 13. *Rudolphiella* sp. from *Callophysus macropterus*; "collar-like" metascolex, partly retracted scolex. SEM. Scale bar 0,200 mm. Original

Amphoteromorphus: Massive metascolex; it consists of a circular wrinkled wall enclosing cavity at the base of which lie four bilobate suckers; when expanded it has a "cauli-flower" aspect, presenting numerous, delicate, adhesive processes, that tend to obscure the biloculate suckers (Figure 14).

Jauella: Cone-shaped metascolex; small, uniloculate suckers, in apical part of scolex; metascolex very retractile, appearing as a structure with circular wrinkles, posterior to scolex. The function of holdfast is very characteristic, it is able to cross the mucosa to the peritoneum; generally the metascolex is dilated, avoiding the retreat of the scolex to the intestinal lumen (Figure 16).

Figure 14. *Amphoteromorphus peniculus* from *Brachyplatystoma flavicans*; holdfast, view from above. SEM. Scale bar 0,200mm. Original

Paramonticellia: Scolex with internal, sac-like suckers; metascolex not well defined, represented by wrinkles covering the entire scolex (Figure 10).

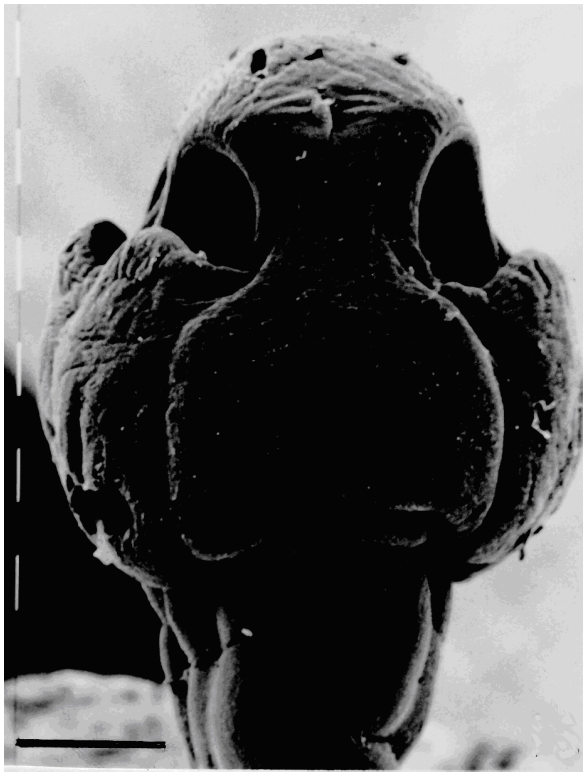

Figure 15. *Choanoscolex abscisus*; note the folds of metascolex partly encircling the suckers. SEM. Scale bar 0,100 mm. Original

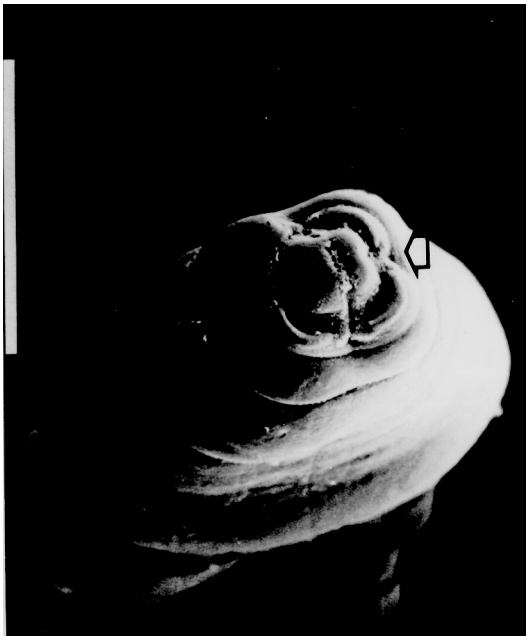

Figure 16. *Jauella glandicephalus* from *Paulicea luetkeni*; apical scolex (arrow) and metascolex posterior. SEM. Scale bar 0.200mm. Original

In recent years, several authors, such as Brooks and Deardorff (1980), Brooks and Rasmussen (1984), Rego (1987, 1989, 1990, 1992, 1994, 1995), Rego and Pavanelli (1992), Chambrier and Paulino (1997), Chambrier and Rego (1995), Pavanelli and Rego (1989, 1991, 1992) have scrutinized the South American proteocephalids, resulting that these forms are becoming better known. Rego (1994) criticized the scheme of Woodland, referring that "the finding of new forms, with intermediate characteristics between the proteocephalids and monticelliids could invalidate the actual taxonomy proposed by Woodland.

Our experience with the group convinced us that the internal characters, the relative cortical/medullar position of gonads and vitellaria to longitudinal internal musculature cannot be the only or the most important characteristics to separate families or subfamilies ranks. However we agree that it is a important character, showing an outstandingly evolved direction. We propose that these characters may be utilized in the definition of genera, with the same importance of scolex characteristics.

In a recent paper, Rego (1995) proposed a radical solution: to eliminate the Woodland's classification and accept only one family, Proteocephalidae for South American Proteocephalids, with two subfamilies, Proteocephalinae and Corallobothriinae, including the species respectively with or without metascolex. To generic value was attributed to the internal characters. Naturally this is a provisional solution as the classification will result insufficient in the future, regarding recent development of phylogenetic systematics and genetical approaches, but its merit lies in pointing out the problems of the taxonomy of the group and compel a reevaluation of some morphological structures, specially the characters of the scolex. As already mentioned it is curious that no South American proteocephalids, as Sandonellinae (with lappets on scolex) and Gangesinae (with rostellum and hooks) deserved more attention to the scolex characterists, as the status of subfamily rank was attributed to these scolex characteristics; curiously in the monticelliids (sensu Woodland), the characteristics of scolex were neglected in benefit to the internal characteristics of gonads. It is necessary to pay more attention to the types of metascolex, characterising each one, and utilizing sections of scolex; and pay attention to the apical glandular structures which occur in other species than non-metascolex species (apical organs were not described from species with metascolex, but with the possible exception of *Jauella glandicephalus*), and the suckers, including sucker's

expansions (described from *Harriscolex kaparari*, *Houssayela sudobim* and other species) and the spines of scolex (microtriches ?).

Some authors are concerned with finding new characters, specially the ones not clearly seen, as for instance, vaginal sphincter or particularities of the copulatory cirrus. Choosing easily observable characters may be very helpful to future classification tasks.

References

Brooks, D.R.; Deardorff, T.L. Three proteocephalid cestodes from Columbian siluriform fishes, including *Nomimoscolex alovarius* sp.n. (Monticelliidae: Zygobothriinae). *Proc. Helminthol. Soc. Washington*, *47*:15-21, 1980.

Brooks, D.R.; Rasmsussen, G. Proteocephalidean cestodes from Venezuelan siluriform fishes with a revised classification of the Monticelliidae. *Proc. Biol. Society Washington, 97*:748-760, 1984.

Chambrier, A. de; Paulino, R.C. *Proteocephalus joanae* sp.n. (Eucestoda: Proteocephalidea) a parasite of *Xenodon neuwiedi* (Serpentes: Colubridae) from South America. *Folia Parasitol.*, *44*:289-296, 1997.

Chambrier, A. de; Rego, A.A. *Mariauxiella pimelodi* n.g., n.sp., (Cestoda: Monticellidae): a parasite of pimelodid sluroid fish from South America. *System. Parasitol.*, *30*:57-65, 1995.

Diesing, K.M. Sechzehn Gattungen von Binnewurmen und ihre Arten. Denkschriften der kaiserlichen Akademie der Wissenschaften. *Mathematisch-Naturwissehschaftliche Klasse Wien*, *9*:171-185, 1855.

Euzet, L. Recherches sur les cestodes *Tetraphyllides* des sélaciens des côtes de France. Montpellier, 1959. (Doctoral Thesis) - Université Montpellier.

Freeman, R.S. Ontogeny of cestodes and its bearing on their phylogeny and systematics. In: Dawes, B. (Ed.). *Advances in parasitology*. London: Academic Press, 1973. v.11, p.481-557.

Freze, V.I. *Proteocephalata, in fish, amphibians and reptiles.* Jerusalem: Israel Program of Scientific Translations, 1965. 597 p. (Essentials of cestodology, 5).

Fuhrmann, O. Eigentuliche Fishcestoden. *Zoolog. Anzeiger*, *46*:385-398, 1916.

Fuhrmann, O. Vier Diesing'sche Typen. *Rev. Suisse Zool.*, *41*:545-564, 1934.

La Rue, G.R. A revision of the cestode family Proteocephalidae. *Illinois Biol. Monogr.*, *1*:1-350, 1914.

Pavanelli, G.C.; Rego A.A. Novas espécies de proteocefalídeos (Cestoda) de *Hemisorubin platyrhynchus* (Pisces-Pimelodidae) do Estado do Paraná. *Rev. Bras. Biol.*, *49*:381-386, 1989.

Pavanelli, G.C.; Rego A.A. Cestóides proteocefalídeos de *Sorubim lima* (Schneider, 1801) Pisces-Pimelodidae) do rio Paraná e reservatório de Itaipu. *Rev. Bras. Biol.*, *51*:7-12, 1991.

Pavanelli, G.C.; Rego, A.A. *Megathylacus travassosi* sp.n. and *Nomimoscolex sudobim* Woodland, 1935 (Cestoda-Proteocephalidea) parasites of *Pseudoplatystoma corruscans* (Agassiz, 1829) (Siluriformes-Pimelodidae) from the Itaipu reservoir and Paraná river, Paraná state, Brazil. *Mem. Instit. Oswaldo Cruz*, *87*, suppl. 1:191-195, 1992.

Rego, A.A. Cestoides proteocefalídeos do Brasil. Reorganização taxonomica. *Rev. Bras. Biol.*, *47*:203-212, 1987.

Rego, A.A. Cestóides proteocefalídeos de "cachara", *Pseudoplatystoma fasciatus* (L.) (Pisces-Pimelodidae) de Mato Grosso. *Mem. Instit. Oswaldo Cruz*, *84*, suppl. IV:455-461, 1989.

Rego, A.A. Cestóides proteocefalídeos parasitas de pintado, *Pseudoplatystoma corruscans* (Agassiz) (Pisces-Pimelodidae). *Ciência e Cultura*, *42*:997-1002, 1990.

Rego, A.A. Redescription of *Gibsoniela mandube* (Woodland, 1935) (Cestoda: Proteocephalidea) a parasite of *Ageneiosus brevifillis* (Pisces: Siluriformes), a reappraisal of the classification of the proteocephalideans. *Mem. Instit. Oswaldo Cruz*, *87*:417-422, 1992.

Rego, A.A. Order proteocephalidea mola, 1928. In: Khalil, L.F.; Jones, A.; Bray, R.A. (Eds.). *Keys to the Cestode parasites of vertebrates*. St. Albans: CAB International, 1994. p. 257-293.

Rego, A.A. Cestóides proteocefalídeos do Brasil. Reorganização taxonômica. *Rev. Bras. Biol.*, *47*:203-212, 1987.

Rego, A.A. A new classification of the cestode Order Proteocephalidea Mola. *Rev. Bras. Zool.*, *12*:791-814, 1995.

Rego, A.A.; Chubb, J.C.; Pavanelli, G.C. Cestodes in South American freshwater teleost fishes: keys to genera and description of species. *Rev. Bras. Zool.*, *16*:299-367, 1999.

Rego, A.A.; Pavanelli, G.C. Check-list of the cestode order Proteocephalidea parasites from South America freshwater fishes. *Rev. Unimar*, *14*:109-137, 1992.

Riggenbach, E. Das Genus *Ichthyotaenia*. *Rev. Suisse Zool.*, *4*:165-275. 1895

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

Scholz, T.; Capellaro, H. The first records of *Corallobothrium parafimbriatum* Befus and Freeman, 1973 (Cestoda: Proteocephalidae) a parsite of North American catfishes (*Ictalurus* spp.) in Europe. *Folia Parasitol., 40*:105-108, 1993.

Wardle, R.A.; McLeod, J.A. *The zoology of tapeworms*. Minneapolis: The University of Minnesota Press, 1952. 780p.

Woodland, W.N.F. On some remarkable new *Monticellia*-like and other cestodes from Sudanese siluroids. *Quart. J. Microscop. Sci.*, *76*:175-208, 1925.

Woodland, W.N.F. On the anatomy of some fish cestodes described by Diesing from the Amazon. *Quart. J. Microscop. Sci.*, *76*:175-208, 1933a.

Woodland, W.N.F. On two new cestodes from the Amazon siluroid fish *Brachyplatystoma vaillanti* Cuv. and Val. *Parasitology*, *25*:485-490, 1933b.

Woodland, W.N.F. On a new subfamily of proteocephalid cestodes-the Othinoscolecinae - from the Amazon siluroid fish *Platystomatichthys sturio* (Kner). *Parasitology*, *25*:491-500, 1933c.

Woodland, W.N.F. On the Amphilaphorchidinae, a new subfamily of proteocephalid cestodes, and *Myzophorus admonticellia*, gen. et sp.n., parasitic in *Pirinampus* spp. from the Amazon. *Parasitology, 26*:141-149, 1934a.

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

Woodland, W.N.F. On six new cestodes from Amazon fishes. *Proc. Zool. Soc. London*, *104*:33-44, 1934c.

Woodland, W.N.F. Additional cestodes from the Amazon siluroids pirarará, dorad and sudobim. *Proc. Zool. Soc. London*, *104*:851-862, 1935a.

Woodland, W.N.F. Some more remarkable cestodes from Amazon siluroid fish. *Parasitology*, *27*:207-225, 1935b.

Woodland, W.N.F. Some new proteocephalids and a ptychobothriid (Cestoda) from the Amazon. *Proc. Zool. Soc. London*, *105*:619-623, 1935c.

Yamaguti, S. *Systema Helminthum*, *2, The Cestodes of Vertebrates*. New York: Interscience, 1959. 860p.

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