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.

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ABSTRACT. The morphology of *Crepidobothrium eirasi* Rego and de Chambrier, 1995 was revised and a cladistic analysis was performed on the six known species of *Crepidobothrium* Monticelli, 1900, namely, *C. eirasi*, *C. gerrardi* (Baird, 1860), *C. viperis* (Beddard, 1913), *C. dollfusi* Freze, 1965, *C. garzoni* de Chambrier, 1988 and *C. lachesidis* (MacCallum, 1921), using 23 characters and one outgroup. This analysis yielded two parsimonious trees with 0.76 consistency index. Both trees concur in the position of *C. eirasi*, and suggest that *Crepidobothrium* is monophyletic only when *C. eirasi* is excluded from the genus. The new genus *Pseudocrepidobothrium* has been built to allocate *C. eirasi*, so that the monophyly of *Crepidobothrium* may be maintained. *Pseudocrepidobothrium eirasi* n. comb. is the only species from a fish with notched suckers, while all *Crepidobothrium* spp. are parasites of South American reptiles.

Key words: *Pseudocrepidobothrium* gen. n., cestode, Proteocephalidea, cladistic analysis, Neotropical region.

RESUMO. *Pseudocrepidobothrium eirasi*, (Rego e de Chambrier, 1995) gen. n., comb. nov. (Cestoda, Proteocephalidea), parasita de um peixe de água doce da América do Sul, e análise cladística comparativa com *Crepidobothrium* spp. Foi revisada a morfologia de *Crepidobothrium eirasi* Rego and Chambrier, 1995, e feita uma análise cladística das seis espécies de *Crepidobothrium* Monticelli, 1900 [viz. *C. eirasi*, *C. gerrardi* (Baird, 1860, *C. viperis* (Beddard, 1913), *C. dollfusi* Freze, 1965, *C. garzoni* de Chambrier, 1988 e *C.lachesidis* (MacCallum, 1921)], utilizando-se 23 caracteres e um grupo externo. Obtiveram-se duas árvores com parcimônia e 0,76 de indice de consistência. Ambas as árvores coincidem na posição de *C. eirasi, o que* sugere que *Crepidobothrium* é monofilético apenas quando *C. eirasi* é excluído do gênero. O novo gênero *Pseudocrepidobothrium* é proposto para alojar *C. eirasi*, e assim a monofilia de *Crepidobothrium* pode ser mantida. *Pseudocrepidobothrium eirasi* comb. n. é a única espécie parasita de peixe que possui ventosas sulcadas, enquanto todas as espécies de *Crepidobothrium* são parasitas de répteis da América do Sul.

Palavras chave: *Pseudocrepidobothrium* gen. n., cestóide. Proteocefalídeo, análise cladística, região neotropical.

Crepidobothrium eirasi Rego and de Chambrier, 1995, Rego and de Chambrier (1995), was described from a siluriform fish, *Phractocephalus hemiliopterus* (Schneider, 1801) from the Brazilian Amazon, and included in *Crepidobothrium* Monticelli, 1900, a genus that groups an arrangement of species from South American snakes. Inclusion of *C. eirasi* in *Crepidobothrium* was basically made on the sucker's morphology with notched edges. However, Rego and de Chambrier (1995) noted the discrepancy in terms of hosts (all *Crepidobothrium* species are parasites from snakes), since *C. eirasi* is a parasite of fish, and in other morphological features. Thus the systematic status of *C. eirasi* should be considered as provisional and must be re-evaluated in the future. We shall study *C. eirasi* and compare it to all other species of the genus *Crepidobothrium*. Further, a phylogenetic analysis of the species in this genus will

be given. Results lead us to establish a new genus, *Pseudocrepidobothrium*, for the allocation of the species.

Material and methods

Mounted slides of *Crepidobothrium* species and *C. eirasi*, from the Helminthological Collection of the Oswaldo Cruz Institute were examined for morphological data. Data from the literature on *Crepidobothrium* species, recently revised by de Chambrier (1988, 1989 a,b), were also analyzed.

A cladistic analysis was performed on 6 recognized species of *Crepidobothrium*, or rather, *C. eirasi*, *C. gerrardi* (Baird, 1860), *C. viperis* (Beddard, 1913), *C. dollfusi* Freze, 1965, *C. garzoni* de Chambrier, 1988 and *C. lachesidis* (MacCallum, 1921). At this point, the sister-group of the genus *Crepidobothrium* is unknown, whereas obvious candidates include members of the Proteocephalinae Mola, 1929. Thus, *Paraproteocephalus parasiluri* (Zmeev, 1936) was arbitrarily chosen. All multistate characters were treated as unordered in the analysis (Table 1). Each character that could not be examined for a particular taxon was coded with a question mark for that taxon within the data matrix. A branch-swapping search was run using the mhennig option of Hennig86 version 1.5.

Table 1. Comparative characters

	Crepidobothrium eirasi	*Crepidobothrium* spp
Worm size	2-8.5 mm long	80-200 mm long*
Number of proglottids	7-12	200-400**
Lappets in proglottids	Present	absent
Testes number	less than 50	100 – 300
Testes distribution	in one field	in two fields or in two fields anteriorly connected
Genital pore	in anterior 1/4	near mid-proglottid
Disposition of vitellaria	Paramuscular	medullar
Scolex-apical organ	absent	present in some species
Longitudinal musculature of proglottids	weakly developed	conspicuously developed
Vitellaria distribution	lacking preporal vitelline follicles	follicles distributed along the entire length of proglottids
Egg morphology	with internal polar structures	polar structures absent

* *C. dollfusi* is 20-30mm long; ** *C. dollfusi* has 25 proglottids

Results

Characters

1. Apical organ in scolex. Two states: 0 = absent; 1= present. Apical organ is present in the scolex of some species of *Proteocephalus* Weinland, 1858; it is therefore considered to be a specific character. In *Crepidobothrium* spp. a vestigial apical organ is reported.
2. Nature of apical organ. Two states: 0 = glandular; 1 = muscular-glandular. The nature of the apical organ is controversial in some species. Whereas in *Crepidobothrium* it seems to be glandular, in other genera, as *Acanthotaenia* von Linstow, 1903, it is both muscular and glandular.
3. Apical sucker. Two states: 0 = present; 1 = absent. Apical sucker is present in many species of *Proteocephalus*; and also in *Crepidobothrium garzoni*.
4. Sucker's shape. Two states: 0 = round, not notched; 1 = notched anteriorly, heart-shaped. The notched condition of suckers eventually could suggest a tendency of the suckers to septate in loculi, as appears in *Peltidocotyle* Diesing, 1850 and *Deblocktaenia* Odening, 1963.
5. Neck length. Two states: 0 = short, or inconspicuous; 1 = long, conspicuous. The neck or proliferating zone is always present in cestodes; in some taxa the neck is very long, while in others it is difficult to observe and the segmentations begin immediately after the scolex.
6. Shape of mature proglottids. Three states: 0 = length greater than width; 1 = more or less square; 2 = width greater than length. This character is generally widely variable in cestodes; polarity of this feature is somewhat pragmatic (Rego *et al*., 1998).
7. Velum or laciniae in posterior margin of proglottids. Two states: 0 = acraspedote; 1 = craspedote.
8. Longitudinal musculature. Two states: 0 = weakly developed; 1 = well developed.
9. Genital pores. Two states: 0 = lateral opening in anterior 1/3 of mature proglottids; 1 = opening near 1/2 of mature proglottids.
10. Opening of vagina. Two states: 0 = posterior to cirrus sac; 1 = opening anterior or posterior in the same strobila.
11. Vaginal sphincter. Two states: 0 = sphincter inconspicuous;1= conspicuous. Difficult to ascertain the plesiomorphic condition; some authors consider the sphincter always present, and a plesiomorphic condition (Rego *et al*., 1998).
12. Distribution of vitelline follicles. Two states: 0 = equally distributed along lateral fields;1 = forming an L-shaped, towards ovary.
13. Extension of vitelline follicles. Two states: 0 = follicles only in posterior part, behind the

level of cirrus sac; 1 = follicles reaching close to the anterior and posterior edges of proglottid.

14. Interruption of vitelline follicles by cirrus sac. Two states: 0 = not interrupted; 1 = interrupted.
15. Shape of ovary. Two states. 0 = bilobate, and more or less compact; 1 = somewhat alate, follicles delicate.
16. Testes, distribution. Three states: 0 = testes in a single field, continuous; 1 = in two fields connected anteriorly; 2 = testes in two separate fields.
17. Number of testes. Three states: 0 = about 50; 1= 100-150 testes; 2 = very numerous, more than 150.
18. Shape of cirrus pouch. Two states: 0 = straight, not recurved; 1 = somewhat bent.
19. Anlagen of uterus. Two states: 0 = uterus appearing in mature proglottids;1 = uterus preformed, appearing in immature proglottids.
20. Shape of uterus. Two states: 0 = tubular, with diverticula well developed, usually occupying most of gravid proglottids;1 = as above, but diverticula not expanded.
21. Position of uterus. Two states: 0 = the uterus develops above the ovary; 1 = uterus develops above and behind the ovary.
22. Egg morphology. Three states: eggs spherical to oval, external hyaline membrane present; 1 = internal polar structures present; 2 = external polar filaments present.
23. Development of uterus. Two states: 0 = uterus develops along a longitudinal axis, in the median line of proglottids;1 = uterus develops horizontally (T-shaped).

Cladistic analysis

Twenty-three characters were identified in the seven taxa included in the analysis. Table 1 shows the distributions of the character states among the seven study taxa in the data matrix. Cladistic analysis resulted in two parsimonious trees, 34 steps long with a consistency index (CI) of 0.76 and a retention index (RI) of 0.52. One of the trees is shown in Figure 1, with the character reconstruction mapped onto each branch as appropriate. Both trees differ only in a switch of position between *C. gerrardi* and *C. garzoni.* The most noteworthy result of this analysis is that the species of *Crepidobothrium*, with the exception of *C. eirasi*, are grouped as a monophyletic clade supported by at least four synapomorphies, two unambiguous (chrs. 5, 13), and two characters with reversals in terminal taxa (chrs. 6 and 9). Lack of support for a close affinity between *C. eirasi* and the remaining *Crepidobothrium* taxa seems to be fairly robust. *C. eirasi* grouped with the outgroup in a basal unresolved polytomy. If the classification is to be consistent with this tree, *C. eirasi* must be removed from the genus *Crepidobothrium*. Therefore a new genus *Pseudocrepidobothrium* is suggested to allocate *C. eirasi* so that the monophyly of *Crepidobothrium* may be maintained.

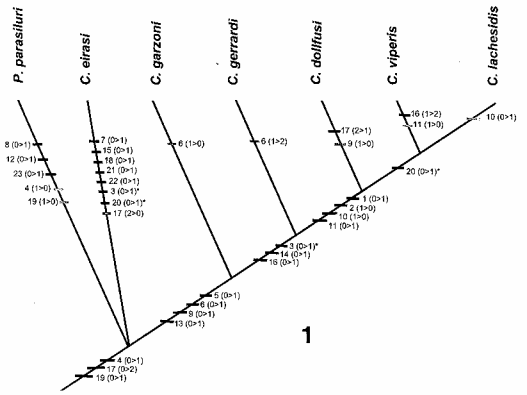

Figure 1. Cladogram showing phylogenetic relationships among *Crepidobothrium* species. Characters and their character states have been mapped onto the tree. Homoplasious occurrences of characters are indicated with asterisks next to the character number. Character reversals are indicated with a grey bar on the appropriate branch of the tree

Table 2. Data matrix used in cladistic analysis of *Crepidobothrium* species

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
C. eirasi	0	?	1	1	0	1	1	0	0	1	0	0	0	?	1	0	0	1	1	1	1	1	0
C.gerrardi	0	1	1	1	1	2	0	0	1	1	0	0	1	1	0	1	2	0	1	0	0	0	0
C.viperis	1	0	1	1	1	0	0	0	1	0	0	0	1	1	0	2	2	0	1	1	0	0	0
C.dollfusi	1	0	1	1	1	1	0	0	0	0	1	0	1	1	0	1	1	0	1	0	0	0	0
C.lachesidis	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	1	2	0	1	1	0	0	0
C. garzoni	0	?	0	1	1	0	0	0	1	1	0	0	1	0	0	0	2	0	1	0	0	0	0
*Paraproteocephalus parasiluri (*outgroup)	0	?	0	0	0	2	0	1	0	1	0	1	0	?	0	0	2	0	0	0	0	0	1

***Pseudocrepidobothrium* n. g.** Diagnosis: small-sized worms, with few proglottids. Scolex bearing 4 heart-shaped suckers, notched anteriorly. Immature proglottids square; mature and gravid proglottids longer than its width. Proglottid appendages (lappets) in ventrolateral position. Genital pores in the anterior third part of proglottids. Vitelline follicles paramuscular (distributed internally, externally, and among longitudinal muscle fibres). Absence of vitelline follicles in preporal region of proglottids. Vagina anterior or posterior to cirrus pouch, having an inconspicuous muscular sphincter. Longitudinal musculature weakly developed,

consisting of small bundles of fibres, most of them situated laterally. Uterus saccular, not expanded. Eggs with internal polar structures and embryophere with hooks. Parasites of Pimelodid Siluriform fish in Amazon.

Type species: *Pseudocrepidobothrium eirasi* (Rego and Chambrier, 1995).

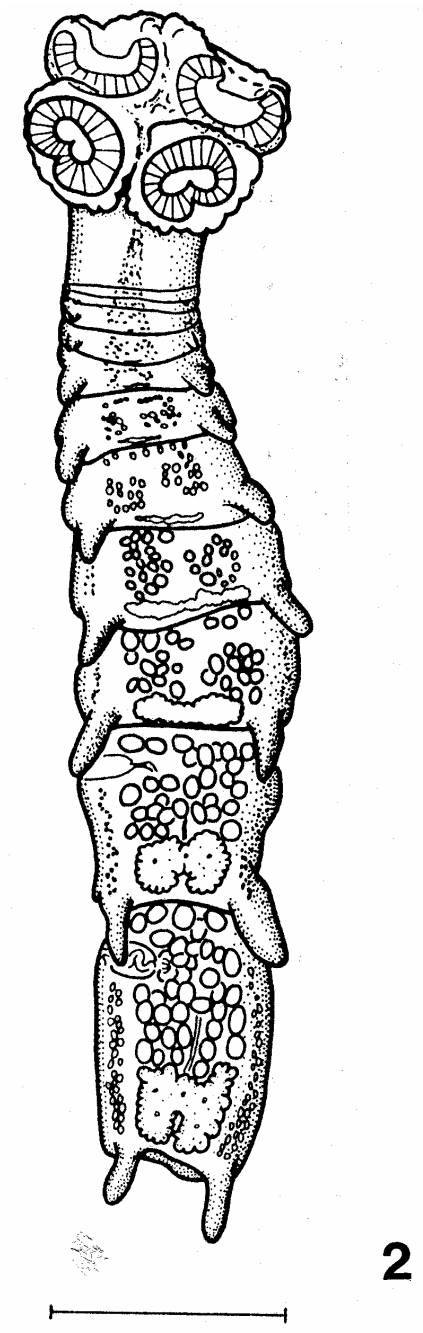

Figure 2. Strobila of *Pseudocrepidobothium eirasi*; note the heart-shaped suckers. Scale bar 0.100 mm

Discussion

Even though a common pattern of four suckers and eventually an apical organ on the scolex exist in species of proteocephalideans, there is a great variety in the morphology of such structures, particularly in proteocephalids from freshwater fishes in South America. Suckers can be biloculate, triloculate, tetraloculate among other forms, and a metascolex (development of folds posterior to sucker´s or between them) of different types could be present (Rego, 1999). Many genera have been described from fishes, especially Siluriforms (about 30), when they are compared to the small number of species (Rego *et al*., 1999). Thus, many genera are monotypic, as in *Pseudocrepidobothrium* n. gen. This situation differs in other zoogeographical regions, as in the Palearctic and Nearctic, where proteocephalid species are grouped in a few genera. For instance, the cosmopolitan genus *Proteocephalus* Weinland, 1858, has more than one hundred species.

The speciation of proteocephalids occurrs mainly in fishes as definitive hosts, even though there are a few genera and species from reptiles. Currently, four genera of proteocephalids parasites of reptiles have been described in South America: *Ophiotaenia* La Rue, 1911, with numerous species parasitizing particularly snakes; *Tejidotaenia* Freze, 1965, from Lacertillids; *Vaucheriella* Chambrier, 1987, with one species from snakes, and *Crepidobothrium*, with five species from snakes.

It is worth noting that, with the exception of *Vaucheriella bicheti* Chambrier, 1987, none of the species of proteocephalids from reptiles have segments with vitellaria and gonads situated in the cortex. A different situation is observed in species from freshwater fishes, mostly having the reproductive organs in medullar position (monticellids sensu lato). If medullar position of vitellaria and gonads form the plesiomorphic condition (Brooks, 1978; Rego *et al*., 1998), and taking into account speciation events of proteocephalids in fishes and reptiles with a greater diversification in the former hosts, a latter colonization of reptile hosts could be suggested.

Pseudocrepidobothrium eirasi is the only species from a fish with notched suckers. The difficulties to include this species in a pre-existing genus, along with results of the phylogentic analysis, and comparative morphology (there are not less than 11 differential characters between *Crepidobothrium* species and *P.eirasi*, Table 1) led us to establish a new genus, *Pseudocrepidobothrium*, for the allocation of this species.

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