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.
dolfiusi 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.

ov. (Cestoda, Proteocephalidea), parasita de um peixe de água doce da Américado Sul, e análise cladistica 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. dolfiusi 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 índice de consistência. Ambas as
árvore 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. Proteocefalideo, análise cladística, região
neotropical.
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, 1989a,b), were also analyzed.

A cladistic analysis was performed on 6 recognized species of *Crepidobothrium*, or rather, *C. eirasi*, *C. gerardi* (Baird, 1860), *C. vaperis* (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 parasituri* (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**

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Crepidobothrium eirasi</em></th>
<th><em>Crepidobothrium spp</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Worm size</td>
<td>2.8-5.5 mm long</td>
<td>80-200 mm long*</td>
</tr>
<tr>
<td>Number of proglottids</td>
<td>7-12</td>
<td>200-400**</td>
</tr>
<tr>
<td>Lappets in proglottids</td>
<td>Present</td>
<td>absent</td>
</tr>
<tr>
<td>Testes number</td>
<td>less than 50</td>
<td>100 – 300</td>
</tr>
<tr>
<td>Testes distribution</td>
<td>in one field</td>
<td>in two fields or in two fields anteriorly connected</td>
</tr>
<tr>
<td>Genital pore</td>
<td>in anterior 1/4</td>
<td>near mid-proglottid</td>
</tr>
<tr>
<td>Disposition of vitellaria</td>
<td>Paramuscular</td>
<td>medullar</td>
</tr>
<tr>
<td>Scolex-apical organ</td>
<td>absent</td>
<td>present in some species</td>
</tr>
<tr>
<td>Longitudinal musculature of proglottids</td>
<td>weakly developed</td>
<td>conspicuously developed</td>
</tr>
<tr>
<td>Vitellaria distribution</td>
<td>lacking preoral vitelline follicles</td>
<td>follicles distributed along the entire length of proglottids</td>
</tr>
<tr>
<td>Egg morphology</td>
<td>with internal polar structures</td>
<td>polar structures absent</td>
</tr>
</tbody>
</table>

* C. dollfusi is 20-30 mm 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...
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.

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

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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).

**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 occurs 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: *Opilotaenia* La Rue, 1911, with numerous species parasitizing particularly snakes; *Tejidotaenia* Freze, 1965, from Lacertilians; *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 phylogenetic 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.
References


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