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The cestode order Rhinebothriidea no longer family-less: A molecular phylogenetic investigation with erection of two new families and description of eight new species of *Anthocephalum*

TIMOTHY R. RUHNKE[1], JANINE N. CAIRA[2] & ALLISON COX[1]

[1]*Department of Biology, West Virginia State University, Institute, WV 25112-1000, USA. E-mail: ruhnketr@wvstateu.edu*
[2]*Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, 06269–3043, USA. E-mail: janine.caira@uconn.edu*

Abstract

The spiral intestines of a total of 30 specimens of 14 species of batoids from around the world were examined for rhinebothriideans. These consisted of *Taeniura grabata, Dasyatis margaritella*, and *Dasyatis* sp. from Senegal, *Dasyatis americana* from Florida, *Dasyatis dipterura* and *Dasyatis longa* from México, *Himantura jenkinsii, Himantura leoparda*, *Himantura uarnak* 2, *Urogymnus asperrimus* 1, and *Neotrygon kuhlii* 4 from Australia, in addition to *Himantura uarnacoides* and *Neotrygon kuhlii* 1 from Borneo. Each of these hosted one or more species of *Anthocephalum*. Eleven of the cestode species were new to science; four represented described species. In addition, *Urotrygon aspidura* from Costa Rica hosted a species of *Escherbothrium*. Sufficient material was available for formal description of the following eight species of *Anthocephalum*: *A. decrisantisorum* **n. sp.**, *A. healyae* **n. sp.**, *A. jensenae* **n. sp.**, *A. mattisi* **n. sp.**, *A. meadowsi* **n. sp.**, *A. odonnellae* **n. sp.**, *A. papefayi* **n. sp.**, and *A. philruschi* **n. sp.** These species differ from their nine described congeners in overall size, number of proglottids and marginal loculi, number and arrangement of testes, apical sucker size, arrangement and distribution of vitelline follicles particularly with respect in the post-poral field, and muscularity of the genital pore. The diagnosis of *Anthocephalum* is emended slightly to accommodate these new species. Material of four previously described *Anthocephalum* species, seven of the novel *Anthocephalum* species described here, 3 undescribed *Anthocephalum* species, and the species of *Escherbothrium* was preserved in 95% ethanol and partial 28S rDNA (D1-D3) and complete 18S rDNA sequence data were generated de novo. These data were combined with data from GenBank for *Anthocephalum* cf. *centrurum* (recognized as *A. mattisi* **n. sp.** below) and 29 species representing 12 other putative rhinebothriidean genera. Phylogenetic analyses using Bayesian Inference and Maximum Likelihood methods were conducted using a total of five representatives of the Lecanicephalidea, Cathetocephalidea and "Tetraphyllidea" as outgroups. The analyses yielded trees that were largely congruent and that supported the existence of four major subgroups of rhinebothriideans. Family designations were established for each of these clades. Echeneibothriidae was elevated from subfamily to family level to accommodate the group consisting of *Echeneibothrium* and *Pseudanthobothrium*; this family is unique in retaining the apical organ (as a myzorhynchus) into adulthood. Rhinebothriidae was elevated from subfamily to family level to accommodate the group consisting of *Rhabdotobothrium*, *Rhinebothrium*, *Rhinebothroides*, *Rhodobothrium*, *Scalithrium* and *Spongiobothrium*. This family is distinctive in its lack of apical suckers and also of a definitive anterior/posterior orientation to the bothridia. Anthocephaliidae n. fam. was established to house *Anthocephalum* and taxa identified as New Genus 1, New Genus 2, and New Genus 4 by previous authors. The bothridia of its members exhibit a conspicuous anterior/posterior orientation signaled by the presence of an apical sucker. In addition, its members bear marginal loculi or one or more rows of facial loculi and vitelline follicles that are usually interrupted by the ovary. Escherbothriidae n. fam. was established to house *Escherbothrium* and the taxon identified as New Genus 3 by previous authors. It most closely resembles Anthocephaliidae but the facial loculi are arranged in columns anteriorly and rows posteriorly, rather than arranged in multiple rows or entirely lacking. A key to the families is also provided.

Key words: *Anthocephalum*, new species, molecular systematics, Rhinebothriidea, Rhinebothriidae, Echeneibothriidae, Anthocephaliidae, Escherbothriidae

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most closely with the "tetraphyllidean" *Caulobothrium opisthorchis.* This discrepancy is intriguing and bears additional investigation, particularly given that *Pentaloculum* remains poorly known and New Genus 7 has yet to be formally described. But, it seems likely that these two genera may not have affinities with the Rhinebothriidea after all. For the present, New Genus 11 **n. sp.** 1, from the dwarf sawfish *Pristis clavata* Garman, has not been assigned to a family. The bothridial morphology of this undescribed genus is unusual in a number of respects (see fig. 6 of Healy *et al*. 2009). Examination of other members of the Rhinopristiformes (sensu Naylor *et al*. 2012a) has yielded additional cestode species that appear to belong to this group. It is possible it will ultimately be found to represent a distinct family-level taxon within the Rhinebothriidea.

The addition of representatives of the seven rhinebothriidean genera not included here (i.e., *Biotobothrium*, *Cairaeanthus*, *Clydonobothrium*, *Notomegarhynchus*, *Pararhinebothroides*, *Phormobothrium*, and *Tritaphros*) to molecular analyses would allow the familial placements assigned here to these genera based on morphological grounds, to be assessed in a molecular context. Also of interest would be inclusion of the genera *Serendip* Brooks & Barriga, 1995 and *Zyxibothrium* Hayden & Campbell, 1981 (see Hayden & Campbell 1981), both of which are clearly candidate members of the Rhinebothriidea. This expanded taxon sampling would also allow for a more robust assessment of the phylogenetic relationships of the order overall. Consideration of additional molecular markers would also be interesting to explore to aid in the further resolution of rhinebothriidean relationships for we note that at this point in time the interrelationships among rhinebothriidean families are poorly resolved.

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