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Phylogenetic analysis and reconfiguration of genera in the cestode order Diphyllidea

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ABSTRACT

The generic boundaries of the Diphyllidea are reassessed based on parsimony and likelihood phylogenetic analyses of 28S rDNA (ribonucleic acid large subunit), 18S rDNA (ribonucleic acid small subunit), and COI (cytochrome oxidase subunit I) sequence data for 31 species representing morphological variation across the order. Trees resulting from these analyses yielded a number of well-supported clades that are congruent with unique morphological features mandating generic revision of the order and erection of at least two new genera. Species originally assigned to *Echinobothrium* van Beneden, 1849 but bearing a corona of spines on the region of the scolex anterior to the bothria and posterior to the apical organ armature are transferred to *Coronocestus* n. gen.; members of this genus typically parasitize triakid sharks, although one report from a hemiscylliid shark exists. Species with lateral hooklets arranged in continuous bands, rather than in two distinct clusters, are transferred to *Halysioncum* n. gen.; all species parasitize batoids, mostly myliobatids and rhinopterids, but a few records also exist from arhynchobatids, rhinobatids, platyrhinids and urotrygonids. Our analyses support transfer of the five species originally assigned to *Macrobothridium* Khalil and Abdul-Salam, 1989 owing to their lack of cephalic peduncle spines to *Echinobothrium*. As a consequence, *Echinobothrium sensu stricto* includes species both with and without spines on the cephalic peduncle, but all members of the genus possess lateral hooklets arranged in clusters on either side of the dorsal and ventral apical hooks. With respect to diphyllideans parasitizing catsharks, *Ahamulina* Marques, Jensen and Caira, 2012 is unique in possessing apical hooks but lacking lateral hooklets and *Ditrachybothridium* Rees, 1959 is unique in entirely lacking scolex armature. By far the majority of species of *Echinobothrium sensu stricto* parasitize skates of the family Rajidae, guitarfish of the family Rhinobatidae, and stingrays of the dasyatid genera *Taeniura* Müller and Henle, *Dasyatis* Rafinesque, and *Himantura* Müller and Henle, although a single species each has been reported from Anacanthobatidae, Rhynchobatidae, Platyrhinidae and Myliobatidae. It now seems clear that while by far the majority of diphyllideans parasitize batoids, the diphyllideans parasitizing sharks, and catsharks in particular, remain problematic. Additional collections from these carcharhiniform hosts are likely to be particularly illuminating.

1. Introduction

Knowledge of the Diphyllidea van Beneden in Carus, 1863, one of the seven orders of cestodes parasitizing elasmobranchs, has grown substantially over the past decade with descriptions of nearly half of the 50 valid species appearing over that time. Collectively members of the order occur in a diversity of elasmobranchs, although the majority of species parasitize batoids. Unlike some of the orders found in elasmobranchs, the monophyly of the Diphyllidea is undisputed (Ivanov and Hoberg, 1999; Tyler, 2006). Its members are united by their possession of a scolex with two bothria, an apical organ that bears apical hooks and lateral hooklets, as well as a cephalic peduncle that may be armed with eight columns of spines, and a mid-ventral common genital pore—although collectively its species exhibit a wide array of configurations of scolex armature (Fig. 1). Three genera are currently recognized. At present, species of *Echinobothrium* van Beneden, 1849 possess three types of armature (apical hooks, lateral hooklets and cephalic peduncle spines), while species of *Ditrachybothridium* Rees, 1959 lack all three types of armature. The currently monotypic *Ahamulina* Marques, Jensen and Caira, 2012 possesses apical hooks but

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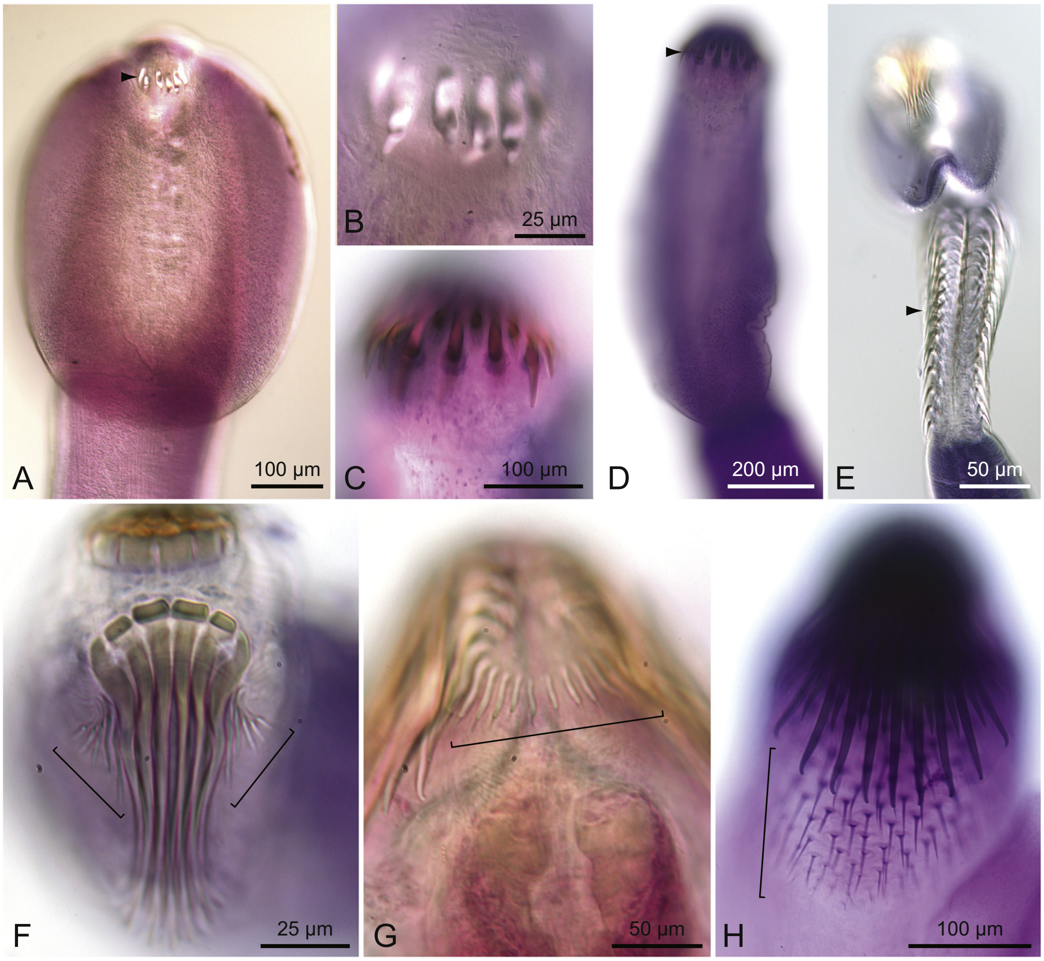

Fig. 1. Light micrographs showing diphyllidean scolex armature. (A) Scolex of *Ahamulina catarina*; arrowhead indicates the single row of apical hooks. (B) Close-up of single row of apical hooks of *A. catarina*. (C) Close-up of single row of apical hooks of *Ahamulina* n. sp. 1; note that apical hooks are unequal in length. (D) Scolex of *Ahamulina* n. sp. 1; arrowhead indicates the single row of apical hooks. (E) Scolex of *Echinobothrium dougbermani*; arrowhead indicates cephalic peduncle spines. (F) Apical organ armature of *E. dougbermani*; brackets indicate two clusters of lateral hooklets. (G) Apical organ armature of *Halysioncum mexicanum*; bracket indicates continuous band of lateral hooklets. (H) Anterior region of scolex of undescribed species of *Coronocestus* from *Iago* sp.; bracket indicates corona of spines.

lacks lateral hooklets and spines on the cephalic peduncle (Fig. 1A). Although now considered a synonym of *Echinobothrium* (see Tyler, 2006; Kuchta and Caira, 2010), *Macrobothridium* Khalil and Abdul-Salam, 1989 was erected for species that exhibit apical hooks and lateral hooklets but lack spines on the cephalic peduncle. The monophyly of all three genera remains to be comprehensively assessed. In fact, diphyllidean phylogenetic relationships have been seriously explored on only two occasions (see Ivanov and Hoberg, 1999; Tyler, 2006), in both cases based solely on morphological data. Although the interrelationships implied by these two studies were consistent in the non-monophyly of *Echinobothrium* relative to *Macrobothridium*, and in the placement of *Ditrachybothridium* as sister to that clade, they differed substantially in other aspects of their topologies. From a molecular standpoint, diphyllideans have been included as outgroups in studies focusing on phylogenetic relationships within other cestode orders (e.g., Olson and Caira, 1999; Olson et al., 1999, 2001, 2010; Littlewood and Olson, 2001; Bray and Olson, 2004; Caira et al., 2005; Brabec et al., 2006; Palm et al., 2009), or as exemplars in broad scale analyses assessing relationships among cestode orders (Waeschenbach et al., 2007, 2012). Diphyllidean interrelationships have not been addressed previously using molecular data.

The primary goals of this study were to (i) investigate the phylogenetic relationships among the diphyllideans from a molecular perspective using data from one mitochondrial (cytochrome oxidase subunit I [COI]) and two nuclear (ribonucleic acid large subunit [28S rDNA] and ribonucleic acid small subunit [18S rDNA]) genes, (ii) assess generic boundaries based on the results of the molecular analyses, (iii) explore morphological attributes that might serve to define the resulting groups, (iv) revise the generic-level classification within the order so as to be consistent with groups supported by both morphological and molecular data, and (v) examine the host associations of the order in the context of the generic-level revision.

2. Materials and methods

2.1. Study taxa

Our analyses included 31 species of diphyllideans consisting of 12 of the 50 valid species and 19 undescribed species. Fifteen of the 31 species were represented by replicates of two to five specimens each, for a total of 54 ingroup specimens. One of the greatest challenges of this study was securing molecular material representing the range of hosts and distinctive morphologies seen across the order. In many cases the only specimens available represented species new to science, many of which came from host species that had not been previously examined. In all cases hologenophores (sensu Pleijel et al., 2008) were sequenced and their associated