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Emendation of *Glyphobothrium* and *Glyphobothrium zwerneri* (Tetraphyllidea) Collected from *Rhinoptera bonasus* (Myliobatiformes: Myliobatidae) in Campeche, México, with Details of the Bothridial Suckers

Scott Monks, Griselda Pulido-Flores, and Scott L. Gardner

Abstract

During a study of the helminth parasites of stingrays from the coast of the Yucatán Peninsula of México, four specimens of *Glyphobothrium zwerneri* were collected from *Rhinoptera bonasus*, Cownose Ray, off the Gulf of Mexico coast of Campeche, México and examined. The scolex of these worms had bothridia with an anterior sucker on each, a feature not reported in the original description but verified as present on type material. The discovery of this feature necessitated an emended diagnosis of the genus, an emendment of the description of the species, and establishment of the family Glyphobothriidae fam. nov. for *Glyphobothrium* and *Duplicibothrium*. The details of the anterior sucker and the scolex that are common to *Glyphobothrium* and *Duplicibothrium* and the possible relationships of *Tiarabothrium* and *Echeneibothrium* with *Glyphobothrium* and *Duplicibothrium* are presented.

Key words: Campeche, Cownose Ray, *Duplicibothrium*, Glyphobothriidae new family, *Glyphobothrium zwerneri*, Gulf of Mexico, México, *Rhinoptera bonasus*

Resumen

Durante el estudio de los helmintos de rayas de la costa de la Península de Yucatán, México, se colectaron cuatro ejemplares de *Glyphobothrium zwerneri,* parásitos de *Rhinoptera bonasus* en la costa de Campeche, en el Golfo de México. El escólex de estos céstodos tiene botridios con una ventosa anterior cada uno. Está característica no fue reportada en la descripción original, pero fue verificada en los ejemplares de la serie tipo. Por el hallazgo de esta característica se realizó una enmienda a las diagnosis del género, la descripión de la especie. Se establecó la familia Glyphobothriidae fam. nov. para *Glyphobothrium* y la *Duplicibothrium*. Los detalles de la ventosa anterior y del escólex son características comunes para *Glyphobothrium* y *Duplicibothrium*; además, se presenta la posible relación de *Tiarabothrium* y *Echeneibothrium* con *Glyphobothrium* y *Duplicibothrium.*

Palabras claves: Campeche, *Duplicibothrium*, Glyphobothriidae nueva familia, *Glyphobothrium zwerneri*, Golfo de México, México, *Rhinoptera bonasus*

INTRODUCTION

As an initial step of a study of the parasites of stingrays off the coast of the Yucatán Peninsula, México, potential hosts were collected and examined for helminths; partial results have been published by Pulido-Flores and Monks (2005, 2008, 2014). However, many of the cestodes that were collected are only now being processed and studied. In this material, specimens of *Glyphobothrium zwerneri* Williams and Campbell, 1977 had been collected from one of nine individuals of the Cownose Ray, *Rhinoptera bonasus* (Mitchill, 1815) (Myliobatiformes: Myliobatidae) (see Pulido-Flores and Monks 2014). The description of *G. zwerneri* was based on 53 specimens taken from four of 11 individuals of *R. bonasus* collected in Chesapeake Bay, Virginia, U.S.A. (Williams and Campbell 1977). Since that date, the species has been mentioned in publications by Brooks and Barriga (1995), Brooks and Evenhuis (1995), Caira et al. (1999a), Olson and Caira (1999), Ruhnke et al. (2000), and Ruhnke (2011), among others, and some of the original material was reexamined using scanning electron microscopy (SEM) (Caira et al. 1999a), but collection of new specimens has not been reported.

This species is particularly interesting because of the structure of the scolex, which is described as globular with four superficial bothridia (i.e., on the surface of the scolex proper) divided into three longitudinal rows of loculi that are separated by narrow longitudinal fissures (Williams and Campbell 1977). Furthermore, in the original description, those authors mentioned the presence of a small "pit" that resembled a vestigial sucker, but they did not include in their figures nor did they mention the presence of bothridial suckers. This condition, lack of bothridial suckers, has been noted by subsequent authors (cited above) and is important as support for its placement in Serendipeidae.

Contrary to our expectations, specimens of *G. zwerneri* that we collected from Mexican stingrays each had a well-developed accessory sucker on the apical end of the bothridia. Paratypes of *G. zwerneri* (Howard W. Manter Laboratory; HWML-20875) were examined and the bothridial suckers were found on each specimen, confirming that this was a characteristic of the species and not just of the specimens from Campeche (see collection locality definition below). Finding these structures necessitated emendation of the original description and a reappraisal of family-level systematics of this group. Herein we provide new information about the scolex, knowledge of which made necessary the emendation of the concept of the genus and the description of the species to include that information, and the establishment of a new family for the genus and its congeners.

MATERIALS AND METHODS

Seven stingrays, *Rhinoptera bonasus* (Mitchill, 1815) (Myliobatiformes: Myliobatidae) (the Cownose Ray), were collected by local fishermen (for open sea localities see Pulido-Flores and Monks 2005); five from north of Ciudad del Carmen (open ocean side), Isla del Carmen, Campeche (18°83'N, 91°49'W) (collected May 2000), one from Champotón, Campeche (19°21'N, 90°54'W) (February 1999), and one from Isla Contoy, Quintana Roo (20°48'N, 86°47'W) (February 1999). Subsequent to these, four stingrays were collected in April 2005 from south of Ciudad del Carmen, Isla del Carmen, Campeche, in Laguna de Términos (center of lagoon located at 18°36'N, 91°33'W). Individual stingrays were killed and maintained on ice until necropsied; the intestinal tract was removed and examined according to Monks et al. (1996). All ecto- and endohelminths were fixed and then transferred to 70% ethyl alcohol. The Monogenea (ectohelminths) were reported by Pulido-Flores and Monks (2005), but the endohelminths remained unprocessed until the present study. One of the nine stingrays (a male collected in May 2000) from the region of Ciudad del Carmen, Campeche, was infected with four specimens of *G. zwerneri*. Worms were stained using Mayer's carmalum, cleared in methyl malicylate, and mounted in Canada balsam for examination as whole mounts. Specimens of *G. zwerneri* were deposited in the Colección Nacional de Helmintos, IBUNAM,

Mexico (CNHE-8838) and the Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, U.S.A (HWML-49760). Specimens of species discussed herein that had been deposited in the United States National Parasite Collection (USNPC) were not available for loan and could not be studied. Measurements are included only when they depart from those of the original descriptions. Taxonomic identification of the hosts were based on current morphological characteristics, although we note that molecular studies have identified potential cryptic species in each taxon that have, to date, not been described formally (Naylor et al. 2012).

The evolutionary transitions leading to *Glyphobothrium* Williams and Campbell, 1977 were not presented on the cladogram of Caira et al. (1999a), so the data matrix from Caira et al. (1999b) and the cladogram (Caira et al. 1999a; their Fig. 87) were entered manually into Mesquite (Maddison and Maddison 2015). The new states for the characters of the taxa discussed herein were changed, as discussed below, in the original data set and the cladogram presented herein was produced using Mesquite.

RESULTS AND DESCRIPTIONS

Based on the type material and the specimens of *Glyphobothrium* collected from the waters of the Gulf of Mexico around Ciudad del Carmen, Campeche, Mexico, the diagnoses of the genus and of the species, *G. zwerneri*, are herein emended and a new family is established.

Glyphobothrium Williams and Campbell, 1977, emended

Fig. 1

Diagnosis (following Williams and Campbell 1977 in all details other than the scolex).—Tetraphylidea. Scolex globular, with four sessile superficial bothridia (i.e., on the surface of the scolex proper) with a single column of horizontal loculi, each loculus secondarily subdivided into three sections by longitudinal muscle bundles; each bothridium surmounted by anteriorly directed sucker. Lacking apical organ (*sensu* Caira et al. 1999a). Ovary consists of multiple lobes radiating in all directions from a central ovarian isthmus. *Type and only known species: Glyphobothrium zwerneri* Williams and Campbell, 1977.

Glyphobothrium zwerneri Williams and Campbell, 1977, emended

Fig. 1

Description (following Williams and Campbell 1977 in details other than the scolex).—Tetraphylidea. Scolex spherical (Fig. 1), with four sessile superficial bothridia with a single column of 10–12 horizontal loculi. Each loculus horizontal, secondarily subdivided into three superimposed quadrangular sections by parallel longitudinal muscle bundles (*sensu* Caira et al. 1999a) (Fig. 1). Horizontal septal borders with distinct external flaps, continuous, not divided by longitudinal muscle bundles. Bothridia surmounted by single anteriorly positioned accessory sucker, width 112–180 μm (mean = 146 μm ± 21 μm; n =13) (Fig. 1). Lacking apical organ but with remnants of vestigial sucker (*sensu* Williams and Campbell 1977; Caira et al. 1999a). Ovary consists of multiple lobes radiating in all directions from a central ovarian isthmus.

Taxonomic Summary

Host.—Rhinoptera bonasus (Mitchill, 1815) (Myliobatiformes: Myliobatidae), the Cownose Ray.

Type locality.—Chesapeake Bay, Virginia.

Other localities.—North of Ciudad del Carmen, Isla del Carmen, Campeche, México (18°83’ N, 91°49’ W).

Type specimens.—United States National Parasite Collection USNPC-74513 (holotype), USNPC-74512 (paratypes); HWML-20875 (paratypes).

Additional specimens.—CNHE-8838 and HWML-49760 (vouchers; this study).

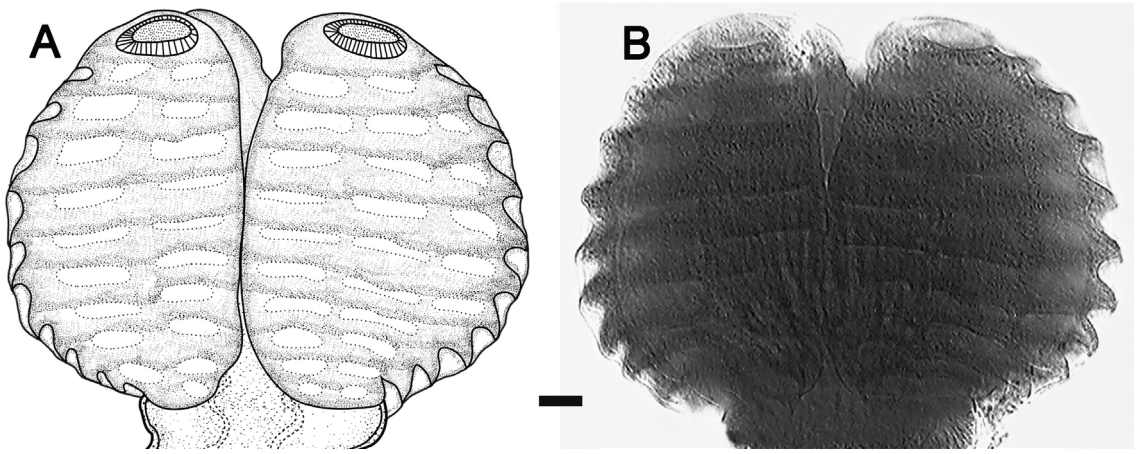

Figure 1. *Glyphobothrium zwerneri* Williams and Campbell, 1977 from *Rhinoptera bonasus.* A. Drawing of the scolex of a specimen of *G. zwerneri* (CNHE 8838). B. Photo of the scolex of a specimen of *G. zwerneri* (CNHE 8838). Scale bar = 100 μm.

Differential Diagnosis

Williams and Campbell (1977) described *Glyphobothrium zwerneri*, the only known member of the genus, and assigned it to the Phyllobothriidae. This species, from elasmobranchs, has a singular type of scolex that is spherical with four bothridia that are fused to the scolex, each divided into horizontal loculi (Fig. 1). The specimens we collected conform to the description of *G. zwerneri* established by Williams and Campbell (1977) in this and the other main features included in their description.

Brooks and Barriga (1995) established Serendipidae (now Serendipeidae Brooks and Evenhuis, 1995) for a putative clade containing *Serendip deborahae* Brooks and Barriga, 1995, and species in the genera *Duplicibothrium* Williams and Campbell, 1978, and *Glyphobothrium*, which they proposed as sister group to *Dioecotaenia* Schmidt, 1969. The members of that putative clade all have fusion of the bothridia (to each other or to the scolex), testes arranged in two layers in the proglottids, and some testes that are postovarian (Brooks and Barriga 1995). Of these three genera, *Glyphobothrium* is distinguishable from *Serendip* Brooks and Barriga, 1995 by having bothridia divided into loculi rather than bothridia that are entire, scolex that is spherical rather than composed of flat bothridia directed anteriorly, and horizontal septal borders rather than radial septal borders (discussed further by Monks et al. 2015). It should be noted that these relationships have not been tested yet in a formal phylogenetic analysis.

Given the observations discussed above and the results of Caira et al. (1999a), *Glyphobothrium* is most similar to *Duplicibothrium*. *Duplicibothrium* was originally diagnosed as having bothridia fused anteriorly into dorsal-ventral pairs and without pedicles. Both taxa possess testes that overlap the ovary and extend into the postovarian space, follicular vitellaria that are joined dorsally and ventrally (i.e., form a continuous sleeve around the reproductive organs, *sensu* Williams and Campbell, 1978), and a weakly craspedote strobila. The ovary of *Duplicibothrium minutum* Williams and Campbell, 1978 was described as bilobed in dorsoventral view, which it clearly is not, although they went on to say it was "…subdivided into digitiform processes radiating from ovarian isthmus…" (Williams and Campbell, 1978; pg. 836), but clarified this by saying "The long digitiform lobes of the ovary combined with its short isthmus made it virtually impossible to determine if the species has a tetralobed or bilobed ovary." (Williams and Campbell, 1978; pg. 837). This is consistent with their figures and with both Caira et al. (1999a), who coded the species as having an ovary that consists of multiple lobes radiating in all directions from a central ovarian isthmus (Character 108, state "4"), and Ruhnke et al. (2000). *Glyphobothrium* and *Duplicibothrium* share these putative synapomorphies, but the two can be distinguished by the form of

the scolex (spherical or bothridia fused into two pairs forming two earlike outgrowths, respectively).

Until this account, *G. zwerneri* has not been reported since the original description and no other species in the genus have been discovered or described. Williams and Campbell (1977) tentatively assigned *G. zwerneri* to Phyllobothriidae because it had multiloculated bothridia, even though they did not have pedicles. However, Williams and Campbell (1977) also commented that the species had features in common with *Dioecotaenia*, a similarity that has not gone unnoticed by recent systematists (see works cited above), but a relationship not supported by recent cladistic analyses (Caira el al. 1999a; Caira el al. 2001).

Brooks and Barriga (1995) suggested that Phyllobothriidae should be divided into two groups: species that have bothridial accessory suckers but lack bothridial loculi, and species that have bothridial loculi but lack bothridial accessory suckers. Thus, as described originally, *Glyphobothrium* could have been assigned to the second group. They further noted that the Triloculariidae was comprised of species that have bothridial accessory suckers and others without accessory suckers, and members of Dioecotaeniidae have bothridial loculi but lack accessory suckers. However, instead of assigning *Glyphobothrium* to either of these, they assigned it to a new family, Serendipeidae, along with *Duplicibothrium* and *Serendip*, because each has bothridia fused to each other, to the scolex, or to both, among other putative synapomorphies.

To support placement of *Glyphobothrium* within the Phyllobothriidae, Williams and Campbell (1977) argued that having multiloculated bothridia was more important in this aspect than having pedunculated bothridia. Confidence in Phyllobothriidae as a natural group remained until at least the studies of Brooks and McLennan (1993), who recognized that the family was not monophyletic. Caira et al. (1999a, 1999b) empirically tested that hypothesis and also could not support the monophyly of Phyllobothriidae, nor was it supported by Healy et al. (2009) or Ruhnke (2011). Although the idea of what it means to be a "phyllobothriid" is still with us, we are at the point, as declared by Ruhnke (2011; pg. 1), that "Of the valid genera historically associated with the family, only *Phyllobothrium* is considered to be an unambiguous member of the family." Caira et al. (1999a) did not assign *Glyphobothrium* to that family, although the study at least did identify it as a "tetraphyllidean (phyllobothriid)" (Caira et al. 1999a; Fig. 87). Based on the new information presented herein and the discussions above, we are unable to assign *Glyphobothrium* to that family.

With the discovery that *Glyphobothrium* has a anterior bothridial sucker, the suite of bothridial characters of the genus does not fit in Serendipeidae, and even a cursory comparison with specimens of *Serendip*, suggests that, while Serendipeidae is ideal for *Serendip*, the other members of that family now seem rather divergent from the type genus (*Serendip*). The members of *Serendip* have a scolex that is plate-like, comprised of four triangular bothridia without pedicels, not fused to the scolex, bothridia subdivided by septa extending radially but not dividing the bothridia into distinct loculi (Brooks and Barriga 1995; Monks et al. 2015). Furthermore, Dioecotaenniidae, with the single member *Dioecotaenia cancellata* (Linton, 1890) Schmidt 1969, proposed as putative sister group to Serendipeidae by Brooks and Barriga (1995), has a scolex with oval bothridia on short pedicels, each divided into 21 loculi, and lacking accessory suckers (Schmidt 1969). Based on the diagnosis and the suite of characters that *Glyphobothrium* possesses, we are convinced that it now is inappropriate to place *G. zwerneri* within that family; as well, a close relationship between *Dioecotaenia* Schmidt 1969 and *Glyphobothrium* has not been supported in phylogenetic analyses (Caira et al. 1999a; Caira et al. 2001). Particularly, although scolex types always were divergent in Serendipeidae, the presence of bothridial suckers marks *Glyphobothrium* as unacceptable for that family. We therefore, create a new family for this group.

Glyphobothriidae fam. nov.

Diagnosis.—Tetraphyllidea. Scolex comprising four elongate bothridia, bothridia fused lengthwise to spherical scolex or dorsal and ventral bothridia fused into two pairs. Each bothridium subdivided into loculi by horizontal septal borders, not subdivided. Longitudinal muscle bundles run continuously throughout the length of acetabulum (*sensu* Caira et al. 1999a). Scolex lacking myzorhynchus, hooks, and apical organ; accessory sucker on bothridia present. Cephalic

peduncle present or absent. Strobila weakly craspedote. Testes distributed in two irregular dorso-ventral fields, overlapping with ovary throughout entire length and extending into postovarian field. Genital pore in anterior third of proglottid, marginal to submarginal, irregularly alternating. Genital atrium present, deep, with vagina and cirrus sac joined to atrium medially. Cirrus weakly developed in terminal proglottids. Ovary consists of multiple lobes radiating in all directions from a central ovarian isthmus. Uterus weakly developed in terminal proglottids. Vitellaria follicular, converging in dorsal and ventral fields, except at level of ovary and cirrus-sac. Parasites of cownose stingrays (*Rhinoptera* spp.).

Type genus.—Glyphobothrium Williams and Campbell, 1977.

Other genera.—Duplicibothrium Williams and Campbell, 1978

Differential Diagnosis

In the description of *G. zwerneri*, Williams and Campbell (1977) noted that the apex of the scolex had a "small pit" that resembled a vestigial sucker, but no other suckers were described or depicted in the figures. No evidence of a pit or vestigial sucker could be seen on any of the specimens examined. However, an anterior accessory sucker is evident on each bothridium of the paratypes deposited in the HWML and our specimens from Campeche (Fig. 1). The bothridia of some of our specimens appeared to be slightly detached at the anterior end, slightly more than the bothridia of *G. zwerneri* and of *Prosobothrium* sp. in the figures of Caira et al. (1999a; their figures 59–60). It is unclear whether this condition (slightly spread apart) in our specimens is normal or if it was caused by a possible but unintentional compression of the scolex during collection and processing.

Williams and Campbell (1977) considered the ovary of their specimens to be bi-lobed in dorsal view and tetra-lobed (i.e., X-lobed) in cross section. However, their figures and our observations do not support that. Caira et al. (1999a) coded *Glyphobothrium* as having an H-shaped ovary (i.e., two lateral lobes connected by a median isthmus) in dorsal view, but the figures of Williams and Campbell (1977), our own observations of the paratypes and of the new material, confirms that the ovary consists of multiple lobes radiating in all directions from a central ovarian isthmus (Character 108, state "4" of Caira et al. 1999a).

In addition to the fusion of the septate bothridia with each other (in *Dioecotaenia*, *Duplicibothrium*, and *Serendip*), Brooks and Barriga (1995) indicated that having the testes arranged in two layers and having post-ovarian testes are synapomorphies for the Serendipeidae. In that diagnosis, Brooks and Barriga (1995) established Serendipeidae as having X-shaped ovaries with lobes that are digitiform—in *Serendip*, the cross-sections that they depicted in their figures (Brooks and Barriga 1995; Fig. 9) could be interpreted thusly, but they are better described as consisting of multiple lobes radiating in all directions from a central ovarian isthmus (also see Monks et al. 2015), the same condition that is present in *Glyphobothrium* (Williams and Campbell 1977; Fig. 4). As well, *Glyphobothrium* also has testes arranged in two layers and post-ovarian testes.

DISCUSSION

The usefulness of phylogenetic trees as predictive tools has come to the point of being common knowledge. Cladograms, as phylogenetic hypotheses, may be used more often for predictions of biographic distributions and for identifying potentially important species in the field of health and the problem of emerging infectious diseases (Brooks 2000; Brooks and Hoberg 2006; Brooks et al. 2014), but they also contain predictions about the characters of the taxa included therein. However, morphologists often do not make use of this same predictive strength (Brooks et al. 2006) in the search for and identification of characteristics that have not been described for particular species.

Our placement of *Duplicibothrium* in Glyphobothriidae is based on the use of the predictive value of the phylogenetic hypothesis presented by Caira et al. (1999a) and Caira et al (2001). We first considered potential synapomorphies for the putative family, and for this we consulted the most recent and most com-

prehensive phylogenetic hypothesis for the tetraphyllidean parasites of elasmobranchs using morphological data, that of the aforementioned studies. Because their study focused on higher-level relationships, Caira et al. (1999a) did not discuss many of the features that identify *Glyphobothrium* as sister to *Duplicibothrium*, or those that might support the recognition of the two genera in a new family.

In that hypothesis, *Glyphobothrium* and *Duplicibothrium* are on a branch (Caira et al. 1999a; figure 87) supported by nine character changes, although these cannot be demonstrated as synapomorphies because the clade to which the two taxa are members is a soft polytomy (unresolved because of lack of included data). The character states, phrased as they were presented in Caira et al. (1999a), shared by the two taxa are: horizontally-oriented loculi that are not subdivided into separate sub-loculi (Character 26; State 1); the number of rows of loculi on the acetabulum greater than three (27; 4); external horizontal septal borders between loculi on acetabulum not present (29; 0); the cephalic peduncle present (41; 1) (although more recently Ruhnke et al. 2000, described *D. paulum* Ruhnke, Curran, and Holbert, 2000 as lacking a cephalic peduncle); armature on the cephalic peduncle not present (42; 0); segmental (*sic*- i.e., proglottid) margins craspedote only (83; 1); number of layers of testes in cross-section anterior to cirrus-sac greater than one (91; 1); cirrus sac shape straight (96; 0); and, the vitelline elements are distributed circum-segmental (*sic*- i.e., proglottid, as above). To these we add the presence of a bothridial sucker (22; 1) (Fig. 2), longitudinal bundles of muscles in the bothridia that run continuously throughout the length of the acetabulum (30; 1) (Fig. 2), and the ovary consisting of multiple digitiform lobes radiating in all directions from a central ovarian isthmus (109; 2). Of course, several of these features are shared with other members of the putative clade and some with distantly related taxa as homoplasies. The complete recognition of synapomorphies for the family must await a study that will resolve the relationships of the 14 members the clade (Fig. 3); however, characteristics of the internode/hypothetical ancestor of the family Glypobothriidae fam. nov. are presented in Figure 3. The internode before the branching off point that represents the hypothetical ancestor of Glypobothriidae (indicated by the number 89), not an actual entity, is inferred to exhibit the traits shared among the taxa above it (*Glyphobothrium* and *Duplicibothrium*) (Prendini 2001).

Species of *Duplicibothrium* currently are diagnosed as not having an apical bothridial sucker, but there are similarities between the two genera (*Glyphobothrium* and *Duplicibothrium*), at least from the published descriptions, that led to our reevaluation of bothridial structures in those taxa. In both the drawings and the SEM micrographs of Ruhnke et al. (2000) there is what we interpret as a weakly developed anterior sucker, and in the micrographs the vertical divisions of the bothridia appear to be like those in *Glyphobothrium*. We examined specimens of *D. minutum*, *D. cairae* Ruhnke, Curran, and Holbert, 2000, and *D. paulum* and observed weak but definite anterior suckers on the bothridia of each specimen (Fig. 2).

Caira et al. (1999a) considered, and argued, that in *Glyphobothrium* the division of the horizontal loculi by the internal muscle bundles is not homologous with the acetabular boundaries that demark each loculus; i.e., they are products of the internal muscle bands that uplift the floor of the loculi, but are not true boundaries. The scanning electron micrographs of Caira et al. (1999a) are not very helpful in that aspect because the scolex is not in its natural form, but our observation that the horizontal septal borders in *G. zwerneri* are not divided completely by the longitudinal muscles bands supports that argument.

Ruhnke et al. (2000) described *D. cairae* and *D. paulum* as having horizontal septal borders with longitudinal divisions. However, the SEMs of Ruhnke et al. (2000) suggest that the vertical divisions of the horizontal loculi of species of *Duplicibothrium* also are the result of longitudinal bands of muscle because they do not completely divide the horizontal loculi as true boundaries. Looking only at their figures (Ruhnke et al. 2000; Figs. 1–16), the resemblance between the bothridia of the members of the two genera is remarkable, despite the bothridia of one being almost completely attached to the scolex and the others fused to each other dorsally and ventrally. The structures dividing the loculi vertically are difficult to draw in order to show the same information as in the micrographs; our line drawing fails completely in the elucidation of these structures. This resemblance of *Duplicibothrium* to

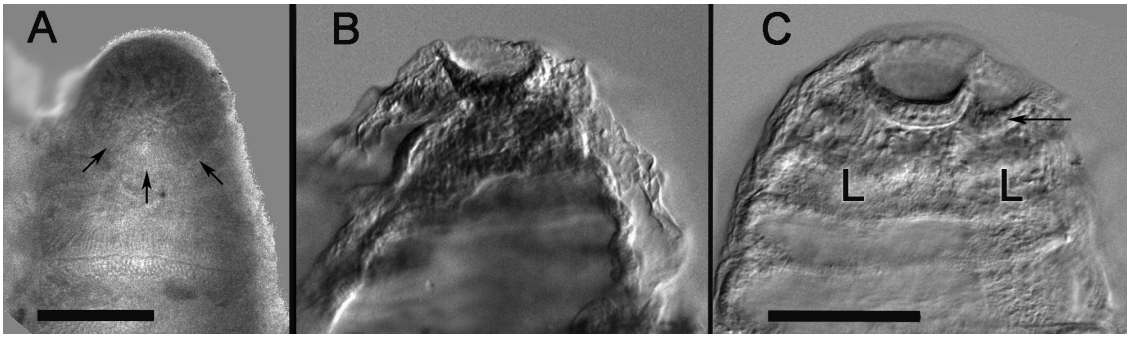

Figure 2. Three species of *Duplicibothrium* Williams and Campbell, 1978 showing the apical sucker and the longitudinal muscle bands. A. *Du. minutum* Williams and Campbell, 1977 (HWML-20884); arrows indicate the postermost limit of the apical sucker. Scale bar = 50 µm. B. *Du. cairae* Ruhnke, Curran, and Holbert, 2000 (HWML-15276). C. *Du. paulum* Ruhnke, Curran, and Holbert, 2000 (HWML-15276); arrow indicates lateral sucker/loculus, "L" indicates longitudinal muscle band. Scale bar for figure A = 50 µm, scale bar for figures B and C = 200 µm.

Glyphobothrium provides another putative synapomorphy for the recognition of the two taxa as members of Glyphobothriidae, although that has not been tested empirically.

Call (2007), in an unpublished thesis, informally redescribed *D. minutum* based on specimens deposited in the HWML (HWML-20884) and the USNPC (Holotype, USNPC-74724; paratypes, USNPC-74725–74726); 13 voucher specimens identified only as USNPC-00000, LRP-00000, and KUNHM-00000) in which he confirmed the presence of longitudinal muscle bundles, and they are depicted in his SEMs (Call 2007; Fig. 11, pg. 48) (KUNHM= Kansas University Natural History Museum; LRP = Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, U.S.A.). The presence of longitudinal muscle bundles was confirmed by Call (2007) in two other species that he described informally (*D. karenae* and *D. mergacephalum*; *nomen nuda*), but which, unfortunately, have not yet been published. Ruhnke et al. (2000) described *D. cairae* and *D. paulum* as having longitudinal muscle bundles, although those authors interpreted these as divisions that formed longitudinal septal borders. However, in their SEMs (Ruhnke et al. 2000; Figs. 6, 14) the longitudinal divisions appear to be homologous with those of *G. zwerneri*. Examination of specimens *D. cairae*, *D. minutum* (HWML-20884), and *D. paulum* (CNHE-3846–3849; HWML-15275–15278) support the recognition of the presence of horizontal bothridial loculi for each species that are undivided by septa and the presence of longitudinal muscle bundles (Fig. 2), all synapomorphies for Glyphobothriidae, as do the figures presented in Ruhnke et al. (2000). The drawings and SEMs of Call (2007) depict the longitudinal muscle bands in all of the species included in his study, including *D. minutum*. Those figures suggest that the divisions of the posteriormost loculi may be divided by the same type of muscular bands rather than locular walls, although cross-sections of the scoleces of these species would be necessary for evaluation of this characteristic.

In the literature there exists ample support for the existence of the structures mentioned above. Examination of specimens of *D. minutum* (HWML-20884), *D. cairae* (CNHE-3846, 3847; HWML-15275, 15276), and *D. paulum* (CNHE-3848, 3849; HWML-15277, 15278) confirmed the presence of a bothridial sucker (Fig. 2). The SEMs and optical photomicrographs of plerocercoids of *D. minutum* presented by de Buron et al. (2013) (identity confirmed by their use of sequences of 28S rRNA) makes their similarity with *Glyphobothrium* even more obvious. First, the bothridial suckers (identified by previous authors as apical loculi) are present (de Buron et al. 2013; Figs. 1–2), and in the plerocercoid the four bothridia are completely fused to the scolex rather that just fused in pairs, pointing to another putative synapomorphy for the members of the Glyphobothriidae. An accessory sucker, as dis-

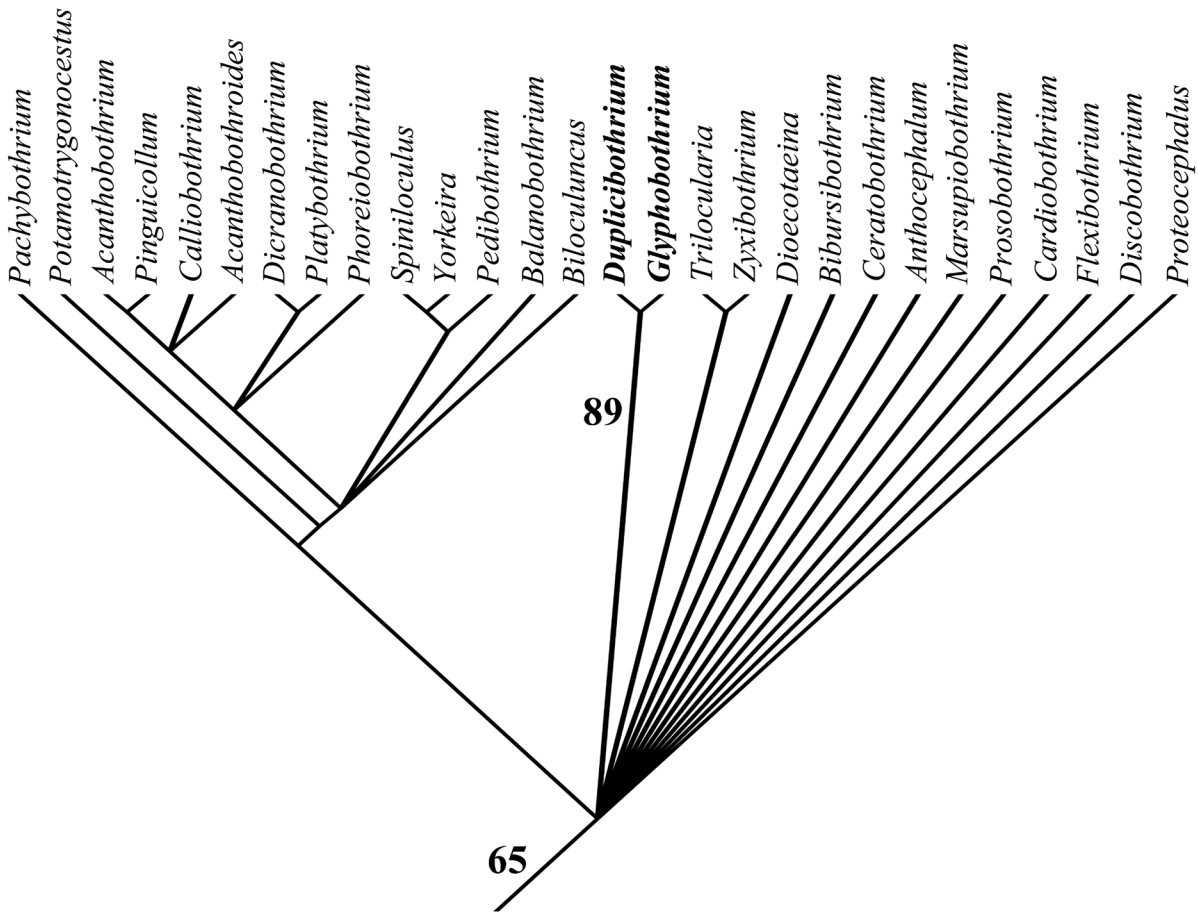

Figure 3. Clade (unresolved; soft polytomy) containing the branch with *Duplicibothrium* and *Glyphobothrium* as sister taxa. The internode/hypothetical ancestor of the family Glyphobothridae n. f. is indicated by the number 89 and the number 65 indicates the hypothetical ancestor of the clade that contains the new family (numbers assigned by Mesquite). Characters and states, taken from Caira et al. (1999b), are given as the number of the character as designated in that work, with the character state at the node as a superscript; * indicates values modified according to the emended descriptions. Figure redrawn from Caira et al. (1999b).

Node 65: 1^{0}, 2^{0}, 3^{0}, 4^{0}, 5^{9}, 6^{9}, 7^{9}, 8^{9}, 9^{9}, 10^{9}, 11^{0}, 12^{9}, 13^{9}, 14^{9}, 15^{9}, 16^{9}, 17^{9}, 18^{9}, 19^{3}, $20^{1,3}$, 21^{0}, 22^{0}, 23^{9}, 24^{9}, $25^{0,9}$, 26^{0}, 27^{0}, 28^{9}, 29^{9}, 30^{0}, 31^{9}, 32^{0}, 33^{0}, 34^{0}, 35^{0}, 36^{0}, 37^{0}, 38^{0}, 39^{0}, 40^{0}, 41^{0}, 42^{9}, 43^{0}, 44^{0}, 45^{0}, 46^{9}, 47^{9}, 48^{9}, 49^{9}, 50^{9}, 51^{9}, 52^{9}, 53^{9}, 54^{9}, 55^{9}, 56^{9}, 57^{9}, 58^{0}, 59^{2}, 60^{9}, 61^{9}, 62^{9}, 63^{9}, 64^{1}, 65^{2}, 66^{1}, 67^{2}, 68^{9}, 69^{9}, 70^{9}, 71^{9}, 72^{9}, 73^{9}, 74^{9}, 75^{9}, 76^{9}, 77^{9}, 78^{9}, 79^{0}, 80^{2}, 81^{0}, 82^{0}, 83^{0}, 84^{2}, 85^{0}, 86^{0}, 87^{1}, 88^{1}, 89^{0}, 90^{2}, 91^{0}, 92^{0}, 93^{0}, 94^{0}, 95^{0}, 96^{1}, 97^{1}, 98^{0}, 99^{0}, 100^{0}, 101^{0}, 102^{0}, 103^{0}, 104^{1}, 105^{0}, 106^{0}, 107^{0},108^{0}, 109^{1}, 110^{2}, 111^{0}, 112^{3}, 113^{1}, 114^{0}, 115^{2}, 116^{0}, 117^{2}, 118^{0}, 119^{1}, 120^{0}.

Node 89: 1^{0}, 2^{0}, 3^{0}, 4^{0}, 5^{9}, 6^{9}, 7^{9}, 8^{9}, 9^{9}, 10^{9}, 11^{0}, 12^{9}, 13^{9}, 14^{9}, 15^{9}, 16^{9}, 17^{9}, 18^{9}, 19^{3}, $20^{0,1,3}$, 21^{0}, 22^{1}*, 23^{9}, 24^{9}, 25^{9}, 26^{1}, 27^{4}, 28^{9}, 29^{0}, 30^{1}*, $31^{0,1,9}$, 32^{0}, 33^{0}, 34^{0}, 35^{0}, 36^{0}, 37^{0}, 38^{0}, 39^{0}, 40^{0}, 41^{1}, 42^{0}, 43^{0}, 44^{0}, 45^{0}, 46^{9}, 47^{9}, 48^{9}, 49^{9}, 50^{9}, 51^{9}, 52^{9}, 53^{9}, 54^{9}, 55^{9}, 56^{9}, 57^{9}, 58^{0}, 59^{2}, 60^{9}, 61^{9}, 62^{9}, 63^{9}, $64^{0,1}$, 65^{2}, 66^{1}, 67^{2}, 68^{9}, 69^{9}, 70^{9}, 71^{9}, 72^{9}, $73^{0,9}$, $74^{2,9}$, 75^{9}, 76^{9}, 77^{9}, 78^{9}, 79^{0}, 80^{2}, 81^{0}, 82^{0}, 83^{1}, 84^{2}, 85^{0}, 86^{0}, 87^{1}, 88^{1}, 89^{0}, 90^{2}, 91^{1}, 92^{0}, 93^{0}, 94^{0}, 95^{0}, 96^{0}, 97^{1}, 98^{0}, 99^{0}, 100^{0}, 101^{0}, 102^{0}, 103^{0}, 104^{1}, 105^{0}, 106^{0}, 107^{0},108^{0}, 109^{2}*, $110^{0,2,3}$, 111^{0}, 112^{3}, 113^{1}, 114^{2}, 115^{2}, 116^{0}, 117^{2}, 118^{0}, 119^{1}, 120^{0}.

cussed above in adults of *Glyphobothrium*, also could be identified. These larvae also are similar to adults of *Glyphobothrium* in having a spherical scolex, with the four bothridia completely attached in one specimen and with the anterior portion extending above the spherical scolex in another (their Figs 1 and 2, respectively). The longitudinal muscle bundles are not obvious in these immature specimens, but that may be because they only become well developed in the adults.

Caira et al. (1999a) coded *G. zwerneri* as lacking an accessory organ (their character 11; 0), having sessile bothridia (char. 20; 0), possessing bothridia with a single column of loculi (char. 26; 1) with longitudinal muscle bundles (char. 30; 1), among other characters not discussed herein. Their SEMs (Caira et al. 1999a, Fig. 75) do not provide any other new information about the structure of the scolex. Although the sagittal sections presented by Caira et al. (1999a, Fig. 59) and Williams and Campbell (1977, Fig. 7) support the recognition of an apical depression, the area between the bothridia is more open in our specimens (Fig. 1) but, as mentioned above, we don't know if this is an artifact of collection. However, histological studies of better-fixed specimens would yield further information.

Specimens of *Tiarabothrium javanicum* Shipley and Hornell, 1906 (*species inquirendum*, *sensu* Ruhnke 2011) were depicted as having a spherical scolex and bothridia with horizontal loculi that are superficially divided by muscle bundles that begin in the peduncle (Shipley and Hornell 1906; Plate IV, Figs. 65–68). These specimens have been lost, but they clearly share scolex characteristics with *Glyphobothrium* and should be assigned to this family, with appropriate taxonomic changes to the diagnosis of the family, if this can be confirmed by the collection of new material. Young (1956), however, thought the genus might be a synonym of *Echeneibothrium* van Beneden, 1850 (as did Euzet 1994). Ruhnke et al. (2000) suggested that *T. javanicum* might belong to Serendipeidae. However, based on Euzet (1994), it is considered to be a *species incertae sedis* and Ruhnke et al (2000) and Ruhnke (2011), in his study of Phyllobothriidae, considered *T. javanicum* to be a *genus inquirendum*. Shipley and Hornell (1906) described *T. javanicum* as having four bothridia, mostly fused to the spherical scolex, with the anterior ends able to separate slightly from the scolex (i.e., "standing out" [*sic*]; Shipley and Hornell 1906, pg. 67), similar to members of Glyphobothriidae and to the specimens of de Buron et al. (2013). Resolution of the taxonomic status of this genus depends on the recovery of the original specimens or the finding of new material.

Of course, with the discovery of new structures and the reinterpretation of known ones, taxa that have or lack those characteristics should be emended or redescribed. Some of the specimens of the species mentioned herein that were deposited in museum collections are not available for study at this time, or they have been lost, although available material and published descriptions were more than sufficient to confirm the presence of the structures used to support our arguments. It is regrettable is that the specimens studied by Call (2007) have not been described formally. This combination of events makes a full redescription of *Duplicibothrium* difficult pending availability of fresh material, particularly those specimens that have been lost to science. However, this is not to say that reevaluation of taxonomic concepts and validity of species cannot be performed based solely on publications; if we cannot trust the publications of competent systematists then there is no reason even to publish, and even less for peer review, but there is no doubt that arguments are stronger when specimens have been deposited. As always, when systematists are struggling to understand the totality of the concept of a taxonomic unit, the one obvious conclusion, as least to the authors of this work, is that "*Ea qua scimus sunt pars minima eorum, qua ignoramus*" (Linnaeus 1758; pg. 823).

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Addresses of authors:

SCOTT MONKS

Universidad Autónoma del Estado de Hidalgo
Centro de Investigaciones Biológicas
Apartado Postal 1-10, CP. 42001
Pachuca, Hidalgo, México
scottmonks@hotmail.com

GRISELDA PULIDO-FLORES

Universidad Autónoma del Estado de Hidalgo
Centro de Investigaciones Biológicas
Apartado Postal 1-10, CP. 42001
Pachuca, Hidalgo, México
gpflores6@hotmail.com

SCOTT LYELL GARDNER

Harold W. Manter Laboratory of Parasitology,
University of Nebraska State Museum, and School of Biological Sciences
W-529 Nebraska Hall
University of Nebraska-Lincoln
Lincoln, Nebraska, USA 68588-0512
slg@unl.edu

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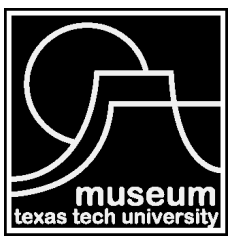

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