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# SERENDIP DEBORAHAE N. GEN. AND N. SP. (EUCESTODA: TETRAPHYLLIDEA: SERENDIPIDAE N. FAM.) IN RHINOPTERA STEINDACHNERI EVERMANN AND JENKINS, 1891 (CHONDRICHTHYES: MYLIOBATIFORMES: MYLIOBATIDAE) FROM SOUTHEASTERN ECUADOR

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ABSTRACT: Cestodes collected in spiral valves of Rhinoptera steindachneri from the southern coast of Ecuador represent an undescribed species of Tetraphyllidea. The new species has bothridia possessing septa but lacking apical suckers. It is diagnosably distinct from all other tetraphyllidean genera by possessing a scolex comprising 4 triangular bothridia that are fused together forming a platelike structure, each of which is subdivided by 2 simple and 1 bifurcating septa radiating from its base and ringed by marginal loculi; therefore, a new genus is proposed for it. By exhibiting some degree of bothridial fusion, testes arranged in 2 layers in the proglottis and postovarian testes, the new species appears to be a member of a clade containing Dioecotaenia, Duplicibothrium, and Glyphobothrium. The new species possesses vitelline fields that converge dorsally in each proglottis, except for the ovarian and terminal genitalia areas, a feature that has been reported previously only in Duplicibothrium and Glyphobothrium. Furthermore, Duplicibothrium and Glyphobothrium, like the new species, are markedly protandric. Therefore, we propose that Duplicibothrium, Glyphobothrium, and the new species comprise the sister group of the Dioecotaeniidae, and propose a new family name for the clade. Tritaphros is rejected as a possible sister group for the clade; suggested alternatives include some species of Caulobothrium, Rhodobothrium, or some members of the Phyllobothrium centrurum group.

Nothing is known about the parasite fauna of elasmobranchs inhabiting the coast of Ecuador. During the initial stages of an inventory of the parasite biodiversity of Ecuadorian elasmobranchs, stingrays were collected from Puerto Bolivar, Puerto Jelí, and Puerto Hualtaco, Provincia de el Oro. Among the parasites collected were specimens of an undescribed and unusual tetraphyllidean eucestode, which we describe and discuss herein.

## MATERIALS AND METHODS

Stingrays were collected by professional fishermen in bottom trawls using bag seines. Cestodes were relaxed in sea water, killed in a relaxed condition with hot water, fixed immediately with AFA, and stored in 70% ethanol. Whole mounts were stained with Mayer's hematoxylin. Serial cross sections of proglottides were cut 7  $\mu$ m thick, stained with Mayer's hematoxylin, and counterstained with eosin. Whole mounts and cross sections were mounted in Canada balsam. All measurements are in  $\mu m$  unless otherwise noted; n = number of specimens examined or measured, MEPN refers to Museo de la Escuela Politecnica Nacional, Quito, Ecuador; MNHG refers to Museum of Natural History, Geneva, Switzerland.

### Serendipidae n. fam.

Diagnosis: Eucestoda; Tetraphyllidea. Scolex comprising 4 rounded or triangular bothridia, each subdivided by septa in various patterns; distinct loculi present or lacking. Bothridia exhibiting some degree of fusion to each other, to scolex, or both. Bothridial apical suckers lacking. Pedicels present or absent. Vestigial apical sucker embedded in tissues of scolex apex or apical pit may be present. Proglottides apolytic or anapolytic; markedly protandric. Testes in 2 or more layers in each proglottis. Postovarian testes present. Cirrus sac spherical to subspherical; cirrus armed. Genital pore preequatorial. Vagina passing anterior to cirrus sac. Ovary X-shaped in cross section; lobes digitiform. Vitellaria follicular, medullary, in 2 lateral fields extending length of proglottis ventrally; vitelline fields converging dorsally in each proglottis, except

for ovarian and terminal genitalia areas, may converge ventrally to form a circummedullary band. Parasites of myliobatid stingrays. Western Atlantic and eastern Pacific oceans.

Type genus: Serendip n. gen.

Other genera: Duplicibothrium Williams and Campbell, 1978; Glyphobothrium Williams and Campbell, 1977.

#### Serendip n. gen.

Diagnosis: Eucestoda; Tetraphyllidea. Scolex comprising 4 triangular bothridia, each subdivided by septa extending radially from base dividing bothridial face but not into distinct loculi, ringed with marginal loculi with thin velum. Bothridial apical suckers lacking. Bothridia fused to form a single platelike structure giving bothridial faces a dorsal rather than lateral aspect; pedicels lacking. Vestigial apical sucker embedded in tissues of scolex apex. Proglottides apolytic, protandric. Testes in 2 layers in each of 2 fields in each proglottid. Some testes postovarian in maturing proglottides, disappearing as ovary develops. Cirrus sac spherical; cirrus armed. Genital pore pre-equatorial. Vagina passing anteriorly to cirrus sac. Ovary X-shaped in cross section; lobes digitiform. Vitellaria follicular, medullary, in 2 lateral fields extending length of proglottis ventrally; vitelline fields converging dorsally in each proglottis, except for ovarian and terminal genitalia areas.

Type and only species: Serendip deborahae.

#### Serendip deborahae n. sp. (Figs. 1-11)

Description (based on 9 specimens, 8 whole mounts and 1 scolex prepared for scanning electron microscopy and strobila cut in serial cross sections): Strobila craspedote, apolytic, up to 60 mm long, composed of approximately 150 proglottides. Scolex 1.5-2.6 mm wide. Vestigial sucker embedded in apical tissue of scolex 106-219 long by 44-56 wide. Pedicels lacking. Bothridia 0.7-1.2 mm long by 0.8-1.6 mm wide, fused at anterior end into 2 pairs, at posterior end into single platelike, anteriorly directed, structure; subdivided by 2 simple and 1 bifurcating, radially diverging, muscular septa not dividing bothridial face into distinct loculi; margins of bothridia with small loculi and thin, contractile, velum-like membrane. Neck 6.9-9.6 mm long. Proglottides slightly craspedote. Immature proglottides wider than long. Mature proglottides squared. Terminal attached proglottides (n = 9) 272-856 long by 546-781 wide. Testes in 2 longitudinal fields and 2 layers extending length of proglottis, 64-116 in total; 3-8 preporal, 25-45 postporal, 36-63 aporal; 47-125 in diameter. Cirrus sac in anterior 1/3 of proglottis, 125-312 long by 156-281 wide, containing spined, eversible cirrus. Vas

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FIGURES 1-4. Scanning electron micrographs of Serendip deborahae n. gen., n. sp. 1. En face view of scolex. Scale bar =  $500 \mu m$ . 2. Enlargement of bothridial face showing the bifurcating and 1 of 2 simple septa. Scale bar =  $250 \mu m$ . 3. Lateral view of scolex. 4. Lateral view of scolex. Magnification of Figures 3 and 4 same as Figure 1.

deferens extensively coiled mostly on poral side of cirrus sac posterior to testes, with some coils aporal to cirrus sac; joining cirrus sac near posterior end. Genital pores alternating irregularly, 23–29% of total proglottis length from anterior end of terminal proglottid. Genital atrium shallow. Vagina anterior to cirrus sac and posttesticular, passing medially, curving posteriorly around aporal side of cirrus sac and around dorsal side of vas deferens. Vaginal sphincter prominent, at junction of genital pore. Ovary fan-shaped in frontal view, X-shaped in cross section, with digitiform processes extending laterally just beyond osmoregulatory ducts, 125–281 long by 375–562 wide. Maturing proglottides with 4–8 postovarian testes. Vitelline follicles medullary, lateral; follicles extending dorsolateral and ventrolateral to osmoregulatory ducts, extending ventrally from anterior extent of testicular fields to near posterior end of ovary, interrupted near genital pore, confluent dorsally except dorsal to ovary and terminal genitalia. Vitelline follicles 20–62 in diameter. Immature uterus a glandular sinuous sac extending ventrally from ovary to near anterior end of proglottis. Mature detached proglottides (n = 2) longer than wide, 1.47-1.84 mm long by 581-644 wide. Genital pore 20-23% of proglottis length from anterior end. Cirrus sac 375-406 long by 281 wide. Few disintegrating testes present. Ovary 344-456 long by 312-375 wide. Vitelline follicles 18-50 in diameter. Gravid detached proglottides not collected.

#### **Taxonomic summary**

*Type host: Rhinoptera steindachneri* Evermann and Jenkins, 1891 (Chondrichthyes: Myliobatiformes: Myliobatidae).

*Type locality:* vic. Puerto Bolivar, Provincia de el Oro, Ecuador. *Site of infection:* Middle  $\frac{1}{3}$  of spiral valve.



FIGURES 5-11. Serendip deborahae n. gen. and n. sp. 5. Schematic representation of a bothridium showing 2 simple and 1 bifurcating septa forming 5 subdivisions of bothridial face. 6. Mature attached proglottis. 7. Dorsal surface of mature proglottis, showing distinctive pattern of distribution of vitelline follicles. 8, 9. Cross sections of mature proglottis. 8. Mid-proglottis, showing testes in 2 layers. 9. Near posterior end of proglottis. 10. Mature detached proglottis. 11. Terminal genitalia. Upper scale bar refers to Figures 3-7; lower scale bar refers to Figures 8. o = Ovary, v = vitelline follicles, t = testes, u = uterus.

Specimens deposited: Holotype: MEPN. Paratypes: MEPN; MNHG no. INVE18254.

Symbiotype specimen: MEPN no. 4569.

*Etymology*: Schmidt (1974) noted that when he found *Dioecotaenia* in *Rhinoptera bonasus* and realized it belonged in its own family, he was tempted to name it *Serendip*, type genus of the family Serendipidae (serendipity) because he was looking for something else at the time he discovered *Dioecotaenia*. We discovered this cestode species living in a species of *Rhinoptera* while looking for something else, so we have named it *Serendip* to honor the memory of Gerald D. Schmidt. The species is named for Deborah A. McLennan.

### Remarks

The Tetraphyllidea lack much rigorous phylogenetic examination. Traditional classifications, e.g., Wardle and McLeod (1952), Euzet (1959), and Yamaguti (1959), divided the order into 2 families, the Phyllobothriidae and the Onchobothriidae, diagnosed on the basis of the presence (Onchobothriidae) or absence (Phyllobothriidae) of hooks associated with the bothridia. Regardless of its convenience, this scheme is weak because the absence of hooks is plesiomorphic (Brooks et al., 1991; Brooks and McLennan, 1993; Berman and Brooks, 1994) and thus not an appropriate character on which to base taxonomic groupings (Wiley et al., 1991). This may have led Schmidt (1986) to recognize the Dioecotaeniidae, comprising 1 genus with 2 species (which Schmidt placed in its own order) and the Triloculariidae, comprising 4 genera with 5 species (Berman and Brooks, 1994).

The "Phyllobothriidae" can be divided into cestodes that have bothridial apical suckers, but lack bothridial septa dividing the bothridial face into distinct loculi (e.g., Anthobothrium van Beneden, 1850; Calyptrobothrium Monticelli, 1893; Clistobothrium Dailey and Vogelbein, 1990; Clydonobothrium Euzet, 1956; Crossobothrium Linton, 1889; Echeneibothrium van Beneden, 1805; Monorygma Diesing, 1863; Orygmatobothrium Diesing, 1863; Rhodobothrium Linton, 1889; and the Phyllobothrium lactuca species group) and species that have bothridial septa dividing the bothridial face into distinct loculi but lack bothridial apical suckers (e.g., Caulobothrium Baer, 1948; Duplicibothrium Williams and Campbell, 1978; Glyphobothrium Williams and Campbell, 1977; Rhabdotobothrium Euzet, 1953; Rhinebothrium Linton, 1889; Rhinebothroides Mayes, Brooks, and Thorson, 1981; Tritaphros Lönnberg, 1889; and the Phyllobothrium centrurum species group). Notably, the Triloculariidae comprises species having bothridial septa and loculi, some possessing bothridial apical suckers (Trilocularia Olsson, 1867 and Escherbothrium Berman and Brooks, 1994) and some lacking them (Zyxibothrium Hayden and Campbell, 1981 and Pentaloculum Alexander, 1953), and the members of the Dioecotaeniidae have bothridial loculi but lack apical suckers.

The absence of bothridial septa by the first group of phyllobothriids is a plesiomorphic trait that renders the taxa a paraphyletic collection of undetermined phylogenetic relationships. Because it contains the paraphyletic *Phyllobothrium*, this group retains the appellation Phyllobothriidae pending a full phylogenetic analysis of the assemblage. The second group, including the Triloculariidae and Dioecotaeniidae, comprises those taxa that have bothridial septa and loculi. If the septa and loculi in all these taxa are homologous, and are nonhomologous with the bothridial loculi in many onchobothriids, the group would form a clade. The form and structure of the bothridia and the septa and loculi in each of the 3 groups are, however, diverse. Bothridia of the Triloculariidae are round to elongate with 3-5 loculi arranged in nonlinear patterns. In the Dioecotaeniidae, the bothridia are rounded with closepacked hexagonal loculi arranged as a central row of loculi surrounded by large marginal loculi. Glyphobothrium is similar to Dioecotaenia, but the loculi are round to squared rather than hexagonal. Duplicibothrium exhibits elongate bothridia with transverse septa anteriorly and a cupshaped posterior end with indistinct radially arranged septa. Finally, members that we will refer to as the "Rhinebothrium group" (e.g., Caulobothrium, Rhabdotobothrium, Rhinebothrium, Tritaphros, Rhinebothroides, and the Phyllobothrium centrurum group), have elongate bothridia with linearly arranged loculi. Members of the Dioecotaeniidae, Duplicibothrium, Glyphobothrium, and the Rhinebothrium group share another apparently derived trait, the absence of bothridial apical suckers. This may indicate that these taxa are more closely related to each other than either is to the Triloculariidae; however, 2 members of the Triloculariidae, Zyxibothrium and Pentaloculum, also lack bothridial apical suckers. The presence or absence of bothridial apical suckers, by itself, may not be a strong indicator of phylogenetic relationship, or the Triloculariidae may be paraphyletic.

Serendip deborahae has bothridia possessing septa, but lacking apical suckers. This would seem to place it with the Dioecotaeniidae, Duplicibothrium, Glyphobothrium, and Rhinebothrium group. Dioecotaenia, Duplicibothrium, and Glyphobothrium exhibit several traits that appear to be apomorphic among the Tetraphyllidea, suggesting a relationship with Serendip. They exhibit some degree of fusion of the bothridia, either with each other (Dioecotaenia, Duplicibothrium, Serendip) or with the scolex (Glyphobothrium). They also possess testes arranged in 2 layers in the proglottis and some postovarian testes. We believe these 3 traits indicate that Dioecotaenia, Duplicibothrium, Serendip, and Glyphobothrium form a clade. Serendip exhibits vitelline fields that converge dorsally in each proglottis, except for dorsal to the terminal genitalia, a feature that has been reported previously only in Duplicibothrium and Glyphobothrium (Williams and Campbell, 1977, 1978). Like Glyphobothrium, Serendip also lacks vitelline follicles dorsal to the ovary. The radial pattern and arrangement of the bothridial septa, lack of distinct loculi, triangular shape of the bothridia, and fusion of the bothridia to form a single platelike structure with dorsal aspect in S. deborahae, however, differ from previously known tetraphyllideans. In this regard, S. deborahae is distinct from Duplicibothrium and Glyphobothrium, supporting the designation of a new genus for it. Furthermore, Duplicibothrium and Glyphobothrium, like Serendip, are markedly protandric; Dioecotaenia exhibits separate male and female strobila. Of secondary significance is the observation that all members of these taxa inhabit only myliobatid stingrays. Therefore, we propose that Duplicibothrium, Glyphobothrium, and Serendip form a clade that is the sister group of Dioecotaenia. Because its sister group is recognized at the family level, we propose a new family name for the clade containing Duplicibothrium, Glyphobothrium, and Serendip.

#### DISCUSSION

Brooks (1982) suggested that if the scolex of *Dioecotaenia* evolved as a result of progressive addition of loculi, a species

like *Tritaphros retzii*, a member of the *Rhinebothrium* group having 3 bothridial loculi, would be its sister species. Given the bothridial morphology of the members of the Serendipidae + Dioecotaeniidae, this assumption is not warranted, and the search for the sister group of this clade begins anew. Below we list some possibilities.

Members of the Dioecotaeniidae + Serendipidae possess cirrus sacs in the anterior half, often in the anterior <sup>1</sup>/<sub>4</sub>, of the proglottis, a trait that is unusual among unarmed tetraphyllideans with loculi and no bothridial apical suckers (although common among members of the basal "Phyllobothriidae"). Species of Caulobothrium, from the Rhinebothrium group, exhibit this trait. At least some species of Caulobothrium also have postovarian testes or testes extending posteriorly between the ovarian lobes (Brooks et al., 1981). These traits may indicate a phylogenetic relationship between Caulobothrium and the Dioecotaeniidae + Serendipidae, although preliminary phylogenetic analysis indicates that Caulobothrium is paraphyletic, comprising 2 clades within Rhinebothrium (Brooks, unpubl. data). In addition, members of the Dioecotaeniidae + Serendipidae possesses marginal bothridial loculi, as do members of the Phyllobothrium centrurum group and Rhinebothroides. Marginal loculi are lacking in all members of the Rhinebothrium group, including all species assigned to Caulobothrium.

Finally, species of *Rhodobothrium* Linton, 1889 also inhabit myliobatid stingrays, are markedly protandric, have testes lying in 2 layers in each proglottis, and possess postovarian testes, suggesting a possible relationship with the Dioecotaeniidae + Serendipidae. Like *S. deborahae*, they possess vestigial suckers embedded in the scolex apex (the "apical pit" of *Glyphobothrium* may also be a vestigial apical sucker). Species of this group lack bothridial septa or marginal loculi, although the bothridial faces are covered with "numerous convolutions forming an irregular pattern" (Campbell and Carvajal, 1979; Mayes and Brooks, 1981). They lack the vitelline configuration diagnostic for the Serendipidae and show no sign of bothridial fusion. If *Rhodobothrium* is a member of the Dioecotaeniidae + Serendipidae clade, it would be the sister group of the other 2.

Phylogenetic systematic studies begin with Hennig's Auxiliary Principle (Hennig, 1966; Brooks and McLennan, 1991, 1993; Wiley et al., 1991) that similarity equals homology. Such initial assumptions are corroborated by congruence with other characters in a phylogenetic analysis and are falsified by incongruence. Testing and supporting the above hypotheses of homology and classification consistent with them will require a larger suite of characters than the few discussed above.

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