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Escherbothrium molinae n. gen. et n. sp. (Eucestoda: Tetraphyllidea: Triloculariidae) in *Urotrygon chilensis* (Chondrichthyes: Myliobatiformes: Urolophidae) from the Gulf of Nicoya, Costa Rica

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ESCHERBOTHRIUM MOLINAE N. GEN. ET N. SP. (EUCESTODA: TETRAPHYLLIDEA: TRILOCULARIIDAE) IN *UROTRYGON CHILENSIS* (CHONDRICHTHYES: MYLIOBATIFORMES: UROLOPHIDAE) FROM THE GULF OF NICOYA, COSTA RICA

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ABSTRACT: Cestodes collected in spiral valves of the stingray *Urotrygon chilensis* from the Pacific coast of Costa Rica represent an undescribed species of Tetraphyllidea. By possessing more than 2 loculi as well as an apical sucker on each bothridium, the new species is diagnosably distinct from all other tetraphyllidean genera; therefore, a new genus is proposed for it. The new species also possesses globular structures irregularly arranged on the surface of the bothridia. We found similar structures on the bothridial faces of *Trilocularia acanthiaevulgaris*, possibly indicating phylogenetic relationships with the new species. This possibility is enhanced by the observation that the bothridia of *T. acanthiaevulgaris* comprise 2 loculi and an apical sucker, rather than 3 loculi.

Little is known about the parasite fauna of elasmobranchs inhabiting the Pacific Ocean coast of Central America. During the initial stages of an inventory of the parasite biodiversity of Costa Rican elasmobranchs, stingrays were collected from several locations in the Gulf of Nicoya and the Guanacaste coast. Among the parasites collected were specimens of an undescribed and unusual tetraphyllidean eucestode, which we describe and for which we propose a new genus.

MATERIALS AND METHODS

Stingrays were collected at night using a beach seine. 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 proglottids and scolices were cut 7 μm thick, stained with Mayer's hematoxylin, and counterstained with eosin. Whole mounts and cross sections were mounted in Canada Balsam. All measurements are in micrometers unless otherwise noted (n = sample size; $\bar{x}$ = mean; SD = standard deviation). USNM Helm. Coll. refers to U.S. National Museum Helminthological Collection, Beltsville, Maryland.

Escherbothrium n. gen.

Diagnosis: Eucestoda: Tetraphyllidea: Triloculariidae. Scolex with 4 pedicellated bothridia. Each bothridium with apical sucker and muscular septa dividing adherent surface of bothridia into loculi. Adherent surface of bothridia with irregular number of irregularly spaced spherical protrusions. Ovary X-shaped in cross section.

Type and only species: Escherbothrium molinae.

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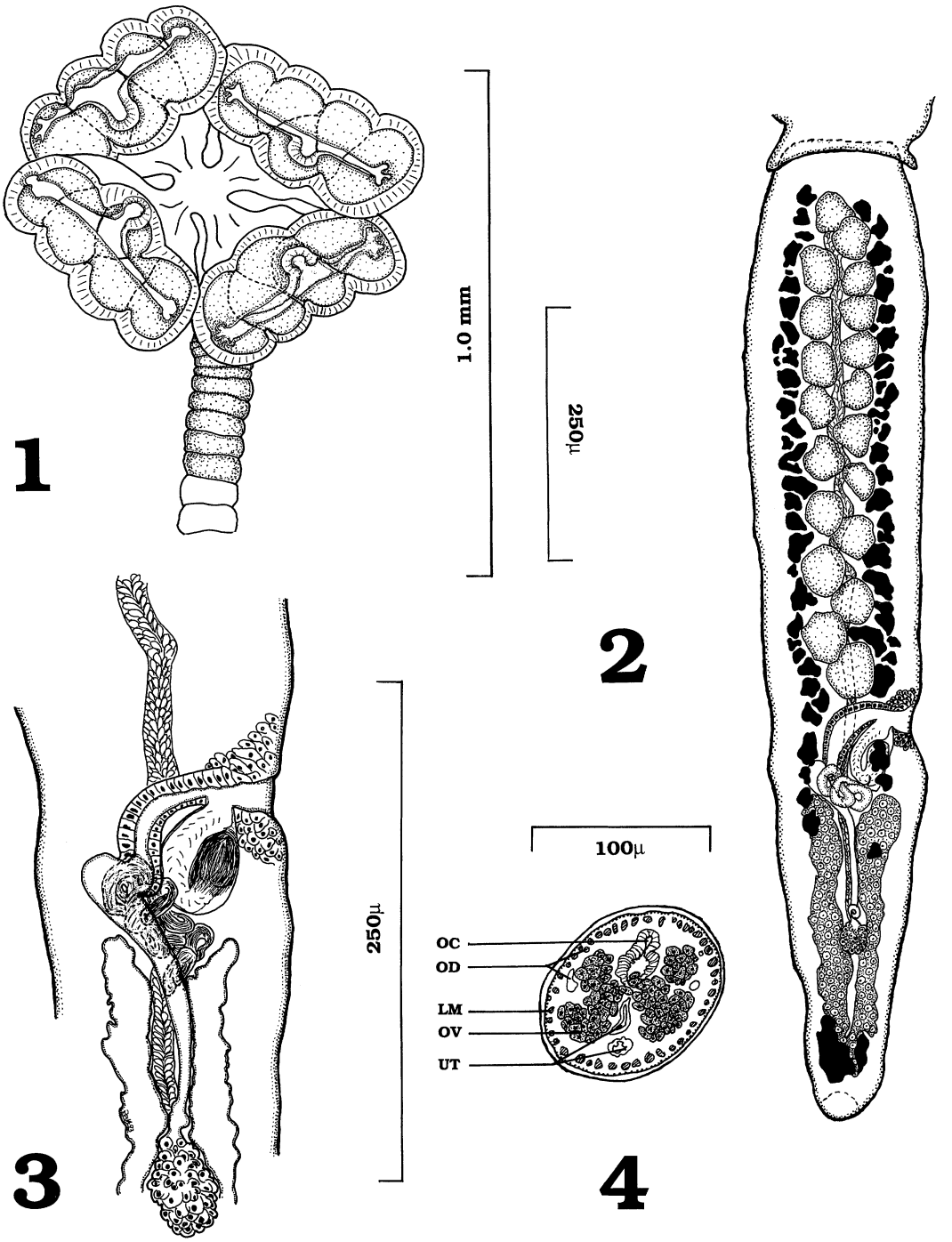
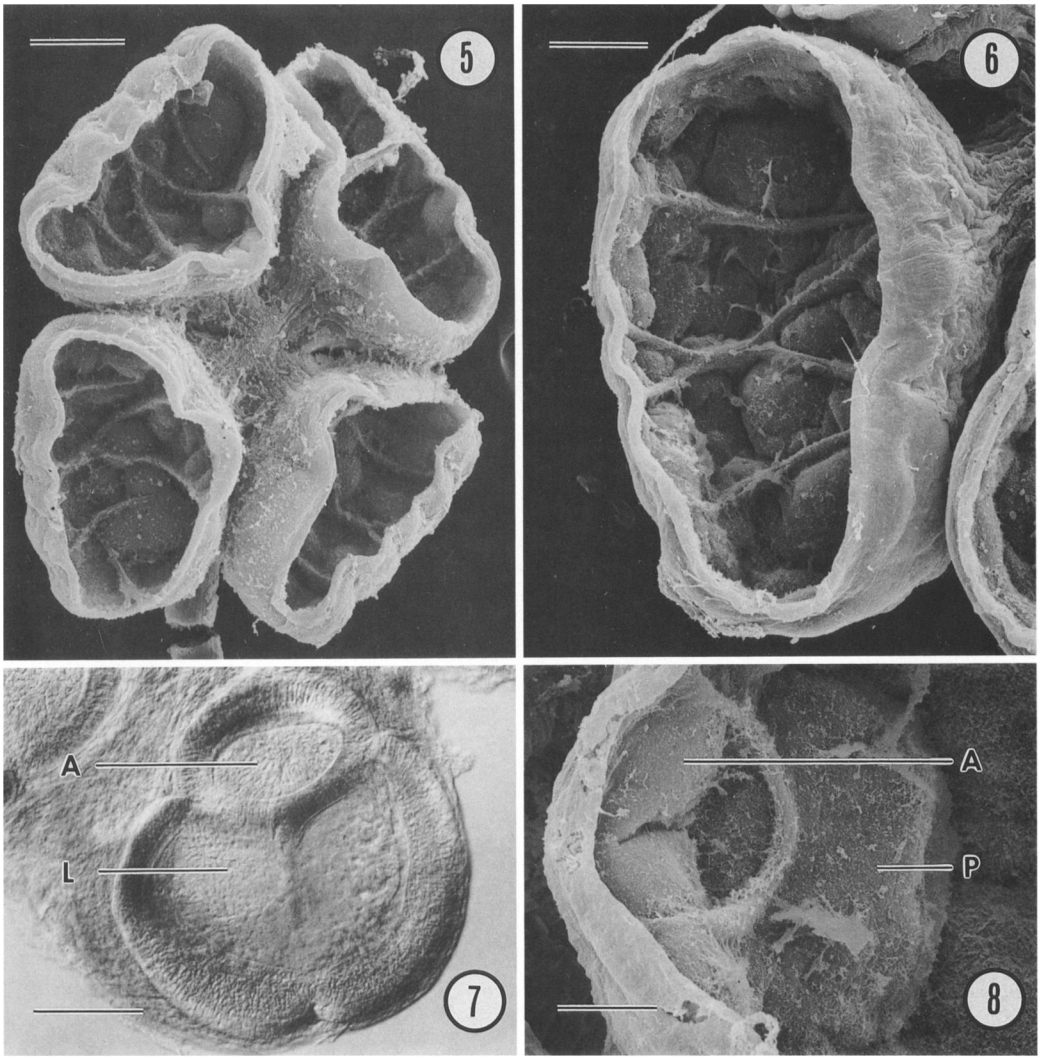
Escherbothrium molinae n. sp.
(Figs. 1–6, 8)

Description (based on 35 specimens): Strobila craspedote, apolytic, up to 8.2 mm long (n = 31, $\bar{x}$ = 5.2, SD = 1.3), composed of 19–39 proglottides (n = 28, $\bar{x}$ = 28, SD = 5.2). Scolex bilaterally symmetrical, with 4 pedicellated, cup-shaped bothridia. Scolex 493–1,019 wide (n = 31, $\bar{x}$ = 748, SD = 112). Pedicels contractile, 34–159 long (n = 103, $\bar{x}$ = 83, SD = 24.2). Bothridia 306–600 long (n = 120, $\bar{x}$ = 463, SD = 52.1) by 144–344 wide (n = 119, $\bar{x}$ = 238, SD = 34.8), subdivided by muscular septa into 4 large and 2 small, somewhat triangular, loculi. Longitudinal edges of bothridia with thin, contractile, velum-like membrane veiling bothridial face when relaxed. Bothridial apical sucker on center of anterior edge of velum, 34–103 in diameter (n = 114, $\bar{x}$ = 66, SD = 10.9). Adherent surface of bothridia with irregular number of irregularly spaced spherical protrusions. Caudal peduncle 141–506 long (n = 30, $\bar{x}$ = 313, SD = 76.5). Immature proglottides square to longer than wide. Mature proglottides longer than wide. Terminal attached proglottides tapered posteriorly, 375–1,469 long (n = 33, $\bar{x}$ = 881, SD = 19.5) by 109–244 wide (n = 33, $\bar{x}$ = 172, SD = 34.6). Internal longitudinal muscles in relatively discrete bundles lying near interior surface of tegument. Testes in 2 longitudinal preporal fields in anterior ⅔ of proglottis, 19–30 in total (n = 24, $\bar{x}$ = 23, SD = 2.8); 9–15 aporal (n = 24, $\bar{x}$ = 11.4, SD = 1.5), 9–15 poral (n = 24, $\bar{x}$ = 11.5, SD = 1.6); 29–73 in diameter (n = 120, $\bar{x}$ = 46, SD = 10.2). Cirrus sac in posterior ⅓ of proglottis, 38–106 long (n = 29, $\bar{x}$ = 70, SD = 12.4) by 23–62 wide (n = 24, $\bar{x}$ = 46, SD = 9.2), containing spined, eversible cirrus. Vas deferens extensively coiled on aporal side of cirrus sac posterior to testes. Genital pores alternating irregularly, 57–75% of total proglottis length from anterior end of terminal proglottid (n = 25, $\bar{x}$ = 62%, SD = 4.2). 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 present. Ovary bilobed, V-shaped in frontal view, X-shaped in cross section, 178–438 long (n = 25, $\bar{x}$ =



FIGURES 1–4. *Escherbothrium molinae* n. gen. et n. sp. **1.** Scolex. **2.** Mature attached proglottis. **3.** Terminal genitalia. **4.** Cross section of mature proglottis at ovarian isthmus, showing X-shaped ovary (OV), oocapt (OC), osmoregulatory ducts (OD), longitudinal muscle (LM), and uterus (UT).



FIGURES 5–8. Photomicrographs of *E. molinae* n. gen. et n. sp. and *Trilocularia* sp. 5–6. Scanning electron photomicrographs of *E. molinae* n. gen. et n. sp. **5.** En face view of scolex. Scale bar = 125 μm. **6.** En face close-up of bothridium showing 6 loculi and numerous rounded protrusions on bothridial surface. Scale bar = 75 μm. **7.** Light photomicrograph of bothridium of *Trilocularia* sp. (USNM Helm. Coll. no. 7678), showing single anterior apical sucker (A) and two loculi (L). Scale bar = 100 μm. **8.** Scanning electron micrograph of bothridial surface of *E. molinae,* showing apical sucker (A) and rounded protrusions (P). Similar structures appear on the bothridial surface of *Trilocularia* sp. Scale bar = 25 μm.

291, SD = 58.9) by 64–130 wide (n = 25, $\bar{x}$ = 92, SD = 17.9), extending anteriorly to level of posterior extent of cirrus sac. Vitelline follicles medullary, lateral, with follicles dorsal and ventral to osmoregulatory ducts, extending from anterior extent of testicular fields to near posterior end of ovary, interrupted near level of ovarian isthmus; follicles not interrupted near genital pore. Vitelline follicles 6–50 in diameter (n = 150, $\bar{x}$ = 24, SD = 6.9). Gravid detached proglottides not collected.

Taxonomic summary

Type host: *Urotrygon chilensis* (Günther, 1871) (Chondrichthyes: Myliobatiformes: Urolophidae).

Type locality: Costa de Pajaros, Gulf of Nicoya, Costa Rica. Other locality: Punta Morales, Gulf of Nicoya, Costa Rica.

Site of infection: Middle ⅓ of spiral valve.

Specimens deposited: Holotype: USNM Helm. Coll. 84064. Paratype: USNM Helm. Coll. no. 84065.

Etymology: The species is named for Helena Mo-

lina-Ureña, Centro de Investigaciones Marinas y Limnologicos (CIMAR) and Escuela de Biologia, Universidad de Costa Rica. The genus is named for M. C. Escher, in light of the unusual arrangement of bothridial loculi, which reminded us of some of Escher's surrealist drawings.

DISCUSSION

The Tetraphyllidea, like most eucestode groups, has a long history of classification but suffers from little rigorous phylogenetic examination, especially at levels of higher taxa. Traditional classifications, e.g., Wardle and MacLeod (1952), Euzet (1959), and Yamaguti (1959), recognize 2 major families, the Phyllobothriidae and the Onchobothriidae. These families are diagnosed on the basis of the presence (Onchobothriidae) or absence (Phyllobothriidae) of hooks associated with the bothridia. This scheme has been convenient from a nomenclatural standpoint but is weak phylogenetically because absence of hooks is a pleisomorphic trait (Brooks et al., 1991) and is thus not a robust character upon which to base a grouping (Wiley et al., 1991). By lacking a diagnosing synapomorphy, the Phyllobothriidae (and associated smaller families) as a whole is a paraphyletic collection of taxa of undetermined phylogenetic relationships. Phyllobothriids can be divided into animals that have apical suckers but lack bothridial loculi, e.g., *Anthobothrium* van Beneden 1850, *Calyptrobothrium* Monticelli 1893, *Clistobothrium* Dailey and Vogelbein, 1990, *Clydonobothrium* Euzet 1956, *Crossobothrium* Linton 1889, *Monorygma* Diesing 1863, *Orygmatobothrium* Diesing 1863, *Rhodobothrium* Linton 1889, and the *Phyllobothrium lactuca* species group (see Brooks and McLennan, 1993; Ruhnke, 1993) and species that have bothridial loculi but lack apical suckers, e.g., *Caulobothrium* Baer, 1948, *Dioecotaenia* Schmidt 1969, *Pentaloculum* Alexander 1953, *Rhabdotobothrium* Euzet, 1953, *Rhinebothrium* Linton, 1889, *Rhinebothroides* Mayes, Brooks and Thorson 1981, *Trilocularia* Olsson 1867, *Tritaphros* Lönnberg 1889, *Zyxibothrium* Hayden and Campbell 1981, and the *Phyllobothrium centrurum* species group (see Brooks and McLennan, 1993). The absence of bothridial loculi is a plesiomorphic trait used to diagnose the first group of phyllobothriids which, like the Phyllobothriidae as a whole, is a paraphyletic collection of taxa of undetermined phylogenetic relationships. The second group, comprised of those taxa that have bothridial loculi but lack bothridial apical suckers (listed above), is a monophyletic group only if the bothridial loculi exhibited by all its taxa are evolutionarily homologous. Some members of the Onchobothriidae, e.g., *Acanthobothrium* van Beneden 1949, *Acanthobothroides* Brooks 1977, *Calliobothrium* van Beneden 1850, and *Onchobothrium* Blainville 1828, also have bothridial loculi. The assumption that the group exhibiting bothridial loculi is monophyletic must therefore be considered tentative as well, pending the documentation of additional characters indicating phylogenetic relatedness among taxa within this group. To complicate matters further, the paraphyletic nature of the Phyllobothriidae makes the use of members of the Onchobothriidae as outgroups in any phylogenetic analysis of phyllobothriid taxa inappropriate (see Brooks and McLennan, 1991; Wiley et al., 1991). The inherent weakness in the 2-family classification of the Tetraphyllidea may have led Schmidt (1986) to recognize 2 smaller families of phyllobothriids, the Dioecotaeniidae Schmidt, 1969, comprising 1 genus with 2 species which Schmidt removed to its own order, and the Triloculariidae, comprising 3 genera with 4 species.

Escherbothrium molinae, because it lacks bothridial hooks and has bothridial loculi, might appear to be a member of the second group of phyllobothriids discussed above. The presence of both bothridial loculi and apical suckers, however, makes *Escherbothrium* diagnosably distinct from all other tetraphyllidean genera and suggests that it might represent a phylogenetically transitional form between some members of the nonseptate phyllobothriids (which have apical suckers) and the septate phyllobothriids (which lack apical suckers). We therefore propose a new genus to accommodate *E. molinae.*

No other tetraphyllideans are currently described as having both apical suckers and septate bothridia. Members of the genus *Trilocularia* Olsson 1867 have been diagnosed as having bothridial faces divided into 3 loculi, e.g., Schmidt (1986). Published line drawings of *Trilocularia* specimens, e.g., Hyman (1951), Euzet (1959), and a scanning electron microscopic (SEM) study by McCullough and Fairweather (1983), show bothridia with 2 large posterior loculi and an anterior smaller structure which McCullough and Fairweather (1983) termed a loculus. Rees (1953) described the scolex morphology of *Trilocularia* as including a bothridium divided into 2 loculi with an apical sucker "lying immediately in front of the bothridium," concurring with earlier ob-

servations (Southwell, 1925). We examined specimens of *Trilocularia* sp. (USNM Helm. Coll. nos. 7678 and 7679) and concur with Rees (1953) that the bothridia comprise 2 loculi and an apical sucker (Fig. 7). Therefore, we believe that *E. molinae* actually represents the second known tetraphyllidean group possessing apical suckers and loculi associated with the bothridia. The shared retention of a plesiomorphic trait, the apical sucker, is not sufficient to suggest any particular hypothesis of phylogenetic relationships between *Escherbothrium* and *Trilocularia*. The specimens of *Trilocularia* and *E. molinae* that we examined, however, possess rounded protrusions on the inner bothridial surface (see Fig. 8 for *E. molinae*). This is a feature that we have not observed or found reported for other tetraphyllideans and which might, therefore, be synapomorphic for *Trilocularia* and *Escherbothrium*. If so, the trait might constitute robust grounds for recognizing the Trilocullariidae as a group distinct from other phyllobothriids. In addition, 1 of the electron photomicrographs of McCullough and Fairweather (1983) depicts a relaxed bothridium of *Trilocularia acanthiaevulgaris*, which is highly reminiscent of those of *Escherbothrium*. *Trilocularia* species, however, differ from *E. molinae* in a number of characteristics, including having posteriorly positioned genitalia and genital pores and dense spines covering the anterior surface of proglottides (Euzet, 1959).

Schmidt (1986) placed *Zyxibothrium* Hayden and Campbell, 1981 and *Pentaloculum* Alexander, 1963 in the Trilocullariidae. We examined the holotype and paratypes of *Zyxibothrium* (USNM Helm. Coll. nos. 75906, 75907–8). There is a marked similarity in the bifurcating structure of the medial bothridial septa of *Escherbothrium* and *Zyxibothrium*, although *Zyxibothrium* lacks apical suckers or rounded protrusions on the inner bothridial surface. We have not been able to obtain any specimens of *Pentaloculum* for examination, but published illustrations of the scolex depict bifurcating bothridial septa similar to those of *Zyxibothrium* and *Escherbothrium*. We thus place *Escherbothrium* in the Trilocullariidae, pending phylogenetic analysis of the group.

Finally, *E. molinae* is a parasite of eastern Pacific Ocean tropical stingrays, whereas *Trilocularia* inhabits sharks and *Zyxibothrium* skates from the northeastern Atlantic Ocean and *Pentaloculum* inhabits sharks from the western Pacific Ocean (New Zealand). Such a wide geographic and host range may indicate that *Escherbothrium*, *Trilocularia*, *Zyxibothrium*, and *Pentaloculum* appear closely related to each other only relative to tetraphyllidean species known at this time.

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