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Calliobothrium evani sp. n. (Tetraphyllidea: Onchobothriidae) from the Gulf of California, with a Redescription of the Hooks of *C. lintoni* and a Proposal for Onchobothriid Hook Terminology

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ABSTRACT: *Calliobothrium evani* sp. n. is described from the spiral valve of an unidentified carcharhinid shark from the Gulf of California. This species is most like *C. lintoni* Euzet, 1954, in that these two are the only species of *Calliobothrium* van Beneden, 1850 with an accessory piece between the axial hook bases. Unlike *C. lintoni,* the accessory piece is oval and broader posteriorly than anteriorly, the axial hooks are dissimilar, and the articular surfaces differ in position and shape. *Calliobothrium evani* also has a larger scolex (approximately twice as large), a more anterior genital pore, and a greater mean number of testes (46 as compared to 34). The hooks of *C. lintoni* are redescribed to include several features omitted from the original description. To facilitate the description of dissimilar hooks a revised hook terminology, applicable to all onchobothriids, is proposed.

A new species of *Calliobothrium* van Beneden, 1850 was collected from the spiral valves of two sharks caught in the Gulf of California near Puertecitos, México. To describe the dissimilar hooks of this species and to compare them with other species of *Calliobothrium,* onchobothriid hook terminology is revised and the hooks of *C. lintoni* Euzet, 1954 are redescribed.

Materials and Methods

The hosts were caught in the Gulf of California and purchased from fishermen at the shore in Puertecitos, México, in March 1982. Photographs of the hosts were taken for further identification, but the negatives were lost during processing. Four worms were collected alive from the spiral valves of two individual sharks and placed in AFA immediately after recovery. In the laboratory they were hydrated in a series of alcohols, stained with Ehrlich's hematoxylin, dehydrated in a series of alcohols, cleared with methyl benzoate and mounted in Canada balsam. Measurements are in micrometers unless otherwise stated, and ranges are given in the text. For each character measured Table 1 provides the mean, the standard deviation, the number of worms examined, and the total number of observations when more than one proglottid or structure per worm was examined. Illustrations were made with the aid of a drawing tube. One specimen, after being mounted in Canada balsam, was unmounted and soaked in xylene followed by several changes of 100% ethanol. It was subsequently prepared for scanning electron microscopy as given in Caira (1985). Measurements of bothridial spines were taken from scanning electron micrographs.

Terminology

In the onchobothriids there are either one or two pairs of bothridial hooks. If there is one pair, the hooks may be either single pronged or multipronged. If there are two pairs, the hooks are single pronged.

The simplest case is one pair of single-pronged hooks per bothridium such as those found in *Potamotrygonocestus* Brooks and Thorson, 1976; *Pachybothrium* Baer and Euzet, 1962; and some species of *Onchobothrium* Blainville, 1828. The hooks of such a pair have been referred to as *outer* and *inner* (Brooks et al., 1981 for *Potamotrygonocestus orinocoensis* Brooks, Mayes, and Thorson, 1981). In *Acanthobothroides* Brooks, 1977 there is one pair of hooks composed of one simple hook and one bipronged hook. Brooks (1977) referred to the hooks of the pair as *inner* and *outer* and also referred to the prongs of the bipronged hook as *inner* and *outer.* A single pair of bipronged hooks per bothridium is found in *Acanthobothrium* van Beneden, 1849; *Pedibothrium* Linton, 1909; *Platybothrium* Linton, 1890; *Uncibilocularis* Southwell, 1925; and some species of *Onchobothrium.* The hooks of a pair have been referred to as *inner* and *outer* (Yamaguti, 1952 for *Platybothrium auriculatum* Yamaguti, 1952); *proximal* and *distal* (Euzet, 1959 for *Platybothrium* spp.); or *larger* and *smaller* (Southwell, 1925 for *Onchobothrium farmeri* (Southwell, 1911) Southwell, 1925). The prongs of the hooks have been referred to as *inner* and *outer* (for example: Yamaguti, 1952; Goldstein, 1964; Campbell, 1969; Williams, 1969, for *Acanthobothrium* spp.; Linton, 1909 for *Pedibothrium* spp.); *small* and *large* (Southwell, 1925 for *Pedibothrium globicephalum* Linton, 1909); or *axial* and *lateral* (Euzet, 1959 for *Acanthobothrium* spp.). A single pair of three-pronged hooks per bothridium exists in *Phoreiobothrium*

Linton, 1889. In this case authors have referred to the prongs as *inner, middle,* and *outer* (Yamaguti, 1954; Campbell, 1975). Euzet (1959) referred to the hooks of a pair in this genus as *distal* and *proximal.*

In the case of two pairs of hooks per bothridium, as found in *Calliobothrium,* the hooks have been referred to as *inner* and *outer* (Yamaguti, 1952 for *C. verticillatum* (Rudolphi, 1819) van Beneden, 1850); *short* and *long* (Southwell, 1925 for *C. leuckartii* van Beneden, 1850); or *axial* and *lateral* (Euzet, 1959). No terminology has been used to distinguish between the hooks of a pair (for example between the two axial hooks) in this genus.

A consistent terminology is needed, particularly for describing onchobothriids with dissimilar hooks in a pair, because differences in size or shape may be of significant systematic value. It is critical in such cases that unambiguous reference to a particular hook be possible. The following terminology is proposed to permit consistent reference to an individual prong or hook for any onchobothriid. This terminology is based on the position of the hook or prong with reference to the axes of the bothridium and scolex.

Each bothridium can be divided into an axial area (along the axis of the bothridium) and two abaxial areas (away from the axis of the bothridium) (Fig. 1). In onchobothriids with two pairs of bothridial hooks such as *Calliobothrium,* the pair of hooks associated with the axis of the bothridium are the axial hooks, and the pair of hooks associated with the abaxial portions of the bothridium are the abaxial hooks (Fig. 1). This system retains the use of the term *axial* (Euzet, 1959) for those hooks associated with the axial portions of the bothridium, and replaces the term *lateral* (Euzet, 1959) with *abaxial* for hooks associated with the abaxial portions of the bothridium. The term *lateral* is reserved for reference to the entire scolex rather than for each bothridium.

The scolex can be divided into one medial area (along the midline of the scolex) and two lateral areas (away from the midline of the scolex) (Fig. 1). Thus, in members of *Calliobothrium,* the axial and abaxial hooks of each bothridium associated with the medial area of the scolex are termed the *medial axial* and *medial abaxial* hooks (collectively, the *medial* hooks), and the axial and abaxial hooks associated with each of the lateral areas of the scolex are termed the *lateral axial* and *lateral abaxial* hooks (collectively,

Table 1. Morphological characters of *Calliobothrium evani* sp. n.

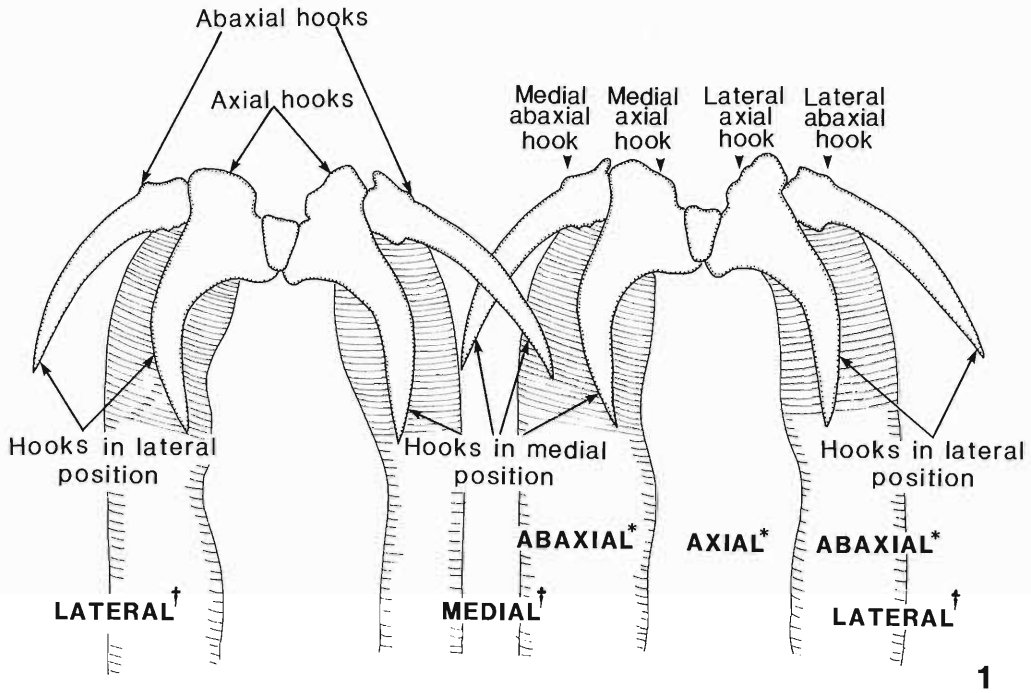
Character	$\bar{x}$	SD	N*	n†
Body:				
Length	10.7 mm	3.2 mm	4	4
Greatest width	770	120	3	3
No. of segments	22	4.9	4	4
Scolex:				
Length	836	49	4	4
Bothridium:				
Length	828	45	4	5
Width	289	43	4	7
Anterior loculus length	265	18.5	3	6
Second loculus length	144	2.5	3	6
Third loculus length	239	42	3	6
Spine length	3.25	0.27	1	10
Lateral axial hooks:				
A	137	18.3	4	11
B	190	11.5	4	12
C	128	5.5	3	10
F	95	6	4	11
Medial axial hooks:				
A′	144	11.3	4	11
B′	186	13	3	10
C′	78	6.5	3	7
F′	57	4.4	4	9
Accessory piece:				
Length	44	4.2	4	11
Width	23	1.7	3	10
Abaxial hooks:				
D,D′	190	12	4	22
E,E′	190	13.8	4	20
Hook tubercle:				
Length	46	3.2	3	11
Posterior proglottid:				
Length	1.29 mm	0.26	3	3
Width	468	87	4	4
No. of testes	46	5.1	3	13
Cirrus pouch:				
Length	247	34	2	4
Width	174	20	2	4
Position of genital pore from posterior end	44%	1.9%	4	11
Length of terminal dilation of cirrus	55	8.7	2	3
Length of vaginal sphincter	61	7.6	2	3

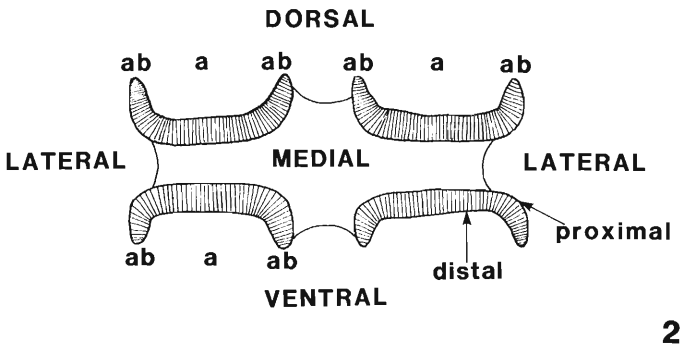
All measurements are in micrometers unless otherwise indicated.

* Number of worms examined.

† Total number of observations (greater than *N* when more than one structure or proglottid per worm was examined).

Letters for hook measurements refer to Figure 6.





Figures 1, 2. Schematic diagrams. 1. Dorsal or ventral view of hooks *in situ* on scolex of *Calliobothrium lintoni* Euzet, 1954 indicating bothridial (*) and scolex (†) axes. 2. Cross section through scolex; a, axial; ab, abaxial.

the *lateral* hooks) (Fig. 1). These terms have been established on a two bothridium basis as, although there are four bothridia per scolex, these bothridia tend to flatten with two positioned dorsally and two positioned ventrally; the terms given above pertain equally to the dorsal and ventral pairs of bothridia (Fig. 2).

For those onchobothriids possessing a pair of bipronged hooks, per bothridium, the hooks of a pair can be identified as medial and lateral, and the prongs of each hook as axial and abaxial. Because a description of the complex hooks of *Phoreiobothrium* involves homology as well as terminology, hook terminology for this genus is discussed in Caira (1985), but is consistent with the above system.

In *Acanthobothroides,* owing to the way the bothridia flatten, the simple hook of the pair is the medial hook, and the bipronged hook is the lateral hook. The prongs of the bipronged hook are axial and abaxial. For those species of onchobothriids with only one pair of simple hooks

per bothridium, such as *Potamotrygonocestus,* the terms axial and abaxial are not necessary; instead the hooks of a pair can be identified on an entire scolex basis as medial and lateral.

Calliobothrium evani sp. n. (Figs. 3-9)

DESCRIPTION (based on 4 specimens): Worms 7.25–14.5 mm long; greatest width 670–900 at anterior end of scolex; 18–29 proglottids per worm; apolytic. Scolex (Fig. 3) 775–880 long, carried on neck; surface of neck and proximal surfaces of bothridia densely covered with fine spines, 2.8–3.7 long, directed posteriorly. Bothridia four, 775–872 long by 220–343 wide; each with three loculi and one inconspicuous apical sucker. Anterior loculus 240–292 long; second loculus 136–144 long; third loculus 192–300 long.

Hooks (Figs. 4, 5) of each bothridium covered with thin layer of tissue. Axial hooks recurved. Lateral axial hooks: distance from point of hook to axial posterior extremity of base (A in Fig. 6) 112–172; distance from point to anterior abaxial extremity of base (B in Fig. 6) 164–204; distance from anterior abaxial extremity of base to posterior axial extremity of base (C in Fig. 6) 120–136; base 88–104 long (F in Fig. 6). Medial axial hooks: distance from point of hook to axial posterior extremity of base (A′ in Fig. 6) 128–164; distance from point to anterior abaxial extremity of base (B′ in Fig. 6) 164–204; distance from anterior abaxial extremity of base to posterior axial extremity (C′ in Fig. 6) 68–88; base 48–64 long (F′ in Fig. 6). Extended bases of axial hooks articulate with, and extend underneath oval accessory piece 40–52 long by 20–25 at widest point (near center) (Fig. 9c). Lateral and medial abaxial hooks of equal curvature and size; 164–208 from point of hook to rounded tubercle (D and D′ in Fig. 6) and 160–220 from point to notched axial extremities (E and E′ in Fig. 6). Notched axial extremities of abaxial hooks articulate with axial hooks on triangular tubercle on proximal side of axial hook base (Fig. 4). Tubercle 40–52 long. Abaxial hooks with rounded tubercle on distal side (Fig. 9a) articulating with anterior notch of axial hooks (Fig. 4). All hooks hollow with internal channel, granular in appearance, opening to outside via pore. In axial hooks channel extending from point of hook past pore into base farther in lateral axial hooks (Fig. 9b) than in medial axial hooks (Fig. 9d). In axial hooks channel opening to outside via pore at center of anterior portion of triangular tubercle. Abaxial hook channels extending from point of hook to anterior portion of hook, opening via pore at axial point of base of rounded tubercle (Fig. 9a). Accessory piece solid.

Proglottids acraspedote. Immature proglottids wider than long, mature proglottids longer than wide. Posterior proglottid mature, 1.08–1.59 mm long by 405–596 wide. Testes 35–53 in number, situated centrally between two lateral fields of ovary and vitellaria. Vas deferens coiled, median, preequatorial in second quarter of proglottid, entering base of cirrus pouch. Cirrus sac (Fig. 7) pyriform, 200–280 long by 148–188 at widest point; containing: coiled cirrus with terminal dilation 50–65 long, lined with spines 2–4 long; entire cirrus up to dilation enveloped in relatively thick cellular layer.

Ovary bilobed, at extreme posterior end of proglottid, poral lobe extending almost to cirrus sac, aporal lobe somewhat shorter. Vagina expanded at base, extending anteriorly to level of cirrus pouch, then laterally along anterior margin of cirrus pouch; small dilation lined with blunt protrusions 2–3 long at base of vaginal sphincter; sphincter 52–66 long, joining genital atrium (Fig. 7). Genital pore lateral 40–47% of proglottid length from posterior end (Fig. 8); pores alternating irregularly. Uterus extending anteriorly along median line from ovarian bridge to anterior third of proglottid; uterine pore not seen. Vitellaria indistinct, granular, extending laterally in narrow band from level of cirrus sac to anterior end of proglottid, somewhat more extensive on aporal side.

HOST: An unidentified shark of the family Carcharhinidae.

SITE OF INFECTION: Spiral valve.

LOCALITY: Gulf of California, vicinity of Puertecitos, Baja California Norte, México.

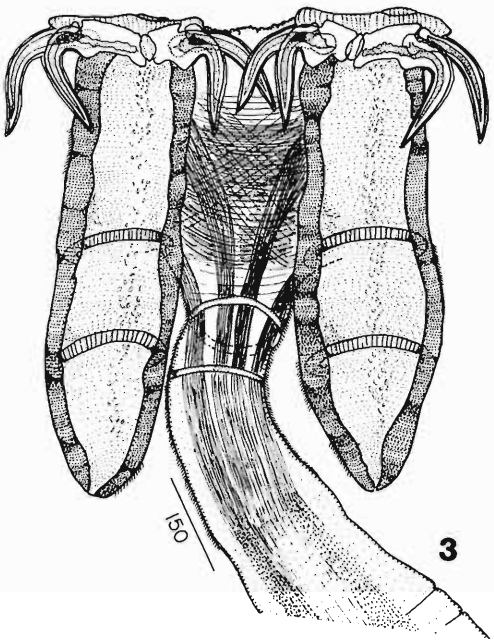
HOLOTYPE: USNM Helm. Coll. No. 78600.

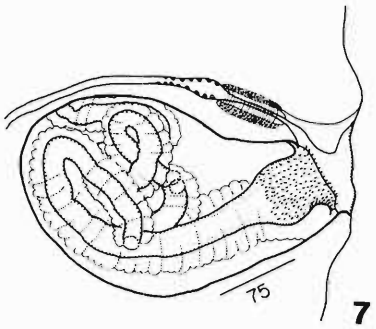
PARATYPES: USNM Helm. Coll. No. 78601, Univ. Neb. State Mus. HWML No. 22536.

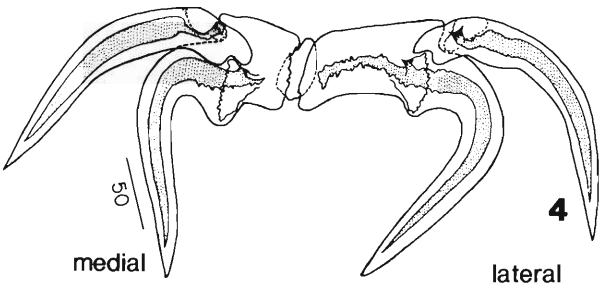
ETYMOLOGY: This species is named in honor of Dr. Evan C. Jolitz whose assistance and encouragement made the Mexican trip possible.

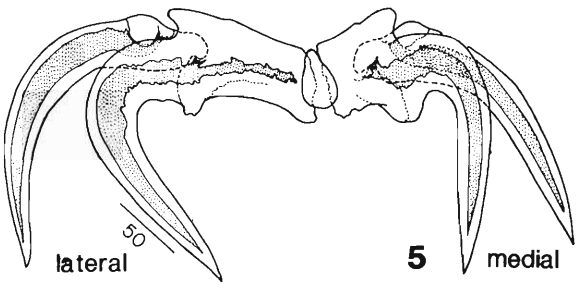
The Hooks of *Calliobothrium lintoni*

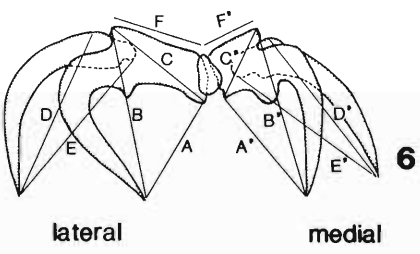
Calliobothrium evani most closely resembles *C. lintoni,* the type material of which was collected by Euzet from *Mustelus laevis* Risso at Sète, France. However, before a comparison of these two species is made, the hooks of *C. lintoni* are redescribed to include several features omitted from the original description. The author was

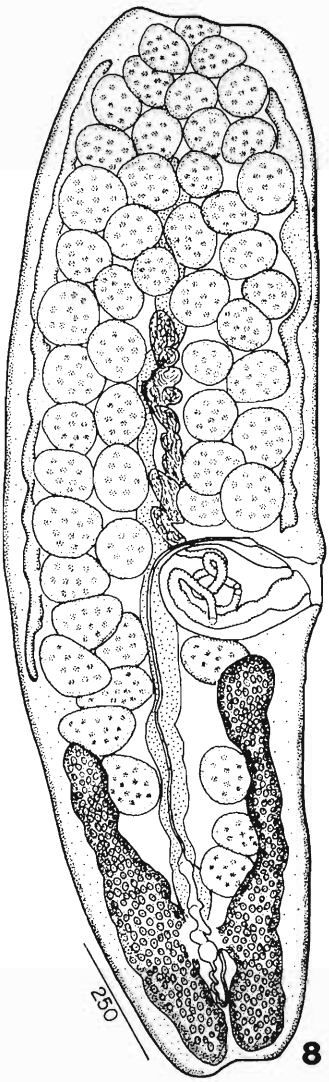












unsuccessful in obtaining the type material of *C. lintoni* from Professor Euzet; however, the following material was available for examination: five voucher specimens collected from *Mustelus canis* (Mitchill) at Wood's Hole, Massachusetts (HWML No. 20933 and USNM Helm. Coll. No. 34831); two voucher specimens collected from *M. canis* at Wood's Hole and identified by Linton (1924) as *Calliobothrium eschrichtii* van Beneden, 1850 (the same specimens that Euzet later [1954] recognized as a synonym of *C. lintoni*) (USNM Helm. Coll. No. 7680); the holotype specimen of *C. pellucidum* Riser, 1955 collected from *M. californicus* Gill in southern California (USNM Helm. Coll. No. 37419); and 15 specimens collected by the author from *M. henlei* Gill near Puertecitos, México (HWML No. 22537).

Euzet (1954, p. 76), at the time of his description of *C. lintoni,* examined his own type material from *M. laevis* in the Mediterranean in conjunction with Linton's specimens from Wood's Hole and wrote:

> . . . elle est identique au parasite de *Mustelus canis* et de *Galeorhinus* (*Mustelus*) *laevis* décrit sous le nom de *C. eschrichtii* par Linton en 1890 et 1924. Nous avons pu nous en assurer en examinant la préparation originale de Linton (U.S.N.M., Helm. Coll. No. 7680).

In addition Euzet (1959) considered *C. pellucidum* from *M. californicus* from the coast of southern California to be a synonym of *C. lintoni.*

Although there is a general similarity in overall morphology, several differences were noted between the specimens collected from Wood's Hole and those collected from California and Mexico. The latter specimens, in general, have smaller hooks and a proportionately smaller accessory piece: for most hook measurements the specimens from Wood's Hole occupy the upper end of the given size range and the specimens from Mexico and California occupy the lower end of the range. In addition, the axial hooks of the specimens from California and Mexico are slightly more recurved than those of the Wood's Hole specimens.

Table 2. Hook measurements of *Calliobothrium lintoni* Euzet, 1954 taken from voucher specimens.*

Character	$\bar{x}$	SD	N‡
Axial hooks:			
A,A′	96	31.3	23
B,B′	144	39.7	22
C,C′	71	20.4	22
Accessory piece:			
Length	25	10.2	23
Width	21	4.9	23
Abaxial hooks:			
D,D′	128	26.6	20
E,E′	127	30	23
Hook tubercle:			
Length	45	15.1	19

All measurements are in micrometers unless otherwise indicated.

* See text for details on specimens.

‡ Number of worms examined.

Letters refer to Figure 11.

Until the type material of *C. lintoni* can be reexamined and, despite the above-mentioned differences, the identity of specimens from Wood's Hole, California, and Mexico is accepted, and information from specimens from all three localities is used to supplement the original description of the hooks. The differences in hook morphology are perhaps attributable to interpopulation variability.

Measurements were taken from either the medial or lateral hooks as there appeared to be no differences between them. The mean, the standard deviation, the number of worms examined, and the total number of observations are given in Table 2.

Calliobothrium lintoni Euzet, 1954 emend. (Figs. 10–13)

The following information should supplement the description of *C. lintoni* Euzet (1954): each bothridium with two pairs of hooks (Fig. 10) covered with thin layer of tissue. Axial hooks recurved. Lateral and medial axial hooks of equal size, 52–155 from point of hook to posterior axial

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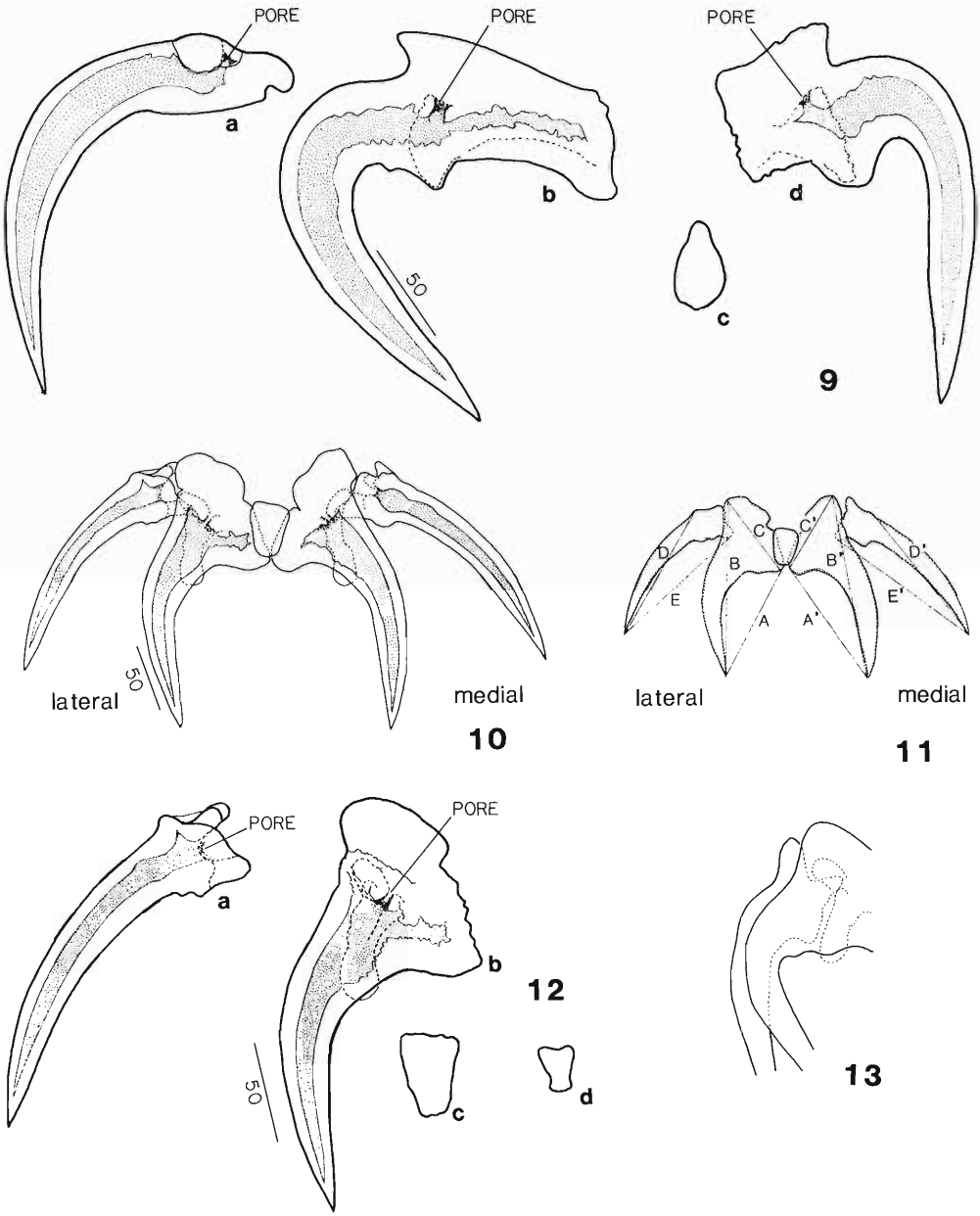
Figures 3–8. ***Calliobothrium evani*** **sp. n. 3. Scolex of holotype indicating two of the four bothridia. 4. Proximal view of hooks; note articulation of abaxial hook with triangular tubercle of axial hook. 5. Distal view of hooks. 6. Hook measurements taken. 7. Terminal genitalia of paratype specimen. 8. Mature proglottid of paratype specimen.**



Figures 9-13. 9. ***Calliobothrium evani*** **sp. n. hook components separated; scale in Figure b applies to a, c, and d also. a. Abaxial hook, b. lateral axial hook, c. accessory piece, d. medial axial hook. 10-13.** ***Calliobothrium lintoni*** **Euzet, 1954. 10. Distal view of hooks of HWML No. 20933. 11. Hook measurements taken. 12. Hook components separated; scale in Figure b applies to a, c, and d also. a. Abaxial hook, b. axial hook, c. accessory piece of a Wood's Hole specimen (HWML No. 20933), d. accessory piece of a Mexican specimen (HWML No. 22537). 13. Distal view of abaxial and axial hook articulation in unflattened Mexican specimen (HWML No. 22537).**

extremity of base (A and A′ in Fig. 11) and 87–214 from point of hook to anterior abaxial extremity of base (B and B′ in Fig. 11). Length of diagonal from anterior abaxial extremity of base to posterior axial extremity of base 43–107 (C and C′ in Fig. 11). Axial hook bases articulate with and extend underneath trapezoidal accessory piece, 11–45 long by 14–30 at widest point (generally at anterior end) (Fig. 12c, d). Abaxial hook bases with four anterior knobs, number visible varying with degree of rotation of hook upon flattening (Fig. 12a). Lateral and medial abaxial hooks of equal curvature and size, 91–176 long from point to beginning of base on abaxial side (D and D′ in Fig. 11), and 80–182 from point of hook to axial extremity of base (E and E′ in Fig. 11). Axial extremity of abaxial hook base articulating with axial hook on elongated tubercle on proximal side of axial hook base (Fig. 13); tubercle 27–71 long. Anteriormost point of abaxial hook resting on notch on proximal side of axial hook tubercle. All hooks hollow, with internal channel granular in appearance, opening to outside via pore. In axial hooks channel extending from point into base to area of abaxial hook articulation and into axial extension of base; pore on proximal side of elongated tubercle (Fig. 12b). Abaxial hook channel extending from point to anterior portion of hook, opening via pore at end of channel (Fig. 12a). Accessory piece solid.

Discussion

The features that characterize *C. evani* are: bases of lateral axial hooks much longer than bases of medial axial hooks; accessory piece oval; articulation of hooks on triangular tubercle on proximal surface of axial hooks. Of the four other species in the genus, *C. evani* most closely resembles *C. lintoni* as they alone share the following features: presence of an accessory piece between bases of axial hooks; and internal channels extended into bases of axial hooks. In addition to the features listed above, *C. evani* differs from *C. lintoni* by possessing: a mean scolex length of 0.83 mm as compared to 0.5 mm; a mean scolex width of 0.77 mm as compared to 0.3 mm; a genital pore that is in the middle of the proglottid as compared to in the posterior one-third; a mean number of testes of 46 as compared to 34; and a total body length that is approximately twice as great.

In addition to the three features listed above, *C. evani* may be distinguished from *C. verticillatum* by its lack of scalloped proglottid overlaps, length of 7.25–14.5 mm as compared to 80–120 mm, 35–53 testes as compared to 110–130, and one accessory sucker per bothridium as compared to three. In addition, *C. evani* differs from *C. leuckartii* by its shorter length (as compared to 50–80 mm), and acraspedote as compared to weakly craspedote proglottids. More testes (as compared to 12–15) and acraspedote as compared to craspedote proglottids also distinguish *C. evani* from *C. eschrichtii* van Beneden, 1850.

It should be noted that the presence of internal channels and pores in hooks are not features limited in occurrence to *C. evani* and *C. lintoni.* These features are present in all species of *Calliobothrium,* and further investigation may reveal a widespread distribution of these traits among the hooked tetraphyllideans.

Acknowledgments

I am grateful to Professor M. H. Pritchard of the H. W. Manter Laboratory, University of Nebraska State Museum, for many stimulating discussions as well as for her helpful criticisms of this manuscript. Special thanks are extended to Gordon Schneider of El Centro, California, who provided transportation down the difficult highway to Puertecitos, and whose knowledge of Spanish made possible the purchase of sharks from the Mexican fishermen. In addition I would like to thank Dr. J. R. Lichtenfels of the Animal Parasitology Institute, USDA, ARS, Beltsville, Maryland for lending specimens; and Scott W. Michaels for identifying the specimens of *M. henlei.*

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