



## Two new species of *Litobothrium* Dailey, 1969 (Cestoda: Litobothriidea) from thresher sharks in the Gulf of California, Mexico, with redescriptions of two species in the genus

P. D. Olson<sup>1,2</sup> & J. N. Caira<sup>2</sup>

<sup>1</sup>Parasitic Worms Division, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

<sup>2</sup>Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, CT 06269-3043, USA

Accepted for publication 5th June, 2000

### Abstract

As part of a survey of the metazoan parasites of elasmobranchs of the Gulf of California, Mexico, the spiral intestines of 10 pelagic thresher sharks *Alopias pelagicus* and one bigeye thresher shark *A. superciliosus* were examined for tapeworms. Eight of the *A. pelagicus* specimens examined were found to host *Litobothrium amplifica* and *L. daileyi*. Both tapeworm species are redescribed based on examination of this new material with light and scanning electron microscopy, and the ranges of most of the measurements for these species are expanded; scanning electron micrographs and detailed illustrations and measurements of their segment anatomy are presented for the first time. An argument is made that the identification of the original host specimens of these species was in error and that *A. pelagicus* is likely to be the correct original host. In addition, *L. nickoli* n. sp., a third species in the genus hosted by *A. pelagicus*, was found in three of the 10 individual hosts examined. This species differs from all six known *Litobothrium* species in the form of the pseudosegments of the scolex, the anterior two being essentially non-cruciform, while the latter three are distinctly cruciform. All other species possess either no non-cruciform or at most one non-cruciform segment anteriorly. The single specimen of *A. superciliosus* examined was found to host the new species, *L. janovyi*. This species differs from *L. coniformis*, *L. gracile* and *L. amsichensis* in its possession of four rather than three, three and five cruciform pseudosegments, respectively. It lacks the modifications of the fourth pseudosegment seen in *L. amplifica* and lacks the anterior non-cruciform fifth pseudosegment found in *L. daileyi*. It most closely resembles *L. alopias* but differs among other features in its greater total length, greater number of segments and longer mature segments. SEM of the four species collected from the Gulf of California as well as material of *L. amsichensis* from the goblin shark *Mitsukurina owstoni* that hosted the type-specimens of this species show that all surfaces of the body of all five species bear a dense covering of long filiform microtriches. *L. amplifica* bears a single row of large spine-like structures throughout most of the posterior margins of the first and second cruciform pseudosegment only. *L. daileyi* possesses one to two rows of overlapping spine-like structures on the posterior margins of the first four pseudosegments with the exception of the medial projections. The fifth pseudosegment lacks these structures. *L. janovyi* n. sp. bears spine-like structures on the lateral margins of only the third and fourth pseudosegments. *L. nickoli* n. sp. bears spine-like structures throughout the posterior margins of the first and second pseudosegments, and throughout the posterior margins of the third and fourth pseudosegments with the exception of the medial projections; the fifth pseudosegment lacks these structures. *L. amsichensis* bears no structures that could be considered to be spine-like on any of its pseudosegments, but possesses a border of densely arranged larger microtriches on the posterior margin of all five pseudosegments. A key to the species is included.

## Introduction

Dailey (1969) erected the order Litobothriidea (Platyhelminthes: Eucestoda), family Litobothriidae and genus *Litobothrium* to house two new tapeworm species, *L. alopias* Dailey, 1969 and *L. coniformis* Dailey, 1969, that were collected from a bigeye thresher shark *Alopias superciliatus* (Lowe) off the coast of California, USA. He later added a third species to the order, *L. gracile* Dailey, 1971, collected from a smalltooth sandtiger shark *Odontaspis ferox* (Risso), also off the California coast (Dailey, 1971). Kurochkin & Slankis (1973) described two more species in the order, *L. daileyi* Kurochkin & Slankis, 1973 and *L. amplifica* (Kurochkin & Slankis, 1973) Euzet 1994 (syn. *Renyxa amplifica* Kurochkin & Slankis, 1973), both purportedly from *A. superciliatus* collected off the Pacific coast of Mexico in the Gulf of Tehuantepec. The sixth and last species described in the order was *L. amsichensis* Cairns & Runkle, 1993 taken from a goblin shark *Mitsukurina owstoni* Jordan collected from the Eastern shores of Australia (Cairns & Runkle, 1993). Ordinal status for this small group of tapeworms continued to be recognised by Wardle et al. (1974) and Schmidt (1986), but Euzet (1994) subsumed the group within the large order Tetraphyllidea. To date, each of the six species is known solely from its original description. This may be explained in part because all six are parasites of lamnid sharks, specifically the families Alopiidae, Mitsukurinidae and Odontaspidae, and these relatively large pelagic sharks are not encountered commonly.

As part of a recent survey of the metazoan parasites of elasmobranchs of the Gulf of California, we were fortunate to work with fishermen in two different localities in the Gulf who were specifically targeting thresher sharks. As a consequence, we were able to examine 10 individuals of the pelagic thresher shark *Alopias pelagicus* Nakamura and a single individual of *A. superciliatus*. Necropsies of these animals resulted in the discovery of two new species of *Litobothrium* described herein. In addition, these collections provided new specimens of *L. amplifica* and *L. daileyi* that were examined using light microscopy and scanning electron microscopy (SEM). Both species are redescribed based on examination of this material. Neither *L. alopias* nor *L. coniformis* were found in either species of thresher sharks examined. For comparative purposes, specimens of *L. amsichensis* taken from the same specimen of *Mitsukurina owstoni* that hosted the type-material, was also prepared and exam-

ined with SEM. The microtriche pattern seen in these five species of *Litobothrium* is described for the first time. This is the first report of litobothriid tapeworms from the Gulf of California, Mexico.

## Materials and methods

All sharks were caught by artisanal fishermen working in the Gulf of California, Mexico. Our sample consisted of five individuals of *A. pelagicus* from Bahia de Los Angeles, collected in August of 1993 using long-lines, three individuals of *A. pelagicus* and one of *A. superciliatus* from Santa Rosalia and two individuals of *A. pelagicus* from Boca de Alamo collected in June, 1996 using small-mesh pelagic shark nets. Spiral intestines were removed from the sharks, opened with a mid-ventral incision and examined preliminarily for tapeworms on site. The majority of the tapeworms discovered at that time were fixed in 10% formalin buffered in sea-water. Spiral intestines were preserved in 10% buffered formalin following the preliminary examination for worms. Cestodes and intestines were transferred to 70% ethanol for storage a minimum of 48 hrs after fixation. Spiral intestines were examined for additional tapeworm specimens upon returning to the lab.

For comparative purposes, the following material was borrowed from the US National Parasite Collection in Beltsville, Maryland (USNPC): two paratypes of *L. alopias* (No. 71325); six paratypes of *L. coniformis* (No. 71365); and three paratypes of *L. gracile* (No. 70731). Six newly prepared specimens of *L. amsichensis* were deposited in the Larry R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut (LRP Nos 2082–87).

Multiple specimens of each of the four litobothriidean species found in the Gulf of California as well as six voucher specimens of *L. amsichensis* and the one unmounted paratype specimen of *L. gracile* were prepared as whole-mounts for light microscopy as follows: they were stained with Gill's haematoxylin, dehydrated in a graded ethanol series, cleared in xylene or methyl salicilate and mounted on glass slides in Canada balsam. Specimens of *L. amplifica*, *L. daileyi* and *L. janovyi* n. sp. were prepared for histological examination as follows: they were embedded in paraplast and cross-sections were cut at 10–12 µm intervals using a rotary microtome. Sections were stained in Gill's haematoxylin, counter-stained in eosin, cleared in xylene and mounted on glass slides in Canada bal-

sam. Multiple specimens of the four species collected in the Gulf as well as previously collected specimens of *L. amsichensis* were prepared for examination by scanning electron microscopy (SEM) as follows: they were hydrated in a graded ethanol series, immersed in 1.5% osmium tetroxide at 20°C overnight, dehydrated in a graded ethanol series, critical point dried in liquid CO<sub>2</sub> or sublimated in hexamethyldisilazane and mounted on aluminum stubs with carbon paint. The dried specimens were sputter-coated with approximately 100-300 Å of gold or palladium and examined with a LEO/Zeiss DSM 982 Gemini field emission scanning electron microscope.

Measurements are given in the text as the range, followed in parentheses by the mean, standard deviation and number of worms examined. The ranges of each measurement that resulted from a combination of the new data with those from the original descriptions of *L. amplifica* and *L. daileyi* are provided in brackets. All measurements are in micrometres unless otherwise stated. Type and representative voucher material was deposited in museums in Mexico (IBUNAM, Istituto de Biologia Universidad Nacional Autonoma de Mexico, Mexico City), the United Kingdom (BMNH, The Natural History Museum, Department of Zoology, Parasitic Worms Division, London) and the United States (LRP and USNPC).

The interpretation and terminology of the anterior region of the body of *Litobothrium* used here follows that of Caira et al. (1999, 2000). Thus, the scolex is considered to consist of an apical sucker and up to five pseudosegments, all, or a subset of which, are cruciform. Until the morphology of the strobila in species of *Litobothrium* is more fully understood, we have considered all segments posterior to the last cruciform pseudosegment, regardless of whether they exhibit evidence of reproductive organs, to be segments of the strobila rather than pseudosegments of the scolex.

***Litobothrium amplifica*** (Kurochkin & Slankis, 1973)  
Euzet, 1994 (Figures 1-5, 17-24)  
Syn. *Renyxa amplifica* Kurochkin & Slankis, 1973

#### *Redescription*

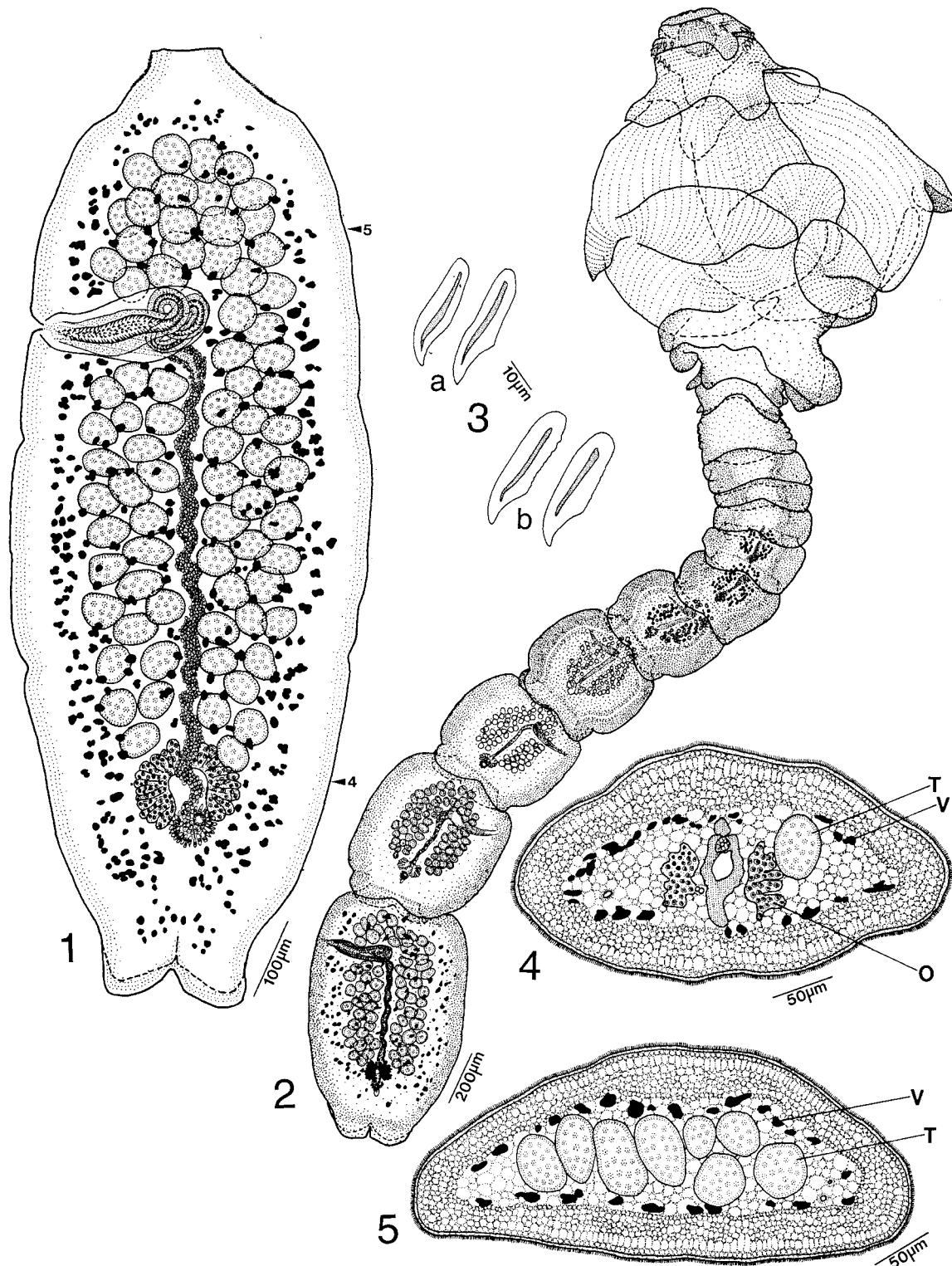
(Based on 17 whole-mounted worms, histological sections of 2 mature segments and 5 worms examined with SEM)

Worms euapolytic, 3.3-6.8 (4.9 ± 0.8; 17) [3.3-8.7] long; greatest width at posterior margin of fourth pseudosegment. Scolex consisting of cup-shaped,

muscular apical sucker, 118-150 (133 ± 8; 16) [90-160] wide by 86-136 (111 ± 16; 15) deep, and 4 cruciform pseudosegments. First 3 pseudosegments cruciform in cross-section, increasing in size posteriorly. Fourth cruciform pseudosegment highly modified. First pseudosegment with inconspicuous dorsomedial and ventromedial projections, diamond-shaped (Figure 20), 80-152 (107 ± 24; 13) [80-160] × 240-320 (292 ± 22; 14) [140-320]; second pseudosegment with conspicuous dorsomedial and ventromedial projections, 72-144 (105 ± 19; 16) [30-144] × 296-464 (406 ± 44; 16) [210-464]; third pseudosegment with conspicuous dorsomedial and ventromedial projections, 160-264 (187 ± 32; 17) [90-270] × 624-880 (731 ± 65; 17) [400-880]; fourth pseudosegment 460-900 (668 ± 138; 17) [460-1,070] × 1,220-1,600 (1,407 ± 124; 17) [940-1,600], with conspicuous dorsomedial and ventromedial projections resembling human nose; lateral margins divided into one small central, one large dorsal and one large ventral projection; dorsal and ventral projections recurved medially (Figure 17). First 3 segments following fourth pseudosegment highly laciniate, non-cruciform; first segment larger than second and third combined; laciniations of first segment extending posterior to third segment. Neck absent.

All regions of body covered with densely packed, relatively elongate, filiform microtriches; filiform microtriches on surfaces of reproductive organ-bearing segments (Figure 23) longer than those on non-reproductive organ-bearing segments. First 2 pseudosegments armed with single row of large spine-like structures (perhaps microtriches) embedded in posterior margins of pseudosegments (Figures 3, 17, 20-22); spine-like structures relatively evenly spaced throughout entire margin of first pseudosegment (Figures 20-21), relatively evenly spaced throughout most of margin of second pseudosegment, interrupted by dorsomedial and ventromedial projections (Figure 17); spine-like structures easily lost; first and second pseudosegment with numerous pits, conspicuous because of uneven arrangement of filiform microtriches around their borders (Figure 24).

Strobila consisting of 13-19 (16 ± 1.6; 17) [13-31], craspedote, weakly laciniate segments. Immature segments 12-19 (15 ± 1.8; 17) in number, initially wider than long, gradually becoming longer than wide. Mature segments 0-2 (0.7 ± 0.6; 17) in number, 664-840 (753 ± 75; 16) [664-850] × 208-560 (429 ± 129; 6). Testes 53-84 (67 ± 9; 8) in number, oval to round, 27-45 (33 ± 3.5; 6) × 36-62 (49 ± 9; 6), extending



Figures 1–5. Line drawings of *Litobothrium amplifica*. 1. Mature free segment (numbered arrows indicate positions of sections in Figures 4 and 5). 2. Entire specimen. 3. Spine-like structures of first (a) and second (b) pseudosegments. 4. Cross-section of segment through ovary. 5. Cross-section of segment anterior to cirrus-sac. Abbreviations: O, ovary; T, testis; V, vitelline follicle.

from anterior margin of segment to anterior margin of ovary, arranged in single field in cross-section. Cirrus-sac pyriform, extending approximately to median line of segment, 180-256 (215 ± 29; 6) × 32-50 (41 ± 6.9; 6), containing highly coiled cirrus. Cirrus armed with spiniform microtriches. Vas deferens looping anterior to cirrus-sac, extending posteriorly around cirrus-sac, convoluted, bifurcating prior to level of the ovary. Ovary posterior, inverted U-shaped, 60-110 (81 ± 22; 6) × 70-164 (112 ± 37; 6), bilobed in cross-section, lobulate. Genital pores lateral, 60-78% (70 ± 6%; 7) of segment length from posterior end, alternating irregularly. Vagina expanded at base, extending anteriorly, then laterally across cirrus-sac, joining genital atrium anterior to cirrus-sac. Mehlis' gland posterior to ovary. Vitellarium follicular; follicles irregular in shape, circum-medullary, distributed throughout length of segment, interrupted by ovary and cirrus-sac. Uterus medial, extending from ovarian bridge approximately to posterior margin of to cirrus-sac. Excretory ducts lateral. Gravid segments not seen.

*Recorded type-host:* *Alopias superciliosus* (Lowe), bigeye thresher shark.

*Probable actual type-host:* *Alopias pelagicus* Nakamura, pelagic thresher shark.

*Site of Infection:* Spiral intestine.

*Type-locality:* Gulf of Tehuantepec, Oaxaca, Mexico.

*Additional localities:* Bahia de Los Angeles (28°55'N, 113°32'W) and Santa Rosalia (27°19'N, 112°17'W), Gulf of California, Mexico.

*Specimens deposited:* 4 voucher specimens (IBUNAM No. 4051); 7 voucher specimens including slides of cross-sections (USNPC Nos 90473-75); 3 voucher specimens (LRP Nos 2074-77); 4 voucher specimens (BMNH No. 2000.3.7.8-10); SEM stub retained in the personal collection of JNC.

#### Remarks

Our data for this species are generally consistent with those of Kurochkin & Slankis (1973). However, we examined a greater number of specimens and have extended the ranges of some features as required. The internal anatomy in our specimens was more clearly visible and thus measurements for the various features of the reproductive organs are presented here for the first time. Drawings of a detail of a segment, cross-sections and ultrastructural descriptions are also presented for the first time. There are two features that we believe have been misinterpreted by Kurochkin &

Slankis (1973). These authors described the ovary of *L. amplifica* as being bilobed. Although sections suggest that the ovary is indeed bilobed in cross-section, the ovary is clearly an inverted U-shape when viewed in whole-mounts. In addition, Kurochkin & Slankis (1973) considered this species to possess five rather than four pseudosegments. These authors suggest that there are concavities on both sides of the ventral and dorsal projections of the fourth pseudosegment that are sealed with the thin semitransparent film that represents an extremely delicate anterior part of the fifth proglottid. They go on to say that the fifth proglottid of the pseudoscolex is strongly flattened, its upper part narrowed and has a small longitudinal thickening in the middle, and the posterior edge forms four petal-shaped projections oriented backward and somewhat deflected laterally. We interpret only the four cruciform pseudosegments, following Caira et al. (1999), to be part of the scolex. This latter interpretation seems to more consistent with detailed information available through examination with SEM.

Kurochkin & Slankis (1973) originally described *L. amplifica* (as *Renyxa amplifica*) from two specimens of *Alopias superciliosus* taken in the Gulf of Tehuantepec, Oaxaca, Mexico. To our knowledge, no additional records of this parasite exist in the literature. Our new collections from the Gulf of California, Mexico (only approximately 12° north of the Gulf of Tehuantepec), lead us to suspect that the identification of the type-host of *L. amplifica* by Kurochkin & Slankis (1973) may have been in error. We discovered specimens of *L. amplifica* in eight of the 10 specimens of *A. pelagicus* examined, whereas we failed to find a single representative of this species in *A. superciliosus*. There are several other pieces of evidence that support this supposition. Despite the existence of keys to aid in the identification of these two shark species (e.g. Compagno, 1984), we initially had difficulty with the specific identification of the thresher shark specimens examined in the Gulf. Particularly problematical was the fact that *A. pelagicus* possesses fairly large eyes, and, in the absence of an example of *A. superciliosus* for direct comparison, misidentification can occur. In fact, we initially identified the thresher shark specimens collected in 1993 as *A. superciliosus*. This error was discovered only after liver tissue from these animals was sent to Andrew Martin (University of Nevada at Las Vegas), who amplified approximately 2,500 bps of the genome, cut it with restriction enzymes and then compared the profiles with those from individuals of *A. pelagicus*, *A. su-*

*percilosus* and *A. vulpinus* whose identities had been confirmed by shark systematist Gavin Naylor (University of Iowa at Ames). This error was confirmed in 1996 when we encountered specimens of *A. pelagicus* and *A. superciliatus* at the same time, allowing direct morphological comparisons to be made between the two species, and causing us to realise that the specimens we had identified in 1993 as *A. superciliatus* were indeed *A. pelagicus*. Finally, if the bigeye thresher shark hosts *L. amplifica*, it is curious that Dailey (1969), who examined specimens of *A. superciliatus* from the West coast of California, found *L. alopis* and *L. coniformis*, but not *L. amplifica*, in either of the animals he examined.

#### ***Litobothrium amsichensis* Caira & Runkle, 1993**

(Figures 25-28)

*Amendment to description* (based on SEM of 4 worms)

All surfaces of body covered with densely packed, elongate, filiform microtriches; posterior margins of 5 cruciform segments and all non-reproductive organ-bearing segments posterior to cruciform segments with larger, densely packed, pointed microtriches (Figure 27) throughout posterior margins, including dorsomedial and ventromedial projections of cruciform pseudosegments.

#### *Remarks*

The only microtriche data provided by Caira & Runkle (1993) for this species was that it possessed filiform microtriches on the ‘pseudoscolex’. Caira et al. (2001) coded this species as possessing segments that lacked filiform microtriches, but bore blade-like microtriches distributed throughout the entire surface of the segments. SEM conducted here leads us to believe that the structures seen on the segments of this species are more appropriately considered to be filiform microtriches. These structures are very densely packed and thus their form is difficult to interpret. Given their similarity in size and form to the structures seen on the segments of *L. amplifica* and *L. daileyi*, however, we believe this to be a more consistent interpretation.

#### ***Litobothrium daileyi* Kurochkin & Slankis, 1973**

(Figures 6-11, 29-34)

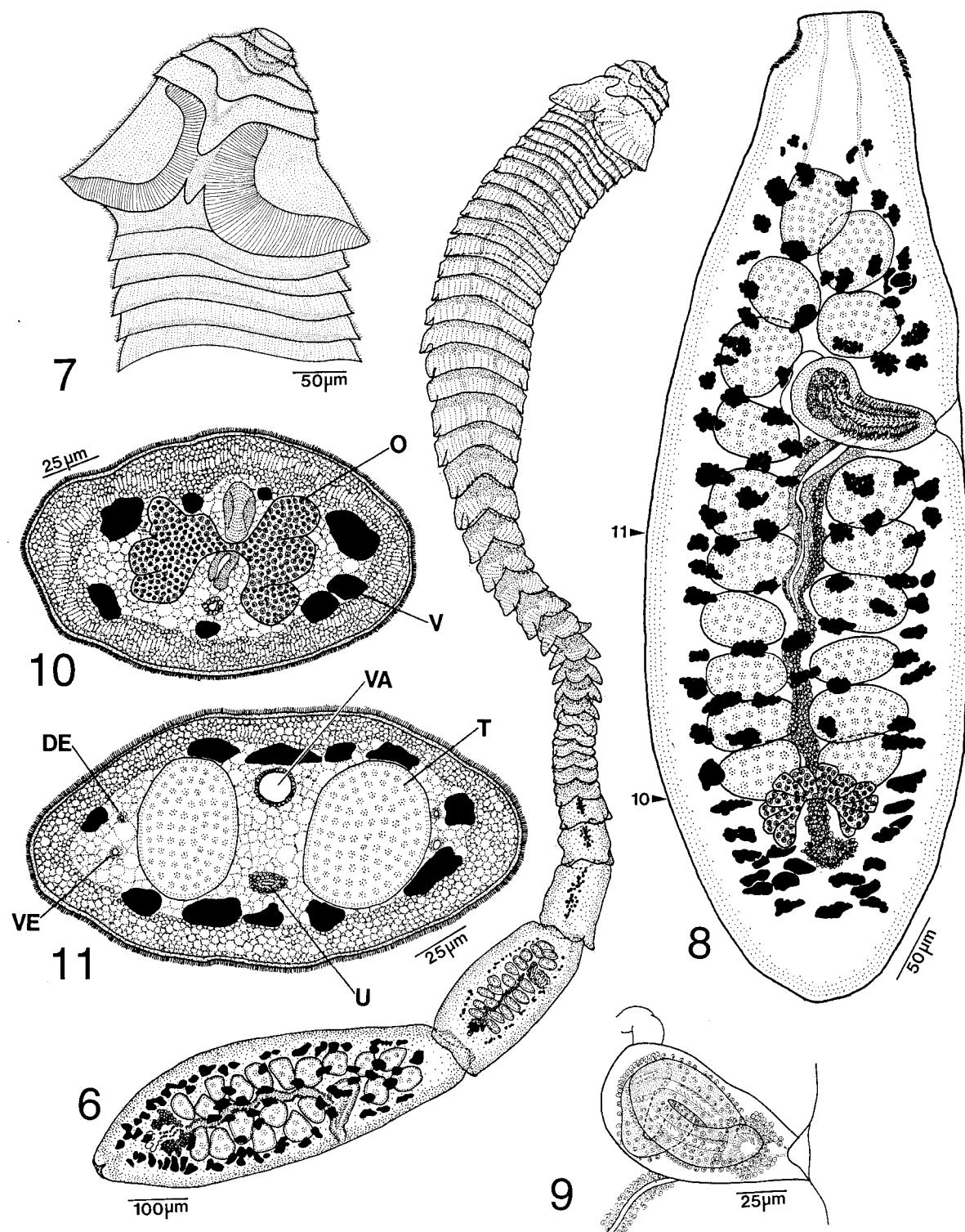
#### *Redescription*

(Based on 18 whole-mounted worms, histological sections of one mature segment and 6 specimens examined with SEM).

Worms euapolytic, 2-4.7 ( $3 \pm 0.8$ ; 18) [1.2-4.7] mm long; greatest width at level of fifth pseudosegment. Scolex consisting of cup-shaped, muscular apical sucker, 35-44 ( $40 \pm 2.1$ ; 18) wide by 25-35 ( $28 \pm 3.4$ ; 18) deep (Figure 30), and 5 pseudosegments; first pseudosegment inconspicuous, round in cross-section, bearing apical sucker; posterior 4 pseudosegments cruciform in cross-section; cruciform pseudosegments increasing in width and conspicuousness of medial projections posteriorly. First pseudosegment essentially equivalent to length and width of apical sucker; second pseudosegment 12-60 ( $30 \pm 12$ ; 15) [12-100]  $\times$  60-101 ( $83 \pm 13$ ; 16) [60-230]; third pseudosegment 24-70 ( $40 \pm 10$ ; 15) [20-90]  $\times$  84-132 ( $107 \pm 15$ ; 17) [84-280]; fourth pseudosegment 48-75 ( $63 \pm 8$ ; 15) [20-90]  $\times$  101-168 ( $135 \pm 21$ ; 17) [101-340]; fifth pseudosegment 94-144 ( $113 \pm 14$ ; 17) [94-370]  $\times$  182-300 ( $239 \pm 34$ ; 16) [182-600]. Fifth pseudosegment longer than pseudosegments 3 and 4 combined; left and right lateral regions often depressed centrally.

All regions of body covered with densely packed, very elongate, filiform microtriches; microtriches on surfaces of non-reproductive organ-bearing segments slightly pointed distally; microtriches on reproductive organ bearing segments rounded distally (Figure 34). Posterior margins of first 4 pseudosegments armed with numerous spine-like structures (possibly microtriches) arranged in 1-2 overlapping rows (Figures 31-32); spine-like structures continuous throughout margin of first 3 pseudosegments, interrupted on dorsomedial and ventromedial projections on fourth pseudosegment; posterior margin of fifth pseudosegment entirely lacking spine-like structures (Figure 33). Spine-like structures easily lost.

Cruciform pseudosegments followed by 30-43 ( $37 \pm 3.6$ ; 17) craspedote, non-cruciform pseudosegments decreasing in width posteriorly along strobila, becoming increasingly laciniate posteriorly. Neck absent. Posterior segments craspedote, slightly laciniate. Immature segments 2-4 ( $2.7 \pm 0.7$ ; 18) in number; terminal segment mature, 2.1-5.1 ( $3.4 \pm 0.8$ ; 18) times longer than wide; 434-1,125 ( $748 \pm 194$ ; 18) [434-1,125]  $\times$  170-285 ( $224 \pm 32$ ; 18) [90-285]. Testes



*Figures 6–11.* Line drawings of *Litobothrium daileyi*. 6. Entire specimen. 7. Scolex. 8. Mature segment (numbered arrows indicate positions of sections in Figures 10 and 11). 9. Detail of terminal genitalia. 10. Cross-section of segment through ovary. 11. Cross-section of segment posterior to cirrus-sac. Abbreviations: DE, dorsal excretory duct; O, ovary; T, testis; U, uterus; V, vitelline follicle; VA, vagina; VE, ventral excretory duct.

15-26 (21 ± 3.2; 11) [15-26] in number, slightly oval, 22-48 (37 ± 8; 13) × 20-58 (43 ± 10; 13), extending from anterior margin of segment to anterior margin of ovary, arranged in 2 lateral columns; in single field in cross-section. Cirrus-sac pyriform, extending to median line of segment, 87-170 (112 ± 24; 15) × 30-45 (35 ± 5.2; 15), containing coiled cirrus. Cirrus armed with spiniform microtriches. Ovary inverted U-shape, 30-75 (50 ± 14; 15) × 41-105 (71 ± 17; 15), bilobed in cross-section, lobulate. Genital pores lateral, 56-72% (65 ± 4; 18) of segment length from posterior end, alternating irregularly. Vagina expanded at base, extending anteriorly then laterally across posterior margin of cirrus-sac, joining genital atrium anterior to cirrus-sac. Mehlis' gland posterior to ovary. Vitellarium follicular; follicles irregular in shape, 9.7-24 (17 ± 4.5; 11) × 13-25 (18 ± 3.6; 11), circum-medullary, distributed throughout segment, interrupted by ovary and cirrus-sac. Uterus medial, extending approximately to posterior margin of cirrus-sac. Excretory ducts lateral. Gravid segments not seen.

*Recorded type-host:* *Alopias superciliatus* (Lowe), bigeye thresher shark.

*Probable actual type-host:* *Alopias pelagicus* Nakamura, pelagic thresher shark.

*Site of Infection:* Spiral intestine.

*Type-locality:* Gulf of Tehuantepec, Oaxaca, Mexico.

*Additional localities:* Bahia de Los Angeles (28°55'N, 113°32'W) and Santa Rosalia (27°19'N, 112°17'W), Gulf of California, Mexico.

*Specimens deposited:* 4 voucher specimens (IBUNAM No. 4050); 8 voucher specimens including slides of cross-sections (USNPC Nos 90470-72); 4 voucher specimens (LRP Nos 2065-68); 2 voucher specimens (BMNH No. 2000.3.7.6-7); SEM stub retained in the personal collection of JNC.

#### Remarks

Our data for this species are generally consistent with those presented by Kurochkin & Slankis (1973). However, we examined a greater number of specimens and have extended the ranges of several features slightly. In the cases of other measurements, such as for example those associated with the pseudosegments, theirs are on the upper ends of the ranges obtained from examination of our specimens. A detailed illustration of the segment anatomy of *L. daileyi* is presented here for the first time. There are two features that

we believe have been misinterpreted by Kurochkin & Slankis (1973). As in *L. amplifica*, we found that although the ovary is indeed bilobed in cross-section, it is clearly an inverted U-shape when viewed in whole-mounts. In addition, Kurochkin & Slankis considered this species to possess four rather than five pseudosegments. Whereas it is true that *L. daileyi* possesses four anterior cruciform pseudosegments, scanning electron microscopy reveals a relatively small, non-cruciform pseudosegment anterior to the first cruciform pseudosegment, which bears the apical sucker of this species (Figure 29), bringing the total number of pseudosegments to five. Finally, Kurochkin & Slankis described the vagina as being situated anterior to the cirrus-sac. In all 18 of our specimens, the vagina would be more accurately interpreted as lying at the same level as, along the posterior margin of the cirrus-sac.

The potential problem with the identity of the type-host of *L. amplifica* described above applies also to *L. daileyi*, as the type-material of this species came from the same host individuals. Whereas the type-host of record is *A. superciliatus*, we believe that it is more likely that the actual host species was *A. pelagicus* for the reasons stated previously in the remarks for *L. amplifica*.

It should be noted that our material of *L. daileyi* includes both specimens consistent in form with the description of this species by Kurochkin & Slankis (1973) (e.g. as in Figure 6), as well as several specimens that exhibit greatly elongated, narrow segments with conspicuous laciniations posterior to the pseudosegments. Because this was the only difference that we were able to detect among this material, we were inclined to consider it a function of the degree of contraction of the specimens and have therefore considered all of this material to represent the single species, *L. daileyi*. This variation, however, may merit further study.

#### *Litobothrium janovyi* n. sp. (Figures 12-16, 35-41)

##### Description

(Based on 20 whole worms, histological sections of 2 mature segments and 5 specimens examined with SEM)

Worms euapolytic, 4.6-9 (6.6 ± 0.3; 20) mm long; greatest width at posterior margin of fourth cruciform pseudosegment. Scolex consisting of anterior clamp-shaped muscular apical sucker 24-36 (29 ± 2.5; 20)

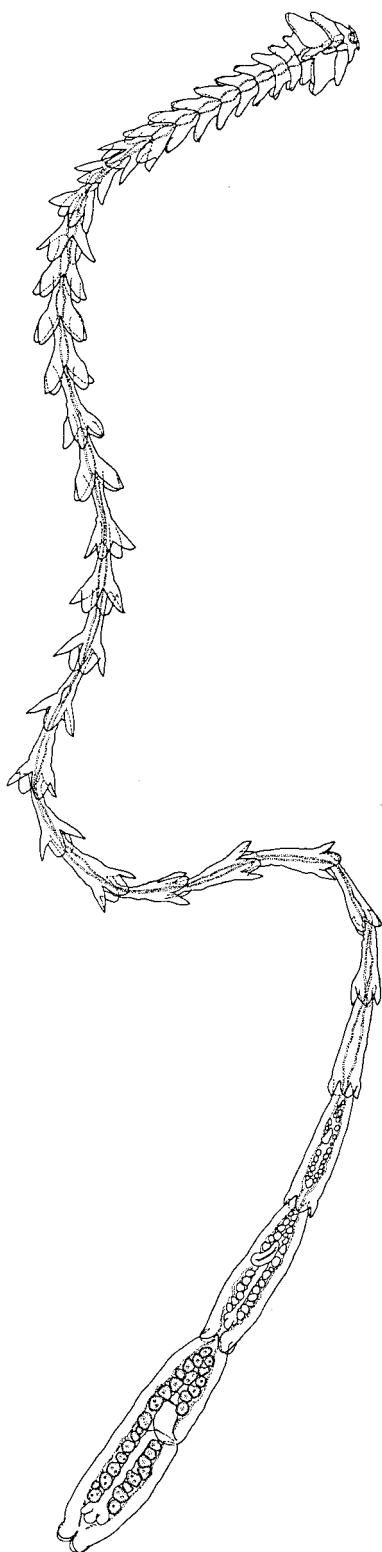
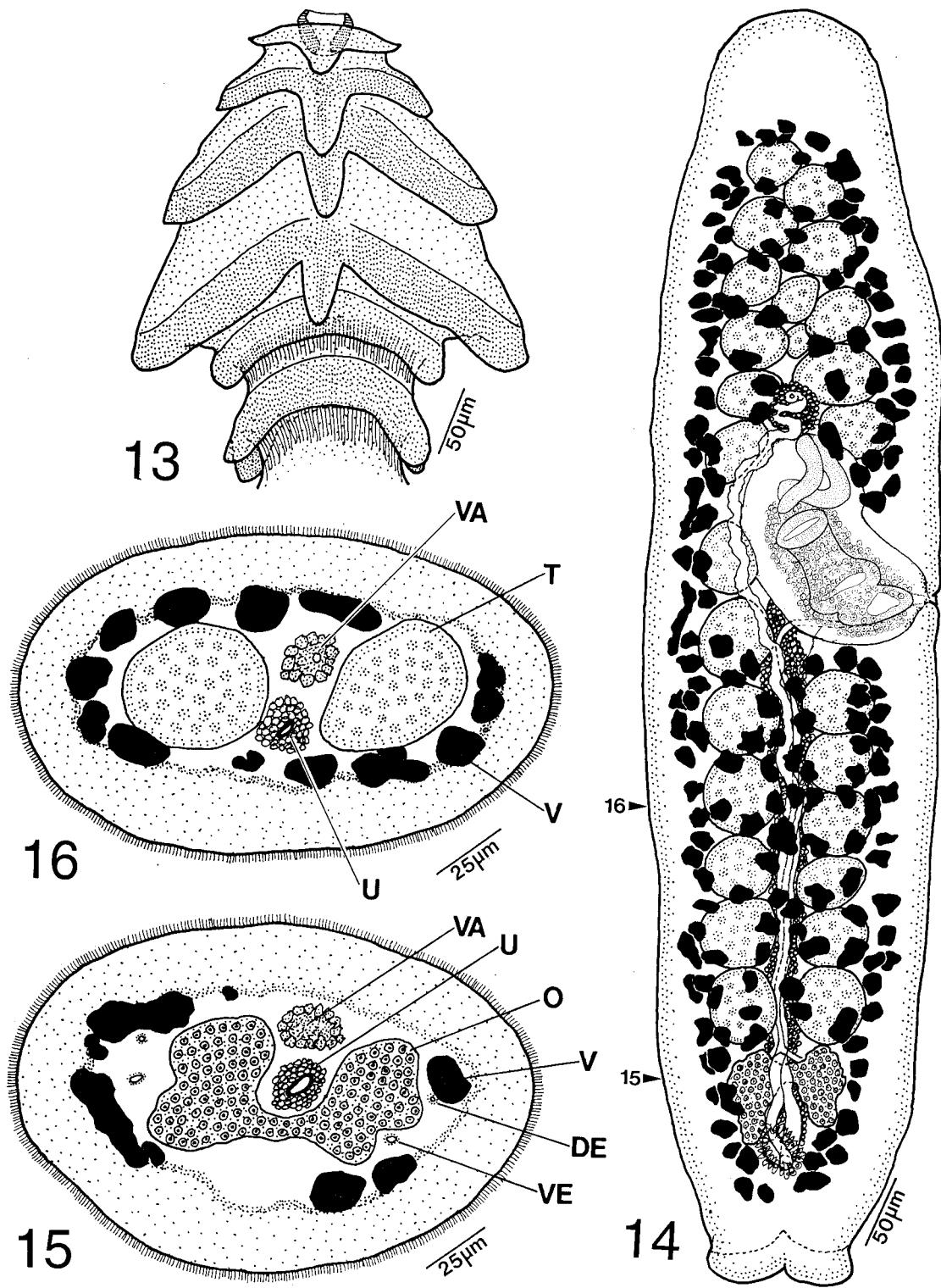


Figure 12. Line drawing of entire specimen of *Litobothrium janovyi* n. sp. (entire worm = 7.8 mm)

$\times$  34-38 ( $36.8 \pm 1.2$ ; 20) (Figure 38) and 4 conspicuously cruciform pseudosegments. First pseudosegment 22-38 ( $30.3 \pm 4.9$ ; 18)  $\times$  68-102 ( $82.7 \pm 9.5$ ; 20); second pseudosegment 50-76 ( $63.6 \pm 8.2$ ; 18)  $\times$  94-162 ( $130 \pm 16.5$ ; 20); third pseudosegment 72-92 ( $82.4 \pm 5.8$ ; 18)  $\times$  170-272 ( $222.8 \pm 29.3$ ; 20); fourth pseudosegment 104-35 ( $121.3 \pm 7.8$ ; 18) (94-370)  $\times$  180-320 ( $261.8 \pm 37.4$ ; 20). Cruciform pseudosegments followed by 24-36 ( $29.7 \pm 2.8$ ; 20) craspedote; non-cruciform segments decreasing in width along strobila, becoming highly lacinate posteriorly. Neck absent.

All regions of body covered with densely packed, relatively elongate, filiform microtriches; microtriches varying in length among surfaces; filiform microtriches on cruciform pseudosegments slightly shorter (Figures 36-37) than those on reproductive organ-bearing segments (Figure 41). Lateral margins of first 2 pseudosegments lacking spine-like structures; spine-like structures found on postero-lateral margins only of third and fourth cruciform pseudosegments; 4 spine-like structures on each side of pseudosegment 3 (Figure 39); up to 8 spine-like structures on each side of pseudosegment 4 (Figure 40).

Segments exhibiting internal anatomy 1-3 ( $2.5 \pm 0.6$ ; 20) in number, craspedote, lacinate. Mature segments 0-2 ( $1.2 \pm 0.5$ ; 20) in number, 2.7-5.6 ( $3.9 \pm 0.8$ ; 20) times longer than wide, 544-1,760 ( $843 \pm 262$ ; 20)  $\times$  164-312 ( $210 \pm 33$ ; 20). Testes 23-35 ( $28 \pm 3.5$ ; 17) in number, oval to round, 22-56 ( $37 \pm 10$ ; 18)  $\times$  26-58 ( $45 \pm 7.8$ ; 18), extending from anterior margin of segment to anterior margin of ovary, arranged in 2 lateral columns, in single field in cross-section (Figure 16). Cirrus-sac pyriform, extending laterally to median line of segment, 130-208 ( $170 \pm 22$ ; 18)  $\times$  40-100 ( $64 \pm 16$ ; 18), containing coiled cirrus. Cirrus microtriches not observed. Vas deferens looping anterior to cirrus-sac, extending posteriorly around cirrus-sac, bifurcating at level of ovary. Ovary inverted U-shaped, 34-94 ( $64 \pm 22$ ; 6)  $\times$  70-124 ( $88 \pm 19$ ; 6), bilobed in cross-section, lobulate. Genital pores lateral, 47-60% ( $54 \pm 3$ ; 19) of segment length from posterior end, irregularly alternating. Vagina expanded at base, extending anteriorly, then laterally across cirrus-sac, joining genital atrium at same level as cirrus-sac. Mehlis' gland posterior to ovary. Vitellarium follicular; follicles irregular in shape, 14-16 ( $15.5 \pm 1$ ; 4)  $\times$  16-22 ( $19 \pm 2.5$ ; 4), circummedullary, distributed throughout length of segment, interrupted by ovary and cirrus-sac. Uterus inconspic-



*Figures 13–16.* Line drawings of *Litobothrium janovyi* n. sp. 13. Scolex. 14. Mature free segment (numbered arrows indicate positions of sections in Figures 15 and 16). 15. Cross-section of segment through ovary 16. Cross-section of segment posterior to cirrus-sac. Abbreviations: DE, dorsal excretory duct; O, ovary; T, testis; U, uterus; V, vitelline follicle; VA, vagina; VE, ventral excretory duct.

uous, extending from ovarian bridge to anterior margin of cirrus-sac. Excretory ducts lateral. Gravid segments not seen.

*Type-host:* *Alopias superciliatus* (Lowe), bigeye thresher shark.

*Site of Infection:* Spiral intestine.

*Type-locality:* Santa Rosalia (27°19'N, 112°17'W), Gulf of California, Mexico.

*Additional localities:* None.

*Specimens deposited:* Holotype and 4 paratypes (IBUNAM Nos 4052 & 4053, respectively); 8 paratypes including slides of cross-sections (USNPC No. 90476); 5 paratypes (LRP Nos 2069-73); 3 paratypes (BMNH No. 2000.3.7.3-5); SEM stub retained in the personal collection of JNC. *Etymology:* This species is named for Professor John Janovy Jr, who has so effectively expanded the horizons of multiple generations of parasitologists.

#### Remarks

*Litobothrium janovyi* n. sp. possesses a total of four pseudosegments, all of which are cruciform. It is thus easily distinguished from *L. coniformis*, *L. gracile* and *L. amsichensis* which exhibit three, three and five cruciform pseudosegments, respectively. It is also easily distinguished from *L. amplifica* in that the fourth pseudosegment is not highly modified. The scolex of *L. janovyi*, at least superficially, resembles that of *L. daileyi* in its possession of four cruciform pseudosegments. However, *L. janovyi* lacks a non-cruciform pseudosegment anterior to the first cruciform pseudosegment, and thus it is pseudosegments 1-4 that resemble pseudosegments 2-5 of *L. daileyi*. In addition, the tegumental features seen in *L. janovyi* differ significantly from those of *L. daileyi*; the filiform microtriches of *L. daileyi* are much longer and more conspicuous than those of *L. janovyi*, and the spine-like structures seen in *L. janovyi* are restricted to the lateral margins of the third and fourth cruciform pseudosegments, whereas in *L. daileyi* the spine-like structures are found on all four of the anteriormost pseudosegments. *L. janovyi* most closely resembles *L. alopias*, a species that has also been reported only from *A. superciliatus*. *L. janovyi* can be distinguished from *L. alopias*, however, in that *L. janovyi* has a greater total length (4.6-6.9 vs 1.65-3.7 mm) and possesses vitelline follicles that are rounded rather than amorphous in form as in *L. alopias*. In addition, the segments of *L. janovyi* are generally much

more elongate and conspicuously lacinate, throughout the entire length of the strobila, than they are in *L. alopias*. Finally, the boundary between the fourth cruciform pseudosegment and the first segment is marked by a conspicuous decrease in width of the latter in *L. janovyi*, whereas the first segment is usually only slightly narrower than the fourth cruciform pseudosegment in *L. alopias*.

It is curious that we did not encounter specimens of either *L. coniformis* or *L. alopias* in the specimen of *A. superciliatus* examined from the Gulf of California. It is equally curious that Dailey (1969) did not report material of *L. janovyi* from either specimen of the *A. superciliatus* he examined. However, figure 4 in Dailey's (1969) original description of *L. alopias* suggests that perhaps specimens of *L. janovyi* were present among his material. He does not mention it in the text, but this figure illustrates a portion of strobila that is consistent with the morphology of *L. janovyi* and that is not seen in any of the type-specimens of *L. alopias* that were deposited by Dailey (1969). Unfortunately, we have been unable to locate the specimen from which that illustration was made.

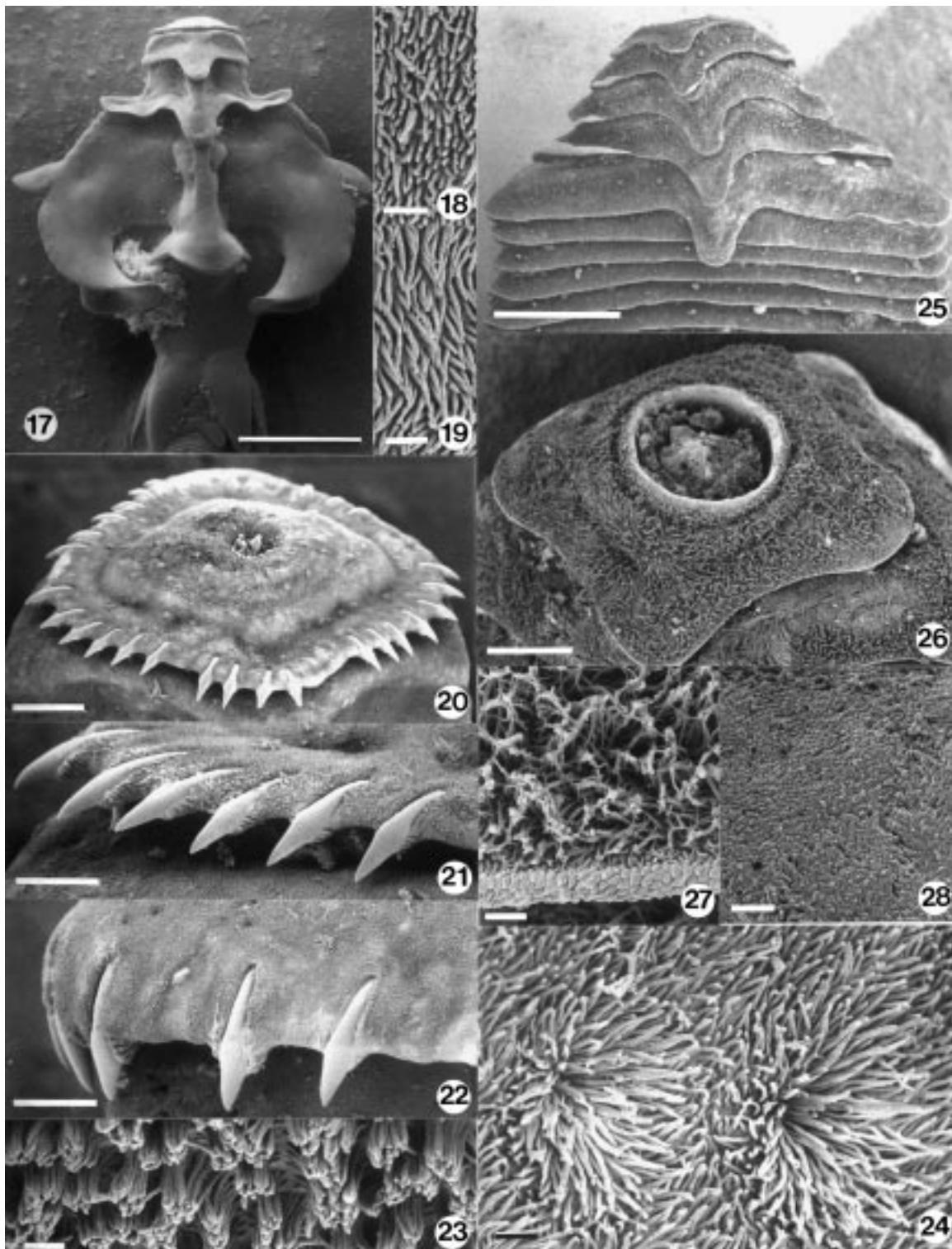
#### *Litobothrium nickoli* n. sp. (Figures 42-50)

##### Description

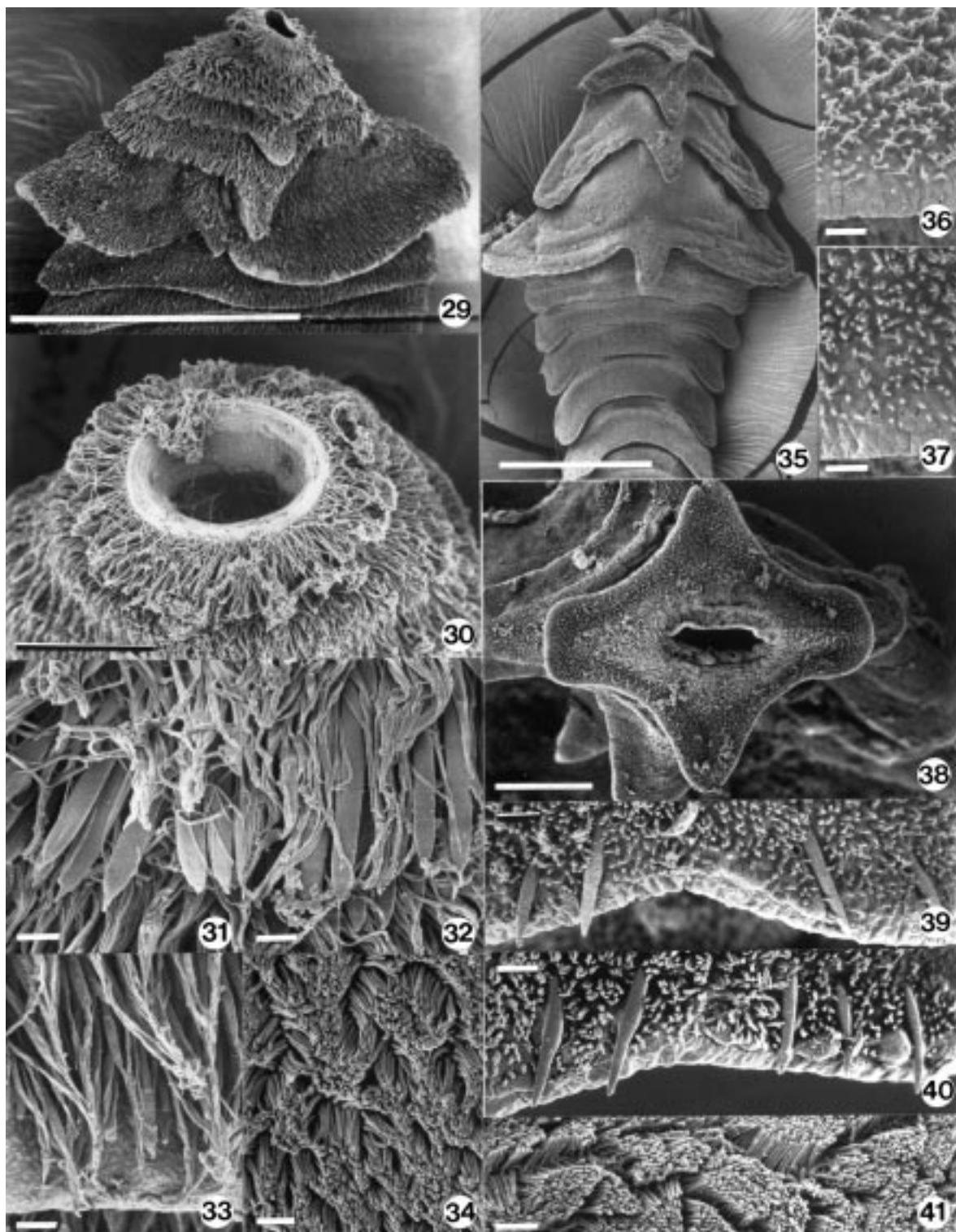
(Based on 12 whole worms and 3 specimens examined with SEM).

Worms euapolytic, 2.5-6.3 ( $4 \pm 0.4$ ; 11) mm long; greatest width at posterior margin of fourth cruciform segment. Scolex consisting of anterior cup-shaped, muscular apical sucker 48-59 ( $54.5 \pm 1.2$ ; 10) deep by 64-77 (70.4  $\pm$  1.4; 10) wide and 5 pseudosegments, posterior 3 of which are conspicuously cruciform. First pseudosegment essentially equivalent to length and width of apical sucker; second pseudosegment 50-70 ( $60.5 \pm 3.1$ ; 8)  $\times$  172-220 (193.1  $\pm$  5.3; 8); third pseudosegment 100-130 (113.9  $\pm$  3.2; 9)  $\times$  199-280 (236  $\pm$  10.1; 9); fourth pseudosegment 175-251 (217  $\pm$  7.1; 9)  $\times$  290-374 (325.7  $\pm$  9.7; 9); fifth pseudosegment 269-384 (316.6  $\pm$  11.6; 9)  $\times$  403-752 (599.1  $\pm$  38; 9). Cruciform pseudosegments followed by 26-35 ( $31.4 \pm 0.9$ ; 8) craspedote, non-cruciform segments decreasing dramatically in width in the first 2-4 segments, becoming slightly lacinate posteriorly. Neck absent.

All regions of body covered with densely packed, relatively elongate, filiform microtriches (Figures 47,



*Figures 17–28.* Scanning electron micrographs of *Litobothrium amplifica* (Figures 17–24) and *L. amsichensis* (Figures 25–28). 17. Scolex of *L. amplifica*. 18. Microtriches four segments posterior to modified cruciform segment. 19. Microtriches five segments posterior to modified cruciform segment. 20. Apex of scolex showing first cruciform pseudosegment and opening of apical sucker. 21. Spine-like structures on margin of first cruciform pseudosegment. 22. Spine-like structures on margin of second cruciform pseudosegment. 23. Filiform microtriches on terminal segment of strobila. 24. Filiform microtriches surrounding “pits” of second cruciform pseudosegment. 25. Scolex of *L. amsichensis*. 26. Apex of scolex showing first, cruciform pseudosegment and opening of apical sucker. 27. Large microtriches on border of third cruciform pseudosegment. 28. Densely packed filiform microtriches on terminal segment of strobila. Scale-bars: 17, 500 µm; 18–19, 23–24, 27–28, 1 µm; 20, 50 µm; 21–22, 26, 20 µm; 25, 100 µm.



*Figures 29–41.* Scanning electron micrographs of *Litobothrium daileyi* (Figures 29–34) and *L. janovyi* n. sp. (Figures 35–41). 29. Scolex of *L. daileyi*. 30. Apex of scolex showing first non-cruciform pseudosegment and opening of apical sucker. 31. Spine-like structures on posterior margin of first pseudosegment. 32. Spine-like structures on posterior margin of third pseudosegment. 33. Posterior margin of fifth pseudosegment (note lack of spine-like structures). 34. Densely packed filiform microtriches on terminal segment of strobila. 35. Scolex of *L. janovyi* n. sp. 36. Posterolateral margin of first pseudosegment (note lack of spine-like structures). 37. Posterolateral margin of second pseudosegment (note lack of spine-like structures). 38. Apex of scolex showing first, cruciform pseudosegment and opening of apical sucker. 39. Spine-like structures on posterolateral margin of third pseudosegment. 40. Spine-like structures on posterolateral margin of fourth cruciform pseudosegment. 41. Densely packed filiform microtriches on terminal segment of strobila. Scale-bars: 29, 100 µm; 30, 38, 20 µm; 31–34, 36–37, 39–41, 1 µm; 35, 100 µm.

50). Posterior margins of first 4 pseudosegments armed with numerous spine-like structures (possibly microtriches) arranged in 1-2 overlapping rows (Figure 49); spine-like structures continuous throughout margin of first 2 pseudosegments, interrupted on dorsomedial and ventromedial projections on third and fourth pseudosegments; posterior margin of fifth pseudosegment lacking large spine-like structures (Figure 50). Spine-like structures easily lost.

Segments exhibiting internal anatomy 2-4 ( $3.2 \pm 0.2$ ; 9) in number, craspedote, slightly laciniate. Mature segments 0-2 ( $1 \pm 0.1$ ; 12) in number; 2.4-4.4 ( $3.8 \pm 0.2$ ; 8) times longer than wide; 625-1,390 (962.8  $\pm$  98.7; 8)  $\times$  179-343 (249.9  $\pm$  17.4; 8). Testes 31-38 ( $34.8 \pm 1.2$ ; 6) in number, oval to round, 22-27 ( $24.5 \pm 1$ ; 4)  $\times$  29-41 ( $36.1 \pm 2.6$ ; 4), extending from anterior margin of segment to anterior margin of ovary, arranged in 2 lateral columns, single field in cross-section. Cirrus-sac pyriform, extending laterally to median line of segment, 129-166 ( $150.6 \pm 11.1$ ; 3)  $\times$  42-51 ( $48 \pm 3$ ; 3), containing coiled cirrus. Vas deferens extensively looping anterior to cirrus-sac, extending posteriorly around cirrus-sac, bifurcating at level of ovary; highly convoluted when filled (Figure 44). Ovary inverted U-shape, 70 (1)  $\times$  160 (1), lobulate. Genital pores lateral, 58-74% ( $65.7 \pm 2.2$ ; 7) of segment length from posterior end, irregularly alternating. Vagina expanded at base, extending anteriorly, then laterally across posterior margin of cirrus-sac, joining genital atrium at same level as cirrus-sac. Mehlis' gland posterior to ovary. Vitellarium follicular; follicles round, becoming ovoid posteriorly, circum-medullary, distributed throughout segment, interrupted by ovary and cirrus-sac. Uterus extending anteriorly from ovarian bridge to posterior margin of the cirrus-sac. Excretory ducts lateral. Gravid segments not seen.

*Type-host:* *Alopias pelagicus* Nakamura, pelagic thresher shark.

*Site of Infection:* Spiral intestine.

*Type-locality:* Bahia de Los Angeles ( $28^{\circ}55'N$ ,  $113^{\circ}32'W$ ), Gulf of California, Mexico.

*Additional localities:* None.

*Specimens deposited:* Holotype and 3 paratypes (IBUNAM Nos 4054 & 4055, respectively); 4 paratypes (USNPC Nos 90477-79); 4 paratypes (LRP Nos 2078-81); 2 paratypes (BMNH No. 2000.3.7.1-2); SEM stub retained in the personal collection of JNC.

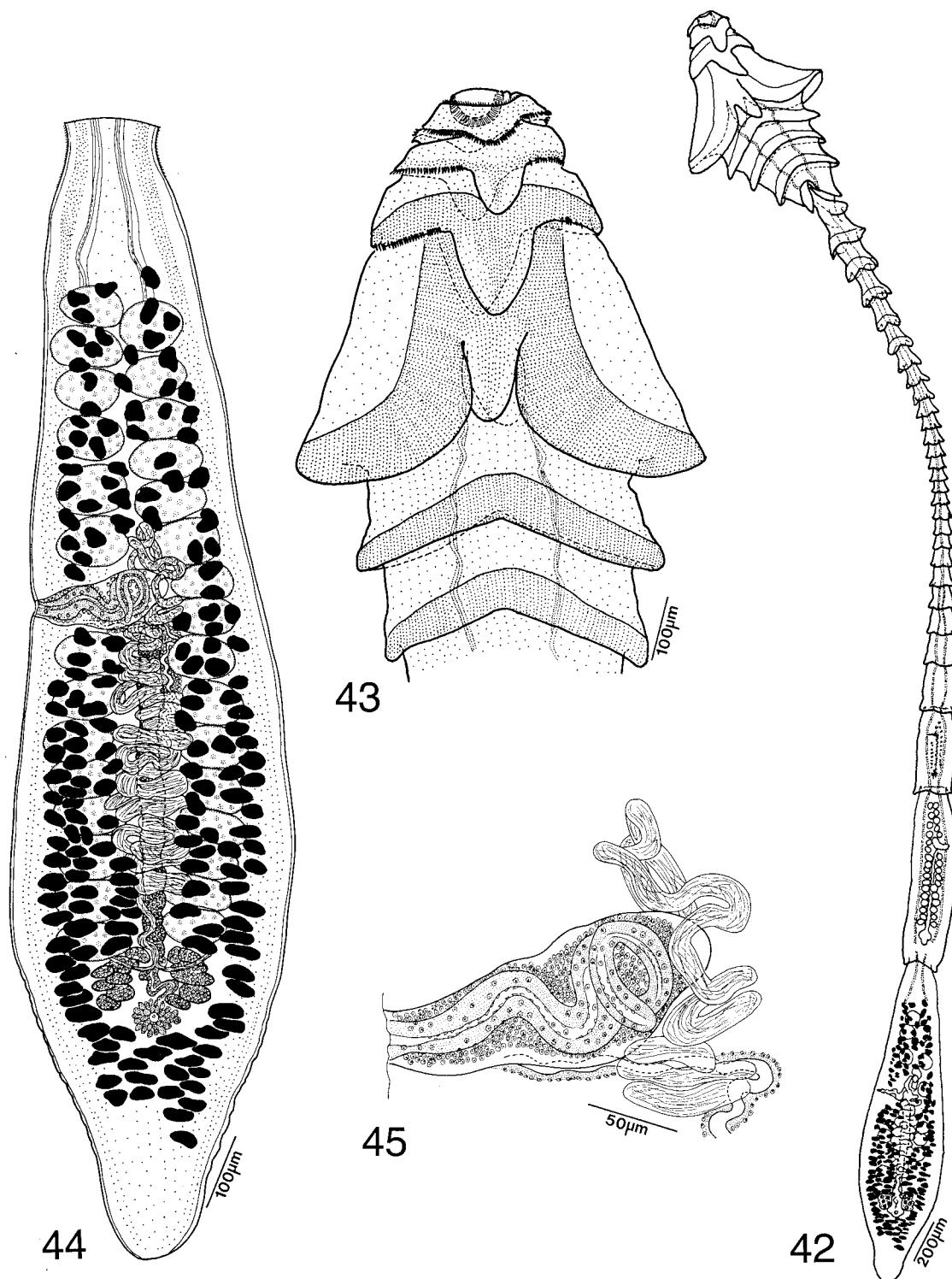
*Etymology:* This species is named for Professor Brent

B. Nickol in grateful recognition of his contributions to the professional development of both authors.

#### Remarks

*Litobothrium nickoli* n. sp. can be distinguished from *L. conformis*, *L. gracilie*, *L. amplifica*, *L. alopias*, and *L. janovyi* n. sp. in its possession of a greater number of pseudosegments (five vs three, three, four, four and four, respectively). It differs from *L. amsichensis* and *L. daileyi*, the other two species in the genus that exhibit five pseudosegments, in that only the posterior three pseudosegments are conspicuously cruciform, whereas in *L. amsichensis* all five pseudosegments are cruciform, and in *L. daileyi* the posterior four are cruciform. It most closely resembles *L. daileyi*, also found in *Alopias pelagicus*, but can be further distinguished from this species in its possession of a greater number of testes (31-38 vs 15-26) and vitelline follicles that are much more densely arranged. In addition, the size of the scolex relative to the strobila is significantly greater in *L. nickoli* than *L. daileyi*, and whereas the segments following the fifth pseudosegment decrease rapidly in width posteriorly along the length of the strobila to form a conspicuous constriction at the level of approximately the fifth segment in *L. nickoli*, the segments of the strobila of *L. daileyi* decrease in width much more gradually so that a region that could be interpreted as a constriction occurs at the level of approximately the 25th segment.

*L. nickoli* was found in three of the 10 specimens of *A. pelagicus*. If our hypothesis is correct that the identification of the hosts examined by Kurochkin & Slankis (1973) may have been in error, the fact that they did not describe specimens consistent with *L. nickoli* in any of the 14 host individuals they examined is problematical. However, the figures of *L. daileyi* provided by Kurochkin & Slankis (1973) shed some light on this issue; of the six figures presented, two (figures 1A & B) illustrate worms that very closely resemble *L. nickoli*. Most conspicuously, the worms portrayed possess a strobila bearing segments that taper dramatically beginning immediately behind the fifth pseudosegment. This is in contrast to, for example, figure 1D of their paper, which portrays a worm much more consistent in morphology with *L. daileyi*. If Kurochkin & Slankis' material of *L. daileyi* did indeed consist of a composite of two different species, the description of *L. daileyi* provided in that paper should be treated with caution, and the redescription provided above for *L. daileyi* might be a more ap-

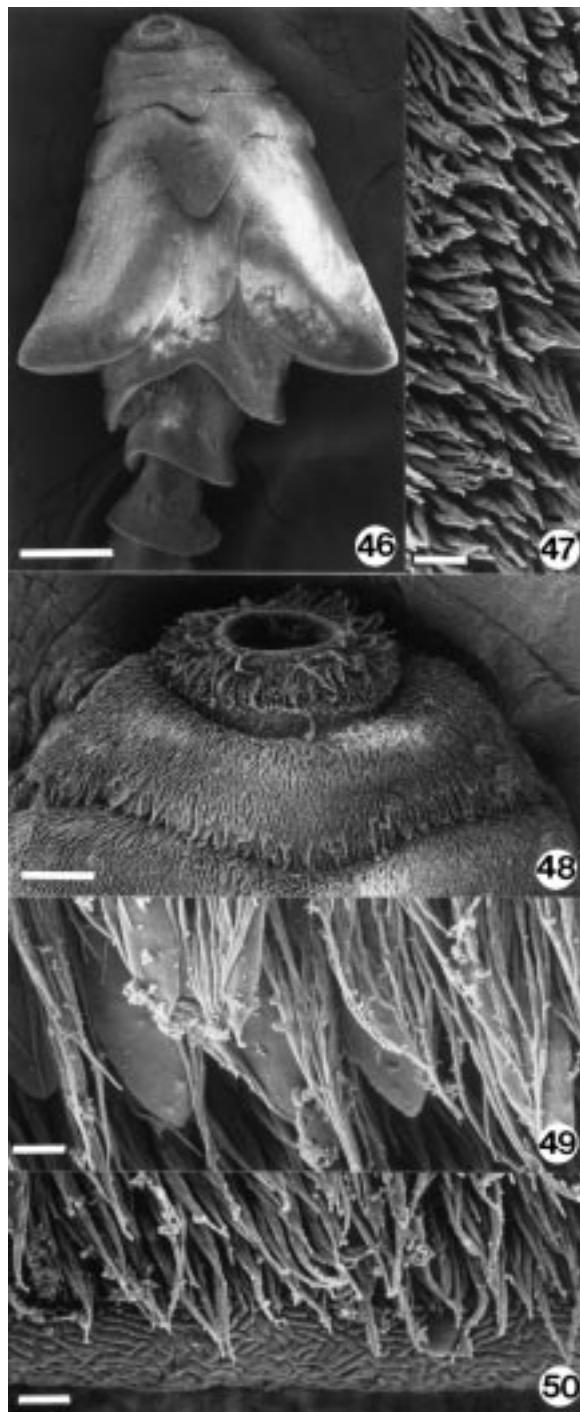


Figures 42–45. Line drawings of *Litobothrium nickoli* n. sp. 42. Entire specimen. 43. Scolex. 44. Mature terminal segment. 45. Detail of terminal genitalia.

ropriate circumscription of that species. It should be noted that we have chosen to redescribe specimens consistent with the worm figured in 1D by Kurochkin & Slankis (1973), rather than those figured in 1A & B, because the former appears to be more consistent with the measurements those authors provided for *L. daileyi*. Unfortunately, we have been unable to locate Kurochkin & Slankis' type-material of *L. daileyi* in order to confirm the presence of both species in their collections.

#### Key to the species of *Litobothrium*

1. a. With five cruciform pseudosegments (Figure 25) ..... *L. amsichensis*
- b. With fewer than five cruciform pseudosegments ..... 2
2. a. With three pseudosegments; all three pseudosegments cruciform ..... 3
- b. With greater than three pseudosegments; with at least three cruciform pseudosegments ..... 4
3. a. Strobila elongate, thread-like in form; segments narrow throughout length of strobila. *L. gracile*
- b. Strobila robust, not thread-like in form; segments immediately posterior to pseudosegments wide, segments gradually narrowing in width until midlevel of strobila to form conspicuous strobilar constriction ..... *L. coniformis*
4. a. Fourth cruciform pseudosegment with recurved lacinations and medial projections resembling a human nose (Figures 2, 17) ..... *L. amplifica*
- b. Fourth cruciform pseudosegment not modified as above ..... 5
5. a. First pseudosegment conspicuously cruciform (Figure 35) ..... 6
- b. First pseudosegment not conspicuously cruciform (Figures 29, 46) ..... 7
6. a. Strobila elongate and thread-like in form (Figure 12); first segment conspicuously narrower than fourth pseudosegment (Figure 13); segments posterior to pseudosegments approximately equal in width throughout length of strobila ..... *L. janovyi*
- b. Strobila robust, not thread-like in form; first segment only slightly narrower than fourth pseudosegment; segments posterior to pseudosegments gradually narrowing in width until midlevel of strobila to form strobilar constriction ..... *L. alopias*



Figures 46–50. Scanning electron micrographs of *Litobothrium nickoli* n. sp. 46. Scolex. 47. Densely packed filiform microtriches on third segment of strobila. 48. Apex of scolex showing first cruciform pseudosegment and opening of apical sucker. 49. Spine-like structures on posterior margin of second pseudosegment. 50. Filiform microtriches on posterior margin of third pseudosegment (note lack of spine-like structures). Scale-bars: 46, 100 µm; 47, 49–50, 1 µm; 48, 20 µm.

7. a. Scolex large relative to the total length of the strobila (Figure 42); anterior segments of strobila gradually narrowing to form conspicuous strobilar constriction at segment 5-6 (Figure 42) ..... *L. nickoli*  
 b. Scolex small relative to the total length of the strobila (Figure 6); segments of strobila gradually narrowing to form inconspicuous strobilar constriction at segment 20-25. (Figure 6) ..... *L. daileyi*

## Discussion

The homologies among the spine-like structures on the pseudosegments of the scolex in *L. amplifica*, *L. daileyi*, *L. janovyi* and *L. nickoli* and the structures seen on all but the reproductive organ bearing segments of *L. amsichensis* remain unclear. In all five species these elements are found in the same position (i.e. associated with the posterior margins) on the pseudosegments and segments. However, in the former four species they are restricted to a subset of the pseudosegments of the scolex, whereas in *L. amsichensis* they are found throughout the body on all but the reproductive organ bearing segments of the strobila. The elements seen in *L. amsichensis* are smaller, much more densely packed and are not nearly as spinose as those seen in the other four species; in fact these structures are quite consistent with the microtriches seen in some other cestode groups (see Caira et al., 1999). Even among the other four species these elements differ significantly in form. The spine-like structures of *L. amplifica* are the least like microtriches in that they are very large, appear to be lightly embedded in the surface rather than representing extensions of the surface itself, and they are hollow (Figure 3). The elements of *L. daileyi*, *L. janovyi* and *L. nickoli* might be interpreted as spiniform microtriches, except that, given their similar position, it is difficult to believe that they are not equivalent to the structures seen in *L. amplifica*. Based on the above differences, until the internal structure of these features can be investigated with transmission electron microscopy, we have considered the elements seen in *L. amsichensis* to be microtriches and the elements in the other four species to be 'spine-like' structures.

Our current understanding of the host associations of the litobothriideans depends heavily on whether Kurochkin & Slankis (1973) original identification of the host of *L. amplifica* and *L. daileyi* was correct.

If so, *Alopias superciliosus* hosts five of the eight known species in the genus: *L. alopia*, *L. amplifica*, *L. coniformis*, *L. daileyi* and *L. janovyi*. *A. pelagicus* hosts *L. nickoli*, as well as *L. amplifica* and *L. daileyi*. *Odontaspis ferox* and *Mitsukurina owstoni* each host one species, *L. gracile* and *L. amsichensis*, respectively. However, if, as suggested here, the original host identification for *L. amplifica* and *L. daileyi* was in error, *A. superciliosus* hosts only three species: *L. alopia*, *L. coniformis* and *L. janovyi*. This would suggest a stronger degree of host-specificity, as each known species of *Litobothrium* is hosted by only a single species of lamniform shark. The host associations of litobothriideans, as inferred herein, are summarised in Table 1.

The known geographical distributions of litobothriideans can be summarised as follows: *L. alopia* and *L. coniformis* are known from Newport Beach and Huntington Beach, California; *L. gracile* is known from San Clemente Island, California; *L. amplifica* and *L. daileyi* are known from the Gulf of Tehuantepec, Oaxaca, on the west coast of Mexico, and also from Bahia de Los Angeles and Santa Rosalia in the Gulf of California, Mexico; *L. janovyi* is known from Santa Rosalia in the Gulf of California, Mexico; *L. nickoli* is known only from Bahia de Los Angeles in the Gulf of California; and *L. amsichensis* is known from the waters off Ulladulla on the east coast of Australia. Thus, the waters off the west coast of North America between 20° and 40°N latitude appear to represent a hot-spot of litobothriidean diversity as seven of the eight species are known only from these waters. Furthermore, litobothriideans remain known only from the Pacific Ocean, which is curious in that all four species of sharks reported to host these worms have been reported from sites bounding essentially all of the major oceans of the world (Compagno, 1984). This distribution may, however, be an artifact caused by the lack of sampling, because we can find no evidence that individuals of any of the lamniform shark species that host litobothriideans have been examined for litobothriideans in any other regions of the world. Indeed, among the specimens of tapeworms from elasmobranchs sent to us by Murray Dailey were two slides of litobothriideans collected from Miami, Florida on July 11, 1979. One slide, with worms identified as having come from *Alopias superciliosus*, includes fragments of several worms, at least two of which are identifiable as *L. coniformis*. The host identification on the other slide is given only as 'thresher shark'. This slide also bears fragments of several worms, at least one of

Table 1. Host associations of litobothriideans.

|   |
|---|
| <i>Alopias superciliosus</i> (Lowe, 1839)                             |
| <i>Litobothrium alopias</i> Dailey, 1969                              |
| <i>Litobothrium conformis</i> Dailey, 1969                            |
| <i>Litobothrium janovyi</i> n. sp.                                    |
| <i>Alopias pelagicus</i> Nakamura, 1935                               |
| <i>Litobothrium amplifica</i> (Kurochkin & Slankis, 1973) Euzet, 1994 |
| <i>Litobothrium daileyi</i> Kurochkin & Slankis, 1973                 |
| <i>Litobothrium nickoli</i> n. sp.                                    |
| <i>Odontaspis ferox</i> (Risso, 1810)                                 |
| <i>Litobothrium gracile</i> Dailey, 1971                              |
| <i>Mitsukurina owstoni</i> Jordan, 1898                               |
| <i>Litobothrium amsichensis</i> Caira & Runkle, 1993                  |

which appears to be a specimen of *L. janovyi*, suggesting that the host individual from which this material came was also *A. superciliosus*. This material is the first, and, at present only, evidence of litobothriideans from the Atlantic Ocean.

Higher level classifications of the eucestodes have been, and remain (e.g. Khalil et al., 1994), based on suites of characters, with the features of the scolex given significant emphasis. The litobothriidean scolex, characterised by cruciform pseudosegments, is unique among all other tapeworms and was the basis of Dailey's (1969) decision to erect a new order to house these species. Although not explicitly stated, the tetraphyllidean-like arrangement of the reproductive organs of these species along with their occurrence in sharks were clearly factors that influenced Euzet (1994) to consider the group to be members of the order Tetraphyllidea. The position of the litobothriideans among the 14 currently recognised orders of the Eucestoda (Khalil et al., 1994) was examined using molecular data by Olson & Caira (1999). Their results, based on the sequences of two independent genes, suggested that the Litobothriidea was distinct from the Tetraphyllidea, often forming a sister-group relationship to the tetraphyllidean clade (that also included a proteocephalidean species). However, the molecular-based study by Olson et al. (1999), which considered only tetraphyllidean, lecanicephalidean and litobothriidean species, supported, albeit weakly, the placement of the litobothriideans among the tetraphyllidean taxa examined. These studies show that litobothriideans indeed have tetraphyllidean affinities evolutionarily, but that further analyses are needed to be more certain of their exact position and its bearing on their classifica-

tion; until that time, we are inclined to recognise the group as being distinct from the order Tetraphyllidea.

Euzet (1994) and Al Kawari et al. (1994) have both linked the species *Eniochobothrium gracile* Shipley & Hornell, 1906 with *Litobothrium* spp. based on a similarity among the uniquely modified anterior segments of their strobila. However, unlike *Litobothrium* spp., *E. gracile* does not possess segments that are cruciform, has a scolex and proglottid anatomy consistent with that of the Lecanicephalidea and is a parasite of rays (see Caira et al., 1999, 2001). Moreover, the molecular works of Olson & Caira (1999) and Olson et al. (1999) strongly supported the affinity of *E. gracile* with a member of the lecanicephalidean genus *Cephalobothrium* Shipley & Hornell, 1906 and not with species of *Litobothrium*.

## Acknowledgements

We thank J. Ralph Lichtenfels for the loan of all type-specimens examined in this study. Paul Cislo, Stephen Curran, Brian Jacobs, Kirsten Jensen and Tim Ruhnke provided assistance with the dissection of the sharks for tapeworms. We are especially grateful to Vasyl Tkach for translating Kurochkin & Slankis (1973) from Russian, Andrew Martin for providing molecular data to aid in the identification of the thresher sharks examined in 1993, and Gavin Naylor for his assistance with identification of thresher sharks examined in 1996. This research was supported by National Science Foundation grants No. DEB 9300796 and No. DEB 9532943. PDO was supported by a Wellcome Trust grant to Tim Littlewood (The Natural History

Museum, London, UK) during the writing of this manuscript.

## References

- Al Kawari, K.S.R., Saoud, M.F.A. & Wanas, M.Q.A. (1994) Helminth parasites of fishes from the Arabian Gulf. 7. On *Eniochobothrium qatarense* sp. nov. (Cestoda: Lecanicephalidea) and the affinities of *Eniochobothrium* Shipley and Hornell, 1906, *Litobothrium* Dailey, 1969 and *Renyxa* Kurochkin and Slankis, 1973. *Japanese Journal of Parasitology*, **43**, 97-104.
- Caira, J.N. & Runkle, L.S. (1993) Two new tapeworms from the goblin shark *Mitsukurina owstoni* off Australia. *Systematic Parasitology*, **26**, 81-90.
- Caira, J.N., Jensen, K. & Healy, C.J. (1999) On the phylogenetic relationships among tetrphyllidean, lecanicephalidean and diphylloidean tapeworm genera. *Systematic Parasitology*, **42**, 77-151.
- Caira, J.N., Jensen, K. & Healy, C.J. (2001) Interrelationships among tetrphyllidean and lecanicephalidean cestodes. In: Littlewood, D.T.J. & Bray, R.A. (Eds) *Interrelationships of the Platyhelminthes*. Taylor & Francis, London, pp. 135-148.
- Compagno, L. (1984) FAO species catalogue. VI. 4, Parts 1 and 2: Sharks of the world. An annotated and illustrated catalogue of shark species known to date. *FAO Fisheries Synopsis*, **125**, 655 pp.
- Dailey, M. (1969) *Litobothrium alopias* and *L. coniformis*, two new cestodes representing a new order from elasmobranch fishes. *Proceedings of the Helminthological Society of Washington*, **36**, 218-224.
- Dailey, M. (1971) *Litobothrium gracile* sp. n. (Eucestoda: Litobothridae) from the sand shark (*Odontaspis ferox*). *Journal of Parasitology*, **57**, 94-96.
- Euzet, L. (1994) Order Tetrphyllidae Carus, 1863. In: Khalil, L.F., Jones, A. & Bray, R.A. (Eds) *Keys to the cestode parasites of vertebrates*. Wallingford: CAB International, pp. 149-194.
- Khalil, L.F., Jones, A. & Bray, R.A. (1994) *Keys to the cestode parasites of vertebrates*, Wallingford: CAB International, 751 pp.
- Kurochkin, Y.B. & Slankis, A.Y. (1973) New representatives and the composition of the order Litobothridae Dailey, 1969 (Cestoidea). *Parazitologiya*, **7**, 502-508. (In Russian).
- Olson, P.D. & Caira, J.N. (1999) Evolution of the major lineages of tapeworms (Platyhelminthes: Cestoidea) inferred from 18S ribosomal DNA and elongation factor-1 $\alpha$ . *Journal of Parasitology*, **85**, 1,134-1,159.
- Olson, P.D., Ruhnke, T.R., Sanney, J. & Hudson, T. (1999) Evidence for host-specific clades of tetrphyllidean tapeworms (Platyhelminthes: Eucestoda) revealed by analysis of 18S ssrDNA. *International Journal for Parasitology*, **29**, 1,465-1,476.
- Schmidt, G.D. (1986) *Handbook of tapeworm identification*. Boca Raton: CRC Press, 675 pp.
- Wardle, R. A., McLeod, J.A. & Radinovsky, S. (1974) *Advances in the zoology of tapeworms*, 1950-1970. Minneapolis: University of Minnesota Press, 274 pp.