

## NEW GENUS WITH TWO NEW SPECIES OF CAPSALID MONOGENEANS FROM DASYATIDS IN THE GULF OF CALIFORNIA

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**ABSTRACT:** We propose *Listrocephalos* n. gen. (Monogenea: Capsalidae: Entobdellinae) for 2 new capsalid species that infect the skin of 2 species of diamond stingrays (Dasyatidae) in the Gulf of California. We also provide additional observations on 2 previously described capsalid species, which infect the external body surface of stingrays and are currently in *Entobdella* Blainville in Lamarck, 1818, and transfer them to the proposed new genus. The members of this genus, *Listrocephalos corona* (Hargis, 1955) n. comb. (type species), *Listrocephalos guberleti* (Caballero and Bravo-Hollis, 1962) n. comb., *Listrocephalos kearni* n. sp., and *Listrocephalos whittingtoni* n. sp., differ from other entobdellines by the combination of having an anterolateral adhesive pad comprising 2 ventral columns of raised ovoid structures, 1 pair of glands that flank the preoral depression, a discoid and aseptate haptor that lacks a marginal valve but has clover-shaped papillae dispersed radially over its entire ventral surface, minute and nonoverlapping median haptoral sclerites, a bizarre chamber yielding a duct that opens on the surface of the penis, separate genital pores, and a gland of Goto located between the testes and ovary. *Listrocephalos kearni* n. sp. infects *Dasyatis brevis* and is most easily distinguished from its congeners by the combination of lacking penis tubules and having a convoluted proximal portion of the vas deferens that occupies the space between the ootype and ovary. *Listrocephalos whittingtoni* n. sp. infects *Dasyatis longa* and is most easily distinguished from its congeners by the combination of having penis tubules and a vaginal pore that is located posterior to the level of the uterus. We report specimens of *L. corona* from the ventral body surface of *Dasyatis say* from a new locality, Mississippi Sound, as well as specimens of *L. guberleti* from the skin of 2 new hosts, *Urobatis maculatus* and *Urobatis concentricus*, and a new locality, Elkhorn Slough, California. We provide a diagnostic key and a table of records for *Listrocephalos* spp.

We propose a new genus of Entobdellinae Bychowsky, 1957 sensu Egorova, 1999 (Capsalidae Baird, 1853 sensu Yamaguti, 1963) for 2 new capsalid species collected from the skin of 2 species of diamond stingrays (Dasyatidae) captured in the Gulf of California. We also provide additional observations on 2 previously described capsalid species currently in *Entobdella* Blainville in Lamarck, 1818 and transfer them to the proposed genus.

### MATERIALS AND METHODS

Stingrays were captured by gill net or spear in the Gulf of California during August–September 1993 and May–June 1996 as well as by hook and line in Mississippi Sound off Ocean Springs, Mississippi, during October 2000; the stingray from Monterey Bay was collected by gill net during March–September 2002. Those from off Marathon, Florida, were collected by hand net or spear during July 2003; those from Alligator Harbor and Saint Joe Bay, Florida, were collected by spear during August 2003 and April 2004, respectively; and those from the Northern Gulf of Mexico off Horn Island and Ship Island, Mississippi, were captured by spear during January–April 2004. For comparison, we also studied specimens of *Entobdella hippoglossi* (Mueller, 1776) Blainville, 1818 (Monogenea: Entobdellinae) collected from the skin of a pacific halibut *Hippoglossus stenolepis* Schmidt, 1904 (Pleuronectidae) caught by hook and line from Cook Inlet off the southern tip of the Kenai Peninsula, Alaska, during 28–29 June 2000. All monogeneans were fixed in 10% neutral buffered formalin. Specimens from Mississippi were heat killed under slight coverslip pressure before fixation. Some whole-mounted specimens were stained in Van Cleave hematoxylin with several additional drops of Ehrlich hematoxylin, alkalized at 70% ethanol with lithium carbonate and butylamine, dehydrated, cleared in clove oil, and mounted in Canada balsam. Others were stained in acetocarmine or fast green and cleared in cedarwood oil or xylene. To elucidate particular aspects of the genitalia, 2–3 specimens of each of the illustrated species were serially sectioned. These specimens were processed routinely, embedded in paraffin, sectioned at 4

µm, and stained with Gill hematoxylin and eosin. Specimens for scanning electron microscopy (SEM) were dehydrated, immersed in hexamethyldisilazane for 15 min, dried under a vacuum for 10 min, and sputter coated with gold–palladium. Drawings of whole-mounted specimens were made with a drawing tube, and measurements are reported in micrometers and given as ranges followed by the sample size in parentheses. Where applicable, anatomical terms are used in accordance with those of Llewellyn and Euzet (1964), Whittington and Horton (1996), and Whittington et al. (2001). The works of Bychowsky (1957), Yamaguti (1963), and Egorova (1999) helped define the diagnostic features of Entobdellinae and Capsalidae. We use “bizarre chamber” and “penis tubules” as provisional terms pending a study of live specimens or an ultrastructural study of these features. We think that such studies could resolve the fine structure and function of these organs in this group of monogeneans. Holotypes and paratypes were deposited in the Instituto de Biología, Universidad Nacional Autónoma de México, México City, México (IBUNAM), and other paratypes were deposited in the United States National Parasite Collection (USNPC) at Beltsville, Maryland. A series of voucher specimens representing the 2 previously named species of this group also were deposited in the USNPC.

### DESCRIPTIONS

#### *Listrocephalos* n. gen.

(Figs. 1–16)

**Description:** Entobdellinae Bychowsky, 1957 sensu Egorova, 1999 (Capsalidae Baird, 1853 sensu Yamaguti, 1963). Body having anterior region with anterolateral adhesive pad comprising 2 ventral columns of raised ovoid structures; preoral depression medial, subterminal, flanked by 1 pair of small glands opening on ventral body surface. Haptor discoid, aseptate, lacking marginal valve, papillate; papillae clover shaped, dispersing radially over entire haptoral ventral surface. Median haptoral sclerites minute, nonoverlapping; accessory sclerites bent, with hooked distal tip, directed anteroventrally; anterior hamuli with bifid proximal end and hooked distal tip, directed posterovertrally; posterior hamuli with hooked distal tip, directed posterovertrally. Hooklets 14 in number, with blade and guard forming C-shaped distal tip, with straight shaft, submarginal. Mouth ventral, near level of eyespots. Pharynx papillate, with intrinsic gland cells. Excretory bladders juxtaposed, longitudinal. Testes

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juxtaposed, intercecal, postovarian, spheroid. Vas deferens extending anteriad in sinistral half of body before entering wall of penis sac dorsomedially. Penis sac posterior to pharynx, orienting anterosinistrad roughly 45° from midline, enclosing bizarre chamber and male accessory gland reservoir; bizarre chamber with distal end yielding narrow duct extending sinistrad and opening on penis surface; male accessory gland reservoir receiving at its proximal end efferent ducts of male accessory gland, joining distally with vas deferens to form ejaculatory duct; ejaculatory duct opening separately from bizarre chamber duct on penis surface. Male pore sinistrolateral, opening at level of or posterior to pharynx. Gland of Goto with 2 ovoid internal cells, occupying space between testes and ovary. Ovary intercecal, pretesticular. Uterine pore sinistral, just dorsal to male pore. Vaginal pore ventral, between uterine pore and vitelline reservoir; vitelline reservoir preovarian. Egg resembling champagne glass, retained singly or clustered, with opercular pole bearing rounded apex; egg anchor discoid with recurved edge. Parasites of external body surface of diamond stingrays (*Dasyatidae*) and round rays (*Urolophidae*).

**Diagnosis:** Anterior region with anterolateral adhesive pad comprising 2 ventral columns of raised ovoid structures; preoral depression flanked by 1 pair of glands. Haptor discoid, aseptate, lacking marginal valve (e.g., Fig. 17), with clover-shaped papillae dispersing radially over entire haptoral ventral surface; median sclerites minute, nonoverlapping. Vas deferens joining with distal portion of male accessory gland reservoir to form ejaculatory duct. Penis with 2 pores: 1 each for bizarre chamber duct and ejaculatory duct. Male pore separate from uterine pore. Gland of Goto occupying space between testes and ovary.

**Type species:** *Listrocephalos corona* (Hargis, 1955) n. comb. (syn. *Entobdella corona* Hargis, 1955) from ventral body surface and gill of the southern stingray, *Dasyatis americana* Hildebrand and Schroeder, 1928 (*Dasyatidae*), Atlantic stingray, *Dasyatis sabina* (Lesueur, 1824), and bluntnose stingray, *Dasyatis say* (Lesueur, 1817), in Alligator Harbor, Florida, Gulf of Mexico.

**Other species:** *Listrocephalos guberleti* (Caballero and Bravo-Hollis, 1962) n. comb. (syn. *Entobdella guberleti* Caballero and Bravo-Hollis, 1962) from gill of Haller's round ray, *Urobatis halleri* (Cooper, 1863) (*Urolophidae*), off Guaymas, Sonora, Gulf of California, Mexico.

**Etymology:** The Greek, "*Listrocephalos*" means shovelhead and refers to "*listron*," meaning shovel and "*cephalos*," meaning head.

## Remarks

The members of *Listrocephalos* n. gen. and *Trimusculotrema* Whittington and Barton, 1990 (*Capsalidae*) have a papillate and circular haptor that lacks a marginal valve (Fig. 17), small and nonoverlapping median haptoral sclerites (Figs. 3, 16), separate genital pores (Figs. 1, 2, 13), a uterine pore that opens dorsally on the left side of the body (Fig. 1), and an egg with an opercular pole bearing a rounded apex (Figs. 8, 14 of Whittington and Barton, 1990). In addition, the members of these genera infect the external body surface of ecologically similar and phylogenetically related fishes: *Trimusculotrema* spp. reportedly infect diamond stingrays only, and *Listrocephalos* spp. reportedly infect diamond stingrays and round rays only. *Listrocephalos*

differs from *Trimusculotrema* by having an anterolateral adhesive pad comprising 2 ventral columns of raised ovoid structures (Fig. 7) as well as clover-shaped haptoral papillae. *Trimusculotrema* has a pair of anterior saucer-like discs and knob-like haptoral papillae (fig. 9 of Whittington and Barton, 1990). Features of the terminal male genitalia also distinguish these genera: *Listrocephalos* has a protrusible intromittent organ, i.e., a penis, whereas *Trimusculotrema* has an eversible intromittent organ, i.e., a cirrus. Furthermore, *Listrocephalos* has 2 openings on the penis, 1 each for the bizarre chamber duct and the ejaculatory duct (Figs. 2, 4, 13), whereas *Trimusculotrema* has only 1 opening on the cirrus (fig. 7 of Whittington and Barton, 1990). In addition, the new genus is unique in that the vas deferens unites with the distal portion of the male accessory gland reservoir to form an ejaculatory duct (Figs. 2, 4, 10).

Despite some morphological similarities, it is difficult to determine which morphological features are primarily the result of ecological constraints, e.g., attachment, egg dispersal, possible spermatophore production and transfer, or locomotion, and which are the result of phylogenetic constraints, i.e., ancestry. The similarity of some morphological features may be because of convergent evolution (Whittington and Barton, 1990) rather than homology because the life histories of capsalids are diverse (Kearn and Whittington, 1992). For this reason and despite the morphological similarities between *Listrocephalos* and *Trimusculotrema* as well as the fact that they infect phylogenetically related hosts, we are hesitant to suggest that the capsalids in these genera are closely related. In the present case, members of both genera reportedly infect the skin of stingrays only, and perhaps they resemble each other because they similarly attach to the host, copulate, disperse eggs, and transfer spermatophores, if present.

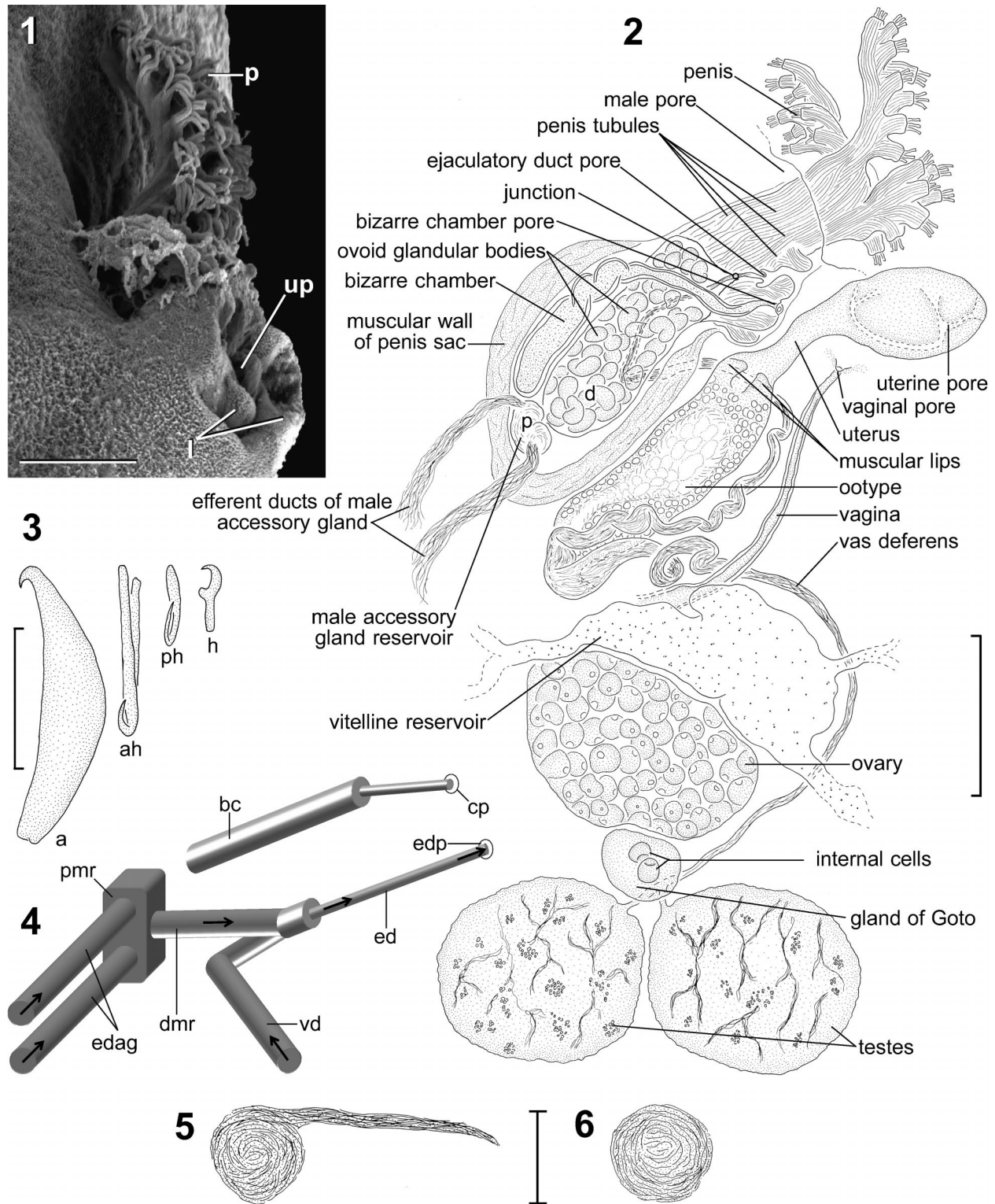
### *Listrocephalos corona* (Hargis, 1955) n. comb.

(Figs. 1–12)

**Adult (measurements based on 8 adult specimens):** Body 3,016–3,586 (5) long, 1,386–1,956 (5) in maximum width or 1.8–2.2 times longer than wide. Anterolateral adhesive pad comprising 11 (5) raised ovoid structures on each side of body (Fig. 7); anterior- and posterior-most raised ovoid structures smallest, easily overlooked in poorly stained specimens. Glands flanking preoral depression 15–25 (5) wide. Haptor 815–978 (5) in diameter or 0.5–0.6 times body width; haptoral papillae 12–37 (4) wide. Accessory sclerites 69–99 (10) long; anterior hamuli 35–62 (10) long or 0.4–0.8 times accessory sclerite length; posterior hamuli 22–37 (6) long; hooklets 14 in number, 15–20 (5) long (Fig. 3). Pharynx 229–338 (5) long, 258–353 (5) wide. Excretory bladders 380–524 (10) long, 40–50 (10) wide or 8.3–11.1 times longer than wide, 880–1,080 (5) or 0.3 times body length from anterior body end.

Testes 1,350–1,473 (5) or 0.4–0.5 times body length from posterior end of body, 218–277 (10) or 0.1–0.2 times body width in diameter (Fig. 2). Vas deferens extending anteriad in sinistral half of body, arching dextrad and traversing midline before becoming convoluted, recurving and extending sinuously anteriad before arching dorsally. Penis sac 465–692 (5) long, 188–287 (5) wide or 2.1–2.5 times longer than wide; wall of proximal portion of penis sac thick, muscular, refractive. Bizarre chamber orienting longitudinally in penis sac, with re-





FIGURES 1–6. *Listrocephalos corona* (Hargis, 1955) n. comb. (syn. *Entobdella corona* Hargis, 1955) (Monogenea: Entobdellinae) from skin of an Atlantic stingray, *Dasyatis say* (Lesueur, 1817) (Dasyatidae). **1.** Scanning electron micrograph: penis (p), uterine pore (up), and external muscular lips (l) flanking uterine pore, dorsal view. Note that ends of penis appear tasseled. Bar = 100  $\mu$ m. **2.** Genitalia labeled for comparative purposes: proximal (p) and distal (d) portions of male accessory gland reservoir. Proximal extent of penis tubules not illustrated. “Junction” denotes site where vas deferens and distal portion of male accessory gland reservoir join to form ejaculatory duct, ventral view. Composite based primarily on voucher specimen, USNPC 94821. Bar = 250  $\mu$ m. **3.** Median haptoral sclerites: accessory sclerite (a), anterior hamulus (ah), posterior hamulus (ph). Hooklet (h). Bar = 30  $\mu$ m. **4.** Composite diagram of terminal male genitalia of *Listrocephalos* spp. based on histological sections and whole-mounted specimens of *L. corona*, *L. guberleti*, *L. kearni*, and *L. whittingtoni*: bizarre chamber (bc), bizarre chamber pore (cp), proximal portion of male accessory gland reservoir (pmr), distal portion of male accessory gland reservoir (dmr), efferent ducts of male accessory gland (edag), vas deferens (vd). **5.** Longitudinal section of a testis. **6.** Cross-section of a testis.

fractive wall, containing granular material, with duct opening near base of penis on posterior aspect of penis surface (Figs. 2, 4). Male accessory gland reservoir occupying much of penis sac volume, with proximal and distal portions; proximal portion dextral, kidney-shaped or ovoid, thin walled, lateral or dorsal to bizarre chamber, receiving efferent ducts of male accessory gland; distal portion larger than proximal portion, joining with proximal portion of male accessory gland reservoir in dextral half of penis sac immediately posterior to proximal end of bizarre chamber (Figs. 2, 4), enclosing ovoid glandular bodies, constricting at distal end about the point at which penis sac wall becomes thinner and less muscular. Vas deferens joining distal portion of male accessory gland reservoir in sinistral portion of penis sac to form ejaculatory duct (Figs. 2, 4, 10); proximal portion of ejaculatory duct laterally expanded if filled with sperm (Fig. 10) or of a consistent diameter throughout length if not swollen with sperm (Fig. 2), extending sinistrad a short distance before opening near base of penis and on posterior aspect of penis just lateral to bizarre chamber pore (Figs. 2, 4). Penis branching, with at least 3 main trunks each having multiple smaller branches, having penis tubules (Figs. 1, 2, 8, 9, 11, 12); penis tubules originating in proximal or medial portion of penis sac and extending sinistrad, approximately 3–6 in diameter; proximal portion of tubules surrounded by a weakly staining granular material, blind ended, thin walled, with lumen containing granular material (Fig. 11); distal portion of tubules with dense, darkly staining material in lumen, with wall 1 thick (Fig. 12), opening on penis branches (Figs. 1, 8). Probable spermatophores observed among penis branches in single whole-mounted specimen, spheroid, with extension (Fig. 5) or not (Fig. 6), lacking obvious outer membrane or jelly coating, containing granular material and dense aggregation of sperm; spheroid portion approximately 14–17 in diameter; extension approximately 46 long (Fig. 5). Male pore 593–943 (5) or 0.2–0.3 times body length from anterior body end. Gland of Goto 84–109 (5) long, 84–104 (5) wide; internal cells 25 (5) in diameter.

Ovary 84–249 (5) long, 234–308 (5) wide or 0.2 times body width (Fig. 2). Vaginal pore minute, at level of male accessory gland reservoir, 791–949 (5) or 0.3 times body length from anterior body end. Vagina extending diagonally posteriad, narrow throughout its length, ventral to vas deferens, dorsal to vitelline reservoir. Connection of vitelline reservoir with vagina not observed. Ootype lateral to and orienting in parallel with penis sac, with proximal region dorsal to vas deferens, 275–425 (5) long, 89–119 (5) wide or 2.7–4.2 times longer than wide, highly glandular, with circular gland cells covering luminal surface, limited distally by pair of flap-like muscular lips. Uterus ventral to vas deferens, extending diagonally anteriad before opening on dorsal surface; uterine pore between level of male pore and vaginal pore, 99–149 (5) from vaginal pore, 742–890 (5) or 0.2–0.3 times body length from anterior body end, with muscular lips flanking opening (Figs. 1, 2). Gland cells near ootype and collecting ducts serving base of ootype

not observed. Egg body 203 (1) long, 79 (1) wide; filament 149 (1) long, 12 (1) wide; anchor 62 (1) in diameter.

*Specimens examined:* A total of 13: 1 paratype from the ventral body surface and gill of either *D. americana*, *D. sabina*, or *D. say* in Alligator Harbor (USNPC 38147; the host species was not specified on the slide label or by Hargis [1955]); 12 specimens from the ventral body surface of *D. say* from Mississippi Sound off Biloxi, Mississippi, Gulf of Mexico (30°26'N, 88°59'W) (a new locality record) (voucher specimens, USNPC 94821–94825).

## Remarks

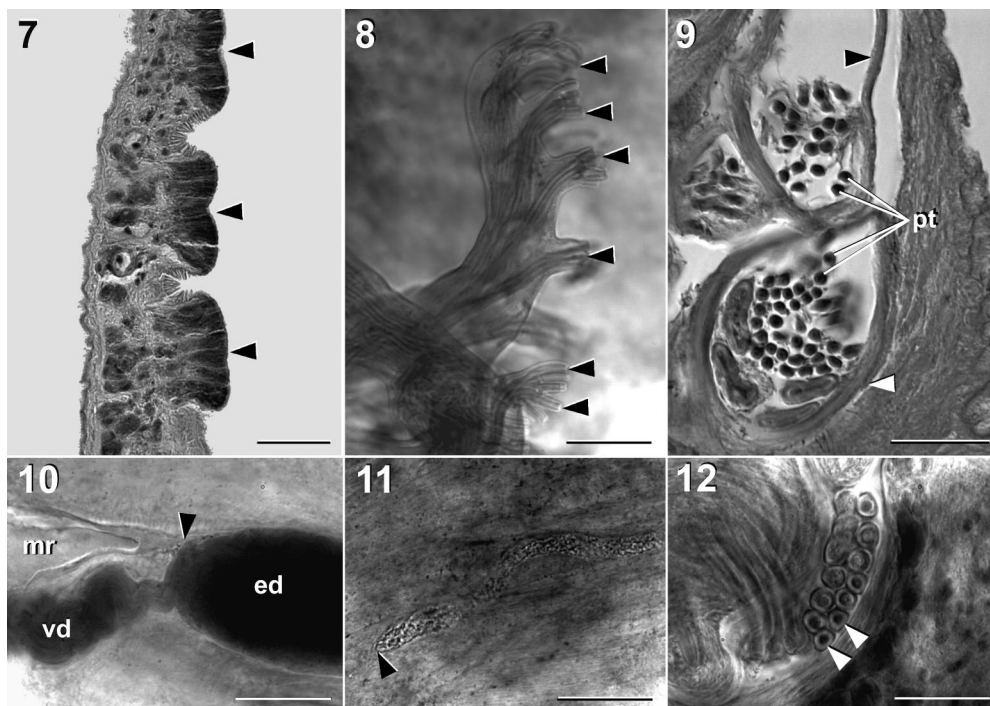
There were discrepancies between our specimens of *L. corona* and the original description of this monogenean (as *E. corona* Hargis, 1955). The “anterolateral pairs of hollow head organs” in the study of Hargis are probably the structures we call raised ovoid structures; however, rather than each raised ovoid structure being hollow and opening marginally as Hargis described, the surface of each of these structures is confluent with the lateral body margin. Furthermore, we did not detect an obvious pore or opening on the surface of any of the raised ovoid structures of the anterolateral adhesive pad (Fig. 7). Regarding the number of raised ovoid structures, we counted 11 on each side of the body, not 9 as previously reported by Hargis (1955). Regarding the preoral depression and mouth, we suspect that Hargis misinterpreted the preoral depression as an opening, i.e., the mouth, to a “buccal funnel” (fig. 28 of Hargis, 1955). The preoral depression of *L. corona* is subterminal and between the anterior-most raised ovoid structures, whereas the mouth is minute and located immediately anterior to the pharynx. We did not observe a buccal funnel. Haptoral papillae were not described previously by Hargis but are present in the haptor of *L. corona*. Regarding the median haptoral sclerites, Hargis illustrated the accessory sclerites as pointed at each end, but we observed that the accessory sclerites have a rounded proximal end and a hooked distal tip. Furthermore, we suspect that Hargis transposed the anterior and posterior hamuli because he illustrated the proximal end of the anterior hamuli as straight and that of the posterior hamuli as bifid (figs. 30–32 of Hargis, 1955); only the anterior hamuli are bifid (Fig. 3).

Details of the terminal genitalia were vague in the original description of *L. corona*. We observed 2 openings on the surface of the penis: one for the bizarre chamber duct and another for the ejaculatory duct. Interestingly, Hargis (1955) may have illustrated these openings (fig. 28 of Hargis, 1955); however, they were omitted from the text and were cryptic in his illustration. Also, we suspect that Hargis misinterpreted the muscular penis sac wall for a cuticle. We did not observe a cuticle, although in this species, the muscles of the penis sac wall are highly refractive and superficially resemble a cuticle. Hargis also did not detail the junction of the vas deferens and the distal portion of the male accessory gland reservoir that forms the ejaculatory duct, but this connection is clearly present in our

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accessory gland (edag), vas deferens (vd), ejaculatory duct (ed), ejaculatory duct pore (edp); arrows represent presumed direction of product flow. Note that there is no arrow associated with bizarre chamber. 5. Comma-shaped probable spermatophore from penis branches. Voucher specimen, USNPC 94821. Bar = 17  $\mu$ m. 6. Spheroid probable spermatophore near site of Figure 5.





FIGURES 7–12. *Listrocephalos corona* (Hargis, 1955) n. comb. (syn. *Entobdella corona* Hargis, 1955) (Monogenea: Entobdellinae) from skin of an Atlantic stingray, *Dasyatis say* (Lesueur, 1817) (Dasyatidae). **7.** Longitudinal section of anterior adhesive pad showing raised ovoid structures (arrowheads) on ventral surface and pits between each structure that have microvilli, hematoxylin and eosin. Bar = 30  $\mu\text{m}$ . **8.** Light micrograph, branched tip of penis showing penis tubules opening on each branch (arrowheads), ventral view. Voucher specimen, USNPC 94821. Bar = 25  $\mu\text{m}$ . **9.** Cross section of branched portion of penis showing penis tubules (t) and muscular wall of penis branches (arrowheads), which are delineated from penis tubules that course through each branch, hematoxylin and eosin. Bar = 20  $\mu\text{m}$ . **10.** Light micrograph of whole-mounted specimen showing junction (arrowhead) of distal portion of male accessory gland reservoir (mr), vas deferens (vd), and ejaculatory duct (ed); the proximal region of the ejaculatory duct is swollen with sperm. Voucher specimen, USNPC 94822. Bar = 25  $\mu\text{m}$ . **11.** Light micrograph of whole-mounted specimen showing blind-ending proximal tip (arrowhead) of a penis tubule. This tubule has a granular material in its lumen. Voucher specimen, USNPC 94823. Bar = 25  $\mu\text{m}$ . **12.** Light micrograph of whole-mounted specimen showing cross-sectional view of penis tubules (arrowheads) near bizarre chamber pore. Voucher specimen, USNPC 94824. Bar = 25  $\mu\text{m}$ .

specimens (Figs. 2, 4, 10). Regarding the female genitalia, we confirm that the uterine pore is separate from the male pore, i.e., there is no internal common atrium, and the vagina does not join the uterus.

A study of live specimens of *L. corona* followed by transmission electron microscopy of those specimens could help resolve the fine structure and function of the bizarre chamber and the penis tubules, both of which were not described previously in *L. corona*. We lack detailed observations of these features in live specimens because when we collected our specimens of *L. corona* we were not studying the anatomy of this species, and subsequent necropsies of dasyatids in the Gulf of Mexico (16 bluntnose stingrays, 36 Atlantic stingrays, and 1 southern stingray from the Northern Gulf of Mexico off Mississippi, 3 Atlantic stingrays from the type locality, and 5 bluntnose stingrays from Saint Joe Bay) failed to produce additional specimens. In lieu of having access to live specimens, we were relegated to using whole-mounted specimens and histological sections. On the basis of that material, the bizarre chamber appears blind ended and yields a duct opening on the penis surface (Figs. 2, 4). That the proximal end of this chamber does not connect with a sperm-carrying duct is bizarre indeed, and this observation certainly raises a question about its function. A detailed study of live specimens may allow the observer to trace the flow of material to and from the chamber, thereby further elu-

cidating the structure of this chamber and perhaps determining its contribution to the male reproductive system. Regarding the penis tubules, at low magnification (Figs. 2, 8), these structures appear as a striated tissue layer resembling muscle fibrils. However, a study of these structures using Nomarski optics with an oil-immersion objective at  $\times 1,000$  magnification reveals that the tubules originate in the proximal portion of the penis sac, wherein they are blind ended and surrounded by a granular and lightly staining material (Fig. 11), and course through the penis (Fig. 9) before opening on the surface of the penis branches (Figs. 8, 12). Proximally, the penis tubules are thin walled and have a granular material in the lumen, whereas distally they are thick walled and have a dense, darkly staining material in the lumen. At present, the function of these tubules is indeterminate, and critical observations of these tubules in a living specimen would constitute a substantial contribution to our understanding of this unusual feature in *L. corona*.

A spermatophore was not described by Hargis (1955), but the 2 bodies we regard as probable spermatophores occurred among the penis branches of a single whole-mounted specimen of *L. corona* (Figs. 5, 6). The single specimen that had the probable spermatophores was fixed under slight coverslip pressure, and it is possible that these probable spermatophores may have been forced out during fixation and before being coated with a surrounding membrane. Indeed, the proximal portion of

TABLE I. Records for *Listrocephalos* spp.

	Host	Site	Locality	Reference
<i>L. corona</i> (Hargis, 1955) n. comb.	<i>Dasyatis americana</i> Hildebrand and Schroeder, 1928, southern stingray	Skin (ventral body surface), rarely gill	Alligator Harbor, Florida, Gulf of Mexico	Hargis, 1955
	<i>Dasyatis sabina</i> (Lesueur, 1824), Atlantic stingray	Skin (ventral body surface), rarely gill	Alligator Harbor, Florida, Gulf of Mexico	Hargis, 1955
	<i>Dasyatis say</i> (Lesueur, 1817), bluntnose stingray	Skin (ventral body surface), rarely gill	Alligator Harbor, Florida, Gulf of Mexico	Hargis, 1955
		Skin (ventral body surface)	Mississippi Sound, Mississippi, Gulf of Mexico (N30°26', W88°59')*	Present study
<i>L. guberleti</i> (Caballero and Bravo-Hollis, 1962) n. comb.	<i>Urobatis halleri</i> (Cooper, 1863), Haller's round ray	Gill	Off Guaymas, Gulf of California, Sonora, Mexico	Caballero and Bravo-Hollis, 1962
		Skin	Bahia de los Angeles, Gulf of California, Mexico (N28°55', W113°32')	Present study
		Skin	Off Moss Landing, Elkhorn Slough, Monterey Bay, California*	Present study
	<i>Urobatis maculatus</i> Garman, 1913, spotted round ray*	Skin	Off Isla San Estaban, Gulf of California, Mexico (N28°42', W112°36')	Present study
	<i>Urobatis concentricus</i> Osburn and Nichols, 1916, spot-on-spot round ray*	Skin	Off Isla San Estaban, Gulf of California, Mexico	Present study
	<i>Urobatis</i> sp.	Skin	Off Isla San Estaban, Gulf of California, Mexico	Present study
<i>L. kearni</i> n. sp.	<i>Dasyatis brevis</i> (Garman, 1880), whiptail stingray	Skin (ventral body surface)	Bahia de los Angeles, Gulf of California, Mexico	Present study
			Off Santa Rosalia, Gulf of California, Mexico (N27°19', W112°17')	Present study
<i>L. whittingtoni</i> n. sp.	<i>Dasyatis longa</i> (Garman, 1880), longtail stingray	Skin	Bahia de los Angeles, Gulf of California, Mexico	Present study
			Off La Paz, Gulf of California, Mexico (N24°08', W110°18')	Present study

\* Indicates a new host record or significant range extension.

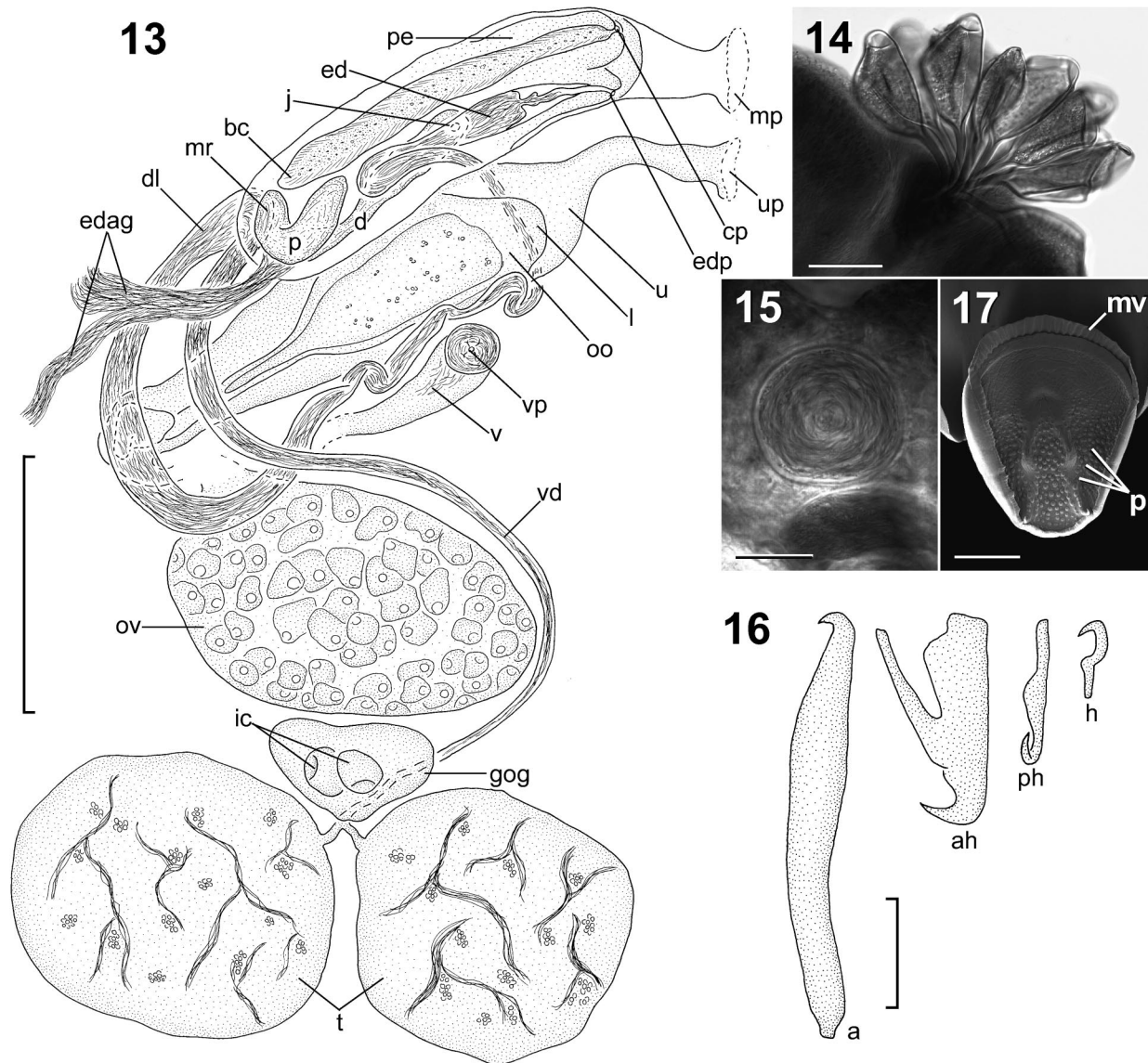
the ejaculatory duct of this specimen was not swollen with sperm, allowing for the possibility that its contents had been squeezed out during fixation. With exception to the absence of a surrounding membrane, these bodies resemble the suspected spermatophore described by Kearns and Whittington (1992) for *Benedenia* sp. 2.

The host range and habitat of *L. corona* needs verification. To date, it has been reported from the skin and gill of 3 sympatric species of *Dasyatis* (Table I). On the basis of our extremely limited data and the previous record of Hargis (1955), adults of *L. corona* seem primarily restricted to the ventral body surface of their hosts. Hargis (1955) reported that specimens of *L. corona* occurred "rarely on the gills," and although we cannot speculate on the exact site where Hargis collected his specimens, we suspect that an adult specimen of *L. corona* residing on the ventral body surface of a stingray could colonize the buccal cavity by crawling through a gill slit. However, it would be of interest to determine if a species of *Listrocephalos* migrates to and matures on the ventral body surface of its host, similar to *Entobdella soleae*, which does so on the ventral body surface of its flatfish host (Kearns, 1963). However, we emphasize that no evidence exists that a specimen of *E. soleae* mi-

grated to the ventral body surface of its host by traveling through the buccal cavity (Kearns, 1984), and in the present study, we did not collect a specimen of *L. corona* from a spiracle or the buccal cavity.

***Listrocephalos guberleti* (Caballero and Bravo-Hollis, 1962) n. comb.**  
(Figs. 13–17)

*Adult (measurements based on 10 adult specimens):* Body 1,326–2,445 (5) long, 516–1,060 (5) in maximum width or 1.9–3.5 times longer than wide. Anterolateral adhesive pad comprising 12–13 (20) raised ovoid structures on each side of body. Glands flanking preoral depression 15 (3) wide. Haptor 534–692 (5) in diameter or 0.6–1.1 times body width; haptoral papillae 10–35 (4) wide. Accessory sclerites 60–77 (10) long; anterior hamuli 30–52 (10) long or 0.5–0.8 times accessory sclerite length; posterior hamuli 25–32 (10) long; hooklets 14 in number, 12 (5) long (Fig. 16). Pharynx 134–224 (5) long, 156–186 (5) wide. Excretory bladders 195–199 (4) long, 45–90 (4) wide or 4.4–5.5 times longer than wide, 524–613 (5) or 0.3 times body length from anterior body end.



FIGURES 13–17. *Listrocephalos guberleti* (Caballero and Bravo-Hollis, 1962) n. comb. (syn. *Entobdella guberleti* Caballero and Bravo-Hollis, 1962) (Monogenea: Entobdellinae) from skin of *Urobatis maculatus*. **13.** Genitalia, vitelline reservoir omitted for clarity: penis (pe), ejaculatory duct (ed), junction (j) of distal portion of male accessory gland reservoir and vas deferens to form ejaculatory duct, bizarre chamber (bc), proximal (p) and distal (d) portions of male accessory gland reservoir (mr), anterior extent of dextral loop (dl) of vas deferens, efferent ducts of male accessory gland (edag), ovary (ov), internal cells (ic), male pore (mp), uterine pore (up), bizarre chamber pore (cp), ejaculatory duct pore (edp), uterus (u), muscular lip (l), ootype (oo), vaginal pore (vp), vagina (v), vas deferens (vd), gland of Goto (gog), testes (t), ventral view. Composite based primarily on voucher specimens, USNPC 94826–94828. Bar = 250  $\mu$ m. **14.** Light micrograph, a bouquetlike cluster of eggs anchored to uterine pore, ventral view. Voucher specimen, USNPC 94827. Bar = 100  $\mu$ m. **15.** Light micrograph, distal portion of vagina containing a spheroid mass of sperm, ventral view. Voucher specimen, USNPC 94826. Bar = 20  $\mu$ m. **16.** Median haptor sclerites: accessory sclerite (a), anterior hamulus (ah), posterior hamulus (ph). Hooklet (h). Bar = 30  $\mu$ m. **17.** *Entobdella hippoglossi* (Mueller, 1776) Blainville, 1818 (Monogenea: Benedeniinae) from skin of *Hippoglossus stenolepis*. Scanning electron micrograph, haptor showing papillae (p) and marginal valve (mv), ventral view. Bar = 500  $\mu$ m.

Testes 583–989 (5) or 0.4–0.5 times body length from posterior end of body, 149–263 (10) or 0.2–0.4 times body width in diameter (Fig. 13). Vas deferens extending anteriorly in sinistral half of body, traversing midline and extending anteriorly in dextral half of body, recurving and extending posteriorly, curving again and extending diagonally anteriorly, passing dorsal to itself thereby completing a wide loop, curving several times before arching dorsally and penetrating penis sac. Penis sac 184–229 (5) long, 55–99 (5) wide or 2.5–3.4 times longer than wide,

thin walled, weakly muscular, lacking any trace of a penis tubule in a whole-mounted specimen or a histological section. Bizarre chamber orienting longitudinally in penis sac, thin walled, with duct opening on penis papilla. Male accessory gland reservoir primarily occupying proximal region of penis sac, ventral to anterior extent of vas deferens, with proximal and distal portions; proximal portion banana-shaped or ovoid, containing dense brownish-yellow and nonstaining material; distal portion extending sinistral before joining with vas defer-



ens to form ejaculatory duct, lacking ovoid glandular bodies. Ejaculatory duct with expanded proximal portion, in some specimens bearing crenulated inner surface, narrowing before opening on surface of a penis papilla. Male pore 299–425 (5) or 0.2 times body length from anterior body end. Gland of Goto 49–79 (5) long, 55–89 (5) wide; internal cells 25 (10) in diameter.

Ovary 109–174 (5) long, 154–210 (5) wide or 0.2–0.3 times body width. Vaginal pore minute, posterior to level of male accessory gland reservoir, 362–544 (5) or 0.2–0.3 times body length from anterior body end (Fig. 13). Distal portion of vagina containing sperm in most specimens, thin walled, appearing as spheroid chamber (Fig. 15); proximal portion of vagina extending diagonally posteriad, thin walled. Connection of vitelline reservoir with vagina not observed. Ootype posterior to or slightly dorsal to penis sac, with proximal region dorsal to vas deferens, 199–318 (5) long, 75–184 (5) wide or 1.7–4.0 times longer than wide, glandular, limited distally by triangular muscular lip. Uterus ventral to vas deferens, extending diagonally anterior and toward sinistral body margin; uterine pore 149–250 (5) from vaginal pore, 362–544 (5) or 0.2–0.3 times body length from anterior end. Gland cells near ootype and collecting ducts serving base of ootype not observed. Egg body 114–199 (5) long, 60–79 (5) wide; filament 50–114 (5) long, 7–12 (5) wide; anchor 32–45 (5) in diameter (Fig. 14).

*Specimens examined:* A total of 85: 1 specimen from the skin of *U. halleri* in Bahia de los Angeles, Gulf of California, Mexico (28°55'N, 113°32'W); 4 specimens from the skin of *U. halleri* in Elkhorn Slough, Monterey Bay, California (a new locality record); 1 specimen from the skin of a spot-on-spot round ray, *Urobatis concentricus* Osburn and Nichols, 1916 (a new host record) from off Isla San Estaban, Gulf of California, Mexico (28°42'N, 112°36'W); 7 specimens from the skin of 2 spotted round rays, *Urobatis maculatus* Garman, 1913 (a new host record) from off Isla San Estaban; and 72 specimens from the skin of *Urobatis* sp. from off Isla San Estaban (voucher specimens, USNPC 94826–94828).

## Remarks

Type material of *L. guberleti* was not available for study because previously the paratypes were lost and the holotype is no longer available for loan from IBUNAM. However, based on the original description of *L. guberleti* as well as our study of the 85 specimens we collected from urolophids in the Gulf of California and Monterey Bay, *L. guberleti* differs from *L. corona* by having a weakly muscular penis sac and a penis that is not branched (Fig. 13). *Listrocephalos corona* has a strongly muscular, refractive penis sac and a penis that is branched (Figs. 1, 2, 8). *Listrocephalos guberleti* further differs from *L. corona* by having a vas deferens with a wide dextral loop that extends anterior to the level of the penis sac (Fig. 13). In addition, histological sections and whole-mounted specimens of *L. guberleti* lacked any trace of a penis tubule, whereas the tubules are a prominent feature in histological sections and whole-mounted specimens of *L. corona* (Figs. 1, 2, 8, 9, 11, 12). The vagina also distinguishes these species: *L. guberleti* has a short vagina with a pore that is located immediately anterior to the vitelline reservoir (Fig. 13), whereas *L. corona* has an elongate, narrow vagina with an opening that is located immediately pos-

terior to the uterine pore (Fig. 2). Specimens of *L. guberleti* also were typically smaller than those of *L. corona*.

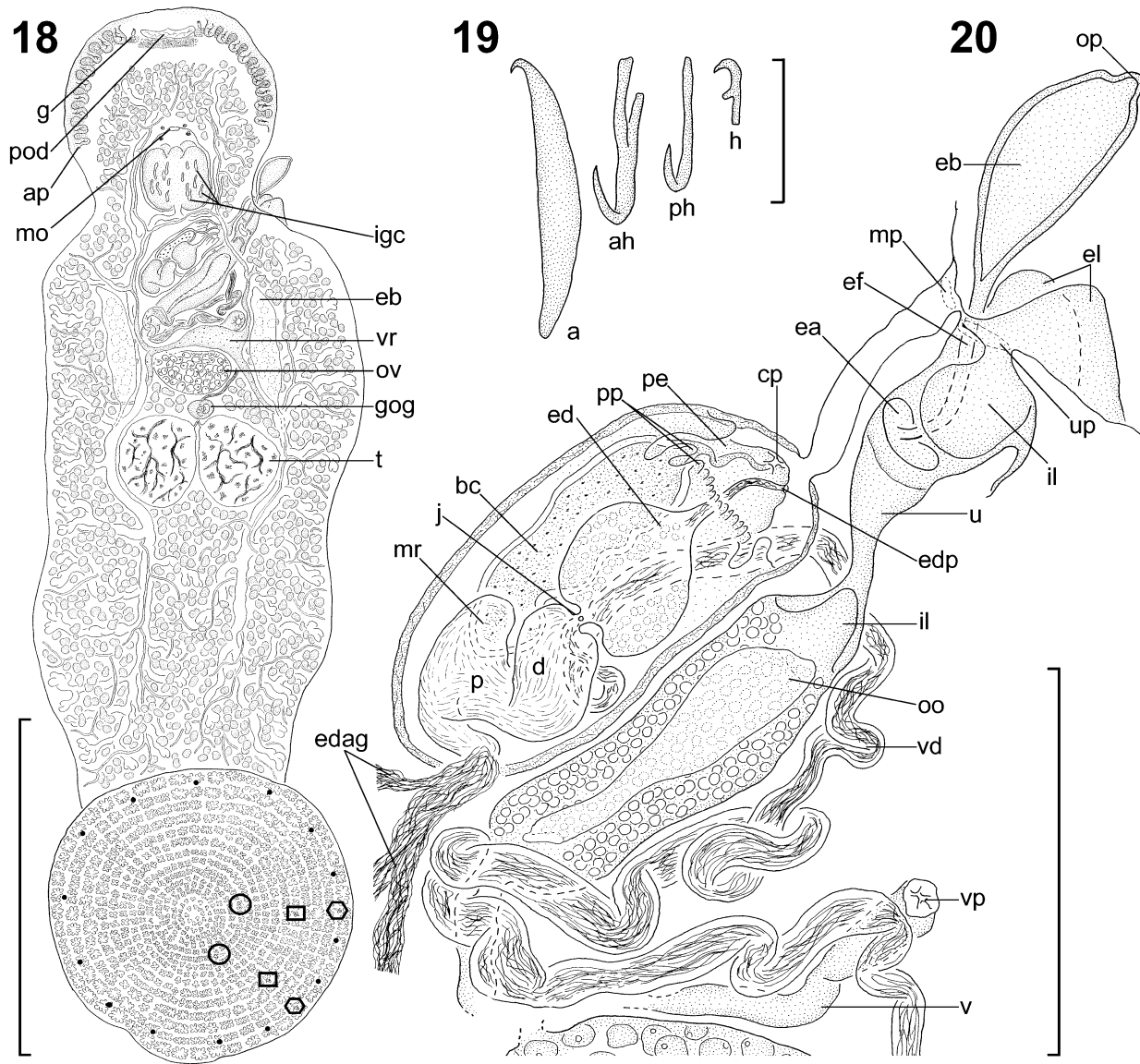
There were discrepancies between our specimens and the original description of this monogenean (Caballero and Bravo-Hollis, 1962). Regarding the number of hooklets, we counted 14, not 12. Also, as demonstrated by figure 7 of Caballero and Bravo-Hollis (1962), the previous authors apparently referred to and labeled the bizarre chamber as a cirrus. We believe that the copulatory organ of *Listrocephalos* spp. is a muscular, non-sclerotized protrusible organ, i.e., a penis, and that the bizarre chamber has its own duct that opens on the surface of the penis. Although the function of the bizarre chamber in *Listrocephalos* spp. is indeterminate, the bizarre chamber, the short duct extending from this chamber, and its opening on the surface of the penis are clearly visible in our whole-mounted specimens of *L. guberleti*. Caballero and Bravo-Hollis (1962) apparently interpreted the proximal portion of the male accessory gland reservoir as a convolution of the vas deferens in the penis sac (see fig. 7, Caballero and Bravo-Hollis [1962]). In our specimens, the vas deferens does not extend dextrad to the proximal end of the penis sac but instead enters the penis sac dorsomedially and soon thereafter unites with the distal portion of the male accessory gland reservoir to form the ejaculatory duct (Figs. 4, 13). Regarding the female genitalia, Caballero and Bravo-Hollis (1962) described the distal portion of the vagina as being highly muscular, but in our specimens, the distal portion of the vagina is thin walled and bulbous and typically contains a mass of sperm, which possibly represents a spermatophore (Fig. 15). We suspect that Caballero and Bravo-Hollis (1962) may have misinterpreted this mass of sperm for muscle, as we did initially.

We identified infections in 2 new host species, i.e., the spotted round ray and the spot-on-spot round ray, and we extend the range of *L. guberleti* to the eastern Pacific Ocean (Table I). *Listrocephalos corona* and *L. guberleti* are seemingly allopatric because their ranges are separated by the Panamanian Isthmus: *L. guberleti* reportedly infects urolophids in the eastern Pacific Ocean and Gulf of California only, whereas *L. corona* reportedly infects dasyatids in the northern Gulf of Mexico only. The yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816) (Urolophidae), is reportedly the only urolophid ranging to the east of the Panamanian Isthmus (McEachran and Fechhelm, 1998). We examined the body surface, buccal cavity, gill filaments, and nasal capsules of 9 yellow stingrays from off Marathon, Florida, but none of these was infected by a species of *Listrocephalos*. Regarding the habitat of *L. guberleti* and as is the case with *L. corona*, the site of infection for *L. guberleti* needs verification: the location of our specimens on the skin was not recorded, and Caballero and Bravo-Hollis (1962) did not specify where their specimens attached in the gill.

## *Listrocephalos kearni* n. sp. (Figs. 18–34)

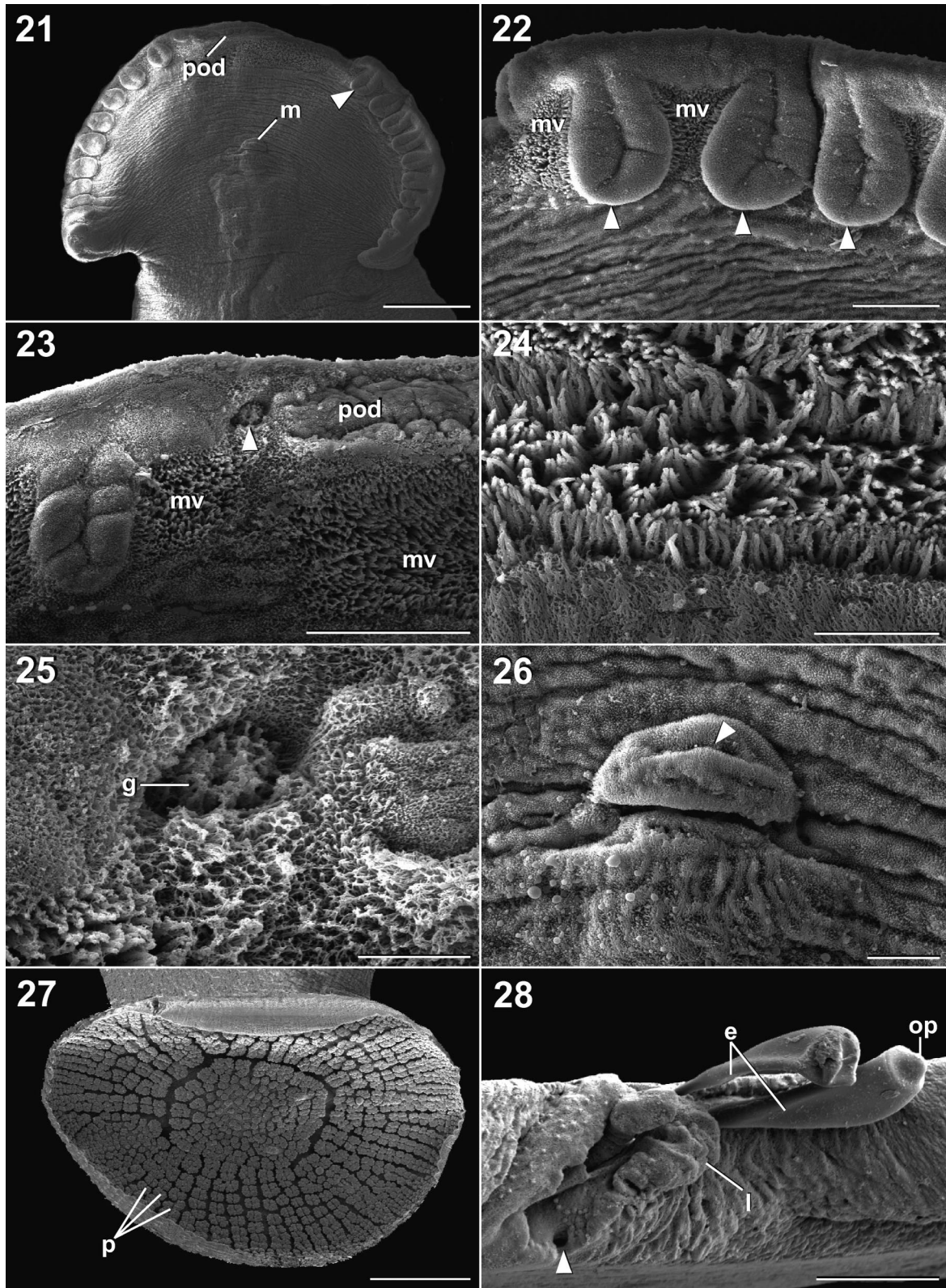
*Adult (measurements based on 20 adult specimens):* Body 2,282–3,994 (10) long, 815–1,467 (10) in maximum width or 2.0–4.0 times longer than wide (Fig. 18). Anterolateral adhesive pad comprising 9 (20) raised ovoid structures on each side of body (Figs. 18, 21–23). Microvilli occupying space between each raised ovoid structure (Figs. 22, 23) and immediately pos-





FIGURES 18–20. *Listrocephalos kearni* n. sp. (Monogenea: Entobdellinae) from skin of *Dasyatis brevis*, ventral view. Paratype, USNPC 94829. **18.** Body: gland (g) flanking preoral depression (pod), dextral column of raised ovoid structures of anterolateral adhesive pad (ap), mouth (mo), intrinsic gland cells (igc), excretory bladder (eb), vitelline reservoir (vr), ovary (ov), gland of Goto (gog), testis (t). Circles, accessory sclerites; squares, anterior hamuli; hexagons, posterior hamuli; dots, hooklets, ventral view. Bar = 1 mm. **19.** Median haptor sclerites: accessory sclerite (a), anterior hamulus (ah), posterior hamulus (ph). Hooklet (h). Bar = 30  $\mu$ m. **20.** Genitalia, vitelline reservoir omitted for clarity: rounded apex of opercular pole (op); egg body (eb), male pore (mp), egg filament (ef), egg anchor (ea), bizarre chamber pore (cp), penis (pe), penis papillae (pp), ejaculatory duct (ed), bizarre chamber (bc), junction (j) of distal portion of male accessory gland reservoir and vas deferens to form ejaculatory duct, proximal (p) and distal (d) portions of male accessory gland reservoir (mr), efferent ducts of male accessory gland (edag), external muscular lips (el), uterine pore (up), internal muscular lip (il), uterus (u), ejaculatory duct pore (edp), ootype (oo), vas deferens (vd), vaginal pore (vp), vagina (v), ventral view. Note that only papillae on ventral aspect of penis are illustrated. Bar = 300  $\mu$ m.

FIGURES 21–28. *Listrocephalos kearni* n. sp. (Monogenea: Entobdellinae) from skin of *Dasyatis brevis*, scanning electron micrographs, all ventral views except Figure 28 that is lateral. **21.** Anterior body end: preoral depression (pod), mouth (m), and anterolateral adhesive pad comprising 9 raised ovoid structures on each side of body. Arrowhead marks sinistral anterior-most raised ovoid structure. Bar = 200  $\mu$ m. **22.** Higher magnification view of Figure 22 showing zones of microvilli (mv) between raised ovoid structures (arrowheads) of anterolateral adhesive pad.



Bar = 50  $\mu\text{m}$ . **23.** Anterior body end: preoral depression (pod), zones of microvilli (mv), and opening of dextral gland flanking preoral depression (arrowhead). Bar = 50  $\mu\text{m}$ . **24.** Higher magnification view of microvilli immediately posterior to preoral depression. Bar = 20  $\mu\text{m}$ . **25.** Higher magnification view of opening of dextral gland (g) flanking preoral depression in Figure 23. The pore resides on a minute papilla in pit. Bar = 10  $\mu\text{m}$ . **26.** Mouth (arrowhead). Bar = 20  $\mu\text{m}$ . **27.** Haptor showing clover-shaped papillae (p) dispersed radially over entire haptoral ventral surface. Bar = 200  $\mu\text{m}$ . **28.** Body margin: male pore (arrowhead), muscular lip (l) flanking uterine pore, eggs (e), rounded apex of opercular pole (op). Ventral surface and anterior end at bottom and right of figure, respectively. Bar = 100  $\mu\text{m}$ .



terior to preoral depression (Figs. 23, 24); glands flanking preoral depression 12–25 (6) wide, opening in shallow pit (Figs. 23, 25). Mouth minute, at level of eyespots (Figs. 18, 26). Haptor 811–1,225 (10) in diameter or 0.8–1.2 times body width; haptor papillae 20–50 (9) wide (Figs. 18, 27). Accessory sclerites 35–55 (20) long; anterior hamuli 27–50 (20) long or 0.6–1.0 times accessory sclerite length; posterior hamuli 22–30 (18) long; hooklets 14 in number, 7–17 (6) long (Fig. 19). Pharynx 174–298 (10) long, 164–273 (10) wide (Fig. 18). Excretory bladders 273–497 (10) long, 30–149 (10) wide or 2.8–9.0 times longer than wide, 692–989 (10) or 0.2–0.3 times body length from anterior body end.

Testes 933–2,013 (10) or 0.4–0.5 times body length from posterior end of body, 197–395 (10) or 0.2–0.3 times body width in diameter, abutting ceca (Fig. 18). Vas deferens extending anteriorly in sinistral half of body in parallel with and near intestine, curving several times before traversing midline, curving several times again before tracking diagonally anteriorly and traversing midline second time, arching dorsally before penetrating penis sac (Figs. 18, 20). Penis sac 273–408 (10) long, 124–224 (10) wide or 1.7–2.4 times longer than wide, with muscular wall (Figs. 20, 32). Bizarre chamber orienting longitudinally in penis sac, with thick and highly refractive wall, containing granular material, with duct opening on penis surface (Figs. 4, 20). Male accessory gland reservoir occupying proximal region of penis sac, slightly ventral to bizarre chamber, containing dense brownish-yellow and non-staining material, with proximal and distal portions; proximal portion irregular in shape; distal portion extending sinistral before joining vas deferens to form ejaculatory duct, lacking ovoid glandular bodies (Figs. 20, 29–32), containing a spheroid mass of sperm in 1 specimen (Fig. 31); ejaculatory duct with proximal portion laterally expanded and possibly functioning as internal seminal vesicle, appearing as a glandular-walled chamber, enclosing spheroid mass of sperm in 3 specimens (Figs. 29–31), containing in some specimens a mixture of granular material similar to that observed in bizarre chamber and sperm (Fig. 30), narrowing in distal portion before opening subterminally on penis surface just posterior to bizarre chamber pore (Figs. 4, 20). Penis papillate, nearly as wide as penis sac, lacking any trace of a penis tubule such as those observed in specimens of *L. corona* (Figs. 9, 11, 12, 32); papillae approximately 30 in number. Male pore 573–791 (10) or 0.2–0.3 times body length from anterior body end (Figs. 18, 20, 28). Gland of Goto 62–100 (10) long, 60–99 (10) wide; internal cells 27 (10) in diameter (Figs. 18, 33).

Ovary 139–227 (10) long, 198–328 (10) wide or 0.2–0.3 times body width. Vaginal pore posterior to level of male accessory gland reservoir, 801–1,399 (10) or 0.3–0.4 times body length from anterior body end (Figs. 18, 20). Vagina arching dorsal to proximal region of vas deferens (Fig. 20). Connection of vitelline reservoir with vagina not observed. Ootype immediately posterior to or slightly dorsal to penis sac, with proximal region dorsal to vas deferens, 298–397 (10) long, 99–124 (10) wide or 3.0–3.8 times longer than wide, with circular gland cells covering luminal surface (Fig. 32), limited distally by triangular and flap-like muscular lip (Fig. 20). Uterus ventral to vas deferens, extending diagonally anteriorly and toward sinistral body margin; uterine pore immediately posterior to level of male pore, 263–348 (10) from vaginal pore, with 1 internal and

2 external muscular lips; internal lip directed medially; external lips directed laterally, flanking uterine pore, mittenlike (Figs. 20, 28, 34). Gland cells near ootype darkly staining, primarily dorsal and posterior to ootype (Fig. 32); collecting ducts serving base of ootype not observed. Eggs attached to adult worm directed anterolaterally; egg body 174–209 (10) long, 65–85 (10) wide; filament 99–129 (10) long, 7–10 (10) wide; anchor 55–85 (10) in diameter (Figs. 20, 28, 34).

### Taxonomic summary

*Type and only known host:* *Dasyatis brevis* (Garman, 1880), whiptail stingray (Dasyatidae).

*Sites:* Skin, ventral body surface.

*Type locality:* Bahia de los Angeles, Gulf of California, Mexico (28°55'N, 113°32'W). Other locality: off Santa Rosalia, Gulf of California, Mexico (27°19'N, 112°17'W).

*Specimens deposited:* Holotype IBUNAM CNHE 5021. Paratypes IBUNAM CNHE 5022; USNPC 94829–94834.

*Prevalence and intensity of infection:* Four of 31 (13%) whiptail stingrays were infected with 5, 6, 11, and 24 specimens of *L. kearni* each.

*Etymology:* The name “*kearni*” honors Graham C. Kearns (School of Biological Sciences, University of East Anglia, Norwich, U.K.) for his exemplary studies that have thrown light on the day-to-day lives of monogeneans.

### Remarks

*Listrocephalos kearni* is most easily distinguished from its congeners by the combination of lacking penis tubules and by having a convoluted proximal portion of the vas deferens that primarily occupies the space between the ootype and ovary, i.e., it lacks a wide dextral loop of the vas deferens (Figs. 20, 32); *L. corona* has penis tubules (Figs. 1, 2, 8, 9) and *L. guberleti* has a vas deferens with a wide dextral loop that extends anteriorly to the penis sac (Fig. 13). The new species further differs from *L. corona* and *L. guberleti* by having a large, glandular chamber that comprises the proximal portion of the ejaculatory duct (Figs. 20, 29–31).

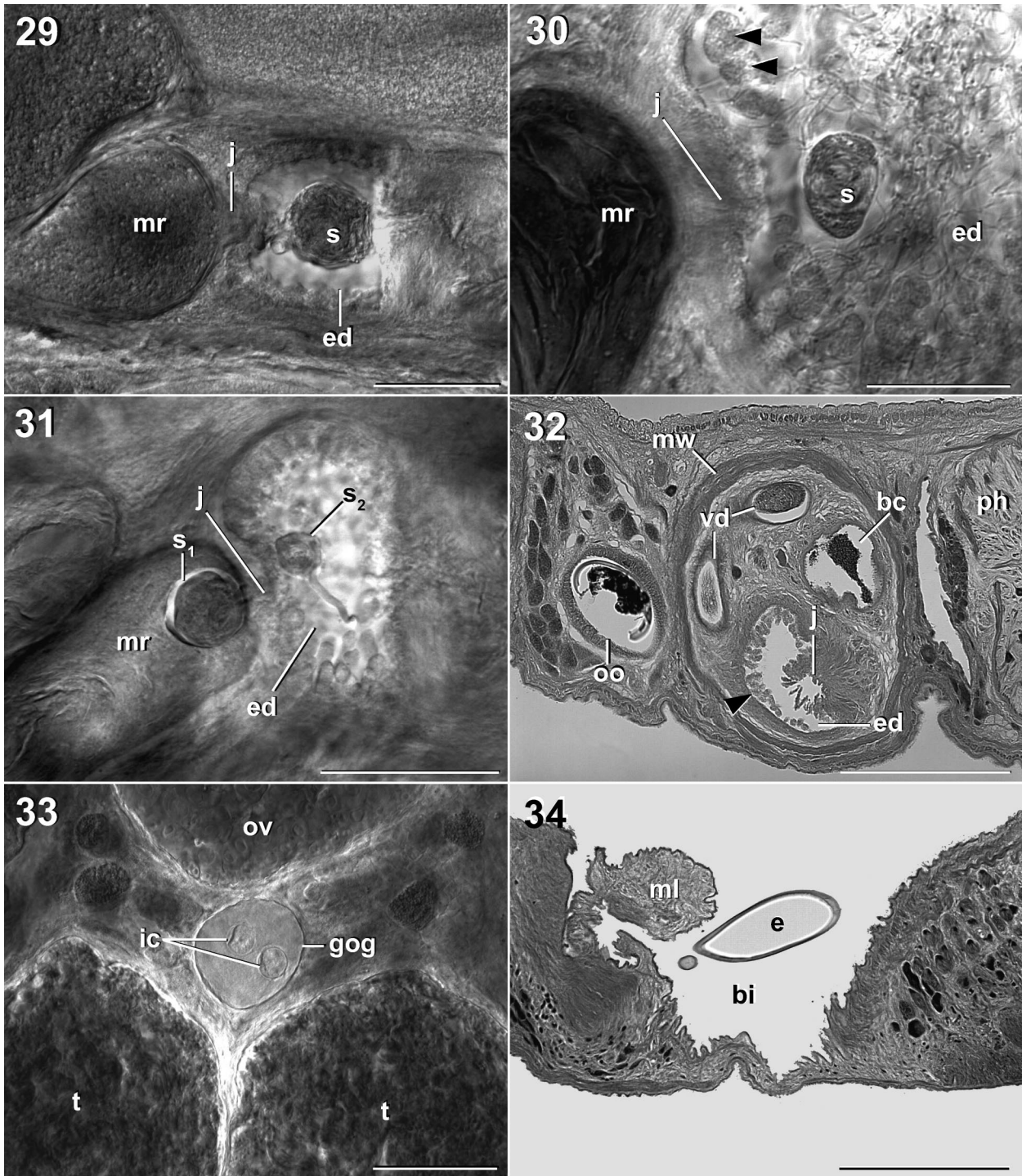
In 3 specimens of *L. kearni*, we observed a spheroid structure comprising a granular material and sperm in the glandular proximal portion of the ejaculatory duct (Figs. 29–31), and, in 1 of these specimens, we observed a similar structure in the distal portion of the male accessory gland reservoir immediately before it joins with the vas deferens (Fig. 31). These spheroid structures resembled the probable spermatophores we observed among the penis branches of the specimen of *L. corona* (Figs. 5, 6) as well as those we observed in the distal portion of the vagina of several specimens of *L. guberleti* (Figs. 13, 15). We suspect that the spheroid masses of sperm depicted in Figures 29–31 may represent spermatophores also, and we speculate that the glandular wall comprising this region of the ejaculatory duct may implicate this region in spermatophore production.

### *Listrocephalos whittingtoni* n. sp.

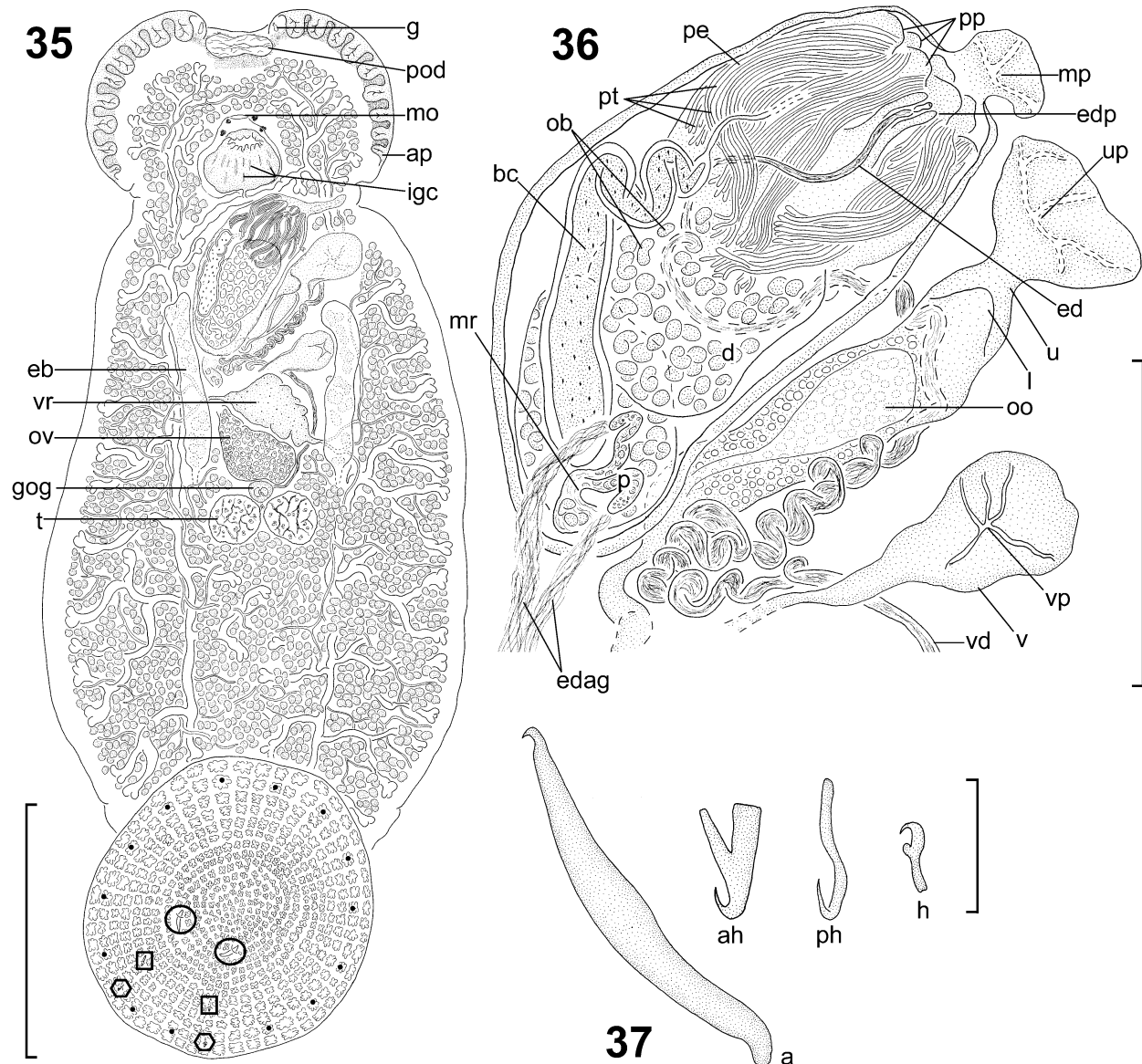
(Figs. 35–41)

*Adult (measurements based on 14 adult specimens):* Body 2,201–4,809 (10) long, 815–1,956 (10) in maximum width or 1.7–3.5 times longer than wide (Fig. 35). Anterolateral adhesive





FIGURES 29–34. *Listrocephalos kearni* n. sp. (Monogenea: Entobdellinae) from skin of *Dasyatis brevis*. **29–31.** Light micrographs showing spheroid masses of sperm (s), possibly spermatophores, and junction (j) of distal portion of male accessory gland reservoir (mr) and vas deferens to form ejaculatory duct (ed), ventral view. Note glandular lumen of laterally expanded proximal portion of ejaculatory duct. **29.** Spheroid mass of sperm in ejaculatory duct. Paratype, USNPC 94830. Bar = 60  $\mu$ m. **30.** Smaller spheroid mass of sperm in ejaculatory duct co-occurring with loose sperm; gland cells marked by arrowheads. Paratype, USNPC 94831. Bar = 40  $\mu$ m. **31.** Two spheroid masses of sperm: one (S<sub>1</sub>) in distal portion of male accessory gland reservoir and another (S<sub>2</sub>) in proximal portion of ejaculatory duct. Paratype, USNPC 94832. Bar = 60  $\mu$ m. **32.** Longitudinal section of genitalia, hematoxylin and eosin: muscular wall (mw) of penis sac encloses bizarre chamber (bc) and distal portion of vas deferens (vd). The pharynx (ph) and ootype (oo) are immediately anterior and posterior to penis sac, respectively. Note where vas deferens joins (j) distal portion of male accessory gland reservoir as well as glandular luminal surface (arrowhead) of proximal portion of ejaculatory duct (ed). Note absence of a penis tubule. Ventral surface and anterior end at bottom and right of figure, respectively. Bar = 125  $\mu$ m. **33.** Gland of Goto (gog) located between testes (t) and ovary (ov) showing 2 internal cells (ic). Paratype, USNPC 94830. Bar = 100  $\mu$ m. **34.** Longitudinal section at level of uterine pore showing muscular lip (ml) and an anchored egg (e) residing in indented portion of body (bi) about uterine pore, hematoxylin and eosin. Ventral surface and anterior end at bottom and right of figure, respectively. Bar = 125  $\mu$ m.

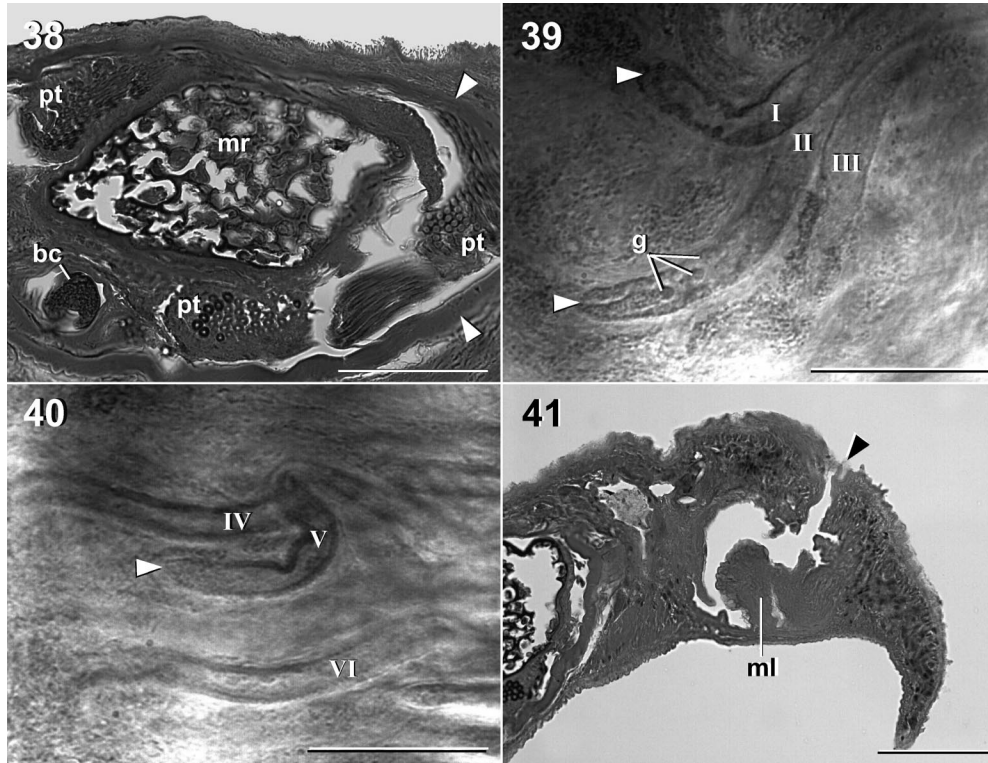


FIGURES 35–37. *Listrocephalos whittingtoni* n. sp. (Monogenea: Entobdellinae) from skin of *Dasyatis longa*. **35.** Body: sinistral gland (g) lateral to preoral depression (pod), mouth (mo), anterolateral adhesive pad (ap), intrinsic gland cells (igc), excretory bladder (eb), vitelline reservoir (vr), ovary (ov), gland of Goto (gog), testis (t). Circles, accessory sclerites; squares, anterior hamuli; hexagons, posterior hamuli; dots, hooklets, ventral view. Paratype, USNPC 94835. Bar = 1 mm. **36.** Genitalia, vitelline reservoir omitted for clarity: penis (pe), penis tubules (pt), ovoid glandular bodies (ob), bizarre chamber (bc), proximal (p) and distal (d) portions of male accessory gland reservoir (mr), efferent ducts of male accessory gland (edag), penis papillae (pp), male pore (mp), ejaculatory duct pore (edp), uterine pore (up), ejaculatory duct (ed), uterus (u), muscular lip (l), ootype (oo), vaginal pore (vp), vagina (v), vas deferens (vd), ventral view. Paratype, USNPC 94836. Bar = 300  $\mu$ m. **37.** Median haptor sclerites: accessory sclerite (a), anterior hamulus (ah), posterior hamulus (ph), hooklet (h). Bar = 30  $\mu$ m.

pad comprising 9 (20) raised ovoid structures on each side of body. Glands flanking preoral depression 15–25 (7) wide, opening in shallow pit. Haptor 897–1,467 (10) in diameter or 0.5–1.3 times body width; haptor papillae 12–35 (10) wide. Accessory sclerites 60–119 (16) long; anterior hamuli 47–60 (17) long or 0.5–0.7 times accessory sclerite length; posterior hamuli 25–32 (18) long; hooklets 14 in number, 15–17 (7) long (Fig. 37). Pharynx 134–336 (9) long, 267–393 (9) wide (Fig. 35). Excretory bladders 297–737 (16) long, 40–147 (16) wide or 4.2–8.7 times longer than wide, 593–1,374 (9) or 0.3–0.4 times body length from anterior body end.

Testes 982–1,964 (10) or 0.4–0.5 times body length from posterior end of body, 158–247 (20) or 0.1–0.2 times body width in diameter, not abutting ceca (Fig. 35). Vas deferens extending anteriorly in sinistral half of body near intestine, arching dextrad and traversing midline, turning numerous times and appearing highly convoluted about midline between ootype and vagina, recurving and traversing midline second time, curving several times before arching dorsally and penetrating penis sac (Figs. 35, 36). Penis sac 544–860 (10) long, 247–445 (10) wide or 1.8–2.5 times longer than wide, with muscular wall (Figs. 36, 38). Bizarre chamber orienting in various planes in penis





FIGURES 38–41. *Listrocephalos whittingtoni* n. sp. (Monogenea: Entobdellinae) from skin of *Dasyatis longa*. **38.** Cross section of penis sac showing penis tubules (pt) surrounding male accessory gland reservoir (mr). Note location of bizarre chamber (bc) and muscular wall of penis sac (arrowheads). Ventral surface at bottom of figure, hematoxylin and eosin. Bar = 65  $\mu$ m. **39.** Light micrograph of whole-mounted specimen showing proximal region of 3 penis tubules (I, II, and III) surrounded by a granular material. Arrowheads mark blind-ending proximal ends of penis tubules I and II. Tubule II has spheroid granules (g) in its lumen. Voucher specimen, USNPC 94837. Bar = 25  $\mu$ m. **40.** Light micrograph of whole-mounted specimen showing another 3 penis tubules (IV, V, and VI) and blind-ending proximal end (arrowhead) of penis tubule V. Note that lumen of each penis tubule appears relatively empty compared with that of penis tubule II. Voucher specimen, USNPC 94838. Bar = 25  $\mu$ m. **41.** Cross section showing internal muscular lip (ml) and dorsal uterine pore (arrowhead). Ventral surface at bottom of figure. Bar = 75  $\mu$ m.

sac, with thick and highly refractive wall, straight or convoluted distally, containing granular material. Male accessory gland reservoir with proximal and distal portions; proximal portion with 2 elongate finger-like extensions each receiving efferent ducts of male accessory gland, dorsal to bizarre chamber; distal portion occupying much of penis sac volume, enclosing ovoid glandular bodies (Fig. 36). Union of vas deferens and male accessory gland reservoir not observed. Penis papillate, nearly as wide as penis sac, having penis tubules; penis tubules originating in proximal or medial portion of penis sac and extending sinistrad, approximately 3–6 in diameter (Figs. 36, 38–40); proximal portion of tubules surrounded by a weakly staining granular material, blind ended, thin walled, with lumen containing granular material (Fig. 39) or not (Fig. 40); distal portion of tubules with dense, darkly staining material in lumen or not, with wall 1 thick (Fig. 38). Male pore 445–884 (10) or 0.4–0.6 times body length from anterior body end. Gland of Goto 50–99 (10) long, 75–109 (10) wide; internal cells 27 (10) in diameter (Fig. 35).

Ovary 139–208 (10) long, 217–346 (10) wide or 0.1–0.3 times body width (Fig. 35). Vaginal pore near sinistral excretory bladder, at level of or posterior to male accessory gland reservoir, 821–1,473 (10) or 0.3–0.4 times body length from anterior body end (Figs. 35, 36). Vagina, a muscular canal extending diagonally posteriad, ventral to vas deferens. Connection of vi-

telline reservoir with vagina not observed. Ootype immediately posterior to penis sac or slightly dorsal to posterior portion of penis sac, with proximal region dorsal to vas deferens, 346–445 (10) long, 98–148 (10) wide or 3–4.1 times longer than wide, highly glandular, with circular gland cells covering luminal surface, limited distally by flap-like muscular lip (Figs. 36, 41). Uterus ventral to vas deferens, extending diagonally anteriad and toward sinistral body margin; uterine pore 247–494 (10) from vaginal pore, between level of male pore and vaginal pore, with muscular lips (Fig. 41). Gland cells near ootype and collecting ducts serving base of ootype not observed. Egg body 146–199 (10) long, 65–99 (10) wide; filament 99–199 (10) long, 10 (10) wide; anchor 50–90 (10) in diameter.

#### Taxonomic summary

*Type and only known host:* *Dasyatis longa* (Garman, 1880), longtail stingray.

*Site:* Skin.

*Type locality:* Bahia de los Angeles, Gulf of California, Mexico (28°55'N, 113°32'W). Other locality: off La Paz, Gulf of California, Mexico (24°08'N, 110°18'W).

*Specimens deposited:* Holotype IBUNAM CNHE 5023. Paratypes IBUNAM CNHE 5024; USNPC 94835–94839.

*Prevalence and intensity of infection:* Three of 9 (33%) long-



tail stingrays were infected with 1, 6, and 9 specimens of *L. whittingtoni* each.

**Etymology:** The name “*whittingtoni*” honors Ian D. Whittington (The South Australian Museum, Adelaide, and The University of Adelaide, South Australia) for contributing to the systematics and biology of capsalid monogeneans.

## Remarks

*Listrocephalos whittingtoni* resembles *L. corona* by having penis tubules (Figs. 2, 8, 9, 11, 12, 36, 38–40) and a male accessory gland reservoir that encloses ovoid glandular bodies (Figs. 2, 36). Penis tubules are a useful diagnostic character for *Listrocephalos* spp. because, as previously stated, they comprise a prominent feature in both *L. corona* and *L. whittingtoni*, whereas we did not detect any trace of a penis tubule in a histological section or a whole-mounted specimen of *L. guberleti* or *L. kearni* (Figs. 13, 20, 32). *Listrocephalos whittingtoni* is most easily distinguished from *L. corona* by having a penis that is not branched as well as a vaginal pore that is located posterior to the level of the uterus (Fig. 36). *Listrocephalos corona* has a penis that is branched (Figs. 1, 2, 8) as well as a vaginal pore that is located at the level of the uterus just posterior to the uterine pore (Fig. 2). In addition to penis characteristics, *L. whittingtoni* further differs from *L. guberleti* by having a vas deferens with a proximal portion that is highly convoluted and occupies the space between the ootype and vagina; *L. guberleti* has a vas deferens with a wide dextral loop that extends anteriorly to the level of the penis sac. *Listrocephalos whittingtoni* further differs from *L. kearni* by having a thin, tube-like ejaculatory duct (Fig. 36), whereas *L. kearni* has a large, ovoid chamber with a highly glandular lumen that comprises the proximal portion of the ejaculatory duct (Figs. 29–32).

## DISCUSSION

The present study, which was based solely on fixed material, has elucidated some unusual characters for Capsalidae, suggested the presence of a spermatophore for members of *Listrocephalos*, and served as a primer for studying the biology of this group of capsalids. A future study of live specimens is needed because it could help resolve particular details about the fine structure and function of the bizarre chamber and penis tubules as well as confirm the presence of a spermatophore. With respect to the spermatophore and except in specimens of *L. whittingtoni*, we observed a spheroid mass of sperm resembling a spermatophore in at least 1 specimen of each species we studied, i.e., among the penis branches of *L. corona* (Figs. 5, 6), in the distal portion of the vagina of *L. guberleti* (Figs. 13, 15), and in the proximal portion of the ejaculatory duct and the distal portion of the male accessory gland reservoir of *L. kearni* (Figs. 29–31). Other capsalids produce a spermatophore, e.g., *E. soleae* (van Beneden and Hesse, 1863) Johnston, 1929 (see Kearns, 1970) and *Entobdella diadema* Monticelli, 1901 (see Llewellyn and Euzet, 1964), or are suspected of producing one, e.g., *Trimusculotrema uarnaki* Whittington and Barton, 1990 (see Whittington and Barton, 1990) and *Benedenia* sp. 2 (see Kearns and Whittington, 1992), but this has yet to be confirmed in a species of *Listrocephalos*.

## Diagnostic key to *Listrocephalos* spp.

1. Vas deferens lacking wide dextral loop reaching penis sac . . . . . 2  
    Vas deferens having wide dextral loop reaching penis sac . . . . .  
    . . . . . *L. guberleti*
2. Penis tubules present . . . . . 3  
    Penis tubules absent . . . . . *L. kearni*
3. Vaginal pore at level of uterus . . . . . *L. corona*  
    Vaginal pore posterior to level of uterus . . . . . *L. whittingtoni*

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