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Levisunguis subaequalis n. g., n. sp., a Tongue Worm (Pentastomida: Porocephalida: Sebekidae) Infecting Softshell Turtles, *Apalone* spp. (Testudines: Trionychidae), in the Southeastern United States

Stephen S. Curran
University of Southern Mississippi, stephen.curran@usm.edu

Robin M. Overstreet
University of Southern Mississippi, robin.overstreet@usm.edu

David E. Collins
Tennessee Aquarium

George W. Benz
Middle Tennessee State University

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Stephen S. Curran,[1] Robin M. Overstreet,[1] David E. Collins,[2]

and George W. Benz[3]

1. Department of Coastal Sciences, University of Southern Mississippi, Ocean Springs, Mississippi, USA
2. Tennessee Aquarium, Chattanooga, Tennessee, USA
3. Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA

Corresponding author – Stephen S. Curran, email stephen.curran@usm.edu

Abstract

A new tongue worm (Pentastomida) belonging to the Sebekidae Sambon, 1922 (Porocephaloidea Sambon, 1922) is described based on exemplars collected from softshell terrapins *Apalone spinifera aspera* (Agassiz) and *Apalone ferox* (Schneider) in the southeastern United States; a new genus is erected to accommodate the new species. The new species belongs in the Sebekidae because adults possess four simple hooks arranged in a trapezoid pattern on the ventral surface of the cephalothorax, a mouth opening between the anterior and posterior pairs of hooks, a terminal anus, an elongated uterus with preanal uterine pore, and a Y-shaped seminal vesicle. Nymphs possess geminate hooks, and the new species has an aquatic life cycle in which nymphs become encapsulated in the body cavity of a freshwater fish and mature in the lungs of a terrapin. The new genus is distinct from other genera in the Sebekidae primarily by differences in hook morphology and the fact that representatives use a terrapin as a definitive host. Nymphs infecting fish and presumed to be the new species matured as postlarval juveniles conspecific with the new species when they were fed to the eastern mud turtle, *Kinosternon subrubrum* (Lacépède). Nymphs of the new species are anatomically

similar to but larger than nymphs of *Sebekia mississippiensis* Overstreet, Self & Vliet, 1985 found in the mesentery of fishes captured in Florida, USA. Adults of the new species differ from those of *S. mississippiensis* based on hook features, chloride cell pore pattern on annuli, body size, and use of a turtle rather than crocodilian definitive host. The new species is the third North American member of the Sebekidae.

Introduction

The subclass Pentastomida Huxley, 1869, which belongs in the phylum Arthropoda Latreille, 1829 (Pancrustacea: Oligostraca: Pentastomida), consists of about 125 species of unusual wormlike obligatory parasites (Regier et al., 2010; Poore, 2012). The Pentastomida in the order Porocephalida Heymons, 1935 generally have a two-host life cycle, in which an intermediate host consumes an egg containing a primary larva. The primary larva hatches and penetrates the intermediate host's digestive tract and enters the viscera where it may become encapsulated. The primary larva subsequently molts and becomes a secondary larva (known as a nymph). The nymph continues to undergo a series of molts, the last resulting in the terminal larva (Storch, 1993). If the intermediate host is consumed by a suitable definitive host, the terminal larva migrates from the stomach through the esophagus into the trachea or lungs and undergoes a final molt resulting in a postlarval juvenile that remains in the lungs and eventually matures (Penn, 1942).

Mammals serve as definitive hosts for a few porocephalidans, but squamatans (scaled reptiles) and crocodilians represent the most common definitive host groups for members of the order (Poore, 2012; Christoffersen & De Assis, 2013). Terrapins represent a particularly unusual definitive host group for the Porocephalida. Only two pentastomatids, both members of the Sebekidae Sambon, 1922, are known to mature in the lungs of terrapins. One of these, *Diesingia megastoma* (Diesing, 1836) Sambon, 1922 matures in the Geoffrey's side-necked turtle, *Phrynops geoffroanus* (Schweigger), and the South American snake-necked turtle, *Hydromedusa tectifera* Cope (both Testudines: Pleurodira: Chelidae) in Brazil (Junker et al., 2003). The second, *Pelonia africana* Junker & Boomker, 2002 matures in the serrated hinged terrapin, *Pelusios sinuatus* (Smith), and the African helmeted turtle, *Pelomedusa subrufa* (Lacépède) (both Testudines: Pleurodira: Pelomedusidae) in South Africa (Junker & Boomker, 2002). Adults of an undescribed porocephalidan were recovered from the lungs of the Gulf Coast spiny softshell turtle, *Apalone spinifera aspera* (Agassiz), in Louisiana, USA, and the Florida softshell turtle, *Apalone ferox* (Schneider), in Florida, USA (both Testudines: Cryptodira: Trionychidae). Additionally, pentastomid nymphs found in the body cavity of the western mosquitofish, *Gambusia affinis* (Baird & Girard), collected in Louisiana and syntopic with the undescribed adult pentastomids were collected and fed to a captive specimen of the eastern mud turtle, *Kinosternon subrubrum* (Lacépède) (Testudines: Cryptodira: Kinosternidae), wherein some matured into postlarval juveniles conspecific with the undescribed adults from *Apalone* spp. Herein we describe the new pentastomid and erect a new genus within the Sebekidae to accommodate it.

Materials and methods

Six adult pentastomids were collected from the lungs of a Gulf Coast spiny softshell turtle captured in Hammond, Louisiana, in May 2000, and pentastomid nymphs were collected from the body cavity of western mosquitofish from near the same location at the same time. During 1995, 27 Florida softshell turtles captured as part of a turtle survey in Palm Beach County, Florida, were frozen and subsequently thawed and examined for parasites. All were found to be infected with numerous pentastomid adults and nymphs. A captive Florida softshell turtle, captured at an unknown locality in Florida and held for an indeterminate period at the Tennessee Aquarium (Chattanooga, Tennessee, USA), was also found to be infected with adults of the same undescribed pentastomid.

Fresh and previously frozen pentastomids were preserved in 70% ethanol. Some specimens were cleared using 85% lactic acid solution and observed intact or partially intact using light microscopy. Other individuals were dissected and the sclerotized structures (hooks, fulcra, oral cadre, copulatory spicules) were removed and mounted on glass slides under coverslips in 85% lactic acid solution for measurement and photography purposes. Hook dimensions measured were blade length (AC), hook length (AD), base length (BC), plateau length (CD), and hook gape (AB); all measurements correspond to those of Fain (1961) and Junker et al. (2003) and are illustrated in Figure 1. All hook measurements were made from hooks oriented on slides with their inside surfaces up. Laterally mounted fulcra were used for fulcrum length measurements, taken along an imaginary straight line between the fulcrum's posterior end and the midpoint between its anterior dorsal and anterior ventral corners (Fig. 1). Several specimens of each sex were permanently mounted for internal observation by hydrating preserved individuals, which were then stained in a hematoxylin solution, dehydrated in a graded ethanol series, cleared in clove oil, and mounted in Canada balsam on glass slides under a coverslip. Two adult specimens (one female, one male) and two western mosquitofish infected with pentastomid nymphs were longitudinally sectioned, and sections were stained with Harris' hematoxylin and eosin using standard methods. Measurements are reported in micrometers as means followed in parentheses by corresponding ranges, unless otherwise noted. For the experimental infection, an eastern mud turtle captured in the Pascagoula River, Jackson County, Mississippi, USA (30°35′57″N, 88°37′22″W, a site where SSC, RMO, and graduate students of RMO had not observed the new pentastomid in dozens of terrapins examined between 1997 and 2013) was held in captivity for one week and fed previously frozen fish. Subsequently, the turtle was fed (daily for one week) four wild-caught western mosquitofish collected in Hammond, Louisiana. Each fish was naturally infected with one to six pentastomid nymphs that were visible through the skin of the fish. The terrapin was fed previously frozen fish over the course of the following week and was then euthanized following the Institutional Animal Care and Use Committee Organization suggested method (Anonymous, 2002). The terrapin was necropsied three hours after euthanasia.

The type-material, as indicated in the description below, is deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA; The Natural History Museum (NHMUK), London, UK; and the Gulf Coast Research Laboratory Museum (GCRLM), Ocean Springs, Mississippi, USA.

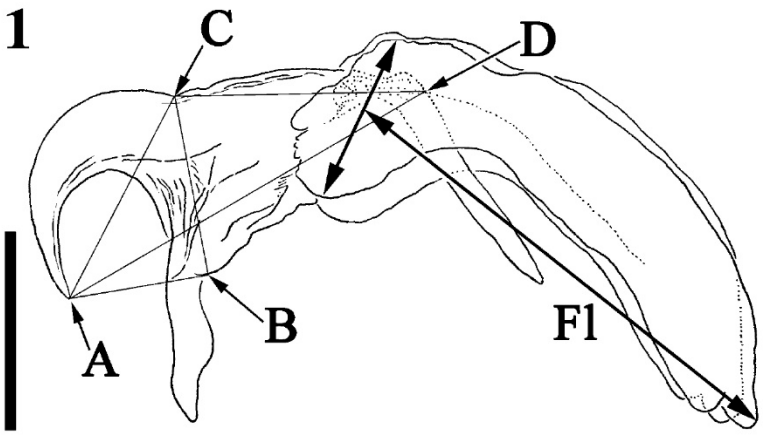


Figure 1. Hook measurement conventions. Posterior right hook and fulcrum of an adult female *Levisunguis subaequalis* n. g., n. sp. AC, blade length; AD, hook length; BC, base length; CD, plateau length; AB, hook gape; Fl, fulcrum length, measured from midpoint between anterior most lateral projections and posterior end. *Scale-bar:* 250 μm

***Levisunguis* n. g.**

Diagnosis

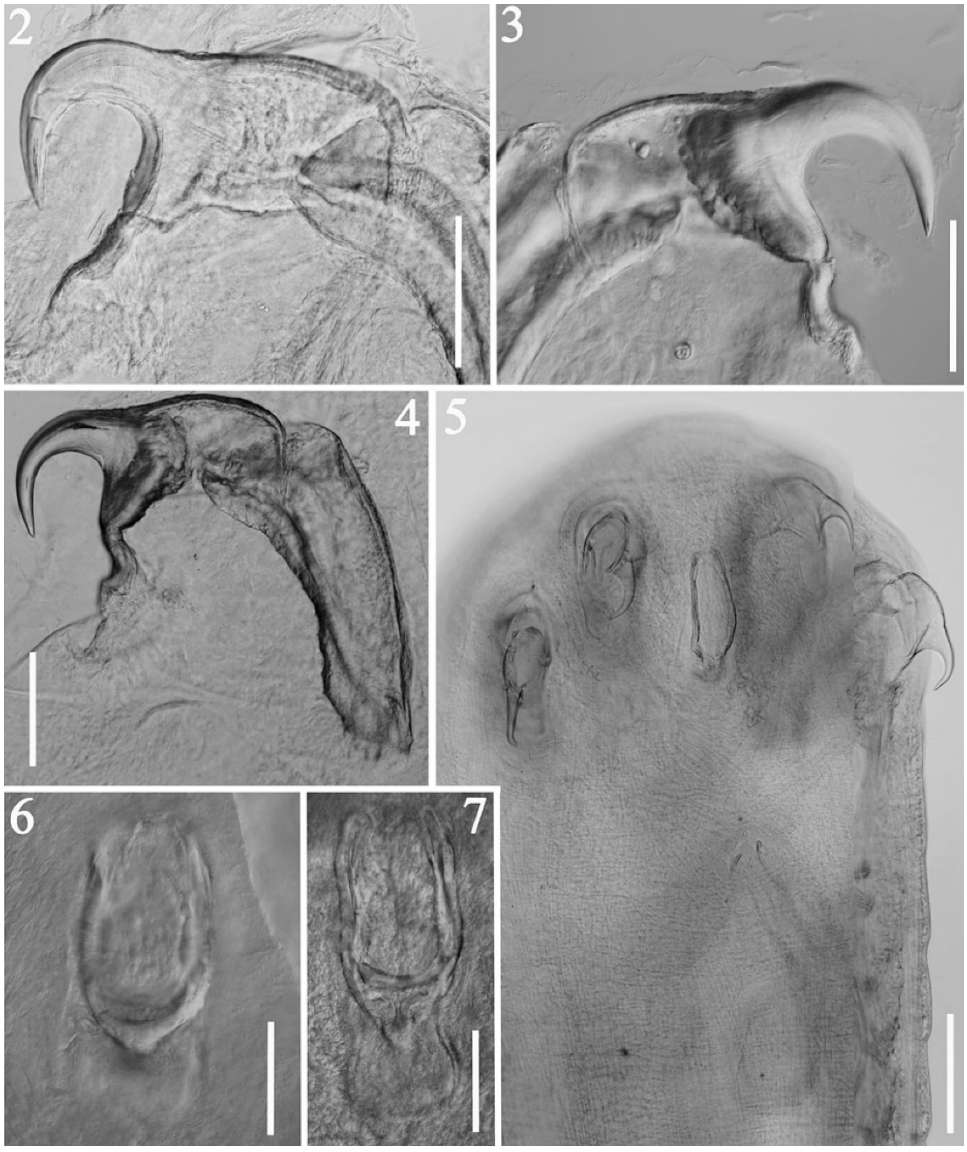
Porocephalida: Sebekidae. Body claviform, comprising approximately 70 annuli; adult male slightly dorso-ventrally flattened with conspicuous lateral line, adult female lacking conspicuous lateral line. Hooks of adults smooth, four in number, strongly recurved (claw-like). Anterior hooks slightly larger than posterior hooks. Fulcra smooth, equal in size regardless of hook position. Mouth opening at level of anterior hooks. Oral cadre U-shaped with sclerotized fibers connecting anterior end, giving appearance of an oval-shaped cadre; small peg-like extension emerging from base of U-shaped opening, with fan-shaped posterior extension extending dorsally into pharynx; extension with rugose surface, lacking pores. Copulatory spicules longer than wide, cowry shell–shaped, with short claw-like anteroventral extension, and longer anterodorsal spatulate extension with minute medial spines. Uterus opening on ventral surface near posterior end of body, separated from anus by three annuli. Adults, post-larval juveniles, and newly migrated nymphs parasitic in lungs of terrapins. Nymphs with geminate hooks, parasitic in viscera of freshwater fishes.

Type-species: Levisunguis subaequalis n. sp.

Etymology: The masculine singular genus name *Levisunguis* is a Latin noun derived from the adjective *levis* (meaning smooth) and the singular noun *unguis* (meaning claw); it denotes the smooth nature of the four claw-like hooks of the species.

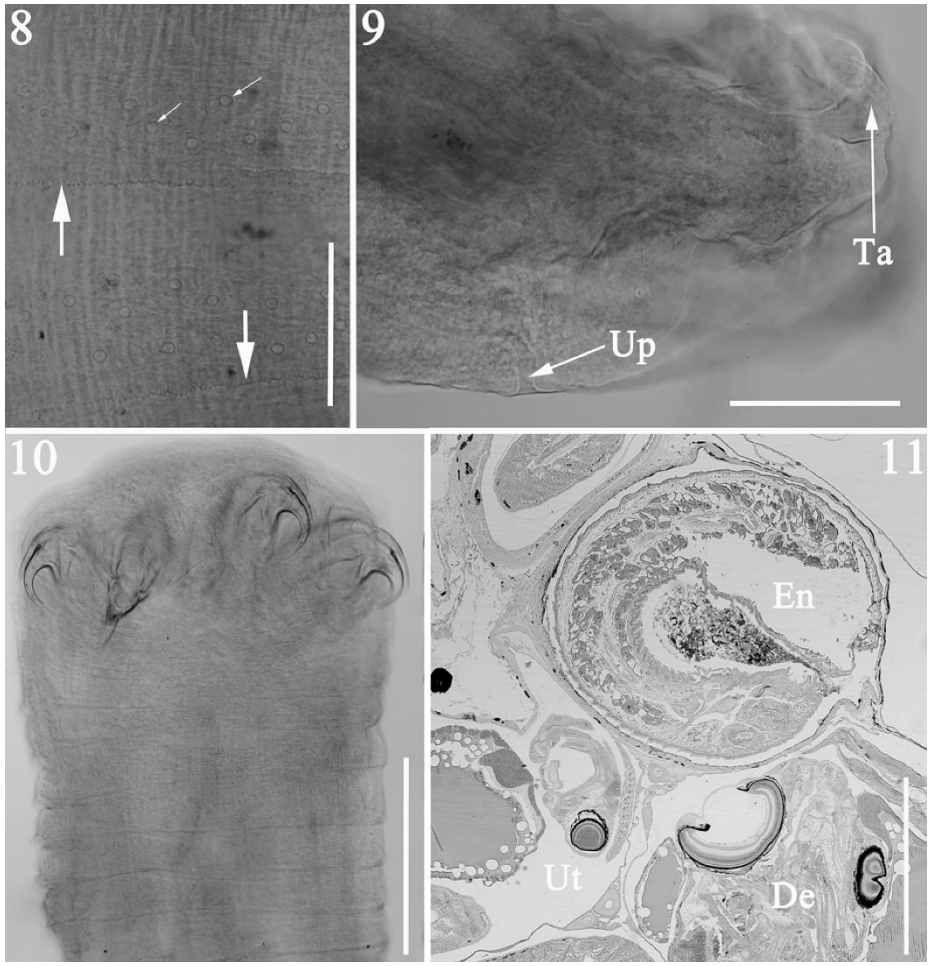
Differential diagnosis

Levisunguis n. g. belongs in the Sebekidae as defined by Fain (1961) because adults of the new genus possess four simple hooks, nongeminate and lacking an accessory piece (Figs. 1–5, 14), arranged in a trapezoid pattern with the mouth opening between the anterior and posterior hooks (Figs. 5, 14), a U-shaped oral cadre (the top of which forms a connection

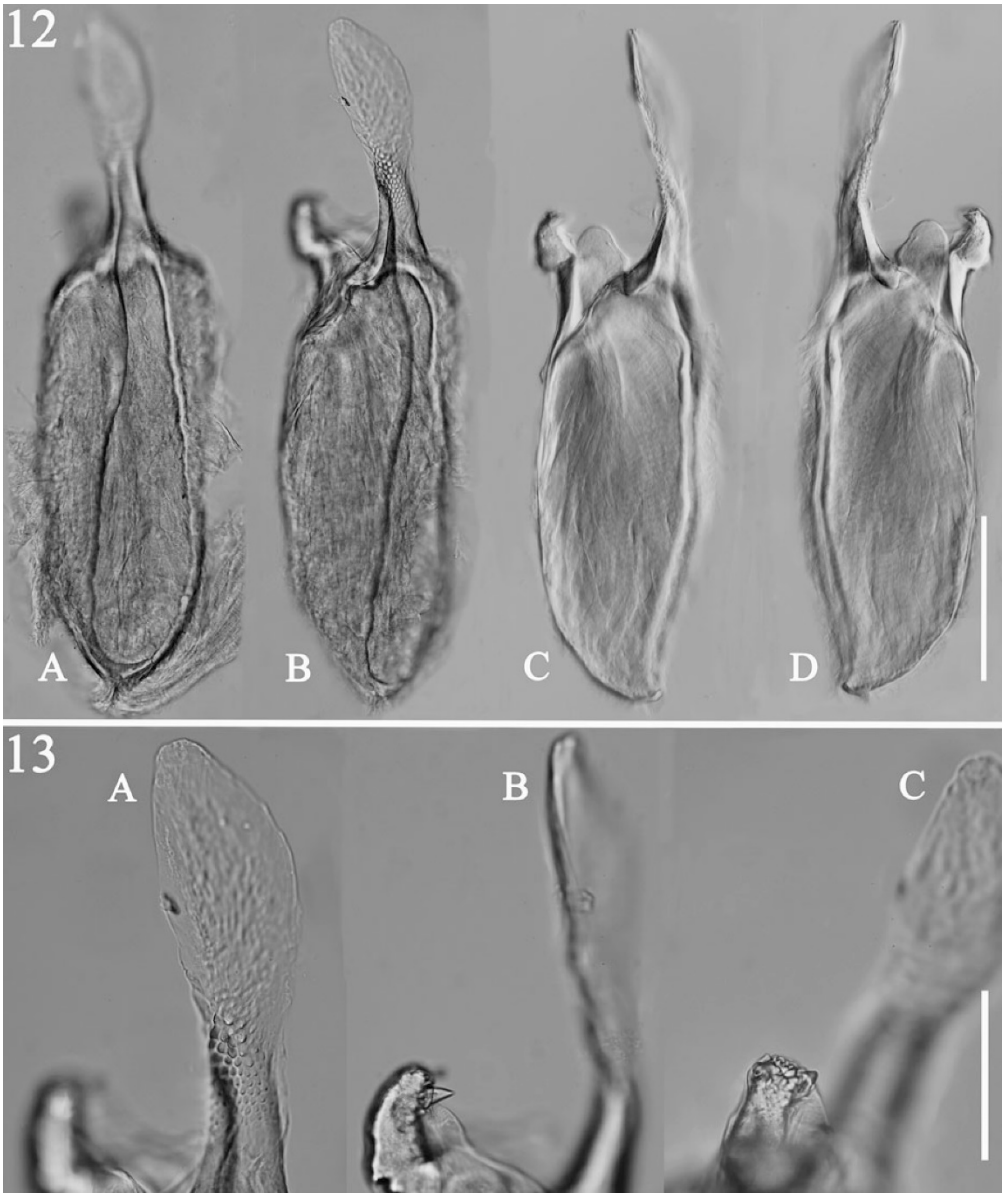


Figures 2–7. *Levisunguis subaequalis* n. g., n. sp. 2, Posterior right hook of adult female; 3, Anterior left hook of adult female; 4, Hook and fulcrum of adult female; 5, Ventral view of cephalothorax of adult male from experimentally infected eastern mud turtle, *Kinosternon subrurbrum*; 6, Ventral view of oral cadre of adult female (*in situ*); 7, Ventral view of oral cadre dissected from adult female. *Scale-bars:* 200 μm

with sclerotized fibers) (Figs. 6, 7), a terminal anus (Fig. 9), a long uterus with pre-anal uterine pore (Fig. 9), and a Y-shaped seminal vesicle (Figs. 14, 18). Nymphs have geminate hooks (Fig. 10) and infect the body cavity of fishes (Fig. 11). Adults infect the lungs of terrapins. Prior to the addition of *Levisunguis* n. g., the Sebekidae comprised seven genera: *Sebekia* Sambon, 1922; *Alofia* Giglioli in Sambon, 1922; *Diesingia* Sambon, 1922; *Leiperia* Sambon, 1922; *Selfia* Riley, 1994; *Agema* Riley, Hill & Huchzermeyer, 1997; and *Pelonia* Junker & Boomker, 2002 (see Poore, 2012).



Figures 8–11. *Levisunguis subaequalis* n. g., n. sp. 8, Cuticle from a nymph collected from the body cavity of the western mosquitofish, *Gambusia affinis*. Large arrows point to spiny ridges that compose posterior margins of annuli. Small arrows point to chloride cell pore caps; 9, Posterior end of adult female. Arrows point to terminal anus (Ta) and ventral uterine pore (Up); 10, Ventral view of cephalothorax of a nymph collected from the body cavity of a western mosquitofish; 11, Histological section through the body cavity of a pregnant female western mosquitofish showing an encysted nymph (En) in the uterus (Ut) of the fish among developing fish embryos (De). *Scale-bars:* 8, 9, 100 μm; 10, 500 μm; 11, 1 mm



Figures 12–13. Various views of right copulatory spicule from mature male *Levisunguis subaequalis* n. g., n. sp. 12A, Dorsal view showing open dorsal groove in cowry shell–shaped base; 12B, Dorsolateral view highlighting dorsal anterior spatulate extension; 12C, Lateral view, left side; 12D, Lateral view, right side; 13A, Dorsolateral view showing detail of dorsal surface of the dorsal spatulate extension; 13B, Lateral view from left side showing detail of ventral claw-like extension; 13C, View showing detail of distal end of ventral claw-like extension from the dorsal aspect with the blurred dorsal spatulate extension in foreground. *Scale-bars:* 12, 200 μm; 13, 100 μm

Figures 14–17. *Levisunguis subaequalis* n. g., n. sp. 14, Anterior end of adult male (specimen under considerable pressure). Note anterior portion of Y-shaped seminal vesicle and position of copulatory spicules; 15, View of copulatory spicules in situ from dorsal aspect of adult male. Lcs, Left copulatory spicule (spicule is slightly turned showing dorsolateral side due to some pressure), Rcs, Right copulatory spicule; 16, Left intromittent organ and associated pouch of adult male; 17, Eggs containing primary larva in distal portion of uterus of an adult female. *Scale-bars:* 14, 500 μm; 15, 250 μm; 16, 50 μm; 17, 100 μm

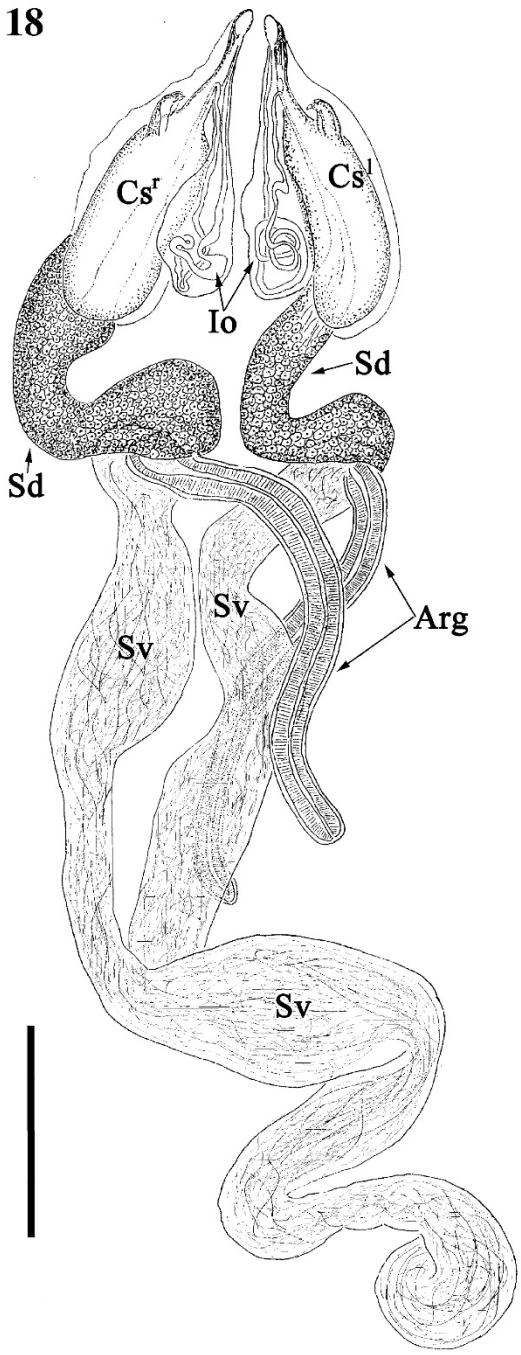


Figure 18. Male reproductive system of *Levisunguis subaequalis* n. g., n. sp. (testis not illustrated). Abbreviations: Arg, accessory reproductive gland; Cpl, left copulatory spicule; Cpr, right copulatory spicule; Io, intromittent organ; Sd, sperm duct (or prostatic duct); Sv, seminal vesicle. *Scale-bar:* 500 μm

Levisunguis n. g. representatives can be differentiated from the species in other genera of the Sebekidae by a combination of hook features, oral cadre shape, shape and size of the copulatory spicules, position of the female genital pore, shape of the female body, and definitive host affiliation. The smooth, strongly curved, slightly dissimilarly sized anterior and posterior hooks of *Levisunguis* n. g. representatives lack spines. Furthermore, there are no spines on extensions of the fulcra (Figs. 1–5), thus making hooks and fulcra fundamentally different than those in *Sebekia* spp. from the New World, which have spines on the anterior region of hooks and spines on extensions of each fulcra (see Riley et al., 1990; Overstreet et al., 1985), and those in *D. megastoma* and *Selfia porosus* Riley, 1994 (both representing monotypic genera) that have spines on the anterior region of the anterior hooks. At least four of the eight nominal species in *Alofia* also have spines associated with the anterior hooks. However, *Alofia nilotici* Riley & Huchzermeyer, 1995 has a dorsal patch of spines on all four hooks, whereas *Alofia simpsoni* Riley, 1994 lacks spines associated with any hook (Riley, 1994; Riley & Huchzermeyer, 1995a). The presence of hook spines is unreported from the remaining two nominal species, *Alofia indica* (Linstow, 1906) Hett, 1924, and *Alofia adriatica* Hirst, 1922, which Riley (1994) considered to be *species inquirendae* (see Giglioli, 1922; Riley, 1994). Nevertheless, all accepted species in *Alofia* possess slender hooks that are canaliculated (i.e., they have a lateral groove) and that exhibit a strong bend (nearly at a right angle) (Giglioli, 1922; Riley & Huchzermeyer, 1995a, b). Hooks of *Levisunguis* n. g. representatives are stout, noncanaliculated, and strongly recurved. As with *Agema silvaepalustris* Riley, Hill & Huchzermeyer, 1997 (representing a monotypic genus), *Levisungius* n. g. representatives exhibit dissimilarly sized anterior and posterior hooks but differ from *A. silvaepalustris* by exhibiting more robust hooks that are more strongly recurved. The oral cadre is U-shaped in *Levisunguis* n. g. representatives whereas it is oval in *A. silvaepalustris*, and the copulatory spicules are equipped with a ventral anterior extension that is absent in *A. silvaepalustris* (see Riley et al., 1997). *Levisunguis* n. g. representatives exhibit a similar morphology of the oral cadre with *Leiperia* spp., but the U-shaped portion of the oral cadre of *Leiperia* spp. is equipped with anterior flanges and pores that enter the pharynx associated with the posterior portion of the oral cadre (Riley & Huchzermeyer, 1996), while such features are absent in *Levisunguis* n. g. representatives (Figs. 6, 7). Furthermore, anterior and posterior hooks are equal in size in *Leiperia* spp., and females of *Levisunguis* n. g. have a slightly claviform, uncoiled body, while the posterior two-thirds of the abdomen is spirally coiled in females of *Leiperia* spp. (see Riley & Huchzermeyer, 1996; Junker et al., 2000). Hooks of *Levisunguis* n. g. representatives appear similar though larger than those of *P. africana* (representing a monotypic genus); however, it is not known if the anterior and posterior hooks are equal or dissimilar in size in *P. africana*. Regardless, the oral cadre of *P. africana* has a prominent bowl-shaped base, which is absent in other sebekids (Junker & Boomker, 2002). *Levisunguis* n. g. is the only pentastomid genus whose representatives mature in terrapins in North America.

Remarks

Self & Rego (1985), Riley et al. (1990), and Riley & Huchzermeyer (1995a, b) noted that while species-level differences within the Sebekidae are fairly straightforward, differenti-

ation of sebekid genera seems arbitrary. These authors mentioned that reliance on particular features such as hook and oral cadre shape is fraught with ambiguities. A subtle but important feature that serves to distinguish *Levisunguis* n. g. representatives from those of other sebekid genera is anterior hooks that are very slightly larger than the posterior pair, whereas anterior and posterior hooks are generally identical in size in other sebekids with the exception of *A. silvaepalustris*. Disparate hook size and presence of stout, smooth, claw-like hooks as well as the use of North America terrapins as the definitive hosts are the main criteria we used to justify the erection of *Levisunguis* n. g. The shape and configuration of the anterior extensions of the copulatory spicules in *Levisunguis* n. g. specimens are unique, but we consider these features to represent species level discriminators.

***Levisunguis subaequalis* n. sp.**

Type-host: Apalone spinifera aspera (Agassiz), Gulf Coast spiny softshell turtle (Testudines: Cryptodira: Trionychidae).

Other host: Apalone ferox (Schneider), Florida softshell turtle (Testudines: Cryptodira: Trionychidae).

Intermediate host: Gambusia affinis (Baird & Girard), western mosquitofish (Cyprinodontiformes: Poeciliidae).

Experimental host: Kinosternon subrubrum (Lacépède), eastern mud turtle (Testudines: Cryptodira: Kinosternidae).

Type-locality: Ponchatoula Creek in Hammond, Louisiana, USA (30°31′37″N, 90°28′35″W).

Other localities: Nymphs collected from *G. affinis* in unnamed ponds near the type-locality (30°31′24″N, 90°28′22″W); adults from *A. ferox* in Palm Beach County, Florida, USA (26°44′N, 80°44′W).

Sites of infection: Lung in definitive hosts; body cavity and viscera in intermediate hosts.

Deposition of specimens: Holotype, intact male in 70% ethanol (NHMUK 2013.1020); allotype, intact female in 70% ethanol (NHMUK 2013.1021); one male paratype, intact in 70% ethanol (NHMUK 2013.1022); three male paratypes, intact in 70% ethanol (USNPC 107471, 107473, 107474); one male paratype, intact in 70% ethanol (GCRLM 06526); seven female paratypes, intact in 70% ethanol (NHMUK 2013.1023-1029); one female paratype, hard parts in 70% ethanol (NHMUK 2013.1030); three female paratypes, intact in 70% ethanol (USNPC 107472, 107475, 107476); one female paratype, intact in 70% ethanol (GCRLM 06525); five female paratypes, intact in 70% ethanol (GCRLM 06527).

Etymology: Species epithet *subaequalis* is a masculine Latin adjective meaning unequal and refers to the unequal size of the anterior and posterior cephalothorax hooks.

Description (Figs. 1–18)

Body widest at base of cephalothorax, with lateral line indistinct in females and distinct in males. Adult hooks lacking spines, canaliculation, or accessory pieces (Figs. 1–5), similar in shape; anterior hooks slightly larger in both sexes (Table 1); hooks supported on fulcra. Fulcra crescent-shaped, equal in size, lacking spines or extensions (Figs. 1, 4; Table 1).

Table 1. *Levisunguis subaequalis* n. g., n. sp. male and female hook and fulcrum dimensions (means followed by ranges in parentheses; both in micrometers)

Sex	Hook position	AC	AD	BC	CD	AB	Fulcrum length
Males (n = 8)	RA	140 (111–156)	279 (255–298)	118 (111–132)	166 (140–211)	105 (80–119)	389 (325–459)
	LA	150 (139–163)	279 (264–293)	118 (107–132)	161 (148–175)	105 (94–111)	423 (389–460)
	RP	136 (114–156)	264 (251–281)	115 (95–130)	162 (143–194)	92 (62–106)	404 (355–459)
	LP	140 (126–157)	271 (246–294)	112 (107–121)	170 (139–228)	92 (67–102)	409 (384–472)
Females (n = 15)	RA	293 (245–347)	512 (459–560)	244 (227–264)	326 (299–376)	159 (141–176)	717 (651–806)
	LA	295 (260–339)	502 (418–538)	244 (226–272)	309 (190–357)	155 (147–165)	696 (629–742)
	RP	285 (251–312)	501 (473–539)	240 (211–255)	329 (271–368)	156 (139–172)	666 (608–720)
	LP	286 (254–334)	499 (455–536)	238 (218–261)	312 (262–357)	142 (124–160)	689 (585–751)

Hook features: AC, hook blade length; AD, hook length; BC, hook base length; CD, hook plateau length; AB, hook gape. *Hook position:* RA, right anterior; LA, left anterior; RP, right posterior; LP, left posterior.

Male [Based on measurements from 8 adults.] Body 11.6 (9.8–11.4) mm long, composed of cephalothorax and 68 (65–76) abdominal annuli. U-shaped frame of oral cadre 234 (194–279) long, 128 (115–150) wide, with overall cadre length 363 (317–398). Testis large and sinuous; proximal portion near terminal end of body; distal portion communicating with Y-shaped seminal vesicle, with each of two anterior arms forming triad with blind accessory reproductive gland and glandular sperm duct (possibly a prostatic duct) (Fig. 18). Each sperm duct communicating with pouch containing a long intromittent organ (Figs. 16, 18). Paired intromittent organs passing into dorsal aspect of right or left copulatory spicule and exiting anterior aspect of respective copulatory spicule. Paired intromittent organs extending into genital atrium. Copulatory spicules paired, sclerotized, cowry shell–shaped, each equipped with 2 anterior extensions (Figs. 12–15). Right spicule cowry shell-shaped base 505 (471–522) long, 202 (184–221) wide; left spicule cowry shell–shaped base 506 (475–537) long, 203 (183–245) wide. Ventral anterior extension short, claw-like, with dorsally directed apical spines emerging from the distal end (Fig. 13B, C). Dorsal anterior extension much longer than wide, spatulate, with minute spines along narrow base (Figs. 12, 13); right spicule dorsal extension 248 (186–283) long; left spicule dorsal extension 278 (237–294) long; overall right spicule length 746 (686–815); overall left spicule length 767 (731–846). Common male genital atrium opens through medial pore on ventral surface of second abdominal annulus.

Female [Based on measurements from 22 adults.] Body 30.1 (26.0–38.0) mm long, composed of cephalothorax and 71 (65–77) abdominal annuli. U-shaped frame of oral cadre 444 (272–

548) long, 243 (203–294) wide, with overall cadre length 603 (530–745). Uterus tubular, extends anteriorly to cephalothorax, then descends posteriorly into abdomen while coiling around intestine. Seminal receptacles paired, communicating with uterus through ducts at junction of cephalothorax and abdomen. Uterine pore opens on mid-ventral surface of abdomen between fourth and fifth annulus from terminus, pore 61 (43–79) from terminal end of body (Fig. 9). Mature eggs near distal end of uterus with a fragile, hyaline, transparent spherical outer layer and primary larva encapsulated by a second layer. Inner layer containing primary larva 86 (75–97) long, 68 (61–76) wide (n = 15) (Fig. 17). Hyaline layer collapsed in preserved specimens (not measured).

Nymph [Based on 6 measured nymphs.] Body 9.7 (9.0–10.8) mm long, composed of cephalothorax and 70 (67–74) abdominal annuli. Cuticular spines minute, present as continuous row on posterior margin of each annulus (Fig. 8). Cuticular chloride cell pores dispersed in loosely organized bands 2 to 3 pores deep; bands arranged in posterior half of each annulus (Fig. 8). Cell pore diameter measuring 7 to 9. Hooks similar in morphology to adult hooks but smaller, each hook with an overlying sclerotized accessory piece extending from anterior end of fulcrum (Fig. 10); accessory piece 180 (160–220) long. U-shaped frame of oral cadre 210 (190–240) long, 90 wide (n = 1), with overall cadre length 320 (300–330).

Experimental infection

Both lobes of the lung of the eastern mud turtle were found upon necropsy to be heavily infected with pentastomid nymphs and post-larval juveniles. One lobe was infected with 11 nymphs and three postlarval juveniles, the other with 12 nymphs. Nymphs were geminate and morphologically identical to those present in the infected western mosquitofish. The three postlarval juveniles were similar in size to the nymphs but lacked accessory pieces associated with their hooks. Cuticular chloride cell pore pattern was identical among nymphs from naturally infected western mosquitofish, nymphs and postlarval juveniles collected from the eastern mud turtle, and adults collected from wild terrapins in Louisiana and Florida. The experimental infection of the eastern mud turtle with wild nymphs confirmed the identity of nymphs found in western mosquitofish as *Levisunguis subaequalis* n. sp. and we believe that if a longer postinfection period had been allowed, all of the pentastomids recovered from the experimentally infected eastern mud turtle would have metamorphosed into adults. The fact that nymphs were found in the lungs indicates that metamorphosis toward adulthood likely takes place in the lungs (rather than in the digestive tract or elsewhere within the definitive host).

Remarks

As the type-species of a monotypic genus, generic level features (see above) uniquely distinguish *Levisunguis subaequalis* n. sp. In addition, numerous other features distinguish the new species from the two sebekids that mature in turtles. *Levisunguis subaequalis* n. sp. may be easily differentiated from *D. megastoma* from terrapins in South America by the absence of a spiny extension of the fulcra on anterior hooks. Furthermore, both anterior and posterior hooks of *L. subaequalis* n. sp. are considerably larger than hooks of *D. megastoma*. The largest hook reported from four females of *D. megastoma* by Junker et al. (2003) had blade

length (AC) 147 μm, hook length (AD) 260 μ, base length (BC) 108 μm, and plateau length (CD) 154 μm. These measurements are nearly half the size of those we report for the new species (Table 1). And, although the copulatory spicules of both species have two anterior extensions, the cowry shell–shaped portion of the spicules is relatively shorter and broader, and has the large opening on the ventral rather than dorsal surface in *D. megastoma* (see Junker et al., 2003). Additionally, the caudal end of both sexes in *L. subaequalis* n. sp. is rounded, whereas it is pointed in females of *D. megastoma* (see Junker et al., 2003).

Levisunguis subaequalis n. sp. may be easily differentiated from *P. africana* collected from terrapins in South Africa by the number of annuli. Whereas males and females are comparably similar in size between the two species, *L. subaequalis* n. sp. has approximately 70 (65–77) annuli compared with 27–30 in *P. africana* (see Junker & Boomker, 2002). Hooks, though similar in morphology in both species, are much larger in *L. subaequalis* n. sp. (see Table 1). Junker & Boomker (2002) reported hook length (AD) 115 μm and fulcrum length 239 μm from an immature female. Additionally, the copulatory spicules of *P. africana* have a single anterior spatulate extension rather than two extensions (Junker & Boomker, 2002). The absence of a thickened bowl-shaped base associated with the U-shaped oral cadre further serves to distinguish the North American species from the South African species.

Discussion

The earliest account of an aquatic North American pentastomid dates to an inadequate description of *Sebekia divestei* Giglioli in Sambon, 1922, a parasite of the American crocodile, *Crocodylus acutus* Cuvier. Aside from being grossly superficial, the description contained no locality data (Sambon, 1922). Knowledge of *S. divestei* was later supplemented by Riley et al. (1990), who examined female specimens from the South Australia Museum (SAM), Adelaide, Australia (SAM N198921-26) and reported that features regarding the adult hooks, body size, and number of annuli (75) indicated that the species was valid; nevertheless, locality data remain unreported and perhaps unknown for the species. Presently, the distribution of the American crocodile in North America is limited to the southern-most portion of Florida, Cuba, and southern Mexico. Riley et al. (1990) noted that the nymph of *S. divestei* (n = 1) possesses a row of minute spines on the anterior margin of each annulus and chloride cell pores arranged in two to three rows per annulus. Holl (1928) discovered geminate nymphs (which he described as *Bdukus ichthyius* Holl, 1928) in the body cavities of fishes collected in North Carolina, USA. Holl's (1928) nymphs exhibited approximately 67 annuli, each with a single row of chloride cell pores about its anterior region, identical to the arrangement present in the sebekid occurring throughout the southeastern United States in the American alligator, *Alligator mississippiensis* Daudin. At the time of Holl's (1928) discovery, the species from the American alligator was thought to be *Sebekia oxycephalum* (Diesing, 1836) Sambon, 1922 (see Heymons, 1935).

Overstreet et al. (1985) described *Sebekia mississippiensis* Overstreet, Self & Vliet, 1985 from adults and nymphs found in the lungs of the American alligator in the southern United States and corresponding nymphs from fishes. Nymphs of *S. mississippiensis* described by Overstreet et al. (1985) were consistent with *B. ichthyius* in having an anterior

ring of chloride cell pores around each annulus. Furthermore, Overstreet et al. (1985) determined that *S. mississippiensis* possessed relatively less robust hooks and hook ornaments than *S. oxycephalum* and that the latter species was confined to South and Central America. Consequently, most parasitologists considered *S. mississippiensis* and *S. divestei* the only sebekids from North America, with *B. ichthyius* being a junior synonym of *S. mississippiensis* (see Overstreet et al., 1985; Poore, 2012).

Nymphs of *L. subaequalis* n. sp. exhibit a cuticular chloride cell pore pattern on abdominal annuli (Fig. 8) consistent with that described from a single nymph of *S. divestei* (see Riley et al., 1990) and nymphs reported as *S. oxycephalum* from Florida by Venard & Bangham (1941). Nymphs of *L. subaequalis* n. sp. are easily distinguished from those of *S. divestei* by having a much longer accessory piece overlying each hook, ranging from 160–220 μm in length (vs. 105 μm). Additionally, the uterus and anus are separated by at least three annuli in *L. subaequalis* n. sp. (observable in adults and nymphs), whereas they are separated by two annuli in the adult female of *S. divestei* (see Riley et al., 1990). The description of nymphs reported as *S. oxycephalum* from Florida by Venard & Bangham (1941) was not sufficient to make comparison with either *L. subaequalis* n. sp. or *S. divestei*, and no specimen was deposited by these authors. Consequently, a more thorough investigation of the pentastomid nymphs from Floridian fishes is necessary to determine the identity of the nymphs studied by Venard & Bangham (1941).

Dukes et al. (1971) suggested that *S. mississippiensis* (as *S. oxycephalum*) may mature in terrapins. Nymphs of *S. mississippiensis* are known to occur in the lungs of various terrapins in North America, but no mature specimens of that species have been reported from such hosts (Dukes et al., 1971; Overstreet et al., 1985). We have observed nymphs of *S. mississippiensis* in the lungs of the snapping turtle, *Chelydra serpentina serpentina* (Linneaus), and the Alligator snapping turtle, *Macrochelys temminckii* (Troost) (both Testudines: Cryptodira: Chelydridae), collected in the Pascagoula River Basin in Mississippi, but have not found adult specimens in these hosts. Foster et al. (1998) reported *Alofia* sp. in *A. ferox* captured in Florida, but the material they studied was conspecific with *L. subaequalis* n. sp. To date, *L. subaequalis* n. sp. represents the only sebekid maturing in North American terrapins and only the third confirmed sebekid from North America.

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