

Gulf and Caribbean Research

Volume 9 | Issue 2

January 1995

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Recommended Citation

Heard, R. W. and J. M. Kinsella. 1995. *Levinseniella deblocki*, New Species (Trematoda: Digenea: Microphallidae) from Salt Marshes along the Eastern Gulf of Mexico with Notes on Its Functional Morphology and Life History. *Gulf Research Reports* 9 (2): 97-103. Retrieved from <https://aquila.usm.edu/gcr/vol9/iss2/3>
DOI: <https://doi.org/10.18785/grr.0902.03>

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**LEVINSENIELLA DEBLOCKI, NEW SPECIES
(TREMATODA: DIGENEA: MICROPHALLIDAE)
FROM SALT MARSHES ALONG THE EASTERN GULF OF MEXICO
WITH NOTES ON ITS FUNCTIONAL MORPHOLOGY AND LIFE HISTORY**

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ABSTRACT *Levinseniella (Austromicrophallus) deblocki*, n. sp., was collected during parasitologic studies of homeothermic vertebrates from salt marshes along the coast of the eastern Gulf of Mexico. Because *L. deblocki* lacks a female pouch, it belongs to the subgenus *Monarrhenos* proposed by Deblock and Pearson (1970). However, since Deblock and Pearson did not explicitly designate a type species for *Monarrhenos*, it is not available and is a *nomen nudum*. The next available name, *Austromicrophallus* Szidat, 1964, a genus synonymized with *Levinseniella* Stiles and Hassall, 1901 by Deblock (1978), is reinstated as a subgenus to receive the species lacking a female pouch and previously assigned to *Monarrhenos*. The adult of *L. deblocki* is found in the lower digestive tracts of the clapper rail (*Rallus longirostris*), rice rat (*Oryzomys palustris*), and raccoon (*Procyon lotor*). Morphologically, *L. deblocki* appears to be most similar to *L. polydactyla* Deblock and Rosé, 1962, known from Europe, and *L. ophidea* (Nicol, Damerec, and Wootton, 1985), described from a freshwater habitat in California. Differences in the life cycle, habitat type, and geographic distribution, plus a combination of distinctive morphological characters (presence of lappets on the oral sucker, number of genital pockets, and body size) separate *L. deblocki* from the other members of the subgenus *Austromicrophallus*. The metacercarial stage of *L. deblocki* occurs in the gonads of fiddler crabs (*Uca* spp.) and the first intermediate host appears to be a hydrobiid gastropod (*Heleobops* sp.). Observations on living and preserved specimens fixed *in copula* indicate that the genital atrium functions as an eversible hermaphroditic organ bearing the male papillae and metraterm. The genital hooks or "Jägerskiöld's bodies" appear to function as holdfast structures during copulation.

INTRODUCTION

During parasitologic studies of homeothermic vertebrates from salt marshes along the coasts of the northeastern and southeastern Gulf of Mexico, we discovered an undescribed species of the microphallid genus *Levinseniella* Stiles and Hassall, 1901. The adult stage of this species occurred in the lower digestive tracts of clapper rails, rice rats, and raccoons. The new species appears to be most closely related to *L. polydactyla* Deblock and Rosé, 1962, known from Europe, and *L. ophidea* (Nicol et al., 1985), described from a freshwater habitat in California.

MATERIALS AND METHODS

Living and fixed specimens of excysted metacercariae from fiddler crabs (*Uca* spp.) and adults from naturally and experimentally infected vertebrate hosts were examined microscopically. Specimens were killed in hot saline with and without coverslip pressure, then immediately fixed in AFA, stained in Erlich's hematoxylin, dehydrated, cleared, and mounted in Permount (TM).

Living metacercariae were excysted in saline-typsin solution at 39°C; excystment usually occurred within 12 hours. Copulating pairs of worms were obtained by placing 50 to 100 excysted metacercariae together in warm saline (39°C). Copulating pairs were examined alive or were heat-killed in hot saline and fixed in AFA (with or without coverslip pressure). Some of the fixed copulating pairs were separated for stained slide preparations, while others were mounted still *in copula*. A Wild-20 drawing tube (camera lucida) aided in the preparation of the illustrations. All measurements are in micrometers (m).

Type material (holotype and paratypes) are deposited in the National Parasite Collection of the US National Museum (USNM), Beltsville, Maryland. Additional paratypes are in the collections of the Museum of the Gulf Coast Research Laboratory (GCRL).

RESULTS: TAXONOMY

Genus *Levinseniella* Stiles and Hassall, 1901

Synonyms. *Austromicrophallus* Szidat, 1964.
Heardlevinseniella Yamaguti, 1971.

Subgenus *Austromicrophallus* Szidat, 1964, n.comb

Synonym. *Monarrhenos* Deblock and Pearson, 1970 (p. 784) *Nomen nudum*.

Diagnosis. Species of *Levinseniella* lacking a female pouch.

Type species. *Levinseniella (Austromicrophallus) anenteron* Szidat, 1964.

Other species referred to *Austromicrophallus*: *L. pellucida* Jägerskiöld, 1907; *L. amnicolae* Etges, 1953; *L. Polydactyla* Deblock and Rosé, 1962; *L. byrdi* Heard, 1968(a); *L. hunterae*¹ Heard, 1968(b); *L. monodactyla* Deblock and Pearson, 1971; *L. capitanea* Overstreet and Perry, 1972; and *L. ophidea* Nicol, Damaree, and Wootton, 1985; *L. ucatanensis* Carnaris and Ching, 1989.

Heard (1968) divided the genus *Levinseniella* into four groups based on the presence or absence of a female pouch and the relative number of atrial pockets ("male pockets" of some authors) present. Deblock and Pearson (1970) proposed the subgenus *Monarrhenos* to receive the species of *Levinseniella* that lack a female pouch. They did not, however, explicitly designate a type species or give a bibliographic reference to a proposed type species for their "new subgenus" as required under Article 13 of the International Code of Zoological Nomenclature. Therefore, *Monarrhenos* cannot be considered an available name and is a *nomen nudum*. In his 1971 treatise, which was in press when Deblock and Pearson's 1970 paper designating the subgenus *Monarrhenos* was published, Yamaguti described the monotypic genus *Heardlevinseniella* to receive *L. byrdi* Heard, 1968. He characterized this genus by the absence of a female pouch and the presence of a postoral muscular ring and oral lappets. Because there are several other species that lack a female pouch (Heard's Groups III and IV), have a large oral sucker, and have a "postoral muscular ring," Overstreet and Perry (1972) synonymized *Heardlevinseniella* with *Levinseniella*. Deblock (1978) redescribed *Austromicrophallus anenteron* Szidat, 1964, a poorly-known monotypic species from gulls collected along the coast of Patagonia (Szidat, 1964). Based on the presence of atrial pockets and the absence of a female pouch, Deblock (1978) synonymized the monotypic *Austromicrophallus* Szidat, 1964 with *Levinseniella* and placed *A. anenteron* in *Monarrhenos*. Thus *Austromicrophallus* Szidat, 1964 is the oldest subgeneric

name available for those species lacking a female pouch and assigned to *Monarrhenos sensu* Deblock and Pearson (1970). *Levinseniella anenteron* and *L. capitanea* are both characterized by the absence of digestive ceca. Following Overstreet and Perry (1972) and Deblock (1978), we consider the absence of digestive ceca to be a highly derived character not warranting generic or subgeneric standing on its own.

***Levinseniella deblocki*, new species**

Figure 1 A-G.

Synonyms. "*Levinseniella* sp. 2 (Heard, comm. écrite)": Deblock and Pearson (1971, p. 787; "*Levinseniella* sp. 2 (Heard, communication écrite)": Deblock (1971, p. 449); "*Levinseniella* sp. 2 (Heard In: Deblock and Pearson, 1971)": Nicol *et al.* (1985, p. 182); *Levinseniella* sp. A: Heard (1976, pp.83-98); *Levinseniella* sp.: Kinsella (1988, pp. 276, 277); *Levinseniella* sp.: Forrester (1992, [in part], p.95, 189).

Description (based on 10 mature worms). Body elongate, 777 to 1020 long by 220 to 278 wide in posterior third of body. Tegument spinose; spines becoming smaller and less conspicuous posteriorly, completely embedded in hindbody tegument. Oral sucker subterminal with well-developed ventrolateral papillae (lappets). Postoral muscular ring immediately posterior to oral sucker. Prepharynx 34 to 62 long. Pharynx 47 to 56 long by 43 to 47 wide. Esophagus 136 to 212 long. Cecae well developed, extending posterolateral and ending near lateral margins of body at level of acetabulum. Acetabulum recessed, 75 to 98 long by 63 to 93 wide. Forebody 63 to 73% of body length.

Testes immediately posterior to acetabulum, symmetrical, usually wider than long; right testis 38 to 67 long by 57 to 78 wide; left testis 44 to 58 long by 67 to 95 wide. Seminal vesicle-pars prostatica complex surrounded by thin membrane, retort-shaped, located intercecally immediately anterior to acetabulum; seminal vesicle 115 to 155 long by 46 to 75 wide; pars prostatica thick walled, 60 to 75 long by 42 to 50 wide, surrounded by numerous prostatic cells. Male genital papilla a relatively small blunt cone, 28 to 32 long by 28 to 30 wide at base, penetrated at base by ejaculatory duct, sperm duct opening at its tip. Genital pockets sinistral to genital pore, 8 to 14 in number; embedded in wall of eversible genital atrium; atrium, when not everted, with male papilla just under upper margin of genital pore; everted atrium protrudes through dilated genital pore, forming large large papilla-like structure, 95 to 110 in diameter (Figure 1, B-E).

¹ Originally, Heard (1968b) named this species for Wanda S. Hunter, but incorrectly gave it the masculine ending "i" instead of the feminine "ae."

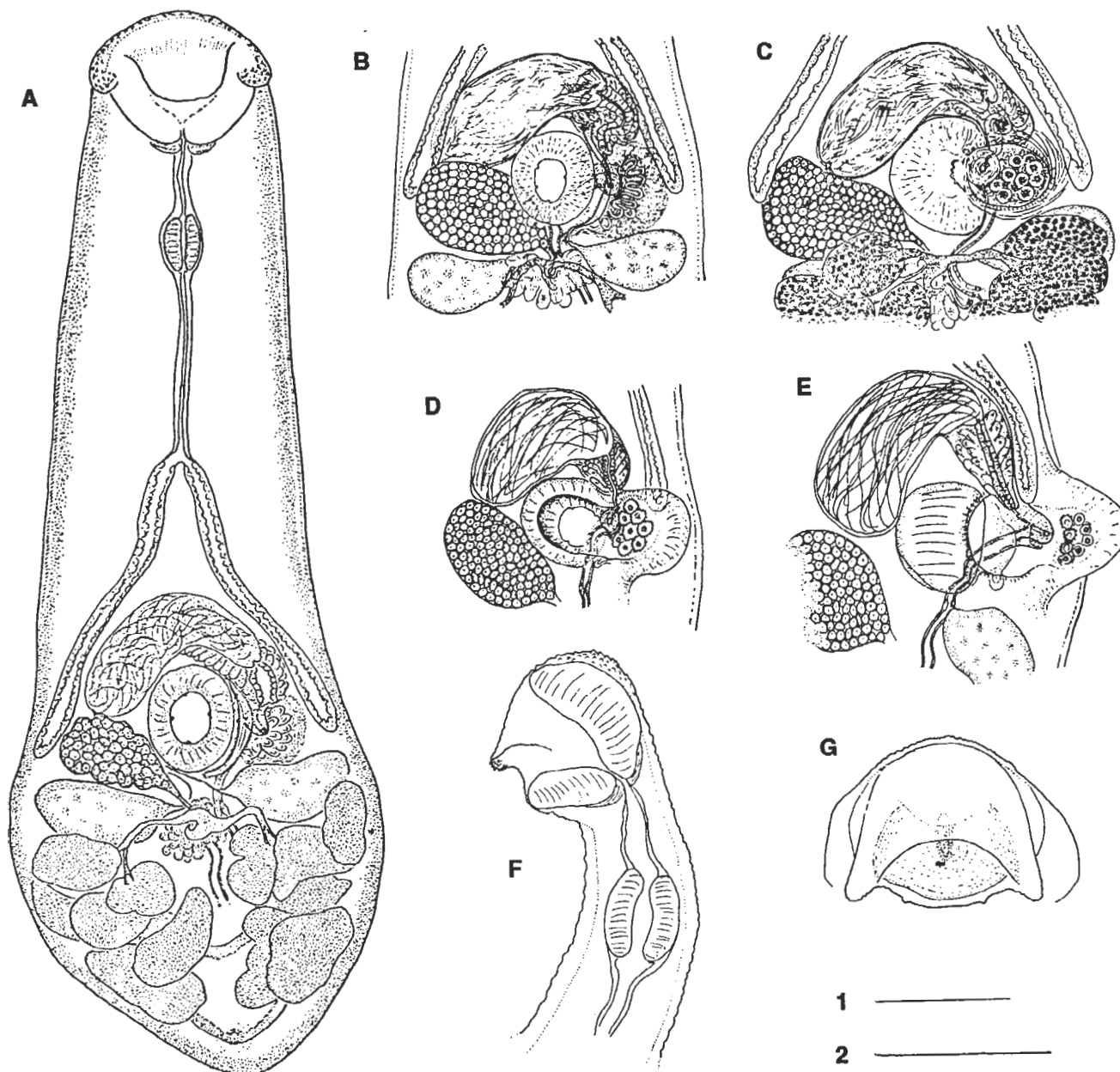


Figure 1. *Levinseniella deblocki*, n.sp.- A, ventral view of entire, mature worm; B-E, ventral views of ventral mid-region of body illustrating acetabulum and associated terminal genitalia. B, hermaphroditic organ retracted, genital pore not dilated; C, genital pore dilated, hermaphroditic organ beginning to protrude from genital pore; D, hermaphroditic organ (everted genital atrium) completely protruded through genital pore; E, protruded hermaphroditic organ under coverslip pressure showing openings of male papilla and metraterm); F, lateral aspect of oral sucker, post oral ring, pharynx, and lateral lappets; G, apical view of specimen showing lateral lappets adjacent to oral sucker. Scales = 100 μ m, scale 1 for A-E, scale 2 for F and G.

Ovary immediately anterior to right testis, dextral to acetabulum, 57-73 long by 73-113 wide. Oötype, Laurer's canal, and Mehlis' gland located in intertesticular region. Vitellariapost-testicular, acinose, six to seven relatively large follicles on each side. Uterine loops not reaching anterior to testes. Metraterm beginning in intertesticular region, passing dorsally along sinistral side of acetabulum, opening into dorsal portion of genital

atrium at base of male papilla. Eggs, 18 to 21 long by 11 to 13 wide.

Excretory bladder U-shaped; pore subterminal, dorsal. Flame cell formula $2[(2+2)+(2+2)]=16$.

Holotype (USNM 84540).

Paratypes. Paratypes: 1 specimen from *Rallus longirostris* (USNM 84541), 1 specimen from *Rallus longirostris* (GCRL 1338); 1 excysted metacercaria from *Uca panacea* (GCRL 1339).

Type host. *Rallus longirostris* Boddaert (clapper rail).

Site of infection. Intestinal ceca.

Type locality. Upper Tampa Bay (June 1965).

Mammalian hosts. *Oryzomys palustris*, rice rat (in large intestine); *Procyon lotor* (L.), northern raccoon (in large intestine).

Localities. Marco Island (Collier County), Florida (*P. lotor*); Cedar Key (Levy County), Florida (*O. palustris*).

Etymology. This species is named for Professor Stéphane Deblock in recognition of his many contributions to the study of the systematics and biology of the family Microphallidae.

Second intermediate hosts (*Uca* spp.). *U. longisignalis* Salmon and Atsides - Little Dauphin Island (Mobile County), Alabama; Cedar Key (Levy County), Florida. *U. panacea* Novak and Salmon-Horn Island, Jackson County, Mississippi.- *U. pugilator* (Bosc)- Cedar Key, Florida (Kinsella, 1988).

Metacercaria. Cysts spherical, 350 to 450 in diameter (from naturally infected *Uca panacea* collected from salt marshes adjacent to Big Lagoon on Horn Island, Mississippi).

Site of infection. Gonads (testes and ovaries).

Taxonomic Remarks. The absence of a female pouch places *Levinseniella deblocki* into the new subgenus *Heardlevinseniella* and the absence of a female pouch coupled with more than five genital pockets ("male pockets") places it in subgroup IV of Heard (1968a). In addition to *L. deblocki*, four other species (*L. polydactyla* Deblock and Rosé, 1962; *L. hunterae*² Heard, 1968; *L. capitanea* Overstreet and Perry, 1972; and *L. ophidea* Nicol, Dameree, and Wootton, 1985) are presently referable to Heard's subgroup. *Levinseniella capitanea* differs from *Levinseniella deblocki* by the former's much larger size, more numerous genital pockets (11-21), and the absence of a pharynx and digestive ceca. Both ecological and morphological differences distinguish *L. deblocki* from *L. ophidea*. *Levinseniella ophidea* utilizes leeches as second intermediate hosts, frogs and snakes as definitive hosts, and occurs in freshwater stream habitats (Nicol, Demaree, and Wootton 1985). Morphologically, *L. ophidea* is distinctly larger than *L. deblocki* and lacks lappets on its oral sucker. The presence of a massive male papilla and the absence of a pair of well-developed ventrolateral lappets on the oral sucker of *L. hunterae* separates it from *L. deblocki*. *Levinseniella deblocki* appears to be most similar to *L. polydactyla*, described from the metacercaria stage in a

marine isopod (*Sphaeroma hoockeri* Leach) from France (Deblock and Rose 1972). It is distinguished from *L. polydactyla* by having an oral sucker with a pair of ventrolateral lappets, a well-developed postoral muscular ring, and a greater range in the number of genital pockets (8 to 14), compared with 10 to 12 in *L. polydactyla*.

OBSERVATIONS

Functional Morphology of the Terminal Genital during Mating

Observations on the possible function of the genital atrium, the acetabulum, and the genital pockets or "Jägerskiöld's bodies" were made on mating pairs of *L. deblocki*. Copulating pairs of worms were obtained by placing 50 to 100 excysted metacercariae together in warm saline (39°C). When these specimens were examined after 1 to 2 hours, usually 25% or more of them were found in copula.

Study of living and permanently mounted specimens in copula demonstrated that during copulation, the entire "genital atrium" everts to form a large hermaphroditic organ bearing the male papilla, the genital pockets and the opening of the metraterm (see Figure 1 B-E). When the hermaphroditic organ was everted, the acetabulum rotated 60 to 80° and partially retracted into the dextral wall of the body, leaving a deep cavity or "acetabular genital atrium" large enough for the insertion of the genital organ of the partner. During copulation, the acetabulum of each worm functions as a true genital sucker by attaching to the lateral wall of the partner's hermaphroditic organ. The genital pockets (Jägerskiöld's bodies) of each worm then partially evert and mesh with those of its partner. While in copula, the terminal genitalia of each worm lies immediately opposite that of its partner, allowing the male papilla of each worm to be inserted simultaneously into the opening of the other's metraterm, thus facilitating the exchange of sperm (cross-fertilization). Excysted worms maintained at 39°C in saline produced eggs within 24 hours.

Life History

The adult stage of *Levinseniella deblocki* appears to be a well-established parasite of both mammals and birds. Clapper rails serve as an avian host for *L. deblocki* along the Gulf Coast of Florida (Heard 1976). Although mammals have been experimentally infected with *Levinseniella* (see Bridgman et al. 1972), the occurrence of *L. deblocki* in rice rats and raccoons from salt marshes along the Gulf Coast of Florida established the first records of the genus in

² Originally, Heard (1968b) named this species for Wanda S. Hunter, but incorrectly gave it the masculine ending "i" instead of the feminine "ae."

naturally infected mammals (Heard 1976; Kinsella 1988; Forrester 1992).

Information on the life cycle of *L. deblocki* is incomplete, but a hydrobiid (*Heleobops* sp.) from salt marshes on Horn Island, Mississippi, shed a microphallid cercaria that appeared to be its larva. In an experiment, this cercaria penetrated *U. longisignalis* and formed cysts in the gonads within 48 hours. Due to high mortality in the fiddler crabs used in this experiment, development to the mature metacercarial stage did not occur. The suspected cercaria of another microphallid *Gynaecotyla* sp. also occurs in *Heleobops* sp. from the same area. These two cercariae can be distinguished from each other by their stylet lengths. The stylet length of the *Levinseniella*-like cercaria was 17 m, whereas the stylet length of the *Gynaecotyla*-like cercaria was 22 m.

The infected fiddler crab and vertebrate hosts of *L. deblocki* occurred sporadically in the higher salinity salt marshes along the edge of the eastern Gulf of Mexico. The distribution pattern of these infected second intermediate

and definitive hosts appears to be directly related to the presence of the suspected gastropod host, *Heleobops* sp.

DISCUSSION

A variety of malacostracan crustaceans, and in one instance a leech, have been reported to be the second intermediate hosts for species of *Levinseniella*. However, more detailed morphological and life history studies are needed, especially for species occurring in Eurasia, before many of these reports can be verified.

Amphipods and isopods have been reported as second intermediate hosts for several species of *Levinseniella* (Villot 1875; Etges 1956; Ouspenskaia 1960, 1963; Deblock and Rosé 1962; Reimer 1963; Rebecq 1964; Heard 1970, 1976; Galaktionov 1988; Galaktionov and Malkova 1993). Besides *L. deblocki*, several other species of *Levinseniella* are known to use decapods as second intermediate hosts (see Table 1).

TABLE 1
Decapod crustaceans reported as hosts of *Levinseniella*.

Species of <i>Levinseniella</i>	Second intermediate host(s)	Reference(s)
<i>L. brachysoma sensu</i> Balozet and Callot*	<i>Palaemonetes punicis</i>	Balozet and Callot (1939)
<i>L. capitanea</i>	<i>Callinectes sapidus</i>	Overstreet and Perry (1972)
<i>L. caracinides**</i>	<i>Carcinides maenas</i>	Rankin (1939)
<i>L. conicostoma</i>	<i>Hemigrapsus penicillatus</i>	Bridgman et al. (1972), Kifune and Takao (1972)
<i>L. cruzi sensu</i> Mortonelli and Shuldt	<i>Palaemonetes argentinus</i>	Martorelli and Schuldt (1990) Schuldt and Lunaschi [1985(1987)]
<i>L. deblocki</i> , n. sp.	<i>Uca longisignalis</i> , <i>U. panacea</i> , <i>U. pugilator</i> , <i>U. rapax</i>	Heard (1976) [as <i>L. sp. A</i>], Kinsella(1988) [as <i>L. sp.</i>], present report
<i>L. cf. pellucida</i> <i>sensu</i> Nikitina	<i>Astacus lepodactylus eichwaldi</i>	Nikitina (1983)
<i>Levinseniella</i> sp.***	<i>Upogebia affinis</i>	Pearse (1945)
<i>Levinseniella</i> sp.	<i>Uca</i> sp.	Cable et al. (1960)
<i>Levinseniella</i> sp. C	<i>Rhithropanopeus harrisi</i>	Heard (1976)
<i>Levinseniella</i> sp.	<i>Portunus pelgicus</i>	Shields (1992)
<i>Levinseniella</i> sp.	<i>Pachygrapsus tansversus</i>	Bush et al. (1993)

* Identification questionable (= *Spelotrema* sp.?)
 ** Identification questionable (= *Spelotrema similis*?).
 *** Generic designation questionable.

Some of the earlier reports of *Levinseniella* metacercaria in decapod crustaceans may be erroneous or need confirmation. Balozet and Callot (1939) reported the shrimp, *Palaemonetes punicis*, from Tunisian waters as an intermediate host of *L. pellucida*; however, Yamaguti (1958, page 886) questioned the identity of their material, indicating that it was probably a species of *Microphallus* Ward, 1901. After studying the description and figure given by Balozet and Callot, we concur with Yamaguti that the Tunisian specimens do not belong to the genus *Levinseniella*. Rankin (1939) suspected the green crab, *Carcinides maenas* (L.), to be the second intermediate host of *L. carcinides* Rankin, 1939, a species described from New England; however, this conjecture has not been confirmed. The metacercaria mentioned by him may have been *Microphallus similis* (Jägerskiöld, 1900), reported from the same crab host by Stunkard (1957). Cable (1956) mentioned the presence of *Levinseniella* metacercariae in fiddler crabs from Puerto Rico, but this record has not been confirmed. Pearse (1945), working at Beaufort, North Carolina, reported a trematode metacercaria from the anomuran mud shrimp *Upogebia affinis* (Say) which he tentatively identified as a species of *Levinseniella*. One of

us (RWH, unpublished data) examined over 100 specimens of this mud shrimp from the Beaufort area and found no microphallid metacercariae.

Levinseniella ophidea is the only species known to use noncrustacean second intermediate hosts. The metacercarial stage of this species was reported in four species of leeches from a California freshwater stream habitat (Nicol et al., 1985).

ACKNOWLEDGMENTS

We wish to thank Robin M. Overstreet and Donald J. Forrester for advice, encouragement, and material support during this study. Jeff Lotz, Robin Overstreet, Jerry McLelland, and Jan Boyd kindly read and made constructive comments on the manuscript. The illustrations were inked by Beryl Story. We also wish to thank the two reviewers for their constructive comments and suggestions. This work was supported in part by NOAA Office of Sea Grant, Department of Commerce under Grant No. 04-6-158-44060 (RWH) and NIH Training Grant TO1-A1-0038-02 from the NIAID (JMK).

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