

***Levinseniella (Levinseniella) gavalanicola* sp. nov. (TREMATODA: MICROPHALLIDAE) IN THE COMMON BLACK HAWK *Buteogallus anthracinus* FROM VENEZUELA, AND NOTES ON ITS LIFE CYCLE**

***Levinseniella (Levinseniella) gavalanicola* sp. nov. (Trematoda: Microphallidae) en el gavilán cangrejero *Buteogallus anthracinus* de Venezuela y nota sobre su ciclo de vida**

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ABSTRACT

A new species, *Levinseniella (Levinseniella) gavalanicola* sp. nov. (Trematoda: Microphallidae) is described from the small intestine, caecum and rectum of naturally infected common black hawk, *Buteogallus anthracinus* (type host), from El Paujil, Cajigal Municipality, Sucre State, Venezuela. The new species is placed into the group III according to Heard (1968) for possessing numerous accessory atrial sacs (7 or more) and presence of female pouch. The new species differs from other species of this group mainly in the number of atrial accessory sac (9 with sclerotized structures) and its arrangement in the wall of the genital atrium. Conspecific adults were obtained from laboratory reared chicks, a duckling, rats and mice after artificially feeding of crabs (*Uca rapax*) which served as the second intermediate host of this newly described trematode from La Ceiba and El Paujil in Sucre State.

Key words: Digenea, microphallidae, *Levinseniella*, *Uca rapax*, *Buteogallus anthracinus*, life cycle, Venezuela.

RESUMEN

Una nueva especie, *Levinseniella (Levinseniella) gavalanicola* sp. nov. (Trematoda: Microphallidae) es descrita a partir del intestino delgado, ciegos intestinales y recto del gavilán cangrejero, *Buteogallus anthracinus* (hospedero tipo) naturalmente infectado, en El Paujil, municipio Cajigal, estado Sucre, Venezuela. La nueva especie, la cual está ubicada en el Grupo III según Heard (1968), debido a la presencia de numerosos sacos auriculares accesorios (7 o más) y de una bolsa femenina, se dife-

rencia de otras especies de este grupo, principalmente en el número de sacos auriculares accesorios (9 con estructuras esclerotizadas) y su disposición en la pared del atrio genital. Adultos conoespecíficos se obtuvieron a partir de hospederos experimentalmente infectados (pollos, un pato, ratas y ratones de laboratorio) alimentados con cangrejos *Uca rapax* de La Ceiba y El Paujil en el estado Sucre, los cuales sirvieron como segundos hospederos intermediarios del nuevo trematodo.

Palabras clave: Digenea, microphallidae, *Levinseniella*, *Uca rapax*, *Buteogallus anthracinus*, ciclo de vida, Venezuela.

INTRODUCTION

The trematodes of the family Microphallidae are small parasites present in the digestive tract of most vertebrates. The genus *Levinseniella* Stiles and Hassall, 1901, contained more than 40 species described mainly from birds around the world, and metacercariae of these species use mainly crustaceans as second intermediate hosts. Of these, only three species have been described from South America: *Levinseniella (M.) cruzi* [13] in *Anas bahamensis* in Brasil; *L. anenteron* Szidat, 1964 [2] in *Larus marinus dominicanus* in Argentina, and *L. (L.) venezuelensis* [6] in *Ereunetes pusillus* in Venezuela. A few studies on life cycles of *Levinseniella* have been previously reported on *L. (L.) brachysoma* (Creplin, 1837) Stiles and Hassall, 1901; *L. (L.) propinqua* Jaegerskiold, 1907; *L. (M.) cruzi* [13]; *L. (M.) amnicolae* [5]; *L. (M.) ophidea* [11]; and *L. deblocki* [9]. To date, only *L. (L.) venezuelensis* has been described in Venezuela. A new microphallid, *L. (L.) gavalanicola* sp. nov. is described in Venezuela from both naturally and experimentally infected vertebrate hosts. The

metacercariae of this species were found in the decapods *Uca rapax*, which served as second intermediate hosts. However, the first intermediate host still needs to be elucidated.

MATERIALS AND METHODS

The study was carried out at Cajigal Municipality, Sucre State, between 1993 and 1999. In 1993, one *Buteogallus anthracinus* (Deppe, 1830; Falconiformes: Accipitridae) was caught using Japanese nets from El Paujil, Cajigal Municipality, Sucre State, Venezuela. Ten digeneans were recovered from the small intestine, intestinal caecum, and rectum of the bird, and the worms were fixed under cover slip pressure in 5% hot formalin and stored in 70% ethanol. In addition, during 1999, 27 specimens of *Uca rapax* (Smith, 1870; Brachyura: Ocypodidae) were collected during the period of low tide from La Ceiba and El Paujil. The carapace of the crab was removed by cutting with scissors around the margin of the dorsal surface, its internal tissues were removed, and examined under dissecting microscope. Metacercarial cysts were collected from the abdominal tissues that cover the insides of the dorsal carapace and hepatopancreas. Cysts were preserved in 0,75% saline for further studies and to be used in experimental infections. Twenty metacercarial cysts were force-fed with pipette to two chicks (*Gallus gallus domesticus* Linnaeus, 1758) and one duck (*Cairina moschata* Linnaeus, 1758). In addition, two rats (*Rattus norvegicus* Berkenhout, 1758) and three mice (*Mus musculus* Linnaeus, 1758) were fed with infected crabs. The experimental animals were examined 4-10 days post-infection and 120 adult trematodes were recovered from small intestine, of which 13 from chicks, 11 from the duck, 28 from rats and 68 from mice. Due to difficulties in studying live metacercar-

iae, these were allowed to excyst naturally in 0.50% solution of trypsin in phosphate buffer at pH 7. 2 and at 26°C. Metacercariae were excysted after four hours in the solution. Some excysted metacercariae and adults were studied live, and other were used for permanent preparations which were killed in hot 0.75% saline, fixed in AFA (alcohol-formalin-acetic acid), and stained in Semichon's acetocarmine after dehydration in a series of ascending alcohols, cleared in oil cloves and mounted in Canada balsam. The drawings were made with a drawing tube on a Carl Zeiss Axioscope microscope attachment, modelo A1, Germany.

All measurements are given in micrometers (µm). The trematodes were identified using the keys of some investigators [3, 7, 8, 14].

RESULTS AND DISCUSSION

Levinseniella (Levinseniella) gavilanicola sp. nov. (FIGS. 1-8).

Description of adult fluke (FIGS. 1-4, 8). Fifty specimens were studied and the measurements are given on TABLE I. Body with linguiform shape, tegument with spines, extending up to the end of intestinal caeca. Preoral lobe weakly developed. Subterminal oral sucker, with two non glandular lateral auricular papillae and a muscular ring at posterior end. Prepharynx, well developed, slightly thick, followed by a muscular pharynx. A long and wide oesophagus bifurcates to two intestinal caeca over anterior to seminal vesicle, extending posteriorly to the lateral margins at the level of acetabulum. Round acetabulum, smaller than oral sucker. Testes symmetrical, post acetabular.

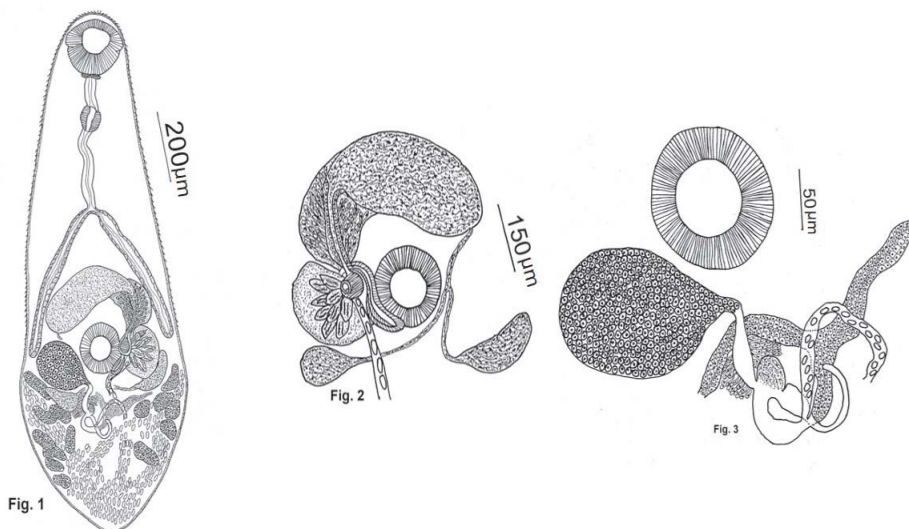


FIGURE 1. *Levinseniella (L.) gavilanicola* sp. nov. ADULT PARASITE FROM *Buteogallus anthracinus*, NATURALLY INFECTED. VENTRAL VIEW.
FIGURE 2. *Levinseniella (L.) gavilanicola* sp. nov. ADULT PARASITE FROM *Buteogallus anthracinus*, NATURALLY INFECTED. MALE GENITALIA.
FIGURE 3. *Levinseniella (L.) gavilanicola* sp. nov. ADULT PARASITE FROM *Buteogallus anthracinus*, NATURALLY INFECTED. FEMALE GENITALIA.

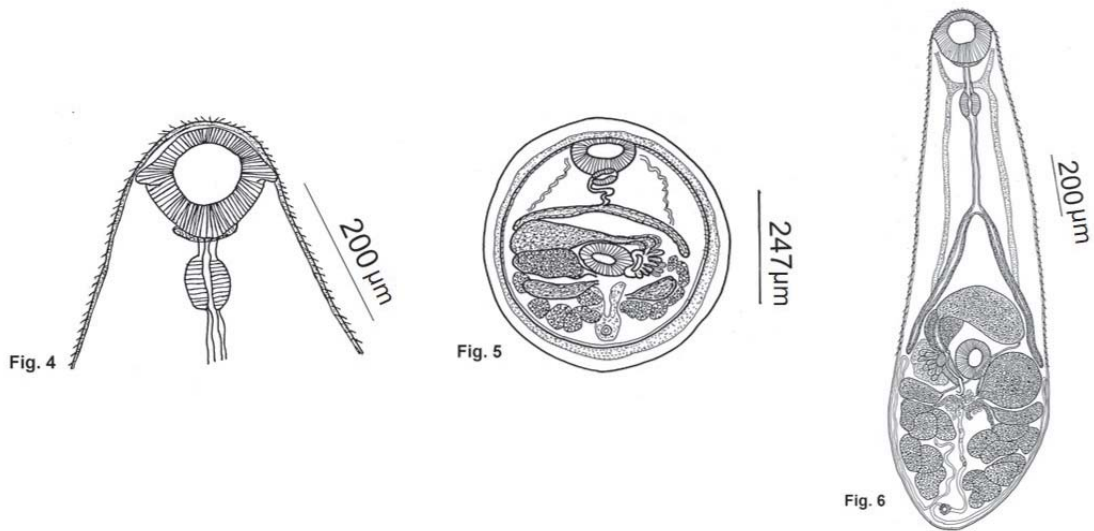


FIGURE 4. *Levinseniella (L.) gavilanicola* sp. nov. ADULT PARASITE FROM *Buteogallus anthracinus*, NATURALLY INFECTED. ORAL SUCKER WITH TWO NON GLANDULAR VENTROLATERAL AURICULAR PAPILLAE AND A POSTERIOR CIRCULAR MUSCLE RING. VENTRAL VIEW.

FIGURE 5. *Levinseniella (L.) gavilanicola* sp. nov. ENCYSTED METACERCARIA FROM *Uca rapax*, NATURALLY INFECTED. DORSAL VIEW.

FIGURE 6. *Levinseniella (L.) gavilanicola* sp. nov. EXCYSTED METACERCARIA FROM *Uca rapax*, NATURALLY INFECTED. DORSAL VIEW.

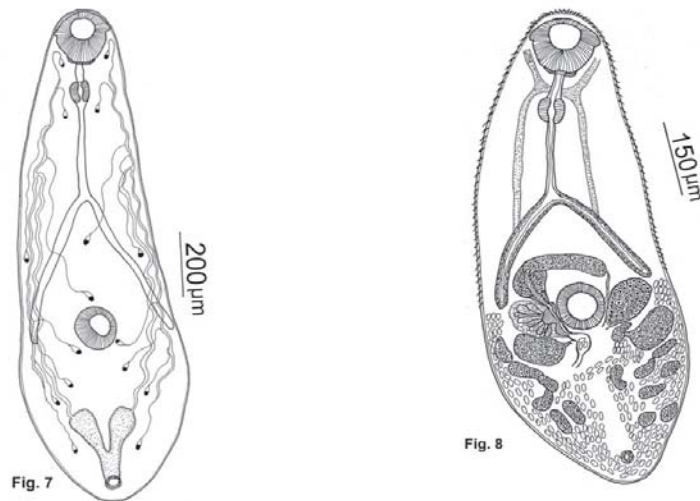


FIGURE 7. *Levinseniella (L.) gavilanicola* sp. nov. EXCYSTED METACERCARIA FROM *Uca rapax*, NATURALLY INFECTED. EXCRETORY SYSTEM.

FIGURE 8. *Levinseniella (L.) gavilanicola* sp. nov. ADULT PARASITE FROM *Rattus norvegicus*, EXPERIMENTALLY INFECTED.

Efferent tubes leave towards anterior part of body at the middle of ventral part of each testis, joining at equatorial level of acetabulum forming deferent duct, entering posterior to seminal vesicle. Elongate seminal vesicle, located anterior to the acetabulum, filled with sperms, covered with a fine non muscular membrane, which continues over the prostatic complex. *Pars prostatica* relatively short, surrounded by prostatic cells. Short ejaculatory duct, ending in a muscular ovoid male genital papilla, projecting into the genital atrium. Genital pore located on

the left of acetabulum, opening to the genital atrium, above or sideways of the male genital papilla. Genital atrium located on the left side and close to acetabulum, containing nine accessory atrial sacs (also called atrial fingerlings), with two rows of sclerotized bars, one with four on the base of atrium and the other with five over it. Well developed female pouch without sclerotic structures, connected with genital atrium by a muscular layer at the base of the male genital papilla. Ovary with smooth or slightly wavy edges, located to the right of acetabu-

TABLE I
MEASUREMENTS OF *Levinseniella (Levinseniella) gavilanicola* sp. nov.

Host	<i>Buteogallus anthracinus</i>	<i>Gallus(Gallus) domesticus</i>	<i>Rattus norvegicus</i>	<i>Mus musculus</i>	<i>Cairina moschata</i>
Number Examined	8	7	28	18	5
Body					
Length	1,045-1,776	1,051-1,358	820-1,666	487-1,333	1,051-1,615
Breadth	476-629	410-43	435-512	282-461	384-512
Oral sucker	147-174 107-161	126-156 131-161	121-182 101-192	136-161 60-161	126-166 131-161
Prepharynx	40-120 20-40	40-50 20-30	50-101 20-40	20-75 15-45	75-101 20-25
Pharynx	60-90 50-80	70-80 50-60	55-85 50-75	30-90 25-70	65-95 50-65
Oesophagus	160-410 10-20	136-166 20-25	101-303 15-25	126-181 15-20	126-328 15-30
Seminal vesicle	60-121 202-262	55-90 227-292	65-111 181-282	50-85 80-287	60-116 151-252
Acetabulum	100-153 112-139	116-121 106-111	101-142 101-142	55-126 55-121	111-136 101-141
Right testis	30-50 80-112	45-50 60-126	40-111 80-126	40-50 40-106	45-55 80-176
Left testis	30-60 80-120	65-85 50-90	45-116 101-151	40-50 60-80	45-70 75-116
Pars prostatica	80-107 90-134	69-75 75-134	43-91 59-134	40-72 59-107	67-99 80-134
Ovary	80-150 140-180	75-111 126-151	55-126 75-176	60-29 65-146	60-146 121-181
Eggs	18-24 10-13	21-24 10-13	18-26 10-16	21-26 10-13	18-24 10-13

lum, immediately anterior to right testis, the oviduct emerges on mid lateral side of ovary, it enlarges immediately to form ootype, surrounded by Mehlis glands, which later forms a small seminal receptacle and Laurer's canal. The oviduct extends posteriorly to unite with the uterus and the main vitellogenic duct. Uterus postacetabular, lateral branches sometimes reach to anterior margin of testes. Lightly muscular metraterm reaches genital atrium close to the male genital papilla. Small eggs, operculate, yellow in color. Vitelline cells localized at postero-lateral to testes, forming two groups of 6-8 follicles on each side. V-shaped excretory bladder, excretory pore subterminal surrounded by glandular cells.

Taxonomic summary

Type host: Natural definitive host: *Buteogallus anthracinus*.

Experimental Hosts: Chick (*Gallus gallus domesticus*), duck (*Cairina moschata*), mouse (*Mus musculus*), rat (*Rattus norvegicus*).

Site of infection: Adults in small intestine, intestinal caecum and rectum.

Second intermediate host: Fiddler crab (*Uca rapax*).

Site of infection: Metacercaria: Tissues that covers the insides of the dorsal carapace and hepatopancreas .

Location: La Ceiba, El Paujil, Cajigal Municipality, Sucre State, Venezuela.

Holotype: It is deposited in the Parasitological Collection of the Institute for Research in Biomedicine and Applied Sciences "Dr. Susan Tai ", Universidad de Oriente, Cumaná, Sucre State, Venezuela, IIBCA No. 1226.

Paratype: IIBCA No. 1227, at the above address.

Etymology: The specific name is derived from the common name of the natural host, gavilan.

Metacercariae (FIGS. 5-7).

Description. (Based on 23 specimens from naturally infected *Uca rapax*, 13 from La Ceiba and 10 from El Paujil, and measurements based on 46 worms and given in micrometers (μm)). Cyst almost spherical, 536-593 X 512-570, double walled, thick outer membrane, whitish, and internally hyaline. Body of

excysted metacercariae elongate, 948-1,871 x 256-589, slightly reduced in the preacetabular region. Integument covered with spines that reach acetabular level. Preoral lobe, 5 x 20, not well developed. Conspicuous nervous system, with a nerve commissure located above the pharynx, two short antero-lateral nerves reach the oral sucker and two long posterior nerves reach the level of seminal vesicle. Oral sucker well developed, 95-181 x 101-191, with a pair of non glandular lateral auricular papillae and a muscular post oral ring. Preacetabular distance: 436-1,096. Short prepharynx: 10-30 x 25-166; Muscular pharynx: 50-166 x 10-65. Oesophagus, 151-414 x 10-30, long and wide; short intestinal caeca, extending to acetabular level. Acetabulum almost spherical, 95-151 x 95-136, postequatorial, spineless. Postacetabular distance: 223-558. Symmetrical testes, right testis: 30-70 x 75-131, and left testis: 35-70 x 35-70. Posttesticular distance: 203-456. Arched seminal vesicle, 50-343 x 50-277, full of sperms, between intestinal caeca and acetabulum. Bulbous pars prostatica, 34-129 x 53-161, well developed with many prostate cells, surrounded by a thin membrane with a short ejaculatory duct ending in a male genital papilla, located over the genital atrium. Genital pore opens to genital atrium. Genital atrium lateral to the acetabulum, containing nine accessory atrial sacs, with sclerotized structures in the form of bars, arranged in two rows, one anterior with five and one posterior with four. Female pouch lies adjacent to the genital atrium. Oval ovary, 40-161 x 75-181, anterior to right testis, oviduct short, ootype cells surrounded by Mehlis glands. Small seminal receptacle. Uterus lies posttesticular, some metacercariae (from La Ceiba) contained some developing eggs, 16-21 x 10-16. Muscular metratermo. Vitelline glands well developed, located in two lateral groups of 6 and 8 follicles each. Excretory bladder "V" shaped, the primary excretory ducts are divided at the junction of the oesophageal bifurcation, excretory duct divided into anterior and posterior, each with four flame cells. Flame cells formula: $[(2 + 2) + (2 + 2)] = 16$. Sub-terminal excretory pore, surrounded by glandular cells.

According to Deblock [3], this new species must be included in the family Microphallidae Ward, 1901, subfamily Levinseniellinae Stiles and Hassall, 1901, and in the genus *Levinseniella (Levinseniella)* Stiles and Hassall, 1901, on the basis of the morphological features of the worms. Members of the genus *Levinseniella* are very similar morphologically, the features of some organs, i.e., the accessory atrial sacs, presence or absence of female pouch are used to differentiate the species. Heard [7] revised the genus *Levinseniella* and divided it into four groups based on the number of accessory atrial sacs and the presence or absence of female pouch. Deblock and Pearson [4] reviewed the genus *Levinseniella* and created two new subgenus, *Levinseniella* and *Monarrhenos* to include those species with or without female pouch. Yamaguti [14] created a new genus, *Heardlevinseniella*, based on the absence of female pouch and having a large oral sucker with lateral auricular papillae and a muscular ring on its posterior part. Overstreet and Perry [12] considered that the diagnostic char-

acters that Yamaguti used to differentiate these two subgenera are not sufficiently viable and synonymised the subgenus *Heardlevinseniella* to *Levinseniella*. According to the classification of Heard [7], *Levinseniella (L.) gavilanicola* sp. nov., clearly belongs to Group III (numerous accessory atrial sacs (7 or more) and presence of female pouch), and according to Deblock [3], the new species falls into genus *Levinseniella (Levinseniella)* Stiles and Hassall, 1901.

Levinseniella (L.) carteretensis [1] is the only species included by Heard [7] into the group III, which is very similar to the new species described herein, but differs due to the absence of a pair of lateral auricular papillae in the oral sucker and a postoral muscular ring; in addition, it bears a weakly developed male papilla and 7-10 accessory atrial sacs. *L (M.) hunteri* [8] is the only species of the genus *Levinseniella* that shares with *L (L.) gavilanicola* sp. nov., the same number of accessory atrial sacs (9) and their arrangement in the wall of the genital atrium. However, the new species can be distinguished from *L (M.) hunteri* by the presence of sclerotized parts in its accessory atrial sacs, a pair of lateral auricular papillae on the oral sucker and female pouch. *Levinseniella (L.) gavilanicola* sp. nov., differs from all the members of the genus *Levinseniella* described in South America, i.e., *L (M.) cruzi* [10] from Brazil. *L. anenteron* (Szidat, 1964) [2] from Argentina and *L (L.) venezuelensis* [6] from Venezuela. *L (M.) cruzi* lacks a pair of lateral auricular papillae, post-oral muscular ring, female pouch and its genital atrium contains 6-10 accessory atrial sacs without sclerotized structures and its vitellaria contains 10-12 follicles on each side of the body. Further, *L (M) cruzi* used the birds, *Rollandia. rolland chilensis* and *Himantopus melanurus* as natural hosts and the freshwater shrimp *Palaemonetes argentinus* as natural intermediate secondary host. The absence of a digestive system and the presence of four accessory atrial sacs in *L. anenteron*, serves to separate it from the new species. *L (L.) venezuelensis* shared with the present species in possessing a pair of lateral auricular papillae on its oral sucker, a postoral muscular ring and the presence of female pouch. However, the more distinct difference between both species are the number of accessory atrial sacs (six in *L (L.) venezuelensis* and nine in *L (L.) gavilanicola* sp. nov.), without sclerotized structures in the former and at least 2-3 row of spines on the acetabulum. According to Fischthal and Nasir [6] the ring of acetabular spines appears unique for the species.

A few studies on the life cycles of *Levinseniella* have been reported on: *L (L.) brachysoma*, *L (L.) propinqua*, *L (M) cruzi*, *L (M.) amnicolae*, *L (M.) ophidea*, and *L. deblocki*. The first two species can be distinguished from *L (L.) gavilanicola* by having three and four accessory atrial sacs and by the absence of lateral auricular papillae and postoral ring (reduced in *L (L.) propinqua*). In addition by their use on of Isopods and Amphipods (only in *L (L.) propinqua*) as second intermediate hosts and Charadriiformes and Anseriformes birds, as definitive hosts. *L (L.) cruzi* has already been differentiated from *L (L.)*

gavilanicola (see above). *L. (M.) amnicolae* is distinguished from the new species by its smaller digestive system, the number of accessory atrial sacs (4), without sclerotized structures, and the absence of female pouch, and an oral sucker without lateral auricular papillae and postoral ring. Additionally, it uses *Asellus communis* as second intermediate host. *L. (M.) ophidea* can be separated from *L. (L.) gavilanicola* sp. nov. by the use of *Thamnophis elegans* (snake) and *Rana catesbeiana* (bullfrog) as natural definitive hosts and several species of leeches: *Glossiphoniaea complanata*, *Helobdella stagnalis*, *Erpodella punctata* and *Dina parva* as second intermediate hosts. Also, by the absence of lateral auricular papillae, postoral ring and female pouch. In addition, *L. (M.) ophidea* possesses 9-15 accessory atrial sacs and its vitellaria has two groups of 5-10 follicles each. *Levinseniella (M.) deblocki*, can be distinguished from the present species by having more than 9 accessory atrial sacs and by lacking a female pouch. Considering the differences described earlier of *L. (L.) gavilanicola* sp. nov. from its closely related species and for being the only species reported from the hawk, *Buteogallus anthracinus*, as its natural host, and the crab *Uca rapax* as second natural intermediate host, it appears reasonable to consider the species of the present study as a new species of the genus *Levinseniella* Stiles and Hassall, 1901.

CONCLUSIONS

A new species, *Levinseniella (Levinseniella) gavilanicola* (Digenea: Microphallidae) and its life cycle is described for the first time in Venezuela from naturally infected crab hawk, *Buteogallus anthracinus* (type host). The new species differs from other species of this group mainly in the number of atrial accessory sac (9 with sclerotized structures) and its arrangement in the wall of the genital atrium. The adults worms were recovered experimentally from chicken, duck, rat and mice after infecting them with metacercariae from the crab *Uca rapax*.

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