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Wallinia chavarriae n. sp. (Trematoda: Macroderoididae) in *Astyanax aeneus* (Günther, 1860) and *Bryconamericus scleroparius* (Regan, 1908) (Osteichthyes: Characidae) from the Área de Conservación Guanacaste, Costa Rica

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WALLINIA CHAVARRIAE N. SP. (TREMATODA: MACRODEROIDIDAE) IN *ASTYANAX AENEUS* (GÜNTHER, 1860) AND *BRYCONAMERICUS SCLEROPARIUS* (REGAN, 1908) (OSTEICHTHYES: CHARACIDAE) FROM THE AREA DE CONSERVACIÓN GUANACASTE, COSTA RICA

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ABSTRACT: *Wallinia chavarriae* n. sp. is described from the small-bodied characids *Astyanax aeneus* and *Bryconamericus scleroparius* in the Area de Conservación Guanacaste, northwestern Costa Rica. The species differs from *W. valenciae* in possessing an acetabulum that is smaller than the oral sucker and vitelline follicles that are ovoid or rounded rather than elongate and tubular. Detailed comparison between these 2 species is handicapped by the less than satisfactory condition of the type and only museum specimen of *W. valenciae*. *Wallinia chavarriae* and *W. valenciae* belong to a subfamily of trematodes, Walliniinae, that arguably includes *Creptotrematina* spp., *Magnivitellum simplex*, and possibly *Margotrema*. The morphology of walliniines suggests that they are macroderoidids, but a clearer understanding of their classification could be gained from their larval morphology or from molecular systematic studies. The host associations of a monophyletic Walliniinae would indicate diversification within 2 groups of freshwater fishes: the neotropical characids for species of *Wallinia*, *Creptotrematina*, and *Magnivitellum* and the endemic central Mexican goodeids for those of *Margotrema*. The biogeography and host associations of these parasites provide a system for studies of potential host switching and vicariance, involving the middle-American and neotropical regions.

The parasites of freshwater fishes in Central America are poorly known. Previous studies are limited to surveys of selected fishes in Nicaragua (Watson, 1976; Aguirre-Macedo et al., 2001) and a few scattered reports of some other parasite species (Sogandares-Bernal, 1955; Caballero and Brenes, 1957; Brenes, 1961; Bravo-Hollis and Arroyo, 1962; Vidal-Martínez et al., 2001). Of these, the reports by Caballero and Brenes (1957) of the trematode *Acanthostomum minimum* Stunkard, 1937, and by Bravo-Hollis and Arroyo (1962), of 2 *Crassicutis* spp. appear to be the only records of freshwater fish parasites from Costa Rica. During June 1998, freshwater fishes from the Area de Conservación Guanacaste (ACG) in northwestern Costa Rica were examined as part of an ongoing biodiversity inventory of the eukaryotic parasites of vertebrates in this area (Brooks and Hoberg, 2000; Brooks et al., 2000). During that study, the 2 common species of characids encountered, *Astyanax aeneus* (Günther, 1860) and *Bryconamericus scleroparius* (Regan, 1908), were found to host a hitherto undescribed species of *Wallinia* Pearse, 1920, which is described here.

MATERIALS AND METHODS

The characids *Astyanax aeneus* (Günther, 1860) and *Bryconamericus scleroparius* (Regan, 1908) were caught in Rio Sapoa (34 *A. aeneus*, 18 *B. scleroparius*) and Quebrada Limonal (16 *B. scleroparius* only) between 6 June and 19 June, 1998; all sampling sites were within the ACG, Guanacaste Province, Costa Rica. Other species of fish (sample sizes in parentheses) examined from Rio Sapoa during the same period were: *Brycon guatemalensis* Regan, 1908 (2), *Astatheros alfari* (Meek, 1907) (6), *Parachromis dovii* (Günther, 1864) (21), *Archocentrus nigrofasciatus* (Günther, 1867) (17), *Gobiomorus dormitor* Lacepède, 1800 (1), *Hypsophrys nicaraguaensis* (Günther, 1864) (16); *Neetroplus nematopus* Günther, 1867 (26), *Poecilia gillii* (Kner, 1863) (13), *Rhamdia guatemalensis* (Günther, 1864) (2), *Rhamdia rogersi* (Günther, 1864) (20). In addition, 26 *A. alfari*, 3 *A. nigrofasciatus*, 1 *Phallichthys amates* (Miller, 1907) and 5 *R. rogersi* were also examined from Quebrada Limonal. Fish were caught with gillnets or by electroshocking, brought back to the laboratory at the Centro de Investigacion of the ACG's Santa Rosa field station, and examined within 1–4 hr of capture. The parasites were recovered live, washed briefly in 0.6% saline, killed by rapid immersion in steaming hot water, and transferred immediately to alcohol–formalin–acetic acid (AFA) or killed and fixed simultaneously in steaming hot 5% formalin, accompanied by brief, vigorous shaking (see also Platt, 2000a, 2000b). The digeneans were stained with acetocarmine, dehydrated, and mounted in balsam on slides or between 2 cover slips. The double cover slip preparations were mounted in aluminum Cobb slides. Specimens were examined using bright-field and differential interference contrast optics on a Nikon BX 50 microscope. Illustrations were made with a drawing tube attached to the microscope.

The following museum specimens were examined (accession numbers are preceded by the acronyms of the museum of their origin): *Wallinia valenciae* Pearse 1920, USNPC 7569 (holotype); *Creptotrematina aguirrepeqenoi*, ex *Astyanax fasciatus mexicanus*, San Marcos River, Texas (loan from Norman Dronen, Texas A&M University); *Creptotrema dispar* de Freitas, 1941 (= *Creptotrematina dispar*), CHIOC 15.357; *Creptotrema dissimilis* de Freitas, 1941 (= *Creptotrematina dissimilis*), CHIOC 10.720; *Margotrema bravoae* Lamothe-Argumedo, 1970, CNHE 869, 1594. USNPC is the U.S. National Parasite Collection, Beltsville, Maryland; HWML is the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska; CHIOC refers to Coleção Helmintológica, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; and CNHE is the Collecion Nacional de Helmintos, Universidad Nacional Autónoma de México, Mexico City, Mexico. In addition, 2 *Magnivitellum simplex*, ex *Astyanax fasciatus* from Nocchon-chunchey (Nicaragua) were examined (loan from Leopoldina Aguirre-Macedo, CINVESTAV, Mérida, México).

Measurements are given in micrometers (μm) and are expressed as the mean ± SD, followed by the range in parentheses. Measurements are from 11 specimens unless otherwise mentioned.

DESCRIPTION

Wallinia chavarriae n. sp.
(Figs. 1–4)

Description: Body 2,739 ± 404 (2,250–3,350) long, maximum width 588.2 ± 130.4 (400–800) at testicular level or at level of cecal ends; anterior end bluntly rounded; body widening gradually behind oral sucker region, narrowing behind testicular field, terminating in narrower bluntly rounded end. Forebody 642.7 ± 65.9 (550–740) long, forebody length: total body length ratio 0.59 ± 0.05 (0.51–0.68); forebody with conspicuous tegumental papillae on lateral sides. Oral sucker 325.5 ± 62.3 (240–400) long, 324.5 ± 56.8 (250–400) wide; oral opening anteriorly directed, outer edge with 2 lateral surface papillae; oral cavity

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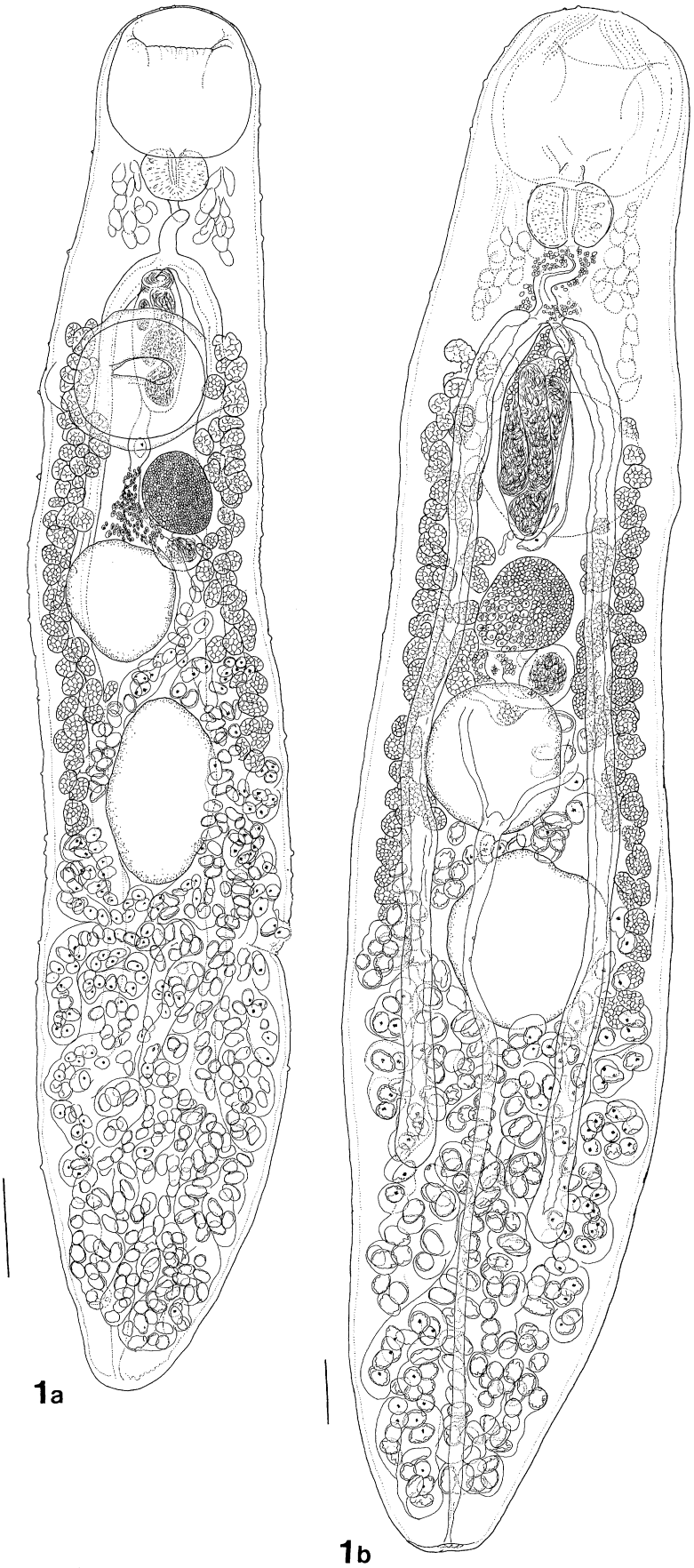
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with 2 conspicuous lateral papillae. Acetabulum 282.7 ± 52.9 (210–340) long, 299.1 ± 56.6 (210–370) wide; acetabular region protruding; opening ovoid or rounded, slightly anteriorly directed. Oral sucker length: acetabulum length 1.14 ± 0.06 (1.1–1.3), oral sucker width: acetabular width 1.08 ± 0.05 (1.03–1.19). Prepharynx absent. Pharynx muscular, 125.4 ± 27.7 (90–160) long, 142.3 ± 24.2 (110–180) wide. Esophagus short, narrow, occasionally winding or looped on itself, extending 99 ± 25.5 (60–130) posterior to pharynx before bifurcating to form ceca. Cecal bifurcation short distance anterior to acetabular margin; ceca situated dorsolaterally in body, terminating blindly posterior to testes, 521.8 ± 133 (360–750) from posterior end (distance measured from end of left cecum); left cecum occasionally longer. Cluster of gland cells on either side in pharyngeal, esophageal region, situated laterally, also deeper dorsally within parenchyma, occasionally few gland cells occurring laterally in acetabular region; narrow gland ducts leading to (opening along?) margin of oral opening, most concentrated dorsolaterally in region of oral sucker. Ovary 177 ± 30.6 (140–220) long, 145 ± 25.5 (110–180) wide (n = 10), generally rounded or pyriform; margin smooth, entire; immediately postacetabular; anterior margin at or overlapping posterior margin of acetabulum; ventrally situated; sinistral (5 specimens), dextral (5 specimens), or medial (1 specimen) in position. Mehlis' gland conspicuous, beside ovary and seminal receptacle, composed of numerous gland cells of various sizes. Seminal receptacle 110 ± 32.5 (70–195) long, 79.5 ± 20.9 (55–120) wide with thick muscular wall, immediately posterior to ovary, slightly overlapping it. Laurer's canal conspicuous; running laterally, slightly anterolaterally, or posterolaterally, opening dorsally over or outside cecal field at level of ovary, seminal receptacle, or anterior testis; opening dextrally when ovary and seminal receptacle sinistral, sinistrally when ovary and seminal receptacle dextral; canal occasionally containing sperm. Uterus tubular, passing posteriorly between intertesticular space or alongside testes; forming convoluted loops, filling posttesticular space; posterior loops in posttesticular region slightly wider than others; descending and ascending uterine loops in posttesticular region not in clearly separated fields; distal arm of uterus winding anteriorly, traversing pretesticular region medially, forming weakly differentiated terminal ventral metraterm leading to gonopore. Eggs numerous, more developed eggs tanned, thin-shelled, ovoid, operculate, 51.73 ± 4.4 (40–60) long, 28.65 ± 2.37 (25–35) wide (n = 26), containing embryos with eyespots. Vitellaria follicular, in 2 ventrolateral fields reaching anterior acetabular margin (n = 3) or extending anteriorly to between anterior margin of acetabulum and gonopore (n = 7); extending posteriorly to posterior margin of posterior testis; follicles overlapping cecal field ventrally, mainly extracecal dorsally; vitelline ducts, 1 from either vitelline field, meeting immediately posterior to Mehlis' gland, forming small vitelline reservoir occasionally containing >50 small vitellocytes (vitellocalcyl cells). Testes 2, tandem or very slightly oblique, ellipsoidal, margins smooth or slightly indented, anterior testis 264.5 ± 41.3 (220–330) long, 226.4 ± 32 (190–300) wide, 60.5 ± 55.3 (0–165) posterior to ovary, position submedial; posterior testis 335.5 ± 63 (260–430) long, 245.5 ± 40.6 (210–310) wide, 970.5 ± 293.4 (675–1650) from posterior end of body, medial in position; distance between testes 34 ± 8.1 (0–75) (n = 10, contiguous in 1 individual). Cirrus sac elongate, median, dorsal, maximum width 100.7 ± 23.7 (60–140), containing: muscular sinuous ejaculatory duct surrounded by scattered gland cells; indistinct vesicular pyriform pars prostatica; unipartite, folded, tubular seminal vesicle occupying most of cirrus sac; cirrus sac reaching posteriorly to within 5–185 of posterior acetabular margin or exceptionally to 5 beyond posterior margin of acetabulum (n = 1). Gonopore median at or immediately posterior to intestinal bifurcation. Excretory bladder narrow, tubular, dorsal, reaching level of anterior testes (anterior, posterior third, or posterior margin of anterior testis), giving off 2 narrow tubular collecting ducts, bladder narrowing posteriorly to slightly dorsoterminal excretory pore.

Taxonomic summary

Type host: Bryconamericus scleroparius (Regan, 1908).
Other host: Astyanax aeneus (Cuvier, 1817).
Site of infection: Anterior third of intestine.
Prevalence: In *B. scleroparius*: 11.1% (2 of 18 fish) in Rio Sapoa, 25% (4 of 16 fish) in Quebrada Limonal. In *A. aeneus*: 5.8% (2 of 34 fish) in Rio Sapoa.
Intensity: Range in *A. aeneus*: 1–2; in *B. scleroparius*: 1–3.
Type locality: Quebrada Limonal, Guanacaste Province, Costa Rica. 10°57′20.5″N, 85°32′43.9″W.
Other locality: Rio Sapoa, Guanacaste Province, Costa Rica. 11°2′42.9″N, 85°36′59.2″W.
Holotype: USNPC 91364.
Paratypes: USNPC 91635–91638; HWML 16431–16433.
Etymology: The species is named after Maria Marta Chavarría Díaz, for her kindness and untiring help during our work at the ACG and for her deep commitment to the ACG and to Costa Rica's biodiversity.

Remarks

Live *Wallinia chavarriae* are moderately active, translucent, dorsoventrally flattened worms with a slight orange tint (fixed specimens become somewhat cylindrical). They were found free or loosely attached to the mucosa of the anterior third of the small intestine of their host.

The type species of the genus, *Wallinia valenciae*, is represented only by the holotype (USNPC 7569), a specimen that unfortunately lies on its side. The original description (Pearse, 1920) appears to have been based on a single worm (see also Pearse, 1920, table VI.D). The worm illustrated by Pearse (1920) is a ventrally mounted specimen and it is possible that, over time, the same specimen has shifted on the slide. Yamaguti (1971) illustrated the holotype in its present condition (i.e., in dorsolateral view), and our observations add little to that illustration. Given the less-than-ideal condition of the holotype (the hindbody appears somewhat contracted), comparisons with *Wallinia chavarriae* are difficult. Nevertheless, *W. chavarriae* appears to differ distinctly from *W. valenciae* in possessing an oral sucker that is larger, rather than smaller, than the acetabulum and in having vitelline follicles that are rounded rather than tubular in shape. Pearse (1920) mentions the distinctly larger acetabulum in his description, and his illustration (Pearse, 1920, fig. 6) of the ventral view of *W. valenciae* confirms this. Both of these characteristics are also clearly visible in the holotype of *W. valenciae*, although it is possible that the tubular shape of the vitelline follicles is an artifact resulting from a specimen that appears to be compressed laterally. Pearse (1920) also stated that the anterior testis is median in position, whereas the posterior testis is displaced submedially, a condition confirmed by Pearse's illustration. This is in contrast to the condition in *W. chavarriae*, where the situation is reversed: the anterior testis is slightly submedial and the posterior testis is always median in position. Unfortunately, other details, such as those of the cirrus sac and of the female reproductive complex are either not visible or extremely difficult to discern with any degree of certainty in *W. valenciae*. Until fresh collections are made from the type host, *Gephyrocharax valenciae* from the type locality, Lake Valencia in Venezuela, little more can be said about *W. valenciae*. From the limited observations one can make from the holotype of *W. valenciae*, the 2 species appear similar in other respects, such as in the shape of the body,

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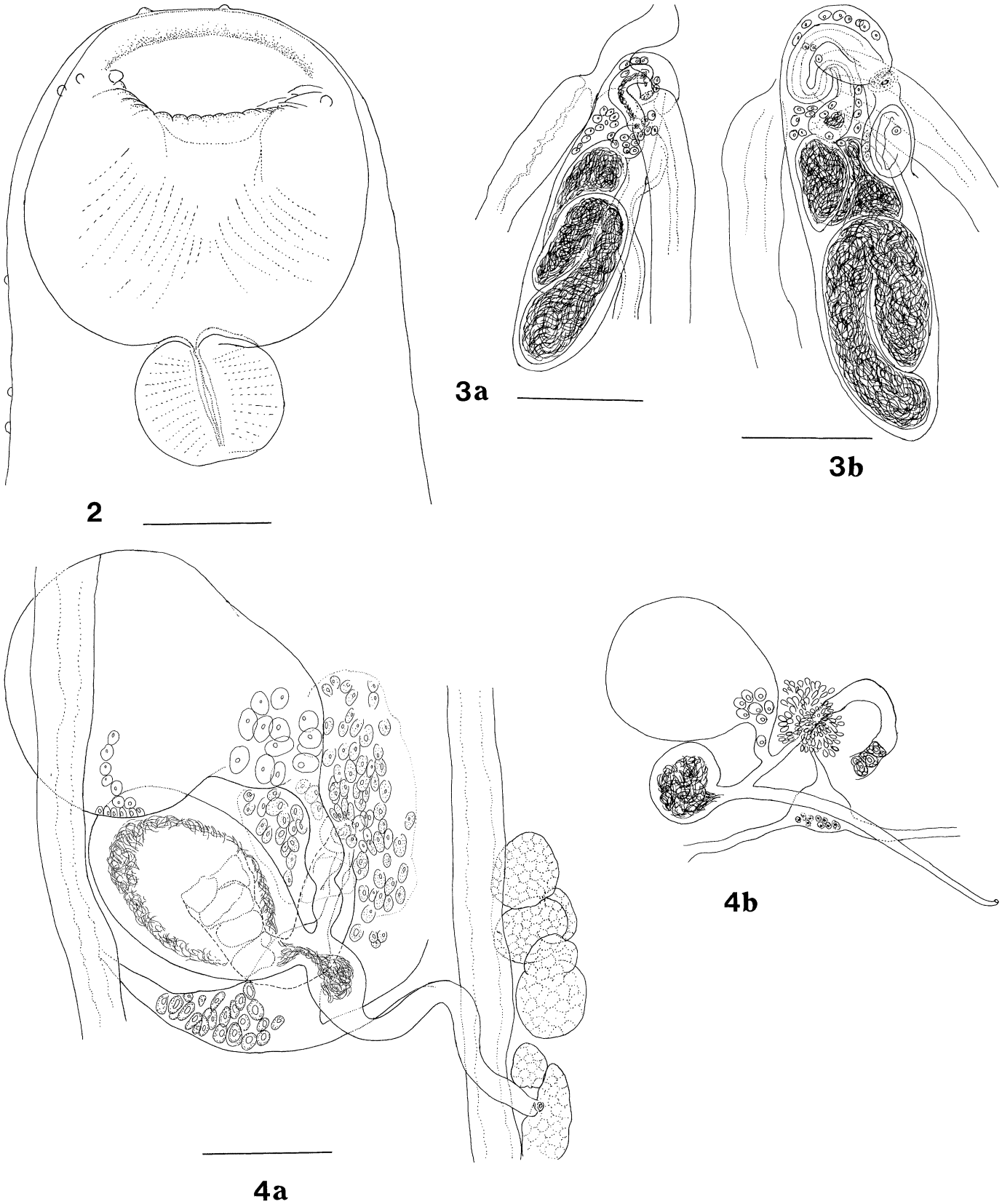
FIGURE 1. *Wallinia chavarriae*. **a.** Ventral view. Scale bar = 200 μm. **b.** Dorsal (slightly dorsolateral) view. Scale bar = 100 μm.



FIGURES 2–4. *Wallinia chavarriae*. **2.** Oral sucker showing paired lateral papillae. **3.** Terminal male reproductive complex. **a.** Dorsal view. **b.** Ventral view. **4.** Female reproductive complex. **a.** Dorsal view. **b.** Schematic diagram. Scale bar = 100 μm.

position of the acetabulum and gonads, extent of the uterus, and so on.

Wallinia chavarriae was not found in any other fish species sampled from Rio Sapoa or Quebrada Limonal. The parasite was not found in *Astyanax aeneus* sampled from 2 other streams, Rio Murcielago (n = 8) and Quebrada Aserradero (n = 9). From the available information, the trematode appears to be specific to the 2 morphologically similar small-bodied characids, *A. aeneus* and *B. scleroparius*.

DISCUSSION

Wallinia chavarriae and *W. valenciae* belong to a subfamily of trematodes, Walliniinae, with a rather controversial taxonomic history (Lamothe-Argumedo, 1970; Yamaguti 1971). An examination of specimens and a perusal of published descriptions of middle-American and neotropical freshwater fish trematodes indicate that, morphologically, the following species are very similar (i.e., *Wallinia* spp., *Magnivitellum simplex*, *Creptotrematina aguirrepeqenoi*, *C. dispar*, and *C. dissimilis*). These species are characterized by an elongate, unspined body; a well-developed hindbody; testes that are generally oblique (varying from almost tandem in *W. chavarriae* to almost symmetrical in *C. dissimilis* and *M. simplex*, although the degree occasionally varies within species); and closer to the ovary, a conspicuous cirrus sac containing a seminal vesicle, a pars prostatica, and an unspined ejaculatory duct, a median preacetabular gonopore, laterally restricted vitellaria, and a uterus that does not cover and obscure the testes but mainly occupies the posttesticular region of the body. Lamothe-Argumedo (1970), recognizing the similarities between his monotypic *Margotrema* Lamothe-Argumedo, 1970 (represented by *M. bravoae* Lamothe-Argumedo, 1970, from a central Mexican goodeid) and *Wallinia valenciae*, placed *Margotrema* in the Walliniinae (see Lamothe-Argumedo, 1970), although the uterus of *M. bravoae* is not as extensively developed in the posttesticular area as in the species mentioned above. Whether or not the aforementioned 7 species form a monophyletic group depends on whether one or more of the characteristics that are common to them are indeed synapomorphies, which requires a phylogenetic analysis. Until then, these species could be considered as comprising the subfamily Walliniinae. Yamaguti (1971) included the marine, monotypic *Walliniella* Yamaguti, 1971, in Macroderoididae McMullen, 1937, in the subfamily Walliniinae, but this is questionable. Among characters diagnosing Macroderoididae, Yamaguti (1971) listed the following: "body usually elongate," "testes tandem or diagonal, rarely symmetrical," and "parasitic as adult in freshwater fishes." In his diagnosis of the monotypic *Walliniella*, Yamaguti (1971) includes "plump body," "symmetrical testes near posterior extremity," and "intestinal parasites of marine fishes" (more accurately, the host of *Walliniella catevaria* (Looss, 1896) is the anadromous *Alosa fallax* (Lacepède, 1803)). Furthermore, it is clear from a perusal of Yamaguti's diagnoses of Macroderoididae and Walliniinae that he had to qualify many of the characters that would otherwise aptly describe other macroderoidids and walliniines to accommodate the very different morphology of *Walliniella*. These include the shape of the body, the form and distribution of the vitelline follicles, the position of the testes, and so on. In view of these considerations, it is unlikely that *Walliniella* belongs in Walliniinae, and we have excluded it from further consideration here.

Uncertainty and confusion have surrounded the classification of the walliniines ever since Yamaguti (1971) placed *C. dispar* and *C. dissimilis* in Lepocreadiidae (Odhner, 1905) and *Magnivitellum simplex* and *Wallinia valenciae* in Macroderoididae. In view of the close morphological similarity between these species, their placement in different families is difficult to justify, particularly the placing of the subfamily Megalogoniinae, to which *C. dispar* and *C. dissimilis* belong (Yamaguti, 1971), in Lepocreadiidae. Yamaguti's (1971) diagnosis of lepocreadiids characterizes them as "Digenea almost exclusively parasitic in digestive tract of marine teleosts" with the body being "usually spinose." Species of Megalogoniinae (*Creptotrematina* spp., *Megalogonia ictlauri* Surber, 1928, etc.) possess unspined bodies and are parasites of freshwater fishes. Furthermore, it is well established that *Megalogonia ictaluri* Surber, 1928 (= *Crepidostomum ictaluri*), the type species of the subfamily Megalogoniinae, is in fact an allocreadiid (Hopkins, 1934; Caira, 1989). Thatcher (1993) placed *Creptotrematina* spp. (as *Creptotrema* Travassos, Artigas and Pereira, 1928) and *Magnivitellum simplex* under Allocreadiidae (Looss, 1902) (*W. valenciae* was apparently missed, possibly because Yamaguti (1971) erroneously listed its host as marine). Lamothe-Argumedo (1970) considered Walliniinae to contain only *Wallinia*, *Walliniella*, and *Margotrema*, a subfamily of Macroderoididae. The morphological evidence leads to a consensus that the walliniines do not belong in Lepocreadiidae, but in a plagiorchiform group such as Allocreadiidae or Macroderoididae. The systematics of the plagiorchiforms are problematic (Brooks and McLennan, 1993) and Allocreadiidae is so broadly defined (Yamaguti, 1971; Gibson, 1996) that a large number of disparate genera and species has been placed in this family. As unspined plagiorchiforms with a well-developed cirrus sac and median gonopore and a tandem arrangement of gonads (ovary and testes) in the hindbody, Walliniinae (as proposed here) may place in Allocreadiidae. However, the oblique placement of the testes, often in the anterior portion (½–⅓) of the hindbody, the laterally restricted vitellaria and the manner in which the uterus passes between the testes on its way to an extensive occupation of the posttesticular area in *Wallinia* spp., *M. simplex*, *Creptotrematina* spp., and *M. bravoae* are all suggestive of macroderoidid affinities. Future studies on the larval stages and life cycle of walliniines or molecular data from the adult worms themselves may provide data useful for phylogenetic studies aimed at resolving the issue.

With the exception of *Margotrema bravoae* (a parasite of a goodeid in central Mexico), all other species under consideration are typically parasites of small-bodied characids. *Astyanax fasciatus* has been reported as a host of at least 3 of these species (*M. simplex*, *C. aguirrepeqenoi*, and *C. dissimilis*) (Kloss, 1966; Jiménez-Guzmán, 1973; Lunaschi, 1985) and *A. aeneus* as 1 of 2 hosts of *W. chavarriae* (this study). A second species of *Margotrema* has been discovered, also in a goodeid in central Mexico (Pérez-Ponce de León, 2001). Goodeids are endemic central Mexican cyprinodontiforms with their closest relatives hypothesized to be in the U.S. Southwest (Parenti, 1981). In contrast, characids are essentially neotropical, although *Astyanax fasciatus* ranges across the middle-American region through the Atlantic coastal drainages of Mexico to

southeastern Texas. Characids are absent from the Mesa Central (where goodeids and *Margotrema* are found) and from central, northern, and northwestern Pacific drainages of Mexico (Espinosa Pérez et al., 1993). If phylogenetic analyses provide support for the monophyly of Walliniinae, then the host associations and biogeography of this group of parasites provide interesting raw material for parascript studies (Brooks and McLennan, 1993) and particularly for an analysis of host switching and vicariance as mediators of diversification.

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