

LARVAL DIGENETIC TREMATODES IN TADPOLES OF SIX AMPHIBIAN SPECIES FROM NORTHEASTERN ARGENTINA

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ABSTRACT: This article presents a survey of metacercariae found in tadpoles of 6 amphibian species collected near the city of Corrientes, Corrientes Province, Argentina. Larval digenetic trematodes of the following species were found: (1) *Travtrema* aff. *stenocotyle* Cohn, 1902 (Plagiorchiidae) from *Physalaemus santafecinus*, *Physalaemus albonotatus*, *Odontophrynus americanus*, *Elachistocleis bicolor*, *Scinax nasicus*, and *Leptodactylus latinasus*; (2) *Styphlodora* sp. (Plagiorchiidae) from *O. americanus* and *E. bicolor*; (3) *Opisthogonimus* sp. (Opisthogonimidae) from *O. americanus* and *P. santafecinus*; (4) *Lophosicyadiplostomum* aff. *nephrocystis* (Lutz, 1928) (Diplostomidae) from *S. nasicus*; (5) *Bursotrema tetracotyloides* Szidat, 1960 (Diplostomidae) from *P. santafecinus* and *S. nasicus*; and (6) an unknown echinostomatid species from *O. americanus* and *S. nasicus*. Metacercariae of these species are reported for the first time in tadpoles of the 6 amphibian species examined. All species are described and illustrated, and their life cycles are briefly discussed. These larvae were found infecting different body parts of tadpoles, but no relationship was observed between the metacercariae and amphibian malformations.

From a parasitological perspective, the study of tadpoles is significant because these animals act as effective links between aquatic and terrestrial ecosystems, transmitting larvae of digenetic trematodes to terrestrial vertebrates (Combes et al., 2002). Tadpoles have also received attention from parasitologists in recent years because some metacercariae have been widely recognized as a probable cause of multiple limb deformities and high mortality in larval, juvenile, and adult amphibian populations under both natural and laboratory conditions (Sessions and Ruth, 1990; Johnson et al., 1999, 2002; Schotthoefler et al., 2003; Johnson et al., 2004). Furthermore, anthropogenic factors and environmental changes, such as cultural eutrophication, may facilitate high parasite intensity (Gilliland and Muzzall, 2002; Belden, 2006) and higher frequency of malformations (Johnson and Chase, 2004; Johnson and Lunde, 2005) in larval and metamorphic stages of amphibians from North America.

In Argentina, despite the existence of a highly diverse amphibian fauna (Frost, 2004), studies of larval digenetic trematodes from naturally and experimentally infected tadpoles are still in their early stages (Ostrowski de Núñez, 1979, 1981; Ostrowski de Núñez et al., 1990, Hamann and Kehr, 1998). Metacercariae infections have been analyzed in adult amphibians (Hamann and Kehr, 1998, 1999; Hamann, González, and Kehr, 2006; Hamann, Kehr, and González, 2006). These studies have reported that the probabilities of infection by larval digenetic trematodes are likely dictated by biotic factors, e.g., feeding strategy, body size, and behavior, as well as abiotic conditions, e.g., habitat variability. The present study assesses the occurrence of metacercariae of the families Diplostomidae Poirier, 1886, Plagiorchiidae (Luhe, 1901), Ophistogonimidae Freitas, 1956, and Echinostomatidae Poche, 1926 in naturally infected tadpoles from Corrientes City, Corrientes Province, Argentina. We also present new information on the morphology and morphometry of metacercariae.

MATERIALS AND METHODS

The tadpoles studied were collected between 3 May and 13 December 2004, from a temporary pond in northwestern Corrientes Province (27°27'S, 58°47'W). A total of 145 tadpoles of the following 6 species was examined: *Scinax nasicus* Cope, 1862 (n = 66; individual tadpoles at Gosner stages [Gosner, 1960]: 5 [30], 6 [31], 8 [32], 3 [33], 3 [34],

5 [35], 8 [36], 10 [37], 3 [38], 13 [41], 2 [42]; Hylidae), *Odontophrynus americanus* (Dumeril and Bibron, 1841) (n = 52; 3 [28], 3 [30], 3 [31], 3 [35], 9 [36], 3 [37], 11 [38], 7 [39], 1 [40], 9 [46]; Cycloramphidae), *Elachistocleis bicolor* (Valenciennes, 1838) (n = 8; 1 [30], 3 [31], 4 [42]; Microhylidae), *Leptodactylus latinasus* Jimenez de la Espada, 1875 (n = 7; 2 [32], 1 [36], 2 [38], 2 [42]; Leptodactylidae), *Physalaemus albonotatus* (Steindachner, 1864) (n = 10; 2 [28], 2 [34], 1 [36], 3 [37], 1 [39], 1 [45]), and *Physalaemus santafecinus* Barrios, 1965 (n = 2; 2 [46]; Leiuperidae). Tadpoles were captured with a 45-cm-diameter dip net and kept alive in the laboratory until dissection 2 days later. All tadpoles were killed with the use of a chloroform (CHCl₃) solution. Esophagus, stomach, gut, lungs, liver, kidneys, body cavity, musculature, integument, and brain were examined for parasites. Metacercariae were counted and isolated from host tissues. Metacercariae were removed from cysts with the use of preparation needles and studied either in vivo mounted in 0.6% saline solution, or killed in hot distilled water, fixed in 70% ethyl alcohol, stained with hydrochloric carmine, cleared in creosote, and mounted in Canada balsam. Measurements are given in micrometers (μm) unless otherwise indicated. Identification of metacercariae was carried out following Yamaguti (1971, 1975), Gibson et al. (2002), and Jones et al. (2005). Infection prevalence and intensity of metacercariae were calculated according to Bush et al. (1997). For examination by scanning electron microscopy (SEM), some specimens were dehydrated through an ethanol series, acetone, and ether. The specimens were gold-coated and examined with the use of a Jeol JSM-35CF SEM. Representative specimens were deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes, Argentina.

DESCRIPTIONS

Lophosicyadiplostomum aff. *nephrocystis* (Lutz, 1928)

(Figs. 1, 11–14)

Metacercaria (based on 10 specimens): Larvae unencysted; body distinctly bipartite, forebody oval, ventrally concave, 455–530 × 185–250, hindbody fusiform, 90–120 × 80–115, body covered with minute spines. Oral sucker elliptical, 71–87 × 60–74, with equatorial muscular ring surrounding it dorsally and laterally. Pharynx small, 23–28 × 18–22. Ventral sucker small, 44–51 × 44–55, in midregion of forebody; holdfast organ circular, 55–69 × 46–53, with central cavity of variable shape.

Taxonomic summary

Family: Diplostomidae Poirier, 1886.

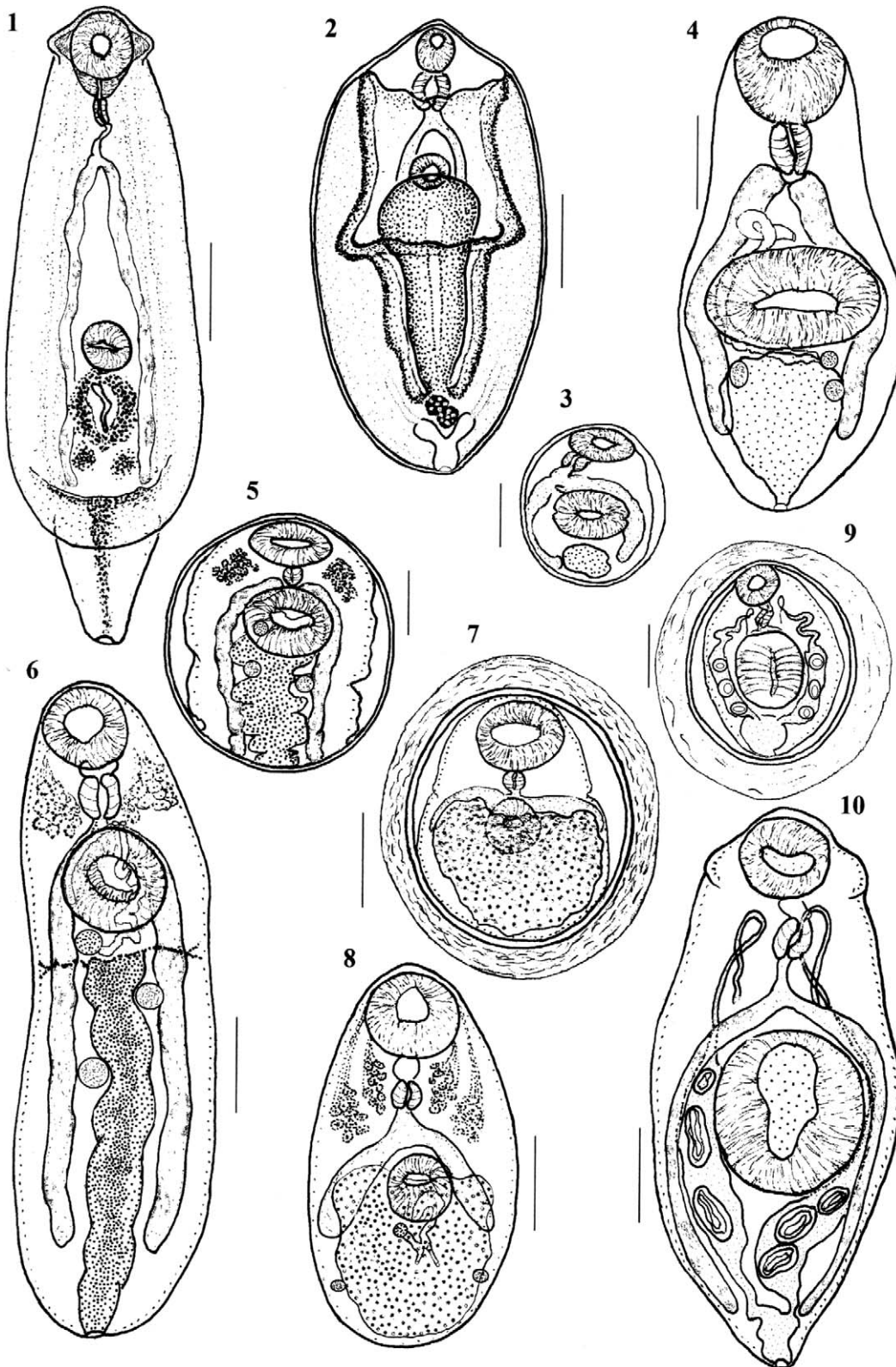
Host: *Scinax nasicus* (prevalence: 1.5%; maximum intensity: 28 larvae; sampling month: May).

Material studied: Thirteen larvae.

Site of infection: Kidneys (Table I).

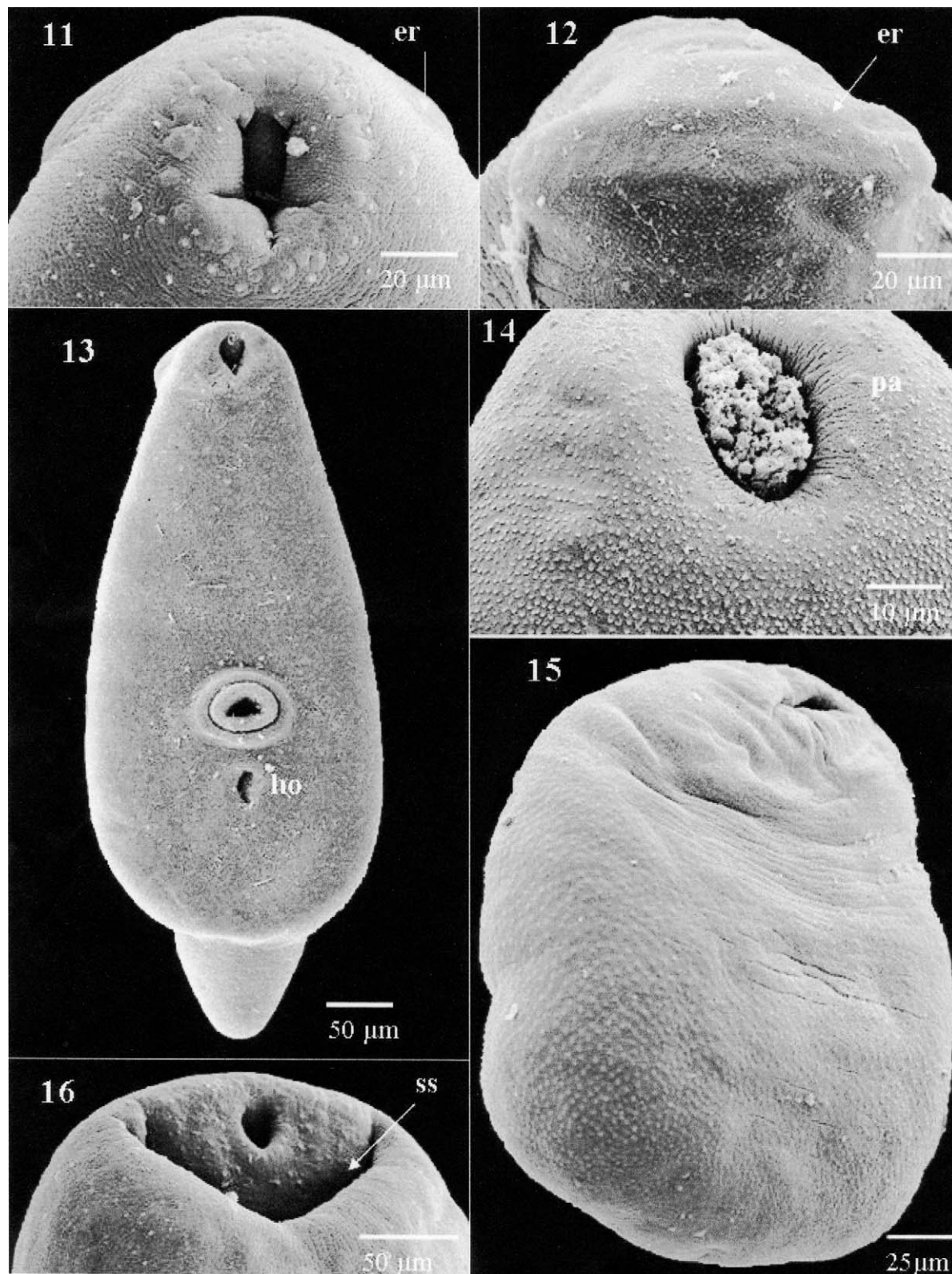
Voucher material: CECOAL 04050347.

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FIGURES 1–10. Metacercariae from *Scinax nasicus*. *Lophosicyadiplostomum* aff. *nephrocystis*. (1) Whole-mount, ventral view. *Bursotrema tetracotyloides*. (2) Whole-mount, ventral view. *Travtrema* aff. *stenocotyle*. (3) Larva encysted; (4) whole-mount, ventral view. *Styphlodora* sp. (5) Larva encysted; (6) whole-mount, ventral view. *Opisthogonimus* sp. (7) Larva encysted; (8) whole-mount, ventral view. Unknown echinostomatid species. (9) Larva encysted; (10) whole-mount, ventral view. Scale bars: 1, 2, 4, 6, 7, 8, 10 = 100 μ m; 3, 5, 9 = 50 μ m.

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FIGURES 11–16. Scanning electron micrographs. *Lophosicyadiplostomum* aff. *nephrocystis*. (11) Cephalic end, ventral view; (12) cephalic end, dorsal view; (13) whole-mount, ventrolateral view; (14) cephalic end, ventrolateral view (note tegumental spines). *Bursotrema tetracotyloides*. (15) Whole-mount, ventrolateral view (note tegumental spines); (16) cephalic end, ventrolateral view. Abbreviations: pa, papilla on oral sucker; ho, holdfast organ; er, equatorial muscular ring; ss, forebody sac-shaped.

TABLE I. Percentage of infection (%) and total number of metacercariae (No.) from different sites of infection in tadpoles (N = 145) from Corrientes, Argentina.

Metacercariae	Site of infections*													
	Bod		Mes		Pha		Mus		Liv		Teg		Kid	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
<i>Travtrema</i> aff. <i>stenocotyle</i>	51.0	74	1.4	16	10.3	67	25.5	99	2.8	4	2.1	4	0.7	7
<i>Bursotrema tetracotyloides</i>	—	—	—	—	—	—	—	—	—	—	—	—	1.4	15
<i>Styphlodora</i> sp.	—	—	—	—	—	—	—	—	4.8	15	—	—	1.4	3
<i>Opisthogonimus</i> sp.	2.1	7	—	—	4.8	10	2.1	6	1.4	2	2.1	6	—	—
Unknown echinostomatid species	—	—	—	—	17.9	79	—	—	—	—	—	—	—	—
<i>Lophosicyadiplostomum</i> aff. <i>nephrocystis</i>	—	—	—	—	—	—	—	—	—	—	—	—	0.7	28

* Site of infections: Body cavity (Bod), mesentery (Mes), pharyngeal zone (Pha), musculature (Mus), liver (Liv), tegument (Teg), and kidneys (Kid).

Remarks

The larvae studied are morphologically similar to the adult of *L. nephrocystis* (Lutz, 1928) Dubois, 1937, reported in the anterior intestine of passerine birds from Venezuela and Brazil (Dubois, 1970). Specific information on the life cycle of *L. nephrocystis* is unknown, but it has been reported that encysted forms occur in the kidneys of frog. Lutz (1928) briefly described this species from “gavilan bermejo” after experimental infection with cysts from the kidneys of *Rana palmipes* Sprix, 1824 from Venezuela. Hamann and Kehr (1998, 1999) found these metacercariae in the kidneys of *Hyla nana* Boulenger, 1889 and *Lysapsus limellus* Cope, 1862 from Corrientes, Argentina.

Bursotrema tetracotyloides Szidat, 1960 (Figs. 2, 15, 16)

Metacercaria (n = 10): Larvae unencysted; body widely oval, 290–440 × 130–240, forebody saccular with oblique aperture, constricted at level of the anterior portion of the holdfast organ; tegument spinous. Oral sucker subterminal, 39–51 × 30–39. Prepharynx absent, pharynx strongly muscular, 30–50 × 21–35; ventral sucker, 23–35 × 28–39. Holdfast organ elliptical, 97–207 × 46–106; genital primordia as 2 masses posterior to holdfast organ. Excretory bladder Y-shaped.

Taxonomic summary

Family: Diplostomidae Poirier, 1886.

Hosts: *Physalaemus santafecinus* (50%; 7; December) and *S. nasicus* (2%; 8; May).

Site of infection: Kidneys (Table I).

Material studied: Seven specimens from *P. santafecinus* and 8 from *S. nasicus*.

Voucher material: CECOAL 04050340.

Remarks

The larvae found in the kidneys of *P. santafecinus* and *S. nasicus* are morphologically identical to those of *B. tetracotyloides* reported by Szidat (1960) from the kidneys of *Leptodactylus ocellatus* (Linnaeus, 1758) from Buenos Aires, Argentina. Hamann, González, and Kehr (2006) and Hamann, Kehr, and González (2006) found these metacercariae in the kidneys of *Leptodactylus chaquensis* and *L. latinasus* from Corrientes, Argentina. Opossums, such as *Didelphis azarae* Temminck, 1825 as reported by Dubois (1976) and *Didelphis albiventris* Lund, 1840 (M. I. Hamann, pers. comm.), are the definitive hosts for this trematode.

Travtrema aff. *stenocotyle* (Cohn, 1902) (Figs. 3, 4)

Metacercaria (n = 10): Cyst almost spherical, thin-walled, 200–380 × 210–390. Excysted larva oval, 380–730 × 210–340. Oral sucker rounded, 69–160 × 85–160, ventral sucker very large, transversally elongated, 96–180 × 110–230. Short prepharynx present; pharynx 32–85 × 35–90. Esophagus very short; ceca terminating some distance short of posterior extremity. Testes symmetrical; cirrus pouch entirely

preacetabular; ovary submedian, posterior to acetabulum. Excretory bladder V-shaped.

Taxonomic summary

Family: Plagiorchiidae (Luhe, 1901).

Hosts: *Scinax nasicus* (65%; 9; May, June, and October), *E. bicolor* (75%; 15; May), *L. latinasus* (57%; 2; May and October), *O. americanus* (62%; 6; June, July, September, and December), *P. albonotatus* (51%; 4; May), and *P. santafecinus* (100%; 4; December).

Site of infection: Body cavity, mesenteries, muscle, pharyngeal zone, liver, and tegument (Table I).

Material studied: Ten specimens from *S. nasicus*, 1 specimen from *P. santafecinus*, 10 specimens from *O. americanus*, 3 specimens from *E. bicolor*, 4 specimens from *L. latinasus*, and 3 specimens from *P. albonotatus*.

Voucher material: CECOAL 04050314.

Remarks

Ostrowski de Núñez (1979) studied cercariae from the freshwater snail *Ampullaria canaliculata* Lamarck, 1801 and was able to produce experimentally metacercariae in *Hyla pulchella* Duméril et Bibron, 1841 from Buenos Aires, Argentina, which possibly corresponded to *T. stenocotyle*. The present material corresponds well to the description he provided. Hamann, González, and Kehr (2006) and Hamann, Kehr, and González (2006) found these larvae in *L. chaquensis* and *L. latinasus* from Corrientes, Argentina. Snakes such as *Philodryas* sp. and *Helicops* sp. act as definitive hosts (M. I. Hamann, pers. comm.).

Styphlodora sp. (most likely *Styphlodora condita* Farias, 1911) (Figs. 5, 6)

Metacercaria (n = 10): Cyst thin-walled, oval, 210–400 × 150–375. Excysted larvae elongated, 550–750 × 150–190, tegument spinous. Oral sucker rounded, 76–97 × 74–101; ventral sucker, 83–110 × 85–115. Pharynx, 39–41 × 39–44. Intestinal ceca long, almost reaching posterior extremity. Testes diagonal; ovary submedian, posterior to acetabulum; genital sac dorsal to acetabulum, with wide genital pore situated immediately anterior to margin of acetabulum. Excretory bladder I-shaped.

Taxonomic summary

Family: Plagiorchiidae (Luhe, 1901).

Hosts: *Elachistocleis bicolor* (13%; 1; May) and *O. americanus* (17%; 6; September).

Site of infection: Kidneys and liver (Table I).

Material studied: Fifteen from *O. americanus*.

Voucher material: CECOAL 04090613.

Remarks

The metacercariae are characterized by the presence of a tubular smooth excretory vesicle, and the ventral sucker in the anterior third of body. The larva studied is morphologically similar to the adult of *S.*

condita, reported from the excretory system (ureters) of snakes from Argentina (Caubisens Poumarau, 1968).

***Opisthogonimus* sp.**

(Figs. 7, 8)

Metacercaria ($n = 10$): Cyst almost spherical 250–370 × 220–350, with inner thin and outer thick walls. Excysted larva oval, 300–430 × 120–240, tegument spinous. Oral sucker rounded, 58–101 × 68–103, ventral sucker, small, 48–69 × 44–78. Short prepharynx present; pharynx 21–28 × 21–30. Esophagus very short; ceca ending some distance from equatorial zone of the body. Testes symmetrical, postacetabular; cirrus pouch large and curved, genital pore in testicular zone; ovary submedian, posterior to acetabulum. Excretory bladder V-shaped.

Taxonomic summary

Family: Opisthogonimidae Freitas, 1956.

Hosts: *Odontophrynus americanus* (29%; 2; June, July, September) and *P. santafecinus* (100%; 4; May).

Site of infection: Body cavity, muscle, pharyngeal zone, and tegument (Table I).

Material studied: Twenty specimens from *O. americanus* and 3 specimens from *P. santafecinus*.

Voucher material: CECOAL 04090620.

Remarks

The morphology of the metacercariae (comparatively large oral sucker, very elongated and curved cirrus pouch, genital pore in testicular zone, etc.) corresponds to the characteristics of *Opisthogonimus* Lühe, 1900 sp. Adult *Opisthogonimus* spp. specimens have been found in snakes from northeastern Argentina by Caubisens Poumarau (1968) and Lunaschi and Drago (2001).

Unknown echinostomatid species

(Figs. 9, 10)

Metacercaria ($n = 10$): Cyst almost spherical, small, covered by transparent thin membrane, outer cyst thick, and 110–200 × 100–190. Excysted larvae oval, 200–300 × 100–128; body covered with spines, without armed head collar. Oral sucker subterminal, 40–48 × 45–50; ventral sucker spherical, 58–90 × 52–80. Pharynx oval, 20–30 × 19–30. Main collecting ducts broadened, filled with 2–3 large refractile corpuscles, 19–35 × 10–25.

Taxonomic summary

Family: Echinostomatidae Poche, 1926.

Hosts: *Scinax nasicus* (3%; 2; June) and *O. americanus* (48%; 11; June, July, and September).

Site of infection: Pharyngeal region (Table I).

Material studied: Three specimens from *S. nasicus* and 35 from *O. americanus*.

Specimens deposited: CECOAL 04062519.

Remarks

Ostrowski de Núñez et al. (1990) described metacercariae of *Cercaria macrogranulosa* Ruiz, 1952 found in tadpoles of an unknown species in Corrientes, Argentina. Those larvae showed few refractile corpuscles within the excretory system. Ostrowski de Núñez (1981) also found other echinostomatid species with similar characteristics, although with eye-spot pigment located close to the pharynx, and cytogenous cells with bar-shaped contents, from *Bufo arenarum* Hensel, 1867, *H. pulchella*, and *Lysapsus mantidactylus* Gallardo, 1961 from Buenos Aires, Argentina. The metacercariae described here show characteristics similar to both echinostomatid species. Echinostomatid trematodes are known to parasitize a range of final hosts, including reptiles, birds, and mammals.

DISCUSSION

Field investigations in the western United States have suggested that infections by larvae of trematodes, specifically *Ri-*

beiroia sp., are linked to limb malformations in natural populations of amphibians (Johnson et al., 2004). In the present study, we report infection by larval digeneans in different body regions of tadpoles, but we did not observe any relationship between the presence of these metacercariae and amphibian limb abnormalities. This could be explained by the absence of *Ribeiroia* spp. in the amphibian populations studied here. Similarly, Gilliland and Muzzall (2002) did not find *Paralechriorchis syntomentera* Byrd et Denton, 1938 or *Ribeiroia* sp. in amphibians in southern Michigan, which may also explain the absence of deformed amphibians in the latter region.

Gastropods are the first intermediate host for all the larval digenetic trematode species found in tadpoles in the present study. Tadpoles act as second intermediate host, and become infected principally by direct penetration of cercariae. The most prevalent (>50%) were *T. aff. stenocotyle* and *Opisthogonimus* sp.; in both of these cases, the definitive hosts for these larval trematodes are snakes. Additionally, frogs are susceptible to infection with cercariae after metamorphosis, suggesting that both frog life stages, tadpole and adult, are suitable second intermediate hosts for these trematodes, as demonstrated by Hamann, González, and Kehr (2006) and Hamann, Kehr, and González (2006) in the helminth communities within the adult populations of 2 frog species (*L. chaquensis* and *L. latinasus*) in northwestern Corrientes province, Argentina.

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