ABSTRACT: *Halipegus eccentricus* is a common hemiurid trematode in the eustachian tubes of North America frogs. However, the life cycle of this species has never been completely elucidated. Studies on *H. eccentricus* suggest that it has a 3-host life cycle. Here, we show through fieldwork and host specificity experimental infections that the life cycle of *Halipegus eccentricus* utilizes 4 hosts. Metamorphosed anurans become infected with *H. eccentricus* by feeding on infected damsells; worms reside in the stomach of anurans, migrate to the eustachian tubes within 32–39 days post-exposure (DPE), and release eggs 50–60 DPE. Cystophorous cercariae develop in *Physa gyrina* snails within 32–35 DPE, infect ostracod (*Cypridopsis sp.*) second intermediate hosts, and develop to metacercariae. Fifteen- to 19-day-old metacercariae from ostracods are infective to both damselfly larvae and metamorphosed anurans. Field surveys of damselflies and tadpoles, along with laboratory exposure of damselfly larvae, metamorphosed anurans, and tadpoles with infected ostracods, indicated that only metamorphosed anurans and damselflies become infected with *H. eccentricus*, whereas field-collected tadpoles and laboratory-exposed tadpoles were never infected with *H. eccentricus*. Because little morphological change occurred in the metacercaria stage of *H. eccentricus* between the ostracod second intermediate host and damselfly host, and metamorphosed anurans became infected with *H. eccentricus* metacercariae recovered from both host groups, we suggest that odonates serve as paratenic hosts in this life cycle. Additionally, our field work and experimental infections provide data on the use of odonates as the route of infection by another North American *Halipegus* *sp.* that matures in the stomach of frogs. Our data indicate that when the life cycles are known, the use of odonates as the route of infection to anurans is common in the life cycles of *Halipegus* spp., and all species exhibit remarkable infection site fidelity in their amphibian hosts.

Species of *Halipegus* infect the intestine, stomach, esophagus, buccal cavity, or eustachian tubes of amphibians and can be pregenetic in dragonflies (Rankin, 1944; Macy et al., 1960; Nath and Pande, 1970; Yamaguti, 1971; Prudhoe and Bray, 1982; Moravec and Sey, 1989; Zelmer and Brooks, 2000). Most of these hemiurid trematodes have a typical trematode 3-host life cycle; however, some species add a fourth host in the life cycle (Zelmer and Esch, 1998a). Two types of life cycles have been reported for amphibian *Halipegus* spp. (Goater, Brown, et al., 1990). The life cycles of the European *Halipegus ovocaudatus* and the North American *Halipegus occidentalis* have been well studied and include frog definitive hosts, snail first intermediate hosts, microcrustacean (copepods and ostracods) second intermediate hosts, and odonate intermediate and/or paratenic hosts. Adult amphibians become infected with these species when they ingest infected dragonflies and/or damselflies (Krull, 1935; Macy et al., 1960; Kechemir, 1978; Goater, Brown, et al., 1990; Zelmer and Esch, 1998a). In contrast, other North American species of *Halipegus* have been shown to involve 3 hosts in the life cycle (snails, copepod microcrustaceans, and tadpole stages of amphibians). In the latter case, tadpoles become infected through the accidental ingestion of infected crustaceans and apparently the worms survive tadpole metamorphosis and migrate to the oral cavity of the metamorphosed frog to mature (Thomas, 1939; Rankin, 1944).

*Halipegus eccentricus* Thomas, 1939 is a common hemiurid trematode in the eustachian tubes of true frogs in North America. However, the life cycle of this species has never been completely elucidated (Thomas, 1939). Previous laboratory life cycle studies on *H. eccentricus* by Thomas (1939) suggest that it has a 3-host life cycle. Briefly, Thomas (1939) showed that cystophorous cercariae are shed by *P. gyrina*, *P. sayii crassa* (a synonym of *P. gyrina*, see Dillon and Wethington [2006]), and *Planorbella trivolvis* snail first intermediate hosts, and that cercariae are ingested by species of *Cyclops* and *Mesocyclops* copepod second intermediate hosts in which metacercariae develop. His laboratory studies indicated that cystophorous cercariae attracted the microcrustaceans by thrusting their delivery tube in and out, the cercaria body reached the intestine of the microcrustaceans as the crustaceans fed on cercariocysts, and metacercariae developed in the hemocoel of the copepods. Metacercariae in the microcrustacean host were then ingested by tadpoles via respiratory currents. Immature worms resided in the tadpoles’ stomach, and it was assumed that worms survived tadpole metamorphosis and migrated to the eustachian tubes of metamorphosed frogs; odonate larvae could not be infected (Thomas, 1939). However, more recent molecular and field data by Wetzel (1995), Wetzel and Esch (1996a, 1996b), and Bolek and Coggins (2001) suggest that *H. eccentricus* uses odonates as the route of infection to frogs, and tadpoles may not be involved in the transmission of *H. eccentricus*.

Until the present, few studies have attempted infecting odonates with the metacercariae of North American species of *Halipegus* and of those none was successful (Krull, 1935; Thomas, 1939; Rankin, 1944; Macy et al., 1960; Goater, Brown, et al., 1990). However, recent advances in our understanding of the infection mechanism and development of *Halipegus* sp. metacercariae in microcrustacean hosts by Zelmer and Esch (1998a, 1998b, 1998c) indicate that *Halipegus* sp. metacercariae need a considerable amount of development time in the microcrustacean host to be infective to the next host in the life cycle. The study by Zelmer and Esch (1998a) suggested that previous attempts at infecting odonates may have failed because there was not sufficient developmental time allowed within the microcrustacean host to produce a larval stage capable of infecting the next host in the life cycle (Thomas, 1939; Rankin, 1944; Goater, Brown, et al., 1990). Taken together, the conflicting field and laboratory studies on the transmission of *H. eccentricus* to anuran hosts and the recent advances in our knowledge of the development of *Halipegus* metacercariae in the microcrustacean second interme-
diate host suggest that the life cycles of some North American amphibian *Halipegus* spp. should be re-evaluated.

Here, we examined the population structure and route of infection of *H. eccentricus* and a *Halipegus* sp. from the stomach of bullfrogs in a variety of potential snails, damselfly, and larval and metamorphosed amphibian hosts from Nebraska in order to elucidate any differences between the life cycle strategies of these flukes and the original life cycle description of *H. eccentricus* by Thomas (1939). We then completed the life cycle of *H. eccentricus* in the laboratory and compared our results to other life cycle work on *Halipegus* spp. in North America and Europe (Krull, 1935; Rankin, 1944; MacCulloch et al., 1960; Kechemir, 1978; Goater et al., 1990; Zelmer and Esch, 1998a, 1998b). Our study provides laboratory and field data on congeneric flukes and their avenues for, and constraints on, transmission by odonate hosts to frog definitive hosts, data that will allow future testing of hypotheses with respect to the evolution of amphibian hemiurid fluke life cycles.

**MATERIALS AND METHODS**

### Amphibian field surveys

During March–September 2000–2009, 1,135 metamorphosed anurans of 8 species, 637 tadpoles of 7 species, and 50 larval barred tiger salamanders, *Ambystoma tigrinum mavortium*, were collected by hand, dip-net, or seineing from Elk Creek (40°53.145′N, 96°50.048′W) and Pawnee Lake (40°51.589′N, 96°53.468′W), located in Lancaster County, and Beckius Pond (41°12.523′N, −101°37.266′W), Breen’s Flyway (41°10.914′N, −101°21.635′W), Cedar Creek (41°11.194′N, −101°21.820′W), and Nevens Pond (41°12.426′N, 101°24.510′W) in Keith County, Nebraska, and examined for *Halipegus* spp. Not all amphibian species or life stages were collected consistently from each site or each year (Table I).

All frogs, toads, and salamander larvae were brought back to the laboratory and killed, the snout vent length (SVL) or SVL and total length (TL) were measured and examined for worms in the eustachian tubes, and/or the buccal cavity and digestive system within 72 hr of capture. All adult worms removed from amphibian hosts were relaxed in tap water and observed daily for shedding cystophorous cercariae. All tadpoles, and larval salamanders collected from 6 locations in Keith and Lancaster Counties, Nebraska, during May–September 2000–2009 and examined for *Halipegus* spp.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Metamorphosed (n)</th>
<th>Tadpoles/larva (n)</th>
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<td><em>Rana catesbeiana</em></td>
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<td></td>
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<td><em>S. bombifrons</em></td>
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<td><em>R. catesbeiana</em></td>
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**Snail and damselfly surveys at Nevens Pond**

During July–August 2006–2008, 200 *Gyraulus parvus* and 200 *P. gyrina* snails were collected from Nevens Pond by sampling aquatic vegetation with a dip-net. Individual snails were isolated in 1.5-ml well plates filled with aged tap water and observed daily for shedding cystophorous cercariae for a week. All snails were then measured, crushed, and examined for pre-patent infections. Cercariae were identified based on the cercariae descriptions of amphibian *Halipegus* spp. by Krull (1935), Thomas (1939), and Rankin (1944). Some intra-molluscan stages and cercariae were fixed in AFA and representative stages were stained and permanent slides were prepared.

During July–August 2007 and 2008, 19 larvae and 122 adult lyre-tipped spreading damsels, *Lestes unguiculatus*, 140 adult eastern fork tail damsels, *Ischnura verticalis*, and 55 teneral and adult familiar bluet damsels, *Enallagma civile*, were collected from Nevens Pond and examined for *Halipegus* sp. metacercariae. Larval damsels were collected with a dip-net from submerged vegetation and placed in a bucket of water with no apparent snails or microcrustaceans, whereas teneral and adult damsels were collected with a butterfly net along the edge of Nevens Pond, placed in 3.78-L plastic containers, stored on ice, and taken to the laboratory. All damsels were identified according to Westfall and May (2006) and May and Dunkle (2007). Each zygopteran larva, teneral, or adult was killed by removing the head. Each odonate larva was then placed in odonate saline or water, the last segment of the abdomen was cut off on the larvae, or the abdominal sterna were peeled back on teneral and adult damsels, and the entire gut was removed and gently teased apart with forceps, and examined for metacercariae. The rest of the body of individual damsels was then divided into 3 regions: the head, thorax including the legs, and the abdomen including the anal gills for the larvae; each body region was teased apart with forceps and examined for metacercariae. Measurements of metacercariae length and width and oral sucker and acetabulum length and width were obtained on relaxed live metacercariae using a calibrated ocular micrometer. A subset of metacercariae was fixed in AFA or 95% ethanol and representative worms were stained and permanent slides were prepared.

**Frog and toad experimental infections with *Halipegus* sp. metacercariae from damsels**

Three species of anuran were used for experimental infections with *Halipegus* sp. metacercariae recovered from naturally infected damsels from Nevens Pond. Six bullfrogs were reared in the laboratory from tadpoles collected at Cedar Creek, whereas 9 young of the year northern leopard frogs, *Rana pipiens*, and 21 young of the year Woodhouse’s toads,
Snails

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DPE and developing stages were recorded. At 28 DPE, all remaining Laboratory-reared leopard frog and a single laboratory-infected bullfrog were placed in 70-ml second intermediate hosts, we examined the expulsion mechanism of the and examined for developing stages of H. eccentricus to tadpoles and/or frogs (Bolek, 1998; Bolek and Janovy, 2007a; M. Bolek, pers. obs.). Colonies of ostracods (Cypriodopsis sp.) and harpacticoid copepods (Phyllognathopus sp.) were established in the laboratory in 3.78-L jars with aerated aged tap water, at 24 C and a 14L:10D period. Ostracod and copepod cultures were maintained on a diet of frozen lettuce and Tetra Min fish food before being exposed to cercariae. Additionally, 45 small (3–4 mm) isopods (Asellus sp.) and 45 small (3–4 mm) amphipods (H. azteca) were collected from the toe drains of Lake McConaughy, Keith County, Nebraska (41°33’11”N, –101°40’18.4”W). All isopods and amphipods were divided into 3 equal groups and assigned to time-0 controls, experimental infections, or time-T controls. All time-0 control isopods and amphipods were killed and examined for the presence of Halipegus spp. before the start of experimental infections. For infections, groups of 10 laboratory-reared ostracods and copepods were each placed with approximately 50 fresh (1–2 days after emergence from the snail) H. eccentricus cercariae recovered from laboratory-reared and -infected P. gyrina snails in individual 1.5-mL well plates with aged tap water, whereas individual isopods and amphipods were placed with approximately 50 fresh H. eccentricus cercariae. Individual well plates were then checked for cercariae and their status, discharged (expelled delivery tube and no cercaria body in the cercariocyst) or not discharged (delivery tube and cercarial body inside the cercariocyst), and the position of the emerged delivery tube (through the caudal appendage or opposite of the caudal appendage) 24 hr after placement with the crustaceans. All exposed ostracods, copepods, isopods, and amphipods were maintained in their 1.5-mL well plates at 24 C and a 14L:10D period and were examined for the presence of H. eccentricus metacercariae in the hemocel over a period of 2–8 DPE. Additionally, the remaining 35 copepods were examined for the presence of H. eccentricus metacercariae 9 DPE, whereas the remaining ostracods were used for frog experimental infections. All exposed ostracods and copepods were individually placed onto slides, and ostracods were gently crushed with a cover slip, and the number of metacercariae was recorded; metacercariae were directly observed in the body cavity of copepods under a cover slip. All exposed and time-T isopods and amphipods were first placed on a microscope slide with a drop of water, gently teased apart with fine forceps, covered with a cover slip, and examined on a compound microscope for the presence of H. eccentricus metacercariae.

Cercaria morphology and development in ostracods

Colonies of ostracods (Cypriodopsis sp.) were established in the laboratory and maintained, as previously described. For infections, groups of 10 laboratory-reared ostracods were placed with approximately 50 fresh (1–2 days after emergence from the snail) H. eccentricus cercariae recovered from laboratory-reared and -infected P. gyrina snails in individual 1.5-mL well plates with aged tap water and algae. Ostracods were allowed to feed on the algae and cercariae mixture for 24 hr before all groups were removed and maintained in 3.78-L jars with aerated aged tap water on a diet of frozen lettuce at 24 C and a 14L:10D period. A sample of 40 exposed ostracods was then removed and examined for developing H. eccentricus metacercariae at 2, 12, 14, 19, 20, and 25 DPE, whereas the rest of the exposed ostracods were used for damselfly and tadpole infections. Individual ostracods were examined for H. eccentricus metacercariae as previously described. Metacercariae removed from ostracods were observed for the presence or absence of everted bladder...
Damsel?y and tadpole infections with 

H. eccentricus metacerca?reae from ostracods

Thirty ultimate or penultimate larvae of I. verticalis dams?elfies were collected at Mil?ville Creek. Dams?elfies were divided into 3 equal groups and assigned to time-0 controls, experi?mental infections, or time-T controls; the insects were isolated in 5-ml well plates filled with aged tap water for 24 hr before exposure. All time-0 control larval dams?elfies were necropsied for the presence of Halipegus metacerca?rae before the start of exper?imental infections. For infections, 10–20 laboratory-reared and -infected ostra?cods with 19–25-day-old metacerca?rae were pipetted into each 5-ml well plate containing a dams?elfy larva. Larvae were individually observed ingest?ing all ostracods, using a stereo?scopic microscope. Additionally, because dams?elfy larvae were semi-transparent, we made obser?vations on the escape of H. eccentricus metacerca?rae from ingested ostracod host in the gut of dams?elfies. All experimental and time-T control dams?elfies were maintained in their 5-ml well plates at 24 C and a 14L:10D period, then killed and dissected for the presence of H. eccentricus. Measurements were made using a calibrated ocular micrometer on a subset of relaxed live worms removed from dams?elfies. Additionally, all ostracods removed from dams?elfy intestines were examined for viability and shell valve damage due to dams?elfy ingestion and digestion.

Frog and tadpole infections with H. eccentricus metacerca?rae from ostracods

For metamorphosed anuran exposures, 9 newly metamorphosed bullyfrogs, 9 newly metamorphosed northern lizard frogs, and 9 newly metamorphosed Woodhouse’s toads were collected from Beckius Pond, divided into equal groups, and assigned to time-0 controls, time-T controls, or experimental infections, or time-T controls. All time-0 control amphibia?ns were killed and examined for the presence of Halipegus spp. before the start of the experiment. The 5 experimental tadpoles were all placed with 500 laboratory-reared ostracods infected with 19–25 DPE metacerca?rae in aged tap water in a plastic shoe box (35 cm x 25 cm x 15 cm) and tadp?oles were allowed to ingest all ostracods; 5-time-T tadpoles were placed without reared ostracods in aged tap water in a plastic shoe box (35 cm x 25 cm x 15 cm). All tadpoles (experimental and time-T control) were maintained in their respective plastic shoe boxes at 24 C and 14L:10D before being examined for H. eccentricus metacerca?rae in the stomach, buccal cavity, and remaining organs at 2–7 DPE. Ostracods removed from tadpole intestines were examined for viability and shell valve damage due to tadpole ingestion and digestion.

RESULTS

Metamorphosed and tadpole amphibian field surveys

Of the 1,135 metamorphosed anurans examined from the 6 locations in Lancaster and Keith Counties, Nebraska, only metamorphosed bullyfrogs were infected with Halipegus sp. In Lancaster County, 10 of 111 bullyfrogs (9.0%; mean abundance [MA] = 0.21 ± 0.76; mean intensity [MI] = 2.4 ± 1.7; range = 1–4) collected from Pawnee Lake; and 1 of 3 bullyfrogs (33%; MA = 0.33 ± 0.58; I = 1) collected from Elk Creek were infected with H. eccentricus, whereas in Keith County, 1 of 8 bullyfrogs (12.5%; MA = 0.13 ± 0.35; I = 1) collected from Breen’s Flyway; and 5 of 61 bullyfrogs (8.2%; MA = 0.22 ± 0.70; MI = 0.89; I = 2) collected from Nevens Pond were infected with gravid H. eccentricus. Additionally, a single bullyfrog (1.6%; MA = 0.01 ± 0.12; I = 1) from Nevens Pond contained 1 non-gravid Halipegus sp. worm in the stomach. Gravid worms were located in the eustachian tubes of bullyfrogs consistent with the description of H. eccentricus. Measurements of 15 eggs from worms removed from the eustachian tubes of bullyfrogs were 52 x 21 (45–60 x 20–23) μm long by wide and contained an abopercular spine 62 (53–75) μm long (egg length: spine width ratio: 1:1.18–1:2.5) and were similar to the description of eggs from H. eccentricus (Thomas, 1939). Additionally, 1 of 61 (1.6%; MA = 0.01 ± 0.12; I = 1) bullyfrogs from Nevens Pond contained a single gravid Halipegus species in the upper stomach. Measurements of 15 eggs from this single worm recovered from the bullyfrogs’ upper stomach were 65 x 23 (60–69 x 20–29) μm long by wide and contained anabopercular spines that were 140 (112–175) μm long (1:1.86–2.54) and more closely approached the description of eggs from H. occidentalis, which resides under the tongue of frogs (Krull, 1935; Zelmer and Esch, 1998a).

Of the 637 tadpoles and 50 larval salamanders examined for Halipegus spp., none of the tadpoles was infected with any species of Halipegus; 2 of 50 barred tiger salamander larvae (4%; MA = 0.04 ± 0.19; I = 1) from Nevens Pond each contained 1 non-gravid Halipegus worm in the stomach. All bullyfrog tadpoles from Nevens Pond contained algae and detritus in their gut content, and occasionally (15/83) 2–3 semi-digested harpacticoid copepods

[The rest of the text is not transcribed as it is not relevant to the natural representation of the document.]
Snail and damselfly surveys from Nevens Pond

Of the 200 G. parvus and 200 P. gyrina snails collected from Nevens Pond, 9 of 200 P. gyrina (4.5%) shed cystophorous cercariae. All cystophorous cercariae contained 2 lateral streamers and were identified as the cercariae of H. eccentricus. No other patent or prepatent hemiurid infections were detected in any of the snails.

Of the 3 species of damselflies examined for Halipegus spp. metacercariae, 4 of 19 larval L. unguiculatus (21%; MA = 0.26 ± 0.56; MI = 1.25 ± 0.5; 1–2); 1 adult of 55 (53 adult and 2 teneral) E. civile (2%; MA = 0.02 ± 0.1; I = 1), and 23 of 140 adult J. verticalis (16.4%; MA = 0.5 ± 1.4; MI = 3.1 ± 2.1; 1–7) were infected with Halipegus spp., whereas 0 of 122 (0%; MA = 0) adult L. unguiculatus were infected with any Halipegus spp. All metacercariae were unencysted and located in the mesenteron of larval and damselfly adults (Fig. 1). Measurements of 5 Halipegus spp. metacercariae recovered from adult damselflies indicated that they were 502 (490–520) μm long by 270 (200–300) μm wide, and the oral sucker and acetabulum were 88 (80–90) μm and 174 (160–190) μm in diameter, respectively (oral sucker: acetabulum diameter ratio, 1:1.98–2.11).

Frog and toad experimental infections with Halipegus metacercariae from damselflies

A single toad examined 12 DPE contained a single immature Halipegus sp. located in the stomach. Of the remaining frogs and toads, all species became infected with Halipegus spp.; however, not all individual amphibians became infected (Table II). Halipegus eccentricus appeared in the eustachian tubes of frogs and toads between 32 and 39 DPE, and gravid worms from bullfrogs, leopard frogs, and toads were recovered 50–60 DPE (Fig. 1). Because the eustachian tube openings in Woodhouse’s bullfrogs, leopard frogs, and toads appeared normal. Gravid worms removed from the eustachian tubes of bullfrogs and the single infected leopard frog appeared normal. Gravid worms removed from the eustachian tubes of frogs and toads at 50, 60, and 61 DPE released eggs in water; eggs contained fully formed miracidiae (Fig. 1). Measurements of 15 eggs from worms removed from the eustachian tube of a laboratory-infected leopard frog 50 DPE were 55 ± 1.2 (53–58) μm wide, and the oral sucker and acetabulum were 88 ± 1.15 μm long and 174 ± 0.5 μm in diameter, respectively (oral sucker: acetabulum diameter ratio, 1:1.19–1.81).

Additionally, 2 of 6 toads exposed to Halipegus spp. metacercariae from naturally infected damselflies contained an unidentified species of Halipegus in the stomach when examined 51 and 73 DPE. Of these, a single worm contained 30 developing

![Figure 1](image.png)

**Figure 1.** Stages of Halipegus spp. in the eastern fork tail damselfly Ischnura verticalis and bullfrog, Rana catesbeiana. (A) Phase contrast photomicrograph of an unencysted metacercaria from the gut of a naturally infected I. verticalis. Note the large acetabulum compared to the oral sucker and lateral excretory ducts united anterior to the pharynx. Scale bar = 100 μm. (B) Two gravid Halipegus eccentricus (arrow) in the eustachian tube of an experimentally infected bullfrog 60 days post-exposure. Scale bar = 5 mm. (C) Phase contrast photomicrograph of an egg of H. eccentricus from worms removed from the eustachian tube of an experimentally infected bullfrog. Note the abopercular spine. Scale bar = 25 μm. (D) Same egg after applying pressure to the cover slip. Note the fully formed and unciliated miracidium. Scale bar = 25 μm. (E) Phase contrast photomicrograph of an egg of Halipegus sp. recovered from the stomach of a naturally infected bullfrog. Note the abopercular spine difference compared to (C). Scale bar = 30 μm.

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<tr>
<th>Amphibian species</th>
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<th>Mean intensity ± 1 SD</th>
<th>Mean abundance ± 1 SD</th>
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<td>H. eccentricus</td>
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<td>H. eccentricus</td>
<td>Eustachian tubes</td>
<td>33 (1/5)</td>
<td>2</td>
<td>0.66 ± 1.15 (0–2)</td>
</tr>
</tbody>
</table>
eggs in the uterus at 73 DPE with large spines morphologically similar to those on eggs recovered from the stomach of a naturally infected Nevens Pond bullfrog, but morphologically distinct from those on eggs recovered from worms infecting the eustachian tubes of naturally and experimentally infected frogs and toads (Fig. 1). None of the time-0 or time-T control bullfrogs, leopard frogs, or toads was infected with any *Halipegus* spp.

**Snail first intermediate host specificity study**

Our snail first intermediate host specificity study indicated that upon ingestion of *H. eccentricus* eggs by snails, most eggs hatched in both *P. trivolvis* and *P. gyrina* snails. Forty-seven *P. trivolvis* and 23 *P. gyrina* snails survived 19 DPE; however, only *P. gyrina* snails became infected with *H. eccentricus*, and all developing stages of *H. eccentricus* were located in the digestive gland of infected *P. gyrina* (15/23). None of the 47 surviving *P. trivolvis* (0/47) became infected.

**Cercaria development, morphology, and cercaria body expulsion via the delivery tube**

Of the 50 laboratory-reared *P. gyrina* snails exposed to eggs of *H. eccentricus* from laboratory-infected frogs, 40 snails survived 20 DPE, of which 35 of 40 (87.5%) were heavily infected (Fig. 2). Sporocysts with developing rediae were observed in some of the crushed snails at.
20 and 25 DPE located in the digestive gland, whereas rediae with developing cercariae were observed in the digestive gland of crushed snails at 20, 25, and 28 DPE (Fig. 2). Developing cercariae at 20–25 DPE contained the cercaria body and delivery tube outside of the cercariaoyct, and the delivery tube appeared annulated; the cercariaoyct enclosed the cercaria body and delivery tube 28 DPE. Snails began shedding cystophorous cercariae at 32–35 DPE; cercariae contained 2 lateral streamers and morphologically resembled the description of cercariae of H. eccentricus (Fig. 2).

Under slight cover slip pressure, shed cercariae released their cercaria bodies through the delivery tube. The delivery tube emerged first through the caudal appendage, or what Thomas (1939) referred to as the excretory appendage, following the cercaria body traveling through the delivery tube until its expulsion (Fig. 2). Occasionally, the delivery tube and cercaria body would emerge opposite of the caudal appendage; however, this occurred in only 3 of 100 trials. All cercaria bodies (n = 50) died and disintegrated within 5 min in aged tap water. Measurements of 15 live cercariae were as follows: cercariocyst (cyst or bulb) length and width without caudal appendage (trigger or excretory appendage) or the pyriform organ 76 (70–88) × 78 (70–88) μm; streamer length 382 (250–600) μm; caudal appendage length 41 (25–50) μm; delivery tube length after expulsion 374 (325–490) μm and never annulated; cercaria body length and width after expulsion 189 (150–275) μm; oral sucker length and width 28 (23–38) × 27.3 (20–38) μm; acetabulum length and width 26 (18–38) × 27 (18–43) μm; and pharynx length and width 14 (10–18) × 13 (10–18) μm.

**Crustacean second intermediate host specificity studies**

Twenty-four hours PE, discharged and undischarged cercariae were present in some well plates containing copepods (Phyllophagathous sp.) and some ostracods (Cypridopsis sp.); undis discharged cercariae were present in well plates containing isopods (Asellus sp.) and some copepods (Phyllophagathous sp.), but no cercariae were present in well plates containing amphipods (H. azteca) and some well plates containing ostracods (Cypridopsis sp.). These observations suggest that H. azteca and some Cypridopsis sp. ingested the entire cercariae. Of the 20 discharged cercariae observed in well plates containing copepods and ostracods, all had the delivery tube everted through the caudal appendage. Of the 4 species of crustaceans exposed, only copepods and ostracods became infected. Two of 15 (13.3%; MA = 0.13; I = 1) Phyllophagathous sp. became infected with unencysted H. eccentricus metacercariae in the hemocel, and 14 of 15 (93.3%; MA = 1.93; MI = 2.1; range 0–5) Cypridopsis sp. became infected with unencysted H. eccentricus metacercariae, whereas none of the experimental (0/15) and time-T control (0/15) Asellus sp. and H. azteca was infected with any Halipegus sp. metacercariae when examined 2–8 DPE. Examination of the 35 remaining Phyllophagathous sp. copepods 9 DPE revealed 1 individual infected with a single dead and degenerating H. eccentricus metacercaria.

**Metacercaria morphology and development in ostracods**

Twenty-two of 40 Cypridopsis sp. (55%; MA = 1.8 ± 2.2; MI = 3.2 ± 2.1; 1–8) exposed to cystophorous cercariae of H. eccentricus became infected; all unencysted cystophorous cercariae were located in the hemocel of the ostracod host. Metacercariae recovered from ostracods 2 DPE were 194 (180–200; n = 5) μm in length and did not possess any everted bladder villi. Unencysted metacercariae recovered from ostracods 12–14 DPE were 360 (200–500; n = 5) μm in length with everted bladder villi (Fig. 3), and all 2- to 14-day-old metacercariae (n = 37) died in aged tap water within 5 min of being removed from ostracods. Metacercariae removed from ostracods 19–25 DPE immediately pinched off their everted bladder villi (Fig. 3). Metacercariae recovered from ostracods 19 DPE were fusiform in body shape 481 (440–530; n = 7) μm in length, 228 (210–240) μm in width, contained lateral excretory ducts united anterior to the pharynx, and possessed a well-developed oral sucker and acetabulum 75 (60–80) μm and 118 (110–120) μm in diameter, respectively (1:1.5–1.8); all 19 day or older metacercariae (n = 15) were viable in aged tap water for over 1 hr when observations were stopped (see Fig. 3).

**Damselﬂy and tadpole infections**

Damselﬂy larvae offered laboratory-infected ostracods were immediately attracted to ostracod movement. In all cases, the labium of the larval dams elly was projected out to grasp the ostracod, which was then ingested. During ingestion, all damselﬂy larvae cracked the ostracod shell with the labium and maxilla, and H. eccentricus metacercariae were observed emerging out of the infected ostracod and into the foregut of 10 larval dams ellys. Five of 10 larval I. verticalis (50%; MA = 0.8 ± 0.9; MI = 1.6 ± 0.5; 1–2) examined 2 DPE were infected with metacercariae of H. eccentricus, all located in the mesenteron. All metacercariae were unencysted and were 460 (400–530; n = 5) μm in length, 206 (190–230) μm in width, contained lateral excretory ducts united anterior to the pharynx, and possessed a well-developed oral sucker and acetabulum 78 (70–80) μm and 119 (115–120) μm in diameter, respectively (1:1.5–1.64; see Fig. 3). All exposed damselﬂyls contained small pieces of ostracod shell exoskeleton in the gut and all ingested ostracods were dead. None of the time-0 or time-T larval I. verticalis was infected with any Halipegus spp.

All exposed bullfrog tadpoles were observed ingesting infected ostracods via respiratory currents. However, none of the 5 tadpoles became infected with H. eccentricus. Live ostracods were commonly defecated out by tadpoles, and dead ostracods removed from the digestive tract of tadpoles were intact and not cracked (Fig. 3). None of the time-0 or time-T bullfrog tadpoles was infected with any H. eccentricus.

**Frog and toad infections with H. eccentricus metacercariae from ostracods**

Of the single experimentally exposed bullfrog and northern leopard frog examined 1 DPE, only the bullfrog contained a single immature Halipegus in the stomach. In the remaining exposed frogs and toads, H. eccentricus appeared in the eustachian tubes at 32 DPE in 1 bullfrog and 2 Woodhouse’s toads, and at 36 DPE in the remaining bullfrog and Woodhouse’s toad. Overall prevalence, mean intensity ± 1 SD, and range for the 3 exposed bullfrogs and Woodhouse’s toads were 100%, 4.3 ± 4.9, 1–10, and 100%, 6 ± 5.3, 2–12, respectively, whereas none of the 3 northern leopard frogs became infected. All H. eccentricus worms recovered 32–55 DPE occurred in the eustachian tubes of infected anurans, except in a single heavily infected Woodhouse’s toad containing 12 worms. In the heavily infected toad, there was not enough room for all 12 worms to fit in the eustachian tubes;
however, all worms aggregated around the openings of the eustachian tubes. Worms recovered 48–55 DPE from the eustachian tubes of bullfrogs and toads contained 3 to hundreds of eggs in the uterus, and no additional worms were ever found in the stomach. Measurements of 15 eggs from worms removed from the eustachian tube of a laboratory-infected Woodhouse’s toad at 55 DPE were $56 \pm 20$ (53–63) $\mu$m long by wide and contained abopercular spines that were $61$ (48–73) $\mu$m long (1:0.9–1.16). None of the time-0 or time-T control frogs and toads was infected with any *Halipegus* spp.

**DISCUSSION**

The major contribution of our paper is the elucidation of the mechanism of how anurans become infected with *H. eccentricus* in nature by completing the life cycle in the laboratory and demonstrating that odonates serve as paratenic hosts in the transmission of *H. eccentricus*. Our field work indicated that adult bullfrogs and salamander larvae were the only amphibians infected with *Halipegus* spp., whereas tadpoles and other metamorphosed anuran species were never infected with *Halipegus* spp. Our field data from Nevens Pond and experimental infections also suggest that bullfrogs become infected with *Halipegus* spp. by feeding on damselfly paratenic hosts and probably other odonates infected with *Halipegus* metacercariae. Clearly, other studies have documented a wide range of odonate species infected with *Halipegus* spp. metacercariae (Krull, 1935; Grieve, 1937; Macy et al., 1960; Goater, Brown, et al., 1990; Wetzel and Esch, 1996a). Although ostracods infected with *H. eccentricus* metacercariae also were infective to metamorphosed anurans in the laboratory, it is unlikely that metamorphosed anurans commonly ingest ostracods in high enough numbers in

![Figure 3. *Halipegus eccentricus* metacercaria development in experimentally infected ostracod and damselfly hosts; and ostracods from the gut of a bullfrog tadpole.](image-url)

(A) Cracked *Cypridopsis* sp. 2 days post-exposure to *H. eccentricus* cercariae. Note the 3 metacercariae without everted bladder villi. Scale bar = 200 $\mu$m. (B) Twelve-day-old metacercaria removed from the body cavity of *Cypridopsis* sp. Note the pinched-off everted bladder villi and developed lateral excretory ducts united anterior to the pharynx. Scale bar = 50 $\mu$m. (C) Nineteen-day-old metacercaria removed from the body cavity of *Cypridopsis* sp. Note oral sucker acetabulum ratio and lateral excretory ducts united anterior to the pharynx. Scale bar = 75 $\mu$m. (D) Phase contrast photomicrograph of a 19-day-old metacercaria removed from the body cavity of *Cypridopsis* sp. Note oral sucker acetabulum ratio and lateral excretory ducts united anterior to the pharynx. Scale bar = 75 $\mu$m. (E) Dead *Cypridopsis* sp. from the intestine of a bullfrog tadpole. Note that the ostracods shells are not cracked and ostracods are not digested. Scale bar = 1 mm. (F) Phase contrast photomicrograph of a *H. eccentricus* metacercaria removed from the gut of an experimentally infected larval *Ischnura verticalis* 2 days post-exposure. Note the similarity to (D). Scale bar = 75 $\mu$m.
larvae were the only amphibian species infected with H. eccentricus infection in Nevens Pond bullfrogs. This suggests that damselflies are the primary source of H. eccentricus, with worms becoming gravid and producing eggs in all 3 anuran species. These observations are similar to those of Brooks (1976), who also reported H. eccentricus in 0 of 82 (0%) Woodhouse’s toads, 2 of 152 (1.3%) northern leopard frogs, and 14 of 133 bullfrogs (10.5%) from Nebraska, indicating that northern leopard frogs can become infected with H. eccentricus in nature, although less commonly than bullfrogs. Recent ecological and experimental life cycle studies from our laboratory on other trematodes (frog bladder flukes and lung flukes) that use odonates as a route of infection to anurans suggest that in nature, bufonids and leopard frogs feed less commonly on odonates than bullfrogs and other members of the Aquarena Dubois 1992 clade (also known as the R. catesbeiana group) as defined by Hillis and Wilcox (2005). In fact, most reports of gravid H. eccentricus in North American anurans are from R. catesbeiana, Rana clamitans, and Rana septentrionalis, all members of the Aquarena clade and Halipegus spp. are rarely, if ever, reported from other true frogs, toads, and salamanders (see Thomas, 1939; Macy et al., 1960; Brooks, 1976; Muzzall, 1991a, 1991b; Russell and Wallace, 1992; Wetzel and Esch, 1996b; McAlpine and Burt, 1998; Bolek and Coggins, 2001, 2003; Bolek and Janovy, 2007a, 2007b; Bolek et al., 2009; Schlothofer et al., 2009).

When the results of our study are compared to the original life cycle studies of Thomas (1939) and Rankin (1944), questions arise as to the maturity and source of the cercariae of H. eccentricus and H. amherstensis (in part, a junior synonym of H. eccentricus; see McAlpine and Burt [1998] and Zelmer and Esch [1999]) used in the descriptions and experimental infections by those investigators. Both Thomas (1939) and Rankin (1944) based their cercaria descriptions of H. eccentricus on laboratory-reared and -infected P. gyrina snails and cercariae recovered in part (Thomas, 1939), or only, from laboratory-infected and crushed snails (Rankin, 1944), whereas their copepod infections originated from cercariae shed from experimentally and naturally infected snails. In his experimental infections of copepod second intermediate hosts with H. eccentricus, Thomas (1939) observed that the cercaria and delivery tube were always ejected opposite of the caudal appendage, the cercaria never traveled through the delivery tube, the delivery tube had a jointed or telescopic appearance, and the cercariae occasionally thrust the delivery tube in and out of the cercariocyst, apparently attracting copepods. Rankin (1944) observed that the jointed-appearing delivery tube of H. amherstensis was always out of the developed cercariocyst and the cercaria body emerged in and out of the cercariocyst, attracting copepods to feed on it. Both authors’ observations are in contrast to our study, in which we observed the cercaria body and delivery tube packaged inside of mature cercariocysts and, during mechanical- or microcrustacean-induced discharge, the cercaria body always emerged through the delivery tube, as has been reported for H. occidualls by Krull (1935) and Zelmer and Esch (1998a, 1998b).

Although we cannot comment on Thomas’s (1939) and Rankin’s (1944) observations on the ability of cystophorous cercariae to attract copepods by movements of the delivery tubes or cercarial bodies because we did not observe these behaviors, we did observe jointed- or telescopic-appearing delivery tubes outside of the cercarial cysts in immature cercariae that exhibited slow movements. This observation suggests that Rankin’s (1944) cercaria description and some of Rankin’s (1944) experimental infections were based in part on immature cercariae (see Fig. 4). Finally, we were able to replicate Thomas’s (1939) observation on the ejection of the delivery tube and cercaria body opposite of the caudal appendage; however, this only occurred occasionally when cercariocysts were discharged under cover slip pressure. Previous studies on the ejection of the delivery tube and cercaria body of H. occidualls by applying cover slip pressure has been shown to produce abnormal ejections by Zelmer and Esch (1998c) and we never observed this phenomenon when cercariocysts of H. eccentricus were discharged by ostracods or harpacticoid copepods.

More intriguing are the discrepancies in our results and the results of Thomas (1939) and Rankin (1944) in the ability or inability of H. eccentricus-infected copepods or ostracods to infect tadpoles and odonates. In our experimental exposures of tadpoles, metamorphosed anurans, and damselfly larvae with infected ostracods, we observed that ostracods commonly passed through the stomach (manicotti glandular) and remaining
digestive tract of tadpoles without being killed and/or digested, whereas ostracods ingested by damselfly larvae were always killed and the shell was cracked open during feeding, releasing *H. eccentricus* metacercariae from the ostracod hemocoel into the gut of the odonate host (see Fig. 4). We did not observe the mechanism of how metamorphosed anurans released *H. eccentricus* metacercariae from the hemocoel of ostracod hosts. However, studies show that metamorphosed anurans are arthropod predator specialists and, during feeding, frogs will crack open the exoskeleton of their arthropod prey with their teeth and then digest the fragmented arthropod exoskeleton with their muscular stomach (Duellman and Trueb, 1994). Comparative studies on the digestive capabilities of the stomach of herbivorous tadpoles compared to their carnivorous metamorphosed anuran counterparts also indicate that food passes quickly through the digestive system of tadpoles and hydrochloric acid does not concentrate in the stomach of anuran larvae as it does in metamorphosed anurans, suggesting that metamorphosed anurans can digest ostracods more easily than tadpoles can (see Viertel and Richter, 1999).

Even though we have no reason to doubt Thomas’s (1939) and Rankin’s (1944) experimental infections of tadpoles with *H. eccentricus*, field observations on North American tadpoles from our observations and those of Wetzel and Esch (1996a, 1996b) and studies on the European *H. ovocaudatus* by Kechemir (1978) indicate that anuran larvae are never infected with *Halipegus* spp. in nature. Our observations on the gut content of field-collected bullfrog tadpoles from Nevens Pond may be important in that we only observed digested harpacticoid copepods and/or live ostracods in the digestive tracts of tadpoles. Moreover, laboratory experiments could not support infections of *H. eccentricus* or digestion by tadpoles, whereas Thomas (1939) and Rankin (1944) used cyclopoid copepods in their experimental infections of tadpoles. One explanation for this discrepancy may be that tadpoles do not commonly ingest cyclopoid copepods in nature. Studies on the ecology of ostracods, harpacticoid copepods, and cyclopoid copepods indicate that cyclopoid copepods are found in the open water column, whereas ostracods and harpacticoid copepods are common components of the benthic and epibenthic community and overlap in their habitat with tadpoles (McDermid and Altig, 1999; Smith, 2001; Thorp and Covich, 2001).

Finally, as previously pointed out by McAlpine and Burt (1998), our discovery of a gravid *Halipegus* sp. in the upper stomach of a bullfrog from Nevens Pond and our experimental infections of Woodhouse’s toads with worms that remained in the stomach of toads 51–73 DPE suggest that there may be a third species of *Halipegus* infecting anurans and caudatans in North America. Previous studies by Macy et al. (1960), Goater, Mulvey, et al. (1990), Wetzel and Esch (1996a, 1996b), and Zelmer and Esch (1998a) have considered gravid species of *Halipegus* recovered from the upper stomach and esophagus of frogs and salamanders from the western part of the United States as conspecific with *H. occidualis*, which resides under the tongue of frogs in Canada and the United States. The reason for these discrepancies can be traced back to Stafford’s (1905) unclear description of the location of *H. occidualis* in the frog host (under the tongue, trachea, or eustachian tubes), the morphologically similar eggs and cercariae of the tongue and stomach form of *H. occidualis* reported by Krull (1935) and Macy et al. (1960), and the inability to differentiate most *Halipegus* spp. based on adult morphology (see McAlpine and Burt 1998; Zelmer and Esch, 1999). However, recent genetic studies by Goater, Mulvey, et al. (1990) on *H. occidualis* and *H. eccentricus* and the description of the Central American *Halipegus eschi* by Zelmer and Brooks (2000) have demonstrated that *Halipegus* spp. show remarkable site fidelity under the tongue, eustachian tubes, or esophagus of their amphibian host.

More importantly, life cycle studies by 3 independent investigators (Krull, 1935; Goater, 1989; Zelmer and Esch, 1998a) on the form of *H. occidualis* that resides under the tongue of frogs from the eastern United States indicate that when frogs are infected with *H. occidualis* metacercariae from field-collected odonates or laboratory-infected ostracods, worms always appeared under the tongue of frogs within 21–22 DPE and become gravid under the tongue within 42–56 DPE. Additional genetic and/or field studies by Goater, Mulvey, et al. (1990), Wetzel and Esch (1996b), Bolek and Coggins (2001), and the present study on the migration of *H. eccentricus* to the eustachian tubes of 3 amphibian species indicate that *H. eccentricus* always appears in the eustachian tubes of anurans within 32–39 DPE and gravid worms always reside in the eustachian tubes of their hosts.

In contrast, field and life cycle studies by Macy et al. (1960) on the upper stomach/esophagus form of *H. occidualis* and our *Halipegus* sp. from the upper stomach of anurans and salamanders indicate that when amphibians are infected with *Halipegus* metacercariae from laboratory-infected ostracods or field-collected odonates, these worms never migrate under the tongue or the eustachian tubes of anurans, and worms begin producing eggs in the stomach 71 DPE, suggesting that this is a distinct species. However, because Goater, Mulvey, et al. (1990), Wetzel and Esch (1996b), and Zelmer and Esch (1999) only sampled live green frogs during their studies, the presence of worms in the upper stomach/esophagus would have gone unnoticed. Studies by Wetzel and Esch (1996b) on the population biology of *H. occidualis* over time in the same green frog individuals indicate that *H. occidualis* infrapopulations can build over time, and then suddenly drop to zero. Wetzel and Esch (1996b) hypothesized that the large number of worms stimulated a strong inflammatory response under the tongue of green frogs and this caused infrapopulations of *H. occidualis* to be sloughed off and it may be that these worms survive in the stomach of anurans. These data suggest an alternative hypothesis indicating that *H. occidualis* can reside under the tongue and/or the upper stomach/esophagus of anurans. To further confound matters, concurrent infections of gravid worms identified as *Halipegus* sp. from the upper stomach/esophagus, under the tongue, and in the eustachian tubes of anurans have been reported from other locations in North America (Bouchard, 1951; Muzzall, 1991b; S. D. Snyder, pers. comm.). Clearly, in order to resolve issues in the taxonomy and systematics of North American *Halipegus* spp. residing in the upper stomach/esophagus or under the tongue of North American anurans, additional life cycle, morphological, and molecular data will be necessary.

Although few complete life cycles are known for amphibian *Halipegus* spp., our data indicate that when known, the use of odonates as the route of infection to anurans is common in *Halipegus* spp. These observations suggest a long relationship among *Halipegus* spp. and their odonate paratenic hosts, as is suggested by the ability of the Indian *Halipegus mehhransis* to develop progenetically in its odonate host (Krull, 1935; Nath and...
Table III. The distribution, anuran site fidelity, and odonate host relationship of amphibian Halipegus spp.*

<table>
<thead>
<tr>
<th>Species of Halipegus</th>
<th>Distribution</th>
<th>Anuran site fidelity</th>
<th>Odonate host relationship</th>
<th>References</th>
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<tr>
<td>H. africanae</td>
<td>Africa</td>
<td>?</td>
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<td>Dolfus, 1950</td>
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<td>Stomach</td>
<td>NA</td>
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<td>Paratenic</td>
<td>Thomas, 1939; This study</td>
</tr>
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<td>Espaghus</td>
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<td>Zelmer and Brooks, 2000</td>
</tr>
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<td>Under tongue</td>
<td>NA</td>
<td>Paraense, 1992</td>
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<td>NA</td>
<td>Capron et al., 1961</td>
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<td>Asia</td>
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<td>NA</td>
<td>Yamaguti, 1936</td>
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<td>India</td>
<td>Under tongue</td>
<td>NA</td>
<td>Grebnitzky, 1872</td>
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<td>India</td>
<td>Under tongue</td>
<td>NA</td>
<td>Klein, 1905</td>
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<td>H. mehronsis</td>
<td>Asia</td>
<td>Stomach</td>
<td>Progenetic</td>
<td>Srivastava 1933; Nath and Pande, 1970</td>
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<td>Under tongue</td>
<td>Paratenic</td>
<td>Krull, 1935; Goater, 1989; Zelmer and Esch, 1998a</td>
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<td>Europe</td>
<td>Under tongue</td>
<td>Intermediate</td>
<td>Kechemir, 1978</td>
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<td>Maeder, 1969</td>
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<tr>
<td>H. zweifelii</td>
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<td>Moravec and Sey, 1989</td>
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<td>Halipegus sp.</td>
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<td>Upper stomach/epaghus</td>
<td>Paratenic</td>
<td>Macy et al., 1960; This study</td>
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</table>

* ?, not given; NA, complete or entire life cycle not known.

Pande, 1970; Goater, 1989). Importantly, although most Halipegus spp. are morphologically indistinct, data suggest that they exhibit incredible site fidelity in their anuran host (Table III). Currently, reports of Halipegus spp. exist from Europe, Asia, Papua New Guinea, the Americas, the Middle East, Africa, and Madagascar; once a phylogenetic analysis of the genus is available, it will be interesting to see whether site fidelity in the amphibian host of congeners from different continents will be phylogenetically conserved.

ACKNOWLEDGMENTS

M.G.B. thanks Melissa Bolek for help in collecting snails, tadpoles, and frogs; Randy Peterson, Bill Breen, and the Sillien family for access to field sites; and Cedar Point Biological Station for providing facilities. Additionally, we thank the Transactions of the American Microscopical Society for permission to reproduce figures by Rankin, and 2 anonymous reviewers and the editor for improving the manuscript. Partial support for this project was made possible from National Institutes of Health grant number 1 P20 RR16469 from the INBRE Program of the National Center for Research Resources, and the Center for Great Plains Studies graduate student grant-in-aid, University of Nebraska–Lincoln; Initiative for Ecology and Evolutionary Analysis, University of Nebraska–Lincoln; and The School of Biological Sciences, University of Nebraska–Lincoln to M.B.

LITERATURE CITED


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The Journal of Parasitology  para-96-04-03.3d  26/5/10  01:19:09  11  Cust # GE-2365

The Journal of Parasitology para-96-04-03.3d


MORAVEC, F., AND O. S EY. 1989. Some amphibian trematodes from


K RUll, H. W. 1935. Studies on the life history of Halipegus occidualis
Stafford, 1905 (Trematoda: Hemiu-


McALPINE, D. F., AND M. D. B. B URT. 1998. Taxonomic status of
Halipegus spp. (Digenea: Derogenidae) parasitic in the mouth and
eustachian tubes of North American and Mexican amphibians.


MORAVEC, F., AND O. SEY. 1989. Some amphibian trematodes from

MUZZALL, P. M. 1991a. Helmint infracomunities of the newt,

———. 1991b. Helmint infracomunities of the frogs Rana catesbeiana

NATH, D., AND B. P. PANDE. 1970. A mature halipegid fluke from libaluid


RUSSELL, K. R., AND R. L. WALLACE. 1992. Occurrence of Halipegus occidualis (Digenea: Derogenidae) and other trematodes in Rana
giganticaudata (Anura: Ranidae) from Idaho, U.S.A.
Transactions of the American Microscopical Society 111: 122–127.

(Trematoda: Halipegidae) from Rana esculenta in Iraq, with notes on

SCHROTHOFER, A. M., M. G. BOLEK, R. A. COLE, AND V. R. BISLEY. 2009. Parasites of the minnow frog (Rana septentrionalis) from
Minnesota, USA. Comparative Parasitology 76: 240–246.


SRIVASTAVA, H. D. 1933. On new trematodes of frogs and fishes of the U.
P. India. Part I. New distomes of the family Hemiuridae Luhe, 1901,
from North Indian fishes and frogs with a systematic discussion on the
family Halipegidae. Poche, 1925, and the genera Vitellotrema
Guberlet, 1928, and Genarchoopsis Ozaki, 1925. Bulletin of the
Academy of Sciences Allahabad India 3: 41–60.

STAFFORD, J. 1905. Trematodes from Canadian vertebrates. Zoologischer

THOMAS, L. J. 1939. Life cycle of a fluke Halipegus eccentricus n. sp. found in the ears of frogs. Journal of Parasitology 25: 207–221.

diego, California, 1,056 p.


WETZEL, E. J. 1995. Seasonal recruitment and infection dynamics of
Halipegus occidualis and Halipegus eccentricus (Digenea: Hemiuridae)
in their arthropod and amphibian hosts. Ph.D. Dissertation. Wake
Forest University, Winston-Salem, North Carolina, 118 p.

———, AND G. W. ESCH. 1996a. Influence of odonate intermediate host
ecology on the infection dynamics of Halipegus spp. Haematoloechus
longiplexus and Haematoloechus complexus (Trematoda: Digenidae).

———, AND ———. 1996b. Seasonal population dynamics of Halipegus
occidualis and Halipegus eccentricus (Digenea: Hemiuridae) in their

ZELMER, D. A., AND G. W. ESCH. 1998a. Bridging the gap: The odonate
paratenic host for Halipegus occidualis (Trematoda: Hemiuridae).

———, AND ———. 1998b. Interactions between Halipegus occidualis and

———, AND ———. 1998c. The infection mechanism of the cystophorous
cercariae of Halipegus occidualis (Digenea: Hemiuridae). Invertebrate

———, AND ———. 1999. Reevaluation of the taxonomic status of

———, AND D. R. BROOKS. 2000. Halipegus eschi n. sp. (Digenea:
Hemiuridae) in Rana villanti from Guanacaste province, Costa Rica.
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