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## HALIPEGUS ESCHI N. SP. (DIGENEA: HEMIURIDAE) IN RANA VAILLANTI FROM GUANACASTE PROVINCE, COSTA RICA

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**ABSTRACT:** *Halipegus eschi* n. sp. is described from the esophagus of *Rana vaillanti* from Guanacaste Province, Costa Rica. The new species differs from other known species of *Halipegus* on the basis of relative testis size, lateral extent of the uterus, vitelline follicle arrangement, egg size, and polar filament length.

During an inventory of parasites of anurans in the Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica, an apparently undescribed species of *Halipegus* with distinguishing adult characters was collected from the esophagus of *Rana vaillanti*.

### MATERIALS AND METHODS

Twenty-seven *R. vaillanti* were collected from a flooded, low-lying area adjacent to the Rio Pizote near the boundaries of the ACG in northwestern Costa Rica and necropsied during June 1998. Five adult *Halipegus* were recovered from 4 frogs. Other anurans necropsied from the same site include *Bufo marinus* (6), *B. leukenii* (4), *B. haematiticus* (1), *Leptodactylus pentadactylus* (11), and *L. melanotis* (3). None was infected with *Halipegus* sp. Live worms either were fixed in hot 5% formalin, or relaxed in tap water with menthol crystals before fixation in 5% formalin. Three worms were postfixed in alcohol–formalin–acetic acid, stained with Meyer's hematoxylin (2) or Semichon's acetic carmine (1), cleared in cedarwood oil after dehydration through 95% ethanol, and mounted in Canada balsam. The remaining 2 worms were postfixed in Bouin's fluid, cleared in cedarwood oil after dehydration through 95% ethanol, and embedded in paraffin. Before embedding, 1 of the worms was mounted in cedarwood oil for whole-mount study. Embedded worms were sectioned at 10  $\mu$ m and stained with Meyer's hematoxylin and eosin in xylene (McLean, 1934 as cited in Lee, 1937). Figures were drawn by hand with the aid of a microprojector to maintain scale. Measurements were taken in micrometers; measurements of the holotype are in parentheses.

### DESCRIPTION

#### *Halipegus eschi* n. sp.

(Figs. 1–3)

Body elongate, rounded at posterior end, 4950–5650 (5300) long, 1370–1450 (1450) wide in posterior third of body. Oral sucker subterminal, 500–550 (550) long, 480–510 (500) wide. Acetabulum muscular, midventral, 560–755 (560) long, 550–730 (550) wide. Ratio of oral sucker:acetabulum width 1:1.09–1.51 (1:1.10). Prepharynx absent; pharynx barrel shaped, 190–210 (205) long, 170–220 (175) wide. Intestinal bifurcation in forebody at level of genital pore in anterior fifth of body. Ceca narrow, dorsal to other organs, extending to near posterior end. Testes paired, subspherical, intercecal, oblique (overlapping 20–30%), not in contact; anterior margin of testis reaching posterior border of acetabulum. Anterior testis 260–440 (330) long, 250–380 (300) wide. Posterior testis 260–510 (360) long, 230–420 (340) wide. Genital sac and sinus organ lacking. Seminal vesicle elongate, and pyriform. Pars prostatica elongate, with a nondelimited sheath of prostatic cells, leading to a short ejaculatory duct (Fig. 2). Common genital pore median, ventral to intestinal bifurcation. Ovary spherical to subspherical, posterior to testes in dorsomedial plane of worm; ovary 385–550 (510) long, 330–510 (510) wide. Laurer's canal opening on dorsal surface posterior to ovary, level with Mehlis' gland (Fig. 3). Proximal end of

Laurer's canal enlarged forming seminal receptacle. Oviduct joining Laurer's canal, forming ootype surrounded by well-developed Mehlis' gland. Uterus passing posteriorly to level of vitellaria, and extending anteriorly, filling intercecal space with predominantly transverse folding, opening to exterior through shared genital pore. Metratrum absent. Hermaphroditic duct lacking. Vitelline follicles irregular, 210–430 long, 140–290 wide, in 2 clusters at posterior end of body; varying in number from 2–5 caudal to posterior testis, and 2–4 caudal to anterior testis; each side always with different numbers of follicles. Eggs operculate, 30–46 long, 10–20 wide; nonoperculate end prolonged into single polar filament 20–32.5 long. Ratio of egg length:polar filament length 1:0.47–0.93. Excretory vesicle Y-shaped, excretory vessel bifurcating at level of posterior margin of acetabulum and united dorsal to oral sucker. Excretory pore terminal.

### Taxonomic summary

*Host:* *Rana vaillanti* (Brocchi) (Anura: Ranidae).

*Site of infection:* Esophagus.

*Holotype:* USNPC no. MT30-18H.

*Paratypes:* USNPC no. MT30-18 i/O.

*Locality:* Rio Pizote, Area de Conservacion Guanacaste, Guanacaste Province, Costa Rica (10°56'2.38"N  $\times$  85°24'4.60"W).

*Etymology:* The species is named for Gerald W. Esch in recognition of his tremendous contributions to the study of parasite community ecology and population biology, including halipegine parasites of frogs.

### DISCUSSION

Faced with species descriptions that often were incomplete, and the resulting poor understanding of variation in the morphology of the terminal genitalia, Gibson and Bray (1979) were forced to maintain a broad concept of *Halipegus*. Recent work on halipegines (Hamann, 1986; Kohn et al., 1990; Lunaschi, 1990; Jackson and Tinsley, 1997; Zelmer and Esch, 1999) has facilitated a more narrowly delimited concept of *Halipegus* that restricts membership to those species lacking genital sacs, permanent sinus organs, and well-developed hermaphroditic ducts.

No phylogenetic analysis has been done within the group as yet, but phylogenetic systematic analysis of the hemiurids (Brooks et al., 1985, 1989) indicates that the sinus organ is a phylogenetically conservative trait. The lack of a genital sac is undoubtedly apomorphic, but only a more thorough phylogenetic analysis will determine whether the other traits also are synapomorphies for *Halipegus*, i.e., they are secondary losses in *Halipegus*, or apomorphies for other members of the clade, i.e., *Halipegus* lacks those traits plesiomorphically. The current concept of *Halipegus* restricts membership to species living in the stomachs, esophagi, mouths, and Eustachian tubes of anurans and, more rarely, urodeles.

Rankin's (1944) revision of *Halipegus* recognized 6 valid species occurring in North America, Europe, and Asia. *H. dubius* Klein, 1905 (in *L. ocellatus* and *L. pentadactylus* from Brazil) is the only other species previously known from am-

Received 10 December 1999; revised 8 May 2000; accepted 8 May 2000.

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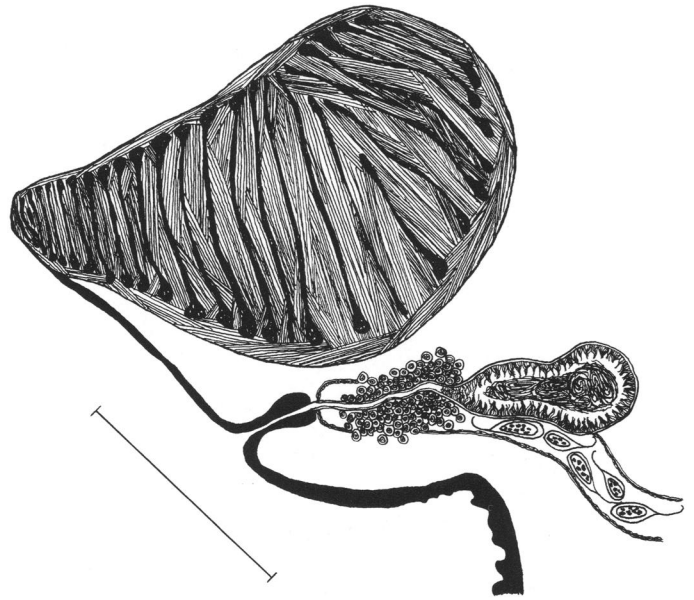
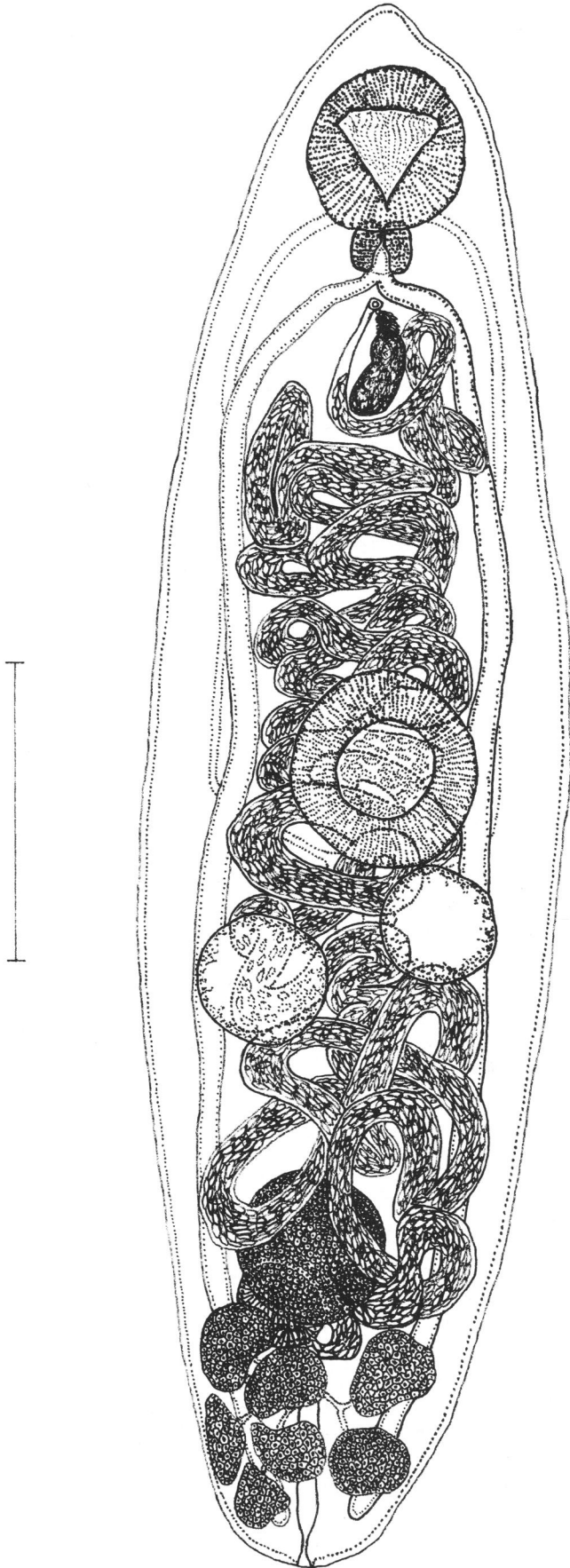


FIGURE 2. Composite drawing of the terminal genitalia of *H. eschi* on the basis of 15 10- $\mu$ m sagittal sections. Scale bar = 200  $\mu$ m.

phibian hosts in the western hemisphere. A recent redescription of *H. dubius* (Paraense, 1992) that relied, in part, on serial sections revealed the presence of an hermaphroditic duct that might have been overlooked in a previous redescription by Kohn and Fernandez (1988). Although this would place *H. dubius* outside the recently narrowed concept of *Halipegus*, it has not been suggested that the status of *H. dubius* be revised.

McAlpine and Burt's (1998) designation of *H. amherstensis* as a junior synonym of *H. occidualis* Stafford, 1905, in part, and *H. eccentricus* Thomas, 1939, in part, despite unique features of the cercaria (Rankin, 1944), reduces the number of valid species of *Halipegus* inhabiting amphibians to 6. Two species of *Halipegus* are known to inhabit frogs and salamanders in the United States and Canada; *H. occidualis* Stafford, 1905, and *H. eccentricus* Thomas, 1939 (McAlpine and Burt, 1998). *H. occidualis* also has been reported from Mexico as *H. amherstensis* in *R. montezumae* from Xochimilco by Caballero (1947), and as *H. lermensis* Caballero, 1941 in *R. montezumae* and "*R. pipiens*" (undoubtedly a different member of the *R. pipiens* clade; see also McAlpine and Burt, 1998) from Lerma (see Rankin, 1944; Caballero, 1947; and McAlpine and Burt, 1998 for synonymies).

Broad anatomical similarity among adults of this genus usually necessitates basing identification on larval characteristics (Paraense, 1992) or, possibly, egg filament length (Goater et al., 1990; McAlpine and Burt, 1998; Zelmer and Esch, 1999). *Halipegus eschi*, however, can be distinguished from other known species on the basis of adult anatomical features. Most notably, both absolute and relative testis size is smaller for *H. eschi* than for any other described species. Figure 4 depicts the relationship between the length and width of the testis and ovary length for *H. eschi* and *H. occidualis*, which was chosen as represen-



FIGURE 1. *H. eschi*. Ventral view. Scale bar = 1 mm.

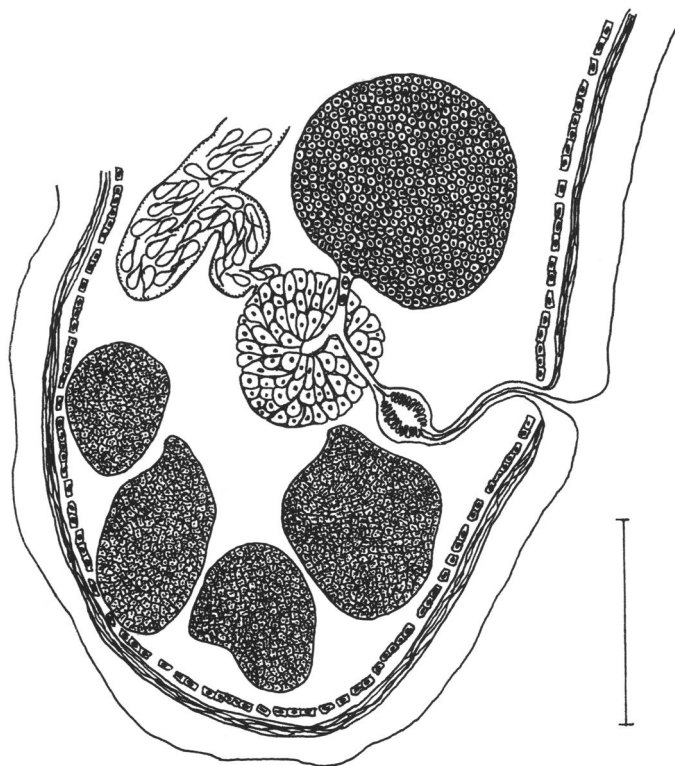


FIGURE 3. Composite drawing of the proximal female genitalia of *H. eschi* on the basis of 18 10- $\mu$ m sagittal sections. Scale bar is 250  $\mu$ m.

tative given the anatomical similarity among the known species of *Halipegus* (data from worms described in Zelmer and Esch, 1999). The slopes (Fig. 4) are not significantly different ( $F = 0.0271$ ;  $df = 3, 28$ ;  $P \gg 0.5$ ), indicating similar allometry between the 2 species. The y-intercepts, however, differ significantly ( $F = 7.89$ ;  $df = 3, 31$ ;  $P \ll 0.001$ ). Tukey's multiple comparison revealed that the only intercepts that did not differ were those for the relation between testis and ovary length and testis width and ovary length for *H. eschi*. This indicates both the spherical nature of the testes of *H. eschi*, and the significant difference in relative testis size between the 2 species.

Previously described species of *Halipegus* have 2 clusters of 4–6 vitelline follicles, with the number never being identical on both sides, and the smaller number occurring on the side where the posterior testis and ovary are situated (Rankin, 1944; Paraense, 1992; Zelmer and Esch, 1999). The number of vitelline follicles in *H. eschi* is more variable, and in 1 specimen, the smaller number was associated with the posterior testis. The uterus of *H. eschi* does not extend laterally beyond the distal margin of the ceca, whereas all other species exhibit extracecal uterine loops. Eggs of *H. eschi* are the smallest of the known *Halipegus* species, as are the polar filaments, although the egg length to filament length ratio (1:0.47–0.93) is similar to that of *H. eccentricus* (see Thomas, 1939).

Most hemiurids, including most derogenines, live in the stomach of their vertebrate hosts. Although adults of *H. occidualis* rarely have been found to inhabit the host esophagus (Macy et al., 1960), the majority of *Halipegus* species live in the floor of the mouth, and *H. eccentricus* inhabits the Eusta-

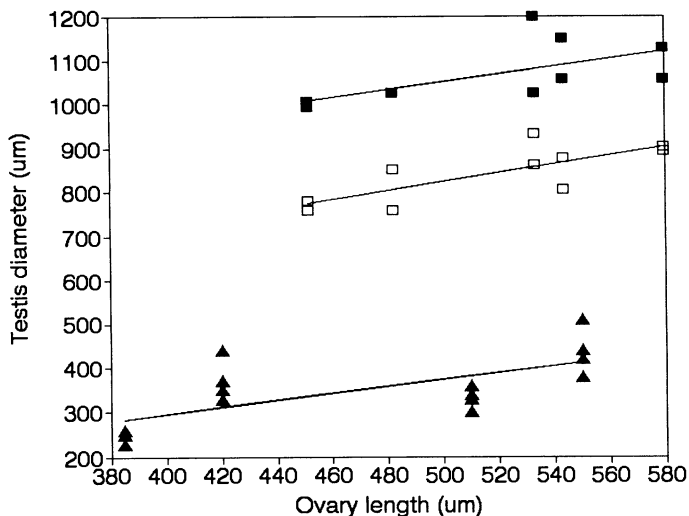


FIGURE 4. Relationship between testis length and width and ovary length for *H. occidualis* (testis length, filled boxes; testis width, open boxes) and *H. eschi* (filled triangles). Lines are least-squares regression lines. The relationship between testis length and ovary length and testis width and ovary length did not differ for *H. eschi*, so only a single regression for all points is depicted.

chian tubes. The presence of *H. eschi* in the esophagus might indicate an evolutionary movement from the stomach to the oral region. A phylogenetic analysis of the genus and its close relatives will facilitate a test of this hypothesis (see Brooks and McLennan, 1991, 1993 for methodology).

#### ACKNOWLEDGMENTS

This study is part of an inventory of eukaryotic parasites inhabiting the 940 species of vertebrates living in the ACG, in northwestern Costa Rica. We thank the scientific and technical staff of the ACG for support of this study, in particular Sifredo Marin, Roger Blanco, Alejandro Masis, Maria Marta Chavarria, Felipe Chavarria, Guillermo Jimenez, Calixto Moraga, Carolina Cano, Elda Araya, Fredy Quesada, Duñia Garcia, Roberto Espinoza, Elba Lopez, and Petrona Rios. We also thank our other international collaborators during June 1998: Thomas Platt, St. Mary's University, South Bend, Indiana, Anindo Choudhury, U.S. Geological Survey, Madison, Wisconsin, and Rita Hartvigsen-Daverdin, Norwegian Institute for Nature Research, Trondheim, Norway. Michael Barger and two reviewers provided helpful comments. Funds for this study were provided by operating grant A7696 from the Natural Sciences and Engineering Research Council (NSERC) of Canada to D.R.B.

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