

# A NEW HELIGMONELLID (NEMATODA) FROM *SYLVILAGUS FLORIDANUS* (LEPORIDAE) IN COSTA RICA, WITH SOME COMMENTS ON SPECIES OF *PARAHELIGMONELLA* DURETTE-DESSET, 1971

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**ABSTRACT:** *Paraheligionella kinsellai* n. sp. (Nematoda: Heligionellidae: Heligionellinae) is described from the small intestine of a cottontail rabbit *Sylvilagus floridanus* (Allen, 1890) (Leporidae) from Costa Rica. The new species is similar to *P. romerolagi* (Gibbons and Kumar, 1980), parasitic in *Romerolagus diazi* from Mexico and to *P. lamothei* Digiani, Carreño, and Durette-Desset, 2008, parasitic in *Sylvilagus floridanus* from Costa Rica, by the characters of the synlophe at mid-body, i.e., 14 cuticular ridges and a single axis of orientation inclined at 30° to the sagittal axis in both sexes. The males of the new species are distinguished from those of *P. romerolagi* by a caudal bursa that is not bell-shaped, a bursal pattern of type 2–3 with a tendency to 2–2–1, and a genital cone that is not hypertrophied. They are also distinguished from the males of *P. lamothei* by having the same bursal pattern in both lobes (in *P. lamothei* the pattern is 2–2–1 on the right lobe and 2–3, with a tendency to 2–2–1, on the left lobe), rays 3 are much longer than rays 2, rays 2 and 3 diverging more proximally, and rays 8 are longer than the dorsal ray. The females of the new species also differ from those of *P. lamothei* by the shape of the posterior extremity (long and strongly curved vs. short and nearly straight) and by the synlophe at the ovejector level (ridge no. 1' hypertrophied and remaining ridges reduced vs. ventral ridges hypertrophied and dorso-lateral ridges reduced).

At present, 4 species of *Paraheligionella* Durette-Desset, 1971 (Trichostrongylina, Heligmosomoidea) have been described, 2 from rodents (Echimyidae and Capromyidae) and 2 from lagomorphs, all in the Neotropical region. These species are: *Paraheligionella interrogans* (Lent and Freitas, 1938) (type species), parasitic in *Thrichomys apereoides* (= *Cercomys cunicularius*) (Echimyidae) from Brazil; *Paraheligionella cubaensis* (Pérez-Vigueras, 1943), parasitic in *Capromys pilorides* (Capromyidae) from Cuba; *Paraheligionella romerolagi* (Gibbons and Kumar, 1980), parasitic in *Romerolagus diazi* (Leporidae) from Mexico, and *Paraheligionella lamothei* Digiani, Carreño and Durette-Desset, 2008, parasitic in *Sylvilagus floridanus* (Leporidae) from Costa Rica. In the same type locality as that of *P. lamothei* (Area de Conservación Guanacaste, Costa Rica), we have discovered a fifth species of *Paraheligionella* from another *S. floridanus*, which is described herein.

## MATERIAL AND METHODS

The specimen of *Sylvilagus floridanus* was collected in the Area de Conservación Guanacaste, Costa Rica, in May 2003. The nematodes recovered were preserved in 70% ethanol. The synlophe was studied following the method of Durette-Desset (1985), and the nomenclature used for the study of the caudal bursa is that of Durette-Desset and Chabaud (1981). The number of cuticular ridges is expressed as: total (dorsal/ventral). Left and right hypertrophied ridges are counted among ventral and dorsal ridges, respectively. The ridges are numbered from left to right as 1 to 6 for the dorsal ridges and as 1' to 8' for the ventral ridges. Measurements (holotype/allotype followed by range and mean of paratypes in parentheses) are in micrometers except where stated otherwise. The parasite classification used, above the family group level, is that of Durette-Desset and Chabaud (1993), and the nomenclature of the hosts at the species level follows Wilson and Reeder (2005). The type material was deposited in the Colección Nacional de Helminthos, San José de Costa Rica, Costa Rica (CHCR), the United States National

Parasite Collection (USNPC), the Helminthological Collection of the Museo de La Plata, La Plata, Argentina (CHMLP), and the Helminthological Collection of the Muséum national d'Histoire naturelle, Paris, France (MNHN).

## DESCRIPTION

### *Paraheligionella kinsellai* n. sp.

(Figs. 1–20)

**General:** Small nematodes, uncoiled, curved along ventral side or forming 1–3 sinistral spirals along ventral side. Cephalic vesicle well developed. In apical view, triangular oral opening surrounded by thick ring; 2 amphids, 6 interno-labial, 4 externo-labial, and 4 cephalic papillae observed. Excretory pore situated between 56 and 93% of esophagus length. Deirids situated at same level as excretory pore, or slightly anterior or posterior to pore (Figs. 1, 2).

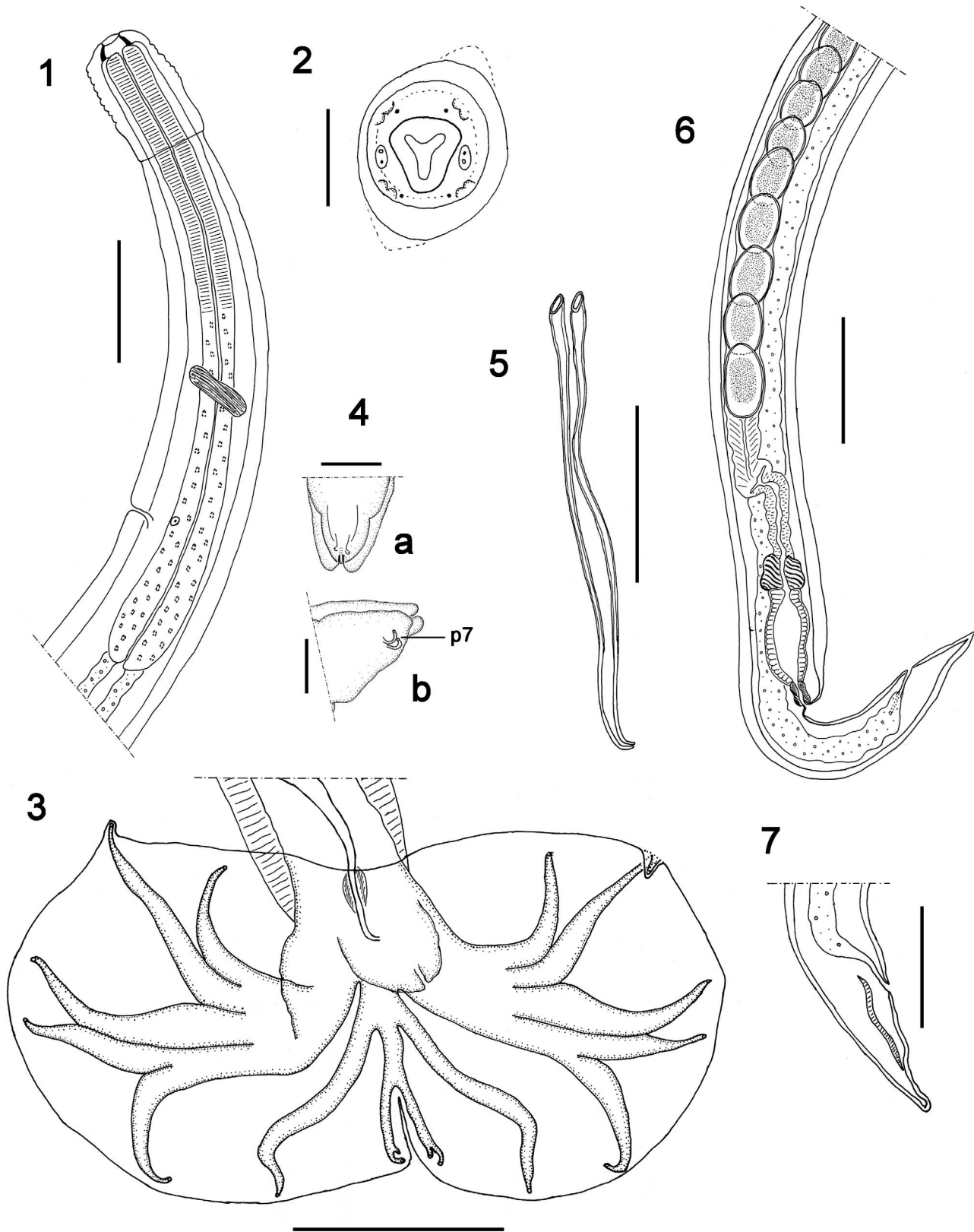
**Synlophe (studied in 2 males and 3 females, paratypes):** In both sexes, body bearing continuous cuticular ridges with chitinoid struts. All ridges, except ventral ridge no. 6', appear just posterior to cephalic vesicle and disappear at different levels anterior to caudal bursa in male and anterior to vulva in female. Ventral ridge no. 6' posterior to excretory pore, near esophago-intestinal junction. Number of ridges: in both sexes 13 (6/7) at level of distal esophagus (Figs. 8, 11), 14 (6/8) at mid-body (Figs. 9, 12), and, in males, 12 (4/8) about 70 anterior to caudal bursa (Fig. 10). In female, ridges decreasing progressively along ovejector region (Figs. 14–20); at level of proximal infundibulum, 11 (5/6); at level of distal infundibulum, 10 (4/6); at level of sphincter 9 (4/5); at level of proximal vestibule, 7 (3/4); and just anterior to vulva, 1 (ventral). At mid-body, ridges adjacent to lateral fields (no. 1' and no. 6) hypertrophied. On dorsal side, gradient of size of ridges latero-median, from right to left. On ventral side, gradient of size from left to right, from left ridge no. 1' up to axis of orientation. Single axis of orientation of ridges directed from right ventral quadrant to left dorsal quadrant inclined at 30° to sagittal axis in both sexes (Figs. 9, 12). Within posterior region of body: in male, lateral ridges well developed, but not hypertrophied, gradient of size preserved, orientation of ridges also preserved (Fig. 10); in fe-

Received 30 July 2008; revised 3 September 2008, 27 October 2008; accepted 17 November 2008.

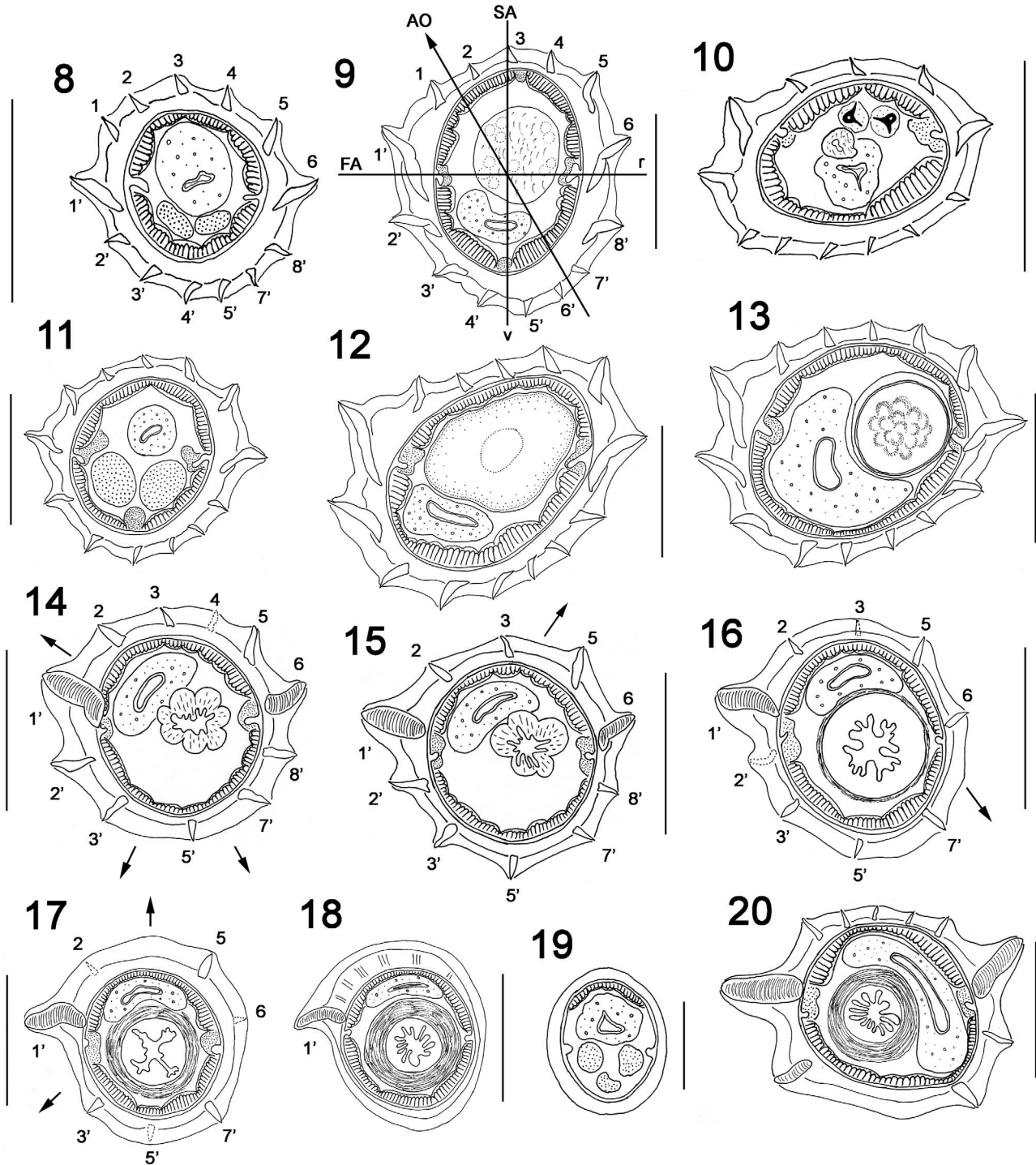
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DOI: 10.1645/GE-1805.1



FIGURES 1–7. *Paraheligmonella kinsellai* n. sp. (1) Male, anterior extremity, left lateral view. (2) Female, head, apical view. (3) Male, caudal bursa, ventral view. (4) (a) Male, genital cone, dorsal view, (b) right latero-ventral view. (5) Male, spicules in situ, right latero-ventral view. (6) Female, posterior extremity, right lateral view. (7) Female, tail, right lateral view. Abbreviations: p7: papillae 7. Scale bars for Figures 2, 4, =20  $\mu$ m; for Figures 1, 7, =50  $\mu$ m; for Figures 3, 5, 6, =100  $\mu$ m.



FIGURES 8–20. *Paraheligmoneella kinsellai* n. sp. Transverse sections of body. (8–10) Male. (8) At esophago-intestinal junction; (9) at mid-body (male 3.3 mm long, section at 1.7 mm from apex); (10) at 70  $\mu$ m anterior to caudal bursa (male 3.15 mm long). (11–19) Female. (11) Just posterior to esophago-intestinal junction, 390  $\mu$ m from apex; (12) at mid-body (female 4.6 mm long, section at 2.4 mm from apex). Female 5.25 mm long, at different levels of ovejector; (13) middle part of uterus (810  $\mu$ m from posterior extremity); (14) proximal infundibulum; (15) distal infundibulum; (16) sphincter; (17) proximal vestibule; (18) distal vestibule; (19) between vulva and anus. (20) Another female, at sphincter level. Arrows indicate disappearance of ridge with respect to the previous section. Ridges in dotted lines indicate disappearance of the ridge along the section. Abbreviations: r: right side, v: ventral side, AO: axis of orientation, FA: frontal axis, SA: sagittal axis. All figures orientated as Figure 9. Scale bars for all figures = 50  $\mu$ m, except for Figure 19, = 25  $\mu$ m.

male, from distal part of uterus, size of ridges progressively decreasing except for ridge no. 1', usually hypertrophied up to its disappearance. In 1 sectioned female, ridges no. 1, no. 4', no. 6', and no. 4 disappearing first, then ridges no. 8', no. 3, and no. 2', then no. 2, no. 5', and no. 6 (Figs. 14 to 17). Last ridges (except hypertrophied ridge no. 1) reduced in size and disappear along vestibule (Figs 17, 18). Ridge no. 1' disappearing just in front of vulva. Pattern may slightly vary in other females, with slight differences in extension of dorsal and ventral ridges and degree of reduction of ridge no. 6; however, certain asymmetry usually maintained, with left ridge no. 1' hypertrophied up to its disappearance and always more developed than other ridges. On tail, no ridges or cuticular thickenings observed in transverse sections.

*Males (based on holotype and 12 paratypes):* 4.35 (3.25–4.95 [3.78]) mm long and 75 (65–100 [79]) wide at mid-body; cephalic vesicle 60 (50–70 [56]) long and 38 (30–45 [35]) wide; nerve ring, excretory pore, and deirids situated at 190 (150–195 [175]), 265 (215–290 [242]), and 260 (210–290 [237]) from apex, respectively; esophagus 290 (250–350 [298]) long. Sub-symmetrical caudal bursa with right lobe slightly more developed than left lobe. Pattern of type 2–3 with a tendency to 2–2–1 for both lobes (Fig. 3). Rays 2 and 3, both joined along basal third of ray 2 then strongly divergent; rays 3 much longer than rays 2, protruding from bursal margin; rays 4 slightly longer than rays 5, both divergent at extremities. Rays 6 arising from trunk 4–6 at about mid-way between divergence of ray 3 and that of ray 5. Rays 8 arising from proximal third of dorsal ray and longer than it. Dorsal ray divided at about mid-length into 2 branches, each branch giving rise to 2 small branches, rays 9 (external branches) slightly longer than rays 10 (internal branches). Spicules 210 (165–250 [190]) long with sharp tips. Tips of spicules bent in a right-angle respect to the main axis of the spicule (Fig. 5). Ratio spicule length/body length: 4.8% (4.5–5.8 [5.0])%. Gubernaculum poorly developed, hardly visible in lateral view, 30 (18–30 [22]) long and 10 (9–15 [11]) wide (n = 5) (Fig. 3). Genital cone well developed, conical, 60 (40–65 [54]) long and 55 (35–60 [46]) wide at base, with sclerotized margins. Ventral lip of genital cone distally indented and longer than dorsal lip. Papilla zero not observed. Pedunculated papillae 7, close to each other, visible on dorsal lip (Fig. 4).

*Females (based on allotype and 13 paratypes):* 5.05 (3.40–6.45 [4.74]) mm long, 80 (62–105 [83]) wide at mid-body; cephalic vesicle 60 (45–70 [54]) long and 40 (30–40 [35]) wide; nerve ring, excretory pore, and deirids at 165 (125–182 [157]), 270 (180–280 [236]), and 250 (177–265 [230]) from apex, respectively; esophagus 325 (245–322 [296]) long. Monodelphic. Vulva at 168 (110–200 [154]) from caudal extremity; vagina vera 13 (13–20 [16]), vestibule 95 (45–105 [74]) long, sphincter 30 (18–55 [28]) long and 35 (25–40 [35]) wide, infundibulum 95 (65–95 [79]) long; uterus length 840 (480–880 [683]) long, taking up 16.6% (11.5–22.1 [14.6])% of body length, containing 31 (12–38 [23]) eggs 50–52 (40–60) long and 27–30 (25–35) wide (Fig. 6). Tail conical, 48 (40–65 [52]) long, bearing 2 flat lateral ridges or cuticular thickenings (Fig. 7).

#### Taxonomic summary

*Type host:* *Sylvilagus floridanus* (Leporidae).

*Site of infection:* Small intestine.

*Type locality:* Sector Quebrada Grande, 2 km west of the village, Guanacaste, Costa Rica, 10°51'25"N, 85°27'09"W. Altitude: 350 m.a.s.l.

*Prevalence and intensity of infection:* One of 3 examined hosts (captured on different dates and from different localities of the same area) harbored 50 males and 152 females.

Co-parasites recorded: Cestodes (unidentified).

*Type material:* holotype, allotype, and 32 paratypes deposited in the CHCR: holotype CHCR No. 288, allotype CHCR No. 289, 7 male paratypes CHCR No. 290, 25 female paratypes CHCR No. 291. Other paratypes: 8 males, 22 females CHMLP 5873; 5 males, 15 females MNHN 408MQ; 15 males, 68 females USNPC No.101164.

*Etymology:* *Parahelimonella kinsellai* n. sp. is named in honor of our colleague, Dr. J. M. Kinsella.

#### Remarks

These specimens possess the main characters of *Parahelimonella* (Heligmonellidae: Heligmonellinae), characterized by a synlophe, with no more than 14 cuticular ridges and hypertrophy of the ridges adjacent to the lateral fields (Durette-Desset, 1971). The new species can be readily differentiated from *P. cubaensis* either by characters of the synlophe and of the caudal bursa: *P. cubaensis* has a synlophe with 11–13 cuticular ridges, with left dorsal and right ventral ridges reduced or poorly developed and an axis of orientation of the ridges passing between ridges 1 and 1', inclined at 65–72° to the sagittal axis. On the other hand, the bursal pattern is of type 1–4 (Pérez-Vigueras, 1943; Baruš and Ryšavý, 1967; Durette-Desset, 1972). It also differs from *P. interrogans*, the type species, mainly by having a different number of ridges in the synlophe (14 vs. 13), a gradient of size of the ridges (absent in *P. interrogans*), and ridges uninterrupted (discontinuous in *P. interrogans* on dorsal side). In addition, the female posterior end in *P. interrogans* is straight, and the tail is invaginable (Durette-Desset, 1968).

The specimens described here resemble both *P. romerolagi* and *P. lamothei*, especially by the synlophe at the mid-body. All 3 species have 14 continuous ridges with a similar gradient of size and the same inclination of the axis of orientation. However, they can be distinguished mainly by characters of the caudal bursa and the female synlophe at the ovejector level.

In *P. romerolagi*, the caudal bursa is bell-shaped, with a rather atypical pattern of type 2–2–1, with ray 6 diverging the most proximally from the common trunk 2 to 6 and the extremities of rays 2 to 5 nearly equidistant; the genital cone is bulbous and hypertrophied (Digiani et al., 2008). In the present specimens, the pattern is type 2–3, with a tendency to type 2–2–1, and the genital cone is conical and moderately developed.

In *P. lamothei*, the bursal rays show a type 2–2–1 pattern on the right lobe and a type 2–3 (with a tendency to type 2–2–1) with a very short common trunk of rays 4 to 6 on the left lobe; rays 2 and 3 diverge distally and both are of equivalent length; the genital cone is blunt and soft (Digiani et al., 2008). In the specimens studied here, the bursal pattern is of type 2–3, with a tendency to type 2–2–1, with a long common trunk of rays 4 to 6; rays 2 and 3 diverge more proximally and ray 3 is longer than ray 2. The genital cone is conical, with chitinized margins.

Other differences among the 3 species relate to the female

synlophe at the ovejector level. In *P. romerolagi*, the lateral ridges no. 1' and no. 6 are hypertrophied and directed dorsally, whereas the dorsal and ventral ridges are reduced. In contrast, in *P. lamothei*, the lateral ridges are reduced and the ventral and latero-ventral ridges are hypertrophied (Digiani et al., 2008). In the specimens studied here, all ridges reduce progressively in size and disappear at vestibular level, except for the ridge no. 1', which is hypertrophied up to its disappearance just in front of the vulva.

Additional differences between *P. kinsellai* and *P. lamothei* relate to the body size and the distance from the tail end to the anus and to vulva. The mean body length in *P. kinsellai* is 3.82 mm in males and 4.76 mm in females, whereas in *P. lamothei* it is 6.45 mm in males and 8.05 mm in females. The proportions of the tail length and the distance from tail end to vulva, with respect to the body length, are also significantly different between both species: 1.09% and 3.29%, respectively, in *P. kinsellai*, and 0.44% and 1.36% in *P. lamothei*.

The finding of 2 congener nematodes (*P. kinsellai* and *P. lamothei*) separately in 2 individual hosts of the same species in the locality is not significant in the sample studied, which was too small. It is likely that, in a sample statistically valuable, both species may be found coexisting in the same individual host—which is actually a frequent situation among the Heligmonellidae (Durette-Desset, 1985). Even in such cases of coparasitism, it is possible for a given species to be absent from some individual hosts, indicating that the host has not been in contact with the infective larvae of such species.

## DISCUSSION

Hypertrophied ridges with strong cuticular support in the posterior region of the female (ovejector level, tail, or both) have been described with different patterns in 4 of the 5 known species of *Paraheligionella* (*P. interrogans*, *P. romerolagi*, *P. lamothei*, and *P. kinsellai*). Such ridges are apparently absent in *P. cubaensis* (Pérez-Vigueras, 1943; Baruš and Ryšavý, 1967; Durette-Desset, 1972). The different patterns observed are recognized as specific characters; however, it is likely that their presence may be a generic character of *Paraheligionella*. Another shared character not previously noted, but also observed in the present study, is the axis of orientation passing between ridges no. 1 and no. 2 on the left dorsal quadrant. Again, this character is present in *P. interrogans*, *P. romerolagi*, *P. lamothei*, and *P. kinsellai*, but is absent in *P. cubaensis*.

The case of *P. cubaensis* is unusual, as this species possesses several other characters of the synlophe and caudal bursa, which place it apart from the remaining species in the genus. These characters include the well-developed, but not hypertrophied, lateral ridges, the presence of comaretes on the ventral side, ridges of the right ventral and left dorsal quadrant strongly

reduced, the axis of orientation of the ridges passing between ridges 1 and 1', inclination of the axis of orientation of the ridges 65° in males and 72° in females, and a type 1–4 pattern of the caudal bursa. These seem to be significant characters at the generic level; further studies involving other Neotropical Heligmonellinae should help in determining the systematic position of *P. cubaensis*.

## ACKNOWLEDGMENTS

The authors wish to thank Daniel Brooks, coordinator of the parasite inventory of the vertebrates from the Area de Conservación Guanacaste (<http://www.parasitesrus.com>), for facilitating the fieldwork, participating in specimen collection, and for other valuable support in this project. We also thank Calixto Moraga, Petrona Ríos, Elda Araya, and María Marta Chavarría for helping us with sample collection and specimen processing. We are grateful for additional technical and scientific assistance from Róger Blanco Segura and Felipe Chavarría. Host specimens were collected under permit 30059 to R.A.C. from the Ministerio del Ambiente y Energía, Costa Rica and resolución OFAU 158-2003. This study was supported, in part, by funds from the Thomas E. Wenzlau Fund, Ohio Wesleyan University, Delaware, Ohio, U.S.A.

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