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Research Note

First Report of Genus *Cosmocercella* Steiner, 1924 (Nematoda: Cosmocercidae) in Amphibians from Argentina

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ABSTRACT: This is the first record of the cosmocercid nematode, *Cosmocercella minor*, in Argentina in the host *Phyllomedusa hypochondrialis*, the orange-legged Leaf Frog. The species was observed using light and scanning electron microscopy (SEM) and relevant features are described, including in males the presence of subventral adanal alae and absence of postanal caudal alae, fine structure of the vesiculated rosettes, and structure of proximal end of spicules; and in females confirmation of their amphidelphic condition.

KEY WORDS: Cosmocercella minor, Nematoda, Phyllomedusa hypochondrialis, Hylidae, Amphibia, Corrientes, Argentina.

Cosmocercella minor (Freitas and Dobbin Jr., 1961) Baker and Adamson, 1977 (described as Raillietnema minor) was reported for the first time occurring in the intestine of Phyllomedusa hypochondrialis (Daudin, 1800) collected in Pernambuco State, Brazil. Gomes (1967), in a revision of the genus Raillietnema Travassos, 1927, reiterated the original description but defined the females as prodelphic. Baker and Adamson (1977) reclassified R. minor as C. minor on the basis of the presence of a vesiculated rosette in the males. Later, Baker and Vaucher (1983) found this species in the same host in the provinces of Concepcion and Amambay, Paraguay.

The hylid *P. hypochondrialis* is distributed in Argentina in the provinces of Salta, Jujuy, Formosa, Chaco, Santiago del Estero, Santa Fe, and Corrientes and also occurs in Colombia, Venezuela, Brazil, Bolivia, and Paraguay (Cei, 1980). This species is a "sit and wait" predator and is found on bank vegetation of permanent and semipermanent ponds (Duré, 1999).

The aim of the present study is to provide morphological details of *C. minor* on the basis of specimens recovered from the type host, captured in Corrientes, Argentina. Moreover, this report extends the geographical distribution of the species and represents the first occurrence of this parasite genus in Argentinean amphibians.

Ten adult specimens of *P. hypochondrialis* (9 males and 1 female) were collected near the city of Corrientes in Corrientes province, Argentina (27°28'S; 58°50'W) between December 2002 and November 2008.

Frogs were transported live to the laboratory and killed using a chloroform (CHCL₃) solution. At necropsy, hosts were sexed and the alimentary canal, lungs, liver, kidneys, urinary bladder, musculature, and integument examined for parasites by dissection. Nematodes were observed in vivo, counted, and killed in hot distilled water and preserved in 70% ethanol, cleared in glycerine or lactophenol, and examined as temporary mounts. Drawings were made with the aid of a drawing tube. Specimens were examined with a scanning electron microscope (SEM), dehydrated in an ethanol series, dried using the critical point technique, coated with gold, and examined with a Jeol 5800LV scanning electron microscope. Measurements are presented in um, unless otherwise stated, as mean ± SD values followed by range values in parentheses. Voucher specimens were deposited in the Helminthological Collection of the Centro de Ecología Aplicada del Litoral (CECOAL 02123302: 20 females; 20 males), Corrientes, Argentina.

One-hundred and sixty-eight specimens of *C. minor* were found in the large intestine of four specimens of *P. hypochondrialis*. Measurements from the present study and those provided by previous authors are presented in Table 1. Morphological details, observed with light microscopy and SEM, are shown in Figures 1–14.

Males and females of *C. minor* possess a finely, transversally striated cuticle. Narrow lateral alae extend from the level of the anterior end of esophagus to the anal level in both sexes. The oral opening is triangular with three lips. One large papilla is present on each subventral lip. Dorsal lip with pair of papillae (Fig. 1). Amphids on subventral lips. The anterior end of the esophagus bears 3 tooth-like projections covered with a thick cuticle. The esophagus com-

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References	Freitas and Dobbin Jr. (1961)	Baker and Vaucher (1983)	Present study
Localities	Pernambuco, Brazil	Amambay and Concepción, Paraguay	Corrientes, Argentina
Males (n)*	?	5	10
Total length	1.10-1.16 mm	0.975-1.23 mm	$1.07 \pm 0.15 \text{ mm} (0.88-1.35)$
Width	63.0	_	$51.1 \pm 4.2 (45-58)$
Pharynx	$21 \times 8 - 13$	20–23	$19.4 \pm 2.1 \ (15-22) \times 14.5 \pm 2.4 \ (11-18)$
Corpus	_	168-203	$194.7 \pm 24.1 \ (172-235) \times 20.1 \pm 3.4 \ (13-26)$
Isthmus	25×13	19–30	$29.4 \pm 4.0 (23-34) \times 12.7 \pm 1.4 (11-15)$
Bulb	$42-46 \times 39-42$	48–56	$47.9 \pm 5.1 (41-58) \times 41.8 \pm 4.6 (37-49)$
Nerve ring†	88-105	90-119	$120.7 \pm 6.9 (110-130)$
Excretory pore†	172–193	174–212	$209.0 \pm 16.1 \; (181 – 230)$
Tail length	100-110	116-126	$89.7 \pm 13.0 (78-119)$
Spicules	105-126	80–97	$87.2 \pm 8.0 (77-100)$
Gubernaculum	21–25	21–25	$28.0 \pm 3.6 (23-32)$
Females (n)	?	5	10
Total length	1.24-1.36 mm	1.29-1.38 mm	$1.26 \pm 0.22 \text{ mm } (0.97-1.75)$
Width	78–100	_	$66.6 \pm 5.8 (57-77)$
Pharynx	$17-21 \times 9-13$	22–27	$22.7 \pm 4.4 (20-34) \times 20.0 \pm 6.3 (14-34)$
Corpus	_	218-231	$223.4 \pm 42.1 \ (192-299) \times 27.9 \pm 5.8 \ (22-37)$
Isthmus	$29-35 \times 13-17$	21-39	$27.2 \pm 7.1 (21-39) \times 19.0 \pm 6.8 (13-34)$
Bulb	$46-52 \times 42-48$	58-63	$55.4 \pm 12.4 (46-80) \times 54.4 \pm 13.6 (45-82)$
Nerve ring†	113-122	131-154	$135.6 \pm 24.5 (100-184)$
Excretory pore†	202–217	221–242	$204.4 \pm 26.6 \; (158 - 257)$
Tail length	120-130	137–144	$162.6 \pm 39.3 (138-270)$
Eggs	$104-157 \times 42-96$	$143-155 \times 72-89$	$143.5 \pm 28.7 (118-207) \times 71.3 \pm 20.9 (55-115)$
Vulva†	470–530	770–850	690 ± 0.091 (600–900)

Table 1. Comparative morphometrics of Cosmocercella minor from Phyllomedusa hypochondrialis from different localities.

prises an anterior cylindrical corpus, narrow isthmus, and esophageal bulb (Fig. 2). There are numerous somatic papillae.

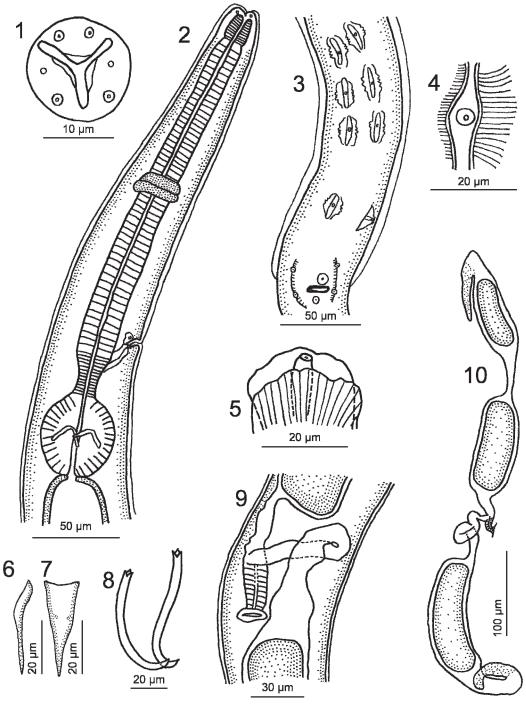
Males with 4 pairs of pre-anal papillae located in small expansions of the cuticle and transversely striated (vesiculated rosette) (Figs. 3-5, 11). First 3 proximal pairs equidistant, separated from each other by 0.5–10; distal pair separated from third by 30–35 and closest to anus (Fig. 12). Distal pair also smaller than first 3. Anterior lip of anus very protruding; unpaired papillae present on anterior margin of anus. Two pairs of rounded adanal papillae, 1 anterior to anus and the other posterior (Fig. 13); in some specimens, 1 additional pair located lateral to anus. These papillae are located in subventral alae lateral to anus (Fig. 13). Unpaired papillae on posterior margin of anus. Four pairs of postanal pedunculated papillae in the second third of the tail; 5-6 pairs of sessile papillae in two rows in midregion of tail, variable in distribution and size; 4 pairs of subdorsal pedunculate papillae, 2 pairs in the second third of the tail and 2 pairs in the last third of the tail. Table 2 shows the distribution of caudal papillae in this species for specimens from different localities. Postanal caudal alae absent. Gubernaculum weakly sclerotized (Figs. 6, 7); proximal end of spicules not rounded but with small, fine projections (Fig. 8).

Females are didelphic and amphidelphic. Vulva lips reduced. Vagina well developed, directed anteriorly, and then divided in 2 uteri; anterior uterus directing anteriorly; posterior uterus is longer, coiled upon itself, and then directed to posterior end of body continuous with posterior ovary (Fig. 9). Ovaries elongate, with reflexed coils; anterior ovary not reaching esophagus—intestine junction, its reflexed part forming 1 coil. Posterior ovary reaching to level of posterior end of intestine (Fig. 10). Uterus with 1 or 2 eggs, some of them with larvae. In mature females, free larvae were observed in uterus. A pair of adanal papillae (Fig. 14).

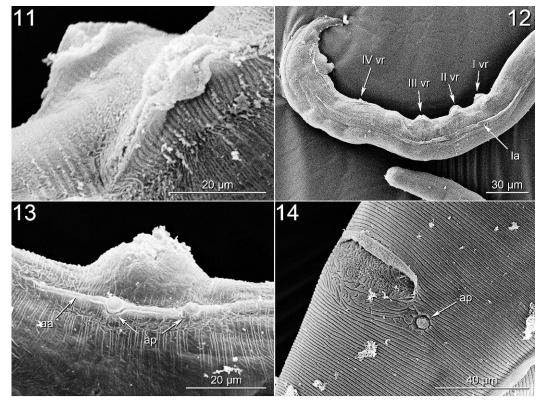
The genus *Cosmocercella* is characterized by vesiculated rosette caudal papillae in the posterior end of males, which are not present in other Cosmocercidae genera, and by the lack of ventral

^{*} Number of specimens examined.

[†] From anterior end.



Figures 1–10. *Cosmocercella minor* from *Phyllomedusa hypochondrialis*. **1.** Cephalic extremity of male, apical view. **2.** Anterior end of male, lateral view. **3.** Disposition of vesiculated rosette, ventral view. **4.** Details of vesiculated rosette, ventral view. **5.** Details of vesiculated rosette, lateral view. **6.** Gubernaculum, lateral view. **7.** Gubernaculum, ventral view. **8.** Detail of spicules. **9.** Detail of the first portion of female reproductive system. **10.** Reproductive system of female.



Figures 11–14. Cosmocercella minor from Phyllomedusa hypochondrialis. **11.** Detail of vesiculated rosette. **12.** Relative placement of vesiculated rosettes. First (I vr), second (II vr), third (III vr) and fourth (IV vr) vesiculated rosette and lateral alae (la), lateral view. **13.** Detail of anus with subventral adanal alae (aa) and adanal papillae (ap), male. **14.** Adanal papillae (ap), female.

plectanes in the pre-anal region of males (Baker and Adamson, 1977).

Nine species of this genus have been reported from amphibians and reptiles in the Americas and Asia

(Mata-López et al., 2008). Two are found in South American hosts: *Cosmocercella minor* in *P. hypo-chondrialis* from Brazil and Paraguay (Freitas and Dobbin Jr., 1961; Baker and Vaucher, 1983) and

Table 2. Distribution of caudal papillae in males of Cosmocercella minor from different localities.

	Freitas and Dobbin Jr. (1961)	Baker and Vaucher (1983)	Present study
Preanal papillae (vesiculated rosette)	4 pairs	4 large pairs	3 + 1 pairs
Adanal papillae	1 pair	4-5 pairs in subventral adanal alae	1 pair anterior to anus; 1 pair posterior to anus (1 pair lateral to anus) in subventral adanal alae
Unpaired papillae anterior to anus	Present	Present	Present
Postanal papillae	_	5–6 lateral or subdorsal in position; 14–16 in 2 sublateral rows in midregion of tail	unpaired sessile papillae posterior to anus; 5–6 pairs of ventral sessile papillae; 4 pairs of sublateral pedunculate papillae; 4 pairs of subdorsal pedunculate papillae
Postanal caudal alae	Present with 8 pairs of papillae	The body cuticle is slightly thickened, but are not interpreted as "true alae"	Absent

Cosmocercella phyllomedusae Baker and Vaucher, 1983 found in *P. hypochondrialis* from Paraguay and in *Phyllomedusa palliata* Peters, 1872, *Phyllomedusa tomopterna* (Cope, 1868) and *Phyllomedusa vaillanti* Boulenger, 1882 from Peru (Baker and Vaucher, 1983; Bursey et al., 2001).

Our C. minor specimens closely resemble the Paraguayan and Brazilian specimens, but we found some differences. Regarding morphometric characters, the spicules of males are slightly shorter that those of the Brazilian specimens but longer than those of specimens from Paraguay; the gubernaculum is longer than the one in both Paraguayan and Brazilian specimens (Table 1). The female specimens collected in this study are longer than the ones examined by Freitas and Dobbin Jr. (1961) and Baker and Vaucher (1983). Also, in females, the vulva is more distant from the anterior end than was reported by Freitas and Dobbin Jr. (1961), but the proportions seem to be more similar to the measurements given by Baker and Vaucher (1983). In general, eggs were larger than those reported by these authors.

According to the original description and redescription made by Gomes (1967), the vesiculated rosettes in the posterior end of males are equidistant from each other. In the specimens analyzed in this study, the first 3 pairs of vesiculated rosettes are equidistant, while the fourth pair is separated from the first 3 by a greater distance. In addition, the fourth pair of distal vesiculated rosettes is smaller than the first 3. Baker and Vaucher (1983) did not report differences concerning the distance between these structures, but the arrangement that these authors present in figure 1A is the same that we found in these males. Freitas and Dobbin Jr. (1961) observed the existence of only one pair of adanal papillae but did not mention the existence of alae lateral to the anus. Baker and Vaucher (1983) established the presence of alae on the sublateral surface of body, extending 30 µm posterior and 40 µm anterior to the anus; each alae containing 4 or 5 small papillae. In our specimens, we observed a very thin alae lateral to the anus with 2 rounded papillae (Fig. 13), although some specimens had 3 pairs of papillae. On the other hand, Freitas and Dobbin Jr. (1961) reported 8 pairs of postanal papillae located in narrow caudal alae that began after the anal opening. When viewed under light microscopy, the postanal pedunculate papillae appear to be located in a fold of cuticle that can be interpreted as a caudal ala. However, when examined using SEM, the specimens did not show lateral caudal alae; this agrees with the fact that Baker and Vaucher (1983) did not identify any structure as being the "true caudal alae" in males. Finally, we describe for the first time the proximal end of the spicules.

In the original description, the females were reported as amphidelphic. Gomes (1967) characterized the females as prodelphic, while Baker and Vaucher (1983) stated that the ovary of the anterior uterus was located anterior to the vulva, and the one corresponding to the posterior uterus was beside it. In this study, we analyzed the reproductive system of female specimens and found it to be amphidelphic.

Here, we provide the first record of the genus *Cosmocercella* in *P. hypochondrialis* from Argentina as well as the description of new features of both sexes of this cosmocercid nematode.

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