A new species of *Aplectana* (Nematoda: Cosmocercidae) parasite of *Pleurodema nebulosum* (Anura: Leptodactylidae) from the Monte desert, Argentina, with a key to Neotropical species of the genus *Aplectana*

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Abstract

Here we describe a new cosmocercid nematode, *Aplectana nebulosa* sp. nov., from the small and large intestines of *Pleurodema nebulosum* (Anura: Leptodactylidae), from the Monte desert of San Juan, Argentina. The new species belongs to the *Aplectana* group that possesses a gubernaculum and unpaired adcloacal papilla anteriorly to cloaca. It resembles *A. membranosa*, *A. paraelenae* and *A. travassosi* by the presence of four adcloacal papillae, but differs from those species by the following characters: number and arrangement of precloacal papillae; number and arrangement of postcloacal papillae; shape and size of spicules and gubernaculum, and by the presence of lateral alae in caudal region of males. The description of the new species is based on light microscopy and scanning electron microscopy (SEM) and we also provide a key to Neotropical species of *Aplectana*.

Key words: Nematode parasite, Amphibians, San Juan Province, Argentina

Introduction

The frog genus *Pleurodema* Tschudi (Leiuperinae) includes 13 valid species widely distributed from Panama to southern South America (Frost 2016), nine of which are present in Argentina (Ferraro and Casagrande, 2009). *Pleurodema nebulosum* and *Pleurodema guayapae* are the species that constitute the *P. nebulosum* clade (Faivovich *et al.*, 2012), and within this clade, *Pleurodema guayapae* inhabits the ‘Chaco Seco’ ecoregion in Argentina, while *P. nebulosum* occurs in the ecoregions ‘Chaco Seco’, ‘Monte de Sierras and Bolsones’, ‘Monte de Llanuras and Mesetas’, and ‘Espinal’ and its distributional limits reach the ‘Pampa’, ‘Puna’, and ‘Estepa’ regions. In Argentina, *P. nebulosum* is the species of this genus with widest altitude range (Ferraro and Casagrande, 2009), and is a common frog in the Monte desert.

The information available on metazoan parasites of the herpetofauna from the Monte desert in Argentina mostly refers to helminth species found in reptiles (Ramallo and Díaz, 1998; Ramallo *et al.*, 2002a; 2002b; 2016a; 2017; Goldberg *et al.*, 2004). Regarding helminths found in amphibians, González *et al.* (2013a) described the nematode *Falcaustra sanjuanensis* González, Sanabria and Quiroga, 2013 from the large intestine of *Odontophrynus* cf. *barrioi* and González *et al.* (2013b) reported the nematode *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931 from the large intestine of *Rhinella arenarum* Hensel, 1867. González *et al.* (2014) reported the presence of the nematodes *F. sanjuanensis* and *Contracaecum* sp. (larvae), from the large intestine and stomach of *Lithobates catesbeianus* (Shaw, 1802). Finally, *F. sanjuanensis* was also reported by Ramallo *et al.* (2016b) from *Leptodactylus latrans* (Steffen, 1815).

However, no helminths have been described for *Pleurodema nebulosum* from Argentina until now. Thus, here

we describe a new cosmocercid species belonging to the genus *Aplectana* Railliet and Henry, 1916. This species was found as parasites of the digestive tract of *P. nebulosum* from Matagusanos, Ullúm Department, San Juan province. We also provide a key of identification for the *Aplectana* species found in the Neotropical Realm.

Materials and methods

Study area: The Matagusanos barreal is located 55 km east of San Juan city, in Ullúm Department (31.11250 S, 68.63587 W, elevation: 600 m). The dominant vegetation in the area includes *Larrea cuneifolia* and *Larrea divaricata* (jarillas), *Prosopis* sp. (algarrobos), and *Capparis atamisquea* (matagusanos). This region is part of the Monte desert, characterized by its arid climate with a mean annual temperature of 17.3 °C, a mean maximum temperature of 25.7 °C, and a mean minimum temperature of 10.4 °C, with a mean annual rainfall of 89 mm during the summer months (wet season) (Cabrera, 1976; Warner, 2004). In this area we collected by hand 41 specimens of *Pleurodema nebulosum* (18 females, 23 males) during the breeding season (January 2012).

Analytical procedure: The frogs were examined for nematode parasites using the standard technique. Intestinal nematodes were fixed in 10% formaldehyde solution and preserved in 70% ethanol and cleared with lactophenol for examination under the light microscope. Illustrations were made using a Leica microscope with the aid of a drawing tube. For examination with scanning electron microscope (SEM), the nematodes were postfixed in 1% OsO₄, dehydrated through an ethanol and an acetone series and critical point dried. The specimens were coated with gold and examined with a JSM-5800 scanning electron microscope. The measurements along the text are given in micrometers unless otherwise indicated, and are presented as the mean $\pm$ 1 Standard Deviation with the range in parentheses; measurements of holotype and allotype are shown in brackets. Amphibian taxonomy follows Frost (2016). Nematodes were deposited in the Helminthological Collection of Museo de La Plata, La Plata, Argentina, and the Helminthological Collection of Centro de Ecología Aplicada del Litoral, Corrientes, Argentina. Symbiotypes are stored in the Herpetological Collection of Fundación Miguel Lillo.

Results

Aplectana nebulosa sp. nov.

(Figs. 1–2)

General morphology. Small, slender nematodes. Cuticle transversely striated. Slight sexual dimorphism, females larger than males (Fig. 1A, B). Narrow lateral alae present in both sexes, beginning at region of nerve ring and ending at the middle of tail in females and at level of first pair of precloacal papillae in males (Figs. 1E–1K). Numerous small somatic papillae present. Mouth with three lips; dorsal lip with two cephalic papillae and each ventrolateral lip with one cephalic papilla and one large amphid (Fig. 1C). Excretory pore anterior of oesophageal bulb (Fig. 1D).

Male (based on holotype and 15 paratypes): Total length 2.9 ± 0.41 mm (2.2–3.5mm) [2.9 mm]; maximum width 136.5 ± 25.4 (100–178) [125]. Pharynx length 40.7 ± 4.2 (35–49.5) [46], width 34.3 ± 5.1 (25–41) [37.7]; oesophagus: corpus length 320.2 ± 31.4 (280–392) [310] width 34.8 ± 5.3 (25–40) [25]; isthmus length 43.7 ± 8.4 (30–56.5) [30], isthmus width 23.8 ± 5.1 (18–35.5) [25]; bulb length 54.2 ± 9 (35.5–68) [60], bulb width 53.3 ± 5.8 (39–61) [55]. Nerve ring 223.0 ± 23.4 (175–255) [240] and excretory pore 331.7 ± 72.9 (191–502.5) [345], from anterior extremity, respectively. Tail length 167.9 ± 31.3 (105–220) [202.2]. Cloacal papillae arranged as follows (Fig. 1E, F): Two rows of 6–7 pairs of precloacal papillae, the last two pairs located laterally to the cloaca (Fig. 2A); 2 pairs of adcloacal papillae in anterior margin of cloaca and one middle, large unpaired papilla between the two anterior pairs (Fig. 2B); 5 pairs of postcloacal papillae (first and second pairs ventral, third pair lateral, fourth pair ventral, fifth pair lateral) (Fig. 2C); papillae pattern: 6–7:2:5+1. Spicules slightly equal, length 104.2 ± 12.4 (85–125.9) [110], pointed at distal end and with variably shaped capitulum (Fig. 1G). Gubernaculum length 52.2 ± 9.1 (37.4–62.9) [55], weakly sclerotized, ovate in ventral view (Fig. 1H).

Female (based on allotype and 13 paratypes): Total length 4.6 ± 0.28 mm (2.9–5.8 mm) [4.54 mm]; maximum width 200 ± 60.1 (100–298) [235]. Pharynx length 58.2 ± 15 (30–83) [70], pharynx width 49.2 ± 12.5 (28–72) [60];

FIGURE 1. *Aplectana nebulosa* sp. nov. from *P. nebulosum*. A. Female, general view. B. Male, General view. C. Male, anterior extremity, apical view. D. Male, anterior extremity, lateral view. E. Male, posterior extremity, ventral view. F. Male, posterior extremity, lateral view. G. Spicule, ventral view. H. Gubernaculum, ventral view. I. Female, vulva and ovejector, ventral view. J. Female, vulva and ovejector, lateral view. K. Female, posterior extremity, lateral view.

FIGURE 2. *Aplectana nebulosa* sp. nov. from *P. nebulosum*. A. Posterior end of male, detail of precloacal papillae. B. Posterior end of male, detail of adcloacal papillae and spicules. C. Posterior end of male, detail of postcloacal papillae. Abbreviations: ap: adcloacal papillae; up: unpaired papilla. In figure A, closed arrows indicate precloacal papillae; open arrows indicate lateral alae; in figure C, closed arrows indicate postcloacal papillae.

oesophagus: corpus length 395.4 ± 65.0 (277–485) [425], width 47.6 ± 13.2 (30–79.1) [60]; isthmus length 48.5 ± 5.3 (41–58) [50], isthmus width 29.5 ± 4.7 (21–38) [40], bulb length 68.4 ± 9.8 (52–84) [80], bulb width 62.9 ± 9.4 (48–75) [70]. Nerve ring 249.4 ± 40.3 (200–325) [285] and excretory pore 417.7 ± 70 (234–506) [450] from anterior extremity, respectively. Vulva post-equatorial, transverse slit, slightly salient, situated 1.7 ± 0.43 mm (1.0–2.5) [1.40 mm] from posterior extremity; muscular ovejector, directed anteriorly (Fig. 11, J), joining 2 uteri, 1

directed anteriorly, 1 posteriorly. Tail, conical, 261.7 ± 41.1 (200–343) [215] in length (Fig. 1K). Eggs, unembryonated, thin shelled oval, length 84.8 ± 15.0 (61.5–105) [90], width 67 ± 14.2 (49–90) [80].

Taxonomy summary

Site of infection: small and large intestine.

Type locality: Matagusanos, Ullúm Department, San Juan Province, Argentina (Fig. 3B).

Deposition of types: Holotype (male), allotype (female) and paratypes (2 males, 2 females) in Helminthological Collection of the Museum of La Plata (Holotype: MLP-He 7156; Allotype: MLP-He 7157; Paratypes: MLP-He 7158); Paratypes (5 males, 5 females) in Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL 16062601).

Symbiotype: *Pleurodema nebulosum* (Burmeister, 1861) (Anura, Leptodactylidae) (Fig. 3A), Herpetology Collection of Fundación Miguel Lillo (Deposited number FML 29422 to 29433).

Etymology. The new species is named in reference to its host.

Host-parasite data. Prevalence 58.5%, twenty-four of the frogs were found to harbour 397 nematodes.

Remarks. The genus *Aplectana* is characterized by possessing lateral alae and somatic papillae, males without rosettes or plectanes on the posterior end, and females with numerous thin-shelled eggs in the uteri, and 2 ovaries anterior to the vulva (Gibbons, 2010). Nematodes of this genus parasitize mainly amphibians and some species of reptiles worldwide (Baker, 1987).

The species of genus *Aplectana* are grouped mainly according to the presence and absence of gubernaculum; other features that distinguish these helminths are the number and arrangement of caudal papillae, and the size of the gubernaculum and spicules.

According to Bursey *et al.* (2011), there are 26 species reported for the Neotropical Realm, 19 of them with gubernaculum: *A. adaechevarriae* Ramallo, Bursey and Goldberg, 2008, *A. albae* Adamson and Baccam, 1988, *A. elenae* Baker and Vaucher, 1986, *A. hamatospicula* Walton, 1940, *A. herediaensis* Bursey, Goldberg and Telford, 2006, *A. hylambatis* (Baylis, 1927) Travassos, 1931, *A. incerta* Caballero, 1949, *A. itzocanensis* Bravo Hollis, 1943, *A. lopesi* Silva, 1955, *A. membranosa* (Schneider, 1866) Miranda, 1924, *A. mexicana* (Caballero, 1933) Ballesteros–Marquez, 1945, *A. micropenis* Travassos, 1925, *A. paraelenae* Baker and Vaucher, 1986, *A. pusilla* Miranda 1924, *A. raillieti* Travassos, 1925, *A. ryšavýi* Barus and Coy Otero, 1969, *A. travassosi* (Gomes and Motta, 1967) Baker, 1980, *A. tucumanensis* Ramallo, Bursey and Goldberg, 2008, and *A. vellardi* Travassos, 1926.

Apart from this, two groups can be identified, following the characteristics of caudal papillae: those with a large unpaired papilla anterior to the cloaca, and those without this character. Of the above mentioned species with gubernaculum, eleven have an unpaired papilla: *A. albae*, *A. elenae*, *A. hamatospicula*, *A. hylambatis*, *A. itzocanensis*, *A. membranosa*, *A. paraelenae*, *A. raillieti*, *A. ryšavýi*, *A. travassosi*, and *A. vellardi*.

The new species described here belongs to the group of species that possess both a gubernaculum and an unpaired papilla anterior to the cloaca, being more related to *A. membranosa*, *A. paraelaenae* and *A. travassosi* for possessing four adcloacal papillae, pre-oesophageal excretory pore, males with tail tapering gradually to spike-like distal portion, and gubernaculum elongated, with posterior end pointed and thickened borders.

However, the new species differs from *A. membranosa* by the following characters: 1) number of precloacal papillae (6–7 pairs vs. 3 pairs); 2) number and arrangement of postcloacal papillae (1° and 2° ventral, 3° lateral, 4° ventral, 5° lateral vs. 1° and 2° ventral, 3° lateral, 4° ventral); 3) shape and size of spicules (no forked spicules, 85–125.9µm vs. forked spicules, 227–234.3µm).

Aplectana nebulosa **sp. nov.** can also be distinguished from *A. paraelenae* by the following characters: 1) number of precloacal papillae (6–7 pairs vs. 3 pairs, respectively); 2) number and arrangement of postcloacal papillae (1° and 2° ventral, 3° lateral, 4° ventral, 5° lateral vs. 1°, 2°, 3° subventral, 4° and 5° subdorsal, 6° lateral); 3) size of spicules (85–125.9 µm vs. 227µm); 4) position of end of lateral alae in males (precloacal, at the level of 3° pair of precloacal papillae vs. postcloacal, at middle of tail).

Finally, *Aplectana nebulosa* **sp. nov.** differs from *A. travassosi* by the following characters: 1) number of precloacal papillae (6–7 pairs vs. 3 pairs); 2) number and arrangement of postcloacal papillae (1° and 2° ventral, 3° lateral, 4° ventral, 5° lateral vs. 1° pair ventral, 2°, 3° and 4° subventral); 3) size of spicules (85–125.9µm vs. 230–245µm); 4) size of gubernaculum (37.4–62.9µm vs. 80–95µm).

We provide a key for the species of *Aplectana* from amphibians and reptiles from Neotropical Realm.

FIGURE 3. A. Adult male of *Pleurodema nebulosum* from Matagusanos. B. Panoramic view of type locality.

Key to Neotropical species of *Aplectana*

1. a. Species with gubernaculum 2
b. Species without gubernaculum 22
2. a. Numerous caudal papillae, variable in number and distribution and indistinguishable from somatic papillae. Anura and Squamata *A. macintoshii* (Steward, 1914) Travassos, 1931
b. Caudal papillae regularly distributed and distinguishable from somatic papillae 3
3. a. Males with unpaired papilla on anterior lip of cloaca 4
b. Males without unpaired papilla on anterior lip of cloaca 14
4. a. Males without adcloacal papillae 5
b. Males with adcloacal papillae 9
5. a. Short gubernaculum ($\leq 32\mu\text{m}$); vulva located at middle of total length of body 6
b. Long gubernaculum ($> 32\mu\text{m}$); post-equatorial vulva 7
6. a. Gubernaculum very short ($12\mu\text{m}$); spicules less than $200\mu\text{m}$ with a basal process approximately one third of total length; two pairs of precloacal papillae and three pairs of postcloacal papillae. Anura *A. vellardi* Travassos, 1926
b. Gubernaculum longer than $12\mu\text{m}$ ($32\mu\text{m}$); spicules longer than $200\mu\text{m}$ without basal process; eight pairs of precloacal papillae and eight pairs of postcloacal papillae. Male tail with short spike-like point. Squamata
. *A. raillieti* Travassos, 1925
7. a. Four pair of precloacal papillae; large spicules ($>230\mu\text{m}$) with unequal distal projections, bluntly pointed, surrounded by a membranous sheath. Anura *A. hamatospicula* Walton, 1940
b. Eight pairs of precloacal papillae; five pairs of postcloacal papillae; spicules less than $230\mu\text{m}$ with blunt capitulum and sharply pointed distal extremity surrounded by transparent rounded sheath 8
8. a. Males and females with maximum length of 2.53 and 3.88mm, respectively (total length is approximately half of that of *A. itzacanensis*); spicules and gubernaculum short ($\leq 143\mu\text{m}$ and $\leq 63\mu\text{m}$, respectively); vulva with papillae. Anura
. *A. incerta* Caballero, 1949
b. Males and females with minimum length of 2.55 and 3.50mm, respectively (total length is approximately twice that of *A. incerta*); spicules and gubernaculum large ($>172\mu\text{m}$ and $>67\mu\text{m}$, respectively); six pairs of postcloacal papillae; vulva without

	papillae. Anura.	<i>A. itzocanensis</i> Bravo Hollis, 1943
9.	a. Vulva with 2–3 mamelon-like protuberances; distal end of spicules covered by a prominent hook-shaped membrane approximately 40–50µm long and usually directed laterally. Anura.	<i>A. hylambatis</i> (Baylis, 1927) Travassos, 1931
	b. Vulva without mamelon-like protuberances; spicules not covered by a hook-shaped membrane.	10
10.	a. Spicules longer than 200µm; three pairs of precloacal papillae.	11
	b. Spicules less than 200µm; more of three pairs of precloacal papillae; five pairs of postcloacal papillae.	12
11.	a. Vulva non-salient; gubernaculum short (53µm); spicules with sharply pointed distal extremity and blunt proximal extremity thickened on dorsal side; five pairs of subventral muscle cells located anterior to cloaca. Anura.	<i>A. paraelenae</i> Baker and Vaucher, 1986
	b. Vulva weakly protruding; gubernaculum longer than 53µm (70–95µm); spicules prominent, curved ventrally at midportion, with blunt capitulum and sharply pointed distal extremity; twelve to thirteen pairs of slender subventral muscle cells located anterior to cloaca. Anura.	<i>A. travassosi</i> (Gomes and Motta, 1967) Baker, 1980
12.	a. Eight pairs of precloacal papillae; only one pair of adcloacal papillae; spicules with markedly large proximal knob and tapering distally into finely attenuated point; lateral alae in males extending beyond cloaca. Anura.	<i>A. elenae</i> Baker and Vaucher, 1986
	b. Precloacal papillae less than 7 pairs; two-four pairs of adcloacal papillae; spicules simple; lateral alae in males ending before cloaca.	13
13.	a. Four pairs of precloacal papillae; spicules less than 85µm (57–63µm) without capitulum; vulva non-salient. Squamata.	<i>A. albae</i> Adamson and Baccam, 1988
	b. Six or seven pair of precloacal papillae; spicules longer than 85µm with capitulum at proximal end; vulva slightly salient. Anura.	<i>Aplectana nebulosa</i> sp. nov.
14.	a. Without adcloacal papillae.	15
	b. With variable number of adcloacal papillae.	17
15.	a. Gubernaculum very short (10µm), crescent-shaped with concavity towards the proximal edge, and a small appendix on the distal convex edge; only one pair of postcloacal papillae; tail of male conical and sharply-pointed; vulva medial; tail of female very long (510µm). Anura.	<i>A. micropenis</i> Travassos, 1925
	b. Gubernaculum longer than 10µm without the abovementioned characteristics; more of one pair of postcloacal papillae; vulva postequatorial; tail of female less than 165µm.	16
16.	a. Long spicules (450µm); three pair of precloacal papillae and five pair of postcloacal papillae; vulva non salient. Gymnophiona.	<i>A. mexicana</i> (Caballero, 1933) Ballesteros-Marquez, 1945
	b. Spicules less than 79µm; nine pairs of precloacal papillae and three pairs of postcloacal papillae; vulva weakly protruding. Squamata.	<i>A. herediaensis</i> Bursey, Goldberg and Telford, 2006
17.	a. Spicules and gubernaculum very long (720µm and 110µm, respectively); female with conical posterior end with three small papillae. Squamata.	<i>A. ryšavýi</i> Barus and Coy Otero, 1969
	b. Spicules and gubernaculum less than 720µm and 110µm, respectively; posterior end of females without papillae.	18
18.	a. Numerous pairs of precloacal papillae (±18 pairs) and 7 pairs of postcloacal papillae; female with posterior end of body rounded, ending in evident slender filament. Anura.	<i>A. lopesi</i> Silva, 1955
	b. Fewer precloacal papillae (9 pairs or less) and less than 7 pairs of postcloacal papillae; posterior end of females without slender filament.	19
19.	a. One pair of adcloacal papillae and six pairs of postcloacal papillae.	20
	b. More than one pair of adcloacal papillae and less than 6 pairs of postcloacal papillae.	21
20.	a. Vulva with 3 mamelon-like protuberances; spicules with blunt capitulum and distal extremity covered by a hook-shaped membrane; eight pairs of precloacal papillae; spicules large (>250µm). Anura.	<i>A. adachevarriae</i> Ramallo, Bursey and Goldberg, 2008
	b. Vulva non-salient without mamelon-like protuberances; spicules without capitulum and without hook-shaped membrane; 9 pairs of precloacal papillae. Squamata.	<i>A. tucumanensis</i> Ramallo, Bursey and Goldberg, 2008
21.	a. Two pairs of precloacal papillae; gubernaculum and spicules long (120–150µm and 320–520µm, respectively); spicules alate and with pointed distal extremity, not bifurcated; 3 toothlike projections in oesophagus. Caudata.	<i>A. uaehi</i> Falcon-Ordaz, Monks, Pulido-Flores and Rodríguez-Amador, 2014*
	b. Five pairs of precloacal papillae; gubernaculum and spicules short (71µm and 227–234µm, respectively); spicules not alate and with distal bifurcated extremity; without toothlike projections in oesophagus. Anura.	<i>A. membranosa</i> (Schneider, 1866) Miranda, 1924
22.	a. Males without unpaired papilla on anterior lip of cloaca.	23
	b. Males with unpaired papilla on anterior lip of cloaca.	24
23.	a. Twelve pairs of precloacal papillae, without adcloacal papillae, six pairs of postcloacal papillae; tail long, slender and sharply pointed in both sexes; in males, the lateral alae extend beyond the cloaca where tail begins to taper. Anura.	<i>A. chilensis</i> Lent and Freitas, 1948
	b. Six pairs of precloacal papillae, one pair of adcloacal papillae, four pairs of postcloacal papillae; tail long, slender and sharply pointed only in males. Anura.	<i>A. crucifer</i> Travassos, 1925
24.	a. Males with adcloacal papillae.	25
	b. Males without adcloacal papillae.	27
25.	a. Numerous pairs of precloacal papillae (>14 pairs); one pair of adcloacal papillae; spicules longer than 300µm.	26
	b. Seven pairs of precloacal papillae; two pairs of adcloacal papillae; spicules shorter than 300µm (130–136µm); tail long, slender and sharply pointed in both sexes. Anura.	<i>A. meridionalis</i> Lent and Freitas, 1948

26. a. Fourteen pairs of precloacal papillae in two longitudinal rows; spicules less than 400µm (322–364µm); tail long, slender and sharply pointed in both sexes; distance from anus to posterior end in females longer than 650µm (690–952µm). Anura.
..... *A. artigasi* Puga and Torres, 1997
b. Variable number of precloacal papillae in four longitudinal rows; spicules 400µm long; distance from anus to posterior end in females less than 650µm (332–380µm). Squamata *A. papillifera* (Araujo, 1977) Baker, 1980
27. a. Numerous pairs of precloacal papillae (>20 pairs); spicules longer than 130µm; posterior end in females tapering abruptly in proximal third, becoming spike-like distal portion. Anura. *A. delirae* (Fabio, 1971)
b. Only one pair of precloacal papillae; spicules less than 130µm; tail of female conical. Anura.
..... *A. tarija* Ramallo, Bursey and Goldberg, 2007

* This record was made in a restricted area of the Mexican Transition zone between the Nearctic and Neotropical Regions.

Discussion

Up to the present, seven species of *Aplectana* have been reported from Argentina: *A. adaechevarriae*, *A. delirae*, *A. hylambatis*, *A. meridionalis*, *A. nebulosa* **sp. nov.**, *A. tarija*, and *A. tucumanensis*. Only *A. tucumanensis* was found in *Amphisbaena bolivica* Mertens, 1929, an amphisbaenid (Squamata) host (Ramallo *et al.*, 2008a), while the other species were found in leptodactylid and bufonid anurans. Three of these species were first described from amphibians collected in Argentina: *A. adaechevarriae* was described from *Rhinella granulosa* (Müller and Hellmich, 1936) and *R. schneideri* (Werner, 1894) (Ramallo *et al.*, 2008b), *A. tarija* from *R. arenarum* (Hensel, 1867) (Ramallo *et al.*, 2007), and the new species, *A. nebulosa* **sp. nov.** was described from *P. nebulosum*. All these species have a limited geographical distribution and have only been found in their respective type-host species. The rest of the abovementioned species, *A. hylambatis*, *A. delirae*, and *A. meridionalis*, were described from amphibian hosts from different countries that have wider geographical distributions. *Aplectana delirae* was described from *Rhinella ornata* (Spix, 1824) (= *Bufo crucifer*) from Rio de Janeiro, Brazil (Fabio, 1971), and was also found in Argentina in *Rhinella major*, *Leptodactylus chaquensis* Cei, 1950, and *L. elenae* Heyer, 1978 (González and Hamann, 2006; 2016; Schaefer *et al.*, 2006), *Aplectana meridionalis* was described from *Odontophrynus americanus* (Duméril and Bibron, 1841) (= *Ceratophrys americana*) from Montevideo, Uruguay (Lent and Freitas, 1948), and reported in Argentina from *Pleurodema borellii* (Baker, 1980). Finally, *A. hylambatis* was originally described from *Leptopelis aubryi* (Duméril, 1856) from Guinea, Africa (see Baker and Vaucher, 1986), and recorded in Argentina in several hosts: *Rhinella achalensis* (Cei, 1972) (= *Bufo achalensis*), *R. arenarum*, *R. major*, *Leptodactylus latinasus* Jiménez de la Espada, 1875, *L. chaquensis*, *L. bufonius* Boulenger, 1894, *Physalaemus santafecinus* Barrio, 1965, and *Trachycephalus venulosus* (Linnaeus, 1758) (González and Hamann, 2015; 2016; Hamann and González, 2015; Draghi *et al.*, 2015).

The frog genus *Pleurodema* is distributed from Panama throughout South America, reaching southern Chile and Argentina, and is currently represented by fifteen species (Faivovich *et al.*, 2012). Helminth parasites in anurans of this genus have been reported from four countries: the nematodes *Aplectana chilensis* Lent and Freitas, 1948, *Rhabdias* sp., and *Oswaldocruzia* sp. were found in *Pleurodema thaul* from Chile (Benavidez *et al.*, 1996), the nematodes *Aplectana hylambatis* and *Oxyascaris* sp. were found in *P. marmoratum* from Peru (Sarmiento *et al.*, 1999), the nematodes *Raillietnema spectans* Gomes, 1964 and *Oxyascaris oxyascaris* Travassos, 1920 (= *Oxyascaris necopinus* Freitas, 1958; see Fabio, 1980), and plerocercoid larvae of the family Proteocephalidae have been found in *P. diplolister* Peters, 1870 from Brazil (Freitas, 1958; Fabio, 1980; Teles *et al.*, 2015), and the monogenean *Polystoma borellii* Combes and Laurent, 1974, and the nematode *Aplectana meridionalis* Lent and Freitas, 1948, found in *P. borelli* (Peracca, 1895) from Argentina (Baker, 1980; Combes and Laurent, 1974). Also, the acanthocephalan, *Pseudoacanthocephalus lutzi* (Hamann, 1891) Arredondo and Gil de Pertierra, 2009 (locality not reported), was found in *P. bibroni* Tschudi, 1838 (Cordero, 1933).

This study represents the first report of nematode parasites in *P. nebulosum*, and *Aplectana nebulosa* **sp. nov.** represents the 28 species assigned to the genus from the Neotropical Realm. More work will be required to determine if the new species is a common component of the helminth community of amphibians from deserts.

Acknowledgments

We would like to thank to Lorena B. Quiroga for field support and critical reading of the manuscript. We thank the

provincial fauna office of San Juan for permission (SA y DS n° 1300–4736–2011) to carry out this research. This research was partially supported by CICITCA–UNSJ–F1003 to EAS.

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