

Taxonomy and systematics

A new species of *Aplectana* (Nematoda: Cosmocercidae) in *Amphisbaena darwinii* (Squamata: Amphisbaenidae) from Argentina

Nueva especie de Aplectana (Nematoda: Cosmocercidae) en *Amphisbaena darwinii* (Squamata: Amphisbaenidae) de Argentina

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Abstract

In the present study, we describe and illustrate *Aplectana nananae* n. sp. from the large intestine of *Amphisbaena darwinii*. *Aplectana nananae* n. sp. is the second species described in Argentinian amphisbaenian hosts and the 60th species assigned to the genus. This species differs from its congeners by a set of unique characters in males: the presence of 1 unpaired preanal papilla and gubernaculum, the papillae pattern (8+1:2:2) and total number (24+1), very conspicuous and prominent excretory pore, located anterior to the esophageal bulb and the size of spicules (60-70 μ m).

Keywords: *Aplectana nananae* n. sp.; Nematoda; Squamata; Argentina; Amphisbaena

Resumen

En el presente estudio, se describe e ilustra a *Aplectana nananae* n. sp. localizada en el intestino grueso de *Amphisbaena darwinii*. *Aplectana nananae* n. sp. es la segunda especie descrita en anfisbenas argentinas y la 60^{ava} especie válida del género. Esta especie se diferencia de las otras por las siguientes características de los machos: la

presencia de una papila impar preanal y gubernaculum, el patrón de papilas caudales (8+1:2:2) y número total (24+1), el poro excretor que es muy conspicuo y prominente, situado anterior al bulbo esofágico y por la longitud de las espículas (60-70 µm).

Palabras clave: *Aplectana nananae* n. sp.; Nematoda; Squamata; Argentina; Anfisbenas

Introduction

Members of the Amphisbaenidae, includes 11 valid genera and more than 160 species, are moderate-sized limbless burrowing lizards that occur in the Greater Antilles, South America and Africa (Vitt & Caldwell, 2014). Twelve species of Amphisbaenidae have been reported from Argentina (Montero & Autino, 2018).

Amphisbaena darwini Duméril & Bibron, 1839 has a wide distribution in Argentina, its conservation status is not threatened, but is absent from 7 provinces: Chubut, Entre Ríos, La Rioja, Neuquén, San Juan, Santa Cruz and Tierra del Fuego (Abdala et al., 2012). Adults range from 250 to 300 mm SVL (Ceí, 1986; Gallardo, 1967; Scrocchi & Giraud, 2005). They reproduce from June to January; are oviparous and lay 2 to 3 eggs; and they have gregarious habits (Ceí, 1986; Gallardo, 1967). Specimens of *A. darwini* are carnivorous, opportunistic predators of underground fauna, although some species feed on vertebrates (Montero, 2019), insects (Cabrera & Merlini, 1990; Gallardo, 1967), and annelids (Autino & Montero 2012; Cusumano & Powell, 1991; Semhan et al., 2010), among other animal groups.

The Cosmocercoidea are mainly parasites of vertebrates and presumably evolved in amphibians and reptiles (Vanderburgh & Anderson, 1987). Studies focusing on helminth parasites of amphisbaenian hosts in the Neotropical region are scarce, with 6 species of *Aplectana* occurring in Neotropical amphisbaenians (Amorim et al., 2017; Ávila & Silva, 2010; Vieira et al., 2020): in Brazil, *Aplectana albae* Adamson & Baccam, 1988 was reported in *Amphisbaena alba* Linnaeus, 1758 and *Amphisbaena ridleyi* Boulenger, 1890, *Aplectana raillieti* Travassos, 1925 in *Amphisbaena alba*, *Aplectana nordestina* Amorim, Da Silva, Morais, Da Silva, Ávila, 2017 in *Leposternon polystegum*, *Aplectana pusilla* (Miranda, 1924) Adamson & Baccam, 1988 in *Amphisbaena* sp., and *Aplectana minaensis* Vieira, Araujo Gonçalves, De Souza Lima, De Sousa & Muniz-Pereira, 2020 in *Amphisbaena alba*. *Aplectana unguiculata* Rudolphi, 1819 in *Amphisbaena* sp. is a species *inquirendae* (Ávila & Silva, 2010). In Argentina, *Aplectana tucumanensis* Ramallo, Bursey & Goldberg, 2008 was found in *Amphisbaena bolivica* Mertens, 1929 (Ramallo et al., 2008). It should be noted that in Argentina, in addition to *A. tucumanensis*, *A. nebulosa*

was recorded in *Pleurodema nebulosum* (Burmeister, 1861) (Anura: Leptodactylidae) from Corrientes Province (Gomez et al., 2017).

This study describes a new species of *Aplectana* parasitizing *A. darwini*, from the municipality of Tañi Viejo, Tucumán Province, Argentina.

Materials and methods

The nematodes were collected from the large intestine of one specimen of *A. darwini* collected in December 2021, from Los Nogales, municipality of Tañi Viejo, Tucumán Province, Argentina; later it was fixed in neutral buffered 10% formalin before preservation in 70% ethanol. The amphisbaenian was identified according to Ceí (1986) and deposited in the Colección de Herpetología (FML), Fundación Miguel Lillo, San Miguel de Tucumán, Argentina.

Nematodes were isolated from the large intestine by utilizing a stereomicroscope, fixed in 5% formaldehyde solution and preserved in 70% ethanol. Nematodes were cleared with lactophenol, and examined under a light microscope. Drawings were made using a camera lucida. All the measurements are given in millimeters unless otherwise stated in the form: mean ± standard deviation (minimum value-maximum value); holotype and allotype measurements shown separately (Table 1). Some specimens were dehydrated throughout an ethanol series, acetone and ether, coated with gold and examined in a scanning electron microscope Zeiss Supra 55VP SEM.

Nematodes were identified to genus according to Anderson et al. (2009). Holotype, allotype and paratypes were deposited in the Colección de Invertebrados (Sección Helmintos y Anélidos) (CH-N-FML), Fundación Miguel Lillo, San Miguel de Tucumán, Argentina.

Description

Aplectana nananae n. sp. Ramallo, Goldberg, Ruiz, 2023 (Figs. 1 - 3)
<http://zoobank.org/urn:lsid:zoobank.org:act:874E0C90-CF42-48CD-B500-6AE96A4D4491>

Small nematodes. Sexual dimorphism evident, with males slightly smaller than females. Lateral alae present in both sexes (0.03 width); beginning anterior to the nerve ring and terminating posterior to the anus in males,

Table 1

Measurements of *Aplectana nananae* n. sp.; holotype (H), allotype (A), paratypes (P).

	Males		Females	
	H	P	A	P
Body length	2.90	2.50 ± 0.12 (2.42-3.00)	3.48	3.41 ± 0.20 (2.88-3.73)
Body width	0.21	0.23 ± 0.03 (0.20-0.30)	0.31	0.32 ± 0.02 (0.27-0.34)
Oesophagus total length	0.68	0.70 ± 0.07 (0.51-0.80)	0.70	0.70 ± 0.04 (0.59-0.75)
Pharyngeal part length	0.06	0.05 ± 0.004 (0.05-0.06)	0.05	0.05 ± 0.003 (0.05-0.06)
Oesophagus bulb length	0.12	0.11 ± 0.02 (0.08-0.14)	0.15	0.14 ± 0.02 (0.10-0.16)
Oesophagus bulb width	0.10	0.10 ± 0.01 (0.08-0.11)	0.11	0.12 ± 0.01 (0.10-0.15)
Nerve ring from anterior end	0.28	0.24 ± 0.02 (0.20-0.28)	0.23	0.25 ± 0.03 (0.23-0.33)
Excretory pore from anterior end	0.64	0.60 ± 0.03 (0.54-0.64)	0.66	0.60 ± 0.03 (0.56-0.66)
Spicule length	0.06	0.07 ± 0.003 (0.06-0.07)	--	--
Gubernaculum length	0.03	0.03 ± 0.003 (0.04-0.04)	--	--
Number and position of caudal papillae	8 + 1:2:2	8 + 1:2:2	--	--
Vulva from posterior end	--	--	1.13	1.20 ± 0.04 (1.13-1.25)
Egg length	--	--	0.10	0.10 ± 0.01 (0.08-0.11)
Egg width	--	--	0.05	0.05 ± 0.004 (0.05-0.06)
Tail	0.10	0.12 ± 0.02 (0.10-0.15)	0.16	0.20 ± 0.03 (0.15-0.25)

and anterior to the anus in females (Fig. 2A, F). Cuticle transversely striated (Fig. 2B, D). Somatic papillae are observed irregularly distributed from the level of the nerve ring to the caudal region in both sexes (Figs. 2B, G, 3C, D). Mouth opening triangular, surrounded by distinct triangular lips. Dorsal lip with 2 large apical sessile papillae; each of the lateroventral lips with large apical ventral sessile papilla, and lateral amphid of circular shape (Figs. 1C, 2B, E). Oesophagus with oesophageal bulb (Fig. 1A, B). Excretory pore located anterior to oesophageal bulb (Figs. 1A, B, 2C, D), very conspicuous and prominent. Nerve ring anterior to the excretory pore, at level of oesophageal corpus (Fig. 1A, B).

Adult males (10 specimens). Caudal papillae preanal: 7 pairs of ventral precloacal papillae, 1 pair lateroventral and large unpaired triangular papilla anterior margin of anus; adanal papillae: 2 ventral pairs; and postanal papillae: 1 ventral pair and 1 lateroventral. Papillae pattern: 8+1:2:2 (Figs 1D; 3A- E). Spicules equal and well developed, with a curved tip at the distal end. Gubernaculum weakly sclerotized, with rhomboidal shape (Fig. 1E).

Gravid females (10 specimens). Vulva post equatorial, muscular ovjector directed anteriorly (Fig 1B). Two prodelphic ovaries. Uterus with non-larvated eggs, with thin shelled oval (Fig 1G). Tail conical slender and sharply pointed (Fig. 1F).

The morphometry of the new species is provided in Table 1.

Taxonomic summary

Type host: *Amphisbaena darwinii* Duméril & Bibron, 1839 (Squamata: Amphisbaenidae) (red worm lizard, cobra de duas cabeças), FML# 31243.

Site of infection: large intestine

Intensity of infection: 121 specimens (54 females, 67 males).

Type locality: gated community Los Nogales (26°42.248' S, 65°12.750' W; 595 m asl), Municipality of Tafi Viejo, Tucumán Province, Argentina.

Type specimens: holotype male: CH-N-FML#07812. Allotype female: CH-N-FML#07813. Paratypes: CH-N-FML#07814 (30 males and 30 females).

Etymology: the specific epithet is named in honor of the nickname “Nanana” of Nilda Vázquez de Ramallo, mother of the first author of this paper.

Remarks

Species of *Aplectana* mainly parasitize amphibians and some species of reptiles worldwide. We assigned the nematodes of the current study to the genus *Aplectana* due to the following characteristics: well developed lateral alae, numerous minute somatic papillae, 3 large

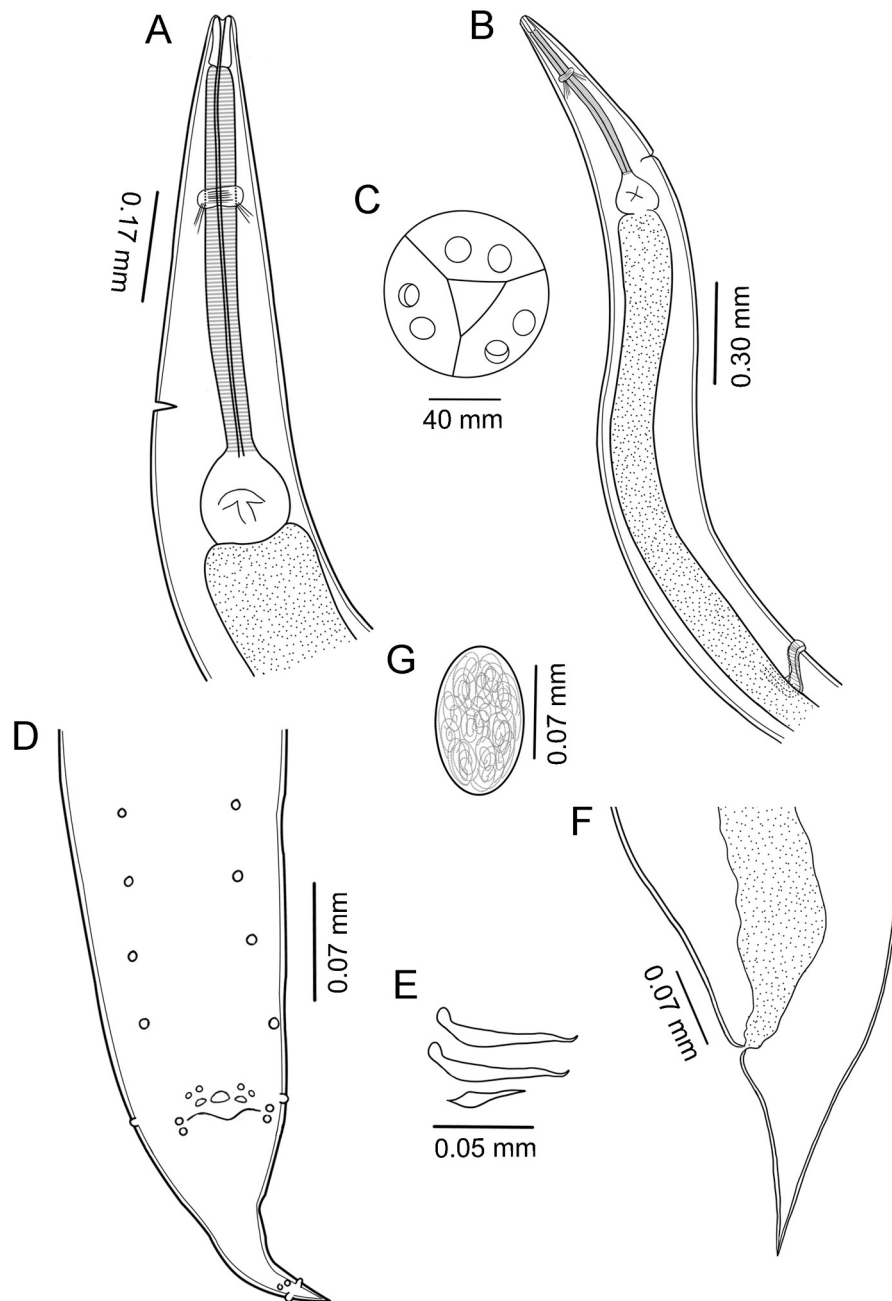


Figure 1. *Aplectana nananae* n. sp. A, Anterior region of body, male, lateral view; B, anterior region of body, female, lateral view; C, anterior end, female, apical view; D, posterior region, male, ventral view; E, spicules and gubernaculum; F, tail, female, lateral view; G, egg.

lips with 4 submedian large cephalic papillae, 1 pair of lateral amphids, an oesophagus with a short cylindrical pharynx, cylindrical corpus and oesophageal bulb, and an excretory pore anterior to the oesophageal bulb. The prodelphic females with many eggs in the uteri and contain ovaries anterior to the vulva, and males present 2 equal

spicules and the tail of males without rosettes or plectanes (Gibbons, 2010; Vieira et al., 2020).

The *Aplectana* species are differentiated by the presence or absence of an unpaired papilla on the cloacal anterior border in males, and the presence or absence of a gubernaculum. The species that present both a

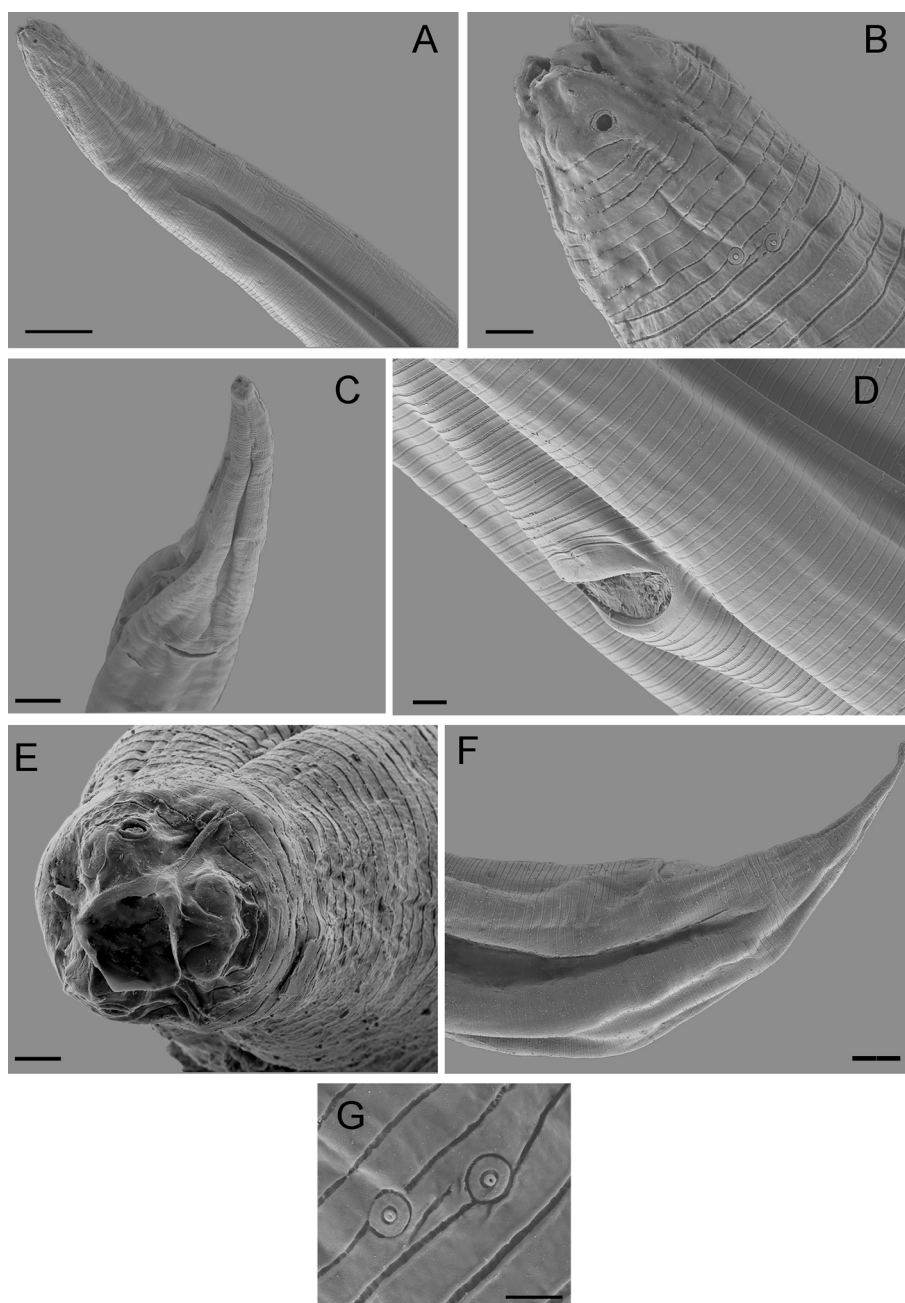


Figure 2. *Aplectana nananae* n. sp. A, Anterior region of body, female, lateral view, scale = 30 μm ; B, anterior end, female, lateroventral view, scale = 4 μm ; C, anterior end, female, ventral view, scale = 40 μm ; D, excretory pore, lateroventral view, scale = 8 μm ; E, female, apical view, scale = 4 μm ; F, posterior region, female, ventral view, scale = 20 μm ; G, detail of somatic papillae, scale = 2 μm .

gubernaculum and an unpaired papilla on the anterior border of the anus are: *A. chamaeleonis* (Baylis), *A. capensis* Baker, *A. degraaffi* Baker, *A. courdurieri* Chabaud & Brygoo, *A. albae* Adamson & Baccam, *A. elenae* Baker & Vaucher, *A. membranosa* (Schneider), *A.*

paraelenae Baker & Vaucher, *A. raillieti* Travassos, *A. zweifeli* Moravec & Sey, *A. leesi* Hristovki & Riggio, *A. linstowi* Yorke & Maplestone, *A. hamatospicula* Walton, *A. nebulosa* Gomez, González & Sanabria, *A. minaensis* Vieira, Araujo Gonçalves, De Souza Lima, De Sousa &

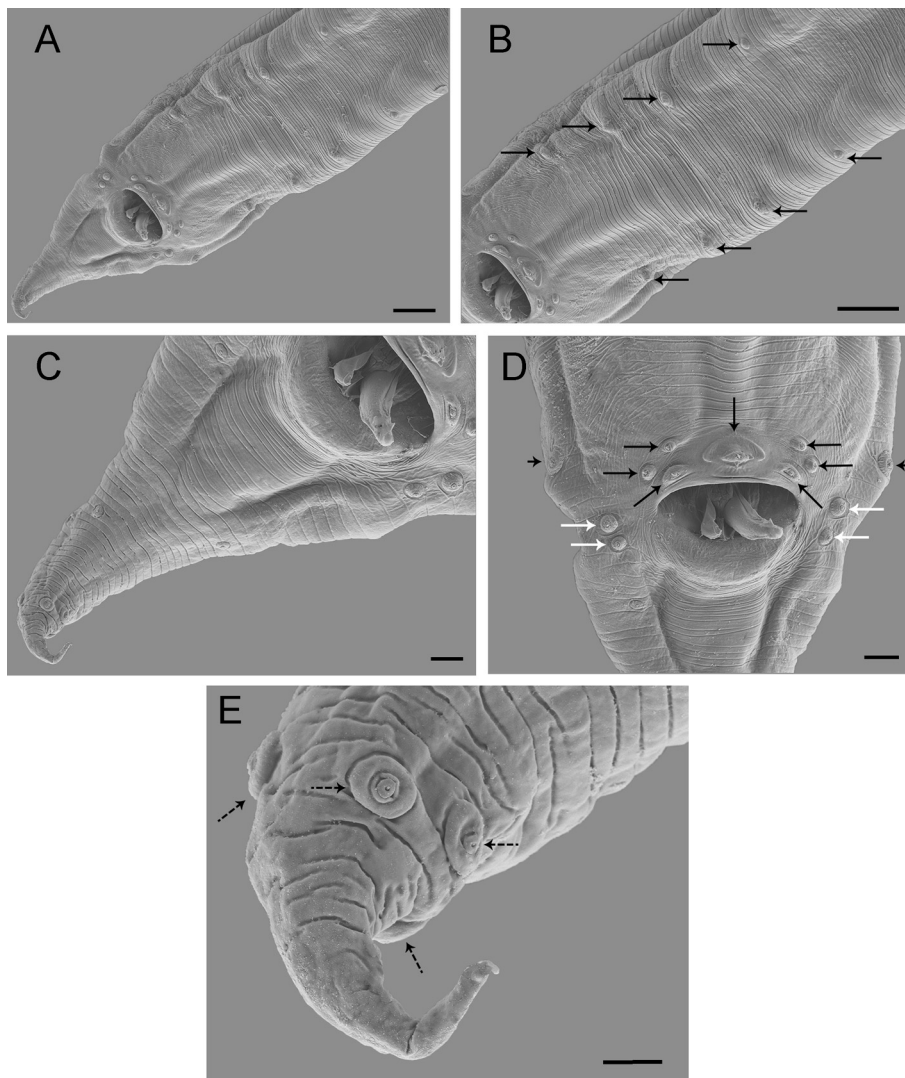


Figure 3. *Aplectana nananae* n. sp. A, Posterior region of body, male, ventral view, scale = 20 μ m; B, male, ventral view, preanal papillae (black arrows), scale = 20 μ m; C, male, ventral view, adanal papillae (white arrows), scale = 4 μ m; D, male, ventral view, preanal and adanal papillae, scale = 6 μ m; E, male, lateroventral view, postanal papillae (discontinuous white arrows), scale = 2 μ m.

Muniz-Pereira and *Aplectana nananae* n. sp. (Vieira et al., 2020; current study). However, the size of male body structures and the number and arrangement of caudal papillae can also differentiate these nematodes (Burse et al., 2018a, b).

Currently, 59 nominal species of *Aplectana* are recognized (Vieira et al., 2020). We can separate the *Aplectana* species presenting gubernaculum and unpaired precloacal papillae in 2 groups: species with less than 20 pairs of caudal papillae and those with more than 20 pairs. In relation to this, 2 of the 3 species recorded in Argentina: *A. nebulosa* in *Pleurodema nebulosum* (Burmeister, 1861)

(Anura: Leptodactylidae) from Corrientes (Gomez et al., 2017) and *A. nananae* n. sp. in *A. darwini* from Tucumán, belong to the first group, since it presents 13-14+1 and 12+1 pairs of caudal papillae, respectively. *Aplectana tucumanensis* Ramallo, Bursey & Goldberg, 2008 described in *Amphisbaena bolivica* Mertens (Amphisbaenidae) from the Province of Tucumán, Argentina, does not contain an unpaired preanal papilla. Likewise, *Aplectana minaensis* in *Amphisbaena alba* L., 1758 (Squamata: Amphisbaenidae) from Brazil (Vieira et al., 2020) also presents unpaired precloacal papillae, a gubernaculum and a posterior region with less than 20 pairs of caudal papillae.

However, *A. nebulosa* differs from *A. nananae* in the number of pairs of caudal papillae in males (13-14+1 vs. 12+1, respectively); the number and arrangement of postanal papillae: 5 pairs: 1st and 2nd ventral, 3rd lateral, 4th ventral, 5th lateral vs. 2 pairs: 1st ventral and 2nd lateral; and by the size of the spicules (85 - 126 vs. 60 - 70 μm). If we compare *Aplectana minaensis*, recorded in Brazil, with *A. nananae* n. sp. they are similar in the number of pairs of caudal papillae 12+1; but they differ in the number and arrangement of the postanal papillae (6 pairs of papillae: the 1st to 4th pair ventral, the 5th laterodorsal, the 6th ventral vs. 2 pairs: the 1st ventral and the 2nd ventral, respectively) and the size of the spicules (413 - 600 vs. 60 - 70 μm , respectively).

Regarding the somatic papillae of the caudal region of males, they are often confused with caudal papillae, especially with the postanal papillae. However, we consider that the somatic papillae differ from the caudal papillae because they are more flattened, small and odd (they are observed from only one side). Therefore, *A. nananae* n. sp. does not resemble any other congeneric species that occurs in amphisbaenians; it is also the 7th species recorded in the Neotropical Realm and the third described from Argentina. The Neotropical Realm contains the highest *Aplectana* richness, totalling 24 species. Fifteen species of *Aplectana* were described over the past 30 years, from which 9 occur in the Neotropical region and 6 in the Oriental region. These data demonstrate the great potential for discovering new species in these 2 biogeographic regions (Vieira et al., 2020; current study).

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