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A NEW SPECIES OF *CYCLOBULURA* (NEMATODA: SUBULURIDAE) FROM *ZAEDYUS PICHIY* AND *CHAETOPHRACTUS VELLEROSUS* (XENARTHRA: DASYPODIDAE) IN ARGENTINA

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ABSTRACT: *Cyclobulura superinae* n. sp. collected from *Zaedyus pichiy* and *Chaetophractus vellerosus* is herein described as the second species in *Cyclobulura* Quentin, 1977, and the first subulurid in armadillos. The species is unique in the spur-like structures present at the tip of both spicules, yet they conform to the description of *Cyclobulura* in the structure of the buccal parts. Specimens of the new species show longer chordal lobes and more conspicuous radial lobes and are smaller than specimens of *C. lainsoni*. In addition, males of *C. superinae* exhibit a spur-like process in the distal end of the spicules and a shorter tail (180 vs. 300 μm) with no spine. Finally, the eggs of *C. superinae* are smaller (52–92 × 41–78 vs. 95–100 × 80–85). To our knowledge, the new species is the first subulurid nematode found in an armadillo.

Armadillos (Cingulata), anteaters, and sloths (Pilosa) constitute the Xenarthra, the basal group of the 4 major clades of placental mammals (Delsuc et al., 2002) and 1 of the most ancient groups represented in the Neotropical Region (Redford and Eisenberg, 1992). More than 21 species of armadillos are present in Argentina alone, occupying different habitats such as the Neotropical forest and the puna, chaco, and semiarid areas in Patagonia (Wetzel, 1985). Several species of parasites, mainly nematodes, have been described from armadillos collected in Argentina (Navone and Lombardero, 1980; Chabaud et al., 1983; Navone, 1983, 1986, 1987a, 1987b; Notarnicola and Navone, 2003). The correlation among helminth communities and food habits of armadillos from Argentina has been studied in detail (Navone, 1990).

The pichi, *Zaedyus pichiy* (Desmarest, 1804) (Dasypodidae), is a small armadillo that inhabits arid and semiarid areas of Argentina and Chile (Wetzel, 1985). This species is omnivorous and opportunistic in its feeding habits (Superina and Boily, 2007). The screaming hairy armadillo *Chaetophractus vellerosus* (Gray, 1865) (Dasypodidae) is a medium-sized species occurring in xeric regions of Argentina, Bolivia, and Paraguay; it is a systematic forager (Cassini, 1993), with habits and diet switching seasonally from insectivorous in the summer to herbivorous in the winter (Greegor, 1985).

Wild populations of *Z. pichiy* in Mendoza province, Argentina, have been monitored to understand their ecology (Superina, 2008). As a result of that research program, Ezquiaga et al. (2009) observed the presence of eggs from nematodes belonging to 3 known species and 1 unidentified *Cyclobulura* sp. Quentin 1977 (Ascaridida: Subuluridae). Subsequently, 4 dead pichis were confiscated from poachers and became available for parasitological examination. Additional species of armadillos from La Rioja province, including *C. vellerosus* Gray, 1865, were examined for parasites.

The aim of the present study is to describe a new species of *Cyclobulura* parasitizing the pichi from Mendoza and *C. vellerosus* from La Rioja, which constitutes the first subulurid nematode found in armadillos and the second known species of the genus.

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MATERIALS AND METHODS

Four individuals of *Z. pichiy* were confiscated from poachers in the following localities from Mendoza province, Argentina: Lavalle 32°50′S, 67°53′W (n = 1); San Rafael 34°21′S, 68°32′W (n = 1); Malargüe 35°38′S, 68°34′W (n = 1); and San Carlos 34°16′S, 69°8′W (n = 1), during 2002–2003. Eight individuals of *C. vellerosus* were collected by M.C.E. from Salina La Antigua 30°02′54″S, 66°03′51″W (n = 6), Departamento Chamical, and Paraje Punta de los Llanos 30°08′S, 66°32′W (n = 2), Departamento Capital, La Rioja, in July 2009.

The digestive tract of specimens was fixed in a 10% formaldehyde solution and dissected in the laboratory. The nematodes found were preserved in 70% ethanol. Specimens were cleared in lactophenol, mounted on a slide under a cover slip, and studied using a compound microscope. A cross section of the anterior end of both male and female was made to obtain an en face view. Drawings were made using an Olympus BX 51 microscope equipped with a drawing tube. Some specimens were dehydrated in a series of 70%, 80%, 90%, 95%, and 100% ethanol, dried with the critical point method, and photographed using a Jeol/SET 100® scanning electron microscope (SEM).

Measurements are given in micrometers, unless otherwise stated; those of paratypes are expressed as the mean ± the standard deviation followed by range values in parentheses (Sokal and Rohlf, 1995). Nematodes were deposited in the Colección de Helmintos Museo de La Plata (CHMLP), La Plata, Buenos Aires, Argentina, the Parasite Collection of the Harold W. Manter Laboratory of Parasitology (HWML) of the University of Nebraska, Lincoln, Nebraska, and the National Parasite Collection (USNPC) Bethesda, Maryland, and hosts in the Colección de Mastozoología Museo de La Plata (MLP).

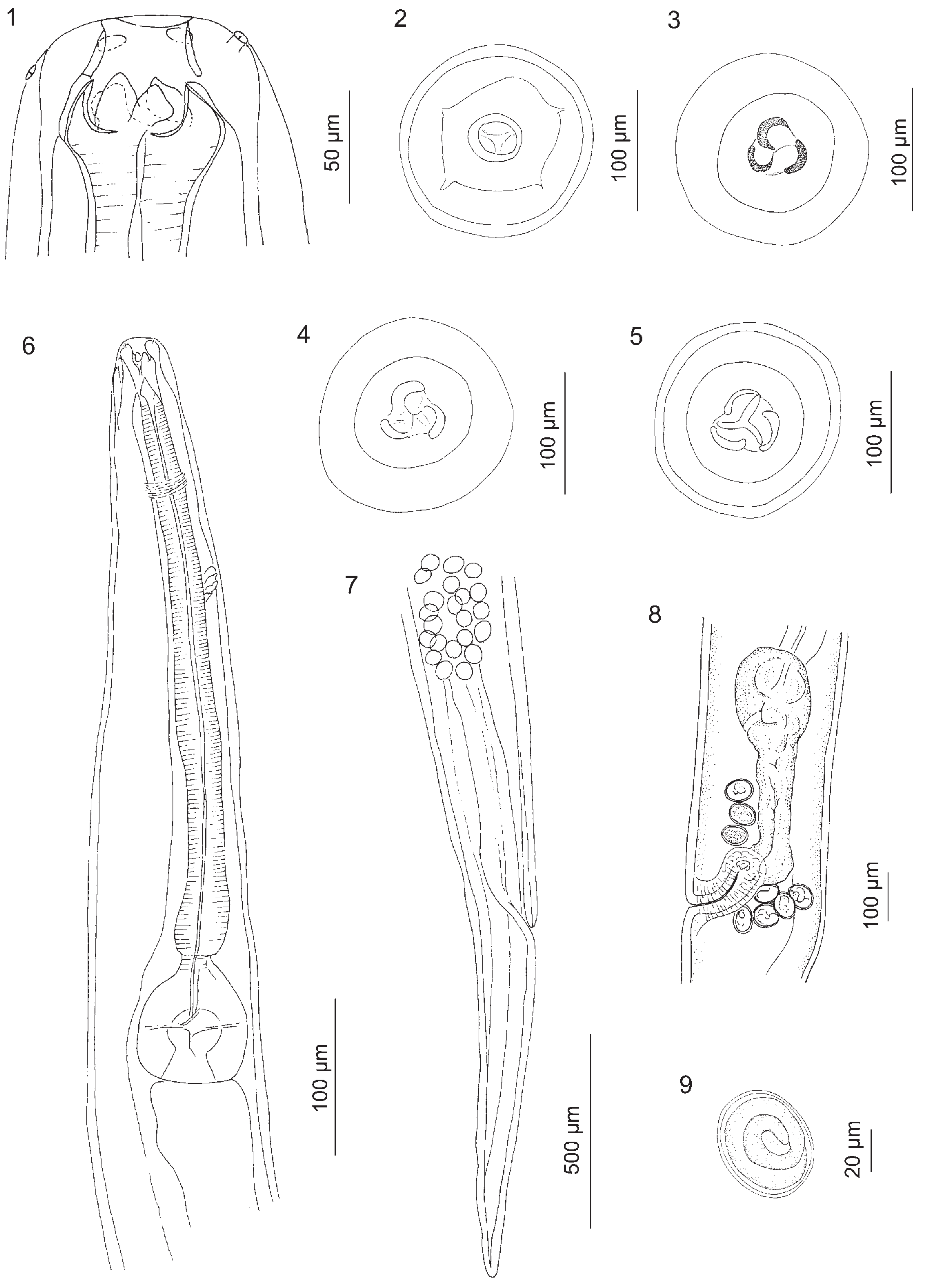
We also studied paratype specimens of *Cyclobulura lainsoni* Quentin, 1977, MNHN 156RL, deposited in the Museum National de Histoire Naturelle, Paris, France.

DESCRIPTION

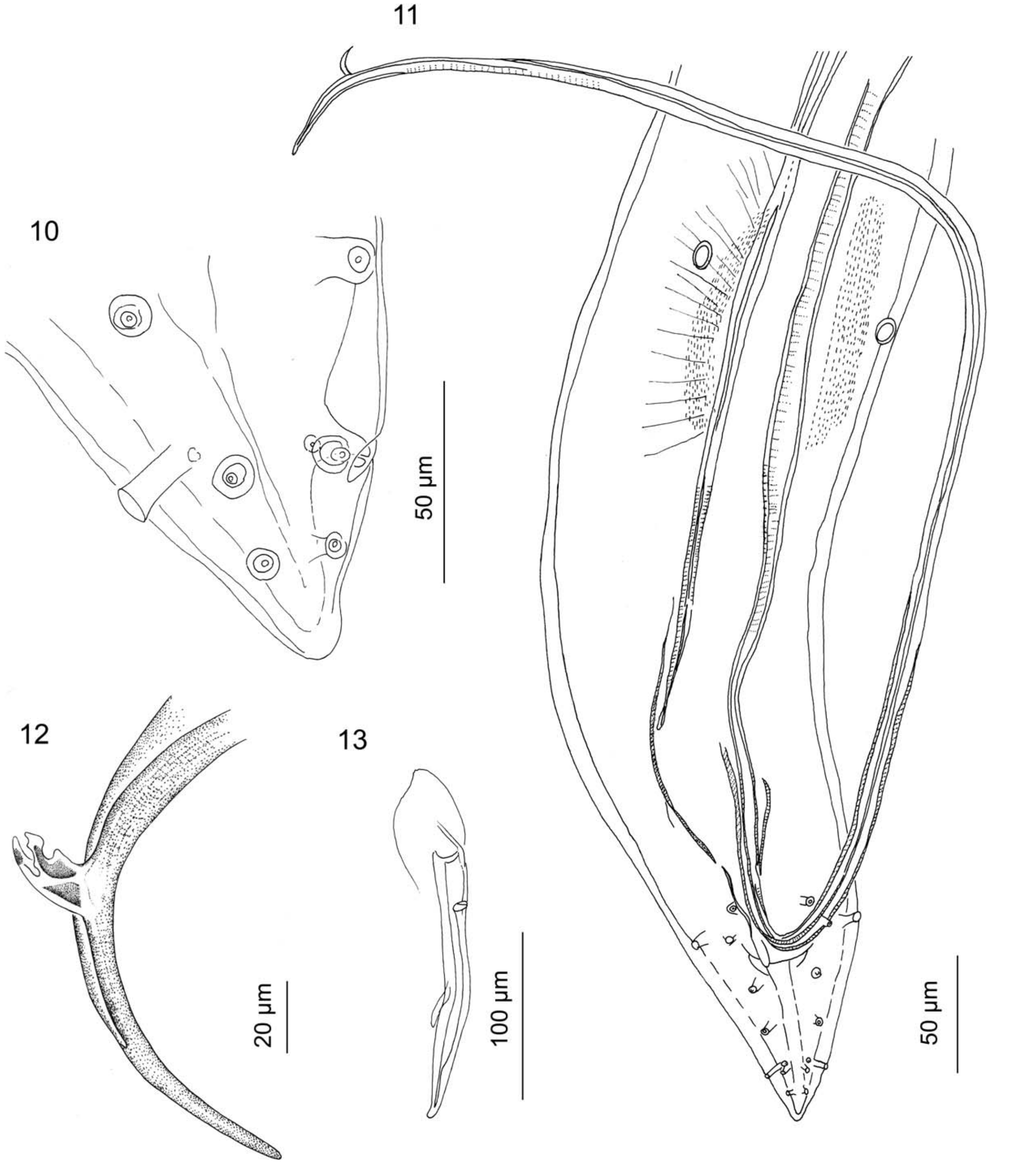
Cyclobulura superinae n. sp.
(Figs. 1–19; Table I)

General diagnosis: Whitish nematodes with both ends gradually tapering. Males and females with identical buccal and pharyngeal structures (Fig. 1). Six inner labial papillae; 4 conspicuous cephalic papillae and lateral amphids (Fig. 14). Circular stoma (Figs. 2, 14). Buccal capsule divided in 2 parts; anterior buccal part with thick cuticularized walls (cheilostome sensu Inglis, 1966), and posterior pharyngeal part (esophagostome sensu Inglis, 1966) with cuticularized walls connected with both pharyngeal lobes and with twisted chordal and radial lobes; chordal lobes rounded and poorly developed, fused on basis with radial lobes (Fig. 1). Buccal cavity with tripartite arrangement in a transverse section (Figs. 3–5).

Males: Tail tapering to a rounded point, without spine (Fig. 10). Spicules subequal, with a dorsal spur-like structure at distal end, structure arises in 90° angle from main axis of spicule, and backwards (Fig. 12). Gubernaculum present (Fig. 13). Ventral precloacal sucker, elongate, with



FIGURES 1–9. *Cyclobulura superinae*. Female: (**1**) Anterior extremity showing chordal, pharyngeal, and radial lobes. (**2–5**) Apical view: (2) at level of stoma. (3–5) Apical view at level of lobes. (**6**) Anterior region, lateral view. (**7**) Posterior extremity, lateral view. (**8**) Detail of vulva. (**9**) embryonated egg.

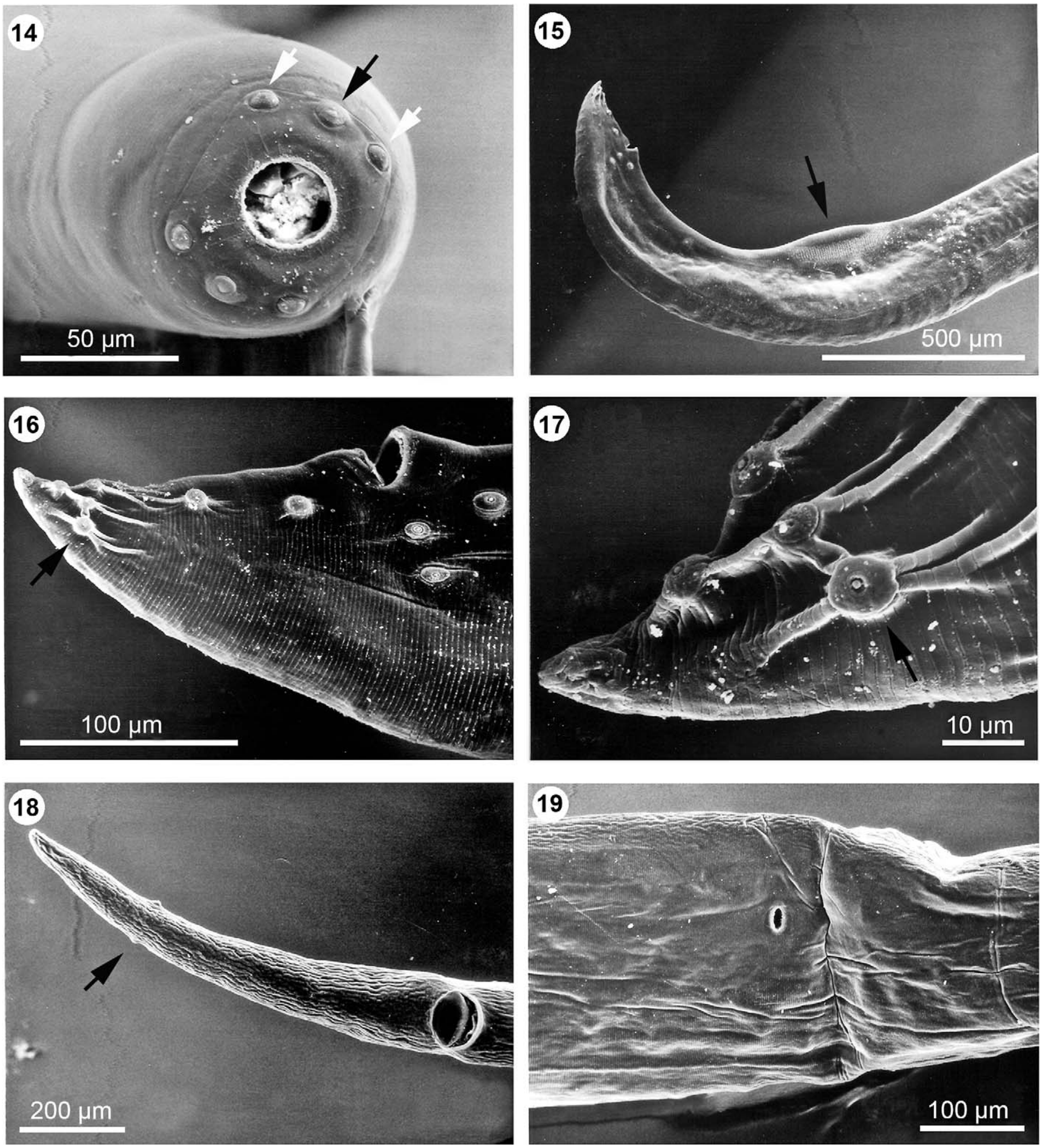


FIGURES 10–13. *Cyclobulura superinae*. Male: (**10**) Caudal extremity showing postcloacal papillae. (**11**) Posterior extremity showing sucker, spicules and papillae. (**12**) Extremity of the spicule with dorsal spur-like structure. (**13**) Gubernaculum.

transversal cuticular striations (Fig. 15). Nine pairs of caudal papillae arranged as follows: 1 pair at level of sucker; 3 subventral lateral pairs; single precloacal median; 4 subventral and 1 postcloacal lateral pair (Fig. 11); phasmids present between 7th and 8th pair of subventral papillae (Figs. 16, 17).

Holotype: Body length 9 mm, width at mid-body 368. Nerve ring and excretory pore located 286 and 503, respectively, from anterior end. Buccal cavity 75 long. Total length of esophagus 1,535. Corpus 1,426 long; esophageal bulb 223 long by 238 at maximum width. Spicules 2,641 and 2,593 long. Gubernaculum 205 long by 16 wide. Sucker located 640 from posterior end and 190 in diameter. Tail length 170; phasmids 54 from tip tail.

Paratypes (n = 18 unless otherwise indicated): Body length 9.4 mm ± 1 (8–12), width at mid-body 381.7 ± 65.4 (277–495) (n = 16). Nerve ring and excretory pore located 328.6 ± 73.8 (180–467) (n = 14) and 591.9 ± 76.6 (498–783) (n = 17) from anterior end, respectively. Buccal cavity 54.3 ± 9.2 (42–68) (n = 10) long. Total length of esophagus 1,727 ± 153.3 (1,303–1,944) (n = 17); corpus 1,438.7 ± 64.3 (1,346–1,570) (n = 11) long; width at level of nerve ring 109.2 ± 35.5 (45–165) (n = 10). Bulb of esophagus 255.4 ± 34.7 (185–332) (n = 16) long by 258.4 ± 36 (190–332) (n = 15) at maximum width. Left spicule 2,452.2 ± 285.6 (1,746–2,822) (n = 16) long, right spicule 1,893 ± 664.5 (1,103.7–2,823) (n = 7) long. Gubernaculum 206.9 ± 23 (150–244) (n = 18) long by 23.4 ± 3.1 (17–27) (n = 9) wide. Sucker 625.2 ± 105 (450–805) (n = 12) from posterior end



FIGURES 14–19. SEM micrographs of *Cyclobulura superinae*. (**14**) Apical view. Black arrow shows amphids, white arrows shows cephalic papillae. (**15**) Male tail showing the ventral sucker. (**16**) Detail of caudal papillae. The arrow shows the left phasmid. (**17**) Detail of phasmids. (**18**) Female, posterior extremity. The arrow shows the phasmids. (**19**) Detail of vulva.

and 198.5 ± 43.4 (150–286) (n = 11) in diameter. Tail length 167 ± 19.9 (133–197) with phasmids at 42 ± 5 (36–45) (n = 3) from end of tail.

Females: Vulva at mid-body. Ovijector simple (Figs. 8, 19). Oval eggs with thin shell, embryonated in the uterus (Fig. 9). Tail elongated, tapering to conical tip (Fig. 7). Phasmids conspicuous (Fig. 18).

Allotype: Body length 13 mm, width at level of vulva 304. Excretory pore located 488 from anterior end. Buccal cavity 74 long. Total length of esophagus including bulb 1,313. Corpus length 1,080 by 117 wide. Bulb of esophagus 264 long by 239 wide. Vulva located 6 mm from anterior end. Tail 948 long. Eggs 73 ± 7.3 (60–89) by 61 ± 5.5 (45–71) (n = 27).

TABLE I. Measurements of males and females of *Cyclobulura superinae* n. sp. from *Chaetophractus vellerosus* collected in La Rioja province, Argentina.

	Males (n = 5)	Females (n = 5)
Body length (mm)	9.2 (8.3–10.6)	13.7 (13–14.1)
Maximum width	457.5 (400–500)	594 (530–650)
Buccal cavity	52.5 (50–60)	69 (60–75)
Excretory pore*	650 (540–720)	756 (520–1,100)
Nerve ring*	490 (350–420)	476 (410–670)
Esophagus length	1,985 (1,870–2,060)	2,298 (2,250–2,400)
Corpus length × width	1,717.5 (1,600–1,800) × 87.5 (70–110)	2,024 (1,970–2,150) × 106 (80–170)
Bulb length × width	267.5 (260–270) × 295 (280–300)	274 (250–330) × 316 (290–350)
Tail	178 (160–200)	1,022 (890–1,170)
Sucker to cloaca	398 (310–450)	—
Sucker length	192 (150–240)	—
Spicule length	3,074 (2,500–3,520)	—
Gubernaculum	208 (180–240)	—
Sucker from post end	576 (480–650)	—
Vulva* (mm)	—	6.7 (5.3–7.7)
Eggs long × wide (n = 11)	—	68.4 (60–79) × 54.9 (48–65)

* Measures to the anterior end.

Paratypes (n = 15 unless otherwise indicated): Body length 12.8 mm ± 1.4 (11–15 mm) (n = 13), width at level of vulva 455.7 ± 80 (347–557) (n = 9). Nerve ring and excretory pore located 369.7 ± 41.7 (300–405) (n = 8) and 617.2 ± 78.6 (515–717) (n = 11) from anterior end, respectively. Buccal cavity 68.1 ± 15.5 (43–84) (n = 7) long. Total length of esophagus including bulb 1,899.9 ± 321.5 (1,265–2,275) (n = 12); corpus 1,526 ± 337.5 (1,034–1,885) long, by 130 ± 45.2 (75–183) (n = 7) wide; esophageal bulb 321 ± 56.2 (224–428) long by 283.4 ± 59.3 (191–390) (n = 14) wide. Vulva located 6.3 mm ± 0.7 (5–8 mm) (n = 9) from anterior end. Tail 825.7 ± 149.9 (637–1,118) (n = 13) long. Eggs 67 ± 10.7 (52–80) (n = 12) by 55 ± 9.5 (39–78) (n = 12). Measurements of specimens collected from *C. vellerosus* are given in Table I.

Taxonomic summary

Type host: Zaedyus pichiy (Desmarest, 1804) deposited at Colección de Mastozoología Museo de la Plata, La Plata, Argentina, MLP 7.V.10.2 (ZP50).

Other hosts: Chaetophractus vellerosus Gray, 1865, MLP 7.V.10.3 (CO36).

Type locality: San Carlos (34°16′S, 69°8′W), Mendoza, Argentina.

Other localities: Salina La Antigua (30°02′54″S, 66°03′51″W), Departamento Chamical, La Rioja, Argentina.

Site of infection: Small intestine and caecum.

Specimens deposited: Holotype CHMLP 6135; allotype CHMLP 6136; paratypes CHMLP 6137 and voucher 6138. Additional specimens: HWML 64573 and USNPC 103073.

Prevalence and mean intensity: In *Z. pichiy:* 75% (3 of 4 prospected specimens); 126 (12–337) parasites per hosts. In *C. vellerosus:* 33% (2 of 6 prospected); 10 worms per host.

Etymology: The species is named after Dr. Mariella Superina, earnest researcher of the biology and conservation of the pichi *Z. pichiy* and other armadillos occurring in Mendoza, Argentina.

Remarks

The general morphology of the specimens herein described conforms to the description of the monotypic *Cyclobulura*, and it resembles *C. lainsoni* in the structures present in the anterior end, including circular stoma, pharyngeal cavity with cuticularized walls, and twisted chordal and radial lobes fused at the base. In addition, they share a similar disposition of ventral sucker, caudal papillae, and length of the spicules (Quentin, 1977). However, specimens of *C. lainsoni* are larger than those of *C. superinae*, and the spicule-body length ratio is greater in the latter than in the former (0.28 vs. 0.08). Males of *C. superinae* possess a proportionally shorter tail, with no caudal spine, and the spicules possess a distinctive spur-like process in the distal end. Moreover, the vulva in specimens of *C. superinae* opens at the mid-body region, while in *C. lainsoni* it opens in the anterior third; the chordal lobes in *C. superinae* are longer, and the radial lobes are conspicuous, as opposed to the atrophied lobes present in *C. lainsoni.*

Cyclobulura is a genus of the Labiobulurinae Quentin, 1969, which is characterized by the cuticularized pharyngeal lobes. The subfamily includes *Labiobulura* Skrjabin and Schikhobalova, 1948, parasites of Australian marsupials, and *Tarsubulura* Inglis, 1958, parasites of tupaids, lorisids, and tarsioids in Asia (Quentin, 1969, 1977; Smales, 2009). The new species also differs from these 2 genera in having a circular stoma, instead of a hexagonal one, or with 6 labial lobes. Other genera in Subuluroidea possess muscular pharyngeal lobes, while they seem to be cuticularized in *Cyclobulura* (Quentin, 1969; Chabaud, 1978; Smales, 2009).

DISCUSSION

Previous to this description, *Cyclobulura* included *C. lainsoni* collected from the silky anteater *Cyclopes didactylus* (Linnaeus, 1758) (Pilosa: Cyclopedidae) from Belem, Brazil (Quentin, 1977). This species is distributed from southern Mexico to southern Brazil, inhabiting the canopy of the rainforest (Best and Harada, 1985). In contrast, the pichi *Z. pichiy* is restricted to central and southern Argentina, living in arid habitats with firm sandy soils, such as the steppe in southern Mendoza; *C. vellerosus* is found inxeric areas of the Gran Chaco of Bolivia, western Paraguay, and Argentina (Redford and Eisenberg, 1992). These species of armadillos do not overlap in their distribution, do not occur in similar habitats, and are somewhat distantly related to the silky anteater. Nonetheless, these mammals are insectivorous, with the armadillos having a wide spectrum diet and the silky anteater being myrmecophagous (Navone, 1990; Redford and Eisenberg, 1992). Subuluroids use arthropods as intermediate hosts, such as larvae of coleopterans, cockroaches, and crickets (Quentin et al., 1977; Anderson, 2000). It is expected that similar invertebrates would serve as intermediate hosts in the life cycle of species of *Cyclobulura*.

Long-term studies on parasites of Dasypodidae from Argentina have been in progress since 1980 (Navone and Lombardero, 1980; Navone, 1986, 1987a, 1987b, 1990; Notarnicola and Navone, 2003; Ezquiaga et al., 2009). The discovery of additional species occurring in armadillos elsewhere (Jiménez-Ruiz and Gardner, 2003; Jiménez-Ruiz et al., 2006) and the parasite herein described suggests that the diversity of parasites of Xenarthrans may be greater than previously thought, and that the discovery of species would necessitate the survey of the diverse biomes across the Americas.

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Authors Queries

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