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A New Species of *Aspidodera* (Nematoda: Heterakoidea) Parasitizing the Giant Anteater *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae) in Brazil and New Key to Species

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ABSTRACT: The relatively recent surge in *Aspidodera* species descriptions suggests that several species are yet to be documented and properly described. We describe *Aspidodera serrata* n. sp. from the giant anteater *Myrmecophaga tridactyla* from the Cerrado biome (Savanna), Brazil. The new species is distinctive in the genus because of the attributes of the cephalic cordons, position of pore-like papillae and a thickening of body cuticle. Because no male specimens were recovered from the host, morphological characteristics that are identical in both sexes and specific for the genus were fundamental during the identification. We emphasize the relevance of hood features and scanning electron microscopy to base descriptions in *Aspidodera*. We provide a new key for the genus to assist in the efforts to identify the species present in multiple infections. This is the first report of a coinfection with 3 congeners in the same individual host involving *Aspidodera*. We urge researchers to preserve and document the metazoan parasites from tetrapod species considered vulnerable, because they can be valuable sources of information on the distribution of parasites in the wild.

KEY WORDS: *Aspidodera serrata* n. sp., Aspidoderidae, Heterakoidea, Nematoda, giant anteater, *Myrmecophaga tridactyla*, São Paulo, Brazil, Cerrado biome, scanning electron microscopy, light microscopy, taxonomic key.

The family Aspidoderidae Freitas, 1956, is a group of nematodes that parasitize the cecum and large intestine of xenarthrans, didelphiomorphs, and hystricognath and sigmodontine rodents (Santos et al., 1990) distributed in the southern Nearctic and Neotropical regions (Jiménez-Ruiz et al., 2008). The genus *Aspidodera* Railliet and Henry, 1912, has 11 known species, which can be diagnosed primarily by the expansion of the anterior end and a hood adorned with cordons (Inglis, 1957). The presence of *Aspidodera* in a number of different mammal taxa indicates the occurrence of multiple events of host switching and continental dispersion (Jiménez-Ruiz et al., 2008). However, the recent surge in species descriptions (Jiménez et al., 2013; Chagas-Moutinho et al., 2014) suggests that many more *Aspidodera* have yet to be discovered and described formally.

The giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758, is a vulnerable Neotropical xenarthran mammal with ample distribution in Central and South America (Miranda et al., 2014). Little is known about the helminth fauna of *M. tridactyla*, and most of the published data are found in articles dealing with the description of its helminth species (Vicente et al., 1997). *Myrmecophaga tridactyla* may be exposed to helminths through the accidental consumption of soil particles contaminated with eggs, such as those of aspidoderids.

Three *Aspidodera* species have been reported parasitizing 2 anteater species in Brazil. In *M. tridactyla*, the nematode *Aspidodera fasciata* (Schneider, 1866) Railliet and Henry, 1913, was collected from *M. tridactyla* in Mato Grosso state (Vicente, 1966), whereas *Aspidodera scoleciformis* (Diesing, 1851) Railliet and Henry, 1912, was collected from *M. tridactyla* examined in São Paulo state (Vicente, 1966). In the case of the southern tamandua, *Tamandua tetradactyla*, *Aspidodera lacombeae* Vicente, 1964, was collected in

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Amazonas state. In the present study, we describe a new species, *Aspidodera serrata* n. sp., infecting an *M. tridactyla* in the savanna biome (Cerrado) of southeastern Brazil and provide a new taxonomic key for the genus *Aspidodera* based on 2 female specimens.

MATERIAL AND METHODS

The carcasses of 2 adult *M. tridactyla*, a male and a female, were found in the Santa Bárbara Ecological Station (SBES, 22°48′S, 49°14′W, state of São Paulo, southeastern Brazil), and examined postmortem in June and July 2015, respectively, after they died from unknown causes. The SBES encompasses 2,712 ha of protected Brazilian savanna biome, characterized by a vegetation mosaic that includes Cerrado sensu lato, gallery forest, patches of semideciduous forest, and tracts of exotic *Pinus* and *Eucalyptus* trees (Melo and Durigan, 2011).

Nematodes were collected from the large intestine, washed in saline solution, and stored in 70% ethanol. They were separated, cleared in lactophenol, and analyzed under an Axion Scope A1 Light Microscope (Zeiss, Göttingen, Germany) for morphological characterization. Drawings were obtained with the aid of a camera lucida attached to a standard Zeiss microscope. The morphological features used for general identification of the specimens were obtained from the diagnoses of Proença (1937), Vicente (1966), Santos et al. (1990), and Vicente et al. (1997). All measurements were recorded in micrometers. Proportions between hood length/body length, esophagus length/body length, distance of nerve ring to anterior end/esophagus length, and distance of excretory pore to anterior end/esophagus length, were expressed as percentages. For egg length and width, the range was given first, followed by sample mean, sample size, and the coefficient of variation (as a percentage), the last 2 in parentheses. Egg measurements were taken only from the holotype, because the paratype was prepared to perform the scanning electron microscopy (SEM) technique.

The morphometric data on the *Aspidodera* species most similar to the new species were included for comparative purposes and were obtained from the literature (Travassos, 1913; Proença, 1937; Pallares and Usher, 1971; Diaz-Ungria, 1979; Santos et al., 1990; Vicente et al., 1997; Jiménez-Ruiz et al., 2006; Jiménez et al., 2013). The relative abundance of each species was calculated as its percentage of the total number of congener specimens collected from an individual host. The specimen of the new species was deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC 38749) in Rio de Janeiro, Brazil.

The specimen prepared for SEM was fixed for 1 hr at room temperature in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer and washed in the same buffer (pH 7.2), postfixed in 2% OsO_4 in 0.1 M Na-cacodylate buffer, and then washed 3 times in the same buffer. This specimen was subsequently dehydrated in a graded series of ethanol (30–100%) in 1-hr steps, critical point–dried in CO_2, sputter-coated with a gold layer about 20-nm thick, and examined with a JEOL JSM-6390 LV microscope (JEOL, Akishima, Tokyo, Japan) with an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

RESULTS

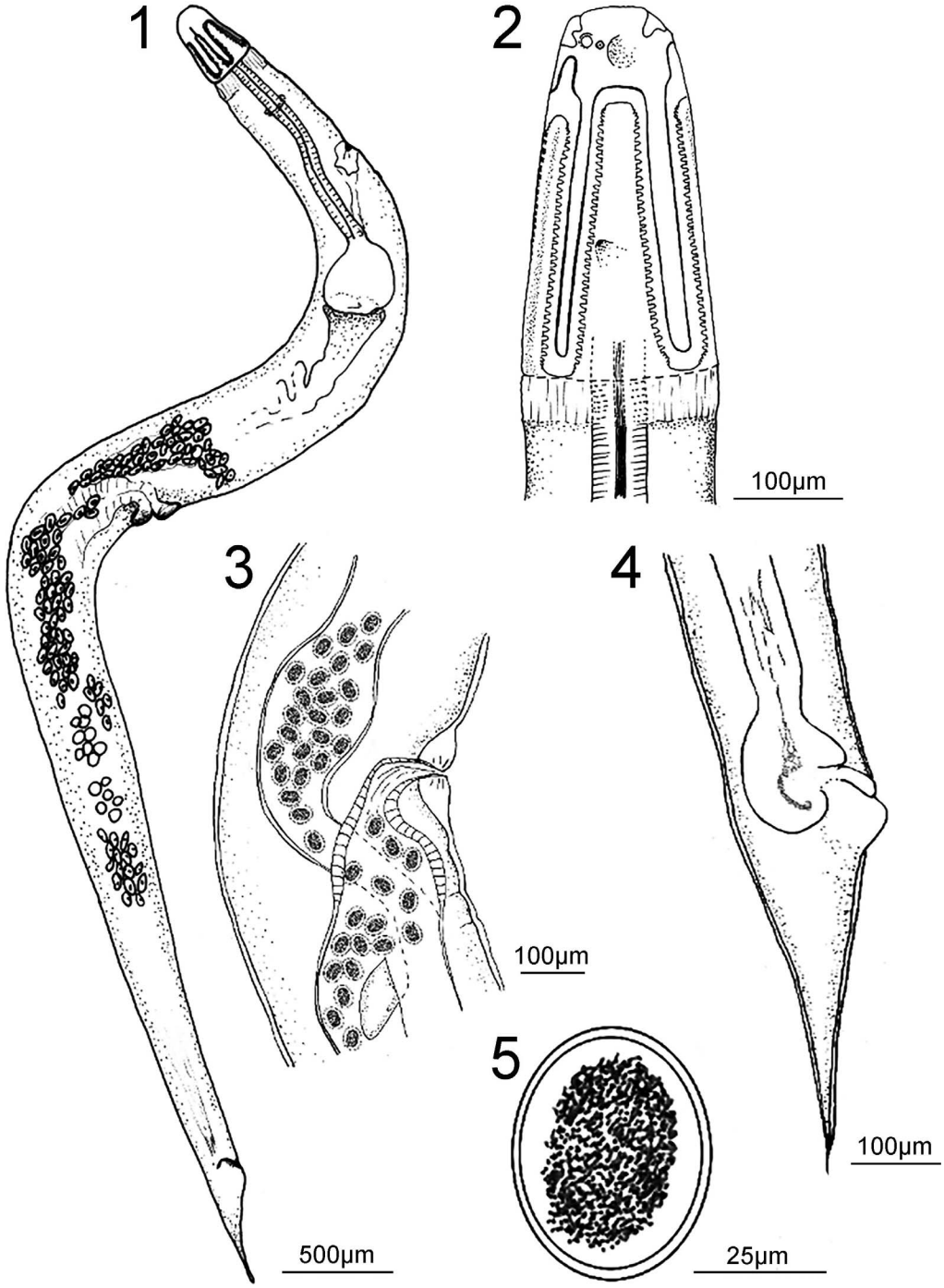
Nematoda Rudolphi, 1808
Secernentea Linstow, 1905
Ascaridida Skrjabin and Schultz, 1940
Heterakoidea Railliet and Henry, 1912
Aspidoderidae Freitas, 1956
***Aspidodera* Railliet and Henry, 1912**
***Aspidodera serrata* n. sp.**
(Figs. 1–12)

Generic diagnosis

Aspidodera: Expanded cephalic end adorned with cordons, lips with papillae, simple mouth leading into a vestibule, and esophagus with a terminal bulb (Inglis, 1957; Santos et al., 1990; Anderson et al., 2009).

Description (based on 2 female specimens)

Body fusiform and tail sharp (Fig. 1). Long hood (or cephalic cap) with anastomosing cordons (Figs. 2, 6, 7). Cephalic cordons with 6 anteriad and 6 posteriad loops, and smooth anterior and serrated posterior margins (Figs. 2, 6, 7). Oral opening with 3 lips (Fig. 8), each presenting 2 slender posterior projections (Fig. 7). Dorsal lip with 2 digitiform projections, lateroventral lips with small, acute projection. Dextroventral lip projection presenting shallow double undulation that fits in sinistroventral lip sockets (Fig. 8). Lateroventral lips with an amphid, a papilla, and a papillary nodule (Figs. 2, 8). Cephalic cordons touching base of hood (Figs. 2, 6, 7). Serrated margin enclosing cuticular expansions from body to hood, named interlabium and labial root. Interlabium long and slender, labial root trapezoidal in shape (Figs. 6, 7). Pore-like papilla on labial root of lateroventral lips at middorsal portion (Figs. 2, 7). Fine transversal cuticular striations along body, thick neck-like cuticle posterior to hood



Figures 1–5. Line drawings of *Aspidodera serrata* n. sp., female, lateral view. **1.** Whole nematode. **2.** Hood. **3.** Genital system. **4.** Terminal portion of the gastrointestinal system. **5.** Unembryonated egg with thick and smooth shell.

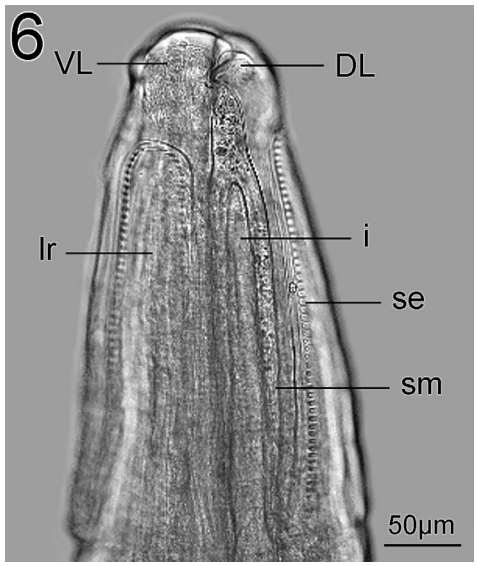


Figure 6. Light micrograph images showing the hood of *Aspidodera serrata* n. sp., female. Abbreviations: DL, dorsal lip; i, interlabium; Ir, interroot expansions; se, posterior serrated cordons; sm, cephalic cordons with anterior smooth cordons; VL, sinistroventral lip.

(Figs. 1, 2, 7). Short vestibulum does not exceed half-length of the hood. Long esophagus with terminal pyriform bulb with valve, followed by long intestine. Nerve ring surrounding esophagus, placed on the anterior half of the entire esophagus (including vestibulum and bulb). Excretory pore located on the posterior half of the entire esophagus (Fig. 1).

Female: Oviparous, didelphas, amphidelphas, with ventral vulva and lightly protuberant lips (Fig. 10) opening in midanterior portion of body. A relatively short and muscular ovojector goes from the vulvar opening to the posterior region of body. Ovojector connects to the uteri through vulvar vestibules. Opposite uteri, a posterior and an anterior (Fig. 3). Elliptical eggs not embrionated in uterus (Fig. 3) with thick and smooth shell (Fig. 5). Anus with a small papilla on each side and posterior protuberance (Figs. 4, 11). Larger papilla and phasmid on both side of tail, in final third of length (Fig. 12). Lateral alae present (Fig. 9), starting posterior to the thick neck-like cuticle to the anus.

Holotype: Body length 7,157, width at level of vulva 152, hood 319 long and 175 wide. Ratio between hood length and body length, 4.5%. Esophagus length with bulb 1,340 (including vestibulum), bulb 205 long and 155 wide. Ratio between esophageal length (from the oral opening to the bulb) and body length, 18.7%. Nerve ring to anterior end 659, excretory pore to anterior end 787. Ratio between nerve ring anterior end and length of the esophagus, 49.2%. Ratio between excretory pore anterior end and length of the esophagus, 58.7%. Vulva to anterior end 3,157, anus to posterior end 521, elliptical eggs range 54–66, 62.1 ($n = 10$, 6.2%) long and 41–50, 46.3 ($n = 10$, 5.6%) wide.

Paratype: Body length 6,105, width at level of vulva 184, hood 361 long and 244 wide. Ratio between hood length and body length, 5.9%. Esophagus length with bulb 1,244 (including vestibulum), bulb 186 long and 171 wide. Ratio between esophageal length (from the oral opening to the bulb) and body length, 20.4%. Nerve ring to anterior end 542, excretory pore to anterior end 659. Ratio between nerve ring anterior end and length of the esophagus, 43.6%. Ratio between excretory pore anterior end and length of the esophagus, 53.0%. Vulva to anterior end 2,815, anus to posterior end 574.

Taxonomic summary

Type host: Giant anteater, *Myrmecophaga tridactyla* Linnaeus, 1758.

Type locality: Santa Bárbara Ecological Station, municipality of Águas de Santa Bárbara, state of São Paulo, Brazil (22°48′59″S, 49°14′12″W).

Site of infection: Large intestine.

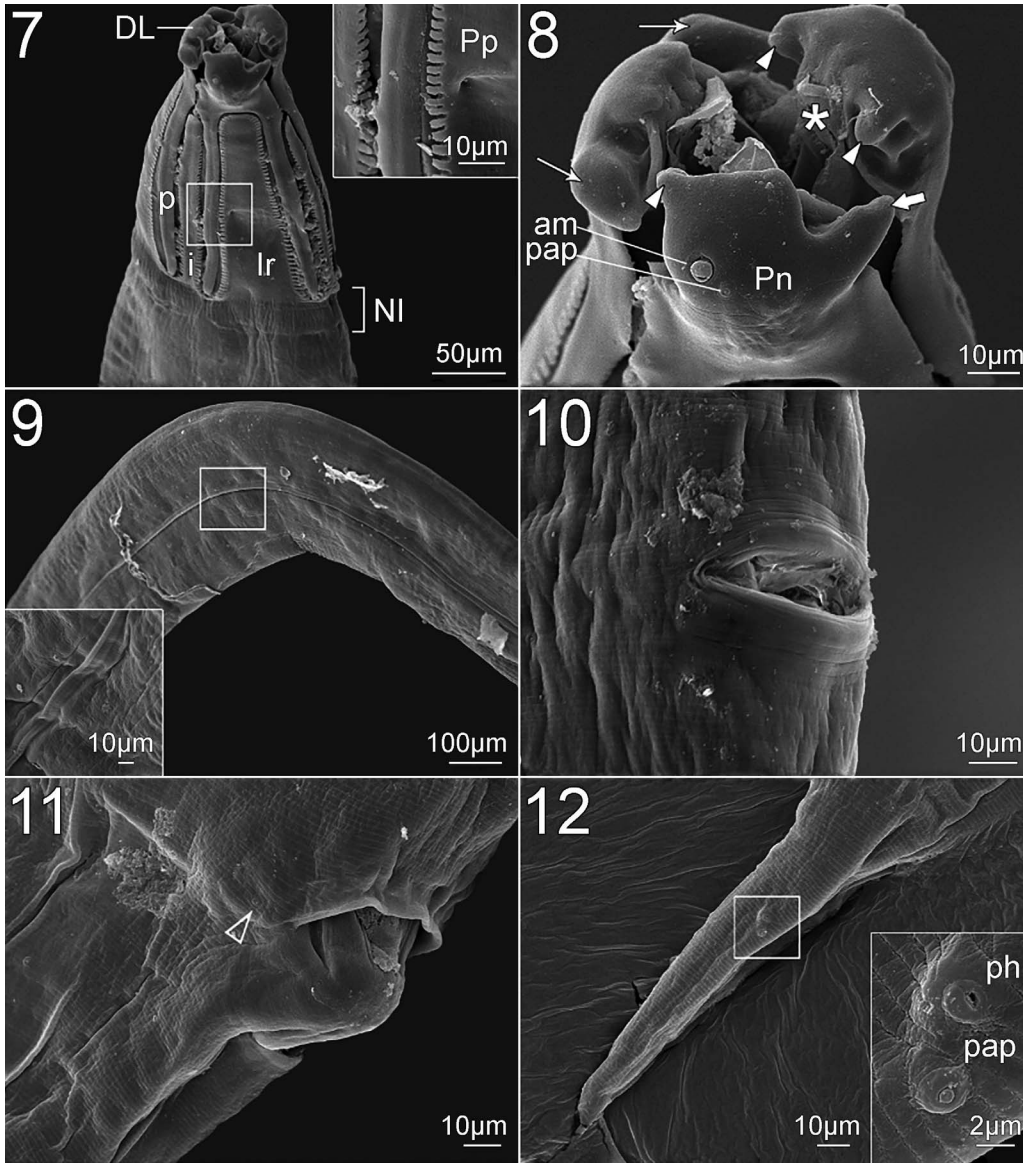
Specimen deposited: Holotype CHIOC 38749 (female).

Etymology: The specific epithet (*serrata*) refers to the most characteristic morphological structure of the new species, the serrated posterior margin of the velum in the cephalic cordons.

Remarks

The nematodes recovered from *M. tridactyla* in the Cerrado biome of São Paulo state, Brazil, presents 3 putative autapomorphies: 1) cephalic cordons with serrated posterior margins, 2) pore-like papillae in the middorsal portion of the labial root in the lateroventral lips, and 3) a neck-like cuticle posterior to the hood. Serrated cordons and neck-like cuticles had not been reported previously in the genus. A pore-like papilla has been observed at the interlabium apex in *Aspidodera raillieti* Travassos, 1913 (Chagas-Moutinho et al., 2007), *Aspidodera lanfredi* Chagas-Moutinho, Sant'Anna, Oliveira-Mendes, and De Souza, 2014, and *A. scoleciformis* (Sant'Anna, personal communication *in* Chagas-Moutinho et al., 2014), but not in the labial root.

Aspidodera serrata n. sp. possesses anastomosed cephalic cordons, distinguishing it from *Aspidodera*



Figures 7–12. Scanning electron micrograph images of *Aspidodera serrata* n. sp., female. **7.** Hood. Increased magnification highlighting cephalic cordons. **8.** Oral aperture with lips. Symbols indicating the following structures: digitiform projections (thin arrows), small acute projections (full arrowheads), dextroventral lip projection (large arrow), cephalic end showing the oral vestibulum lined with cuticular membranes with an external smooth edge (asterisk). **9.** Lateral alae. **10.** Vulva. **11.** Anus with small papillae indicated by empty arrowhead. **12.** Posterior end of tail. Increased magnification highlighting phasmid and papilla. Abbreviations: am, amphid; DL, dorsal lip; i, interlabium; Ir, interroot expansions; Nl, neck-like cuticle; p, slender projections of lips; pap, papilla; ph, phasmid; Pn, papillary nodule; Pp, pore-like papilla.

ansirupta Proença, 1937, in which the cephalic cordons are not anastomosed. The shape of the cephalic cordons also distinguishes the new species from *Aspidodera binansata* Railliet and Henry, 1913, *A. lacombeae*, *A. scoleciformis*, and *Aspidodera subulata* (Molin, 1860) Railliet and Henry, 1912. The number and position of the amphids, papillae, and papillary nodules, as well as the trapezoidal labial root and slender interlabium of *A. serrata* n. sp., are all similar to the configuration found in *Aspidodera kinsellai* Jiménez, Carreno, and Gardner, 2013, *Aspidodera sogandaresi* Jiménez-Ruiz, Gardner, and Varela-Stokes, 2006,

Aspidodera vazi Proença, 1937, and *A. fasciata*. The digitiform projection of the dextroventral lip of *A. serrata* n. sp. is similar to that found in *A. kinsellai* and *A. sogandaresi* but contrasts with that found in *A. fasciata*, which has distinctly undulating projections. A lateral alae is present in *A. serrata* n. sp., *A. binansata*, *A. ansirupta*, and *A. raillieti*, although it is absent in *A. kinsellai*, *A. sogandaresi*, *A. fasciata*, *A. scoleciformis*, *A. subulata*, *A. lacombeae*, and *A. lanfredi*.

Morphometric features overlap considerably between *A. serrata* n. sp. and its most similar congeners (*A. kinsellai*, *A. sogandaresi*, *A. vazi*, and *A. fasciata*), primarily because of the considerable amplitude of the parameters. Even so, *A. kinsellai* appears to be the species most similar to *A. serrata* n. sp., especially in body length and hood length and width (Table 1). The most divergent values were found in *A. vazi* (hood and bulb) and *A. sogandaresi* (nerve ring to anterior end and vulva to anterior end).

Key for the species

1. Cephalic cordons nonanastomosed anteriorly in labial root..... ***A. ansirupta***
   Cephalic cordons anastomosed anteriorly in labial root.....2
2. Dorsal lip with 2 anterior loops and 3 posterior loops.....***A. binansata***
   Dorsal lip with 1 anterior loop and 2 posterior loops 3
3. Cephalic cordons with serrated aspect.....
   .....***A. serrata* n. sp.**
4. Cephalic cordons with smooth aspect 4
5. Cephalic cordons square-shaped in labial root.....***A. scoleciformis***
6. Cephalic cordons trapezoid- or round-shaped in labial root.....5
7. Long caudal spine, above 0.1 mm length.....6
8. Short to medium caudal spine, up to 0.07 mm length.....7
9. Constriction between caudal spine and tail.....***A. vazi***
   Without constriction between caudal spine and tail.....***A. kinsellai***
10. Cephalic cordons surpass the level of vestibulum, always touching the base of the hood.....8
11. Cephalic cordons do not surpass vestibulum and do not touch the base of the hood .....***A. subulata***
12. Hosts are marsupials and hood is up to 0.19 mm length.....9

Table. 1. Comparative measurements* for female specimens of *Aspidodera serrata* n. sp. (this study) and the most similar *Aspidodera* species (literature compilation).

Aspidodera species	Body (length × width)	Hood (length × width)	Esophagus	Bulb (length × width)	Excretory pore†	Nerve ring†	Vulva†	Egg (length × width)
A. serrata n. sp.	6,105–7,157 × 152–184	319–361 × 175–244	1,244–1,340	186–205 × 155–171	659–787	542–659	2,815–3,157	54–66 × 41–50
A. kinsellai	5,307–6,906 × 346–557	362–440 × 175–258	1,324–1,640	166–244 × 172–235	700–955	504–611	2,431–3,112	57–78 × 37–61
A. sogandaresi	4,711–6,886 × 219–385	213–312 × 114–258	1,038–1,504	158–221 × 123–238	549–757	361–523	1,811–2,807	60–92 × 40–70
A. vazi	5,000–9,200 × 380–570	344–530 × —	1,060–1,650	186–260 × 180–240	736–1,100	780–1,100	2,550–3,367	50–72 × 36–54
A. fasciata	5,400–10,000 × 270–660	200–304 × —	1,200–1,850	190–330 × 150–263	660–1,000	480–900	2,350–3,760	52–60 × 40–48

* All measurements are reported in micrometers.
† Distance to anterior end.

Hosts are xenarthrans and hood is longer than 0.17 mm 10

13. Small acute projection in sinistroventral lip, base of interlabium wider than the apex, papillary nodule present on lateroventral lips ..... ***A. lanfredi***
    Digitiform projection in sinistroventral lip, slender interlabium, papillary nodule absent on lateroventral lips ..... ***A. raillieti***
14. Short caudal spine up to 0.014 mm length ..... ***A. lacombeae***
    Medium caudal spine above 0.025 mm length 11
15. Conspicuous digitiform projection of dextroventral lip with marked undulations ..... ***A. fasciata***
    Blunt projection of dextroventral lip with shallow undulations ..... ***A. sogandaresi***

Aspidodera species recovered from the host

We recovered 3 species of *Aspidodera* from the adult female *M. tridactyla* host, including the new species described here. The adult male *M. tridactyla* host did not contain any *Aspidodera*. *Aspidodera fasciata* was the most abundant, with 86.9% of the specimens collected (139/160) and a sex ratio of 1.2 : 1 (76 males vs. 63 females). *Aspidodera scoleciformis* provided a further 11.9% of the specimens, with a sex ratio of 0.58 : 1 (7 males vs. 12 females). *Aspidodera serrata* n. sp. was represented by only 2 females (1.2%).

DISCUSSION

The aspidoderid hood is an important diagnostic character for the detection of morphological variability and conservatism (Inglis, 1957; Jiménez-Ruiz et al., 2008) and is illustrated perfectly in *A. serrata* n. sp., whose unique characteristics provide important insights into the variability in the cephalic structures that are nevertheless typical of the genus. These structures permitted the description of the new species based only on female specimens, with the use of the SEM. It is important to note, however, that other well-established morphological and morphometric features were fundamental to the diagnosis of the new species and its insertion in the taxonomic key.

The 2 known species we found in *M. tridactyla*, *A. fasciata* and *A. scoleciformis*, as well as *A. raillieti*, occur in the greatest diversity of host species among the congeners. However, whereas *A. scoleciformis* and *A. raillieti* seem to be more generalist, in that they occur in xenarthrans and marsupials (in the first case) and in marsupials and rodents (in the second case), *A. fasciata* has only been reported, so far, in xenarthrans (armadillo and giant anteater). Other hosts for *A. fasciata* are the southern naked-tailed armadillo (*Cabassous unicinctus* Linnaeus, 1758), 9-banded armadillo (*Dasypus novemcinctus* Linnaeus, 1758), 6-banded armadillo (*Euphractus sexcinctus* Linnaeus, 1758), giant armadillo (*Priodontes maximus* Kerr, 1792), southern 3-banded armadillo (*Tolypeutes matacus* Desmarest, 1804), and Brazilian 3-banded armadillo (*Tolypeutes tricinctus* Linnaeus, 1758) (Santos et al., 1990; Vicente et al., 1997). *Aspidodera scoleciformis* also parasitize *C. unicinctus*, large hairy armadillo (*Chaetophractus villosus* Desmarest, 1804), *D. novemcinctus*, *E. sexcinctus,* murine mouse opossum (*Marmosa murina* Linnaeus, 1758), Tate's woolly mouse opossum (*Micoureus paraguayanus* Tate, 1931), gray short-tailed opossum (*Monodelphis domestica* Wagner, 1842), and *T. tricinctus* (Santos et al., 1990; Vicente et al., 1997).

The coinfection of a host by multiple parasite species is a common pattern in natural populations (Petney and Andrews, 1998; Griffiths et al., 2011). Associations have been reported between a number of pairs of *Aspidodera* species, including *A. fasciata* and *A. scoleciformis* (Travassos, 1913; Vicente, 1966; Fujita et al., 1995), *A. fasciata* and *A. binansata* (Cameron, 1939), *A. raillieti* and *A. subulata* (Vicente, 1966; Santos et al., 1990), and *A. kinsellai* and *A. sogandaresi* (Jiménez et al., 2013). A coinfection of *M. tridactyla* by *A. fasciata* and *A. scoleciformis* was already recorded (Vicente, 1966), and in the present study, both these species were found in sympatry with *A. serrata* n. sp. As in all previous cases of sympatry, *A. fasciata* was the most abundant species, reflecting its dominance in the different hosts in which it occurs.

This study presents the description of the twelfth *Aspidodera* species known to science, *A. serrata* n. sp., distinguished on the basis of the serrated pattern of its cephalic cordons. The study also updates the taxonomic key for the species of the genus, including features that can only be discerned by electron microscopy. The study also documents the first reported case of triple coinfection by *Aspidodera* species in a single host specimen. This discovery of a new *Aspidodera* species in *M. tridactyla* reinforces the need for the more systematic sampling of the biodiversity of the Neotropical region and underscores the importance of examining every single individual parasite found in an infected organism. This practice will help scientists detect rare species, as well as provide information on associations among the species found

within the same ecosystem. The very low intensity of *A. serrata* n. sp. found in the *M. tridactyla* specimen may suggest that this mammal is not the principal host, although *Aspidodera* seems to be poorly host-specific in general.

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