# A Third New Species of *Aporcelinus* Andrássy, 2009 (Dorylaimida, Aporcelaimidae) from Vietnam, with the First SEM Study of a Representative of the Genus

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Abstract: A new species, the third one from Vietnam, of the genus Aporcelinus is described from natural areas. Aporcelinus falcicaudatus sp. n. is characterized by its 1.28 to 1.63 mm long body, lip region offset by weak constriction and 16 to 18  $\mu$ m broad, odontostyle 18 to 21  $\mu$ m at its ventral side, neck 354 to 406  $\mu$ m long, uterus tripartite and 61 to 95  $\mu$ m long, V= 50 to 55, tail strongly recurved dorsad and conical (23–31  $\mu$ m, c = 43–58, c' = 0.7–0.9) with finely rounded tip, and male absent. Scanning electron microscope (SEM) study, the first of a representative of the genus, shows a lip region pattern significantly different from that observed in the typical aporcelaimid taxa.

Key words: aporcelaims, description, free-living nematodes, southeast Asia, taxonomy.

The genus *Aporcelinus* Andrássy, 2009 is a worldwide distributed, free-living nematode taxon, mainly dwelling soils of both natural and agricultural habitats and, more exceptionally, freshwater sediments too. It hitherto contains 18 valid species, but many of them certainly remain to be discovered. This is the second contribution in a short series devoted to studying the abundant material of this genus collected in Vietnam, the first one (Nguyen et al., 2016) focused on the description of two new species, namely *Aporcelinus paramamillatus* and *Aporcelinus paraseychellensis*.

Recently, Vinciguerra et al. (2014) raised some doubt about the taxonomy of *Aporcelinus*. It was originally classified under Aporcelaimidae Heyns, 1965, but also shows some morphological affinities with representatives of Qudsianematidae Jairajpuri, 1965. The study of a nondescribed species, the third one from Vietnam, with the first available SEM pictures of a representative of the genus, will probably provide additional evidence to clarify its taxonomical position.

## MATERIALS AND METHODS

Sampling: Soil samples were collected in a natural area on Cat Ba Island of northern Vietnam and temporally stored in plastic bags to transport them to the laboratory. Nematode extraction was made following the methods by Baermann (1917) and Flegg (1967). Then, the worms were relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerol

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according with Siddiqi's (1964) technique, and mounted on permanent glass slides for their handling and observation under light microscope.

*Light microscopy:* Measurements, line drawings, and pictures were taken using a Nikon Eclipse 80i light microscope provided with differential interference contrast optics, a Nikon Digital Sight DS-U1 camera and a drawing tube. Morphometrics include Demanian ratios as well as other relevant measurements, and are presented in Table 1. Raw photographs were edited using Adobe Photoshop CS.

Scanning electron microscopy: After their examination and identification, one specimen in good condition was selected to its observation under SEM following the protocol by Abolafia (2015). The nematode was hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope.

## RESULTS

## Aporcelinus falcicaudatus<sup>\*</sup> sp. n. (Figs. 1-3; Table 1)

*Material examined:* Nine females, in variable state of preservation.

#### Description

*Female*: Moderately slender (a = 23-28) nematodes of medium size, 1.28 to 1.63 mm long. Habitus visibly curved ventrad on fixation, C- or G-shaped. Cuticle two-layered, 3 to 4 µm thick at anterior region and midbody and 6 to 7 µm on tail; outer layer thin and bearing fine but conspicuous transverse striation throughout the body, specially visible under SEM; inner layer thicker and more refractive than the outer layer. Lateral chord 4 to 9 µm broad or 5% to 13% of midbody diameter. Body pores often obscure, but two cervical pores are visible at both dorsal and ventral sides at level of odontostyle plus odontophore. Lip region angular, offset by a shallow constriction, 2.7 to 3.0 times as wide

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Population	Cat Ba National Park(Hai Phong Province)		
	Secondary forest		
	Holotype (♀)	Paratype (7♀♀)	Primary forest ♀
L	1,554	$1.41 \pm 0.12 \ (1.28 - 1.62)$	1,508
a	27	$25.8 \pm 3.5 \ (16.8-27.9)$	23
b	4.3	$3.7 \pm 0.2 \ (3.6-4.3)$	3.7
с	52	$48.8 \pm 5.2 \ (42.7 - 58.1)$	50
c'	0.8	$0.8 \pm 0.1 \ (0.7-0.9)$	0.9
V/T	54	$53.1 \pm 1.9 \ (50.2 - 55.3)$	51
Lip region diameter	16	$17 \pm 0.7 (16 - 18)$	16
Odontostyle length at ventral side	20	$20 \pm 1.0 (18-21)$	21
Odontostyle length at dorsal side	22	$21.5 \pm 1.1 \ (20-23)$	22
Odontophore length	37	$39.5 \pm 5.6 (37 - 53)$	39
Guiding ring from anterior end	10	$10.5 \pm 0.7 (10.0-12)$	11
Neck length	360	$374 \pm 18.4 \ (354-406)$	411
Pharyngeal expansion length	200	$200 \pm 14.1 \ (177 - 218)$	183
Diameter at neck base	51	$52.5 \pm 4.1 \ (46-57)$	59
At midbody	58	$56 \pm 8.8 \ (50-77)$	67
At anus	37	$35 \pm 2.4 (33 - 39)$	35
Prerectum length	51	$40 \pm 9.2 (33-59)$	62
Rectum length	41	$37 \pm 1.7 (35 - 40)$	35
Tail length	30	$29 \pm 2.5$ (23–31)	35

TABLE 1. Morphometrics of *Aporcelinus falcicaudatus* sp. n. measurements in  $\mu$ m (except L, in mm), and in the form: mean  $\pm$  standard deviation (range).

as high and 27% to 37% of body diameter at neck base; lips (under SEM) moderately separated by marked interlabial depressions; labial papillae somewhat protruding, the inner ones a little bigger than the outer, all of them button-like, each surrounded by a ring-like incisure; cephalic papillae less protruding than the labial papillae, bearing a short transverse opening and not surrounded by a ring-like incisure; oral field poorly demarcated, with coarse radial incisures (striations) starting at the oral field and running along the interlabial depressions. Amphideal fovea cup-shaped, its opening 10 to 12 µm broad or more than one-half of lip region diameter. Cheilostom nearly as long as wide, with no specialization. Odontostyle strong, 1.2 to 1.4 times longer than lip region diameter, 5.8 to 7.3 times as long as wide, and 1.29% to 1.72% of total body length; aperture 9 to 10 µm or 43% to 50% of its total length. Guiding ring simple but distinct and somewhat plicate, located at 10 to 12 µm or 0.6 to 0.7 times the lip region diameter from the anterior end. Odontophore rod-like, 1.7 to 2.3 times the odontostyle long. Pharynx consisting of a slender but muscular anterior section enlarging very gradually in the posterior expansion that is 7.3 to 10.0 times as long as wide, 3.3 to 4.3 times the corresponding body diameter and occupies 49% to 56% of total neck length; gland nuclei located as follows (n = 1): DO = 57, DN = 61, S<sub>1</sub>N<sub>1</sub> = 71, S<sub>1</sub>N<sub>2</sub> = 78, S<sub>2</sub>N = 88. Nerve ring at 114 to 143 µm or 30% to 39% of total neck length from the anterior end. Pharyngo-intestinal junction bearing a distinctly developed dorsal lobe; cardia conical, 10 to  $13 \times 11$  to 16 µm; a dorsal cell mass is present at level of pharyngo-intestinal junction. Genital system didelphic-amphidelphic, with both

branches equally and moderately developed, 122 to 214 µm long or 9% to 13% of total body length: ovaries variably sized, 41 to 183 µm long, often surpassing the oviduct-uterus junction; oviduct 45 to 94 µm long or 0.8 to 1.6 times the body diameter, and consisting of a slender portion and a moderately developed pars dilatata; a narrowing separates oviduct and uterus; uterus 61 to 95  $\mu$ m long or 1.0 to 1.7 times the corresponding body diameter long, tripartite, that is consisting of a short and wider proximal region, a narrower intermediate section with no lumen, and a nearly spherical distal part; vagina extending inward 24 to 30 µm or 40% to 51% of body diameter, with pars proximalis 15 to  $20 \times 14$  to 18 µm and somewhat sigmoid walls surrounded by weak musculature, *pars refringens* consisting of two drop-shaped to trapezoidal pieces 4 to  $6 \times 4$  to 6 µm and a combined width of 8 to 13 µm, and pars distalis 3 to 5 µm long and visibly refractive; vulva a transverse slit. Prerectum 1.1 to 1.6, rectum 0.9 to 1.1 times the anal body diameter long. Tail conical with rounded tip, ventrally convex, dorsally first convex, and then concave, always strongly recurved dorsad; cuticle at ventral side much thicker than at dorsal side, bearing a small space (lacuna) between the cuticle layers at the anterior part of the dorsal concavity; hyaline portion easily perceptible in most specimens, up to 10 µm long; caudal pores two pairs, subdorsal, at the middle of tail.

Male: Unknown.

*Diagnosis:* The new species is characterized by its 1.28 to 1.63 mm long body, lip region offset by weak constriction and 16 to 18  $\mu$ m broad, odontostyle 18 to 21  $\mu$ m at its ventral side of 1.2 to 1.4 times the lip region diameter, neck 354 to 406  $\mu$ m long, pharyngeal



FIG. 1. Aporcelinus falcicaudatus sp. n. (Female). A. Lip region in surface, lateral view. B. Anterior region in median, lateral view. C. Neck region. D. Anterior genital branch. E. Entire. F–H. Posterior body region.

expansion 177 to 218  $\mu$ m long or 49% to 56% of total neck length, a dorsal cell mass present at the pharyngointestinal junction, uterus tripartite and 61 to 95  $\mu$ m long or 1.0 to 1.7 times the corresponding body diameter long, V = 50 to 55, tail (23–31  $\mu$ m, c = 43–58, c' = 0.7–0.9) strongly recurved dorsad and conical with finely rounded tip, and male absent.

*Relationships:* The new species is easily recognizable by the morphology of its caudal region (Fig. 2H–N): strongly recurved dorsad, cuticle at ventral side much thicker than

at dorsal side, the presence of a hyaline small space (lacuna) between the two cuticle layers at level of the dorsal concavity, and a conspicuous terminal hyaline portion.

In its general morphometrics (medium-sized body, lip region up to 18  $\mu$ m broad, odontostyle ca. 20  $\mu$ m long) and caudal region with a distinct dorsal concavity, this species resembles *A. irritans* (Cobb in Thorne and Swanger, 1936) Andrássy, 2009, *A. jiaonanensis* (Zhao and Zhao, 2010) Álvarez-Ortega and Peña-Santiago, 2013, and *A. seychellensis* (Andrássy, 2011) Andrássy, 2009, but it differs



FIG. 2. Appreclinus falcicaudatus sp. n. (Female, light microscopy). A. Entire. B. Anterior region in median view. C. Pharyngeal expansion and pharyngo-intestinal junction. D. Posterior genital branch. E. Lip region in lateral, surface view. F. Vagina. G. Oviduct-uterus junction. H. Posterior body region. I–N. Caudal region. (Scale bars: A = 200 μm; B, E, F, I–N = 10 μm; C = 50 μm; D, G, H = 20 μm.)

from these in its strongly (vs. not distinctly) recurved dorsad caudal region. Besides, it can be distinguished from A. irritans, a poorly known species, in its broader lip region (16-18 vs. 14-15 µm) and much shorter caudal region (23–31  $\mu$ m, c = 47–58, c' = 0.7–0.9 vs. 39–42  $\mu$ m, c = 36, c' = 1.3-1.4); from A. *jiaonanensis* in its much shorter caudal region (23–31  $\mu$ m, c = 47-58, c' = 0.7-0.9 vs.  $38-46 \,\mu\text{m}, c = 32-40, c' = 1.1-1.4$ ; and from A. seychellensis in its shorter caudal region (23–31  $\mu$ m, c = 47-58 vs. 33–43  $\mu$ m, c = 25-42) with rounded (vs. very acute) tip.

# Type locality and habitat

Holotype: Northern Vietnam, Cat Ba National Park, Hai Phong Province (GPS coordinates: 20° 47'45" N

and 107° 00'40"E, elevation 170 m), in soil of a secondary forest with Cinnamomum sp., Machilus sp., and Dimocarpus sp. as dominant plants. Deposited on slide Cat Ba 13.02 in the nematode collection of the University of Jaén, Spain.

Paratypes: Same locality and habitat that holotype. Seven paratypes deposited on slides Cat Ba 13.01 and 13.02 in the nematode collection of the University of Jaén, Spain. One paratype on slide Cat Ba 22.02 in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Etymology: The specific epithet is a compound Latin term meaning "sickle-shaped tail."



FIG. 3. Aporcelinus falcicaudatus sp. n. (Female, scanning electron microscope). A, B. Anterior region in sublateral view. Entire. C. Lip region in face view. D. Caudal region in lateral view. E. Vulva in ventral view. F. Caudal region in subdorsal view. (Scale bars:  $A-C = 5 \mu m$ ;  $D-F = 10 \mu m$ .)

#### DISCUSSION

As mentioned in the introductory section, Andrássy (2009) originally classified *Aporcelinus* under Aporcelaimidae, an action that has been recently questioned by Vinciguerra et al. (2014). The first SEM study of a representative of *Aporcelinus* is herein provided. Its results show that the lip region pattern significantly differs from that observed in the typical aporcelaims, which is characterized by having a biradial symmetry, oral aperture a large, dorsoventral, hexagonal slit, and a distinctly demarcated oral field (for instance, see Álvarez-Ortega et al. [2012] for *Aporcelaimellus communis*; Nguyen et al. [2016] for *Sectonema* spp.). Then, Vinciguerra et al.'s opinion is herein supported. Nevertheless, further data should be obtained, especially molecular analyses, to solve definitively the matter.

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