Morphological and molecular characterization of *Epidorylaimus procerus* sp. n. (Dorylaimida: Qudsianematidae) from Vietnam

Thi Anh Duong Nguyen\(^1,3,5,*\) and Reyes Peña-Santiago\(^2\)

\(^1\)Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

\(^2\)Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus ‘Las Lagunillas’ s/n, Edificio B3, 23071, Jaén, Spain.

\(^3\)Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

\(*\)E-mail: nad2807@gmail.com

This paper was edited by Zafar Ahmad Handoo.

Received for publication June 18, 2020.

---

The genus *Epidorylaimus* Andrássy, 1986 is a widespread taxon in Dorylaimida. Originally proposed to accommodate 12 species previously included in *Eudorylaimus* Andrássy, 1959 its taxonomy was later updated by several authors (Andrássy, 1992; Jairajpuri and Ahmad, 1992; Vinciguerra, 2006; Andrássy, 2009). More recently, Ahmad et al. (2016) presented an emended diagnosis, a list of 15 valid species with a key to their identification, and a compendium of their more relevant morphometrics.

In spite of the nearly cosmopolitan distribution of the genus, none of its species was hitherto recorded in Vietnam. However, a nematological survey conducted to study nematode fauna associated with natural habitats in the country located an interesting *Epidorylaimus* species. Its study revealed that it belonged to an unknown species that is hereunder described.

---

**Abstract**

*Epidorylaimus procerus* sp. n., collected from a natural habitat in Vietnam, is described and illustrated. It is distinguishable by its 2.16 to 2.46-mm-long body, lip region offset by depression and 15 to 17-µm broad, odontostyle 32 to 35-µm long, neck 415 to 461-µm long, pharyngeal expansion occupying 47 to 52% of the total neck length, uterus 76 to 130-µm long or 1.0 to 1.5-body diameters, vulva transverse (\(V=40-43\)), caudal region conical elongate (157-186 µm, \(c=12.1-14.4\), \(c^\prime=4.4-5.5\)) with blister-like bodies, and hyaline portion occupying one-fourth its length, and males absent. Molecular analysis shows a close relationship of the new species and *E. lugdunensis*, supporting monophyly of the genus *Epidorylaimus*.

**Keywords**

D2-D3 28S-rRNA, Description, Morphology, Morphometry, Nematode, New species, Taxonomy, Vietnam.

---

**Materials and methods**

**Nematode extraction and processing**

Soil samples were collected in Cao Bang Province, a natural area of Northern Vietnam, and temporarily stored in plastic bags for transport to the laboratory. Nematode extraction was done following the methods of Baermann (1917) and Flegg (1967). Specimens were relaxed and killed with heat, fixed in 4% formaldehyde, processed to anhydrous glycerin according to Siddiqi’s (1964) technique, and mounted on permanent glass slides for handling and observation with light microscopy.

**Light microscopy (LM)**

Specimens were measured, drawn, and identified with a Nikon Eclipse 80i light microscope equipped
Epidorylaimus procerus sp. n. from Vietnam: Nguyen and Peña-Santiago

DNA extraction, PCR, and sequencing

DNA was extracted from single individuals using the proteinase K and Worm Lysis Buffer protocol (William et al., 1992). Each nematode was transferred to a 0.5-ml Eppendorf tube containing 18µl of Worm Lysis Buffer (WLB) (50 mM KCl, 10 mM Tris, pH 8.3, 2.5 mM MgCl₂, 0.45% NP 40, and 0.45% Tween 20) and 2 µl of proteinase K (600 µg ml⁻¹) (Thermo Scientific). The tubes were incubated at 65°C (1 hr) and then at 95°C (15 min). PCR was performed in a 30-µl final volume containing 24.9 µl of sterile water, 0.6 µl of each PCR primer, 0.6 µl of dNTP, 0.3 µl of Taq-polymerase, 3 µl of Buffer 10x Thermo Scientific Green, and 1 µl of DNA-extracted solution. The PCR amplification profile consisted of 4 min at 94°C, 35 cycles of 1 min at 94°C, 1.5 min at 55°C, and 2 min at 72°C, followed by a final step of 10 min at 72°C. The primers used for amplification were D2A (5´-ACAAGTACCGTGAGGGAAAGTTA-3´) and D3B (5´-TCCTCGGAAGGAACCAGCTACTA-3´) for amplification of the D2-D3 region of 28S (Subbotin et al., 2006).

PCR products were purified with the GeneJET PCR Purification Kit (#K0701, Thermo Scientific, USA), following the manufacturer’s manual. The sequencing reaction was performed with 15 ng of purified template, 4 µl of BigDye Terminator v3.1 Ready Reaction Mix, 2 µl of 5X Sequencing Buffer, and 3.2 pmol of forward/reverse primers for a total of 10-µL volume. The mixture was heated for 10 sec at 96°C, then 5 sec at 55°C, repeated for 32 cycles followed by 4 min at 60°C. The sequencing was performed on 3500xL Genetic Analyzers (Applied Biosystems, Foster City, California) at the National Key Laboratory of Gene Technology (IBT – VAST, Hanoi). The sequences obtained were submitted to the GenBank database under accession numbers MT612084 and MT612088.

Phylogenetic analysis

The obtained sequences were aligned with 35 other D2-D3 expansion segments of 28S rDNA gene sequences available in GenBank, using SeaView with the muscle algorithm followed by manual refining (V4.5.3, Gouy et al., 2010). Outgroup taxa were chosen according to previously published data (Holterman et al., 2008; Álvarez-Ortega et al., 2013). The sequence dataset was analyzed with Bayesian inference (BI) and maximum likelihood (ML) using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We chose the commonly used model GTR+I+F that then was used to calculate phylogenetic trees in PhyML 3.1 with 100 replicates and MrBayes with a burn-in of 25% and the final split frequencies of less than 0.01 (settings: mcmc ngen = 1,000,000; sample freq = 500; print freq = 500; diag freq = 5,000; Ronquist and Huelsenbeck, 2003; Altekar et al., 2004). Both trees where then combined into one reconstructed phylogeny in Adobe Illustrator® (Creative Suite 6).

Results

Epidorylaimus procerus sp. n. (Figs. 1 and 2)

Material examined

In total, there were 15 females from one location in excellent state of preservation.

Morphometrics

see Table 1.

Description

Female

There were moderately slender (a = 29-33) nematodes of medium size, 2.16-2.46-mm long. Their body was cylindrical, tapering anteriorly, extended posteriorly as an elongate, conical, and had a curved tail. Upon fixation, the habitus slightly curved ventrad, C- to hook-shaped. The cuticle was two-layered, 2 to 3-µm thick at the anterior region, 3.5 to 4 µm in mid-body, and 6.5 to 8.5 µm on the tail; the outer layer was thin and smooth, with constant thickness throughout the body; the inner layer was much thicker than the outer layer, which was especially conspicuous at the tail. The lateral chord was 15 to 19-µm broad, occupying 21 to 27% of mid-body diameter. Body pores were obscure. The lip region was truncate, offset by a distinct constriction, 1.9 to 2.3 times broader than high and one-fifth to one-fourth (21-26%) of body diameter at the neck base; the lips were amalgamated, with visibly protruding labial and cephalic papillae. Amphid fovea appeared to be cup-like, its aperture measured 4 to 5.5 µm, occupying up to one-third (28-33%) of
Figure 1: *Epidorylaimus procerus* sp. n. (female). A: Entire. B, C: Anterior region in lateral median view. D: Lip region in lateral surface view. E: Pharyngo-intestinal junction. F: Vagina. G: Neck region. H: Anterior genital branch. I: Posterior body region [scale bars: A=500 µm; B, C=10 µm; D, F=5 µm; E=20 µm; G-I=50 µm].
Figure 2: *Epidorylaimus procerus* sp. n. (female, LM). A: Entire. B-D: Anterior region in lateral median view. E: Neck region. F: Pharyngo-intestinal junction. G: Lip region in lateral surface view. H: Anterior genital branch. I: Uterine egg. J: Vagina. K: Caudal region. L: Anterior portion of the caudal region in median view. M: Same in submedian view, showing the blister-like elements mainly subventrally and ventrally [scale bars: A = 500 µm; B-D = 10 µm; E, H, K = 50; F, I, L, M = 20 µm; G, J = 5 µm].
lipo-region diameter. Cheilostom was a truncate cone, without any particular differentiation. Odontostyle was relatively long and slender, 10.7 to 11.5 times longer than wide, length about twice (1.9-2.1 times) the diameter of the lip-region diameter, and about 1.38 to 1.51% of the total body length, and about 1.38 to 1.51% of the total body length. The aperture measured 6.5 to 8.0-µm long, occupying up to one-fourth (20-25%) of odontostyle length. Odontophore was rod-like, nearly equal (0.8-1.0 times) in length to odontostyle. The pharynx was entirely and conspicuously muscular, with its slender portion enlarging gradually, and the basal expansion being 5.4 to 6.5 times as long as wide, 3.0 to 3.6 times longer than body diameter at the neck base, and occupying ca one-half (47-52%) of neck length; gland nuclei are located as follows: DO = 56 to 63, DN = 59 to 65, S, N = 77 to 80, S, N = 78 to 83, and S, N = 89 to 90. The nerve ring was situated at 152 to 165 µm or 34 to 37% of the total neck length from the anterior end. The pharyngo-intestinal junction consisted of a well-developed, conical cardia measuring 19 to 29×11 to 15 µm, enveloped by the intestinal wall. The intestine lacked any relevant differentiation. The genital system was didelphic-amphidelphic, with both branches equally developed, anterior 212 to 278 µm or 10 to 13%, posterior 206 to 324 µm or 9 to 14% of the total body length. The ovaries were reflexed, the anterior measured 122 to 165 µm, and the posterior 140 to 229-µm long, often reaching and surpassing the oviduct-uterus junction, with oocytes arranged first in two or more rows and then in a single row. The oviduct was 91 to 132-µm long or 1.3 to 1.8-body-diameter long, consisting of a distal, slender section made of prismatic cells and a poorly developed proximal pars dilatata with visible lumen inside. A narrowing surrounded by a muscular ring (sphincter) separates the oviduct and the uterus. The uterus is a simple, tube-like structure measuring 76 to 130 µm or

---

Table 1. Morphometrics of *Epidorylaimus procerus* sp. n. from Vietnam.

| Character                      | Holotype       | Paratypes |
|-------------------------------|----------------|-----------|
|                               | ♀              | 15♀♀      |
| Lip-region diameter           | 17             | 15.9±0.6 (15-17) |
| Odontostyle length            | 32             | 33.4±1.2 (32-35) |
| Odontophore length            | 32             | 30.2±1.5 (28-33) |
| Neck length                   | 432            | 435±18 (415-461) |
| Pharyngeal expansion length   | 204            | 212±13 (194-238) |
| Body diam. at neck base       | 61             | 65.0±4.0 (61-73) |
| Mid-body                      | 77             | 72.6±3.4 (68-79) |
| Anus/cloaca                   | 36             | 35.2±1.3 (32-36) |
| Distance vulva – anterior end  | 962            | 938±43 (885-1012) |
| Prerectum length              | 91             | 91.8±18.4 (70-123) |
| Rectum length                 | 42             | 42.5±4.6 (35-49) |
| Tail length                   | 182            | 171±9.2 (157-186) |

Note: Measurements are in µm, except *L* in mm, and paratype measurements are average±sd (range).
Figure 3: Combined Bayesian inferred and maximum likelihood tree of *Epidorylaimus procerus* sp. n. based on sequences of the 28S rDNA region.
1.0 to 1.5-body-diameter long. The vagina extended inward from 28 to 34 µm, occupying 39 to 46% of body diameter: pars proximalis 18-25 × 14-21 µm, and was surrounded by moderately developed circular musculature; pars refringens consisted of (in lateral view) two trapezoidal sclerotized pieces, 3.5-6 × 6-7 µm, with a combined width of 12.5 to 14.5 µm; pars distalis measured 4.5 to 6.5-µm long. Vulva was a transverse slit. Prerectum 2.0-3.4x, rectum 1.0-1.4x times the anal body diameter long. The tail was conically elongated, gradually tapering to a finely rounded tip, and strongly curved ventrad, with abundant saccate- or blister-like bodies inside the cuticle at its anterior third; two pairs of caudal pores, one subventral, another subdorsal, at about one anal-body diameter behind the level of the anus; the inner core could not reach the tail tip; therefore, a hyaline portion exists measuring 36 to 48-µm long and occupying ca one-fourth (22-28%) of the total tail length.

Male
Unknown.

Diagnosis
The new species is distinguished from other Epidorylaimus spp. by a combination of the following character states and morphometrics: body 2.16 to 2.46-mm long, lip region offset by constriction, width 15 to 17-µm broad, odontostyle 32 to 35-µm long with aperture occupying 20 to 25% of its length, neck 415 to 461-µm long, pharyngeal expansion 194 to 238-µm long (47-52% of the total neck length); the female genital system was described as follows: didelphic-amphidelphic, uterus simple and 76 to 130-µm long (1.0-1.5 body diameters), and vulva transverse (V = 40-43); the caudal region was conically elongated (157-186µm, c = 12.1-14.4, c’ = 4.4-5.5) with blister-like bodies and hyaline portion occupying one-fourth its length.

Relationships
Epidorylaimus procerus sp. n. resembles E. mellenbachensis (Alther, 1974) Andrássy, 1986 and E. rivalis Gagarin, 1991., and can be easily distinguished from other species of the genus (see key and table compendium by Ahmad et al., 2016) in having odontostyle longer than 25 µm. It differs from the German freshwater species E. mellenbachensis in its larger general size (body length 2.16-2.46 vs 1.70-2.00), less slender body (a = 29-33 vs 42-50), longer odontostyle (32-35 vs 28-29µm; 1.9-2.1 vs 1.25 times lip-region diameter), much more anterior vulva (V = 39-42 vs V = 50-55), and relatively longer tail (c = 12-14 vs c = 19-38; c’ = 4.4-5.5 vs c’ = 3-4). From the Russian species E. rivalis, it differs in being longer (2.16-2.46 vs 1.46-2.29 mm, n = 15), the lip region is less differentiated (weak vs deep constriction), and it has a longer odontostyle (32-35 vs 28-30µm). Males of E. rivalis are as abundant as females, whereas they are unknown in E. procerus n. sp. and thus likely to be rare or absent.

Evolutionary relationships, as derived from the analysis of D2-D3 28S-rRNA gene sequences, are presented in a molecular tree (Fig. 3). The new species forms part of a highly supported (99.9%) clade with E. lugdunensis de Man, 1880, the type species of the genus, supporting monophyly of Epidorylaimus. Both species appear closely related (100% support) to Crassolabium circuliferum Loof, 1961, a member of the family Dorylaimidae, and form part of a highly supported larger clade that includes members of the families Nordiidae and Qudsianematidae.

Type locality and habitat
Vietnam, Cao Bang Province, Cao Bang Natural Reserve (GPS coordinates: 22° 34′ 07″ N, 105° 52′ 34″ E), in a tropical evergreen forest soil with Dipterocarpus sp. and Cinnamomum sp. as dominant plant species.

Type material
Female holotype and nine female paratypes were deposited with nematode collection of the University of Jaén, Spain. Five female paratypes were deposited with the nematode collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Etymology
The specific epithet is the Latin term procerus = slim or svelte, and refers to the slender figure of these nematodes.

Acknowledgments
The authors thank the Director of Cao Bang Nature Reserve for providing facilities to collect soil samples, and the financial support received from the projects NAFOSTED (National Foundation for Science and Technology Development, Vietnam) 106.05–2017.330 and ‘PAIJAVA 2019/2020: EI_RNM02_2019’
of the University of Jaén, Spain. The assistance of Dr. O. Holovachov (Stockholm, Sweden) in the translation of a paper from the Russian language is also much appreciated.

References

Ahmad, W., Imran, Z. and Araki, M. 2016. *Epidorylaimus monhystera* sp. n., an atypical species of the genus *Epidorylaimus* Andrásy, 1986 (Dorylaimida: Qudsianematidae) from Japan. Zootaxa 4072:90–100.

Altekar, G., Dwarkadas, S., Huelsenbeck, J. P. and Ronquist, F. 2004. Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20:407–15, doi: 10.1093/bioinformatics/btg427.

Altherr, E. 1974. Nématodes de la nappe phréatique du réseau fluvial de la Saale (Thuringe), II. Limnologica 9:81–132.

Álvarez-Ortega, S., Subbotin, S. A. and Peña-Santiago, R. 2013. Morphological and molecular characterization of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). Nematology 15:431–9, doi: 10.1163/15685411-00002691.

Andrássy, I. 1959. Taxonomische Übersicht der Dorylaimen (Nematoda). I. Acta Zoologica Hungarica 5:191–240.

Andrássy, I. 1986. The genus *Eudorylaimus* Andrásy, 1959 and the present status of its species (Nematoda: Qudsianematidae). Opuscula Zoologica Budapestinensis 22:1–42.

Andrássy, I. 1992. The superfAMILY Dorylaimoidea (Nematoda) – a review. Family Qudsianematidae, II. Opuscula Zoologica Budapestinensis 24:3–55.

Andrássy, I. 2009. Free-living nematodes of Hungary. Ill. Pedozoologica Hungarica no. 5 Hungarian Natural History Museum, Budapest, Hungary, 608 pp.

Baermann, G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneeskundig Tijdschrift voor Nederlandsch-Indiën 57:131–7.

Flegg, J. J. M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb’s decanting and sieving technique. Annals of Applied Biology 60:429–37.

Gagarin, V. G. 1991. [Seven new species of freshwater nematodes.] Zoologicheskii Zhurnal 70:20–7. (In Russian.)

Gouy, M., Guindon, S. and Gascuel, O. 2010. SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27:221–4, doi: 10.1093/molbev/msp259.

Holtermann, M., Rybarczyk, K., van den Elsen, van Mengen, S. H., Mooyman, P., Peña-Santiago, R., Bongers, T., Bakker, J. and Helder, J. 2008. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. Molecular Ecology Resources 8:23–34, doi: 10.1111/j.1755-0998.2007.01963.x.

Holzinger, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:515–25.

Jairajpuri, M. S. and Ahmad, W. 1992. Dorylaimida. Free-living, predaceous and plant-parasitic Nematodes. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India, 458 pp.

Ronquist, F. and Huelsenbeck, J. P. 2003. MrBAYES3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–4, doi: 10.1093/bioinformatics/btg180.

Siddiqi, M. R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Zeitschrift für Zoologische Systematik und Evolutionsforschung 2:174–84, doi: 10.1111/j.1439-0469.1964.tb00720.x.

Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N. and Baldwin, J. G. 2006. Phylogenetic analysis of *Tylenchida* Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology 8:455–74.

Vinciguerra, M. T. 2006. “16. Dorylaimida Part II: Superfamily Dorylaimoidea”, In Abebe, E., Traunspurger, W. and Andrássy, I. (Eds), Freshwater Nematodes: Ecology and Taxonomy. Wallingford: CAB International, pp. 392–467.

Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. and Waterston, R. H. 1992. A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Genetics 131:609–24.