

**NEW RECORDS OF RING NEMATODES, *Bakernema enormese* sp. n.  
AND *Criconema (Nothocriconemella) graminicola* Loof, Wouts & Yeates, 1997  
(Nematoda: Criconematidae) FROM NATURAL FORESTS OF VIETNAM**

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**ABSTRACT**

*Bakernema enormese* sp. n., collected from rhizosphere of forest wood trees in Muong Phang, Dien Bien Province (north Vietnam) is described and illustrated. The new species is characterized by large body size and stylet. In general, this new species is close to two existing species of the same genus, *B. inaequale* and *B. dauniense* by cuticle structure in transparent membranous projections which appear in lateral view as spine-like structures on each annulus. These structure arranged into several rows along the body. In morphology, the new species differs from *B. inaequale* and *B. dauniense* by body and stylet length, i.e. 609–842  $\mu\text{m}$  and 143.5–150  $\mu\text{m}$  vs. 391–578  $\mu\text{m}$  and 59–74  $\mu\text{m}$  for *B. inaequale* and vs. 391–461  $\mu\text{m}$  and 65–74  $\mu\text{m}$  for *B. dauniense*. In addition, new species can be distinguished from *B. inaequale* by the longer membranous projection, 8–12 vs. 6–10  $\mu\text{m}$  and vagina shape, curved vs. sigmoid. From *B. dauniense*, the new species differs by the much longer membranous projection, 8–12 vs. 1.4–2.2  $\mu\text{m}$  and less number annules between vulva and tail end (RV), 3–4 vs. 7.8 annules. The presence of *Criconema (Nothocriconemella) graminicola* Loof, Wouts & Yeates, in Vietnam with morphometrics, illustrators and remarks given.

**Keywords:** *Bakernema*, *Criconema*, morphology, taxonomy.

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## INTRODUCTION

Plant-parasitic nematodes of the family Criconematidae Taylor, 1936 (Nematoda: Tylenchida), commonly known as ring nematodes due to their very strongly annulated body cuticle with complex ornamentation in some species, are considered ectoparasites causing damage to host plants which may result in serious loss. Taxonomically, this nematode group is of moderate diversity in Vietnam. Currently, seven genera of ring nematodes have been recorded in Vietnam, including *Criconemella* de Grise & Loof, 1965 (9 species), *Criconema* Hofmann & Menzel (1 species), *Ogma* Southern, 1914 (2 species), *Discocriconemella* de Grise & Loof, 1965 (4 species), *Hemicriconemoides* Chitwood & Birchfield, 1957 (5 species), *Caloosia* Siddiqi & Goodey, 1957 (1 species) and *Hemicycliophora* de Man, 1921 (1 species) with a total of 23 species (Eroshenko & Nguyen, 1981; Eroshenko et al., 1985, Nguyen & Nguyen, 2001). Of these, four species were recorded as new species, e.g. *Criconemella helica* Eroshenko & Nguyen, 1981; *C. magnifica* Eroshenko & Nguyen, 1981; *Hemicriconemoides magnificus* Nguyen & Nguyen, 2001 and *Hemicycliophora vietnamense* Nguyen & Nguyen, 2001.

Recently, during surveys and sampling on plant parasitic and entomopathogenic nematodes in natural forest ecosystems in Vietnam, two species of ring nematodes were recorded from the rhizosphere of forest trees in Muong Phang forest (Dien Bien Province) and Nam Cat Tien National Forest Park (Dong Nai Province). Of these two, an unknown species named as *Neobakernema enormese* is described and illustrated with drawings and microphotographs. The other species *Criconema* (*Nothocriconemella*) *graminicola* Loof, Wouts & Yeates, 1997, a first record for Vietnam, is also described, illustrated and compared with the literature.

## MATERIALS AND METHODS

*Field survey and sampling:* For each composed sample, 250 ml of rhizosphere soil

and roots within the area of 20 square centimeters around tree base was taken using a sampling shovel.

*Nematode extraction:* Nematodes from 250 m soil samples were extracted using a modified Cobb's sieving-decanting technique with a final sieve with 75 µm mesh size, followed by static filtering of living nematodes using a special sieve 80 mm in diameter and 15 mm high with a cylindrical shaft 10 mm high and three supporting legs 5 mm high (Nguyen & Nguyen, 1993). The bottom of the sieve is made of nylon mesh with openings 75 µm in size. The sieve containing nematode sediment obtained after decantation was placed in a petri box 90 mm in diameter for stationary filtering of living nematodes. For the sieve containing nematode sediment, enough tap water was added to cover the layer of sediment and the sieve was left for 48 hours at room temperature, allowing nematodes to be removed through sieving into the petri box below.

Nematodes were killed in hot water at 65-70°C, then fixed in TAF and mounted in anhydrous glycerin using the slow method of Hooper and Evans (1993). Abbreviations used are defined following Siddiqi (1986) and Wouts (2006). All morphometrics were performed with a camera lucida drawing tube. Measurements are presented in micrometers (except for ratios) and expressed as the mean ± standard deviation followed by the range.

All nematode specimens examined were deposited at the Nematode Collection of the Department of Nematology, Institute Ecology and Biological Resources (IEBR) Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam.

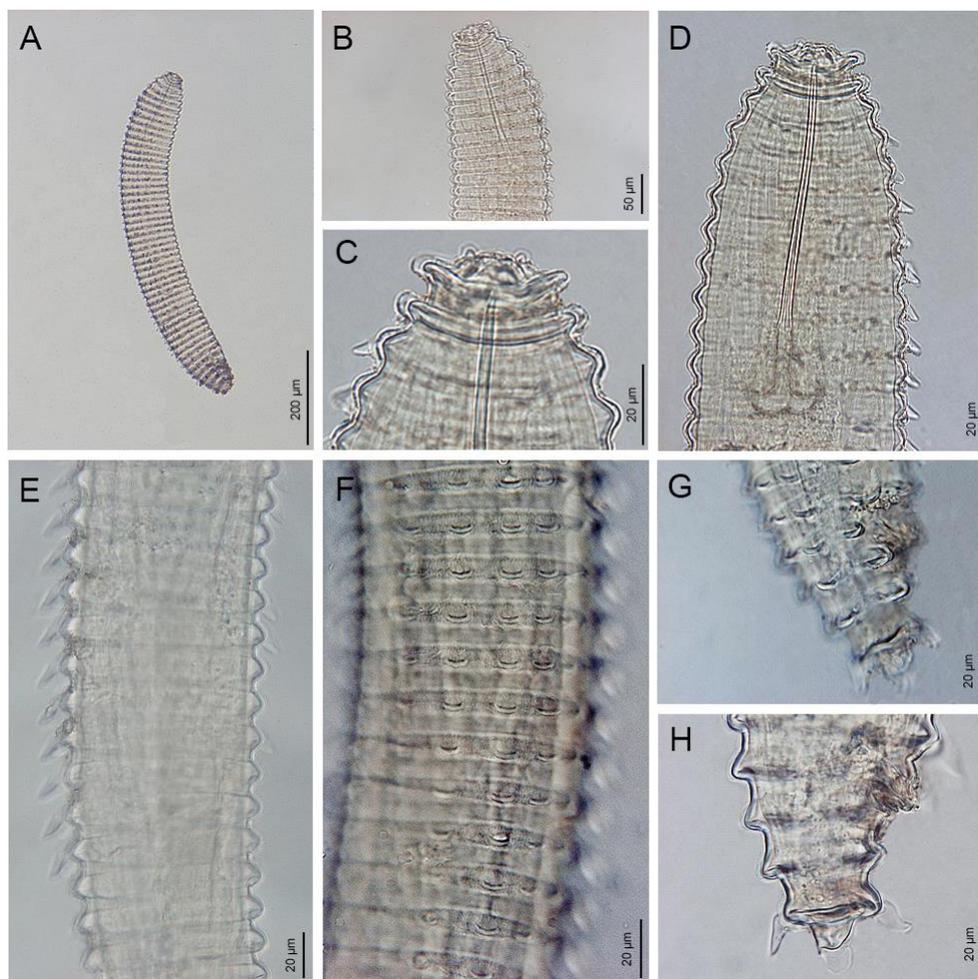
## DESCRIPTION

*Bakernema enormese* sp. n. (Figs 1, 2)

### Measurements

*Holotype (female):* L = 740 µm; body width = 10; esophagus length = 204.5; excretory pore = 268.0; V = 95%; O = 14.6; head diameter = 46.8 µm; head height = 26.9

$\mu\text{m}$ ; stylet = 150.5  $\mu\text{m}$ ; St%L = 20.5; stylet knob width = 27.4  $\mu\text{m}$ ; stylet cone length = 109.6  $\mu\text{m}$ ; DGO = 36.0  $\mu\text{m}$ ; nerve ring = 257.9  $\mu\text{m}$ ; hemizonid = 254.9  $\mu\text{m}$ . tail length = 77  $\mu\text{m}$ ; a = 7.7; b = 3.3; c = 25.0; c' = 0.8; R = 60; Rst = 12; Res = 18; Rex = 22; RV = 3; RV-an = 1; VL/VB = 0.85.



**Figure 1.** Photomicrographs of *Bakernema enorme* sp. n. A: Entire female (holotype). B: Anterior region showing esophagus (paratype). C: Lip region showing enface view with six papillae (paratype). D: Anterior region showing stylet (paratype). E: Mid-body region showing transparent spine-like appendages (membranous projections) on annules (paratype). F: Middle body showing membranous projections arranged in longitudinal rows (paratype). G–H: Posterior region showing vulva, anus and terminal cuticular membranous projections (paratype)

*Paratype females* (n = 6): L = 609.0–842.0 (730.0  $\pm$  75.5)  $\mu\text{m}$ ; body width = 90.5–106 (96  $\pm$  6)  $\mu\text{m}$ ; eso. length = 152.5–204.5 (179.6  $\pm$  20.7)  $\mu\text{m}$ ; exc. pore = 244.5–275 (261.1  $\pm$  14.7)  $\mu\text{m}$ ; stylet = 143.5–150.0 (146.7  $\pm$  3.4)  $\mu\text{m}$ ; stylet knob width = 16.5–27.5 (22.2  $\pm$  5.5)  $\mu\text{m}$ ; St%L = 17–24.5 (20.3  $\pm$  2.4)  $\mu\text{m}$ ; stylet cone length = 107.3–114.5 (110.7  $\pm$  3.5)  $\mu\text{m}$ ; O = 12.3–16.2 (14.3  $\pm$  3.8); head diameter = 36.6–46.8 (43.6  $\pm$  4.7)  $\mu\text{m}$ ; head height = 22.1–26.9 (24.6  $\pm$  2.5)  $\mu\text{m}$ ; stylet knob width = 16.5–27.4 (22.2  $\pm$  5.5) ; V = 91–95 (93.2  $\pm$  1.7) %; DGO = 25.6–36 (31.1  $\pm$  5.2)  $\mu\text{m}$ ; a = 7–8.8 (7.7  $\pm$  0.7); b = 3–

3.5 (3.3 ± 0.2); c = 19.7–25.0 (22.1 ± 2); c' = 10–13 (11.3 ± 1); Res = 17–19 (17.8 ± 0.8); 0.7–0.9 (0.8 ± 0.1); nerve ring = 235.4–267 (250 ± 15.1) μm; hemizonid = 228.4–260 (244 ± 16) μm. R = 52–70 (64.5 ± 2.9); Rst = 19–22 (20 ± 1.4); RV = 3–4 (3.7 ± 0.5); RV-an = 1–2 (1.2 ± 0.4); VL/VB = 0.8–0.9 (0.8 ± 0.6).

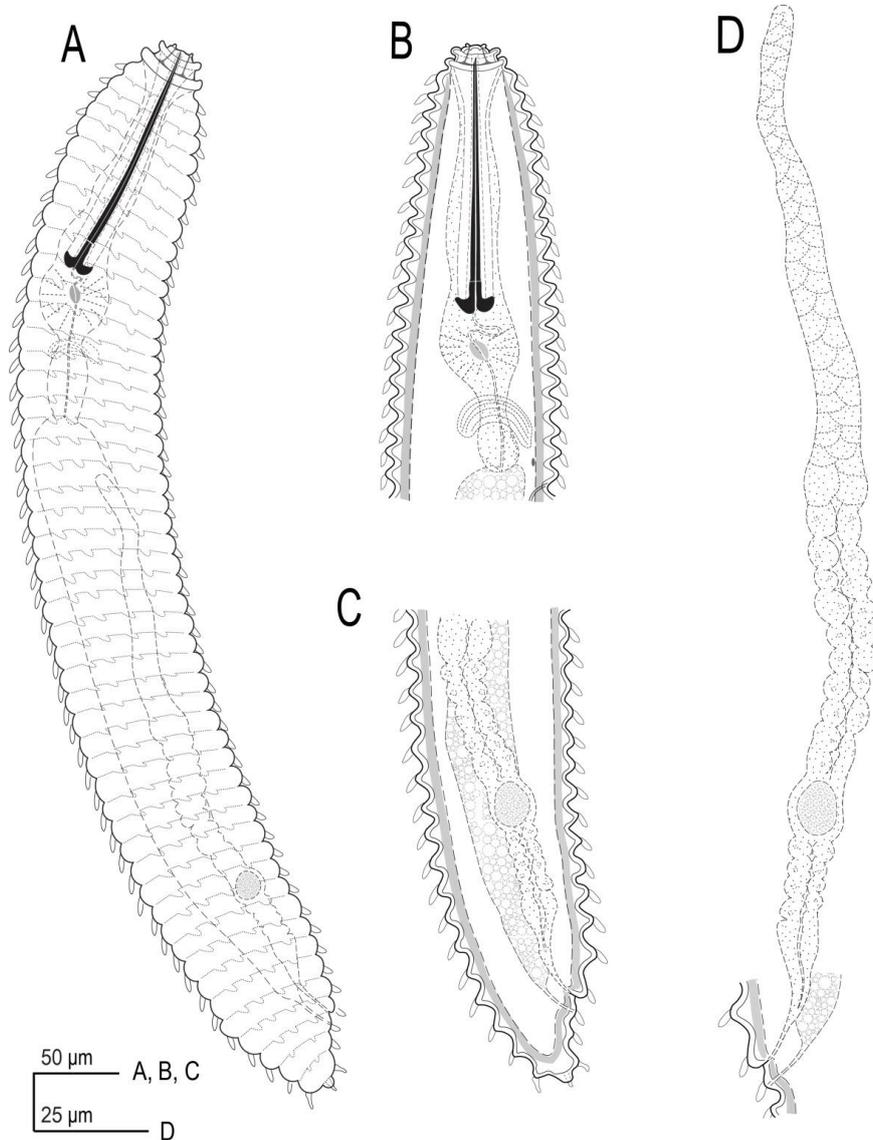


Figure 2. *Bakernema enorme* sp. n. Females. A: Entire female (holotype). B: Anterior end showing stylet (paratype). C: Posterior end (paratype). D: Female reproduction system

### Morphological characteristics

*Females*: body cylindrical upon relaxation, straight or slightly ventrally arcuate. Body tapering from stylet base to anterior end and from vulva to tail terminus. Head with two

annules, posterior one being larger than the anteriormost annule; lip region with six pseudolips surrounding the oral opening (fig. 1C). Inside head, a well-developed and strongly sclerotized cephalic framework. Inside the head, strong sclerotization of six rigid

sectors (hexaradiate) of cephalic framework. Body cuticle thick, annuli rounded and not retrorse, 5.2–5.9  $\mu\text{m}$  wide, posterior edges bearing thin, transparent membranous projections, spine-like in lateral view and 12–14  $\mu\text{m}$  long, more or less arranged in 12–14 longitudinal rows at mid-body; number of projections decreasing towards both ends of the body (figs 1E, 2A). Terminal annule without membranous projections but previous annule clearly bearing three or four projections. Stylet straight or slightly concave dorsally, stout, 140–150  $\mu\text{m}$  long or 19% of body length, with knobs concave or anchor-shaped. Esophagus typical criconematoid. Secretory-excretory pore located 1 or 3 annules posterior to esophagus base. Female genital tract monodelphic. Ovary outstretched, sometimes extending to stylet knobs. Spermatheca spherical and filled with sperms. Vulva closed, located three or four annules from tail terminus. Anterior vulval lip slightly overlapping the posterior lip. Vagina straight to slightly arcuate. Anus distinct, pit-like, located one annule posterior to vulva and two anterior to tail terminus. Tail bluntly conoid, terminal annule conoid rounded not bearing projection.

*Male:* not found.

*Type host and locality:* Specimens collected from rhizosphere soil of wood tree (unidentified) at Muong Phang conservation forest, Muong Phang commune, Dien Bien province.

*Type specimens:* Holotype female and 6 female paratypes (mounted in slide number 882 MF-DB) deposited in the Nematode Collection of the Department of Nematology, IEBR-VAST, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam.

*Diagnosis and relationships:* *Bakernema enormese* n. sp. is characterized by large body (609–842  $\mu\text{m}$ ) and very long stylet (143.5–150.0  $\mu\text{m}$ ), body rings provided with transparent membranous projections, spine-like in lateral view and 10–12  $\mu\text{m}$  long, and 3–4 annules between vulva and tail terminus (RV). Currently, the genus *Bakernema* has only two species, *B. inaequale* (Taylor, 1936) Mehta & Raski, 1971, the type species and *B. dauniense*

Vovlas, 1992. The new species differs from both known species by its larger body length (vs 457–578  $\mu\text{m}$  in *D. inaequale* and 390–460  $\mu\text{m}$  in *B. dauniense*) and its much longer stylet (vs 58.5–70  $\mu\text{m}$  in *B. inaequale* and 65–74  $\mu\text{m}$  in *B. dauniense*). *B. enormese* n. sp. is most similar to the type species of the genus, *B. inaequale* in cuticular ornamentation with long projections (figs 1D, 2 B) but differs from it in addition to longer body and stylet length also by a short anterior vulva lip and a straight to slightly arcuate vagina.

*B. enormese* n. sp. differs from *B. dauniense* Vovlas, 1992 in addition to the longer body and stylet length, also in cuticular ornamentation with very short (1.5–2  $\mu\text{m}$  long) numerous projections, in fewer RV (vs. 7–8 in *B. dauniense*) and in Rst (number of annules from posterior end to stylet), Res (number of annules counted from anterior end to esophagus) and shorter VL/VB (length of postvulval part/body width at vulva) (10–13 vs. 13–16; 17–19 vs. 20–25 and 0.8–0.9 vs. 1–1.4, respectively).

*Discussion:* The genus *Bakernema* characterized by its transparent spine-like cuticular projections, a cephalic region of 2–3 annules and provided with an elevated labial disc, poorly developed pseudolips and weakly developed submedian lobes and a strongly developed anterior vulva lip and a sigmoid vagina. Juveniles lack cuticular appendages in both known species but annules are smooth or crenate in the type species while showing beaded margins in *B. dauniense*. Unfortunately no juveniles nor males were found of the new species.

Species in *Bakernema* show quite some interspecific differences (Geraert, 2010) which makes characterization of the genus more difficult. In a molecular analysis based on ITS1-rDNA sequences by Cordero *et al.* (2012) *Bakernema inaequale* clustered with strong support as a sister species to *Criconema petasum* (Wu, 1965) Raski & Luc, 1985 and *C. warrenense* Cordero, Robbins & Szalanski, 2012.

The new species appears to possess the longest stylet and body length within the

family. Information on the *en face* view of females and the study of juveniles, males would better support the characterization of the genus within *Bakernema*.

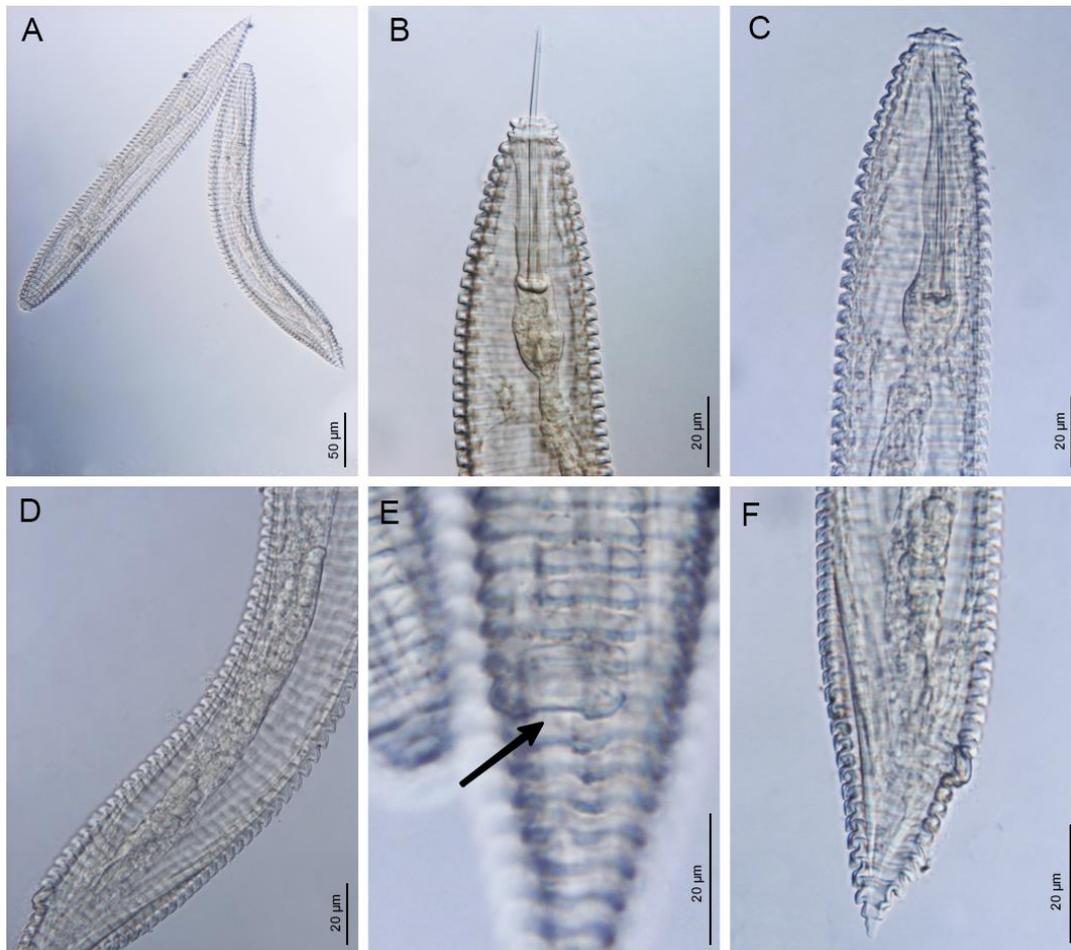
**Etymology:** The name of species *B. enormese* is derived from latin word referring to the aberrant long body length and stylet length of the nematode specimens.

***Criconema (Nothocriconemella) graminicola* Loof, Wouts & Yeates, 1997 (Figs 3, 4)**

**Measurements**

*Females* (n=7): L = 300–355 (328.9 ± 23) µm; body width = 42.0–44.5 (43.7 ± 1) µm; esophagus length = 84.0–103.5 (95.3 ± 10.1)

µm; excretory pore = 94.5–129.5 (109.4 ± 13.8) µm; nerve ring = 75.0–94.5 (86.2 ± 9.5) µm; stylet = 58.0–79.5 (72.0 ± 8.1) µm; stylet knob width = 7.5–9.0 (8.3 ± 1) µm; St%L = 19.4–23.9 (21.9 ± 1.7) µm; stylet cone length = 48.3–65.5 (58.8 ± 7.2) µm; head diameter = 12.9–16.4 (14.2 ± 1.3) µm; head height = 6.5–7.3 (6.9 ± 0.4) µm; V = 85.8–87.5 (86.4 ± 0.7) %; a = 7.1–8.0 (7.5 ± 0.4); b = 3.2–3.7 (3.5 ± 0.2); c = 8.9–11.7 (10.6 ± 1.2); c' = 1.1–1.6 (1.4 ± 0.2); R = 70–79 (76 ± 4); Rst = 12–19 (16.4 ± 2.9); Res = 21–25 (22.8 ± 1.8); Rex = 22–28 (24.8 ± 2.4); RV = 11–12 (11.6 ± 0.5); Ran = 8–11 (9.6 ± 1.1).



*Figure 3. Criconema (Nothocriconemella) graminicola. Females. A: Entire females. B, C: Anterior end showing stylet. D: Reproduction system. E: Anterior vulval lip overlapping posterior lip (arrow), ventral view. F: Posterior end*

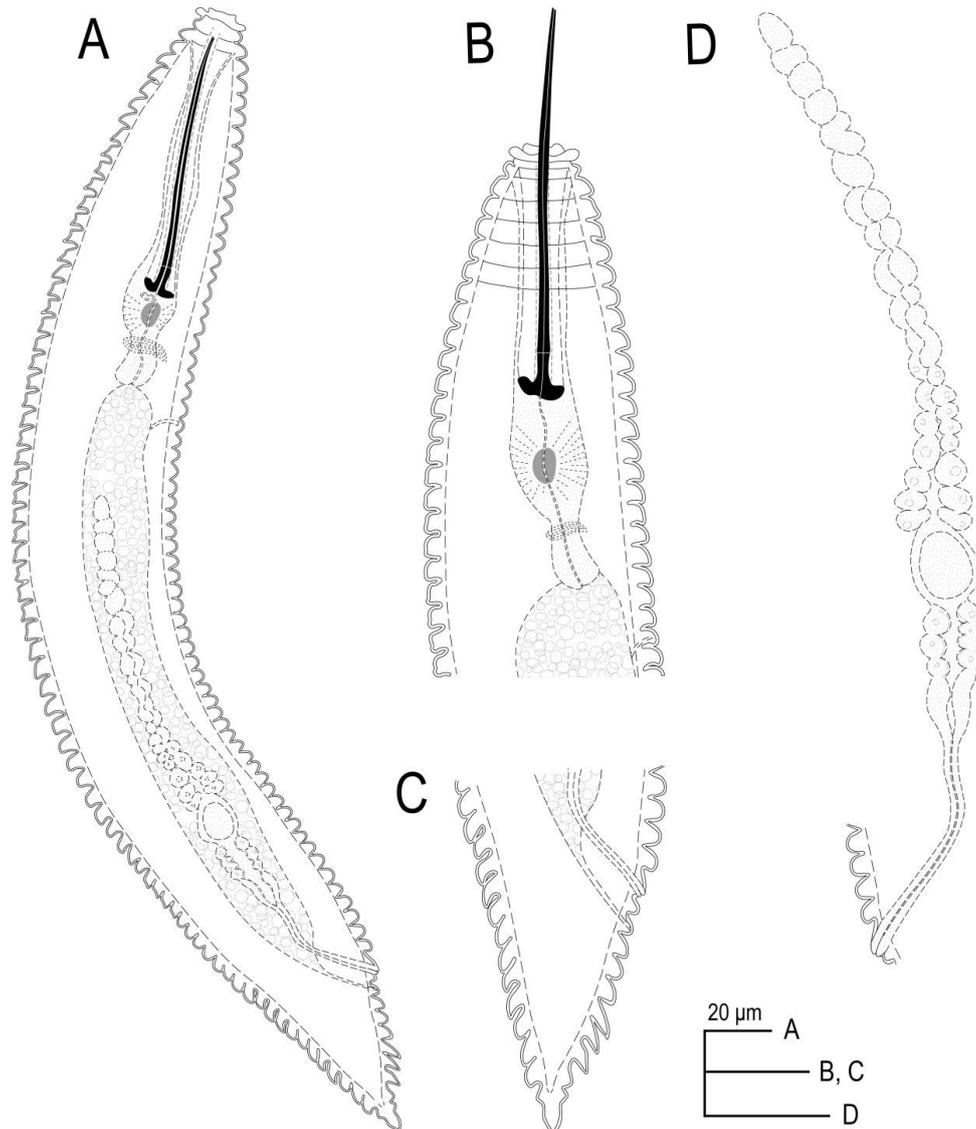


Figure 4. *Criconema (Nothocriconemella) graminicola*. Females. A: Entire female. B: Anterior end showing stylet. C: Posterior end. D: Female reproduction system

#### **Morphological characters**

Body straight or slightly curved ventrally. Lateral line lacking. Lip region low, wide, with 2 annules, first annule directed sideways or anteriorly, second annule distinct, pseudolips present retrorse. At the mid-body annules, margin parallelogram-shaped posteriorly. Stylet robust, straight, curved ventrally or dorsally, 58–79.5 μm long. Vulva closed, anterior vulval lip protruding and overlapping posterior lip, vagina sigmoid,

spermatheca larger with empty. Tail conically pointed, annules not fused. Males not found.

*Remarks:* In general, all morphological characters and morphometrics are in agreement with original description by Loof, Wouts & Yeates, 1997 with only some minor differences, e.g. stylet slightly curved ventrally, more tail annules, being 8–11 (9.6) in Vietnamese population vs. 6–8 (7.1) in original population. In addition, some variation of stylet knob width (7.5–9.0 vs.

10.5), V= 85.8–87.5 vs. 82–88, R=70–79 vs. 73–86.

**Recorded locality:** Nam Cat Tien National Forest Park (Dong Nai Province), Southern Viet Nam.

**Distribution:** *Criconema (N.) graminicola* is relatively common in cooler areas of New Zealand, and this record occurred outside of New Zealand in tropical forests in Vietnam.

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