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THREE NEW SPECIES OF THE SPIRAL NEMATODE GENUS *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) FROM VIETNAM

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ABSTRACT

Investigations on entomopathogenic and plant parasitic nematodes in some natural forests in the Ngoc Linh mountain (Quang Nam province) and the Thuong Xuan forest (Thanh Hoa province) revealed three new species of plant parasitic nematodes belonging to the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae). These new species were named *Helicotylenchus castanus* sp. n., *Helicotylenchus madhucus* sp. n. and *Helicotylenchus digitus* sp. n. with morphological characteristics described and illustrated. The new species *Helicotylenchus castanus* sp.n. is characterized by the highly hemispherical shape of the lip region, extremely short and rounded tail without projection. The second new species *Helicotylenchus madhucus* sp. n. is characterized by high lip region with 4–5 annuli, hemispherical and not set off from body contour, stylet 31.3–35.9 (33.3 ± 1.3) µm long, tail with a short ventral projection and the presence of males in the population. The third new species, *Helicotylenchus digitus* sp.n. was recognized by broad rounded tail with strong annulated projection in the digital shape, and also the presence of males in population.

Keywords: Nematoda, Hoplolaimidae, *Helicotylenchus*, new species, taxonomy, Vietnam.

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BA LOÀI TUYẾN TRÙNG KÝ SINH THỰC VẬT MỚI GIỐNG *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) TỪ VIỆT NAM

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TÓM TẮT

Khi nghiên cứu tuyến trùng ký sinh và thực vật ở một số khu rừng tự nhiên ở núi Ngọc Linh (tỉnh Quảng Nam) và Thường Xuân (tỉnh Thanh Hóa) đã phát hiện ba loài tuyến trùng ký sinh thực vật mới thuộc giống *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimida), được đặt tên là *Helicotylenchus castanus* sp. n., *Helicotylenchus madhucus* sp. n. và *Helicotylenchus digitus* sp. n., được mô tả và minh họa bằng ảnh vẽ và ảnh chụp hiển vi. Loài mới *Helicotylenchus castanus* sp.n. đặc trưng bởi cấu trúc vùng môi cao, bán cầu, đuôi cực ngắn và tròn mà không có mấu. Loài *Helicotylenchus madhucus* sp. n. đặc trưng bởi vùng môi hình bán cầu với 4–5 vòng cu tin và không tách biệt với đường viền cơ thể, kim hút khỏe, dài 31,3–35,9 (33,3 ± 1,3) µm, tận cùng đuôi có mấu ngắn về phía bụng và có sự hiện diện của con đực trong quần thể. Loài *Helicotylenchus digitus* sp.n. được phân biệt với các loài khác của giống bởi đuôi tròn rộng với mấu đuôi lớn hình ngón tay ở mút đuôi, có sự hiện diện của con đực trong quần thể.

Từ khóa: Nematoda, Hoplolaimidae, *Helicotylenchus*, tuyến trùng ký sinh thực vật, khóa định loại, Việt Nam.

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INTRODUCTION

With about 230 valid nominal species (Uzma, Nasira, Firoza & Shahina, 2015), the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) is one of the most species rich and widely distributed migratory ectoparasitic or semi-endoparasitic nematode genera of the order Tylenchida (*sensu* Siddiqi, 2000). They are associated with a variety of crops of agricultural importance (Decraemer & Hunt, 2006; Subbotin *et al*., 2015). In Vietnam, the genus *Helicotylenchus* is also the most diverse genus and significant in agriculture and forest ecosystems. Eroshenko et al. (1985) described seven new species of *Helicotylenchus* from different agriculture crops in Northern provinces of Vietnam. In 2000, Nguyen N. Chau & Nguyen V. Thanh published a book entitled "Plant Parasitic Nematodes in Vietnam" (Fauna of Vietnam, Vol. 4) in which 160 species of plant parasitic nematodes from agriculture ecosystems of Vietnam were recorded and described. Among them, 30 species of the genus *Helicotylenchus* were described and illustrated with species identification keys. A 1996–1997 survey on plant parasites associated with natural ecosystems in Vietnam recorded four new species of *Helicotylenchus* from agricultural and nature forest ecosystems in the Northern and Central Vietnam, of which two species were recorded from agriculture and the other two from forest ecosystems (Nguyen V.T. & Nguyen N.C. 2001). This newly published description increased the total number of spiral nematodes recorded from Vietnam to 34 species, most of which were recorded from

agriculture and only two species recorded from forest, and 11 of them were new species.

During more recent survey in 2002–2005 on plant parasitic and entomopathogenic nematodes in natural forests of Central Vietnam, three new species of spiral nematodes, *Helicotylenchus* were found from soil samples. Herein, they are described and illustrated as *Helicotylenchus castanus* n. sp., *Helicotylenchus madhucus* n. sp. and *Helicotylenchus digitus* n. sp.

MATERIALS AND METHODS

Survey and sampling: For entomopathogenic nematodes and plant parasitic nematodes from several natural forests in Central Vietnam were conducted during 2002–2005. For each composed sample, 250 ml rhizosphere soil and root within the area of 20 cm^2 around tree base was taken using a sampling shovel.

Nematode extraction: Nematodes from 250 ml soil samples were extracted using a modified Cobb's sieving-decanting technique with final use of a sieve with 75 µm mesh size for filtering living nematodes. This was followed with static filtering of living nematodes, using a special sieve 80 mm in diameter and 15 mm high, including 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 dish 90 mm in diameter for stationary filtering of living nematodes. For the filter sieve containing nematode sediment, 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 bottom of Petri dish.

Nematodes were killed in hot water at 65–70^oC, then fixed in Triethanolamin-Alcohol-Formalin (TAF) solution and mounted in anhydrous glycerin using the slow method of Hooper and Evans (1993). 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 as slides numbered 545TX1 to 545TX4); 546TX1 to 546TX4; 691N1 to 691NL3 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.

Descriptions

***Helicotylenchus castanus* sp. n.** (Figs 1–2)

Measurements

Holotype female: 615 µm; a = 17.9; b = 5.8; b' = 4.1; c = 89.2; c' = 0.5; V = 67.6%; O = 41.2; head diameter = 6 µm; head height = 3.5 µm; stylet = 31.5 µm; stylet knob width = 4 µm; stylet cone length = 10 µm; DGO = 10 µm; Exc. Pore = 109.5 µm; nerve ring = 82.5 µm; hemizonid = 101.5 µm.

Paratype females (n = 16): L = 569–658 (610 ± 30) µm; a = 17–24.5 (21 ± 2.4); b = 4.5–6 (5 ± 0.4); b' = 3.6–4.8 (4 ± 0.3); c = 63.3–91.2 (77 ± 9); c' = 0.5–0.7 (0.6 ± 0.1); V = 66.4–70.8 (67.6 ± 1.2)%; O = 33.3–42.4 (39.3 ± 2.1); head diameter = 4.5–6 (5 ± 0.9) µm; head height = 3–5 (4.1 ± 0.7) µm; stylet = 28.5–32.5 (30 ± 1) µm; stylet knob width = 3–5.5 (4 ± 0.9) µm; stylet cone length = 10–13.5 (11 ± 1.2) µm; DGO = 9.5–12.5 (10.5 ± 1.1) µm; Exc. Pore = 95–115 (102.5 ± 6) µm; nerve ring = 81.5–86.5 (83 ± 1.9) µm; hemizonid = 95.5–114.5 (106 ± 5.1) µm.

Morphological characteristics

Female: Body short and thin, arcuate ventrally to open C-shape after treatment by gentle heat; cuticle finely striated with 1.3–1.4 µm wide annuli at mid-body. Lip region continuous with body contour, hemispherical in shape or rounded with developed labial framework and 4–5 annuli. Stylet strong, conus length 35% of stylet length. Basal knobs with one side anteriorly convex, the other anteriorly concaved.

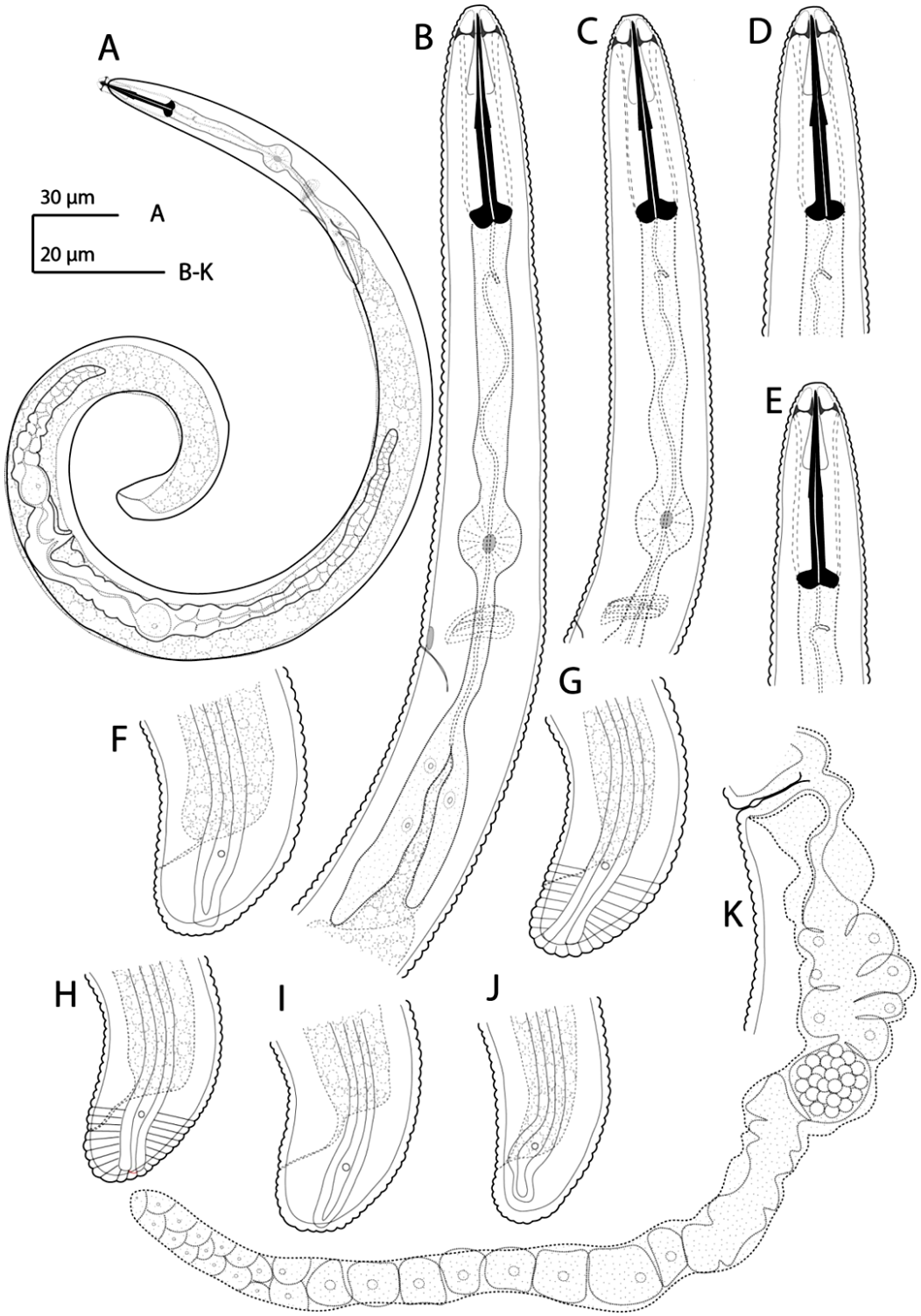

Figure 1. Drawing of *Helicotylenchus castanus* sp. n. A. Entire female (holotype). B. C. D. Anterior end showing stylet and esophageal region. K. Female posterior genital branch. F. G. H. I. J. Female posterior end with variation of tail terminus, phasmid position and lateral field

Figure 2. Photomicrographs of *Helicotylenchus castanus* sp. n. A. Entire female (holotype). B, C. Anterior end showing stylet. D. Esophageal region. E. Reproduction system. F. Lateral field showing phasmid. G. Posterior end

Esophagus with well-developed oval metacorpus, esophageal gland 112.7–130.6 (122.6 ± 6.3) µm long and overlapping intestine ventrally. Nerve ring around isthmus and located anterior excretory pore more than 10–15 µm. Hemizonid situated at 0–2 annuli anterior the excretory pore. Lateral field four lines occupying about one fourth of corresponding body diameter, with these lateral fields usually expanded at phasmid region, closing rounded near tail terminal. Reproductive system didelphic amphidelphic 72.5–101.1 (84.5 ± 9.4) µm long, oocytes usually arranged in one row, vulva a transverse slit in ventral view. Spermatheca oval or rounded and filled with sperms. Round tail, 19.1–29.9 (23.2 ± 3.3) µm long with 7–11 ventral annuli dorsally convex and without

mucro. Phasmids pore-like situated at the anus level or 3–4 annuli anterior to anus.

Male: not found.

Type habitat and locality: *Helicotylenchus castanus* n. sp. was extracted from rhizosphere soil of sweet chestnut (*Castanea sativa*) in **the** Thuong Xuan natural Forest, Thanh Hoa province (North Central Coast of Vietnam).

Diagnosis and relationship: *Helicotylenchus castanus* sp. n. is characterized by the high hemispherical shape of the lip region, short and rounded tail. In morphology, the new species belongs to the group of rounded tail species, including *H. canadensis* Waseem, 1961, *H. cavenessi* Sher, 1966, *H. limari* Eroshenko et al., 1985, *H. multicintus* (Cobb, 1893) Golden, 1956, *H. pseudodigonicus* Szczygiel, 1970 and *H. whiteheadi* (Ganguly & Khan, 1987) Firoza & Maqbool, 1994. The new species, however, differs from all these species by much shorter tail with c = 63–91 (c' = 0.5–0.7), whereas other species c = 33–65 and c' index is larger, being 0.8. In addition, stylet is much longer, being 28–33 µm whereas other ones have stylet length below 30 µm. In addition, one by one comparison showed other differences. For example, compared to *H. canadensis*, the new species has smaller V index (61–66 vs 66.4–70.8), shorter DGO (6–9 vs 9.3–12.4 µm), phasmid position (12-3 vs 3-4 ann.) and tail rounded vs. convex-conoid. To *H. multicinctus*, the new species has larger average body length (610 vs 470–530 µm), c index smaller (35–46 vs 63–91), stylet shorter (22–24 vs 28.5–32.5 µm) and phasmid position (10-4 vs 3-4 ann.). To *H. limari*, the new species also differs by body length, stylet length, indeces a, V.

Type specimens: Holotype female and sixteen female paratypes were deposited at the Department of Nematology, Institute Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam.

Etymology: The species name is derived from the scientific name of the host plant (*Madhuca pasquieri*), the Thuong Xuan forest, Thanh Hoa province.

***Helicotylenchus madhucus* sp. n.** (Figs 3–4)

Measurements

Holotype female: 806 µm; a = 27.4; b = 6.8; b' = 5.3; c = 51.3; c' = 0.8; V = 60.1 %; O = 35.6; Head diameter = 5.5 µm; Head height = 3.5 µm; stylet = 33.5 µm; stylet knob width = 3.5 µm; stylet cone = 10.6 µm; DGO = 8.7 µm; exc. pore = 123 µm; nerve ring = 87.5 µm; hemizonid to anterior end = 104 µm;

Paratype females (n = 16): L = 637–898 (801 ± 58) µm; a = 21.3–27.4 (24.5 ± 1.8); b = 5.4–7.1 (6.2 ± 0.5); b' = 4.3–5.5 (4.8 ± 0.4); c = 50–78 (61.5 ± 7.8); c' = 0.6–0.8 (0.7 ± 0.1); V = 56.5–62 (59 ± 1.6) %; O = 30.6–38.4 (33.8 ± 2.5); head diameter = 5.0–7 (5.9 ± 0.6) µm; head height = 3.5–5.0 (4.2 ± 0.5) µm; stylet = 31.5–36 (33.3 ± 1.3) µm; stylet knob width = 3.0–4 (3.4 ± 0.3) µm; stylet cone length = 9–11.5 (10.2 ± 0.9) µm; DGO = 7–9 (8.0 ± 0.5) µm; exc. pore = 103.5–123 (116 ± 6); nerve ring = 86–90 (87.7 ± 1.4) µm; hemizonid to anterior end = 101.5–122 (111.4 ± 7.8) µm.

Paratype males (n = 3): L = 675–731.5 (705.9 ± 28.7) µm; a = 38.6–43.8 (40.4 ± 2.9); b = 6.4–7.0 (6.7 ± 0.3); b' = 6.4–6.9 (6.6 ± 0.3); c = 88.8–134.2 (109.2 ± 23.1); c' = 0.3–0.4 (0.4 ± 0.1); O = 78.1–94.8 (85.2 ± 8.6); head diameter = 5–5.5 (5.5 ± 0.1) µm; head height = 3.5–4 (3.9 ± 0.3) µm; stylet = 20–23 (21 ± 1.5) µm; stylet knob width = 3.5–4 (3.5 ± 0.2) µm; stylet cone = 8.5–90 (8.6 ± 0.2) µm; DGO = 6–6.5 (6.3 ± 0.2) µm; exc. pore = 75.5–94.5 (84 ± 9.6) µm; nerve ring = 75.5–82 (78.5 ± 3) µm; hemizonid = 75–94 (84 ± 9.5) µm; spicule = 23.6–23.9 (23.8 ± 0.2) µm; gubernaculum = 7.4–8.3 (7.7 ± 0.5) µm.

Morphological characters

Female: Body short and thin, 16.4–19.7 (18.0 ± 1.0) µm wide at mid-body, usually C-shaped upon fixation. Cuticle distinctly striated with 0.9–1.8 (1.4 ± 0.3) µm wide annuli at midbody. Lateral field with four incisures occupying 1/3 of body diameter and elongated to terminal of tail. Inner two

incisures not fused at terminal of tail. Lip region hemispherical not set off from rest of body contour, 5.0–6.9 (5.9 ± 0.6) µm wide and 3.4–5.0 (4.2 ± 0.5) µm high, with developed labial framework and 4–5 annuli. Stylet robust with one rounded and one anteriorly flattened basal knob 3.0–3.9 (3.4 ± 0.3) µm wide. Dorsal esophageal gland located posterior basal knob at 25–30% stylet length. Esophagus 105.3–110.8 (108.3 ± 1.6) µm long, with long procorpus and isthmus and oval metacorpus. Hemizonid distinct, usually about 1–1.5 annuli wide and situated 1–2 annuli anterior to excretory pore. Reproductive system didelphic amphidelphic, well developed with anterior branch 76.0–87.6 (80.6 ± 4.3) µm long and posterior branch 83.4–100.6 (92.9 ± 5.8) µm long, oocytes usually arranged in one row, but anterior in two rows until the cap cell. Vulva a transverse slit. Spermatheca axial, rounded, 9.2–12.4 µm diameter with sperm. Tail short 5–7.5 (6.8 ± 0.7) µm and ventrally arcuate, with 5–8 annuli and terminated by broad ventral projection. Phasmids distinct, pore-like, usually situated at 2–10 annuli posterior to anus level.

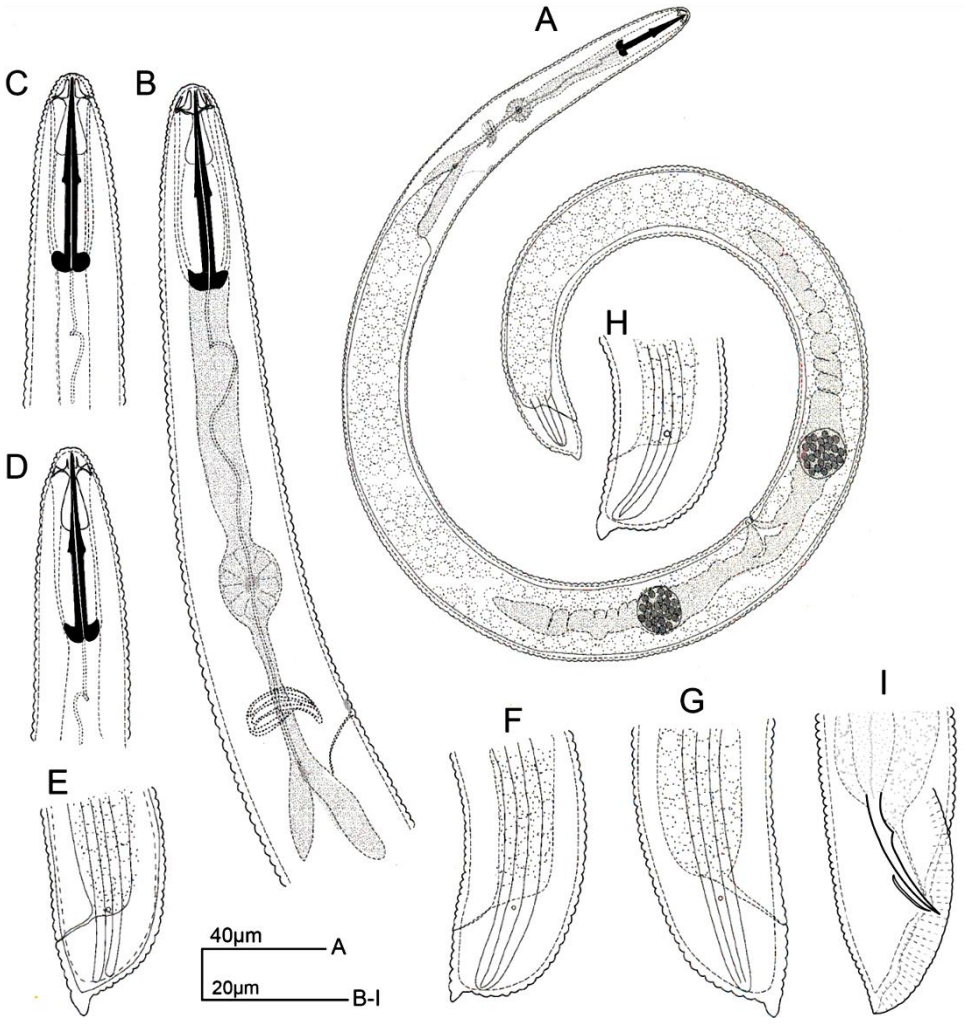

Figure 3. Drawing pictures of *Helicotylenchus madhucus* sp. n. A. Entire female (holotype). B. Oesophageal region. C-D. Anterior end showing stylet. E-G Variation of tail showing phasmid and lateral field

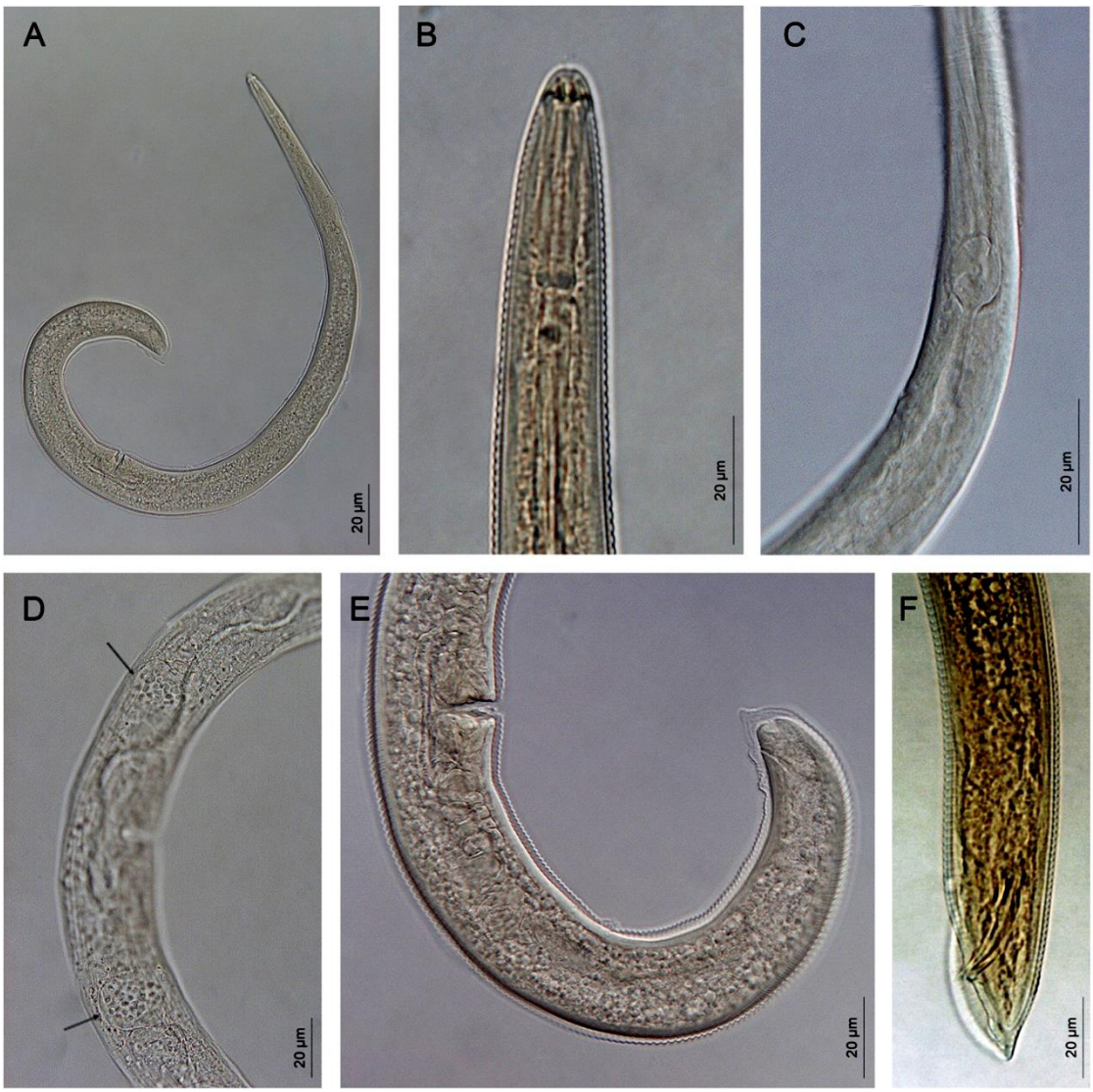

Figure 4. Microphotographs of *Helicotylenchus madhucus* sp. nov. A. Entire female (Paratype). B. Anterior body end. C. Oesopageal region showing median bulb and esophageal glands. D. Vulval region showing portion of two genital branches with spermatheca. E. Female posterior body with short tail

Male: Habitus C-shape or straight. Lip region hemispherical, 5.5–6 (5.5 ± 0.1) µm wide and 3.5–4 (3.9 ± 0.3) µm high. Labial framework extending 3–5 annuli posterior from basal plate. Stylet knobs 3–3.5 (3.5 ± 0.2) µm anterior faces flattened or indented. Ventral overlap of esophageal lobes 14.5–16.5 (15.5 ± 1.1) µm long. Excretory pore situated opposite posterior part of isthmus. Hemizonid situated 1–2 annuli anterior to excretory pore. Testis single, anteriorly outstretched and 194–234 µm long. Spicule moderately slender with a narrow distal portion bearing a ventral flange and an expanded tip. Gubernaculum curved in lateral view. Bursa crenate, extending from opposite the proximal end of the retracted spicule to the tail terminus. Phasmids pre-cloacal 14–16 annuli from the anterior cloacal lip.

Type habitat locality: Nematode specimens were collected from rhizosphere soil of bullet wood (*Madhuca pasquieri)* in the Thuong Xuan forest, Thanh Hoa province (in North Central Coast of Vietnam).

Diagnosis and relationnship: The new species, *Helicotylenchus madhucus* sp.n. can be recognized by short tail with a broad ventral projection. Lip region with 4–5 annuli, hemispherical not set off from body contour. Stylet strong with knob rounded in one side to slightly anterior concave in other side. In morphology, *Helicotylenchus madhucus* sp.n. is close to *H. atlanticus* Fernandez, Razjivin, Ortega & Quincosa, 1980, *H. canalis* Sher, 1966, *H. notabilis* Eroshenko & Nguyen, 1981 and *H. crassatus* Anderson, 1973. To *H. atlanticus*, the new species rather differs by longer body (637–898 µm vs 620–710 µm) and stylet (31–36 vs 26–27.4), but smaller V (56–62 vs 65–66%). To *H. notabilis* and *H. crassatus*, the new species differs by longer stylet (31–36 vs 21–23 and 26–30 µm), smaller V value (56–62 vs 62–66 and 61–74), head anulus (4–5 vs. and 4 annuli). In addition, the new species differs from others by the presence of male in population.

Type specimens: Holotype female, 16 paratype females and 3 males are deposited at the Nematode Collection of the Department of Nematology, Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, 122100 Hanoi, Vietnam.

Etymology: The species name is derived from the scientific name of the host plant (*Madhuca pasquieri*) the Thuong Xuan forest, Thanh Hoa province.

***Helicotylenchus digitus* sp. n.** (Figs 5–6)

Measurements

Holotype female: 550 µm; a = 27.4; b = 5.7; b' = 4.8; c = 79.7; c' = 0.8; V = 61.2%; O = 53.1; m = 43.8; head diameter = 6 µm; head height = 4.5 µm; stylet = 22.5 µm; stylet knob width = 4 µm; stylet cone length = 10 µm; exc. pore = 96 µm; nerve ring = 75.5 µm; hemizonid = 82.5 µm.

Paratype females (n = 15): L = 535–550 (540 ± 6.2) µm; a = 24.1–28.3 (26.0 ± 1.8); b = 5.4–7.3 (6.1 ± 0.7); b' = 4.2–4.8 (4.5 ± 0.3); c = 55.1–80.8 (73.3 ± 10.9); c' = 0.8–0.9 (0.8 ± 0.1); V = 58.9–63.1 (61.8 ± 1.8) µm; O = 48.5–58 (53.9 ± 3.4); m = 41.6–47.7 (43.9 ± 2.3); stylet = 21.5–24 (22.4 ± 0.9) µm; stylet knob width = 3.5–4.5 (4.1 ± 0.3) µm; stylet cone length = 9–10.5 (9.8 ± 0.7) µm; exc. pore = 86–92 (89.6 ± 2.3) µm; nerve ring = 73–80 (75.9 ± 2.4) µm; hemizonid = 82.5–90 (86.5 ± 3) µm.

Paratype males (n = 3): L = 446.5–494 (466.6 ± 24.5) µm; a = 24–28 (26.1 ± 2.1); b = 5.3–6.0 (5.7 ± 0.5); b' = 4.6–4.8 (4.7 ± 0.1); c = 32.3–37.1 (34.0 ± 2.7); c' = 1.1–1.2 (1.1 ± 0.1); O = 43.4–47.4 (45.4 ± 2.8); head diameter = 60–6.5 (6 ± 0.3) µm; head height = 4.5–5 (4.6 ± 0.1) µm; stylet = 19.5–23 (21.5 ± 2) µm; stylet knob width = 4–5 (4.5 ± 0.5) µm; stylet cone length = 8.5–9 (8.5 ± 0.3) µm; exc. pore = 75–76.5 (7.6 ± 1.1) µm; nerve ring = 72.5–73.5 (73 ± 0.8) µm; hemizonid = 73.5–74.5 (74 ± 0.8) µm; spicule = 20.8–22.3 (21.6 ± 0.8) µm; gubernaculum = 6.6–7 (6.8 ± 0.2) µm.

Morphological characters

Female: Habitus spiral when relaxed. Cuticle coarsely annulated, annuli 1.1–1.4 (1.3 ± 0.2) µm wide at mid-body. Lateral fields 4.2–5.1 µm wide and 20–30% of body diameter. Lip region almost conical, truncate, continuous with body contour and marked by four distinct annuli, with dimension of 5.5–6.5 (6.3 ± 0.4) µm wide at base and 4–5 (4.4 ± 0.2) µm high. Cephalic framework strongly sclerotized, its outer margins extending posteriorly 1–2 annuli from basal plate. Stylet robust with knobs well developed, with flattened anterior surfaces, 3.7–4.4 (4.1 ± 0.3) µm across. Orifice of dorsal pharyngeal gland located more than one half of stylet length from knobs. Median bulb oval, gradually enlarging with moderately developed valve. Nerve ring 73.5–79.5 (76 ± 2.4) µm from anterior end of body. Hemizonid generally distinct, one to two body annuli anterior to excretory pore. Reproductive system didelphic-amphidelphic with both branches about equally developed, length of anterior

branch 99.5–106 (102.4 ± 3.2) µm and posterior branch 94.2–104.5 (101.0 ± 4.5) µm. Spermatheca rounded, 8.5–10 (9.2 ± 0.8) µm in diameter, full of sperms. Vagina with uniformly thin wall, 7.5–8.5 (8.0 ± 0.4) µm long and about 1/3 to 1/2 of the corresponding body diameter. Tail short, with 6–11 annuli, conoid, convex on the dorsal side, and flat in the ventral side with a sub-digitate peg-like terminus. Phasmids conspicuous, five to six annuli anterior to level of anus.

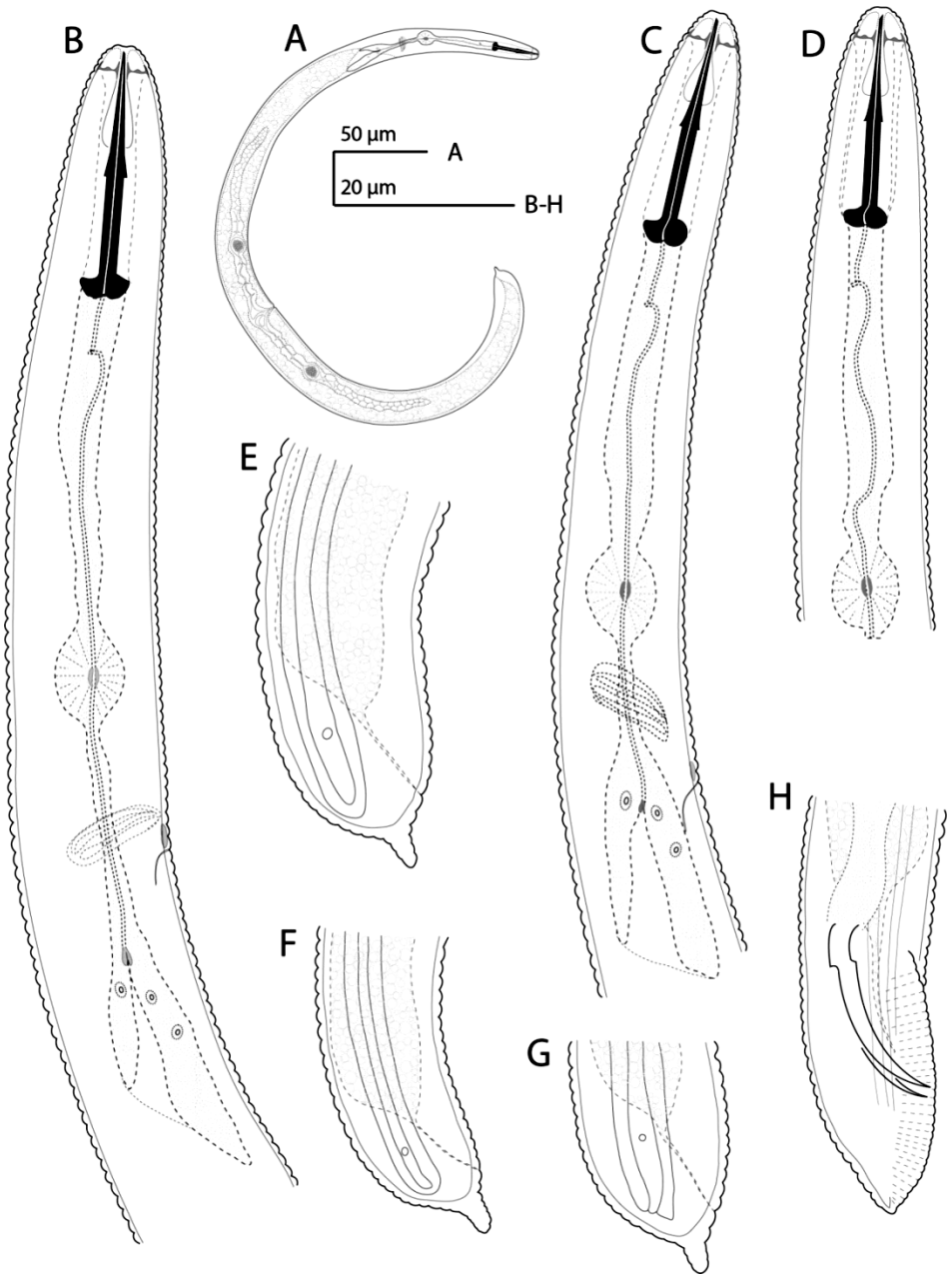

Figure 5. Drawing of *Helicotylenchus digitus* sp. n. A. Entire female. B. Anterior end. C-D. Oesopageal region showing stylet. E.F.G. Variation of female tails showing phasmid and lateral field. H. Male tail

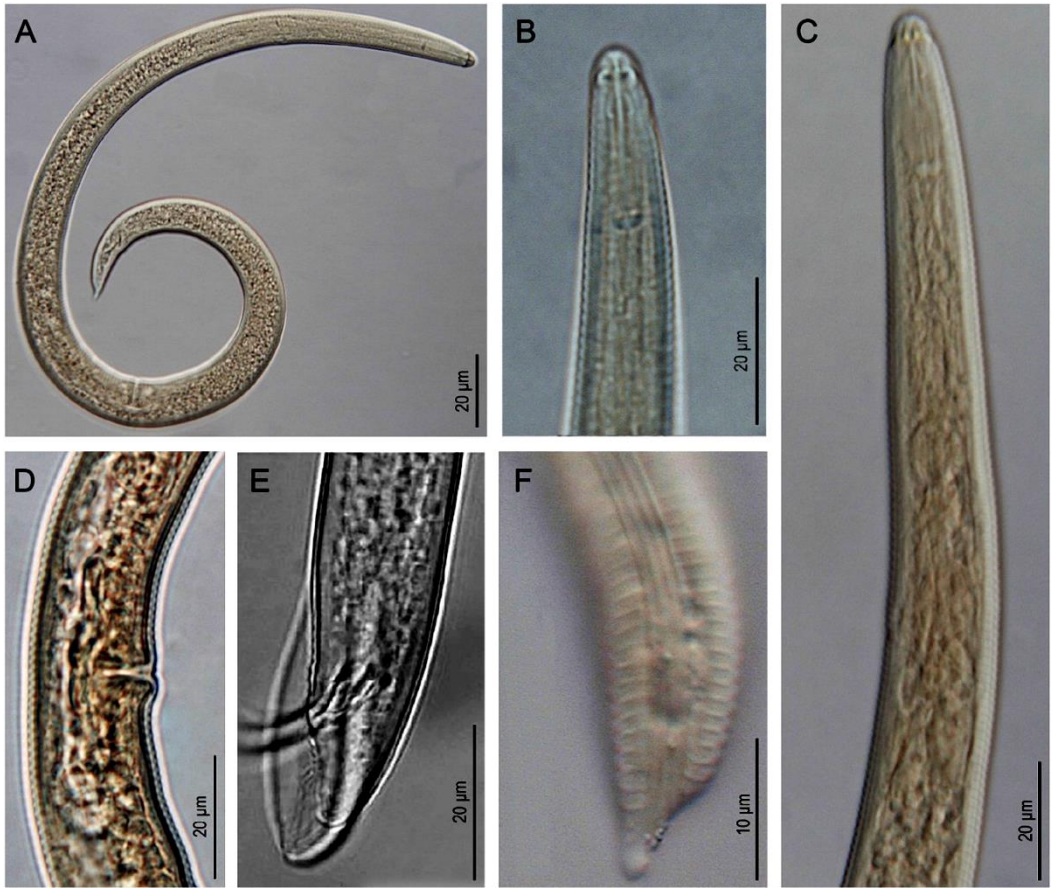

Figure 6. Microphotographs of *Helicotylenchus digitus* sp. now. A. Entire female (holotype). B. Anterior end. C. Oesopageal region showing stylet. D. Reproduction system. E. Male tail. F. Female tail. G, H. Female tail showing phasmid and laterial field

*Male***:** Body ventrally arcuate or straight when killed by heat. Lip region anteriorly flattened with rounded sides, continuous with the body, 6–6.5 (6.0 ± 0.3) µm wide and 4.5–5 (4.6 ± 0.1) µm high. Labial framework extending 4–5 annuli posterior from basal plate. Stylet knobs 4–5 (4.4 ±0.4) µm anterior faces flattened or indented. Orifice of dorsal oesophageal gland located 10–11 (10.5 ± 0.8) µm behind stylet base. Oesophageal glands overlapping the intestine, with the longest overlap ventrally situated, 16–17.5 (16.7 ± 1.2) µm in length. Excretory pore situated opposite posterior part of isthmus. Hemizonid not prominent, occupying about two body annuli. Lateral field about 1/4 of mid-body diameter with four incisures. Testis single, anteriorly outstretched and 129–158 µm long. Spicule has a narrow distal portion bearing a ventral flange and an expanded tip. Gubernaculum curved in lateral view. Bursa crenate, extending from opposite the proximal end of the retracted spicule to the tail terminus. Phasmids pre-cloacal 5–7 annuli from the anterior cloacal lip.

Type habitat and locality: *Helicotylenchus digitus* n. sp. was extracted from rhizosphere soil of wood tree (non-identified name) at the Ngoc Linh mountain forest, Quang Nam Province (Central Vietnam).

Diagnosis and relationship: The new species, *Helicotylenchus digitus* sp.n. can be recognized by broad tail with strong annulated projection. Lip region almost conical, truncate, and continuous with four distinct annuli. In

morphology, the new species is most close to *H. inifatis* Fernandez, Razjivin, Ortega & Quincosa, 1980, *H. gerti* Marais, Mekette & Tiedt, 2005, *H. similis* Fernandez, Razjivin, Ortega & Quincosa (1980), *H. amplius* Anderson & Eveleigh (1981) and *H. nigeriensis* Sher (1966). The new species, however, differs from all these species by smaller body (535–550 vs. 710–750, 520–680, 690–890 and 690–860 µm). To *H. inifatis*, *H. amplius* and *H. nigeriensis*, the new species has shorter stylet (21.5–23.5 vs. 27–33 µm).

Type specimens: Holotype female, 15 female paratypes and 3 allotype males are 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, 122100 Hanoi, Vietnam.

Etymology: The species name is derived from the digitate shape of the nematode tail terminus.

Updated key to the species of genus *Helicotylenchus* from Vietnam

1 - Tail hemispherical or broad conical, no projection……..2
- Tail conical with projection……...23
2 - Tail terminus broad rounded……..……..3
- Tail terminus conical or rounded …….....13
3 - Tail curved dorsally……4
- Tail symmetrical……..6
4 - Stylet 23 µm, phasmid anal level……..…*H. vietnamiensis*
- Stylet more than 26 µm, phasmid pre-anal…….....5
5 - Stylet 29–33 µm, phasmid pre-anal 3–4 annules……..*H. castanus* n. sp.
- Stylet 27–29 µm, phasmid pre-anal 11–17 annules.…..……..*H. concavus*
6 - Phasmid pre-anal……7
- Phasmid post-anal 3–5 annules ……...*H. ferus*
7 - Stylet knobs rounded or flattened anteriorly, phasmid pre-anal 4 annules……*H. limarius*
- Stylet knobs concave anteriorly, phasmid pre-anal 6–14 annules…..……...8
8 - Inner incisures of lateral field fused distally……...9
- Inner incisures of lateral field not fused distally……...10
9 - Stylet 25–28 µm, phasmid pre-anal 9–13 annules……..……*H. dignus*
- Stylet 24.5–25 µm, phasmid pre-anal 5–9 annules……..…..*H. rotundicauda*
10 - Stylet 22–24 µm, male present……*H. multicinctus*
- Stylet longer 25 µm, no male……11
11 - Stylet less than 26 µm……...……..*H. retusus*
- Stylet 29–33 µm……..…..12
12 - Head truncate, phasmid pre-anal 3 or post-anal 2 annules……...*H. canadensis*
- Head hemispherical, phasmid pre-anal 5–11 annules……..…..*H. varicaudatus*
13 - Phasmid anal level……14
- Phasmid pre-anal or rarely post-anal……16
14 - Tail terminus annulated, stylet 31 µm……...*H. pasohi*
- Tail terminus not annulated, stylet less than 22 µm……..15
15 - Lip region not annulated or indistinct, inner incisures of lateral field fused distally……
.……*H. falcatus*
- Lip region annulated, inner incisures of lateral field not fused distally...……..*H. curvatus*
16 - Stylet 30–34 µm……..*H. vulgaris*
- Stylet less than 30 µm……...17
17 - Spermatheca functional, male present…… ……*H. exallus*
- Spermatheca non functional, no male……...18

18 - Lip region truncated……..19
- Lip region hemispherical…….20
19 - Stylet knobs anterior flattened, phasmid 1–5 pre-anal to 3 post-anal annules……..
……..*H. digonicus*
- Stylet knobs rounded to sloping backward, phasmid post-anal 5–6 annules.....
……..*H. magnification*
20 - Tail terminus not annulated……..……..*H. rotundicauda*
- Tail terminus annulated…….21
21 - Stylet 23–24 µm, inner incisures of lateral field not fused distally……..*H. caribensis*
- Stylet more than 24 µm, inner incisures of lateral field fused distally…….22
22 - Phasmid post-anal 3–4 annules……*H. indicus*
- Phasmid pre-anal 4–6 annules……*H. cavenessi*
23 - Tail projection short, blunt……24
- Tail projection long, pointed……..25
24 - Stylet 25–28 µm, inner incisures of lateral field not fused distally… …… .....*H. crastinus*
- Stylet 21–23 µm, inner incisures of lateral field fused distally……. ... .*H. notabilis*
25 - Stylet 21–23 µm… ……..*H. laevicaudatus*
- Stylet more than 25 µm…….26
26 - Stylet 31.5–36 µm…….. ……..*H. madhucus* n. sp.
- Stylet 25–27 µm……...27
27 - Phasmid pre-anal 2 to 3 post-anal annules…. ……..*H. crassatus*
- Phasmid pre-anal more than 5 annules…….. ……..28
28 - Tail terminus concave…… ……*H. crenacauda*
- Tail terminus not concave…….29
29 - Tail with long projection……...30
- Tail with short projection……..31
30 - Tail projection digit-shaped, annulated……. ……..*H. digitus* n. sp.
- Tail projection pointed, not annulated…. ……..*H. marinus*
31 - Inner incisures of lateral field fused distally……..32
- Inner incisures of lateral field not fused distally……...33
32 - Body 680–840 long, lip region truncated……..……*H. cornurus*
- Body 540–590 long, lip region hemispherical…..……...*H. bambesae*
33 - Spermatheca non functional, no male …….. ……..34
- Spermatheca functional, male present ……..*H. erythrinae*
34 - Phasmid pre-anal 3–11 annules…….35
- Phasmid pre-anal 1–2 annules……..……..*H. coffeae*
35 - Tail without mucro……..36
- Tail with mucro…… ……..*H. paramucronatus*
36 - Phasmid pre-anal 2–6 annules…….. ……..*H. pseudorobustus*
- Phasmid pre-anal 7–11 annules…….. .....*H. dihystera*

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