

TWO NEW SPECIES OF PLANT PARASITIC NEMATODES *Hirschmanniella bananae* n.sp. (Nematoda: Pratylenchidae) AND *Scutellonema tanlamense* n.sp. (Nematoda: Hoplolaimidae) ASSOCIATED WITH BANANA IN VIETNAM

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ABSTRACT: During investigation on the plant parasitic nematodes on bananas in North and Central provinces of Vietnam carried out in 1995 two new species were found, *Hirschmanniella bananae* n.sp. and *Scutellonema tanlamense* n.sp. *Hirschmanniella bananae* n.sp. is characterized by having 6 annuli in lip region, areolation of entire lateral field and presence of a mid-terminal mucro at the tail end. The new species is close to *H. shamini* Ahmad, 1972 but differs from *H. shamini* by longer body length in females (1450-1750 μm vs 1190-1360 μm), longer stylet longer (18-20.5 μm vs 16-19 μm) and longer spicule longer (28-29.5 μm vs 22-25 μm). *Scutellonema tanlamense* n.sp. is characterized by lip region with three annuli and numerous longitudinal ridges (18) in basal annulus and structure of lateral field at the tail region with inner lines extending to tail terminus. The new species is closest to *S. brachyurus* type B (African populations) described by Van Den Berg *et al.* in 2013 but can be distinguished by body length shorter (643-708 μm vs 777-820 μm), stylet length shorter (24.5-26.5 μm vs 28-29.5 μm), and also by structure of lateral field with two inner lines going through at tail region and vulval edge distinct with epitygma appearing double and folded into vagina.

Keywords: *Hirschmanniella*, *Scutellonema*, bananas, North and Central Vietnam, plant parasitic nematodes, new species, taxonomy.

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INTRODUCTION

Banana (*Musa paradisiaca* L.) is one of the important fruit crops in Vietnam and is used as desert fruit as well as staple food. Banana is grown everywhere; apart from some state farms, every household at the countryside grows banana. Up to 2014 in total 50,200 ha bananas were planted with about 1.4 million tons of fruit produced. Most of these products is consumed domestically for dessert and food cooking, one fourth of the production is exported to China and Japan.

The preliminary surveys on plant-parasitic nematodes in Vietnam were carried out in North Vietnam during 1977-1978, by joint collaboration of Russian-Vietnamese scientists. Ninety species of plant-parasitic nematodes

were recorded, among them 24 species associated with banana (Eroshenko *et al.*, 1985). Another survey to assess the occurrence and potential damage of parasitic nematodes on bananas in Vietnam was carried out in 1995. The latter investigation revealed 53 species of plant-parasitic nematodes associated with bananas in North and Central Vietnam. Some species considered to be most common in distribution and detrimental to bananas were *Pratylenchus coffeae*, *Helicotylenchus multicinctus*, *H. dihystra*, *Meloidogyne incognita* and *Rotylenchulus reniformis* (Nguyen *et al.*, 1997). Among these plant parasitic nematodes, *Hirschmanniella* spec. n. and *Scutellonema* spec. n. were revealed as new species but were not putting scientific named for official publication so far. This paper deals

with the description of these two new species named as *Hirschmanniella bananae* n.sp. and *Scutellonema tanlamense* n.sp.

Materials and Methods

Survey and sampling. A total of 47 combination samples that included soils and roots associated with planed banana varieties AAA and AAB were collected from five localities from Northern and Central Vietnam including: Yen Nghia and Ba Vi districts (surround Hanoi city), Tam Thien Mau farming (Ha Bac province), Van Giang district (Hai Hung province) and Tanlam Faming (Quang Tri province). Each combination sample was taken 250 mL soil and roots within a rhizosphere with facial profile 20 × 20 × 20 cm at base of banana tree (Speijer & De Waele, 1997).

Nematode extraction. Nematodes from 250 mL soil samples were extracted by a modified Cobb's sieving-decanting technique (ref?). Firstly, the 250 ml soil sample was transferred into a 5L capacity container in 3 liters of water, gently stirred to homogenize the sample in the water, and then poured over a standard sieve (22 cm diameter) with a mesh size of 0.5 mm into a second container to remove coarse soil and plant particles. This decantation was repeated about 5-7 times, and then the soil solution is filtered over a standard sieve with mesh size 75 µm, so as to collect sediment with nematodes. The latter is rinsed over a small sieve described by Nguyen & Nguyen (1993). Nematodes were killed in water at 65-70°C, fixed in TAF and mounted in anhydrous glycerin using the slow method of Hooper & Evans (1993).

All morphometrics were performed with a camera lucida drawing tube. In the measurement and description of the species, the De Man's formulae (1884) are used. Of these some abbreviated letter used as: L = total body length in µm; a = body length/maximum body width; b = body length/esophageal length; b' = body length/distance from anterior end to posterior end of esophageal glands; c = body length/anal body width; c' = body length/body width at anus; V = distance from anterior end to vulva × 100/body length; T = distance from cloacal aperture to anterior of testis × 100/body

length; m = length of conus as percentage of total stylet length; O = distance between stylet base and orifice of dorsal esophageal gland in µm. All measurements are presented in micrometers and expressed as the mean ± standard deviation followed by the range.

SEM photographs. The nematodes used for SEM observation were fixed in a 4% formaldehyde solution and mounted on slide in dehydrated glycerin following Seinhorst's rapid method (1959). When glycerin embedded nematodes were used for SEM. Specimens were first transferred into a small dish filled with a drop of glycerin. Distilled water is added drop by drop until the nematodes are in pure distilled water. Particles which might adhere to the nematodes are removed by ultrasonic treatment for about 5 min. The nematodes are dehydrated using a gradual series of ethanol concentration of 30%, 50%, 75% and 95%. After this initial dehydration which lasts for about 8 hours in total. The specimens are left overnight in 100% ethanol. The standard critical point drying procedure is applied with CO₂ this temperature of 31.1°C and the pressure at 72.9 bar. During the transportation the specimens are kept into absolute ethanol. The dried nematodes are individually mounted on standard specimens stubs and sputter-coated with gold. The nematodes are picked by hand one by one and put on the specimen table, so that the head of nematodes lies on the glass rod and can be studied en face. The surface of the specimen table consists of an auto-adhesive conducting aluminum tape stick to the nickel-print (glue). The scanning of nematode specimens were observed and taken photographs with the JSM-840 (Eisenback, 1991).

All examined nematode specimens was 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, 10000 Hanoi, Vietnam.

Descriptions of new species

Hirschmanniella bananae n.sp. (Figs 1 & 2)

Measurements

Holotype (female). L = 1600 μ m; a = 67.2; b = 12.8; c = 17; c' = 6.8; V (%) = 52.6; stylet length = 19.1 μ m; m = 50.

Female paratypes (n = 17). L = 1582 \pm 98.7 (1450-1750) μ m; body width 23.6 \pm 1 (22.0-24.5) μ m; a = 66.8 \pm 2.4 (64.0-71.0); esophageal length = 133.1 \pm 9.5 (122.5-144.0) μ m; b = 11.9 \pm 0.8 (10.5-13.0); tail length = 88.9 \pm 6.0 (77.5-93.5) μ m; c = 17.9 \pm 2.4 (16.0-22.5); c' = 6.7 \pm 0.9 (5.5-8.1); V = 53.7 \pm 1.6 (52.5-57.0); stylet length = 19.2 \pm 0.8 (18.0-20.5) μ m; m = 50.0 \pm 0.6 (49.0-51.0); O = 2.5 \pm

0.1 (2.2-2.6) μ m.

Male paratypes (n = 12). L = 1250 \pm 87.6 (1150-1400) μ m; body width 20 \pm 1.4 (18-22.5) μ m; a = 62.6 \pm 3 (57.5-65.5); oesophageal length = 114 \pm 13 (93-133) μ m; b = 10.9 \pm 0.7 (10.3-12.3); tail length = 84 \pm 4.5 (77-90) μ m; c = 14.4 \pm 1.0 (13.0-15.5); c' = 6.6 \pm 1.0 (5.5-8.5); testis length = 34.7 \pm 2.7 (30-37.5) μ m; stylet length = 18 \pm 0.4 (18-19) μ m; m = 49 \pm 1 (47-50); O = 2 \pm 0.5 (1.5-3) μ m; spicule length = 28 \pm 0.6 (28-29.5) μ m; gubernaculum length = 10 \pm 0.5 (10-11) μ m.

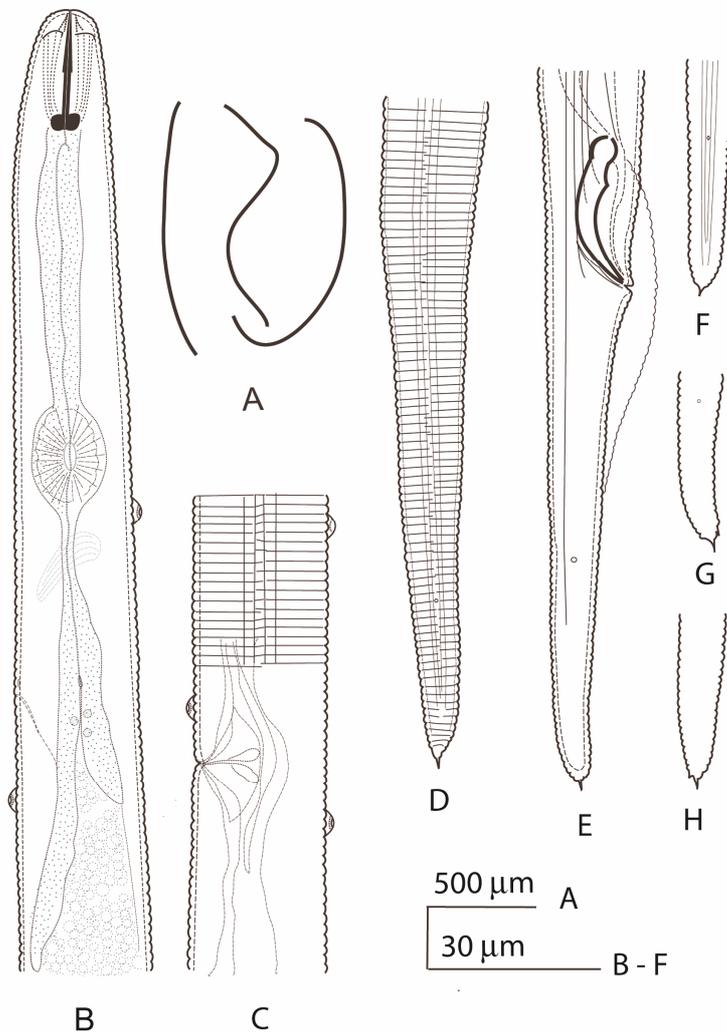


Figure 1. *Hirschmanniella bananae* n. sp.

A. Body habitus of heat-relaxed specimens; B. anterior region; C. lateral view at the vulva region with showing infecting of parasitic bacteria (*Pasteuria penetrans*); D. female tail; E. male tail; F, G, H. variation of the tail terminus.

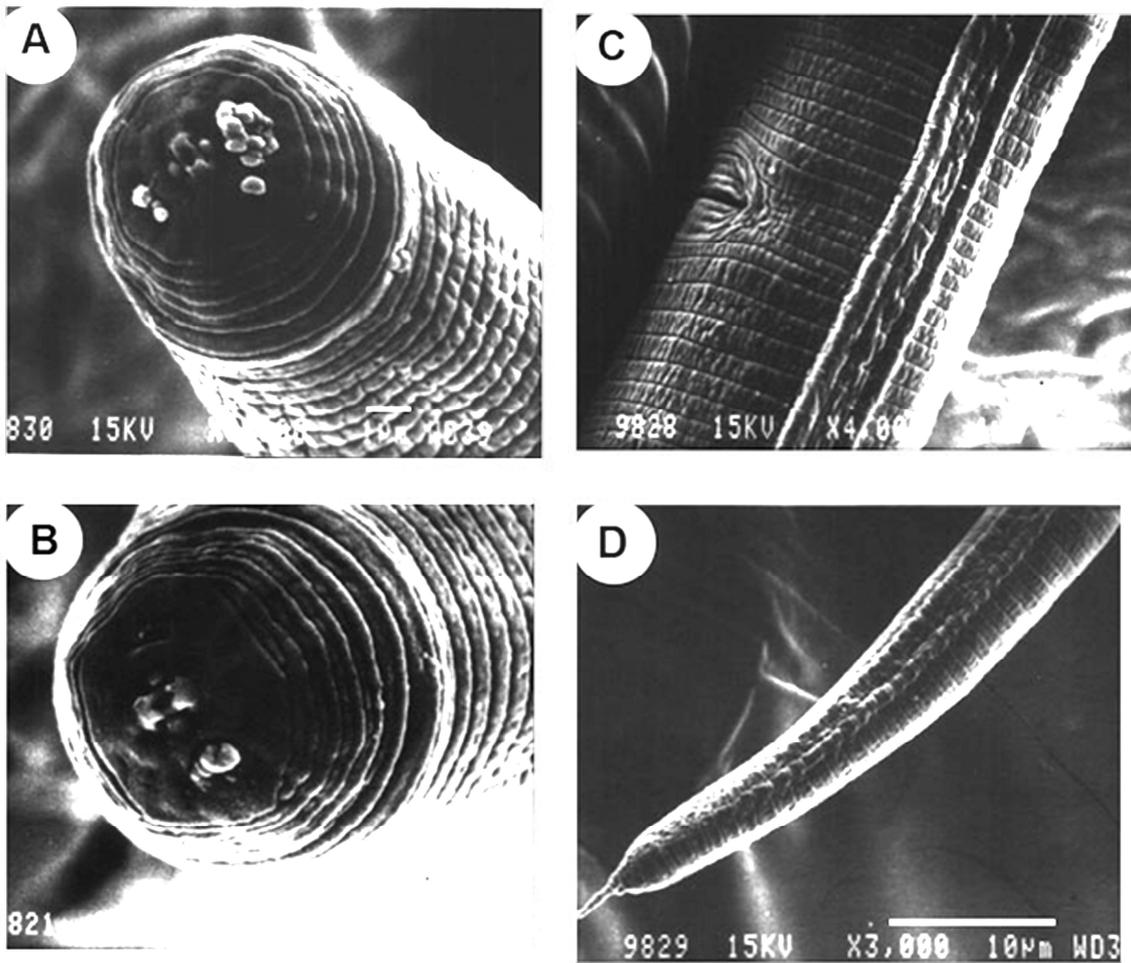


Figure 2. SEM photographs of *Hirschmanniella bananae* n.sp. A: anterior end in lateral view; B: lip region in en-face view; C: lateral view at vulva showing areolation on entire lateral field; D: tail region. Scale bars are showed on the pictures.

Morphological characterization keep telegraphic style

Female. Body slender, usually straight, sometimes in irregular curved shape when heath relaxed. Annuli fine, 1.1 -1.2 μm wide at middle body. Areolation present over entirely lateral field that composed of three ridges. Of these two outer ridges crossed by annulation, but inner ridge with irregular annulation (fig. 2C). Lateral field with 4 lines starting from anterior esophageal region and running in parallel up to the mid-tail, where both inner lines join at 6-9 annuli posterior to phasmid, ending at 11-12 annuli from tail end; the outer lines end 4-8

annuli from tail end. At the beginning of lateral field near lip region very indistinct, but apparently starting body diameter posterior to stylet knobs, comprising three bands (i.e., four lines) areolated along entire length of body, more distinct in anterior and posterior region under light microscope whereas SEM photographs showing all three longitudinal bands areolated over entire length of body with anastomoses as twisted lines in middle band (fig. 2 D). Lip region moderately low, rounded and apex flattened, continuous with body contour and composed of five annuli (indistinct in some specimens). SEM photographs showing

no longitudinal lines on lip annuli; an oblong labial disc, slightly raised above lip region at laterally and fused with labial plate dorsally and ventrally. Amphidial openings forming two slits on lateral sides of labial disc. Cephalic framework moderately sclerotised, extending posteriorly for two or three annuli from basal plate. Stylet short and strong, knobs anteriorly somewhat curved but varying from sloping to anteriorly and posteriorly rounded. Hemizonid distinct, located one to two annuli anterior to the secretory-excretory (SE) pore. SE- pore located 1-2 annuli posterior to level of esophago-intestinal junction or at 115-136 μm from anterior end. Esophageal glands are slender and overlapping intestine on ventral side with outstretched point far 171-188 μm from anterior end. Reproductive system didelphic-amphidelphic. Ovaries outstretched, spermathecae oval shaped, 9-10 \times 13-14 μm in size and filled with plural sperms, but sometime empty. Vulva at about mid-body to slightly posterior with vulval edge depressed into body contour. Vagina small, straight, and connected with oval vaginal glands. Tail conoid and elongated with terminal mucro. Apart from middle terminal mucro, sometimes a ventral projection present. Phasmids located at 30-31 μm or at 20-21 annuli from tail terminus.

Male. Similar to female in general characters except for sexual differences and smaller body size. Spicules well developed and slightly accurate ventrad. Gubernaculum simple and slightly curved at distal end, not protruding. Tail with 78-84 ventral annuli, straight or slightly curved dorsad with a ventral mucro. Phasmids situated at 35-45 μm and 29-36 annuli to tail tip. In the posterior body four lines of lateral field not joined but expanded and ending at the spicule region.

Bionomics. Both males and female specimens of new species are often infected by parasitic bacteria, *Pasteuria penetrans* with number from 18 to 36 endospores observed on the body cuticle of each nematode individual. Although in almost banana samples, specimens of *H. banana* often occurs together with specimens of *H. mucronata*, only *H. banananae*

is infected with bacteria *P. penetrans*.

Type locality. Specimens of *Hirschmanniella banananae* n.sp. were collected from banana soils and banana roots *Musa paradisiaca* L. (AAA group) at Yen Nghia commune, a suburban region of Hanoi city. The new species is also recorded in several other banana regions around Hanoi as Ba Vi district (Hanoi city). Tam Thien Mau farming of bananas (Ha Bac Province) and banana areas in Van Giang district (Hai Hung Province).

Type specimens. Holotype female, 17 female paratypes and 12 male paratypes 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, 10000 Hanoi, Vietnam.

Differential diagnosis. The new species is readily separated from most species by the areolation of the entire lateral field and presence of a mid-terminal mucro at the tail terminus. In morphology and morphometrics, the new species is similar to *H. kwazuna* Van Den Berg et al., 2009; *H. anchoryzae* Ebsary et Anderson, 1982; *H. caudacrena* Sher, 1968, *H. miticausa* Bridge, Mortimer et Jacson, 1983 and *H. shamini* Ahmad, 1972. However, each of these species differs from the new species in the extent of areolation. From *H. kwazuna* the new species differs by a combination characters through a slightly shorter female length at 1450-1750 μm vs 1522-2049 μm in females and 1150-1400 μm vs 1314-1960 μm in males, shorter esophageal length (distance from anterior end to oesophageal valve) at 122.5-144 μm vs 233-437 μm , longer distance from phasmids to tail end at 30-31 μm vs 15-26 μm . In addition, the new species does not appeared angular crystal-like inclusions in body cavity and the tail terminus often has a ventral projection. The new species is separated from *H. areolata* by having a shorter stylet in males with 18.5-22 μm vs 25-27 μm and females at 18-20.5 vs 24-27 μm ; the excretory pore is situated more anteriorly at 66-121 μm vs 121-147 μm ; and the male tail is strongly curved dorsad in most specimens vs

ventrally arcuate. The new species can be distinguished from *H. anchoryzae* in females are shorter at 1582 μm (1450-1750 μm) vs 1784 μm (1462-2100 μm); stylet longer at 18-20.5 μm vs 16-17 μm ; and position of the phasmids are more close to the tail terminus at 30-31 μm vs 37-57 μm . From *H. caudacrena* and *H. miticausa* the new species differs by having tail terminus with typical mucro and annulation to the end of tail. In size body, the new species might be closest to *H. shamini* (Ahmad, 1972) which is also commonly appeared in flood rice in Vietnam (Eroshenko et al., 1985) but it differs by longer body length in females at 1450-1750 μm vs 1190-1360 μm , longer stylet at 18-20.5 μm vs 16-19 μm and longer spicule at 28-29.5 μm vs 22-25 μm .

Etymology. The new species is named after common name of host plant, e.g. banana which the new species are infected.

***Scutellonema tanlamense* n.sp.** (Figs 3 & 4)

Measurements

Holotype (female). L = 620 μm ; a = 28.6; b = 7.1; b' = 5.5; c = 61.4; V = 58.7; stylet length = 24.5 μm ; O = 23; m = 43.

Female paratypes (n = 11 females). L = 673 \pm 34.6 (643-708) μm ; a = 30.3 \pm 1.4 (28.6-32.4); b = 7.9 \pm 0.4 (7.1-8.2); c = 63.5 \pm 2.3 (61.2-66.5); V = 57.5 \pm 1.1 (55.0-58.7); stylet length = 25.5 \pm 0.6 (24.5-26.5) μm ; O = 23 \pm 2.5 (20.5-27.5) μm ; m = 43.5 \pm 1.1 (42-45).

Morphological characterization

Female. Body usually spiral shaped. Annulation distinct with 1.3-2.0 μm (average 1.7 μm) wide at middle body. Lip region rounded and mostly not set off, with distinct labial disc rounded and small amphid openings laterally. SEM microphotographs showing clearly three lip annuli and basal annulus arranged with irregular blocks that defined about 18 -20 longitudinal lines over entire annulus. Stylet robust with rounded knobs. Stylet robustus with anterior conical part (as

metenchium) usually shorter than shaft (as telenchium). Lateral fields with four lines in which two outer lines areolated anteriorly opposite to pharyngeal region and posteriorly at the scutellum region. In the SEM photographs areolation in the scutellum region often asymmetric variation posterior to scutellum two inner lines of lateral field continuing till tail end. Tail slightly narrower posteriorly and often dorsally rounded with 9-10 annuli. Median bulb ovoid to moderate rounded, 13-14 μm \times 14-15 μm in size. Esophageal glands outstretched, posteriorly with three gland nuclei and overlapping intestine dorsally. Pharyngeal-intestinal junction distinct and located closely to neve ring. Intestine slightly overlaps rectum. Secretory- excretory pore located opposite to esophageal glands in 8-10 annuli posterior to esophageal intestinal transition valve. Hemizonid two annuli long and situated just anterior to the SE-pore. Scutellum moderate with 3.5-4 μm diameter equivalent two annuli and situated at three annuli posterior to anus and at 6-7 annuli from tail terminus. Reproductive system didelphic-amphidelphic. Vagina straight and connected with two small glands. Two vaginal dilatator muscles distinct but no vaginal constrictor muscles observed. Epiptygma appearing double and folded into vagina. In ventral view, vulva surrounded by oval prominent cuticular border. Ovaries outstretched; spermathecae rounded, with or without sperms.

Male: not found.

Type locality. A total of 12 specimens of *Scutellonema tanlamense* n.sp. was extracted from soil around the roots of banana, *Musa paradisiaca* L. (AAA group) in Tan Lam black paper Farm, Quang Tri province.

Type specimens. Holotype female and 11 female paratypes 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, 10000 Hanoi, Vietnam.

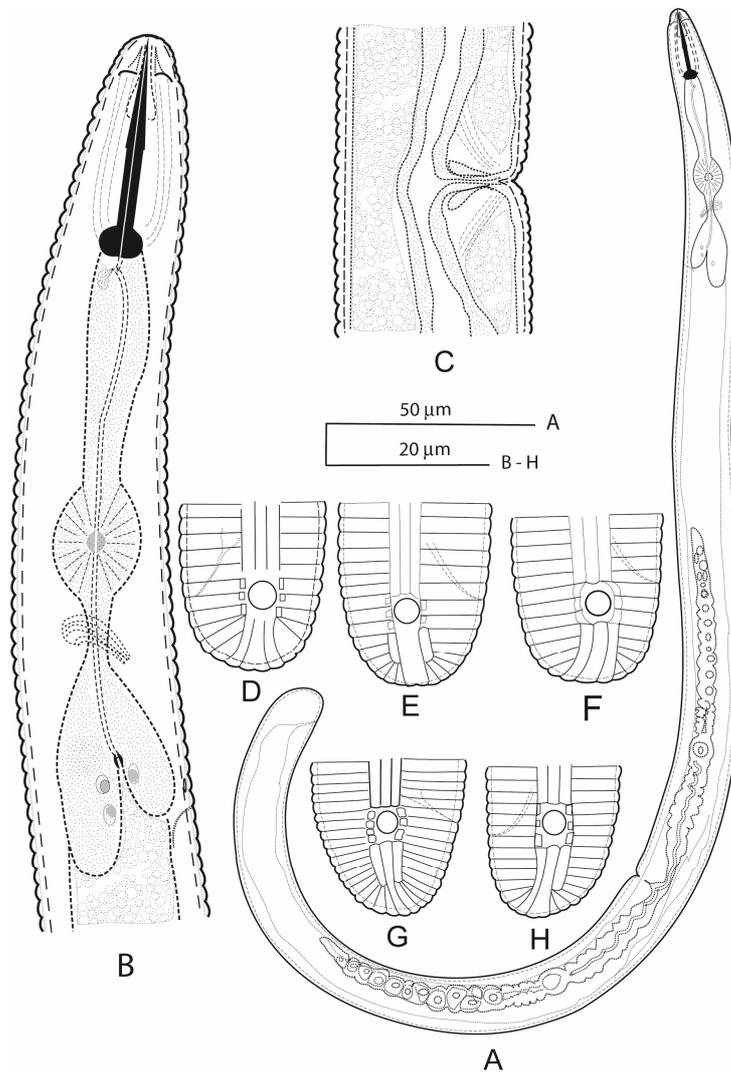


Figure 3. *Scutellonema tanlamense* n.sp. (Female)

A. entire view; B. esophageal region; C. vulva region. D, E, F, G, H. variation of the tail terminus.

Differential diagnosis. In general morphology, the new species is differentiated from all species of the genus by the number of longitudinal ridges in basal annulus with 18 -20 ridges, structure of lateral field at the tail region and inner lines going through out at terminus of the tail. In morphology, the new species come close to *S. brachyurus* (Steiner, 1938; Eroshenko et Nguyen, 1981) Andrassy, 1958; *S. brevistyletum* Siddiqi, 1972 and *S. vietnamense* Eroshenko et Nguyen, 1981. The new species differs from *S. brachyurus* in original description by lip region lower and slightly set off, with 3 annuli vs well set off with 4-6 annuli; more longitudinal ridges on basal

annulus i.e. 18 vs 4-12; shorter stylet, 24.5-26.5 μm vs 26-31 μm ; distinct spermathecae and separation of the inner lines of lateral field at the scutellum region with variation but two inner lines always going through out at terminus of the tail. The new species might be closest to *S. brachyurus* type B described by Van Den Berg et al., 2013 based on three populations collected from South African by a combination characters such as lip region is not set off, with 3 annuli at *Scutellonema tanlamense* n.sp. vs lip region broadly rounded, well set off with 4 at *S. brachyurus* type B, moreover the new species distinguished to by body length shorter (643-708 μm vs 777-820 μm), stylet length shorter

(24.5-26.5 μm vs 28-29.5 μm , and also by structure of lateral field with two inner lines going through at tail region and vulval edge distinct with epiptygma appearing double and folded into vagina. In addition, the new species is differentiated from 8 female populations recorded formerly from vegetable crops and coffee in Vietnam by structure of lip region rounded and mostly not set off vs lip region strongly set off, body length shorter (643-708 μm vs 720-780 μm), stylet length shorter (24.5-26.5 μm vs 28-31 μm ; vulva position located more anterior with $V= 57.5$ (55-58.7) vs $V= 61$

(57.5-65). Also by structure of lateral field with two inner lines going through at tail region and vulval edge distinct with epiptygma appearing double and folded into vagina. The new species is separated from *S. brevistyletum* in having a longer stylet at 24.5-26.5 μm vs 21-23 μm ; more numerous of longitudinal ridges at basal annulus with 18 vs 10 in *S. brevistyletum*. The new species differs from *S. vietnamense* by shorter length of body at 643-708 μm vs 810-920 μm and stylet shorter at 24.5-26.5 μm vs 31-33 μm .

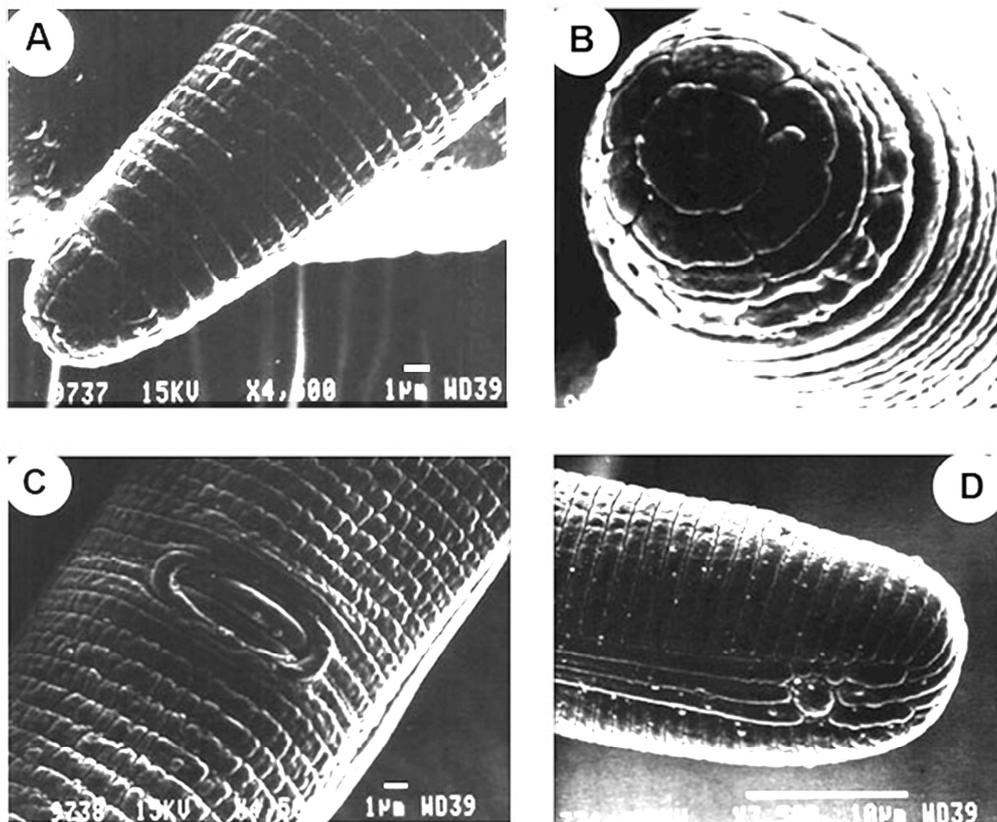


Figure 4. SEM photographs of *Scutellonema tanlamense* n.sp. (Female) A: anterior region in lateral view; B: lip region en-face view; C: vulva region showing paired epiptygma; D: posterior end showing phasmid and structure of lateral field at tail terminus. Scale bars are showed on the pictures.

Etymology. The species name is derived from the Tan Lam black paper Farm where nematode specimens of new species were found in banana.

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