AN UNDESCRIBED SPECIES OF *STEINERNEMA* (RHABDITIDA: STEINERNEMATIDAE) FROM CHUMOMRAY NATIONAL PARK (VIETNAM)

Phan Ke Long

Institute of Ecology and Biological Resources,
Vietnamese Academy of Science and Technology

Abstract. An undescribed species of *Steinernema* (Rhabditida: Steinernematidae) was isolated from forest soil of the Chumomray National park (Kontum prov., Sa Thay distr., Sa Son municipality) Vietnam. Morphological and morphometrical studies revealed that this species clearly differs from other known *Steinernema* species. It has very large spicule as well as in *S. intermedium* but can be separated by the longer IJ tail length, lower ratio E%, shorter spicule, shape of spicule, the number of genital papillae at caudal region and the presence of mucro in male. Its lateral fields resemble the ones of *S. sangi* but can be separated by higher E% and D%, larger and shorter spicule, the morphology of spicule head (manubrium) and dorsal lobe of spicule. Morphometrics of IJs of this species are closed to *S. monticolum* but differ by the position of excretory pore, shorter and larger spicule and the morphology of spicule head.

1. Introduction

Entomopathogenic nematodes (EPN) of the genus *Steinernema* Travassos, 1927 and *Heterorhabditis* Poinar, 1976 have great potential for biological control of insect pests. Currently, 33 species of the genus *Steinernema* and 11 species of the genus *Heterorhabditis* are described. Four species of the genus *Steinenema*, *S. tami* (Pham *et al.*, 2000), *S. sangi* (Phan *et al.*, 2001a), *S. loci* and *S. thanhi* (Phan *et al.*, 2001b), and one species of the genus *Heterorhabditis*, *H. baujardi* (Phan *et al.*, 2003a) have been described from Vietnam. Obviously, Vietnam has a high species diversity of entomopathogenic nematodes that may provide good potential for biological control of insects. During a nematological survey carried out in the Chumomray National Park (Phan *et al.*, 2003b) an unknown steinernematid was detected. This isolate is distinguished from other *Steinernema* species by its morphology and morphometric characters.

2. Materials and methods

2.1. Nematodes

The entomopathogenic nematodes were isolated from soil samples taken in the forest of Chumomray national park (Kontum prov., Sathay distr., Sason municipality) by the *Galleria mellonella* L. baiting method and infective juveniles (IJs) were collected from *Galleria* cadavers using White trap (Phan *et al.*, 2001a) and stored at 15^0 C in aerated

water. Co-ordinates and altitudes of the sampling sites were registered using GARMIN GPS 12 CX.

2.2. Morphological observations

Nematodes were reared on *G. mellonella*. We used IJs collected during a week after their first emergence from the insect cadavers; adults of the first generation were dissected from the cadavers. Nematodes were killed and fixed in hot 4% formalin (50-60^0 C), and kept in this solution for 48 h (Phan *et al.*, 2001a). Fixed nematodes were transferred to anhydrous glycerine and mounted on slides. All measurements were made using a drawing tube attached to an Olympus BX50 light microscope (LM).

3. Description

3.1. Male

Body curved ventrally, C-shaped when heat-killed (Figure 1A). Cuticle looks smooth under LM. Head rounded, slightly offset from the body. Head with six pointed labial papillae and four cephalic papillae. Amphids inconspicuous. Mouth opening funnel-shaped or cup-shaped. Stoma shallow. Oesophagus muscular; procorpus cylindrical; metacorpus slightly swollen non-valvate; isthmus distinct; basal bulb pyriform, valve distinct. Nerve ring just above basal bulb. Cardia prominent and protruding into intestine lumen. Excretory pore at middle of oesophagus. Excretory duct cuticularised; excretory gland swollen and elongated. Monorchic gonad reflexed. Spicule paired, yellow-brownish in colour, well curved and large (Figure 1G). Ratio SL/SPW about 4.5 (3.8-5.6). Spicule head (manubrium) as long as wide. Blade arcuate with spicule tip straight. Three lobes on blade well defined. Anterior, dorsal lobe enlarged dorsally and well curved, terminate posterior to spicule tip. Lateral lobe prominent, usually enlarged anteriorly in width and terminate at spicule tip. Ventral lobe enlarged anteriorly at the ventral side, to form a prominent rostrum and terminate at spicule tip. Velum large, not covering spicule tip. Spicule tip blunt. Gubernaculum about 70% of spicule length. In lateral view, gubernaculum boat-shaped, swollen at middle and proximal end with knob ventrally curved (Figure 1G). In ventral view, cuneus long, bifurcate, not reaching to the end of corpus. Corpus separated posteriorly. A single ventral precloacal papilla and eleven pairs of genital papillae present and arranged as follows: five pairs subventrally preanal, one pair lateral at the same level of the single ventral precloacal papillae. One pair subventral ad-anal. Three pairs caudal, subventral and one pair caudal, subdorsal. Tail conoid with mucron. Phasmids inconspicuous.

3.2. Female

Body robust, C-shaped when heat-killed. Cuticle looks smooth under LM. Head broadly rounded. Head with six pointed labial papillae and four cephalic papillae. Amphids inconspicuous. Mouth opening funnel-shaped or cup-shaped. Stoma shallow. Oesophagus with cylindrical procorpus; metacorpus slightly swollen and non-valvate; isthmus indistinct; basal bulb pyriform, valve observed. Excretory pore at middle of oesophagus (Figure 1C). Excretory duct cuticularised and excretory gland swollen. Cardia prominent protruding into intestine lumen. Didelphic, amphidelphic gonad reflexed and tightly filled

with eggs. Vulva a transverse slit, protrunding from the body, without epiptygma (Figure 1F) and at middle of body. Vagina short, oblique with muscular walls. Post-anally slightly swollen (Figure 1H). Tail dome shaped, shorter than anal body width with terminal peg.

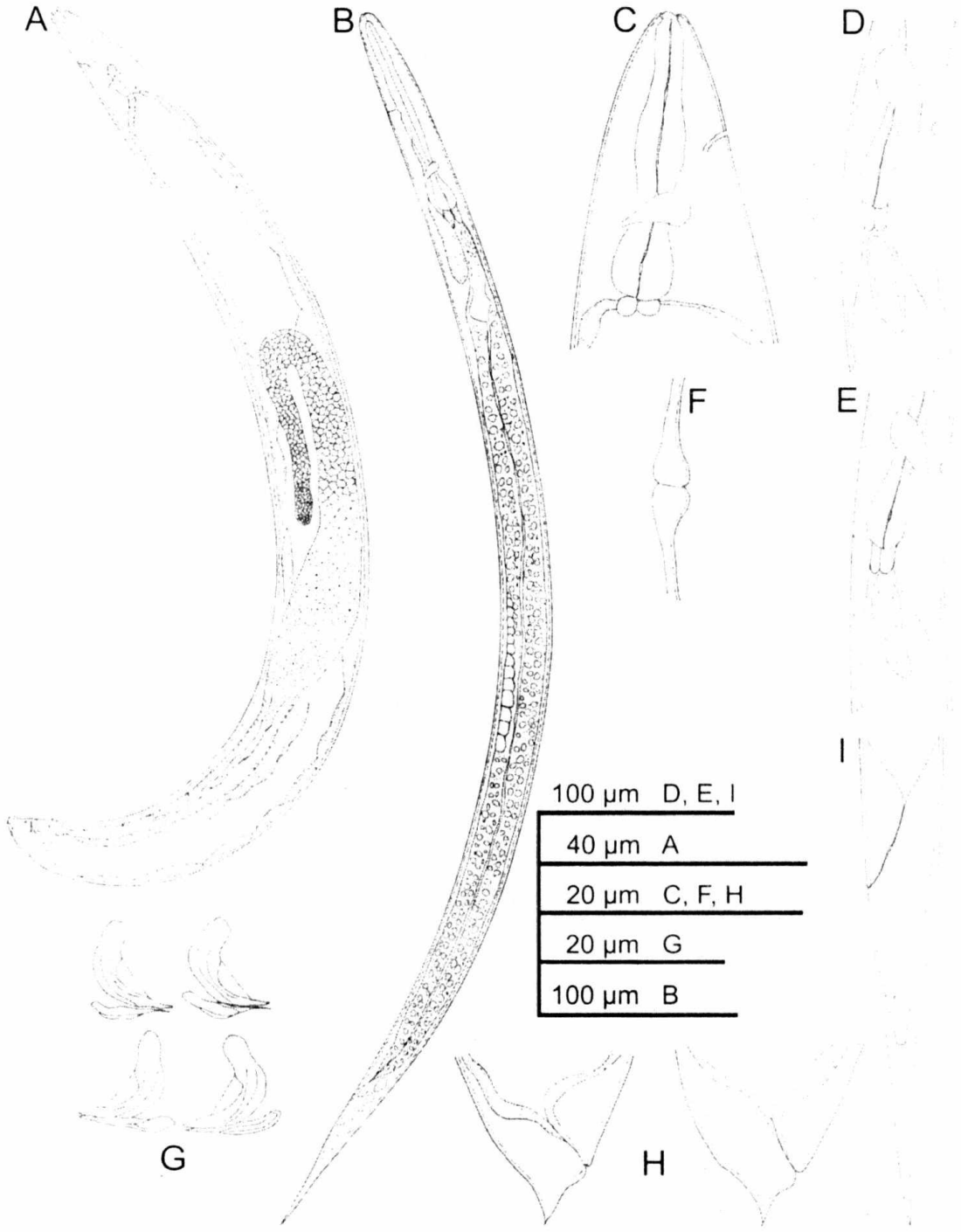

Figure 1. Drawing of the undescribed species of ***Steinernema*** from Chumomray National Park (Vietnam). A & G: Male first generation. A. Entire view; G. Spicule & Gubernaculum. B, D, E & I: Infective juveniles. B. Entire view; D & E. Bacterial vesicle; I. Tail in lateral view. C, F & H: Female first generation. C. Oesophagus region; F. Vulva region; H. Tail in lateral view.

3.3. Infective juvenile

When heat killed, body moderately C-shaped (Figure 1B); often enclosed in cuticle of second-stage; tapering regularly from base of oesophagus to anterior end and from anus to terminus. Mouth and anus closed. In the head, labial papillae not observed; pore-like amphids situated below labial disc just above cephalic papillae. Oesophagus long and narrow, isthmus distinct and surrounded by nerve ring, basal bulb elongated with valve. Cardia prominent. Excretory pore at middle of oesophagus. Hemizonid distinct and located anteriorly to basal bulb. Bacterial vesicle elliptical or elongate (26-28 àm long and 7-10 àm wide) (Figure 1D, E). Lateral field with eight ridges (at mid-body), submarginal and central pair less distinct, sometimes the submarginal not observed. Tail long and constricted at hyaline portion, especially on the dorsal side. Hyaline portion well pronounced about 54% of tail length. Phasmids distinct and located in anterior half of tail (Figure 1I).

3.4. Differential diagnosis

The undescribed species is characterised by the body length about 712 (642-778) àm, the distance from anterior end to excretory pore about 56 (50-68) àm, the tail length about 75 (68-92) àm, the E% about 75 (67-87)%, and the lateral field at mid-body with eight ridges (submarginal and central pair less distinct) of the IJs, as well as by the large spicules of the males (SL/SPW about 4.5) (Table 1).

The morphometrics of IJs of the undescribed species are close to those of *S. monticolum* (Stock *et al.*, 1997) except for the position of the excretory pore (at 1/2 *vs* at anterior 1/3 of oesophagus). Moreover, the new species can be distinguished from *S. monticolum* by male characters such as a shorter spicule length [58 (51-65) *vs* 70 (61-80) µm], larger spicule [SL/SPW = 4.5 (3.8-5.6) *vs* 8.75 (8.0-8.71)] and the spicule head (manubrium) elongated *vs* round (Table 1).

As *Steinernema intermedium* (Poinar, 1985), this undescribed species has very large spicules but can be separated from this species by the longer IJ tail length [75 (68-92) *vs* 66 (53-74) àm], the lower ratio E% [75 (67-87) *vs* 96 (89-108)%]; shorter spicules [58 (51-65) *vs* 91 (84-100) àm], the shape of the spicules (arcuate *vs* well curved anteriorly, posterior almost straight), the number of genital papillae at the caudal region (4 pairs *vs* 6 pairs), and the presence of a mucron on the male tail (Table 1).

The undescribed species has a lateral field resembling to the one of *S. sangi*, also found in Vietnam, but can be separated from this latter species by a higher E% [75 (67-87) *vs* 62 (56-70)], higher D% [46 (43-59) *vs* 40 (36-44)], larger spicule [ratio SL/SPW = 4.5 (3.8-5.6) *vs* 5.25 (5.71-5.8)], shorter spicule length [58 (51-65) *vs* 63 (58-80) àm], the spicule head (manubrium) (elongated and about 1/4 spicule length *vs* short, blunt and about 1/5 spicule length), and the dorsal lobe of the spicule (not terminated at spicule tip *vs* terminated at spicule tip) (Table 1).

Table 1. Morphometric characters (in àm) of the undescribed species. Measurement in form: mean ± SD (range)

Character*	1st generation male	1st generation female	Infective juvenile
n	20	20	25
Body length (L)	1433 ± 106 (1320-1665)	3206 ± 249 (2745-3765)	712 ± 43 (642-778)
Body width (W)	127 ± 15 (105-150)	193 ± 18 (165-240)	28 ± 3 (26-35)
Stoma length	4 ± 1 (3-5)	6 ± 1 (5-8)	-
Stoma width	6 ± 1 (5-8)	10 ± 1 (8-12)	-
EP	96 ± 5 (89-104)	108 ± 8 (90-117)	56 ± 4 (50-68)
NR	120 ± 5 (110-129)	148 ± 9 (132-165)	84 ± 4 (80-100)
ES	173 ± 7 (162-186)	226 ± 6 (216-239)	120 ± 7 (115-152)
Testis flexure	264 ± 58 (165-360)	-	-
Tail length	29 ± 5 (23-33)	54 ± 5 (47-63)	75 ± 5 (68-92)
H%	-	-	54 ± 3 (49-62)
Anal body width (ABW)	46 ± 4 (39-56)	73 ± 9 (59-87)	16 ± 1 (14-48)
Spicule length (SP)	58 ± 3 (51-65)	-	-
Spicule width (SPW)	13 ± 1 (11-15)	-	-
Gubernaculum length (GU)	41 ± 3 (36-44)	-	-
Gubernaculum width	6 ± 1 (5-8)	-	-
SP/SPW	4.6 ± 0.4 (4.2-5.6)	-	-
Vulva (%)	-	55 ± 2 (50-58)	-
a (L/W)	11 ± 1 (9-13)	17 ± 1 (15-19)	25 ± 3 (18-29)
b (L/ES)	8 ± 1 (7-9)	14 ± 1 (13-16)	6 ± 1 (3.6-6.3)

* EP = distance from anterior end to excretory pore; NR = distance from anterior end to nerve ring; ES = oesophagus length; H% = hyaline part/tail length × 100.

c (L/T)	50 ± 8 (40-67)	60 ± 7 (49-70)	10 ± 1 (6-11)
D%=EP/ES ì 100	56 ± 5 (50-63)	48 ± 3 (39-53)	46 ± 3 (43-59)
E%=EP/T ì 100	-	-	75 ± 5 (67-87)
SW=SP/ABW	1.29 ± 0.12 (1.11-1.50)	-	-
GS=GU/SP	0.7 ± 0.05 (0.64-0.79)	-	-
Mucron	2.4 ± 0.56 (1.5-3.0)	-	-

4. Discussion

Precise identification of any organism is of outmost importance. Identification of *Steinernema* and *Heterorhabditis* species by standard methods using morphology and morphometrics is rarely straightforward (Hominick *et al.*, 1997) because that kind of investigation requires the examination of numerous characters, some being difficult to observe. Moreover, morphometrics of IJ vary within species and between populations (Miduturi *et al.*, 1996). Some morphological characters are useful for distinguishing species or groups of species of *Steinernema*, *e.g.* lateral fields (Hominick *et al.*, 1997), amoeboid cells (Spiridonov *et al.*, 1999), and morphology of spicula and gubernaculums (Nguyen & Smart, 1997). As a conclusion of their study of the morphometrical characters of several populations of *Heterorhabditis*, Phan *et al.* (2003b) suggested that the morphometrical characters, and the ratio e, ratio f and body diameter of IJ as well as spicule length, gubernaculum length and ratio SW of male along with the morphology of gubernaculums should be considered when identifying and describing *Heterorhabditis* spp.

Hominick *et al.* (1996) argued that molecular techniques could be an addition to traditional identification methods. Distinctions based on molecular characterisation may elucidate species and groupings, which then can be studied for morphological characters that distinguish them from each other. Several modern techniques have been used for identification of entomopathogenic nematodes. They include isozyme patterns (Akhurst, 1987), total protein patterns (Poinar & Kozodoi, 1988) or immunological techniques (Jackson, 1965). Initial research in molecular taxonomy and diagnostics of entomopathogenic nematodes utilised cloned DNA probes and restriction fragment length polymorphisms (RFLPs) as discriminatory methods (Roland & John, 1998). The internal transcribed spacer region (ITS) is an ideal region for molecular taxonomic purposes. The ribosomal genes flanking this region are highly conserved allowing the construction of primers that enable PCR amplification of the highly variable ITS region between them (Reid *et al.*, 1997). Sequence variation in this region yields many RFLP, which can be used for taxonomy. By comparison of the bands generated after restriction digests it was possible to construct a provisional tree showing the relatedness of the *Steinernema* species studied (Reid *et al.*, 1997). DNA sequences of ITS regions yield more detailed information about variation within and among nematodes species than PCR-RFLP approaches. These spacer sequences have been used successfully to diagnose species and populations of nematodes

(Phan *et al.*, 2003a). Analyses of ITS rDNA sequences also have been used to reconstruct phylogenetic relationships of *Steinernema* and *Heterorhabditis* species (Stock *et al.*, 2001; Phan *et al.*, 2003a). The ongoing study in molecular characterisation of this undescribed species may yield more interesting results for complete the description of this species.

The study of other characters of this interesting species including the molecular ones is going on in order to completely describe it in the near future.

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REFERENCE

1. Akhurst, R.J. Use of starch gel electrophoresis in the taxonomy of the genus *Heterorhabditis* (Nematoda: Heterorhabditidae). *Nematologica* **33** (1987), pp 1-9.
2. Hominick, W.M., Reid, A.P., Bohan, D.A. & Briscoe, B.R. Entomopathogenic nematodes: biodiversity, geographical distribution and the Convention on Biological Diversity. *Biocontrol Science and Technology* **6** (1996), pp 317-331.
3. Hominick, W.M., Briscoe, B.R., Del-Pino, F.G., Heng, J., Hunt, D.J., Kozodoy, E., Mracek, Z., Nguyen, K.B., Reid, A.P., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C. & Yoshida, M. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *Journal of Helminthology* **71** (1997), pp 271-298.
4. Jackson, G.J. Differentiation of three species of Neoplectana (Nematoda: Rhabditida) grown axenically. *Parasitology* **55** (1965), pp 571-578.
5. Miduturi, J.S., Matata, G.J.M., Waeyenberge, L. & Moens, M. Naturally occurring entomopathogenic nematodes in the province of East Flanders, Belgium. *Nematologia Mediterranea* **24** (1996), pp 287-293.
6. Nguyen, K.B. & Smart, G.C. Scanning electron microscope studies of spicules and gubernacula of *Steinernema* spp. (Nemata: Steinernematidae). *Nematologica* **43** (1997), pp 465-480.
7. Pham, V.L., Nguyen, K.B., Reid, A.P., Spiridonov S.E. & Sturhan, D. *Steinernema tami* sp. n. (Rhabditida: Steinernematidae) from Cat Tien forest, Vietnam. *Russian Journal of Nematology* **8** (2000), pp 33-43.
8. Phan, K.L., Nguyen, N.C. & Moens, M. *Steinernema sangi* sp. n. (Rhabditida: Steinernematidae) from Vietnam. *Russian Journal of Nematology* **9** (2001a), pp 1-7.
9. Phan, K.L., Nguyen, N.C. & Moens, M. *Steinernema loci* sp. n. and *Steinernema thanhi* sp. n. (Rhabditida: Steinernematidae) from Vietnam. *Nematology* **3** (2001b), pp 503-514.
10. Phan, K.L., Subbotin, S.A., Nguyen N.C. & Moens, M. *Heterorhabditis baujardi* sp. n. (Rhabditida: Heterorhabditidae) from Vietnam with morphometric data for *H. indica* populations. *Nematology* (2003a), pp 367-382.
11. Phan, K.L., Nguyen, N.C. & Moens, M. Natural distribution of entomopathogenic nematodes (Rhabditida: *Steinernema* and *Heterorhabditis*) in Vietnam. *Proceedings of the 2nd National conference in life science*. Science & Technics Publishing House, Hanoi. 2003b, pp 670-673.

12. Poinar, G.O. and Kozodoi, E. M. *Neoaplectana glaseri* and *N. anomali*: sibling species or parallelism. *Revue de Nematologie* **11** (1988), pp 13-19.
13. Poinar, G. O., Jr. *Neoaplectana intermedia* n. sp. (*Steinernema*tidae: Nematoda) from South Carolina. *Revue de Nematologie* **8** (1985), pp 321-327.
14. Reid, A.P., Hominick, W.M. & Briscoe, B.R. Molecular taxonomy and phylogeny of entomopathogenic nematode species (Rhabditida: Steinernematidae) by RFLP analysis of the ITS region of the ribosomal DNA repeat unit. *Systematic Parasitology* **37** (1997), pp 187-193.
15. Roland, N.P & John, T.J. The use of molecular biology techniques in Plant Nematology: Past, present and future. *Russian Journal of Nematology* **6** (1998), pp 47-56.
16. Spiridonov, S.E., Hominick, W.M. & Briscoe, B.R. Morphology of amoeboid cells in the uterus of *Steinernema* species (Rhabditida: Steinernematidae). *Russian Journal of Nematology* **7** (1999), pp 49-56.
17. Stock, S.P., Choo, H.Y. & Kaya, H.K. *Steinernema monticolum* sp. n. (Rhabditida: *Steinernema*tidae), an entomopathogenic nematode from Korea with a key to other species. *Nematologica* **43** (1997), pp 15-29.
18. Stock, S.P., Campbell, J.F. & Nadler, S.A. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters, *Journal of Parasitology* **87** (2001), pp 877-889.

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VỀ MỘT LOÀI CHƯA ĐƯỢC MÔ TẢ THUỘC STEINERNEMA (RHABDITIDA: STEINERNEMATIDAE) PHÂN LẬP ĐƯỢC TỪ ĐẤT RỪNG CỦA VƯỜN QUỐC GIA CHƯ MOM RAY (VIỆT NAM)

Phan Kế Long

Viện Sinh thái và Tài nguyên sinh vật

Viện Khoa học và Công nghệ Việt Nam

Loài chưa được mộ tả thuộc giống Steinernema này (Rhabditida: Steinernematidae) phân lập được từ đất rừng của Vườn Quốc gia Chư Mom Ray (tỉnh Kon Tum: huyện Sa Thầy, xã Sa Sơn). Các nghiên cứu về hình thái và số đo cho thấy nó khác biệt rõ ràng với tất cả các loài đã biết của giống *Steinernema*. Gai giao cấu của nó rất lớn giống như ở *S.intermedium* nhưng khác loài này vì có IJ đuôi dài hơn, tỷ lệ E% thấp hơn, gai giao cấu ngắn hơn, ở kích thước của gai giao cấu, số lượng của nhú sinh dục ở vùng đuôi và ở sự có mặt của mấu đuôi ở con đực. Loài chưa được mô tả này có các vùng bên giống như ở *S.sangi* nhưng phân biệt với nó vì có E% và D% lớn hơn, gai giao cấu to hơn và ngắn hơn, ở hình thái của đầu và thùy lưng của gai giao cấu. Các số đo IJ của loài chưa được mô tả này gần như ở *S.monticolum* nhưng khác bởi vị trí của lỗ bài tiết, gai giao cấu ngắn hơn và to hơn, và ở hình thái đầu của gai giao cấu.