

Four new species of *Steinernema* Travassos, 1928 with short infective juveniles from Vietnam

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Summary. The length of the infective juveniles (IJs) of four new species (*Steinernema cumgarensense* sp. n., *Steinernema eapokense* sp. n., *Steinernema backanense* sp. n. and *Steinernema sasonense* sp. n.) is less than 600 µm. The IJ have lateral fields with four central ridges and two prominent marginal ridges (at least in anterior body half). As the IJs have no horn-like appendages on the cephalic end, these species belong to the 'carpocapsae-scaterisci-tami' group. The length of IJ of *S. cumgarensense* sp. n. is similar to *S. eapokense* sp. n. and *S. siamkayai*, but differs from *S. sasonense* sp. n., *S. carpocapsae*, *S. scapterisci* and *S. tami*. This species can be distinguished from the closely related species *S. eapokense* sp. n. by the D% value, and the hyaline part of IJ tail. *Steinernema cumgarensense* sp. n. IJs have thicker body than *S. siamkayai*. The spicules and gubernaculum of this species are longer than *S. backanense* sp. n. Moreover, *S. cumgarensense* sp. n. differs from *S. sasonense* sp. n. in IJ pharynx and tail length. The E% value of *S. eapokense* sp. n. helps to distinguish this species from *S. siamkayai*. IJs of *S. eapokense* sp. n. are shorter than those of *S. sasonense* sp. n., *S. carpocapsae* and *S. scapterisci*. The spicule of *S. eapokense* sp. n. is shorter than that in *S. backanense* sp. n. Males of *S. backanense* sp. n. can be distinguished from *S. cumgarensense* sp. n., *S. eapokense* sp. n. and *S. sasonense* sp. n., and also from *S. tami*, *S. scapterisci* and *S. siamkayai* by their short spicules. *Steinernema backanense* sp. n. also differs from *S. carpocapsae* in D% value of IJs. *Steinernema sasonense* sp. n. can be distinguished from *S. scapterisci* by the IJ length; and from *S. cumgarensense* sp. n. and *S. eapokense* sp. n. by longer IJs. *Steinernema sasonense* sp. n. can be distinguished from *S. carpocapsae* by the shorter pharynx length and D% value of IJs. In addition, four new species can be differentiated further from each other and from closely related species of the 'carpocapsae-scaterisci-tami' group by characteristic sequences of their ITS-rRNA gene. The phylogenetic relationships within clade 'carpocapsae-scaterisci-tami' of the genus *Steinernema* with inclusion of newly sequenced *S. cumgarensense* sp. n., *S. eapokense* sp. n., *S. backanense* sp. n. and *S. sasonense* sp. n. are presented on the basis of ITS-rRNA gene sequence analysis.

Key words: Entomopathogenic nematodes, ITS-rDNA, phylogenetic, *Steinernema*, *S. cumgarensense* sp. n., *S. eapokense* sp. n., *S. backanense* sp. n. and *S. sasonense* sp. n., taxonomy, Vietnam.

Several groups of species are recognised in the genus *Steinernema* Travassos, 1928 (Reid et al., 1997); 'steinernematids with short juveniles' group are widely accepted as separate evolutionary branch of these nematodes (Hominick et al., 1997, Nguyen et al., 2001; Stock et al., 2001) and designated as 'carpocapsae' group of *Steinernema*. Analysing the ITS-RNA gene sequence Spiridonov

et al. (2004) proposed to distinguish the 'carpocapsae-scaterisci-tami' group, which represents a separate clade on the *Steinernema* phylogenetic tree. This group contains steinernematids with mean infective juveniles (IJ) length of less than 550-600 µm. Members of this group are rare in temperate regions; e.g. Sturhan (1999) reported *S. carpocapsae* only once from 584

samples that were positive for the presence of steinernematids. The general opinion is that the '*carpocapsae-scaterisci-tami*' is more common in tropical and subtropical areas. The first species of this group, *S. carpocapae* was described nearly fifty years ago (Weiser, 1955); the second species, *S. scapterisci* was found only a decade ago (Nguyen & Smart, 1990). Several new species of these steinernematids were recently described from tropical regions: *S. siamkayai* (Stock *et al.*, 1998), *S. thermophilum* (Ganguly & Singh, 2000), *S. tami* (Pham *et al.*, 2000) and *S. asiaticum* (Anis *et al.*, 2002). The validity of some of these species is still to be evaluated but it is possible to assume that the real diversity of these steinernematids is quite high and that the '*carpocapsae-scaterisci-tami*' group includes many more species than at present. Several new steinernematid species were recently described from Vietnam (Phan *et al.*, 2001 a, b), *S. tami* was the only species of the '*carpocapsae-scaterisci-tami*' group found in this country. Descriptions of four new species of this group from Vietnam are presented below.

MATERIAL AND METHODS

The entomopathogenic nematodes were isolated from soil samples taken in the forest of Backan, Daklak, Kontum and Vinhphuc provinces by the *Galleria mellonella* (L.) baiting method (Bedding & Akhurst, 1975). IJs were collected from *Galleria* cadavers using the method of White (1927) and stored at 15°C in aerated water. Coordinates and altitudes of the sampling sites were registered using GARMIN GPS 12 CX.

Morphological observations. For light microscopy, nematodes were reared on *G. mellonella*. We used IJs collected during the week after their first emergence from the insect cadavers; adults of the first generation were dissected from the cadavers (Nguyen & Smart, 1995). Nematodes were killed and fixed in hot 4% formalin (50–60°C), and kept in this solution for 48 h. Fixed nematodes were transferred to anhydrous glycerine according to Seinhorst's (1959) rapid method as modified by De Grisse (1969) and mounted on permanent slides, and studied with a LEITZ "Diaplan" microscope provided DIC optics.

DNA extraction, PCR, DNA purification and sequencing. DNA was extracted from a single female using a modification of the method of Joyce *et al.* (1994). For each isolate, a specimen was cut in 8 µl of worm lysis buffer (500 mM KCl, 100 mM Tris-Cl pH 8.3, 15 mM MgCl₂, 10 mM DTT, 4.5% Tween 20, 0.1% gelatin). The nematode fragments were transferred in 4 µl of the

buffer to an Eppendorf tube to which 5 µl of double distilled water and 1 µl of proteinase K (600 µg/ml) were added. After freezing (–70°C for 1 h) the tubes were incubated at 65°C for 1h and then at 95°C for 10 min.

After centrifugation (1 min; 13000 g) of the tubes, 10 µl of the DNA suspension were added to a PCR reaction mixture containing 4 µl 10x PCR buffer, 1 µl MgCl₂ (25 mM), 1 µl dNTP mixture (10 mM each), 0.2 µl (500 nM) of each primer, 1.5 U Taq polymerase and 33.3 µl double distilled water to a final volume of 50 µl. The forward primer 18S (5'-TTG ATT ACG TCC CTG CCC TTT-3') and the reverse primer 26S (5'-TTT CAC TCG CCG TTA CTA AGG-3') were used in the PCR reaction for amplification of the complete ITS region (Vrain *et al.*, 1992). The amplification profile was carried out using a PTC-100 thermocycler, with 2 min of preheating at 92°C followed by 35 cycles of 92°C for 30 s, 54°C for 30 s and 72°C for 2 min, and then 72°C for 10 minutes. After DNA amplification, 5 µl product was loaded on a 1% agarose gel for DNA checking. Amplified products were purified using a Qiaquick Gel Extraction Kit (Qiagen GmbH, Leusden, the Netherlands). DNA fragments were sequenced with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, USA) and run on ABI PRISM 310 Genetic Analyser (PE Applied Biosystems, USA). The original ITS sequences of *Steinernema* were deposited at GenBank under accession numbers: AY487918 (*S. backanense* sp.n.), AY487919 (*S. sasonense* sp. n.), AY487920 (*S. cumgareense* sp. n.), and AY487921 (*S. eapokense* sp. n.).

Sequence alignment and phylogenetic analysis. The DNA sequence of new *Steinernema* species were aligned using Clustal X 1.64 with the ITS1-5.8S-ITS2 sequences of other *Steinernema* species (Nguyen *et al.*, 2001; Stock *et al.*, 2001; Spiridonov *et al.* 2004; Qiu, L. *et al.*, unpublished) belonging to the Clade II ('*carpocapsae-scaterisci-tami*') and two outgroup *Steinernema* taxa (Spiridonov *et al.*, 2004). The alignment is available by request from the third author.

Two types of phylogenetic analyses were applied to analyze the alignment: maximum parsimony (MP) and maximum likelihood (ML) with PAUP* 4b4a (Swofford, 2003). We conducted equally weighted MP analyses. The gaps were coded as missing data and molecular characters were assessed as independent and unordered. Heuristic search settings were 10 replicates of random taxa, addition, tree bisection-reconnection, branch swapping, multiple tree

retained and without steepest descent. For ML, all necessary parameters were estimated from the data using ModelTest based on Akaike Information Criterion (Posada & Crandall, 1998). Robustness of the clades was assessed by the bootstrap analysis yielding bootstrap percentage (BS) for each node estimated from 1000 and 100 replicates for MP and ML analyses, respectively.

DESCRIPTION

Steinernema cumgarens sp. n. (Fig. 1, Fig. 5 B)

Measurements. See Table 1.

Male. Body fusiform, obese, 'C'-shaped in heat-killed specimens, tapering toward anterior end. Cuticle annulation 1-1.3 μm wide poorly distinguishable throughout the body. No lateral fields visible but irregular anastomoses of cuticle annulation present along body lateral side. Anterior extremity rounded with flattened area just around the mouth. Six labial papillae and more prominent cephalic sensillae present. Cheilostom cuticle connected to a thick strongly cuticularised 2-3 μm long ring of 6-6.5 μm diameter, forming the middle part of stoma walls. Additional thinner cuticular ring situated behind thicker one, embedded into pharynx tissue. Pharynx corpus cylindrical, without visible metacorporeal swelling. Basal bulb subpyriform. Nerve ring situated on anterior slope of basal bulb in most males. Valves in shape of platelets of thin folded cuticle. Cardium spherical, protruding for 18-22 μm into intestine proventriculus. Deirids of 4-5 μm diameter situated behind level of pharynx-intestine junction. Excretory channel strongly cuticularised in 10 μm from the pore. Intestinal lumen open up to mid-body. Separate spermatocytes poorly distinguishable even in the reflexed testis part, being replaced with complex rachis structure in testis middle, with characteristic patterns of spermatogenesis up to 'vas deferens'. Posterior part of intestinal lumen open, forming a wide cavity. Characteristic steinernematid set of genital papillae with a row of usually 9 pairs of subventral papillae extending from tail terminus to body middle, one unpaired large precloacal papilla in mid-ventral position, one pair of papillae in lateral position at manubrium level when spicules retracted, and one pair of papillae in dorso-lateral position in post-cloacal area. This latter pair usually closer to equatorial part of tail. A pair of postdeirids situated dorso-laterally on the level of anteriormost pair of precloacal papillae. Spicules with elongated,

funnel-shaped manubrium, tapering toward distal end, with round extremity and more or less pronounced terminal duct. Bifurcation of outer line of velum and ventral rib of spicule visible near rostrum. All four main 'ribs' of spicule discernible, separated by gaps visible in lateral aspect. Spicule body gradually narrowing from rostrum to distal end. Cloacal opening is covered with flap when spicules retracted. Two papillae-like structures on the cloacal opening edges. Gubernaculum usually straight with ventrally curved proximal end, often with cuticular knobs on inner surface. Tail mucron spike-like.

Female. Cuticle annulation 1.5 μm wide, well discernible near vulva lips. Cephalic end with 6 labial and 4 cephalic papillae. Cuticular ring forming main part of stoma walls 5-6 μm thick and 7-7.5 μm long. Stomatal cavity 10 μm wide. Excretory duct strongly cuticularised, entering body at nearly right angle. Equatorial vulva with epyptygma. Vulva lips protruding up to 20-25 μm , equal in size. Tail end dome-shaped, with peg-like 3-5 μm long mucron.

Third-stage infective juvenile. Body of heat-killed juveniles quite obese, moderately C-shaped, tapering toward anterior end. Cuticular annulation about 1 μm wide visible throughout body. Lateral field in anterior body half with 4 ridges in the centre, two pronounced marginal ridges and poorly discernible submarginal lines. Such regular pattern not observed in body posterior half, with ventral gap between central and marginal ridges disappearing. Lateral field 8.5-9.5 μm wide at mid-body. Cuticle swollen near anterior end forming 4-5 μm long cephalic capsule. Frontal surface of anterior end always regularly round. Labial papillae indistinct, four cephalic sensilla and amphidial pouches occasionally visible. Pharynx corpus slender, slightly widening to metacorporeal part. Nerve ring always around corpus-isthmus junction. Hemizonid on isthmus level, often with additional fibres in 1-3 μm behind main one. Basal bulb with poorly visible valves and large nucleus in dorsal anterior half. Cardia about 10 μm long, narrowing to posterior end. Bacterial vesicle elliptical, 10-13 μm long and 5-6 μm wide. Rectum about two anal body diameter long. Genital primordium 50 μm long. Phasmids at one third of tail length behind anus (phasmid - mucron distance about 25-26 μm), with aperture near ventral edge of ventral marginal line. Lateral field lines disappearing at level of phasmids. Tail with pronounced ventral concavity, ventrally curved with terminus pointed to finely rounded. Hyaline portion occupying less than half of tail length.

Table 1. Morphometrics (in μm) of *Steinernema cumgarens* sp. n. Mean \pm SD (range).

Characters ¹⁾	Holotype male	1st generation ♂♂ (Paratypes)	1st generation ♀♀	Infective juveniles
n		20	20	25
L	1320	1307 \pm 75 (1185-1470)	5178 \pm 760 (4275-6045)	402 \pm 14 (384-432)
Body diam.	90	96 \pm 10 (90-120)	222 \pm 29 (180-270)	22 \pm 1 (21-25)
Stoma length	5	4.7 \pm 0.5 (4.5-6)	8 \pm 1 (7-10)	—
Stoma width	8	6.8 \pm 0.8 (6.0-7.5)	12 \pm 2 (10-14)	—
Excretory pore	77	76 \pm 7 (62-86)	100 \pm 9 (84-113)	35 \pm 2 (33-38)
Nerve ring	101	102 \pm 5 (90-108)	156 \pm 8 (146-173)	58 \pm 2 (54-60)
Pharynx	144	140 \pm 6 (128-150)	222 \pm 12 (204-240)	86 \pm 3 (80-91)
Testis flexure	396	330 \pm 38 (283-396)	—	—
Tai length	17	16 \pm 2 (14-20)	21 \pm 3 (17-26)	36 \pm 2 (34-38)
H%	—	—	—	53 \pm 3 (48-59)
Anal body diam.	29	28 \pm 2 (24-32)	64 \pm 7 (50-77)	11 \pm 1 (10-12)
Spicule length	75	76 \pm 4 (68-81)	—	—
Spicule width	11	12 \pm 1.2 (11-15)	—	—
Gubernaculum length	57	58 \pm 5 (48-66)	—	—
Gubernaculum width	8	6 \pm 1 (4.5-7.5)	—	—
V%	—	—	54 \pm 2 (51-57)	—
a	15	14 \pm 1 (12-15)	23 \pm 2 (21-29)	18 \pm 1 (17-19)
b	9	9 \pm 0.3 (8.9-10)	23 \pm 2 (20-26)	4.7 \pm 0.2 (4.4-4.9)
c	80	82 \pm 7 (69-93)	259 \pm 66 (162-354)	11 \pm 0.4 (10.5-12.1)
D%	53	45 \pm 5 (35-52)	45 \pm 5 (35-52)	41 \pm 2 (38-43)
E%	—	—	—	99 \pm 5 (87-103)
SW	2.63	2.77 \pm 0.2 (2.5-3.13)	—	—
GS	0.76	0.76 \pm 0.04 (0.71-0.83)	—	—
Mucron	1.5	1.91 \pm 0.62 (1.5-3.0)	4 \pm 1 (2-5)	—

¹⁾ D% = distance from anterior end to excretory pore/pharynx length \times 100; E% = distance from anterior end to excretory pore/tail length \times 100; SW = spicule length/anal body diam.; GS = gubernaculum length/spicule length.

Morphological diagnosis and relationships with other species. Nematodes of this species have 402 (384-432) μm long IJs and lateral fields with four central ridges and two prominent marginal ridges (at least in anterior body half) but no horn-like appendages on the cephalic end, and thus belong to the '*carpocapsae* group'. In IJ body length, this species is similar to *S. eapokense* sp. n., 402 (370-434) μm and *S. siamkayai*, 446 (308-495) μm , but differs from *S. sasonense* sp. n., 471 (437-516) μm ,

S. carpocapsae, 546 (427-640) μm , *S. scapterisci*, 572 (517-609) μm and *S. tami*, 530 (400-600) μm . This new species can be distinguished from the closely related species *S. eapokense* sp. n. by the D% value (38-43 vs 32-37%) and hyaline part of IJ tail [38 (38-43) vs 53 (47-57)%]. *Steinernema cumgarens* sp. n. IJs have thicker body than *S. siamkayai* (17-19 vs 19-23 μm). *Steinernema cumgarens* sp. n. differs from *S. backanense* sp. n. by the length of spicule and gubernaculum (68-81

vs 53-62 μm) and (48-66 vs 36-48 μm). *Steinernema cumgarensense* sp. n. also differs from *S. sasonense* sp. n. by the length of IJ pharynx (80-91 vs 94-104 μm) and tail length (34-38 vs 43-49 μm).

Type locality. Specimens of this new species were collected near the village Cu M'Gar in the Daklak province of Vietnam (longitude 108°02'E, latitude 12°29'N, altitude 600 m above sea level) from the basalt red soil of highland coffee plantations.

Other localities. Recently, the ITS-rRNA gene sequence of an unidentified *Steinernema* from Guangdong, China was deposited in the GenBank by Qiu, L. et al. (unpublished) under accession number AY170339. Our analyses revealed very high similarity of this sequences with that of *Steinernema cumgarensense* sp. n. from Vietnam and based on this results, we concluded that Chinese *Steinernema* belong to *S. cumgarensense* sp. n.

Etymology. *Steinernema cumgarensense* sp. n. is named after the type locality.

Deposition of type material. Holotype male and seven paratype males are deposited at Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 119071, Russia. Slides with paratype males, first generation females and infective juveniles deposited in Department of Nematology, Institute of Ecology and Biological Resources, National Center for Science and Technology, Hoang Quoc Viet 18, Nghiado, Cauaiay, Hanoi, Vietnam.

Steinernema eapokense sp. n. (Fig. 2, Fig. 5 C)

Measurements. See Table 2.

Male. Body fusiform, obese, 'C'-shaped in heat-killed specimens, tapering toward anterior end. Cuticle annulation 1-1.4 μm wide poorly distinguishable throughout the body. No lateral fields visible but irregular anastomoses of cuticle annulation present along body lateral side. Anterior extremity rounded without prominent flattened area around mouth. Six labial papillae and 4 more prominent cephalic ones present. Cheilostom cuticle connected to a thick strongly cuticularised 2 μm long ring of 6-6.5 μm diameter, forming the middle part of stoma walls. Additional thinner cuticular ring situated behind thicker one, being embedded into pharynx tissue. Pharynx corpus cylindrical, with visible metacorporeal swelling and swelling around basal part of stoma. Basal bulb subspherical. Nerve ring situated on anterior slope of basal bulb in most males. Cardium flat, 8-10 μm long. Deirids of 2 μm diameter sitting in the centre of 4-5 μm diameter round spot of smooth cuticle behind level of pharynx-intestine

junction. Excretory channel strongly cuticularised up to 12-13 μm from pore. Intestinal lumen open throughout body length. Separate spermatocytes poorly distinguishable even in the reflexed testis part, being replaced with complex rachis structure in testis middle, with characteristic patterns of spermatogenesis up to vacuolated '*vas deferens*'. Characteristic steinernematid set of genital papillae with a row of usually 9 pairs of subventral papillae extending from tail terminus to body middle, one unpaired large precloacal papilla in midventral position, one pair of papillae in lateral position at manubrium level when spicules retracted, and one pair of papillae in dorso-lateral position in post-cloacal area. This latter pair usually closer to equatorial part of tail. A pair of postdeirids situated dorso-laterally on the level of anteriormost pair of precloacal papillae. Spicules with elongated, funnel-shaped manubrium, tapering toward distal end, with round extremity and more or less pronounced terminal duct. Bifurcation of outer line of velum and ventral rib of spicule visible near rostrum. All four main 'ribs' of spicule discernible, separated by gaps visible in lateral aspect. Spicule body gradually narrowing from rostrum to distal end. Cloacal opening is covered with flap when spicules retracted. Two papillae-like structures on the cloacal opening edges. Gubernaculum usually straight with ventrally curved proximal end, and well visible dorsal 10-12 μm long "hole". Tail mucron often cylindrical, with globular inclusion.

Female. Cuticle annulation 1.5 μm wide. Cephalic end with 6 labial and 4 cephalic papillae. Cuticular ring forming main part of stoma walls 5 μm thick and 5-5.5 μm long. Stomatal cavity 10 μm wide. Excretory duct strongly cuticularised, entering body at angle about 45°. Equatorial vulva with petal-like 1012 μm long structures protruding from its opening. Vulva lips less than 12-15 μm high, equal in size. Tail end dome-shaped, with conical 10-15 μm long spike-like mucron.

Third-stage infective juvenile. Body of heat-killed juveniles quite obese, moderately 'C'-shaped, tapering toward anterior end. Cuticular annulation about 1 μm wide visible throughout body. Lateral field in anterior body half with 4 ridges in the centre, two pronounced marginal ridges and poorly discernible submarginal lines. Lateral field 8-9 μm wide at mid-body. Such regular pattern not observed in body posterior half, with ventral gap between central and marginal ridges disappearing. Cuticle swollen near anterior end forming cephalic capsule about 5 μm long. Frontal surface of anterior end always regularly round. Labial papillae indistinct,

Table 2. Morphometrics (in μm) of *Steinernema eapokense* sp. n. Mean \pm SD (range).

Characters ¹⁾	Holotype male	1st generation ♂♂ (Paratypes)	1st generation ♀♀	Infective juveniles
n		20	20	25
L	1095	1096 \pm 50 (1035-1200)	3584 \pm 232 (3210-3930)	402 \pm 24 (370-434)
Body diam.	90	87 \pm 6 (75-90)	180 \pm 11 (165-195)	19 \pm 1 (18-21)
Stoma length		4 \pm 1 (3-6)	8.4 \pm 1 (7.5-11)	—
Stoma width		6 \pm 1 (5-6)	10 \pm 1.5 (7.5-12)	—
Excretory pore	62	57 \pm 3 (51-62)	81 \pm 9 (69-96)	29 \pm 1 (26-31)
Nerve ring	98	98 \pm 3 (93-104)	143 \pm 9 (134-161)	58 \pm 3 (54-62)
Pharynx	129	134 \pm 5 (127-146)	200 \pm 9 (182-209)	84 \pm 3 (79-88)
Testis flexure	218	232 \pm 53 (190-288)	—	—
Tail length	20	20 \pm 2 (15-23)	47 \pm 6 (38-58)	39 \pm 3 (35-45)
H%	—	—	—	53 \pm 3 (47-57)
Anal body diam.	30	28 \pm 1 (26-30)	58 \pm 7 (44-69)	10 \pm 0.6 (8.6-11)
Spicule length	69	69 \pm 4 (63-74)	—	—
Spicule width	11	11 \pm 1 (9-12)	—	—
Gubernaculum length	50	50 \pm 2 (47-56)	—	—
Gubernaculum width	8	6 \pm 1 (5-8)	—	—
V%	—	—	55 \pm 2 (52-57)	—
a	12	13 \pm 1 (12-14)	20 \pm 1 (18-22)	21 \pm 2 (18-23)
b	8	8.2 \pm 0.3 (7.8-8.8)	18 \pm 1 (16-21)	4.8 \pm 0.3 (4.3-5.3)
c	56	57 \pm 7 (49-71)	78 \pm 8 (63-90)	10.2 \pm 0.5 (9.6-11.4)
D%	48	43 \pm 3 (37-48)	40 \pm 4 (33-47)	34 \pm 2 (32-37)
E%	—	—	—	73 \pm 5 (66-80)
SW	2.3	2.46 \pm 0.19 (2.21-2.82)	—	—
GS	0.72	0.73 \pm 0.04 (0.69-0.8)	—	—
Mucron	3	2.94 \pm 0.48 (2.25-3.75)	—	—

¹⁾ D% = distance from anterior end to excretory pore/pharynx length \times 100; E% = distance from anterior end to excretory pore/tail length \times 100; SW = spicule length/anal body diam.; GS = gubernaculum length/spicule length.

four cephalic sensilla and amphidial pouches occasionally visible. Pharynx corpus slender, slightly widening to metacorporeal part. Nerve ring always around corpus-isthmus junction. Excretory pore encircled with refractive ring of cuticle 1-1.5 μm deep along duct. Hemizonid on isthmus level, usually protruding from body with 3-4 annulation rings along. Basal bulb with poorly visible valves. Deirids round subcuticular structures on hemizonid level. Cardia about 7 μm long, with

rounded posterior end. Bacterial vesicle elliptical, 5-7 μm wide and 10-14 μm long. Postdeirids well visible, situated in one third of body length from tail end under dorsal part of lateral field (looking just as deirids, i.e. circular subcuticular area with ending or duct approaching cuticle surface). Rectum about 1.5 anal body diameter long. Genital primordium 50 μm long. Phasmids at one third of tail length behind anus (phasmid-mucron distance about 25-26 μm), with aperture near

ventral edge of ventral marginal line. Lateral field lines disappearing at level of phasmids. Tail with pronounced dorsal concavity, dorsally curved with terminus pointed to finely rounded. Hyaline portion occupying less than half of tail length.

Morphological diagnosis and relationships with other species. As mentioned above, *Steinernema eapokense* sp. n. differs from the closely related species *S. cumgarensense* sp. n. in D% and H% values of IJs. The E% value helps to distinguish *S. eapokense* sp. n. from *S. siamkayai* (66-80 vs 85-112%). IJs of *S. eapokense* sp. n. (370-434 μ m) are shorter than those of *S. carpocapsae* (438-650 μ m), *S. scapterisci* (517-609 μ m) and *S. sasonense* sp. n. (437-516 μ m). Spicules of *S. eapokense* sp. n. are shorter than *S. backanense* sp. n. (63-74 vs 47-62 μ m).

Type locality. Type isolate of this new species was collected in Eapok, Daklak province (longitude 106°04'E, latitude 12°48'N, altitude 600 m above sea level) from the basalt red soils of coffee plantations on the submontane plateau. Steinernematids of this species were also recorded from a similar habitat near Krongana, Daklak province. Both localities are situated at 600 m altitude.

Etymology. *Steinernema eapokense* sp. n. is named after the type locality.

Deposition of type material. Holotype male and six paratype males are deposited at Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 119071, Russia. Slides with paratype males, first generation females and infective juveniles are deposited in Department of Nematology, Institute of Ecology and Biological Resources, National Center for Science and Technology, Hoang Quoc Viet 18, Nghiado, Caugiay, Hanoi, Vietnam.

Steinernema sasonense sp. n. (Fig. 3; Fig. 5 D)

Measurements. See Table 3.

Male. Body fusiform, obese, 'C'-shaped in heat-killed specimens, tapering toward anterior end. Cuticle annulation 1.2-1.5 μ m wide poorly distinguishable throughout the body. No lateral fields. Anterior extremity rounded with flattened area just around the mouth. Six labial and four prominent 2-3 μ m high cephalic papillae. Cheilostom cuticle connected to a thick strongly cuticularised 2.5 μ m long ring of 6-6.3 μ m diameter, forming the middle part of stoma walls. Additional thinner cuticular ring situated behind thicker one, being embedded into pharynx tissue. Pharynx corpus cylindrical, without visible swelling

around stoma, metacorporeal swelling up to 18-19 μ m with corpus diameter 16 μ m. Basal bulb subspherical. Nerve ring situated on anterior slope of basal bulb in most males. Valves in shape of platelets of thin folded cuticle. Cardium with vacuoles, protruding for 8 μ m into intestine proventriculus. Deirids as thin channel situated behind level of pharynx-intestine junction. Excretory channel strongly cuticularised in 10 μ m from the pore. Intestinal lumen wide in 150-200 μ m from bulb, then only narrow meandering lumen up to 130-160 μ m long posterior to ventricle. Separate spermatocytes disappearing soon in flexed testis part. 'Vas deferens' walls vacuolated. Characteristic steinernematid set of genital papillae with a row of usually 9 pairs of subventral papillae extending from tail terminus to body middle, one unpaired large precloacal papilla (not so protruding in this species as other ones described here) in midventral position, one pair of papillae in lateral position at manubrium level when spicules retracted, and one pair of papillae in dorso-lateral position in postcloacal area. This latter pair and third from tail subventral pair close to equatorial part of tail. A pair of postdeirids with pointed subcuticular ending situated dorsolaterally on level of anteriormost pair of precloacal papillae. Spicules with elongated, funnel-shaped manubrium, tapering toward distal end, with round extremity and more or less pronounced terminal duct. No bifurcation of outer line of velum and ventral rib of spicule observed near rostrum. All four main 'ribs' of spicule discernible, but ventralmost one tightly adpressed to inner one. Spicule body gradually narrowing from rostrum to distal end. Cloacal opening is covered with flap when spicules retracted. Two papillae-like structures on the cloacal opening edges. Distal half of velum edge is strongly cuticularised. Gubernaculum usually straight with slightly curved proximal end and well visible 10-12 μ m long hole. Tail mucron as 2 μ m short round spike.

Female. Cuticle annulation poorly discernible. Cephalic end with 6 labial and 4 cephalic papillae. Cuticular ring forming main part of stoma walls 5-6 μ m thick and 7-7.5 μ m long. Stomatal cavity 12 μ m wide. Excretory duct strongly cuticularised, entering body at nearly angle about 45°. Equatorial vulva with petal-like 12-14 μ m long structures protruding from its opening. Vulva lips less than 10 μ m high, equal in size. Tail end dome-shaped, with 12-14 long spike-like mucron.

Third-stage infective juvenile. Body of heat-killed juveniles quite obese, moderately 'C'-shaped, tapering toward anterior end. Anterior end

Table 3. Morphometrics (in μm) of *Steinernema sasonense* sp. n. Mean \pm SD (range).

Characters ¹⁾	Holotype male	Sason, Sathay, Kontum			Thachnham, Mangla, Kontum		
		1st generation ♂♂ (Paratypes)	1st generation ♀♀	Infective juveniles	1st generation ♂♂	1st generation ♀♀	Infective juveniles
n		15	15	25	15	15	25
L	1365	1344 \pm 59 (1245-1440)	4417 \pm 343 (3930-5070)	471 \pm 15 (437-502)	1237 \pm 86 (1110-1380)	3214 \pm 373 (2370-3915)	486 \pm 16 (456-516)
Body diam.	120	113 \pm 8 (105-120)	224 \pm 18 (195-240)	21 \pm 1 (20-22)	102 \pm 13 (90-135)	171 \pm 25 (150-255)	22 \pm 1 (19-23)
Stoma length	3	3.5 \pm 0.7 (3-4.5)	8.2 \pm 1 (6-11)	—	3.4 \pm 0.7 (3-4.5)	5.6 \pm 1 (4.5-7.5)	—
Stoma width	6	6.3 \pm 0.8 (4.5-7.5)	11 \pm 1 (9-14)	—	5.7 \pm 0.8 (4.5-7.5)	8.7 \pm 1 (7.5-11)	—
Excretory pore	65	58 \pm 4 (53-65)	85 \pm 14 (50-105)	30 \pm 1 (27-34)	55 \pm 4 (45-60)	66 \pm 12 (48-86)	32 \pm 1 (30-34)
Nerve ring	102	89 \pm 7 (80-102)	135 \pm 11 (111-155)	68 \pm 2 (65-72)	83 \pm 7 (74-96)	127 \pm 11 (105-143)	69 \pm 2 (65-72)
Pharynx	149	145 \pm 5 (134-152)	227 \pm 19 (205-248)	100 \pm 3 (94-104)	133 \pm 5 (123-144)	199 \pm 14 (183-221)	100 \pm 2 (96-104)
Testis flexure	360	355 \pm 52 (225-420)	—	—	338 \pm 39 (275-405)	—	—
Tai length	17	18 \pm 2 (15-21)	29 \pm 5 (21-36)	46 \pm 2 (43-49)	16 \pm 2 (14-20)	27 \pm 5 (20-39)	47 \pm 2 (42-52)
H%	—	—	—	42 \pm 3 (35-50)	—	—	43 \pm 3 (38-48)
Anal body diam.	30	29 \pm 2 (26-32)	62 \pm 6 (53-71)	11 \pm 0.4 (10-11)	28 \pm 2 (23-30)	61 \pm 7 (50-71)	11 \pm 0.5 (10-13)
Spicule length	71	71 \pm 3 (66-78)	—	—	63 \pm 4 (54-68)	—	—
Spicule width	12	12 \pm 1 (11-14)	—	—	11 \pm 1 (9-14)	—	—
Gubernaculum length	54	53 \pm 2 (50-56)	—	—	47 \pm 3 (42-51)	—	—
Gubernaculum width	6	6.3 \pm 0.8 (4.5-7.5)	—	—	5.8 \pm 0.5 (5-7)	—	—
V%	—	—	54 \pm 1 (52-56)	—	—	55 \pm 1 (53-57)	—
a	11	12 \pm 1 (11-13)	20 \pm 2 (17-25)	22 \pm 1 (21-24)	12 \pm 1 (10-14)	20 \pm 2 (17-24)	22 \pm 1 (21-24)
b	9	9.3 \pm 0.4 (8.4-9.9)	20 \pm 2 (17-22)	4.7 \pm 0.2 (4.4-5.0)	9.3 \pm 0.6 (8.3-10)	16 \pm 2 (13-21)	4.8 \pm 0.2 (4.6-5.1)
c	83	77 \pm 8 (64-94)	213 \pm 47 (146-327)	10 \pm 0.3 (9.7-11)	78 \pm 12 (62-102)	157 \pm 35 (105-221)	10 \pm 0.4 (9.4-11)
D%	43	40 \pm 3 (37-45)	37 \pm 6 (22-48)	30 \pm 1 (28-33)	41 \pm 3 (34-46)	33 \pm 6 (25-44)	32 \pm 1 (30-34)
E%	—	—	—	66 \pm 4 (59-74)	—	—	69 \pm 3 (61-76)
SW	2.35	2.5 \pm 0.2 (2.19-3.06)	—	—	2.28 \pm 0.2 (2-2.67)	—	—
GS	0.77	0.75 \pm 0.04 (0.69-0.82)	—	—	0.75 \pm 0.04 (0.67-0.86)	—	—
Mucron	2.25	2.7 \pm 0.5 (1.5-3)	—	—	2.3 \pm 0.7 (1.5-3)	—	—

¹⁾ D% = distance from anterior end to excretory pore/pharynx length \times 100; E% = distance from anterior end to excretory pore/tail length \times 100; SW = spicule length/anal body diam.; GS = gubernaculum length/spicule length.

is hemispherical with 4 protruding cephalic papillae. The lining of the buccal cavity forms a protruding plug with triradiate lumen. Cuticular annulation about 1 μm wide visible throughout body. Lateral field in anterior body half with 4 ridges in the centre, two pronounced marginal ridges and poorly discernible submarginal lines. Such regular pattern not observed in body

posterior half, with ventral gap between central and marginal ridges disappearing. Lateral field 8-9 μm wide at mid-body. Frontal surface of anterior end always regularly round. Labial papillae indistinct, only four cephalic sensilla poorly visible. Pharynx corpus slender, slightly widening to metacorporeal part. Nerve ring slightly behind corpus-isthmus junction. Hemizonid on isthmus

level. Basal bulb with poorly visible valves. Cardia about 10 μm long, cylindrical, slightly narrowing to posterior end. Bacterial vesicle round or nearly round, with length about 5-8 μm long, loosely filled with 2-4 μm long rod-like bacterial cells. Rectum about 2 anal body diameter long. Genital primordium 60 μm long. Phasmids at one quarter of tail length behind anus (phasmid-mucron distance about 35-36 μm), with aperture near ventral edge of ventral marginal line. Lateral field lines disappearing at level of phasmids. Tail with slightly pronounced ventral concavity or without it, conical, with some irregularities in shape, terminus sharply pointed. Hyaline portion occupying about half of tail length.

Morphological diagnosis and relationships with other species. Nematodes of this species can be distinguished from *S. scapterisci* by the IJ length (437-502 vs 517-609 μm), from *S. cumgarensis* sp. n. and *S. eapokense* sp. n. by longer IJs. Pharynx is shorter in *S. sasonense* sp. n. IJs than in *S. carpocapsae* (94-104 vs 103-190 μm); D% value differs in these two species, 28-33% in *S. sasonense* sp. n. against 23-28% in *S. carpocapsae* (Hominick *et al.*, 1997).

Type locality. Specimens of this new species were isolated on the Southern slope of the Chumomray mountain near Sason, Sathay in the Kontum province (longitude 107°43', latitude 14°27', altitude 700 m above sea level). Soils were humi, fernalie acrisols of primary evergreen tropical broad-leaved forest growing on the slopes of granite mountains. This species was also found in primary closed evergreen tropical broad-leaved forest mixed with some conifers. This habitat was situated on the slope of a sandstone and gneiss mountain in Thachnham (Mangla) near Konplong, Kontum province (longitude 108°25', latitude 14°38', altitude 1210 m above sea level). Soils also were humi, fernalie acrisols.

Etymology. *Steinernema sasonense* sp. n. is named after the type locality.

Deposition of type material. Holotype male and six paratype males are deposited at Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 119071, Russia. Slides with paratype males, first generation females and infective juveniles deposited in Department of Nematology, Institute of Ecology and Biological Resources, National Center for Science and Technology, Hoang Quoc Viet 18, Nghiado, Caugiay, Hanoi, Vietnam.

Steinernema backanense sp. n. (Fig. 4; Fig. 5 E)

Measurements. See Table 4.

Male. Body fusiform, obese, 'C'- or broadly 'J'-shaped in heat-killed specimens, tapering toward anterior end. Cuticle annulation 1.3-1.5 μm wide poorly distinguishable throughout the body. Anterior extremity rounded. Six labial spike-like papillae and four more protruding cephalic ones present. Cheilostom cuticle connected to a thick strongly cuticularised 2 μm long ring of 5 μm diameter, forming the middle part of stoma walls. Additional thinner cuticular ring situated behind thicker one, being embedded into pharynx tissue. Pharynx corpus cylindrical, without visible collar around stoma, but with prominent metacorporeal swelling (mid-corpus diameter about 16 μm , metacorporeal diameter about 19-20 μm). Basal bulb subspherical. Nerve ring situated on anterior slope of basal bulb in most males. Cardium very flat, only 5 μm long. Deirids of 2 μm diameter situated behind level of pharynx-intestine junction. Excretory channel strongly cuticularised in 4-5 μm from the pore. Intestine with wide lumen up to mid-body but with only narrow meandering lumen up to rectum. Separate spermatocytes visible up to flexure, being replaced with complex rachis structure in growth zone. 'Vas deferens' walls not vacuolised, but with spherical inclusions of glandular appearance. Characteristic steinernematid set of genital papillae with a row of usually 9 pairs of subventral papillae extending from tail terminus to body middle, one unpaired large precloacal papilla in mid-ventral position, one pair of papillae in lateral position at manubrium level when spicules retracted, and one subterminal pair of papillae in dorso-lateral position in postcloacal area. Third from tail tip pair of subventral papillae near equatorial part of tail. A pair of postdeirids situated sublaterally anteriorly to first pair of precloacal papillae. Spicules with elongated, funnel-shaped manubrium, tapering toward distal end, with round extremity and more or less pronounced terminal duct. No bifurcation of outer line of velum and ventral rib of spicule observed near rostrum. All four main 'ribs' of spicule discernible, but ventralmost one tightly adpressed to inner one. Spicule body gradually narrowing from rostrum to distal end. Cloacal opening is covered with flap when spicules retracted. Two papillae-like structures on the cloacal opening edges. Distal half of velum edge is strongly cuticularised. Gubernaculum usually straight with ventrally curved proximal end, often with cuticular knobs on inner surface and well visible 10-12 μm long hole. Tail mucron spike-like.

Female. Cuticle annulation 1.5 μm wide, discernible only near vulva lips. Cephalic end with

Table 4. Morphometrics (in μm) of *Steinernema backanense* sp. n. Mean \pm SD (range).

Characters ¹⁾	Holotype male	Lungchu, Babe, Backan			Thacbac, Tamdao, Vinhphuc		
		1st generation $\sigma\sigma$ (Paratypes)	1st generation ♀♀	Infective juveniles	1st generation $\sigma\sigma$	1st generation ♀♀	Infective juveniles
n		15	15	25	15	15	25
L	1125	1138 \pm 93 (1005-1320)	3184 \pm 363 (2610-3990)	470 \pm 28 (420-557)	1412 \pm 70 (1275-1530)	4484 \pm 596 (3330-5460)	438 \pm 20 (391-473)
Body diam.	105	101 \pm 14 (75-135)	168 \pm 14 (150-195)	20 \pm 1 (19-22)	129 \pm 18 (90-150)	229 \pm 24 (195-270)	20 \pm 1 (18-22)
Stoma length	5	4,3 \pm 0,5 (3-4,5)	6,8 \pm 1 (4,5-9)	—	3,5 \pm 0,7 (3-4,5)	6,8 \pm 1 (4,5-7,5)	—
Stoma width	6	6 \pm 1 (4,5-7,5)	9,8 \pm 0,8 (9-11)	—	6,3 \pm 0,7 (6-7,5)	10 \pm 1 (9-12)	—
Excretory pore	51	56 \pm 5 (45-63)	74 \pm 11 (60-99)	32 \pm 2 (29-38)	58 \pm 6 (48-69)	82 \pm 11 (63-104)	31 \pm 1 (28-33)
Nerve ring	81	91 \pm 5 (81-99)	135 \pm 17 (105-168)	66 \pm 4 (58-75)	86 \pm 5 (77-93)	132 \pm 10 (116-149)	62 \pm 2 (58-65)
Pharynx	137	138 \pm 7 (123-150)	203 \pm 19 (164-236)	95 \pm 5 (90-115)	139 \pm 5 (131-150)	225 \pm 11 (206-248)	91 \pm 3 (87-97)
Testis flexure	284	256 \pm 45 (156-330)	—	—	342 \pm 55 (183-399)	—	—
Tai length	17	19 \pm 2 (15-21)	28 \pm 3 (24-33)	42 \pm 3 (36-52)	18 \pm 2 (15-21)	26 \pm 6 (15-38)	42 \pm 2 (39-46)
H%	—	—	—	44 \pm 2 (40-48)	—	—	42 \pm 2 (37-46)
Anal body diam.	26	26 \pm 2 (24-29)	65 \pm 5 (59-72)	11 \pm 1 (10-12)	29 \pm 3 (24-35)	66 \pm 9 (54-81)	11 \pm 1 (10-11)
Spicule length	59	58 \pm 3 (53-62)	—	—	58 \pm 2 (54-62)	—	—
Spicule width	11	10 \pm 1 (9-12)	—	—	10 \pm 1 (9-12)	—	—
Gubernaculum length	47	43 \pm 3 (36-48)	—	—	46 \pm 2 (44-51)	—	—
Gubernaculum width	8	6 \pm 1 (4,5-7,5)	—	—	6 \pm 0,8 (4,5-7,5)	—	—
V%	—	—	57 \pm 1 (54-59)	—	53 \pm 1 (51-54)	—	—
a	11	11 \pm 1 (9,8-15)	19 \pm 2 (16-21)	23 \pm 1 (22-26)	11 \pm 2 (9,2-15)	20 \pm 3 (13-24)	22 \pm 1 (20-27)
b	8	8,2 \pm 0,6 (7,2-9,4)	16 \pm 1 (14-19)	4,9 \pm 0,2 (4,5-5,4)	10 \pm 0,5 (9,4-11,3)	20 \pm 2 (15-23)	4,8 \pm 0,2 (4,5-5,2)
c	58	61 \pm 8 (51-74)	152 \pm 25 (114-194)	11 \pm 0,4 (10-12)	79 \pm 8 (65-92)	241 \pm 53 (156-332)	10 \pm 0,4 (9,8-11)
D%	41	41 \pm 5 (30-48)	37 \pm 4 (30-43)	33 \pm 1 (29-35)	42 \pm 5 (35-52)	36 \pm 5 (29-46)	34 \pm 2 (30-37)
E%	—	—	—	77 \pm 5 (66-87)	—	—	73 \pm 4 (64-83)
SW	2.05	2.19 \pm 0.18 (1.84-2.56)	—	—	2.05 \pm 0.23 (1.68-2.41)	—	—
GS	0.79	0.74 \pm 0.05 (0.66-0.79)	—	—	0.8 \pm 0.03 (0.73-0.84)	—	—
Mucron	1.5	2 \pm 0,7 (1,5-3)	—	—	2,4 \pm 0,6 (1,5-3)	—	—

¹⁾ D% = distance from anterior end to excretory pore/pharynx length \times 100; E% = distance from anterior end to excretory pore/tail length \times 100; SW = spicule length/anal body diam.; GS = gubernaculum length/spicule length.

6 labial and 4 cephalic papillae. Cuticular ring forming main part of stoma walls 5-6 μm thick and 2.5-3 μm long. Stomatal cavity 10 μm wide. Excretory duct strongly cuticularised, entering body at angle less than 30°. Equatorial vulva without discernible epyptygma in some specimens, and often with unequal vulva lips protruding up to 30-

35 μm . Tail end subconical, with spike-like 7-9 μm long mucron.

Third-stage infective juvenile. Body of heat-killed juveniles quite obese, slightly 'C'-shaped, tapering toward anterior end. Cuticular annulation about 1 μm wide visible throughout body. Lateral field in anterior body half with 4 ridges in the

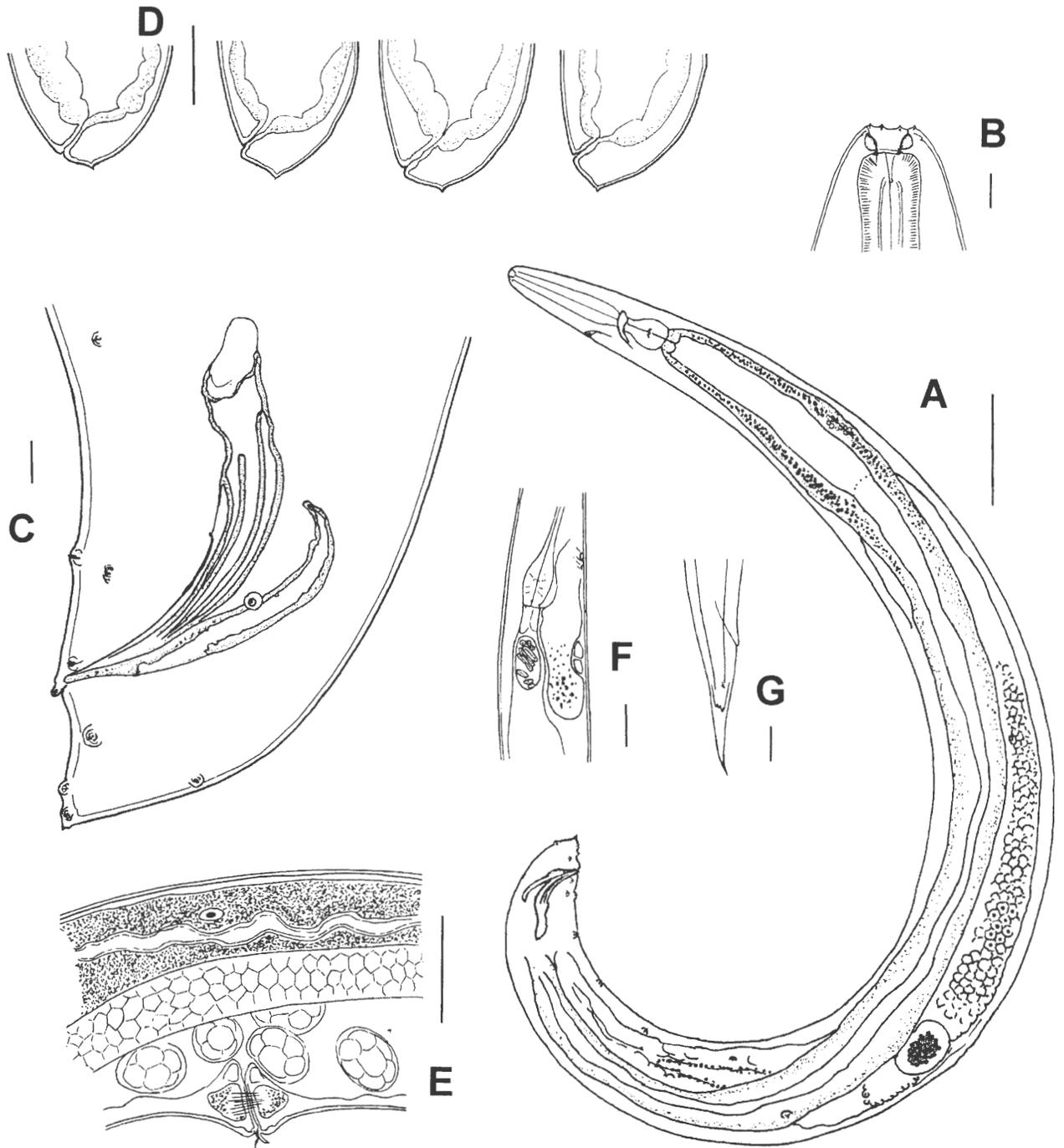


Fig. 1. *Steinernema cumgarensis* sp. n. A: first generation male, total view; B: male, anterior end; C: male tail; D: first generation female tail shapes; E: female, vulvar region; F: pharyngeal region of infective juvenile; G: tail of infective juvenile (all in lateral view), scales A, E – 100 μ m, B, C, F, G – 10 μ m, D – 50 μ m).

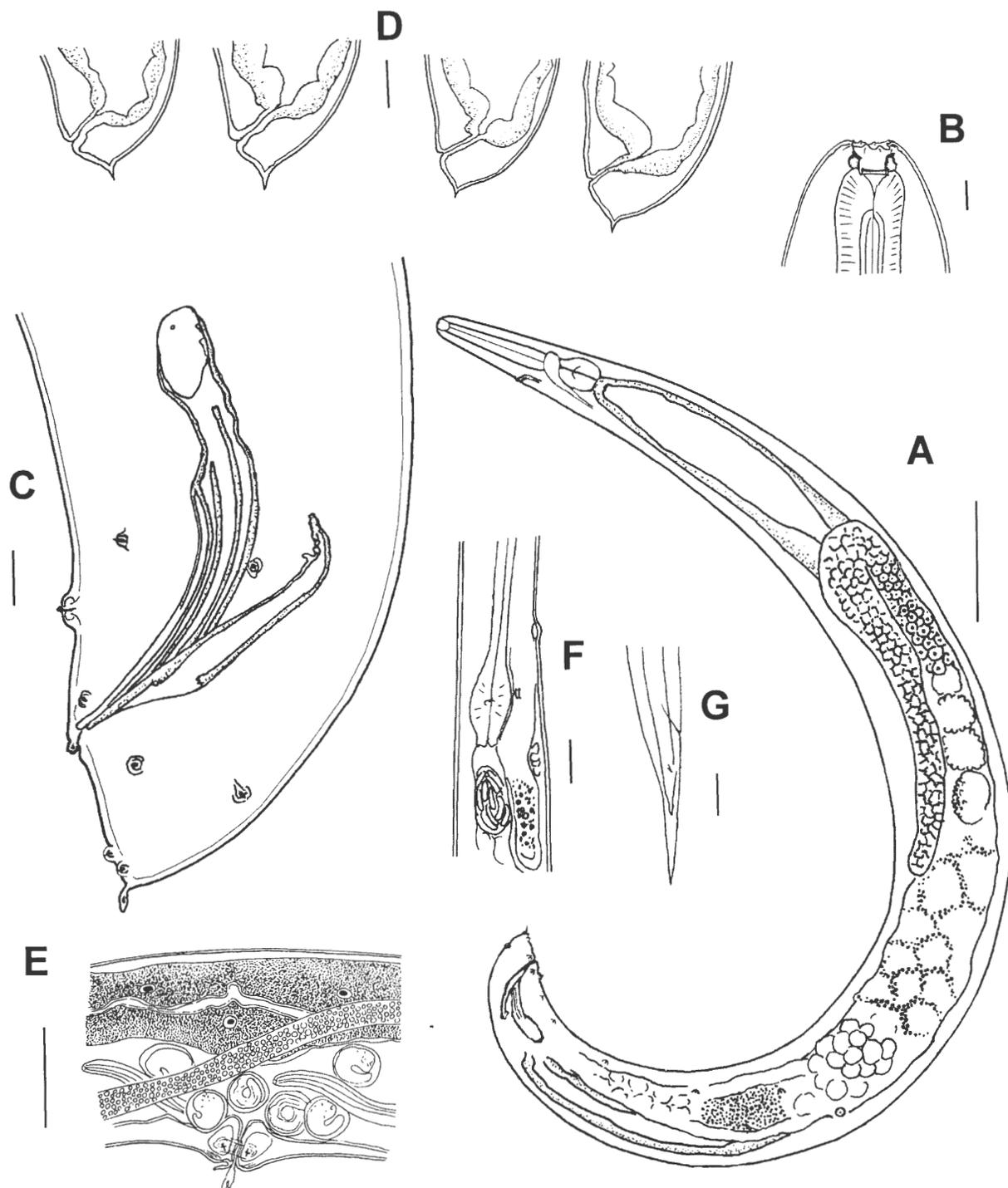


Fig. 2. *Steinernema eapokense* sp. n. A: first generation male, total view; B: male, anterior end; C: male tail; D: first generation female tail shapes; E: female, vulvar region; F: pharyngeal region of infective juvenile; G: tail of infective juvenile (all in lateral view), scales A, E – 100 μ m, B, C, F, G – 10 μ m, D – 50 μ m).

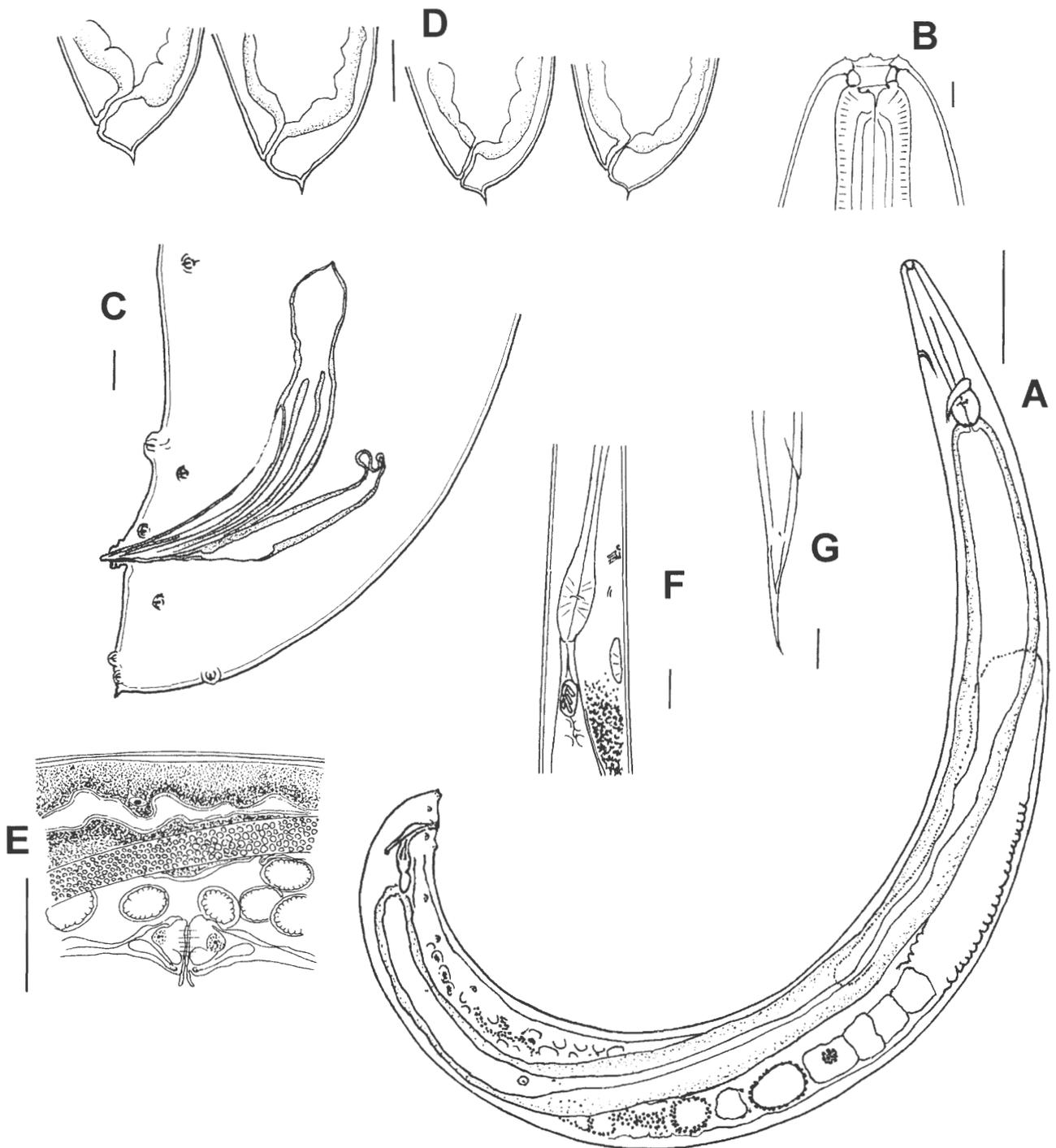


Fig. 3. *Steinernema sasonense* sp. n. A: first generation male, total view; B: male, anterior end; C: male tail; D: first generation female tail shapes; E: female, vulvar region; F: pharyngeal region of infective juvenile; G: tail of infective juvenile (all in lateral view), scales A, E – 100 μm, B, C, F, G – 10 μm, D – 50 μm).

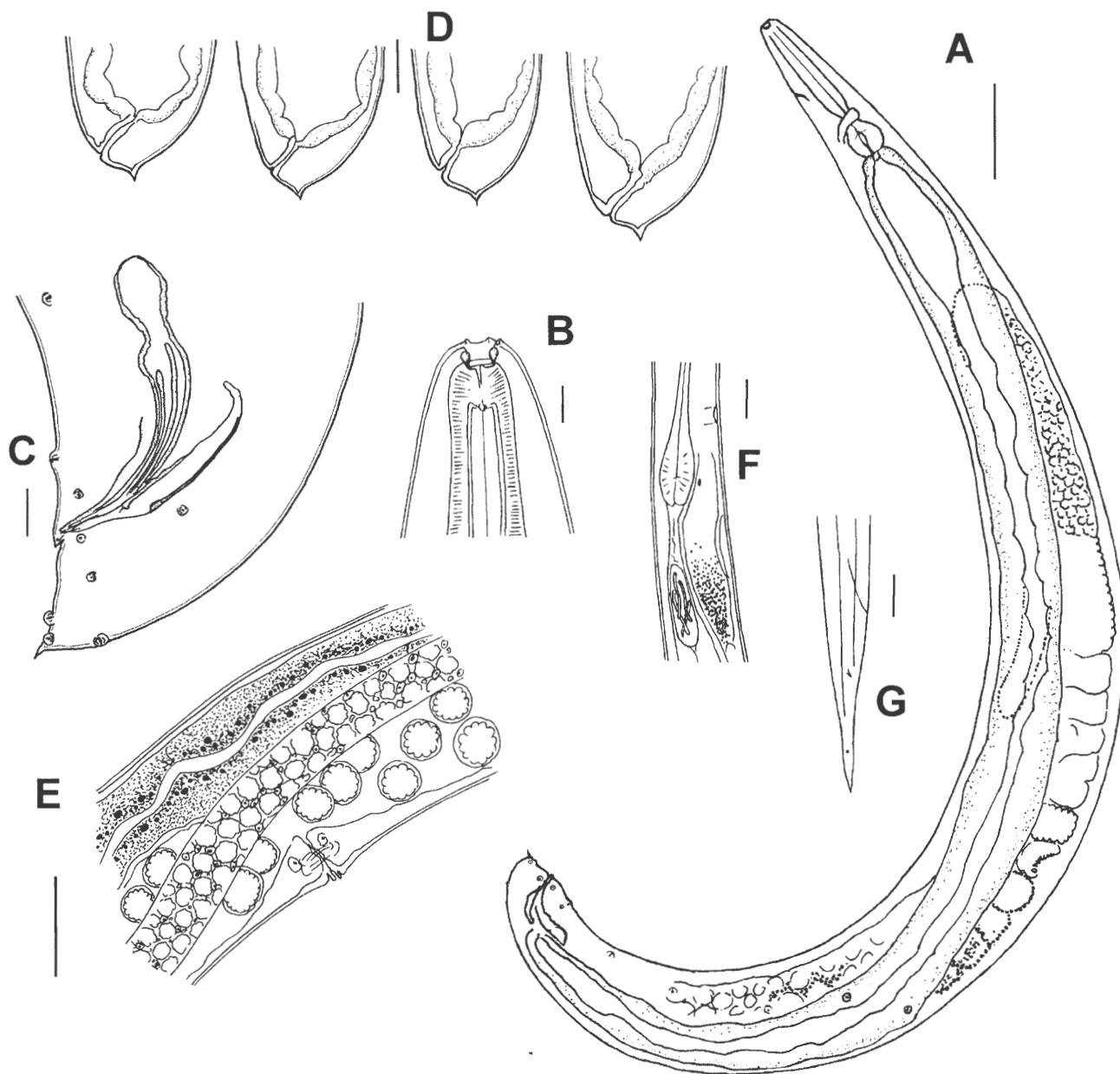


Fig. 4. *Steinernema backanense* sp. n A: first generation male, total view; B: male, anterior end; C: male tail; D: first generation female tail shapes; E: female, vulvar region; F: pharyngeal region of infective juvenile; G: tail of infective juvenile (all in lateral view), scales A, E – 100 μ m, B, C, F, G – 10 μ m, D – 50 μ m).

centre, two pronounced marginal ridges and poorly discernible submarginal lines. Such regular pattern not observed in body posterior half, with ventral gap between central and marginal ridges disappearing. Lateral field 5-6 μm wide at mid-body. Frontal surface of anterior end always regularly round. Labial papillae indistinct, four cephalic sensilla and amphidial pouches occasionally visible. Pharynx corpus slender, only slightly widening to metacorporeal part. Nerve ring slightly behind corpus-isthmus junction. Hemizonid on isthmus level, flattened. Round deirid body beneath lateral field on hemizonid level. Basal bulb with poorly visible valves. Cardia about 5 μm long, conical, narrowing to posterior end. Bacterial vesicle elliptical with poorly visible walls, about 12 μm long, loosely filled with 10 μm long curved bacterial cells. Rectum about two anal body diameter long. Genital primordium 50 μm long. Phasmids at one third of tail length behind anus (phasmid-mucron distance about 30 μm), with aperture near ventral edge of ventral marginal line. Lateral field lines disappearing at level of phasmids. Tail with slightly pronounced ventral concavity, regularly conical, without prominent ventral curvature, with terminus sharply pointed. Hyaline portion occupying less than half of tail length.

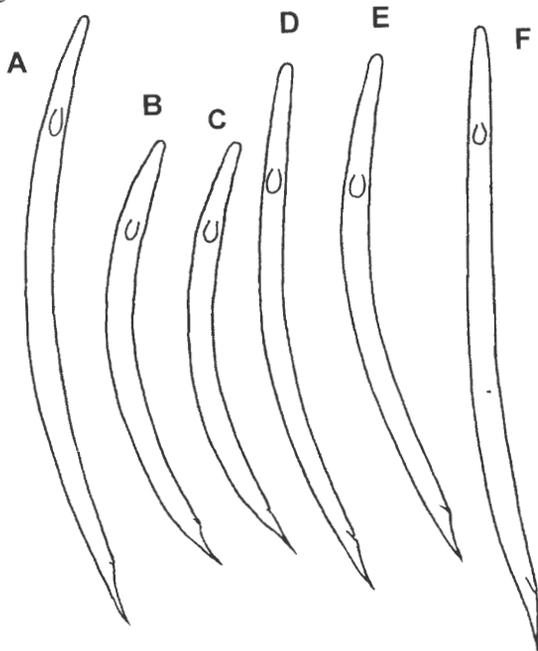


Fig. 5. Body shape and comparative size of IJs in steinernematid species of 'carpocapsae' group. A: *S. tami*, type culture; B: *S. cumgarensis* sp. n.; C: *S. eapokense* sp. n.; D: *S. sasonense* sp. n.; E: *S. backanense* sp. n.; F: *S. carpocapsae*, 'Pieridarum', scale 100 μm .

Morphological diagnosis and relationships with other species. Nematodes of this species can be distinguished from all three other species described in this contribution, and also from *S. scapterisci*, *S. siamkayai* and *S. tami*, by its short spicules 56 (47-62 μm). Spicules shorter than 63 μm were sometimes reported only for *S. carpocapsae* with a reported range of 58-77 μm (mean 66 μm), but *S. backanense* sp. n. differs from this latter species in D% value of IJs (29-35 vs 23-28%).

Type locality. Nematodes of the new species were collected in Lungchu (Babe lake) in Backan province (longitude 105°34'E, latitude 22°26'N, altitude 700 m above sea level) from lixisols soil of the primary closed evergreen tropical seasonal forest. This habitat of broad-leaved forest was situated on the slope of limestone mountain. Another finding for this species is on the banks of the Thacbac stream in the Tamdao mountains of Vinhphuc province (longitude 105°35'E, latitude 21°27', altitude 900 m above sea level). The habitat was primary closed evergreen tropical broad-leaved forest on slopes of rhyolite mountains.

Etymology. *Steinernema backanense* sp. n. is named after the type locality.

Deposition of type material. Holotype male and nine paratype males are deposited at Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow, 119071, Russia. Slides with paratype males, first generation females and infective juveniles deposited in Department of Nematology, Institute of Ecology and Biological Resources, National Center for Science and Technology, Hoang Quoc Viet 18, Nghiado, Cauaiay, Hanoi, Vietnam.

MOLECULAR DIFFERENTIATION AND PHYLOGENETIC RELATIONSHIPS

The DNA fragment including the ITS-5.8S-ITS2 region of rRNA for the new species ranged in length from 723 (*S. eapokense* sp. n.) to 742 bp (*S. sasonense* sp. n.). Alignment used for phylogenetic analyses included 779 positions. Strict consensus of twelve maximum parsimonious tree and maximum likelihood trees showed the same general topology and are presented in Fig. 6. South American *S. scapterisci* occupied a basal position. The relationships of two of the species, *S. sasonense* sp. n. and *S. backanense* sp. n. with *S. carpocapsae*, and with a clade including other four species from Asia are not resolved. The two new species, *S. cumgarensis* sp. n. and *S. eapokense* sp. n. formed a highly supported clade with the tropical species *S.*

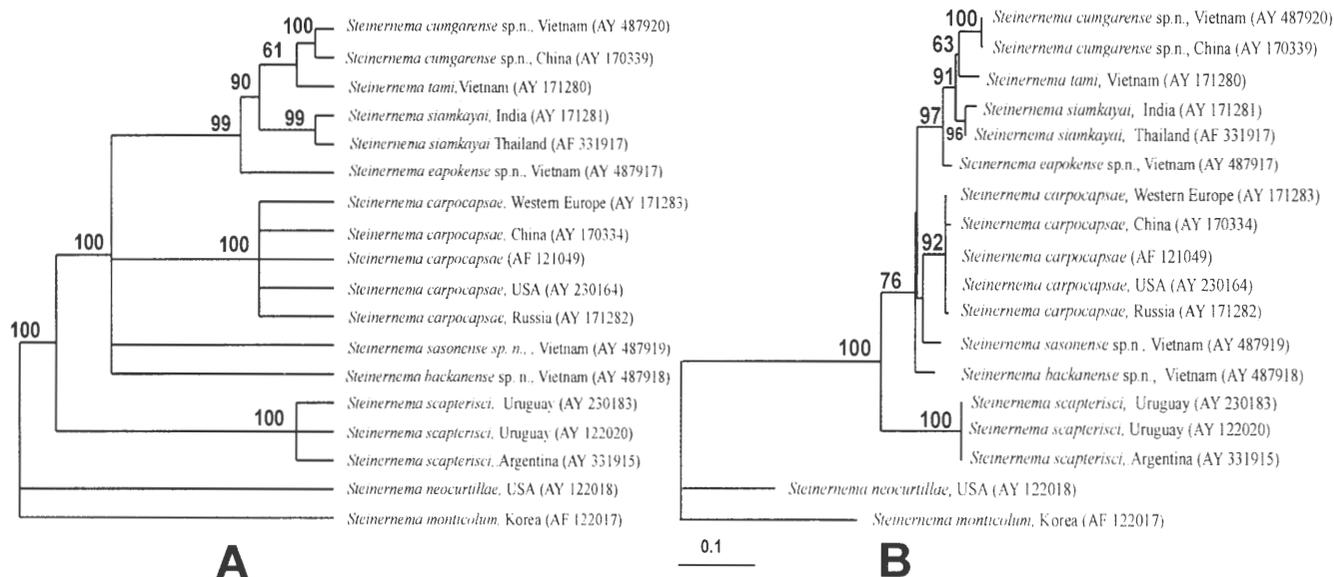


Fig. 6. A: strict consensus of twelve most parsimonious trees obtained from parsimony analysis of the ITS sequence alignment with 18 *Steinernema* species (tree length = 504, CI excluding uninformative character = 0.7662; HI excluding uninformative characters = 0.2338; RI = 0.8676; RC = 0.7368); B: A maximum likelihood tree resulting from the analysis of analysis of the ITS sequence alignment with 18 *Steinernema* species under TVM+G model. Bootstrap values exceeding 50% are given on appropriate clades.

siamkayai and *S. tami* (Stock *et al.*, 1998; Spiridonov *et al.*, 2004). The pairwise divergence between the 16 species of ‘*carpocapsae-scateriscitami*’ group ranged from 3.5 to 14.4% (63–102 nucleotides). The inter-specific variability of *S. carpocapsae* sequences ranged from 0 to 1.1%, and between two populations of *Steinernema cumgarensis* sp. n was 1.2% (Table 5). Spiridonov *et al.* (2004) analyzing ITS *Steinernema* sequences found that the greatest level of inter-specific variation was 2.8%. The sequences for species described here are different from each other and from those of known steinernematid species by more than 3.4% variation. Thus, the presence of autapomorphies and the substantial sequence differences of the ITS-rRNA gene region support the independence of each of the new species.

DISCUSSION

The discovery of several new isolates of steinernematids with short IJs in South East Asia could be predicted as this group of the genus *Steinernema* is common in tropical habitats. In fact, two species of this group were already described from the area (*S. siamkayai* and *S. tami*). This diversity of steinernematids in the Asian tropics is making the problem of species differentiation more complicated.

Obviously, several morphological features (body

size and shape of IJs, spicular morphology) of the steinernematids described here are of diagnostic value and help to differentiate some of the species. However, the molecular data enabled the definite establishment of the new species. The high level of nucleotide differences in ITS1–5.8S–ITS2 rDNA was observed between these four species found in Vietnam, and was considered as a main evidence of species independence. Nevertheless, the absence of morphological differences is an alarming trend, leading steinernematid taxonomy to the situation in which clusters of sibling, or presumably sibling species, could be differentiated only through sequence analysis. Sometimes such a situation is considered as normal and inevitable. In the recent review by De Ley and Blaxter (2002), for example, the authors stated that “morphology does not suffice, even for morphospecies”. Adams (1998) argued that in nematology the species concept is still very unstable, and the procedures proposed to discover and characterise species are not satisfactory.

Even though molecular methods will play an increasing role in steinernematid taxonomy, we still hope that further morphological studies can reveal additional features to characterise or at least separate groups of species inside the genus. We made an attempt to seek for morphological features distinguishing between newly found and

previously described species of the 'carpocapsae-scaterisci-tami' group. Gravid females usually have few characters that are useful for species separation in steinernematids. In the four newly described species, the only difference between females is the tail, with tails of *S. cumgarensis* sp. n. being dome-shaped with minute peg-like mucron (Fig. 3 C), whereas the three remaining species have a more or less pronounced conical tail mucron (Fig. 3 A, B, D). Such differences can be revealed only after the examination of a statistically representative sample. Moreover, the female tail shape can be affected by the fixation and mounting procedure. The differences in IJ body posture and size are more stable (Fig. 3 E-J); juveniles of *S. cumgarensis* sp. n. and *S. eapokense* sp. n. are the shortest ones with pronounced 'C'-shaped curvature of the longitudinal axis (Fig. 3 F-G). Additional differences were discovered in the morphology of IJs. The tail ends of juveniles in the 'carpocapsae' group are usually ventrally concave; dorsal concavity, however, is visible in the majority of IJs of *S. eapokense* sp. n. (Fig. 4 J). Also the hemizonid is protruding from the ventral side in the majority of *S. eapokense* sp. n. IJs (Fig. 4 D). The bacterial vesicle is the smallest in *S. sasonense* sp. n. (4-8 µm), and larger than 8-12 µm in the three other new species from Vietnam, also in *S. carpocapsae* and *S. tami*. The vesicles of *S. cumgarensis* sp. n. and *S. eapokense* sp. n. are mainly elliptical in shape with a maximum length of about 1.5 times its diameter. The vesicles in *S. backanense* sp. n. are more elongated, with a length to width ratio of approximately 3. Additional morphological studies are needed to obtain similar data for the other species of the 'carpocapsae-scaterisci-tami' group, before these criteria can be considered as valid. The independence of some nominal species of this group should be questioned. For example, *S. asiaticum* (Anis *et al.*, 2002) was considered as conspecific with an undescribed isolate from Sri Lanka (Amarasinghe *et al.*, 1994) and was separated from other species of the group by a set of morphological and RFLP differences. An analysis of *S. siamkayai* sequence (AF331917) with WebCutter® 2.0, as well as comparison of RFLP profiles for this species provided by Pham *et al.* (2000) revealed that, for the majority of endonucleases, the count of characteristic restriction sites was the same as obtained on a gel with PCR product from *Steinernema* sp. from India (Hussaini *et al.*, 2001), identified here as *S. siamkayai* (AY171281), and from *S. asiaticum* (Anis *et al.*, 2002). It seems that the specific

independence of *S. asiaticum* from *S. siamkayai* still needs additional support.

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Phan K.L., Spiridonov S.E., Subbotin S.A., Moens M. Четыре новых вида *Steinernema* Travassos, 1928 с короткими инвазионными личинками из Вьетнама.

Резюме. Дано описание четырех новых видов штейнернематид, длина инвазионных личинок у которых менее 600 μm . Латеральные поля инвазионных личинок всех 4-х видов с четырьмя центральными ребрами и хорошо выраженными маргинальными ребрами (по крайней мере в передней части тела). Рожковидные выступы на головном конце личинок отсутствуют, что позволяет отнести все эти новые виды к группе '*carpocapsae-scapterisci-tami*'. Длина инвазионных личинок *S. cumgarensis* sp. n. близка к таковой у *S. eapokense* sp. n. и *S. siamkayai*, но отличается от *S. sasonense* sp. n., *S. carpocapsae*, *S. scapterisci* and *S. tami*. От близкого *S. eapokense* sp. n. этот вид отличается значением индекса D% и длиной гиалиновой части хвостового конца. У личинок *Steinernema cumgarensis* sp. n. тело толще, чем у *S. siamkayai*. Спикулы и губернакулюм этого вида длиннее, чем у *S. backanense* sp. n. К тому же *S. cumgarensis* sp. n. отличается от *S. sasonense* sp. n. по длине пищевода и хвоста личинок. Значение индекса E% *S. eapokense* sp. n. позволяет отличить их от *S. siamkayai*. Инвазионные личинки *S. eapokense* sp. n. короче, чем у *S. sasonense* sp. n., *S. carpocapsae* и *S. scapterisci*. Спикулы *S. eapokense* sp. n. короче, чем у *S. backanense* sp. n. Самцы *S. backanense* sp. n. отличаются от самцов *S. cumgarensis* sp. n., *S. eapokense* sp. n. и *S. sasonense* sp. n., а также *S. tami*, *S. scapterisci* и *S. siamkayai* своими короткими спикулами. *Steinernema backanense* sp. n. также отличается от *S. carpocapsae* по значению индекса D% у личинок. *Steinernema sasonense* sp. n. отличается от *S. scapterisci*, *S. cumgarensis* sp. n. и *S. eapokense* sp. n. по длине инвазионных личинок. *Steinernema sasonense* sp. n. отличается от *S. carpocapsae* более коротким пищеводом и значением индекса D% личинок. К тому же все четыре новых вида отличаются друг от друга и от других видов группы по характерным последовательностям ITS-rRNA. Представлена новая филограмма взаимоотношений видов группы '*carpocapsae-scapterisci-tami*'.
