# Additional data on *Steinernema cameroonense* Ngo Kanga, Phap Quang Trinh, Wayenberge, Spiridonov, Hauser & Moens, 2012

**Françoise Ngo Kanga[1], Elena S. Ivanova[2], Nadezhda S. Shepeleva[2] and Sergei E. Spiridonov[2]**

[1]International Institute of Tropical Agriculture (IITA) Cameroon, P.O. Box: 2008 Messa, Yaoundé, Cameroon, e-mail: kangafrancoise7@yahoo.fr
[2]Centre of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninskii prospect 33, 119071.

Accepted for publication 20 March 2014

**Summary.** An isolate of *Steinernema cameroonense* Ngo Kanga et al., 2012, which differs from type one in 2 bp of the ITS rDNA region, has been cultured on *Galleria mellonella* since 2011. The body length and pharynx length of the infective juveniles of this isolate differ from those reported for the type isolate (ranges do not overlap). The taxonomically important structures (male and female posterior end, juvenile lateral fields) are described and illustrated. The sequence data are also provided for the symbiotic bacterium of the genus *Xenorhabdus*, isolated from this nematode species. According to the analysis of 16S DNA, *Rec*A and *Ser*C genes the symbionts of *S. cameroonense* differ from all known representatives of the genus but show similarity with *X. miraniensis* from Australia, *X. khoisanae* and an undescribed *Xenorhabus* sp. R001-293 from Africa.

**Key words:** bacterial symbiont, morphology, morphometrics, intraspecific differences, SEM, *Steinernema*, *Xenorhabdus*.

*Steinernema cameroonense* Ngo Kanga, Phap Quang Trinh, Wayenberge, Spiridonov, Hauser & Moens, 2012 was recently described from Western Africa. The description was based on single isolate of this species, but another isolate of this species, provided by Dr Francoise Ngo Kanga in 2010, was kept in the collection of the Centre of Parasitology of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow. Morphological and molecular examination of this second strain of *S. cameroonense* Ngo Kanga et al., 2012 was carried out and showed that the nematodes of this isolate are similar in general morphology and body proportions to those described by Ngo Kanga *et al.* (2012) but differ in some details, which are discussed below.

## MATERIALS AND METHODS

**Cultivation.** The culture of *S. cameroonense* studied here originates from the soil sample collected from a teak tree plantation in the Obala region of the Central province of Cameroon in March 2009. The collector provided the following coordinates of the collection place: N 4°12'49", E 11°35'10". This position is near the road N1 'Obala-Olambe'. Isolated steinernematids were kept in the laboratory of the Centre of Parasitology of the Severtsov Institute of Ecology and Evolution since 2010 and once a year recycled through *Galleria mellonella* using the injection method. Attempts to rear the culture using infection with infective juvenile suspension on filter paper or in the sand did not succeed.

**Morphological studies.** To obtain adult stages of the present isolate of *S. cameroonense*, *Galleria* larvae were injected with 10 infective juveniles (IJ) larva$^{-1}$ using a microsyringe. First and second generation males and females were obtained by dissecting *Galleria* cadaver 3 and 8 days after infection, respectively. Infective juveniles were collected after 6 months storage at 12°C. Measurements and drawings were taken on formalin-fixed nematodes mounted on permanent slides after processing to glycerin according to Seinhorst (1959).

Compound microscopes, Zeiss Jenaval and Nikon Eclipse E200, with drawing attachment were used. Illustrations were finished with WACOM Intuos A4 USB drawing tablet and Adobe Illustrator CS5 following Coleman (2003). For scanning electron microscopy (SEM), material was re-hydrated after formaldehyde fixation, dehydrated in a graded ethanol series, critical-point dried using a HCP-2 HITACHI dryer, mounted on aluminium stubs and coated with gold in a BIO-RAD SC502 sputter coater. Specimens were studied in a JCM-6380 LA SEM.

**Abbreviations**: L, body length; ABD, anal body diameter; Ph, pharynx length; NR, the distance from the anterior extremity to the nerve-ring; EP, distance from anterior end to excretory pore; T, length of tail; Sp (chord), spicule length measured on the chord; Gub, length of gubernaculum; GW, width of gubernaculum; H, length of hyaline region, V%, distance from the anterior extremity to the vulva in relation to body length (%); D%, EP/Ph x 100; E%, EP/T x 100; GS, Gub/Sp; H%, H as % of T; SW, Sp/ABD; a, b, c, c', De Man indices.

**Molecular characterisation.** DNA was obtained from the suspension of living juveniles and adults with Wizard® SV Genomic DNA Purification System columns according to the manufacturer's protocol (Promega Benelux, Leiden, The Netherlands). About 1-1.2 µl of the DNA solution obtained from Wizard® columns, were used as template in a PCR reaction. For amplification of ITS rDNA the following primers were used: 18S (5'-TTG ATT AGG TCC CTG CCC TTT-3') and 26S (5'-TTT CAC TCG CCG TTA CTA AGG-3') (Vrain et

**Table 1.** Comparative morphometrics of infective juveniles of two strains of *Steinernema cameroonense* Ngo Kanga *et al*., 2012. All measurements are in µm and in the form: mean ± standard deviation (range).

| Characters | IJs (present study, 6 months old) n=10 | IJs (original description) n=20 |
|---|---|---|
| Body length (L) | 780 ±24.3 (736-819) | 622 ±61 (490-694) |
| Body diameter (W) | 41 ±2.5 (35-44) | 30 ± 2.8 (24-35) |
| pharynx length (Ph) | 141.5 ±4.9 (135-150) | 113 ± 5.6 (105-125) |
| nerve ring (NR) | 104.2 ± 5.3 (96-110) | 85 ± 8.3 (69-100) |
| excretory pore (EP) | 64.1 ± 2 (62-68) | 54 ± 4.9 (45-64) |
| tail length (T) | 77.8 ± 8.2 (66-95) | 76 ±18.7 (52-107) |
| hyalin portion (H) | 42.7 ± 4.1 (36-51) | 31 ± 4.8 (22-38) |
| H% (H/T) × 100 | 55.2 ± 0.1 (44.2-65.3) | 50 ± 4.7 (40-59) |
| D% (EP/Ph) × 100 | 45.4 ± 2.3 (42-49.6) | 48 ± 4 (42-56) |
| E% (EP/T) × 100 | 83 ± 7 (70.5-93.3) | 75 ± 18.3 (48-116) |
| a (L/W) | 18. 9 ± 1.6 (17.5-23.1) | 21 ± 1.9 (17-25) |
| b (L/Ph) | 5.5 ± 0.2 (5.2-6.1) | 6 ±0.5 (5-6) |
| c (L/T) | 10.1 ± 0.9 (8.3-11.1) | 9 ± 1.6 (6-12) |

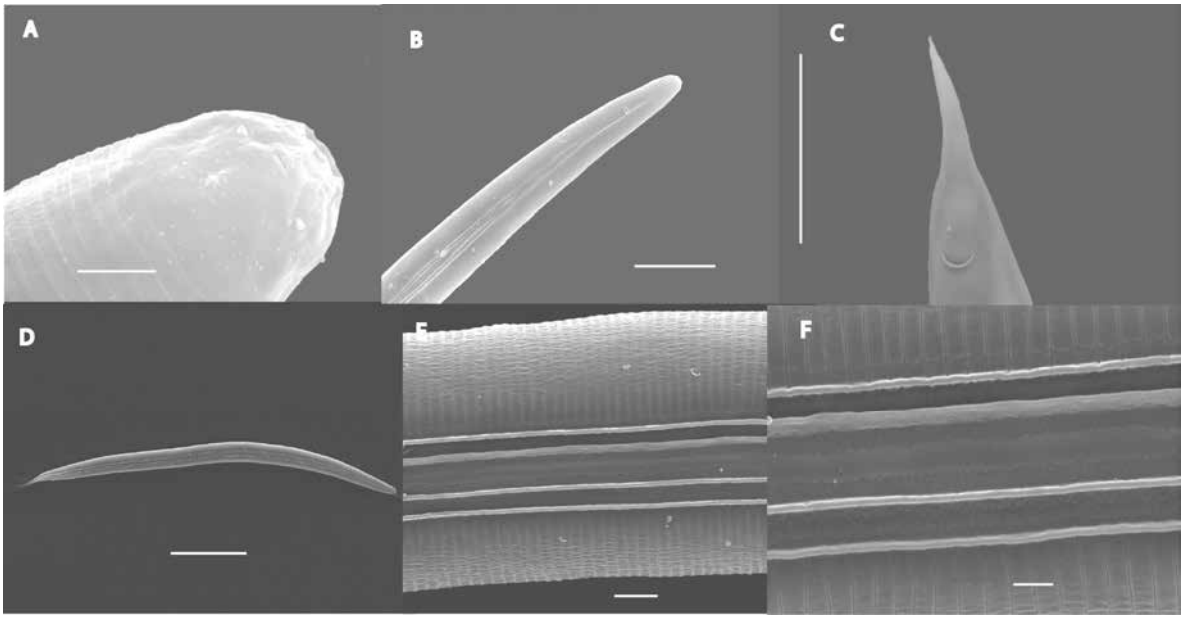


**Fig. 1.** *Steinernema cameroonense*. Infective juveniles. A, head, lateral view; B, anterior, lateral view; C, tail ventral view; D, entire juvenile, lateral view; E, F, lateral field at mid-body. Scale bars: A, 3 µm; B, C, 30 µm; D, 100 µm; E, 5 µm; F, 2 µm.

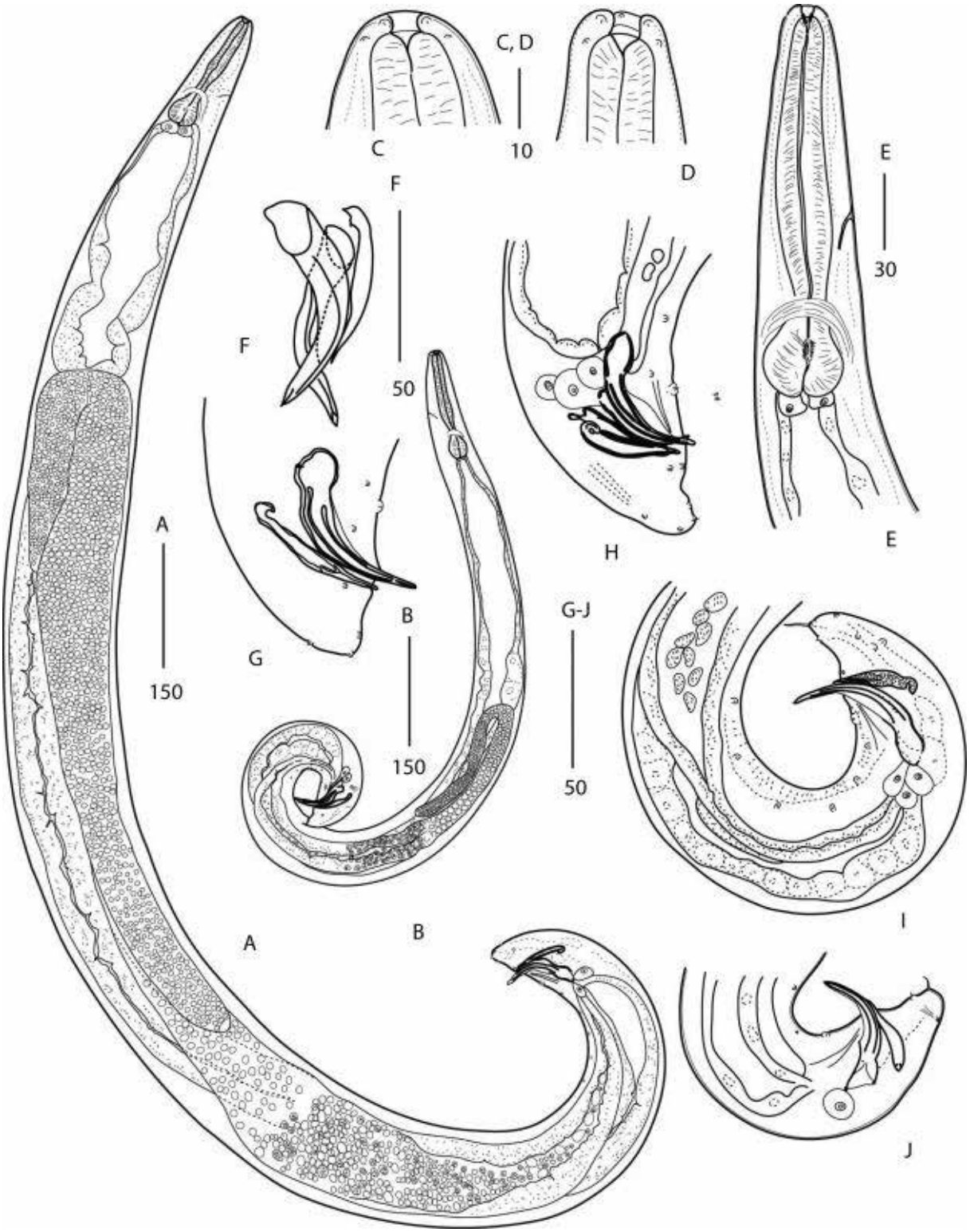


**Fig. 2.** *Steinernema cameroonense*. Males of the first generation: A, C, F, G, H; second generation males: B, D, E, I, J. A, B, entire view; C, D, head end; E, pharynx region; F, spicules and gubernaculum; G-J, tail. All in lateral position. Scale bars in μm.

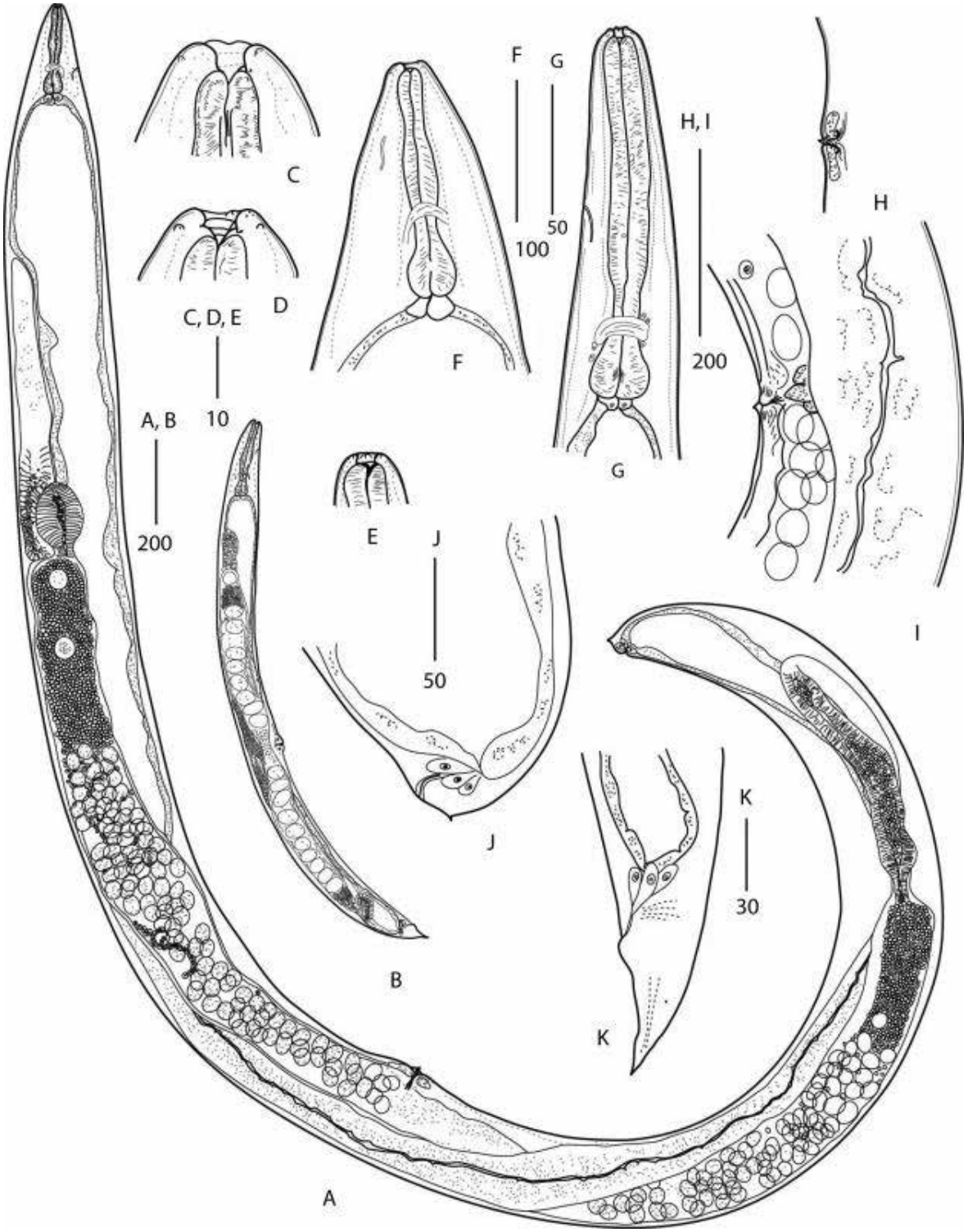


Fig. 3. *Steinernema cameroonense*. Females of the first generation: A, C, D, F, H, I, J ; second generation females: B, E, G, K. A, B, entire view; C-E, head end; F, G, pharynx region; H, I, vulva region; J, K, tail. All in lateral position. Scale bars in µm.

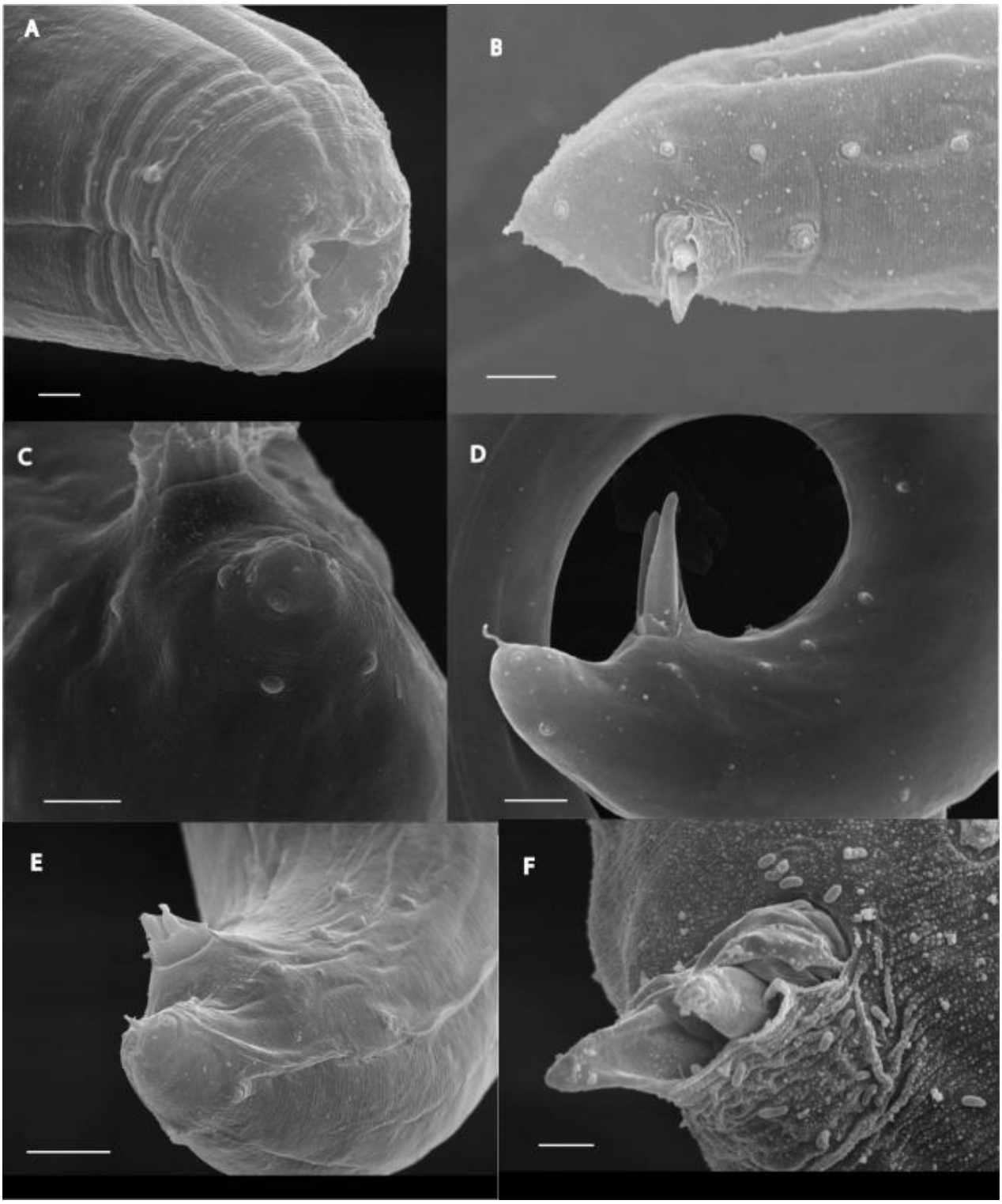


**Fig. 4.** *Steinernema cameroonense*. Males of the first generation: B, C, F; second generation males: A, D, E. A, head; B, tail ventral view; C, tail tip *en face* view; D, tail, lateral view; E, posterior end of tail, dorso-lateral view; F, cloaca *en face*.

Scale bars: A, 3 µm; B, 10 µm; C, 3 µm; D, E µm, 10 µm; F, 3 µm.

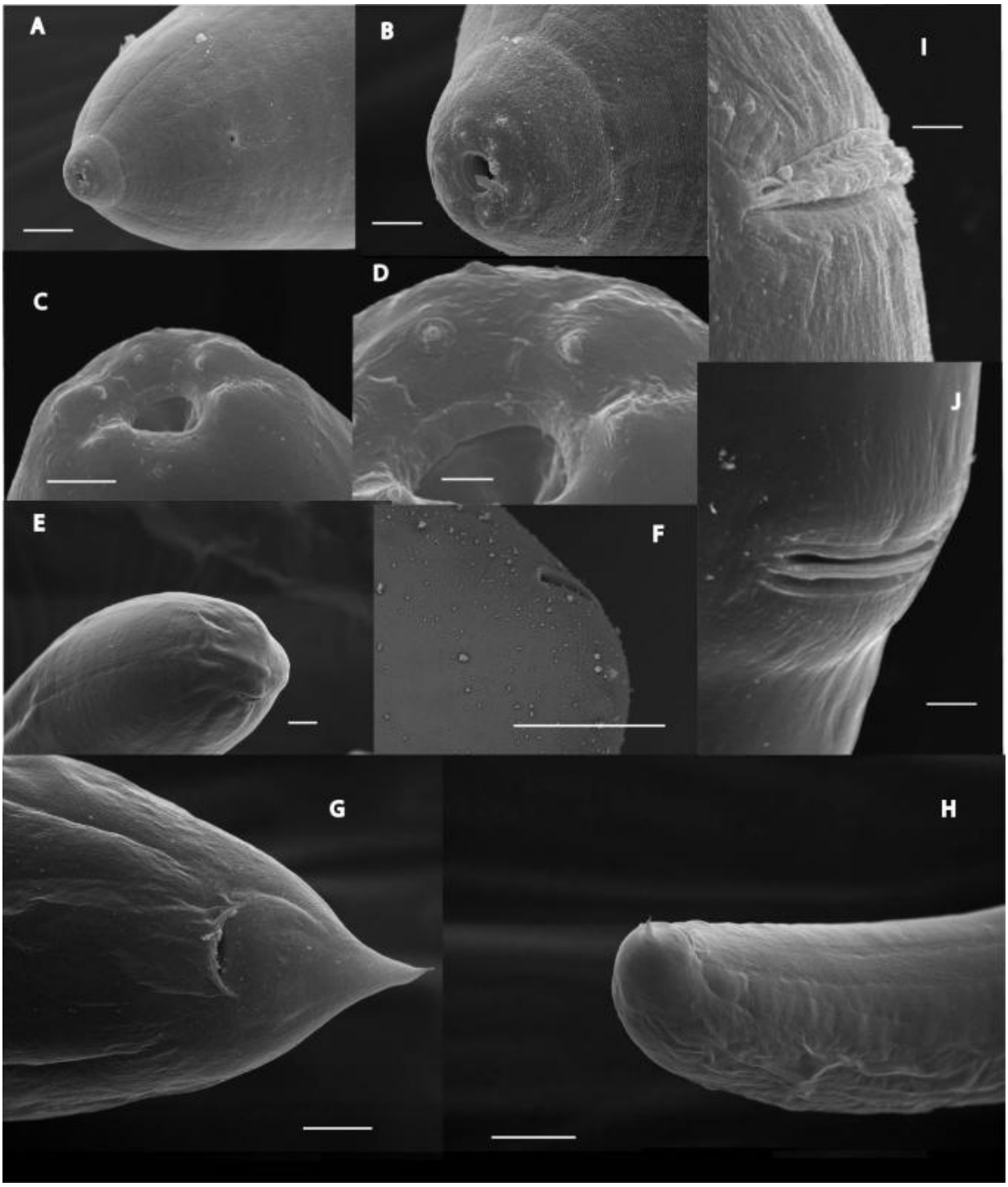


**Fig. 5.** *Steinernema cameroonense*. Females of the first generation: A, B, E, F, I; second generation females C, D, G, H, J. A, B, C, D, head end; E-H, tail; H, I, vulva region; J, K, tail.
Scale bars: A, 30 µm; B, C, 10 µm; D, 3 µm; E, F, 30 µm; G, 10 µm; H, 30 µm; I, 3 µm; J, 5 µm.

al., 1992). The PCR cycling parameters included a primary denaturation step at 94ºC for 5 min followed by 34 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, followed by a post-amplification extension step at 72°C for 6 min. Following electrophoresis (100V, 10mA) on a 0.8% agarose gel, PCR products were cleaned using Promega® Wizard SV Gel and PCR Clean-Up System (Promega Benelux, Leiden, The Netherlands). LSU rDNA products were

sequenced directly with PCR primers.

**Study of symbiotic bacteria.** Symbiotic bacteria were isolated from a suspension of IJs after 2-3 h of incubation in 0.1% mertiolate. Surface-sterilised IJ were homogenised in a glass micro-mortar and the mortar content spread over the surface of NBTA agar (Koppenhöfer, 2007). Bacteria from blue colonies were transferred to LB broth, which was shaken for 2-3 days at room temperature. After the centrifugation of the bacterial culture (2000 *g*, 5 min), the sediment of bacterial cells was transferred to 500 µl of lysis buffer (10mM Tris-HCl, pH: 8.0; 1mM EDTA; 1% Triton X-100) and incubated at 95ºC for 30 min. After centrifugation (7000 *g*, 2 min), 2 µl of the supernatant was used for PCR (Babic *et al.,* 2000) with following primer pairs: for 16S DNA - (5'-GAA GAG TTT GAT CAT GGC TC-3'), and (5'-AAG GAG GTG ATC CAG CCG CA-3'), for *Rec*A gene (recA - CCA ATG GGC CGT ATT GTT GA and recA-R - TCA TAC GGA TCT GGT TGA TGA A) as proposed by Sergeant *et al*. (2006) The obtained PCR product was directly sequenced using the same primers. Obtained sequences were deposited in GenBank as: partial 16S (KJ413066), partial sequences of *Rec*A (KM040764) and *SerC* genes (KM040765).

**Phylogenetic analysis.** For comparative purposes and construction of the phylogeny, the set of nematode and bacterial rDNA sequences deposited in GenBank was used. BLAST option was used to trace out related forms (Altschul *et al.* 1990). Bacterial sequences of 16S DNA, and genes *Rec*A and *Ser*C of closest bacterial forms were choosen for comparison. Bacterial species next to the set of the closest species was selected as an 'outgroup'

**Table 2.** Comparative morphometrics of 1st generation males of two strains of *Steinernema cameroonense* Ngo Kanga *et al*., 2012. All measurements are in µm and in the form: mean ± standard deviation (range).

| Characters | Males (present study); n=8 | Males (original description) n=20 |
|---|---|---|
| Body length (L) | 1907 ±288 (1323-2228) | 1331 ±193 (1019-1718) |
| Body diameter (W) | 143 ±24 (98-170) | 90 ± 21 (65-124) |
| pharynx length (Ph) | 154 ±10.3 (135-160) | 146 ± 8.8 (127-160) |
| nerve ring (NR) | 116 ± 9.4 (100-130) | 114 ± 7.3 (104-126) |
| excretory pore (EP) | 97 ± 10.1 (84-112) | 93 ± 11.5 (65-109) |
| tail length (T) | 36 ± 6.3 (27-47) | 38 ±2.9 (34-43) |
| Spicule length (S) | 76.5 ± 7 (65-87) | 69 ± 7.9 (51-85) |
| Gubernnaculum length (G) | 59 ± 7.3 (50-67) | 45 ± 6.4 (37-57) |
| D% (EP/Ph) × 100 | 63.1 ± 0.1 (56.4-76.2) | 64 ± 6.3 (48-76) |
| SW% (G/anal body diam.) × 100 | 169.1 ± 0.2 (144.4-206.4) | 170 ± 16.8 (131-201) |
| GS% (H/T) × 100 | 65.9 ± 0 (60.4-74.4) | 64 ± 6.8 (47-76) |
| a (L/W) | 13. 5 ± 1.4 (11.6-15.5) | 32 ± 4.1 (26-40) |
| b (L/Ph) | 12. 3 ± 2 (7.7-14.1) | 9 ±1.2 (7-12) |
| c (L/T) | 54.1 ± 9 (38.0-70) | 35 ± 4.5 (28-43) |

**Table 3.** Comparative morphometrics of 1st generation females of two strains of *Steinernema cameroonense* Ngo Kanga *et al*., 2012. All measurements are in µm and in the form: mean ± standard deviation (range).

| Characters | Females 1st generation (present study) ; n=7 | Females 1st generation (original description); n=20 |
|---|---|---|
| Body length (L) | 4791 ±1802 (1783-7313) | 3168 ±606 (2423-4607) |
| Body diameter (W) | 202 ±24 (160-220) | 172 ± 25 (110-231) |
| pharynx length (Ph) | 186 ±31 (160-250) | 173 ± 11.9 (152-198) |
| nerve ring (NR) | 148 ± 23 (135-188) | 135 ± 9.8 (111-152) |
| excretory pore (EP) | 85 ± 10 (65-85) | 104 ± 21 (74-140) |
| tail length (T) | 30 ± 7.5 (20-42) | 38 ± 5.2 (30-52) |
| V% (V/L) × 100 | 53 ± 2.4 (49.7-56.4) | 52 ± 2 (48-57) |
| D% (EP/Ph) × 100 | 46.7 ± 0.1 (32.8-55.2) | 60 ± 10.6 (43-79) |
| a (L/W) | 23.6 ± 8.3 (11.1-34) | 18 ± 2 (15-22) |
| b (L/Ph) | 25.5 ± 7.9 (9.9-32.5) | 18 ±2.7 (14-24) |
| c (L/T) | 162.2 ± 61.1 (66.0-272.9) | 85 ± 17.4 (58-112) |

taxon. The NCBI accession numbers of sequences of bacterial species are cited in the Table 4. Sequence alignments were generated using Clustal X under default values for gap opening and gap extension penalties. Alignments were analysed using PAUP* 4.0b10 (Swofford 1998) for maximum parsimony (MP) with option of search for nucleotide differences.

## RESULTS

**Infective juveniles.** Infective juveniles of the present population have noticeably larger body size and longer pharynx, while tail length and H% value differ insignificantly (Table 1). Ridges 3-4 are lower and less distinct (Fig. 1 E, F) compared with the original description (Fig. 3C in Ngo Kanga et al., 2012). Swollen phasmids (Fig. 3 D, E in Ngo Kanga et al., 2012) were never observed in our material (Fig. 1C).

**Males 1st generation.** Larger in size than in the original description with longer spicules and a gubernaculum (Table 2). The rest of the measurements are similar to those presented by Ngo Kanga et al. (2012). In the majority of specimens, a mucron is absent (Fig. 2 A, G, H; Fig. 4 C). When present, its length does not exceed 3 µm (Fig. 4B). Ngo Kanga et al. (2012) indicated that a mucron is usually present.

**Males 2nd generation.** Mucron 3-10 µm long is always present (Fig. 2 B, I & J; Fig. 4 D, E).

**Females 1st generation**. Perioral disc is present in larger specimens. Excretory pore is located closer to anterior extremity (mean 85 µm *vs* 104 µm, Table 3), whereas tail is shorter (mean 30 µm *vs* 38 µm, Table 3). Tail is rounded with a short mucron (1-3 µm long) (Fig. 3 A, J; Fig. 5 E, F). Post-anal swelling is not expressed.

**Females 2nd generation**. Tail conical, pointed or rounded with mucron ca. 10 µm long (Fig. 3 B, K; Fig. 5 G, H).

**Molecular characterization.** ITS rDNA sequence of the studied isolate of *S. cameroonense* was found to be nearly identical with that of the type isolate with a difference in 2 bp only.

**Molecular characterisation of the bacterial symbiont.** Partial sequences of 16S DNA, *Rec*A and *Ser*C gene were obtained for the symbiotic bacteria and deposited in NCBI GenBank under accession numbers KJ413066, KM040764 and KM040765, respectively. BLAST search for the closest species revealed that all three sequences of *S. cameroonense* symbiont are significantly different from corresponding sequences of all previously studied *Xenorhabdus* species. An analysis of each of these three sequences provided different nearest forms. The 16S sequences of *Xenorhabdus miraniense* from an undescribed steinernematid (strain Q1) from Australia was the most similar to that of the *S. camerooniense* symbiont. The *Rec*A sequences of *X. khoisanae* from *S. khoisanae* were the closest according to analysis of this gene. The sequence of *Xenorhabdus* sp. from steinernematid strain R001-293 from South Africa was the closest according to the *Ser*C gene analysis.

## DISCUSSION

The difference in two nucleotide positions was detected between ITS rDNA sequences of the *S. cameroonense* strain studied here and the type strain used for first the description (Ngo Kanga *et al*., 2012). As such minor nucleotide differences are reported even for the conspecific strains isolated at the same locality (Addis *et al.*, 2011), it is no wonder that differences were found between the steinernematid cultures isolated in two distant points: 4°30' N, 12°01' E for the type isolate (Ngo Kanga *et al*., 2012) and 4°12'49", 11°35'10" E for the isolate used in the present study. The differences in some morphometric parameters were also found. For example, the length of infective juveniles in these two isolates of *S. cameroonense* differ, with a range of 490-694 µm for the type isolate and 736-819 µm for the second isolate studied (Table 1). The ranges for pharynx length also do not overlap (105-125 µm *vs* 135-150 µm). Unlike juvenile morphometric features, these of the adult stages of both isolates are similar (Tables 2, 3). Both juvenile body length and juvenile pharynx length are important features used in descriptions of steinernematid species. Some morphological features (swollen phasmids, equal four central ridges in the lateral field) reported for the type isolate were not found in second one. Such observations indicate a significant plasticity of both morphological and morphometric characteristic in steinernematids.

The symbiotic *Xenorhabdus* bacterium of *S. cameroonense* was molecularly characterised in the course of this study. The nucleotide differences in 16S DNA between this bacterium and known representatives of the genus *Xenorhabdus* are on the level of interspecific differences. Thus, the difference with the *X. miraniensis* (the closest species according to BLAST search) is 11 bp, which is approximately equal to the difference of this latter species with the closest species *X. szentirmaii*. The sequences of *Rec*A and *Ser*C genes of *S. cameroonense* symbiont are most similar to that of two African *Xenorhabdus* species, *X. khoisanae* and *Xenorhabdus* R001-293, both from South Africa. It can be concluded that the symbiotic bacterium of *S. cameroonense* is resembling several *Xenorhabdus*

**Table 4.** Pairwise distances between *Xenorhabdus* sp. – symbiotic bacteria of *Steinernema cameroonense* Ngo Kanga, Phap Quang Trinh, Wayenberge, Spiridonov, Hauser & Moens, 2012 and other *Xenorhabdus* species. Below diagonal - total character differences, above diagonal – differences, expressed as percentage of the length of compared sequences.

| 16S (alignment length 1383 bp, parsimony informative characters – 24) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1. *Xenorhabdus szentirmaii,* Argentina, strain Sargento Cabral, GU480989 | – | 0 | 0.8 | 2.1 | 2.1 | 1.0 | 2.4 |
| 2. *Xenorhabdus szentirmaii,* Argentina, strain K77, DQ211712 | 0 | – | 1.0 | 1.0 | 2.2 | 1.0 | 2.4 |
| 3. *Xenorhabdus miraniensis,* Australia strain Q1, NR_043644 | 12 | 14 | – | 2.5 | 2.2 | **0.7** | 2.1 |
| 4. *Xenorhabdus stockiae,* China, strain HN_DS02, JQ219854 | 29 | 29 | 35 | – | 2.6 | 1.6 | 3.3 |
| 5. *Xenorhabdus indica*, India, strain CICR-WG, JN558595 | 30 | 31 | 30 | 36 | – | 1.8 | 3.0 |
| 6. *Xenorhabdus sp. ex S. cameroonense,* present study | 14 | 14 | **11** | 23 | 26 | – | 2.2 |
| 7. *Xenorhabdus beddingii*, Australia, strain DSM 4764, NR_042822 | 34 | 34 | 30 | 46 | 42 | 31 | – |
| *RecA* (alignment length 390 bp, pasrimony informative characters – 6) | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1. *Xenorhabdus khoisanae*, South Africa, strain SF80, JX623967 | – | 0 | 0.2 | 0.2 | 0.1 | **2** | 7 |
| 2. *Xenorhabdus khoisanae,* South Africa, strain SF87, AB685736 | 0 | – | 0.2 | 0.2 | 0.1 | **2** | 7 |
| 3. *Xenorhabdus khoisanae,* South Africa, strain SF362, JX623979 | 1 | 1 | – | 0.5 | 0.18 | 2.3 | 8 |
| 4. *Xenorhabdus khoisanae,* South Africa, strain 106-C, JX623973 | 1 | 1 | 2 | – | 1.8 | 2.3 | 8 |
| 5. *Xenorhabdus miraniensis*, Australia, strain Q1, FJ823414 | 6 | 6 | 9 | 9 | – | 2.3 | 7 |
| 6. *Xenorhabdus sp. ex S. cameroonense*, present study | **8** | **8** | 9 | 9 | 9 | – | 6.4 |
| 7. *Xenorhabdus beddingi,* Australia, strain Q58, FJ823415 | 29 | 29 | 30 | 30 | 27 | 25 | – |
| *SerC* (alignment length 609 bp, pasrimony informative characters – 94) | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1. *Xenorhabdus vietnamense,* Viet Nam, ex type isolate of *Steinernema sangi*, GU481021 | – | 6 | 10 | 12 | 14 | 15 | 18 |
| 2. *Xenorhabdus ishibashii,* Japan, strain IkWj136, AB630952 | 36 | – | 10 | 12 | 14 | 14 | 18 |
| 3. *Xenorhabdus griffinae,* Malaysia, strain T87, GU481009 | 62 | 59 | – | 11 | 13 | 14 | 19 |
| 4. *Xenorhabdus kozodoii*, Italy, strain 'Apulia', GU480999 | 76 | 74 | 69 | – | 13 | 14 | 19 |
| 5. *Xenorhabdus* sp., South Africa, strain R00I-293, GU481012 | 87 | 84 | 80 | 81 | – | **3.7** | 17 |
| 6. *Xenorhabdus sp. ex S. cameroonense*, present study | 91 | 84 | 81 | 87 | **23** | – | 17 |
| 7. *Xenorhabdus bovienii,* Turkey ex *Steinernema weiseri* , GU481025 | 112 | 111 | 111 | 115 | 104 | 105 | – |

strains isolated in tropical regions, but most probably represents a separate bacterial species.

## ACKNOWLEDGEMENT

This work was supported by RFBR grant No 14-04-01513 a. We would like to thank the staff of Laboratory of electron microscopy of Moscow State University for their help.

## REFERENCES

ADDIS, T., MULAWARMAN, M., WAYENBERGE, L., MOENS, M., VIAENE, N. & EHLERS, R.-U. (2011). Identification and intraspecific variability of *Steinernema feltiae* strains from Cemoro Lawang village from Eastern Java, Indonesia. *Russian Journal of Nematology, 19*, 21-29.

ALTSCHUL, S.F., GISH, W., MILLER, W, MYERS, E.W. & LIPMAN, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*, 403-10.

BABIC, I., FISCHER-LE SAUX, M., GIRAUD, E. & BOEMARE, N. (2000). Occurence of natural dixenic associations between the symbiont *Photorhabdus luminescens* and bacteria related to *Ochrobactrum* spp. in tropical entomopathogenic *Heterorhabditis* spp. (Nematoda, Rhabditida). *Microbiology*, *145*, 709-718.

COLEMAN, C. O. (2003). "Digital inking": How to make perfect line drawings on computers. *Organisms, Diversity and Evolution 3, Electronic Supplement, 14*, 1–14.

KOPPENHÖFER, H. S. (2007). Bacterial symbionts of *Steinernema* and *Heterorhabditis*. In: K.B. Nguyen & D.J. Hunt (Eds). *Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts*. Nematology Monographs and Perspectives, Volume 5 (Hunt, D.J. & Perry, R.N., series editors: Brill, Leiden, The Netherlands, pp. 735–808.

NGO KANGA F., PHAP QUANG TRINH, WAEYENBERGE, L., SPIRIDONOV S.E., HAUSER, S. & MOENS M. 2012. Two new species of *Steinernema* Travassos, 1927 from the humid forest of southern Cameroon. *Russian Journal of Nematology*, 20: 15-26.

SEINHORST, J. W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica, 4*, 54–60.

SERGEANT, M., BAXTER, L., JARRETT, P., SHAW, E., OUSLEY, M., WINSTANLEY, C. & MORGAN, J.A.W. (2006). Identification, typing and insecticidal activity of *Xenorhabdus* isolates from entomopathogenic nematodes in United Kingdom soil and characterization of the xpt toxin loci. *Applied and Environmental Microbiology*, *72*, 5895–5907.

Swofford, D.L. (1998) *PAUP*. Phylogenetic analysis using parsimony.* Version 4. Sunderland, Massachusetts, Sinauer.

**Ngo Kanga, F., E. S. Ivanova, N. S. Shepeleva, S. E. Spiridonov.** Дополнительные сведения о *Steinernema cameroonense* Ngo Kanga, Phap Quang Trinh, Wayenberge, Spiridonov, Hauser & Moens, 2012.

**Резюме.** С 2011 года в культуре на *Galleria mellonella* поддерживается изолят *Steinernema cameroonense* Ngo Kanga et al., 2012 отличающийся от типового на 2 нуклеотида последовательности ITS rDNA. Длина тела и пищевода инвазионных личинок этого изолята отличается от таковых у типового изолята (пределы изменчивости этих признаков у данных двух изолятов неперекрываются). Приводится описание важных в таксономическом отношении морфологических структур (хвостовые конца самцов и самок, латеральные поля инваазионных личинок). Получены нуклеотидные последовательности симбиотической бактерии рода *Xenorhabdus*, ассоциированной с данными видом штейнернематид. По результатам анализа нуклеотидных последовательностей 16S DNA и генов *Rec*A и *Ser*C симбионты *S. cameroonense* отличаются от всех известных представителей этого рода, показывая определенное сходство с *X. miraniensis* из Австралии, а также *X. khoisanae* и *Xenorhabus* sp. R001-293 из Южной Африки.