# Addenum to the description of *Steinernema jollieti* Spiridonov, Krasomil-Osterfeld & Moens, 2004

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**Summary.** Additonal morphological data are provided for *Steinernema jollieti* Spiridonov, Krasomil-Osterfeld, Moens, 2004. A light and scanning electron microscopy were used to particularize taxonomically important characters missing in the first decsription. The GS% and SW% indices are provided for males. The distribution of genital papillae on male tail is illustrated with SEM images. Structure of female tail and vulvar area are decsribed for females of the first and second generation. The relationships of *S. jollieti* with other steinernematid *feltiae/kraussei* group species are discussed.

**Key words:** entomopathogenis nematodes, infective juveniles, morphology, Steinernematidae.

*Steinernema jollieti* Spiridonov, Krasomil-Osterfeld & Moens, 2004 was described from a soil sample collected close to a stream waterbed in the Bush-Augusta State Park region, Missouri valley near St. Louis (Spiridonov *et al*., 2004). This species belongs to the *kraussei/feltiae* group (Nguyen et al., 2007). The species description presented all important morphological, morphometric and genetic data; however, the microphotographic documentation of valuable characters are represented only by a scanning electron microscopy (SEM) of infective juvenile (IJ) lateral field. The main goal of our paper is to add SEM and DIC microphotography of adult and infective juvenile nematodes and particularize some taxonomically important characters according to suggestion published in Hominick *et al*. (1997).

## MATERIALS AND METHODS

The *S. jollieti* living culture was recieved from the collection of Institute of Parasitology RAS, Moscow. The nematodes were reared on the late instar larvae of *Galleria mellonella* L.. For examination with SEM the first generation males, females and IJ were used. Larvae of *G. mellonella*, infected with 50-70 IJ per insect at 22.5±0,5°C, were dissected in Ringer's solution (Woodring & Kaya, 1988) 4-5 days after the nematode and host established contact. Males, females and IJ thus obtained were collected in centrifuge tubes, heat relaxed (55°C, 3-5 min), fixed for 24-36 h in 2% glutaraldehyde solution (POCH) in 0.15 M cacodylate buffer (pH 7.2, POCH) at 4°C, rinsed in cacodylate buffer four times (10 min each change) and post-fixed for the next 12-24 h in 1% osmium tetroxide (Sigma Aldrich) with the same buffer and temperature. Fixed nematodes were rinsed in cacodylate buffer four times again (10 min intervals) and dehydrated in ethanol series (POCH) (10, 30, . . . , 90% at 10 min intervals, 100% at 20 min intervals with three changes). After exchanging the ethanol for acetone (POCH), specimens were critical point-dried (Polaron Range CPD 7501) in liquid $CO_2$, mounted on SEM stubs, sputter-coated with 20 nm gold-palladium layer (Polaron Range SC 7620) and examined in a LEO 1430VP scanning electron microscope at an accelerating voltage of 15 kV in high vacuum mode.

For a light microscopy nematodes were fixed in 2% glutaraldehyde (25% glutaraldehyde diluted with Ringer's solution), processed to anhydrous glycerin according to Seinhorst method (Seinhorst, 1959) and observed using a Leica 5500B.

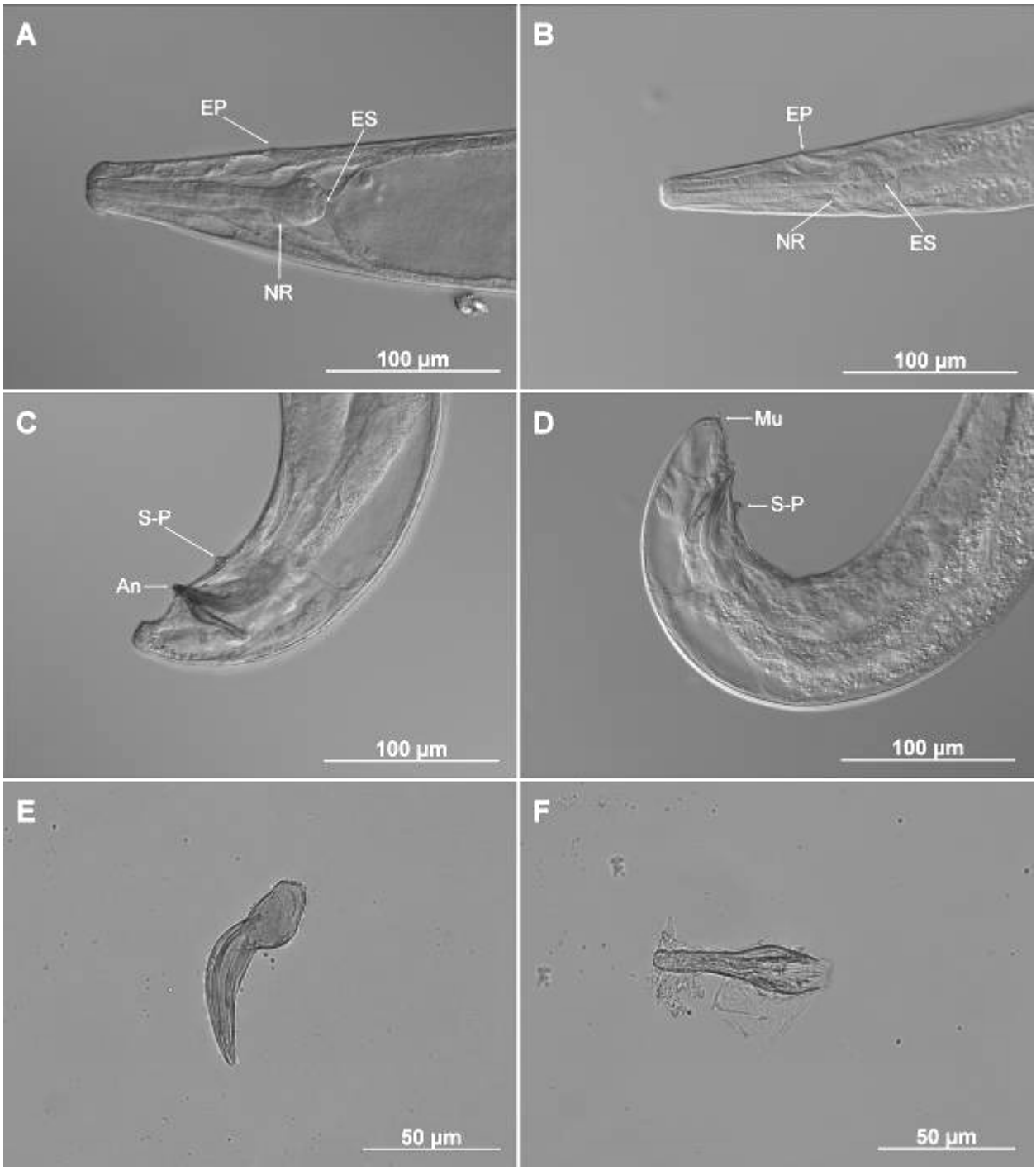


**Fig. 1.** *Steinernema jollieti.* A-F: Male. A: First generation head; B: Second generation head; C: First generation tail with genital papillae (laterally); D: Second generation tail with genital papillae (laterally) and mucron; E: First generation spicule; F: First generation gubernaculum (ventrally). EP – excretory pore, ES – oesophagus, NR – nerve ring, An- anus, S-P – single preanal papilla, Mu – mucron.

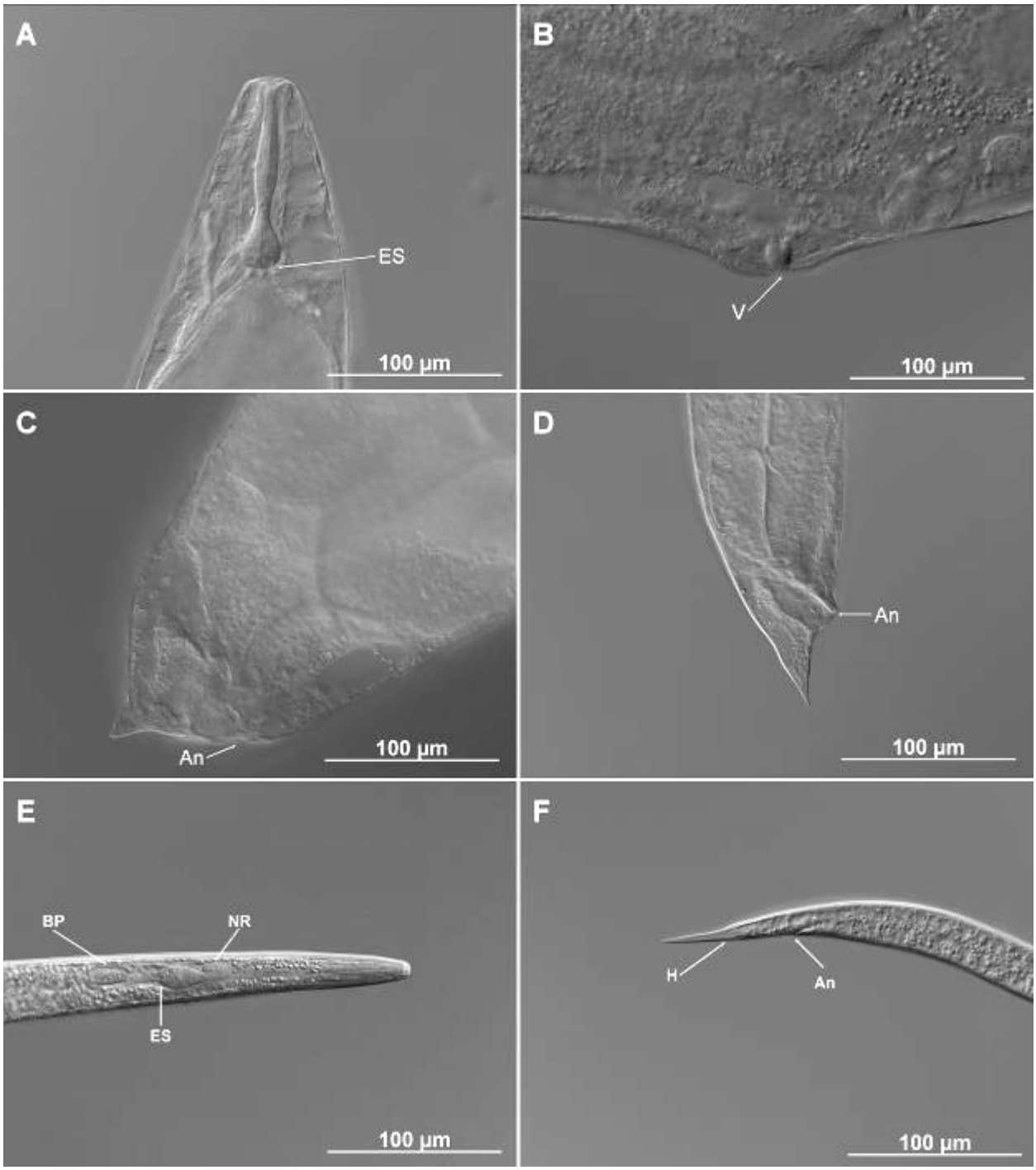


**Fig. 2.** *Steinernema jollieti.* A-D: Female. A: First generation head; B: First generation vulva; C: First generation tail; D: Second generation tail; E-F: Infective juvenile. E: Head with esophagus; F: Tail with hyaline layer. ES – oesopahgus, V – vulva, An – anus, NR - nerve ring, BP – bacterial pouch (receptacle), H – hyaline portion of tail.

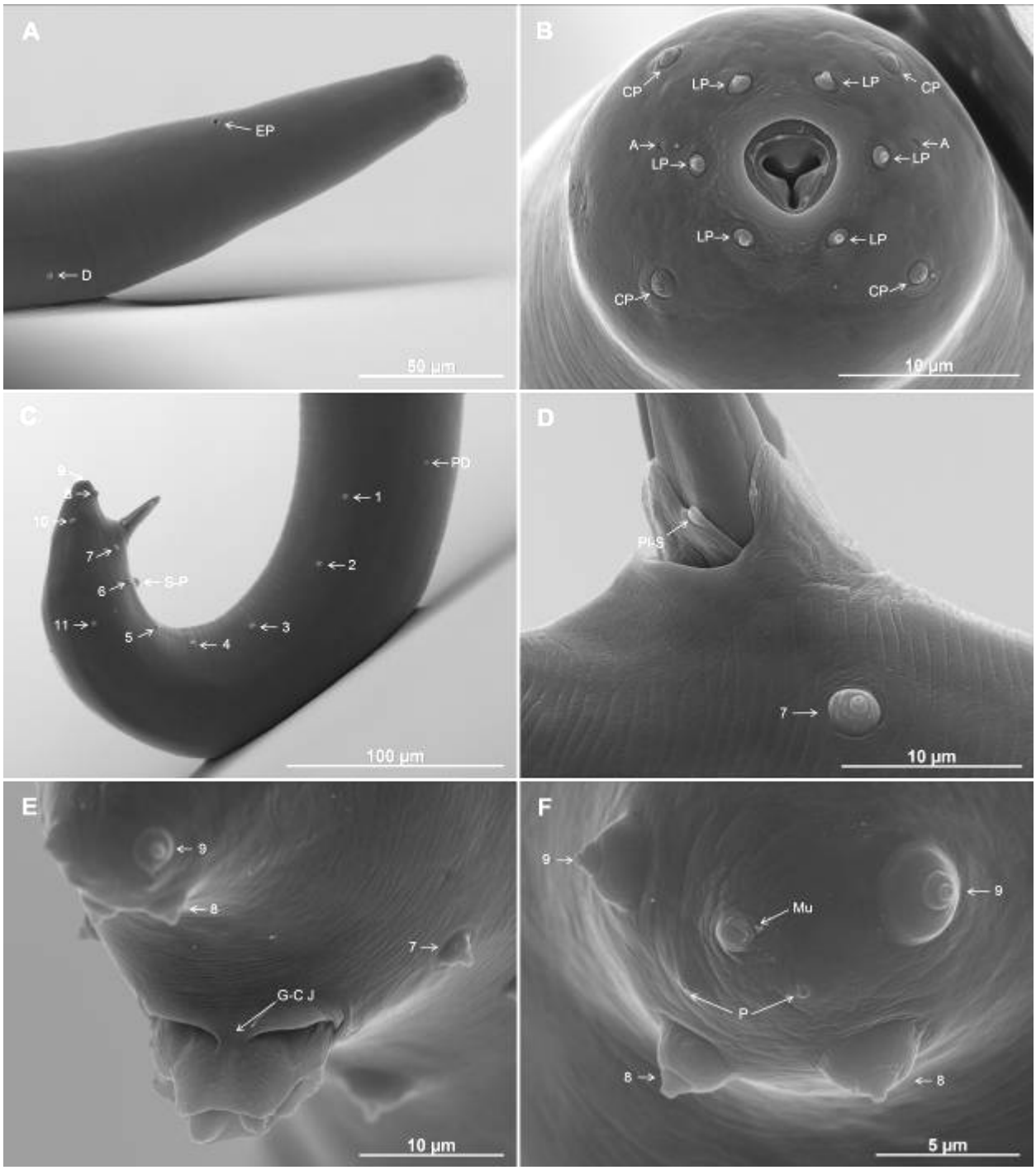


**Fig. 3.** *Steinernema jollieti*. A-F: Male first generation. A: Head "en face view"B: head region; C: Tail region with genital papillae; D: Tail region around cloaca with a papilla-like structure at the cloaca base; E-F: Tail tip. EP – excretory pore, D – deirid, CP –cephalic papilla, LP – labial papilla, A – amphid opening, 1-11 – genital papillae, PD- postdeirid, S-P – single preanal papilla, Pl-S – papilla-like structure, G-C J – gubernaculum-cuticle junction, Mu – mucron, P – phasmid opening.

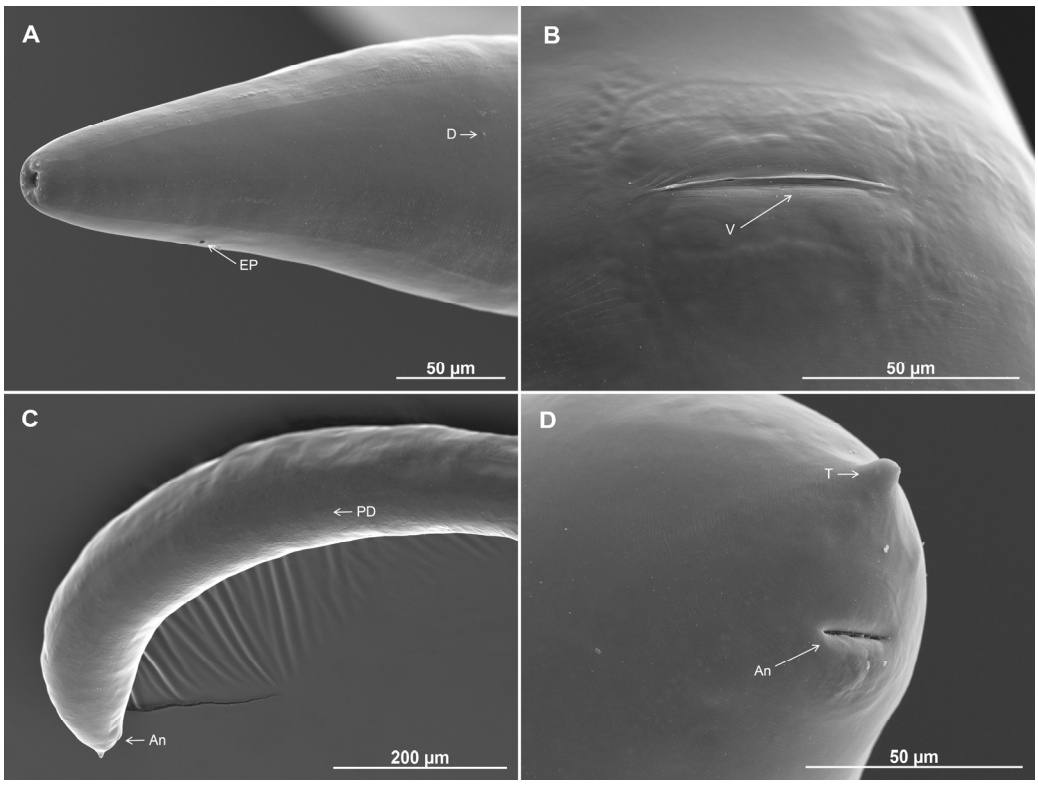


**Fig. 4.** *Steinernema jollieti*. A-D: Female first generation. A: Head region; B: Vulva; C: Hind body portion; D: Tail with a conical peg. EP – excretory pore, D – deirid, V – vulva, An – anus, PD – postdeirid, T – tail.

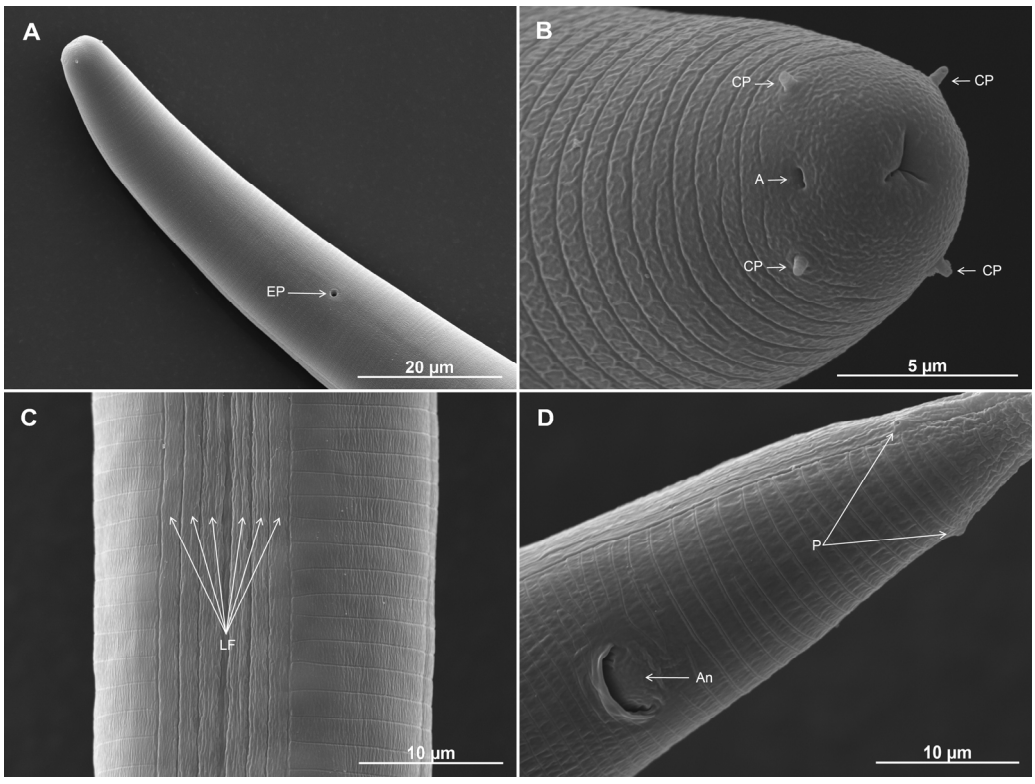


**Fig. 5.** *Steinernema jollieti.* A-D: Infective juvenile. A: Forebody portion; B: Head; C: Lateral field; D: Tail with anus and phasmids. EP- excretory pore, CP –cephalic papilla, A – amphid opening, LF – laterail fields, An – anus, P – phasmid opening.

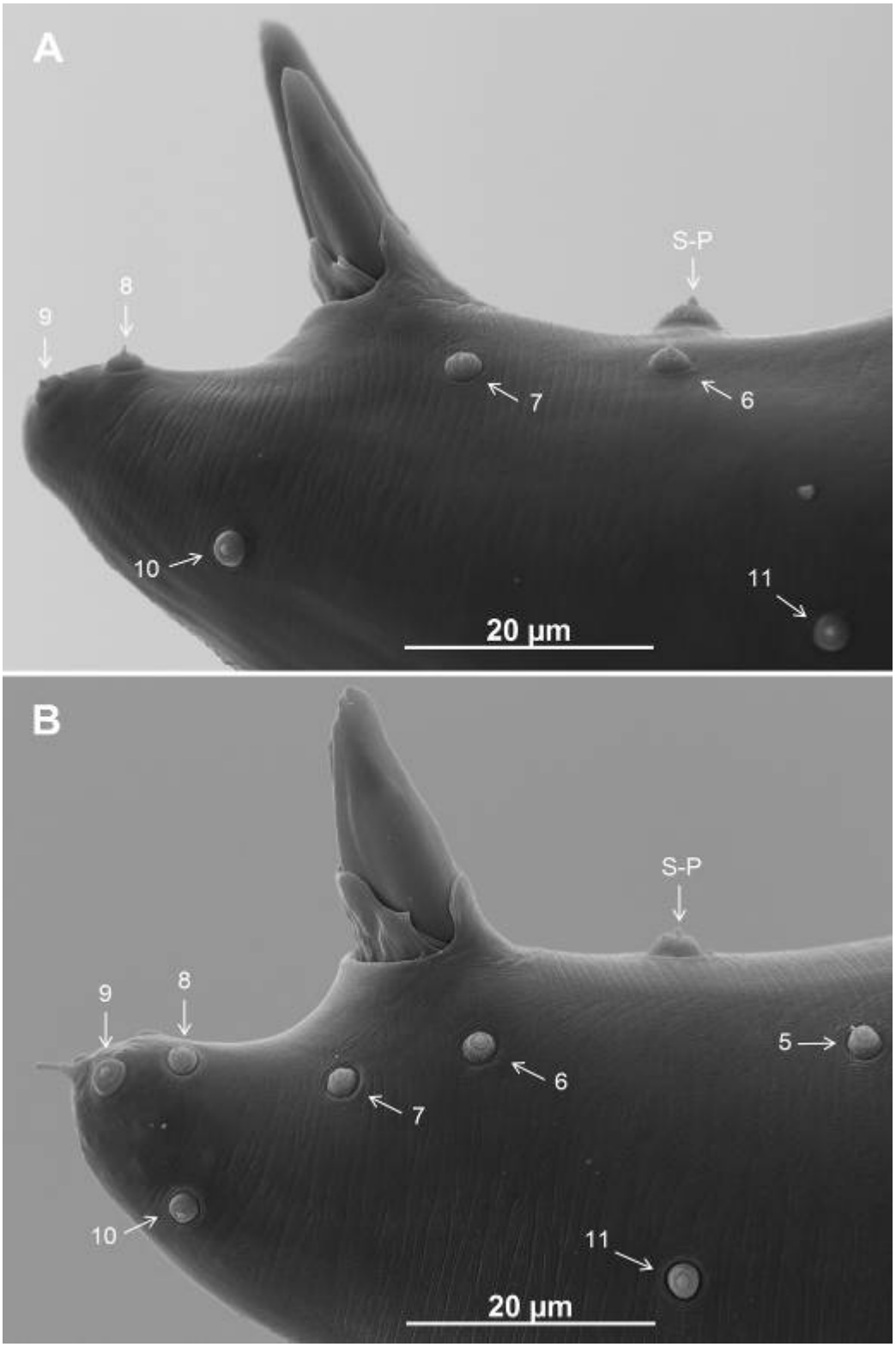


**Fig. 6.** An arrangement of adcloacal genital papillae. A: *Steinernema jollieti*; B: *S. feltiae*.
A – amphid, An – anus, BP – bacterial pouch, CP – cephalic papilla, EP – excretory pore, ES – esophagus, H – hyaline layer, LF – lateral field, LP – labial papilla, Mu – mucron, NR – nerve ring, P – phasmid, S-P – single preanal papilla, V - vulva, G-C J – gubernaculum-cuticule junction, D – deirid, PD – postdeirid, Pl-S – papilla-like structure, 1-11 – genital papillae.

## RESULTS

**Comparison of the description data and topotype strain.** There are several discrepancies and missing data in the original description of *S. jollieti.* With respect to the published drawings (Spiridonov *et al*., 2004) it is evident that several characters must be corrected and added.

**Males** (Figs. 1, 3). Spicules slightly to moderately, not strongly, curved. Manubrium elongated, robust in the first generation with ratio length/width 1.5-2 : 1. Second generation manubrium slender with ratio at least 2:1. Gubernaculae very variable in their shape, often with a depressed dorsal side on the proximal portion forming narrow neck. Proximal end slightly bent, never hooked. Typical mucron absent in the first generation; however, papilla-like (mucron-like) structure visible in 50% of specimens under SEM (Fig. 3F).

Second generation males possess either conical in younger males (length 2-3 μm) or filamentous mucron in older specimens, (length 5-8 μm).

Important ratios in the first generation GS% 84 *vs* 79 (76-81) and SW% 145 *vs* 169 (154-183) were recalculated from the original description *vs* measured from specimens of our living culture, respectively. In the second generations the GS% 72 *vs* 79 (76-83) and SW% 187 *vs* 149 (128-164) were recalculated *vs* measured, respectively.

In the original description the arragement of genital papillae in *S. jollieti* was mentioned as specific due to postcloacal position of only three pairs of papillae, whereas other species of the *feltiae/kraussei* group possess five postcloacal pairs. However, in Nguyen *et al*. (2007) only three postcloacal pairs are mentioned, also for *S. kraussei* (Steiner, 1923) Travassos, 1927 and from five postcloacal pairs in *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 two pairs are more adcloacal. Generally, the correct position of genital papillae can be resolved by using SEM. The position of papilae that are close to the cloaca may be influenced by the bend of fixed male tail and two adcloacal pairs may vary slightly in their position. In our observation (Fig.6), the arrangement of three postcloacal papillae in *S. jollieti* was found to be relatively constant and different from *S. kraussei* and *S. feltiae*. However, more SEM studies of genital papillae in species of the *feltiae/kraussei* group are needed to resolve if the postcloacal papillae arrangement can be considered as a specific trait distiguishing *S. jollieti* from other species of the *feltiae/kraussei* group. The single-preanal papilla is bigger than that in *S. feltiae* and *S. kraussei*. Moreover, there is a papilla-like structure at the base of cloaca that is characteristic for some species of *feltiae/kraussei* group.

**Infective juveniles** (Figs 2 E-F, 4 and 5). Head rounded, not offset from the body contour. Important ratio c´4.5 and %E 88 were recalculated from the original description *vs* our measurements of living culture, 4.2 (3.9-4.3) and 92 (81-98), respectively. Ratio H 55% (46-60) ranks *S. jollieti* among species that have hyaline layer longer than 50% of tail length. Described mucron on the IJ tail tip was not found either in living or fixed specimes and this character cannot be considered taxonomically important. Tail was found slightly dorsally depressed.

**Females** (Figs. 2 A-D and 4). First generation possess the smooth conical tail with a peg on the tip bearing three minute indistinct papilla like structures, one on the tip and two situated laterally that may represent an inconspicuous phasmid openings. Postcloacal swelling not developed. Second generation females possess the conical tail extending to the filamentous mucron. Anal opening is surrounded by the moderately protruding preanal fold and postcloacal swelling.

**Table 1**. Infective juvenile characters distinguishing some Holarctic species of the *feltiae/kraussei* group of *Steinernema.*

| character/species | *jollieti* | *feltiae* [x] | *kraussei* [x] | *oregonense* [x] | *weiseri* [x] |
|---|---|---|---|---|---|
| head | rounded | rounded | **flattened** | **flattened** | **flattened** |
| lateral ridges | 6 | **8** | **7** | **7** | **8** |
| c´ | 4.5 [x] | 4.8 (4.5-5.1) [y] | **3.8** | 4.7 | **3.7 (3.2-4.1)** |
| % D | 48 (46-50) | 46 (44-50) [y] | 47 | 50 (40-60) | 51 (44-55) |
| % E | 88 [x] | **74 (67-81)** [y] | 80 | **100 (90-110)** | 95 |
| % H | 55 (46-60) | **44 (37-51)** [y] | **38 (35-40)** | 47 | **36 (34-39)** |

Bold– character different from *S. jollieti;* [x] recalculated from the species description, [y] after Nguyen et al., (2007), c' – tail length divided by anal body width, %D – distance from anterior end to excretory pore divided by pharynx length ×·100%, %E - distance from anterior end to excretory pore divided by tail length ×·100%, %H – hyaline posrtion of tail divided by tail length × 100%.

**Relationship.** *Steinernema kraussei, S. feltiae, S. oregonense* Liu & Berry, 1996, and *S. weiseri* Mráček, Sturhan & Reid, 2003 represent *feltiae/kraussei* group species with a Holoarctic distribution. Of these *S. kraussei* and *S. feltiae* were found in both Palearctic and Neoarctic regions. *Steinernema jollieti* infective juvenile differs from these species by characters in Table 1. Of these characters we consider as the most important six ridges in the lateral field, whereas other mentioned species have seven or eight ridges, respectively. Rounded head of *S. jollieti* differs from those flattened in *S. kraussei*, *S. oregonense* and *S. weiseri*. Ratio c´4.5 is different from *S. kraussei* 3.8 and *S. weiseri* 3.7 (3.2-4.1), respectively. H% is much higher in *S. jollieti* 55 (46-60) when compared with *S. feltiae* 44 (37-51), *S. kraussei* 38 (35-40) and *S. weiseri* 36 (34-39), respectively. Second generation females possess characteristically formed anal surroundings. While a postanal swelling is common in many steinernematid species, preanal fold offset from body contour seem to be quite unique for *S. jollieti*.

The SW% value of the first generation male of *S. jollieti* is higher than that of *S. oregonense* and *S. weiseri* at 169 (154-183) *vs* 110 and 140, respectively, whilst the GS% value did not bring significant differences.

## ACKNOWLEDGEMENT

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**H. W. Skrzypek, W. S. Kazimierczak, A. M. Kreft, S.E. Spiridonov, Z. Mráček.** Дополнения к описанию *Steinernema jollieti* Spiridonov, Krasomil-Osterfeld & Moens, 2004 .

**Резюме.** Приводятся дополнительные данные по морфологии *Steinernema jollieti* Spiridonov, Krasomil-Osterfeld & Moens, 2004, отсутствующие в первоописании. Для их получения была использована световая и сканирующая электронная микроскопия. Приводятся значения индексов GS% и SW% для самцов. Распределение генитальных папилл на хвостовом конце самцов иллюстрируется с помощью СЭМ. Приводятся сведения об организации хвостового конца и вульвы у самок первого и второго поколения. Обсуждаются филетические отношения *S. jollieti* с другими видами штейнернематид.