Addendum to the description of *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982

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Summary. In the light of current morphological knowledge, the *Steinernema carpocapsae* morphology is not satisfactorily described, some important characters of this species are missing and no thorough molecular characterisation of this species has been performed. *Steinernema carpocapsae* represents a type of the '*carpocapsae*' group and thus a main objective of this study was to add characters such as, infective juvenile hyaline layer with %H, tail morphometry with ratio c', shape and protuberances on the F1 tail *etc*., to the general knowledge about this species. We further aimed to sequence ITS region and D2-D3 regions of the ribosomal DNA, and compare them with the sequences for *S. carpocapsae* available in the GenBank NCBI database. Four *S. carpocapsae* isolates (strains) were studied (USA – DD136 and All, Russian – Agriotos and Swiss – DOK83). Morphology was identical for all isolates and morphometry close each to other. Infective juveniles possess rather narrow, continuous and hemispherically rounded head, lateral field with six ridges (seven lines) with the less prominent central pair, hyaline layer occupies about 40-44% of the tail length, ratio c' varies from 4.0 (3.8-4.1) to 4.2 (3.9-4.4) and ratio %E from 71 (59-82) to 79 (74-83). First generation females possess a characteristic blunt tail with a terminal, conical sharply ended peg and no other minute protuberances and rounded vulva slightly protruding from the body contour. All male generations possess the only minute mucron and there was little size difference between the first and second (other) generations. Both studied genetic markers effectively characterize *S. carpocapsae* as a species, showing very low interspecific variation. However, the status of the closely related species *S. anatoliense* and *S. websteri* seems to be doubtful.

Key words: entomopathogenic nematodes, Steinernematidae, morphology.

Recently, the number of steinernematid species has increased rapidly. Even though significant differences in morphological characters between these species exist, all are classified in the genus *Steinernema* Travassos, 1927. To enable easier species characterisation, five groups of morphologically similar species were established (*e.g*., Hominick *et al*., 1997). Later, molecular data supported these groups as phylogenetic groups (*e.g*., Stock *et al*., 2001; Spiridonov *et al*., 2004).

The steinernematid species forming the '*carpocapsae*' group are characterised by a short body length, finely rounded head, six (eight) ridges in the lateral field and anterior position of excretory pore of infective juveniles (IJ). Males possess slender, finely arcuate spicules with a prominent rostrum. The first described species of this group, *S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982 was redescribed by Poinar (1967). However, in the light of current morphological knowledge, the *S. carpocapsae* morphology is not satisfactorily described and some important characters of this species are missing. Therefore, *S. carpocapsae* represents a type of this group so we added characters, such as, IJ hyaline layer with %H, tail morphometry with ratio c', shape and protuberances on the F1 tail *etc*., to the general knowledge about this species and complete Weiser's (1955) description and the redescriptions by Poinar (1967) and Nguyen *et al*. (2007), and emphasise those characters that should be compared with other species belonging to the '*carpocapsae*' group.

A large number of ITS and D2-D3 of *S. carpocapsae* and related unidentified isolates are available in the GenBank NCBI database. Some sequences attributed to this species are doubtful, and

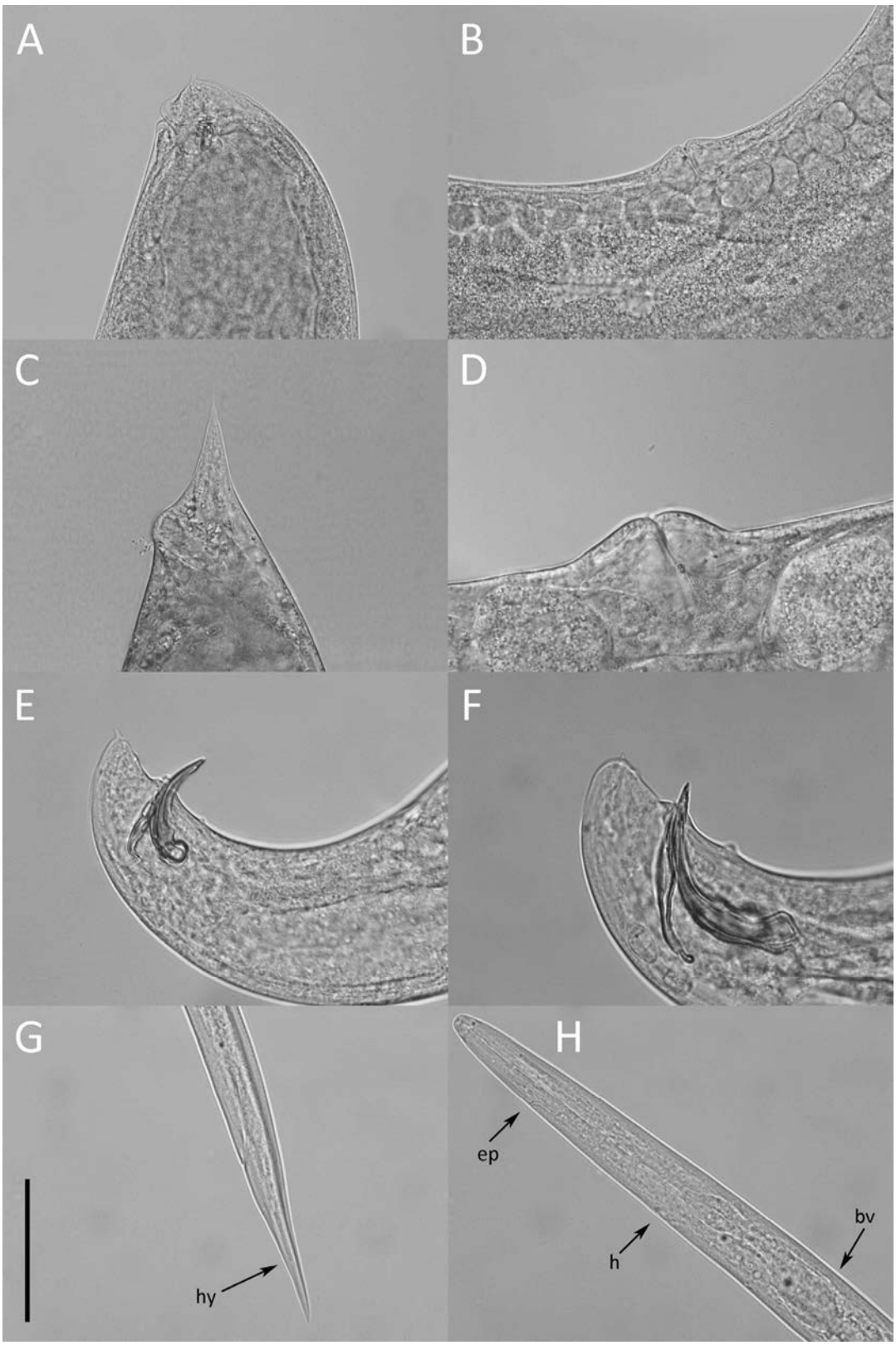

Fig. 1. *Steinernema carpocapsae* (light microscopy). A & B first generation female: A – tail with conical peg, B – vulva; C & D second generation female: C – tail, D – vulva; E & F male: E – first generation, F – second generation; G & H infective juvenile: G – tail, H – anterior portion. (Scale bars: A-B = 300 μm; C-D = 87 μm; E-F = 60 μm; G-H = 27 μm.). Abbreviations: bv – bacterial vesicle, ep – excretory pore, h – hemizonid, hy – hyaline layer beginning.

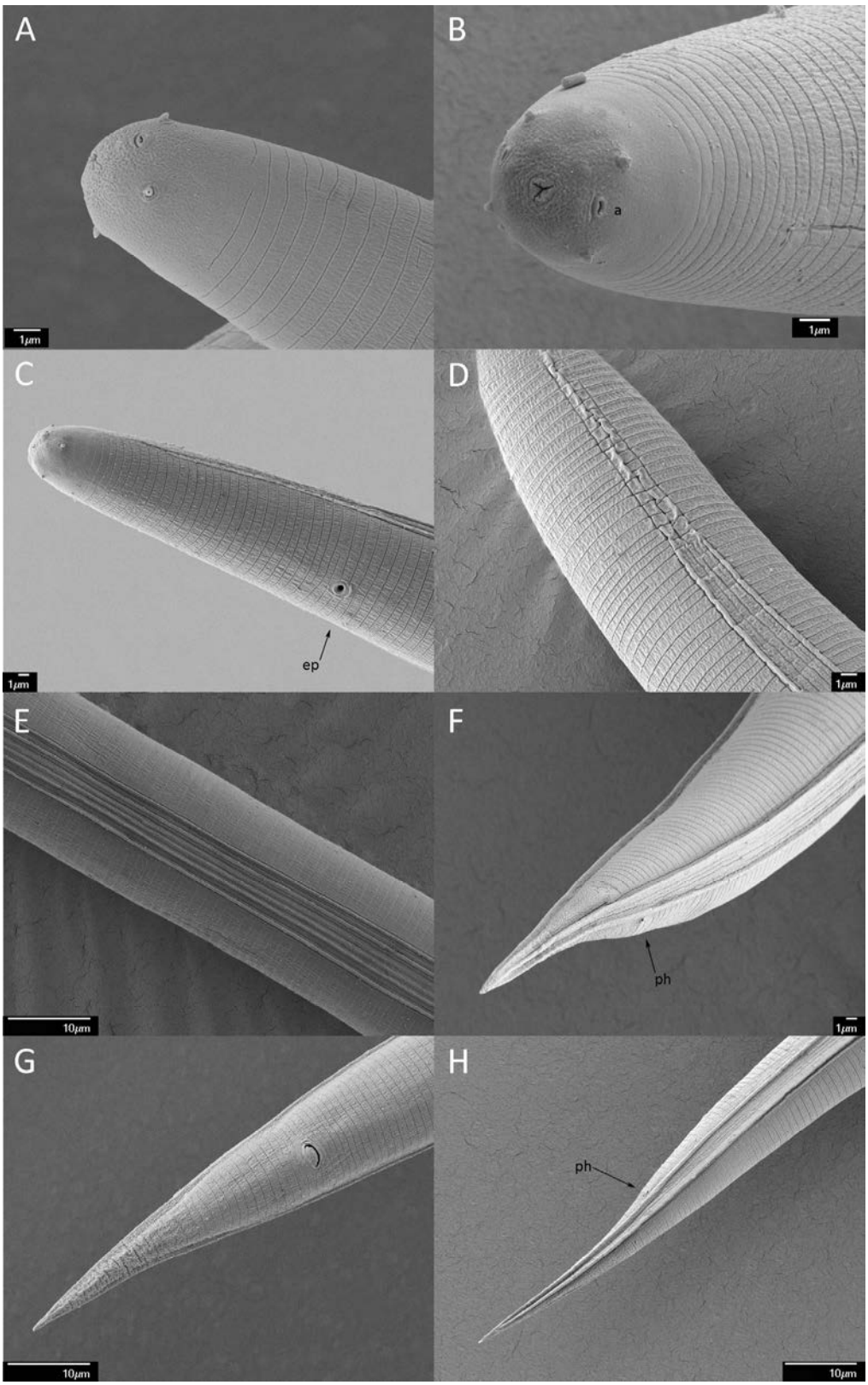

Fig. 2. *Steinernema carpocapsae* (scanning electron microscopy). A-H infective juvenile: A-C – head portion; D – lateral field in anterior portion; E – lateral field in the mid body; F & H – lateral field in the tail portion; G – tail ventrally. Abbreviations: ep – excretory pore, ph – phasmid.

some sequences attributed to other species show very high similarity to *S. carpocapsae*. Thus, we aimed to revise relevant sequences in the GenBank NCBI database.

MATERIALS AND METHODS

Steinernema carpocapsae has a Holarctic distribution (Hominick, 2002); however, its recovery from fields is rare (Mráček *et al.*, 2005). The original topotype culture of Weiser (1955) is lost. Therefore, we studied the following cultures: *S. carpocapsae* strain Agriotos, which came from the original culture of Dr Veremtchuk (1969) and it has been kept for 30 years in our living stock, US strains DD-136 and All, received from the collection of Dr Ralf Ehlers, Christian Albrechts University Kiel, Germany, and a Swiss strain DOK83 from the collection of Dr Raquel Campos-Herrera, University of Neuchâtel, Switzerland. All these strains were propagated at the Laboratory of Entomopathogenic Nematodes, Institute of Entomology, Biology Centre ASCR in České Budějovice.

Light microscopy (LM) and morphometry. IJ and adult nematodes were studied either heat-killed on glass slides in a drop of water or fixed in a hot TAF (Courtney *et al.*, 1955) and transferred to glycerin by a slow evaporation method (Seinhorst, 1959). The measurements and examination of morphology were performed with an AMPLIVAL light microscope, Carl Zeiss Jena, and a Leitz DIAPLAN with Nomarski optics.

Scanning electron microscopy (SEM). Nematodes were fixed in 4% formalin buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 h at 4-6°C and post-fixed with 2% osmium tetroxide solution for 12 h at 25°C. After dehydratation in a graded ethanol series, critical point drying with liquid COB_{2B}, mounting on SEM stubs, and coating with gold (Nguyen & Smart, 1995) the nematodes were observed under SEM JEOL JSM 7401F.

Molecular characterisation. DNA was extracted from single females. Each female was transferred into a sterile Eppendorf tube (500 µl) with 20 µl of extraction buffer (17.7 µl of ddH_2O, 2 µl of 10 × PCR buffer, 0.2 µl of 1% tween and 0.1 µl of Proteinase K). Buffer and nematode were frozen at –20°C for 20 min and then immediately incubated at 65°C for 1 h, followed by 10 min at 95°C. The lysates were cooled on ice, and then centrifuged (2 min, 9000 *g*) and 2 µl of supernatant was used for PCR. A fragment of rDNA containing the internal transcribed spacer regions (ITS1, 5.8S, ITS2) was amplified using primers 18S: 5'-TTGATTACGTCCCTGCCCTTT-3' (forward), and 28S: 5'-TTTCACTCGCCGTTACTAAGG-3' (reverse) (Vrain *et al*., 1992). The other fragment containing D2-D3 regions of 28S rDNA was amplified using primers D2F: 5'-CCTTAGTAACGGCGAGTGAAA-3' (forward) and 536: 5'-CAGCTATCCTGAGGAAAC-3' (reverse) (Nguyen, 2007). The PCR master mix consisted of ddH_2O 7.25 µl, 10 × PCR buffer 1.25 µl, dNTPs 1 µl, 0.75 µl of each forward and reverse primers, polymerase 0.1 µl and 1 µl of DNA-extract. The PCR profiles were used as follows for ITS: 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 60 s, 50°C for 60 s, 72°C for 60 s and a final extension at 72 °C for 7 min (Nguyen, 2007) and for 28S rDNA: 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s and a final extension at 72°C for 10 min (Mráček *et al*., 2006). PCR was followed by electrophoresis (45 min, 120 V) of 2 µl of PCR product in a 1% TAE buffered agarose gel stained with ethidium bromide (20 µl ETB per 100 ml of gel).

PCR-products were sequenced and the sequences were edited and deposited in the GenBank NCBI database under accession numbers KJ950291 (ITS sequence, strain NCR), KJ950292 (D2-D3 sequence, strain NCR) and KJ950293 (D2-D3 sequence, strain Dok83). ITS sequence of the strain Dok83 (KJ818295) and ITS and D2D3 sequences of the strains All (AY230164, AF331900) and DD136 (HM140694, HM140688) were downloaded from the GenBank NCBI database. An alignment of our samples together with sequences of the species of the '*carpocapsae*' group, including other isolates of *S. carpocapsae* present in GenBank NCBI database, was produced for each amplified DNA-region using default ClustalW parameters in MEGA 5.05 (Tamura *et al*., 2011). Alignments were optimised manually in BioEdit (Hall, 1999) and dubious sequences (*e.g*., those with marked differences only at the edges) were removed. Pairwise distances between species of the '*carpocapsae*' group were computed using MEGA (Tamura *et al*., 2011). Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding.

The phylogenetic trees were obtained by the Minimum Evolution method (Rzhetsky & Nei, 1992) in MEGA 5.05 (Tamura *et al*., 2011). Representatives of other Steinernematid groups *S. riobrave*, *S. abbasi*, *S. glaseri*, *S. feltiae*, *S. texanum*, *S. litorale*, *S. affine*, *S. intermedium* and *S. sichuanense* were added to illustrate the position of the '*carpocapsae*' group within the family and *Caenorhabditis elegans* was used as the outgroup taxon. The Minimum Evolution trees were searched

using the Close-Neighbour-Interchange (CNI) algorithm (Nei & Kumar, 2000). The Neighbour-joining algorithm (Saitou & Nei, 1987) was used to generate the initial trees. The evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000) and are expressed as the number of base differences per site.

RESULTS

IJ head and neck region (Figs 1H, 2A-C). The shape of head varies in its lip region in the steinernematid species. The fore part can be finely rounded to flattened. In the neck region, the head is continuous or slightly offset from the body contour. However, a detailed description of the *S. carpocapsae* head has not yet been published. Stanuszek (1974) reported the head of *S. carpocapsae* (erroneously identified as *S. feltiae*) was narrow, elongated, separated with lateral and ventral constriction. Sturhan (pers. comm.) described it as rather narrow, continuous and hemispherically rounded. This description seems to be characteristic for all species of the '*carpocapsae*' group. Lens-like hemizonid is between the nerve ring and terminal bulb beginning.

IJ lateral fields (Figs 2D-H). Lateral field represents an important morphological character. Procedures of IJ preparation for a scanning electron microscopy may result in some cuticular artifacts and therefore the structure of the lateral field must be studied carefully. In our observation, all strains possessed six ridges (seven lines) with the less prominent central pair, the same number as reported by Poinar (1967). The lateral fields begin in the neck region as a single depression shortly splitting in three depressions. From these depressions the first pair of ridges raises with a wide flat space between them. In the flat space the remaining ridges increase gradually up to six. In the tail portion the two central pairs merge before the anus, whereas the prominent lateral pair leads to its tip.

IJ hyaline layer and %H (Fig. 1G). Valuable taxonomical characters recommended by Dr Sturhan (pers. comm.) that, unfortunately, are often forgotten in recent species descriptions. In *S. carpocapsae*, the hyaline portion is not well seen in a LM. In exsheathed IJ the length of layer (measured from the tip of hypodermal lining) varied between 19.1 and 28.7 µm depending on the body length. Longer IJ had longer hyaline layer, but the ratio %H, which varied from 40 to 44, was not influenced.

IJ tail, ratio c' and %E. Ratio c' (tail length/tail width) is an often overlooked and little used character that may separate species quite precisely, even within one group. For example, for '*carpocapsae*' group species, it varies from 3.1 to 4.6 (Phan *et al.*, 2014). In our measurements and calculations, the tail length varied from 48 to 57 µm and when divided by the tail width, the average ratio c' of our four isolates varied from 4.0 (3.8-4.1) in the Swiss strain to 4.2 (3.9-4.4) in the Agriotos strain. In Nguyen *et al.* (2007), the ratio %E is reported to be 60 (54-66). By contrast, our results varied from 71 (59-82) in the DD 136 strain to 79 (74-83) in the Swiss strain.

Female tail and vulvar structure (Figs 1A-D, 3C & D). Female tail morphology provides two characters valuable in steinernematid taxonomy. Small papilla-like protuberances (mucrons) in the first generation females vary in number (from 0 to 4) and arrangement. Postanal swelling may absent or form slight, moderate or prominent protrusion. In *S. carpocapsae* the blunt tail possesses characteristic terminal conical sharply ended peg and no other minute protuberances. Postanal swelling not or very slightly developed. Second generation females possess conical tail without postanal swelling and with characteristic depression (All). In both generations, the rounded vulva is slightly protruding from the body contour.

Male mucron (Figs 1E & F, 3E-H). Male tail with a terminal mucron, either filamentous or spine-like, belongs to the important taxonomical characters. Generally in steinernematids, the mucron can be missing or present. Usually, if the mucron is developed, the first generation possesses a shorter mucron compared with the second generation, which is much longer. Stanuszek (1974) reported similar average length of the *S. carpocapsae* (erroneously identified as *S. feltiae*) mucron when compared first and second generations, 2.3 *vs* 2.4 µm, respectively. In our measurements, in all generations only the minute mucron was developed and there was a little size difference between the first and second (other) generations, 2.2 *vs* 2.6 µm, respectively. Ratios % GS and % SW were comparable to previously published data.

Molecular characterisation. ITS region. For the ITS region, the variability among different *S. carpocapsae* strains ranged from 0-1.1% with the exception of *S. carpocapsae* C101 (EU914605), which differed from other strains by 1.2-2.4% (Table 2). Such intraspecific variation in the ITS region is comparable to other steinernematid species (Spiridonov *et al.*, 2004). The distance between *S. carpocapsae* strains and the closest species *S. sasonense* was between 4.3-6% (Table 2). All *S. carpocapsae* strains formed a highly supported monophyletic group (Fig. 4). The general topology

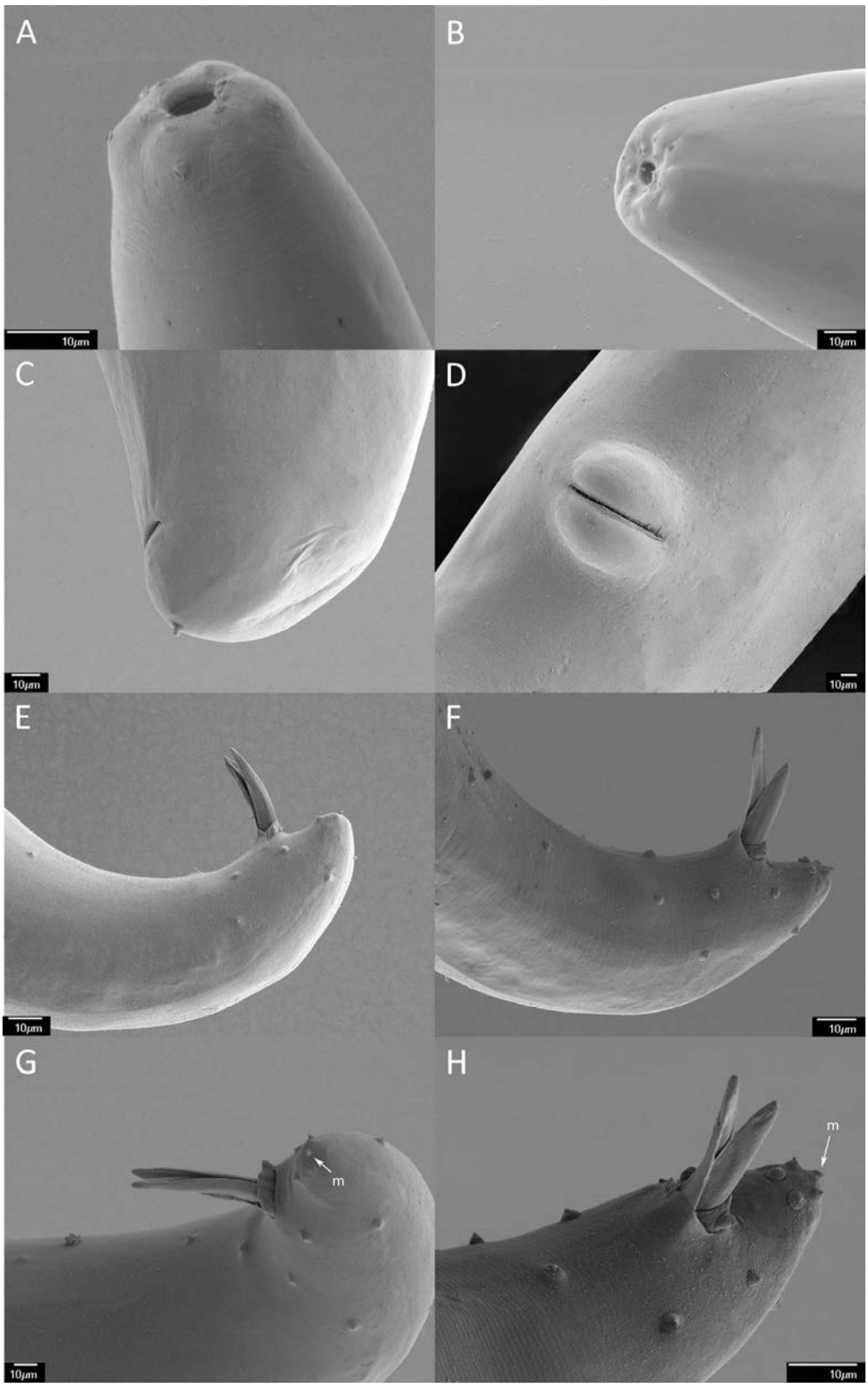

Fig. 3. *Steinernema carpocapsae* (scanning electron microscopy). A-D first generation female: A & B – head portion with labial and cephalic papillae, C – tail with conical peg, D – vulval portion; E-H males; E & F lateral view, G – ventrolateral view, H – ventral view. Abbreviation: m – mucron.

Table 1. Comparative morphometrics of different *Steinernema carpocapsae* strains (IJ – infective juvenile and M1 – first generation male). Data are in the form: mean (range).

Strain	IJ BL	IJc'	IJ%D	IJ%E	IJ%H	LF ridges	M 1[s] %GS	M 1%SW
DD 136	525 (404-606)	4.1 (3.8-4.6)	33 (28-35)	71 (59-82)	37 (33-42)	6	82 (79-88)	155 (148-166)
All	594 (545-626)	4.1 (3.9-4.5)	32 (29-34)	73 (69-78)	38 (34-44)	6	79 (79-79)	Yy (143-156)
Agriotos	566 (485-626)	4.2 (3.9-4.4)	33 (29-36)	77 (69-84)	42 (40-44)	6	82 (77-86)	150 (144-177)
Dok83	626 (545-667)	4.0 (3.8-4.1)	35 (32-37)	79 (74-83)	39 (36-41)	6	77 (71-80)	161 (152-168)
Pieridarum [x]	556 (465-631)	3.9	38	81			60	173
carpocapsae [y]	558 (438-650)[y]	4.6[z]	26 (23-28)[y]	60 (54-66)[y]	42[z]	6[y]	72[y]	151[y]

[s] spicules measured along the chord; [x] recalculated, according to Stanuszek (1974); [y] from Nguyen *et al*. (2007); [z] from Phan *et al*. (2014). BL = body length, c' = tail length divided by tail width, %D = length to excretory pore divided by pharynx length, %GS = gubernaculum length divided by spicule length, %E = length to excretory pore divided by tail length, %H = hyaline divided by tail length, LF – lateral field, %SW = spicule length divided by tail width.

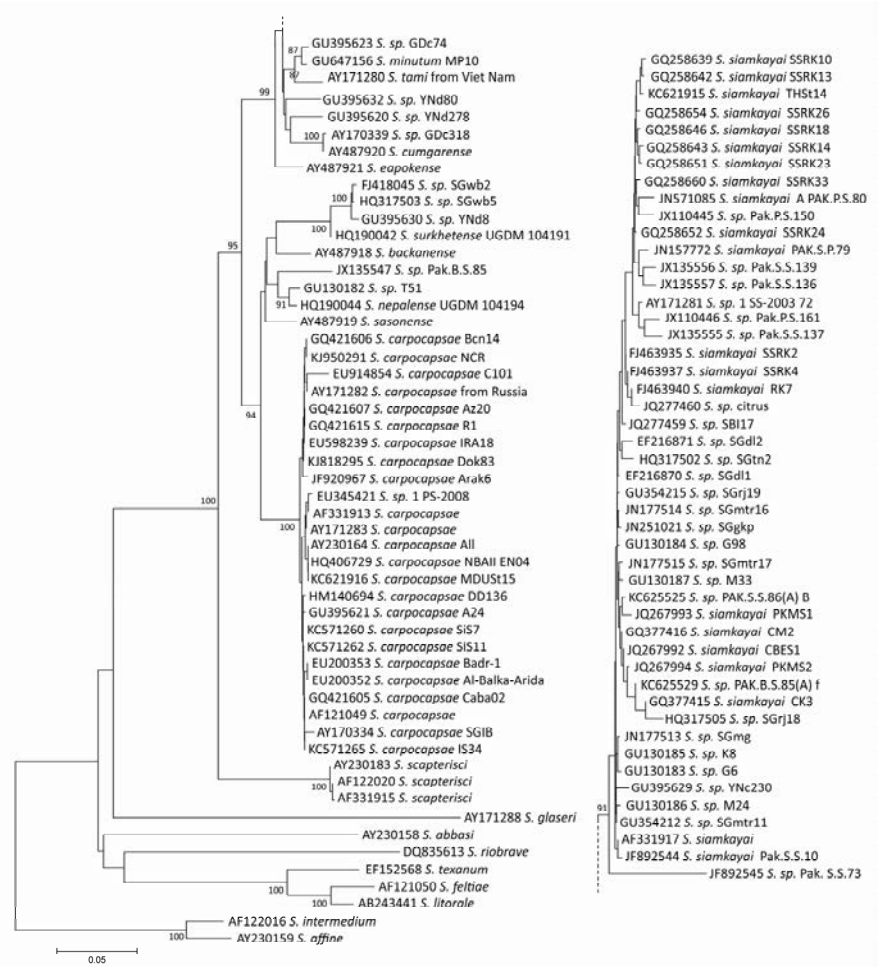

Fig. 4. Phylogenetic relationships of the species of the '*carpocapsae*' group and other selected steinernematid species based on analysis of ITS rDNA regions. *Steinernema affine* and *S. intermedium* were used as outgroup taxa. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches. Branch lengths indicate evolutionary distances and are in the units of the number of base differences per site.

Table 2. Pairwise distances of ITS region between selected strains of *Steinernema carpocapsae* and other related species (percentage distances) (*Sc* = *S. carpocapsae*, *Ssa* = *S. sasonense*, *Ssc* = *S. scapterisci*).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	EU200353 *Sc* Badr-1																									
2	EU200352 *Sc* Al-Balka-Arida-1	0.0																								
3	AF121049 *Sc*	0.2	0.2																							
4	AF331913 *Sc*	0.4	0.4	0.1																						
5	AY170334 *Sc* SGIB	0.8	0.8	0.6	0.5																					
6	AY171282 *Sc* Russia	0.7	0.7	0.4	0.5	1.1																				
7	HM170694 *Sc* DD136	0.3	0.3	0.0	0.1	0.7	0.4																			
8	AY230164 *Sc* All	0.7	0.7	0.5	0.1	1.1	0.4	0.0																		
9	KJ818295 *Sc* DOK83	0.6	0.6	0.4	0.5	1.0	0.0	0.4	0.4																	
10	KJ950291 *Sc* NCR	0.7	0.7	0.5	0.7	1.0	0.1	0.5	1.0	0.1																
11	EU345421 *S. sp.* 1 PS-2008	0.5	0.5	0.3	0.1	0.9	0.4	0.0	0.3	0.6	0.8															
12	EU598239 *Sc* IRA18	0.7	0.7	0.4	0.5	1.0	0.0	0.4	0.4	0.0	0.1	0.4														
13	HQ406729 *Sc* NBAII EN04	0.7	0.7	0.5	0.1	1.1	0.4	0.0	0.0	0.4	1.0	0.3	0.4													
14	EU914854 *Sc* C101	2.0	2.0	1.7	1.7	2.4	1.2	1.6	1.7	1.3	1.2	1.7	1.3	1.7												
15	GQ421605 *Sc* Caba02	0.2	0.2	0.0	0.1	0.6	0.4	0.0	0.5	0.4	0.5	0.3	0.4	0.5	1.7											
16	GQ421606 *Sc* Bcn14	0.7	0.7	0.5	0.7	1.0	0.1	0.5	1.0	0.1	0.0	0.8	0.1	1.0	1.2	0.5										
17	GQ421607 *Sc* Az20	0.6	0.6	0.3	0.5	0.9	0.0	0.4	0.9	0.0	0.1	0.6	0.0	0.9	1.3	0.3	0.1									
18	GQ421615 *Sc* R1	0.6	0.6	0.3	0.5	0.9	0.0	0.4	0.9	0.0	0.1	0.6	0.0	0.9	1.3	0.3	0.1	0.0								
19	GU395621 *Sc* A24	0.5	0.5	0.2	0.4	0.8	0.7	0.3	0.7	0.6	0.7	0.5	0.7	0.7	2.0	0.2	0.7	0.6	0.6							
20	JF920967 *Sc* Arak6	0.9	0.9	0.7	0.9	1.1	0.4	0.8	1.3	0.4	0.5	1.0	0.4	1.3	1.6	0.7	0.5	0.4	0.4	0.9						
21	KC571260 *Sc* SiS7	0.2	0.2	0.0	0.1	0.6	0.4	0.0	0.5	0.4	0.5	0.3	0.4	0.5	1.7	0.0	0.5	0.3	0.3	0.1	0.7					
22	KC571262 *Sc* SiS11	0.2	0.2	0.0	0.1	0.6	0.4	0.0	0.5	0.4	0.5	0.3	0.4	0.5	1.7	0.0	0.5	0.3	0.3	0.1	0.7	0.0				
23	KC571265 *Sc* IS34	0.2	0.2	0.0	0.1	0.6	0.4	0.0	0.5	0.4	0.5	0.3	0.4	0.5	1.7	0.0	0.5	0.3	0.3	0.2	0.7	0.0	0.0			
24	KC621916 *Sc* MDUSt15	0.8	0.8	0.5	0.1	1.1	0.4	0.0	0.0	0.4	1.0	0.3	0.4	0.0	1.7	0.5	1.0	0.9	0.9	0.8	1.3	0.5	0.5	0.5		
25	AY487919 *Ssa*	4.5	4.5	4.3	4.5	5.0	4.7	4.3	4.4	4.7	4.9	4.3	4.7	4.4	6.0	4.3	4.9	4.7	4.7	4.6	4.8	4.3	4.3	4.3	4.4	
26	AF122020 *Ssc*	11	11	11	12	11	12	12	12	12	11	12	12	12	13	11	11	11	11	11	11	11	11	11	12	12

Table 3. Pairwise distances of D2D3 region between selected strains of *Steinernema carpocapsae* and other related species (percentage distances) (*Sw* = *S. websteri*, *Sc* = *S. carpocapsae*, *Sa* = *S. anatoliense*, *Sh* = *S. huense*, *Ssu* = *S. surkhetense*, *Sn* = *S. nepalense*, *Ssc* = *S. scapterisci*).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 JF503100 *Sw* AS1																						
2 GU569058 *Sw* Alcazar	0.1																					
3 EF217323 *Sw* JCL006	0.0	0.1																				
4 EF217324 *Sw* JCL015	0.0	0.1	0.0																			
5 EF217325 *Sw* JCL030	0.0	0.1	0.0	0.0																		
6 EF217326 *Sw* JC1032	0.0	0.1	0.0	0.0	0.0																	
7 AY841762 *Sw*	0.0	0.1	0.0	0.0	0.0	0.0																
8 KJ950292 *Sc* NCR	0.0	0.1	0.0	0.0	0.0	0.0	0.0															
9 KJ950293*Sc* DOK83	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0														
10 HM140688 *Sc* DD136	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0													
11 AY169552 *Sc* BW	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2												
12 GU395640 *Sc* BW	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2											
13 GU395641 *Sc* A24	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0										
14 EU200356 *Sa*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.2	0.4	0.4									
15 GU569043 *Sa*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.2	0.4	0.4	0.0								
16 AY841761 *Sa*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.2	0.4	0.4	0.0	0.0							
17 HQ190043 *Ssu*	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	1.1	0.7	0.7	1.3	1.0	1.3						
18 HQ190045 *Sn*	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.5	1.4	1.4	1.8	1.4	1.4	2.0	1.7	2.0	0.7					
19 KF857582 *Sh*	1.5	1.3	1.4	1.4	1.4	1.4	1.4	1.4	1.6	1.6	1.8	1.4	1.4	2.1	1.7	2.1	1.1	1.5				
20 GU395646 *Ssc*	5.5	5.6	5.5	5.5	5.5	5.5	5.5	5.6	5.5	5.5	7.0	5.6	5.6	5.7	5.7	5.7	4.8	5.2	5.6			
21 AF331900 *Sc* All*	34	33	33	33	33	33	33	33	34	34	37	34	34	33	33	33	30	30	34	34		
22 EU598241 *Sc* IRA18*	34	34	34	34	34	34	34	34	34	34	37	34	34	34	34	34	31	32	35	34	0.0	
23 DQ145660 *Sc**	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	38	37	0.4	0.4

*sequence belongs to *Heterorhabditis*

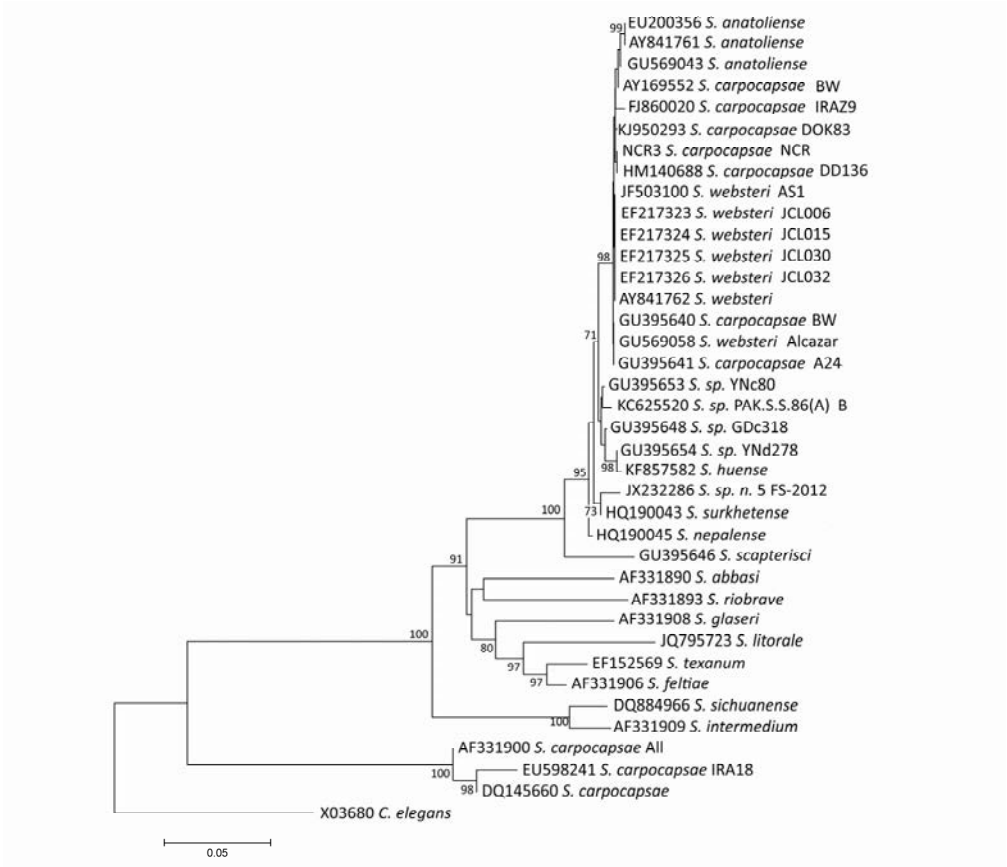

Fig. 5. Phylogenetic relationships of the species of the '*carpocapsae*' group and other selected steinernematid species based on analysis of D2D3 regions of the 28S rDNA. *Caenorhabditis elegans* was used as outgroup taxon. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches. Branch lengths indicate evolutionary distances and are in the units of the number of base differences per site.

with two well supported main clades and *S. scapterisci* occupying a basal position corresponds to the results of Phan *et al.* (2014). Monophyly of all the species represented by more than one population suggests that ITS region accurately characterises the species within the '*carpocapsae*' group.

D2-D3 expansion of the 28S rDNA. The variability among different *S. carpocapsae* strains ranged from 0-0.2%. Surprisingly, the D2-D3 sequences attributed to *S. anatoliense* and especially *S. websteri* showed a minimum distance from the sequences of *S. carpocapsae* strains (Table 3). The other closest species *S. surkhetense* was separated by 0.7-1.3%.

In the phylogenetic analysis based on this region (Fig. 5), *S. websteri* and *S. anatoliense* fell within the well supported group with *S. carpocapsae* strains. It thus seems that these nematodes do not represent independent evolutionary lines, and are conspecific with *S. carpocapsae*. Unfortunately, sequences of the ITS region or other markers are not available for these nematodes. Thus, the status of *S. websteri* and *S. anatoliense* seems to be doubtful and should be further examined.

Both the pairwise comparisons and phylogenetic tree further showed that D2-D3 sequences with accession numbers AF331900 (*S. carpocapsae*, strain All), EU598241 (*S. carpocapsae*, strain IRA18) and DQ145660 *S. carpocapsae*, strain All) belong in fact to the nematodes of the genus *Heterorhabditis*. Unfortunately, these sequences were used in some phylogenetic studies of the Steinernematidae family, causing placement of the '*carpocapsae*' group to the basal position. Studies based on the ITS region sequence usually support the basal position of '*affine*' group (*e.g.*, Spiridonov *et al.*, 2004). To prevent inappropriate usage, these sequences should be removed from the database or adequately renamed.

In any new species description of steinernematid nematodes, the sequences of both ITS and D2-D3 regions should be included.

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Резюме. Морфология энтомопатогенных нематод *Steinernema carpocapsae* описана недостаточно полно, и некоторые важные особенности этого вида остаются неизученными. Недостает и подробного молекулярно-таксономического исследования. *Steinernema carpocapsae* представляет собой типовую форму группы «*carpocapsae*», поэтому дополнение первоописания данными о гиалиновой части и других морфометрических особенностях хвостового конца личинок, наличии выступов на оконечности хвостового конца самок первого поколения представляются необходимыми для понимания взаимоотношений в этой группе штейнернематид. Были получены последовательности ITS и D2-D3 участков рибосомальной ДНК и проведено их сравнение с другими последовательностями *S. carpocapsae*, имеющимися в ГенБанке (NCBI). Было исследовано 4 изолята *S. carpocapsae* (из США – DD136 и All, России – Agriotos, а также Швейцарии – DOK83). Морфологические особенности и морфометрия оказались сходными у всех изолятов. Инвазионные личинки характеризуются зауженным и полусферическим головным концом и контурами, продолжающими контуры тела; латеральными полями с 6-ю ребрами (семь линий), с менее различимой центральной парой. Гиалиновая часть хвостового конца составляет 40-44% от его общей длины, индекс c' варьирует от 4.0 (3.8-4.1) до 4.2 (3.9-4.4), а индекс %E от 71 (59-82) до 79 (74-83). Самки первого поколения характеризуются тупой оконечностью хвостового конца с терминальным конически завершающимся отростком (без дополнительных мелких выступов). Округлые губы вульвы слабо выступают над поверхностью тела. У самцов всех поколений имеется небольшой мукрон, лишь незначительно различающийся по длине между 1-м и 2-м поколениями. Оба генетических меркера показывают видовую самостоятельность и целостность *S. carpocapsae* как вида, выявляя лишь низкую внутривидовую нуклеотидную изменчивость. Сомнительным, правда, остается видовой статус близких видов *S. anatoliense* и *S. websteri*.