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179

SEM and systematic studies of *Steinernema abbasi* Elawad et al., 1997, and *S. riobrave* Cabanillas et al., 1994 (Rhabditida: Steinernematidae)

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Abstract

SEM studies of the two nematodes S*teinernema abbasi* and *S. riobrave* revealed that they have two horn-like structures on the labial region similar to those of *S. bicornutum, S. ceratophorum, S. pakistanense,* and *S. thermophilium*. The structures were not mentioned in the original descriptions. In addition , *S. riobrave* has the twelfth pair of genital papillae at the edge of the cloaca. The papillae at this position were reported only in *S. scapterisci.* The formula of the lateral field pattern of *S. abbasi* is 2, 6, 8, 7, 6, 2, and that of *S. riobrave* is 2, 7, 8, 6, 2. Phylogenetic trees from sequences of D2/D3, and ITS regions of *Steinernema* species show that the four species *S. abbasi, S. bicornutum, S. ceratophorum,* and *S. riobrave* comprise a monophyletic group. These relationships support the present morphological studies.

Key words: Entomopathogenic nematodes, D2/D3, ITS, morphology, *S. abbasi*, *S. riobrave*, systematics

Introduction

Morphological studies of *Steinernema abbasi* Elawad, Amad & Reid, 1997 and *S. riobrave* Cabanillas, Poinar & Raulston, 1994 showed that there are some structures which are important in taxonomy but were not reported when the nematodes were described. Since *S. abbasi* may have a good potential for biological control of insects in subtropical region, and *S. riobrave* may be used to control insect pests such as the citrus root weevils, mole crickets etc, accurate identification of these nematodes becomes important. The purpose of this paper is to present SEM structures of the two nematode species. Some of these structures are important for their differentiation.

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Materials and methods

Scanning electron microscopy: The population of *S. abbasi* used in these studies was obtained from S. Elawad, University of Reading, UK; *S. riobrave* from E. Cabanillas, USDA, Weslaco, Texas, USA. The nematodes were maintained in last instar larvae of the greater wax moth, *Galleria mellonella* (L). To obtain nematodes of different stages, ten *G. mellonella* were exposed to 5000 infective juveniles in a petri dish (100 x 15 mm) lined with two moistened filter papers. First-generation adult nematodes were obtained by dissecting infected insects 3 to 4 days after the insects died. Third-stage infective juveniles (IJ) were obtained when they emerged from the cadavers (7-10 days after the insect died). The methods reported by Nguyen and Smart (1995, 1997) were used in this study. Twenty males and females, and 50 infective juveniles were used for observation.

Phylogenetic analysis: In order to show that the new finding is in agreement with phylogenetic systematic, phylogenetic trees were created using sequences of D2/D3 and the ITS regions of *Steinernema* species available in the GenBank. ITS regions of *S. abbasi*, and *S. rarum* USA strain were sequenced by us. The two species, *S. pakistanense* and *S. thermophilum* that have horn-like structures could not be obtained for this study.

Alignment of multiple sequences: The processes of alignment used in these studies were reported by Nguyen et al. (2001). The sequences of the D2/D3 region used for the alignment were from: *S. abbasi* (accession # AF331890), *S. affine* (AF331899), *S. arenarium* (AF331892), *S. bicornutum* (AF331904), *S. carpocapsae* (AF331900), *S. ceratophorum* (AF331888), *S. cubanum* (AF331889), *S. feltiae* (AF331906), *S. glaseri* (AF331908), *S. intermedium* (AF331909), *S. karii* (AF331902), *S. kraussei* (AF331896), *S. kushidai* (AF331897), *S. longicaudum*, China strain (AF331894), USA strain (AF331901), *S. monticolum* (AF331895), *S. oregonense* (AF331891), *S. puertoricense* (AF331891), *S. rarum*, Argentina strain (AF331905), USA strain (AY253296), *S. riobrave* (AF331893), *S. scapterisci* (AF331898), *S. siamkayai* (AF331907), and *Panagrellus redivivus* (AF331910).

ITS regions used for the alignment were from: *S. abbasi* (AY248749), *S. affine* (AF331912), *S. bicornutum* (AF121048), *S. carpocapsae* (AF121049), *S. ceratophorum* (AF440765), *S. diaprepesi* (AF440764), *S. feltiae* (AF121050), *S. glaseri* (AF1220115), *S. intermedium* (AF122016), *S. monticolum* (AF122017), *S. neocurtillae* (AF122018), *S. oregonense* (AF122019), *S. riobrave* (AF192988), *S. scapterisci* (AF122020), *Steinernema diaprepesi*. (AF122021), and *S. siamkayai* (AF331917).

Phylogenetic analysis: Maximum parsimony analyses were performed using D2/D3 and ITS alignments. Maximum parsimony trees were constructed using Paup* 4.0b10 (Swofford, 2001). All steps suggested by Nguyen et al. (2001) were followed in this analysis. *Panagrellus redivivus* was used as the out group taxon for the D2/D3 region (Stock et al. 2001), and *S. intermedium* for the ITS regions (Nguyen et al. 2001).

Result and Discussion

Steinernema abbasi

Males: Head region slightly swollen. Six labial, and four cephalic papillae prominent. Amphids small (Fig. 1C). Posterior region with one single and 11 pairs of genital papillae as described for most *Steinernema* species (Fig. 1D,E). Spicule head wider than long, ventrally directed; shaft present but short; velum present; spicule tip conoid, bluntly pointed (Fig. 1F,G).

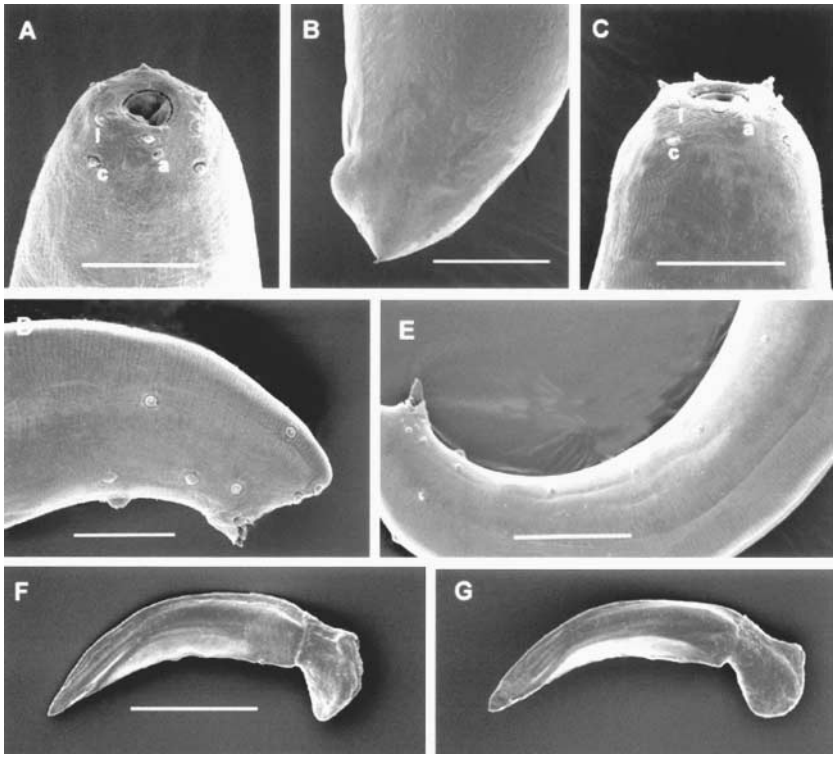

FIGURE 1. SEM of female and male of *S. abbasi*. A, anterior region of a female showing mouth, labial papillae (l), amphid (a) and cephalic papillae (c); B, posterior region of a female showing postanal swelling. C, anterior region of a male showing mouth, labial papillae (l), amphid (a) , and cephalic papillae (c). D, posterior region of a male showing genital papillae. E, Posterior region of a male showing preanal papillae. F,G, variation in shape of spicules. Scales: A = 10 µm; B = 30 µm; C = 10 µm; D = 20 µm; E = 37.5 µm; E and F = 23 µm.

Females: Head region of female as reported for other *Steinernema* species. Six labial and four cephalic papillae prominent, amphids rounded, small but obvious (Fig. 1A). Tail conoid with ventral postanal swelling and prominent mucron (Fig. 1B).

Infective juveniles: Labial region of exsheathed IJs with two horn-like structures (Fig. 2A,B) are difficult to see on ensheathed IJs, especially soon after the IJs emerging from

Galleria mellonella cadaver (Fig. 2C,D). Perhaps, this is the reason why the structures were not reported in the original description. Lateral field pattern begins anteriorly with one line usually at the third or fourth annule (Fig. 3A). At annules 14 or 15, two other lines appear to form 2 ridges. Near excretory pore, the lateral field changes gradually from two to 6 (Fig. 3B). A short distance posteriorly, the two external ridges divide into two (Fig. 3B) making a total of 8 ridges [=9 incisures, 8 incisures was reported in the original description (Alawad et al., 1997)], the maximum number of the lateral field (Fig. 3B,D,E). Sometimes, the number of ridges in lateral field is not very clear as in Fig. 3C (ridge second from the left is fainted). A short distance anterior to anus (Fig. 3E, arrow), the number of ridges reduces to 7. From that point, the two submarginal ridges become large, then just anterior to phasmid, the number of ridges in lateral field becomes 6 (Fig. 3F). Posterior to the phasmid, the lateral field changes into two large ridges (Fig. 3 F). In general, the formula of lateral field pattern (Adams & Nguyen, 2002) of *S. abbasi* is 2, 6, 8, 7, 6, 2.

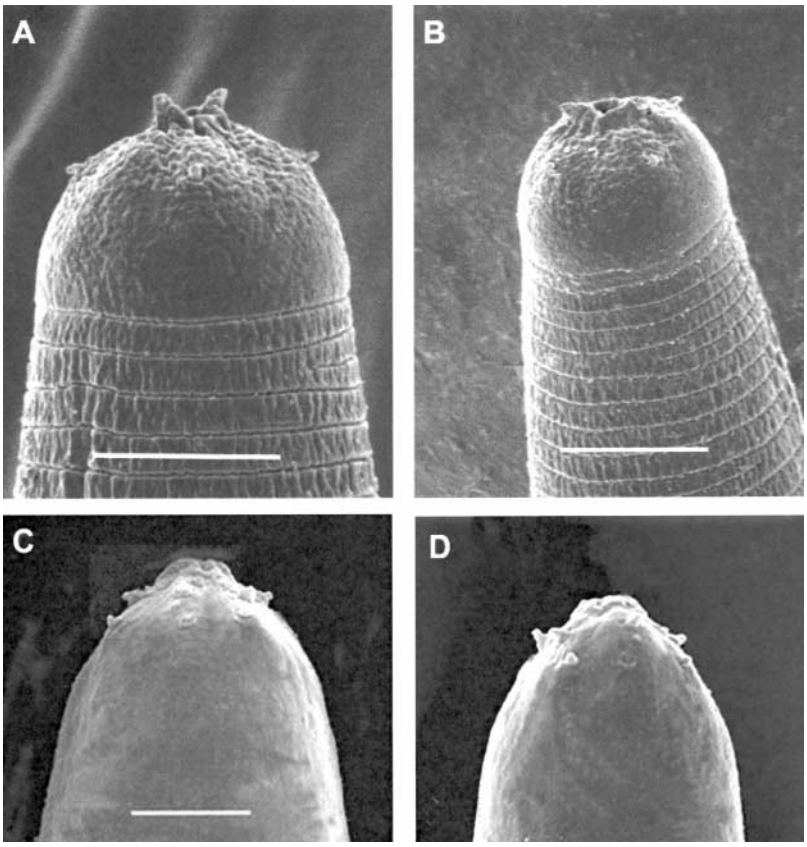

FIGURE 2. SEM of anterior region of infective juveniles (IJ) of *S. abbasi*. A,B, labial region of exsheathed IJ showing 2 horn-like structures in labial region. C,D, labial region of ensheathed infective juveniles showing labial region without 2 horn-like structures. Scales: A = 4.3 μm; B = 5.0 μm; C,D = 3.0 μm.

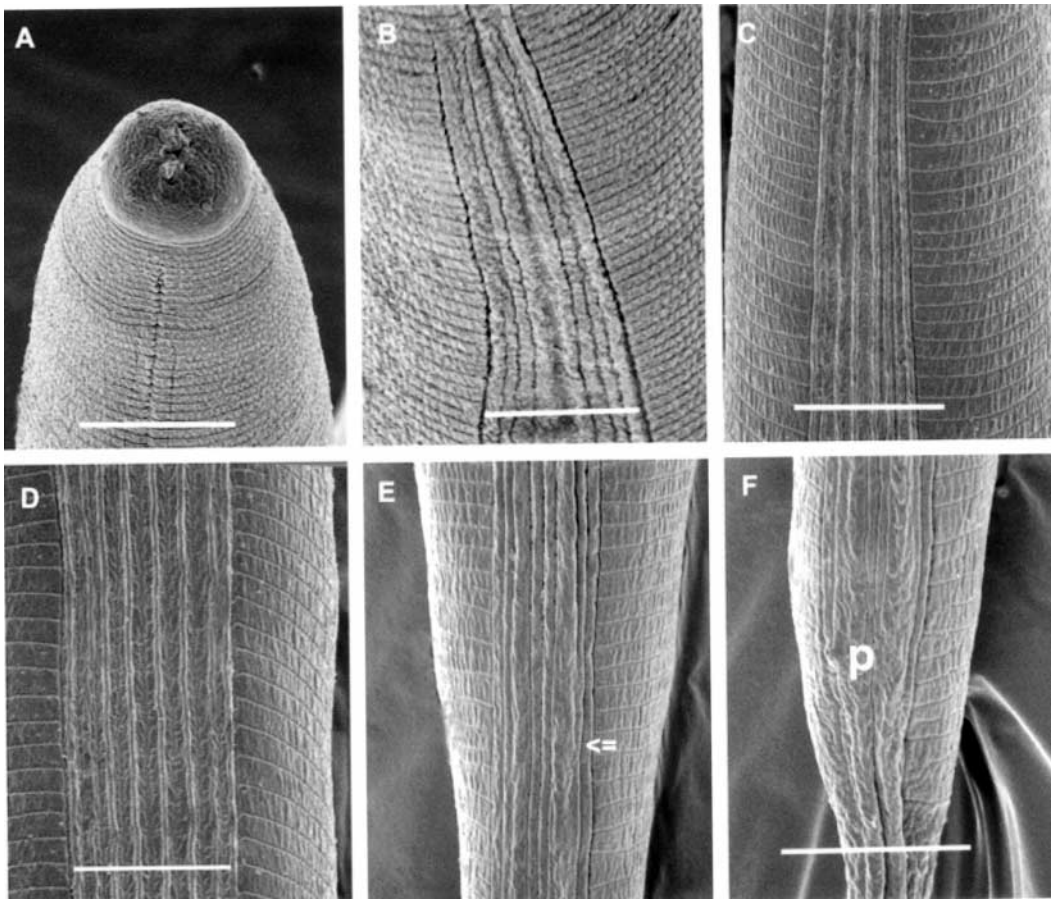

FIGURE 3. SEM of lateral field of infective juveniles of *S. abbasi*. A, anterior region of an infective juvenile showing the beginning of lateral field. B, anterior region of an infective juvenile showing the changes of lateral field from 2 to 6 then to 8 ridges. C, lateral field with 8 ridges but the second ridge from the left is fainted. D, lateral field with 8 ridges. E, lateral field with 8 ridges then 7. F, posterior part of lateral field showing phasmid (p) and the change of ridge number from 6 to 2. Scales: A=7.5 µm; B=5 µm; C=7.5 µm; D=6.6 µm; E,F=6.7 µm.

Steinernema riobrave

Males: Anterior end similar to that of female in Fig. 4A but smaller. Posterior region curved, having one single and 12 pairs of genital papillae in which one pair is located at the edge of cloaca (Fig. 4B-E). This pair of papillae has been observed only in *S. scapterisci* (Nguyen & Smart, 1992) and *S. riobrave*.

Females: Female face view is very close to other *Steinernema* species with two amphids, four cephalic papillae and 6 prominent labial papillae (Fig. 4A).

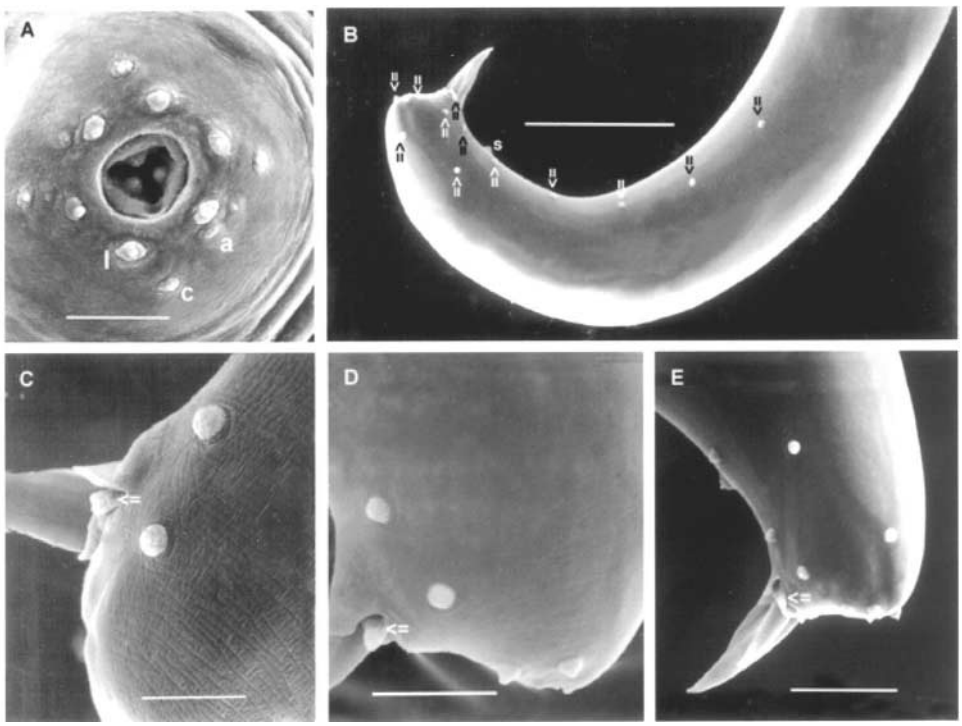

FIGURE 4. SEM of female and males of *S. riobrave*. A, labial region showing the mouth, labial papillae (l), amphid (a), and cephalic papillae (c). B, posterior region of *S. riobrave* showing 12 pairs (arrows) and a single (s) genital papillae. C-D posterior region of males showing the presence of a special pair of genital papillae at the edge of the cloaca (arrow). Scales: A = 7.5 μm; B = 60 μm; C = 8.6 μm; D = 10.0 μm; E = 17.6 μm.

Steinernema riobrave

Males: Anterior end similar to that of female in Fig. 4A but smaller. Posterior region curved, having one single and 12 pairs of genital papillae in which one pair is located at the edge of cloaca (Fig. 4B-E). This pair of papillae has been observed only in *S. scapterisci* (Nguyen & Smart, 1992) and *S. riobrave*.

Females: Female face view is very close to other *Steinernema* species with two amphids, four cephalic papillae and 6 prominent labial papillae (Fig. 4A).

Infective juveniles: As in *S. abbasi*, labial region of exsheathed IJ with two horn-like structures (Fig. 5A,B). These structures on ensheathed IJs are not observed, especially soon after the IJs emerge from *G. mellonella* cadavers (Fig. 5C,D). Lateral field pattern begins anteriorly with one line usually at the second or third annule (Fig. 5B). At annules 8 or 9, two other lines appear to form 2 ridges (Fig, 6A). Near excretory pore, the lateral field changes gradually from two to seven (Fig. 6A,B). A short distance posteriorly, the central ridge divides into two (Fig. 6C) making a total of 8, the maximum number of the

lateral field. Near anus, two dorsal marginal ridges reduce in width then disappear making a total of 6 ridges (Fig. 6D). Finally near phasmid, two submarginal ridges on both sides fused, the ventral external ridge, and two central ridges disappear forming two large ridges in the lateral field (Fig. 6E,F). In general, the formula of lateral field pattern of *S. riobrave* is 2, 7, 8, 6, 2. Phasmids present, ventral to lateral field (Fig. 6E,F). The number of ridges in the lateral field was not reported in the original description (Cabanillas et al. 1994).

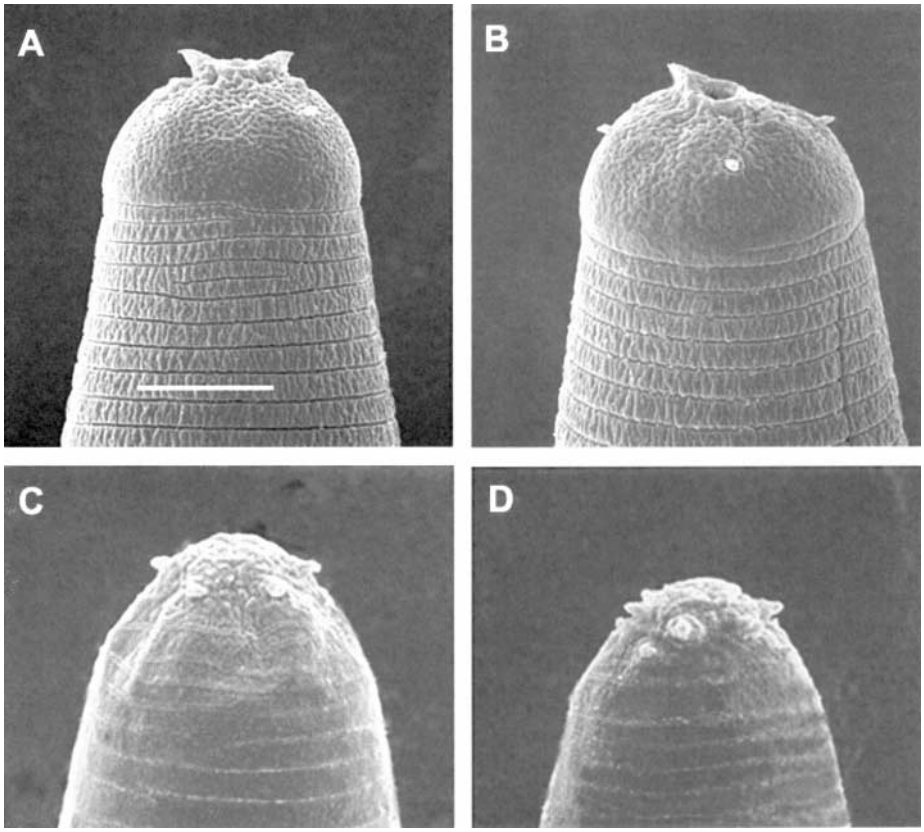

FIGURE 5. SEM of anterior region of exsheathed and ensheathed infective juveniles of *S. riobrave*. A,B, labial region of exsheathed IJ showing 2 horn-like structures in labial region. C,D, labial region of ensheathed infective juveniles showing labial region without 2 horn-like structures. Scales: A - D = 4.3 μm.

Phylogenetic analysis

Phylogenetic relationships between species of *Steinernema* are shown in Fig. 7 and 8. In both trees, the four nematode species, *S. abbasi*, *S. bicornutum*, *S. ceratophorum*, and *S. riobrave* comprise a phylogenetic group. The presence of horn-like structures on the labial region of these four species constitutes an apparent synapomorphy that adds additional support for this relationship.

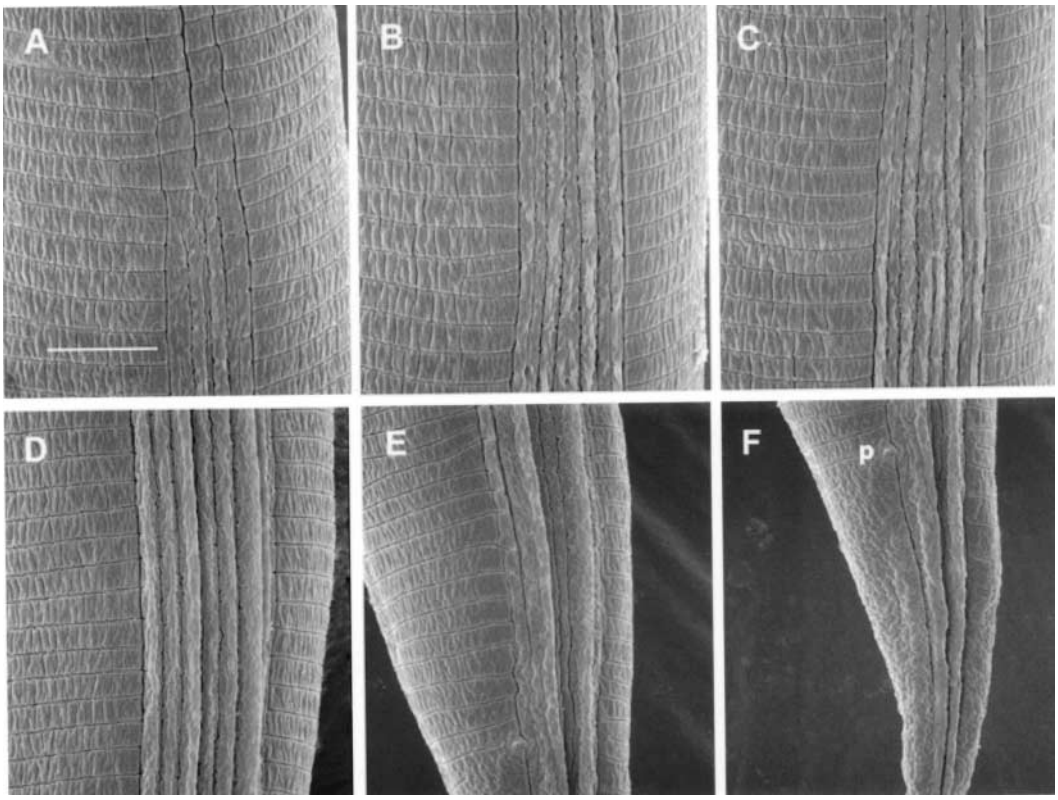

FIGURE 6. SEM of lateral field of infective juvenile of *S. riobrave*. A, anterior region of an infective juvenile showing the changes of lateral field pattern from 2 to 7. B,C, anterior region of an infective juvenile showing the changes of lateral field from 7 to 8 ridges. D, lateral field changes from 8 to 6 ridges. E,F, posterior region of lateral field of infective juvenile showing phasmid (p), and the change of the ridge number from 6 to 2. Scales: A-F= 4.3 μm.

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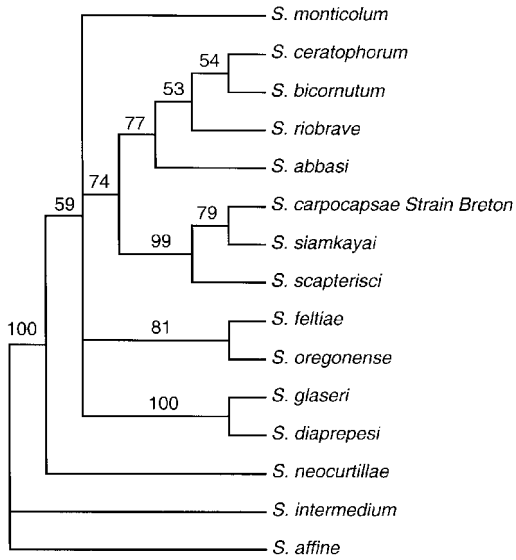

FIGURE 7. Phylogenetic relationships between 15 species of *Steinernema* with bootstrap analysis (500 replicates) of ITS regions. Note that the four species with horn-like structures (*S. abbasi, S. bicornutum, S. ceratophorum, S. riobrave*) form a monophyletic group. The numbers represent bootstrap proportions.

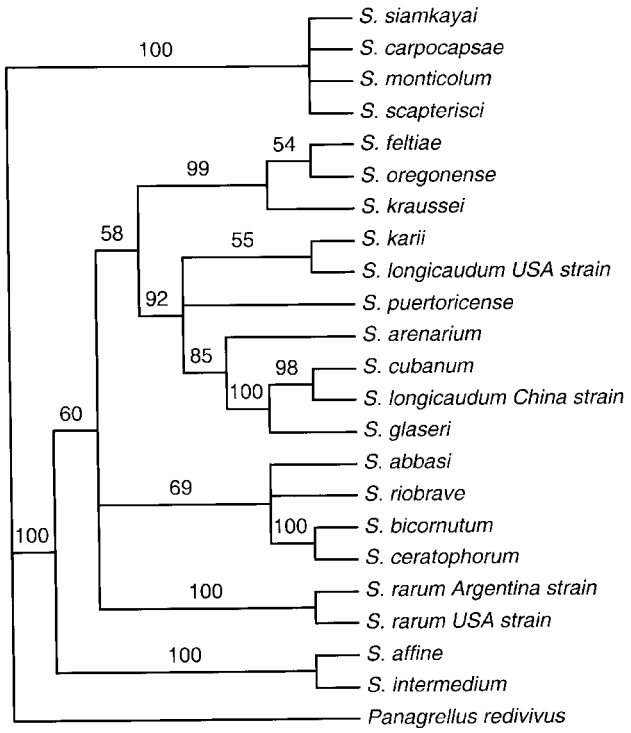

FIGURE 8. Phylogenetic relationships between 22 species of *Steinernema* with bootstrap analysis (500 replicates) of D2/D3 regions. A heuristic search was conducted using maximum parsimony analysis (PAUP). Note that the four species with horn-like structures (*S. abbasi, S. bicornutum, S. ceratophorum, S. riobrave*) comprise a monophyletic group. The numbers represent bootstrap proportions.

References

Adams, B.J., & Nguyen, K.B. (2002) Taxonomy and systematics. In: Gaugler, R. (ed) *Entomopathogenic Nematology.* CABI Publishing, Oxon, UK**,** pp. 1-33.

Nguyen, K.B. & Smart, G.C. Jr. (1992) Addendum to the morphology of *Steinernema scapterisci. Journal of Nematology,* 24, 478-481.

Nguyen, K.B. & Smart, G.C. Jr. (1995) Scanning electron microscope studies of *Steinernema glaseri* (Nematoda: Steinernematidae). *Nematologica,* 41, 183-190.

Nguyen, K.B. & Smart, G.C. Jr. (1997) Scanning electron microscope studies of spicules and gubernacula of *Steinernema* spp. (Nemata: Steinernematidae). *Nematologica*, 43, 465-480.

Nguyen, K.B., Maruniak, J. & Adams, B.J. (2001) The diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of Steinernema. *Journal of Nematology,* 33, 73-82.

Stock, S.P., Campbell, J.F. & Nadler, S.A. (2001) Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *Journal of Parasitology,* 87, 877-889.

Swofford, D.L. (2001) PAUP*. Phylogenetic Analysis Using Parsimony. (*and other methods), vers 4.0b8. Sunderland, MA: Sinauer Associates.

