Steinernema feltiae (Rhabditida: Steinernematidae) from hilly areas of Kashmir valley, India with a note on its geographical distribution

Tarique Hassan Askary[1], Aashaq Hussain Bhat[2], Mohammad Jamal Ahmad[3], Ashok Kumar Chaubey[2] and Sergei E. Spiridonov[4]

[1]Division of Entomology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Wadura, Sopore-193201, Jammu and Kashmir, India
[2]Nematology Laboratory, Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, 250004, India
[3]Division of Entomology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar-190025, Jammu and Kashmir, India
[4]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii Prospect 33, 119071, Moscow, Russia
e-mail: tariq_askary@rediffmail.com

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Summary. Four strains of *Steinernema feltiae* were isolated from agricultural fields in Anantnag district, the hilly areas of Pir Panjal region of Jammu and Kashmir Union territory of India. Phylogenetic analysis of the Internal Transcribed Spacer rDNA sequences revealed that three isolates belong to the well defined 'Himalayan clade' of *S. feltiae*, whereas one isolate belongs to the cosmopolitan group of haplotypes of this species. Morphometric data for one of these Himalayan isolates are provided and the phylogeography of *S. feltiae* is discussed. Finally, analysis of the GenBank records has shown that *S. feltiae* is ubiquitous in most geographical regions of the globe.

Key words: cosmopolitan, entomopathogenic nematode, first report, ITS rDNA, Himalayan, morphology, phylogeography.

The entomopathogenic nematodes (EPN) of the family Steinernematidae are an intensively studied group of soil inhabiting nematodes (Labaude & Griffin, 2018). They are considered effective biocontrol agents of insect pests (Lacey *et al.*, 2015). These nematodes are also interesting as an example of evolutionary progress of a group of soil rhabditids, which developed a peculiar life-style through symbiosis with associated enterobacteria (Dziedziech *et al.*, 2020). The nematodes of the genus *Steinernema* Travassos, 1927 are nearly cosmopolitan, and their widespread presence, especially in harsh conditions like polar or mountain ecosystems, is especially useful as it can reveal the adaptive potential of these organisms and the evolutionary history of this taxon. *Steinernema feltiae* Filipjev, 1934 is probably the most promising subject for such studies. Although the majority of *Steinernema* species have a limited range, the representatives of this species are found on almost all continents with developed vegetation (Hunt & Nguyen, 2016). In temperate regions, *S. feltiae* is very common in field and grassland soils (Sturhan & Liskova, 1999; Adams *et al.*, 2006). The association of this species with cultivated soil could partially explain its nearly global distribution, but studies in some regions of the planet demonstrate that these nematodes can be found in locations where neither agriculture nor signs of it in previous époques can be found (Tarasco *et al.*, 2011) (*e.g.*, on the mountain slopes in scree soils). Such preliminary observations invite further search for *S. feltiae* isolates in mountainous areas.

MATERIAL AND METHODS

Collection of soil samples. In order to isolate EPN, one hundred soil samples were collected from the rhizosphere of walnut trees and tomato plants grown in Anantnag district, the hilly areas of Pir

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Panjal Range of Kashmir valley (33°72' North, 75°14' East and 1,601 m above sea level), India. The soil samples were collected from agricultural fields using a trowel. Each sample contained 1,000 g soil, that constituted a mixture of five subsets of soil samples taken from a depth of 10-15 cm in case of tomato plants and 20 cm in case of walnut trees (Bhat *et al.*, 2020). The soil was clayey loam with a pH of 6.8-8.9. The predominant climate in the sampling areas is mild, generally warm and temperate monsoon, covered with snow during the winter season.

Soil sample analysis. Soil samples were brought to the laboratory, Division of Entomology, SKUAST-K. Rice moth *Corcyra cephalonica* eggs were obtained from Biological Control Laboratory of SKUAST-K, Shalimar campus, Srinagar. The eggs were spread on sterilised crushed maize grain filled in a wooden box having the size 15 × 30 × 45 cm and rearing of insect was done as described by Patel (2011). *Corcyra cephalonica* larvae were used to recover nematodes from soil samples by baiting technique (Bedding & Akhurst, 1975). The cadavers of *C. cephalonica* obtained from baits were rinsed with dH_2O, disinfected with 0.1% sodium hypochlorite and transferred to White traps (White, 1927) to obtain emerging infective juveniles (IJ). IJ thus collected were surface sterilised with 0.1% sodium hypochlorite and washed with dH_2O, and finally stored in tissue culture flasks in BOD incubator at 15°C for further studies (Bhat *et al.*, 2019). IJ, maximum 7 days old, were used in the experiments.

Nematode morphology and morphometry. Nematodes were reared on last instar larvae of the greater wax moth, *Galleria mellonella*. First and second generation nematodes were obtained by dissecting nematode infected cadavers 3rd to 4th and 5th to 6th days after infection, respectively. However, IJ were collected directly from White traps after their emergence. All the nematodes were heat-killed in Ringer's solution and fixed in triethanolamine formalin (Courtney *et al.*, 1955). Nematodes were dehydrated by the Seinhorst method (Seinhorst, 1959) and processed further as described by Bhat *et al.* (2017). Briefly, the nematodes were kept in pure glycerol. Adult males and females of the first and second generations as well as IJ were mounted separately in a drop of glycerol poured on a clean glass slide. Paraffin wax was used to seal and prevent the flattening of nematode specimens (Bhat *et al.*, 2020). The morphology and morphometric analysis of the specimens was conducted using light compound microscope (Magnus MLX) and phase-contrast microscope (Nikon Eclipse 50i). Morphometric analyses were carried out with the aid of in-built software of the phase-contrast microscope (Nikon DS-L1). The slides and live specimens of the recovered nematodes were maintained at the Division of Entomology, Sher-e Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar and also deposited in the Nematology Laboratory of the Department of Zoology, Chaudhary Charan Singh University, Meerut.

The terminology used for the morphology of pharynx and spicules/gubernaculum follow the proposals of Baldwin *et al.* (2004) and Abolafia & Peña-Santiago (2017), respectively. The formulae used to calculate ratios and percentages are presented in Table 1.

Molecular analysis of nematodes. Genomic DNA was isolated from IJ (n > 500) by using the Qiagen Blood and Tissue Analysis Kit (Qiagen, Germany) following the manufacturer's protocol. A fragment of the rDNA gene containing the ITS regions (ITS1, 5.8S, ITS2) and D2-D3 regions of the 28S rDNA gene was amplified using primers as given by Vrain *et al.* (1992) and Nadler *et al.* (2006), respectively. The PCR protocol for ITS rDNA amplification was followed as described by Bhat *et al.* (2018, 2019). Five µl PCR product was electrophoresed (45 min, 100 V) in 1% TAE (Tris-acetic acid-EDTA) buffered agarose gel stained with EtBr (Suman *et al.*, 2020). The amplified products were purified and Sanger sequenced in both directions. The obtained sequences were manually curated, trimmed and submitted to the National Centre for Biotechnology Information (NCBI), USA (for ITS gene, 'P1' as MK256355, 'P2-P4' as MN044868-044870). Steinernematid ITS rDNA sequences similar to the ones obtained were searched for in NCBI GenBank with NBLAST algorithm (Altschul *et al.*, 1990). The sequences obtained in this study and those downloaded from GenBank were used to obtain alignment. If several identical or nearly identical (1 bp difference) *S. feltiae* sequences were obtained from the same locality, only one was left in the final alignment. Obtained alignment of a limited set of data was analysed in MEGA7.0.14 (Kumar *et al.*, 2016) with three methods: maximum parsimony (MP), neighbour joining (NJ) and maximum likelihood (ML).

RESULTS AND DISCUSSION

The topology of phylogenetic trees obtained for the complete set of all *S. feltiae* sequences found that were similar to those of Kashmiri isolates after

Table 1. Morphometrics of *Steinernema feltiae* isolate 'P3' (MN044869).

Characters	First generation		Second generation		Infective Juveniles
	Male	Female	Male	Female	
n	**20**	**20**	**20**	**20**	**25**
Body length (L)	1212 ± 75 (1115-1398)	4190 ± 492 (3182-5248)	901 ± 50 (807-976)	2552 ± 277 (1899-2896)	766 ± 63 (689-913)
a (L/BD)	10.3 ± 1.1 (7.9-12.0)	18 ± 2.0 (15.6-22)	9.4 ± 1.0 (8.2-12.1)	12.8 ± 1.9 (8.8-16.7)	31 ± 2.8 (25-36)
b (L/PS)	8.9 ± 0.8 (7.4-11.0)	23 ± 2.1 (19-28)	7.1 ± 0.6 (6.2-9.0)	16.5 ± 1.7 (13.2-20)	6.4 ± 0.6 (5.2-8.0)
c (L/T)	32 ± 3.5 (23-39)	64 ± 10.7 (40-88)	31 ± 3.7 (28-45)	53 ± 9.8 (32-73)	9.6 ± 0.7 (8.3-10.9)
c' (T/ABW)	1.1 ± 0.1 (0.9-1.8)	1.0 ± 0.1 (0.7-1.2)	0.8 ± 0.1 (0.3-1.2)	1.1 ± 0.2 (0.8-1.4)	4.6 ± 0.4 (4.2-6.2)
V (AV/L × 100)	–	52 ± 2.3 (46-58)	–	49 ± 2.6 (46-57)	–
Body diam. (BD)	131 ± 10.8 (110-151)	234 ± 27 (195-285)	96 ± 8.8 (74-109)	201 ± 15.1 (172-225)	26 ± 2.4 (23-32)
Excretory pore-ant. end (EP)	71 ± 8.3 (50-82)	82 ± 7.1 (68-97)	58 ± 5.9 (44-69)	79 ± 9.3 (65-96)	62 ± 5.7 (51-74)
Width at EP (WEP)	50 ± 6.9 (39-68)	77 ± 9.6 (60-95)	41 ± 5.0 (31-49)	78 ± 11.9 (64-107)	17 ± 1.7 (12-20)
Nerve ring (NR)	69 ± 8.7 (55-87)	84 ± 7.9 (70-97)	62 ± 5.0 (49-69)	79 ± 7.8 (66-97)	108 ± 8.2 (97-116)
Pharynx length (PS)	139 ± 8.04 (117-153)	198 ± 14 (169-223)	126 ± 8.5 (100-137)	155 ± 8.3 (141-168)	131 ± 8.7 (126-143)
Bulb length (EBL)	42 ± 3.4 (37-49)	54 ± 4.0 (45-61)	38 ± 4.3 (27-43)	43 ± 3.4 (38-40)	20 ± 2.2 (14-24)
Bulb width (EBW)	31 ± 2.5 (27-36)	44 ± 3.4 (37-50)	29 ± 2.7 (23-35)	35 ± 3.8 (28-39)	9.3 ± 1.0 (6.7-10.6)
Tail length (T)	42 ± 4.7 (36-51)	64 ± 7.9 (52-77)	29 ± 2.6 (21-35)	49 ± 6.7 (35-59)	84 ± 5.1 (75-89)
Anal body width (ABW)	37 ± 5.4 (23-46)	67 ± 6.5 (51-76)	32 ± 3.8 (22-38)	44 ± 6.2 (29-56)	17 ± 1.8 (15-20)
Spicule length (SPL)	72 ± 6.4 (58-86)	–	49 ± 5.0 (39-58)	–	–
Gubernaculum length (GL)	47 ± 5.6 (34-56)	–	38 ± 5.0 (29-47)	–	–
D% (EP/PS × 100)	51 ± 5.9 (36-58)	46 ± 4.2 (40-54)	46 ± 7.2 (34-69)	51 ± 5.7 (43-65)	50 ± 4.7 (42-62)
E% (EP/T × 100)	170 ± 30 (107-219)	126 ± 20 (93-171)	203 ± 30 (141-282)	164 ± 25 (127-217)	74 ± 7.8 (64-97)
SW% (SL/ABD × 100)	201 ± 45 (144-336)	–	152 ± 52 (226-226)	–	–
GS% (GL/SL × 100)	65 ± 5.9 (55-79)	–	79 ± 8.2 (65-91)	–	–
Width at Vulva	–	235 ± 23 (191-270)	–	196 ± 14.6 (179-217)	–
Anterior to vulva (AV)	–	2207 ± 258 (1678-2732)	–	1259 ± 150 (982-1607)	–
Posterior to vulva (PV)	–	1983 ± 273 (1504-2516)	–	1293 ± 160 (916-1539)	–
H	–	–	–	–	28 ± 2.4 (23-38)
H%	–	–	–	–	35 ± 3.2 (28-42)
Mucron	9.7 ± 1.8 (6.8-13.9)	–	6.3 ± 0.6 (5.1-7.6)	–	–

Measurements are in µm (except n, ratio and percentage) and in the form: Mean ± SD (range).

BLAST search and resulted from analysis of the limited set of data were concordant. The phylogenetic trees based on limited set of data (Fig. 1) and obtained with three methods of analysis (MP, NJ and ML) demonstrate identical topology too, apart from one inner weakly supported subclade of *S. feltiae* sequences (indicated with an arrow on Fig. 1).

Steinernema feltiae is one of the most intensively studied entomopathogenic nematode species and the number of deposited sequences is high. It is obvious from the analysis of these sequences that the majority of deposited sequences are identical or very similar with nucleotide differences in the range of 1-2 per about 1000 bp sequence, which is the level of error of amplification with standard PCR. The majority of the subclades, uniting isolates of *S. feltiae*, were strongly supported. The sequence of the isolate 'P1' clustered with a group of Asian, one Australian and several European isolates; the three remaining sequences ('P2'-'P4') formed a well-defined clade together with isolate 'JC1' from Nepal.

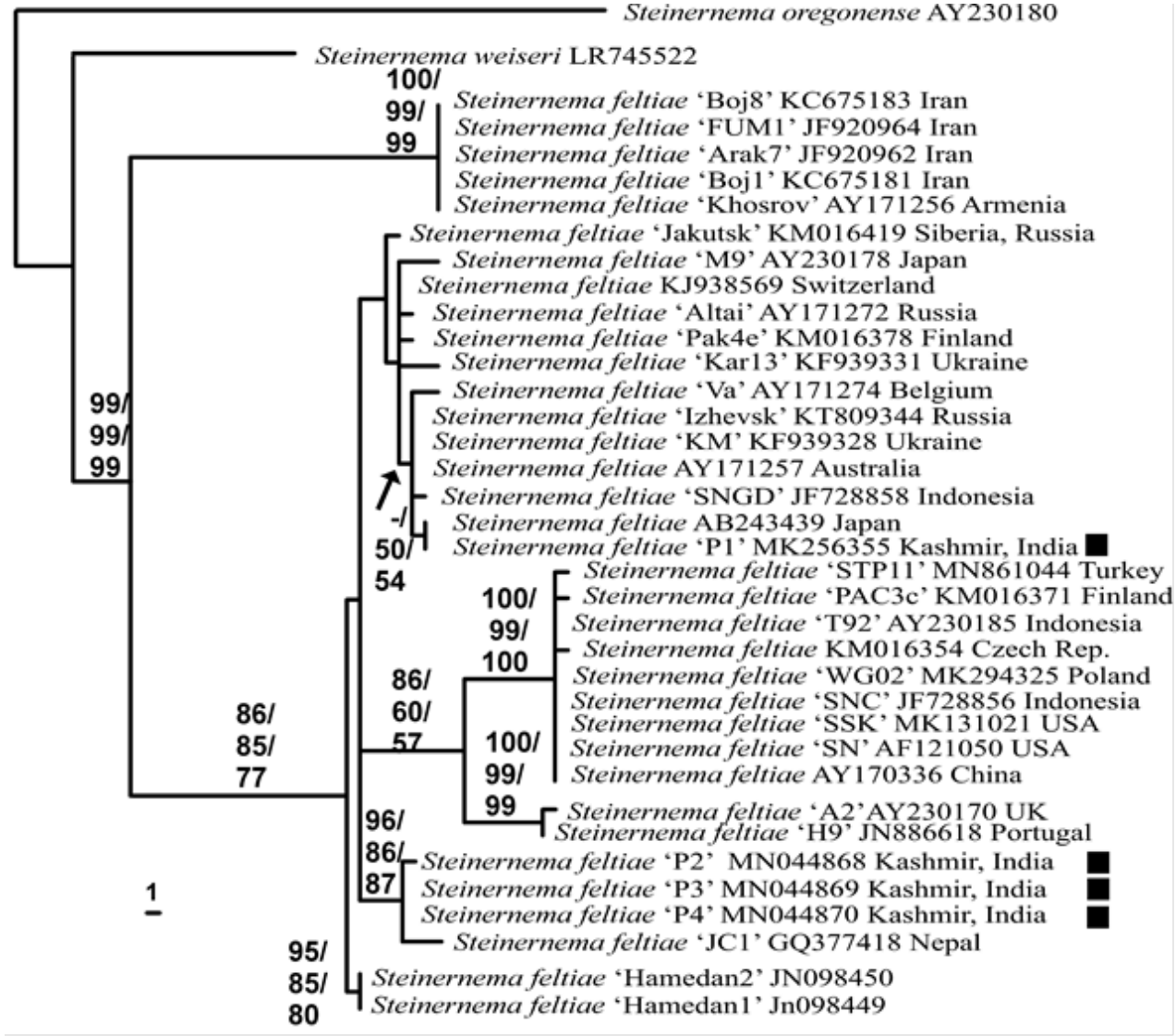

Fig. 1. Phylogenetic relationships of Kashmiri strains of *Steinernema feltiae*. Bootstrap supports presented in format MP/NJ/ML were obtained with 1000 pseudo-replicates for MP and NJ, and 500 for ML. Scale is for MP tree. Model for ML: Kishino-Hasegava-Yano with Gamma distributed sites (HKY+G). Black squares indicate the Kashmiri isolates of *S. feltiae* from this paper. An arrow indicates the clade, which is absent in MP analysis.

In our opinion, it is good example of the complicated process of local fauna formation. Some strains of the same species (in this case – 'P1') were introduced into the area of this study only recently (*e.g.*, by the agricultural activity of two last centuries), whereas other strains ('P2'-'P4') are the result of long processes, *e.g.*, existing in local natural habitats for long periods of time (thousands of years) and their sequences reflect long-term isolation of these strains. The clustering of Kashmiri and Nepalese strains looks reasonable – indeed, it is the same mountainous ridge. Localities in such regions can have similar abiotic (soil structure) and biotic factors (same hosts for these nematodes), which can support isolation and independent evolution of these strains.

Morphological structures of the specimens belonging to one of the Kashmiri isolates ('P3') were studied. The morphology of these specimens corresponds to the published descriptions of *S. feltiae* (Fig. 2) and no distinct differences in the measurements were detected between studied isolates 'P2', 'P3' and 'P4' from Kashmir. The measurements for the main features of one isolate ('P3') are presented in Table 1.

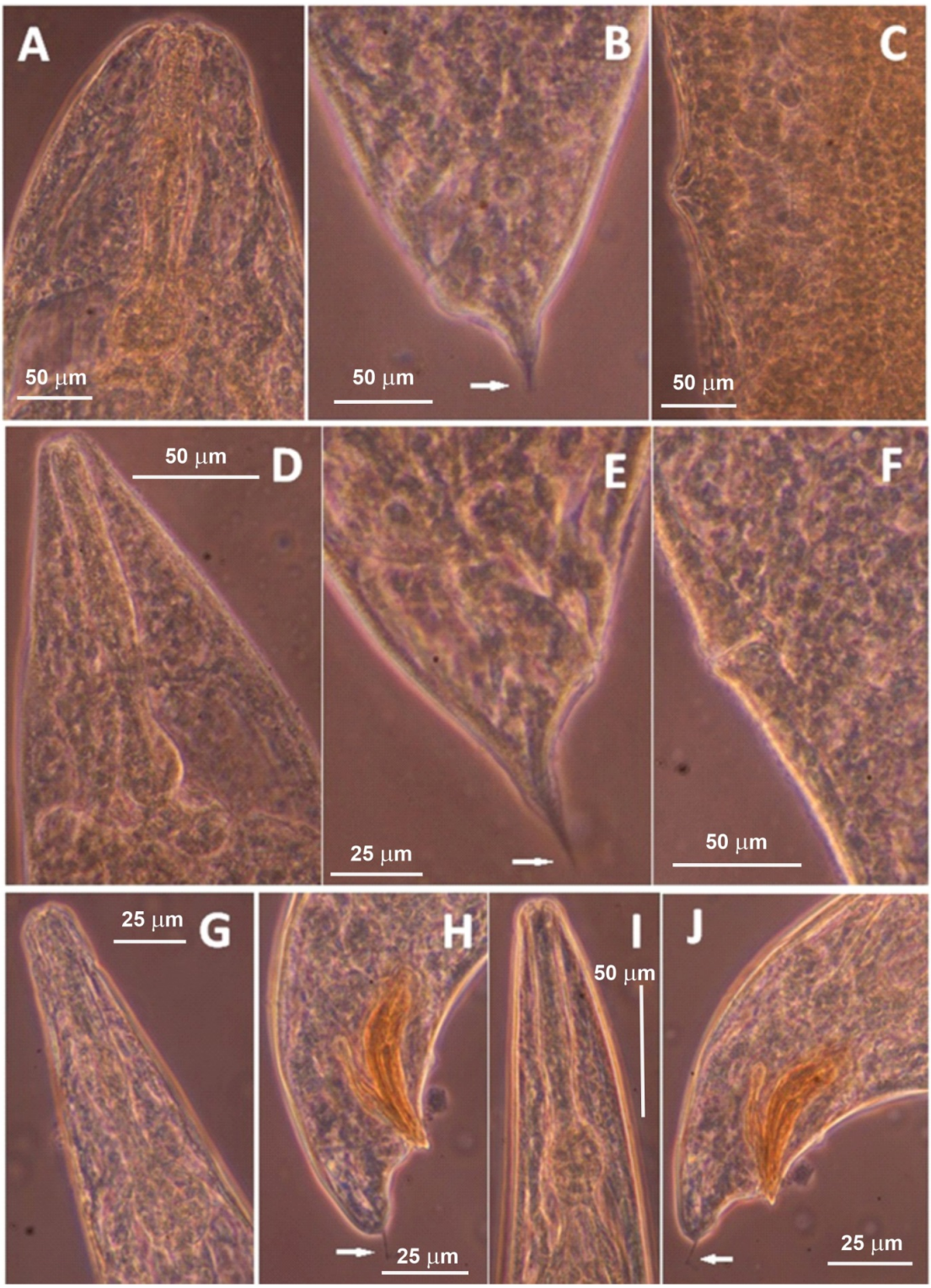

Fig. 2. *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 (light microscopy). A-C: First generation female. A: Neck region; B: Posterior end (arrow pointing the mucron); C: Vulval region. D-F: Second generation female. D: Neck region; E: Posterior end (arrow pointing the mucron); F: Vulval region. G, H: First generation male. G: Neck region; H: Posterior end (arrow pointing the mucron). I, J: Second generation male. I: Neck region; J: Posterior end (arrow pointing the mucron).

The ITS-region of ribosomal repeats is a variable part of the *Steinernema* nuclear genome, and some level of intraspecific differences has been reported (Spiridonov *et al.*, 2004). It was experimentally demonstrated that crossing of two Belgian strains with 2.5% difference in nucleotide sequences of ITS rDNA produce viable and fertile progeny (Spiridonov *et al.*, 2004). Analysis of our data demonstrates that *S. feltiae* strains differ in 2.5-2.7% of ITS rDNA nucleotides, when the nucleotide differences between strains of *S. feltiae* and the closest species *S. weiseri* are in the range of 3.9-5.0%. In our analysis, the sequences of two closely related species (*S. weiseri* and *S. oregonense*) were used as out-groups. The bootstrap support for the node uniting all *S. feltiae* isolates was strong under all methods of analysis. Strong or medium support was also characteristic for the majority of basal nodes in the *S. feltiae* tree (Fig. 1). Some of the inner subclades of the *S. feltiae* tree comprise the isolates from the same region of the globe; *e.g.*, the basal subclade consists of isolates from Armenia and Iran. The first one was obtained from the soil on the mountain slope in Khosrov National Park of Armenia in 1990. The slope was nearly free of vegetation with only scattered *Juniperus* sp. trees. Several other subclades also contain strains isolated in the same region: strains from Southern Iran (Hamedan 1 and 2), strains from UK and continental Portugal ('A2' and 'H9') and, finally, three Kashmiri strains and 'JC1' from Nepal. One strongly supported clade unites the strains from very different places from Western and Northern Europe up to China and the USA. Our strain 'P1' clustered with numerous strains mainly from Europe but with inclusion of Asian and Australian ones. The reason may be the same as explained above for the isolate 'JC1' from Nepal. The contemporary picture of strain distribution is a result of several biotic (*e.g.*, same hosts for these nematodes) and abiotic factors (*e.g.*, soil structure). These factors were acting for different period of time: recent introduction *vs* long process of isolation and local adaptation.

If we consider today's sampling of *S. feltiae* strains as representative, the origin of this species can be hypothesised. The basal clade of *S. feltiae* consists of strains from a limited area in the Middle East (Armenia and Iran). It is reasonable to note that this geographical region was not affected by glaciations and at the same time, it is a cradle of agriculture in the Old World. We can imagine that the natural mechanisms and processes are responsible for the spread of steinernematids and their ability to colonise new territories (*e.g.*, after the end of glaciation period). The probable role of their dipteran imago hosts cannot be excluded, as was demonstrated by Bovien (1937). At the same time, the transfer in the small amounts of soil with seedlings or tubers is also possible. Hypothetically, we can suspect that the presence of genetically close haplotypes of *S. feltiae* in such remote places as Northern Europe and Australia is a result of unintended transportation in the course of human activity. With little knowledge of natural hosts of steinernematids and mechanisms ensuring the genetic entity of local populations we can only speculate about the evolutionary history of this species. Still, the basal position of Middle East strains in the *S. feltiae* phylogenetic tree is observed under different methods of analysis and with different strains involved into analysis.

Geographical distribution of *Steinernema feltiae*. The presence of *S. feltiae* in different regions was assessed using GenBank records of ITS rDNA in NCBI database and literature search. It is noteworthy to mention that this approach has some limitations as the absence of records does not mean that the organism is not present in a particular area, but provides, however, a suitable tool to determine where they actually occur.

For BLAST search query, we used the sequence ander NCBI accession number AF121050, as the first *S. feltiae* isolate used to describe this species was characterised only morpho-taxometrically but not molecularly. BLASTN analysis shown that *S. feltiae* occurs in Turkey (53 reported isolates); Portugal (40); Italy (31); Czech Republic (19); Finland (16); UK (9); Lebanon (8); Belgium (8); Russia (6); Iran (5); Ukraine (4); Indonesia (4); Spain (4); India (4); Switzerland (3); New Zealand (3); USA (3); Israel (3); Poland (2); China (2); Croatia (2); Japan (2), Armenia (1); Australia (1); France (1); Canada (1); Slovenia (1); Bulgaria (1), Nepal (1), Jordon (1) and Nigeria (1). Data from the literature confirmed that this species has also been found in Austria, Denmark, Estonia, Finland, France, Hungary, Ireland, Sweden, The Netherlands, Norway, UK and Pakistan based on RFLP or simple morphological studies (Hominick, 2002).

In India, GenBank records for *S. feltiae* shows it is known only from Jammu and Kashmir (4 isolates) submitted during the present study and this report is the first valid proof of existence of this species from India. The number of sequences in GenBank from a particular region reflects not only the abundance of the organism within the area, but also the actual sampling effort. This report is the first molecularly confirmed finding of *S. feltiae* in India.

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REFERENCES

ABOLAFIA, J. & PEÑA-SANTIAGO, R. 2017. On the identity of *Chiloplacus magnus* Rashid & Heyns, 1990 and *C. insularis* Orselli & Vinciguerra, 2002 (Rhabditida: Cephalobidae), two confusable species. *Nematology* 19: 1017-1034. DOI: 10.1163/15685411–00003104

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. & LIPMAN, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410.

BALDWIN J. G., NADLER S.A. & ADAMS, B. 2004. Evolution of plant parasitism among nematodes. *Annual Review of Phytopathology* 42: 83-105.

BEDDING, R.A. & AKHURST, R.J. 1975. Simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110. DOI: 10.1163/187529275X00419

BHAT, A.H., ISTKHAR, CHAUBEY, A.K., PŮŽA, V. & SAN-BLAS, E. 2017. First report and comparative study of *Steinernema surkhetense* (Rhabditida: Steinernematidae) and its symbiont bacteria from subcontinental India. *Journal of Nematology* 49: 92-102.

BHAT, A.H., CHAUBEY, A.K. & PŮŽA, V. 2018. The first report of *Xenorhabdus indica* from *Steinernema pakistanense*: co-phylogenetic study suggests co-speciation between *X. indica* and its steinernematid nematodes. *Journal of Helminthology* 92: 1-10. DOI: 10.1017/S0022149X14000583

BHAT, A.H., CHAUBEY, A.K., SHOKOOHI, E. & MASHELA, P.W. 2019. Study of *Steinernema hermaphroditum* (Nematoda, Rhabditida) from the West Uttar Pradesh, India. *Acta Parasitologica* 64: 720-737. DOI: 10.2478/s11686-019-00061-9

BHAT, A.H., ASKARY, T.H., AHMAD, M.J., SUMAN, AASHA & CHAUBEY, A.K. 2020. Description of *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) isolated from hilly areas of Kashmir Valley. *Egyptian Journal of Biology Pest Control* 29: 96. DOI: 10.1186/s41938-019-0197-6

BOVIEN, P. 1937. Some types of association between nematodes and insects. *Videnskabelige meddelelser fra den Naturhistoriske Forening i Kjöbenhavn* 101: 1-114.

COURTNEY, W.D., POLLEY, D. & MILLER, V.L. 1955. TAF, an improved fixative in nematode technique. *Plant Disease Reporter* 39: 570-571.

DZIEDZIEH, A., SHIVANKAR, S. & THEOPOLD, U. 2020. *Drosophila melanogaster* responses against entomopathogenic nematodes: focus on hemolymph clots. *Insects* 11: 62.

FILIPJEV, I.N. 1934. Miscellanea Nematologica 1. Eine neue Art der Gattung *Neoaplectana* Steiner nebst Bermerkungen über die systematische Stellung der letzteren. *Magasin de parasitologie de l'Institut Zoologique de l'Academie de l'USSR* 4: 229-240.

HOMINICK, W.M. 2002. Biogeography. In: *Entomopathogenic Nematology* (R. Gaugler Ed.). pp. 115-143. Wallingford, UK, CAB International.

HUNT, D.J. & NGUYEN, K.B. 2016. *Advances in Entomoparasitic Nematode Taxonomy and Phylogeny. Nematology Monographs and Perspectives, Volume 12* (D.J. Hunt & R.N. Perry Series Eds). The Netherlands, Brill. 438 pp.

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: molecular evolutionary genetics analysis. Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874.

LABAUDE, S. & GRIFFIN, C.T. 2018. Transmission success of entomopathogenic nematodes used in pest control. *Insects* 9: 72. DOI: 10.3390/insects9020072

LACEY, L.A., GRZYWACZ, D., SHAPIRO-ILAN, D.I., FRUTOS, R., BROWNBRIDGE, M. & GOETTEL, M.S. 2015. Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology* 132:1-41. DOI: 10.1016/j.jip.2015.07.009

NADLER, S.A., DE LEY, P., MUNDO-OCAMPO, M., SMYTHE, A.B., STOCK, S.P., BUMBARGER, D., ADAMS, B.J., DE LEY, T., HOLOVACHOV, I.O. & BALDWIN, J.G. 2006. Phylogeny of Cephalobina (Nematoda): molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Molecular Phylogenetics and Evolution* 40: 696-711. DOI: 10.1016/j.ympev

PATEL, R.K.M. 2011. *Comparative biology and mass production of Corcyra cephalonica (Stainton) (Pyralidae: Lepidoptera) on different food materials*. Ph.D. Dissertation, Anand Agricultural University, Anand, India.

SEINHORST, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69. DOI: 10.1163/187529259X00381

SPIRIDONOV, S.E., REID, A.P., PODRUCKA, K., SUBBOTIN, S.A. & MOENS, M. 2004. Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida)

as inferred from analyses of sequences of the ITS-5.8S-ITS2 region of rDNA and morphological features. *Nematology* 6: 547-566. DOI: 10.1163/1568541042665304

STURHAN, D. & LISKOVA, M. 1999. Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. *Nematology* 1: 273-277. DOI: 10.1163/156854199508261

SUMAN, BHAT, A.H., AASHA, CHAUBEY, A.K. & ABOLAFIA, J. 2020. Morphological and molecular characterisation of *Distolabrellus veechi* (Rhabditida: Mesorhabditidae) from India. *Nematology* 22: 439-452. DOI: 10.1163/15685411-00003315

TARASCO, E., ALVAREZ, C., TRIGGIANI, O. & QUESADA, E. 2011. Laboratory studies on the competition for insect haemocoel between *Beauveria bassiana* and *Steinernema ichnusae* recovered in the same ecological niche. *Biocontrol Science and Technology* 21: 693-704. DOI: 10.1080/09583157.2011.570428

TRAVASSOS, L. 1927. Sobre o genero *Oxysomatium*. *Boletim Biologico, Sao Paulo* 5: 20-21.

VRAIN, T.C., WAKARCHUK, D.A., LÉVESQUE, A.C. & HAMILTON, R.I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15: 563-573.

WHITE, G.F. 1929. A method for obtaining infective nematode larvae from cultures. *Science* 66: 302-303. DOI: 10.1126/science.66.1709.302-a

T.H. Askary, A.H. Bhat, M.J. Ahmad, A.K. Chaubey and S.E. Spiridonov. *Steinernema feltiae* (Rhabditida: Steinernematidae) из горных районов в долине Кашмира в Индии с замечаниями о географическом распространении этого вида.

Резюме. Из сельскохозяйственных почв района Анантнаг, в горном районе Пир Панджал (Pir Panjal) в Джамму и Кашмире (союзная территория Индии) были выделены нематоды вида *Steinernema feltiae*. Филогенетический анализ ITS-участка рибосомальных повторов (ITS rDNA) показал, что три из выделенных четырех изолятов образуют особую «гималайскую» группу в пределах вида *S. feltiae*, тогда как еще один изолят относится к космополитной группе гаплотипов данного вида. Представлены морфометрические данные для одного из изолятов «гималайской» группы. На основе анализа нуклеотидных данных, депонированных в ГенБанке NCBI, продемонстрировано широкое распространение вида *S. feltiae*.