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Research Paper

Molecular phylogeny of three variable size juvenile isolates of *Steinernema abbasi* (Rhabditida: Steinernematidae) from the western part of Uttar Pradesh, India by the application of conserved genes sequence analyses

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Abstract: Three isolates of *Steinernema* sp. (Nematoda: Steinernematidae) named as CS_3, CS_6 and CS_{21} were recovered by *Galleria* baiting technique from soil samples collected from Saharanpur district of western part of Uttar Pradesh, India. The worms were characterized as member of bicornutum group due to the presence of two horn-like cephalic structures in 3rd stage infective juveniles (IJs). The morphometric comparative analyses with earlier described nine bicornutum group species reflected that the isolated specimens are closer to *Steinernema abbasi* and *S. thermophilum*. However, the results obtained through the molecular phylogeny based on the ITS1-5.8S-ITS2 gene sequence analyses and numerical applications were proved that these newer worms were similar to *S. abbasi* but not identical. Variations within the discovered isolates with the validated species were hypothetical to be due to the influence of extrinsic or environmental factors.
Keywords: molecular phylogeny, *Steinernema abbasi*, ITS1-5.8S-ITS2 gene, conserved genes, 3rd stage infective juveniles.

INTRODUCTION

EPNs, the abridged term used for entomopathogenic nematodes because of their major virulence to insect pests. They belong to families Heterorhabditidae and Steinernematidae and are obligate parasites to insects. They are often used as biological control agents against economically important insect pests. The two major genera *Steinernema* Poinar and *Heterorhabditis* Travassos, are globally distributed except Antarctica (Hominick, 2002) with more than 26 and 100 identified species respectively.

Most of the identified species of *Steinernema* indicate the polymorphisms within genera; however, others show several morphotaxometric resemblances. Therefore, based on these features they are classified into five groups (Nguyen and Hunt, 2007) but their identification is always a difficult task for nematologists. The internal transcribed spacers (ITS) of rDNA comprises ITS1, 5.8S and ITS2, are the most important molecular markers used in the taxonomy and phylogeny of entomopathogenic nematodes. Attention to ITS rDNA study were made by earlier workers on its phylogenetic efficacy at the species and population level for taxonomic purposes as these regions evolved at much higher rates as comparison to 18S and 28S genes (Chilton et al., 1995; Cherry et al., 1997). Indigenous species of any biological control agent that are adapted to local environmental and climatic conditions are especially good candidates and entomopathogenic nematodes are not a different case for such activities. The applicability of these species is not restricted to only the area from which they are recovered but also active to same mode at different places. The objective of the present study is to validate the existence of three EPNs isolates of *S. abbasi* (Elawad et al., 1997) from western part of Uttar Pradesh, India, based on morphological features, numerical taxonomy and comprehensive analyses of conserved gene locus. So that the newly discovered roundworms can be utilize as biological control agents.

MATERIALS AND METHODS

Collection and processing of nematodes:

The nematode populations were isolated during two years (2012-2014) continuous soil surveys conducted in western part (Saharanpur district) of Uttar Pradesh, India (Lon 29.97ºN, Lat 77.55ºE, MSL 269m) by soil baiting technique (Bedding and Akhurst, 1975). The last instar larvae of *Galleria mellonella* were used for the maintenance of nematode populations. The emerging infective juveniles (IJs) were stored in tissue culture flasks at 15±1ºC in BOD incubator. The 1st and 2nd generation male and female worms were recovered after 2nd and 4th day of post mortality period. The emerging 3rd stage juveniles (IJs) came into water first were isolated from the prepared white traps (White, 1927). All the stages were killed by gentle heat, fixed in TAF and processed to glycerol (Sienhorst, 1959) and mounted in glycerine for morphometric measurements. All the measurements are given in micrometers (µm) as mean±standard deviation (SD) followed by ranges in parentheses. The microphotographs were taken by trinocular light and phase contrast microscope (Nikon Eclipse 50i).

Molecular analysis:

Total genomic DNA of fresh IJs was extracted in Nematology Laboratory from DNeasy Blood and Tissue Kit (Qiagen) after Upadhyay (2012) and instructions of manufacturer. The presence of DNA in the extracted sample was confirmed by the 0.8% agarose gel electrophoresis (AGE). The DNA bands were observed under UV light in Geldoc system and quantified by the nanodrop. The amplification of ITS regions was performed by polymerase chain reaction (PCR) by modified primers (Joyce et al., 1994). The 25µl of PCR reaction volume were contains; DTG master mix 2X (Tsci) 12.5µl, primer 0.7µl each, template DNA 2µl, water (mq) 9.1µl. The PCR reaction was set for 35 cycles in theromcycler with initial temperature 94ºC for 5 minutes, followed by denaturation at 94ºC for 30 seconds, annealing at 64ºC for 30 seconds, primer extension at 72ºC for 30 seconds, final extension at 72ºC for 8 minutes and storage temperature 4ºC for infinity. The proper amplification of gene was confirmed

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with 100bp DNA ladder through 1% AGE. The purification of PCR products was done by the use of purification kit (Qiagen) and sequenced by the autosequencer (Applied Biosystems). Obtained sequences were aligned for phylogeny in Clustal W (Tamura et al., 2004) and compositions of the sequences were calculated through BioEdit (Hall, 1999). Phylogenetic trees and distance matrix were constructed by molecular evolutionary genetics analysis (MEGA) software version 6 (Tamura et al., 2013).

RESULTS

Morphology and Taxometry:

The worms isolated from the selected sites were characterized as member of bicornutum group due to the presence of two horns like cephalic armature (Fig. 1).

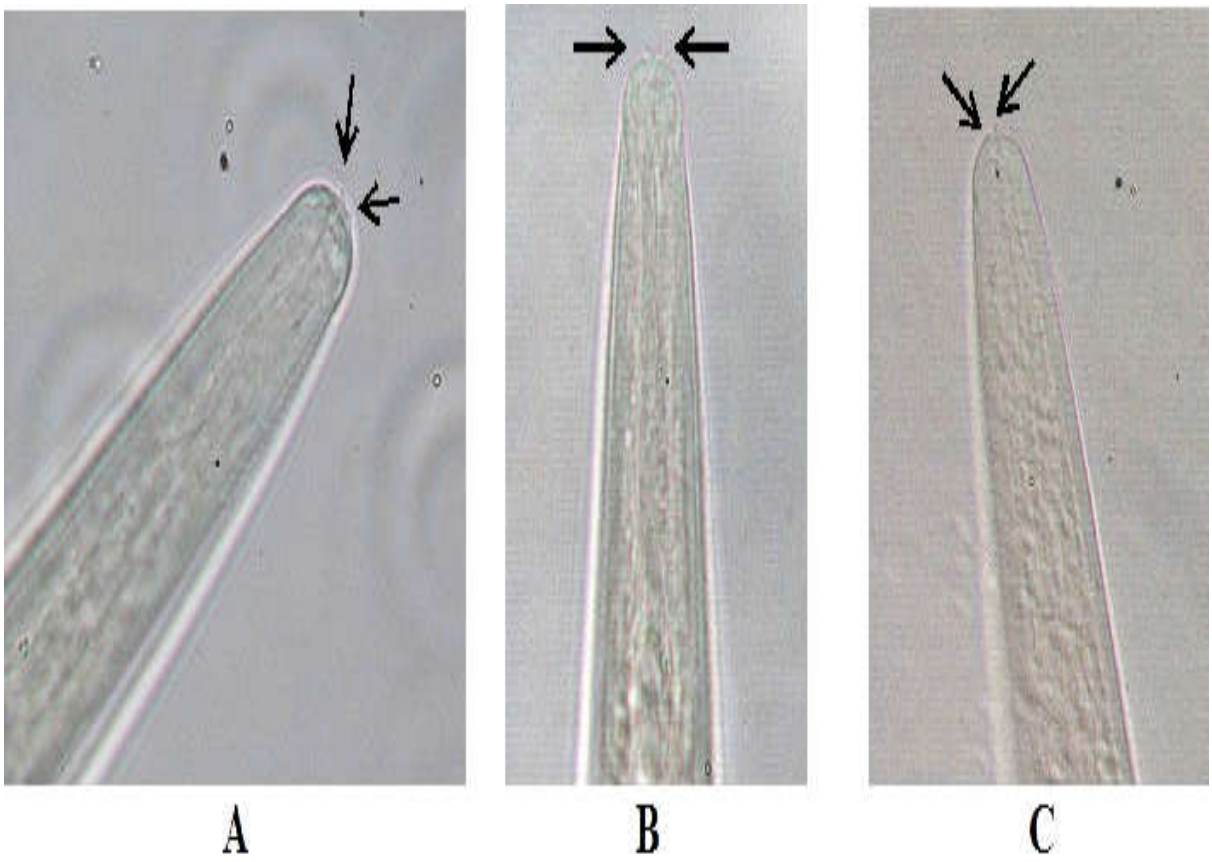

Figure 1. Third stage juveniles of *Steinernema abbasi* isolates with cephalic armature (1000X): (A) CS_3, (B) CS_6, (C) CS_{21}.

The detailed morphotaxometric calculations of recovered 3rd stage juveniles and 1st generation males of three isolates were summarised in Table 1 and Table 2 respectively.

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Table 1. Comparative taxometry of 3rd stage juveniles of isolates CS_3, CS_6 and CS_{21} with other *Steinernema* species of bicornutum group.

Characters	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. pakistanense*	*S. ribobrave*	*S. thermophilum*	*S. yirgalemense*	*S. costarecense*	*S. paillatum*	Isolate CS_3	Isolate CS_6	Isolate CS_{21}
n				20	20	20	25	20	20	20	20	20
L	541±24 (496-579)	770±52 (648-873)	706±62 (591-800)	683±21 (649-716)	622±39.5 (361-701)	555±34 (480-620)	635±36 (548-693)	1696.5±62 (1600-1774)	652±39.6 (572-720)	631±26 (583- 680)	581±59 (458- 663)	529±32 (455- 578)
a	18±0.9 (17-20)	26.5±1.5 (23-29)	25.9 (23.7-27.9)	24±1.5 (21-27)	22±1.1 (19.9-23.5)	26±0.9 (24-28)	21±1.3 (20-25)	20±1.4 (17-23)	27±2.5 (22-30)	23±1 (22- 26)	23±1 (21- 25)	22±2 (18- 26)
b	6±0.3 (5.5-6.6)	6.2±0.3 (5.6-6.9)		6±0.3 (5-6)	5.4±0.3 (4.9-6)	6.4±0.4 (5.8-7.1)	5.2±0.3 (4.8-5.9)	7.1±0.5 (6.1-7.9)	5.9±0.4 (5-6.4)	6±0.5 (5- 7)	6±0.6 (5- 7)	5±0.5 (4- 6)
c	9.8±0.8 (8.1-10.8)	10.7±0.66 (9.7-12)	10.6 (8.8-12.9)	11±0.5 (10-12)	11.6±0.7 (10.1-12.4)	12.3±0.4 (11.5-12.8)	10.3±0.6 (9.2-11.2)	12±0.5 (11-13)	12.1±1.3 (8.3-15)	11±0.5 (10- 12)	11±1 (9- 12)	12±2 (9- 15)
c'			4.2 (3.3-5.1)			3.4±0.3 (3-3.9)	3.3			4±0.3 (3- 4)	4±0.3 (3- 4)	3±5 (3- 4)
GBW	29±1 (27-30)		27±3 (23-34)	27±1.2 (24-29)	27.6±1.7 (25.6-30)	21±0.7 (21-23)	29±2.2 (24-33)	39±2.5 (23-45.5)	24.4±3.2 (21-31)	28±1 (24- 29)	25±2 (21- 28)	25±2 (22- 29)
EP	48±1.5 (46-51)		55±5 (47-70)	54±2.2 (49-58)	56.2±3.2 (51.2-63.3)	40±2 (37-46)	51±3.4 (45-59)	25±2.3 (20-30)	50±3.3 (44-58)	48±4 (41- 55)	47.5±2 (44- 53)	48±4.5 (39- 60)
NR	68±2.4 (64-72)		92±6 (79-103)	80±2.1 (76-83)	77.2±1.4 (83.7-88.7)	71±4 (65-79)	88±3.6 (82-93)	54±8.4 (46-69)	88±4.1 (81-96)	83±7 (78- 109)	79±5 (20- 88)	76±3 (70- 81)
ES	89±1.8 (85.92)		123±7 (108-144)	113±4.2 (108-122)	113.5±2.1 (108.7-116.2)	87±6 (80-100)	121±3.7 (115-128)	93.5±5.3 (81-103)	110±4.9 (103-121)	112±6 (106- 131)	98±5 (90- 108)	105±3 (100- 115)
Tail	56±3.2 (52-61)	72±5 (63-78)	66±5 (56-74)	58±2.1 (53-62)	53.5±3.5 (46.2-58.7)	45±3 (40-52)	62±2.7 (57-67)	54±2.3 (51-59)	54±6.7 (40-78)	58±3 (51- 63)	53±4 (46- 60)	45±6 (33- 56)
ABW			15±2 (9-18)		16±0.4 (15-16.5)	13±1 (12-15)	19±4.3 (17-21)	17±0.8 (15-18)		17±1 (14- 18)	14±2 (11- 16)	13±1 (10- 16)
D%	53±2 (51-58)	50±30 (40-60)	45±3 (40-56)	47±2.7 (42-53)		46±3.5 (42-53)	42±2.7 (38-48)	42±6.8 (25-50)	46±3.3 (40-52)	43±3 (37- 49)	48±3 (44- 59)	46±5 (36- 57)
E%	86±5 (79-94	80±6 (80-100)	84±6 (74-96)	91±5 (87-102)		90±6 (81-102)	83±7 (67-98)	44±5 (35-56)	93±10.8 (66-121)	84±6 (72- 95)	90±6 (78- 99)	110±22 (79- 170)

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Table 2. Comparative taxometry of 1st generation males of isolates CS_3, CS_6 and CS_{21} with other *Steinernema* species of bicornutum group.

Characters	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. pakistanense*	*S. ribobrave*	*S. thermophilum*	*S. yirgalemense*	*S. costarecense*	*S. papillatum*	Isolate CS_3	Isolate CS_6	Isolate CS_{21}
n				20	10	20	20	19	20	15	15	15
L	1252±189 (999-1534)	1353±149 (945-1539)	1385±134 (1136-16-94)	1357±89 (1163-1505)	1700±9 (1500-1900)	1197±236 (990-780)	1566±39 (1331-1777)	2282±201 (2076-2683)	1146±127 (851-1321)	1682±110 (1394-1823)	1367±125 (1155- 1561)	1605±147 (1336- 1872)
GBW	87±6.7 (82-98)	108±11 (80-128)	146±21 (104-185)	102±10.2 (80-128)	133±13.5 (116-160)	77±15.2 (60-100)	112±11 (97-138)	128±18 (89-157)	69±9.9 (54-870)	148±11 (126- 165)	101±9 (83- 115)	148±21 (109- 177)
EP	80±7.8 (68-89)	82±7.8 (67-98)	85±11 (50-104)	81±4.8 (72-92)	103±4.8 (94-111)	75±8.5 (64-92)	86±9 (74-107)	117±9.8 (104-136)	73±9.1 (54-96)	93±6 (84- 104)	83±6 (68- 93)	100±7 (189- 109)
NR	103±6.5 (99-123)	123±8 (108-137)	123±14 (90-147)	99±6.3 (88-107)	103±7.6 (106-134)	93±8.2 (80-110)	108±10 (98-136)	165±9 (155-178)	104±11.5 (74-125)	121±10 (104- 135)	105±10 (15- 124)	123±10 (111- 145)
ES	133±6 (121-144)	156±7 (138-67)	165±10 (149-190)	132±5.8 (126-146)	144±7.9 (128-154)	125±20 (97-165)	148±12 (132-165)	211±8 (202-227)	136±15.6 (94-63)	157±7 (143- 172)	132±6 (119- 142)	149±7 (135- 163)
TR	274±33 (234-319)		393±94 (163-574)		226 ±26.5 (185-257)		278±17 (205-331)		340±54.3 (238-454)	235±57 (134- 347)	226±46 (171-289)	228±46 (137-275)
Tail	26±3 (20-31)	32±2.5 (25-35)	30±4 (23-38)	25±0.8 (24-27)	31±1.7 (29-35)	22.4±4.7 (19-34)	20±4 (17-27)	45±6.7 (38-63.5)	25±4.9 (16-35)	30±2 (25- 34)	24±3 (20- 29)	27±2 (23- 31)
ABW	43±5 (37-55)		52±5 (45-70)	36±2.3 (32-40)	59±4.7 (50-64)	37±7 (28-49)	38±6 (32-45)	55±4.3 (47-65)	34±4.7 (26-440)	41±3 (35- 46)	34±5 (27- 43)	38±3 (34- 43)
SPL	65±5.7 (57-74)	65±4.3 (53-70)	71±7 (54-90)	68±3.6 (62-73)	67±4.2 (63-65)	61±7 (44-72)	64±8 (51-77)	92±5.5 (81-101)	58±4.6 (47-66)	74±4 (64- 82)	72±4.6 (63-80)	75±4.9 (66-87)
SPW	12±1.3 (10-14)		9±1 (7-11)		12.4±0.7 (11.2-13.7)		14±3.6 (12-15)		12±1.3 (9-14)	8±1 (8- 10)	9.2±1.5 (6.7-12.7)	8.5±1 (6.3-10)
GL	45±4.3 (35-50)	47±3.5 (38-50)	40±4 (25-45)	41±3.2 (36-45)	51±2.6 (48-56)	36±4 (30-42)	48±6.9 (42-54)	46±3 (40.5-50.5)	31±3.2 (23-36)	44±6 (36- 54.7)	47±3.5 (42-54)	54±2.6 (48-59)
GW	7±0.1 (6-8.5)		7±1 (5-9)		8.1±0.6 (7.1-8.7)		11±3 (9-12)		7±1.2 (4-9)	8±1 (6- 9)	7.3±1 (5.8-8.6)	7.1±0.9 (5.9-9.5)
D%	60±5 (51-58)		51±7 (33-69)	60±3 (50-60)		63±10 (50-87)	58±7.6 (50-66)	53±4.5 (50.5-66)	54±5.3 (43-65)	59±4 (52- 66)	63±4 (52- 72)	67±5 (61- 76)
E%			283	310±40 (210-370)		343±43 (269-400)		262±41 (165-301)		306±25 (271- 363)	350±45 (283- 423)	4±0.5 (3- 4)
F%										488±61 (418- 646)	427±52 (308- 513)	549±69 (423- 678)
SW	156±22 (107-187)			189		1.7±0.4 (1.2-2.8)	171±13 (121-213)	1.6±0.05 (1.5-1.7)	156±21.2 (125-194)	181±14 151- 201)	215±32 (163-279)	197±19 (166-233)
GS	70±7 (58-85)	72		60.3		0.6-0.1 (0.5-0.7)	74±8 (65-85)	0.49±0.04 (0.45-0.55)	59±6.5 (48-70)	60±9 (47- 78)	65±4.8 (58-75)	198±24 (153-236)

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Type species: *Steinernema abbasi* (Elawad et al., 1997).
Type specimens: *Steinernema abbasi* isolates viz. CS_3, CS_6 and CS_{21}.
Host locality: Soils of Saharanpur, Uttar Pradesh, India (isolate CS_3 from fields of *Solanum* sp.; CS_6 from Grass field and CS_{21} from garden of *Mangiferra* sp.).

Gene sequence analysis:

The obtained aligned sequences containing partial 18S and 28S rDNA and complete ITS1-5.8S-ITS2 regions submitted to gene bank under the accession number KP189195, KP036919 and KP189196 for *Steinernema* sp. isolate C_{S3}, CS_6 and CS_{21} respectively. The respective read length of individual isolate for the complete ITS1-5.8S-ITS2 region was 827bp for C_{S3}; 808bp for CS_6 and 800bp for CS_{21} with flanking regions of 18S and 28S rDNA. A comparative account on sequence length variation within ITS-5.8S-ITS2 among the compared species was analysed (Table 3).

Table 3. Nucleotide variations within ITS rDNA of newly recovered isolates with reference species of bicornutum group.

Species	ITS1	5.8S	ITS2	GC%	AT%	A	C	G	T	TOTAL
Isolate CS_3	269	157	314	36.89	63.11	175	111	162	292	740
Isolate CS_6	268	157	314	36.94	63.06	175	111	162	291	739
Isolate CS_{21}	267	157	315	36.94	63.06	175	111	162	291	739
S. riobrave	281	157	316	34.75	65.25	204	98	164	288	754
S. pakistanense	291	157	300	36.9	63.1	216	118	158	256	748
S. bicornutum	281	157	330	37.5	62.5	201	123	165	279	768
S.ceratophorum	243	157	341	36.17	63.83	192	117	151	281	741
S. yirgalemense	270	157	284	35.72	64.28	191	93	161	266	711
S. abbasi	268	157	314	36.94	63.06	175	111	162	291	739
S. thermophilum	267	157	314	36.99	63.01	175	111	162	290	738
S. costaricense	280	157	296	39.02	60.98	167	116	170	280	733
S. papillatum	314	157	323	35.14	64.86	223	110	169	292	794

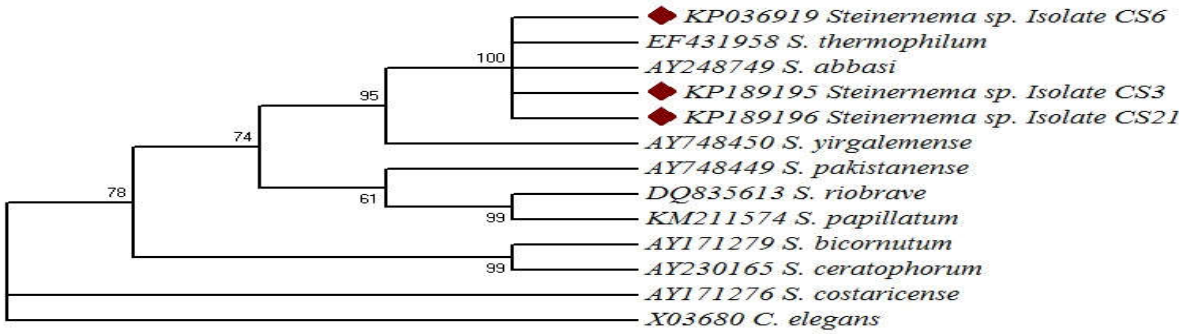

Figure 2. Phylogenetic tree based on ITS-rDNA sequences of isolates CS_3, CS_6 and CS_{21} with *Steinernema* species of bicornutum group by the Maximum Parsimony

method. *Caenorhabditis elegans* was used as outgroup. The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.

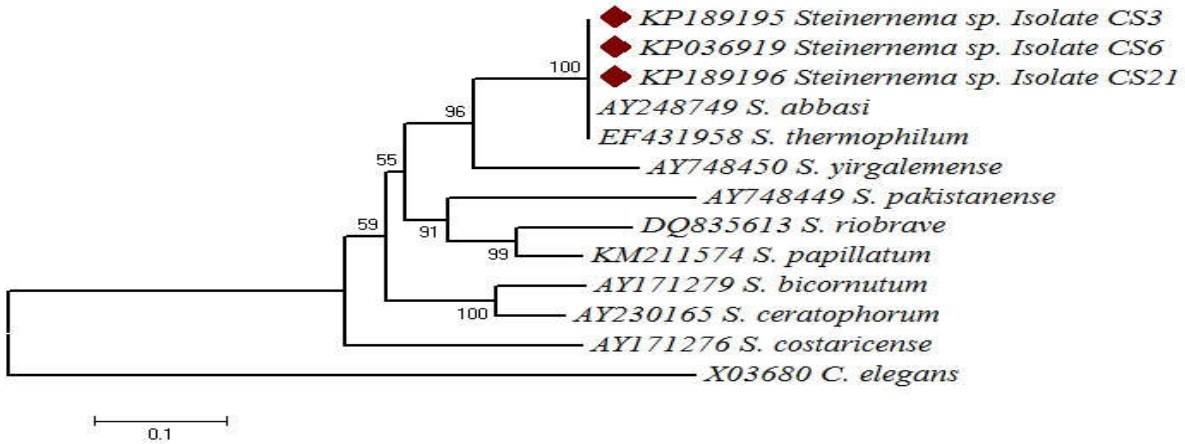

Figure: 3. Neighbour-joining (nj) tree based on ITS gene sequences of newly discovered three isolates (CS_3, CS_6, CS_{21}) with available *Steinernema* species of bicornutum group through K2P model. *Caenorhabditis elegans* was used as outgroup.

A phylogenetic relationship was worked out by the Maximum Parsimony (Fig. 2), neighbour joining (Fig. 3) and nucleotide distance matrix was calculated by Kimura 2 Parameter (K2P) model (Table 4).

Table 4. Pairwise nucleotide distance matrix of recovered isolates with species of bicornutum group based on ITS-rDNA sequences (K2P model).

Species	**CS_3**	**CS_6**	**CS_{21}**	**RIO**	**PAK**	**BIC**	**CER**	**YIR**	**ABB**	**THE**	**COS**	**PAP**
Isolate CS_3												
Isolate CS_6	0.000											
Isolate CS_{21}	0.000	0.000										
S. riobrave	0.326	0.326	0.326									
S. pakistanense	0.361	0.361	0.361	0.322								
S. bicornutum	0.306	0.306	0.306	0.328	0.393							
S.ceratophorum	0.294	0.294	0.294	0.292	0.381	0.120						
S. yirgalemense	0.213	0.213	0.213	0.340	0.383	0.340	0.332					
S. abbasi	0.000	0.000	0.000	0.326	0.361	0.306	0.294	0.213				
S. thermophilum	0.000	0.000	0.000	0.326	0.361	0.306	0.294	0.213	0.000			
S. costaricense	0.376	0.376	0.376	0.406	0.443	0.317	0.312	0.410	0.376	0.376		
S. papillatum	0.270	0.270	0.270	0.137	0.299	0.294	0.263	0.310	0.270	0.270	0.360	
C. elegans	0.946	0.946	0.946	0.968	1.070	1.003	0.990	1.040	0.946	0.946	0.959	0.989

The analysis involved 13 nucleotide sequences. The numerical variations in sequences within ITS regions of discovered isolates with the pre-existing members of bicornutum group were found to be remarkable (Table 5). All positions containing gaps and missing data were eliminated.

Table 5. Numerical variations in sequences within ITSrDNA of recovered isolates with species of bicornutum group.

Species*	Region	CS_3	CS_6	CS_{21}	RIO	PAK	BIC	CER	YIR	ABB	THE	COS	PAP
Isolate CS_3	ITS1-5.8S-ITS2	0	-1	-1	14	8	28	1	-29	-1	-2	-7	54
	ITS1	0	-1	-2	12	22	12	-27	1	-1	-2	11	45
	ITS2	0	0	1	2	-14	16	27	-30	0	0	-18	9
Isolate CS_6	ITS1-5.8S-ITS2	1	0	0	15	9	29	2	-28	0	-1	-6	55
	ITS1	1	0	-1	13	23	13	-26	2	0	-1	12	46
	ITS2	0	0	1	2	-14	16	27	-30	0	0	-18	9
Isolate CS_{21}	ITS1-5.8S-ITS2	1	0	0	15	9	29	2	-28	0	-1	-6	55
	ITS1	2	1	0	14	24	14	-25	3	1	0	13	47
	ITS2	-1	-1	0	1	-15	15	26	-31	-1	-1	-19	8
S. riobrave	ITS1-5.8S-ITS2	-14	-15	-15	0	-6	14	-13	-43	-15	-16	-21	40
	ITS1	-12	-13	-14	0	10	0	-39	-11	-13	-14	-1	33
	ITS2	-2	-2	-1	0	-16	14	25	-32	-2	-2	-20	7
S. pakistanense	ITS1-5.8S-ITS2	-8	-9	-9	6	0	20	-7	-37	-9	-10	-15	46
	ITS1	-22	-23	-24	-10	0	-10	-49	-21	-23	-24	-11	23
	ITS2	14	14	15	16	0	30	41	-16	14	14	-4	23
S. bicornutum	ITS1-5.8S-ITS2	-28	-29	-29	-14	-20	0	-27	-57	-29	-30	-35	26
	ITS1	-12	-13	-14	0	10	0	-39	-11	-13	-14	-1	33
	ITS2	-16	-16	-15	-14	-30	0	11	-46	-16	-16	-34	-7
S.ceratophorum	ITS1-5.8S-ITS2	-1	-2	-2	13	7	27	0	-30	-2	-3	-8	53
	ITS1	26	25	24	38	48	38	-1	27	25	24	37	71
	ITS2	-27	-27	-26	-25	-41	-11	0	-57	-27	-27	-45	-18
S. yirgalemense	ITS1-5.8S-ITS2	29	28	28	43	37	57	30	0	28	27	22	83
	ITS1	-1	-2	-3	11	21	11	-28	0	-2	-3	10	44
	ITS2	30	30	31	32	16	46	57	0	30	30	12	39
S. abbasi	ITS1-5.8S-ITS2	1	0	0	15	9	29	2	-28	0	-1	-6	55
	ITS1	1	0	-1	13	23	13	-26	2	0	-1	12	46
	ITS2	0	0	1	2	-14	16	27	-30	0	0	-18	9
S. thermophilum	ITS1-5.8S-ITS2	2	1	1	16	10	30	3	-27	1	0	-5	56
	ITS1	2	1	0	14	24	14	-25	3	1	0	13	47
	ITS2	0	0	1	2	-14	16	27	-30	0	0	-18	9
S. costaricense	ITS1-5.8S-ITS2	7	6	6	21	15	35	8	-22	6	5	0	61
	ITS1	-11	-12	-13	1	11	1	-38	-10	-12	-13	0	34
	ITS2	18	18	19	20	4	34	45	-12	18	18	0	27
S. papillatum	ITS1-5.8S-ITS2	-54	-55	-55	-40	-46	-26	-53	-83	-55	-56	-61	0
	ITS1	-45	-46	-47	-33	-23	-33	-72	-44	-46	-47	-34	0
	ITS2	-9	-9	-8	-7	-23	7	18	-39	-9	-9	-27	0

*RIO, *S. riobrave;* PAK, *S. pakistanense;* BIC, *S. bicornutum;* CER, *S.ceratophorum;* YIR, *S. yirgalemense;* ABB, *S. abbasi;* THE, *S. thermophilum;* COS, *S. costaricense;* PAP, *S. papillatum.*

DISCUSSION

The topological investigations and numerical morphotaxometric correlations and comparisons of newly recovered 3rd stage juveniles (Table 1) and 1st generation males (Table 2) of three isolates with the earlier described taxa of same group like *S. abbasi* (Elawad et al., 1997), *S. bicornutum* (Tallosi et al., 1995), *S. ceratophorum* (Jian et al., 1997), *S. pakistanense* (Shahina et al., 2001), *S. riobrave* (Cabanillas et al., 1994), *S. thermophilum* (Ganguly and Singh 2000), *S. yirgalemense* (Nguyen et al., 2004), *S. costarecense* (Uribe et al., 2007) and *S. Paillatum* (San-Blas et al., 2015) reflected that all the isolates were closely related to the bicornutum group and particularly nearer to *S. abbasi* and *S. thermophilum*. The present isolates can be differentiated from the *S. abbasi* by the application of numerical taxonomic parameters. The isolate CS_{21} was 2.2% and 4.68% shorter than the *S. abbasi* and *S. thermophilum* respectively. On contrary CS_3 was 17% and 14% whereas CS_6 was 7.4% and 4.7% larger as compare to *S. abbasi* and *S. thermophilum* respectively. The tail length and maximum body width were found close to *S. abbasi*, while the position of nerve ring and length of pharynx was significantly different. Life history events diminish the divergence of present specimens with *S. abbasi* with noticeable visibility of mucronate process in 1st generation male and presence of giant females.

It was evident by the gene sequence alignment out of 1032 characters 200 were constant, 333 were parsimony informative and 449 were parsimony uninformative. The maximum parsimony analysis involved 13 nucleotide sequences, in which 590 positions were in final data set with 0.64 consistency index, 0.654 retention index and 0.419 composite index (Fig. 2). The molecular phylogeny by maximum likelihood and Neighbor-Joining placed all the isolates in one clade with *S. abbasi* and *S. thermophilum* with significant bootstrap numbers (Fig. 3). The nucleotide variations in ITS regions of isolate CS_3, CS_6 and CS_{21} were noticeably similar to each other in numbers and compositions with a little variation in 1 or 2 nucleotides have been proven as good diagnostic numerical tool to differentiating the closely related species or isolates and producing valuable data for molecular phylogeny (Nguyen et al., 2001). The length of ITS-5.8S-ITS2 regions was greatly variable in comparison to *S. yirgalemense* (711bp in) and *S. papillatum* (794bp). The 5.8S region flanked with ITS regions has highly conserved quality in the isolates than the earlier described 13 *Steinernema* sequences with 157 residues of nucleotides corroborated the similar results as described by the yesteryear workers (Szalanski et al,. 2000; Nguyen et al., 2001). The composition of purine residues (adenine and thymine) was highly variable than the pyrimidine (guanine and cytocine) in bicornutum group and similar to *S. abbasi* and *S. thermophilum*. The distance matrix illustration and analyses reflected that the present specimens were not identical to anyone but closer to *S. abbasi* and *S. thermophilum* with little fluctuations in the sequences of conserved regions than the other member of bicornutum group (Fig. 3). The findings in polymorphism of newly recovered isolated were more for ITS1 as compared to ITS2 was supported by the study of Hunt (2007) in which he has synonymised *S. thermophilum* as junior synonym of *S. abbasi*. Therefore, authors are able to conclude the present specimens (isolate CS_3, CS_6 and CS_{21}) of Saharanpur districts as isolates of *S. abbasi*. The laboratory trials of infectivity and reproductive potential varying within the isolates similar to *Steinernema* (Hominick et al., 1996) and exhibit differences in host range, pattern and burden of infectivity,

environmental tolerances and it has to be stimulated for many surveys, seeking new strains and species of EPNs.

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