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Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Meghalaya, NE India

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ABSTRACT

In the present study, the entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples collected from 20 sites representing ecologically diverse types of habitats in Meghalaya, India. Out of 89 positive samples, the frequency of occurrence of *Steinernema* spp. was found to be more (73.03%) than *Heterorhabditis* sp. (26.97%). All the EPN positive samples were from the forest soils except the one isolated from banana plantations near teak forest in Jorabat. No entomopathogenic nematodes were isolated from dry-land, jhumland (burned and cultivated land) and wet land (water-saturated soil). *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil. The isolates were identified as *Heterorhabditis indica, Steinernema thermophilum* and *S. glaseri* using morphometric analysis and scanning electron microscopic studies.

Key words: Infective juveniles; entomopathogenic nematodes; *Heterorhabditis indica*; *Steinernema thermophilum*; *Steinernema glaseri*.

INTRODUCTION

Entomopathogenic nematode (EPN) species of the genera *Steinernema* Travassos (Rhabditida: Steinernematidae) and *Heterorhabditis* Poinar (Rhabditida: Heterorhabditidae) have attracted the attention of entomologists (and of nematologists) for a number of years for use in controlling economically important insect pests. With increasing restrictions on the use of chemicals and the mounting problem of resistance, such nematodes are valuable addition to the range of biological control agents available for insect pest management since they possess many of the attributes of effective biological control agents.

Steinernematid and heterorhabditid nematodes are obligate pathogens that infect a wide range of insects, and are characterized by their association with symbiotic bacteria carried in their digestive tract; *Xenorhabdus* spp. Thomas and Poinar (Enterobacteriaceae) in steinernematids, and *Photorhabdus* spp. Boemar *et al.* (Enterobacteriaceae) in heterorhabditids.[1] They have a similar life cycle, except for a few differences. Their entry into the host is usually through natural openings such as the mouth, anus or spiracles.[2,3] The bacteria released by

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steinernematid and heterorhabditid nematodes rapidly multiply and the kills the host by 'septicemia' within 24-48.[4] The nematodes feed on the bacteria and nutrients made available because of bacterial digestion.

EPNs have been reported to occur in the tropical, subtropical and temperate countries.[5] The only continent where they have not been found to occur is Antarctica.[6] Numerous surveys for EPNs have been conducted throughout the world by using baiting technique.[7] There is an intense interest to isolate these nematodes from different regions of the world, which are climatically adapted and have the potential for biological control of pests in that area. Many countries are concerned about the introduction of exotic species of EPNs, because of their negative impacts on non-target organisms. Hence, surveys have been conducted in many parts of the world demonstrating their wide spread occurrence and providing an indication of which species are indigenous for a given area.

The work on EPNs in India was first started in 1966 by Rao and Manjunath[8] on biocontrol potentials of an exotic EPN species, *Steinnernema carpocapsae* Weiser, and for about another 2 decades, the same trend existed where several imported strains of EPNs were studied mainly for biocontrol point of view. However, due to poor adaptability of these strains under Indian conditions, the search for indigenous strains to suit to the agro-climatic conditions prevailing in the country was felt necessary.

MATERIALS AND METHODS

Nematode source

Surveying for nematodes

Soil samples were collected from different localities from Ri-Bhoi District of Meghalaya for a period of two years, i.e., during 2004-2006. The samples were collected from different habitats *viz*. dry land, wet land, jhum land and forest land, at a depth of 10-15 cms at each site covering an area about 1 sq. m, pooled and made up to 500 gms and transported in polyethene bags to laboratory. Information on sampling months, location, soil type was noted for each sample.

Baiting of soil samples

Nematodes were isolated by baiting techniques[7] where the samples were baited in 500 ml container. Ten numbers of last instar larvae of wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) were used as baiting agent. Three replicates were maintained for each soil samples. Larval mortality was observed daily for 10 days.

The dead larvae were washed twice or thrice in distilled water. The dead larvae were examined for the presence of entomopathogenic nematodes by colour change and smell emanating form the dead larvae. The infected larvae were transferred to modified white trap[9] for extraction.

Culture of entomopathogenic nematodes

Infection of G. mellonella larvae

Laboratory cultures of all the EPNs were maintained using final instar larvae of *G. mellonella*. Nematodes were multiplied using the methods of Dutky *et al.*[10] The nematode suspension was applied to a double layer of Whatman #1 filter paper in a petri dish. Insect larvae were introduced to the Petri dish, which was sealed and incubated at 25°C.

Extraction and storage of nematodes

Infected larvae were transferred to modified White traps[9] and incubated at 25°C. The emerged infective juveniles (IJs) were extracted in a beaker and cleaned two or three times with distilled water by sedimentation, followed by decantation. The extracted nematodes were stored at 10°C in distilled water for future processing.

Morphological characterization

Light microscopy

All nematodes used in this study were produced in *G. mellonella* larvae. First and second generation adult steinernematids were obtained by dissecting infected insects 2 to 3 days and 5 to 7 days, respectively, after the insects died while hermaphrodite and male/female of heterorhabditids were obtained by dissecting 4 to 5 days and 6 to 8 days respectively, after insects died. The infective juveniles (IJs) used for measurements were collected 3 days after the first emergence of IJs. All nematode samples, including IJs, the first and second generation males and females, were killed by gentle heat and then fixed in triethanolamine–formalin (TAF) fixative[11] and processed to anhydrous glycerol using the method described by Seinhorst.[12] Permanent slides were made using glass slide; cover glass supports were used in all cases to avoid flattening of specimens. At least 50 each of female, male and infective juvenile were observed and measured. Measurements and their photography were performed using a Leitz-Dialux 20 EB microscope with 10x, 20x or 40x differential interference contrast lens. All measurements, taken with the help of ocular and stage micrometers are in µm and based on fifty specimens of each stage unless otherwise stated.

Scanning electron microscopy

For scanning electron microscopic (SEM) examination, method described by Dey *et al.*[13] was followed. The specimens were mounted and positioned on stubs, coated with a thin layer of gold in a Fine Coat Ion Sputter and examined using JEOL (JSM – 6360) scanning electron microscope, operating at electron accelerating voltage of 10-15 KeV.

OBSERVATIONS AND RESULTS

In the present study, the entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples collected from 20 villages representing ecologically diverse types of soils (Table 1). Out of 89 positive samples, the frequency of occurrence of *Steinernema* spp. (73.03%) was found to be more than *Heterorhabditis* sp. (26.97%). All the entomopathogenic nematode positive samples were from the forest soils except one *H. indica* isolated from banana plantations near teak forest in Jorabat. No entomopathogenic nematodes were isolated from dryland, jhumland (burned and cultivated land) and wet land (water-saturated soil). *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil.

On the basis of light, scanning electron microscopical studies (Fig 1-3), morphometric analysis of different stages of nematodes (Table 2-4) and behavior (colour of dead insect larvae killed by the nematode, i.e., brick red in heterorhabditids and pale yellowish or brownish in steinernematids) the entomopathogenic nematodes recovered in this study were identified as *Heterorhabditis indica* Poinar *et al*. *Steinernema thermophilum* Ganguly and Singh and *S. glaseri* Glaser.

Table 1. Prevalence and distribution of entomopathogenic nematodes in Ri-Bhoi District, Meghalaya.

Habitats	No. of samples collected	No. of samples positive for EPNs			
		Heterorhabditis sp.	*Steinernema* spp.	Total	%
Dryland	480	-	-	-	-
Wetland	216	-	-	-	-
Forestland	576	24 (4.17 %)	65 (11.28 %)	89	15.45
Jhumland	384		-	-	-
TOTAL	**1656**	**24 (1.45 %)**	**65 (3.93 %)**	**89**	**5.37**

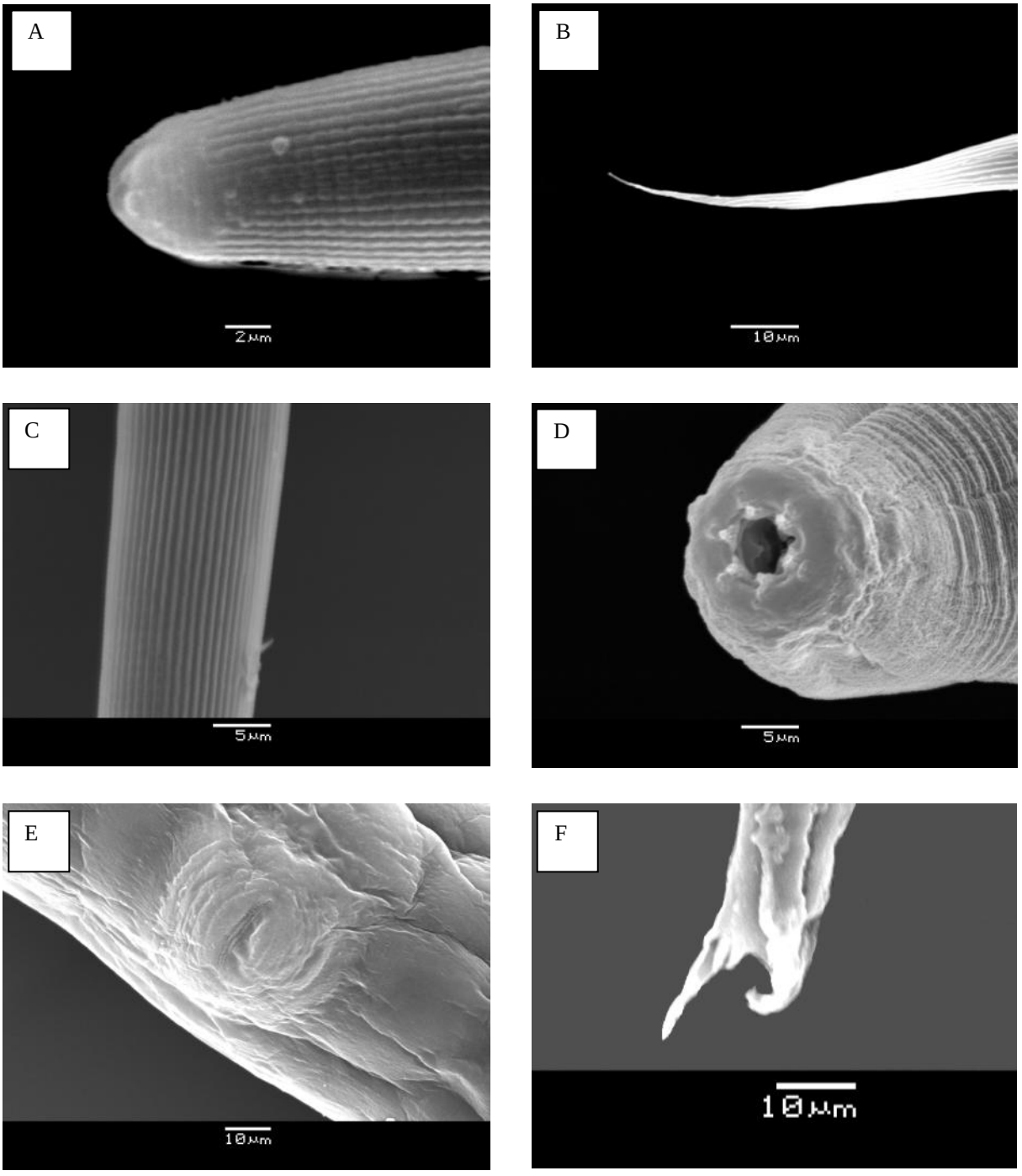

Figure 1. SEM of *H. indica.* A. anterior region of infective juvenile; B. tail of infective juvenile; C. longitudinal ridge of infective juvenile; D. anterior end of hermaphrodite female; E. Vulvar opening of female; F. posterior end of male showing spicule.

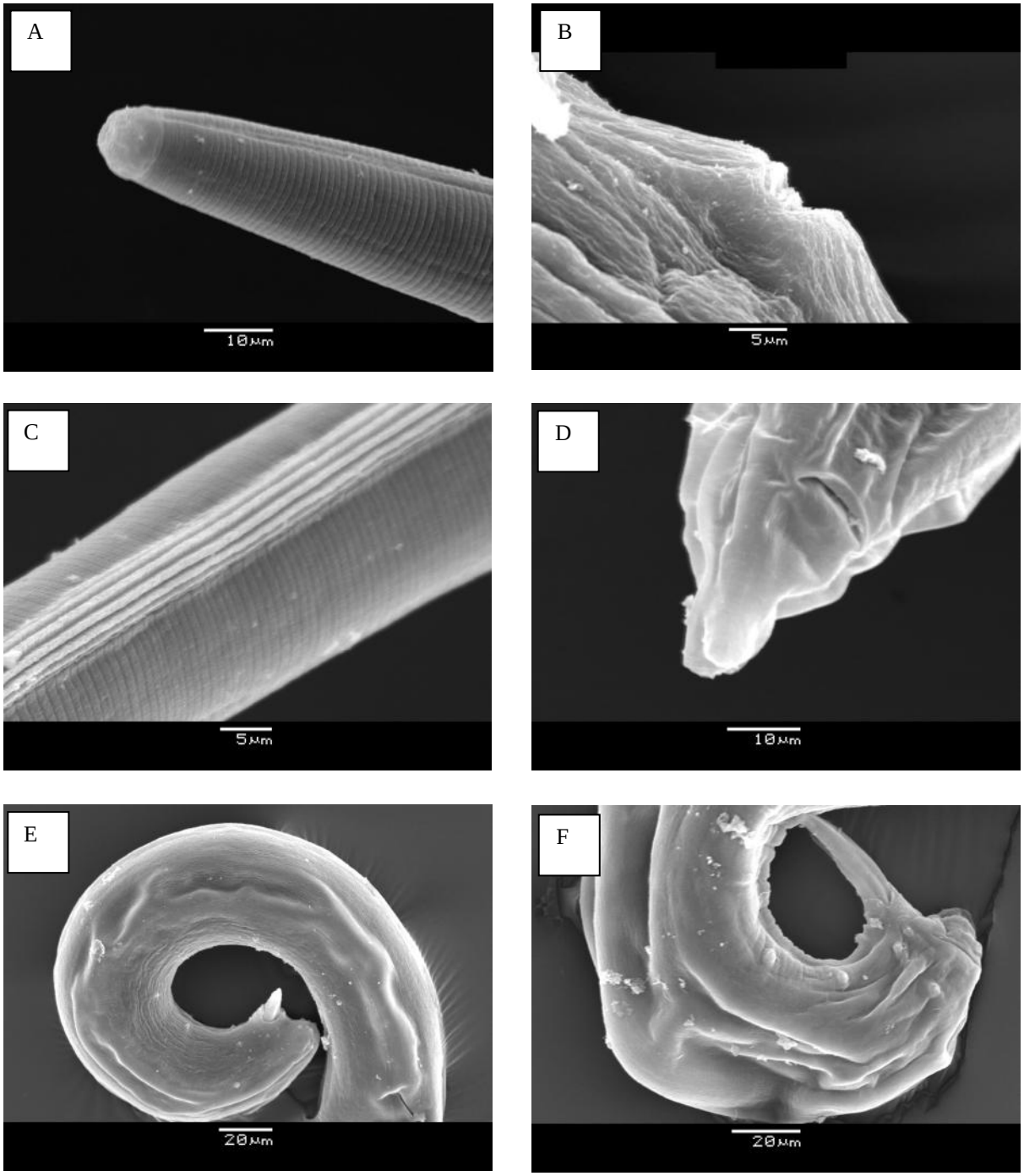

Figure 2. SEM of *S. thermophilum.* A. anterior region of infective juvenile showing 2 horn like structures; B. ridges of infective juvenile; C. vulvar opening of female; D. posterior end of male showing preanal, genital papillae and spicules; E. anterior portion of female; F. posterior end of 2nd generation female showing mucron.

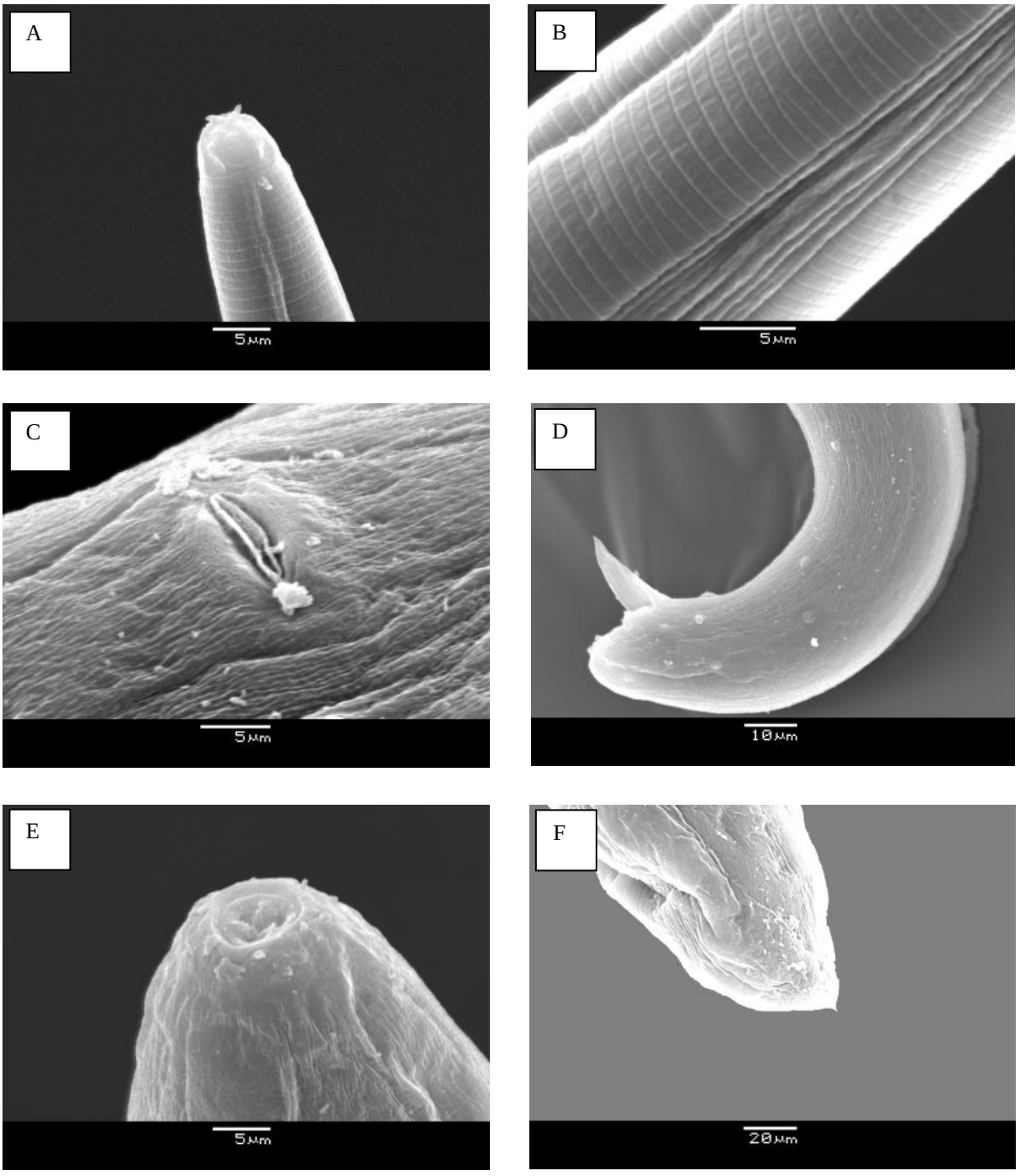

Figure 3. SEM of *S. glaseri.* A. anterior region of infective; B. vulvar opening of female; C. ridges of infective juvenile; D. posterior end of female showing anal pore; E. posterior portion of male showing genital papillae and spicules; F. posterior end of male showing preanal genital papillae and spicules.

Table 2. Morphometric measurements (mean ± SE) of *H. indica* (in µm).

	Infective Juveniles	Hermaphrodite female	Amphimictic female	Male
BL	542.78 ± 3.13 (478.80 - 587.10)	3075.09 ± 51.94 (2280 - 3933)	1488.17 ± 27.18 (1199.85 – 2109)	686.91 ± 10.79 (521.55 – 798)
BW	20.32 ± 0.15 (18.52 - 22.80)	160.46 ± 2.40 (119.70 - 190.95)	81.23 ± 1.91 (59.85 – 116.85)	41.54 ± 0.27 (37.05 – 45.6)
StL			5.77 ± 0.07 (5.7 – 8.55)	6.04 ± 0.37 (2.85 – 11.40)
StW			6.93 ± 0.17 (5.7 – 8.55)	5.89 ± 0.07 (5.70 – 7.13)
ES	117.25 ± 0.53 (108.30 - 125.40)	175.9 ± 1.13 (159.60 - 190.95)	137.99 ± 0.95 (128.25 – 159.6)	108.98 ± 0.59 (96.90 – 119.70)
EP	91.96 ± 0.62 (85.50 - 102.60)	163.78 ± 1.38 (142.50 - 182.40)	123.04 ± 0.76 (111.15 – 136.8)	114.15 ± 0.90 (99.75 – 128.25)
NR	79.23 ± 1.07 (76.95 - 82.65)	118.75 ± 1.20 (114.00 - 122.50)	87.59 ± 1.05 (85.5 – 91.5)	
ABW	13.28 ± 0.20 (11.40 - 17.10)	54.67 ± 0.78 (37.05 - 65.55)	23.94 ± 0.43 (19.95 – 31.35)	23.11 ± 0.23 (19.95 – 25.65)
TL	97.75 ± 1.84 (94.05 - 108.30)	75.92 ± 1.66 (51.30 - 105.45)	62.53 ± 0.90 (48.45 – 71.25)	28.15 ± 0.23 (25.56 – 31.35)
A	26.75 ± 0.17 (23.88 - 28.75)			
B	4.63 ± 0.03 (4.24 - 5.10)			
C	5.48 ± 0.03 (5.06 - 6.10)			
D	0.79 ± 0.01 (0.72 - 0.87)		0.89 ± 0.01 (0.73 – 1.00)	1.05 ± 0.01 (0.95 – 1.17)
E	0.93 ± 0.01 (0.84 - 1.06)		1.99 ± 0.03 (1.64 – 2.58)	4.08 ± 0.04 (3.5 – 4.67)
F	0.20 ± 0.001 (0.18 - 0.23)			
SPL				42.64 ± 0.39 (37.05 – 54.15)
GL				19.91 ± 0.77 (17.10 – 22.8)
SW				1.84 ± 0.02 (1.18 – 2.29)

BL= Body length; BW = Body width; ES = Oesophagous length; EP = Excretory pore position from anterior end; NR = nerve ring from anterior end; ABW = Anal body width; TL = Tail length; A = BL/BW; B = BL/ES; C = BL/TL; D = EP/ES; E = EP/TL; F = BW/TL; SPL = Spicule length; GL = Gubernaculum length; SW = SPL/ABW

DISCUSSIONS

The present study revealed the presence of three entomopathogenic nematode species in the area, namely *H. indica, S. thermophilum* and *S. glaseri*. These species are being reported for the first time from northeast region of India, in general, and from the state of Meghalaya, in particular. Out of 1656 soil samples collected from ecologically diverse type of habitats, 89 (5.37%) were found to be positive for EPNs. Further, the study revealed a pre-dominance of steinerne-

Table 3. Morphometric measurements (mean ± SE) of *S. thermophilum* (in µm).

	Infective Juveniles	**1st gen. female**	**2nd gen. female**	**1st gen. male**
BL	542.75 ± 6.64 (513.0 - 658.30)	4190.87 ± 185.11 (3063.75 - 6099)	2567.82 ± 187.45 (2137.50 - 4349.10)	1057.92 ± 27.69 (983.25 - 1140)
BW	23.77 ± 0.31 (22.80 - 28.50)	170.86 ± 2.96 (148.20 - 210.90)	161.03 ± 7.41 (122.55 - 199.50)	88.35 ± 1.81 (85.5 - 94.05)
ES	98.04 ± 0.74 (91.20 - 102.60)	168.15 ± 2.90 (151.05 - 216.60)	148.20 ± 4.67 (136.80 - 159.60)	128.25 ± 2.89 (116.85 - 133.95)
EP	44.12 ± 0.37 (39.90 - 45.60)	83.46 ± 2.34 (62.70 - 99.75)	68.40 ± 0.57 (59.85 - 76.95)	76.95 ± 0.91 (74.10 - 79.80)
NR				88.35 ± 1.23 (85.5 - 96.90)
ABW	14.36 ± 0.20 (11.40 - 17.10)	62.29 ± 0.99 (51.30 - 71.25)	51.30 ± 1.43 (31.35 - 71.25)	37.62 ± 0.57 (37.05 - 39.90)
TL	52.48 ± 0.95 (39.90 – 57.00)	30.81 ± 0.51 (25.65 - 34.20)	28.67 ± 0.33 (25.65 - 39.90)	28.5 ± 0.91 (25.65 - 31.35)
A	22.86 ± 0.21 (20.00 - 25.25)			
B	5.54 ± 0.07 (5.11 - 6.42)			
C	10.42 ± 0.19 (9.25 - 12.9)			
D	0.45 ± 0.01 (0.42 - 0.5)	0.51 ± 0.01 (0.39 - 0.61)	0.46 ± 0.04 (0.35 - 0.61)	0.61 ± 0.02 (0.57 - 0.68)
E	0.85 ± 0.02 (0.70 - 1.07)	2.74 ± 0.09 (1.83 - 3.50)	2.39 ± 0.15 (1.46 - 3.50)	2.71 ± 0.11 (2.45 to 3.00)
F	0.46 ± 0.01 (0.40 - 0.57)			
V%		52.43 ± 0.63 (47.79 - 57.64)	51.09 ± 2.15 (47.14 - 55.04	
SPL				62.7 ± 0.91 (59.85 - 65.55)
GL				31.35 ± 0.02 (31.35 - 34.20)
SW				1.67 ± 0.03 (1.57 - 1.77)

matids (73.03%) over heterorhabditids (26.97%) in EPN positive soil samples. Rosa *et al*.[14] have summarized the rate of recovery of EPNs from various soil surveys conducted throughout the world. Most surveys showed their recovery rate from soils between 6 and 35%.[14] Other surveys with 5% or less recovery of EPNs includes, 2% in Turkey by Hazir *et al*.,[15] 2.20% in Scotland by Boag *et al*.,[16] 3.8% in Northern Ireland by Blackshaw,[17] 4.6% in Korea by Choo *et al*.,[18] 4.7% in Turkey by Ozer *et al*.[19] and 5% in Italy by Ehlers *et al*.[20]

In the present study, the steinernematids were found to be significantly predominating than heterorhabditids. Many other workers have also reported dominance of steinernematids recovery over heterorhabditids.[21,22,23,24,25] In contrast, the dominance of heterorhabditids over steinernematids has been found in rather few surveys. For example, Rosa *et al*.[14] in a study in nine islands of the Azorean archipelago noticed that *Heterorhabditis* spp. were present on 30 sites from six islands, whereas *Steinernema* spp. were found only on 16 sites from three islands.

Table 4. Morphometric measurements (mean ± SE) of *S. glasseri* (in µm).

	Infective Juveniles	**1st gen. female**	**2nd gen. female**	**1st gen. male**
BL	1386.09 ± 19.95 (1074.4 - 1556.1)	5424.39 ± 251.39 (4212.3 - 7182)	2815.6 ± 105.04 (2399.7 – 4959.00)	1413.32 ± 31.26 (1291.05 - 1573.20)
BW	45.37 ± 0.79 (39.9 - 54.15)	229.90 ± 6.85 (202.5 - 276.45)	151.27 ± 4.29 (139.65 - 182.40)	69.26 ± 2.95 (48.45 - 82.65)
ES	157.21 ± 0.88 (148.20 - 165.30)	275.03 ± 4.08 (259.35 - 302.10)	154.15 ± 2.9 (139.65 - 185.25)	165.3 ± 5.09 (136.8 - 190.95)
EP	104.88 ± 1.14 (85.50 - 116.85)	177.41 ± 5.67 (139.65 - 208.05)	74.91 ± 3.14 (59.85 - 76.95)	117.99 ± 5.41 (91.2 - 145.35)
NR		182.4 ± 11.52 (159.6 - 196.65)		
ABW	29.98 ± 0.29 (28.50 - 31.35)	73.15 ± 2.63 (59.85 - 85.50)	58.67 ± 1.63 (51.30 - 71.25)	42.18 ± 1.19 (34.20 - 48.45)
TL	89.38 ± 1.65 (68.40 - 102.60)	51.06 ± 1.51 (42.75 – 57.00)	27.19 ± 0.26 (25.65 - 37.05)	37.62 ± 1.02 (34.20 - 42.75)
A	30.68 ± 0.49 (23.56 to 34.87)			
B	8.82 ± 0.12 (6.98 - 9.80)			
C	15.62 ± 0.34 (12.86 – 20.00)			
D	0.67 ± 0.01 (0.56 - 0.71)	0.64 ± 0.54 (0.52 - 0.73)	0.51 ± 0.01 (0.39 - 0.61)	0.71 ± 0.02 (0.60 - 0.81)
E	1.19 ± 0.02 (1.03 - 1.54)	3.48 ± 0.02 (2.88 - 3.94)	2.74 ± 0.09 (1.83 - 3.50)	3.16 ± 0.18 (2.40 - 4.25)
F	0.51 ± 0.01 (0.40 - 0.66)			
V%		55.39 ± 0.09 (52.57 - 58.32)	57.03 ± 2.11 (51.54 - 59.44)	
SPL				73.53 ± 3.15 (59.85 - 94.05)
GL				44.18 ± 1.14 (39.9 - 48.45)
SW				1.75 ± 0.07 (1.40 - 2.20)

Similarly, Griffin *et al*.[26] also reported a dominance of heterorhabditids over steinernematids in Britain and Ireland. Predominance of heterorhabditids over steinernematids has also been reported by Hara *et al.*[27] in Hawaiian Islands and by Roman and Figueroa in Puerto Rico.[28]

Further, our findings were comparatively average to studies in India, where Raj Kumar *et al*.[29] showed that out of 105 soil samples collected from Rajasthan, only 5 (4.76%) were found to be positive for steinernematids and heterorhabditids. Subsequently, Parihar *et al*.[30] undertook another survey in Rajasthan and reported the presence of *Steinernema* sp. and *Heterorhabditis* sp. from 8 (1.68%) samples out of 477 samples studied. They further mentioned that out of 8 positive samples, 5 (62.5%) were positive for *Heterorhabditis* sp. and the other 3 (37.5%) constituted *Steinernema* sp. Josephrajkumar and Sivakumar[31] in their study in Tamil Nadu reported the prevalence of steinernematids to be (94.44%) and of heterorhabditids (5.55%). In contrast to this, Singh *et al*.[32] reported a very low prevalence (1.82%) of *Steinernema* sp. at ICRISAT centre, Hyderabad. Kaushal *et al*.[33] examined 207 soil samples from diverse areas of India (Uttar Pradesh, Himachal Pradesh, Gujarat), of these 17 (8.21%) were found EPN positive, and 10 (58.82%) comprised steinernematids while 7 (41.18%) samples constituted

heterorhabditids.

H. indica was originally described by Poinar from sugarcane fields at Coimbatore (Tamil Nadu).[34] The species had been commonly isolated from subtropical and tropical region of the world such as Sri Lanka,[35] Cuba,[36] Egypt,[37] and Guadeloupe Island.[38] In context of India, the species has been reported to occur in Coimbatore and Kanyakumari districts,[39] Kerala,[40] and Bangalore.[41] Ganguly and Singh originally described *S. thermophilum* from the fields of Indian Agricultural Research Institute (IARI), New Delhi.[42] The species was named '*thermophilum'* because of its adaptability to high temperature condition (30-35°C) for its reproduction and multiplication. In this study, *S. thermophilum* was isolated from forest soils having less vegetation bordering Assam, where the temperature ranges from 27-35°C. The other *Steinernema* sp. encountered in the present study include, *S. glaseri,* which was recovered from Korhadem forest having sandy loam soil. In India, Gulsarbanu *et al*. reported the presence of this species from Kerala soils.[40] The present study extends the range of occurrence of this species in India.

An important indicator determining whether EPNs occur in the environment is the soil type. Soil texture influences nematode survival and mobility. Generally, higher clay content results in lower nematode survival. This is due to decreased pore size and reduced oxygen availability.[43,44] Nematodes are generally more mobile in sandy soil and mobility decreases as the percentage of clay and silt increases and these factors greatly contribute to the distribution of nematodes in particular habitat.[45,46] We agreed with the above statements as all the steinernematids isolated in this study were from sandy loam soil.

Interestingly, in the present study, *H. indica* was recovered from red loamy soils near banana plantations that contradict other studies, which generally document its frequent occurrence from sandy soils at coastal sites in other subtropical and tropical regions of the world.[47] The present study thus adds new information on the soil and habitat preference of this species.

In the present study, EPNs were detectable only from forest soils and no nematodes were isolated from other habitats studied. The reason could be attributed to that, the forest trees and shrubs have many defoliators from the orders Lepidoptera, Hymenoptera and Coleoptera, which pupate in the soil and thus serve as the host for EPNs. Similarly, many fly larvae (Bibionidae, Sciaridae and Tipulidae) feed on organic matter and roots in the soil.[48] All these insects may create an ideal environment for EPNs for their persistence and occurrence. In agreement with our findings, Stock *et al*.[49] reported that majority of nematodes were recovered from woodlands (coniferous forest) (33.8%) and oak forests (33.8%) and no nematodes were recovered from soil samples of chaparral or any of the desert habitats.

In conclusion, the present study constitutes the first report of EPNs in the northeast region of India, in general, and in the state of Meghalaya, in particular. Even though EPNs have been proved as potential biocontrol agents against a number of insect pests world over, in India it is in developing stage. The nematode species of EPNs exhibit differences in survival, search behavior and infectivity. Therefore, there is a greater interest in finding populations with traits suitable to local conditions. Although a number of EPN surveys has been conducted in many parts of the world, not much is known about the natural diversity of EPNs in India. There is a need for intensive surveys for isolation of EPN species from different agro climatic zones of India. The information generated from present study may open the prospects for using EPN species in the biological control programs against insect pests in the area because indigenous EPNs are adapted to the local environmental conditions and are natural regulators of insect populations.

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