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Isolation of entomopathogenic nematodes from Assam Agricultural University, Jorhat, Assam

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Abstract
Entomopathogenic nematodes (EPN) are lethal parasites of insects, used as biocontrol agents. The objective of this work was to survey the presence of EPN in Assam Agricultural University, Jorhat, Assam and to characterize the different species. Out of 200 soil samples, 8 were positive for entomopathogenic nematodes (4%), with 4 (2%) *Steinernema* isolates, 2 (1%) *Heterorhabditis* isolates and 2 (1%) *Oscheius* isolates. These EPNs have been identified on the basis of morphological and morphometric character. The identified species were *Heterorhabditis bacteriophora* and *Oscheius chongmingensis*. Entomopathogenic nematodes were not recovered from the fallow land.

Keywords: *Galleria mellonella*, *Heterorhabditis*, *Steinernema*, survey

1. Introduction
Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae have considerable potential for biological control of insect pests. The non feeding infective juvenile (IJ), carrying cells of the symbiotic bacteria, *Xenorhabdus* spp. in gut, migrate through the soil, enter a susceptible host and release the symbiont into the haemocoel. Proliferation of the bacteria leads to death of the insect within days, followed by nematode growth and reproduction. Surveys for EPNs have been conducted in many parts of the world, both for the purpose of recovering potentially useful isolates and gaining an insight into the ecology of the nematodes. An understanding of the factors governing the natural occurrence and abundance of the nematodes is of importance in formulating a rational approach to their utilization as bio-control organism. It is known that EPN distribution depends on temperature and precipitation and is closely related to vegetation type and presence of insect hosts. The Jorhat Campus of Assam Agricultural University has a wide diversity of crops, such as fruit trees, cereals and vegetables, tea and natural habitats. These habitats are subject to insect pests which every year cause significant losses in agricultural production. Entomopathogenic nematodes show significant variation in behaviour, host range, infectivity, reproduction and tolerance to adverse environmental conditions and therefore it is of major interest to fully characterize natural populations [1]. Keeping all these views in mind, extensive survey on distribution of entomopathogenic nematodes have been conducted demonstrating their occurrence and providing an indication of species which are indigenous for a given particular area.

2. Materials and Methods
2.1. Sample collection
A systematic survey was undertaken in Assam Agricultural University, Jorhat for the presence of entomopathogenic nematodes during the year 2015-16. A total 200 numbers of soil samples were collected randomly during the period November 2015 to November 2016 from four habitats (vegetation type) viz., Instructional Cum Research (ICR) Farm (total area of 69.61 hectares), Experimental farm of Department of Horticulture (total area of 16.43 hectares), Experimental farm for plantation crops (total area of 52 hectares.) and fallow land. For each location, GPS coordinates and soil physical characteristics such as soil texture were recorded (Table 1).

2.2. Nematode isolation and identification
Entomopathogenic nematodes were recovered from soil samples using the insect baiting method described by Bedding and Akhurst [2].

The dead larvae were thoroughly rinsed in distilled water and placed in modified White traps [3] until emergence of third-stage infective juveniles. The adults and the IJs (20 each) were then mounted in dehydrated glycerin using appropriate sized glass support. The mounted specimens were used for detailed microscopic studies [5]. In addition to the deMan formula, the other characters studied were: D% (Distance from Head to Excretory pore/ oesophageal length x 100), E% (Distance from Head to Excretory pore/tail length x 100), F% (Body width/tail length x 100), SW (Spicule length / cloacal body width), GS (gubernaculums length / spicule length). Morphological and morphometrical data of the isolates were compared with the original description of the type species. As *G.mellonella* was used as the laboratory bait insect, it was reared on artificial diet as per the procedure described by David and Kurup [6].

3. Results and Discussion

Survey data revealed that out of 200 soil samples, eight soil samples were positive for entomopathogenic nematodes (4%). Among the extracted populations, 4 isolates were assigned to the genus *Steinernema* (2%) designated as EPN-S-J-1, EPN-S-J-2, EPN-S-J-3.The isolates were found from rhizosphere of mung bean, arahar and cowpea respectively from ICR Farm, AAU, Jorhat. Another isolate of steinernematid (designated as EPN-S-J-4) was found from rhizosphere of citrus from the Experimental farm of the Department of Horticulture. Two isolates were assigned to the genus *Heterorhabditis* (1%), designated as EPN-H-J-1, was found from rhizosphere of citrus in the Experimental farm of the Department of Horticulture, another isolate designated as EPN-H-J-2 was found from tea plantation area of Experimental farm for plantation crops of AAU. Two isolates were assigned to the genus *Oscheius* (1%), one isolate designated as EPN-O-J-1 was found from rhizosphere of coconut in the Experimental farm of the Department of Horticulture, another isolate designated as EPN-O-J-2 were found from tea plantation area of Experimental farm for plantation crops of AAU, Jorhat. Entomopathogenic nematodes were not recovered from the fallow land (Table 1). The present study recorded for the first time the occurrence of entomopathogenic nematodes in the fields of AAU, Jorhat. Although, EPN were recovered from only 8 out of 200 samples (4%), this highlights the importance of conducting a more intensive survey. Kumar *el al.*, [8] also found entomopathogenic nematodes in only 5 samples out of 105 samples. Similar findings have been reported by Lorio *et al.*, [9] who found *Steinernema* spp. in only 12.3% samples. EPN distribution depends on temperature, precipitation and soil type and is closely related to vegetation type and presence of insect hosts [10 -12]. Assam Agricultural University has a wide diversity of crops, such as fruit trees, cereals, vegetables, tea and natural habitats. These habitats are subject to various insect pests. The recorded soil temperature was ranged from 20-30 ºC and the average rainfall was 135.77 mm per month. The soils are sandy or sandy loam with a good amount of organic matter. The organic matter content of soils ranged from 0.58 to 0.60% and pH 5.87. The nematode presence and abundance were varied from four different habitats of most of the sampling sites. Positive samples were most commonly found in agricultural field with 4% frequency of occurrence (Table 1). Although EPN were recovered at a low rate in present study, three different species were isolated from three different habitat (vegetation) viz., ICR field, Experimental farm of Department of Horticulture, and Experimental farm for plantation crops. Four isolates of Steinernema spp. (2%), two isolates of *Heterorhabditis bacteriophora* (1%), and two isolates of *Oscheius chongmingensis* (1%) were recovered. It may be resulted due to condition of the crop land in terms of irrigation of the field, where the temperature and the soil moisture was suitable for their persistence. One reason for the low recovery rate obtained in the present study, could be the fact that only one insect, *G. mellonella*, was used as bait insect and it may not be the appropriate host for all EPN species [13]. Furthermore, the choice of sampling sites may contribute to differences in EPN recovery percentage [14]. Lower percentage of EPNs probably also due to chemical control of insect pests in experimental fields which partially reduces the abundance of natural biocontrol agents. However, this low recovery percentage is not unusual, and it has already been reported from other surveys [13-15]. Rosa *et al*., [16] reported that most of the surveys showed their recovery rate from soil varies from 6% to 35% in Northern Ireland. Raj Kumar *et al*., [17] reported that out of 105 soil samples collected from Rajasthan, 5(4.76%) were found to be positive for EPNs. Mracek and Becvar [18] and Bruck [19] reported that recovery frequency of EPNs may vary from 0.7% to 70.1%. Entomopathogenic nematodes were not recovered from the fallow land that was covered by long grass, shrubs and weeds. Akhurst and Brooks [20] and Griffin *et al.*, [21] observed that entomopathogenic nematodes were more prevalent in agricultural fields than in natural habitats. All the eight EPN positive soil samples were from sandy loam soil and this finding was in agreement with the findings of the surveys conducted by Ambika and Sivakumar [22] which revealed that the occurrence of EPNs was more in light soils like sandy loam, sandy, loamy sand, loam soils rather than in heavy soils. However EPNs are present in heavy soils like clay soil also as recorded by Shyamprasad *et al*., [23] and Sosamma and Rasmi [24] in the South Andamans and Kerala, respectively. In Sri Lanka, *Heterorhabditis sp*. was reported to be restricted to sandy soils within 100 m of the sea [25]. *H. bacteriophora* is highly mobile, responding to chemical signals from the host, and being adapted to infect less mobile insects that are found in lower soil layers [26]. Since our samples were collected from the upper soil layer, this could explain the low recovery of *H. bacteriophora*.

Morphological studies of the two heterorhabditid isolates were undertaken and it was found to be similar to each other. Therefore detailed morphometrical studies of the isolate EPN-H-J-1 were undertaken. Morphological and morphometrical studies of different life stages (infective juveniles, adults of both generations) of EPN-H-J-1 revealed that it is closely resemble with *H. bacteriophora* [7] in most of the characters (Table 2 and Table 3). The head of the third-stage infective juvenile (IJ) bears dorsal tooth with mouth and anus are closed. Stoma appears as a closed chamber. The head is with sheath (cuticle of second-stage juvenile). Esophagus and intestine are reduced. The excretory pore is posterior to nerve ring. The tail is long, pointed and covered with a sheath. The male of second generation had slightly round head. They possess a tubular stoma and pharynx with a cylindrical corpus. The isthmus is distinct with a globose basal bulb and a prominent valve. The nerve ring surrounding the isthmus is located near the basal bulb. The excretory pore is located near the middle of the basal bulb. The reproductive structure is monarchic; anteriorly reflexed. The spicules are paired, symmetrical and separate, with pointed tips, slightly curved

ventrally. The gubernaculum is flat and narrow, bursa peloderan, open, with nine pairs of genital papillae, tail is pointed. The hermaphroditic female of first generation body curved ventrally like C- shaped when heat-killed. Head region is slightly rounded, six lips are prominent. They possess a tubular stoma and pharynx with a cylindrical corpus. The isthmus is distinct and short. Nerve ring surrounding isthmus is just anterior to basal bulb. Excretory pore is posterior to the basal bulb. Gonads amphidelphic and reflexed. Vulva is near mid-body. Vulva protruding outward and functional for oviposition. Tail is longer than anal body width, conoid with pointed terminus. Anal region is slightly protruding. The amphimictic females of second generation body curved ventrally like C- shaped when heat-killed, smaller in size than hermaphroditic females. Head region is subconical, six lips are prominent. They possess a tubular stoma and pharynx with a cylindrical corpus. The vulva does not protrude outward, surrounded by a hardened deposit. Anal region is slightly protruding.

The IJs of EPN-H-J-1 showed close similarity with *H. bacteriophora* with respect to head shape, ratio b, D% and E%, but exhibited minor differences from the type measurements by having lower tail length (84 vs. 91), which were considered as intraspecific variations of *H. bacteriophora* (Table 2). The males of this isolate showed close similarity with *H. bacteriophora* with respect to head shape, anal body width, gubernaculum length but exhibited minor differences from the type measurements by having higher esophagus length (110 vs. 103) and tail length (32 vs. 28), which are considered as intraspecific variations of *H. bacteriophora* (Table 2). The hermaphroditic and amphimictic females of this isolate showed close similarity with *H. bacteriophora* with respect to head shape, tail length and vulval position but exhibited differences from the type measurements by having lower body length, lower body width and lower anal body width which are considered as intraspecific variations of *H. bacteriophora* (Table 3). The heterorhabditid isolate was similar to *H. bacteriophora* in original description with respect to third stage infective juvenile in characters like greatest width; distance from anterior end to excretory pore; distance from anterior end to pharynx base; body width at anus; ratio a; ratio b; ratio c; D%; E%. However, the isolate showed variation in body length of IJs (550 vs.570) and tail length (84 vs.91). Variation also observed with respect to adult stage of both male and female generations in some characters like body length, position of pharynx, position of excretory pore, tail length, spicule length and gubernaculum length, etc. Nguyen and Smart [27] observed variations in body length, position of excretory pore, tail length and value of E% of *H. bacteriophora* in relation to time of harvest. It was observed that body length of infective juvenile was 605µm (579µm-634µm) on 3rd day of harvest where as body length 565µm (524 µm -604 µm) on 15th day of harvest. In the present investigation the third stage IJs were obtained when they emerged from the cadavers after 7 to 10 days. Poinar [7] isolated and described *H. bacteriophora* from Brecon, South Australia. The nematode was isolated from the body cavity of *Heliothis punctigera* Hall (Noctuidae: Lepidoptera). *H. bacteriophora* is distributed in America, Southern and Central Europe, Australia and East Asia [28]. In Europe it has been reported from Spain, Italy, Moldova, Hungary, Southern France [29] the Azores, Germany, Switzerland [30], South Russia [31] the European part of Turkey [15] and Slovenia [32]. *H. bacteriophora* isolates were found in neutral (vertisol) or acidic (oxysol) soils in croplands, orchards, and woodland habitats in Guadeloupe (Grande Terre, Basse Terre) and neighbouring islands. *H. bacteriophora* was reported from India by Sivakumar *et al*., [33] and Hussaini *et al*., [34]

Morphological studies of the two *Oscheius* isolates were undertaken and it was found to be similar to each other. Therefore detailed morphometrical studies of the isolate EPN-O-J-1 were undertaken. Morphological and morphometrical studies of different life stages (infective juveniles, males of second generation) of EPN-O-J-1 revealed that it is closely resemble with *Oscheius chongmingensis* in most of the characters (Table 4 and Table 5). Infective juveniles body elongate, sheath is present immediately after harvesting. The head of the infective juvenile (IJ) do not bear dorsal tooth. Stoma appears as a closed chamber. The excretory pore is posterior to basal bulb. Exsheathed IJs body is annulated. The tail is long and pointed. The male of second generation body curved ventrally like J shaped when heat-killed. Head is slightly swollen, six conical lips prominent. They possess a tubular stoma and pharynx with a cylindrical corpus, metacarpus swollen. The isthmus is distinct with a globose basal bulb and a prominent valve. The nerve ring surrounding the isthmus is located anterior to isthmus, cardia present, protruding into intestine. The excretory pore is posterior to the basal bulb. The reproductive structure is monarchic; anteriorly reflexed. The spicules are paired, symmetrical and separate, slightly curved ventrally, head of spicules with rounded anterior end. The gubernaculum is flat and narrow, about 50% length of spicule, curved ventrally. Bursa is peloderan, open and surrounding male cloaca. Tail is pointed. The hermaphroditic female of first generation body curved ventrally like C- shaped when heat-killed. Head region is tapering anteriorly, six conical lips are prominent. They possess a tubular stoma and pharynx with a cylindrical corpus. The isthmus is distinct and short. The nerve ring surrounding the isthmus is located anterior to isthmus. Valve of basal bulb is prominent. Vulva with a transverse slit, situated on a protruding area, posterior to mid body and without cuticular flaps. Tail is longer than anal body width, conoid with pointed terminus. Post-anal swelling is well distinguished. The amphimictic females of second generation body curved ventrally like C- shaped when heat-killed, smaller in size than hermaphroditic females. Head region is tapering anteriorly, six conical lips are prominent. They possess a tubular stoma and pharynx with a cylindrical corpus. The isthmus is distinct and short. The nerve ring surrounding the isthmus is located anterior to isthmus. Valve of basal bulb is prominent. Vulva is not protruding, covered with copulation plug after mating. Tail is longer than anal body width, conoid with pointed terminus. Post-anal swelling is well distinguished. The IJs of EPN-O-J-1 showed close similarity with *Oscheius chongmingensis* with respect to head shape, ratio a, ratio b, D% but exhibited minor differences from the type measurements position of excretory pore (102 vs. 90) and lower tail length (71 vs. 111), higher E% (148 vs. 83) and ratio c (6.9 vs. 3.9) which were considered as intraspecific variations of *Oscheius chongmingensis* (Table 4). The males of this isolate showed close similarity with *Oscheius chongmingensis* with respect to head shape, tail length, anal body width, but exhibited minor differences from the type measurements by having lower body length (962 vs. 1115), higher body width (59 vs. 46) and GS (65 vs. 48) which are considered as intraspecific variations of *Oscheius*

chongmingensis (Table 4). The hermaphroditic females and amphimictic females of this isolate showed close similarity with *Oscheius chongmingensis* with respect to head shape, anal body width but exhibited differences from the type measurements by having lower body length, lower body width, higher tail length which are considered as intraspecific variations of *Oscheius chongmingensis* (Table 5). The isolate EPN-O-J-1 was thus identified as *Oscheius chongmingensis*, which is a new record of this species from Assam, India.

The *Oscheius* isolate was similar to *Oscheius chongmingensis* in original description of *Heterorhabditidoides chongmingensis* with respect to third stage infective juvenile in characters like distance from anterior end to excretory pore; distance from anterior end to nerve ring, distance from anterior end to pharynx base; body width at anus; ratio a and ratio b. However, the isolate showed variation in body length (484 vs. 428), body width (28 vs. 22), tail length (71 vs. 111), E% (118 vs. 83) of infective juvenile which were considered as intraspecific variations of *Oscheius chongmingensis*. Variation also observed with respect to adult stage of both male and female in some characters like body length, distance from anterior end to nerve ring, excretory pore, tail length. Zhang *et al*., [35] isolated the new entomopathogenic nematode species from soil samples of Chongming Island in Eastern China and described as *Heterorhabditidoides chongmingensis*. Phylogenetic tree of 18S rDNA and ITS rDNA showed that the nematode is a monophyletic group and is a closer relative of the genus *Oscheius*, *Rhabditis* and *Pellioditis* in the family Rhabditidae. Phenotypic characters and phylogenetic analysis based on 16S rDNA sequence data indicated that the symbiotic bacterium of *Heterorhabditidoides chongmingensis* belong to genus *Serratia* (Superfamily Enterobacteriaceae). Based on a phylogenetic tree of 18S rDNA sequences, Ye *et al*., [36] and Liu *et al*., [37] suggested that *Heterorhabditidoides*, the first identified entomopathogenic genus in the family Rhabditidae, was a junior synonym of *Oscheius*, and proposed that the name of the type species of *Heterorhabditidoides* should be changed to be *Oscheius chongmingensis* n. comb. Efficacy of *Oscheius* sp. against spindle bug and red ant in arecanut [38], rice yellow stem borer in India [39-42] have been documented. Ali *et al*., [43] isolated and described *Oscheius amsactae* n. sp. as a necromenic associate of red hairy caterpillar *Amsacta moori* from *Vigna radiata* in cultivated field at Indian Institute of Pulses Research, Kanpur, India.

Table 1: Occurence of Entomopathogenic nematodes in AAU campus, Jorhat, Assam

Agro-climatic regions	District	Habitat (vegetation)	No. of sample	Soil texture	No. of +ve sample	Crop	Species (Nematode Isolate)	Latitude, Longitude
Upper Brahmaputra Valley	Jorhat	ICR Farm, AAU, Jorhat	50(soil)	Sandy loam	1	Mungbean	*Steinernema* sp. (EPN-S-J-1)	26^{0}43'7.896"N 94^{0}11'40.098"E
					1	Arahar	*Steinernema* sp. (EPN-S-J-2)	26^{0}43'07.9"N 94^{0}11'40.1"E
					1	Cowpea	*Steinernema* sp. (EPN-S-J-3)	26^{0}43'27.3"N 94^{0}12'01.5"E
		Experimental farm, Deptt. Of Horticulture, AAU, Jorhat	50(soil)	Sandy loam	1	Coconut	*Oscheius* sp. (EPN-O-J-1)	26^{0}43´42.82″N 94^{0}12´02.09″E
					1	Citrus	*Steinernema* sp. (EPN-S-J-4)	26^{0}43.356´ N 94^{0}11.936´ E
					1	Citrus	*Heterorhabditis* sp. (EPN-H-J-1)	26°43.353´ N 94°11.930´E
		Experimental farm for plantation crops, AAU, Jorhat	50(soil)	Sandy loam	1	Tea	*Heterorhabditis* sp. (EPN-H-J-2)	26^{0}43´26.40″N 94^{0}12´21.13″E
					1	Tea	*Oscheius* sp. (EPN-O-J-2)	26^{0}43´02.28″N 94^{0}11´53.68″E
		Fallow land, AAU, Jorhat	50(soil)	Sandy loam	-	-	-	-

Table 2: Morphometrics of *Heterorhabditis* sp. (EPN-H-J-1) from fields of AAU, Jorhat, Assam in comparison with original description of *Heterorhabditis bacteriophora* of infective juvenile and second generation male. Measurements in µm and in the form: mean± SD (range).

Character	*Heterorhabditis sp.* (EPN-H-J-1) (IJ) (n=20)	Type measurement *H. bacteriophora* (IJ) (Poinar,1976) (n=15)	Type measurement *H. bacteriophora* (IJ) (Poinar 1990) (n=25)	*Heterorhabditis* sp. (EPN-H-J-1) (male) (n=20)	Type measurement *H. bacteriophora* (male) (Poinar, 1976) (n=15)
Body length (L)	550.2±12.1 (480-580)	570 (520-600)	558 (512–671)	831±55 (750-970)	820 (780-960)
Body width (W)	25±0.5 (13-30)	24 (21-31)	23 (18–31)	48±3.9 (44-50)	43 (38-46)
Anterior end to excretory pore (EP)	105.4±1.7 (80-112)	104 (94-109)	103 (87–110)	117±6.2 (109-130)	121 (114-130)
Anterior end to nerve ring (NR)	88.7±1.3 (60-102)	83 (81-88)	85 (72–93)	77±3.1 (74-84)	72 (65-81)
Anterior end to esophagus base (ES)	115.8±1.9 (88-130)	125 (119-130)	125 (100–139)	110±8.5 (99-130)	103 (99-105)
Testis reflexion				82±5.2 (76-90)	79 (59-87)
Tail length (T)	84.9±1.2 (60-90)	91 (83-99)	98 (83–112)	32±2.8 (30-36)	28 (22-36)
Anal body width (ABW)	15.8±0.2 (8-20)	-		22±1.9 (20-25)	23 (22-25)
Ratio a= (L/W)	25.0±5.5 (11.5-32.1)	25 (17-30)	25 (17–30)		

Spicule length (SL)				43±2.6 (40-50)	40 (36-44)
Gubernaculum length (GL)				21±1.5 (20-25)	20 (18-25)
D%=(EP/ES)x100	85.5±7.7 (72-93)		84 (76–92)	87.6±7.6 (77.8-97.4)	117
SW%=SL/ABW×100				180.3±40.5 (100-250)	174
GS%=GL/SL×100				0.48 ± 0.05(0.40-0.50)	50

Table 3: Morphometrics of *Heterorhabditis* sp. (EPN-H-J-1) from fields of AAU, Jorhat, Assam in comparison with original description of Heterorhabditis *bacteriophora* of hermaphroditic and amphimictic female. Measurements in µm and in the form: mean± SD (range).

Character	***Heterorhabditis* sp. (EPN-H-J-1) Hermaphroditic females (n=12)**	**Type measurement *H. bacteriophora* Hermaphroditic females (Poinar,1976) (n=15)**	***Heterorhabditis sp.* (EPN-H-J-1) Amphimictic female (n=12)**	**Type measurement *H. bacteriophora* Amphimictic female (Poinar,1976) (n=15)**
Body length (L)	1895.8±93.8 (1230-2020)	4030 (3630-4390)	1696.5±191.2 (1700-1960)	3500 (3180-3850)
Body width (W)	150.4 ± 7.2 (138-160)	165 (160-180)	149.9±4.1 (120-155)	190 (160-220)
Anterior end to excretory pore (EP)	162.4±9.7 (120-210)	209 (189-217)	147.5±7.6 (105-169)	192 (174-214)
Anterior end to nerve ring (NR)	120.6±2.5 (100-130)	126 (121-130)	116.1±2.4 (100-120)	103 (93-118)
Anterior end to esophagus base (ES)	177.5±10.6 (150-212)	197 (189-205)		
Tail length(T)	90.6±3.6 (72-99)	90 (81-93)	79.8±9.9 (76-85)	82 (71-93)
Anal body width (ABW)	18.7±4.4 (15-30)	46 (40-53)	23.4±3.4 (20-30)	28 (22-31)
V%= distance from anterior end to vulva as percentage of length	49.8±3.0 (44.0-54.0)	44 (41-47)	49.8±4.8 (41.5-57.5)	47 (42-53)

Table 4: Morphometrics of *Oscheius* sp. (EPN-O-J-1) from fields of AAU, Jorhat, Assam in comparison with original description of *Oscheius chongmingensis* of infective juvenile and second generation male. Measurements in µm and in the form: mean± SD (range)

Character	***Oscheius* sp. (EPN-O-J-1) (IJ) (n=20)**	**Type measurement *Oscheius chongmingensis* (IJ) (Zhang *et al.*, 2008) (n = 25)**	***Oscheius* sp. (EPN-O-J-1) (Male) (n=12)**	**Type measurement *Oscheius chongmingensis* (Male) (Zhang *et al.*, 2008) (n=20)**
Body length(L)	484.5±59.4 (350-560)	428±25 (395-474)	962.2±172.1 (700-1220)	1115±151 (822-1400)
Body width(W)	28±10.0 (20-40)	22.6±3.1 (19-29)	59.5±9.4 (50-70)	46±5.9 (37.7-62)
Anterior end to excretory pore(EP)	102.8 ± 5.7 (82-110)	90±7.5 (80-105)	174.4±10.4 (150-185)	169±18 (124-193)
Anterior end to nerve ring (NR)	93.5±7.4 (70-100)	74±10.5 (63-100)	143.2±5.6 (140-160)	115±14 (88-133)
Anterior end to esophagus base (ES)	114.0±7.5 (90-120)	104±8.2 (92-120)	189.7±6.7 (180-200)	157±18 (113-186)
Anal body width(ABW)	11.2±2.2 (10-15)	12±1.6 (10-15)	26.0±4.8 (20-32)	26±3.1 (21-33)
Tail length(T)	71.7±12.2 (60-100)	111±18.9 (89-159)	27.9±3.2 (23-30)	29±4.4 (22-38.8)
Spicule length(SL)			50±2.5 (40-55)	51±8.2 (37-68)
Gubernaculum length (GL)			25±0.6 (20-35)	24.6±3.8 (20-33)
SW% (SL/ABW)x100			202.5±41.8 (150.5-270)	195±33.6 (112-269)
GS% (GL/SL)x100			65.7±4.9 (61.4-77.3)	48±3.2 (43.2-54.5)
Ratio a(L/W)	19.8±7.6 (10-28)	19.1±1.8 (15-21)		
Ratio b(L/ES)	3.6±0.9 (3.0-4.6)	4.1±0.3 (3.6-4.4)		
Ratio c(L/T)	6.9±1.3 (5.6-11.6)	3.9±0.5 (2.9-4.9)		
D%(EP/ES)x100	90.4±4.9 (81.6-95.4)	86±1.4 (84-88)	91.8±4.0 (81.0-97.2)	107±1.4 (103-110)
E%(EP/T)x100	118.4±32.4 (98-153.3)	83±8.7 (67-97)		

Table 5: Morphometrics of *Oscheius* sp. (EPN-O-J-1) from fields of AAU, Jorhat, Assam in comparison with original description of *Oscheius chongmingensis* of hermaphroditic female and Amphimictic female. Measurements in µm and in the form: mean± SD (range)

Character	***Oscheius* sp (EPN-O-J-1) (Hermaphroditic female) (n=12)**	**Type measurement *Oscheius chongmingensis* Hermaphroditic female. (Zhang *et al.* 2008) (n=20)**	***Oscheius* sp. (EPN-O-J-1) (Amphimictic female) (n=12)**	**Type measurement *Oscheius chongmingensis* Amphimictic female (Zhang *et al.*2008) (n=20)**
Body length(L)	1586.6±183.0 (1380-2020)	1921±251 (1640-2220)	1062.7±167.7 (700-1348)	1143±141 (809-1351)
Body width(W)	93.5±17.9 (80-140)	104±19.6 (76.5-135)	53.1±14.3 (23-85)	55±6.6 (44-67)

Anterior end to excretory pore (EP)	192.5±13.9 (170-220)	207±27 (176-276)	175.1±19.8 (145-210)	158±14 (127-180)
Anterior end to nerve ring(NR)	164.3±20.2 (110-200)	143±22 (105-176)	140±23.67 (100-175)	123.5±15 (102-156)
Anterior end to esophagus base (ES)	218.1±15.5 (180-234)	179±22.6 (152-235)	191±20.7 (160-220)	180±14 (154-202)
Anal body width(ABW)	26±6.6 (20-40.5)	28±5.5 (23-42.2)	21.5±2.8 (20-30)	22.6±1.7 (20-27)
Tail length(T)	136.8±16.7 (110-160)	90±11.8 (75.3-117)	106.3±21.7 (75-170)	81±10.9 (67-102)
V%= distance from anterior end to vulva as percentage of length	48.6±2.2 (42.9-52.3)	52±1.5 (50.2-54.4)	53.6±5.9 (50.0-67.1)	51±1.5 (50-54.8)

4. Conclusion

Further work emphasis on the determination of its efficacy and potential use of isolated nematodes in the field.

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