Int.J.Curr.Microbiol.App.Sci (2020) *9*(4): 2474-2487

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 4 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.904.296

Isolation, Morphometrics and Morphological Characterization of *Oscheius chongmingensis* from Assam, India

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Keywords

Oscheius chongmingensis, Tea and *Galleria*

Article Info

Accepted:
18 March 2020
Available Online:
10 April 2020

A B S T R A C T

A total 200 soil samples were collected from tea plantation areas of district, Jorhat, Assam and were assessed for entomopathogenic nematodes using the *Galleria* baiting technique. Out of 200 soil samples, EPNs were found in 1 soil samples collected from Experimental farm for plantation crops, Section-4,10,19AAU, Jorhat with 0.5% frequency of occurrence. The measurements were expressed in % ratios and Means ± SD ranges. The IJs of EPN-O-J-1 isolate were characterized by the position of excretory pore (97 vs. 90), lower tail length (87 vs. 111), higher E% (111vs. 83) and ratio c (5.22 vs. 3.9). The male of this second-generation body is curved ventrally like J shaped when heat-killed; lower body length (1042 vs. 1115), higher body width (54.8vs. 46) and GS (48.8vs.48) and the first-generation hermaphroditic female of this isolate body was curved ventrally when heat-killed and measured about 1742±288µm(1362-2340µm) in body length, 98.2±18.4µm(77-138µm) in body width, 109.6±18.0µm(83-136µm) in tail length, 27.5±4.4µm(21-39µm) in anal body width and 50.11±4.06µm(43.2-55.4µm) in V%, respectively. The The second generation amphimictic females of isolate EPN-O-J-l were also showed ventrally curved body when heat killed and measured about 1085.7±174.9µm(753-1392µm) in body length, 55.1±10.1µm(34-76µm) in body width, 98.5±21.8µm(72-158µm)in tail length, 28±5.5µm(23-42.2µm) and 52.72±3.69µm(48-62µm) in V%. Comparative analysis revealed that an EPN-O-J-l isolates belongs to the *Oscheius chongmingensis* as earlier described by Zhang *et al.*, 2008from Chongming Island in eastern China in respect of body length, body width, tail length, higher E%, GS, ratio c and V%, respectively.

Introduction

Nematodes which are capable of killing, sterilizing or hampering the development of insect and completing at least one stage of their life cycle in the host are called entomopathogenic nematodes (EPNs). Entomopathogenic nematodes, *Steinernema* and *Heterorhaditis* in the family Steinernematidae and Heterorhabditidae of the order Rhabditida are obligate parasites of insect pests. They are dispersed in natural and agricultural soils. They have enormous potential as biocontrol agent against a wide range of insect pests. The other genus *Oscheius*, in the family Rhabditidae is also recently been isolated and found to be entomopathogenic. EPNs are considered as

one of the most significant non-chemical alternatives to insect pest control due to their high reproductive potential, ease of mass production and their harmlessness to microbes, animals, humans and plants. Only the third juvenile stage is the infective juvenile that is free-living in the soil, non-feeding, encased in a double cuticle with closed mouth and anus and capable of surviving for several weeks in the soil, before infecting a new host individual.

The infective juveniles actively penetrate through the mid gut wall or tracheae into the insect body cavity (hemocoel) containing insect blood. EPNs have a mutualistic partnership with gram-negative gamma-proteobacteria in the family Enterobacteriaceae. *Xenorhabdus* bacteria are associated with steinernematid nematodes while *Photorhabdus* are symbionts of heterorhabditids. *Photorhabdus* and *Xenorhabdus*, are carrying in their intestines, whereas *Heterorhabditidoides* (=*Oscheius*) carry symbiotic bacterial strains of *Serratia* in both the intestine and the cuticle (Kaya and Gaugler, 1993; Torres-Barragan *et al.,* 2011; Zhang *et al.,* 2008, 2009, 2012). Nematode and bacteria overcome the insect immune system and the host insect is killed within 24-48 hours post infection (Adams and Nguyen, 2002). The life cycle of heterorhabditidis similar to that of steinernematids except for the fact that the IJs always develop into self-reproducing hermaphrodites (Poinar, 1990). Strauch *et al.,* (2000) observed that offspring of the first-generation hermaphrodites can either develop into amphimictic adults or into automictic hermaphrodite both can occur simultaneously. The cycle from entry of IJs into a host until emergence of new IJs is dependent on temperature and varies for different species and strains. Generally, life-cycle of EPNs (infective juvenile penetration to infective juvenile emergence) is completed within 12-15 days.

The optimum temperature for growth and reproduction of nematodes is between 25°C and 30°C.Tea (*Camellia sinensis* L.) belonging to the family Theaceae and tribe Gordonaceae is a long duration perennial crop grown under monoculture. Tea leaves are mostly plucked for the making of tea beverages like green tea and black tea and it contain the polyphenols such as catechins and epicatechins. Such compounds act as antioxidant and provide health benefits like reduction of weight loss, the risk of diabetes and cardiovascular disease. The crop is extensively cultivated in 13 states of India out of which Assam, West Bengal, Tamil Nadu and Kerala are the largest producers.

In Assam, it is cultivated on 765 tea gardens covering an area of 307.08 thousands hectares and more than 17.00 percent workers of Assam are engaged in the tea industry (Anonymous, 2018). The total annual production of tea in Assam is 676.31 million kg in year 2017-18, which is more than 50 percent of India's total tea production (Anonymous, 2018). Crop loss in tea due to pests, diseases and weeds varies between 7-15% (Borthakur *et al.,* 1992). Pest infestation is a major problem associated with tea cultivation that caused reduction not only in the quantity but also in quality of tea. Tea plantation provides a permanent ecosystem for more than1034 arthropods (Chen and Chen, 1989).

Das (1965) reported that 167 species of arthropods have been noted from the northeast India and cause 11.00-55.00 percent annual yield loss. Among them, tea mosquito bug, *Helopeltistheivora* (Waterhouse) (Hemiptera: Miridae) is one of them. *H.theivora* becomes the greatest enemies of tea planters in Africa and Asia causing 55.00 percent and 11.00-100.00 percent crop loss, respectively (Wilson and Clifford 1992; Anonymous, 1994; Sundararaju and Sundara

Babu, 1999). In Assam *H. theivora* caused 15.00-20.00 percent crop loss (Hazarika *et al.*, 2009; Anonymous, 2010). This particular pest causes damage to tea plant throughout the year but the incidence is more severe during the months of May-September.

Bunch caterpillars, *Andracabipunctata* Walk. (Lepidoptera: Bombycidae) is a well-known pest of tea (Watt and Mann, 1903) and mostly occurs in India, Indonesia, Taiwan, China, and Vietnam. In India, the pest is reported from Assam, Sikkim, and West Bengal.

The hatched larvae feed on the shoot, bud, young leaves, and matured leaves in a group and completely defoliate the tea plant causing 15.00 percent yield loss in Assam (Anonymous, 2010). EPNs are not only exhibited in wide range of habitats but also showed species variation in respect of morphology, reproduction, infectivity, host range and conditions for survival(Bedding, 1983).

In the northeastern region of India, a few surveys against EPNs have been conducted in various habitats but and no survey has been conducted to document the occurrence of EPNs in tea habitats. It was the first documentation of EPN fauna in Tea habitats of Assam for that a study was undertaken to isolate and identify EPNs from tea infested by *H. theivora* and *A. bipunctata.*

Materials and Methods

Survey and sample collection

A survey was undertaken in the tea plantation areas of Jorhat district of Assam for the presence of entomopathogenic nematodes (EPNs) during the year 2017-18. A total of 200 soil samples were collected randomly during the period Nov 2017 to Nov 2018. Each soil sample, weighing approximately 1 kg, was a composite of five random subsamples collected at least 100m apart at each site at a depth of 10–20 cm in an area of $20m^2$. Information regarding date of sampling, and soil type along with GPS (Global Positioning System) location was recorded.

Samples were packed in polythene bags and maintained at refrigerated conditions in the laboratory for further processing. The soil was thoroughly mixed on a plastic sheet and half of each sample was used for extraction of EPNs.

Rearing of bait insect

Greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) larvae is used as a bait insect for the trapping of EPNs. *G. mellonella* was collected from the Department of Entomology, AAU, Jorhat. The culture of *G. mellonella* was maintained on a semi-synthetic diet in the P.G laboratory, Department of Nematology, AAU, Jorhat (Plate 4).All solid ingredients, *viz.*, corn flour (400 gm), wheat bran (150 gm), wheat flour (200 gm), wheat germ (50 gm), yeast and milk powder (200 gm) were mixed thoroughly in a clean flat plastic tray. Honey and glycerin (200 gm) dissolved in lukewarm water (200 gm) was added slowly to the solid mixture followed by the addition of streptomycin sulphate (100 gm).

The mixture was kneaded thoroughly until semisolid light yellow coloured dough was obtained. This diet was transferred to 2 L capacity wide mouth jars and filled up to 1/4th of its volume. They were inoculated with 20-25 egg masses (each containing 500 eggs). The jars were covered with white muslin cloth, secured by rubber band and incubated at 25ºC in the BOD incubator. Incubated eggs hatched within a week, emerging neonates fed voraciously and

underwent 5 moults before pupation in 25 days. Pupae were transferred to a clean adult rearing cage. Adult moths emerged from pupae within 10-12 days. The emerging moths were collected every morning, sorted into males and females based on their size and shape and released into the egg laying chamber in the ratio of 1:6 (male : female).

Tissue papers made into folds were hanged inside the chamber for egg laying. Adults were feed on 20-30% honey solution through cotton wick in a small plastic dish.

Within few days of mating, females laid eggs on the tissue paper. These eggs were then transferred to a fresh culture medium kept at 28°C in the BOD incubator for next generation. The 4th instar larvae were used for baiting.

Extraction of entomopathogenic nematodes (EPNs) from soil samples

Entomopathogenic nematodes were isolated from the soil samples by the method described by Fan and Hominick (1991) with larvae of the greater wax moth (*Galleria mellonella*). Before processing, soil samples were homogenized and then baited. Ten last instar larvae of *G. mellonella* were released into the plastic container containing 200g of soil sample.

Baited samples were stored in the dark at room temperature. Samples were inverted at regular intervals and monitored for mortality up to 7-8 days. Insect cadavers from each soil sample were taken out and examined for infection.

Collected *G. mellonella* larvae were transferred to White traps (White, 1927) and infected cadavers were placed on a 9 cm Whatman No.1 filter paper over a small Petri dish (50 mm × 17 mm) which was then placed in bigger Petri dish (100 mm × 20 mm) containing water. IJs recovered for the 5-12 following days were collected.

IJs that emerged were pooled from each sample and used to infect fresh last instars of *G. mellonella* larvae to verify their pathogenicity and allow the production of progeny for identification at the genus level, considering the characteristic colour of the *G. mellonella* cadavers (Kaya and Stock, 1997).

Isolation of adults

In nature, the adults of first and second generation are found only in the haemocoel of cadaver; hence they were extracted by dissection in Ringer's solution. The dissection was done at 2-4 and 4-5 days after inoculation (DAI) for recovering the first generation and second-generation adults, respectively. The recovered nematodes were kept in clean ringer's solution for further processing.

Processing of nematodes

Third stage infective juveniles in sterile distilled water and freshly dissected out first and second-generation adults in Ringer's solution were killed and fixed by pouring equal volumes of hot tri-ethanolamine formalin (TAF) fixative over the EPN suspension (Kaya and Stock,1997).

After 24 hrs, the specimens were handpicked individually and transferred to 100% TAF and fixed for a week. Killed and fixed nematodes were further processed with Seinhorst's slow glycerol dehydration method (Seinhorst, 1959).

Permanent mounts were prepared by transferring the nematodes to a drop of anhydrous glycerin on a clean glass slide supported by radially placed 3 small pieces of

glass wool supports with thickness approximately equal to the diameter of the nematode to prevent flattening of specimens. The slides were sealed with paraffin wax and labeled with adequate information including locality, slide number, sex and stage of nematode.

Light microscopic studies

The permanent slides were examined for detailed morphological characters and body dimensions were studied using de Man's formula (De Man, 1880) and additional ratios to establish their taxonomic identity. The morphological identification was performed on the basis of characters of third stage infective juveniles and male individuals (Poinar, 1976; Poinar and Georgis, 1990; Nguyen and Smart, 1995; Stock *et al.,* 2002).

Morphological characters

The following morphological characters were taken into consideration for identification at species level.

Shape of head
Presence or absence of epiptygma
Shape and size of spicules
Shape and size of gubernaculum
Presence or absence of post anal swelling in adult females
Tail shapes of both adults and infective juveniles
Presence or absence of mucron in adults of both sexes

Morphometrical measurements

In addition to the morphological characters, morphometric measurements also have taxonomic significance in differentiation of species. Morphological characters of 20 specimens each of infective juveniles, males and females of first and second generation were observed. Morphological observations and quantitative measurements were made by advanced stage compound microscope (Olympus).

Liner body dimensions recorded were as follows

Body length (L)
Body width (W)
Oesophageal length (ES)
Distance from anterior end to excretory pore (EP)
Distance from anterior end to nerve ring (NR)
Spicule length (SL)
Gubernaculums length (GL)
Anal body width (ABW)
Tail length (T)

The following ratios were computed

Ratio a = Body length/Greatest body width
Ratio b = Body length/ Oesophageal length
Ratio c = Body length/Tail length
V = Distance of vulva from anterior end/Body length × 100
D% = Distance from anterior end to excretory pore / Oesophageal length × 100
E%= Distance from anterior end to excretory pore/Tail length × 100
SW% = Spicule length/anal body with × 100
GS% = Gubernaculum length/ Spicule length × 100

Comparison with known species

The morphological character and body dimension of *Oscheius* sp. identified in the present study were compared with the original descriptions given by Zhang *et al.,* (2008). Variations in the morphometrical characters

of the existing species were recorded and described.

Results and Discussion

A random survey was conducted for the natural occurrence of EPNs during 2017-18 from tea plantation area of the district, Jorhat, Assam. A total of 200 samples were collected fromExperimental farm for tea plantation crops, Section-4, 10, 19 AAU, Experimental farm for plantation crops, Section-14 AAU, Jorhat, Experimental Farm, Tocklai Tea Research Station and Chetiagaon, Jorhat. Survey data revealed that out of 200 samples, 1 sample was found positive for EPNs with 1 sample containing *Oscheius* sp.(0.25%)(Table 1). *Oscheius* isolates was designated as EPN-O-J-1, were isolated from rhizosphere of tea from experimental farm for tea plantations crops Section-4, 10, 19 AAU, Jorhat. Morphological and morphometrical characters were used for the identification of nematode isolates.

Morphological and morphometrical studies of different life stages (infective juveniles, males of second generation) of EPN-O-J-l revealed that it is closely resemble with *Oscheius chongmingensis* in most of the characters (Table 2, 3 and Fig. 1). Infective juvenile's body is elongate sheath is present immediately after harvesting. The head of the infective juvenile (IJ) do not bear dorsal tooth (Figure 2). Stoma appears as a closed chamber (Figure 2). The excretory pore is posterior to basal bulb (Figure 2). The tail is long and pointed (Figure 3). The male of second-generation body is curved ventrally like J shaped when heat-killed. Head is slightly swollen. They possess a tubular stoma and pharynx with a cylindrical corpus, metacarpus is swollen. The isthmus is distinct with a globose basal bulb and a prominent valve. The nerve ring is surrounding the isthmus is located anterior to the isthmus, cardia is present and protruding into intestine. The excretory pore is posterior to the basal bulb. The reproductive structure is monarchic and anteriorly reflexed. The spicules are paired, symmetrical and separate, slightly curved ventrally, head of spicules with rounded anterior end (Figure 8). The gubernaculum is flat and narrow, curved ventrally. Bursa is pleoderan and open (Figure 8). Tail is pointed.

The hermaphroditic female of first-generation body curved ventrally when heat-killed (Figure 4). Head region is tapering anteriorly. It possesses a tubular stoma and pharynx with a cylindrical corpus (Figure 4). Theisthmus 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 is situated on a protruding area and posterior to mid body and without cuticular flaps (Figure 6). Tail is longer than anal body width and conoid with pointed terminus (Figure 6). Post anal swelling is well distinguished. The amphimictic females of second-generation body curved ventrally when heat killed, smaller in size than hermaphroditic females. Head region is tapering anteriorly (Figure 5).

It possesses a tubular stoma and pharynx with a cylindrical corpus (Figure 5). The isthmus is distinct and short (Figure 5). The nerve ring surrounding the isthmus is located anterior to isthmus (Figure 5). Valve of basal bulb is prominent. Vulva is not protruding and covered with copulation plug. Tail is longer than anal body width and conoid with pointed terminus. Post-anal swelling is well distinguished. The IJs of EPN-O-J-l showed close similarity with *O.chongmingensis* with respect to head shape, ratio a, ratio b, D% but exhibited minor differences from the type measurements position of excretory pore (97 vs. 90) and lower tail length (87 vs. 111), higher E% (111vs. 83) and ratio c (5.22 vs. 3.9)

which were considered as intraspecific variations of *O.chongmingensis* (Table 4.1 B). The males of this isolate showed close similarity with *O.chongmingensis* with respect to head shape, tail length, anal body width, but exhibited minor differences from the type measurements by having lower body length (1042 vs. 1115), higher body width (54.8vs. 46) and GS (48.8vs. 48) which are considered as intraspecific variations of *O.chongmingensis* (Table 4.1 B). The hermaphroditic females and amphimictic females of this isolate exhibited differences from the type measurements by having lower body length, lower body width, higher tail. The isolate EPN -O-J-1wasthus identified as *O.chongmingensis* (Table 3).

A total of two isolates of entomopathogenic nematodes from 200 soil samples collected from Tea plantation areas of district Jorhat, Assam with a per cent recovery of 1%. EPNs distribution depends on temperature, precipitation and soil type and is closely related to vegetation type and presence of insect host (Nielsen and Philipsen, 2003; Puza and Mracek, 2005; Campos-Herrera *et al.*, 2007; Campos-Herrera *et al.,* 2011; El Borai *et al.,* 2012). The soil is sandy or sandy loam with a good amount of organic matter. The nematode presence and abundance were low in different tea fields of most of the sampling site. Although EPNs were recorded at a low rate in present study, one isolate of *O.chongmingensis* (0.5%) was recorded. 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, *Galleriamellonella*, was used as bait insect may not be the appropriate host for all EPN species (Kary *et al.,* 2009). Furthermore, the choice of sampling sites may contribute to difference in EPN recovery percentage (Mracek *et al.,* 2005). Lower percentage of EPNs probably also due to chemical control of insect pest in tea fields which partially reduces the abundance of natural biocontrol agents.

Table.1 Occurrence of Entomopathogenic nematodes in tea plantation areas of district, Jorhat, Assam

Locality	No. of samples	No. of +ve samples for EPN	Crop	EPN isolate		Latitude, Longitude
				Oscheius sp.	Frequency of occurrence (%)	
Experimental farm for plantation crops, Section-4,10,19 AAU, Jorhat	50	1	Tea	EPN-O-J-1	0.5	26°71'96.63"N 94^{0}19'73.03"E
Experimental farm for plantation crops, Section-14 AAU, Jorhat	50	0	Tea	-	-	
Experimental Farm, Tocklai Tea Research Station	50	0	Tea	-	-	
Chetiagoan	50	0		-	-	
Total	**200**	**1**			**0.5**	

Table.2 Morphometrics of *Oscheius sp*. (EPN-O-J-1) infective juveniles and second-generation male in comparison with original description of *O.chongmingensis*

Character	*Oscheius sp.* (EPN-O-J-1) (IJ) (n=40)	Type measurement *O.chongmingensis* (IJ) (Zhang *et al.*, 2008) (n=25)	*Oscheius sp.* (EPN-O-J-1) (Male) (n=20)	Type Measurement of *O.chongmingensis* (Male) (Zhang *et al.*, 2008) (n=20)
Body length (L)	453.7±45.6 (372-520)	428±25 (395-474)	1042.20±204 (773-1392)	1115±151 (822-1400)
Body width (W)	25.3±4.4 (19-36)	22.6±3.1 (19-29)	54.85±6.98 (46-69)	46±5.9 (37.7-62)
Anterior end to excretory pore (EP)	97.05±6.9 (78-106)	90±7.5 (80-105)	172.5±12.12 (148-187)	169±18 (124-193)
Anterior end to nerve ring (NR)	86.08±10.6 (65-103)	74±10.5 (63-100)	131.90±15.7 (106-153)	115±14 (88-133)
Anterior end to esophagus base (ES)	110.2±8.8 (86-124)	104±8.2 (92-120)	181.80±13.4 (158-206)	157±14 (113-186)
Tail length (T)	87.8±10.1 (74-128)	111±18.9 (89-159)	28.7±3.24 (24-36)	29±4.4 (22-38.8)
Anal body width (ABW)	11.1±1.5 (9-14)	12±1.6 (10-15)	26.6±3.9 (20-32)	26±3.1 (21-33)
Ratio a= (L/W)	18.2±2.6 (11-24)	19.1±1.8 (15-21)	-	-
Ratio b(L/ES)	4.12±0.27 (3.1-4.6)	4.1±0.3 (3.6-4.4)	-	-
Ratio c(L/T)	5.22±0.74 (3.73-6.53)	3.9±0.5 (2.9-4.9)	-	-
Spicule length (SL)	-	-	49.00±5.10 (38-55)	51±8.2 (37-68)
Gubernaculum length (GL)	-	-	23.80±3.52 (20-34)	24.6±3.8 (20-33)
D%= (EP/ES)×100	88.16±3.6 (75-94)	86±1.4 (84-88)	94.94±1.24 (90-96)	107±1.4 (103-110)
SW%=SL/ABW×100	-	-	186.5±23.8 (143.3-228)	195±33.6 (112-269)
GS%=GS/SL×100	-	-	48.8±6.8 (37.7-64.1)	48±3.2 (43.2-54.2)
E%(EP/T)×100	111.58±13.2 (81-134)	83±8.7 (67-97)	-	-

Measurements in µm and in the form: mean± SD (range)

Table.3 Morphometrics of *Oscheius sp*. (EPN-O-J-1) hermaphroditic and amphimictic female in comparison with original description of *O.chongmingensis*

Character	*Oscheiussp.* (EPN-H-J-1) (Hermaphroditic females) (n=12)	Type measurement *Oscheius chongmingensis*(Hermaphroditic females) (Zhang *et al.*, 2008) (n=20)	*Oscheiussp.* (EPN-H-J-1) (Amphimitic females) (n=12)	Type Measurement of *Oscheius chongmingensis* (Amphimitic females) (Zhang *et al.*, 2008) (n=20)
Body length (L)	1742±288 (1362-2340)	1921±251 (1640-2220)	1085.7±174.9 (753-1392)	1143±141 (809-1351)
Body width (W)	98.2±18.4 (77-138)	104±19.6 (76.5-135)	55.1±10.1 (34-76)	55±6.6 (44-67)
Anterior end to excretory pore (EP)	198.9±23.3 (172-266)	207±27 (176-276)	167.1±18.6 (132-195)	158±14 (127-180)
Anterior end to nerve ring (NR)	152.5±24.7 (108-193)	143±22 (105-176)	129.65±19.9 (104-170)	123.5±15 (102-156)
Anterior end to esophagus base (ES)	223.5±23.0 (185-274)	179±22.6 (152-235)	186.05±18.0 (157-213)	180±14 (154-202)
Tail length (T)	109.6±18.0 (83-136)	90±11.8 (75.3-117)	98.5±21.8 (72-158)	81±10.9 (67-102)
Anal body width (ABW)	27.5±4.4 (21-39)	28±5.5 (23-42.2)	22.85±2.8 (20-29)	22.6±1.7 (20-27)
V%= distance from anterior end to vulva as percentage of length	50.11±4.06 (43.2-55.4)	52±1.2 (50.2-54.4)	52.72±3.69 (48-62)	51±1.5 (50-54.8)

Measurements in µm and in the form: mean± SD (range)

Figure.1 Infective juveniles of *Oscheius chongmingensis*

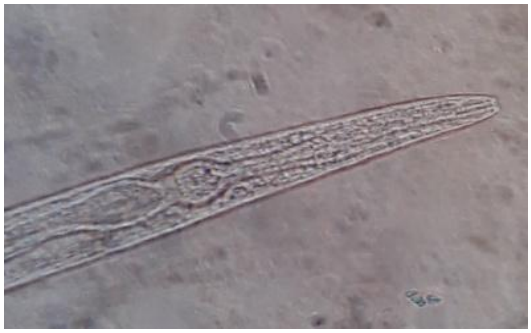

Figure.2 Anterior region of infective juvenile of *Oscheius chongmingensis*

Figure.3 Tail of infective juveniles of*Oscheius chongmingensis*

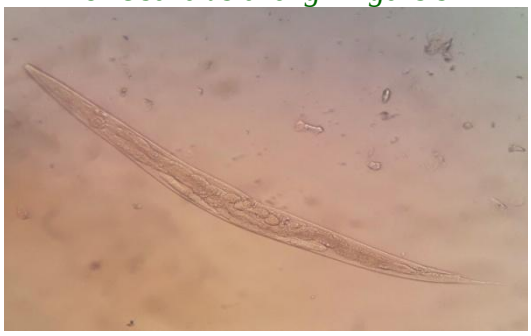

Figure.4 Hermaphroditic female of*Oscheius chongmingensis*

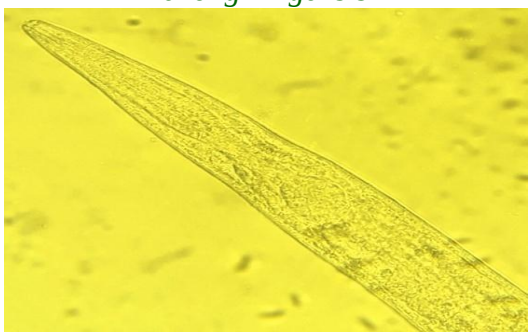

Figure.5 Anterior part of Amphimictic female of *Oscheius chongmingensis*

Figure.6 Tail region of Hermaphroditic female of *Oscheius chongmingensis*

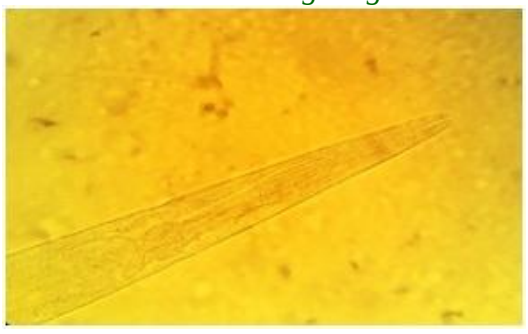

Figure.7 Oesophagus of Hermaphroditic female of *Oscheius chongmingensis*

Figure.8 Vulva of Hermaphroditic female of *Oscheius chongmingensis*

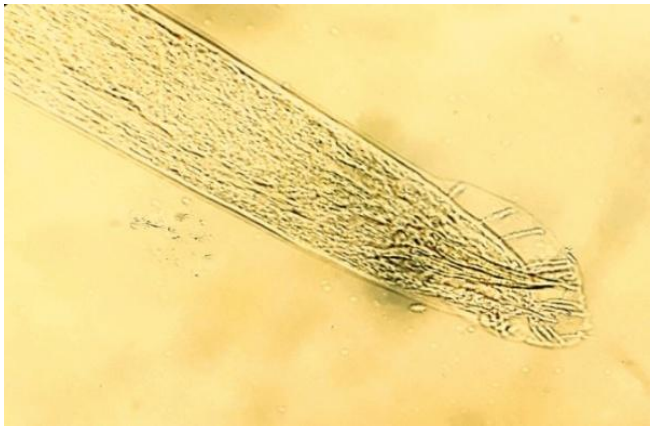

Figure.8 Spicule of *Oscheius chongmingensis*

However, this low recovery percentage is not unusual and it has already been reported from other surveys (Hazir *et al.,* 2003; Kary *et al.,* 2009). Rosa *et al.,* (2000) reported that most of the surveys showed their recovery rate from soil varies from 6% to 35% in Northern Ireland. Raj kumar *et al.,* (2001) reported that out of 105 soil samples collected from Rajasthan, 5(4.76%) were found to be positive for EPNs. Mracek and Beavar (2000) reported that recovery frequency of EPNs may vary from 0.7% to 70.01%. Akhurst and Brooks (1984) and Griffin *et al.,* (1991) observed that entomopathogenic nematodes were prevalent in agricultural fields than in natural habitats.

All the two EPN isolates positive soil samples were from sandy loam soil and this finding was in agreement with findings of the surveys conducted by Ambika and Sivakumar (2000), which revealed that the occurrence of EPNs was more in light soils like sandy loam, sandy, loamy sand, and loam soil rather than in heavy soils. However, EPNs are present in heavy soils like clay soil also as recorded by Shyamprasad *et al.,* (2001) and Sosamma and Rasmi (2002) in the South Andaman and Kerala, respectively.

The *Oscheius* isolate was similar to *O.chongmingensis* in original description of *Heterorhabditidoides chongmingensis* with respect to third stage infective juvenile in characters like distance from anterior and 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 (453 vs. 428), body width (25 vs. 22), tail length (87vs.111), E%(111 vs. 83) of infective juvenile which were considered as intraspecific variations of *O. chongmingensis* .The 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 and tail length. Zhang *et al.,* (2008) isolated the new entomopathogenic nematode species from soil samples of Chongming Island in Eastern China and described as *H.chongmingensis*. Ye *et al.,* (2010) and Liu *et al.,* (2012) 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 *O.chongmingensis*n. comb. Ali *et al.,* (2011) isolated and described *O.amsactae* n. sp. as a necromemioc associate of red hairy caterpillar *Amsactamoori* from *Vigna radiate* in cultivated fields of Indian Institute of Pulse Research, Kanpur, India.

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How to cite this article:

Bharath Amuri and Gitanjali Devi. 2020. Isolation, Morphometrics and Morphological Characterization of *Oscheius chongmingensis* from Assam, India. *Int.J.Curr.Microbiol.App.Sci.* 9(04): 2474-2487. doi: https://doi.org/10.20546/ijcmas.2020.904.296