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Isolation of insect parasitic nematode, *Oscheius rugaoensis* from Assam

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Abstract
An insect parasitic nematode belonging to the genus *Oscheius* was recovered from the vegetable growing fields from the Jorhat district in Assam, India. Survey data revealed that out of 200 soil samples collected, six samples were tested positive for *Oscheius* isolate with 3% frequency of occurrence. Morphological and morphometrical studies on this species exhibited its high resemblance with *Oscheius rugaoensis*.

Keywords: Entomopathogenic nematodes, *Oscheius* species, *Oscheius rugaoensis*, morphological and morphometrical studies

Introduction
Oscheius, in the family Rhabditidae is considered as entomopathogenic nematode (EPNs) are parasitic and lethal to some insect pests for insect pest control (Torres-Barragan *et al*., 2011) [13]. In contrast to Steinernematidae and Heterorhabditidae, EPNs of Heterorhabditidoides carry specific mutualistic bacteria belonging to the genus *Serratia* in their intestine or on their cuticle (Kaya and Gaugler, 1993; Zhang *et al*., 2008; Zhang *et al*., 2009; Lephoto *et al*., 2015; Torrini *et al*., 2015) [6, 9, 17, 8, 14]. There are some controversies about the status of the genus *Heterorhabditidoides*. Zhang *et al*., (2008) [16] defined the genus *Heterorhabditidoides* and described the type species *Heterorhabditidoides chongmingensis*, whereas Ye *et al*., (2010) [15] and Liu *et al*., (2012) [18] considered the genus *Heterorhabditidoides* as a junior synonym of *Oscheius*. The genus *Oscheius* Andrássy, 1976 comprises two main groups, Dolichura and Insectivorus (Sudhaus & Hooper 1994) [11] and until now EPNs were found only in the Insectivorus-group. So far, 43 species were described in *Oscheius* with 29 in Insectivora and 14 in Dolichura (Tabassum *et al*., 2016) [12]. Infective juveniles enter in the host body through natural openings such as mouth, trachea into the insect body cavity. Afterwards juveniles release the symbiotic bacteria into the hemocoel. Nematode and bacteria overcome the insect immune system and the host insect is killed within 24-48 hours post infection (Adams and Nguyen, 2002) [2].
The survey was conducted in different vegetable field of Jorhat district of Assam, India to find the isolates of EPNs and to identify the species present which are probably act as a biological control agents against important insect pests of vegetables.

Materials and Methods
Survey and sample collection: A total of 200 soil samples were collected from different vegetable fields of Jorhat district of Assam for the presence of entomopathogenic nematodes. From each field 10 numbers of soil samples were collected randomly. Soil samples collected from different vegetable fields includes cucumber, chilli, bhendi, brinial, cowpea, ridge gourd, cabbage, cauliflower, pumpkin, tomato, ivy gourd and carrot. *Oscheius* isolates were designated as EPN-O-J-1, EPN-O-J-2 and EPN-O-J-3 and were found from rhizosphere of chilli, cowpea, bhendi respectively from ICR Farm, AAU, Jorhat. *Oscheius* sp. designated as EPN-O-J-4 was isolated from rhizosphere of chilli from Experimental farm, Department of Horticulture, AAU, Jorhat. *Oscheius* isolate designated as EPN-O-J-5 was isolated from rhizosphere of bhendi from Hostel areas, AAU, Jorhat. Another *Oscheius* isolate designated as EPN-O-J-6 was found from rhizosphere of pumpkin and ivy gourd from Allengmora, Jorhat. Morphological and morphometrical characters were used in the identification of nematode isolates.

Extraction of entomopathogenic nematodes (EPNs) from soil samples

Nematode isolation: soil samples collected from different vegetable growing fields were baited using fifth instar larvae of wax moth, *Galleria mellonella* for the presence of entomopathogenic nematodes (Bedding and Akhurst, 1975) [3]. First generation of EPNs were consists of hermaphroditic females and infective juveniles. Second generation contains male, infective juveniles and amphimictic females. All stages of EPNs were collected separately and processed for further studies.

Morphological and morphometric characterization

First and second generation adults and IJs were collected from the cadaver of *Galleria mellonella.* Different stages were killed and fixed by pouring equal volumes of hot tri-ethanolamine formalin (TAF) fixative over the EPN suspension (Kaya and Stock, 1997) [7]. The specimens were handpicked individually and transferred to 100% TAF and fixed for a week. further processing were done with Seinhorst's slow glycerol dehydration method (Seinhorst, 1959) [10]. For descriptive purposes, 20 specimens for each stage were fixed in TAF and mounted into a small drop of glycerin. Morphological observations were made using light compound microscope (Magnus MLX). The permanent slides were examined for detailed morphological characters and body dimensions were studied using de Man's formula (De Man, 1880) [5] and additional ratios to establish their taxonomic identity. The following characters were measured: total body length; maximum body diameter; anal body diameter; excretory pore position; distance from anterior end to nerve ring position; distance from anterior end to base of pharynx; gubernaculum length; spicule length. 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), and F% (Body width/tail length x 100). 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*., (2012) [18].

Entomopathogenicity of *Oscheius* isolate was evaluated using host insects, *Galleria mellonella*, in pathogenicity bioassays. A Petri dish (3.5 cm diam.) with two layers of filter paper (Whatman No. 1) containing one last instar larva of *G. mellonella* was used as experimental unit. A distilled water suspension of 250 µl containing about 400 Infective Juveniles (IJs) was inoculated into each Petri dish. Only distilled water was used as control. Petri dishes were stored at 24 °C in darkness. Mortality of larvae was evaluated every 24 h for a week. Fifteen larvae/host insect were used for each bioassay and the experiments were repeated three times. Half of the dead larvae were dissected to evaluate the presence and development of nematodes inside and half were transferred to White traps to determine nematode emergence. In virulence tests, mortality data were corrected for natural mortality in controls using Abbott's formula (Abbott, 1925) [1]. Lethal time to 50% mortality (LT50) was estimated.

Results and Discussion

Morphological studies of the six *Oscheius* isolates were undertaken and it was found to be similar to each other. Therefore detailed morphological and morphometrical studies of the EPN-O-J-1 were undertaken and presented in the Table 1 and Table 2. Morphological and morphometrical studies of different life stages of EPN-O-J-1 revealed that it is closely resemble with *Oscheius rugaoensis* in most of the characters. IJs have an elongated C-shaped body when killed by heat. Their cuticles are well-annulated. The tail is long and pointed and covered with a sheath. It is approximately five times longer than the body width at the anus and has one phasmid. The head has no prominent dorsal tooth and has a triangular slit. The labial region has six labial papillae, lacking visible cephalic papillae, and the mouth is closed. The stoma is tubular, and the cheilorhabdions are not cuticularized. The metarhabdions show no hemispherical swellings. Males have a J-shaped body when killed by heat. Male head shows no constriction, and has six separate well-developed lips, each with one or two conical terminal papillae. Bursa ridges are well- developed, and the spicules are paired, separate, often asymmetrical, and slightly curved ventrally. Male have a pharynx with a cylindrical corpus. The metacorpus is swollen and the esophageal collar is very long. The isthmus is distinct with a globose basal bulb and a prominent valve. The nerve ring surrounding the isthmus is located posterior to it, with the cardia protruding into the intestine and the excretory pore usually located near the middle of the basal bulb. The testis is monorchic and reflexed. The gubernaculum is boat-shaped (lateral view) and ventrally curved, and its length approximately 40% of the spicule length. Bursa peloderan, extends on both sides and surrounds the male cloaca. The head of the spicule tip is round, crochet-needle-shaped. The males have a small, pointed tail. Hermaphroditic females have a C-shaped body when killed by heat. The stoma is tubular, and the cheilorhabdions are well cuticularized. The isthmus is long and distinguishable, and the nerve ring surrounding the isthmus is located posterior to it. The valve of the basal bulb is prominent. Gonads are didelphic, amphidelphic, and reflexed. The vulva is a transverse slit situated on a protruding area usually near midbody. It is protected by two symmetrical cuticular flaps, and the vagina is short. The tail is approximately three times longer than the body width at the anus and has a conoid or post-anal swelling with a pointed terminus. Amphimictic females are similar to hermaphroditic females but are smaller. The reproductive system is amphidelphic and reflexed. The vulva is a transverse slit situated on a protruding area. It is covered with exudates after mating.

The IJs of EPN-O-J-1 showed close similarity with *Oscheius rugaoensis* with respect to ratio a, ratio b, ratio c and D%. The males of this isolate showed close similarity with *Oscheius rugaoensis* with respect to head shape, body length, ratio b, ratio c, but exhibited minor differences from the type measurements by having lower body width (48 vs. 62), position of excretory pore (290 vs. 281). The hermaphroditic females and amphimictic females of this isolate showed close similarity with *Oscheius rugaoensis* with respect to head shape, anal body width but exhibited difference from the type measurements by the position of excretory pore (242 vs. 257), oesophagus length (254 vs. 278), tail length (184 vs. 198), and anal body width (25 vs. 32). The isolate EPN-O-J-1 was thus identified as *Oscheius rugaoensis.*

Zhang *et al*., (2012) [18] isolated and described *Heterorhabditidoides rugaoensis* n. sp. RG081015, collected from soil samples using the *Galleria mellonella* baiting method from Rugao, Jiangsu Province, China. Darsouei *et al*., (2014) [4] reported two new species, *Oscheuis rugaoensis* isolated from the white grub larvae *Polyphylla adspersa* from Iran.

Oscheius rugaoensis infected 86% of *Galleria* larvae in one week: LT_{50}: 6.8 days. IJs of the nematodes emerged from cadavers within 72 h after host death, and from all the dissected dead larvae nematodes of different developmental stages were recovered. Preliminary experiments showed that these nematodes had the potential to infect and kill the *Galleria* larvae, under laboratory conditions. This potentially makes these species viable biological control agents.

Table 1: Morphometrics of infective juvenile and second generation male of *Oscheius* sp. (EPN-O-J-1) in comparison with original description of *Oscheius rugaoensis* Measurements in µm and in the form: mean± SD (range)

Character	*Oscheius* sp. (EPN-O-J-1) (IJ) (n=20)	Type measurement of *Oscheius rugaoensis* (IJ) (Zhang *et al.*, 2012) [18] (n=20)	*Oscheius* sp. (EPN-O-J-1) (male) (n=20)	Type measurement of *Oscheius rugaoensis* (male) (Zhang *et al.*, 2012) [18] (n=20)
Body length (L)	586.3±48.4 (500-580)	591.7±44.3 (524.9-665.6)	1369±201.9 (980-1610)	1396.2±103.2 (1195-1692)
Body width (W)	25.8±3.1 (20-30)	28.9±2.6 (25.1-33.2)	48.2±6.1 (40-60)	62.4±10.6 (46.3-66.2)
Anterior end to excretory pore (EP)	125.4±9.7 (110-140)	135.4±11.2 (119.7-150.7)	290.8±59.9 (190-390)	281.1±93.3 (201.9-411.5)
Anterior end to esophagus base (ES)	140.7±13.6 (124-160)	149.3±11.6 (134.5-166.4)	322.4±6402 (200-400)	304.4±99.9 (219.1-443.9)
Tail length(T)	70.6±13.3 (50-90)	82.5±9.5 (69.3-101.4)	140±14.6 (115-160)	151±14.3 (128.4-163.1)
Anal body width (ABW)	-	-	26.06±10.1 (10-45)	30.3±8.6 (16.9-52.5)
Spicule length (SL)	-	-	46.3±9.7 (32-60)	49.2±6.8 (35.2-60.9)
Gubernaculum length (GL)	-	-	16.2±6.1 (8-25)	19.7±4.6 (9.9-26.5)
Ratio a= (L/W)	22.8±1.1 (21.6-25.5)	20.5±1.2 (18.9-21.3)	28.3±1.8 (24.5-31)	22.8±3.75 (15.8-26.3)
Ratio b= (L/ES)	4.1±0.15 (4.01-4.4)	4±0.2 (3.6-4.3)	4.2±0.3 (4.1-5)	4.8±1.0 (3.8-6.4)
Ratio c= (L/T)	8.4±0.9 (7.2-10.2)	7.2±0.4 (6.6-7.6)	9.7±0.5 (8.5-10.5)	9.3±1.2 (7.3-10.5)
D%= (EP/ES) × 100	89.3±2.9 (85.5-94.4)	90.1±2.1 (86.9-93.3)	90.4±6.4 (77.4-100)	92.3±0.84 (90.9-93.0)
SW% = SL/ABW × 100	-	-	191.4±44.4 (133-320)	173±42 (91.5-241.3)
GS% = GL/SL × 100	-	-	33.8±6.6 (25-41.6)	4. ±8.2 (24.1-50.5)

Table 2: Morphometrics of Hermaphroditic and Amphimictic female of *Oscheius* sp. (EPN-O-J-1) in comparison with original description of *Oscheius rugaoensis* Measurements in µm and in the form: mean ± SD (range)

Character	*Oscheius* sp. (EPN-O-J-1) Hermaphroditic female (n=20)	Type measurement of *Oscheius rugaoensis* Hermaphroditic female (Zhang *et al.*, 2012) [18] (n=20)	*Oscheius* sp. (EPN-O-J-1) Amphimictic female (n=20)	Type measurement of *Oscheius rugaoensis* Amphimictic female (Zhang *et al.*, 2012) [18] (n=20)
Body length(L)	2031±307.7 (1600-2410)	1923±179.6 (1639.1-2259.4)	1017±75.5 (900-1100)	1042±101.1 (920.6-1179.9)
Body width(W)	81.5±15.2 (65-110)	87.3±12.5 (70.8-106)	41.2±7.3 (30-50)	49.5±6.9 (39.8-58.2)
Anterior end to excretory pore (EP)	242.6±21.4 (215-280)	257.2±19 (222-278)	155.8±17.4 (125-180)	191.08±11.7 (173-204.6)
Anterior end to esophagus base (ES)	254.6±14.5 (235-285)	278.4±22.1 (237.4-303.8)	191±9.3 (175-200)	209±11.5 (189.3-222.1)
Tail length (T)	184.2±11.1 (170-200)	198.7±14.4 (179.6-215.6)	84.9±7.4 (80-105)	131±14.6 (113-154.9)
Anal body width (ABW)	25.3±4.1 (20-32)	32.5±4 (26.1-39.3)	23±5.3 (15-30)	26.2±3.3 (21-31.7)
V%= distance from anterior end to vulva as percentage of length	48.7±1.4 (47.3-51.4)	50.8±2.2 (47.7-53.6)	50.04±5.02 (37.8-52.2)	54.8±5.4 (54.2-66.9)
Ratio a = (L/W)	25±1.2 (21.9-26.3)	23±2.9 (19.4-27.9)	25.1±3.03 (21.8-30.3)	21±3.1 (17.7-25.8)
Ratio b = (L/ES)	7.9±0.8 (6.8-9.1)	7.1±0.8 (6-9.4)	5.3±0.16 (5.1-5.50	5±0.3 (4.9-5.6)
Ratio c = (L/T)	10.9±1.03 (9.4-12.3)	12.9±0.3 (12.6-13.2)	12±1.3 (8.5-13.6)	8.5±0.7 (7.6-9.3)
D%= (EP/ES) × 100	95.1±3.8 (88.7-100.7)	92.4±4.4 (90.3-93.6)	81.4±6.7 (71.1-94.2)	91.7±1.1 (89.9-92.9)

Fig 1: Infective juvenile of *Oscheius rugaoensis*

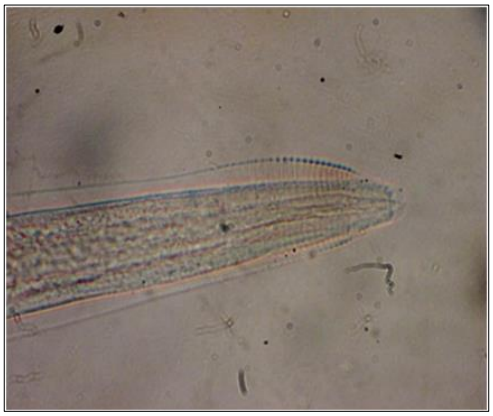

Fig 2: Anterior region of infective juvenile of *Oscheius rugaoensis*

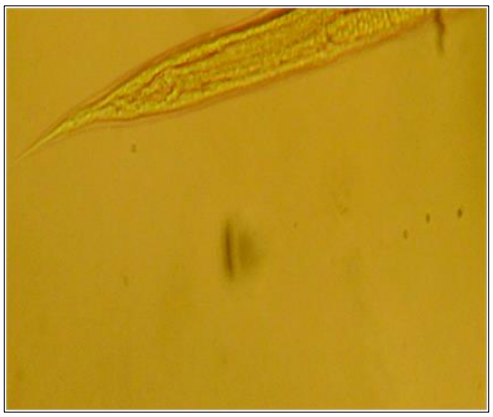

Fig 3: Tail of infective juvenile of *Oscheius rugaoensis*

Fig 4 Anterior part of hermaphroditic female of *Oscheius rugaoensis*

Fig 5: Oesophagus of Amphimictic female of *Oscheius rugaoensis*

Fig 6: Tail region of Hermaproditic female of *Oscheius rugaoensis*

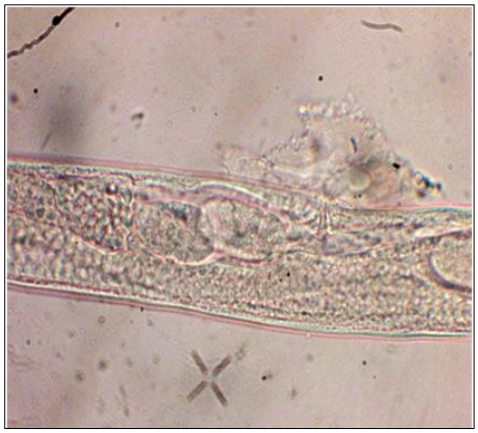

Fig 7: Vulva of Hermaphroditic female of *Oscheius rugaoensis*

Fig 8: Spicule of *Oscheius rugaoensis*

Conclusion

For the identification of EPNs, the combination of morphological, morphometric, molecular observations are very important. In this study entomopathogenic nematodes were found only in six soil sample out of 200. The isolation of *Oscheius rugaoensis* underscores the need for a more thorough assessment in Assam. The native isolates of entomopathogenic nematodes will enhance the ecological compatibility and thereby help us in using biological control programs against insect pests.

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References

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925;18:265-267. http://dx.doi.org/10.1093/jee/18.2.265a
2. Adams BJ, Nguyen KB. Taxonomy and systematics. In: Entomopathogenic nematology, Gaugler, A. (ed.) CABI Publishing, Wallingford: UK, 2002, 1-33.
3. Bedding RA, Akhurst RJ. A simple technique for the detection of insect parasitic rhabditid nematode in soil. Nematologica. 1975;21(1):109-110.
4. Darsouei R, Karimi J, Shokoohi E. *Oscheius rugaoensis* and *Pristionchus maupasi*, two new records of entomophilic nematodes from Iran. Russ. J Nematol. 2014;22(2):141-155.
5. De Man JG. Die Einheimischen, frei in der reinen Erde und im sussen Wasser lebende Nematoden. 1880;1:1-104.
6. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annual Review of Entomology. 1993;38:181-206.
7. Kaya HK, Stock SP. Techniques in insect nematology. Manual of Techniques in Insect Pathology, 1997, 281-324.
8. Lephoto TE, Featherston J, Gray VM. Draft whole-genome sequence of Serratia sp. strain TEL, associated with *Oscheius* sp. TEL-2014 (Nematoda: Rhabditidae) isolated from a grassland in South Africa. Genome Announc. 2015;3(4):e00747-e00715.
9. Liu QZ, Mráček Z, Zhang LJ, Půža V, Dong LM. Re-description of *Oscheius chongmingensis* (Zhang *et al.*) (Nematoda: Rhabditidae) and its entomopathogenicity. Nematology. 2008;14:139-49.
10. Seinhorst JW. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica. 1959;4(1):67-69.
11. Sudhaus W, Hooper DJ. *Rhabditis* (Oscheius) *guentheri* sp. n., an unusual species with reduced posterior ovary, with observations on the Dolichura and Insectivora-groups (Nematoda: Rhabditidae). Nematologica. 1994;40:508-533. http://dx.doi.org/10.1163/003525994X00391
12. Tabassum KA, Shahina F, Nasira K, Erum YI. Description of six new species of Oscheius Andrassy, 1976 (Nematoda: Rhabditida) from Pakistan with a key and diagnostic compendium to species of the genus. Pakistan. Journal of Nematology. 2016;34:109-61.
13. Torres-Barragan A, Suazo A, Buhler WG, Cardoza YJ. Studies on the entomopathogenicity and bacterial associates of the nematode *Oscheius carolinensis.* Biological Control. 2011;59:123-129.
14. Torrini G, Mazza G, Carletti B, Benvenuti C, Roversi PF, Fanelli E, *et al*. *Oscheius onirici* sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave. Zootaxa. 2015;3937(3):533-548.
15. Ye W, Torres-Barragan A, Cardoza YJ. *Oscheius carolinensis* n. sp. (Nematoda: Rhabditidae), a potential entomopathogenic nematode from vermicompost. Nematology. 2010;12:121-135.
16. Zhang C, Liu J, Xu M, Sun J, Yang S, An X, *et al*. *Heterorhabditidoides chongmingensis* gen. nov., sp. Nov. (Rhabditida: Rhabditidae), a novel number of the entomopathogenic nematodes. Journal of Invertebrate Pathology. 2008;98(2):153-168.
17. Zhang CX, Yang SY, Xu MX, Sun J, Liu H, Liu JR, *et al*. A novel species of *Serratia*, family Enterobacteriaceae: *Serratia nematodiphila* sp. nov., symbiotically associated with entomopathogenic nematode *Heterorhabditidoides chongmingensis* (Rhabditida: Rhabditidae). International Journal of Systematic and Evolutionary Microbiology. 2009;59:1603-1608.
18. Zhang KY, Liu XH, Tan J, Wang Y, Qiao L, Yedid G, *et al*. *Heterorhabditidoides rugaoensis* n. sp. (Rhabditida: Rhabditidae), a novel highly pathogenic entomopathogenic nematode member of Rhabditidae. Journal of Nematology. 2012;44:348-360.