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Morphological Identification and Description of *Osccheius* from Srinagar, Pauri Garhwal Uttarakhand, India

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Abstract: The present study was conducted on the isolation, identification and morphometrical description of Entomopathogenic nematodes of Srinagar (Pauri Garhwal) Uttarakhand, India. Srinagar is located in the district Pauri Garhwal of Uttarakhand, India. Its geographical coordinates are approximately 30.2250° N latitude and 78.7828° E longitude. During the study, few soil samples were collected from selected locality of Srinagar. The isolation techniques used was Cobb's decanting and sieving technique in which the soil is mixed in water by stirring with hand or paddle which was then allowed to stand until water almost stops spinning. All the sediment is poured through different sieves and finally to the beaker which is then subjected to the observation. Around 10 samples were collected and based on morphological and morphometrical studies of the samples the nematodes were identified as of *Osccheius*, then after processing around 20 females, 20 males, and 20 13th stage juveniles were found. For morphometry Seinhorst method was followed in which the nematodes were first heat killed and then fixed in a fixative like TAF (Triethanolamine). The average female body length was 1557±70 µm. Female body is larger than that of male, tail is elongated and conoid with tapering ends, cuticle is transversely annulated and the lip region possess 6 separate lips. Stoma is tubular and pharynx is of rhabditoid type that encircles the nerve ring. The average male body length was 1179 ± 104 µm, they differ from female in having small size and curved posterior region. Tail is conical and reduced, anteriorly present bursa. Bursa opens anteriorly; leptoderan does not enclose the tail spine. Gubernaculum is well developed and boat shaped, it consists of manubrium corpus that are straight. The average 13th stage juvenile length was roughly 534±21 µm, body is elongated with curved posterior end and encased in 2nd stage juvenile cuticle, however, in 3rd stage juvenile sheath is free anteriorly yet tightly connected to body rear portion. Pharynx is present and clearly visible having pharyngeal corpus. Smooth cuticle and lip region.

Keywords: Entomopathogenic nematodes, Morphometrical description, Insects, *Osccheius*, Cobb's decanting and sieving Technique

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Introduction

Nematodes are basic roundworms which are free-living, predatory, or parasitic. They are colourless, unsegmented, and lack appendages (Vashishta *et al.*, 2013). If nematodes meet the following criteria for entomopathogenicity, like carrying a pathogenic bacterium, the release of the pathogenic bacteria within the host by a dauer (also known as infective) juvenile nematode, active host-seeking and penetration by dauer juveniles, rapid insect death, nematode and bacterial reproduction, reassociation of the pathogenic bacteria with new generations of dauer juveniles, and the emergence of IJs from the corpse so that the cycle can be repeated they are deemed to be entomopathogenic (Dillman *et al.*, 2012).

The phrase entomopathogenic nematodes (EPN), which derives from the Greek vocables “entomos,” “insects,” “pathê,” “disease,” and “guenos,” “generating,” refers to a type of nematodes that have the capacity to infect insects with disease by weakening their immune systems (Sharma *et al.*, 2021). The first EPN to be discovered in 1923 was *Aplectana kraussei*, which is now known as *Steinernema kraussei* (Nguyen, 2007).

The two families to which these nematodes belong are *Steinernematidae* and *Heterorhabditidae*. Only 18 species of *Heterorhabditis* (*Heterorhabditidae*) and 101 species of *Steinernema* (*Steinernematidae*) have been discovered globally till date (Machado *et al.*, 2021). The life cycles of *Steinernema* and *Heterorhabditis* are identical. A balance exists between the free-living and parasitic stages in both genera (Sharma *et al.*, 2021).

Nematodes of the genera *Steinernema* and *Heterorhabditis* have been utilised as biocontrol agents to reduce pest populations because they are frequently entomopathogenic (Kaya and Gaugler 1993). Except for Antarctica, these nematodes have been discovered on every continent. They are deadly to a variety of insect pests (Poinar and Thomas, 1984).

These nematodes and Enterobacteriaceae, the bacteria they coexist with, have a potent insecticidal complex or symbiotic connection that kills a range of insect species (Orozco *et al.*, 2014). Two bacterial taxa, *Xenorhabdus* and *Photorhabdus*, are symbiontally linked to particular nematodes from the families *Steinernematidae* and *Heterorhabditidae*, respectively (Poinar, 1990). Despite being extremely destructive to many significant insect pests, entomopathogenic nematodes are safe for non-target organisms (Georgis *et al.*, 1991).

A free-living EPN known as *Oscheius*, which is a member of the phylum Nematoda, class Secernentea, subclass Rhabditia, order Rhabditida, and family Rhabditidae, can be found in saprophytic biotypes such as detritus, dung, and agricultural soils (Kumar *et al.*, 2019). The lip area and stoma, tail form, male posterior body region and bursa, spicules, and gubernaculum are the characteristics that distinguish the genus *Oscheius* (Andrássy, 1976 (Scholze and Sudhaus, 2011)).

Oscheius was distinct from other Rhabditinae by having very small buccal tube. Later, Andr ssy (1976) established the genus *Oscheius* designating *O. insectivorus* as its type species. *Oscheius* was split into the Insectivora and Dolichura groups (Sudhaus and Hooper, 1994).

Dolichura differs from the Insectivora group in that Dolichura has a spicule with a slender tubular tip and a peloderan bursa, whereas the Insectivora group has a bursa leptoderan or pseudopeloderan (Kumar *et al.*, 2019).

Pathogenicity of *Oscheius* species on insects is primarily associated with endosymbiont bacteria from the genera *Serratia* and *Enterococcus* (Thiercelin and Jouhaud, 1903). Infected *Steinernematids* nematode larvae change colour to a creamy/dark brown hue, while *Heterorhabditis* nematode larvae change colour to a reddish/purplish hue (Kumar *et al.*, 2020). In contrast, *Oscheius* cadaver colour remains the same. Over 13 of the approximately 30 recognised

species in the genus *Oscheius* have insect-killing abilities (Loulou *et al.*, 2022).

The aim of the present study was to characterise the *Oscheius* genera that were isolated and identified from the Srinagar (Pauri Garhwal) Uttarakhand, India, morphologically and morphometrically.

Materials and Methods

The present study was conducted on isolation and identification of nemic fauna of Srinagar (Pauri Garhwal), of few selected sites. During the study only 10 samples were collected from different localities of Srinagar region. Srinagar is located in the district Pauri Garhwal of Uttarakhand, India. Its geographical coordinates are approximately 30.2250° N latitude and 78.7828° E longitude, Due to differences in height and climatic conditions, the area is distinguished by a rich flora.

Soil sample collection:

Soil sampling method was followed as per protocols of Orozco *et al.* (2014); each sampling site was covered to a minimum of 2 to 4 metres square. A hand shovel was used to gather soil samples that were at least 15 cm deep. To prevent sample leakage, samples were double bagged and stored in autoclave plastic bags. The samples were marked with a waterproof marker; containing information about each sampling site, the cleaning and disinfection processes for collection tools included washing them in water and/or using a 70% ethanol or 0.5 per cent bleach solution. Samples were transported to the lab while being stored at 8 to 15 °C.

Nematodes isolation:

The Cobb's sieve and decanting procedure was used to isolate nematodes (Rana *et al.*, 2020). The soil samples were placed in the beaker after it had been half-filled with water. For a few minutes, the beaker was left motionless to allow the heavy particles to settle. The suspension was decanted onto many fine sieves (350, 400), each of which retained nematodes of various sizes, after the heavy silt particles settled in 10–20 seconds

(based on their body sizes). Each sieve's nematode suspension was cleaned before being poured through a fine wire gauze sieve that had already been placed on a beaker to catch the fine silt. The beaker was then left overnight. Small pores in the tissue paper would have allowed the nematodes to get through and congregate at the beaker's bottom. The beaker's contents were then poured into the hollow block and examined under binocular microscopy after being strained through a 400 mm sieve strainer.

Morphometry:

Nematodes were handled using Seinhorst's method (1959) after being heat-killed and fixed in TAF (Triethanolamine fixative) for 5-7 days (Seinhorst, 1959). Nematodes were put on glass slides for microscopy permanently. The specimens were inspected and measured under a light microscope. They were captured on camera with a 1000x magnification CANON Power Shot S50.

Results

During the study a total of 10 samples were taken from Srinagar Garhwal (Pauri Garhwal), from which 60 individuals were identified out of these 20 were female, 20 were male and 20 were in juvenile stage (Table 1, Fig. 1). Many other organisms were also found but the current study is restricted to the Entomopathogenic nematodes.

Female:

The average of female body length was 1557 µm; body was slightly bent on ventral side. Pharynx was 175 µm long. The total length of nerve ring from anterior end was 153 ± 13.3 µm. The total neck length that comprises stoma and pharynx was 195 µm. Body width at base of neck was 54 µm.

The total length of the excretory pore from anterior end was 202 µm. It is conspicuous and located ventrally at the level of basal bulb. Uterus is well developed with lengthy glandular and muscular areas. Length of anterior and posterior spermatheca was 43 µm and 37 µm, respectively. Vagina is 25 µm long, it comprises of thick and

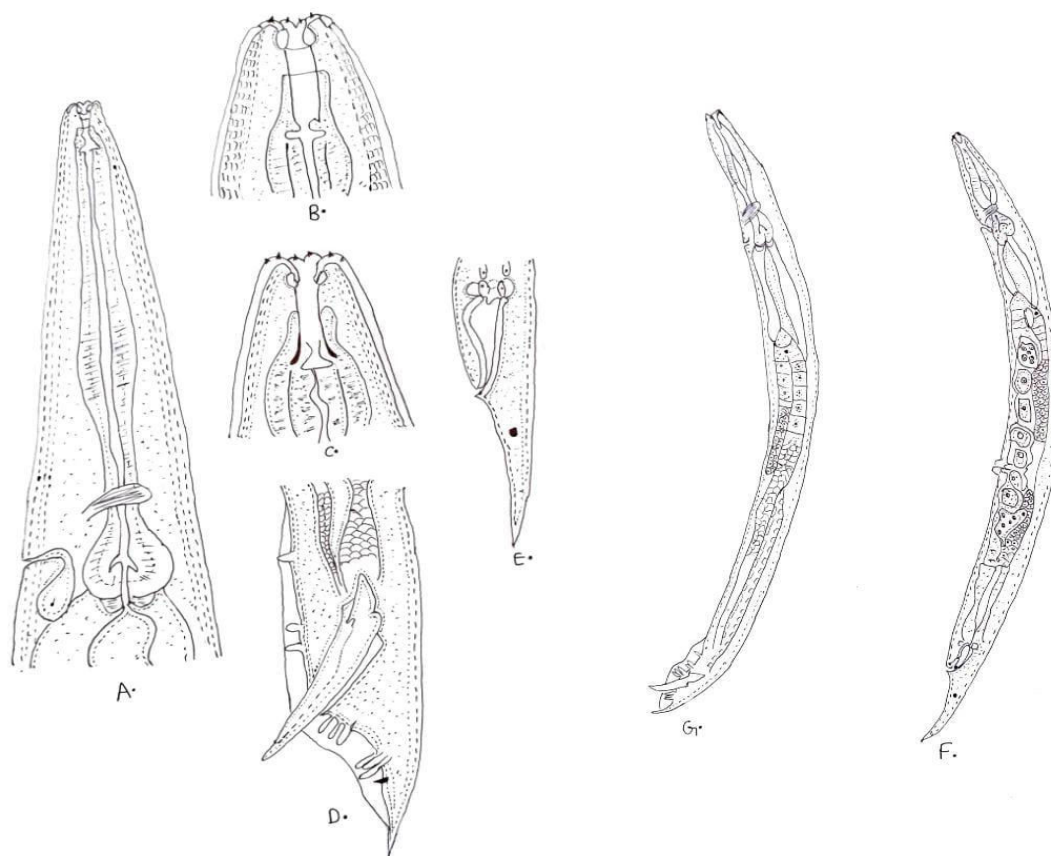


Fig.1: *Oscheius* general body structure. (A) Anterior region of female, (B) Stoma female, (C) Stoma male, (D) Male posterior region, (E) Female posterior region, (F) Adult female, (G) Adult male.

cuticularized wall. The length of vulva from anterior end was 750 μm . Rectum was short with rectal glands at its junction, measuring about 2.3 times (70 μm) the diameter of anal body (30 μm). Conoid tail is gradually narrowed down to a fine termination measure about 172 μm .

Male:

They were smaller than female having length of 1179 μm . Spicules were present having length of around 45 μm . Gubernaculum is flattened on ventral side and follows the spicules' outline, it measures around 30 μm , and Pharynx was 160 μm long.

The total length of nerve ring from anterior

end was 135 μm . The total neck length that comprises stoma and pharynx was 180 μm . Body width at base of neck was 40 μm . The length of the excretory pore from anterior end was 192 μm . Testis was single measuring about 610 μm in length. Anal body diameter was 22 μm . Tail is conical and small than that of female, measuring about 42 μm .

Juvenile:

They measure about 534 μm in length. Bulb was 102 μm long. The total length of nerve ring from anterior end was 72 μm . The total neck length, which comprises stoma and pharynx, was 118 μm . Body width at base of neck was 25 μm . The length

Table 1: Morphometric of *Oscheius* n. sp. All measurements are in micrometres (except n, ratio, and percentage) and in the form: mean $\pm$ SD

S. No.	Characters	Female	Male	Juveniles
1.	N	20	20	20
2.	Body length (L)	1557 $\pm$ 70 (1459–1687)	1179 $\pm$ 104 (936–1441)	534 $\pm$ 21 (502–574)
3.	a (L/BD)	14.5 $\pm$ 1.8 (12–20)	20 $\pm$ 2.2 (16.4–25)	20 $\pm$ 1.0 (18.3–22)
4.	b (L/NL)	7.8 $\pm$ 0.5 (6.8–8.7)	6.5 $\pm$ 0.5 (5.2–7.6)	4.3 $\pm$ 0.2 (3.4–4.8)
5.	c (L/T)	8.0 $\pm$ 1.0 (6.5–10.6)	26 $\pm$ 3.0 (19–32)	7.1 $\pm$ 0.7 (5.6–8.7)
6.	c' (T/ABW)	5.6 $\pm$ 1.1 (2.7–8.0)	1.7 $\pm$ 0.3 (1.2–2.4)	5.0 $\pm$ 0.7 (4.8–7.1)
7.	V (VA/L*100)	46 $\pm$ 2.3 (43–52)		
8.	Bulb length (BL)	35 $\pm$ 2.2 (30–37)	30 $\pm$ 3.0 (25–35)	102 $\pm$ 7.2 (92–125)
9.	Pharynx length (PL)	175 $\pm$ 9.2 (165–195)	160 $\pm$ 11.1 (132–191)	
10.	Nerve ring – ant. end (NR)	153 $\pm$ 13.3 (132–173)	135 $\pm$ 10.4 (108–160)	72 $\pm$ 6.5 (60–84)
11.	Excretory pore– ant. end (EP)	202 $\pm$ 15.9 (173–240)	192 $\pm$ 10.6 (165–210)	115 $\pm$ 6.3 (98–125)
12.	Neck length(stoma+pharynx, NL)	195 $\pm$ 9.5 (185–215)	180 $\pm$ 12.2 (162–214)	118 $\pm$ 6.1 (108–135)
13.	Body width at neck base	54 $\pm$ 5.2 (45–62)	40 $\pm$ 3.2 (34–45)	25 $\pm$ 2.0 (23–30)
14.	Anterior ovary or testis	178 $\pm$ 14.6 (150–196)	610 $\pm$ 55 (525–720)	–
15.	Anterior genital branch	330 $\pm$ 40 (255–405)	–	–
16.	Vagina length	25 $\pm$ 6.2 (16.6–34)	–	–
17.	Vulva – ant. end (VA)	750 $\pm$ 35 (662–815)	–	–
18.	Rectum length	70 $\pm$ 10.2 (45–84)	–	30 $\pm$ 3.1 (25–36)
19.	Anal body diam. (ABD)	30 $\pm$ 7.5 (15–50)	22 $\pm$ 2.0 (18.3–25)	10.8 $\pm$ 1.3 (8.9–12.5)
20.	Tail length (T)	172 $\pm$ 18.9 (142–203)	42 $\pm$ 3.6 (32–44)	74 $\pm$ 9.1 (57–96)
21.	Hyaline part of tail (H)	–	–	40 $\pm$ 6.1 (30–52)
22.	Phasmid to anus distance	38 $\pm$ 6.1 (32–55)	21 $\pm$ 3.5 (14–23)	–
23.	Spicule length (SL)	–	45 $\pm$ 5.7 (32–54)	–
24.	Gubernaculum length (GL)	–	30 $\pm$ 4.9 (24–39)	–
25.	H% (H/T)*100			54 $\pm$ 9.8 (40–82)
26.	Ant. Sperm theca Length	43 $\pm$ 9.3 (30–62)		
27.	Post. Sperm theca Length	37 $\pm$ 7.2 (30–52)		

– characters absent or not measured

of the excretory pore from anterior end was 115 μm . Rectum length was 30 μm . Anal body diameter was 10.8 μm . Conoid tail with pointed tip is present that measure about 74 μm .

Discussion

In the present study, a number of isolates of soil nematodes were found in the Srinagar Garhwal Uttarakhand and effectively identified as belonging to the *Oscheius* genus. Entomopathogenic nematodes (EPNs), free-living rhabditid nematodes of the families Steinernematidae and Heterorhabditidae are utilized as biocontrol agents against a variety of insect pests. However, additional Rhabditida species have also demonstrated the ability to kill insects, making them potentially effective new candidates for biocontrol of insect pests. Many *Oscheius* species are known for their insect-killing prowess and are often frequently referred to be EPNs. The soil was collected from the various localities of Srinagar Garhwal region into a small plastic bags. The soil, is of alluvial type making it particularly appropriate for farming that is both rain-fed and irrigated. After collection the decanting and sieving technique was followed by using sieve of mesh 350 μm and 400 μm , around 20 females, 20 males and 20 13th stage juvenile were isolated, they were then processed for the morphometric study which revealed the genera to be *Oscheius*, the average of female body length was 1557 ± 70 μm , the male was 1179 ± 104 μm long, while the average of 13th stage juvenile length was about 534 ± 21 μm . The De Man formulas or indices were used to calculate other metrics.

Oscheius are extremely beneficial to agricultural culture because they are parasitic and deadly to several insect pests. This study's principal objective was to shed light on the indigenous nematode fauna of the Srinagar Garhwal Uttarakhand. Additionally, in the future, molecular characterization will reveal the species that predominate in the specific area where the nematode was isolated.

Similar work was done by Bhat *et al.* (2021) in

the Hapur district of western Uttar Pradesh, India. They described an insect parasitic nematode of the genus *Oscheius* in the agricultural soils, this species had significant degree of similarity to the Pakistani species *Oscheius siddiqii* and *O. niazii* which was revealed through morphological research. Also after molecular characterization, Ali *et al.* (2011), from the Kanpur district of India isolated the *Oscheius* from a larva of the red hairy caterpillar *Amsacta moori* and named it the *O. amsacta*.

The present study may have paved a path for more discoveries of the same genera of Entomopathogenic nematodes in the nearby regions whose species is yet to be confirmed after molecular characterization.

Conclusion

Srinagar being surrounded by Garhwal Himalayas serve as an agricultural land and has variety of flora and fauna. An entomopathogenic nematode was isolated from the soil of Srinagar, Pauri Garhwal district. The soil was collected from the various localities of Srinagar region around 10 samples were collected into small plastic bags. After collection the decanting and sieving technique was followed by using sieve of mesh 350 μm and 400 μm , 20 females, 20 males and 20 13th stage juvenile were isolated, they were then processed for the morphometric study which revealed the genera to be *Oscheius*, the average of female body length was 1557 ± 70 μm , the male was 1179 ± 104 μm while, the average of 13th stage juvenile length was about 534 ± 21 μm . The De Man formulas or indices were used to calculate other metrics.

The ability of *Oscheius* nematodes to adapt and endure in many situations is a noteworthy trait. They are renowned for their toughness and ability to tolerate extreme temperatures and desiccation. They are useful for researching evolutionary processes and mechanisms of stress responses because of their versatility. Overall, the genus *Oscheius* includes nematodes that have made a considerable contribution to our understanding of

a variety of biological processes and continue to be crucial study organisms in a wide range of scientific fields.

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