Morphometric study of *Oscheius* spp. isolated from Karanprayag region (Chamoli), Uttarakhand, India

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Abstract: The present study was conducted on isolation and identification of entomopathogenic nematodes. During the study soil samples were collected from different locality of Karnprayag. Entomopathogenic nematode were isolated from agricultural field of Karanprayag District Chamoli, Uttarakhand India. Based on morphological and morphometrical studies the nematodes were identified as *Oscheius* sp. This work represents the first report of this genus in the Karanprayag region. Around 20 male, 20 female and 20 13th stages juvenile were isolated following the Cobb's decanting and sieving technique. Furthermore in future the molecular characterization will bring out the species dominant in the particular region of Karanprayag from where the Nematodes were isolated.

IndexTerms: Entomopathogenic nematodes, Karanprayag, *Oscheius*, Cobb's decanting and Sieving Technique.

I. INTRODUCTION:

Nematodes, are simple tiny, colourless, unsegmented roundworms belonging to Phylum Nematoda, can be free living, predaceous or parasitic [1]. Entomopathogenic nematodes (also known as EPN) are a class of nematodes that live in soil and parasitize a variety of insects. *Aplectana kraussei* (now known as *Steinernema kraussei*) was the first EPN to be identified in 1923 [2]. Travassos (1927) renamed *Aplectana, Steinernema* [3].

Steinernematidae and Heterorhabditidae are two families to which these nematodes belong. Till date only 101 species of *Steinernema* (Steinernematidae) and 18 of *Heterorhabiditis* (Heterorhabiditidae) species have been discovered around the world [4]. They are found naturally, in the soil setting and respond to carbon dioxide, vibration and other chemical cues to seek their host [5].

EPN'S have powerful insecticidal complex or symbiotic relationship with Enterobacteriaceae bacteria that kills variety of insect species [6]. *Steinernema* is in mutual symbiotic relationship with bacterium *Xenorhabdus* while *Heterorhabiditis* is mutually symbiotically related to *Photorhabdus* bacteria [7].

Oscheius that belong to phylum Nematoda, class Secernentea, subclass Rhabditia, order Rhabditida, and Family Rhabditidae, is a free living EPN that can be found in saprophytic biotype like debris, dungs and agricultural soils [8]. As per their feeding habit is concerned, they are bacterivores that feed on bacteria found in the disintegrating insect carcass (necronemic) or free-living bacterivores. They can be distinguished by having short buccal tube whose length and width is nearly similar and is marked by absence of pharyngeal enlargement [9].

The life cycles of Steinernematids and Heterorhabditids are very similar. Their infective juveniles (IJ'S) are free-living, non-feeding, developmentally halted and have both insect and nematode characteristics. In case of *Oscheius,* they possess only a single generation. Infected larvae of Steinernematids nematode turns creamy/dark brown colour and that of Heterorhabiditis nematodes turn reddish/purplish colour [10] whereas in *Oscheius* cadaver colour remains the same.

Oscheius comprises of two main group dolichura and insectivora [11]. Insectivora group differ from Dolichura in having bursa leptoderan or pseudopeloderan and spicule with a crochet needle shaped point, while in Dolichura,spicule with the thin tubular tip along with peloderan bursa is present [8]. Till now 43 valid species under genus *Oscheius* were found, out of which 29 belong to the insectivora group and 14 belong to dolichura group (Tabassum *et al.,* 2016). Metastom with warts, missing median bulb, rectum longer than anal body diameter, spicules distinct and distally hooked are all characteristics of *Oscheius* morphology [12].

Recent evidence has made evident that *Oscheius* species pathogenicity on insects, is a behaviour that is primarily correlated with the endosymbiont bacteria that belong to two genera *Serratia* [13] and *Enterococcus* [14]. In most of the cases *Oscheius* are entomophilic in nature.

II. MATERIALS AND METHODS:

To achieve the goals of the investigation following materials and methods were applied. The present study was conducted on morphological and morphometrical characterization of *Oscheius* genera isolated from the Karanpragyag (Chamoli district) of Uttarakhand, India.

Samples were taken from agricultural soil of Karanpragyag (Latitude: 30° 16' 12.00" N Longitude: 79° 15' 0.00" E, 860ASL) region of Chamoli district Uttarakhand.

Soil sample collection

Soil sample collection was done by following the method of [6]

1. For each sampling site, a minimum of 2 – 4meter square was covered.
2. Soil samples were collected at least 15 cm deep using a hand shovel. Samples were kept in autoclave plastic bag, to avoid sample leakage, double bagging of samples were done. Using a waterproof marker, the samples were labeled, including the details for each sampling location, Collection tools were cleaned by washing them with water and/or disinfecting them with 70% ethanol or 0.5% bleach solution.
3. During transfer to the laboratory, samples were kept in a cooler (8 – 15 °C)

Isolation of Nematodes-

Nematodes were isolated by following the Cobb's sieving and decanting technique [15].

1. The beaker was filled halfway with water and the soil samples were added to it.
2. Beaker was kept still for few minutes, allowing the heavy particles to settle down.
3. The heavy silt particles settled in 10–20 seconds, and the suspension was then decanted onto a series of fine sieves (350, 400), each retaining different sized nematodes (based on their body sizes).
4. Each sieve's nematode suspension was washed and poured through a fine wire gauze sieve containing double layered tissue paper already put on a beaker to keep the fine silt, then left overnight in the beaker .The nematodes would have passed through the tissue paper's minute gaps and were collected at the bottom of the beaker
5. Finally, the contents of the beaker were strained through a 400 mesh the nematode containing contents were then poured into the hollow block and were observed under binocular microscopy.

Morphometry-

Nematodes were first heat-killed, and were fixed in TAF (Triethanolamine fixative) for 5-7 days, and then processed by Seinhorst's method (1959) [16]. Nematodes were permanently mounted on microscope glass slides. A light microscope was used to inspect and measure the specimens. They were photographed with CANON Power Shot S50 at 1000x magnification. A drawing was done to create the illustration.

III. OBSERVATION AND RESULTS:

Present study was conducted on Morphological and Morphometrical characterization of *Oscheius* genra isolated from Karanpragyag (Chamoli district) Uttarakhand, India. During studies a total of 15 samples were collected and analyzed. The isolation was done by the method of Cobb's sieving and decanting technique [15].

SN	Characters	Female	Male	13th stage Juvenile
1.	N	20	20	20
2.	Body length	1354±125(1104-1408)	1122±116(816-1221)	524±11(402-474)
3.	*a*(L/Mbd)	15.7±1.1(13.2-18.4)	23±3.7(12.7-2.4)	18±1.0(17-21)
4.	*b*(L/NL)	6.7±0.8(5.1-7.9)	5.7±0.6(4.3-6.7)	3.3±0.3(2.7-3.9)
5.	*c*/(L/T)	8.4±1.3(5.5-17.2)	25±2.1(18.1-32)	5.4±0.5(6.0-7.2)
6.	*c'*(T/ABW)	4.8±0.8(3.5-6.3)	1.8±0.2(1.5-2.5)	6.0±0.7(4.9-7.2)
7.	V%(AV/L×1000)	48±2.1(43-52)	-	-
8.	Bulb length	34±3.2(22-32)	28±2.0(24-35)	2.0±2.1(16-20)
9.	Pharynx length	178±6.4(167-196)	162±7.2(129-163)	102±7.4(88-115)
10.	Nerve ring- ant.end (NR)	143±8.6(129-165)	128±8.6(110-168)	70±6.4(59-78)
11.	Excretory pore-ant end (EP)	185±16(161-222)	179±21.4(158-201)	110±6.4(101-122)
12.	Neck length (stoma pharynx, NL)	210±7.0(202-224)	173±6.2(162-182)	122±3.7(112-126)
13.	Body width at neck base	52±5.3(28-56)	32±3.4(34-48)	26±2.1(23-28)
14.	Mid body diameter (MBD)	84±6.5(71-112)	47±7.6(36-62)	23±1.2(21-22)
15.	Uterus or testis	67±12.5(51-82)	547±32(462-586)	-
16.	Ant. Spermatheca length	42±7.0(28-59)	-	-
17.	Anterior genital branch	311±48(210-388)	-	-
18.	Post. Spermatheca length	36±7.2(29-52)	-	-
19.	Posterior genital branch	250±38(198-322)	-	-
20.	Vagina length	22±2.1(16-28)	-	-
21.	Vulva- ant.end (VA)	734±52(608-858)	-	-
22.	Rectum length	66±9.7(55-78)	-	28±2.4(23-32)
23.	Anal body diameter (ABD)	28± (24-38)	18±2.8(19.1-28)	11.8±1.3(10.9-14.5)
24.	Tail length	147±20(123-169)	38±3.1(34-44)	52±10.1(58-94)
25.	Plasmid to anus distance	38±6.1(31-55)	22±1.2(18-24)	-

26.	Spicule length (SL)	-	42±5.1(32-52)	-
27.	Gubernaculum length (GL)	-	20±3.1(20-22)	-
28.	Hyaline part of tail (H)	-	-	31±5.3(46-48)
29.	H% (H/T×100)	-	-	52±3.0(48-61)

All samples were morphometrically and meristically analysed, the nematodes were identified on the basis of morphometry and identification keys. The studies revealed that the nematodes are of *Oscheius* genera.

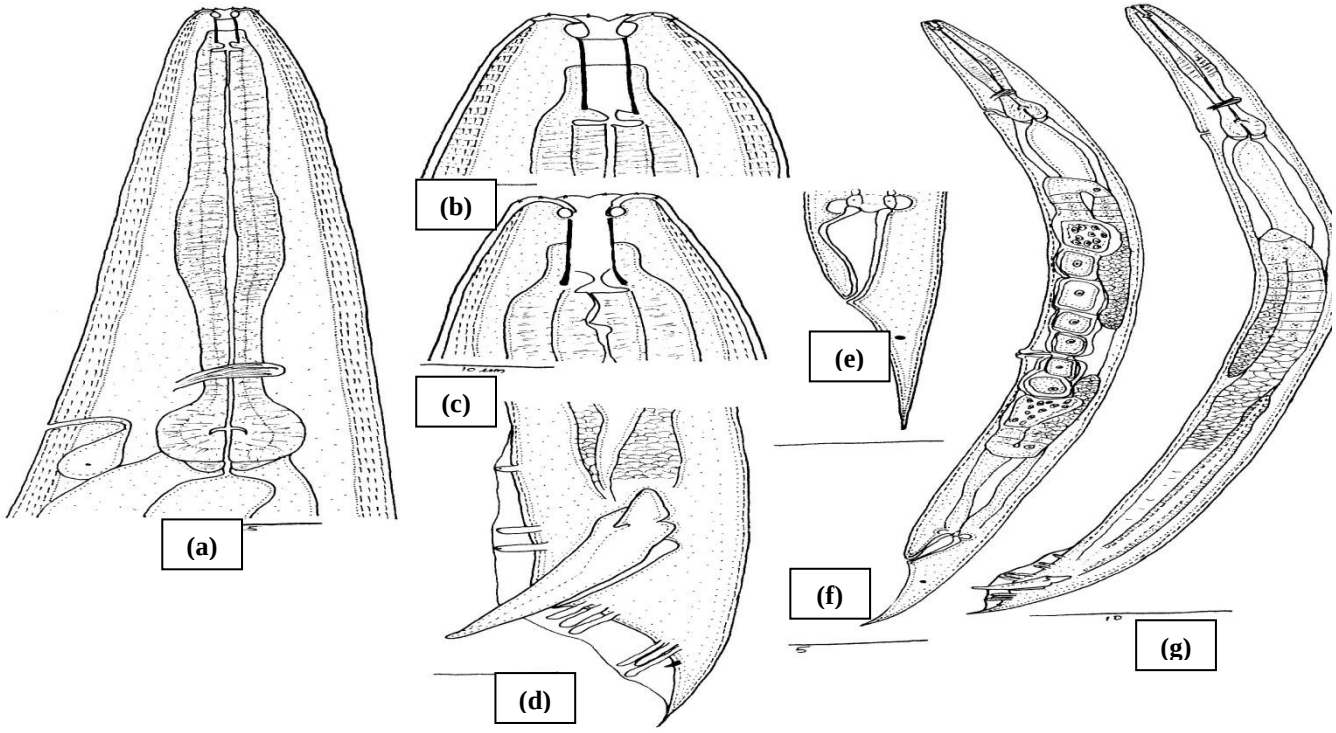

Figure 1: *Oscheius* spp. general body structure
A. Pharyngeal region; B. male anterior region. C. female anterior Region, D. male posterior Region, f. female, g male
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Female- The average of female body length was 1354µm; body was slightly bent on ventral side. Pharynx was 178µm long. The total length of nerve ring from anterior end was 143µm and the standard deviation was 8.6. The total neck length that comprises stoma and pharynx was 210µm. Mid body diameter was 84µm. Body width at base of neck was 52µm.
The total length of the excretory pore from anterior end was 185µm; it is conspicuous and located ventrally at the level of basal bulb. Uterus is well developed with lengthy glandular and muscular areas with length of 67µm. Length of anterior and posterior sperm theca was 42µm and 36µm. Vagina is 22µm long, it comprises of thick and cuticularized wall. The length of vulva from anterior end was 734µm.
Rectum was short with rectal glands at its junction, measuring about 2.3 times (66µm) the diameter of anal body (28µm). Conoid tail that is gradually narrowed down to a fine termination measures about 147µm.
Male- They were smaller than female having length of 1122µm Spicules were present having length of around 42µm. Gubernaculum is flattened on ventral side and follows the spicules' outline, it measures around 20µm, and Pharynx was 162µm long.
The total length of nerve ring from anterior end was 128µm. The total neck length that comprises stoma and pharynx was 173µm. Mid body diameter was 47µm. Body width at base of neck was 32µm. The length of the excretory pore from anterior end was 179µm. Testis was single measuring about 547µm in length. Anal body diameter was 28µm. Tail is conical and small than that of female, measuring about 38µm.
Juvenile (3rd stage) they measure about 524µm in length. Pharynx was 102µm long. The total length of nerve ring from anterior end was 70µm. The total neck length that comprises stoma and pharynx was 122µm.
Mid body diameter was 23µm. Body width at base of neck was 26µm. The length of the excretory pore from anterior end was 110µm. Rectum length was 28µm. Anal body diameter was 11.8µm. Conoid tail with pointed tip is present that measure about 52µm.

Ⅳ. Discussion:

The genus *Oscheius* was discovered by Andrassy (1976), while Sudhaus had divided the species in two groups- dolichura group and insectivora Group, as mentioned in historical reviews. *Oscheius* has species in large number that are similar in accordance to their morphology.

During the present study *Oscheius* genera was isolated from the soil of Karanpragyag region, Chamoli district, Uttarakhand, it is the first time that *Oscheius* was isolated from the soil samples of Karanpragyag region, to confirm the species further work on the molecular level is to be done.

Ali *et al.*, 2011 had isolated the *Oscheius* from a larva of red hairy caterpillar *Amsacta moori* and described it as the *O. amsacta* after molecular characterization, from Kanpur district India [17].

Similar work was done by Kumar *et al.*, 2019where they isolated *Oscheius* from the soil samples collected from Cachar District, Assam, after morphometrical study using the morphological attributes like body length, bulb length, pharynx length, and nerve ring- ant. end (NR), excretory pore-ant end (EP), neck length (stoma+pharynx,NL), body width at neck base, mid body diameter (MBD), uterus or testis length, vagina length, vulva- ant.end (VA), rectum length, anal body diameter (ABD), tail length, phasmid to anus distance, spicule length (SL), gubernaculum length (GL), hyaline part of tail (H) etc, all these measurements, along with molecular characterization confirmed the species to be *indicus.* They have reported 14 paratype females of length 1272.3µm and the standard deviation was 111.7 and 14 paratype males of length 1062.7µm and the standard deviation was 104.5 [8].

Bhat *et al.*, 2021 had isolated the *Oscheius* genera from the agricultural soil of Hapur District in western U.P that share the similar resemblance with *O. siddiqui* and *O. niazii*, the species form Pakistan [18]

V. SUMMARY:

Karanpragyag being a small town was never explored for the study of Entomopathogenic nematodes; it was the first time that the study was conducted on it. An Entomopathogenic nematode was isolated from the soil of Karanpragyag region (Chamoli district) Uttarakhand.

The soil was collected from the various localities of Karanpragyag region into a small plastic bags, soil is of sandy loam type & humus rich which is extremely suitable for rain fed and irrigated type of farming, pH varies from 5.0-6.5, after collection the decanting and sieving technique was followed by using sieve of mesh 350 mm and 400 mm, around 28 females, 24 males and 18, 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 1354µm and the standard deviation was 125, the male was 1122µm long and had the standard deviation of 116 while, the average of 13th stage juvenile length was about 524µm with standard deviation of 11 other measurements were calculated by following the De Man formulae or the De Man indices.

Oscheius being parasitic and lethal to some insect pests make them extensive useful to the agricultural society. The future perspective of this thesis is to bring light over the Nemic fauna of the Karanpragyag (Chamoli district) Uttarakhand. Furthermore, in future the molecular characterization will bring out the species dominant in the particular region of Karanpragyag from where the nematode was isolated.

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