

Description of *Oscheius karachiensis* n.sp. (Nematoda: Rhabditidae), a new entomopathogenic nematode from chicko (*Manilkara zapota*), saeedabad, Sindh Pakistan

Nasir Mehmood and Tabassum Ara Khanum

Abstract— *Oscheius karachiensis* n. sp., was isolated from fruit growing areas of Sindh, identified using a combination of morphological and morphometric characters. *O. karachiensis* n. sp., belongs to dolichura group by having peloderan bursa. The new species is characterized by the presence of lateral field marked by 4 ridges and 5 incisures, that is a key diagnostic feature. On the basis of spicule length *O. karachiensis* n. sp., distinguished all species of dolichura group having larger size of spicule i.e. (50-52) μm except *O. pherosphi*. In female *O. karachiensis* n. sp. differs from all species having larger tail (116-160) μm except *O. guentheri* (126-162) μm and *O. pherosphi* (120-183) μm but differs from both species having longer pharynx (208-233) VS (126-162); (158-184) μm respectively. Entomopathogenicity bioassay revealed that this nematode is capable of infecting larvae of *Galleria mellonella* and *Tribolium castaneum*.

Index Terms—*Oscheius karachiensis* n. sp., entomopathogenic, nematode, morphometric character, dolichura, mortality, *Galleria mellonella*, *Tribolium castaneum*

1 INTRODUCTION

FROM Pakistan many species of the genus *Oscheius* Andrassy, 1976 were described and reported. Tabassum and Shahina described *O. maqbooli* in 2002, *O. andrassyi* in 2008, *O. niazi* and *O. siddiqi* in 2010, whereas Tabassum *et al.*, (2016) described six new species viz., *O. citrai*, *O. cobbi*, *O. esculentus*, *O. cynodonti*, *O. saccharai* and *O. punctata* species. All above mentioned species belong to insectivore group, during the present studies (2016-2018) extensive surveys were conducted of various fruits of Sindh, more than 50 soil samples were collected out of which many rhabditid nematodes were isolated. In which one new species of the genus *Oscheius* of dolichura group was found, detailed description based on morphological and morphometrical characteristics with illustration and photomicrography is presented here.

MATERIALS AND METHODS

Nematode source: A new isolate was recovered from soil using the insect baiting method Bedding and Akhurst, (1975). Five last instar *Galleria mellonella* larvae were placed in 250 ml plastic containers with moistened soil from samples, each being covered with a lid turned upside down and kept at 27-30 °C.

Soil assay were checked daily, dead larvae were picked out, rinsed in distilled water and placed in Petri dish until the nematode progeny emerged and harvested into a beaker. For mass rearing, the insect parasitic nematode species were maintained on nutrient agar as per Nuchanart *et al.*, (1999). Adult nematodes were harvested after 2-3 days at room temperature and stored in distilled water in a flask at 15-20 °C.

2. Morphology and morphometrics of the isolated nematodes: Nematodes were cultured on *G. mellonella* at 25±5 °C according to Baliadi *et al.*, (2009). Mortality of *G. mellonella* larvae was recorded after 24-48 hrs. For morphological observation IJs, female and males were killed by hot water, then fixed in TAF, Courtney *et al.*, (1955) and processed to glycerine by the Seinhorst method, Seinhorst, (1959). Permanent slides were made using glass support to avoid flattening of specimens. At least 25 specimens of each stage (male, female and IJs) were randomly selected and measured using the Nikon E400 light microscope (LM). Photomicrographs were also recorded with the Nikon E400 light microscope.

Description:

Measurements: Holotype male: L= 846 μm ; a = 18; b = 5; c = 26; c' = 1; Tail length = 32 μm ; Anal body width = 28 μm ; Spicules = 52 μm ; Gubernaculum = 23 μm ; Maximum body width = 48 μm .

- Nasir Mehmood, National Nematological Research Centre, University of Karachi, Karachi- 75270, Pakistan, PH-03323933166. E-mail: nasir00576@gmail.com
- Tabassum Ara Khanum National Nematological Research Centre, University of Karachi, Karachi- 75270, Pakistan, PH-03322278012. E-mail: tabassumak@uok.edu.pk (corresponding author)

Paratype males: L= 822-980 (890 ± 46.4) μm ; a = 14-19 (17.1 ± 1.3); b = 4-5 (4.2 ± 0.2); c = 24-37 (27.5 ± 3.7); c' = 1-2 (1.1 ± 0.3); Tail length = 24-36 (32.6 ± 3.4) μm ; Anal body width = 26-30 (27.1 ± 2.8) μm ; Spicules = 50-52 (51.3 ± 0.8) μm ; Gubernaculum = 23-25 (23.6 ± 0.9) μm ; Maximum body width = 46-64 (52.5 ± 5.3) μm .

Paratype females: L= 1171-1470 (1308 ± 85.9) μm ; a = 14-20 (15.8 ± 1.9); b = 5-6 (6 ± 0.7); c = 9-12 (12 ± 2.5); c' = 3-5 (4.3 ± 0.6); Tail length = 116-160 (121 ± 14.4) μm ; Anal body width = 24-48 (28.4 ± 6.1) μm ; V% = 46-50 (49 ± 1.5); Maximum body width = 86-108 (84.7 ± 11.5) μm .

Adult: Body cylindrical, anterior and posterior ends tapered. Cuticle 1-1.5 μm thick with fine annulation. Lateral field with four ridges and five incisures occupying about 0.8-10 μm of the corresponding body diameter at mid body. Head continuous with body contour, six separate well developed lips each with one- minute labial papilla. Amphid pore like on lateral lips. Stoma short rhabditoid with distinct chilo, gymno and stegostome, metastegostome bearing small denticles. Glottoid apparatus well developed, isomorphic. Pharyngeal collar present, about 8-10 μm long enveloping up-to 70 % of stoma. Pharyngeal corpus cylindrical containing metacarpus with valvated basal bulb. Median bulb absent. Nerve ring encircling isthmus at middle, near or base of terminal bulb. Deirids conspicuous. Excretory pore located at the junction of isthmus and basal bulb. Cardia present protruding into intestine.

Male: Body cylindrical, J shaped after killing with hot ($65-75^{\circ}\text{C}$) water. Lateral field with four ridges. Testis monorchic reflexed ventrally, vas deference well developed filled with sperms, without demarcation of seminal vesicle. Ejaculatory gland not present. Spicule paired of equal length, separate with shape of manubrium varying from rounded to beak shape, lamina as long as manubrium velum extending from neck to distal tips present. Distal tips rounded. Gubernaculum boat shaped 20-23 μm in length about *ca* 1.5-2 shorter than spicules. Tail conical somewhat concave ventrally, tail tip reaching the edge of bursa. Bursa open, wide peloderan. Nine pairs of genital papillae (Gps) incorporated into bursa (formula 1+1+1+2/1+3). First pair shortest, distance from 2nd pair Gp2. Gp4-6 and 7-9 move closely spaced than Gp1-3. Gp5 and 8 curved dorsally and not reaching rim of bursa.

Female: Body straight to slightly arcuate ventrally tapering at both extremities. Cuticle smooth finely annulated, 0.8-1.5 μm at mid body. Head continuous with body contour, six separate well developed lips each with one- minute terminal papilla. Stoma tubular, 4 times as long as broad, about 16-20 μm long. Pharyngeal corpus 120-144 μm long, procorpus cylindrical; metacarpus not clearly differentiated. Basal bulb rounded,

nerve ring usually surrounding mid-part of isthmus. Excretory pore conspicuous, usually ventrally located at level of basal bulb. Hemizonid not clearly observed. Reproductive system didelphic, amphidelphic. Vulva middle in position vagina thin wall 20-25 % of corresponding body diameter. Gonad reflexed with tips sometimes reaching level of vulva, unreflexed part of anterior branch 400-500 μm long, reflexed part measuring 320-375 μm . Unreflexed part of the posterior gonad measuring 350-400 μm and reflexed part 300-350 μm long. Oviduct forming spermatheca, containing large sperm cells of about 2 μm . Ovoviviparous species, uterus with 8-10 eggs at the same time. Rectum 2.5 ± 0.3 (2.0-3.0) anal body diam., long. Tail elongate, slender, tapering gradually, 3-5 μm anal body diam., long. Phasmids prominent, position variable, located at one-third of tail region posterior to anus. Tail elongate with pointed terminus.

Infective Juveniles: Third stage juvenile unsheathed in cuticle of second stage juvenile. Body short, slender tapering gently toward both ends. Cuticle with longitudinal striations; head rounded lip region smooth, mouth closed. Stoma and pharynx morphology similar to adult. Cardia protruding into intestine. Excretory pore located at basal bulb. Nerve ring surrounding isthmus. Tail elongate conical, tapering to a pointed terminus. Clumps of juveniles observed in agar plate cultures, juveniles usually being attached to one another by both ends.

Type host and locality: Soil around the roots of chicko (*Manilkara zapota*) from Saedabad, Karachi, Sindh Pakistan.

Type Specimen: Holotype male and 15 paratype male and female specimens deposited in the Nematode collection of the National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

Etymology: This species is named after the city (Karachi) where it was collected.

Differential Diagnosis: *Oscheius karachiensis* n. sp. is characterized by the presence of lateral field marked by 4 ridges and 5 incisures, that is a key diagnostic feature. *Oscheius karachiensis* n. sp. belongs to *dolichura* group by Sudhaus, (2011) by having peloderan bursa. Comparing *Oscheius karachiensis* n. sp. with 15 species of *O. dolichura* group, it differs to all species except *O. dux* by Gorgadze, (2010) Sudhaus, (2011) and *O. latus* by Cobb, (1906) Sudhaus, (2011), by having 4 ridges or 5 incisures. On the basis of spicules length *O. karachiensis* n. sp. distinguished all species of *dolichura* group having larger size of spicules i.e. (50-52) μm except *O. pherosphi* by Smart and Nguyen, (1994) Sudhaus, (2011).

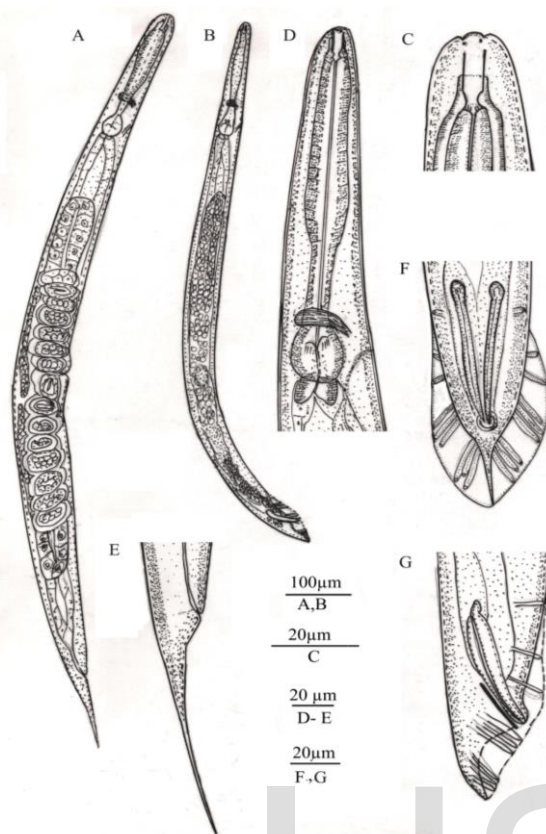


Fig. 1 (A-G). *Oscheius karachiensis* n. sp. Female (A,C-F): A. Whole body; C. Anterior region; D. Pharyngeal region; E. Tail region; Male (F, G): F. lateral view of tail region showing papillae; G. Ventral view of tail region showing papillae.

In female *O. karachiensis* n. sp. differs from all species having larger tail (116-160) μ m except *O. guentheri* (126-162) μ m by Sudhaus and Hooper, (1994) and *O. pherosophi* (120-183) μ m but differs from both species having longer pharynx (208-233) vs (126-162); (158-184) μ m respectively.

The new species can be distinguished from *O. dux* by having in male larger body in size (822-980) vs (520-720) μ m; Pharynx (164-193) vs 121-136) μ m; tail (24-36) vs (22-30) μ m, spicules (50-52) vs (22-30) μ m. In female larger body size, pharynx and tail [body size (1171-1470) vs (860-1100) μ m; pharynx (208-233) vs (152-167) μ m and tail in length (116-160) vs (95-106) μ m respectively]. *Oscheius karachensis* n. sp. also differs from *O. latus* by having in male larger body size (822-980) vs 500 μ m; shorter a and longer c value {(a= 16-19) vs 26; (C= 24-37) vs 10}. In female by having larger body size (1171-1470) vs 600; lower V% (46-50) vs 65; shorter 'a' value (14-20) vs 23; higher 'b' value (5-6) vs 3.9.

Nematode Entomopathogenicity: *Oscheius karachiensis* n. sp. infected 70% of *Galleria* and 60 % of *Tribolium castaneum* while *S. pakistanense* showed 80% and 75% respectively, of these insects within 24 h. Due to high temperature IJs of both nematodes emerges within 24-48h.

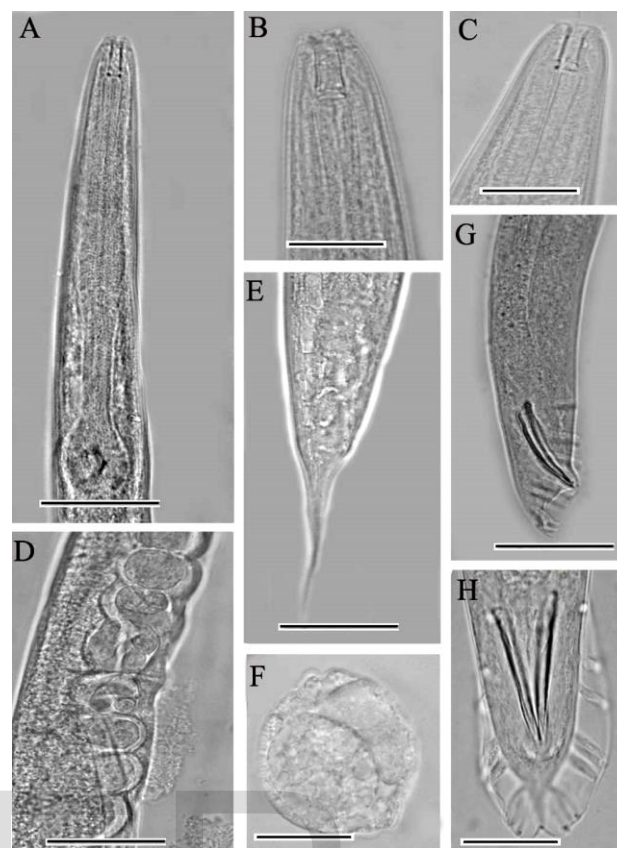


Fig. 2 (A-H). Light microphotographs of *Oscheius karachiensis* n. sp. Female (A-F): A. Pharyngeal region; B, C. Anterior region; D. Vulval region covered with exudates; E. Tail region; F. T.S of mid region of nematode showing lateral field having 5 incisures; Male (G, H): G. Anterior region; H. Ventral view of tail region showing papillae (scale: A, G-I=10 μ m; B-F=20 μ m).

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