

Description of six new species of *Oscheius* Andrassy, 1976 (Nematoda: Rhabditida) from Pakistan with a key and diagnostic compendium to species of the genus

K. A. Tabassum, F. Shahina[†], K. Nasira and Y. I. Erum

National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan

[†]Corresponding author: shahinafayyaz@gmail.com

Abstract

Six new species of the genus *Oscheius* Andrassy, 1976 viz., *Oscheius citri* n. sp., *O. cynodonti* n. sp., *O. cobbi* n. sp., *O. esculentus* n. sp., *O. punctata* n. sp. and *O. sacchari* n. sp., are described by both morphological and molecular means from different agro-climatic regions of Sindh, Punjab and Azad Jammu & Kashmir, Pakistan. All species belong to insectivora group on the basis of leptoderan bursa, crochet needle-shaped spicules, normal rectum, lateral field with six separate lines and had unique ribosomal DNA-ITS, sequence. A compendium of the genus *Oscheius* (of both insectivora and dolichura groups) based on the following characters: body length, tail, spicules and gubernaculum length, ratios a, b, c, c' and vulva percentage is given. The morphometric and allometric characters with anterior and posterior regions were derived from the original descriptions. An identification key to 42 valid species of both insectivora and dolichura group of the genus *Oscheius* is given.

Keywords: *Oscheius*, insectivora, dolichura group, new species, taxonomy, molecular, phylogenetic relationship, key, compendium.

In 1976, Andrassy described *Oscheius* as a sister taxa of *Rhabditis* (Dujardin, 1845) with *O. insectivorus* as its type species. Sudhaus (1976) placed *Oscheius* in Rhabditidae and divided it into two groups insectivora and dolichura; he synonymized the genus *Dolichorhabditis* Andrassy, 1983 with *Oscheius* in dolichura group as *O. dolichura*. The species of insectivora group have crochet needle-shaped spicules, leptoderan bursa and normal rectum whereas dolichura group have peloderan bursa, absence of crochet needle-shaped spicules and expansile rectum. In 2005, Andrassy classified superfamily Rhabditoidea which comprises of Mesorhabditidae, Rhabditidae, Diploscapteridae and Protorhabditidae. Sudhaus (2011) gave bauplan of Rhabditidae on morphological and molecular basis, which consists of three groups namely Pleiorhabditis, Synrhabditis and Anarhabditis and placed the genus *Oscheius* in

Synrhabditis with 28 nominal species within the genus *Oscheius*; among them 15 species belong to insectivora group and 13 species in dolichura group. Four species of insectivora group viz., *O. carolinensis* Ye *et al.*, 2010, *O. amsactae* Ali *et al.*, 2011, *O. niazii* and *O. siddiqii* Tabassum & Shahina, 2010 and one species of dolichura group, *O. onirici* Torrini *et al.*, 2015 have been recognized as entomopathogenic nematodes. So far, 5 species of insectivora group have been described and reported from Pakistan. Tabassum & Shahina (2002) described *O. maqbooli* from sugarcane plantation of Chatta goth, Balochistan, while *O. andrassyi* was described as a new species from sugarcane field of Jhang, Punjab by Tabassum & Shahina (2008). Later on, Tabassum & Shahina (2010) described two new species, *O. niazii* and *O. siddiqii* from soil of palm tree and rose, respectively along with *O. shamimi* Tahseen & Nisa (2006) as a new record

on palm tree from Karachi, Sindh, Pakistan. More recently, several *Oscheius* species have been described bringing it a total of 42; of which 22 species belong to insectivora group and 14 to dolichura group along with 6 new species described in this paper.

During the present studies (2013-2014) of various agro-climatic zone of Sindh, Punjab and Azad Jammu & Kashmir, 45 soil samples were collected, which contained a large number of rhabditid nematodes; most of them closely resemble the insectivora group of *Oscheius*, based on morphological and DNA sequence data. Six new species were recovered as follows: *O. citri* n. sp., *O. cynodonti* n. sp., *O. cobbi* n. sp., *O. esculentus* n. sp., *O. punctata* n. sp. and *O. sacchari* n. sp. Detailed descriptions based on morphological and molecular characteristics, illustrations and SEM of these species are presented herein.

In the compendium format eleven characters (allometric and morphometric) viz., body length, tail, spicules, gubernaculum length, ratios a, b, c, c' and vulva percentage are used to separate *Oscheius* species. Drawings of anterior and posterior ends of 31 available species were taken from original publications along with 6 new species. An up to date list of 42 nominal species along with their diagnostic key, is also provided.

Materials and Methods

Nematode source: Six new isolates PAK. S. N. 1 (*Oscheius punctata* n. sp.); PAK. S. N. 2 (*O. cynodonti* n. sp.); PAK. S. N. 5 (*O. sacchari* n. sp.); PAK. S. N. 10 (*O. cobbi* n. sp.); PAK. S. N. 17 (*O. esculentus* n. sp.) and PAK. S. N. 24 (*O. citri* n. sp.) were recovered from soil using the insect baiting method (Bedding & 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 assays

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.

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 were randomly selected and measured using the Nikon E400 light microscope (LM). The potomicrographs were taken with a Nikon DS-Fi1 camera attached to microscope.

Molecular characterization and phylogenetic relationship: DNA was extracted from 50-100 females. New species were characterized by molecular assessment of the internally transcribed spacer (ITS) ribosomal DNA genes. The ITS1-5.8S-ITS2 regions were amplified using the forward primer TW81 (5' GTTTCCGTAGGTGAAACCTGC 3') and the reverse primer AB28 (5' ATATGCTTAAGTTCAGCGGGT 3') described by Joyce *et al.*, (1994). The PCR profile were used as denaturation at 3 min at 94 °C followed by 37 cycles at 94 °C for 30 sec, re-annealing 50 °C for 30 sec and extension at 72 °C for 45 sec followed by 7 min incubation at 72 °C, respectively (Nadler *et al.*, 2006). PCR product was separated by 1.0 % agarose gel electrophoresis stained with ethidium bromide and images were obtained in UV-transilluminator.

PCR products were purified using the QIA quick Gel Extraction Kit (Qiagen, Valencia, CA) and cloned into PGemT easy vector System I (Promega, Madison, WI). The plasmids were transformed into *Escherichia coli* strain JM109 competent cells (Promega, Madison, WI) according to the manufacturer's protocols. Clones containing putative rRNA inserts were identified through blue/white color selection. The plasmid preparations were extracted using the QIAquick Spin Miniprep Kit (Qiagen, Valencia, CA). From the positive resulted plates, two clones for each nematode individual were sequenced using an ABI Prism 377 sequencer (PE Applied Biosystem, Foster City, CA, USA) and dye terminator sequencing reagents at the Centralized Lab, University of Karachi, Karachi, Pakistan.

These sequences were compared with already registered or published sequences available in NCBI Genbank database using Blast (<http://www.blast.ncbi.nlm.nih.gov>), multiple alignments of the new sequence data together with others sequences selected from NCBI databases were performed using Clustal W2 software (Larkin *et al.*, 2007). On the basis of ITS regions, phylogenetic analysis was performed by maximum parsimony (MP) method in PAUP, 4.0b8 (Swofford, 2001). Trees were evaluated statistically by bootstrap analysis based on 1000 re-samplings of the dataset. The resulting trees were visualized by using Mr Ent 2.0 (Zuccon & Zuccon, 2010).

***Oscheius citri* n. sp.
(Fig.1: A-I, 2: A-I)**

Measurements: Table 1

Description

Female: Body straight to slightly arcuate ventrally, tapering at both extremities, more towards posterior end. Cuticle smooth, finely annulated, annules 1-1.5 μm at mid body. Lateral field with five longitudinal ridges (six lines evenly spaced with each other). Head

continuous with body contour, six separate well developed lips each with one terminal papilla. Amphids pore like located on lateral lips just posterior to labial sensilla. Stoma tubular 3-4 times longer than diameter. Cheilostome not cuticularized, stegostome (pharyngeal collar) prominent, enveloping 50% of stoma length. Glottoid apparatus well developed, isomorphic meta stegostomatal flaps difficult to observe under light microscope. Pharyngeal corpus cylindrical, 32.9-35% of pharyngeal length.

Median bulb absent. The metacorpus swollen and the pharyngeal collar is very long. Isthmus distinct, cylindrical, slightly narrower 50-54 μm long, about one body width long with pyriform basal bulb 34-36 x 27-30 μm in diameter with well-developed valves. Nerve ring usually surrounding mid part of isthmus at 51.2-69.5% of pharyngeal length.

Excretory pore conspicuous located at level of basal bulb ranging 79.9-97% of pharyngeal length. Excretory duct cuticularized, curved anteriorly than posteriorly. Hemizonid and deirids not clearly observed. Cardia present, protruding into intestine. Vulva near mid body, lips protruding, vulva usually covered with exudate, vagina cuticularized, one third of body width long. Gonad didelphic, amphidelphic ovaries reflexed, anteriorly often extending as far as vulva, uterus large, mature females with 7-15 eggs in various embryonic stages. Spermatheca filled with sperms. Rectum about twice as long as anal body width. Tail elongate (4-6) times longer than the body width at anus, sharply pointed. Phasmids pore like located at one third of tail region, posterior to anus.

Male: Tail conical somewhat concave ventrally with a short part of tail protruding beyond bursa, 10-12 μm long. Bursa leptoderan and bearing nine pairs of genital papillae of different length arranged as 1+1+1/3+3, (three of them pre-cloacal and six pairs post-cloacal). First pair at the level posterior to spicules head or capitulum. The distance between p1 and p2 slightly greater (14-17 μm) than between p2 and p3 (7-10 μm)

Table 1. Morphometric values of *Oscheius citri* n. sp. (n=25 each). All measurements are in μm .

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|----------------------------|-----------------------------|
| | | Female | Male |
| Total body length | 1427 | 1601 \pm 274 (1173-2015) | 1249 \pm 96.8 (1097-1464) |
| Maximum body width | 58 | 80 \pm 17.3 (52-105) | 53 \pm 6.8 (43-68) |
| Stoma length | 16 | 18.8 \pm 1.25 (17-20) | 17.2 \pm 1.3 (15-20) |
| Stoma width | 05 | 5.5 \pm 0.5 (5-6) | 4.6 \pm 0.4 (4-5) |
| EP | 210 | 217 \pm 26.5 (187-265) | 197 \pm 15.3 (165-215) |
| NR | 170 | 160 \pm 19.8 (120-190) | 164 \pm 13 (140-190) |
| ES | 240 | 252 \pm 10.7 (234-273) | 224 \pm 14.9 (200-250) |
| Tail length | 40 | 143 \pm 12.7 (120-160) | 43.5 \pm 3.5 (35-50) |
| Anal body width | 32 | 25 \pm 3.5 (22-28) | 31.3 \pm 1.7 (28-35) |
| V% | -- | 49.3 \pm 1.2 (47.4-51.2) | -- |
| a | 24.6 | 20.8 \pm 2.8 (17.7-28.5) | 23.4 \pm 2.8 (18-28) |
| b | 5.9 | 6.3 \pm 1.0 (4.8-7.9) | 5.5 \pm 0.3 (5-6.1) |
| c | 35.6 | 11 \pm 1.5 (9-13) | 28.6 \pm 2.5 (25-35.6) |
| c' | 1.25 | 4.8 \pm 0.5 (4-5.9) | 1.3 \pm 0.1 (1.2-1.6) |
| D % | 87.5 | 86 \pm 9.3 (76.9-104) | 87 \pm 5 (79-97) |
| Spicules | 70 | -- | 63.7 \pm 7.4 (57-70) |
| Gubernaculum | 30 | -- | 27.8 \pm 2.0 (25-30) |
| SW | 2.18 | -- | 2.0 \pm 0.17 (1.7-2.5) |
| GS % | 42.8 | -- | 44 \pm 3.7 (34-50) |

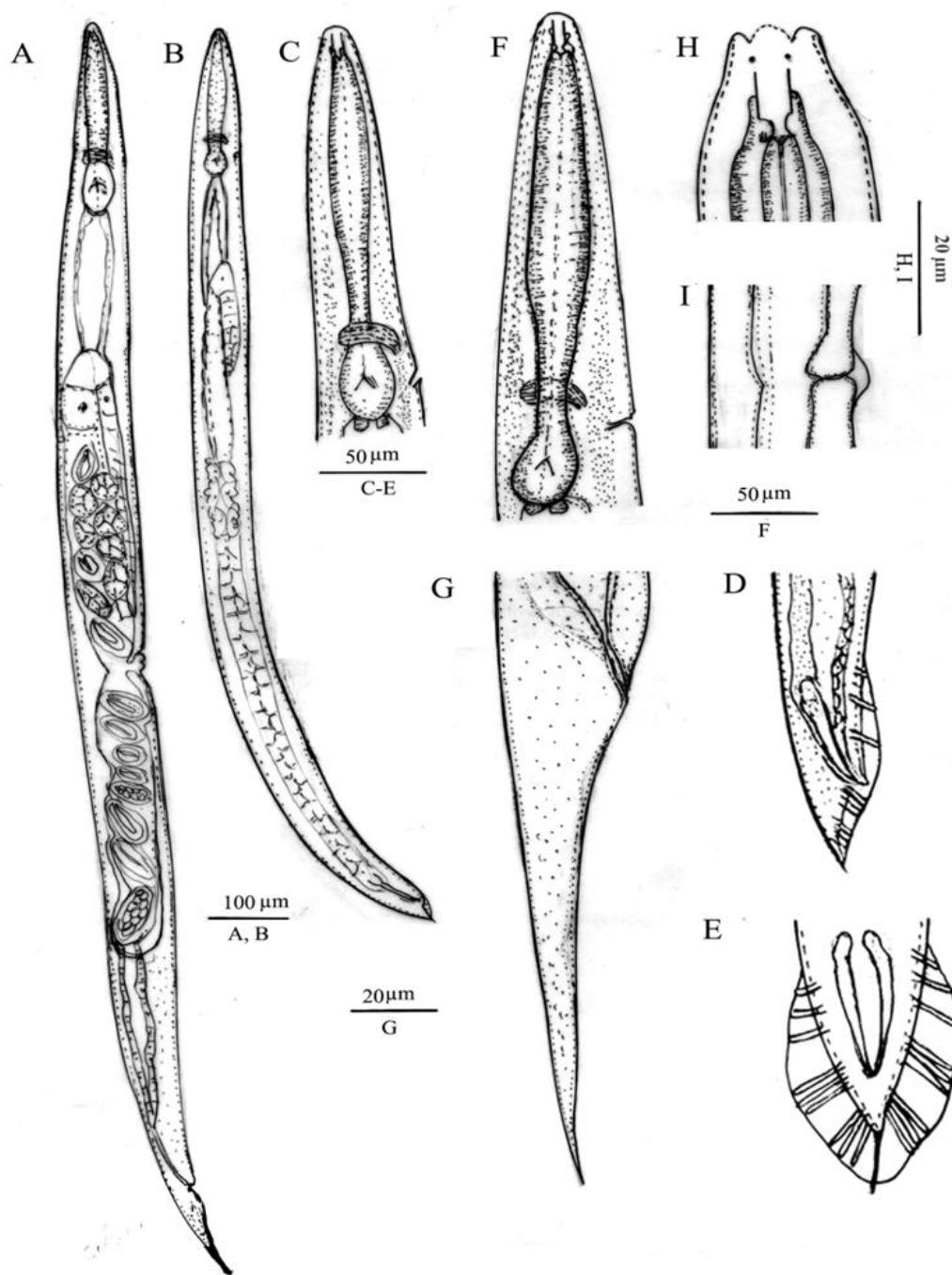


Fig. 1 (A-I). *Oscheius citri* n. sp. Female (A, F-I): A. Entire body; F. Pharyngeal region; G. Posterior region; H. Anterior region showing stoma; I. Lateral view of vulval region; Male (B-E): B. Entire body; C. Pharyngeal region; D. Lateral view of tail showing bursa and spicules; E. Ventral view of tail showing bursa, spicules and papillae.

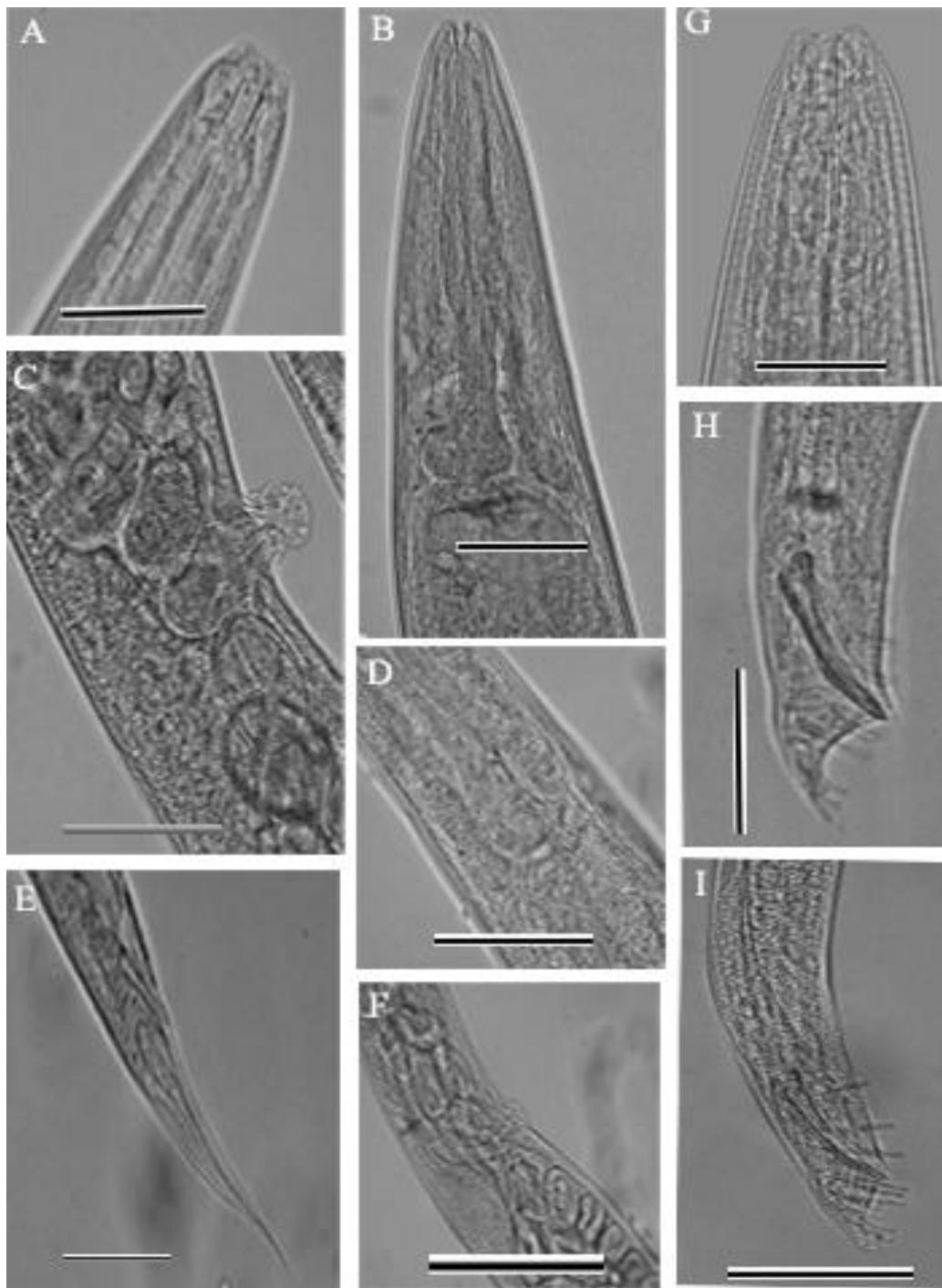


Fig. 2 (A-I). Light microphotographs of *Oscheius citri* n. sp. Female (A-F): A. Anterior region; B. Pharyngeal region; C, F. Vulval region covered with exudates; D. Posterior end of basal bulb; E. Tail region; Male (G-I): G. Anterior region; H-I. Lateral view of tail region showing papillae (scale: A, G-I=10 μ m; B-F=20 μ m).

and post-cloacal papillae arranged in two groups of 3 papillae each, spaced equi-distance. Pair 5 and 8 curved dorsally and not reaching rim of bursa (Fig. 13A). Spicule paired, long, separate, symmetrical, slightly curved ventrally with crochet needle-shaped tips. Spicules with prolonged manubrium, bent ventrally, calomus short and offset, lamina expanded in its proximal part, curved ventrally with one internal rib and thinner distally. Velum prominent extending 80-83% of spicules length. Gubernaculum almost straight, slightly bent distally, thin and elongate about 42-43% of spicules length (Fig. 13-15).

Juvenile: Body straight when heat killed. Stoma and pharynx morphology similar to adult. Tail elongate conical tapering to a hyaline part.

Type habitat and locality: Soil around the roots of citrus (*Citrus* spp.) from Gadap, 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 citrus plant (*Citrus* spp.), from where it was collected.

Diagnosis and relationship: *Oscheius citri* n. sp. belongs to insectivora-group on the basis of leptoderan bursa and crochet needle-shaped spicules. The new species is characterized by its unique ribosomal DNA-ITS sequence, amphimictic reproduction, lateral field with six separate lines, male medium size (average: 1249 μm), large spicules (average: 63.7 μm), bursa open, leptoderan leaving spike free, bursal papillae arranged as 1+1+1/3+3.

Morphologically, the new species is most closely related to *Oscheius cobbi* n. sp. and *O.*

cynodonti n. sp. However, it can be distinguished from *O. cobbi* n. sp. in the following characters: having longer males and females (average: 1249 vs 941 μm and 1601 vs 1197 μm), respectively; longer pharynx of males and females (average: 224 vs 161 μm and 252 vs 157 μm), respectively; longer tail of males and females (average: 43.5 vs 28.45 μm and 143 vs 93.6 μm), respectively and in having a longer spicules (average: 63.7 vs 54 μm).

The new species *O. citri* n. sp. is also distinguished from *O. cynodonti* n. sp. in having longer body of males (average: 1249 vs 1129 μm) and females (average: 1601 vs 1457 μm), longer pharynx of male and female (average: 224 vs 191.8; 252 vs 149.5 μm), respectively, slightly longer stoma of male (average: 17.2 vs 14.4 μm) and female (average: 18.8 vs 15 μm), lower 'b' value of male and female (average: 5.5 vs 5.8; 6.3 vs 9.7), respectively. Longer spicules and gubernaculum (average: 63.7 vs 56.3; 28 vs 23 μm), respectively. The detail species comparison of insectivora group of *Oscheius* are given in Table 9 (a, b).

It also resembles morphologically with *O. chongmingensis* Zhang et al., (2008) and *O. rugaoensis* Zhang et al., (2012); but it differs from *O. chongmingensis* in greater body width of male (average: 53 vs 46 μm), longer pharynx and tail length (average: 224 vs 157; 43.5 vs 29 μm), respectively. In female, longer body length (average: 1601 vs 1143 μm), more robust body (average: 80 vs 104 μm), longer stoma (average: 18.8 vs 9.7 μm), pharynx (average: 252 vs 180 μm) and tail length (average: 143 vs 81 μm).

O. citri n. sp. differs from *O. rugaoensis* in smaller pharynx of male (average: 224 vs 304 μm) and tail length (average: 43.5 vs 151 μm), in higher 'b' and 'c' ratios (average: 5.5 vs 4.8, 28.6 vs 9.3), respectively. From female, it differs in longer body length (average: 1601 vs 1042 μm); greater body

width (average: 80 vs 49.5 μm), longer pharynx (average: 252 vs 209 μm) and tail length (average: 143 vs 131 μm), more anteriorly located vulva (average: 49.3 vs 54.8 %) and higher 'c' value (average: 11 vs 8.5).

Molecular phylogenetic tree based on rDNA-ITS sequence shows that *O. citri* n. sp. belongs to insectivora group. This species is more closely related to the species *O. cobbi* n. sp. and *O. cynodonti* n. sp. of the same group under the traditional classification of *Oscheius*. *O. citri* n. sp. appears to be 65% bootstrap value to *O. cobbi* n. sp. and 38% to *O. cynodonti* n. sp. for maximum parsimony (Fig. 16).

***Oscheius cynodonti* n. sp.**
(Fig. 3: A-G, 4: A-I)

Measurements: Table 2

Description

Female: Medium sized nematodes. Body slender, 1252-1662 μm long, slightly curved, ventrad after fixation. Cuticle finely annulated. Lateral field with 5 ridges (6 incisures). Head region continuous, labial papillae minute. Amphids pore like on lateral lips. Stoma rhabditoid, cheilostome not sclerotized, gymnostome a sclerotized cylinder, stegostome about half of stoma length, glottoid apparatus well developed, metastegostome bearing small denticles. Pharyngeal collar present, surrounding about two third of stoma from its base.

Pharyngeal corpus cylindrical, 125.5 (110-135 μm) long or 81.4-84.9% of pharyngeal length, distinctly separate from isthmus, isthmus cylindrical 38 (35-45) μm long or 25-28% of pharyngeal length, basal bulb spherical 28-32 \times 24-27 μm in diameter with well-developed valve. Nerve ring 74-78% of pharyngeal length usually surrounding middle of isthmus.

Excretory pore conspicuous, usually located at level of basal bulb or slightly posterior to oesophagus, 96.3-100% of pharyngeal length. Hemizonid obscure. Cardia present, protruding into intestine. Reproductive system didelphic, ovaries reflexed, oviduct short, vagina thin walled surrounded by glands. Vulva equatorial with prominent protruding lips. Tail elongate with acute terminus. Phasmids 40-45% of tail length.

Male: Testis monarchic, reflexed ventrally. Vas deference well developed filled with sperms, without demarcation of seminal vesicle. Spicules paired, separate, symmetrical slightly curved ventrally with hooked tips. Head of spicules with rounded anterior end, lamina expanded in its proximal part velum prominent. Guberna-culum boat shaped in lateral view, about 37-45% of spicules length, slightly curved ventrally. Bursa closed, leptoderan type, with a short part of tail protruding beyond bursa (Fig. 13-15).

Nine pairs of bursal papillae arranged as 1+1+1/3+3. Pair 1 is well anterior to the cloaca, pairs 2 and 3 immediately anterior to cloaca whereas pair 4-6 and 7-9 posterior to cloaca and more closely spaced to each other. Pair 5th and 8th curved dorsally and not reaching rim of bursa. Tail 1.1-1.8 anal body diameter long (Fig. 13B).

Daur juvenile: Body elongate sheath present immediately after harvesting. Head without prominent dorsal tooth. Stoma and pharynx morphology similar to adult. Tail elongate conical tapering to a hyaline part. Tail length with sheath is about twice as long as the tail without sheath.

Type habitat and locality: Found from soil around the roots of bermuda grass (*Cynodon dactylon* L.) from Islamabad.

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.

Table 2. Morphometric values of *Oscheius cynodonti* n. sp. (n=25 each). All measurements are in µm

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|------------------------|------------------------|
| | | Female | Male |
| | | | |
| Total body length | 1182 | 1457 ± 122 (1252-1662) | 1129 ± 220 (863-1560) |
| Maximum body width | 48 | 81.7 ± 11.7 (65-100) | 48.6 ± 4.1 (43-58) |
| Stoma length | 17 | 15 ± 1.9 (12-18) | 14.4 ± 3.4 (14-17) |
| Lip width | 13 | 11 ± 1.4 (10-15) | 14 ± 2.6 (11-18) |
| EP | 210 | 140 ± 6.5 (130-160) | 179 ± 25.4 (153-210) |
| NR | 170 | 110 ± 4.5 (100-125) | 149 ± 12.4 (130-170) |
| ES | 202 | 149.5 ± 7.6 (135-159) | 191.8 ± 13.6 (168-208) |
| Tail length | 50 | 91 ± 17 (60-108) | 44.6 ± 3.9 (32-50) |
| Anal body width | 28 | 21.6 ± 5 (15-30) | 29 ± 3.4 (25-33) |
| V% | -- | 48.8 ± 2.8 (41-52.6) | -- |
| a | 24.6 | 17.9 ± 1.3 (16-20) | 23.4 ± 5.2 (17.4-34.6) |
| b | 5.8 | 9.7 ± 0.7 (8.5-10.7) | 5.8 ± 0.9 (4.5-6.8) |
| c | 23.6 | 16.4 ± 3.9 (13-24) | 25.4 ± 5.2 (19.3-34) |
| c' | 1.78 | 4.7 ± 0.9 (4-6.7) | 1.5 ± 0.2(1.14-1.8) |
| Spicules | 58 | -- | 56.3 ± 2.4 (54-60) |
| Gubernaculum | 26 | -- | 23.6 ± 2.4 (20-27) |
| SW | 2.0 | -- | 1.9 ± 0.2 (1.6-2.2) |
| GS % | 44.8 | -- | 43.5 ± 5. 8 (37.7-45) |

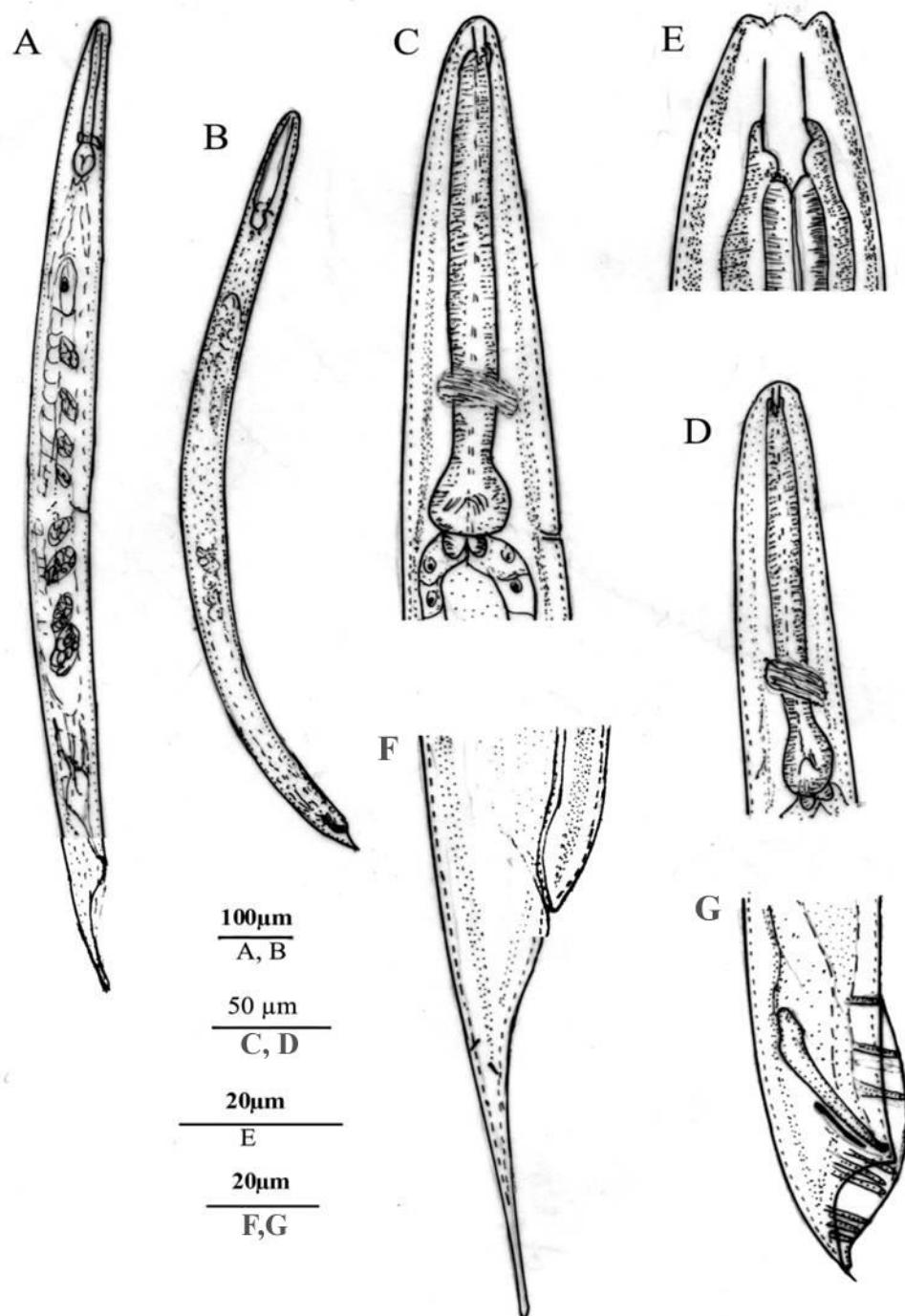


Fig. 3 (A-G). *Oscheius cynodonti* n. sp. Female (A, C, E, F): A. Entire body; C. Pharyngeal region; E. Anterior region; F. Tail region. Male (B, D, G): B: Entire body; D. Pharyngeal region; G. Lateral view of tail region showing papillae.

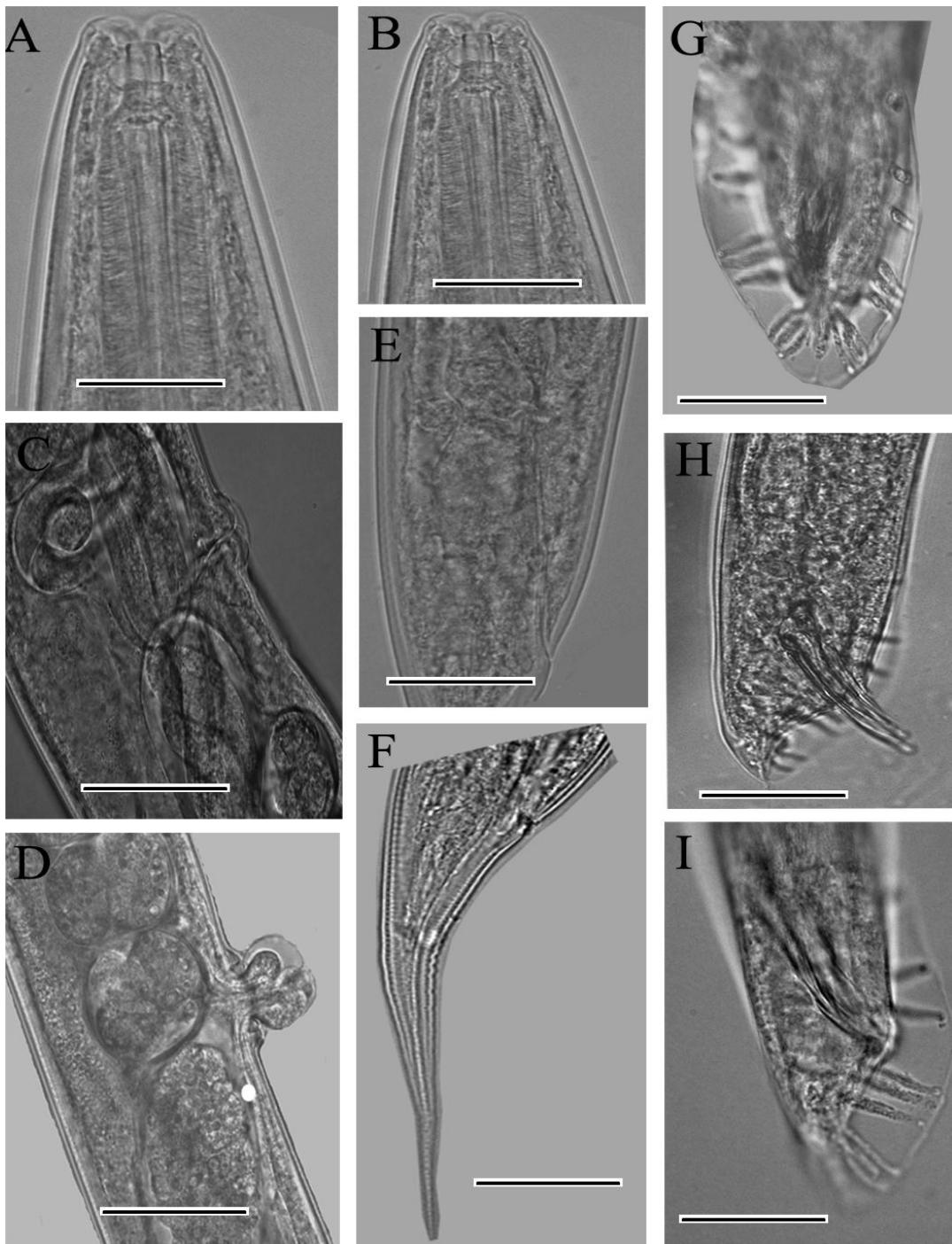


Fig. 4 (A-I). Light microphotographs of *Oscheius cynodonti* n. sp. Female (A-F): A, B. Anterior region; C-D. Vulval region covered with exudates; E, F. Tail region; Male (G-I): G. Ventral view of tail region showing papillae; H, I. Lateral view of tail region showing papillae (scale: A, B, G-I=10 µm; C-F=20µm).

Etymology: The species is named after its isolation from grass (*Cynodon dactylon* L.).

Diagnosis and relationships: *Oscheius cynodonti* n. sp. belongs to insectivora group on the basis of leptoderan bursa and crochet needle-shaped spicules. The new species is characterized by its unique ribosomal DNA-ITS sequence, amphimictic reproduction, lateral field with six incisures, male medium size (average: 1129 μm), large spicules (average: 56.3 μm), bursa open leptoderan, leaving spike free, bursal papillae arranged as 1+1+1/3+3.

New species *Oscheius cynodonti* n. sp. comes close to *O. cobbi* n. sp. and *O. citri* n. sp. in morphological characteristics. However, morphologically it can be distinguished from *O. cobbi* n. sp. in longer male and pharynx (average: 1129 vs 941 and 191.8 vs 162 μm), respectively, longer tail (average: 44.6 vs 28.4), more robust body (average 23.4 vs 19.8), lower c ratio (average: 25.4 vs 33.9). In female, longer body (average: 1457 vs 1199 μm), short pharynx (average: 149.5 vs 157 μm), shorter tail length (average: 91 vs 93.5 μm), shorter anal body width (average: 21.6 vs 25.7 μm) higher a, b, c, and c' ratio (average: 17.9, 9.7, 16.4, 4.2 vs 15.3, 7.8, 12.8, 3.7), respectively.

The new species also differs from *Oscheius citri* n. sp. in more slender body of male (maximum body width average: 48.6 vs 53 μm), smaller pharynx (average: 191.8 vs 224 μm), longer tail (average: 45 vs 43.5 μm), lower c ratio (average: 25.4 vs 28.6), higher c' ratio (average: 1.5 vs 1.3), small spicules and gubernaculum (average: 56.3 vs 63.7 and 23.6 vs 27.8 μm), respectively.

New species is most closely related to *O. chongmingensis* Zhang *et al.*, (2008) and *O. rugaoensis* Zhang *et al.*, (2012) but it differs from *O. chongmingensis* in longer pharynx, tail and spicules in male (average: 191.8 vs 157; 44.6 vs 29 and 56.3 vs 51 μm), respectively. In female, longer body, stoma and tail length (average: 1457 vs 1143; 15 vs 9.7; 91 vs 81 μm), respectively,

more slender body (average: 81.7 vs 104 μm) and short pharynx (average: 149.5 vs 180 μm). From *O. rugaoensis* the new species differs in small body, lesser body width, shorter pharynx and tail in male (average: 1129 vs 1396; 48.6 vs 62.4; 191.8 vs 304 and 44.6 vs 151 μm), respectively, and higher 'c' ratio (average: 25.4 vs 9.3). In female, longer body and greater body width (average: 1457 vs 1042; and 81.7 vs 49.5 μm), respectively; and in having higher 'b' and 'c' ratio (average: 9.7 vs 5; 16.4 vs 8.5), respectively.

The test of the phylogenetic position of *O. cynodonti* n. sp. stands it as a new species. It comes under the branching system of *Oscheius* and form a separate clade with closely linked species *O. cobbi* n. sp. and *O. citri* n. sp. on the basis of rDNA-ITS sequences. *O. cynodonti* n. sp. confirms diverging position at 38% bootstrap proportional value from *O. cobbi* n. sp. and *O. citri* n. sp. (Fig. 16).

Oscheius cobbi n. sp. (Fig. 5: A-G, 6: A-I)

Measurements: Table 3

Description

Female: Body robust, 1119 (950-1712) μm long, slightly curved ventrad after fixation. Cuticle finely annulated. Lateral field with six longitudinal incisures. Lip region continuous with six rounded lips, labial papillae 6 each bearing one papilla. Amphids opening circular. Stoma rhabditoid, with distinct cheilo, gymno and stegostome, cheilostome finely cuticularized, gymnostome short, having well cuticularized walls, metastegostome bearing small denticles. Pharyngeal collar present, surrounding about half of stoma from its base. Pharyngeal corpus 96 (74-170) μm long, distinctly separate from isthmus, isthmus cylindrical 35 (29-50) μm long, basal bulb spherical with well-developed valve. Nerve ring surrounding middle of isthmus, 85-97% of pharyngeal length.

Table 3. Morphometric values of *Oscheius cobbi* n. sp. (n=25 each). All measurements are in µm

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|------------------------|-----------------------|
| | | Female | Male |
| Total body length | 1010 | 1199± 258.6 (950-1712) | 941 ± 92.2 (789-1110) |
| Maximum body width | 55 | 80.3 ± 14.4 (59-115) | 47 ± 6.4 (36-58) |
| Stoma length | 15 | 14.7 ± 1.4 (14-22) | 14 ± 1.2 (13-16) |
| Lip width | 10 | 11 ± 0.5 (10-12) | 10 ± 0.8 (8-12) |
| EP | 158 | 148 ± 15 (130-280) | 158 ± 17 (130-170) |
| NR | 145 | 160 ± 12 (130-240) | 162 ± 12.3 (120-170) |
| ES | 155 | 157 ± 21 (134-282) | 161 ± 29.2 (130-220) |
| Tail length | 32 | 93.6 ± 32.8 (80-170) | 28.4 ± 5.7 (20-32) |
| Anal body width | 25 | 25.7 ± 5.9 (20-38) | 20 ± 5.9 (10-25) |
| V% | -- | 48.2 ± 5.8 (42-57) | -- |
| a | 18 | 15.3 ± 2.8 (12-21.6) | 19.8 ± 3.3 (17-21) |
| b | 6.4 | 7.8 ± 1.2 (6.6-8.8) | 6.3 ± 0.6 (5.8-7.4) |
| c | 22 | 12.8 ± 2.7 (9-14.8) | 33.9 ± 7 (22-38) |
| c' | 1.8 | 3.7 ± 0.5 (2.8-4.3) | 1.4 ± 0.4 (1.2-2.5) |
| Spicules | 59 | -- | 54 ± 4.4 (47-62) |
| Gubernaculum | 26 | -- | 24.5± 4.4 (20-35) |
| SW | 2.3 | -- | 2.9 ± 0.89(2.3-5) |
| GS % | 44 | -- | 45.8 ± 10.8(38-60) |

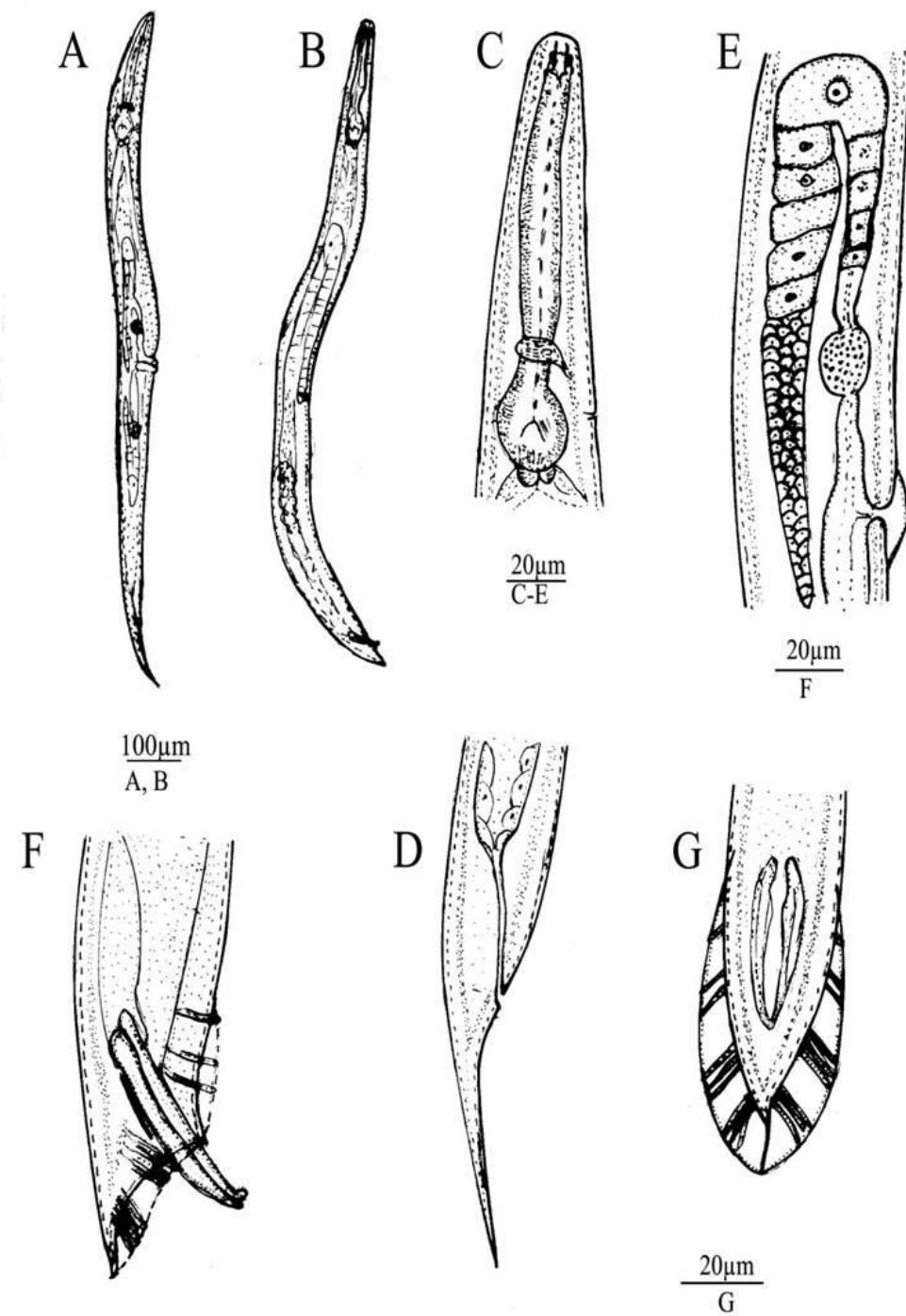


Fig. 5 (A-G). *Oscheius cobbi* n. sp. Female (A, C, D, E): A. Entire body; C. Pharyngeal region; D. Posterior region; E. Vulval region; Male (B, F, G); B. Entire body; F. Lateral view of tail showing bursa and spicules; G. Ventral view of tail showing bursa and papillae.

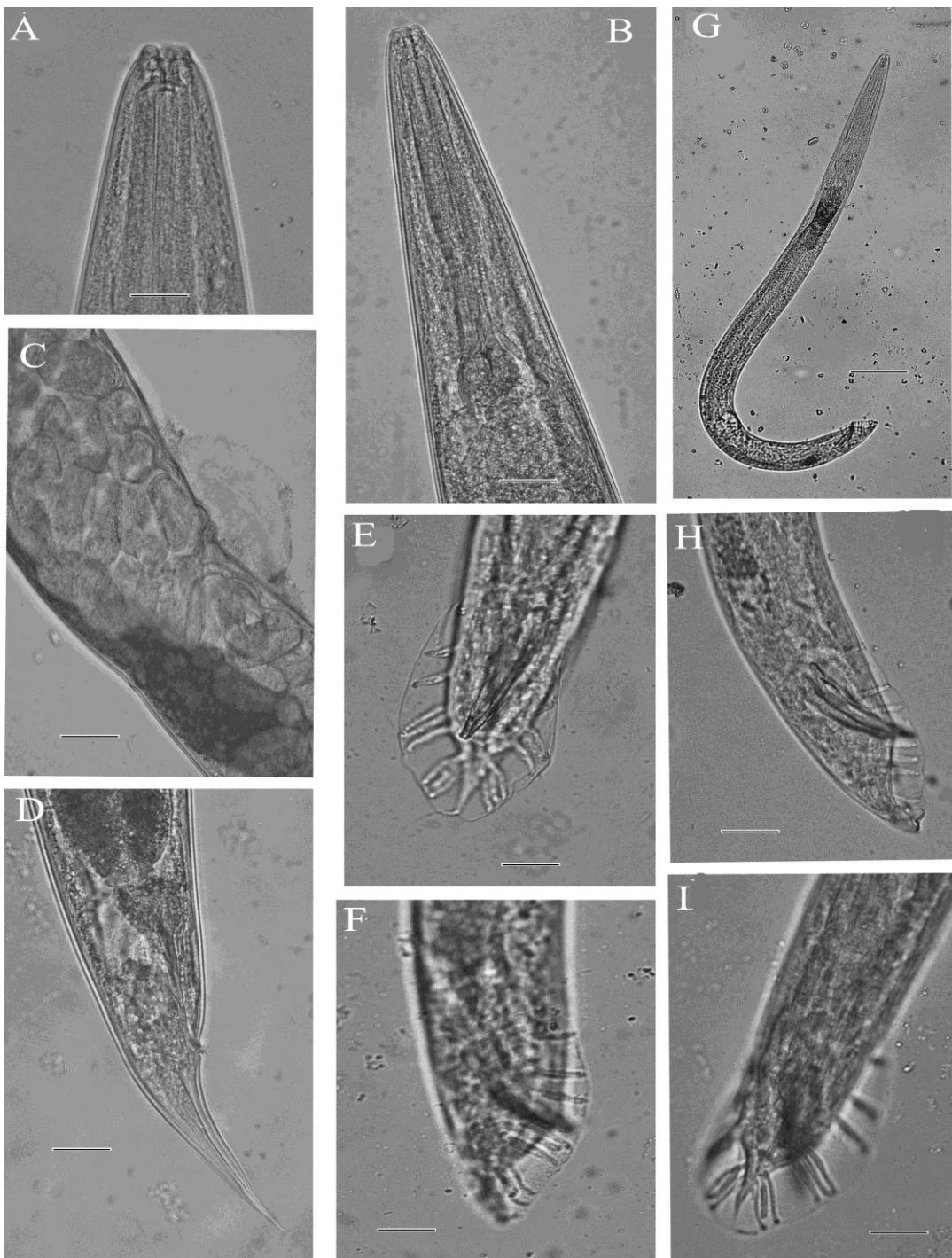


Fig. 6 (A-I). Light microphotographs of *Oscheius cobbi* n. sp. Female (A-D): A. Anterior region; B. Pharyngeal region; C. Vulval region; D. Posterior region; Male (E-I): E, F. Tail showing papillae; G. Entire body; H, I. Tail region showing bursa and arrangement of papillae. (A, E, F, I= 10 μ m; B, C, D, H=20 μ m; G= 50 μ m).

Excretory pore located at anterior to middle portion of the basal bulb 97-99.3% of pharyngeal length. Cardia present, protruding into intestine. Reproductive system didelphic, ovaries reflexed, oviduct short, vagina thin walled surrounded by glands. Vulva equatorial with prominent protruding lips. Tail conical with acute terminus. Phasmids 25-30% of tail length.

Male: Gonads monarchic, reflexed ventrally. Vas deference well developed filled with sperms, without demarcation of seminal vesicle. Ejaculatory gland not present. Spicules paired, separate, symmetrical slightly curved ventrally with hooked tips. Head of spicules with rounded anterior end, lamina expanded in its proximal part, velum prominent. Gubernaculum boat shaped in lateral view, about 35-40% of spicules length, curved ventrally. Bursa closed, leptoderan type, with a short part of tail protruding beyond bursa (Fig. 13-15). Nine pairs of bursal papillae at different length arranged as 1+1+1/3+3. First pair (p1) is well anterior to the cloaca, p2 and p3 immediately anterior to cloaca whereas p4-p6 and p7-p9 posterior to cloaca and more closely spaced to each other. pairs, p5 and p8 curved dorsally and not reaching rim of bursa. Tail 1.4 (1.2-2.5) anal body diam. long protruded outside the bursa (Fig. 13C).

Daur juvenile: Body straight when heat killed. Stoma and pharynx morphology similar to adult. Tail elongate conical tapering to a hyaline part.

Type habitat and locality: Found from soil around the roots of bermuda grass (*Cynodon dactylon* L.) of International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, 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: The species is dedicated to Dr. N. A. Cobb known as “Father of Nematology” for his excellent contributions to Nematology.

Diagnosis and relationships: *Oscheius cobbi* n. sp. belongs to insectivora group on the basis of leptoderan bursa and crochet needle-shaped spicules. This new species is characterized by its unique ribosomal DNA-ITS sequence, amphimictic reproduction, lateral field with six separate lines, male medium size (average: 941 μm), medium size spicules (average: 54 μm) and distally hooked tip, bursa open, leptoderan leaving tail spike free, bursal papillae 1+1+1/3+3.

New species *Oscheius cobbi* closely resembled to *Oscheius citri* n. sp. and *Oscheius cynodonti* n. sp. (Fig. 16). However, it can be distinguished from *Oscheius citri* by having shorter length of male and female (average: 941 vs 1249; 1199 vs 1601 μm), respectively, short pharynx (average: 161 vs 224; 157 vs 252 μm), shorter tail (average: 28.4 vs 43.5; 93.6 vs 143 μm), respectively, short spicules (average: 54 vs 63.7 μm) and gubernaculum (average: 24.5 vs 27.8 μm). In female, stoma is short (average: 14.7 vs 18.8 μm), shorter distance of excretory pore from head end (average: 148 vs 217 μm) and lower value of ‘a’ ratio (average: 15.3 vs 20.8).

The new species also differs from *Oscheius cynodonti* n. sp. in shorter length of male and female (average: 941 vs 1129; 1197 vs 1421 μm), respectively. Shorter pharynx (average: 161 vs 191.8 μm), and tail (average: 28.4 vs 44.6 μm), lower value of ‘a’ ratio (average: 19.8 vs 23.4) of male. In female, longer pharynx (average: 157 vs 149.5 μm) and lower values of ‘a’, ‘b’, ‘c’ and ‘c’ ratio (average: a= 15.3 vs 17.9; b= 7.8 vs 9.7; c= 12.8 vs 18.4; c'= 3.7 vs 4.7).

New species shows resemblance with *O. chongmingensis* Zhang et al., (2008) and *O. rugaoensis* Zhang et al., (2012) belonging to insectivora group but *O. cobbi* n. sp. differs from it in male having smaller body, longer pharynx and spicules (average: 941 vs 1115; 161 vs 157; 54 vs 51 μm), respectively. In female, shorter body width (average 80 vs 104 μm) and pharynx (average: 157 vs 180 μm), longer stoma (average: 14.7 vs 9. μm), and tail length (average: 93.6 vs 81 μm).

From *O. rugaoensis* the new species differs in male by having smaller body length and body width (average: 941 vs 1396; 47 vs 62.4 μm), respectively, shorter pharynx (161 vs 304 μm), and tail (average: 28.4 vs 151 μm) and higher ‘b’ and ‘c’ values (average: 6.3 vs 4.8; 33.9 vs 9.3), respectively. In female, longer body (average: 1199 vs 1042 μm), wider body (average: 80.3 vs 49.5 μm), shorter pharynx (average: 157 vs 209 μm), and tail (average: 93.6 vs 131 μm), more anteriorly located vulva (average: 48.2 vs 54.8 %), lower ‘a’ value (average: 15.3 vs 21) and higher ‘b’ and ‘c’ ratios (average: 7.8 vs 5; 12.8 vs 8.5), respectively.

The separated entity of *O. cobbi* n. sp. is justified on the basis of rDNA-ITS sequence. *O. cobbi* n. sp. comes close to *O. citri* n. sp. under monophyletic extend and confirming *O. cobbi* n. sp. and *O. citri* n. sp. sister species at 65% bootstrap value. It next comes close to *O. cynodonti* n. sp. at significant proportional value (38%) for maximum parsimony (Fig. 16).

***Oscheius esculentus* n. sp. (Fig.7: A-H, 8: A-H)**

Measurements: Table 4

Description

Female: Body robust, 1249-1798 μm long, slightly ventrally curved after fixation. Cuticle

finely annulated. Lateral field with six longitudinal incisures. Lip region continuous with six rounded lips, labial papillae 6 each lip bearing one papilla. Amphids opening circular. Stoma rhabditoid, with distinct cheilo, gymno and stegostome, cheilostome finely cuticularized, gymnostome short, having well cuticularized walls, metastegostome bearing small denticles.

Pharyngeal collar present, surrounding about half of stoma from its base. Pharyngeal corpus 123 (105-150 μm) long, distinctly separate from isthmus, isthmus cylindrical 40 (33-48) μm long, basal bulb spherical with well-developed valve. Nerve ring surrounding middle of isthmus, 78-112.6% of pharyngeal length. Excretory pore located at middle portion of the basal bulb and 84-112.6% of pharyngeal length. Cardia present, protruding into intestine. Reproductive system didelphic, oviduct short, vagina thin walled surrounded by glands. Vulva almost equatorial with prominent protruding lips. Tail conical, straight with pointed terminus. Phasmids tubular located at 20-25% of tail length.

Male: Similar to female in general morphology except for smaller size and body more arcuate posteriorly. Gonads monorchic, reflexed ventrally on left side of intestine. Vas deference well developed filled with sperms, without demarcation of seminal vesicle. Ejaculatory gland not present. Spicules paired, separate, symmetrical, slightly curved ventrally with hooked tips, lamina expanded in its proximal part, velum prominent (Fig. 13-15).

Gubernaculum boat shaped in lateral view, about 30-40% of spicules length, curved ventrally (Fig. 13-14). Bursa open, leptoderan type. Nine pairs of bursal papillae of different length arranged as 1+1+1/3+3. Pairs 1-3 evenly spaced papillae, pair 1 and 2 anterior to cloaca whereas pair 3 is adanal while pairs 4-6 and 7-9 posterior to cloaca and

Table 4. Morphometric values of *Oscheius esculentus* n. sp. (n=25 each). All measurements are in μm .

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|----------------------------|----------------------------|
| | | Female | Male |
| Total body length | 1230 | 1512 ± 169 (1249-1798) | 1161 ± 158 (875-1370) |
| Maximum body width | 60 | 83 ± 13.7 (70-115) | 53.6 ± 6.7 (46-64) |
| Stoma length | 17 | 15 ± 1.7 (13-18) | 15 ± 0.9 (14-17) |
| Lip width | 12 | 15.3 ± 1.9 (14-20) | 12 ± 0.5 (11-12) |
| EP | 160 | 188 ± 32 (140-240) | 160.8 ± 15 (135-185) |
| NR | 130 | 156 ± 38 (129-240) | 122.5 ± 9.8 (110-140) |
| ES | 180 | 188 ± 14 (165-213) | 172.8 ± 14.6 (155-200) |
| Tail length | 50 | 105 ± 11.3 (90-120) | 48.3 ± 6.8 (30-60) |
| Anal body width | 28 | 30 ± 2.5 (26-35) | 29.6 ± 1.2 (28-31) |
| Vulva | -- | 777 ± 94.8 (650-978) | -- |
| a | 20.5 | 18 ± 1.1 (15.6-19.5) | 21.7 ± 2.7 (19-27.7) |
| b | 6.8 | 7.6 ± 0.6 (6.8-8.9) | 6.6 ± 0.5 (5.6-7.5) |
| c | 24.6 | 14.2 ± 0.7 (13-15.3) | 24.4 ± 4.8 (18.9-34) |
| c' | 1.7 | 3.5 ± 0.4 (2.9-4.2) | 1.5 ± 0.2 (1.3-1.9) |
| V% | -- | 51.7 ± 1.9 (49.3-55) | |
| Spicules | 50 | -- | 51 ± 3.5 (45-57) |
| Gubernaculum | 23 | -- | 22.3 ± 1.2 (20-24) |
| SW | 178 | -- | 170.5 ± 126 (150-185) |
| GS % | 46 | -- | 43.6 ± 2.6 (38-46) |

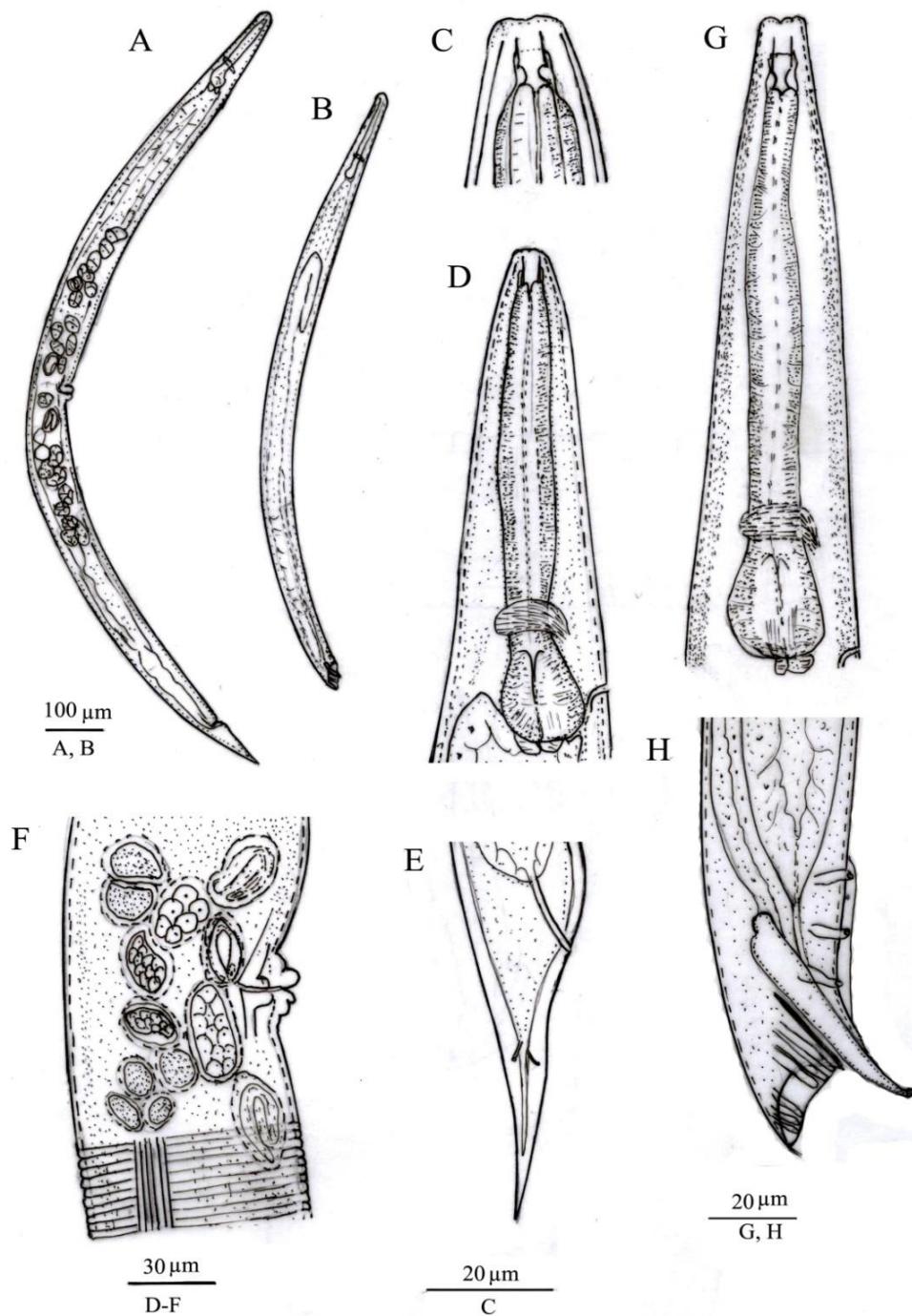


Fig. 7 (A-H). *Oscheius esculentus* n. sp. Female (A, C-F): A. Entire body; C. Anterior region; D. Pharyngeal region; E. Tail region showing tubular phasmids; F. Vulval region with lateral lines; Male (B, G, H): B. Entire body; G. Pharyngeal region; H. Tail region showing arrangement of papillae and leptoderan bursa.

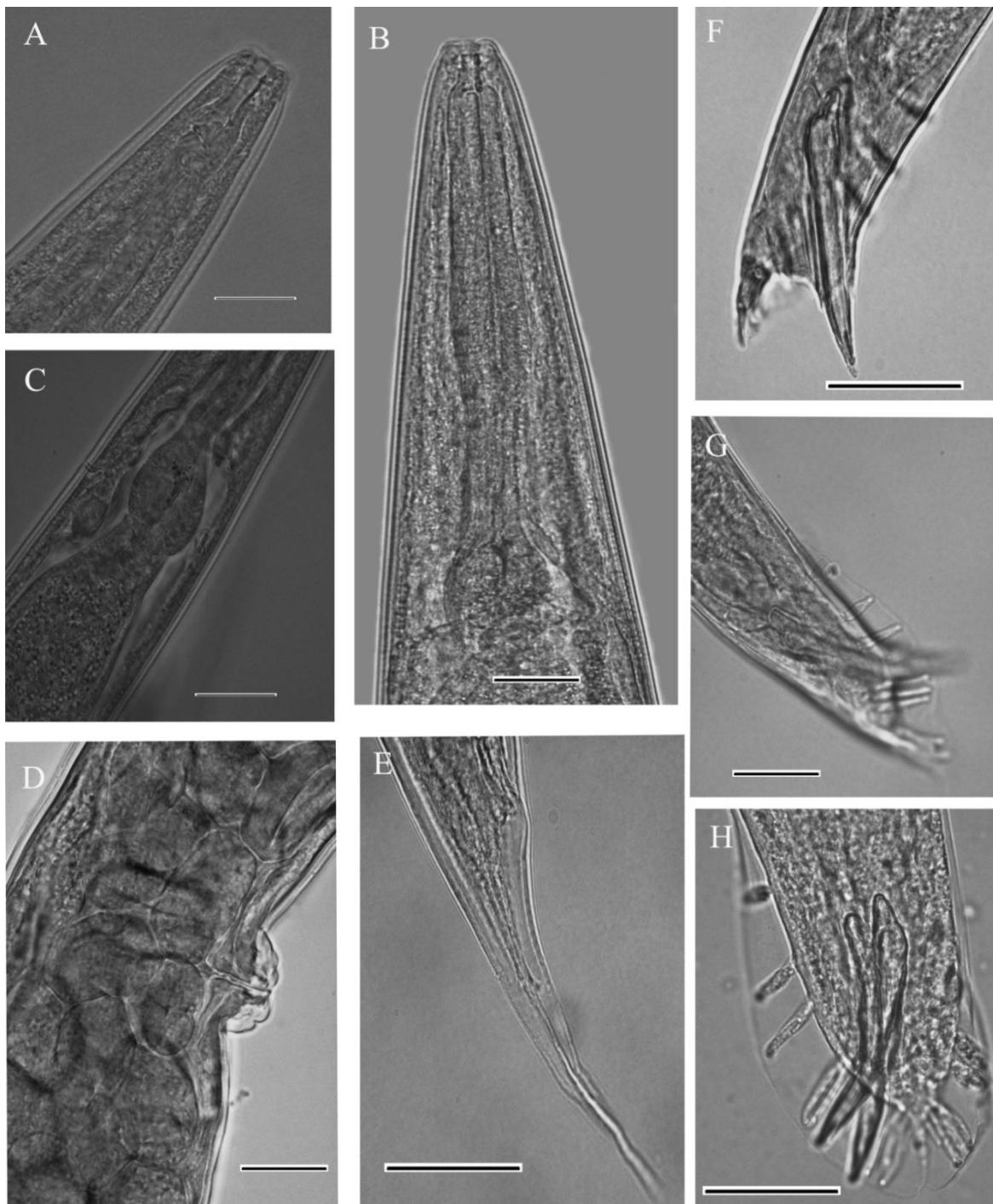


Fig. 8 (A-H). Light microphotographs of *Oscheius esculentus* n. sp. Female (A-E): A. Anterior region; B. Pharyngeal region C. Posterior pharyngeal region showing excretory pore; D. Vulval region; E. Tail region showing tubular phasmids; Male (F-H): F-H. Tail region showing arrangement of papillae and leptoderan bursa (scale: A-C, E= 20 μ m; D, F-H= 10 μ m).

more closely spaced to each other (Fig. 13D). Tail conoid curved ventrally, length more than 1.3-1.9 anal body width.

Daur juvenile: Body slender tapering regularly from base of pharynx to anterior end and from anus to terminus. Stoma and pharynx morphology similar to adult. Tail elongate conical tapering to a hyaline part.

Type habitat and locality: Found from soil around the roots of okra (*Abelmoschus esculentus* L.) from Azad Jamu & Kashmir, 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 *Oscheius esculentus* n. sp. is named after its isolation from soil around the roots of okra (*Abelmoschus esculentus* L.) field.

Diagnosis and relationships: *Oscheius esculentus* n. sp. belongs to insectivora group on the basis of leptoderan bursa and crochet needle-shaped spicules. The new species is characterized by its unique ribosomal DNA-ITS sequence, amphimictic reproduction, lateral field with six separate lines, male medium size (average: 1161 μm), medium size spicules (average: 51 μm), bursa open leptoderan leaving spike free, bursal papillae arranged as 1+1+1/3+3.

New species is most closely related to *Oscheius punctata* n. sp. and *O. sacchari* n. sp. in morphologically characteristics. However, it can be distinguished from *Oscheius punctata* n. sp. in longer body of male and female (average: 1161 vs 944.7; 1512 vs 1421 μm), respectively, slender body (average: 53.6 vs 60; 83 vs 91.3 μm) and higher value of 'a' ratio (average: 21.7 vs 15.6; 18 vs 15), respectively; longer stoma

(average: 15 vs 12.7 μm); pharynx (average: 172 vs 145 μm) and tail (average: 48.3 vs 35.4 μm).

The new species *Oscheius esculentus* n. sp. is also distinguished from *Oscheius sacchari* n. sp. in males having longer body (average: 1161 vs 862 μm); lesser body width (average: 53.6 vs 61.6 μm); shorter stoma (average: 15 vs 17.8 μm), longer pharynx (average: 172 vs 163 μm), and tail (average: 48.3 vs 35.3 μm); greater value of 'a' ratio (average: 21.7 vs 17.6) and lower value of 'c' ratio (average: 24.4 vs 32.5). In female, differs in having a shorter body, pharynx and tail length (average: 1512 vs 1607 μm ; 188 vs 196.8 μm and 105 vs 122 μm), respectively and in the lower 'c' ratio (average: 3.5 vs 4.9).

Morphologically the new species is closely related to *O. chongmingensis* Zhang et al., (2008) and *O. rugaoensis* Zhang et al., (2012) by having in the insectivora group but it can be differentiated from *O. chongmingensis* in longer pharynx and male tail (average: 172 vs 157; 48.3 vs 29 μm), respectively and longer body length (average: 1512 vs 1143 μm); more body width (average: 83 vs 104 μm), longer stoma (average: 15 vs 9.7 μm) and female tail (average: 105 vs 81 μm).

From *O. rugaoensis* the new species differentiated in having smaller body length, body width, pharynx and tail length in male (average: 1161 vs 1396 μm ; 53 vs 62.4 μm ; 172 vs 304 μm and 48.3 vs 151 μm), respectively; and in having a higher 'c' value (average: 24.4 vs 9.3). In female by the longer and robust body (average: 1512 vs 1042 μm ; 83 vs 49.5 μm), respectively, short pharynx and tail length (average: 185 vs 209 μm ; 105 vs 131 μm), respectively and higher 'c' ratio (average: 14.2 vs 8.5).

The independent origin of *O. esculentus* n. sp. is significantly supported from maximum parsimony test of our data. *O. esculentus* n. sp.

seems to be genetically closed to *O. punctata* n. sp. under phylogenetic tree assessment. It nodes out from *O. sacchari* n. sp. at 47% that represents bootstrap proportion for MP (Fig. 16).

***Oscheius punctata* n. sp.
(Fig. 9: A-H, 10: A-G)**

Measurements: Table 5

Description

Female: Body slender, quite straight including tail after fixation. Cuticle finnely annulated. Lateral field with six longitudinal incisures. Lip region continuous with six rounded lips, labial papillae minute. Amphids pore like on lateral lips. Stoma rhabditoid, with cheilo, gymno and stegostome, cheilostome finely cuticularized, gymnostome short, having well cuticularized walls, metastegostome bearing small denticles. Punctations present along body wall. Pharyngeal collar present, surrounding about half of stoma from its base. Pharyngeal corpus cylindrical, 107 (92-125) μm long, distinctly separated from isthmus 58-65% of pharyngeal length, isthmus cylindrical 36.8 (28-45) μm long, basal bulb spherical about 34-40 \times 34-37 μm in size, with well-developed valve.

Nerve ring surrounding the posterior region of isthmus, 74.5-78% of pharyngeal length. Excretory pore 84-97.6% of pharyngeal length located at anterior to middle portion of the basal bulb. Cardia present, protruding into intestine. Reproductive system didelphic, ovaries reflexed, oviduct short, vagina thin walled surrounded by glands. Vulva equatorial with prominent protruding lips. Tail conical with acute terminus. Papillae for phasmids present at middle of tail.

Male: Similar to female in general morphology except for smaller size 944.7(800-1118 μm). Body straight after fixation. Testis monorchic,

reflexed ventrally. Vas deference well developed filled with sperms, without demarcation of seminal vesicle. Ejaculatory gland not present. Spicules paired, separate, symmetrical slightly curved ventrally with hooked tips. Head of spicules with rounded anterior end, lamina expanded in its proximal part, velum prominent. Gubernaculum boat shaped in lateral view, about 35-40% of spicules length, curved ventrally (Fig. 13-15). Bursa anteriorly open, leptoderan. Nine pairs of bursal papillae of different length arranged as 1+1+1/3+3. Pair 1 is well anterior to the cloaca, pair 2 and 3 immediately anterior to cloaca whereas pairs 4-6 and 7-9 posterior to cloaca and more closely spaced to each other. Pairs 5th and 8th curved dorsally and not reaching rim of bursa (Fig. 13 E). Tail 1-1.7 anal body diam. long.

Daur juvenile: Body straight when heat killed. Similar to adult in general morphology. Tail elongate tapering to a pointed terminus.

Type habitat and locality: Found from soil around the roots of bermuda grass (*Cynodon dactylon* L.) of International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi.

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: The specific name *punctata* refers to the punctations present on whole body along with body wall.

Diagnosis and relationships: *Oscheius punctata* n. sp. belongs to insectivora group on the basis of leptoderan bursa and crochet needle-shaped spicules. The new species differs from all species of the genus in having punctuation along the body wall. The new species is characterized by amphimictic reproduction,

Table 5. Morphometric values of *Oscheius punctata* n. sp. (n=25 each). All measurements are in μm .

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|----------------------------|----------------------------|
| | | Female | Male |
| Total body length | 977 | 1412 \pm 126 (1217-1680) | 944.7 \pm 113 (800-1118) |
| Stoma length | 13 | 14 \pm 1.3 (12-15) | 12.7 \pm 2.1 (10-17) |
| Lip width | 11 | 13 \pm 1.4 (12-15) | 11.6 \pm 0.7 (11-12) |
| Maximum body width | 62 | 91.3 \pm 11.7 (70-112) | 60 \pm 7.6 (53-72) |
| EP | 125 | 159.6 \pm 13.7 (132-185) | 135 \pm 14.7 (105-160) |
| NR | 110 | 134.6 \pm 9.8 (117-150) | 116 \pm 15.9 (95-140) |
| ES | 135 | 174 \pm 9.3 (157-190) | 145 \pm 14.8 (125-169) |
| Tail length | 33 | 110 \pm 21.6 (80-170) | 35.4 \pm 4.4 (26-45) |
| Anal body width | 28 | 28 \pm 6.5 (14-35) | 27.2 \pm 3.6 (20-30) |
| V% | -- | 51.3 \pm 3.2 (46-61) | -- |
| a | 15.7 | 15 \pm 1.3 (12.5-18.4) | 15.6 \pm 1.6 (12.2-18.6) |
| b | 7.2 | 8 \pm 0.6 (7-9.6) | 6.5 \pm 0.8 (5.2-7.7) |
| c | 29.6 | 10 \pm 2.3 (8.2-16.8) | 27 \pm 5.2 (19.5-36.3) |
| c' | 1.17 | 3.9 \pm 0.5 (3.3-5.7) | 1.3 \pm 0.23 (1.03-1.7) |
| Spicules | 60 | -- | 54.4 \pm 3.7 (50-60) |
| Gubernaculum | 22 | -- | 21 \pm 1.6 (20-25) |
| SW | 2.1 | -- | 2.09 \pm 0.37 (1.7-2.9) |
| GS % | 36.6 | -- | 39.2 \pm 3.1 (36-47) |

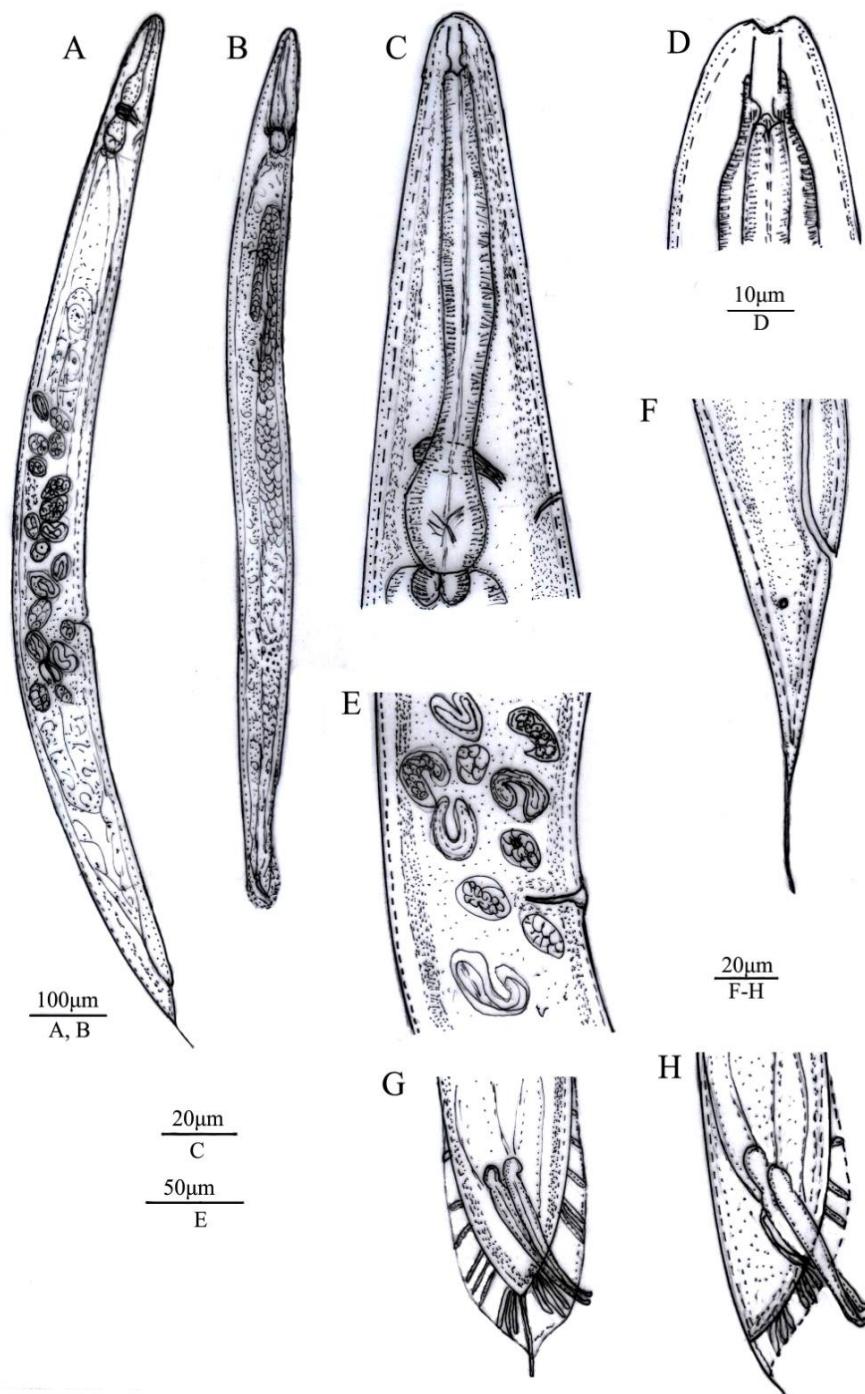


Fig. 9 (A-H). *Oscheius punctata* n. sp. Female (A, C-F): A. Entire body; C. pharyngeal region; D. Anterior region; E. Vulval region; F. Tail region showing phasmid; Male (B, G, H): B. Entire body; G, H. Tail region showing arrangement of papillae and leptoderan bursa.

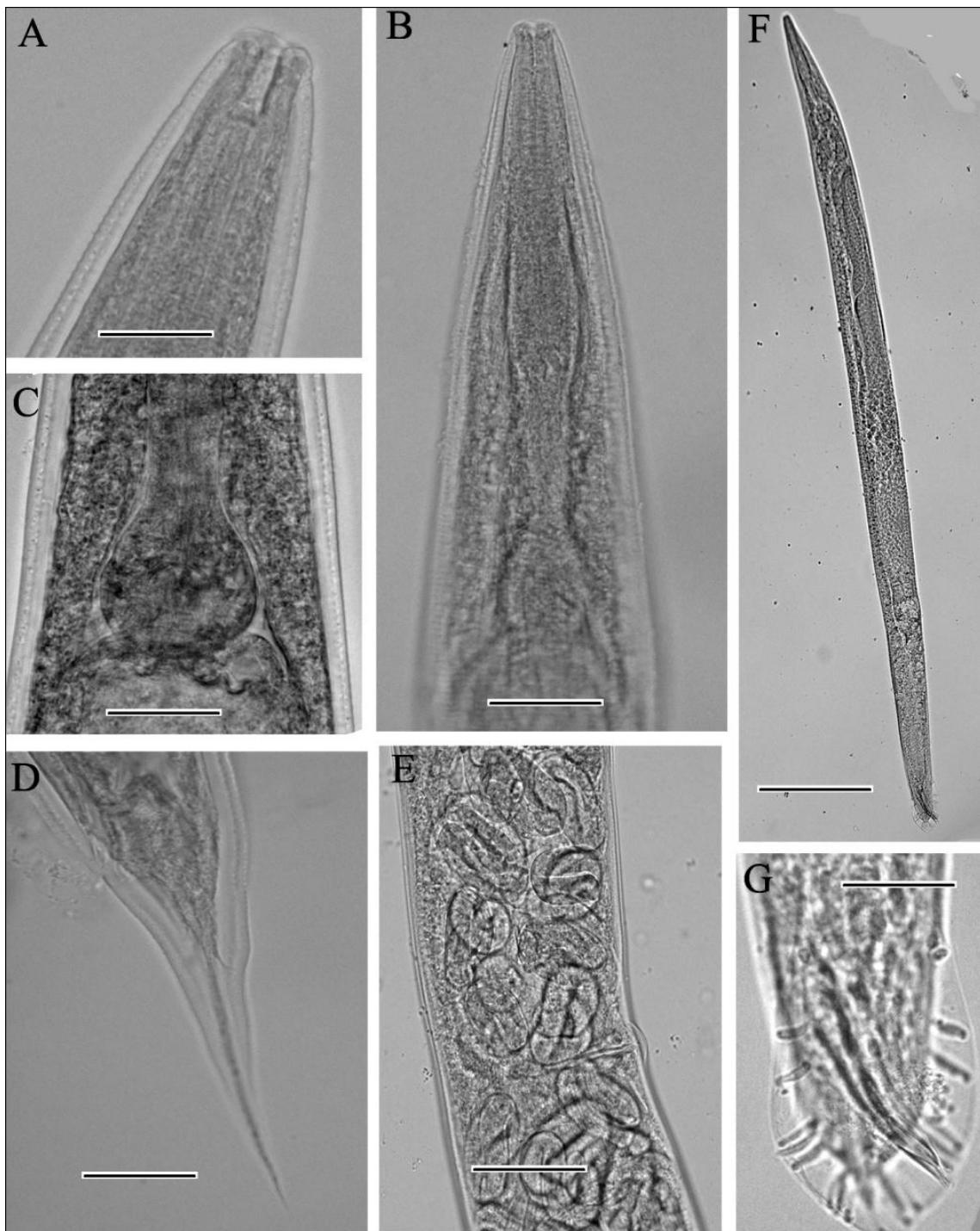


Fig. 10. (A-G). Light microphotographs of *Oscheius punctata* n. sp. Female (A-E): A. Anterior region; B. Pharyngeal region; C. Basal bulb; D. Tail region; E. Vulval region; Male (F-G): F. Entire body; G. Ventral view of tail region showing papillae. (scale: A, C, G= 10 μm ; B, D, E = 20 μm ; F= 100 μm).

lateral field with six separate lines, male medium size (average: 944.7 μm), medium size spicules (average: 54.4 μm), bursa open, leptoderan, leaving spike free, bursal papillae arranged as 1+1+1/3+3.

New species is most closely related to *Oscheius esculentus* n. sp. and *O. sacchari* n. sp. morphologically. However, it can be distinguished from *Oscheius esculentus* n. sp. in having shorter body, pharynx and tail length in male (average: 944.7 vs 1161; 145 vs 172; 35.4 vs 48.3 μm), respectively, greater body width (average: 60 vs 53.6 μm) and higher ‘c’ ratio (average: 27 vs 24.4). In female, shorter body and pharynx length (average: 1421 vs 1512; 174.9 vs 188 μm), respectively; greater body width (average: 91.3 vs 83 μm); longer distance from head end to excretory pore (average: 159.6 vs 148 μm) and in having slightly longer tail length (average: 110 vs 105 μm).

The new species *Oscheius punctata* also differentiated from *Oscheius sacchari* n. sp. in longer body length of males (average: 944.7 vs 862 μm); smaller stoma (average: 12.7 vs 17.8 μm); shorter pharynx (average: 145 vs 163 μm), longer spicules (average: 54.4 vs 50 μm), lower values of ‘a’ and ‘c’ ratio (average: 15.6 vs 17.6 and 27 vs 32.5), respectively. In female, smaller body length, pharynx and stoma length (average: 1421 vs 1607; 174.9 vs 196.8; 14 vs 17.5 μm), respectively, greater body width (average: 91.3 vs 88 μm), shorter distance of excretory pore from head end (average: 159.6 vs 186 μm) and lower value of ‘a’ ratio (average: 15 vs 18.4).

New species is also resembles with *O. chongmingensis* Zhang *et al.*, (2008) and *O. rugaoensis* Zhang *et al.*, (2012) by having in the insectivora group but it can be differentiated from *O. chongmingensis* in longer body, stoma and tail length in male (average: 1421 vs 1143 μm ; 14 vs 9.7 μm ; 110

vs 81 μm), respectively. In females, smaller body length (average: 944.7 vs 1115 μm); wide body (average: 60 vs 46 μm); shorter pharynx (average: 145 vs 157 μm) and longer tail (average: 35.4 vs 29 μm).

The new species also differs from *O. rugaoensis* in males by having shorter body, pharynx and tail length (average: 944.7 vs 1396 μm ; 145 vs 304 μm ; 35.4 vs 151 μm), respectively. In female, longer body and shorter pharynx (average 1421 vs 1042 μm ; 174.9 vs 209 μm), respectively; body more wider (average: 91.3 vs 49.5 μm), and shorter tail length (average: 110 vs 131 μm). *Oscheius punctata* n. sp. cluster under the group insectivora in arrangement with its peculiarities of morphology and molecular characterization as well. *O. punctata* n. sp. comes close to *O. esculentus* n. sp. at 62% proportional value for maximum parsimony. On the basis of rDNA-ITS sequence analysis its separated position is well resolved (Fig. 16).

Oscheius sacchari n. sp. (Fig. 11: A-I, 12: A-I)

Measurements: Table 6

Description

Female: Body ventrally arcuate when relaxed by gentle heat. Cuticle smooth, finely annulated. Lateral field with five longitudinal ridges (6 lines) occupying about 3-4 μm of the corresponding body diameter at mid body. Six un-fused lips each bearing one terminal papillae continuous with body contour. Amphidial aperture pore like on lateral lips. Stoma tubular 2-3 times longer than diameter. Cheilostome with indistinct cheilarhabdians. Stegostome (pharyngeal collar) comprising 50-60% of stoma. Promeso-stegostome with parallel wall, meta-stegostome swelling each with three minute warts. Glottoid apparatus isomorphic. Corpus cylindrical, occupying 60-65% of pharyngeal length.

Table 6. Morphometric values of *Oscheius sacchari* n. sp. (n=25 each). All measurements are in μm .

| Characters | Holotype Male | Paratypes | |
|--------------------|------------------|----------------------------|----------------------------|
| | | Female | Male |
| Total body length | 1250 | 1607 \pm 227 (1362-2015) | 882.9 \pm 79 (760-1390) |
| Maximum body width | 70 | 88 \pm 20.7 (72-125) | 61.6 \pm 9.5 (53-75) |
| Stoma length | 20 | 17.5 \pm 0.83 (16-18) | 17.8 \pm 1.7 (15-21) |
| Lip width | 12 | 12.8 \pm 0.75 (12-14) | 12.6 \pm 0.8 (12-16) |
| EP | 160 | 186 \pm 11 (170-200) | 161 \pm 19 (142-200) |
| NR | 128 | 150 \pm 10 (128-175) | 122 \pm 7.2 (110-135) |
| ES | 170 | 196.8 \pm 24.9 (165-232) | 163 \pm 8.4 (154-178) |
| Tail length | 35 | 122 \pm 13.4 (100-135) | 35.3 \pm 2.9 (26-40) |
| Anal body width | 25 | 26.6 \pm 4.8 (21-33) | 27.2 \pm 25 (24-30) |
| V% | -- | 48.7 \pm 2.6 (45-52) | -- |
| a | 17.8 | 18.4 \pm 1.8 (16-19.8) | 17.6 \pm 1.0 (16.3-24) |
| b | 7.3 | 8.2 \pm 1.38 (6.7-10.3) | 7 \pm 0.7 (6.2-8) |
| c | 35.7 | 13.4 \pm 2.9 (7.6-16.8) | 32.5 \pm 4.1 (28-36.6) |
| c' | 1.4 | 4.9 \pm 1.0 (3.5-5.9) | 1.35 \pm 0.19 (1-1.6) |
| D % | 94 | 99 \pm 15.6 (77-119) | 98.4 \pm 14.4 (87-117) |
| E% | 457 | 132.6 \pm 19 (87-171) | 464 \pm 58.4 (405-512) |
| Spicules | 50 | -- | 50 \pm 4.4 (47-55) |
| Gubernaculum | 22 | -- | 20.3 \pm 1.1 (18-22) |
| SW | 2 | -- | 1.88 \pm 0.4 (1.6-2) |
| GS % | 0.44 | -- | 0.39 \pm 0.1 (0.35-0.44) |

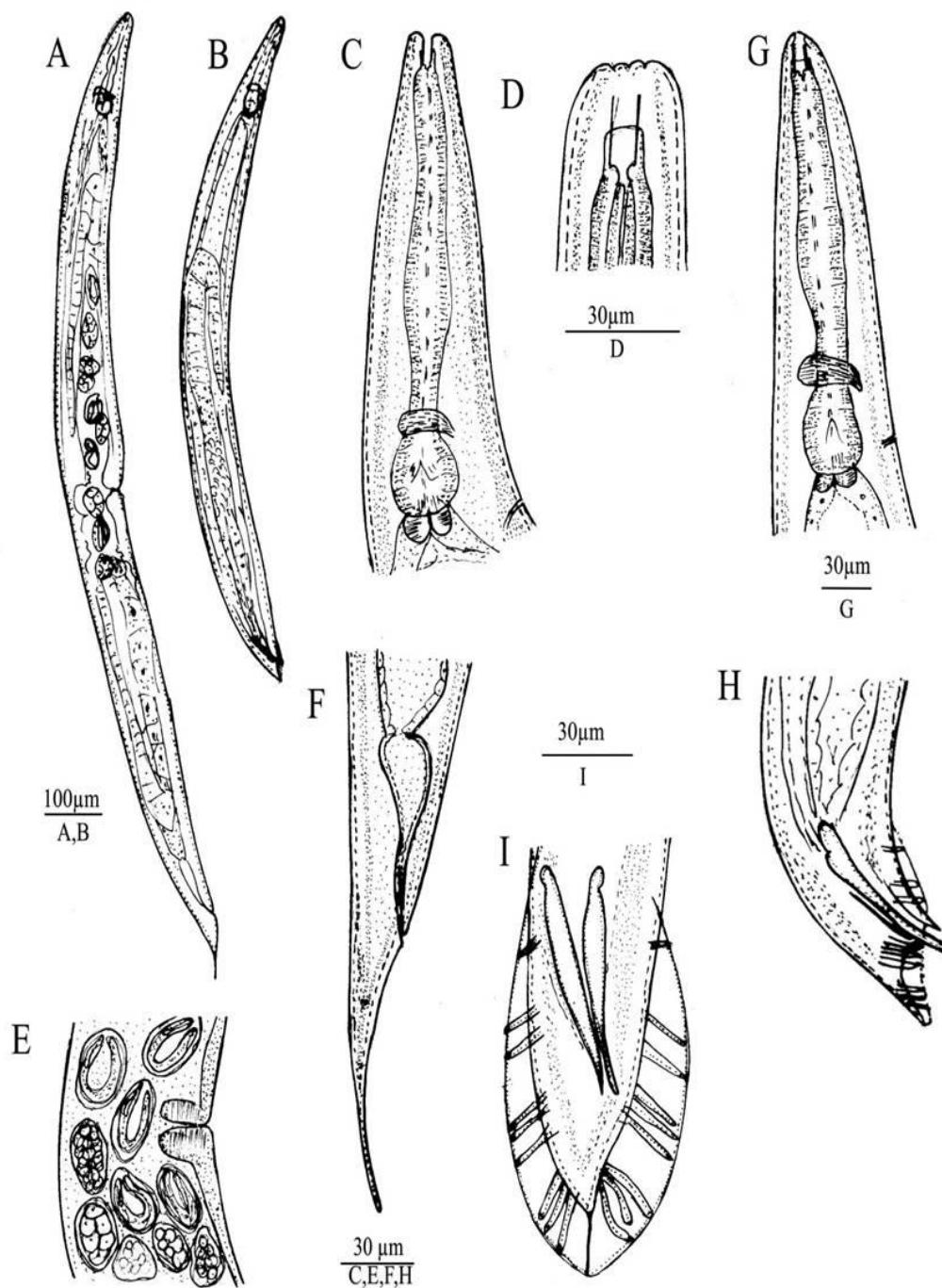


Fig. 11 (A-I). *Oscheius sacchari* n. sp. Female (A, C-F): A. Entire body; C. Pharyngeal region; D. Anterior region; E. Vulval region; F. Posterior region; Male (B, G-I); B. Entire body; G. Pharyngeal region; H. Lateral view of tail showing bursa and spicules; I. Ventral view of tail showing bursa and spicules.

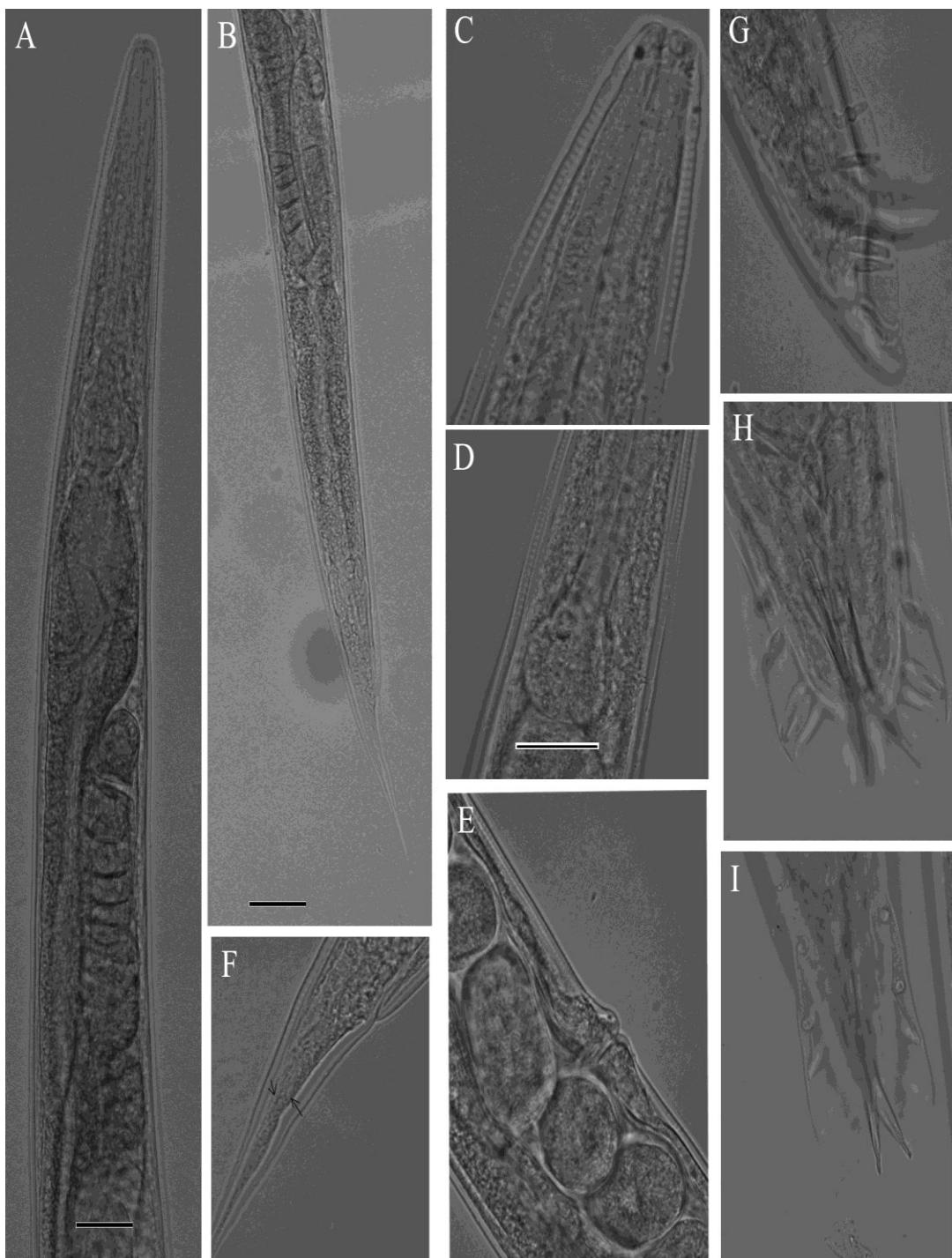


Fig. 12 (A-I). Light microphotographs of *Oscheius sacchari* n. sp. Female (A-F): A. Anterior region; B. Posterior region; C. Head; D. Posterior pharyngeal region; E. Vulval region; F. Tail region showing phasmid; Male (G-I): G. Lateral view of tail region showing papillae; H, I. Ventral view of tail region showing bursa, spicules and papillae (scale: A, B= 20 μm ; C-I= 10 μm).

Metacorpus strongly developed, isthmus short distinct, basal bulb spherical, $32-36 \times 30-34$ μm in diameter with well-developed valve. Nerve ring surrounding middle of isthmus, 75.4-77.5% of pharyngeal length. Excretory pore located at basal portion of basal bulb 82-103% of pharyngeal length. Cardia present, protruding into intestine. Gonad didelphic, amphidelphic ovaries reflexed anteriorly, vulva in a form of transverse slit. Tail conical, gradually tapering to a fine point. Phasmid pore like located behind anus at 50% of tail length.

Male: The testes monorchic, reflexed ventrally. Vas deference well developed filled with sperms, without demarcation of seminal vesicle. Ejaculatory gland not present. Spicule paired, separate, symmetrical slightly curved ventrally with hooked tips. Head of spicules with rounded anterior end, lamina expanded in its proximal part, velum prominent. Gubernaculum boat shaped in lateral view, about 35-40% of spicules length, curved ventrally (Fig. 13-15).

Bursa well developed open, leptoderan type, bursal cuticle transversely striated. Nine pairs of bursal papillae of different lengths arranged as 1+1+1/3+3. Pairs 1-2 evenly spaced papillae whereas pairs 4-6 and 7-9 more closely spaced. Pairs 5th and 8th curved dorsally and not reaching rim of bursa. Tail 1.6 anal body diameter long, protruded slightly outside the bursa (Fig. 13F).

Daur juvenile: Body straight when heat killed. Stoma and pharynx morphology similar to adult. Tail elongate conical tapering to a hyaline part.

Type habitat and locality: Found from unidentified dead moth from sugarcane (*Saccharum officinarum* L.) field at Nawabshah, Sindh.

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 its isolation from unidentified dead moth from sugarcane field from sugarcane (*Saccharum officinarum* L.) field.

Diagnosis and relationships: *Oscheius sacchari* n. sp. belongs to insectivora group on the basis of leptoderan bursa and crochet needle-shaped spicules. This new species is characterized by its unique ribosomal DNA-ITS sequence, amphimictic reproduction, lateral field with six separate lines, male medium size (average: 882 μm), medium size spicules (average: 50 μm), bursa open, leptoderan, leaving spike free, bursal papillae arranged as 1+1+1/3+3.

New species is closely related morphologically to *Oscheius esculentus* n. sp. and *O. punctata* n. sp. However, it can be distinguished from *Oscheius esculentus* n. sp. in greater body width in male (average: 61.6 vs 53.6 μm), lower 'a' and higher 'c' ratio (average: 17.6 vs 21.7; 32.5 vs 24.4), respectively and shorter tail (average: 35.3 vs 48.3 μm). In female longer pharynx and tail (average: 196.8 vs 188; 122 vs 105 μm), respectively, higher 'b' and 'c' ratio (average: 8.2 vs 7.6; 4.9 vs 3.5), respectively and lower c' value ratio (average: 13.4 vs 14.2).

The new species *Oscheius sacchari* also distinguished from *Oscheius punctata* n. sp. in shorter body length (average: 882 vs 944 μm), longer pharynx (average: 163 vs 145 μm) and higher 'a' and 'c' ratios (average: 17.6 vs 15.6 and 32.5 vs 27), respectively. In female, longer body length (average: 1607 vs 1421 μm), longer stoma (average: 17.5 vs 14 μm), longer pharynx (average: 196.8 vs 174.9 μm), longer tail (average: 122 vs 110 μm), longer distance of excretory pore from head end (average: 186 vs 159.6 μm) and higher 'a' and 'c' ratio (average: 18.4 vs 15 and 4.9 vs 3.9), respectively.

New species also resembled with *O. chongmingensis* Zhang et al., (2008) and *O. rugaoensis* Zhang et al., (2012) by having in the insectivora group but it can be differentiated from *O. chongmingensis* in males by having smaller body (average: 882.9 vs 1115 μm), wider body (average: 61.6 vs 46 μm), longer pharynx and tail length (average: 163 vs 157; 35.3 vs 29 μm), respectively. In females, it differs by the longer body (average: 1607 vs 1143 μm); shorter body width and longer stoma (average: 88 vs 104; 17.5 vs 9.7 μm), respectively, longer pharynx and tail length (average: 196.8 vs 180; 122 vs 81 μm), respectively.

From *O. rugaoensis* the n. sp. differs in males by shorter body, pharynx and tail length (average: 882.9 vs 1396; 163 vs 304; 35.3 vs 151 μm), respectively and higher value of ‘c’ ratio (average: 32.5 vs 9.3). In female the new species differs in longer and wider body (average: 1607 vs 1042; 88 vs 49.5 μm), respectively, shorter pharynx and tail length (average: 196.8 vs 209; 122 vs 131 μm), respectively, more anterior located vulva (average 48.7 vs 54.8 %); lower ‘a’ and higher ‘b’ and ‘c’ ratios (average: 18.4 vs 21; 8.2 vs 5; 13.4 vs 8.5), respectively.

The phylogenetic tree based on rDNA-ITS sequence confirms the diverging position of *O. sacchari* n. sp. It erects out from the clade comprises of 2 species (*O. punctata* n. sp. and *O. esculentus* n. sp.) and forms monophyletic origin. From the results of molecular phylogeny and data interpretation under maximum parsimony maximum proportional value 47% has been gained for *O. sacchari* (Fig. 16).

Comparative morphology of tail region and spicules of *Oscheius* new species

Dichotomous keys are used for species identification that is based on their

morphological and morphometric characters but practically pictorial keys have more advantage (Scholze & Sudhaus, 2011). To facilitate the identification of *Oscheius* new species, drawing of male posterior region, showing genital papillae, bursa, spicules and gubernaculum (Fig. 13), drawing and SEM of spicules (Fig. 14 & 15, respectively) are also incorporated.

Tail region of all the six new species illustrates nine pairs of bursal papillae having 1+1+1/3+3 bursal formula. Their arrangement is as follows: first pair is more or less at the level of spicule’s head in all species except *O. citri* n. sp. The distance between p1 and p2 is slightly more than p2 and p3; p4-p6 and p7-p9 are at equidistance; p5 and p8 curved dorsally and not reaching rim of bursa. However, some differences have been observed in tail shape and free tail tip.

Tail is conical in all species but differs in its length and ventral curvature. Some species have sharp ventral curvature after cloacal opening (Fig. 13 A, B and C) while in others the curvature is not as sharp (Fig. 13 D, E and F). Length of free tail tip also varies from species to species. *O. cobbi* n. sp. has long free tail tip (Fig. 13C) followed by *O. punctata* n. sp., *O. citri* n. sp. and *O. cynodonti* n. sp. (Fig. 13E, A and B). While, *O. sacchari* n. sp. bears the smallest length of free tail tip (Fig. 13 F).

Fig. 14 and 15 depicted the shapes of spicules of all six new species with obvious differences in their proximal ends (head and shoulder). Spicules slender, paired, separated, weakly arcuate with typical slanted shoulder behind the head; having a rounded and broadly rounded manubrium, usually elongated, shape variable. Lamina with one internal rib. Distinct ventral velum present. The spicule tip distally hooked with a crochet-needle. Gubernaculum slender, boat-shaped in lateral view.

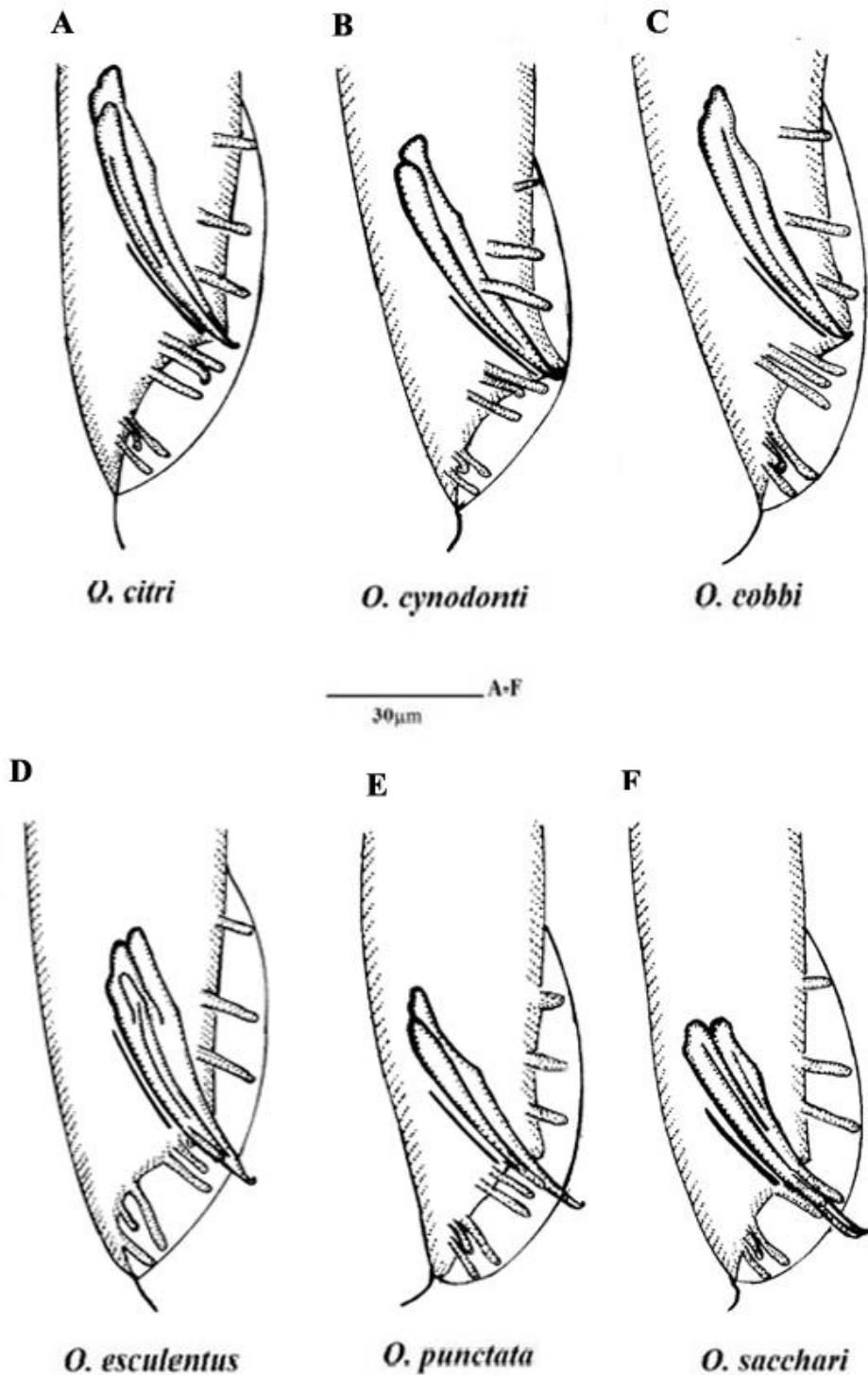


Fig. 13 (A-F). Male posterior regions of *Oscheius* new species.

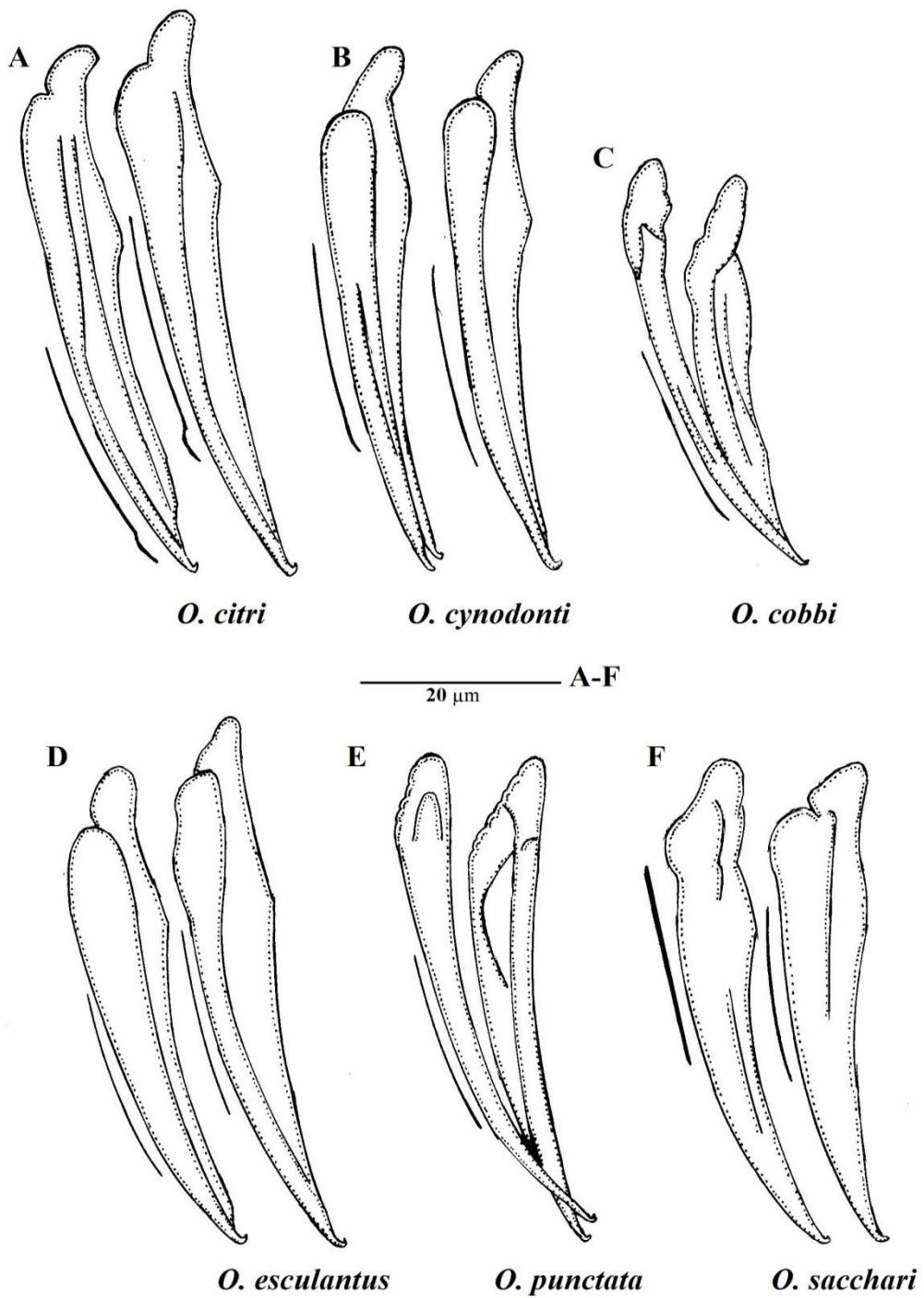


Fig. 14 (A-F). Spicules and gubernaculum of *Oscheius* n. sp.

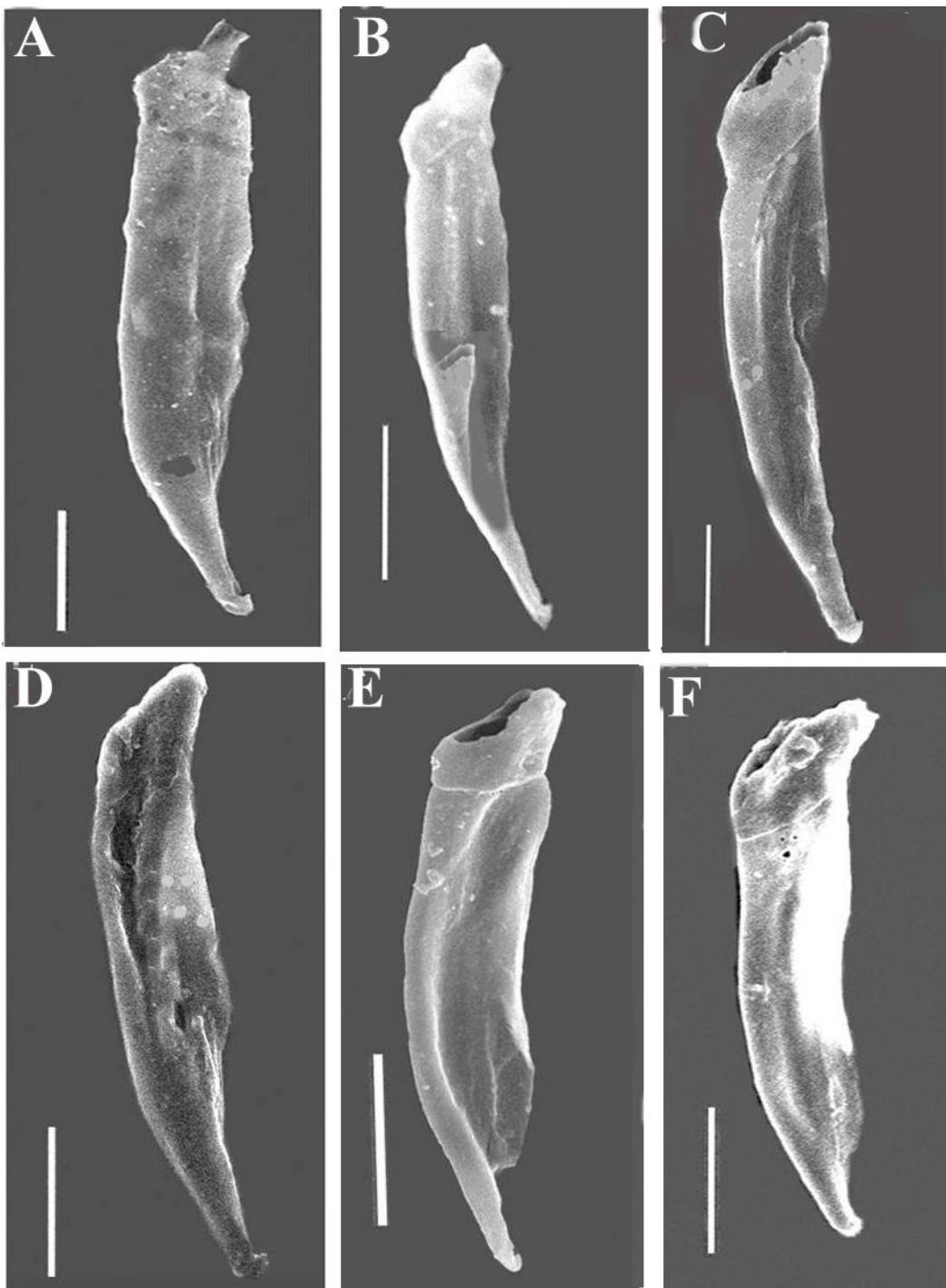


Fig. 15 (A-F). Scanning Electron Microscopy (SEM) of spicules of *Oscheius* n. sp. (scale: A-F 10 μm). A. *O. citri*; B. *O. cynodonti*; C. *O. cobbi*; D. *O. esculentus*; E. *O. punctata*; F. *O. sacchari*.

Molecular characterization and phylogenetic relationships among new and known species of *Oscheius*

The sequence of the *Oscheius citri* n. sp. (KT250509), *O. cynodonti* n. sp. (KU997024), *O. cobbi* n. sp. (KT878513), *O. esculentus* n. sp. (KT878514), *O. punctata* n. sp. (KU284845) and *O. sacchari* n. sp. (KT250510) are characterized genetically by the sequence of ITS-rDNA sequence, having 872 bp, 881 bp, 885 bp, 844 bp, 727 bp and 853 bp, respectively.

The comparison of sequence length and base pair composition of ITS regions of new species and other members of *Oscheius* are given in Table 7. Using *Rhabditella* and *Rhabditis* as an outgroups taxon, this tree inferred many highly supported monophyletic groups and compared with the ITS sequence of all other species of the genus *Oscheius*, present in GenBank sequence database (<http://www.ncbi.nlm.nih.gov/>) shown in Fig.16.

Maximum parsimony analysis showed that the alignment resulted in 3340 characters of which 1667 characters are constant, 605 variable characters are parsimony-uninformative and 1068 characters are parsimony informative.

The phylogenetic relationship of 16 species of *Oscheius* based on maximum parsimony (Fig.16), tree length = 4207, CI = 0.7894, HI= 0.2106, CI excluding uninformative characters = 0.7272, HI = excluding uninformative character=0.2728, RI=0.7890 RC = 0.6228) is presented. In MP tree all new species form a clade within the insectivora group.

Clade I consists of nine species. The members of CI distributed in the three highly supported subclades (SC). SC I comprises of two species *Oscheius chongmingensis* and *O. rugaoensis*

with a strongly supported clade (100% by MP bootstrap) confirming them sister species. SC II consists of *O. cynodonti* n. sp., *O. cobbi* n. sp., and *O. citri* n. sp., among them *O. cynodonti* diverse from the rest two species with maximum proportional value. SC III also comprises of three species viz., *O. punctata* n. sp., *O. esculentus* n. sp. and *O. sacchari* n. sp. The unique rDNA-ITS sequence of *O. sacchari* n. sp. justify it to be the new species that erected out from the cluster with more bootstrap proportion value as compare to *O. punctata* n. sp. and *O. esculentus* n. sp. The remaining two species of the same clade cluster under a node with closed but separate entities.

Clade II and III consists of known species. Bootstrap support overall for this clade is 100 in MP tree. Pairwise distances between closely related species show that *O. chongmingensis*, *O. rugaoensis* and *O. carolinensis* are the most divergent species from all new species with 374, 375 and 377 bp, respectively, while *O. dolichura*, *O. dolichuroides* and *O. tipulae* are the least divergent species with 77, 86 and 89 bp, respectively. From these results, the conclusion can be drawn that the morphological and molecular characteristics were found sufficient to allow *Oscheius citri* n. sp., *O. cynodonti* n. sp., *O. cobbi* n. sp., *O. esculentus* n. sp., *O. punctata* n. sp. and *O. sacchari* n. sp. to be regarded as new species.

Oscheius Andrassy, 1976

syn. *Heterorhabditoides* Zhang et al., 2008
H. chongmingensis Zhang et al., 2008
Dolichorhabditis Andrassy, 1983
Leptoderadolichura A. Schneider, 1866

Diagnosis

Synrhabditis: Body length between 1.2-3.2 mm. Cuticle finely annulated. Head not offset, lips separate, amphids pore like on the lateral lips. Buccal tube short, at most one and a half times

Table 7. Comparison of sequence length and base pair composition of ITS regions of new and known species of *Oscheius* and other members of Rhabditids.

| Nematode species | A | C | G | T | Sequence length (bp) |
|-----------------------------|---------|---------|---------|---------|----------------------|
| <i>Rhabditella axei</i> | 0.24805 | 0.17629 | 0.27301 | 0.30265 | 1669 |
| <i>O. chongmingensis</i> | 0.19501 | 0.22621 | 0.26053 | 0.31825 | 1007 |
| <i>O. punctata</i> n. sp. | 0.21373 | 0.23245 | 0.25897 | 0.29485 | 727 |
| <i>O. cynodonti</i> n. sp. | 0.21529 | 0.22777 | 0.24961 | 0.30733 | 881 |
| <i>O. sacchari</i> n. sp. | 0.21529 | 0.23089 | 0.25273 | 0.30109 | 853 |
| <i>O. cobbi</i> n. sp. | 0.21373 | 0.23245 | 0.24961 | 0.30421 | 885 |
| <i>O. esculentus</i> n. sp. | 0.21685 | 0.22933 | 0.25117 | 0.30265 | 844 |
| <i>O. citri</i> n. sp. | 0.21529 | 0.23401 | 0.24961 | 0.30109 | 872 |
| <i>O. rugaoensis</i> | 0.19657 | 0.22465 | 0.26209 | 0.31669 | 1040 |
| <i>O. carolinensis</i> | 0.20125 | 0.24493 | 0.26677 | 0.28705 | 1176 |
| <i>O. dolichura</i> | 0.24337 | 0.18097 | 0.26365 | 0.31201 | 1683 |
| <i>O. dolichuroides</i> | 0.25585 | 0.17629 | 0.26053 | 0.30733 | 1709 |
| <i>O. tipulae</i> | 0.24649 | 0.19189 | 0.26989 | 0.29173 | 1677 |
| <i>O. guentheri</i> | 0.24337 | 0.18097 | 0.27145 | 0.30421 | 1706 |
| <i>Rhabditis</i> | 0.24337 | 0.18253 | 0.27769 | 0.29641 | 1707 |
| <i>O. myriophila</i> | 0.26989 | 0.21061 | 0.26521 | 0.25429 | 1018 |
| <i>Oscheius</i> | 0.29173 | 0.18565 | 0.28081 | 0.24181 | 1708 |
| <i>O. insectivora</i> | 0.25968 | 0.21685 | 0.28081 | 0.24805 | 1715 |

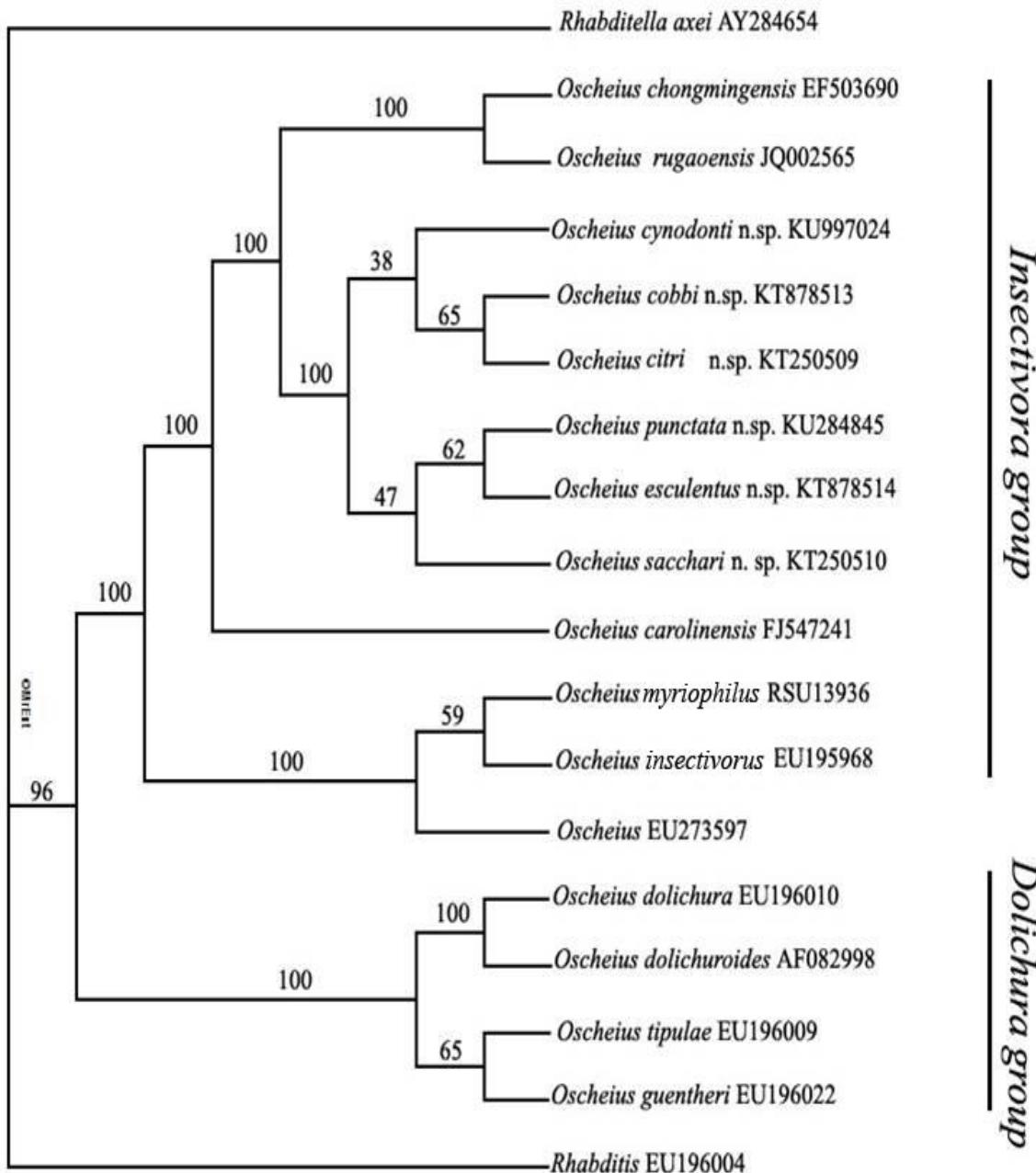


Fig.16. Phylogenetic relationships of *Oscheius citri* n. sp., *O. cobbi* n. sp., *O. cynodonti* n. sp., *O. esculentus* n. sp., *O. punctata* n. sp., *O. sacchari* n. sp. with 12 rhabditids based on rDNA-ITS sequences. *Rhabditella axei* (AY284654) was used as an outgroup. Numbers at the nodes represent bootstrap proportion for MP (50%).

longer than wide. Chielostome not cuticularized, promesostome with parallel walls, metastomatal dwellings each with three minute denticles. Oesophageal collar present. Corpus cylindrical, median bulb absent, terminal bulb strong. Excretory duct strongly sclerotized.

Female gonads paired. Vulva middle of body amphidelphic, rectum longer than anal body diameter. Tail conical in both sexes. Testis reflexed ventrally, bursa peloderan or leptoderan with a short free tail tip, anteriorly open, nine pairs of papillae present, arranged as 1+1+1/3+3. Spicules separate distally hooked or without hooked. Gubernaculum adjacent to spicules, about half of their length.

Insectivora group: Body large and wide; lateral field with three to five ridges (4-6 incisions). Bursa pseudopeloderan/leptoderan (with short free tail tip). Phasmid posterior to the last genital papillae. Spicules distally hooked like a crochet-needle. 14 species belong to insectivora group (Sudhaus, 2011).

Recent investigations have added a number of *Oscheius* species in this group which has now become twenty two. In the present studies, six new species are described that belong to insectivora group, raising the total number of species twenty eight.

Dolichura group: Body slender; female has expansile rectum; bursa peloderan, phasmids inconspicuous, posterior; spicules tip straight not hooked. 13 species placed in this group (Sudhaus, 2011). Later on, one species has been described in this group (Torrini *et al.*, 2015).

Species identification of the genus is very challenging since morphological characters are very similar. The genus *Oscheius* includes 42 valid species belonging to insectivora (28) and dolichura (14) groups (Table 8). A key has

been presented to identify all the known species of the genus *Oscheius*. Illustrations of anterior and posterior regions of the 37 available species are given in Fig. 17 (a-d) while in Table (9 a, b & 10 a, b) compiled morphometrics data have been furnished.

Acknowledgements

The authors thanks Dr. Zafar A. Handoo, USDA ARS Nematology Laboratory, Beltsville, MD, USA for critical review of the manuscript. The authors would also like to thanks Dr. W. Sudhaus from Germany for providing useful literature.

References

- Andrassy, I. (1976). *Evolution as a basis for the systematization of nematodes*. Pitman Publishing, Budapest, London, San Francisco, 288 pp.
- Andrassy, I. (1983). *A taxonomic review of the suborder Rhabditina (Nematoda: Secernatia)*. Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Paris, 241p.
- Andrassy, I. (2005). *Free living nematodes of Hungary*, Vol. 1. (*Nematoda errantia*). Hungarian Natural History Museum, Budapest, 495pp.
- Baliadi, Y., Kondo, E. & Yoshiga, T. (2009). The continual forming and contribution of infective juveniles produced via endotokia matricida of entomopathogenic nematodes in the family of Steinernematidae and Heterorhabditidae. *Indonesian Journal of Agricultural Science*, 10, 26-33. Doi: 10.1303/aez.2004.61
- Bedding, R. A. & Akhurst, R. J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica*, 21, 109-110. Doi: 10.1163/187529275x00419.
- Courtney, W. D., Pooley, D. & Miller, V. L. (1955). TAF, an improved fixative in nematode technique. *Plant Disease Reporter*, 39, 570-571.

Table 8. Valid species of *Oscheius* of insectivora and dolichura group.

| <u>Insectivora group</u> | |
|---|---|
| <i>Oscheius amsactae</i> Ali et al., 2011 | <i>O. wohlgemuthi</i> (Volk, 1950) Sudhaus, 2011 |
| <i>O. andrassyi</i> Tabassum & Shahina, 2008 | <i>O. citri</i> n. sp. |
| <i>O. carolinensis</i> Ye et al., 2010 | <i>O. cobbi</i> n. sp. |
| <i>O. caulleryi</i> (Maupas, 1919) Andrassy, 2005 | <i>O. esculentus</i> n. sp. |
| <i>O. ciceri</i> Azra et al., 2011 | <i>O. cynodonti</i> n. sp |
| <i>O. chongmingensis</i> Zhang et al., 2008 | <i>O. sacchari</i> n. sp. |
| <i>O. colombianus</i> Stock et al., 2005 | <i>O. punctata</i> n. sp. |
| <i>O. esperancensis</i> (Stock, 1991) Sudhaus, 2011 | <u>Dolichura group</u> |
| <i>O. gingeri</i> Pervez et al., 2013 | <i>Oscheius bengalensis</i> Timm, 1956 |
| <i>O. hussainii</i> Azra et al., 2011 | <i>O. dolichura</i> Schneider, 1866 |
| <i>O. insectivorus</i> (Korner, 1954) Andrassy, 1976 | <i>O. dolichuroides</i> Anderson & Sudhaus, 1985 |
| <i>O. lucianii</i> (Maupas, 1919) Andrassy, 2005 | <i>O. dux</i> (Gorgadze, 2010) Sudhaus, 2011 |
| <i>O. maqbooli</i> Tabassum & Shahina, 2002 | <i>O. guentheri</i> Sudhaus & Hooper, 1994 |
| <i>O. myriophilus</i> (Poinar, 1986) Sudhaus & Hooper, 1994 | <i>O. janeti</i> (Lacaze-Duthiers in Janet, 1894) Sudhaus, 2011 |
| <i>O. nadarajani</i> Ali et al., 2011 | <i>O. latus</i> (Cobb, 1906) Sudhaus, 2011 |
| <i>O. necromenus</i> Sudhaus & Schulte, 1989 | <i>O. onirici</i> Torrini et al., 2015 |
| <i>O. niazii</i> Tabassum & Shahina, 2010 | <i>O. pheropsophi</i> (Smart & Nguyen, 1994) Sudhaus, 2011 |
| <i>O. rupaekramae</i> (Khan et al., 2000) Sudhaus, 2011 | <i>O. pseudodolichura</i> Korner, 1954 |
| <i>O. rugaoensis</i> Zhang et al., 2012 | <i>O. sechellensis</i> Potts, 1910 |
| <i>O. shamimi</i> Tahseen & Nisa, 2006 | <i>O. tereticorpus</i> (Kito & Ohyama, 2008) Sudhaus, 2011 |
| <i>O. siddiqii</i> Tabassum & Shahina, 2010 | <i>O. tipulae</i> Lam & Webster, 1971 |
| | <i>O. zarinae</i> (Khan et al., 2000) Sudhaus, 2011 |

Key to species of the genus *Oscheius* Andrassy, 1976

| | | |
|-----|--|---------------------------|
| 1. | Body slender; female has expansile rectum; bursa peloderan, phasmids inconspicuous, posterior; spicules tip straight not hooked..... | 2 |
| | Body large and wide, female has normal rectum, bursa leptoderan, spicules tip hooked..... | 15 |
| 2. | Female with posterior genital branch reduced..... | <i>O. guentheri</i> |
| | Female with both genital branches of similar size and well developed | 3 |
| 3. | Lateral field absent..... | <i>O. zarinae</i> |
| | Lateral field present..... | 4 |
| 4. | Female tail elongate with clavate terminus, marine species..... | <i>O. bengalensis</i> |
| | Female tail conical to form filiform with acute terminus, terrestrial species..... | 5 |
| 5. | Spicules with differentiated calamus and manubrium | 6 |
| | Spicules with non-differentiated calamus and very reduced manubrium..... | 7 |
| 6. | Lip region continuous with adjacent body, spicules of equal length..... | <i>O. dolichuroides</i> |
| | Lip region wider than adjacent body, spicules of unequal length..... | <i>O. dolichura</i> |
| 7. | Stoma narrower about four times longer than wide..... | <i>O. sechellensis</i> |
| | Stoma wider three times longer than the wide..... | 8 |
| 8. | Lateral field with 8 incisures..... | <i>O. pheropsophi</i> |
| | Lateral field with 6 or below 6 incisures..... | 9 |
| 9. | Lateral field with 4 incisures..... | 10 |
| | Lateral field with 6 incisures..... | 11 |
| 10. | Female body length < 0.80mm, spicules of equal length..... | <i>O. tipulae</i> |
| | Female body length > 0.90mm, spicules of unequal length..... | <i>O. pseudodolichura</i> |
| 11. | Female body length < 1000µm..... | 12 |
| | Female body length > 1000µm..... | 14 |
| 12. | V% < 50% | 13 |
| | V% > 50%..... | <i>O. latus</i> |
| 13. | In female a ratio < 20, c ratio < 10..... | <i>O. dux</i> |
| | In female a ratio > 20, c ratio > 10..... | <i>O. tereticorpus</i> |
| 14. | Male length < 1000 µm, a ratio > 20..... | <i>O. janeti</i> |
| | Male length >1000 µm, a ratio < 20..... | <i>O. onirici</i> |
| 15. | Hermaphroditic (antogonic), males are rare..... | 16 |
| | Bisexual (gonochoristic), males and females are equal in population..... | 21 |
| 16. | Lateral field with 6 incisures | 17 |
| | Lateral field with 4 or 5 incisures | 18 |
| 17. | Small female < 1500 µm long, longer tail, lower b value (3.9-5.3), anterior lips of male elongate horn like..... | <i>O. maqbooli</i> |
| | Small female >1500 µm long, shorter tail, higher b value (6.2-7), anterior lips of male not elongate..... | <i>O. andrassyi</i> |
| 18. | Lateral field with 5 incisures | <i>O. rugaoensis</i> |
| | Lateral fields with 4 incisures..... | 19 |
| 19. | Large female > 1800 µm, male >1000 µm long..... | <i>O. caulleryi</i> |
| | Small female < 1800 µm, male < 1000 µm long..... | 20 |
| 20. | Male medium sized 671-950 µm long, spicules average length < 40 µm, female stoma \leq 18 µm..... | <i>O. necromenus</i> |
| | Male large sized 841-1125 µm long, spicules average length > 40 µm, female stoma \geq 18µm..... | <i>O. myriophilus</i> |
| 21. | Lateral field with six or above six incisures..... | 22 |
| | Lateral field with five or below five incisures..... | 33 |
| 22. | Lateral field with 10 incisures..... | 23 |
| | Lateral field with 6 incisures..... | 24 |
| 23. | Female body length >1000µm, tail length <100 µm, spicules length >30 µm..... | <i>O. amsactae</i> |

| | |
|---|-----------------------------|
| Female body length < 1000 μm , tail length > 100 μm , spicules length < 30 μm | <i>O. gingeri</i> |
| 24. Male body length > 1000 μm | 25 |
| Male body length < 1000 μm | 28 |
| 25. Spicules length \geq 60 μm , longer stoma | 26 |
| Spicules length \geq 50 μm , shorter stoma..... | 27 |
| 26. Male body length < 1200 μm , spicules length 54-60 μm | <i>O. cynodonti</i> n. sp. |
| Male body length > 1200 μm , spicules length 57-70 μm | <i>O. citri</i> n. sp. |
| 27. Female with free lips, short stoma, and c ratio > 14 μm | <i>O. niazii</i> |
| Female with fused lips to form three doublets, long stoma, c ratio < 14 μm | 29 |
| 28. Male body length > 1000 μm , stoma short (4-4.5) μm long..... | <i>O. rupaekramae</i> |
| Male body length < 1000 μm , stoma > 10 long..... | 30 |
| 29. Spicules length < 55 μm long, c, ratio < 24 and stoma short..... | <i>O. siddiqii</i> |
| Spicules length > 55 μm long c, ratio > 24 and stoma long..... | <i>O. shamimi</i> |
| 30. Average body length of male < 950 μm , tail length > 40 μm | 31 |
| Male body length > 900 μm , tail length \leq 40 | 32 |
| 31. Maximum body width of male 36-58 μm , pharynx length 130-220 μm and tail length 20-32 μm | <i>O. cobbi</i> n. sp. |
| Maximum body width of male 53-72 μm , pharynx length 125-169 μm and tail length 26-45 μm | <i>O. punctata</i> n. sp. |
| 32. Longer pharynx in female average length 196 μm , longer tail average 122 μm , male tail short 26-40 μm | <i>O. sacchari</i> n. sp. |
| Shorter pharynx in female average length 188 μm , shorter tail average 105 μm , male tail long 30 - 60 μm | <i>O. esculentus</i> n. sp. |
| 33. Lateral field with 5 incisures, medium size male and female..... | <i>O. wohlgemuthi</i> |
| Lateral field with 4 incisures | 34 |
| 34. Shorter stoma (18.5-19) μm , bursal notch absent..... | <i>O. nadarajani</i> |
| Longer stoma (21-28) μm , bursal notch present..... | 35 |
| 35. Median bulb absent, long rectum and medium size spicules..... | <i>O. columbianus</i> |
| Median bulb present, medium size rectum and long spicules..... | 36 |
| 36. Medium size male and female, spicules length < 80 μm and gubernaculum < 30 μm | 37 |
| Large size male and female, spicules length > 80 μm and gubernaculum > 30 μm | 40 |
| 37. Spicules length > 50 μm and gubernaculum > 20 μm | 38 |
| Spicules length < 50 μm and gubernaculum < 20 μm | 41 |
| 38. Total body length of female > 1000 μm , short stoma (average 16 μm) and short male tail..... | <i>O. esperancensis</i> |
| Total body length of female < 1000 μm , long stoma and long male tail..... | 39 |
| 39. Stoma \leq 20 μm long, pharynx \leq 200 μm long, male tail < 60 μm | <i>O. ciceri</i> |
| Stoma \geq 20 μm long, pharynx \geq 200, μm long, male tail > 60 μm | <i>O. hussainii</i> |
| 40. Female body length > 1500 μm anal body width > 25 μm | <i>O. lucianii</i> |
| Female body length < 1500 μm anal body width < 25 μm | <i>O. chongmingensis</i> |
| 41. Tail of female shorter 1/8-1/10 of entire body length, rectum 2-3 times as long as anal body diameter, body short 1.5-2.5 mm long..... | <i>O. carolinensis</i> |
| Tail of female shorter 1/12-1/15 of entire body length, rectum 3-4 times as long as anal body diameter, body large 2-6 mm long..... | <i>O. insectivorus</i> |

Table 9 a. Comparison of males in the insectivora group of *Oscheius*. All measurements are in µm.

| S. No | Species | Total body length | Max. body width | Stoma | Pharynx | Tail length | Anal body width | a | b | c | c' | Spicules | Gubernaculum |
|----------|-------------------------|----------------------|---------------------|---------------------|------------------------|---------------------|------------------------|-------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 1 | <i>O.amsactae</i> | -- (594-804) | -- (31.6-45) | -- (15-16.9) | -- (134-169) | -- (41-55) | -- (15.8-19.7) | -- (16.6-19.3) | -- (4-5) | -- (10.7-17.8) | -- (1.4) | -- (30.8-35) | -- (13.4-16.5) |
| 2 | <i>O.andrassyi</i> | 1096 (1025-1132) | 50.2 (45-51) | 15.7 (15-17) | 168 (158-178) | 49.2 (45-63) | 37.2 (30-40) | 21.8 (20-25) | 6.3 (6.2-7) | 21 (17.9-24.9) | 1.4 (1.1-2.1) | 46 (45-51) | 22 (20-25) |
| 3 | <i>O.carolinensis</i> | 1499 (1000-2000) | 73 (52-89) | 20 (18-23) | 214 (165-245) | 48 (32-64) | 34 (25-40) | 20.4 (15.8-24.3) | 7 (4.9-8.9) | 32.3 (18.9-50) | 1.4 (1-2.3) | 65 (50-81) | 29.4 (20-35) |
| 4 | <i>O.caulleryi</i> | -- (1054-1310) | -- (55-72) | -- -- | -- -- | -- -- | -- -- | -- (5.9-6.3) | -- (23.1-30.5) | -- -- | -- (39-48) | 28 -- | |
| 5 | <i>O.chongmingensis</i> | 1115 (822-1400) | 46 (37.7-62) | -- -- | 157 (113-186) | 29 (22-38.8) | 26 (21-33) | -- -- | -- -- | -- -- | -- (37-68) | 51 (20-33) | 24.6 -- |
| 6 | <i>O.ciceri</i> | 820 (753.6-972.9) | 41 (38.8-46.5) | 19.49 -- | 158.3 (149-171) | 56.8 (55-58) | 28 (26-30) | 20 (19.4-20.8) | 5.3 (5-5.6) | 15 (13.6-16.7) | 2.4 (2.1-2.7) | 39 (34-43) | -- -- |
| 7 | <i>O.colombianus</i> | 915 (665-1163) | 49 (23-72) | 21 (19-24) | 183 (168-213) | 63.5 (51-70.5) | 26 (15-32) | 18 (16-29) | 4.9 (3.9-5.4) | 14.5 (13-16) | 2.4 (2.1-3.5) | 56 (43-68) | 20 (16-24) |
| 8 | <i>O.esperancensis</i> | -- (1125-1210) | 74.4 (65-83) | 16.4 (15.3-17) | 203 (186-212) | 20.7 (18.4-21.9) | -- -- | (16-20.3) (5.8-7.09) | -- (15.9-20.6) | -- -- | -- (35.3-42.3) | 39.4 (8.4-11.7) | 9.8 -- |
| 9 | <i>O.gingeri</i> | 739.5 (673-821.5) | 35.2 (31.6-38.9) | 17.9 (16.8-19.2) | 171.4 (142.5-187.5) | 51.8 (43.5-59.3) | 17.7 (15.5-19.2) | 20.6 (18.3-24) | 4.7 (4.3-5.3) | 14.6 (11.5-16.7) | -- -- | 25.7 (24-27.3) | 9.3 (8.7-9.8) |
| 10 | <i>O.hussainii</i> | 862.9 (855-889) | 33.2 (30-35) | 22.5 (22-23) | 226 (223-228) | 63 (61-65) | 19.9 (19.4-20.5) | 26 (25.3-28) | 3.81 (3.83-3.89) | 13.6 (13.6-13.9) | 3.16 (3.15-3.17) | 41.5 (40.7-43.8) | -- -- |
| 11 | <i>O.insectivorus</i> | - (1587-3252) | -- (78-91) | -- -- | -- -- | -- -- | (20-24.8) (7.3-9.6) | -- (21-24) | -- -- | -- (88-95) | -- (38-54) | -- -- | |
| 12 | <i>O.lucianii</i> | -- (1215-1900) | -- (57-66) | -- -- | -- -- | -- -- | -- (6.6-7.3) | -- (28.3-45.2) | -- -- | -- (57-66) | -- -- | 60 -- | |
| 13 | <i>O.maqbooli</i> | 983.5 (720-1165) | 57.6 (40-69.6) | 15.6 (10.4-17.6) | 204.1 (184-248) | 43.8 (32-48) | 31.8 (28-36) | 17.1 (12.8-19) | 4.7 (3.9-5.3) | 22.5 (18.6-27) | 1.3 (1.3-1.6) | 54.8 (48-60) | 27.8 (25.6-29.6) |
| 14 | <i>O.myriophilus</i> | -- (841-1175) | -- (52-72) | -- -- | -- -- | -- -- | -- (4.0-6.1) | -- (14.4-21.8) | -- -- | -- (27-39) | 28 (19-32) | -- -- | |

Contd...

Description of six new species of *Oscheius* Andrassy, 1976 (Nematoda: Rhabditida) from Pakistan

| S. No. | Species | Total body length | Max. body width | Stoma | Pharynx | Tail length | Anal body width | a | b | c | c' | Spicules | Gubernaculum |
|-----------|-----------------------------|----------------------|---------------------|-----------------|--------------------|---------------------|---------------------|---------------------|-------------------|---------------------|---------------------|---------------------|--------------------|
| 15 | <i>O. nadarajani</i> | 1280 (1191-1395) | 44 (40-47.5) | 19.4 (--) | 250 (242-256) | 34.3 (32.5-35.9) | 26 (23.5-29.3) | 25.8 (23.5-27.7) | 4.9 (4.5-5.3) | 36 (34.2-37.9) | 1.15 (1.31-1.85) | 60.5 (58.5-62.9) | -- |
| 16 | <i>O. necromenus</i> | 790 (671-950) | 46 (36-50) | -- | -- | -- | -- | 17.4 (14.4-19.2) | 4.5 (4.3-5.0) | 17.4 (11.5-17.6) | -- | 40 (34-44) | 15 (12-23) |
| 17 | <i>O. niazii</i> | 884.8 (715-1020) | 52.8 (45-57.5) | | 167 (160-175) | 36.2 (35-40) | 27 (20-37.5) | 16.7 (15.6-17) | 5 (4.4-5.8) | 22 (18-25.5) | 1.4 (1-1.8) | 47.5 (42.5-52) | 23.5 (17.5-30) |
| 18 | <i>O. rupaekramae</i> | 1120 (816-1250) | -- | 4.4 (4-5) | -- | -- | -- | 17.8 (14.9-23.6) | 5.17 (4.3-7.1) | 27.2 (22.5-34.9) | 1.3 (1.4-2.0) | 52 (44-60) | (10-12) |
| 19 | <i>O. rugaoensis</i> | 1396 (1195-1692) | 62.4 (46.3-66.2) | -- | 304 (219-443.6) | 151 (128.4-163) | 30.3 (16.9-52.5) | 22.8 (15.8-26.3) | 4.8 (3.8-6.4) | 9.3 (7.3-10.5) | -- | 49.2 (35.2-60.9) | 19.7 (9.9-26.5) |
| 20 | <i>O. shamimi</i> | 1012 (938-1118) | 53.8 (49-57) | 19.2 (18-22) | 190 (181-203) | 35.4 (29-38) | 28 (25-31) | 18.8 (17-20) | 5.4 (5.1-5.5) | 28.2 (25.1-31.1) | 1.3 (1.1-1.5) | -- | 25.6 (22-28) |
| 21 | <i>O. siddiqii</i> | 919 (828-998) | 47.2 (43-53) | 14.4 (14-15) | 178 (165-180) | 40.7 (38-45) | 28.6 (28-30) | 19.4 (18-21) | 5.1 (4.3-5.6) | 22.4 (20-23.6) | 1.4 (1.3-1.5) | 51.7 (50-55) | 22.5 (20-25) |
| 22 | <i>O. wohlgemuthi</i> | -- (1170-1520) | 59 -- | 19 -- | -- (20-235) | -- (46-57) | 34 -- | -- (17.7-20.3) | -- (5.6-7.3) | - (21.6-30) | 2.1 -- | -- (54-66) | 16 -- |
| 23 | <i>O. citri</i> n. sp. | 1249 (1097-1464) | 53 (43-68) | 17.2 (15-20) | 224 (200-250) | 43.5 (35-50) | 31.3 (28-35) | 23.4 (18-28) | 5.5 (5-6.1) | 28.6 (25-35.6) | 1.3 (1.2-1.6) | 63.7 (55-70) | 27.8 (25-30) |
| 24 | <i>O. cobbi</i> n. sp. | 941 (789-1110) | 47 (36-58) | 14 (13-16) | 161 (130-220) | 28.4 (20-32) | 20 (10-25) | 19.8 (17-21) | 6.3 (5.8-7.4) | 33.9 (22-38) | 1.4 (1.2-2.5) | 54 (47-62) | 24.5 (20-35) |
| 25 | <i>O. esculentus</i> n. sp. | 1161 (875-1370) | 53.6 (46-64) | 15 (14-17) | 172 (155-200) | 48.3 (30-60) | 29.6 (28-31) | 21.7 (19-27.7) | 6.6 (5.6-7.5) | 24.4 (18.9-34) | 1.5 (1.3-1.9) | 51 (45-57) | 22.3 (20-24) |
| 26 | <i>O. sacchari</i> n. sp. | 862 (760-1390) | 61.6 (53-75) | 17.8 (15-21) | 163 (154-178) | 35.3 (26-40) | 27.2 (24-30) | 17.6 (16.3-24) | 7 (6.2-8) | 32.5 (28-36.6) | 1.35 (1-1.6) | 50 (47-55) | 20.3 (18-22) |
| 27 | <i>O. cynodonti</i> n. sp. | 1129 (863-1560) | 48.6 (43-58) | 14.4 (14-17) | 191.8 (168-208) | 44.6 (32-50) | 29 (25-33) | 23.4 (17.4-34) | 5.8 (4.5-6.8) | 25.4 (19.3-34) | 1.5 (1.1-1.8) | 56.3 (54-60) | 23.6 (20-27) |
| 28 | <i>O. punctata</i> n. sp. | 944.7 (800-1118) | 60 (53-72) | 12.7 (10-17) | 145 (125-169) | 35.4 (26-45) | 27.2 (20-30) | 15.6 (12-18) | 6.5 (5.2-7.7) | 27 (19.5-36) | 1.3 (1.0-1.7) | 54.4 (50-60) | 21 (20-25) |

Table 9b. Comparison of females in the insectivora group of *Oscheius*. All measurements are in µm.

| S. No | Species | Total body length | Max. body width | Stoma length | Pharynx | Tail length | Anal body width | V% | a | b | c | c' |
|----------|--------------------------|-----------------------|---------------------|---------------------|----------------------|----------------------|--------------------|-----------------------|---------------------|--------------------|---------------------|------------------|
| 1 | <i>O. amsactae</i> | -- (658-786) | -- (32-39) | -- (15.8-18) | -- (159.5-177) | -- (64.7-80.5) | -- (15.8-17.4) | -- (49.7-58.4) | -- (19.7-22.9) | -- (4.0-4.8) | -- (8.9-12) | -- |
| 2 | <i>O. andrassyi</i> | 1601 (1322-1962) | 90.7 (70-122) | 15.7 (14-18) | 190.6 (175-215) | 111.5 (100-130) | 32.4 (25-40) | 50.3 (48.9 - 52.9) | 17.7 (15-21) | 8.2 (6.7-9.4) | 14.4 (11-17.9) | 3.3 (3-4.3) |
| 3 | <i>O. carollinensis</i> | 1728 (1360-2420) | 95.7 (67-123) | 23 (18-27) | 247 (228-264) | 160 (108-206) | 36 (28-43) | 50 (47.6-55.6) | 18.2 (14.9-23.2) | 7 (5.4-9.5) | 11 (8.4-17.8) | 4.4 (3.2-6.1) |
| 4 | <i>O. caulleryi</i> | -- (1910-2364) | -- (111-122) | -- (16-20) | -- | -- | -- | -- | -- (9.1-9.7) | -- (19.9-24.6) | 2.5 -- | |
| 5 | <i>O. chongmingensis</i> | 1143 (809-1351) | 104 (76-135) | 9.7 (8.9-10.3) | 180 (154-202) | 81 (67-102) | 22.6 (20-27) | 51 (50-54.8) | -- | -- | -- | -- |
| 6 | <i>O. ciceri</i> | 996 (964-1018) | 41.7 (38.8-45.5) | 18.9 (18.4-19.4) | 171 (166-176) | 89.5 (74-96) | 23.6 (20-28) | -- | 21.6 (20-22) | 5.8 (5.7-5.9) | 11 (10.3-12.9) | 3.8 (3.6-4.3) |
| 7 | <i>O. colombianus</i> | 1288 (923-1805) | 81.5 (49-106) | 23 (21-28) | 205 (176-225) | 140 (110-167) | 31 (22-38) | 51 (47-57) | 17 (15-19) | 6.5 (5.2-8) | 9.2 (8.3-10) | 4.5 (3.5-5) |
| 8 | <i>O. esperancensis</i> | 1475.8 (1273-1800) | 87.5 (70-106) | 17.6 (14.1-20) | 219.5 (192-260) | 127.8 (104-160) | 34.8 (21.1-44) | 46.3 (44.6-52.6) | -- (15-22.5) | -- (5.9-7.5) | -- (9.1-17.3) | -- |
| 9 | <i>O. gingeri</i> | 1638 (1418-1813) | 81.6 (75-89) | 19.5 (18.7-20.8) | 253 (189-283) | 121.3 (115.2-129) | 26.4 (25.2-28) | 54 (51.3-60.2) | 20.3 (18.5-21) | 6.2 (5.15-5.35) | 13.8 (12-13.2) | -- |
| 10 | <i>O. hussainii</i> | 946.8 (902-989) | 36.8 (32.5-40.5) | -- (22-23) | 222.7 (219-225) | 81.4 (77.6-87.3) | 21 (19-23) | -- | 24.9 (24.4-25.5) | 4 (3.8-4.3) | 11.5 (10.2-12.7) | 4 (3.3-4.8) |
| 11 | <i>O. insectivorus</i> | -- (2000-3200) | -- (125-169) | -- | -- | -- | -- (45-50.5) | -- (14-19) | -- (8.8-13.5) | -- (12-15) | -- | |
| 12 | <i>O. lucianii</i> | -- (1601-2871) | -- (85-143) | -- (10-18) | -- | -- | 32 (--) | -- | -- (6.2-10.2) | -- (14.5-17.8) | -- | |
| 13 | <i>O. maqbooli</i> | 1123.2 (942-1342) | 68.9 (53-92) | 15 (12-19.6) | 211.5 (182-237.6) | 124.7 (112-148) | 23.7 (20-29.6) | 51 (49.6-52.8) | 16.4 (14.1-18) | 5.2 (4.8-5.6) | 9 (6.7-11) | 5.3 (4.6-5.9) |
| 14 | <i>O. myriophilus</i> | -- (792-1530) | -- (52-100) | 20 (18-21) | -- | -- | -- | -- | -- (4.2-8.5) | -- (8.9-13.0) | -- (3-4) | |

Contd...

Description of six new species of *Oscheius* Andrassy, 1976 (Nematoda: Rhabditida) from Pakistan

| S. No | Species | Total body length | Max. body width | Stoma length | Pharynx | Tail length | Anal body width | V% | a | b | c | c' |
|----------|-----------------------------|----------------------|---------------------|-----------------|--------------------|----------------------|--------------------|---------------------|----------------------------|--------------------|--------------------|-------------------|
| 15 | <i>O. nadarajani</i> | 1507 (1358-1606) | 78.5 (77.6-79.5) | -- (18.5-19) | 260 (256-267) | 122 (121.2-123) | 24.3 (23-25.5) | | 18.7 (17.5-20) | 5 (5-5.2) | 12.2 (11.1-13) | 5.13 (5.1-5.2) |
| 16 | <i>O. necromenus</i> | 1179 (830-1500) | 69 (54-90) | 17 (14-18) | -- | 106 (81-131) | 45 | 50 (41-56) | 17.2 (15.3-20.3) | 5.5 (4.2-6.3) | 11.2 (9.7-13.9) | -- (3-4) |
| 17 | <i>O. niazii</i> | 1197 (837-1487) | 72.5 (50-100) | 19.3 (15-25) | 189 (180-200) | 97.9 (75-175) | 27 (20-35) | 51.4 (48-54) | 16.2 (14.6-17.3) | 6.2 (4.4 - 7.8) | 12 (10.8-14) | 3.6 (2.8-4) |
| 18 | <i>O. rupaekramae</i> | 2000 (1800-2400) | -- | -- (4-4.5) | -- | -- | -- | 49.7 (48.8-50.5) | 15.9 (14.7-17.3) | 10.7 (8.8-11.5) | 16.7 (13.5-20) | 3.1 (2.6-3.7) |
| 19 | <i>O. rugaoensis</i> | 1042 (920.6-1179) | 49.5 (39.8-58.2) | -- | 209 (189-222) | 131 (113-154.9) | 26.2 (21-31.7) | 54.8 (51.2-66.9) | 21 (17.7-25.8) | 5 (4.9-5.6) | 8.5 (7.6-9.3) | -- |
| 20 | <i>O. shamimi</i> | 1114 (760-1524) | 67.2 (58-97) | 20.5 (19-23) | 211 (181-241) | 118 (110-132) | 24 (21-32) | 49.5 (45.6-51.3) | 17.6 (12-20) | 5.3 (4.2-6.3) | 10 (6.8-13.8) | 5.1 (4.1-5.8) |
| 21 | <i>O. siddiqii</i> | 1294 (1130-1390) | 80.5 (73-90) | 15.5 (13-17) | 196.7 (185-204) | 119 (100-132) | 27.8 (24-31) | 47.7 (40-52.3) | 16.5 (15-18) | 6.6 (6-6.9) | 11.2 (10-13) | 4.3 (3.5-5.4) |
| 22 | <i>O. wohlgemuthi</i> | -- (1570-2100) | 49 (40-69) | 24 -- | (270-284) | 108-176 (108-176) | 20 (18-25) | -- (48-53) | (16.0-19.3) (16.0-19.3) | -- (6.4-7.6) | -- (10.1-14.2) | 5.2 -- |
| 23 | <i>O. citri</i> n. sp. | 1601 (1173-2015) | 80 (52-105) | 18.8 (17-20) | 252 (234-273) | 143 (120-160) | 29.8 (22-37) | 49.3 (47.4-51) | 20.8 (17.7-28.5) | 6.3 (4.8-7.9) | 11 (9-13) | 4.8 (4-5.9) |
| 24 | <i>O. cobbi</i> n.sp. | 1199 (950-1712) | 80.3 (59-115) | 14.7 (14-22) | 157 (134-282) | 93.6 (80-170) | 25.7 (20-38) | 48.2 (42-57) | 15.3 (12-21.6) | 7.8 (6.6-8.8) | 12.8 (9-14.8) | 3.7 (2.8-4.3) |
| 25 | <i>O. esculentus</i> n. sp. | 1512 (1249-1798) | 83 (70-115) | 15 (13-18) | 188 (165-213) | 105 (90-120) | 30 (26-35) | 51.7 (49.3-55) | 18 (15.6-19.5) | 7.6 (6.8-8.9) | 14.2 (13-15.3) | 3.5 (2.9-4.2) |
| 26 | <i>O. sacchari</i> n. sp. | 1607 (1362-2015) | 88 (72-125) | 17.5 (16-18) | 196.8 (165-232) | 122 (100-195) | 26.6 (21-33) | 48.7 (45-52) | 18.4 (16-19.8) | 8.2 (6.7-10.3) | 13.4 (7.6-16.8) | 4.9 (3.5-6.2) |
| 27 | <i>O. cynodonti</i> n. sp. | 1457 (1252-1662) | 81.7 (65-100) | 15 (12-18) | 149.5 (135-159) | 91 (60-108) | 21.6 (15-30) | 48.8 (41-52.6) | 17.9 (16-20) | 9.7 (8.5-10.7) | 16.4 (13-24) | 4.7 (4-6.7) |
| 28 | <i>O. punctata</i> n. sp. | 1421 (1226-1680) | 91.3 (72-116) | 14 (12-17) | 174.9 (157-190) | 110 (80-170) | 28 (14-35) | 51.3 (46-61) | 15 (12.5-18.4) | 8 (7-9.6) | 12.9 (8.2-16.8) | 3.9 (3.3-5.7) |

Table 10 a. Comparison of males in the dolichura-group of *Oscheius*. All measurements are in µm.

| S. No | Species | Total body length | Max. body width | Stoma | Pharynx | Tail length | a | b | c | c' | Spicules | Gubernaculum |
|----------|---------------------------|----------------------|--------------------|---------------|------------------|----------------|---------------------|------------------|---------------------|------------------|---------------|---------------|
| 1 | <i>O. bengalensis</i> | 940 (710-1170) | -- (28-63) | -- (15-18) | -- (163-210) | -- (24-34) | 29.9 (22.2-39) | 5.5 (4.4-6.7) | 27.8 (21.6-33) | -- (1.1-1.5) | -- (30-33) | -- (14-19) |
| 2 | <i>O. dolichura</i> | -- (528-886) | -- (27-51) | -- | (108-197) | -- (24-32) | -- (16-22) | -- (4.5-5.8) | 20.3 -- | -- (1.1-1.7) | -- (32-35) | -- (20-26) |
| 3 | <i>O. dolichuroides</i> | 1118 (1045-1286) | 37 (30-41) | -- | 228 (216-241) | 66 (60-74) | 30 (27-35) | 4.9 (4.5-5.7) | 43 (32-56) | 1.3 (1.0-1.7) | 46 (41-48) | 26 (22-29) |
| 4 | <i>O. dux</i> | 640 (520-720) | 36 (30-41) | -- | 127 (121-136) | 27 (22-30) | 17.6 (16-21.3) | 5 (4.2-5.7) | 23.7 (20-30) | -- | 26 (22-30) | 8 (--) |
| 5 | <i>O. guentheri</i> | 745 (655-799) | 38 (36-39) | -- | 136 (134-140) | 38 (36-40) | 19.7 (18.4-20.4) | 5.5 (4.9-5.8) | 19.5 (18.4-20.8) | -- | 19 (17-21) | -- |
| 6 | <i>O. janeti</i> | 930 | -- | -- | -- | -- (25-26) | -- 3-5 | -- (32-33) | -- | -- | -- | -- |
| 7 | <i>O. latus</i> | 500 | -- | -- | -- | 26 | 3.6 | 10 | -- | -- | -- | -- |
| 8 | <i>O. onirici</i> | 510 (443-553) | 32 (29-35) | -- | 125 (117-134) | 21 (18-25) | 16 (14.6-18.2) | 4.1 (3.8-4.6) | 24.2 (21.2-30.4) | 1.2 (0.9-1.5) | 22 (20-24) | 10 (9-13) |
| 9 | <i>O. pheropsophi</i> | 872 (805-1000) | 44 (36-52) | -- | 146 (127-152) | 36 (31-42) | 20 (18-23) | 6 (5.5-6.8) | 24.7 (21-28) | 1.4 (1.1-1.6) | 47 (34-55) | 23 (19-28) |
| 10 | <i>O. pseudodolichura</i> | 764 | 38 | -- | -- | 20.1 | 6.4 | 25.5 | -- | 27 | -- | |
| 11 | <i>O. sechellensis</i> | -- (496-500) | -- | -- | -- | 31 -- | -- | -- | -- | -- (21-22) | -- | |
| 12 | <i>O. tereticorpus</i> | unknown | | | | | | | | | | |
| 13 | <i>O. tipulae</i> | 676 | 39 | -- | 126 | 28 | 17.2 | 5.4 | 24 | -- | 25 | -- |
| 14 | <i>O. zarinae</i> | 1020 (820-1140) | -- | -- | -- | -- | 18.3 (15.3-25.4) | 4.7 (4-5.5) | 28.9 (26.8-34) | 1.3 (1.0-1.5) | 37 (32-40) | -- (10-12) |

Table 10 b. Comparison of females in the dolichura-group of *Oscheius*. All measurements are in μm .

| S. No | Species | Total body length | Max. body width | Stoma length | EP | Pharynx | Tail length | V% | a | b | c | c' |
|----------|---------------------------|----------------------|--------------------|------------------|------------------|------------------|------------------|---------------------|---------------------|-------------------|--------------------|------------------|
| 1 | <i>O. bengalensis</i> | -- (1100-1730) | -- (57-96) | -- (18-24) | -- | -- (197-234) | -- (83-117) | 50.7 (48-54) | 29.2 (26-34.3) | 6.3 (5-9.3) | -- (10-16.3) | 3.6 -- |
| 2 | <i>O. dolichura</i> | 686 (720-1100) | -- (54-74) | -- (20-22) | -- | -- (130-197) | -- (73-116) | 49-55 (49-55) | -- (14-20) | -- (5.3-8.6) | -- (8-11) | -- (3-4) |
| 3 | <i>O. dolichuroides</i> | 1355 (1167-1580) | -- (16-21) | 255 (231-273) | 144 (121-172) | 54 | -- (51-57) | 27 (22-31) | 5.4 (4.6-6.2) | 9.4 (8.2-11.2) | 7 (5.9-8.2) | |
| 4 | <i>O. dux</i> | 980 (860-1100) | 63 (53-68) | 15 | 147 (133-159) | 160 (152-167) | 101 (95-106) | 49 (44.4-53.7) | 15.5 (14.6-16.2) | 5.8 (5-6.9) | 9.6 (8.2-10.7) | -- |
| 5 | <i>O. guentheri</i> | 863 (733-1071) | 47 (38-61) | -- | -- (126-162) | 145 (116-172) | 53 (50-56) | 18.3 (15.8-20.7) | 6.1 (5.6-6.6) | 6.1 (5.4-7.0) | 7.7 (6.9-9.6) | |
| 6 | <i>O. janeti</i> | 1130 | -- | -- | -- | -- | -- | -- (21-22) | 5.5 | -- (11-19) | -- | |
| 7 | <i>O. latus</i> | 600 | -- | -- | -- | -- | -- | 65 | 23 | 3.9 | 8.1 | -- |
| 8 | <i>O. onirici</i> | 671 (584-801) | 36 (30-51) | 14 (12-16) | 108 (92-146) | 137 (126-146) | 69 (63-81) | 51 (47.3-52.6) | 19 (15.8-22.5) | 4.9 (4.4-6.0) | 9.7 (8.6-11.8) | 4.3 (3.5-5.0) |
| 9 | <i>O. pheropsophi</i> | 1217 (1097-1360) | 68 (29-82) | 16.6 (14-18) | 167 (152-181) | 170 (158-184) | 146 (120-183) | 48 (45-51) | 18 (15-21) | 7 (6.5-7.7) | 8.4 (6.9-10) | 6 (4.7-7.1) |
| 10 | <i>O. pseudodolichura</i> | -- (915-1216) | -- (46-79) | 17 | -- -- | 156 | 108 | -- (45.5 - 49) | -- (15.4 - 19.9) | -- (6.6 - 8.0) | -- (7.4 - 8.3) | 6.0 -- |
| 11 | <i>O. sechellensis</i> | 680 | -- | -- | -- | 128 | 120 | 50.6 | -- | 5.3 | 5.7 | -- |
| 12 | <i>O. tereticorpus</i> | 821 (703-1070) | 35.1 (25-46) | 18.3 (17-21) | 119 (94-163) | 159 (146-179) | 81.3 (70-92) | 52.9 (49.9-54.3) | 24 (17.9-29.3) | 5.2 (4.5-6) | 10.2 (7.6-12.7) | -- |
| 13 | <i>O. tipulae</i> | 603 (505-691) | 33 (29-42) | -- (17-20) | 100 (81-109) | 140 (129-151) | 105 (84-120) | 48.6 (46.4-51.7) | 18.4 (16.3-19.9) | 4.3 (3.9-4.9) | 7.0 (6.2-8.5) | 5.2 (4.2-6.4) |
| 14 | <i>O. zarinae</i> | 1230 (1090-1560) | -- | 21.3 (16-25) | -- | -- | -- | 51.5 (51.5-53.2) | 23.7 (20.8-28.0) | 5.2 (4.7-6.4) | 9.5 (7.8-12) | 5.1 (4.3-6.8) |

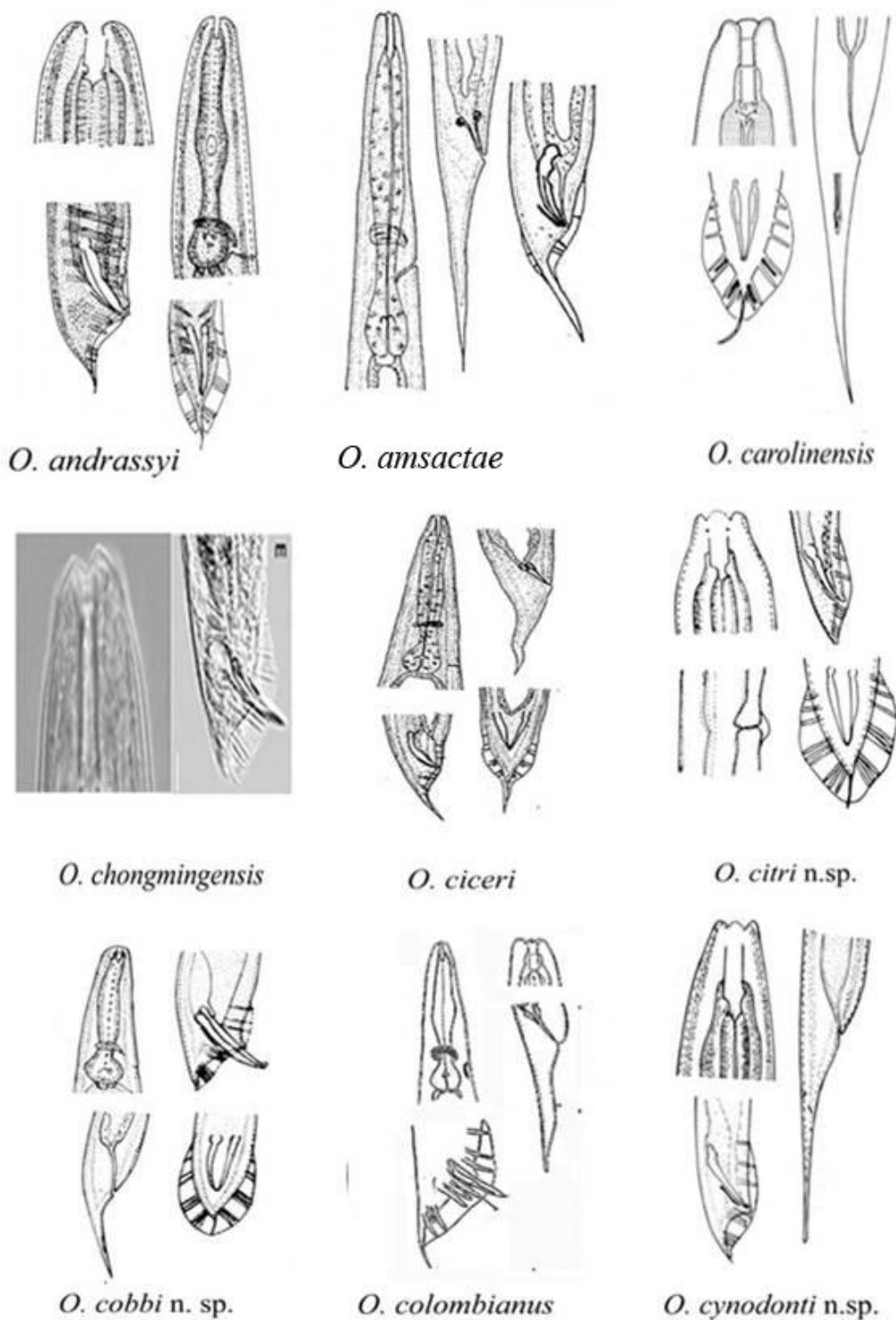


Fig. 17 a. Anterior and posterior regions of insectivora group of *Oscheius* (from original description)

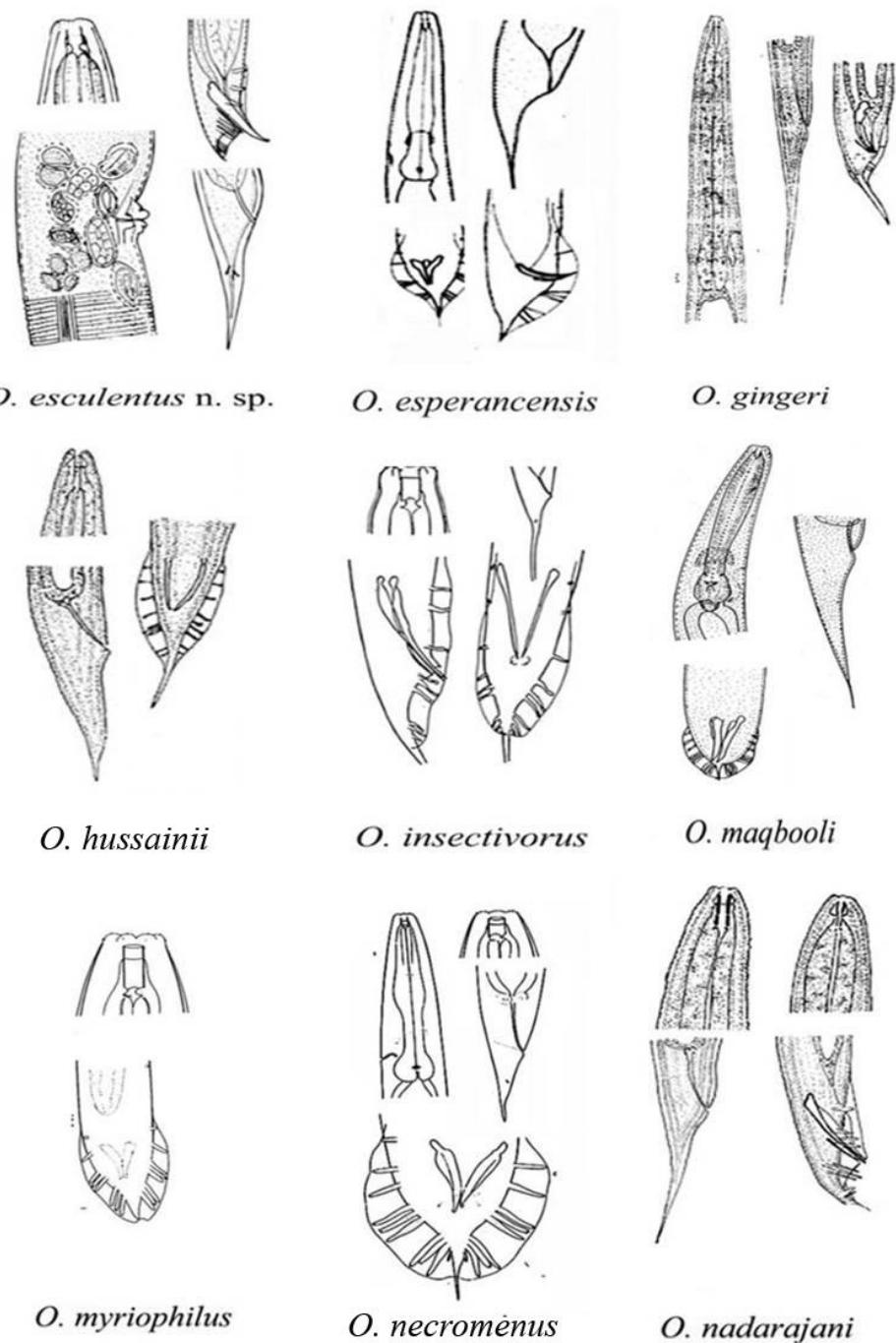


Fig. 17 b. Anterior and posterior regions of insectivora group of *Oscheius* (from original description)

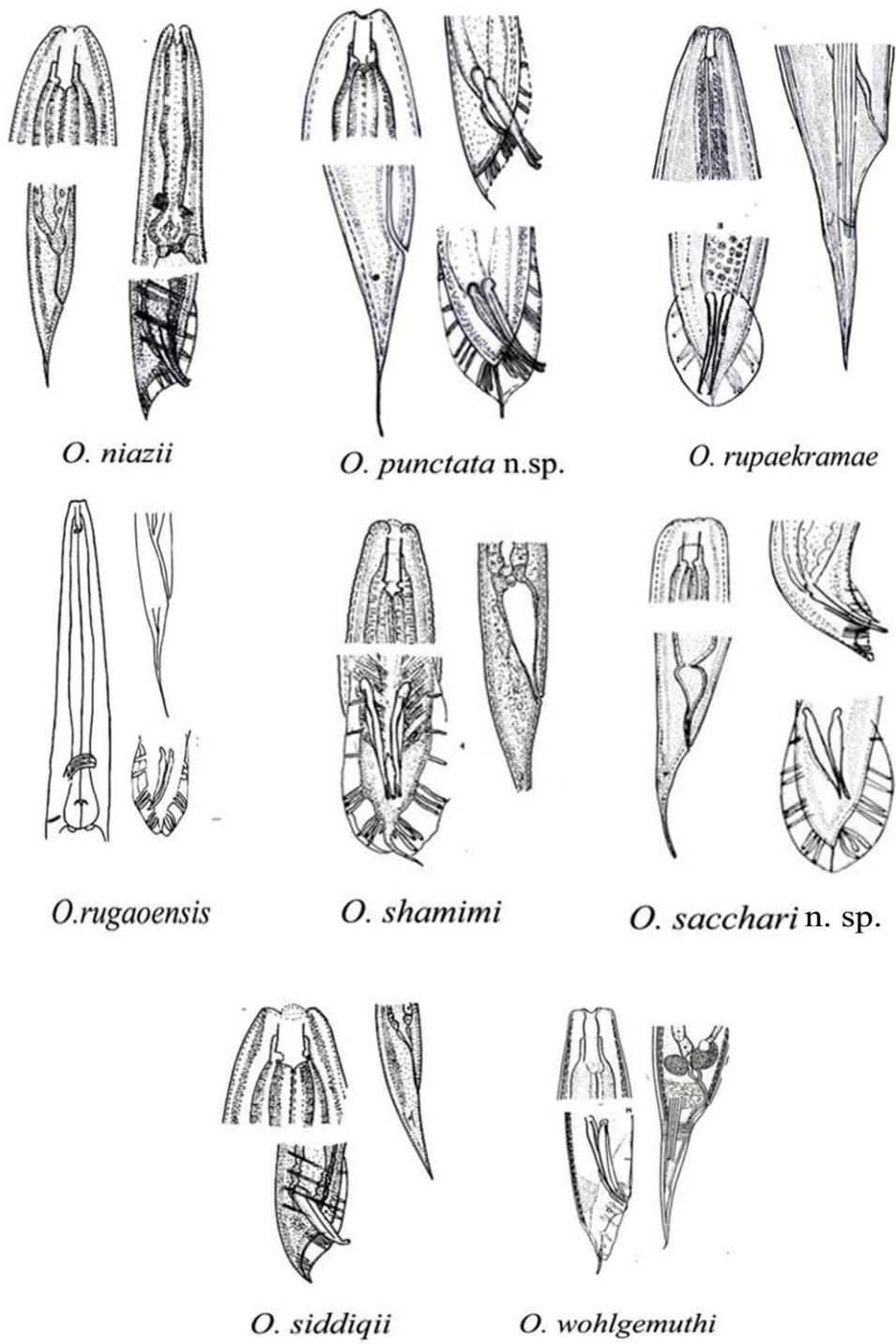


Fig. 17 c. Anterior and posterior regions of insectivora group of *Oscheius* (from original description)

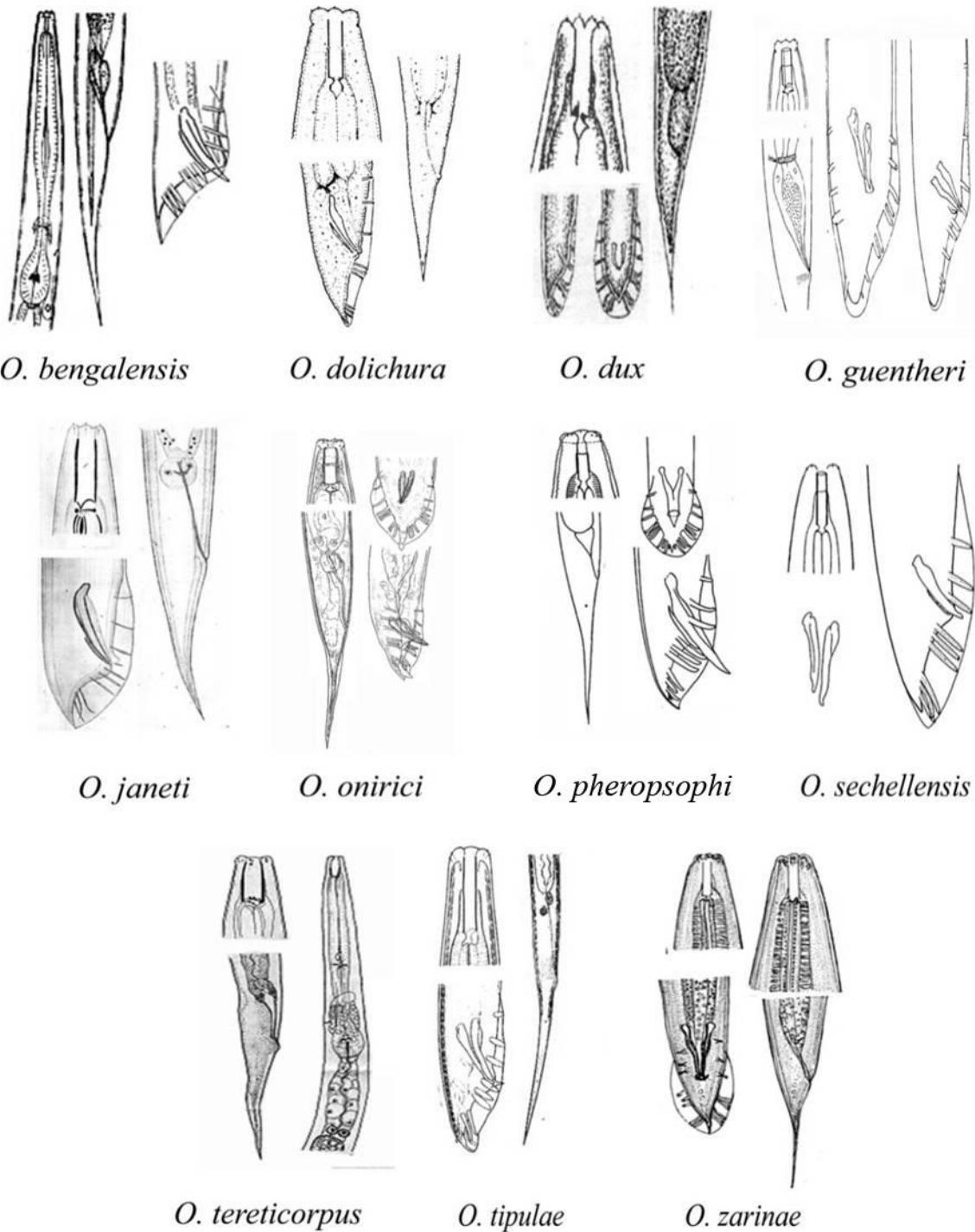


Fig. 17 d. Anterior and posterior regions of dolichura group of *Oscheius* (from original description).

- Dujardin, F. (1845). *Histoire naturelle des helminthes ou vers intestinaux*. Paris, 654pp.
- Joyce, S. A., Reid, A. P., Driver, F. & Curran, J. (1994). Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: Burnell, A. M., Ehlers, R. U. & Masson, J. P. (Eds.) *COST 812 Biotechnology: Genetics of Entomopathogenic Nematodes-Bacterium Complexes, Proceedings of Symposium & Workshop*. St. Patrick's College, Maynooth, Co., Kildare, Ireland. Luxembourg: European Commission, DG XII, pp. 178-187.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGgettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson T. J. & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0 *Bioinformatics*, 23, 2947-2948.
- Doi: 10.1093/bioinformatics/btm404.
- Liu, Q. Z., Mraćek, Z., Zhang, L. J., Puzačk, V. & Dong, L. M. (2012). Re-description of *Oscheius chongmingensis* (Zhang *et al.*, 2008) (Nematoda: Rhabditidae) and its entomopathogenicity. *Nematology*, 14, 139-149.
- Nadler, S. A., Bolotin, E. & Stock, S. P. (2006). Phylogenetic relationship of *Steinernema* (Cephalobina, Steinernematidae) based on nuclear, mitochondrial and morphological data. *Systematic Parasitology*, 63, 159-179.
- Doi: 10.1007/s11230-005-9009-3.
- Nuchanart, T., Sontirat S. & Chanpaiseang, J. (1999). Monoxenix culture of a new Thai strain of entomopathogenic nematodes (*Steinernema* sp. KB strain) on artificial media. *Thammasat International Journal of Science & Technology*, 4, 49-53.
- Schneider, A. (1866). *Monographie der Nematoden*. Berlin, 37pp.
- Scholze, V. S. & Sudhaus, W. (2011). A pictorial key to current genus groups of "Rhabditidae". *Journal of Nematode Morphology and Systematics*, 14, 105-112.
- Seinhorst, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, 4, 67-69. Doi: 10.1163/187529259x00381.
- Swofford, D. L. (2001). PAUP: *Phylogenetic Analysis using Parsimony (and other methods)*, version 4. Sunderland, MA, USA: Sinauer Associates (DVD). Available from: <http://paup.csit.fsu.edu>,
- Sudhaus, W. (1976). Vergleichende Untersuchungen zur phylogenie, Systematik, Okologie, Biologie and Ethologie der Rhabditidae (Nematoda). *Zoologica*, 43, 1-229.
- Sudhaus, W. (2011). Phylogenetic systemization and catalogue of paraphyletic "Rhabditidae" (Secernentea: Nematoda). *Journal of Nematode Morphology and Systematics*, 14, 113-178.
- Tabassum, K. A. & Shahina, F. (2002). *Oscheius maqbooli* n. sp. and observation on three know *Rhabditis* species (Nemata: Rhabditida) from sugarcane fields of Balochistan, Pakistan. *Pakistan Journal of Nematology*, 20, 1-21.
- Tabassum, K. A. & Shahina, F. (2008). *Oscheius andrassyi* sp. n. (Nematoda: Rhabditidae) with its key and embryonic and postembryonic development from Jhang, Pakistan. *Pakistan Journal of Nematology*, 26, 125-140.
- Tabassum, K. A. & Shahina, F. (2010). *Oscheius siddiqii* and *O. niazii*, two new entomopathogenic nematode species from Pakistan, with observation on *O. shamimi*. *International Journal of Nematology*, 20, 75-84.
- Tahseen, Q. & Syim-ul-Nisa (2006). Embryology and gonad development in *Oscheius shamimi* sp. n. (Nematoda: Rhabditidae). *Nematology*, 8, 211-221.
- Timm, R. W. (1956). Marine nematodes from The Bay of Bengal. I. Phasmigia. *The Journal of the Bombay Natural History Society*, 54, 87-90.
- Torriani, G., Mazza, G., Carletti, B., Benvenuti, C., Roversi, P. F., Fanelli, E., Luca, F. D., Troccoli, A. & Tarasco, E. (2015). *Oscheius*

- onirici* sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave. *Zootaxa*, 26, 533-548. Doi: 10.11646/zootaxa.3937.3.6.
- Ye, W., Torres-Barragan, A. & Cardoza, Y. J. (2010). *Oscheius carolinensis* n. sp. (Nematoda: Rhabditidae), a potential entomopathogenic nematode from vermicompost. *Nematology*, 12, 121-135. Doi: 10.1163/156854109X458464.
- Zhang, C., Liu, J., Xu, M., Sun, J., Yang, S., An, X., Gao, G., Lin, M., Lai, R., He, Z., Wu, Y. & Zhang, K. (2008). *Heterorhabditoides chongmingensis* gen. nov., sp. nov. (Rhabditida: Rhabditidae), a novel member of the entomopathogenic nematodes. *Journal of Invertebrate Pathology*, 98, 153-168. Doi: 10.1016/j.jip.2008.02.011.
- Zhang, K. Y., Liu, X. H., Tan, J., Wang, Y., Qiao, L., Yedid, G., Dai, C. S., Qiu, R. L., Yan, X. W., Tan, H. W., Su, Z. Y., Lai, R. & Gao, G. F. (2012). *Heterorhabditoides rugaoensis* n. sp. (Rhabditida: Rhabditidae), a novel highly pathogenic entomopathogenic nematode member of Rhabditidae. *Journal of Nematology*, 44, 348-360.
- Zuccon, A. & Zuccon, D. (2010). *Mr Entv.* 2.1. Program distributed by the authors.

(Accepted: May 25, 2016)