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Oscheius amsactae n. sp. (Nematoda: Rhabditida), a necromenic associate of red-hairy caterpillar, *Amsacta moori* (Lepidoptera: Arctiidae) from Kanpur district, India

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Oscheius amsactae n. sp. is described as a necromenic associate of red-hairy caterpillar *Amsacta moori* (Lepidoptera: Arctiidae) from Vigna radiata ((L.) Wilczek) cultivated field at Indian Institute of Pulses Research, Kanpur, India. This new species resembles with Rhabditis (O.) columbiana (Stock et al. 2005), however, it differs in having smaller body length (♀ $L = 1100$–1288 μm), lesser b value ($b = 4.9$–6.1), much smaller body length ($c = 14$–20.5), smaller spicule length (56–57 μm) and small gubernaculum length (20–27 μm), number of genital papillae and position of excretory pore. *O. amsactae* n. sp. can be compared with R. (O.) guentheri (Sudhaus and Hooper 1994) but differs in having a leptoderan bursa, different shape of spicule, number and arrangement of genital papillae. It also differs from R. (O.) necromena (Sudhaus and Schulte 1989) in having longer and narrower stoma and difference in shape of glottoid apparatus, smaller body length and shape of bursa. The new species closely resembles *O. shamimi* (Tahseen and Nisa (2006)) but differs in having narrower lip region (lip region diameter 7.1–7.9 μm *vs.* 11–12 μm in *O. shamimi*); shorter males ($L = 594$–804 μm *vs.* $L = 1012$ μm in *O. shamimi*). Moreover, the shape of spicule is also different, *O. amsactae* n. sp. has crochet-needle shaped tip of spicule whereas it is slightly curved in *O. shamimi*. Bursa is leptoderan in *O. amsactae* n. sp. whereas it is pseudopeloderan in *O. shamimi*. Additionally, molecular characterisation and electron microscopic studies confirm that it is distinctive from other species of the genus and it is a new species.

Keywords: entomopathogenic nematodes; morphology; molecular characterisation; taxonomy; Oscheius

Introduction

Several nematode species of the genus Rhabditis (Dujardin 1845) are associated with soil invertebrates. Andrássy (1976) erected genus Oscheius in the family Rhabditinae with type species *Oscheius insectivora* new combination for Rhabditis insectivora. Sudhaus and Hooper, 1994 accepted seven species of member of subgenus of Rhabditis (Oscheius) under Dolichura group. R. (O.) dolichura (Schneider 1866), R. (O.) dolichuroides (Anderson and Sudhaus 1985), R. (O.) guentheri (Sudhaus and Hooper 1994), R. (O.) pseudodolichura (Osche 1952), R. (O.) sechellensis

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(Potts 1910) and R. (O.) tipulae (Lam and Webster 1971) gave diagnosis of the subgenus Oscheius (Andrássy 1976) of Rhabditis. While in insectivora group they recognised five known species, R. (O.) caulleryi, (Maupas 1919), R. (O.) insectivora, (Korner 1954), R. (O.) lucianii, Maupas 1919 (syn. R. wohlgemuthi Völk 1950), R. (O.) myriophila (Poinar 1986), R. (O.) necromena (Sudhaus and Schulte 1989). Tabassum and Shahina (2002) as well as Tahseen and Nisa (2006) recognised Oscheius as genus and described *Oscheius maqbooli* and *O. shamimi*, respectively. In this article, we have followed the classification as proposed by Andrássy, 1976 and described the present new species under the genus Oscheius.

The genus Oscheius (Nematoda: Rhabditidae) comprises several free living mostly hermophroditic species. A number of species of this genus were recorded from cadaver/soil like Rhabditis (O.) tipulae re-described Sudhaus, 1993 associated with leather jackets larva of Tipula paludosa (Diptera: Tipulidae), R. (O.) myriophila (Poinar 1986); Rhabditis (O.) columbiana (Stock et al. 2005) associated with burrower bug, Cyrtomenus bergi (Hemiptera: Cydnidae). R. (O.) necromena (Sudhaus and Schulte 1989) associated with millipede *Oncocladosoma castaneum* (Diplopoda: Paradoxosomatidae), *R. caulleryi* (Maupas 1919) and *R. myriophila* (Poinar 1986) also cultured from millipedes (Sudhaus and Schulte 1989), R. (O.) pheropsophi (Smart and Nguyen 1994) associated with bombardier beetle, *Pheropsophus aequinoctialis* L. are shown to be of economic importance as a biological control agent. While R. (O.) maqbooli (Tabassum and Shahina 2002) and R. (O.) shamimi (Tahseen and Nisa 2006) were recovered from soil and R. (O.) guentheri (Sudhaus and Hooper 1994) was isolated from decaying rice plants. This new species was also recovered from cadaver of red-hairy caterpillar.

Whilst searching for natural enemies, a saprophagous rhabditid was isolated from a larva of red-hairy caterpillar, *Amsacta moori* Butler (Lepidoptera: Arctiidae) and described here as *Oscheius amsactae* n. sp. Red-hairy caterpillar, *A. moori* is one of the major pests of mungbean (Vigna radiata (L.) Wilczek) and urdbean (Vigna mungo (L.) Hooper) and is widely distributed in India. This insect feeds particularly all sorts of vegetation growing during rainy season (Chhabra et al. 1993). Its attack is particularly serious on other crops of economic importance, including soybean (Glycine max), pigeonpea (Cajanus cajan), sesame (Sesamum indicus), sunhemp (Crotalaria juncia), maize (Zeamay), clusterbean (Cyamopsis psoralioides) grown in various pulse growing regions of the country. Fields after fields are devastated by moving army of caterpillar and in the year of severe infestation, there may be a complete failure of the crop. The pest is active from mid-June to end of August and passes the rest of the year in pupal stage in soil.

Morphological and molecular evidence indicated that this is a new species of the genus Oscheius. The description of this species is provided herein for further studies to formulate a biopesticide in future for management of red-hairy caterpillar infesting mungbean and urdbean.

Material and methods

Nematode source

Nematodes were isolated from dead red-hairy caterpillar, *A. moori*, obtained from the rhizosphere of mungbean (Vigna radiata) crop cultivated at Indian Institute of Pulses Research Farm, Kanpur, Uttar Pradesh, India in March 2006 when temperature ranges from 35 to 40°C. The dead cadaver was placed on moistened

filter paper in Petri plate (100 × 15 mm^2). The Infective juveniles (IJs) isolated then multiplied in laboratory on last instar larvae of *Galleria mellonella* (L.) (Dutky et al. 1964) at room temperature 35 ± 2°C. The juveniles were obtained within 1 week after emerging from insect cadaver. The extracted nematodes were reared *in vivo* on *G. mellonella* larvae to test pathogenicity and confirmed Koch's postulates.

Morphological characterisation

Nematodes of different stages were killed in warm water at 60°C and fixed in triethanolamine formalin (TAF) and processed to anhydrous glycerine for mounting (Seinhorst 1959). The specimens were mounted on glass slides, rods support to avoid flattening. Observations were made from Leica DMLB research microscope equipped with differential interference optics. Specimens were measured and line drawing were made by Camera Lucida and calibrated with stage micrometre, while photographs of different stages were taken by digital Camera attached to the microscope.

Molecular characterisation

Molecular characterisation of the new species was done by analysis of the 18S internally transcribed spacer (ITS) ribosomal RNA genes; 100 to 500 specimens were pooled and used for DNA extraction. The genomic DNA extraction was carried out by Madani et al. (2004) method. Primers 5′ GTTTCCGTAGGTGAACCTGC 3′ and 5′ ATATGCTTAAGTTCAGCGGGT 3′ were used to amplify the ribosomal genes of the nematodes. The PCR conditions used were initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 1 min; 55°C for 1 min 30 s; 72°C for 2 min and a final extension of 72°C for 7 min. The 50 μl reaction mixture contained 1X PCR buffer, 200 μM each dNTP, 1.5 mM $MgCl_2$, 25 pmol of each primer, 1–10 ng of DNA and 2.5 U of Taq DNA polymerase. Five microlitres of the amplified product was run on 1% (w/v) agarose gel for determination of molecular weight and visualisation of the product. The amplified products were purified by Microcon columns (Millipore, USA) and sequenced with the help of ABI Prism 310 Genetic Analyser (ABI, USA) as per the instructions of the manufacturer using the Big Dye Terminator sequencing kit v. 2.0. The DNA sequences of ~350 bases thus obtained were compared with the sequences present in the public Domain (GenBank) for homology studies by BLASTN programme (Altschul et al. 1997).

Results and discussion

Oscheius amsactae *n. sp. (Figures 1 and 2)*

Measurements

See Tables 1 and 2.

Description

Adults: Cuticle finely striated, about 1 μm thick, lateral field pattern with nine ridges (10–11 longitudinal lines evenly spaced from each other) visible from

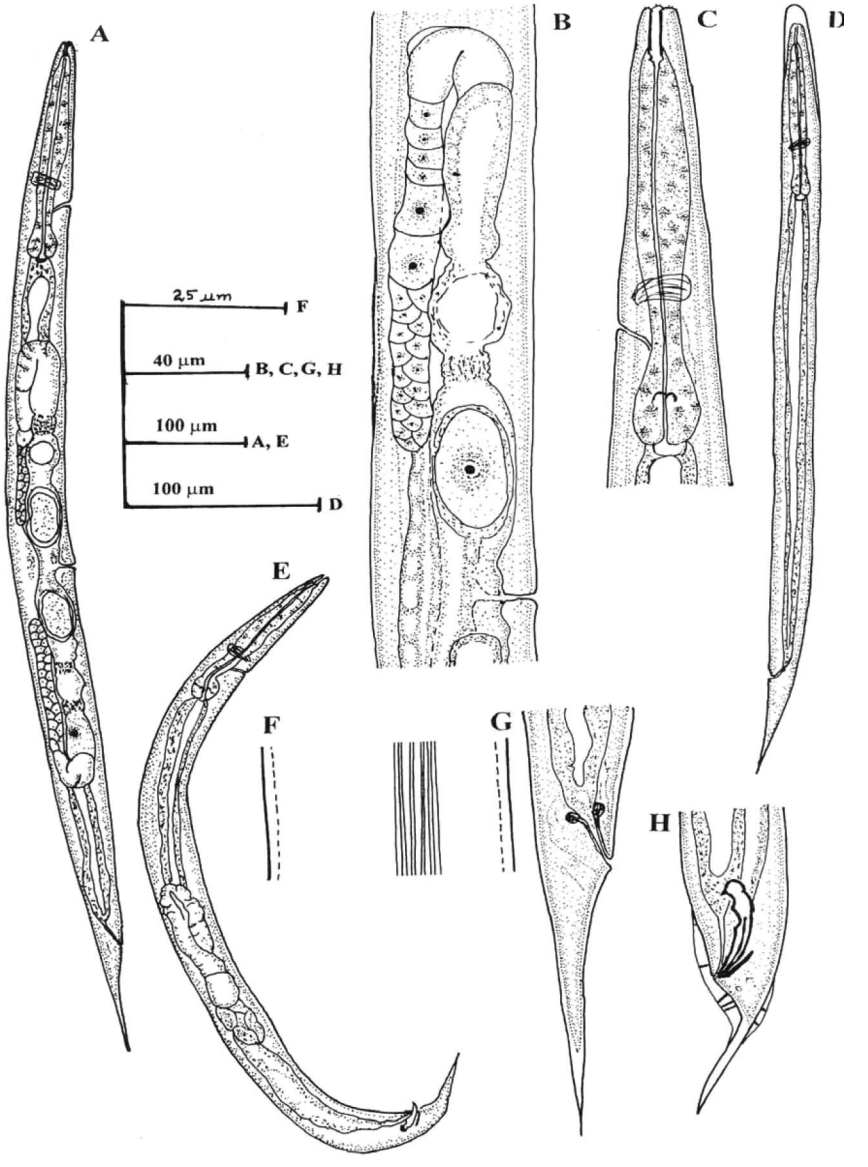

Figure 1. *O. amsactae* sp. n. Female (A–C, F,G): A, entire body; B, anterior reflexed gonad; C, anterior region; F, lateral field; G, posterior region; D, ensheathed juvenile; Male (E–H): E, entire body; H, posterior region.

mid-carpus to near phasmids in female or in bursal region of male. Six unfused lips each bearing two terminal sensillae. Lip region diameter 7–8 μm. Amphidial apertures elliptical, amphidial pouch pocket-like. Stoma long, narrow 5–6 times longer than diameter. Cheilostom with indistinct cheilorhabdions. Stegostom (pharyngeal collar) comprising 30% of stoma length. Glottoid apparatus isomorphic. Corpus cylindrical, occupying 60–65% of pharynx length. Median bulb absent. Isthmus forming 20–25% of pharynx length. Basal bulb spherical with well-developed valve, comprising 15–20% of pharynx length. Excretory pore

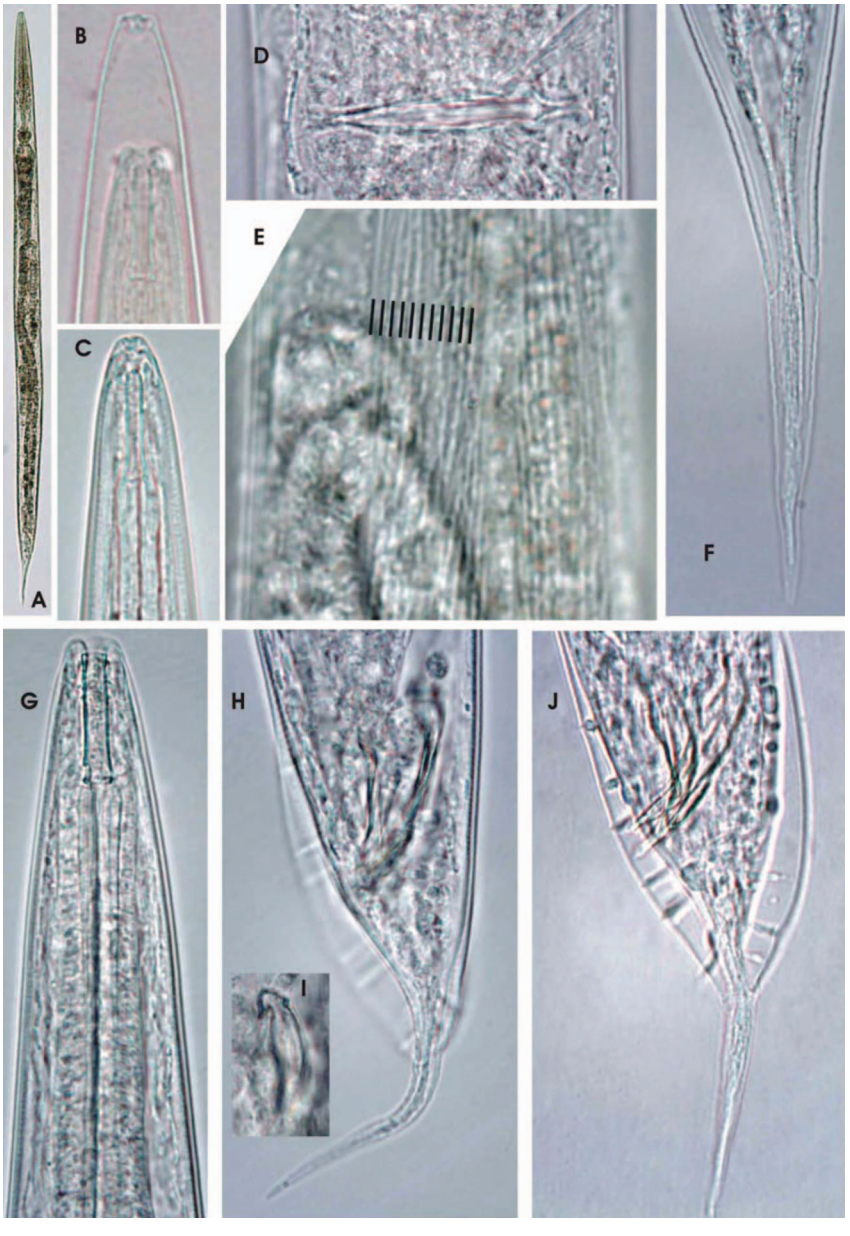

Figure 2. *O. amsactae* n. sp. Juvenile (A and B): A: Entire body of 3rd stage. B: 3rd stage with cuticle of 2nd stage; Female (C–F): C, anterior region; D, vulval region; E, lateral lines indicated by bar and reflexed gonad; F, posterior region; Male (G–J): G, anterior region; H, bursal rays lateral view; I, crochet needle-shaped tip of spicule; J, bursal rays in dorso-ventral view. Magnification, A: ×20, B–J: ×100.

located posterior to nerve ring, before basal bulb. Nerve ring located at 60% of pharynx. Phasmids conspicuous.

Female: Gonads didelphic, anterior branch situated on right of intestine, posterior branch also on right side. Dorsally reflexed ovaries often extending as far

Table 1. Morphometrics of *O. amsactae* sp. n. (all measurements in μm).

		Male			Female	
	Third stage juvenile	Holotype	First generation	Second generation	Normal form	Second generation
N	10		10	10	10	10
L	335–409	711.0	594–804	683.3–782.1	658.1–786.1	888.7–929.8
Greatest width	14.2–16.5	32.3	31.6–45.0	30.8–37.1	32.3–39.5	43.4–50.5
Stoma length	12.6–14.2	15.8	15.0–16.9	16.5–18.1	15.8–18.1	15.8–17.4
Stoma width	1.6	3.9	3.16–3.9	3.2–3.5	3.5–3.9	3.2–3.9
EP	68.7–82.0	121.7	86.9–113.7	114.5–131.1	109.0–130.3	135.8–138.2
EPW	11.8–15.8	28.4	27.6–35.5	30.0–31.6	25.2–31.6	30.8–31.2
NR	59.2–69.5	102.7	79.0–108.2	92.4–110.6	98.7–112.2	112.2–113.8
ES	90.8–97.9	166.6	134.2–169.0	135.0–177.7	159.5–177.7	165.1–173.8
Testis reflection (%)		10.5	10–26	15–16		
Anal body width	7.9–11.1	15.8	15.8–19.7	16.5–18.2	15.8–17.4	18.2–20.5
Tail length	48.9–60.4	47.4	41.1–55.3	59.2–87.6	64.7–80.5	64.7–83.7
Spicule length		26.9	30.8–35.5	25.2–27.6		
Gubernaculum length		10.3	13.4–16.5	10.3–11.8		
V					49.7–58.4	44.2–48.5
a	21.8–26.5	21.9	16.6–19.3	21.0–23.0	19.7–22.9	17.5–21.4
b	3.5–4.3	4.3	4.0–5.0	4.3–5.0	4.1–4.8	5.38
c	6.6–7.4	15.0	10.7–17.8	8.9–11.5	8.9–12.1	11.1–13.7
d (EP/ES) %	66.3–80.0	73.0	66.3–79.4	84.2–85.3	69.0–74.0	78.2–83.7
e (EP/tail)	113–192	256.6	157.1–275.0	192.0–193.2	146.0–185.3	162.2–213.4

EP, excretory pore; EPW, body width at level of excretory pore; NR, nerve ring; ES, total pharyngeal length.

Table 2. Comparative morphometrics of *O. amsactae* sp. n. with other known species of Oscheius (all measurements in μm).

	O. amsactae n. sp.	*O. shamimi*	*O. columbiana*	*O. necromena*	*O. guentheri*
Female					
L	658.1–786.1	760–1524	923–1805	830–1500	733–1071
b	4.1–4.9	4.2–6.3	5.2–8.0	4.2–6.3	5.6–6.6
c	8.9–12.1	6.8–13.8	8.3–10.0	9.7–13.9	5.4–7.0
Max. body weight	32.3–39.5	58–97	49–106	54–90	38–61
Stoma length	15.8–18.1	19–23	21–28	14–18	15–18
Stoma width	3.5–3.9	4.6–5.7	4.5	4.5	–
abd	15.8–17.4	21–32	22–38	45	–
Male					
L	594–804	938–1118	665–1163	671–950	655–799
b	4.0–5.1	5.1–5.5	3.9–5.4	4.3–5.0	4.9–5.8
c	10.7–17.8	25.1–31.1	13–16	11.5–17.6	18.4–20.8
Max. body weight	31.6–45.0	49–57	23–72	38–50	36–39
Spicule length	30.8–35.5	53–67	42–68	34–44	17–21
Gubernaculum length	13.4–16.5	22–28	16–24	12–23	NA
Length	335–409	422–563	439–535	535–670	305–355

Abd, anal body diameter.

as vulva. Vulva in form of a transverse slit. Young females with slightly protruding and symmetrical vulval lips. Mating plug absent in mature females. Rectum 1.3–1.5 anal body diameter long. Anal lips not salient. Tail conical, gradually tapering to a fine point, 4–5 anal body diameter long.

Male: Gonad monarchic, situated to left of intestine. Bursa closed, leptoderan with a short part of tail protruding beyond bursa. Eight pairs of bursal rays arranged as two pairs pre-cloacal and six pairs post-cloacal. First pair almost at the level of spicule head, 3rd, 4th and 5th forming a continuous pattern, and again 6th, 7th and 8th form a bench or continuous pattern. Pairs, 1st, 4th, 6th, 7th and 8th reach upto rim of bursa, 2nd and 3rd curved dorsally not reaching upto rim of bursa. Spicules slender, with crochet-needle-shaped tip of head. Lamina with one internal rib. Gubernaculum thin, elongate following contour of spicule.

Juveniles: Third stage juveniles ensheathed in cuticle of second stage juvenile. Sheath loose anteriorly, tightly attached to posterior region of body of third stage. Body slender, from anus to tail terminus. Cuticle with transverse striae. Lip region smooth; mouth closed. Stoma long and narrow, forming 13–14% of pharynx length. Pharynx and isthmus both long and narrow. Basal bulb valvate. Nerve ring located at isthmus level. Excretory pore located at middle pharynx level. Tail conoid with pointed terminus. Clumping of juveniles not seen.

Molecular characterisation

CGCCGGTTCCTGTTTTATATGTTACCACAAATTACGCTTCTTGGAAATG
ACGCCGACGCAGTCGGCATGTTCCCTTGAAGTGTGTCGTATCTCCACG
CAGTGTGCAGGAGTGAACGTCGTTCGCTAACTTGGGGAGGTTAGCTG
CTTCTGTCGGGGGCAACCTTGGCAGAAGACGATTGTTTGCGACTCGA
AAGAGTTGCTTAAAGGTCAGCTTTGACCGCACTGCAAACCAGCTGCT
GGCTAATGCGCCTTAATGACTTGATCAATGGCGGCAGCTCGGTTATT
TTCACATTCGAACTTTTCAATTTTGAAACTTTTAAAAAGATTAGCATT
AGTGTTGGATCGGTCGATTCGTAATCGATGAAGAA

Doesn't show significant homology with any sequences in the public domain, which shows it is to be a distinct and new species at molecular level.

Diagnosis and relationships

O. amsactae n. sp. is characterised by finely striated cuticle, lateral field pattern with 10–11 longitudinal lines in adult, 6 unfused lips each bearing 2 terminal sensillae, amphidial apurture elliptical, stoma long, cheilostom with indistinct cheilorhabdion, stegostem 30% of the stoma length, gloittoid apparatus isomorphic. Basal bulb spherical with well developed wall. Excretory pore posterior to nerve ring. Phasmids conspicuous, female gonad didelphic, dorsally reflexed ovary, vulva transverse slit with slightly protruding vuval lips, female tail conical gradually tapering to a fine point. Male gonad monarchic, bursa closed leptoderan with a short part of the tail protruding beyond bursa. Eight pairs of bursal rays arranged two pairs pre-cloacal and six pairs post-cloacal. Spicule slender with crochet-needle shaped tip of head. Lamina with one internal rib, gubernaculum thin elongate, following contour of spicule. Juvenile slender, mouth closed, stoma long and narrow, tail conoid with pointed terminus.

O. amsactae n. sp. closely resembles with *O. columbiana* (Stock et al. 2005), however, differs in having smaller body length (♀ $L = 1100–1288$ μm), lesser *b* value

(i.e. $b = 4.9–6.1$), much lesser c value ($c = 14–20.9$), smaller spicule length (56–57 μm) and smaller gubernaculum (20–27 μm). Number of genital papillae also differs from *O. columbiana* (No. of papillae = 9 *vs.* *O. amsactae* n. sp.). New species also differs from *O. columbiana* in position of excretory pore which is at level of basal bulb in *O. columbiana*.

New species can also be compared with *O. guentheri* (Sudhaus and Hooper 1994) but differs in having a leptoderan bursa, completely different shape of spicule and presence of a notch interiorly to vulva and number and arrangement of genital papillae. It also differs from *O. necromena* (Sudhaus and Schulte 1989) in having longer and narrower stoma and difference in shape of glottoid apparatus. New species is smaller in body length (828–1498 μm) and shape of bursa (serrated margin posteriorly in *O. necromena*).

The new species closely resembles *O. shamimi* (Tahseen and Nisa 2006) but differs in having narrower lip region (lip region diameter 7.1–7.9 μm *vs.* 11–12 μm in *O. shamimi*); shorter males ($L = 594–804$ μm *vs.* $L = 1012$ μm in *O. shamimi*); shorter female tail (tail length = 64–83 μm *vs.* 122–118 μm in *O. shamimi*); very short rectum (rectum = 22.9–26.8 μm *vs.* rectum = 68–77 μm in *O. shamimi*) and shorter spicule (31.2–35.5 μm *vs.* spicule = 60.3 μm in *O. shamimi*). Moreover the shape of spicule is also different, *O. amsactae* n. sp. has crochet-needle shaped tip of spicule whereas it is slightly curved in *O. shamimi*. Bursa is leptoderan in *O. amsactae* n. sp. whereas it is pseudopeloderan in *O. shamimi*.

Dauer juveniles of some species of this genus enter the body openings of insects, millipedes or other invertebrates get integrated by them and nematodes complete their life cycle within the host body. Maturation and reproductive phase of the nematodes depend on the bacteria associated with the decaying carcass.

Type host and locality

Type host: Red-hairy caterpillar, *Amsacta moori* (Lepidoptera: Arctiidae).
Type locality: Mungbean field, Main farm, Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India.
Type specimens: Holotype male, five paratype males, five paratype females and two paratype juveniles deposited at CABI Bioscience, UK.
Etymology: The new species is named after its host *Amsacta moori*

Bionomics

The life cycle of *O. amsactae* n. sp. is comparable with existing Oscheius species including an egg, four juvenile stages, and adult (male, female) stage. *O. amsactae* n. sp. was isolated from larva of red-hairy caterpillar. Third stage of juveniles of this species ingested while crawling aboveground soil strata. In case of *Galleria mellonella* and *Corcyra cephalonica* larvae, third stage juvenile enters though mouth and/or spiracles and then penetrate into haemocoel. Once in haemocoel, juveniles release symbiotic bacteria which kill the larvae at 30°C. The infected larva turns grey in colour.

Third stage juveniles carry symbiotic bacteria in their intestine. The bacteria was isolated from haemolymph of *O. amsactae*-infected C. cephalonica larva and streaked on MacConkey agar. Single colonies were isolated and subcultured subsequently. The colonies were light yellow in colour. Identification of bacteria has not been done. The presence of pathogenic bacteria in the intestine of third stage

juvenile is reported in Steinernematidae, Heterorhabditidae, in *Phasmorhabditis hermaphroditis* (Schneider 1866) and in many species of Oscheius like R. (*O. nacromena*) (Sudhaus and Schulte 1989) and R. (O.) columbiana (Stock et al. 2005).

Moreover, *in vitro O. amsactae* sp. n. can easily be cultured in great quantity in Wouts medium and Indian Institute of Pulses Research (IIPR) medium. The bioefficacy studies of *O. amsactae* sp. n. were carried out against final instar larvae of pod borer *Helicoverpa armigera* at room temperature 25–30°C. The mortality of *H. armigera* larvae by *O. amsactae* occurred within 96 h. This nematode can be used as potent biocontrol agent against lepidopteran and other group of insects.

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