Research Article

A New Species of Entomopathogenic Nematode *Oscheius anaseuli* n. sp. (Nematoda: Rhabditidae) from West Georgia (Caucasus)

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Abstract | A new species of the entomopathogenic nematode *Oscheius anaseuli* n. sp. was isolated from a soil sample from a nut orchard in the village Anaseuli, Guria Region, West Georgia. Morphological studies were carried out using an optical microscope, which confirmed the anatomy-morphological similarity of the nematode to the genus *Oscheius*. The main features to assign the new species to the *insectivorus*-group of the genus are leptoderan bursa and hook-like ends of spicula. *Oscheius anaseuli* n. sp. characterised by: didelphic-amphidelphic reproductive system, six separate lips with one sensilla-like brush on each lip, 8 lateral lines, open bursa and bursal papillae (1+1+1/3+3+ph). The most distinctive morphological feature distinguishing the new species from *insectivorus* species-group is the presence of 8 ridges and 9 incisures. An updated key to the species of the *insectivorus*-group is provided. *Oscheius anaseuli* n. sp. is morphologically most similar to the following species of *insectivorus*-group: *O. colombianus*, *O. muriophilus, O. punctata* and *O. insectivorus*. As the laboratory studies evidence, the new species has proved to be pathogenic for test insects *Galleria mellonella* and *Tenebrio molitor*. The nematodes caused insect mortality after 24 hours, and infective juveniles emerged from insect carcasses 36 hours after the insects died. Nematode inoculation resulted in 63.5% mortality of *G. mellonella* within 5 days and 100% mortality of *T. molitor* within 48 hours. The entomopathogenicity of nematodes was evaluated by considering the different effects of nematodes on insects. Nematodes showed the best results against *T. molitor*. Thus, the new nematode species has potential as a biological control agent.

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Introduction

Most nematodes of the Rhabditidae family are free-living hermaphroditic species. Some are ecologically related to bacteria, fungi, and insects (Lieutier and Laumond, 1978; Poinar, 1986; Smart and Nguyen, 1994; Stock *et al.*, 2005; Adams *et al.*, 2006). Nematodes in this group have developed specific symbiotic relationships with genera *Xenorhabdus* (Adams *et al.*, 2006; Campos-Herrera *et al.*, 2015) and *Photorhabdus* (Blackburn *et al.*, 2016) of the family Enterobacteriaceae. As compared to *Steinernema*

and *Heterorhabditis*, entomopathogenic nematodes (EPNs) of genus *Oscheius*, have similar life cycles and stages, such as non-feeding infective juveniles, which can act as carriers of insect pathogenic bacteria of the genus *Serratia* (Poinar, 1990; Stock *et al*., 2005). Some strains of *Serratia marcescens* bacteria are pathogenic to insects and cause the death of host insects within 24-48 hours (Ye *et al*., 2010). To identify the species of the genus *Oscheius*, the classifications of nematodes by Andrássy (1976) and Sudhaus (1976) were used. Within the genus *Oscheius*, Andrassy (1976) distinguished a group of insectivores; then, Sudhaus and Hooper (1994) placed *Oscheius* in the family Rhabditidae, and the genus *Oscheius* was further divided into *Dolichura* and *insectivorus*-groups based on the bursa's structure. Species of the *insectivorus*-group have a leptoderan bursa, while *dolichura*-group species have a peloderan bursa. The phylogeny of the genus Oscheius has recently been reviewed and its content revised by several authors (Abolafia and Pena-Santiago, 2019). Currently, according to Sudhaus (2023), there are 16 valid species in the Dolichura group and 23 in the Insectivora-group.

It should be noted that only six species from the *insectivorus*-group: *O. carolinensis* (Ye *et al*., 2010), *O. niazii, O. siddiqii* (Tabassum and Shahina, 2010), *O. amsactae* (Ali *et al*., 2011), *O. microvilli* (Zhou *et al*., 2017) and *O. safricana* (Serepa-Dlamini and Grey, 2018), and only one species *O. onirici* from group *dolichura* were considered EPNs (Torrini *et al*., 2015). The isolation of local strains or the identification of species adapted to local climatic conditions is important to improve the biocontrol potential (Stock *et al*., 2005). Entomopathogenic nematodes (EPNs) are soil-dwelling nematodes that serve as potent biological control agents (BCAs) against pests of various crops (Kaya and Gaugler, 1993; Shapiro-Ilan *et al*., 2012). Recent studies (Gorgadze *et al*., 2025) confirmed the presence of *O. cyrus, O. insectivorus*, and *H. bacteriophora* in hazelnut orchards of Georgia. The obtained data indicate that Georgian soils are rich in entomopathogenic nematodes. Hazelnut is noteworthy not only as a valuable food and export commodity for Georgia, but also for the global market. To improve its quality and ensure the production of environmentally friendly products, the aim of our study was to isolate entomopathogenic nematodes from local hazelnut plantations that could be utilized in the biological control of hazelnut pests.

Materials and Methods

Collection and isolation of nematodes

Nematodes were isolated from soil samples collected in 2022 in the Anaseuli village, Guria Region, West Georgia. The soil samples were taken from the nut orchard at a depth of 10-15 cm in a zigzag manner, 20-25 meters apart (Metlitsky, 1985). Each volume of soil sample (300-400 g) was placed in a plastic bag, labeled, and sent to the laboratory for nematode analysis. Entomopathogenic nematodes were detected in soil samples using the *Galleria mellonella* L. (Lepidoptera: Pyralidae) baiting method (Bedding and Akhurst, 1975; Kaya and Stock, 1997). Twenty-six microplastic containers (14 x 7 x 8 cm) were used to isolate nematodes from the soil, and 250-300 g of soil sample and 6 specimens of the last instar larvae of *G. mellonella* were placed in each container. After 72 hours of the exposition, the dead insects were placed in White's traps (White, 1927) for nematode incubation.

Laboratory culture of Oscheius anaseuli n. sp.

To obtain a pure population of nematodes, one adult female was cultivated on a damaged *G. mellonella* larval body for 8–10 days at 24°C.

The population of adult and invasive nematode larvae obtained from the adult female were used for morphological and morphometric studies.

For the experiment, nematodes were cultured in large numbers on fourth- and fifth-stage *G. mellonella* larvae (Kaya and Stock, 1997). For morphometric studies, 25 invasive larvae were inoculated per Galleria larva. To determine the nematode species, adult nematodes (females, males, and invasive larvae) were isolated from the Galleria corpse on days 3 and 5. The isolated nematodes were used to prepare slides for microscopic examination. The grown invasive larvae were washed three times by sedimentation in distilled water and placed in a refrigerator at 7 °C for further morphological and morphometric studies.

Morphology and morphometrics of nematodes

Under a light microscope (Motic Digital Microscope, DMB Series B1 Advanced Series), nematodes were examined in a drop of water to which agar was added from time to time, or softened in Ringer's solution by heating to 60 °C (Filipjev, 1934). Twenty-seven exemplars of infectious juvenile, 20 females and 21 males of adult nematodes were used to make

temporary preparations for nematode measurement. The nematodes were placed on a slide in a drop of water and covered with a coverslip. Permanent slides were prepared by fixing nematodes in hot TAF (Courtney *et al*., 1955) and processed according to the Seinhorst (1959) by gradually displacing water from glycerol. To better observe the morphology of nematode features such as stoma structure, lateral lines, spicula, and gubernaculum, a 100x magnification was used. The cover-glasses were supported by glass filaments to prevent nematode flattening. The morphometric features were selected according to (Hominick *et al*., 1997). Measurements were taken in micrometers (µm) and expressed as mean±standard deviation. All observations on slides, drawings, and photographs were made with a light microscope.

Entomopathogenicity of nematodes

The entomopathogenicity of a newly isolated nematode species from a nut orchard was evaluated in vitro using the Petri dish method (Ali *et al*., 2005) against fifth-instar larvae of *Galleria mellonella* and sixth- to seventh-instar larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Each 9-cm Petri dish contained two layers of moistened filter paper and ten test larvae. Dishes were inoculated with 3,000 infective juveniles (equivalent to 300 nematodes per insect), while control dishes received only distilled water. For each insect species, five replicates and one control were performed. Treated larvae were maintained at 24 °C in a thermostat, and mortality was recorded every 24 hours following Abbott's Method (1925). Half of the cadavers were transferred to modified White traps (White, 1927; Nguyen, 2005) to assess nematode reproduction, while the remaining half were dissected to confirm nematode presence. Residual cadavers were subsequently placed in White traps to monitor nematode emergence. Emerged nematodes were stored at 7 °C for subsequent morphological identification.

Results

Oscheius anaseuli n. sp

(Figures 1 and 2)

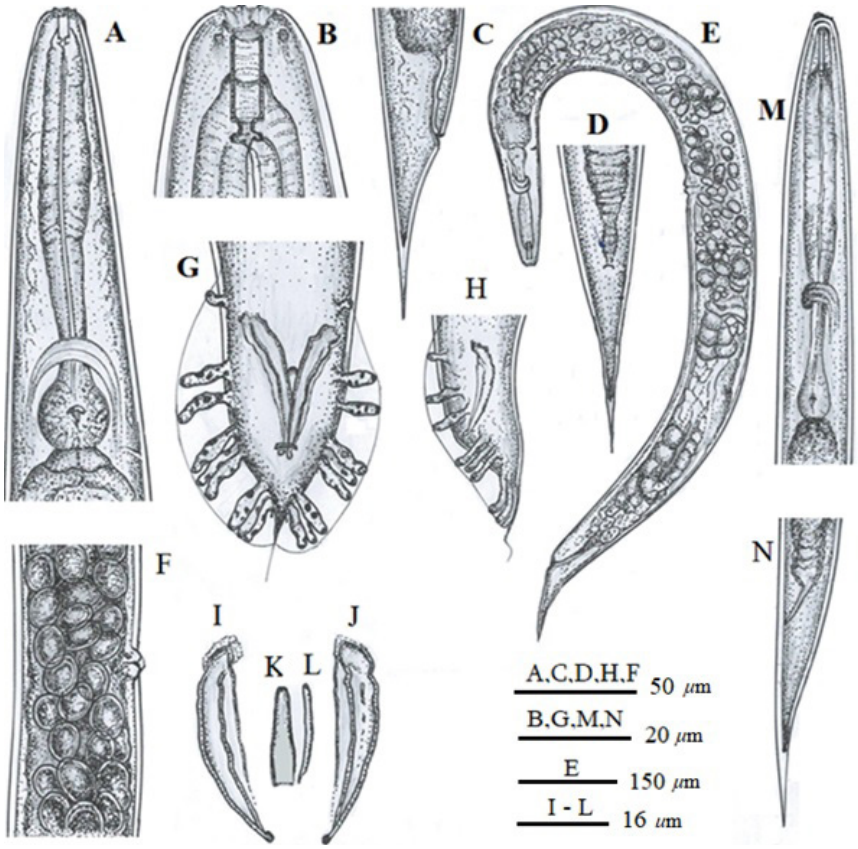

Figure 1: *Oscheius anaseuli n. sp. A-F: Female. A: Pharyngeal region, lateral view; B: Anterior end with a stoma; C, D: Tail in lateral and ventral view, respectively; E: Entire female; F: Vulva region, lateral view. G-L: Male. G, H: Tail with a bursa and a pointed stem in ventral and lateral view, respectively; I, J: Spicula, right and left lateral views; K, L: Gubernaculum, dorsal and ventral view, respectively; M, N: Third-stage juvenile. M: Pharyngeal region, unsheathed in cuticle of second-stage juvenile; N: Tail, lateral view.*

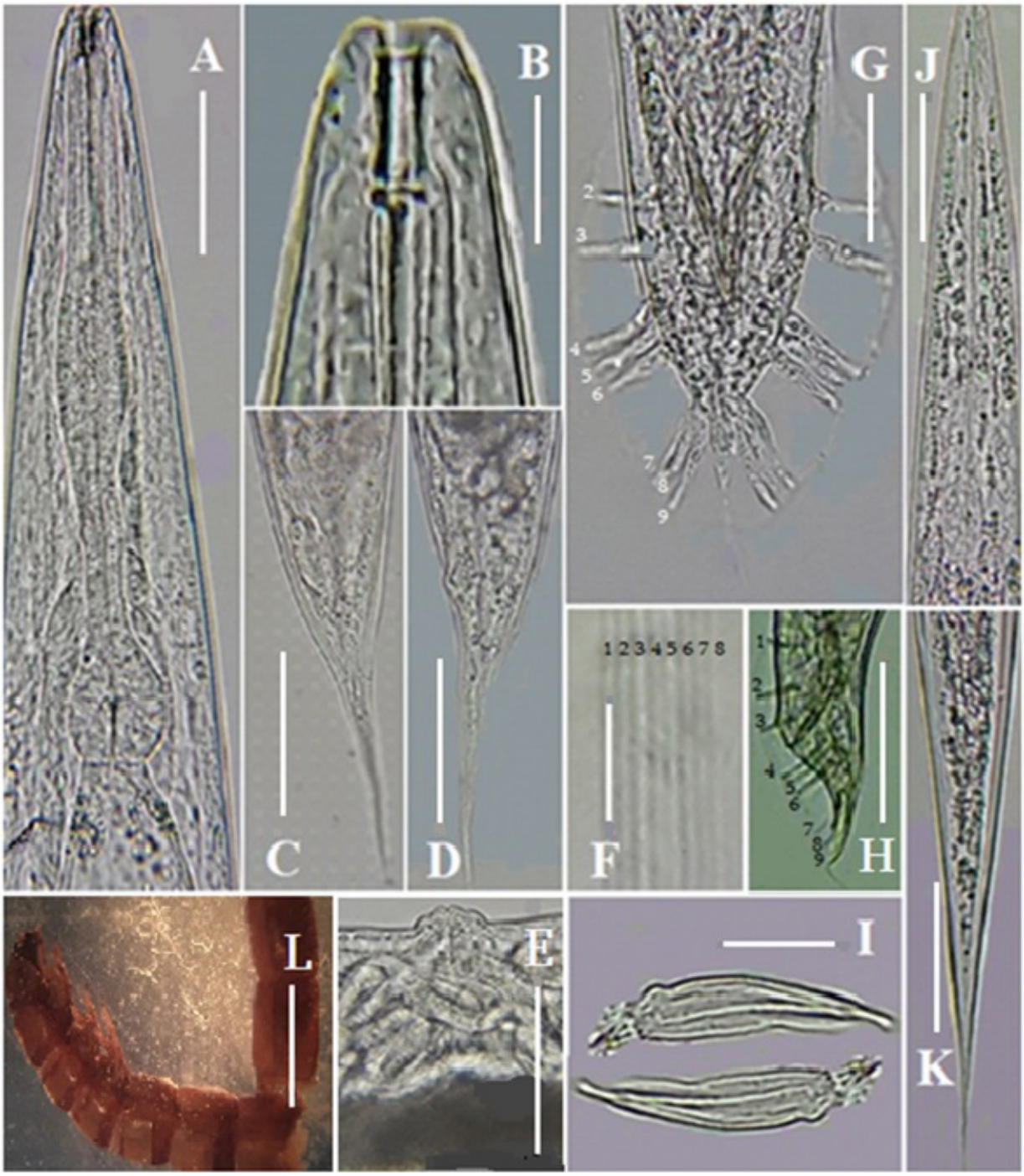

Figure 2: *Oscheius anaseuli n. sp. A-F: Female. A: Pharyngeal region, lateral view; B: Anterior and; C, D: Tail in lateral and ventral view, respectively; E: Vulva region, lateral view; F: Lateral field in mid-body region. G-I: Male. G, H: Tail with bursa in ventral and lateral view, respectively; I: Spicules, ventral view; J, K: Infective juvenile. J: Pharyngeal region; K: Tail lateral view. (Scale bar: A, C-E=50 µm; B, G, J, K=20 µm; F, I=16 µm).*

Measurements
See Tables 1, 2

Description
Hermaphroditefemales: Body straight when heated. Anterior and posterior parts of body equally conical. Cuticle smooth and its thickness in the head and tail is greater (4.6 µm) than in the middle part of the body (2.5 µm). It has 8 lateral lines of equal thickness and 13 µm wide. Body diameter at labial lips 16.2 µm (15.6-18.2). It has six small lips on the head, each with a single lip sensillum. Oval-shaped amphids well-marked; just below the labial sensillum, their canals are situated between the lateral lips. Stoma long and tubular; its length is nearly four times its width. Stoma wall structures are isomorphic; it is undifferentiated, except for gymnostome and stegostome; telostome is narrow and funnel-shaped. Thin pharyngeal collar 55%

Table 1: *Measurements of adults and infective juveniles of Oscheius anaseuli n. sp. (All measurements are in µm and the form: mean ± s. d. range).*

Character	Male		Hermaphrodite Female	Infective juvenile
	Holotype	Paratypes	Paratypes	Paratypes
n	1	21	20	27
Total body length (L)	1123	1121 ± 123	1862 ± 197	602 ± 53
	-	(915-1456)	(1487-2204)	(520-738)
Maximum body diameter (D)	73	75 ± 4	111± 12	27 ± 2
	-	(65-86)	(83-132)	(26-34)
Stoma length	18.2	17.2 ± 1.2	19.1 ± 1.6	18.2 ± 1.3
	-	(15.0-18.2)	(15.6-20.8)	(17.9-18.8)
Stoma diameter	4.9	4.8 ± 0.7	5.0 ± 0.1	2.6
	-	(3.9-5.2)	(4.9-5.2)	-
Excretory pore from anterior end (EP)	179	182 ± 11	224 ± 14	109 ± 4
		(163-197)	(192-247)	(101-119)
Nerve ring from anterior end (NR)	138	134 ± 12	145 ± 8	94 ± 4
		(101-151)	(130-161)	(83-99)
Esophagus length (ES)	190	194 ± 10	214 ± 10	135 ± 4
		(182-234)	(197-234)	(130-145)
Tail length (TL)	57	60 ± 10	129 ± 11	87 ± 7
		(47-67)	(109-156)	(78-106)
Anal body diameter (ABD)	33	32 ± 4	39 ± 3	15 ± 1.4
		(28-41)	(34-47)	(13-20)
V% = (AV/L) x 100	-	-	49.6 ± 1.1	-
	-	-	(46.5-51.7)	-
a = L/D	15.3	14.8 ± 1.0	16.6 ± 1.1	21.7 ± 1.0
		(13.0-17.0)	(15.0-19.2)	(19.7-23.5)
b = L/ES	5.9	5.7 ± 0.6	8.5 ± 0.6	4.4 ± 0.3
		(4.8-7.7)	(7.5-9.5)	(3.8- 5.2)
c = L/T	19.7	18.4 ± 1.9	14.3 ± 1.0	6.8 ± 0.4
		(14.2-21.7)	(12.6-17.1)	(6.0-7.7)
c' = T/ABD	2.9	2.8 ± 0.3	2.0 ± 0.1	2.5 ± 0.2
		(2.3-3.7)	(1.9-2.3)	(1.9-2.9)
D% = (EP/ES) × 100	94	93 ± 7	103 ± 5	80 ± 4
		(77-101)	(94-114)	(69-91)
Spicula length (SL)	42	44 ± 3	-	-
		(36-49)	-	-
Gubernaculum length (GU)	18	18 ± 1	-	-
		(17-21)	-	-
SW % = (SL/ABD) × 100	127	136 ± 19	-	-
		(100-164)	-	-
GS% = (GL/SL) × 100	43	41 ± 3	-	-
		(36-47)	-	-
E% = (EP/TL) × 100	314	301 ± 21	174 ± 12	125 ± 9
		(259-348)	(150-192)	(95-146)
H% = (HT/TL) × 100	-	-	-	37 ± 6
	-	-	-	(25-49)

Table 2: *Comparison of morphometric data for female, male and Infective juvenile of species in the insectivorus-group of Oscheius (All measurements are in µm and in the form: mean ± s. d. range).*

Character	*O. anaseuli* n. sp.	*O. colombianus*[1]	*O. muriophilus*[2]	*O. punctata*[3]	*O. insectivorus*[4]
Female					
L	1862	1288	1320	1412	-
	(1487-2204)	(923-1805)	(1200-1500)	(1217-1680)	(1375-3205)
a	16.6	17	-	15	-
	(15.0-19.2)	(15-19)	(19.1-21.3)	(12.5-18.4)	-
b	8.5	6.5	-	8	-
	(7.5-9.5)	(5.2-8.0)	(6.8-7.7)	(7.0-9.6)	(8.8-13.5)
c	14.3	9.2	-	10	-
	(12.6-17.1)	(8.3-10.0)	(2.2-3.5)	(8.2-16.8)	(12.4-23)
Max. body diam.	111	81.5	62	91.3	-
	(83-132)	(49-106)	(57-70)	(70-112)	(125-186)
Stoma length	19.1	23	20	14	-
	(15.6-20.8)	(21-28)	(18-21)	(12-15)	
Stoma diam.	5.0	4.5	3.2	-	-
	(4.9-5.2)	(3.5-7)	-	-	
Pharynx length	214	205	185	174	-
	(197-234)	(176-225)	174-193	(157-190)	-
Tail length	129	140	117	110	-
	(109-156)	(110-167)	(108-135)	(80-170)	-
ABW	39	31	25	28	-
	(34-47)	(22-38)	(22-28)	(14-35)	-
Lateral line	8	4	-	6	-
Male					
L	1121	915	1270	944.7	-
	(915-1456)	(665-1163)	(830-1470)	(800-1118)	(1587-3252)
a	14.8	18	-	15.6	-
	(13.0-17.0)	(17-29)	(18.4-21.9)	(12.2-18.6)	(20-28)
b	5.7	4.9	-	6.5	-
	(4.8-7.7)	(3.9-5.4)	(5.16-7.36)	(5.2-7.7)	(7.3-13.4)
c	18.4	14.5	-	27	-
	(14.2-21.7)	(13-16)	(14.8-20.4)	(19.5-36.3)	(21.1-46.9)
Max. body diam.	75	49	63	60	-
	(65-86)	(23-72)	(38-80)	(53-72)	(76-153)
Spicula length	44	21	47	54.4	-
	(36-49)	(19-24)	(32-54)	(50-60)	(85-118)
Gubernaculum length	18	20	47	21	-
	(17-21)	(16-24)	(32-54)	(20-25)	(37-54)
Infective juvenile					
L	602	505	564	-	-
	(520-738)	(439-535)	(504-611)	-	(666-725)

References: [1]after Stock et al., 2005; [2]after Poinar, 1986; [3]after Tabassum et al., 2016; [4]after Körner, 1954.

of the stoma length. Pharynx cylindrical and well-developed. Basal bulb is clearly shaped and rounded, with a well-developed flap bulb; Basal bulb length 36 (31-39) µm and 34 long (28-36) µm. Procorpus well developed, constituting 58% (50-62) of the length of pharynx. Nerve ring at posterior part of isthmus or

at 68-74% of the length of the pharynx. Excretory aperture at 103-112% of the length of the pharynx. Reproductive system didelphic-amphidelphic. Eggs oval (64.5 µm long and 38.5 µm wide). Uterus large with 95.6 eggs. Vulva located in the middle of the body with a transverse incision; each individual has a slightly recessed vulvar area, compared to the width of the body; in sexually mature females, the lips of the vulva are elevated and protected by a transverse flap. Rectum broad and 84 µm long or twice longer than anal body width. Tail-conical and elongated, with a pointed end. Phasmids located at one-third tail length from anus on the ventral side of the body.

Males

After heat-relaxing body is predominantly J-shaped. Cuticle is smooth. Number, position and morphology of the lips, sensilla, and amphids on the head are the same as in females. The body diameter at the labial lips is 16 (14-17) µm. The testis is monodelphic, and its anterior section is bent ventrally. The spermatocytes are positioned in several rows. Spicula paired, broad and wide, separate and slightly curved ventrally. Head of the spicula has triangular contours, and its distal part has hook-shaped ends. Gubernaculum thin and slender, making up 39.2% of length of spicula. Bursa open and leptoderan, and has nine pairs of papillae; 3 pairs of bursa papillae precloacal, and six pairs 4-6 and 7-9 postcloacal; 3 precloacal pairs at different distances from each other; first pair slightly separated from 2nd and 3rd pairs; pairs 4-6 and 7-9 closer together than pairs 1-3; they located according to formula 1+1+1/3+3+ph. Phasmids small and located at base of the ninth pair of papillae. Filiform terminus 13.5 (7.8-19.5) µm long; Bursa has a depression near terminus.

Infective juvenile

Body of the heat-killed juveniles is straight and slender. Body gradually narrows from base of pharynx to anterior part of body and from anus to end of tail. Cuticular larval outgrowths of second stage located asymmetrically on external terminal surface of head. Oral cavity closed. Cuticle is smooth. Stoma long and narrow, with its length 7 times its width. Compared to adults, amphidial diaphragms more behind lips. Esophagus and isthmus long and narrow. Basal bulb oval and elongated. Excretory duct located at level of basal bulb. Rectum wide and long. Anal canal does not open outward. End of tail of second stage of juvenile elongated and pointed, while that of third stage of juvenile relatively blunt.

Type host and locality

Natural host unknown, the nematode species was identified in the soil of the nut orchard. It was collected in the village of Anaseuli, Guria Region, West Georgia. The soil material was sampled at an altitude of 132 m above sea level (GPS coordinates: 41° 54′ 05″ N, 41° 59′ 11″ E).

Type material

Holotype: male (one slide ISUZI0004409); Paratype: males (four slides with 21 specimens; ISUZI0004410-ISUZI0004413); Paratype: females (four slides with 20 specimens; ISUZI0004414- ISUZI0004417) and Infective juveniles (Three slides with 27 specimens; ISUZI0004418-ISUZI0004420) are stored in the Collection Museum of the Institute of Zoology, Ilia State University, Tbilisi, Georgia (Figures 1, 2 and Table 1).

Differential diagnosis

Oscheius anaseuli n. sp. belongs to the *insectivorus*-group. The new species has a valvate bulb, didelphic-amphidelphic reproductive system, 8 ridges and 9 incisures, medium-sized male (vs. 1121 µm), medium-sized spicula (vs. 44 µm), leptoderan, open bursa without notch, 9 pairs of bursal papillae positioned according to formula 1+1+1/3+3, and spicula with hook-shaped distal ends. Owing to these typical features, *O. anaseuli* n. sp. has been attributed to the *insectivorus*-group (Sudhaus and Hooper, 1994; Sudhaus and Fitch, 2001). The list of species in this group is compiled and updated (Stock *et al.*, 2005) and 5 (Tabassum *et al.*, 2016).

Oscheius anaseuli n. sp., with its morphology, is closest to *O. colombianus* (Stock *et al.*, 2005), *O. muriophilus* (Poinar, 1986), *O. punctata* (Tabassum *et al.*, 2016), *O. insectivorus* (Körner, 1954) species.

Within the *insectivorus*-group, *O. anaseuli* n. sp. is very similar to *O. colombianus* but differs in having the shorter female and male bodies (vs. 1288 and 915 µm, respectively) (see Table 2) and smaller 'c'-index values in both female and male (vs. 9.2 and 14.5, respectively), longer female tail and smaller male body diameter (vs. 140 and 49 µm, respectively), shorter spicula (21 µm), less number (4) of lateral lines, and shorter infective juveniles (505 µm).

Oscheius anaseuli n. sp. also resembles *O. muriophilus*, but differs from it in having a shorter female body and longer male body (vs. 1320 and 1270 µm, respectively), smaller female and male body diameters (vs. 62 and 63 µm, respectively), shorter pharynx of female (vs. 185 µm), higher male and female "a" coefficient (vs. 19.1-21.3 and 18.4-21.9, respectively), longer spicula and gubernaculum (vs. 47 and 28 µm, respectively), and shorter infective juveniles (vs. 564 µm).

The new species is very similar to *O. punctata*, but differs in having shorter female and male bodies (vs. 1412 and 944.7 µm, respectively), less female "c" coefficient and higher male "c" coefficient (vs. 10 and 27, respectively), maximum width of male and female less bodies (vs. 91.3 and 111 µm respectively), shorter length of stoma and esophagus of female (vs. 14 and 174 µm), smaller body width at anus (vs. 28 µm), less lateral lines (6), higher male "c" coefficient (27) and larger spicula and gubernaculum (vs. 54.4 and 21 µm, respectively).

Oscheius anaseuli n. sp. is also very similar to *O. insectivorus*, but differs in having maximum body diameter of males and females (vs. 125-186 µm and 76-153 µm, respectively), longer male body (vs. 1587-3252 µm, respectively), larger male "a" and "c" coefficients (vs. 20-28 and 21.1-46.9, respectively), larger male body diameter (vs. 76-153), and larger spicula and gubernaculum (vs. 85-118 µm, respectively) (Table 2).

Entomopathogenicity of nematodes

The results showed that the new species of nematodes were pathogenic for *G. mellonella* and *T. molitor*. The nematodes caused death of the insects within 24 hours. The mortality rate of *G. mellonella* was 63.5% within 5 days after inoculation with the nematodes, while the highest rate was observed for *T. molitor,* where 100% mortality was achieved within 48 hours after inoculation with the nematodes. Nematodes began to emerge from the insect corpse 36 hours after death. The entomopathogenicity of the nematodes was assessed by the various effects of the nematodes on the insects. In both experiments, high mortality of the larvae of the experimental insects was observed, especially *T. molitor. G. mellonella* demonstrated higher nematode productivity, although it took longer for the nematodes to emerge from the host compared to *T. molitor*, which demonstrated lower nematode productivity but emerged from the insect carcasses faster. The mortality rate for *T. molitor* was higher than that of *G. mellonella*. Research suggests that *O. anaseuli* may be used as an effective biological control agent for pests, particularly in agriculture.

Discussion

The presence of *Oscheius anaseuli* n. sp. in Georgian hazelnut orchards confirms the prevalence of entomopathogenic nematodes in local agroecosystems. Isolation using the Galleria bait method underscores the suitability of this approach for detecting nematode diversity in soil environments. However, species-level identification within the genus Oscheius remains challenging due to pronounced morphological similarities, which frequently result in misidentifications. Such inaccuracies hinder biodiversity assessments and obscure the evolutionary relationships of the genus.

To address these challenges, the *O. anaseuli* n. sp. population from a walnut orchard was characterized through combined morphological and morphometric analyses. Morphological similarities were observed with *O. colombianus* (Stock *et al.*, 2005) and *O. muriophilus* (Poinar, 1986), reflecting close resemblance in structural features. Comparable traits were also noted in *O. punctata* (Tabassum *et al.*, 2016), while diagnostic overlap was evident with *O. insectivorus* (Körner, 1954). Despite these affinities, morphometric measurements revealed distinctive diagnostic traits in the Georgian population, including female body width, oesophagus length, medium-sized spicules, and a lateral field with 8 ridges and 9 incisures. These features support recognition of *O. anaseuli* n. sp. as a distinct species.

Pathogenicity assays confirmed the high virulence of *O. anaseuli* n. sp. against *T. molitor* and *G. mellonella*. Mortality reached nearly 100% within five days, with *T. molitor* larvae succumbing more rapidly than *G. mellonella*. Interestingly, recovery of invasive juveniles was greater in *G. mellonella*, suggesting host-specific differences in nematode reproduction and survival. These findings highlight the potential of *O. anaseuli* n. sp. as a biological control agent, particularly in agricultural contexts where sustainable pest management strategies are urgently needed.

Despite the robustness of morphological and morphometric analyses, molecular studies remain

essential for precise taxonomic placement and confirmation of species identity. Future research should integrate molecular approaches with ecological and pathogenicity studies to fully evaluate the biocontrol potential of *O. anaseuli* n. sp. in both laboratory and field conditions. Such investigations will contribute to a deeper understanding of nematode biodiversity in Georgia and expand opportunities for environmentally friendly pest management.

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Novelty Statement

The article describes a new species of entomopathogenic nematode, which represents a novelty for Georgia.

Authors Contribution

OG: Methodology, conceptualization, data collection, analysis, writing, visualization. MKO: Data analysis, manuscript writing, review and editing. MKU: Fieldwork or sample collection, Laboratory analysis.

Generative AI and AI-assisted technology statement
Generative AI and AI-assisted technologies were used solely as supportive tools for language refinement and technical corrections, while all research content, analysis, and conclusions remain the original work and responsibility of the author.

Conflict of interest
The authors have declared no conflict of interest.

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