

# Asian Journal of Research in Biological and Pharmaceutical Sciences

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## REVEAL ENTOMOPATHOGENIC AND PARASITIC NEMATODES FROM THE TERRITORY OF EAST GEORGIA

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### ABSTRACT

Entomopathogenic and parasitic nematodes are lethal parasites of insects, used as biocontrol agents and have occupied a wide range of habitats. Isolation and identification of native nematodes are necessary for successful control of endemic insect pests in a particular location. The aim of this study was to reveal native nematodes from Eastern Georgia and their potential, which were found from soil and insects and for used them against pest insects. For this study, we chose two parasitic and one entomopathogenic nematode. *Amphimermisthesamica* sp. n. (Nematoda: Mermithidae) was isolated from the soil of gardening plot, located in village Tezami of Mtskheta-Mtianeti Region. Among many species of nematodes, we found out specimens of nematodes of the genus *Labronemella* Andrassy, 1985, that was described as a new species *Labronemellageorgiensis* sp. nov. From the restored ecosystems of Tbilisi environs. Entomopathogenic nematode - *Steinernemaborjomiense* n. sp. was isolated in the territory of Borjom-Kharagauli Reserve, from the host-insect cadaver. Morphological, anatomic and morphometric analysis of nematodes, isolated from different location has shown that the described nematodes are a new species. All above-mentioned nematodes were found from Eastern Georgia.

### KEYWORDS

Entomopathogenic and Parasitic nematodes, Biological control agents and Insect pests.

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### INTRODUCTON

Insects have many types of natural enemies. As with other organisms, insects can become infected with disease-causing organisms, called pathogens. Soil serves as a natural home and reservoir for many kinds of insect pathogens, including viruses, bacteria, protozoa, fungi, and nematodes. We can take advantage of these natural enemies of insects to help manage insect pests. The use of natural enemies to manage pests is called biological control<sup>1</sup>.

Biological control is the beneficial action of parasites, pathogens, and predators in managing pests and their damage. Biocontrol provided by these living organisms, collectively called natural enemies, is especially important for reducing the numbers of pest insects and mites<sup>2</sup>.

Some groups of nematodes can survive under disturbed environmental conditions such as global climate change which also in the last decades influenced the water regimes of soil, which is crucial for a nematode survival<sup>3,4</sup>.

Entomopathogenic nematodes. Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential as biological control agents<sup>5,6</sup>. These nematodes have a symbiotic association with bacteria of the genus *Xenorhabdus*<sup>7</sup>. The bacteria convert the insects into a suitable environment for development and reproduction of the nematodes' parasitic stages<sup>8</sup>. The only function of the infective juveniles is to locate and parasitize new host<sup>9</sup>.

Parasitic nematodes are divided into two groups: endoparasites and ectoparasites. Endoparasites are parasites that live inside the body of the host, whereas ectoparasites are parasites that live on the outer surface of the host and generally attach themselves during feeding<sup>10</sup>.

Endoparasites have two groups of parasites: intercellular and intracellular parasites. Intercellular parasites live in spaces within the host, whereas intracellular parasites live in cells within the host. Rather than requiring adaptations to penetrate the host, as ectoparasites do, endoparasites are in a nutrient-rich location so they instead have adaptations to maximize nutrient absorption<sup>11</sup>. Endoparasites have various anatomical and biochemical adaptations, typically at the host-parasite interface, to maximize nutrient acquisition. One such adaptation is the tegument, a metabolically active external cover that plays an important role in nutrient extraction from the host<sup>10</sup>. Endoparasites have various anatomical and biochemical adaptations, typically at the host-parasite interface, to maximize nutrient acquisition. One such adaptation is the tegument, a metabolically active external cover that plays an important role in nutrient extraction from the host<sup>10</sup>.

The parasite tegument is permeable to various organic solutes and has transporters for the facilitated or active uptake of nutrients. Various studies have attempted to characterise these transporters in a number of parasites e.g. The amino acid transporter molecules in protozoa<sup>12,13</sup>.

Ectoparasites live on the outer surface of the host. This group includes ticks, leeches, mites and the tsetse fly. Ectoparasites do not have a readily available source of nutrients available on the outer surface of the host, so they need adaptations to access host nutrients. This requires penetrating features they can insert into the host, as well as the ability to secrete digestive enzymes and the presence of a gut to digest host-derived nutrients<sup>10,11</sup>. Ectoparasites also have a variety of parasite transporters and permeases to enable them to acquire nutrition from their host, across numerous membranes. Many ectoparasites are known pathogen vectors, so they transmit these pathogens during nutrient acquisition<sup>14</sup>.

The aim of this work was reveal local and new entomopathogenic and parasitic nematodes from the territory of East Georgia and used them as a biological control agent.

The search of endemic and new species of nematodes is very important for revealing their genetic diversity and optimization because the Caucasus regions have a rich diversity of biologic species which are well adapted to the local climatic conditions and insect organisms.

## MATERIAL AND METHODS

Collection Insects and Nematodes. To fulfill this task expedition were organized in 2018 in East Georgia. The work was conducted in different locations, such as: Lagodekhi National Reserve, the bordering forest zone of Georgia and Azerbaijan, village Thezami and neighboring areas of Tbilisi city - River Vere.

On the whole field work was carried out from June to September. In order to carry out work dealing with collection of material were used, microscopes, Petri dishes, traps, small glass vessels etc. To reveal nematodes from the soil *Galleria mellonella*, *Tenebrio molitor* and *Bombyxmori* larvae were used as a trap. Traps were laid at different depths of the soil layer: the first layer was about 1-3 cm deep; the

second –about 1-5 cm deep and the third – about 5-10 cm deep. About 22 traps of *Galleria mellonella*, *Tenebrio molitor* and *Bombyxmory* were set in order to catch nematodes in the soil on the territory of the reserve. Traps were set in soil in a zigzag line 20-25 m apart from one another. The obtained material was kept in a special vessels and were labeled. In order to study the nematofauna of soil and to find out entomopathogenic nematodes, 18 soil samples were taken.

Scientific research and processing of the obtained material was carried out in the Laboratory of Entomonematology, on the whole, 80 specimens of 12 species of pest insects were processed.

Entomopathogenic as well as ectoparasitic and endoparasitic nematodes (larvae of age I-III) were isolated from soil samples according to the method of Bedding<sup>15</sup>. In order to reveal entomopathogenic nematodes, some forest soil samples were taken from neighboring forest areas.

Collected material was placed in special test-tubes and plastic bags, which were labelled. On the labels there were indications of place, from which were samples taken, including date.

## RESULTS AND DISCUSSION

Such harmful insects of plants, as: *Cerambyx dux*, *Melolontha pectoralis*, *Melolonthahyppocastani*, *Amphymalonsolstitialis*, *Erannisdefoliaria* and *Ocneriadispar* were gathered in the Lagodekhi National Reserve and between bordering area between Georgia-Azerbaijan. In addition to this insects, were: *Operophterabrumata*, *Caprocapsapomonella* and *Scolutusmali* which were collected from fruit trees in the villages - Chiauri and Giorgeti as well. Some pests were collected from cultures of vegetable gardens (e.g. from potatoes, cabbages, egg-plants, etc.) and from various decaying deciduous trees (e.g. beech-tree, horn-beam, etc.) as well as from different parts of plants - leaves, stems and, in some cases, from plant roots as well.

In June, when the average temperature of air was 22,5°C and air humidity equaled 72,5%, the largest number of nematodes was revealed in traps in the first layer (1-3 cm deep) of the soil (20,5 individuals per larva). In traps of the second layer (1-5 cm deep) the number of nematodes was approximately

4,5 individuals per larva. Nematodes were not revealed at all in the third layer (5-10 cm deep). But in July, when temperature was +25°C and air humidity equaled 67,5%, the position of nematodes in the soil changed. The number of nematodes in traps became larger in the second layer (14,3 individuals) than in the first layer (6,7 individuals). Nematodes were scanty in the third layer.

At the end of September different number of nematodes were taken from the soil and insect's body. Nematodes of the family: *Rabhditidae*, *Mermithidae*, *Diplogasteridae*, *Steinernematidae* and *Tylenchidae* were found among them (Table No.1). Native species of parasitic and entomopathogenic nematodes were identified using morphological and molecular characteristics<sup>16,17</sup>. Virulence and reproductive potential of nematodes were assessed using by larvae - *G. mellonella*, *T. molitor* and *B. mori*.

### **Amphimermisthezamica Sp. N. (Nematoda: Mermithidae) a New Species of Nematode from Georgia**

Female and male individuals of *Amphimermisthezamica* sp. n. (Nematoda: Mermithidae) have been described. The host organism is unknown. The material was collected from the soil of private gardening plot, located in village Tezami of Mtskheta-Mtianeti Region (East Georgia). A new species is characterized by the combination of the following features: amphids cup-shaped, less oval and with average size, vagina prolonged, S-shaped, round eggs with smooth surface and thick envelope, 56 papillae, arranged in three rows in genital part, long double spicule, intertwined insome sections. By morphological and morphometric data, a new species is close to the group of *A. bogongae*, especially to *A. litoralis*. New species resembles *A. litoralis* by the shape of amphids, S-shaped vagina, ending of a tail and twisted spicule. It differs from *A. Litoralis* by the length of vagina, structure of spicule and twisted parts, presented in its different sections, by the length of twisted and untwisted parts; by the shape of stoma. We present the list of species of the genus *Amphimermis*, distributed in Holarctic, with brief information on morphological characters, hosts and places of distribution.

The description of the new species is based on the forms of nematodes that were taken in the East Georgia region in the village of Tezami. This is the second record of this kind in Georgia. New species of mermithid are morphologically different from *Amphimermis lagidzae* which was recorded in Georgia, as well as from all species united in this genus.

**Labronemellageorgiensis sp. nov. (Nematoda, Dorylaimida) from Eastern Georgia**

In 2018 we studied the soil nematodes of restored ecosystems on the left side of the river Vere in environs of Tbilisi. Among many species of nematodes, we found out specimens of nematodes of the genus *Labronemella* Andrassy, 1985, which we describe as a new species from order Dorylaimida (Nematoda) was described.

***Labronemellageorgiensis* sp. nov.**

Measurements: Female:

L=2.27-2.6 mm; a=23-30.9; b=3.8-4.2; c=51-66; V=52-55%.

Male: L=2.2-2.9 mm; a=24.4-29.1; b=3.8-4.6; c=55-61; c1=0.9;

Spic=67-67.5 mkm; Suppl=17-20.

Cuticle very finely radially striated, not annulated, with weak but clear longitudinal lines, about 2.5 mkm thick on mid-body and 3.5 on the tail. Head strongly set off by constriction, 21.5 mkm wide and 5.5 mkm high. Body at posterior end of oesophagus 3 times as wide as the head, outer lips discaloid or ear-like. Oral field deeply sunk in the head contour, with six small liplets. Field of inner liplets 12 mkm wide, about half as wide as entire head diameter, 2, 5 mkm high. Amphids funnel shaped or gently cup like, about half as wide (10 mkm) as corresponding body diameter.

Odontostil 25-30 mkm long, 1.3 times head diameter, 2.5 mkm thick in the middle part i. e., about 12 times as long as thick; the orifice equal to 1/3 of spear length; guiding ring double, somewhat anterior to middle of spear.

Oesophagus muscular, expanding in the middle, with total length 570-630 mkm; prerectum 81-89 mkm long. 2-2.5 times as long as anal (cloacal) body diameter.

Female gonads pair, bent towards 1/3 way to the vulva; vulval labia sclerotized; vagina deep, its length about 40% of vulval body diameter. The vulva-anus distance 1.5 times the oesophagus length.

Male testes two. Spicula 67-67.5 mkm long, dorilaimoid, rather weakly cuticularized. In addition to the adanal (precloacal) pair 18-20 supplementary organs are present; they are almost contiguous, 110-120 mkm long in a row. Tail 38-39 mkm, only 0.8-0.9 anal body diameter, with 5 pairs of small papillae.

Type Locality: 6 females, 5 males and some juvenils are found in environs of Tbilisi, in the canyon of the river Vere, on the restored ecosystems on the left bank, in the soil (Figure No.5).

***Steinernemaborjomiense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Georgia**

A new species of entomopathogenic nematode, *Steinernemaborjomiense* n. sp., was isolated from the body of the host insect, *Oryctes nasicornis* (Coleoptera: Scarabaeidae), in Georgia, in the territory of Borjomi-Kharagauli. Morphological characters indicate that the new species is closely related to species of the feltiae-group. The infective juveniles are characterised by the following morphological characters: body length of 879 (777-989)  $\mu\text{m}$ , distance between the head and excretory pore = 72 (62-80)  $\mu\text{m}$ , pharynx length = 132 (122-142)  $\mu\text{m}$ , tail length = 70 (60-80)  $\mu\text{m}$ , ratio a = 26.3 (23.0-29.3), H% = 45 (40-51), D% = 54 (47-59), E% = 102 (95-115), and lateral fields consisting of seven ridges (eight incisures) at mid-body. *Steinernemaborjomiense* n. sp. was molecularly characterized by sequencing three ribosomal regions (the ITS, the D2-D3 expansion domains and the 18S rRNA gene) and the mitochondrial COI gene. Phylogenetic analyses revealed that *S. borjomiense* n. sp. differs from all other known species of *Steinernema* and is a member of the monticolum-group (Figure No. 1,2).

Morphological and molecular data confirm that *S. borjomiense* n. sp. is a new species closely related to *S. schliemanni* and all species of the monticolumgroup. This study confirms that the ITS sequences among *Steinernema* species are highly variable and contain more informative sites than either the D2-D3 expansion domains or the 18S rRNA gene.

Local entomopathogenic nematodes —*S. thesami*<sup>16</sup>, *S. disparica*<sup>19</sup> and *S. borjomiense*<sup>17</sup>, were used

against pest's insects: *Pieris brassicae*,  
*Operophterabrumata*, *Erannis defoliaris*,  
*Leptinotarsa decemlineata* etc.

Spraying of plants with nematode suspension was carried out in cloudy days and in the evenings.

We counted the number of dead insects killed by nematodes and the number of remained live insects according to the method of Abbot<sup>19</sup>(1925). To raise the bioeffectiveness of the nematode suspension we enlarged the doze of glycerin in nematode suspension to reduce its rapid evaporation.

**Table No.1: The distribution of nematode species in the insects**

S.No	Insect species	Nematode species
1	<i>Cerambyx dux</i>	<i>Rhabditis acarta</i> ; <i>Mesorhabditis quercophila</i> ; <i>Eudiplogaster leptospiculum</i> ; <i>Diplogaster</i> sp.
2	<i>Melolontha pectoralis</i>	<i>Thelestomakorsakov</i> ; <i>Heterorhabditis poinar</i> ; <i>Gryllonema bispiculata</i> ; <i>Cephalobellus melolonthae</i> Leisbersperg ; <i>Peloderateres</i> Schneider
3	<i>Melolontha hypocaustani</i>	<i>Heterorhabditis poinar</i> ; <i>Gryllonema bispiculata</i> ;
4	<i>Amphymalonsolstitialis</i>	<i>Hexamermissolstitialis</i> sp., <i>Mesorhabditis solstitialis</i> sp., <i>Parasitorhabditis solstitialis</i> sp., <i>Cephalobellus leukarti</i> , <i>Diplogaster coronata</i>
5	<i>Erannis defoliaria</i>	<i>Mermis nigrescens</i> Schultz
6	<i>Ocneriadirpar</i>	<i>Hexamer misalbicans</i> ; <i>Steinernema disparica</i>
7	<i>Operophterabrumata</i>	<i>Complexomermiselegans</i> ; <i>Hexamer misalbicans</i> Siebold; <i>Steinernema thesami</i>
8	<i>Carpocapsa pomonella</i>	<i>Diplogaster nubialis</i> ; <i>Parasitorhabditis pomonella</i>
9	<i>Scolytus mali</i>	<i>Parasitorhabditis mali</i> ; <i>Panagrolaimus rigidus</i> ; <i>Panagrellus redivivus</i> ; <i>Bursaphelenchus eucarpus</i>
10	<i>Pieris brassicae</i>	<i>Steinernema thesami</i> , <i>Amphimermiselegans</i>
11	<i>Leptinotarsa decemlineata</i>	<i>Hexamer misdecemlineata</i> sp.; <i>Amphimermiselegans</i> ; <i>Psaumomermis decemlineata</i>
12	<i>Agriotes gurgistanus</i>	<i>Protorhabditis elaphri</i> Daughert ; <i>Steinernema</i> sp.

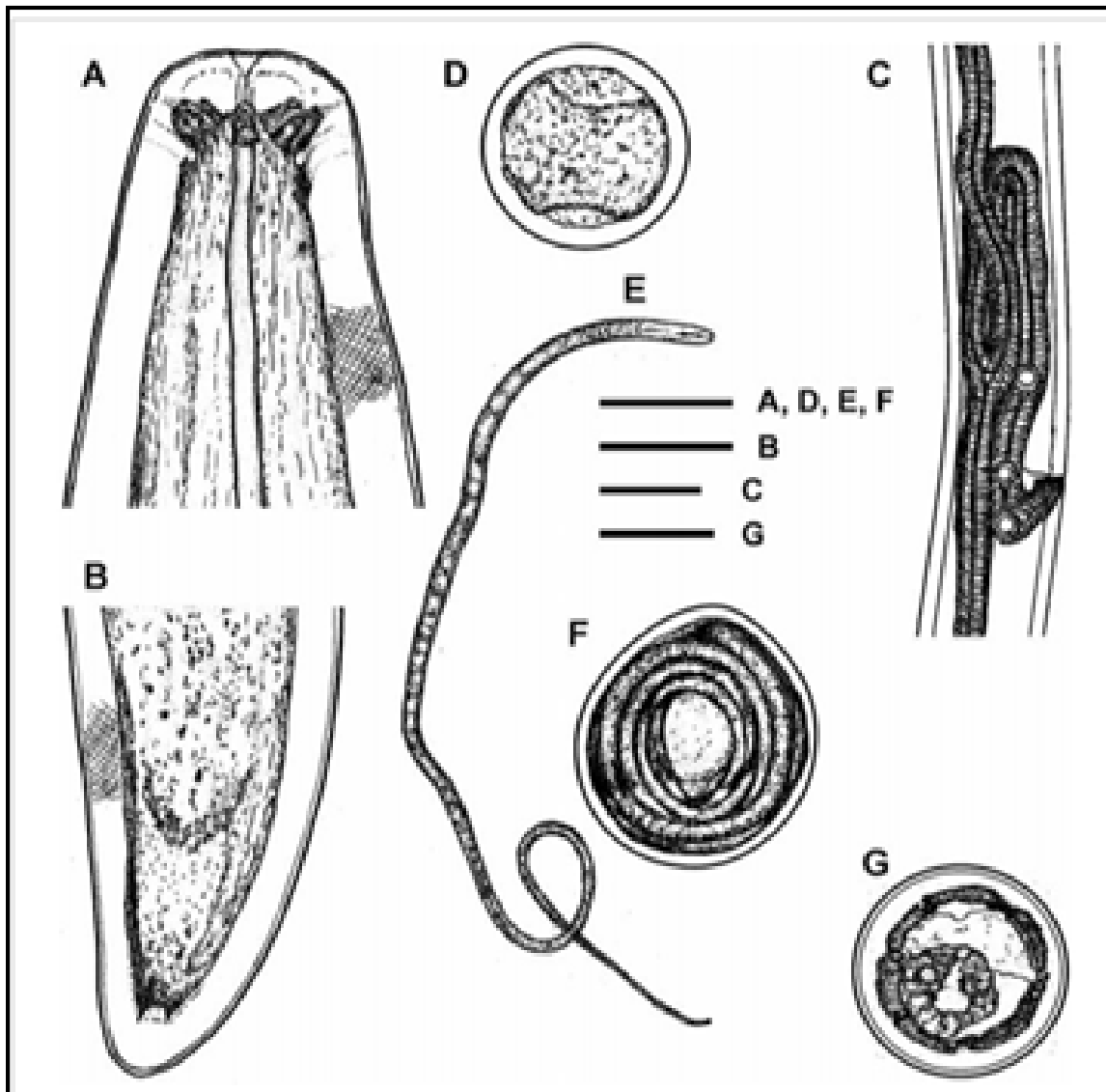
**Table No.2: The distribution number of registered nematodes in the insects**

Host insects	Number of insects	Body surface		Intestine		Inside the body	
		Number of infection insects	Intensive infection of insects	Number of infection insects	Intensive infection of insects	Number of infection insects	Intensive infection of insects
<i>Cerambyx dux</i>	35	10	14				
<i>Melolontha pectoralis</i>	60			5	4	10	8
<i>Melolontha Hippocastani</i>	40	8	30				
<i>Amphymalonsolstitialis</i>	50	12	7			17	35
<i>Leptinotarsa decemlineata</i>	110					20	15
<i>Erannis defoliaria</i>	80					10	5
<i>Operophterabrumata</i>	70					7	3
<i>Ocneriadirpar</i>	45	30	10			14	12
<i>Formica rufu</i>	20					5	4
<i>Agriotes gurgistanus</i>	78	18	33			11	20
<i>Anthonomus pomorum</i>	70	25	10			15	7
<i>Scolytus mali</i>	56	18	25			20	35

**Table No.3: Bioeffectiveness of local Steinernematidae nematodes against various insect species in field conditions (dozes: 2000±150 nematodes per 1 ml. of water)**

Order	Species	Life Stage	Steinernema nematode effectiveness			
			<i>S. tesami</i>	<i>S. dispica</i>	<i>Steinernema sp.</i>	<i>S. borjomiense</i>
Lepidoptera	Pieris brassicae	II-III	70,5	72,3	77,6	73,8
	Operophterabrumata	III-IV	58,8	67,4	65,9	67,4
	Erannis defoliaris	II-III	67,3	74,6	80,2	78,1
Coleoptera	Leptinotarsa decemlineata	III-IV	56,4	51,0		
Diptera	Tipulapaludosa	II-III	62,3	65,3	68,2	72,5
Homoptera	Aphis pomi	II-III	62,3	87,6	78,7	76,9

As the table shows, Steinernema nematode effectiveness on pests fluctuates between 67, 4% and 78, 1%.



**Figure No.1: *Amphimermisthezamica* sp. n., female, holotype. A: Anterior region of the body, lateral view; B: Posterior end, lateral view; C: Vagina, lateral view; D: Uterine egg; E: stage -2 (infective), whole body; F: Last stage of larva in egg; G: Mid-body, cross section. Scale for A, D, E, F = 50 µm; B = 100 µm; C=400 µm; G=200 µm**

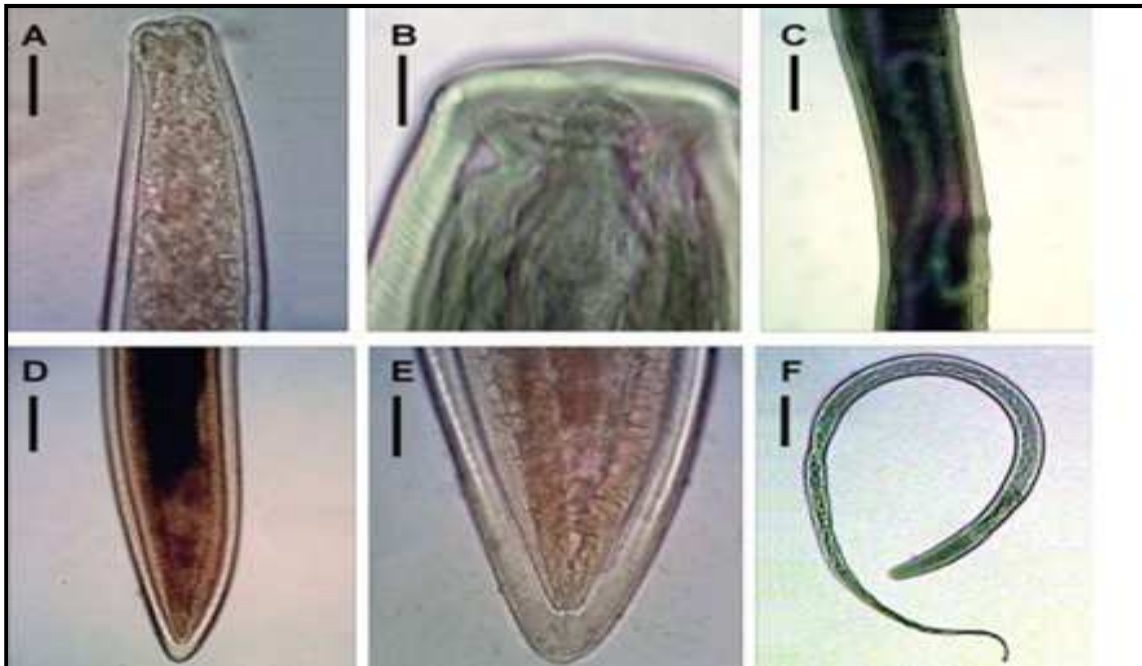


Figure No.2: *Amphimermisthezamica* sp. n., female, holotype. A: Anterior region of the body, nerve ring, and pharyngeal tube, lateral view; B: Head, lateral view; C: Vagina, lateral view; D: Posterior region of the body, lateral view; E: Tail end; F: stage -2 (infective juveniles) whole body. Scale for A, E=25  $\mu$ m; for B=30  $\mu$ m; for C, D=100  $\mu$ m; for F=100  $\mu$ m

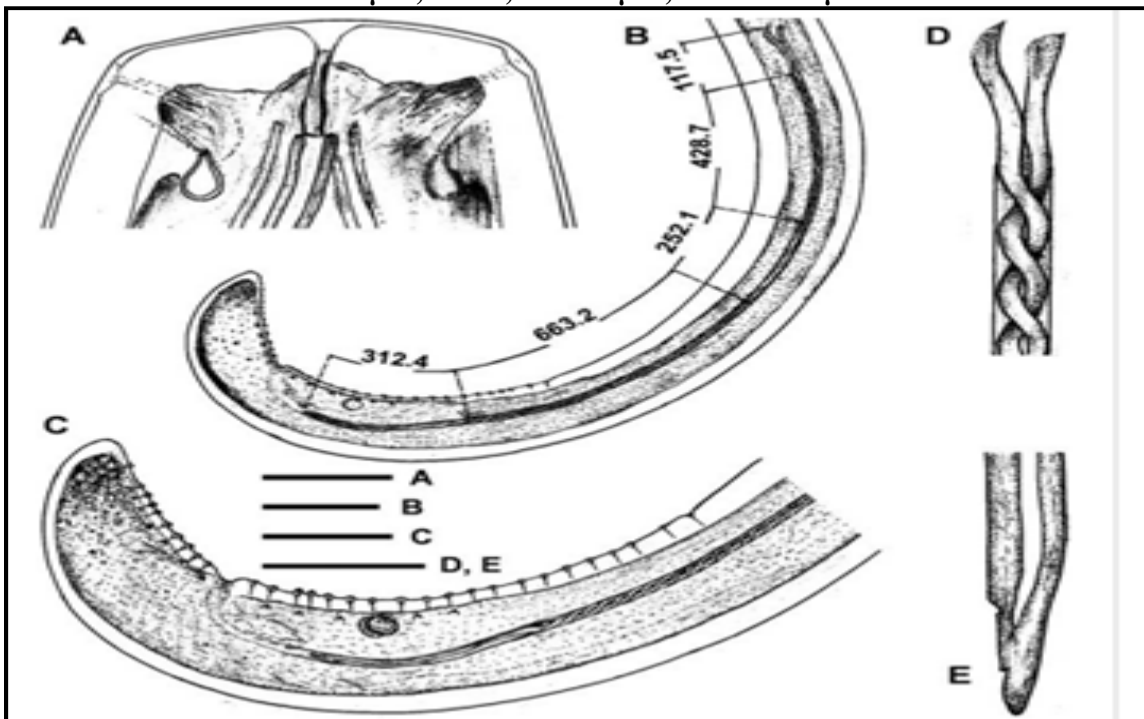


Figure No.3: *Amphimermisthezamica* sp. n., male, holotype. A: Head, wide of the cuticle, lateral view; B: Tail, with whole spicula, lateral view; C: An enlarged tail with a half spicule; D: Non twisted and twisted head of spicule; E: Last part of non-twisted spicule. Scales for A=20  $\mu$ m; B=150  $\mu$ m; C=100  $\mu$ m; D, E=25  $\mu$ m



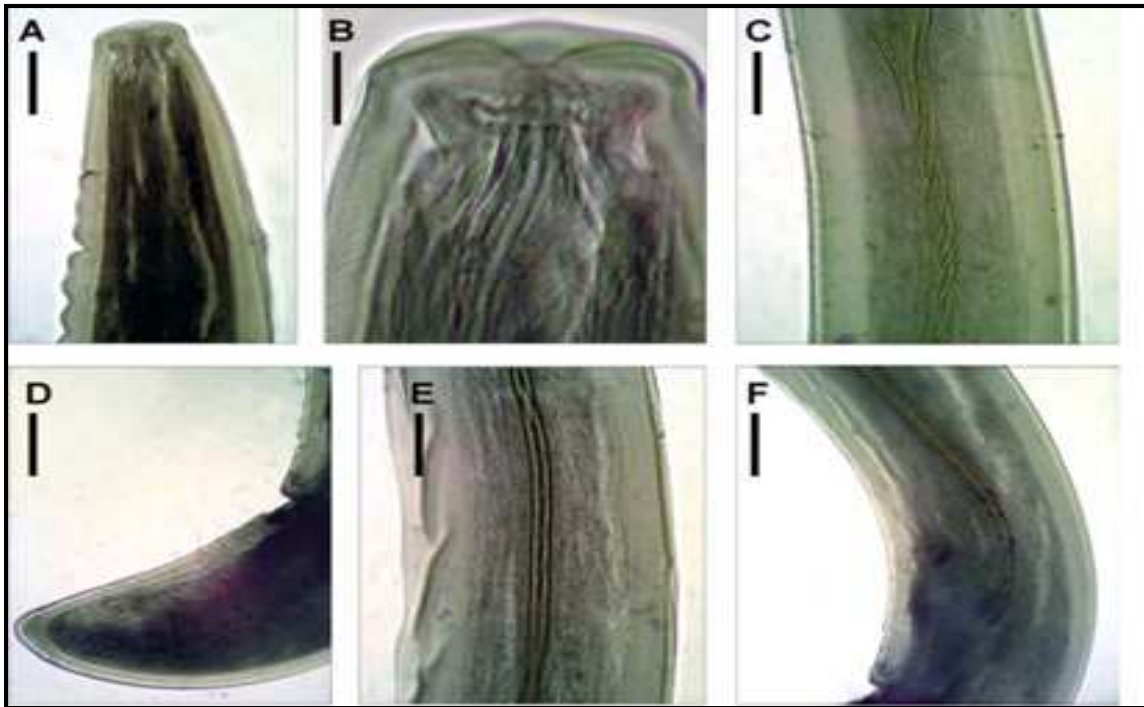


Figure No.4: *Amphimermisthezamica* sp. n., male, holotype. A: Anterior region of body, lateral view; B: Head, lateral view; C: Non - twisted and twisted part of spicule head; D: Tail, lateral view; E: Middle part of non-twisted spicule; F: End of non-twisted spicule, lateral view; Scales for A, C, D, E, F=25  $\mu$ m; for B=30  $\mu$ m

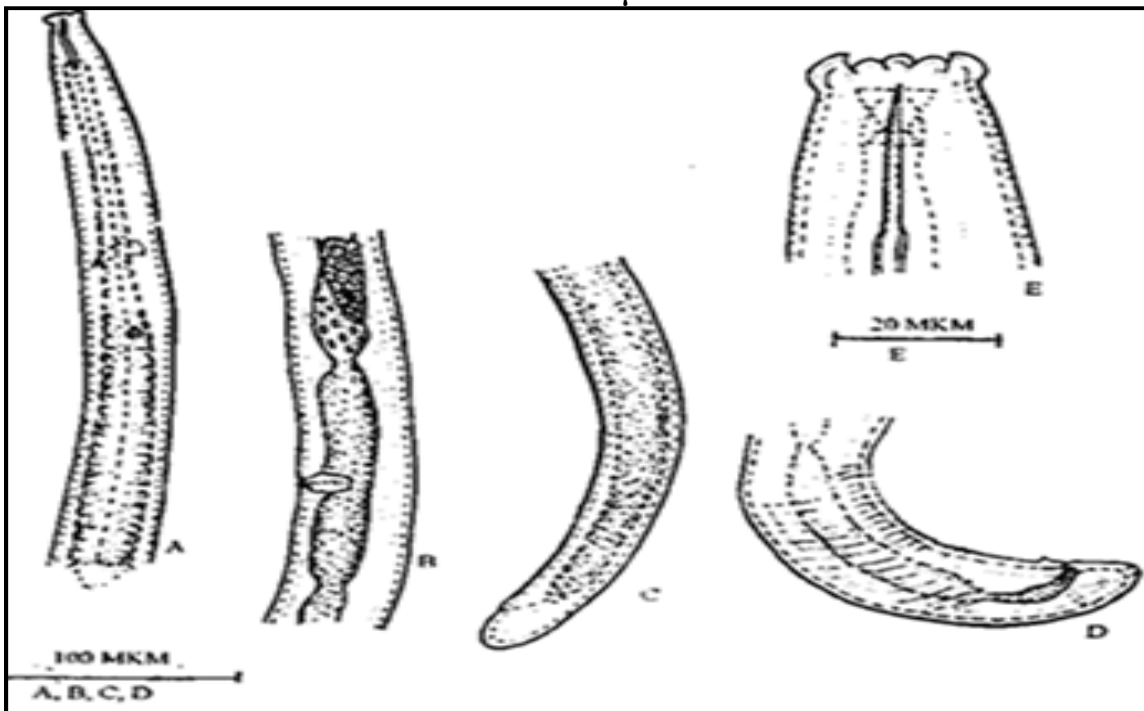


Figure No.5: Differential diagnosis: The new species *Labronemellageorgiensis* is similar to *Labronemella labiate* Andrassy, 1985 (I) –both are large species of genus, having similar shape of tail and orifice of spear equal to 1/3 of spear length, but differs in some signs: 1. The female of new species is known; 2. Oesophagus of new species is much longer, 3. Body plumper, a= 23-30, against 48 by *L. labiate*; 4. Prerectum of new species is much shorter; 5. Supplements 18-20 (against 15 by *L. labiate*).



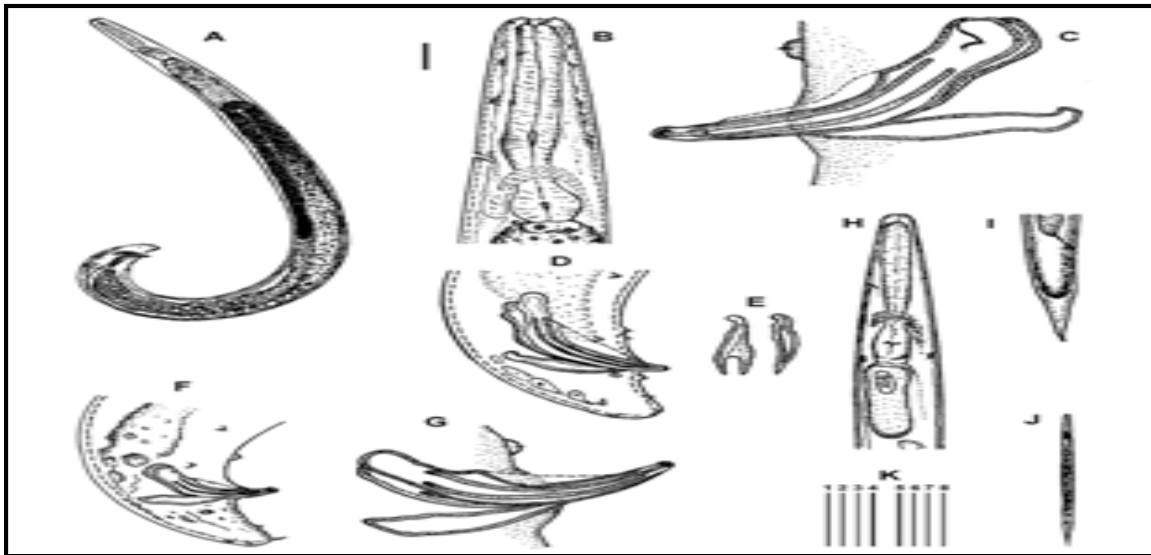


Figure No.6: *Steinerneborjomiense* n. sp. First-generation male (A-E). A: Entire body; B: Anterior end; C: Spicules with gubernaculum (lateral view); D: Posterior end; E: Gubernaculum (ventral and lateral view). Second-generation male (F, G). F: Posterior end (lateral view); G: Spicules with gubernaculum (lateral view). Infective juvenile (H-K). H: Anterior region; I: Tail; J: Entire body; K: Lateral field in mid-body region. (Scale bar: A = 100  $\mu$ m; B, D-F, H, I = 25  $\mu$ m; C, G = 10  $\mu$ m; J = 250  $\mu$ m; K = 12  $\mu$ m.)

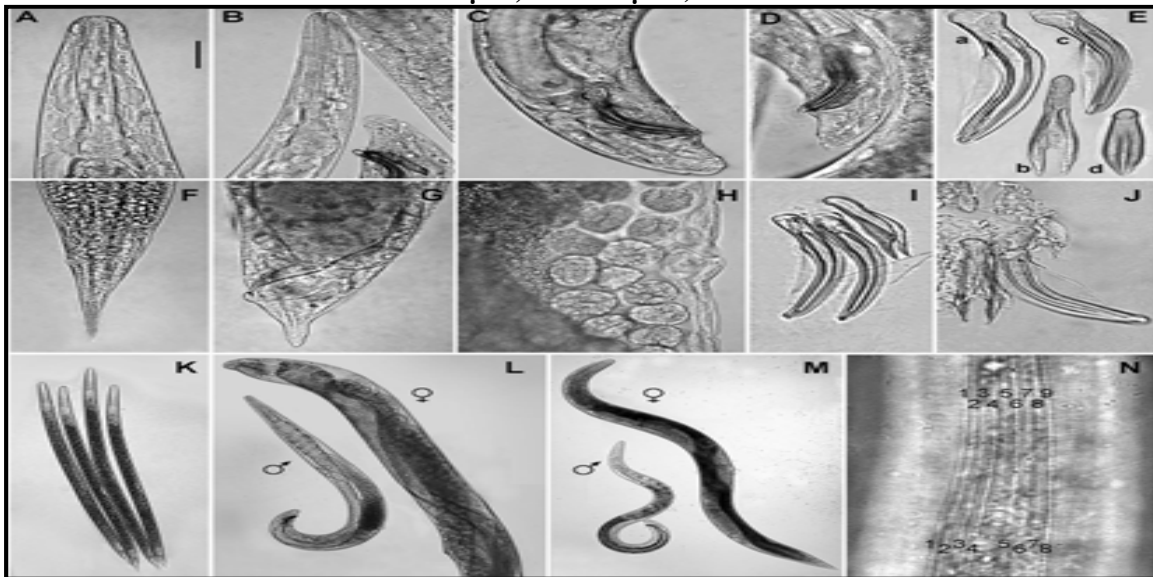


Figure No.7: Light microscope (LM) photographs of *Steinerneborjomiense* n. sp. First-generation male (A, C, E, J, L). A: Pharyngeal region (lateral view); C: Posterior end (lateral view); E: Lateral and ventral view of spicule and gubernaculum, respectively (a, b); J: Gubernaculum (ventral) and spicule (lateral view); L: Entire body (on left). Second-generation male (B, D, E, I, M). B: Pharyngeal region; D: Posterior end (lateral view); E: Lateral and ventral view of spiculum and gubernaculum, respectively (c, d); I: Spicules and gubernaculum (lateral view); M: Entire body (on left). First-generation female (G, H, L). G: Posterior end (lateral view); H: Vulval region (lateral view); L: Head to vulval region of female (on right). Second-generation female (F, M). F: Posterior end (lateral view); M: Entire body (on right). Infective juveniles (K, N). K: Entire body; N: Lateral field at mid-body region showing seven ridges (eight striae, numbered) below but with an additional stria at the top. (Scale bars: A-D, F-H = 25  $\mu$ m; E, I, J = 15  $\mu$ m; K = 100  $\mu$ m; L, M = 200  $\mu$ m; N = 5  $\mu$ m.)

## CONCLUSION

This study focuses on reveal of the endemic and new entomopathogenic and parasitic nematodes from the territory of East Georgia. The search of endemic and new species of nematodes is very important for revealing their genetic diversity and optimization because the Caucasus regions have a rich diversity of biologic species which are well adapted to the local climatic conditions and insect organisms.

## ACKNOWLEDGEMENT

This study was prepared in the framework of the Project: No. 832128641/83273372 "Introducing environmentally friendly pest-control for bio-protection of agricultural crops in the border areas Azerbaijan (Sheki - Zagatala) and Georgia (Lagodekhi)" of the Ea PTC Cooperation program Azerbaijan - Georgia. We gratefully acknowledgement support from donor organization - German Society for International Cooperation (GIZ) GmbH.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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**Please cite this article in press as:** Manana Lortkipanidze et al. Reveal entomopathogenic and parasitic nematodes from the territory of east Georgia, *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 6(3), 2018, 94-104.