



Amphimermis thezamica Sp. N. (Nematoda: Mermithidae) a New Species of Nematode from Georgia

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Keywords: *amphimermis thezamica* sp.n., *nematoda*, *mermithidae*, *parasitic*, *Georgia*.

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Amphimermis thezamica Sp. N. (Nematoda: Mermithidae) a New Species of Nematode from Georgia

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Abstract- Female and male individuals of *Amphimermis thezamica* 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 in some 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.

Keywords: *amphimermis thezamica* sp.n., nematoda, mermithidae, parasitic, Georgia.

I. INTRODUCTION

Nematodes, united in the genus *Amphimermis* are polyphages; they are of economic importance as agents of biological control of harmful insects (Poinar & Welch, 1981; Chen & Yang, 1985). They parasitize on both, terrestrial and soil insects belonging to orders Orthoptera, Lepidoptera, Coleoptera, Hymenoptera and cause their death (Poinar, 1975; Rubtsov, 1977, 1978; Ipatieva & Pimenova, 1985; Poinar et al., 2006). Development of nematodes of this group takes place mainly in the soil. They differ from mermithids of other genera by a very long, twisted spicule and bent S-shaped long vagina (Rubtsov, 1978). More than 20 species are described from this genus worldwide. In Georgia, in particular in West Georgia, only one species of the genus *Amphimermis* - *Amphimermis lagidzae* Rubzov (Rubtsov, 1975) was registered so far. Morphological, anatomic and morphometric analysis of nematodes, isolated from soil has shown that the described nematode is a new form and it belongs to the genus *Amphimermis*.

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II. MATERIAL AND METHODS

Mermithid nematodes have been collected using the shovel from the soil of private gardening plot, located in village Tezami [41°53'2"C, 44°57'28" B] of Mtskheta-Mtianeti Region (East Georgia) in April of 2015. The nematodes were found at 20-25 cm depth from the soil surface. Adult female and male nematodes were observed alive and then killed by immersion in 60°C distilled water for 2 min, fixed in TAF (7 ml formalin, 2 ml triethanolamine, and 91 ml distilled water) (Poinar, 1975) and processed to glycerol by Seinhorst's method for taxonomic studies (Polozhentsev & Artyukhovski, 1963; Curran & Hominick, 1980). Drawings and pictures have been done using both alive and dead individuals of nematodes. Measurements were made using the light microscope (Motic-DMB1) on 4x, 10x, 40x and 100x magnification. Some small-size details of nematodes, significant for the diagnosis (stoma, amphids, etc.) were examined using the immersion objective of 100x magnification. Drawings and photos of nematodes mounted on slides were performed using a digital video camera Genius (G-Shot) DV 1110. Host of the nematode is unknown. The range of the characters is given following the mean value. The range of the characters is given following by the mean value. Type material deposited in the collection of the museum 16 of the Institute of Zoology of Ilia State University, Georgia

III. RESULTS

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.

a) *Diagnosis of genus Amphimermis Kaburaki & Imamura, 1932*

= *Complexomermis* Filipjev, 1934, Diagnosis (Kaiser, 1991 emend.)

Mermithids are of medium and big size – from 13 to 260 mm. Body is thin and long, frontal part is significantly narrowed, while the posterior part is less narrowed. The six head papillae are arranged in the

same circle. Stoma opening is terminal; sometimes slightly displaced to the abdominal side. Stoma tube is long; it makes 60% of the body length. The distal part of adult, sexually mature forms does not extend beyond the inner head capsule. Amphids are big in size, located at the level of papillae or are bent backwards. Cavity of amphids is whether sharply prolonged or barrel-shaped. Cuticle is thick, with well-defined fibrous crossed filaments. Hypodermal chordae six in number. Vagina is S-shaped by shape, with three nodes and very long; very often the frontal part is widened. Vulva has a shape of an oblique fissure. Eggs numerous, of medium size and round, with thick envelope, without villi. Spicules long (from 1.0 mm to 3.6 mm), partly twisted. Mermithids have three orders of sexual papillae: middle papillae doubles in the region of sexual sphere. At the end of rounded tail under the cuticle they have uneven margins. Terminus of a tail of parasite and postparasite larvae is equipped with mucro.

b) Taxonomy

i. *Amphimermis thezamica* sp. n. (Figures 1- 4).

Type Host and locality: Host of the described nematode is unknown. It was collected from the soil of private gardening plot, located in village Tezami of Mtskheta-Mtianeti Region (East Georgia) [41°53'2"C, 44°57'28" B]. The species *Amphimermis thezamica* sp.n. 4 was named according to the place of collection.

Type material: slide of the holotype (adult female and male): Mtskheta-Mtianeti Region (East Georgia), village Tezami, April 2015 (the code villi. Tezami -2015 0-01); paratypes (18 females -16 slides; 7 males- 5 slides and 20 invasive larvae - 6 slides) have the same characters as the holotypes. Holotypes of a new species are preserved in the collection of the museum of the Institute of Zoology of the Ilia State University.

ii. Measurements

Allotype (female): length=205 mm; head diameter at the level of cephalic papillae: 68 (μ m); body diameter at the level of the nerve ring: 144 (μ m); maximum body diameter at vulva: 372 (μ m); body diameter at the posterior end of trophosome: 253 (μ m); body diameter at vulva: 405 (μ m); distance from the anterior end to the nerve ring: 322 (μ m); V%=47.7; length of vagina: 2.1

mm; width of vagina: 114 μ m.

Female (paratypes; n=18): length=242 \pm 77 (71-282) mm; diameter of head at the level of cephalic papillae: 68 \pm 5 (64-79) (μ m); body diameter at the level of nerve ring: 148 \pm 7 (133-152) (μ m); maximum body diameter at vulva: 390 \pm 24 (349-420) (μ m); body diameter at the posterior end of trophosome: 247 \pm 22 (228-260) (μ m); distance from the anterior end to the nerve ring: 360 \pm 24 (292-360) (μ m); distance from the end of trophosome to the end of tail 480 \pm 96 (315-580) (μ m); V%=47.5 \pm 3 (41.8-47.7); length of vagina: 2.2 \pm 0,8

(1.8-2.7) mm; width of vagina: 120 \pm 11 (100-140) μ m.

Holotype (male): Length=35 mm; head diameter at the level of cephalic papillae: 60 μ m; body diameter at the level of the nerve ring: 114 μ m; maximum body diameter:178 μ m; body diameter at the level of anus: 159 μ m; distance from the anterior end to the nerve ring: 228 μ m; length of the spicule: 1.773 mm; width of the spicule: 16 μ m.

Male (paratypes; n=7):Length=40 \pm 8 (29-54) mm; head diameter at the level of cephalic papillae: 62 \pm 3 (56-68) μ m; body diameter at the level of the nerve ring: 112 \pm 4 (107-118) μ m; maximum body diameter: 182 \pm 12 (151-188) μ m; body diameter at the level of anus: 169 \pm 23 (132-195) μ m; distance from the anterior end to the nerve ring: 232 \pm 3 (224-232) μ m; length of the spicule: 1.770 \pm 0.4(1.668-1.776) mm; width of the spicule: 15 \pm 0.8 (14-16) μ m.

Invasive larva: (n=20). Body length 1.1 \pm 0.1 (1-1.3) mm; maximum width 22 \pm 2 (19-22) μ m; larva has a style, which length fluctuates from 25 to 26 μ m. Body diameter at the level of the head papillae 10 \pm 1 (9-11) μ m; at the nerve ring - 19 \pm 3 (15-19) μ m; distance from the apical end of the head to the nerve ring makes 53 \pm 4 (53-57) μ m, while from the rectum from 83 \pm 6 (79-86) μ m length till the end of tail larva is spindle-shaped. It is characterized by the movement of plectoidnematodes. Anterior part of the body is wide, but is very narrow at the terminal part and ends with micro bulb-like tail. Tail terminus is used by the larva to attach to the substrate.

iii. Description

a. Morphology

Female. Nematode is of white colour. Body is thin and long. Body diameter increases at the 45.5 mm distance from the apical end of anterior part and then it is of nearly even width till the end of tail. Head is rounded and slightly flattened (Figures 1A, 2A,B). Diameter of the head capsule 9.2 μ m. Cuticle is thick, it contains crossed fibrous threads; stoma is symmetrically situated at the end of the head. The 6 head papillae are well defined. Each papilla has 2-3 sensils. Amphids have shape of cup, are less oval and middle size (length of amphids 15.2 μ m, width 9.6 μ m). Hollows of amphids open behind the papillae at 15 and 19 μ m distance. Stoma is narrow (1.5 μ m wide); length of stoma 19.4 μ m. Stoma is without collar. Oesophagus 5.4mm long, diameter 5.5 μ m. Channel of vagina starts obliquely. It is long 1.5 mm. (Figures 1C, 2C) cylindrical and bent S-shaped, nearly of even width along the whole length. Eggs round (length fluctuates from 83 to 87 μ m, width 79-87 μ m); with smooth surface; envelope thick - 4.2 μ m wide. Size of cells of trophosome fluctuates from 27 to 140 μ m. Lateral chordae narrow, size of chordae, located at dorsal and ventral sides fluctuates from 15 to 19 μ m. Tail is slightly conical (Figures 1B, 2D,E); its terminal part is rounded and unevenly immersed under the cuticle.

Male. In comparison with female, male is smaller in size. Oesophagus is 1.5 mm long. Long, twisted, double spicule is characteristic of the male (Figure 3D). It contains twisted and non-twisted parts (Figures 3B, C, D, E, 4C,E,F). Length of distal untwisted part of spicule is 117.5 μm (see Figure 3B), length of the next, twisted part is 428.7 μm , length of the central, untwisted part is 252.1 μm ; the next, twisted part is 663.2 μm long, and length of the proximal, untwisted part makes 312.4 μm . Terminal part of spicule is conical and its end is rounded. In the terminal part of spicule 2 notches are visible (Figure 3E), which is characteristic to this species. Cloaca diameter 159.6 μm . In postanal part 2 well defined nucleoli are visible (38 μm long and 30 μm wide), which are not found in other Amphimermitids. Sexual papillae are arranged in three orders; in preanal part 10 quite big papillae are situated medially, though on each of lateral parts 7-7 small papillae are presented. In postanal part 20 papillae are situated medially (among them the first, second, nineteenth and twentieth papillae are small in size, though the rest papillae are big in size) and on each of its lateral part 6-6 papillae are situated. Total 56 papillae are situated in the genital part. Size of spermatocytes fluctuates from 7 to 11 μm . Tail is 266.5 μm long. It is slightly conical (Figures 3C, 4D), rounded at the end.

c) *Diagnosis and Relationships*

By its morphological and morphometric characters a newly described species - *A. thezamica* sp. n. belongs to the genus *Amphimermis*. By the character of twisting of spicule and shape of amphids the nematodes, united in the genus *Amphimermis* are divided into four groups (Bacer & Poinar, 1994): 1. *volubilis* (*A. volubilis*); 2. *avoluta* (*A. avoluta*, *A. acridiorum*, *A. buraki*); 3. *bogongae* (*A. bogongae*, *A. maritime*, *A. litoralis*, *A. tinui*, *A. bonaerensis*, *A. mirabinda*); 4. *elegans* (*A. elegans*, *A. zuimushi*, *A. tongaensis*, *A. artyukhovskii*, *A. australoelegans*).

A new species, which is characterized by a twisted spicule, was compared with nematodes of all four groups. It turned out that, by morphological and morphometric characters it is the most close to the group of *A. bogongae*, especially to the species *A. litoralis*.

A. thezamica sp. n. Resembles *A. litoralis* by the shape of amphids (both species have cup-shaped amphids), S-shaped vagina and end of tail; (both *A. thezamica* sp. n. and *A. litoralis* are characterised by the uneven deepening under the cuticle at the end of the tail, twisted spicule and diametric dimensions of the body.

A. thezamica sp. n. differs from *A. litoralis* by the following characters: size of the body (in a new species male $L=40$ (29-54) mm, in *A. litoralis* – 57 (34-76) mm; in female – correspondingly 242 mm and 136

mm; by the diameter of the body - (in a new species diameter of the body is 182 (151-188) μm , though that of *A. litoralis* 267 (232-471) μm ; by the length of spicule - in a new species length of spicule is 1.773 (1.668-1.776) mm – in *A. litoralis* - correspondingly 3.200 (2.900-3.600) mm; by the length of twisted parts of spicule and their positioning: length of the first untwisted part of spicule in a new species is 117 μm ; in *A. litoralis* it is equal to 194 (41-336) μm . Length of the first twisted part of spicule in a new species is 428 μm , in *A. litoralis* – 647 μm ; length of a central untwisted part in a new species is 253 μm ; in *A. litoralis* – 302 μm ; length of the next twisted part in a new species is 663 μm , though in *A. litoralis* it is 646 μm long. Terminal part of spicule in a new species is untwisted (length 312.4 μm), though in *A. litoralis* it is twisted till the end. Vagina of the female of a new species is longer 1.5 mm, than that of *A. litoralis* (960 μm). Part of stoma, which from the apical part of head connects to the esophagus, is slightly widened in *A. thezamica* sp.n., though this is not a case in *A. litoralis*. Terminal part of a head is rounded in *A. litoralis*, though in a new species it is flattened. These two species differ from each other by the positioning of vulva towards the body: $V\%$ for *A. litoralis* is 50, though vulva of a new species is located slightly ahead ($V\%=47$). *A. thezamica* sp.n., and *A. litoralis* differ also by the thickness of the cuticle: thickness of cuticle at the head capsule - 13 μm ; at vulva - 12 μm ; at the end of tail - 51 μm ; in a new species these indexes are correspondingly 30, 38 and 41 μm . Comparison has shown that despite the fact that by morphological and morphometric characters new species, described by us, is quite close to *A. bogongae*, by the key diagnostic characters it differs from the latter: by the length of vagina, structure of spicule and presence of twisted segments at different places; by the length of twisted and untwisted parts; by the shape of stoma and thickness of the cuticle. Based on the above we consider that the mermithid, described by us can be regarded as a new species.

IV. DISCUSSION

Female individual of *A. thezamica* sp. n. is of white colour. Body is thin and long. At the 45.3 mm distance from the apical end of the body its diameter increases, and then body is nearly of the same width till the tail. Head is rounded and slightly flattened. Cuticle is thick and it contains crossed fibrous threads. Stoma is situated symmetrically at the end of the head. Number of head papillae – 6. Amphids are cup-shaped. Stoma is narrow, without collar. Channel of vagina starts obliquely. It is long, cylindrical, bent S-shaped, and has the same width along the whole length. Eggs are round and have smooth surface. Tail is slightly conical. Its terminal part is rounded and unevenly deepened under the cuticle.

Male individual is smaller in size than the female one. Male is characterized by the long, double spicule. It is composed of twisted and untwisted parts. The 2 notches are well defined at the end of the spicule, which is characteristic to this species. Total 56 papillae present in the genital part. Tail is slightly conically, rounded at the end.

Despite the resemblance of morphological and morphometric characters of *A. thezamica* sp.n. with those of species, belonging to the genus *Amphimermis*, by the structures of spicule and presence of twisted segment at its different parts, length of twisted and untwisted parts, shape of stoma, a new species differs from Holarctic species, united in this genus, such as: *A. zuimushi* Kaburaki & Imamura, 1932; *A. acridiorum* Baker & Poinar, 1994; *A. artyukhovskii* Artyukhovski & Karchenko, 1965; *A. australoelegans* Baker & Poinar, 1994; *A. avoluta* Rubtsov & Koval, 1975; *A. bogongae* Welch, 1963; *A. bonaerensis* Miralles & Camino, 1982; *A. buraki* Baker & Poinar, 1994; *A. elegans* (Hagmeier, 1912) Welch, 1963; *A. lagidzae* Rubtsov, 1975; *A. litoralis* Artyllkhovski & Karchenko, 1971; *A. longiscapus* Rubtsov, 1976; *A. maritime* Rubstov, 1971; *A. mirabinda* Baker & Poinar, 1994; *A. mongolica* Rubtsov, 1976; *A. polaris* Spiridonov & Lantsov, 1996; *A. tinyi* Nickle, 1972; *A. tongaensis* Spiridonov, 1987; *A. volubilis* Rubtsov & Koval, 1975;

A. zuimushi - parasitizes on Lepidoptera: Noctuidae-*Agrotis infusa* (Boisd.), Pyralidae- *Chilo simplex* (Butler). Cavity of amphidin a lateral position is extended along the longitudinal axis of the body. It differs from all genera by a very long and stretched spicule and long, S-shaped bent vagina, Spicules 1.02-1.45 mm long. Distribution: Japan.

A. acridiorum- parasitizes on Orthoptera: Acrididae-*Phaulacridium vittatum* (Sjostedt); *Oedaleus australis* (Saussure); *Chortoicetes terminifera* (Walker)., Amphid opening posterior to lateral cephalic papilla. Vagina short and broad (in lateral view), vagina long and narrow (in lateral view). Distribution: Australia.

A. artyukhovskii - parasitizes on Lepidoptera: Lymantriidae - *Lymantria dispar* (L), Geometridae - *Operopthera brumata* (L)., Spicule 1.6-1.8 mm. Body length 26-53 mm. Tail conoid, rounded. Female body length 36-190 mm. Distribution: Voronezh region, (Russia).

A. australoelegans- parasitizes on Orthoptera: Acrididae-*Phaulacridium vittatum* (Sjostedt), *Chortoicetes terminifera* (Walker); Coleoptera : Scarabaedae - *Sericesthis* sp., Tail conoid, pointed terminus. Vagina long (1.5 mm), thin walled. Distribution: Australia.

A. avoluta - parasitizes on Coleoptera: Chrysomelidae-*Leptinotarsa decemlineata* Say. Proximal half of spicule untwisted (=avoluta group), vagina medium (0.5 mm). Distribution: Ukraine.

A. bogongae - parasitizes on Lepidoptera: Noctuidae-*Agrotis infusa* (Boisd.). Tail bluntly rounded, distance of proximal papillae from cloaca=20% of spicule length; medium sized amphids in relation to head diameter, thick-walled. Distribution: Australia.

A. bonaerensis -parasitizes on Orthoptera: Acrididae-*Laplatacris dispar* Rhen., Tail pointed, distance proximal papillae to cloaca≤spicule length. Vulva with flanges. Distribution: Australia.

A. buraki - parasitizes on Orthoptera:Tettigoniidae-*Conocephalus* sp. ; Body short (30 mm), spicule length 750-975 μm, terrestrial, amphids thick - walled, situated anterior to neck region with opening immediately posterior to lateral head papillae. Distribution: Australia.

A. elegans - parasitizes on Orthoptera: Acrididae: *Stenobothrus* sp. Tail bluntly rounded. Body length 195-260 mm. Distribution: Germany.

A. lagidzae - the host organism and male individuals are not known. Amphids situated in neck region with opening well posterior to lateral head papillae. Body 74 mm. Distribution: Georgia.

A. litoralis - unknown. Spicule long (2.9-3.6 mm) in relation to the body length (45-70 mm). Body length 136 mm, terrestrial. Distribution: Voronezh Region (Russia).

A. longiscapus - parasitizes on Lepidoptera: Lymantriidae: *Lymantria dispar* (L). Male individuals are not known. Amphids situated anterior to the neck region with opening, immediately posterior to the lateral head papillae. Body 43 mm. Distribution: Kirgizstan.

A. maritime - parasitizes, unknown. Spicule 2.2 mm. Body 53 mm. Junction of vagina and uterus at an obtuse anterior-ventral angle. Distribution: Primorsk Region (Russia).

A. mirabinda - parasitizes on Orthoptera: Acrididae: *Phaulacridium vittatum* (Sjostedt). Spicule 1.2-1.5 mm. Body 35-42 mm. large amphids in relation to the head diameter, thin-walled. Distribution: Australia;

A. mongolica - parasitizes, unknown. Amphids thin-walled, situated in neck region with opening well posterior to lateral head papillae. Distribution: Mongolia;

A. polaris- parasitizes on Diptera: Tipulidae -*Tipula (Savtshenkia) glaucocinerea* Lundtsr. Body length of male - 6.065 mm, length of spicule – 1.648 mm, length of females – 10.740 mm; usually on the tail terminus is developed 12 μm long cuticular appendix. Distribution: Taimyr, Dolgano-Nenetskyi Autonomous region.

A. tinyi - parasitizes on Odonata: Coenagrionidae: *Ischuraposita* (Hagen), *Annomalagrion bastatum* (Say). Male body short (11-17 mm), spicule 700-860 μm, aquatic. Female body length 30 mm, aquatic. Distribution: USA.

A. tongaensis - parasitizes, unknown. Diameter of amphid is greater than two thirds of head width. Female unknown. Distribution: Tonga.

A. volubilis - parasitizes on Coleoptera: Chryso-melidae: *Leptinotarsa decemlineata* Say. Spicule twisted entire length (= volubilis group), vagina long (0.7 mm).

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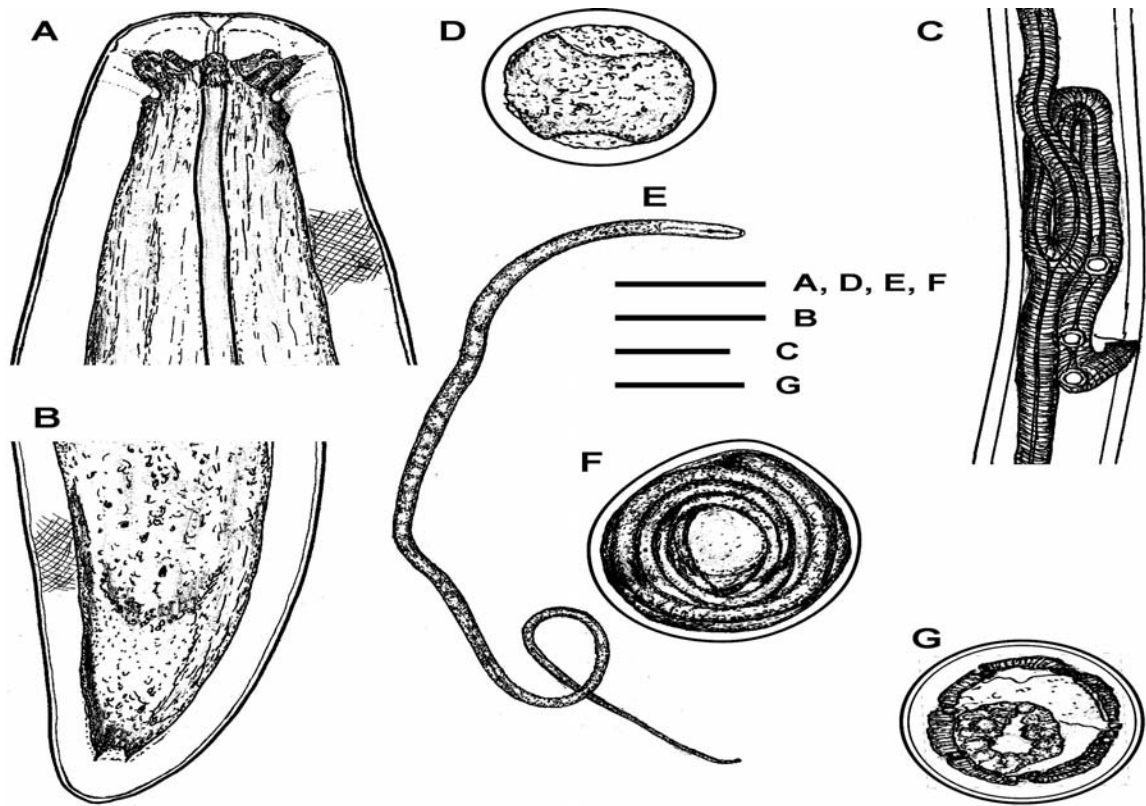


Figure 1: *Amphimermis thezamica* sp. n., female, holotype. A: Anterior region of the body, lateral view; B: Posterior end, lateral view; C: Vagina, lateral view; D: Uterine egg; E: stage -2 (infective), whole body; F: Last stage of larva in egg; G: Mid-body, cross section. Scale for A, D, E, F = 50 μ m; B = 100 μ m; C = 400 μ m; G = 200 μ m.

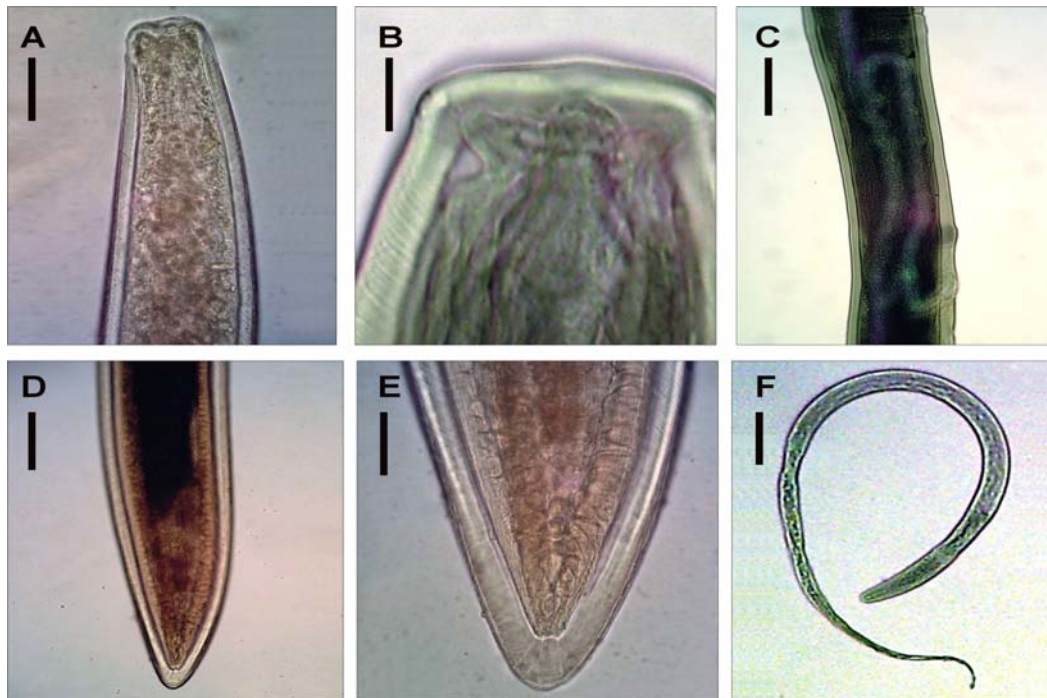


Figure 2: *Amphimermis thezamica* sp. n., female, holotype. A: Anterior region of the body, nerve ring, and pharyngeal tube, lateral view; B: Head, lateral view; C: Vagina, lateral view; D: Posterior region of the body, lateral view; E: Tail end; F: stage -2 (infective juveniles) whole body. Scale for A, E = 25 μ m; for B = 30 μ m; for C, D = 100 μ m; for F = 100 μ m.

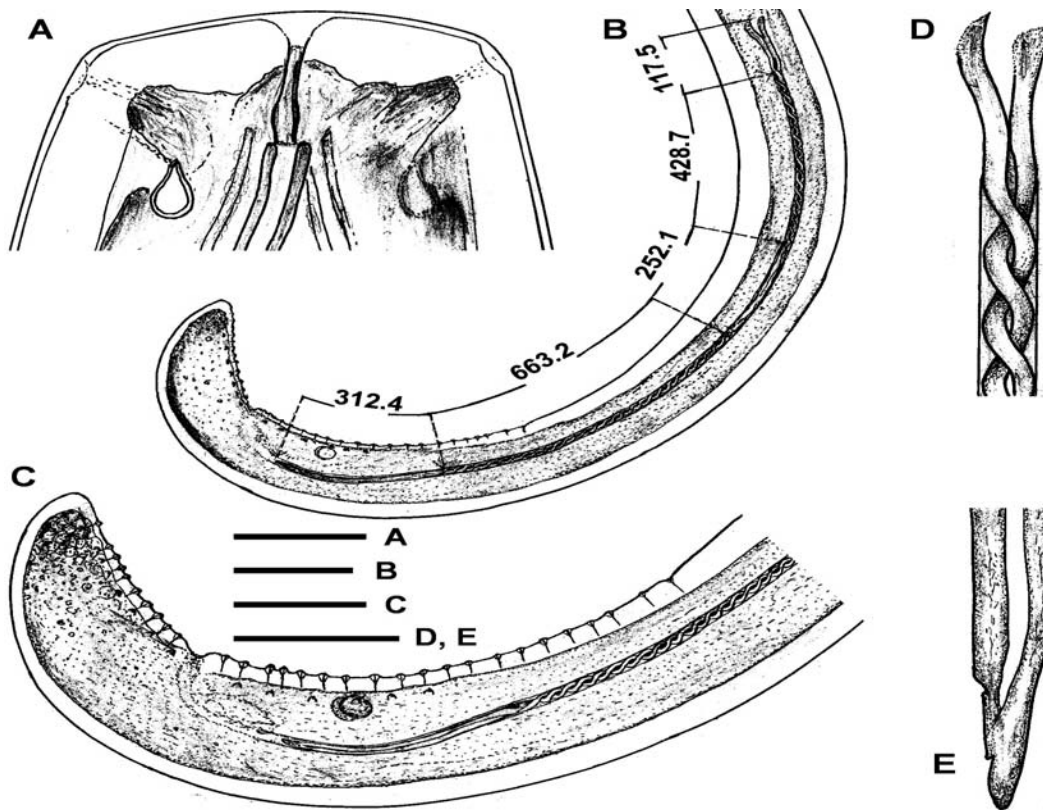


Figure 3: *Amphimermis thezamica* sp. n., male, holotype. A: Head, wide of the cuticle, lateral view; B: Tail, with whole spicula, lateral view; C: An enlarged tail with a half spicula; D: Non twisted and twisted head of spicule; E: Last part of non-twisted spicule. Scales for A=20 μ m; B=150 μ m; C=100 μ m; D,E=25 μ m.

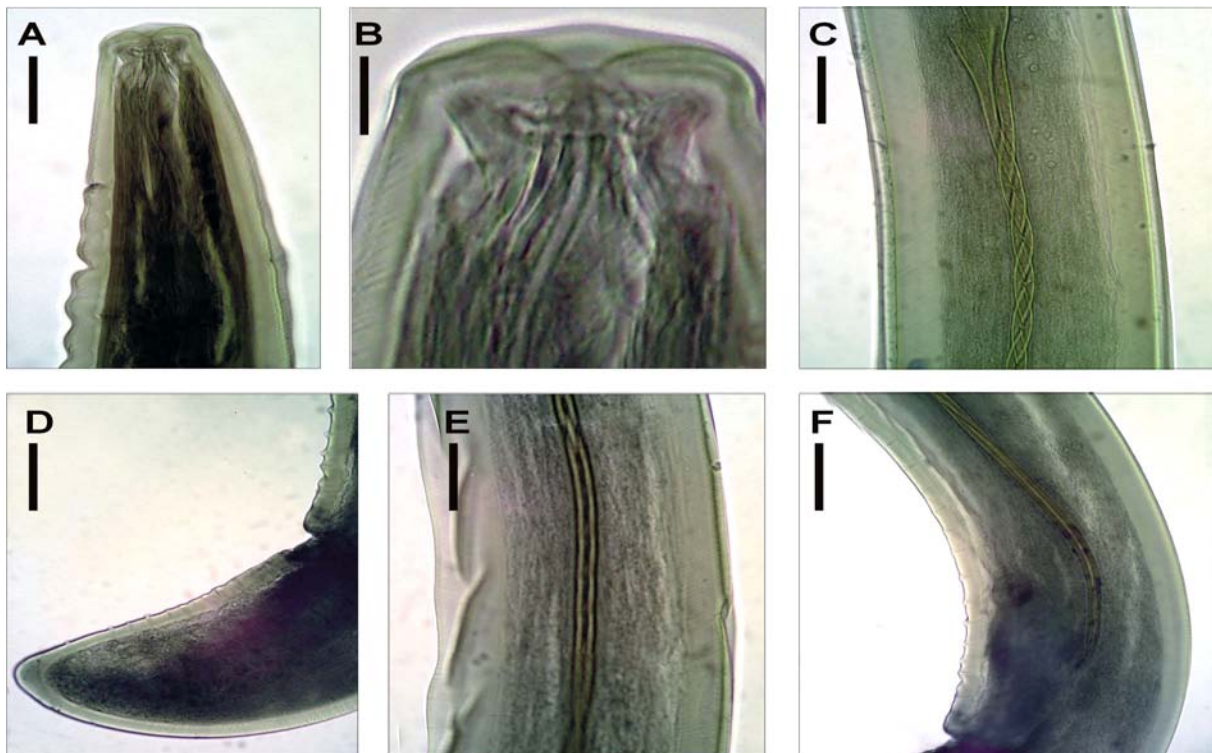


Figure 4: *Amphimermis thezamica* sp. n., male, holotype. A: Anterior region of body, lateral view; B: Head, lateral view; C: Non - twisted and twisted part of spicula head; D: Tail, lateral view; E: Middle part of non-twisted spicule; F: End of non-twisted spicule, lateral view; Scales for A,C,D,E,F=25 μ m; for B=30 μ m.