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Zoology

Isomermis halyomorphae n. sp. (Nematoda: Mermithidae) Parasite of Brown Marmorated Stink Bug *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) in the West Georgia (Caucasus)

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(Presented by Academy Member Tinatin Sadunishvili)

The paper reports on *Isomermis halyomorphae* n. sp. (Nematoda: Mermithidae) revealed from the brown marmorated stink bug -*H. halys* (Stål, 1855) (Hemiptera: Pentatomidae) in the West Georgia. This species is an invasive, highly polyphagous and aggressive pest from Asia. It feeds and damages more than 170 different plant species. In Georgia pest appeared for the first time in 2015 and has caused losses of about 200 million dollars to the hazelnut production. During biological studies from 2019 to 2020 *H. haly* adults were collected from hazelnut orchards of Guria and Samegrelo regions (West Georgia) and studied in laboratory conditions. From pest body not known species of nematode was identified, which belongs to the family of Mermithidae. Representatives of this family are obligate parasites. They are found in Orthoptera, Lepidoptera, Coleoptera, Hymenoptera, Hemiptera and in invertebrates of different, other genera and cause pest mortality. Only post parasitic larvae of female Mermithidae are described. Male is not known. Anatomically and morphologically the new species is most closely related to species of I. rossica, I. tansaniensis, I. brevis, I. Wisconsinensis. This Mermithidae differs from the previously described species of the genus by the long body, large size of stomatal collar, short esophagus tube, amphidoid form, and slightly S-shaped short and strong vagina. *© 2024 Bull. Georg. Natl. Acad. Sci.*

Isomermis haliomorphae n. sp., *Halyomorpha halys*, Nematoda, Mermithidae, parasitism, morphology, taxonomy

Brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) ((Hemiptera: Pentatomidae) is a highly polyphagous and aggressive pest. It feeds on and damages more than 170 different plant species, including economically important fields, vegetables, fruit trees, and ornamental plants [1-3]. *H. halys* is widespread in forested and agricultural areas as well as in urban areas of West Georgia, and it is becoming a serious pest for economically important hazelnut crops. According to the Natio-

nal Food Agency of the Ministry of Environmental Protection and Agriculture of Georgia, since 2015, *H. halys* has caused losses of about 200 million dollars to the hazelnut sector [4]. During biological studies of the mentioned pest, which was collected from hazelnut orchards of Guria and Samegrelo regions (West Georgia), an unknown species of nematodes belonging to the family Mermithidae was identified from their bodies. Representatives of this family are obligate parasites and are found in Orthoptera, Lepidoptera, Coleoptera, Hymenoptera, Hemiptera and invertebrates of many other genera [5-9]. The larvae of mermithid parasites, which live freely in the external medium, penetrate into the host organism by different routes (per-os, per-rectum, per-kutaneum) and continue their parasitic life there [10]. After reaching the parasitic stage, the larva makes a hole in the body of the insect and exits the insect, resulting in the death of the host due to intoxication, whereas the post-parasitic stage mermithid larva continues to grow and develop as an independent form in the environment [11]. At this stage, the parasite does not feed, but only selects a suitable habitat for maturation [12]. The goal of the study was to identify and study the pathogens of *H. halys* common in West Georgia. In the present work, unknown forms of mermithids recorded in the pest were investigated. The detected nematodes were identified using morphological and morphometric analysis. The research analysis showed that the described mermithid is a new parasitic form and belongs to the genus Isomermis.

Materials and Methods

The brown marmorated stink bug (*Halyomorpha halys*) was collected in the regions of Guria and Samegrelo, West Georgia. In Guria Region, adults and nymphs of *H. halys* were obtained in private hazelnut gardens in Anaseuli village (41°54'05" N; 41°59'11" E, 123 m a.s.l), and in village Shamgona in Samegrelo region (42°31'09" N; 41°46'10" E, 60 m a.s.l). Sampling of the study insects was conducted in both regions during summer and fall 2019/2020. Live forms of *H. halys* collected from the walnut orchard were placed in the containers with meshed caps, labeled and transported to the laboratory for pathogen testing. Prior to the bio-analysis, insects were incubated for two days at room temperature 21-22°C and relative humidity 68-72%. Up to 100 live adult insects and young nymphs were placed in each container. To survive, the insects were fed raw grass, apple slices, pear and some other fruits. The containers were sprinkled with tap water from inside and periodically checked for post-parasitic mermithid larvae emerging from the body of the insects [13]. A total of 2780 live specimens of *H. halys* were checked for the presence of pathogens. Nematode specimens isolated from the insect were washed twice with distilled water and preserved in 96.5% alcohol. For several weeks, the nematodes from the alcohol were being transferred to a closed vessel at room temperature where the nematodes were gradually dehydrated by the method of glycerol-alcohol mixture (70 ml 95.5% alcohol, 5 ml glycerol, 25 ml distilled water) [14]. The dehydrated nematodes were placed on slides in paraffin rings and labeled [15]. Female postparasitic mermithid larvae were examined individually. Sketches, photographs, morphological and morphometric studies were performed using a Motic–DMB-1 light microscope under 10-, 40- and 100-fold magnification. Morphological identification was carried out mainly according to the originals of Welch [16], Rubzov [17, 13] and later described species [18-20].

Morphological Analysis, Results

Mermithid from *Halyomorpha halys*

Family Mermithidae Braun, 1883

Genus Isomermis Coman, 1953

Diagnosis. It is of medium size. Cuticle is relatively thick, without transverse filaments. Its head has 6 papillae, usually with 2-3 sensillae. Amphidia are small, rarely large oval and elongated; Amphid apertures open near the papillae. It has 8 longi-

tudinal chordae. Mouth opening are always terminal, and are sometimes slightly bent to the ventral side. Collar is slightly pronounced. The esophagus is ¼ of the body length. Stichosomes are with 14-16 large stichocytes assembled in groups of 2, 4, 6, and 8. Vagina is slightly curved in an S shape and located obliquely. Spicules are of medium length, in pairs, curved sickle-shaped, with parallel margins. The tail is blunt and rounded. The larva has a very short caudal appendage. It is an inhabitant of running water.

Typical species: ***Isomermis rossica*** **Rubzov, 1968**

Isomermis halyomorphae **n. sp.**

Description (Figs. 1 and 2)

Measurement:

Allotype: (n = 1); Post-parasitic juvenile female; L = 64.0 mm; a = 212.3; b = 15.3; V % = 57.4. Body diameter at head papillae is 91 μm, is 183 μm at nerve ring, 301 μm at vaginal rudiment, and 208 μm at last part of trophosoma; distance from head to nerve ring is 310 μm and 36 mm to vaginal rudiment. Distance from end of trophosome to end of tail is 218 μm. Cuticle thickness on the anterior side of head capsule is 9.1 μm, 10.9 μm at vaginal rudiment, and 25 μm at the end of tail capsule.

Paratype: (n = 3); Post-parasitic juvenile female; L = 64.0±3 (mean ± standard deviation) (60.5-67.5) mm; a = 212.3±7 (202.4-216.7); b = 15.3±1 (13.1-16.4); V% = 57.4±2 (55.6-59.8). Body diameter at head papillae is 91±6 (82-93) μm, 183±13 (161-184) μm at nerve ring, 301±7 (299-312) μm at vaginal rudiment, and 208±18 (182-218) μm at the end of trophosoma; Distance from the head to the nerve ring is 310±15 (291-322) μm, and 36±2 (33-38) mm to vaginal rudiment. Distance from the end of trophosoma to end of tail is 218±43 (213-291) μm. Cuticle thickness on the anterior side of head capsule is 9.1±1 (8 -10) μm, 10.9±1 (10-11) μm at vaginal rudiment and 25±1 (25-26) μm at the end of tail.

Post-parasitic juvenile female: Female mermitid is very long and slender. It is growing. Apical end of the head is roundly obtuse. Neck constriction is absent or expressed slightly under the cuticle. Cuticle is without transverse fibers. Mouth opening is terminal. Its head is with 6 cephalic papillae arranged in a row and a pair of medium-sized oval-cup-shaped amphidia 15.6± 0.7 (14.5-16.1) μm long and 14.3±0.9 (14.7-15.6) μm wide; diameter of amphidian aperture is wide 9.8±0.7 (9.1-10.5) μm. Amphidial sensors open slightly behind the cephalic papillae. Head lobes are rounded and protrude to the head surface. Esophagus is short, 4.3±0.6 (4.1-4.6) mm long and 5.2±0.6 (4.6-5.9) μm in diameter. Esophageal tube constitutes 2. 56% of body length and is enclosed in a broad sheath along its entire length. Stoma is broad; stomatal tube shows differentiation of prorabdione, mesorhabdione and metarhabdione. Length of stomatal tube is 26 μm, it tapers at the head and tip and is of equal (2.6 μm) width, while the middle part is wider (5.2 μm). Collar is prominent, occupying 80% of the length of stoma. Diagonal tissue cells of the nerve ring can be observed in the anterior part of the body (Figs. 1, 2 - A). Stichosomes are heterogeneous glandular cells are located along esophagus. Body is also followed by trophosome protein granules that do not reach the end of tail as a slightly dark line. Larvae at this stage have not yet fully formed a vulva, but a partial vagina is present, and the vulva is thought to be at the base of the vagina, which is its normal position. Lips of vulva from outside of body are unnoticeable, not protruding; vaginal rudiment is short and strong, and slightly eakly S-shaped; vaginal branches are very short (Fig. 1, 2 - E). Vagina is located somewhat behind middle part of body. Posterior part of the body is slightly narrowing. End of tail is rounded and ends in a small barbed appendage 65 (60-67) μm long (with mucro) (Fig.1, 2-B, D).

Male. Not known.

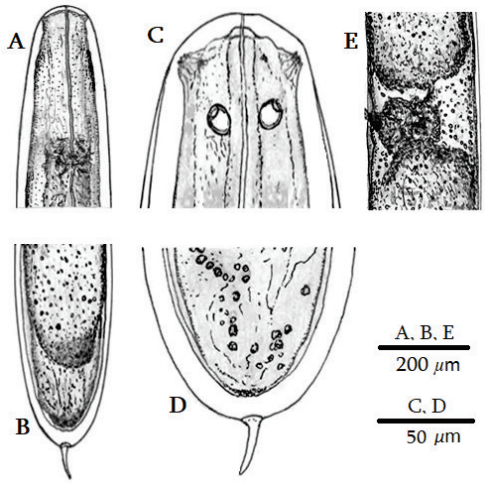

Fig. 1. *Isomermis halyomorphae* n. sp., Post-parasitic juvenile female, holotipe. A: Anterior region of the body, nerve ring and pharyngeal tube, lateral view; B: Posterior region of the body, (with a trophosome) lateral view; C: Head, with papillae and amphids, ventral view; D: Tail end; E: Vagina, lateral view.

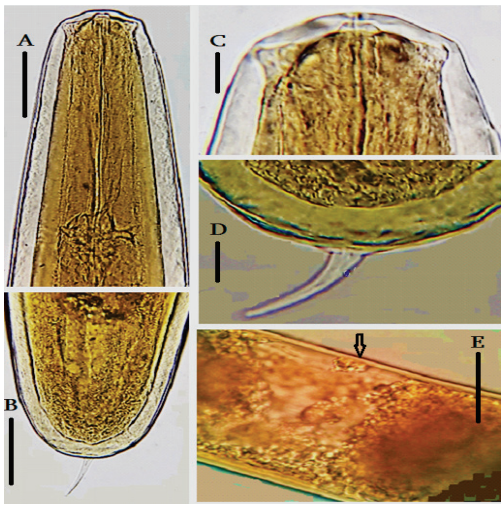

Fig. 2. *Isomermis halyomorphae* n. sp., Post-parasitic juvenile female, holotipe. A: Anterior region of the body, nerve ring, and pharyngeal tube, lateral view; B: Rear end with tail appendage, lateral view. C: Head, with a stoma collar and an esophageal plug; D: Tail end; E: Vagina, lateral view. (Scale bars: A, B, E=50 μm; C, D=12.5 μm).

Type host and locality. *The host is Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae). It is a highly polyphagous and aggressive pest. It feeds on and damages more than 170 different plant species, including economically important fields, vegetables, fruit trees, and ornamental plants [1-3]. Study insects were collected in village Anaseuli in Guria Region (GPS coordinates: 41°54'05" N; 41°59'11" E, 123 m a.s.l) and village Shamgona in Samegrelo Region (42°31'09" N; 41°46'10" E, 60 m a.s.l) in West Georgia.

Type material. Allotype: Female (slide ISUZI0004841); and 2 paratypes: female (slide ISUZI0004842 and slide ISUZI0004843) are stored in the Collection Museum of the Institute of Zoology, Ilia State University, Tbilisi, Georgia.

Etymology: Specific name is taken from the host genus *Halyomorpha.*

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Diagnosis and Relationships

Genus Isomermis, like the entire Family Mermithidae, needs a complete revision. Many symbols are used to describe species. They show intraspecific and ontogenetic variability [15]. Therefore, quite often, the initial diagnosis of a species is not precise [21]. Camino [19] considers approximately 12 species of the genus as recognized: *Isomermis balcarcensis* [19], *I. benevolus* [22], *I. bipapillatus* [18], *I. brevis* [23], *I. gallica* [24], *I. herculanensis* [25], *I. lairdi* [26], *I. papilata* [27], *I. riparia* [28], *I. rossica* [17], *I. sierrensis* [20], *I. solenamphidis* [29], *I. tanzaniensis* [23], *I. ventania* [19], *I. wisconsinensis* [16].

Isomermis halyomorphae n. sp. was identified and subject to differential studies using the samples of postparasitic young larvae of females united in the genus Isomermis (features of adult forms were also taken into account).

The main features for attributing the new species to the genus Isomermis are: medium-sized body, thick cuticle, 6 cephalic papillae, medium-sized amphids, amphid sensors located close behind the papillae, terminal mouth opening, stoma collar, short and wide esophagus, slightly obliquely curved S-shaped vagina, blunt rounded tail and spike-like appendage (mucro) on tail.

With its anatomy and morphology, *I. Halyomorphae* n. sp. is closest to species *I. rossica, I. tansaniensis*, *I. brevis* and *I. wisconsinensis*.

I. halyomorphae n. sp. is very similar to *I. rossica,* but differs significantly from it in body length (average 64.0 and 14.5 mm, respectively), thicker cuticle (average 9.1-18.5 and 10-16 µm, respectively), smaller amphidia (average 14.3 x 14.3 and 16 x 20 µm respectively), with the location of the vulva in the posterior part of the body (respectively V%=57.4 and V%=50), with the host (*H. halys and Simulium morsitans* Edw., *Eusimulium latipes* Mg. respectively). *I. rossica* was found in different regions of Russia, Belarus [17], Ukraine [30] and France [24].

The new species is very similar to *I. Tansaniensis* and differs from it by a longer body (64.0 and 13 mm on average, respectively), greater "a" and "b" coefficients (a=212.3, b=15.3 and a=60, b=3.1, respectively), with thicker cuticle in the posterior part of the body near the tail (average 26.1 და 15.5 µm, respectively), absence of transverse filaments in the cuticle. larger body diameter near the head papillae, nerve ring, in the middle of the body (91, 183, 301 µm და 80, 125, 240 µm, respectively). *I. tansaniensis* was found in Tanzania; its host is *Simulium damnosum* Theo.

I. halyomorphae n. sp. is very similar to *I. brevis* and differs from it by a larger body (average 64.0 and 8.1 mm respectively). The new species has vulva in the posterior of its body (V% = 57.4), while *I. brevis* has it in the anterior of the body (V% = 48), *I. brevis* has a narrowed neck, while the new species does not have it. The new species has a larger diameter of the amphidian orifice (on average 9.8 µm larger than *I. brevis* (4-6 µm). *I. brevis* was found in Luginsky district, St. Petersburg region, Russia. The host is *Simulium morsitans* Edw.

The new species is very similar to *I. Wisconsinensis* and differs from it by a larger body (average 64.0 და 22.8 mm, respectively), length of the barbed appendage on the tail (65 and 18 µm, respectively), and greater distance from the head to the nerve ring (291-322 and 140-250 µm, respectively), with a larger body diameter at the vaginal rudiment (299-312 and 130-230 µm, respectively), with the vaginal rudiment located posterior to the body (V%=55-59 and V%=42-50, respectively). with the host (*H. halys* and *Simulium vittatum* Zett., Respectively). *I. wisconsinensis* was found in the state of Wisconsin in Canada.

The new species differs from the species of the genus Isomermis in having the largest body size, a sharply pronounced stomatal collar, a short esophagus, a short and powerful vagina, and a different host (Halyomorpha halys). Morphologically, the new species is also close to the genus *Meximermis ectatomics* [7] and *Mesomermis melusinae* [23].

Between 2018 and 2019 the entomopathogenic microsporidia bacterium *Nosema maddoxi* and the parasitoid *Anastatus bifasciatus* (Hymenoptera: Eupelmidae) have been found as biological control agents in the *Halyomorpha halys* population in Georgia [31-33].

ზოოლოგია

აზიური ფაროსანას *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) პარაზიტი *Isomermis halyomorphae* n. sp. (Nematoda: Mermithidae) დასავლეთ საქართველოში (კავკასია)

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აზიური ფაროსანა არის მაღალპოლიფაგური და აგრესიული მავნე მწერი. ის იკვებება და აზიანებს 170-ზე მეტ სხვადასხვა სახეობის მცენარეს, მათ შორის, ეკონომიკურად მნიშვნელოვან მინდვრებს, ბოსტნეულს, ხეხილს, დეკორატიულ მცენარეებს. მავნებელი პირველად 2015 წელს იქნა რეგისტრირებული დასავლეთ საქართველოს ტყისა და სასოფლო-სამეურნეო, ასევე ქალაქის ტერიტორიებზე. ის გახდა ეკონომიკურად მნიშვნელოვანი თხილის კულტურების სერიოზული მავნებელი. მან საქართველოში თხილის სექტორს დაახლოებით 200 მილიონი დოლარის ზარალი მიაყენა. 2019-2020 წლებში, მავნებლის ბიოლოგიური კვლევისას, რომელიც შეგროვდა გურიისა და სამეგრელოს რეგიონის თხილის ბაღებში (დასავლეთ საქართველო), მათი სხეულიდან გამოვლინდა ნემატოდის უცნობი პარაზიტული ფორმა, რომელიც მიეკუთვნება Mermithidae-ს ოჯახს. ამ ოჯახის წარმომადგენლები ობლიგატური პარაზიტებია და გვხვდება Orthoptera-ს, Lepidoptera, Coleoptera, Hymenoptera, Hemiptera-ს და სხვა მრავალი რიგის უხერხემლოებში და მათ სიკვდილს იწვევენ. ლაბორატორიაში მერმიტიდების გამოყოფა მოხდა ცოცხალი ზრდასრული აზიური ფაროსანას სხეულიდან (დაყოვნების მეთოდის გამოყენებით). ნემატოდებზე გამოკვლეული იქნა აზიური ფაროსანას 2780 ეგზემპლარი, რომელშიც გამოვლინდა 3 პოსტ-პარაზიტული მდედრი მერმიტიდის ლარვა, ხოლო მამრი მერმიტიდები არ გამოვლენილა. მერმიტიდების იდენტიფიცირება ჩატარდა მხოლოდ მორფოლოგიური და მორფომეტრული მეთოდების გამოყენებით. გამოკვლეული მერმიტიდების დიაგნოსტიკური ნიშნებია: ძალიან გრძელი და წვრილი სხეული, მომრგვალო ბლაგვი თავი, ექვსი ცეფალური პაპილა, ტერმინალური პირის ხვრელი, ოვალურ-ფინჯნისებრი ფართოხვრელიანი ამფიდები, S-ფორმის განიერი საშო და მომრგვალებული კუდის ბოლო-წვეტიანი ეკლისმაგვარი დანამატი. აღწერილი მერმიტიდის ახალი სახეობა, მსგავსებით ყველაზე ახლოსაა Isomermis-ის გვარის სახეობებთან, როგორიცაა: *Isomermis rossica, I. tansaniensis, I. brevis* და *I. wisconsinensis*. ეს მერმიტიდი განსხვავდება გვარში ადრე აღწერილი სახეობებისგან, ყველაზე გრძელი სხეულით, სტომის საყელოს სიდიდით, მოკლე საყლაპავით და ოდნავ ოვალური ამფიდების ფორმით. კვლევითმა ანალიზმა აჩვენა, რომ აღწერილი ნემატოდა არის ახალი სახეობა *Isomermis haliomorphae* n. sp. (Nematoda: Mermithidae).

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