

***Stammerinema hyalinum* n. comb. for *Filaria hyalina* von Linstow, 1890 and its recognition as a senior synonym of *Stammerinema rhopalocephalum* (Sołtys, 1952) (Spirurida: Acuariidae), a parasite of shrews**

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Abstract Based on a re-examination of type-specimens of *Filaria hyalina* von Linstow, 1890 from *Sorex araneus* L. (Mammalia: Soricidae) and morphological studies (light and scanning electron microscopy) of specimens collected from the same host species in Bulgaria and previously identified as *Stammerinema rhopalocephalum* (Sołtys, 1952), both these forms are considered to be conspecific. Accordingly, *F. hyalina* is transferred to the genus *Stammerinema* Osche, 1955 as *Stammerinema hyalinum* n. comb. and the species originally described as *Synhimantus rhopalocephalus* Sołtys, 1952 is considered its junior synonym.

Introduction

The nematodes of the family Acuariidae Railliet, Henry & Sisoff, 1912 form a diverse group

comprising mainly parasites of birds, rarely of mammals (Bain et al., 2014). The genus *Stammerinema* Osche, 1955 includes parasites of Soricidae (Mammalia) in the Holarctic Region. Quentin & Wertheim (1986) considered three valid species within this genus: *S. soricis* (Tiner, 1951) Osche, 1955 (type-species), a parasite of *Sorex monticolus* Merriam (= *Sorex obscurus alascensis* Merriam) in Alaska, *S. rhopalocephalum* (Sołtys, 1952) Quentin & Wertheim, 1986, a parasite of diverse European shrews, and *S. globocephalum* Quentin & Wertheim, 1986, a parasite of *Crocidura suaveolens* (Pallas) (= *Crocidura russula monacha* Thomas) in Israel. Another spirurid species, *Filaria hyalina* von Linstow, 1890, was described from *Sorex araneus* L. (= *Sorex vulgaris* Nilsson) (see von Linstow, 1890). It was suspected to be a member of *Stammerinema* by Guerrero & Bain (2011); however, due to the limited morphological information obtained from the type-specimens (posterior fragments of two males), they considered this species as *incertae sedis*.

In this article, we discuss the generic allocation of *Filaria hyalina* and its synonymy in view of new information obtained after our re-examination of the type-specimens from the collection of the Museum für Naturkunde, Berlin (ZMB), and its comparison with specimens of acuariid nematodes from shrews from Bulgaria identified as *Stammerinema rhopalocephalum* and studied by means of light and scanning electron microscopy (SEM).

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Materials and methods

The type-material of *Filaria hyalina* von Linstow, 1890 (ZMB Q3905, syntypes, from “*Sorex vulgaris*”, type-locality not mentioned, posterior fragments of two male specimens) from the Helminthological Collection of the ZMB, was redescribed. In addition, the following samples from the stomach of *Sorex araneus* from Bulgaria deposited in the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER-BAS), Sofia, were studied:

- 1 entire male and posterior fragment of another male collected at Borovets (Rila Mountains) on 15.iv.1968, reported by Jančev (1974) as *Skrjabinochlava soricis* (Tiner, 1951) (IBER-BAS N001.119);
- 2 entire females, two posterior fragments of females and 8 posterior fragments of males collected at Borovets (Rila Mountains) on 28.viii.1968, reported by Jančev (1974) as *Skrjabinochlava soricis* (Tiner, 1951) (IBER-BAS N001.120);
- posterior fragments of 4 males collected at Borovets (Rila Mountains) and reported by Jančev (1974), mounted on a SEM stub (IBER-BAS N001.121);
- 3 males collected at Krastatitsa Village (Rhodope Mountains) (41°38'9"N, 24°57'19"E) on 5.xi.2007 by YM and BBG, IBER-BAS N001.123 (2 males, preserved in 70% ethanol), IBER-BAS N001.121 (1 male mounted on a SEM stub);
- 1 male collected at Krastatitsa Village (Rhodope Mountains) (41°38'9"N, 24°57'19"E) on 2.xi.2007 by YM and BBG, used for molecular studies.

For light-microscopy, specimens were cleared and examined as temporary mounts in glycerol. Specimens used for SEM observations were transferred from 70% ethanol to 40% ethanol (10 min), rinsed in 0.1 M cacodylate buffer, post-fixed in 1% OsO₄ for 2 h and dehydrated in an ethanol series. They were coated with gold in fine coater JEOL JFS 1200 and examined using a JEOL JSM 5510 microscope at 10 kV. Metrical data are given as the range, with the mean and the number of measurements taken (n) in parentheses. Measurements are in micrometres unless otherwise indicated.

DNA was extracted from a single male specimen and dissolved in 100 µl TE buffer. A fragment of the mitochondrial cytochrome oxidase *c* subunit I (COI)

gene was amplified using the newly-developed for the purposes of the present study on the basis of unpublished COI gene sequences of acuariid nematodes primers COI-F Acu2 (forward; 5'-TTT CCT CGT GTT AAT GCT TT-3') and COI-R Acu3 (reverse; 5'-CAA ACA AAC GCT CCT TAT CAG A-3'). Polymerase chain reaction (PCR) amplifications were performed in a final volume of 50 µl containing 1× CoralLoad PCR buffer (Qiagen), 0.2 mM of each dNTP, 10 pmol of each primer, 1.5 U Taq polymerase (Qiagen) and 1 µl of DNA extract. The PCR cycling conditions were: 94°C for 1 min followed by 5 cycles of 94°C for 50 s, 47°C for 50 s and 72° for 60 s, followed by 30 cycles of 94°C for 50 s, 50°C for 50 s, 72°C for 60 s, and a final extension step of 72°C for 5 min. Sequencing was performed by Macrogen Inc. using the amplification primers.

Mammalian nomenclature follows Wilson & Reeder (2005).

Stammerinema hyalinum (von Linstow, 1890) n. comb.

Syns *Filaria hyalina* von Linstow, 1890; *Synhimantus rhopalocephalus* Sołtys, 1952 (new synonymy); *Stammerinema rhopalocephalum* (Sołtys, 1952) Quentin & Wertheim, 1986

Redescription of the type-specimens (Fig. 1)

[Based on posterior fragments of two male syntype specimens of *Filaria hyalina* (ZMB Q3905); measurements of the first specimen are followed by those of the second one given in parentheses.] Posterior body fragments 3.25 (5.99) mm long, with maximum width 274 (248). Cuticle 8–10 thick, with transverse striations 4–6 apart. Tail 207 (256) long; body width at cloaca 114 (137). Left spicule thin, 530 (519) long, with pointed distal end and composed of equal in length lamina and handle. Right spicule short and robust, 166 (174) long. Caudal alae 347 (416) long. Precloacal papillae represented by single ventral precloacal papilla and 4 pairs of subventral pedunculate papillae arranged in 2 groups each containing 2 pairs (Fig. 1A, B). Postcloacal papillae 6 pairs: 5 pairs of subventral pedunculate papillae and 1 pair of sessile papillae, situated between bases of last pedunculate papillae. Phasmids subterminal.

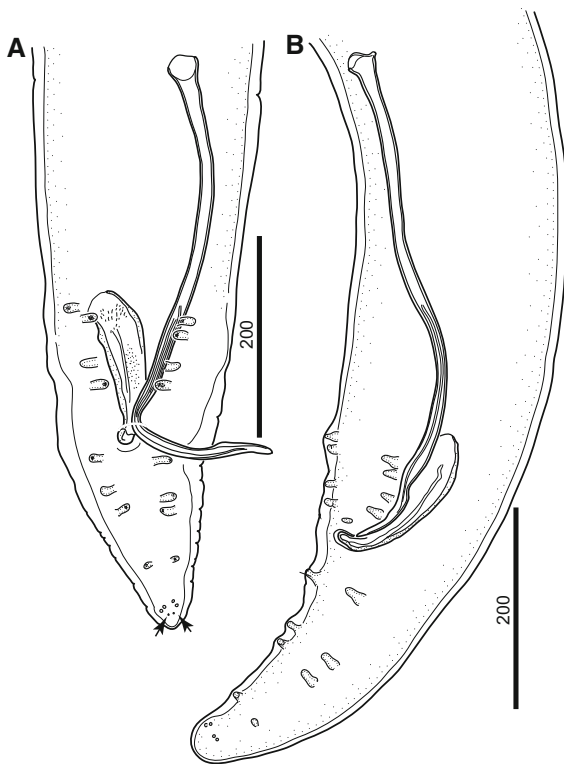


Fig. 1 *Stammerinema hyalinum*, syntypes (ZMB Q3905). A, Posterior end, ventral view, note phasmids (arrows); B, Posterior end, lateral view. All scale-bars are in micrometres

Description of comparative materials (Figs. 2–3)

General. [Based on specimens ex *Sorex araneus* from Bulgaria.] Anterior end with two small triangular pseudolabia, each bearing single amphid and pair of cephalic papillae (Fig. 3B). Body posterior to pseudolabia markedly swollen (Figs. 2A, B, 3A). Cords arise dorsally and ventrally between pseudolabia, extending posteriorly beyond nerve-ring, recurrent in anterior direction and anastomosing (or may not anastomose) at level of mid-length of buccal cavity. Each cord consists of 2 rows of cuticular plates with slightly serrate posterior rim, delimiting deep longitudinal gutter (Fig. 3B). Deirids small, spine-like, at level of posterior loop of cords (Fig. 3C). Two lateral rows of small cuticular spines extend posterior to cords and fade before mid-level of glandular oesophagus (Figs. 2A, 3C, D).

Male [Based on materials from Krastatitsa; $n = 3$, unless otherwise indicated.] Body 6.5–7.8 (7.3) mm

long. Anterior end swollen, 385–424 (405) long, 312–393 (351) wide. Maximum body width 197–210 (204), measured at level of middle and posterior third of body length. Body width at level of cloaca 82–104 (92). Tail 195–225 (207) long. Cords extended to 313–358 (338) from anterior body end, recurrent in anterior direction to 89–134 from anterior extremity, with maximum width 34–39 (36). Excretory pore at 376–425 (401, $n = 2$) from anterior end. Buccal cavity 184–206 (196) long, 11–13 wide. Muscular oesophagus 633–654 (644, $n = 2$) long, 54–57 (56) wide. Glandular oesophagus 2,910–3,011 (2,961, $n = 2$) long, 79–100 (91) wide. Nerve-ring at 250 ($n = 1$) from anterior body end. Cuticle 7–9 thick, with transverse striations 5–6 apart. Caudal alae 335–375 (351) long. Precloacal papillae represented by single ventral precloacal papilla and 4 pairs of subventral pedunculate papillae arranged in 2 groups, each group consisting of 2 pairs (Figs. 2C, 3E). Postcloacal papillae 6 pairs: 5 pairs of subventral papillae and 1 pair of small papillae situated between bases of last pair of subventral papillae (Fig. 3F). Left spicule 479–523 (499) long, with pointed distal end; lamina and handle of equal length. Right spicule short, robust, 156–169 (161) long (Fig. 2D). Ratios: length of muscular oesophagus/length of glandular oesophagus 0.210–0.225; length of oesophagus/body length 0.456–0.540; length of caudal alae/body length 0.045–0.053; length of left spicule/length of right spicule 2.935–3.353.

Female [Based on materials from Borovets; $n = 2$, unless otherwise indicated.] Body length 11.7–13.1 mm. Anterior body end markedly swollen, 1,032–1,089 long, 688–722 wide. Maximum body width measured posteriorly to swollen anterior end, 459–516, posterior to vulva. Body width 459–482 at level of vulva, 197–210 at level of anus. Cords 516–528 long, 45–54 wide. Deirids and excretory pore not observed. Buccal cavity 258 ($n = 1$) long. Muscular oesophagus 81 ($n = 1$) wide. Glandular oesophagus 3,300 ($n = 1$) long, 121 ($n = 1$) wide. Cuticle 5–6 thick, with transverse striations 8–9 apart. Vulva at 6.6–7.0 mm from anterior body end. Reproductive system didelphic-amphidelphic, extending in anterior direction to anterior swollen part of body. *Vagina vera* short, separated from short *vagina uterina* by well-developed sphincter (Fig. 2E). Tail 179–233 long (Fig. 2F). Eggs oval, with developed first-stage larva,

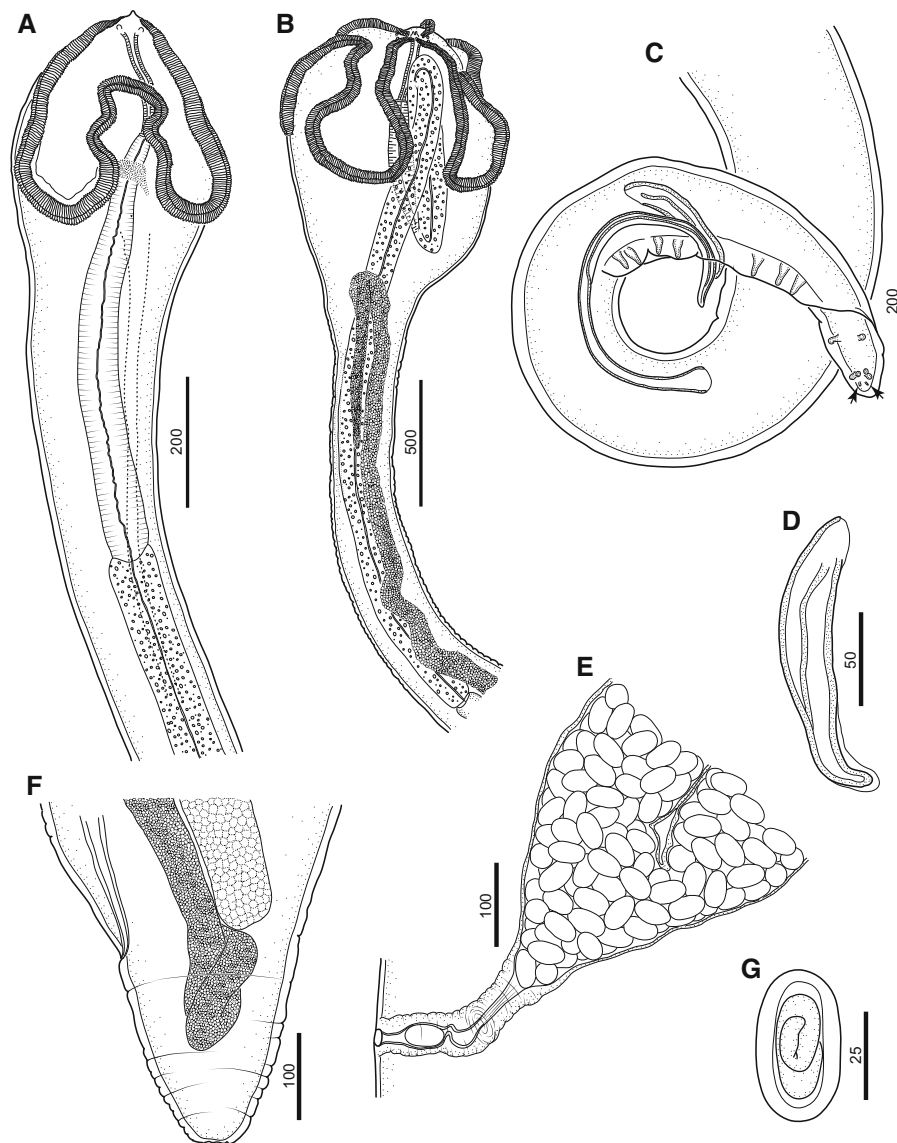


Fig. 2 *Stammerinema hyalinum*, comparative materials from Bulgaria. A, Anterior end, male, lateral view; B, Anterior end, female, lateral view; C, Posterior end, male, sinistral view, note phasmids (arrows); D, Right spicule, dextral view; E, Vagina, lateral view; F, Posterior end, female, lateral view; G, Egg. All scale-bars are in micrometres

43–44 × 22–24 (n = 10) (Fig. 2G). Ratio distance from vulva to posterior end/body length 0.537–0.563.

Molecular identification

A fragment of 481 bp of the COI gene was amplified. The nucleotide sequence is available in the GenBank database under accession no. KP059294. The closest available match after a query in the GenBank was a fragment of

COI gene of another acuariid species, *Proyseria petterae* Mutafovich, Mariaux & Georgiev, 2014, accession no. KJ995862, published by Mutafovich et al. (2014).

Discussion

Osche (1955) synonymised *Synhimantus rhopalcephalus* Soltys, 1952 with *Dispharynx soricis* Tiner,

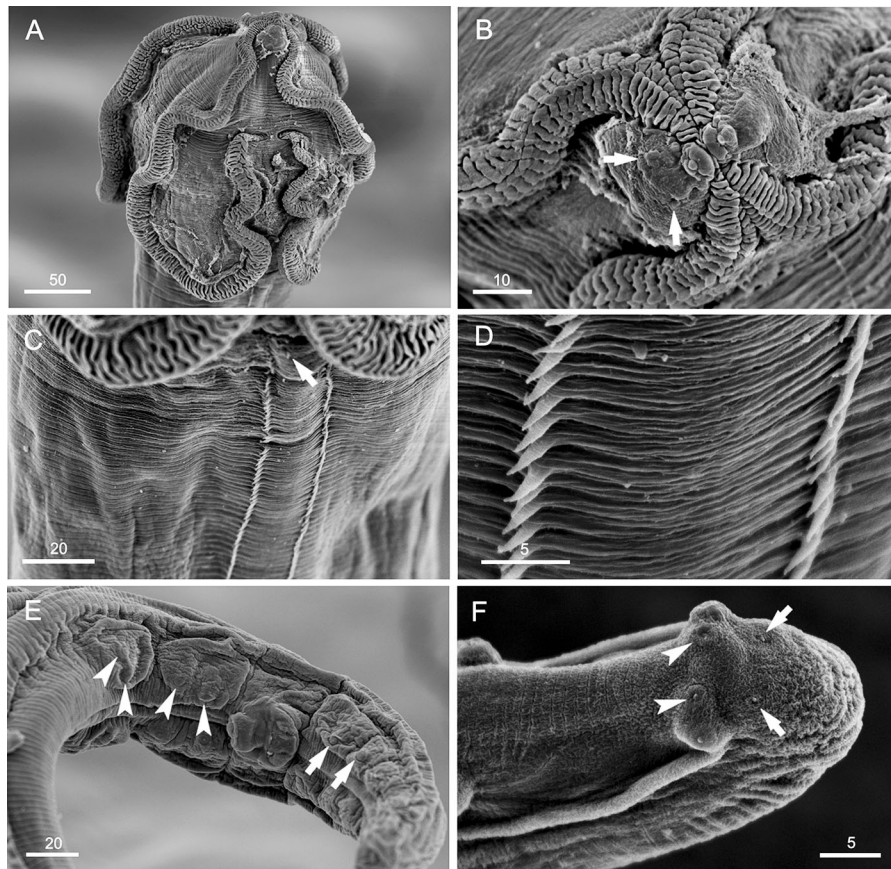


Fig. 3 *Stammerinema hyalinum*, comparative materials from Bulgaria, male, SEM. A, Anterior end, lateral view; B, Anterior end, apical view, note cephalic papillae (arrows); C, Detail from posterior end of cordons, lateral view, note two rows of cuticular spines and deirid (arrow); D, Lateral cuticular rows of spines; E, Posterior end, ventral view, note precloacal papillae (arrow heads) and postcloacal papillae (arrows); F, Tail extremity, ventral view, note a pair of small papillae (arrowheads) situated between bases of last pair of subventral papillae and phasmids (arrows). All scale-bars are in micrometres

1951 and erected the genus *Stammerinema* as monotypic. Skrjabin et al. (1965) did not accept *Stammerinema* as valid and considered it as a synonym of *Skrjabinoclava* Sobolev, 1943; they followed the synonymy of *Dispharynx soricis* and *Synhimantus rhopalocephalus* as proposed by Osche (1955). The subsequent records of nematodes belonging to this acuariid group and parasitising European shrews were referred as either *Stammerinema soricis* or *Skrjabinoclava soricis* (Tiner, 1951) Skrjabin, Sobolev & Ivashkin, 1965 (see Merkusheva, 1966; Jančev, 1974; Jančev & Stoykova-Hadjinikolova, 1980; Mészáros et al., 1982; Genov, 1984; Quentin & Beveridge, 1986; Smogorzhevskaya, 1990; Matskási et al., 1996; Portolés et al., 2004). Quentin & Wertheim (1986) recognised *Synhimantus rhopalocephalus* as valid and considered it as parasitic in European shrews only.

We agree with their decision for distinguishing *S. soricis* as described by Tiner (1951) and *S. rhopalocephalus* from European shrews as well grounded. Our observations based on Bulgarian material are in agreement with those by Quentin & Wertheim (1986) that females of *Stammerinema* recorded in Europe differ from those of the other two congeneric species by the anterior swollen extremity, which takes smaller part of the body length and is not occupied by parts of the reproductive system.

The type-specimens of *Filaria hyalina* re-examined here correspond well to *S. rhopalocephalus* as described by Sotys (1952) in the arrangement of the caudal papillae, the shape of the spicules as well as in the length of the right spicule. The left spicule of the specimens described by Sotys (1952) is slightly shorter (450–480 vs 519–530 μm), however this

difference falls within the limits of intraspecific variation known for the acuariid nematodes (see Table 1). On this basis, we recognise *S. rhopalcephalum* as a junior synonym of *Stammerinema hyalinum*.

The specimens from *S. araneus* collected in Bulgaria and studied by us exhibit close morphology to *S. hyalinum* as well as to the previously described samples from Poland and the Ukraine (Table 1). Although they could be distinguished from those reported in France and Belgium by their smaller body dimension and shorter left spicule, they resemble them by the swollen anterior part, which is not occupied by the reproductive system.

The host range of *S. hyalinum* includes *Sorex alpinus* Schintz in Slovakia (Mészáros et al., 1982); *Sorex*

araneus in Belgium (Bernard, 1961), France (Quentin & Beveridge, 1986), Germany (Osche, 1955), Poland (Sołtys, 1952), Hungary (Matskási et al., 1996), Slovakia (Prokopič, 1956), Bulgaria (Jančev, 1974; Genov, 1984), Ukraine (Smogorzhevskaya, 1990), Belarus (Merkusheva, 1966); *S. minutus* L. in Germany (Osche, 1955), Poland (Sołtys, 1954), Hungary (Matskási et al., 1996), Bulgaria (Jančev, 1974; Genov, 1984); *S. caecutiens* Laxmann (= *Sorex macropygmaeus karpinskii* Dehnel) in Poland (Sołtys, 1954); *Neomys fodiens* (Pennant) in Germany (Osche, 1955), Poland (Sołtys, 1954); as larvae in the intestine and the abdominal cavity in *Crocidura russula* Hermann (see Portolés et al., 2004) and in the liver and the mesentery of one *Crocidura suaveolens* (Pallas) in Bulgaria (Jančev & Stoykova-

Table 1 Metrical data of *Stammerinema hyalinum* ex *Sorex araneus* from various localities

Source	Sołtys (1952)	Bernard (1961)	Quentin & Beveridge (1986)	Smogorzhevskaya (1990)	Genov (1984)	Present study	
Country	Poland	Belgium	France	Ukraine	Bulgaria	Bulgaria	
						Krastatitsa	Borovets
Male	na	na	na	na	na	(n = 3)	(n = 1)
Body length (mm)	4.5–6.0	8.6	9.5	6.5	5.9–7.4	6.5–7.8	6.4
Tail, length	–	229	175–335	250	226–241	195–225	159
Buccal cavity, length	–	202	190		189–194	184–206	161
Muscular oesophagus, length	–	797	730	590	362–417	633–654 ^a	465
Glandular oesophagus, length	–	3,580	2,850	2,600	2,811–2,920	2,910–3,011 ^a	2,149
Cordons, length	–	476	430	410	–	313–358	282
Left spicule, length	450–480	567	580	460	466–492	479–523	483
Right spicule, length	160–170	148	160–175	150	154–172	156–169	152
Female	na	na	na		na		(n = 2)
Body length (mm)	11.0–14.0	16.6	15.8	–	9.5–15.7	–	11.7–13.1
Tail, length	–	600	360	–	–	–	179–233
Buccal cavity, length	–	357	250	–	290–323	–	258
Muscular oesophagus, length	–	1,011	800	–	878–989	–	–
Glandular oesophagus, length	–	4,339	4,600–4,900	–	2,460–4,200	–	3,300
Cordons, length	–	833–960	800	–	–	–	516–528
Vulva, distance from anterior body end	4.2–5.5	7.8	6.5	–	5.7–6.9	–	6.6–7.0
Eggs	38–42 × 25–29	41–43 × 22–24	43–26	–	42–46 × 24–28	–	43–44 × 22–24 ^b

^a n = 2; ^b n = 10; na data on the number of specimens examined not available

Hadjinikolova, 1980). Based on these records, the predilection hosts of *S. hyalinum* seem to be insectivores of the genus *Sorex* L., whereas those from the genera *Neomys* Kaup and *Crociodura* Wagler are accidental.

The original spelling proposed by Quentin & Wertheim (1986) for the Palaearctic species of the genus *Stammerinema* is “*Stammerinema rhopalocephala*” and “*Stammerinema globocephala*”. According to Article 30 (30.1.2) of the International Code of Zoological Nomenclature (ICZN, 1999), the generic names ending in “-nema” are of neuter grammatical gender. Therefore, the proper spelling of the two species mentioned above is *Stammerinema rhopalocephalum* and *Stammerinema globocephalum*.

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