

Observations on the nematode fauna of terrestrial molluscs of the Sofia area (Bulgaria) and the Crimea peninsula (Ukraine)

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Summary. Short surveys on the nematode fauna of terrestrial molluscs in Sofia, Bulgaria and Crimea, Ukraine were performed. The composition of nematode fauna was compared with data from the literature and in our records of nematode fauna from different regions of continental Europe and the British isles. Two nematode species, *Angiostoma dentiferum* Mengert, 1953 and *Agfa tauricus* Korol & Spiridonov, 1991 were found parasitising three and four, respectively, out of a total of 23 host species. The composition of nematode fauna was similar in both surveys, though the dominant nematode species in each region were different, with *A. dentiferum* in Bulgaria and *A. tauricus* in Crimea. Both morphological and molecular methods have demonstrated the complete identity of material from the two regions. The re-examination of our own material of *A. dentiferum* from Belgium has confirmed its similarity to that from Bulgaria and Crimea by molecular characteristics, and slightest morphological differences. Morphologically, *A. dentiferum* from Bulgaria, Crimea and Belgium corresponds well with its original description from Germany (Mengert, 1953). We assume that the re-description of *A. dentiferum* by Morand & Spiridonov, 1989, based on the material from France, is that of a closely related but different species, which is distinguished by presence vs absence of lateral alae and shorter spicules. Although the data on the distribution of parasitic nematodes of terrestrial molluscs in Europe are inconsistent, it seems that *A. tauricus* tends to be present in the south-east of Europe and absent from more northern territories where it is replaced by *A. flexilis* though the host range for both species is similar.

Key words: *Angiostoma dentiferum*, *Agfa tauricus*, morphology, morphometrics, r-DNA, SEM.

Land snails and slugs are known to host nematode parasites belonging to four of the six main clades of Nematoda (Ross *et al.*, 2010a). A number of nematode species, all from the Rhabditidae family, while not being true parasites are known to associate with terrestrial molluscs forming relationships ranging from pathogenic to necromenic. On the territory of continental Europe, surveys on nematodes associated with terrestrial molluscs were carried out in Germany (Mengert, 1953), France (Morand, 1986, 1992; Morand & Hommay, 1990; Morand & Petter, 1986), Crimea (Korol & Spiridonov, 1991; Vorobjeva *et al.*, 2008), Slovenia (Laznik *et al.*, 2009) and Belgium (own unpublished data). We carried out short surveys on nematode fauna of terrestrial snails and slugs in Bulgaria in 2012 and Crimea in 2011. The data obtained were compared with the data in the

literature. The identification of nematode species was made using morphological studies and by the comparison of partial sequences of 18S and D2-D3 regions of DNA. In this study, the specific names of *Angiostoma dentiferum* Mengert, 1953 and *A. kimmeriense* Korol & Spiridonov, 1991 were spelled according to the gender of the genus following Ivanova & Wilson, 2009.

MATERIALS AND METHODS

In Bulgaria, molluscs were collected from five localities in Sofia in May, 2012: Yuzhen park, Loven park, the park zone in Academy of Science campus, and two sites outside Sofia: Vitosha near Boyana, 8 km to the south and Pancharevo, 20 km to the south-east of Sofia. Additionally, 20 specimens of *Helicella obvia* (Menke, 1828) from Veliko Tŭrnovo were examined. In

total, 256 land snails and slugs of 17 species (Table 1) were collected and examined on the presence of parasitic nematodes.

In Crimea, the sampling was done near Yalta in September, 2011 by D. Kuznetsov. Collected material was examined in Moscow by the present authors (Table 2).

Molluscs were kept in 1 l containers lined with wet filter paper at 12°C. Prior to dissection, molluscs were rinsed with tap water to get rid of surface-dwelling nematodes. Animals were killed by cutting off the head and then dissected through the body wall. Internal organs were removed and examined separately from the body. The alimentary tract and reproductive system and their parts were also examined separately in Ringer's solution.

Several specimens of each nematode species and each locality were preserved in ethanol for DNA study and the rest in hot 4-5% formaldehyde for light microscopy and SEM. Formaldehyde-fixed nematodes were then processed into anhydrous glycerol for mounting according to Seinhorst (1959). Light microscopy studies and measurements were performed using a Zeiss Jenaval and Nikon microscopes. Abbreviations used: a, b, c – de Manian Indexes, V% – percentage of anterior – vulva distance to body length, GP – genital papillae. For SEM studies, material was re-hydrated after formaldehyde, dehydrated in a graded ethanol series, critical-point dried in the critical-point dryer HCP-2 HITACHI, mounted on aluminium stubs and coated with gold in a BIO-RAD SC502 sputter coater. Specimens were studied in a JCM-6380 LA SEM.

Nematode specimens fixed in 95% ethanol were rehydrated in water overnight before DNA extraction. The DNA was extracted from single nematodes according to the protocol of Holterman *et al.* (2006). The PCR reactions were performed using kits of different producers according to the manufacturers' instructions. Primer pairs D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') were used to amplify D2-D3 expansion segment of 28S rDNA fragment (Nunn, 1992) and two pairs of primers G18S4-26R (GCT TGT CTC AAA GAT TAA GCC and CAT TCT TGG CAA ATG CTT TCG) and 24F-Q39 (AGR GGT GAA ATY CGT GGA CC and TAA TGA TCC WTC YGC AGG TTC ACC TAC) were used to amplify 18SrDNA (Blaxter *et al.*, 1998). PCR products were directly sequenced by a commercial company.

For comparative purposes, the following D2-D3 expansion segment of LSU rDNA sequences deposited in GenBank were used: *A. dentiferum* (GQ167726), *A. limacis* Dujardin, 1845 (GQ167725), *A. milacis* Ivanova & Wilson, 2009 (FJ949063), *A. glandicola* Ivanova & Spiridonov, 2010 (GQ167724).

Newly obtained sequences of D2-D3 expansion segment of 28S rDNA for *Agfa tauricus* Korol & Spiridonov, 1991 and *A. flexilis* Dujardin, 1845 were deposited in NCBI GenBank (KF157965 and KF157966, respectively). Sequence alignments were generated using Clustal X (Thompson *et al.*, 1997) under default values for gap opening and gap extension penalties. All alignments were analysed using PAUP* 4.0b10 (Swofford, 1998) for maximum parsimony (MP) to calculate nucleotide differences.

Table 1. Prevalence and abundance of parasitic nematodes of terrestrial molluscs in Sofia, Bulgaria in May, 2012.

Mollusc species	Number collected	Nematode species	Prevalence (%)	Abundance Mean (range)
<i>Helix pomatia</i>	67	–	–	–
<i>Chondrula tridens</i>	24	–	–	–
<i>Cepaea vindobonensis</i>	3	–	–	–
<i>Monacha cartusiana</i>	7	–	–	–
<i>Oxychilus</i> sp.	2	–	–	–
<i>Fruticicola fruticum</i>	47	–	–	–
<i>Limax maximus</i>	9	<i>Angiostoma dentiferum</i>	80	4.4 (1-26)
		<i>Agfa tauricus</i>	11	0.7 (0-6)
<i>Limax maculatus</i>	2	<i>Angiostoma dentiferum</i> *	100	6.5 (3-10)
		<i>Agfa tauricus</i> *	50	1 (0-2)
<i>Limacus flavus</i>	3	<i>Agfa tauricus</i>	33	0.3 (0-1)
<i>Arion fasciatus</i>	53	–	–	–
<i>A. hortensis</i>	6	–	–	–
<i>A. vulgaris</i>	10	–	–	–
<i>A. subfuscus</i>	1	–	–	–
<i>Deroceras reticulatum</i>	5	–	–	–
<i>Tandonia kusceri</i>	12	–	–	–
<i>T. budapestensis</i>	4	–	–	–
<i>Tandonia</i> sp.	1	–	–	–

* New host association

Table 2. Prevalence and abundance of parasitic nematodes of terrestrial molluscs in Yalta, Crimea (Ukraine) in September, 2011.

Mollusc species	Number collected	Nematode species	Prevalence (%)	Abundance Mean (range)
<i>Eobania vermiculata</i>	37	–	–	–
<i>Monacha fruticola</i>	25	–	–	–
<i>L. flavus</i>	7	<i>Angiostoma dentiferum</i> <i>Agfa tauricus</i>	43	0.6 (1-3)
<i>Oxychilus deilus deilus</i>	3	<i>Agfa tauricus</i>	100	18.7 (11-29)
		<i>Phasmarhabditis</i>	66	2.7 (0-6)
		<i>neopapillosa</i>	33	0.3 (0-3)
<i>Helix albescens</i>	25	–	–	–
<i>Xeropicta sp.</i>	28	–	–	–

RESULTS

Distribution of parasitic nematodes of terrestrial molluscs by hosts and geographical region. In Bulgaria, 18 mollusc species in total were found and examined: *Helix pomatia* Linnaeus, 1758, *Chondrula tridens* (Müller, 1774), *Cepaea vindobonensis* (Férussac, 1821), *Monacha cartusiana* (Müller, 1774), *Oxychilus sp.*, *Fruticicola fruticum* (Müller, 1774), *Limax maximus* Linnaeus, 1758, *L. maculatus* (Kaleniczenko, 1851), *Limacus (Limax) flavus* (Linne, 1758), *Arion fasciatus* (Nilsson, 1822), *A. hortensis* (Férussac, 1819), *A. vulgaris* Moquin-Tandon, 1855, *A. subfuscus* (Drapanaud, 1805), *Deroceras reticulatum* (Müller, 1774), *Tandonia kusceri* (H. Wagner, 1931), *T. budapestensis* (Hazay, 1881), *Tandonia sp.* in Sofia and *Helicella obvia* in Velyko Tyrnovo. The sampling sites were characterised by slightly different ranges of terrestrial molluscs, though *Helix pomatia* was dominant and very numerous in all sampling sites. Apart from it, in both public parks “forest” species of slugs (*Arion fasciatus*, *A. hortensis* and *A. subfuscus*) were prevalent while the invader slug species, *A. vulgaris*, or Iberian slug, was scarce. The park zone in the campus of the Academy of Science was characterised by the absence of arionids; instead, it was inhabited by agriolimacids (*Deroceras reticulatum*), milacids (*Tandonia kusceri*, *T. budapestensis*, *Tandonia sp.*), and by limacids (*Limax maximus*, *Limacus flavus*, *Limax maculatus*), typically associated with human habitat.

Only three slug species, all of them being limacids, were found infected, namely *L. maximus*, *L. maculatus* and *L. flavus*. All host species were found in one of four sampling sites, on the institute territory. Nearly all collected slug specimens were mature animals. Two host species, *L. maximus* and *L. maculatus*, were infected by *Angiostoma dentiferum* and *Agfa tauricus* whereas a single specimen of *L. flavus* was infected by *A. tauricus*

only (Table 1). *Angiostoma dentiferum* were recovered from the slug oesophagus and *A. tauricus* from the ducts of the salivary glands.

In Yalta, Crimea, only six mollusc species were collected and examined in 2011, namely *Eobania vermiculata* (Müller, 1774), *Monacha fruticola* (Krynicky, 1833), *L. flavus*, *O. deilus deilus* Bourguignat, 1857, *Helix albescens* (Rossmässler, 1839), and *Xeropicta sp.*, and the same nematode species (*A. dentiferum* and *A. tauricus*) were recovered. Also, the pathogenic nematode *Phasmarhabditis neopapillosa* (Schneider, 1859) was found in the pallial cavity of *O. deilus deilus*. In the previous study in Crimea with sampling in Simferopol, Bakhchisarai and Alushta (Vorobjeva *et al.*, 2008), 16 mollusc species in total (*Helix albescens*, *Phenacolimax annularis* (Studer, 1820), *Tandonia cristata* (Kaleniczenko, 1851), *Deroceras reticulatum*, *D. caucasicum* (Simroth, 1901), *Monacha fruticola*, *Eobania vermiculata*, *Xeropicta sp.*, *Helicopsis sp.*, *Mentissa canalifera* (Rossmässler, 1836), *Oxychilus diaphanellus* (Krynicky, 1833), *O. deilus deilus*, *L. flavus*, *L. maculatus*, *Bilania boettgeri* Clessin, 1883) were collected vs 17 in Bulgaria with seven species (*H. albescens*, *D. caucasicum*, *O. deilus deilus*, *L. flavus*, *O. diaphanellus*, *E. vermiculata*, *Helicopsis sp.*) vs three serving hosts to nematodes. Larger helicids (*Helix albescens*, *H. pomatia*) were dominant in both surveys but were free of infection. In Bulgaria, no *Phasmarhabditis* invasion was recorded while in Crimea, three slug and one snail species (*T. cristata*, *D. reticulatum*, *D. caucasicum*, *E. vermiculata*) have shown susceptibility to this pathogenic nematode (Vorobjeva *et al.*, 2008). In Crimea, slugs *L. flavus*, *D. caucasicum* and a snail *O. deilus* (the two latter species are endemic) were heavily parasitised by *A. tauricus*. The total number of nematodes recorded per animal was 22 specimens, which can be considered as quite a burden given the size of nematodes. The width of larger specimens is comparable to the width of the

ducts of the salivary glands, which makes it time consuming to recover nematodes from ducts when a mollusc is dissected. Several nematodes in a salivary gland completely filled the whole length of ducts of this small organ; these blocked ducts are presumably not able to function properly. Although the same species of *Angiostoma* and *Agfa* were found in both regions, the patterns of their distribution were different with prevalence of *Angiostoma* in Bulgaria and *Agfa* in Crimea (Tables 1 & 2). Another species of *Angiostoma*, *A. kimmeriense* Korol & Spiridonov, 1991, described from native Crimean *O. deilus*, has not been found again since the time of its description.

Identification and taxonomy of the nematodes. The obtained partial D2-D3 28S rDNA nucleotide sequences were tested using BLAST (Altschul *et al.*, 1990) against the sequences deposited in NCBI GenBank. Partial D2-D3 28S rDNA sequence of Bulgarian and Crimean *Angiostoma* demonstrated 100% identity with the sequence of *A. dentiferum* GQ167726. This latter was obtained by the first and the last authors in 2006-2007 in Belgium from *L. flavus*. This nematode species was also registered in Belgium in another limacid slug, *Lehmania marginata* (Müller, 1774).

We compared the sequences of D2-D3 28S rDNA obtained for two *Agfa* species: *A. tauricus* from Bulgaria and Crimean peninsula (Ukraine) and *A. flexilis* from the United Kingdom. In the analysis, the previously obtained unpublished data for *A. tauricus* from Crimea were used. The specimens of *A. tauricus* from Bulgaria and Crimea were identical in D2-D3 28S rDNA, whereas the nucleotide difference between *A. tauricus* (Bulgaria & Crimea) and *A. flexilis* (United Kingdom) was in nine positions of D2-D3 28S rDNA.

***Angiostoma dentiferum* Mengert, 1953. (Table 3, Fig. 1)**

Angiostoma dentiferum was originally described from intestines of *Limax cinereoniger* Wolf, 1803 and *L. maximus* by Mengert (1953) in Germany. The brief description stated the presence of six lips each bearing a single papilla, tubular, cuticularised stoma, which is as wide as long and lacking cheilostom and bearing a small but distinct dorsal tooth, pharynx anteriorly enveloping half of stoma, valvate basal bulb, pointed tails in both sexes, males with peloderan bursa, three precloacal and six postcloacal genital papillae with GP4 and GP5 closely situated and located at cloaca level, boat-like gubernaculum with jagged margins and females

ovoviparous. Later this species was re-described by Morand & Spiridonov (1989) based on the material from the same host species from France. They found that *Angiostoma* from France differed from that from Germany by the lack of a stomatal tooth, presence of lateral alae, presence of adcloacal papillae and leptoderan vs peloderan bursa. They regarded the presence of a stomatal tooth as a single trait that was not sufficient to establish a different species. Other traits mentioned were not considered. However, the spicule length, which is usually treated as an important and stable character, differed remarkably compared with the material from France (av. 60 µm vs 84 µm in Mengert, 1953).

The specimens of *A. dentiferum* from Bulgaria and Crimea were characterised by the absence of lateral alae; presence of lip as well as cephalic papillae; small, triangular mouth aperture; almost indiscernible excretory pore; stoma a wide tube; dorsal tooth present but faint; long, conical tail in both sexes; bursa reduced, low; nine GP arranged as 3/3+3 with GP4 and GP5 clustered and GP5 and GP8 dorsal and spicule length 87 µm on average; lateral margins of gubernaculum smooth or jagged. The specimens of this species from Belgium showed a little difference in morphometrics (Table 3) but the rest of the morphology was similar.

Ross *et al.* (2010b) had found *A. dentiferum* in the native European slug *Limax marginatus* (= *Lehmania marginata*) collected by J. Ross in the USA in 2009 which by its morphology and morphometrics was close to the specimens from Bulgaria, Crimea and Belgium (Table 3).

The presence of lip papillae occurred in the material from Bulgaria, Crimea and Belgium was reported neither by Mengert nor by Morand & Spiridonov. As they are minute structures only seen under SEM, they could be overlooked by Mengert. However, in the PhD Dissertation of Morand on which the re-description was based there were SEM images of *A. dentiferum* showing no lip papillae. Apart from this, the head of the nematode from Morand's study shows the same shape of cephalic papillae, amphids and mouth aperture. The arrangement of genital papillae in our material was similar to that of Morand & Spiridonov (1989) though unpaired precloacal papilla was less prominent than shown in Morand's dissertation.

Within the *Angiostoma*, the morphological traits of absence/presence of lateral alae and particular spicule length are definitely the stable characters displayed the same way in different populations, as shown above.

Thus, we are inclined to consider the presence vs absence of lateral alae and spicule length of av. 60

μm vs av. 82 μm in *A. dentiferum* sensu Morand & Spiridonov, 1989 vs all other available material as a clear indication that Morand & Spiridonov (1989) have dealt with another, though very close species of *Angiostoma*.

Mengert (1953) reported a peloderan bursa of the species in the original description. A peloderan bursa, as it is usually understood, is a quite wide, fan-like structure characteristic for relatively short-tailed nematodes; *A. dentiferum* as shown by Mengert (1953) and seen from Figure 1 is a rather long-tailed nematode and a caudal bursa is reduced to the extent that its end cannot be determined.

Agfa tauricus Korol & Spiridonov, 1991 (Fig. 2)

Specimens of *Agfa* found in Bulgaria and Crimea were identical by both D2-D3 and 18S partial sequences as well as by their morphology and represented *A. tauricus*. This species was described by Korol & Spiridonov (1991) from the Crimean endemics *Oxychilus deilus* (snail), *Krynycillus melanocephalus* (Kaleniczenko, 1851) (slug) and cosmopolitan *L. flavus*. Later, the invasion of this nematode species in other regions of Crimea (Simferopol, Bakhchisarai and Alushta) was registered in slugs *L. flavus* and *Deroceras caucasicum* and a snail *O. deilus* by Vorobjeva *et al.* (2008). Korol & Spiridonov (1991) differentiated *A. tauricus* from *A. flexilis*, the only species of *Agfa* known at the time, on the basis of much longer stoma and spicules. However, later Morand & Hommay (1990) when re-describing *A. flexilis*, reported the

spicule length of 120 μm , as being equal to *A. tauricus*. It is also seems likely that the stoma length of 10 μm in *A. flexilis* males was reported erroneously as the relevant scale bar on the drawing indicated that it was 25 μm . In *A. tauricus*, a stoma is nearly twice as long (ca 40 μm) with close similarity of general morphology of both species.

DISCUSSION

The surveys have shown that nematode fauna of terrestrial molluscs in Bulgaria and Crimea contains the same species of parasitic nematodes, though the second Crimean species of *Angiostoma*, *A. kimmeriense*, was not found during either surveys. Also, the prevalence of *A. dentiferum* over *A. tauricus* was observed in Bulgaria with the opposite situation in Crimea. No *Phasmarhabditis* infection so far was recorded in Bulgaria, whereas in Crimea several mollusc species were shown to be susceptible for this nematode (*O. deilus*, *T. cristata*, *D. reticulatum*, *D. caucasicum*, *E. vermiculata*) (Table 2 and Vorobjeva *et al.*, 2008).

There are presently eight species of *Angiostoma* and three species of *Agfa* reported from terrestrial molluscs in continental Europe and the UK. The members of the genus *Angiostoma*, which presently numbers 17 species, are known to associate with terrestrial molluscs (13 species) and salamanders and reptiles (4 species), all associated with vertebrates of other than European provenance.

In Europe, there are *A. limacis* Dujardin, 1845, *A. dentiferum*, *A. stammeri* Mengert, 1953, *A. aspersae* Morand, 1986, *A. kimmeriense*, *A. spiridonovi* Morand, 1992, *A. milacis* Ivanova & Wilson, 2009 and

Table 3. Main morphological traits of *Angiostoma dentiferum* Mengert, 1953 from different populations.

Origin	Lateral alae	Body length (male), mm (av.)	Pharynx length (male), μm (av.)	Tail length (male/female) μm (av.)	Spicule length μm (av.)	Gubernaculum length μm (av.)	Reference
<i>Limax cinereoniger</i> <i>Arion subfuscus</i> Germany	–	4.6-6.7	Ca. 300	188/294-342	84	31	Mengert, 1953
<i>L. cinereoniger</i> France	+	4.7	255	170/250	60	30	Morand & Spiridonov, 1989
<i>Lehmania marginata</i> Belgium	–	5.1	325	142/191	78	33	Own data
<i>Limax maximus</i> Bulgaria	–	6.4	322	150/203	87	26	Own data
<i>Lehmania marginata</i> USA (coll. by Dr J. Ross)	–	4.1	343	160/221	87	30	Own data

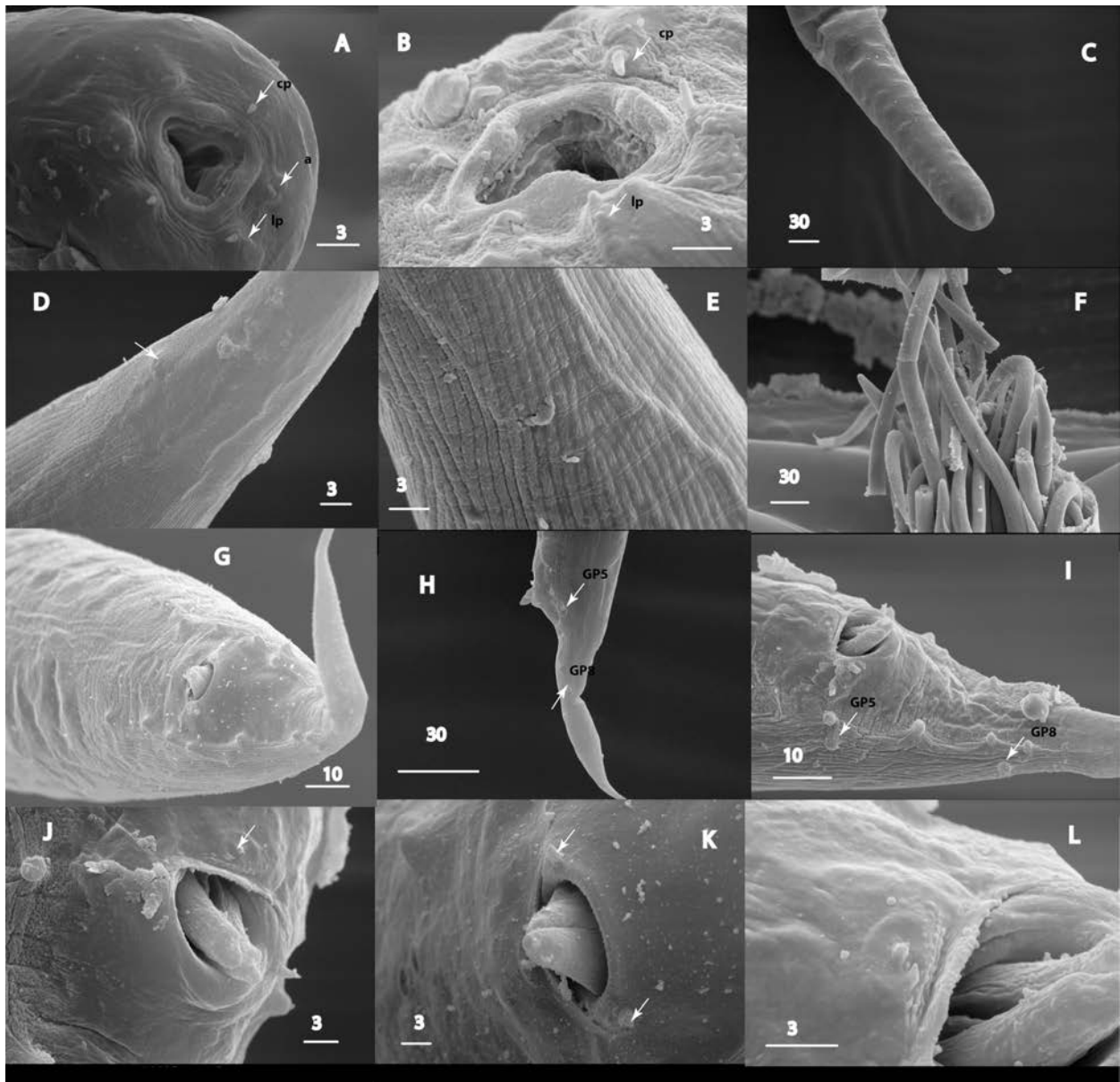


Fig.1. SEM images of *Angiostoma dentiferum* from Sofia. A: female head, a – amphid, cp – cephalic papilla, l – lip papilla; B: male head, cp – cephalic papilla, l – lip papilla; C: anterior end of female; D: female posterior end, ph – phasmid; E: female, cuticle; F: intra-uterine juveniles; G-L: male posterior, GP5 and GP8 – dorsal papillae; J: arrow indicates unpaired precloacal papilla; K: arrows indicate adanal papillae. Scale bars in μm .

A. zonitidis Ivanova & Wilson, 2009. Among them, *A. dentiferum*, *A. stammeri* and *A. spiridonovi* were reported as being associated with several species of limacid slugs, *A. limacis* with arionid and, rarely, agriolimacid slugs, *A. kimmeriense* and *A. zonitidis* with zonitid snails, *A. aspersae* with helicids and *A. milacis* with milacid slugs with the occasional infection in agriolimacids. In the original description of *A. dentiferum* Mengert (1953) had also given the arionid slug *Arion subfuscus* as a host of the nematode. The fact that angiostrongylid species parasitise hosts from remote families is rather

unusual because other species tend to associate with hosts from a single family or two closely related families (Limacidae + Agriolimacidae; Milacidae + Agriolimacidae, which are all members of the Limacoidea).

Arionid slugs are absent from the mollusc fauna of Crimea but were collected and examined in Bulgaria. However, we did not find the infection of *A. limacis* in Bulgaria, though it can be expected because its distribution in Germany and Belgium is based on the same host species as in Bulgaria and this species was found there along with *A.*

dentiferum. *A. limacis* is apparently the most common angiostrongylid found also in France (Morand & Spiridonov, 1989) and UK (Ivanova & Wilson, 2009). The wide distribution of *A. limacis* is apparently linked with the higher population density of arionid slugs compared with limacids. Although *A. dentiferum* was registered far to the north from the southern borders of Europe, it was never abundant.

All species of *Agfa* were reported from Europe where native limacid slugs can be considered as their principal hosts. *Agfa tauricus* was so far reported only from Crimea and Bulgaria, where it can also parasitise agriolimacid slug *D. caucasicum* and zonitid snail *O. deilus*. The type species of the genus, *A. flexilis*, is quite common in France (Morand & Hommay, 1990), Belgium and UK (own unpublished data), where nearly 100% of the most common host, *L. flavus*, are infected with this nematode. However, in our surveys in Crimea and

Bulgaria this host species was parasitised by *A. tauricus*. *Agfa tauricus* distribution is seemingly restricted by the southern regions of Eastern Europe though some mollusc hosts of the nematode inhabit also north of Europe.

Although Dujardin (1845) had described *A. flexilis* from salivary glands of the mollusc, all subsequent authors had reported species of *Agfa* being found in the genital tract of molluscs. The particular part of this complex structure inhabited by nematodes was never specified. It is apparent from the anatomy of molluscan genital tract that some parts of it hardly suitable for parasitising by nematodes. Having seen two species of these nematodes only in the ducts of salivary glands of the mollusc host we can assume that the reported location of nematodes in the genital tract is erroneous and occurred from poor knowledge of molluscan anatomy.

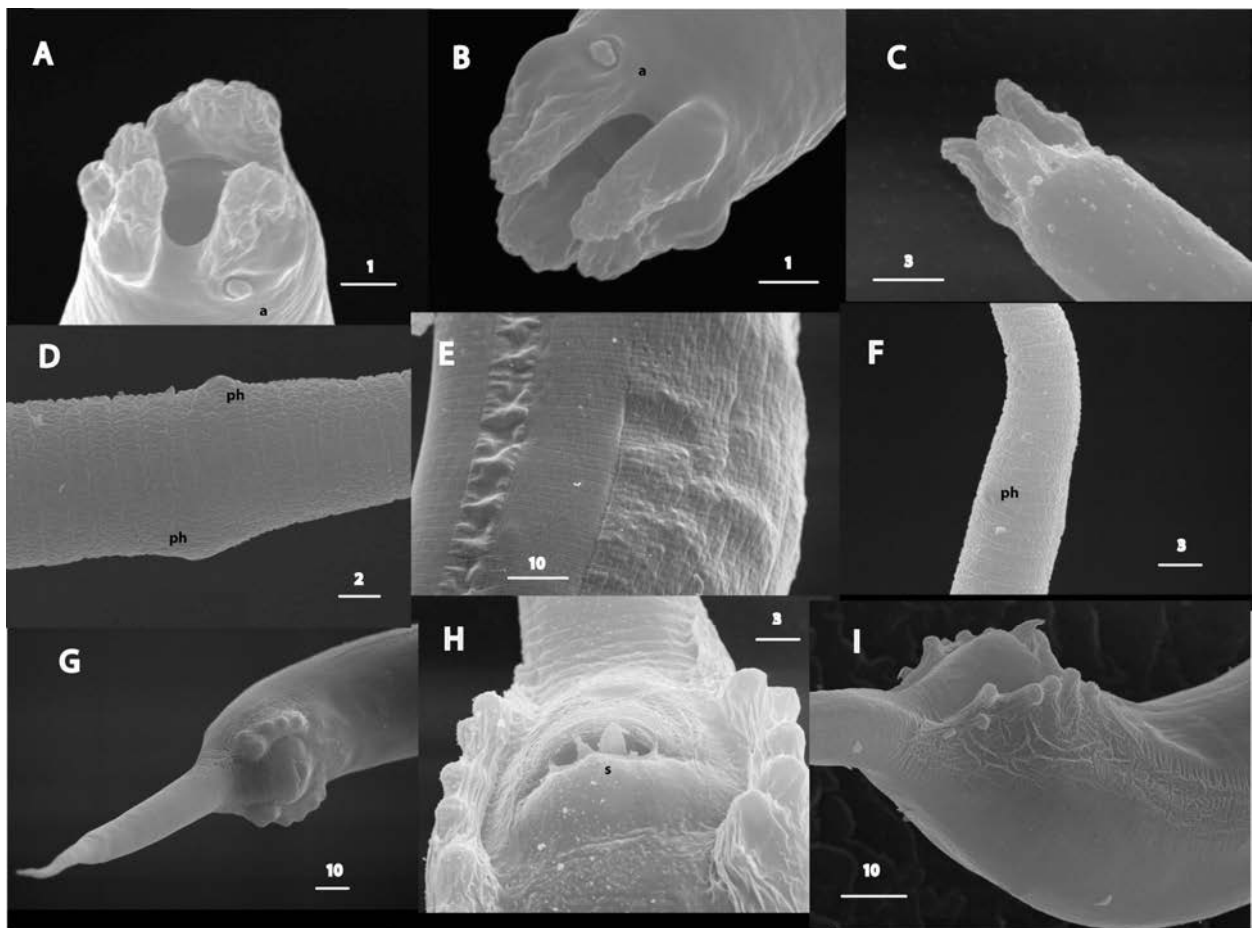


Fig.2. SEM images of *Agfa tauricus* from Sofia and Crimea. A: female head, a – amphid; B: male head, a – amphid; C: male anterior end; D, F: female tail, ph – phasmid; E – male, cuticle at mid-body; G-I: male tail, s – single precloacal papilla. Scale bars in μm .

Both nematode species belong to the same clade IV according to Blaxter *et al.* (1998) classification and are closely related genetically, although nominally belonging to different rhabditid families, Angiostomatidae and Agfidae. Although the size of angiostomatid and agfid nematodes and the number of nematodes per host is generally similar, it can be expected that the impact of the nematodes on their hosts will be different because of the different habitat within the host's body.

Both surveyed areas are rich in molluscs and, in terms of endemism, are one of the richest areas in Europe. According to Leonov (2009), the mollusc fauna in Crimea accounts to 111 species with 19 species endemic. Dedov (1998) has reported 236 species with 57 endemic ones in Bulgaria. As only a small part of the molluscan diversity was explored in our studies, a greater diversity of nematodes associated with terrestrial molluscs in Bulgaria and Crimea can be expected.

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Е. Иванова, М. Панайотова-Пенчева и С. Спиридонов. О фауне нематод наземных моллюсков Софии (Болгария) и Крымского полуострова (Украина).

Резюме. Обсуждаются результаты проведенных в Софии и на территории Крыма обследований наземных моллюсков. Анализируется состав фауны паразитических нематод в этих районах в сравнении с фауной континентальной Европы и Британских островов. Соответственно, у 3-х и 4-х из 23 видов моллюсков обнаружены нематоды *Angiostoma dentiferum* и *Agfa tauricus*, с доминированием первого вида в Болгарии и второго – в Крыму. Изучение морфологии и сравнение полученных последовательностей участков рибосомальной ДНК показали тождество материала из Болгарии и Крыма. Морфологически *A. dentiferum* из этих двух регионов и Бельгии отвечает первоописанию этого вида, сделанному Менгертом (Mengert, 1953), а его переописание (Morand & Spiridonov, 1989), видимо, относится к другому близкому виду, так как у описанных этими авторами нематод имеются латеральные крылья и более короткие спикулы. Хотя данные по распространению нематод моллюсков на территории Европы неполны, кажется вероятным, что распространение *A. tauricus* ограничено территорией юга Европы, а в более северных районах этот вид замещен на *A. flexilis*, при сходстве круга хозяев.
