Vestnik Zoologii, **53**(1), 65–74, 2019.
DOI 10.2478/vzoo-2019-0007

sciendo

UDC 595.132.7

MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF *AMIDOSTOMUM ANSERIS* (NEMATODA, AMIDOSTOMATIDAE) FROM *ANSER ANSER DOMESTICUS*

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Morphological and Biological Characteristics of *Amidostomum anseris* (Nematoda, Amidostomatidae) from *Anser anser domesticus*. Yevstafieva, V. A., Stybel, V. V., Melnychuk, V. V., Prijma, O. B., Yatsenko, I. V., Antipov, A. A., Bakhur, T. I., Goncharenko, V. P., Pidborska, R. V., Shahanenko, V. S., Dzhmil, V. I. — Morphological characteristics were studied in adult and embryonic *Amidostomum anseris* (Zeder, 1800) obtained from domestic goose *Anser anser domesticus* Linnaeus, 1758. The studied characters included species-specific morphometric indices of male and female specimens and differential characters of sex-related dimorphism in that species. Stages and periods of embryonic development, and viability of the nematodes were studied at laboratory conditions. Size dimorphism in *A. anseris* was considerable, females were significantly larger (by 10.09–27.98 %) than males by 11 parameters. Additional metric characters were proposed to enhance effectiveness of differentiation of female and male *A. anseris* specimens. Under laboratory conditions, embryonic development of *A. anseris* occurs in four stages: blastomere cleavage; larval formation; formation of non-infective larvae I and II; formation of infective larva III which hatches from the egg. Infective larvae develop at 23 °C in six days, and their viability was up to 78.33 ± 2.08 %.

Key words: *Amidostomum anseris*, domestic goose, morphological characters, metric characters, embryonic development

Introduction

Nematodes of the genus *Amidostomum* Railliet & Henry, 1909 (Nematoda, Amidostomatidae) are most widely distributed helminthes of waterfowl. These roundworms evolved to parasitize under the gizzard cuticle,

and during winter they can enter the mucous tissues of the glandular stomach. They often occur in both wild and domestic anseriform birds (Anseriformes Wagler, 1831) (MacNeill, 1970; Nowicki et al., 1995; Purvis et al., 1997; Fedynich et al., 2005; Yoshino et al., 2009; Syrota and Kharchenko, 2015; Amundson et al., 2016).

The genus *Amidostomum* is currently comprised of 17 species, ten of which (*Amidostomum anseris* (Zeder, 1800), *A. acutum* (Lundahl, 1848), *A. chevreuxi* Seurat, 1916, *A. cygni* Wehr, 1933, *A. fulicae* (Rudolphi, 1819), *A. henryi* Skrjabin, 1915, *A. monodon* (Linstow, 1882), *A. orientale* Ryjikov et Pavlov, 1959, *A. similis* Freitas & Mendonca, 1954, *A. spatulatum* Baylis, 1932) are recognized by most specialists (Czaplinski, 1962; Kobulej and Ryzhikov, 1968; Borgsteede, 2005; Borgsteede et al., 2006; Kavetska et al., 2015). However, the species composition of the genus *Amidostomum* remains an open question. According to some reports, *A. chevreuxi* is synonymic of *A. acutum*, and *A. similis* is synonymic of *A. cygni* (Czaplinski, 1962; de Jong et al., 2014).

Many species of this genus are pathogenic. One of those is *A. anseris*, one of the most widely distributed helminthes of domestic goose (*Anser anser domesticus* Linnaeus, 1758). This parasitic species is found on all continents except for Australia. In Poland, 50.0 % of domestic geese were found infected with *A. anseris* (Kornaś et al., 2015), in Nakhchivan Autonomous Republic (Azerbaijan) this proportion was 15.9 % (Seyidbeyli and Rzayev, 2018), 3.7 % at the territory of former Czechoslovakia (Busta, 1980), and 2.4 % in Spain (Figuerola et al., 2005). Infection prevalence of domestic geese in different regions of Russia varied from 9.7 to 100.0 % (Bamba Sidiki et al., 1995; Sergushin, 2000). In several regions of Ukraine, the prevalence varied from 34.6 to 56.9 % (Shevtsov, 1961; Ljulin, 2006; Yevstafieva and Mykhailiutenko, 2011).

The morphology of the buccal capsule of *Amidostomum*, particularly the number of teeth is taxonomically important. Several species have three esophageal teeth in the buccal capsule, other only one tooth, depending on the structure of the gizzard cuticle in infected birds. In nematodes of the genus, the number of teeth increases with thickness and roughness of the cuticle (as in geese). The main diagnosticcharacters in *Amidostomum* species also include the length of body in males and females; morphology and measurements of the spicules, gubernaculum, caudal bursa in males; morphology of vulvar area and position of the vulva relative to the tail end in females. Length and width of the esophagus, diameter of the buccal capsule, maximum width of vulvar area and egg size are also used in species differentiation (Skrjabin et al., 1952; Ryzhikov, 1967; Kobulej and Ryzhikov, 1968). The biology of *Amidostomum* nematodes is also considered as important characteristic (Enigk and Dey, 1968; Stradowski, 1977).

There is a considerable amount of works dealing with the morphology, systematics and biology of some species of the genus *Amidostomum*, in particular *A. anseris*. However, the species-level morphometric characters of their adult and embryonic stages of development in domestic goose remain insufficiently studied. Clarification of these aspects will add data on the morphological and biological features of these helminthes.

Material and methods

The studied materials were collected in 2016–2018. Altogether, 427 specimens of domestic goose from farms of Poltava, Kharkiv, Kyiv and Lviv Regions of Ukraine were investigated. Nematodes were collected during helminthological investigation of the gizzard and glandular stomach of dead or euthanized birds (Skrjabyn, 1928). Nematodes were identified according to the keys by Skrjabyn et al. (1952) and Ryzhikov (1967). Morphology of 862 adult nematodes (313 male and 549 female specimens) of *A. anseris* was analyzed.

To study the peculiarities of embryogenesis and cultivation of *A. anseris*, adult female nematodes were obtained from avian stomachs and kept, 20 per Petri dish, in NaCl 0.9 % solution. The females laid eggs which were then cultured in thermostat at 23 °C for 10 days until the formation of infective larvae (L III). Every day, the cultures were examined under a microscope to study the level of embryonic development, morphology, and numbers of destroyed and no longer developing eggs and dead larvae. The experiment was conducted in triplicate.

Morphometric parameters of adult and embryonic stages of development of *A. anseris* were analyzed using ImageJ for Windows® (version 2.00) in interactive mode using 10×, 40× objectives and 10× photo eyepiece. Image analyzer was calibrated with MikroMed object-micrometer. Photomicrographs were taken using a 5 Mpix digital camera mounted on the MikroMed (China) microscope.

Statistical processing of the experimental data was carried out using MS Excel program; mean value (M) and standard deviation (SD) were calculated. Statistical significance of difference between mean values in studied groups was determined by Fisher's criterion. Results were considered significant at $P < 0.05$.

Results and discussion

Nematodes *A. anseris* in domestic geese were found in all studied regions of Ukraine. Prevalence of infection was 54.09 % on average. Interestingly, 28.14 % of infections were registered in glandular stomach of the host.

Morphological study revealed the characters common for both female and male specimens of *A. anseris*. Nematode body is thin, filiform, pale pink, with dense cuticle. Body size

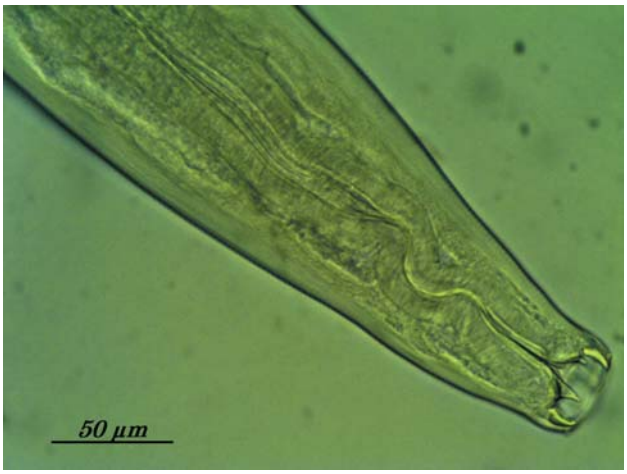


Fig. 1. Head end of *Amidostomum anseris.*

ranges from 11.20 to 20.70 mm. On the anterior end, a wide sclerotized capsule is present; the circumoral lips are indistinct. Three esophageal teeth include two small (almost a half of capsule's height in length, 4.12–6.68 µm) and one large (up to the frontal end of the capsule, 9.71–16.82 µm in length). Esophagus widens posteriorly. In 77.96 % of studied specimens, esophagus was slightly curved (fig. 1). In studied males this curve is slightly more frequent (86.90 %) than in females (72.86 %).

The sex-related size differences in *A. anseris* were found to be significant (table 1).

Female nematodes are larger than males by 11 measurements of body and morphological structures ($P < 0.05$). Females are longer by 27.98 % (18.30 ± 1.37 mm) and wider (at the buccal capsule area, middle and posterior esophagus, middle of body) by 12.11–20.65 % (49.29 ± 2.33–196.24 ± 3.39 µm) than males (13.18 ± 1.26 mm and 39.11 ± 1.50–167.73 ± 5.01 µm, respectively). Buccal capsule is also longer by 10.09 % (24.56 ± 1.79 µm) and wider by 17.24 % (36.07 ± 1.75 µm) in females than in males (22.08 ± 1.09 and 29.85 ± 1.40 µm). Correspondingly, esophageal teeth are longer in females than in males. The large tooth is

Table 1. Morphometric characters and sex-related dimorphism of ♀ and ♂ *Amidostomum anseris*, n = 20

Characters	♀			♂		
	M ± SD	Min	Max	M ± SD	Min	Max
Length of body, mm	18.30 ± 1.37*	16.20	20.70	13.18 ± 1.26	11.20	15.30
Width of body (µm), at:						
– the middle	196.24 ± 3.39*	190.11	201.12	167.73 ± 5.01	160.25	178.58
– buccal capsule base	49.29 ± 2.33*	43.59	55.98	39.11 ± 1.50	36.57	41.97
– esophageal curve	58.78 ± 2.37*	52.81	62.99	69.82 ± 2.28	64.97	73.18
– middle esophagus	152.07 ± 7.09*	138.18	162.17	131.20 ± 3.06	125.66	136.95
– posterior part of esophagus	187.36 ± 3.21*	182.46	192.17	164.67 ± 5.71	151.24	172.18
Width of buccal capsule, µm	36.07 ± 1.75*	32.09	38.85	29.85 ± 1.40	27.13	32.04
Length of buccal capsule, µm	24.56 ± 1.79*	21.22	26.88	22.08 ± 1.09	20.45	23.85
Length of large tooth, µm	15.49 ± 1.00*	13.85	16.82	11.67 ± 1.07	9.71	13.74
Length of small tooth, µm	5.78 ± 0.53*	4.69	6.68	4.76 ± 0.37	4.12	5.24
Length of esophagus, mm	1.33 ± 0.02*	1.29	1.36	1.04 ± 0.11	0.91	1.25
Width of esophagus at the middle, µm	46.87 ± 1.77*	42.85	49.52	55.92 ± 3.05	50.22	60.40
Maximum width of esophagus, µm	80.72 ± 3.35*	75.61	86.22	68.48 ± 1.47	65.08	70.18

* $P < 0.05$ —compared to values for ♂.

Table 2. Morphometric parameters of ♂ *Amidostomum anseris*, n = 20

Characters	M ± SD	Min	Max
Width of body at caudal bursa base	171.68 ± 9.30	153.39	187.42
Width of caudal bursa	509.86 ± 13.01	483.42	534.12
Length of spicule	331.26 ± 21.3	297.87	364.16
Width of spicule at the middle	30.12 ± 1.07	28.12	31.86
Length of gubernaculum	130.79 ± 14.78	108.04	152.05
Width of proximal end of gubernaculum	6.18 ± 0.45	5.19	7.08
Width of distal end of gubernaculum	14.84 ± 0.71	13.11	15.91
Length of anteroventral rays	87.65 ± 4.54	80.22	95.46
Width of anteroventral rays at the base	11.28 ± 0.59	10.22	12.64
Width of anteroventral rays at the middle	7.56 ± 0.75	6.00	8.45
Length of posteroventral rays	154.33 ± 8.49	140.00	167.32
Width of posteroventral rays at the base	21.55 ± 1.32	19.28	23.35
Width of posteroventral rays at the middle	17.47 ± 1.04	15.84	19.47
Length of anterolateral rays	139.47 ± 6.14	127.70	147.02
Width of anterolateral rays at the base	25.53 ± 0.92	24.00	26.91
Width of anterolateral rays at the middle	21.50 ± 1.08	18.59	22.80
Length of mediolateral rays	194.97 ± 7.72	180.55	209.57
Width of mediolateral rays at the base	13.56 ± 0.56	12.64	14.44
Width of mediolateral rays at the middle	12.75 ± 0.86	11.00	13.85
Length of posterolateral rays	193.89 ± 6.55	182.04	201.67
Width of posterolateral rays at the base	11.76 ± 0.82	9.85	12.94
Width of posterolateral rays at the middle	14.11 ± 1.03	12.41	15.71
Length of external dorsal rays	131.93 ± 2.11	128.60	135.71
Width of external dorsal rays at base	21.16 ± 1.00	19.61	22.74
Width of external dorsal rays at the middle	14.25 ± 1.57	11.55	17.32
Length of dorsal ray from base to bifurcation point	63.20 ± 3.01	58.44	69.83
Width of dorsal ray at base	27.86 ± 1.71	24.63	30.77
Width of dorsal ray at the middle	10.91 ± 0.56	10.25	11.98
Width of dorsal ray at bifurcation point	13.03 ± 0.97	11.76	14.93

Note. All measurements are given in μm.

longer by 24.66 % (15.49 ± 1.00 μm), the small teeth are longer by 17.65 % (5.78 ± 0.53 μm). Length and maximum width of esophagus are 1.33 ± 0.02 and 80.72 ± 3.35 μm, which is more by 16.18 and 15.16 % respectively than in males (1.04 ± 0.11 and 68.48 ± 1.47 μm). Simultaneously, female nematodes are smaller than males by two characters. In male nematodes, body at the area of esophageal curve is wider by 15.81 % (69.82 ± 2.28 μm), and esophagus at the middle is wider by 16.18 % (55.92 ± 3.05 μm) than in females.

Details of caudal bursa structure and spicules in males can also be used as typical differential morphological and metric characters (table 2).

The caudal bursa is wide (509.86 ± 13.01 μm), trilobate, with long lateral lobes and a short dorsal lobe. Its cuticle is markedly ornamented. Width of body at the base of caudal bursa is 171.68 ± 9.30 μm. Each ray of lateral lobes has an individual base. The longest rays are the mediolateral (194.97 ± 7.72 μm) and posterolateral (193.89 ± 6.55 μm) ones. The posteroventral rays are slightly shorter (by 20.40–20.84 %, 154.33 ± 8.49 μm). The anterolateral and external dorsal rays are even shorter (by 28.07–28.47 %, 139.47 ± 6.14 μm and by 31.96–32.33 %, 131.93 ± 2.11 μm, respectively). The shortest rays are the anteroventral ones (by 54.79–55.04 %, 87.65 ± 4.54 μm). Ray width also varies. The anterolateral rays are the widest (ranging from 21.50 ± 1.08 to 25.53 ± 0.92 μm). The posterolateral rays are slightly narrower (by 15.59–18.74 %, 17.47 ± 1.04–21.55 ± 1.32 μm), similarly to the external dorsal rays (which are by 17.12–33.72 %, 14.25 ± 1.57–21.16 ± 1.00 μm). The mediolateral rays are

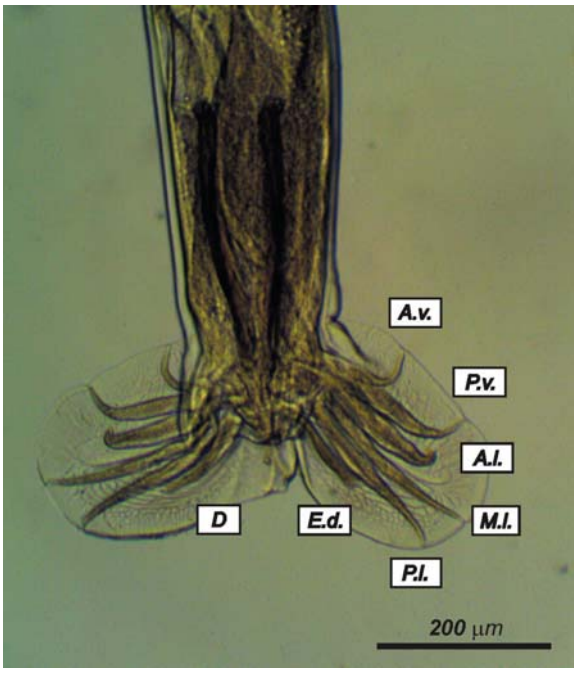


Fig. 2. Caudal end of ♂ *Amidostomum anseris: A.v.* — anteroventral ray; *P.v.* — posteroventral ray; *A.l.* — anterolateral ray; *M.l.* — mediolateral ray; *P.l.* — posterolateral ray; *E.d.* — external and dorsal ray; *D* — dorsal ray.

even narrower (by 40.69–46.89 %, 12.75 ± 0.86–13.56 ± 0.56 μm), as are the posterolateral rays (by 34.37–53.94 %, 11.76 ± 0.82–14.11 ± 1.03 μm). The narrowest rays are anteroventral (by 55.82–64.84 %, 7.56 ± 0.75–11.28 ± 0.59 μm). The dorsal rays of caudal bursa of males bifurcate, and each branch also splits into two short branches (fig. 2). The width of a dorsal ray at its base is 27.86 ± 1.71 μm, then it narrows to 10.91 ± 0.56 μm and conversely, slightly widens at the point of bifurcation (to 13.03 ± 0.97 μm). The dorsal ray is the shortest one in the caudal bursa, 63.20 ± 3.01 μm from base to bifurcation.

The two spicules are very alike, 331.26 ± 21.3 μm in length and 30.12 ± 1.07 μm wide in the middle. Distal end of spicule is split in three small branches. Proximal end of spicule is thickened, with curved, hooked spur. Shape of gubernaculum is elongated prism, proximal end of gubernaculum is narrow (6.18 ± 0.45 μm), the distal end is 2.4 times wider (14.84 ± 0.71 μm) (fig. 3).

Differential morphological characters of *A. anseris* females may also include vulval

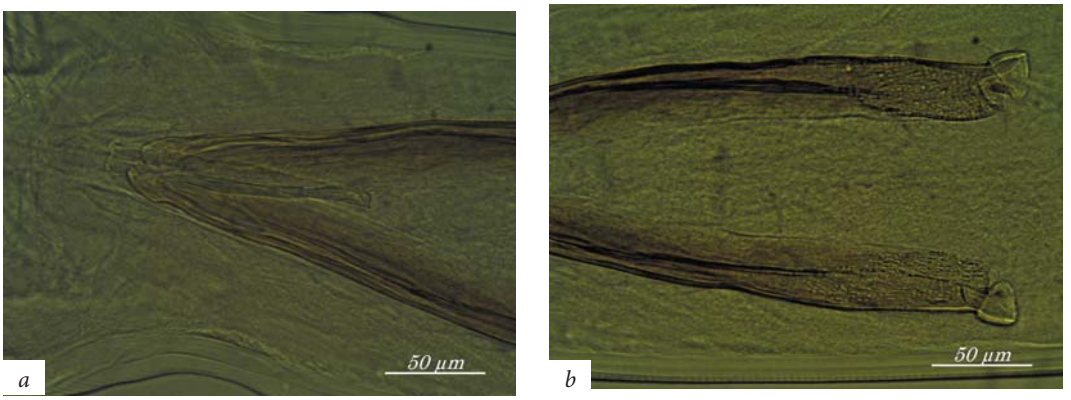


Fig. 3. ♂ *Amidostomum anseris*: *a* — distal ends of spicules, and gubernaculum; *b* — proximal ends of spicules.

Table 3. Morphometric characters of ♀ *Amidostomum anseris*, n = 20

Characters	M ± SD	Min	Max
Distance from head end to vulva, mm	15.05 ± 1.39	12.85	17.42
Distance from vulva to caudal end, mm	3.25 ± 0.10	3.06	3.39
Distance from vulva to anus, mm	2.95 ± 0.13	2.52	3.18
Width of body near vulva, μm	300.69 ± 13.27	283.17	321.18
Length of ovejector, μm	403.19 ± 5.36	390.67	409.87
Distance from anus to caudal end, μm	0.34 ± 0.03	0.28	0.36
Distance from anus to base of the digitate process of tail end, μm	0.14 ± 0.02	0.10	0.21
Distance from base of the digitate process to tail end, μm	0.19 ± 0.03	0.14	0.24
Width of body near anus, μm	88.10 ± 5.71	79.18	97.14
Width of body at base of the digitate process of tail end, μm	48.32 ± 1.82	44.36	50.25
Width of the digitate process, μm	37.76 ± 2.24	33.00	40.25
Egg length, μm	103.55 ± 2.60	99.14	108.33
Egg width, μm	58.31 ± 3.39	52.64	64.85

region and caudal end, and their metric indices (table 3).

Tail end in females is conical, with a digitate process 37.76 ± 2.24 μm wide (fig. 4, *a*). The body is 48.32 ± 1.82 μm wide in the area of the digitate process. Anus is distinct (fig. 4, *b*) at 0.14 ± 0.02 μm to the base of digitate process, and 0.34 ± 0.03 μm to the tail end. Width of body near anus is 88.10 ± 5.71 μm. Distance from the base of digitate process to tail end is 0.19 ± 0.03 μm.

Vulva is in the posterior part of body, at 15.05 ± 1.39 μm from the head end and 3.25 ± 0.10 μm from the tail end, and at 2.95 ± 0.13 μm from anus. Width of body at vulval is 300.69 ± 13.27 μm. Vulvar opening is covered by a process shaped as an oval valve directed

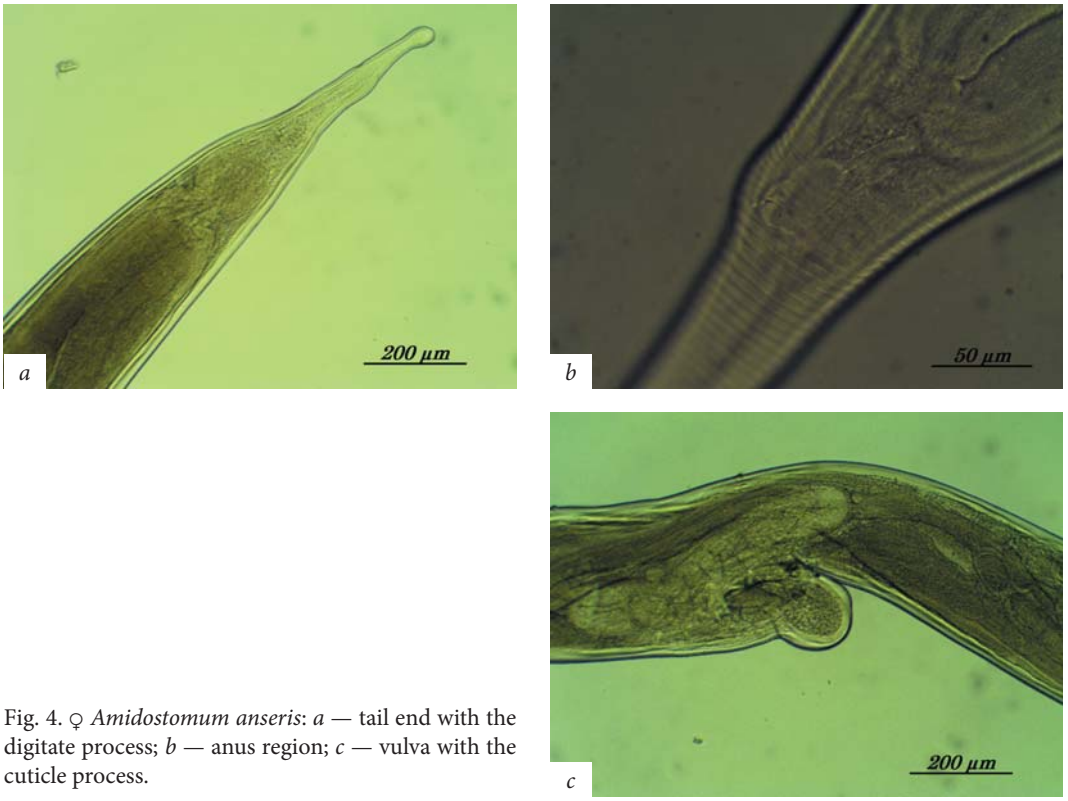
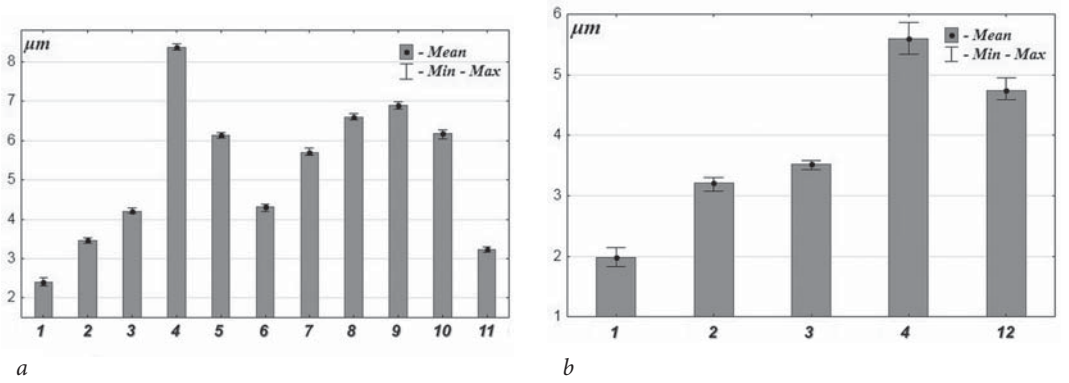


Fig. 4. ♀ *Amidostomum anseris*: *a* — tail end with the digitate process; *b* — anus region; *c* — vulva with the cuticle process.



a *b*

Fig. 5. Transverse striation of cuticle in *Amidostomum anseris* (n = 20) ♀ (*a*) and ♂ (*b*) at: *1* — anterior esophagus; *2* — middle esophagus; *3* — posterior esophagus; *4* — middle of body; *5* — base of cuticle process; *6* — middle of cuticle process; *7* — posterior to vulva; *8* — between vulva and anus; *9* — anus; *10* — between anus and base of the digitate process; *11* — base of the digitate process; *12* — spicule area.

toward the tail end (fig. 4, *c*).

The ovejector is 403.19 ± 5.36 μm in length. Eggs are average, oval, grey, thin-shelled, 103.55 ± 2.60 μm in length and 58.31 ± 3.39 μm in width.

The transverse striation of cuticle is different in separate body regions in males and females. This can be used in species identification. In females, this striation is 2.39 ± 0.07 μm at head end, 8.36 ± 0.04 μm in the middle, 5.70 ± 0.05 μm in the area of vulva and cuticle process, 6.90 ± 0.05 μm between vulva and anus, and from anus to the digitate process it decreases from 6.17 ± 0.08 to 3.22 ± 0.04 μm (fig. 5, *a*).

In males the striation also increases from head end (1.98 ± 0.11) to the middle of body (5.60 ± 0.17 μm). In spicule area it decreases to 4.74 ± 0.10 μm (fig. 5, *b*).

The embryogenesis of *A. anseris* at 23 °C in laboratory culture occurs in four stages: blastomere cleavage (fig. 6, *a*); larval formation (fig. 6, *b*); formation of larvae L1 и L2 (fig. 6, *c*); formation of infective larva L3 and its hatching from egg (fig. 6, *d*). The L3 stage occurs at sixth day. Viability of embryonic stages of development in the studied laboratory culture of *A. anseris* is 78.33 ± 2.08 % (table 4).

Eggs obtained from female nematodes were all (100 %) at the stage of blastomere cleavage (morula stage). Their further development was rather fast. On the second day of cultivation, 72.33 ± 2.31 % of eggs were at the stage of larval development, and only 27.67 ± 2.31 % were at the blastomere cleavage stage. On the third day, 59.00 ± 6.24 % of developing eggs contained larvae L1 and L2, 11.00 ± 2.65 % of eggs hatched releasing L3 larvae. On fourth to sixth days, the number of infectious larvae increased from 24.33 ± 3.06 to 78.33 ± 2.08 %, and 21.67 ± 2.08 % of embryonic nematodes died.

To summarize, nematodes *Amidostomum anseris* (Zeder, 1800) are common parasites

Table 4. Embryonic development of *Amidostomum anseris* in laboratory culture, n = 100, M ± SD

Day of culture	Stages of development				Mortality of eggs (larvae)
	Blastomere cleavage	Larval formation	Formation of L1, L2	Formation of L3	
1	100.00	–	–	–	–
2	27.67 ± 2.31	72.33 ± 2.31	–	–	–
3	11.33 ± 2.52	18.67 ± 1.53	59.00 ± 6.24	11.00 ± 2.65	–
4	–	4.67 ± 1.15	59.67 ± 4.51	24.33 ± 3.06	11.33 ± 2.52
5	–	–	3.67 ± 1.53	77.00 ± 2.65	19.33 ± 2.52
6	–	–	–	78.33 ± 2.08	21.67 ± 2.08

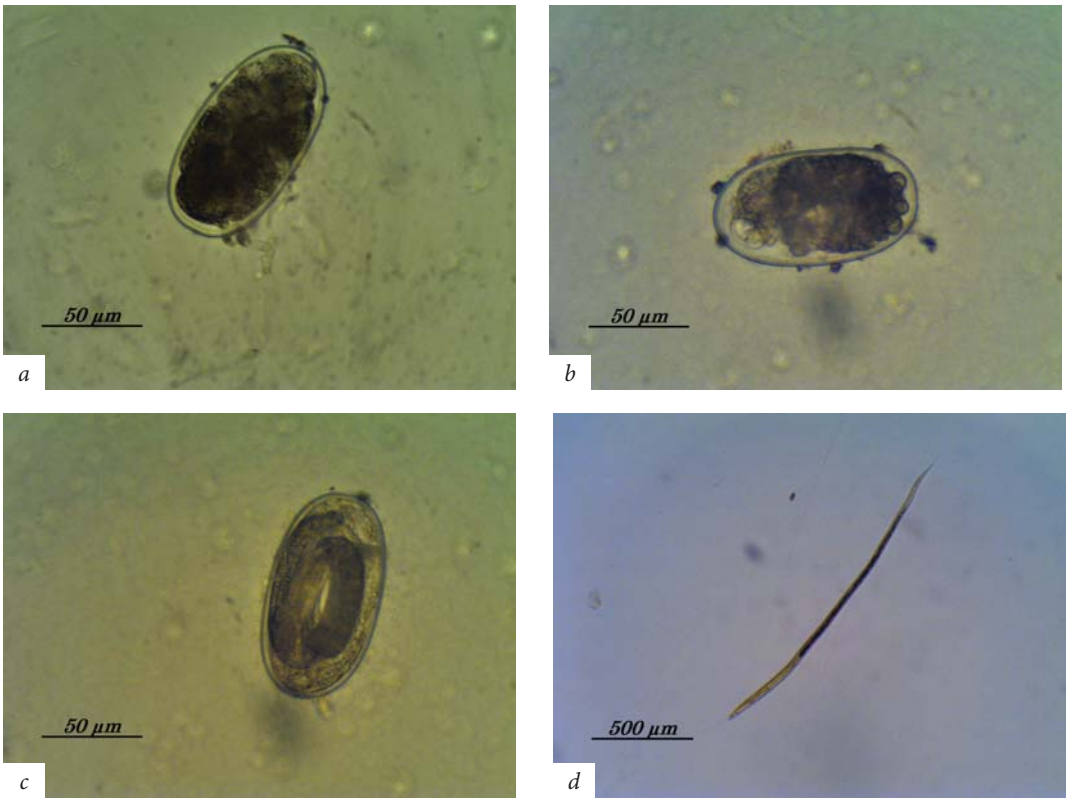


Fig. 6. Stages of embryonic development of *Amidostomum anseris*: *a* — blastomere cleavage; *b* — larval formation; *c* — formation of L1 and L2; d — L3 (infective larva).

of domestic geese in Poltava, Kharkiv, Kyiv and Lviv Regions of Ukraine, and infection rates are as high as 54.09 %. This significant level of infection was also observed in other studies (Seyidbeyli and Rzayev, 2018; Sergushin, 2000; Ljulin, 2006). Nematodes were located in gizzard and glandular stomach of 28.14 % of studied birds, indicating high adaptability of *A. anseris* to domestic goose.

Morphometric examination of nematodes revealed the morphological character, esophageal curve in anterior esophagus. It was found in 77.96 % of studied nematodes, more often in males (86.90 %) than in females (72.86 %). Considering that this character was highly frequent, it can be used in species identification.

In most publications on identification of *Amidostomum* nematodes, the morphological and metric characters include body size, shape of buccal capsule, presence and number of esophageal teeth; shape and length of spicules and gubernaculum, and morphology of caudal bursa in males; morphology of vulval area, shape and size of ovejector and eggs in females (Skrjabin et al., 1952; Ryzhikov, 1967; Kobulej and Ryzhikov, 1968; Kavetska et al., 2011).

We describe the sex-related dimorphism of body size and morphology of *A. anseris* nematodes in more details. The females are significantly larger than males by 11 characters. Also in males, 29 metric indices are suggested for species identification. The length and width and the ratio of length to width in different rays of caudal bursa are considered. Previously, the position of rays was used in identification (Skrjabin et al., 1952; Ryzhikov, 1967). Changes in transverse striation of cuticle in males and females at different body areas are described, adding to the data on morphology of *A. anseris*.

The study of development of *A. anseris* eggs revealed four stages of embryogenesis: blastomere cleavage; larval formation; formation of early-stege larvae (L1 and L2); forma-

tion of infective larvae L3, and hatching. Similar findings were published in Enigk and Dey (1968) and Stradowski (1977), but the stages have not been determined. It was also recorded that at 23 °C the larvae in eggs reached the third stage in six days, and their viability was 78.33 ± 2.08 %. Our data on the embryogenesis of *A. anseris* (fast development in the outer environment and high viability) indicate high adaptability of the parasite.

Conclusion

Nematodes *Amidostomum anseris* (Zeder, 1800) are well-adapted to parasitizm in domestic geese (*Anser anser dom.* Linnaeus, 1758) in the Central, North-Eastern and Western Ukraine. Infection rates reached 54.09 % in studied regions. Characters used in species identification of adult *A. anseris* specimens are morphometric. For both female and male nematodes, common morphological characters are identified. The size dimorphism is described: female nematodes are larger than males by 11 parameters ($P < 0.05$). We suggest using the metric parameters (length and width of tail bursa rays) for reliable identification of *A. anseris* males. Variability in the cuticle striation at various body areas is described for male and female specimens. Embryogenesis of *A. anseris* in laboratory culture is characterized by four stages of development: blastomere cleavage, larval formation, formation of non-infective larva, formation of infective larva. At 23 °C, larvae become infective at the sixth day of culture, and viability of embryonic *A. anseris* is 78.33 %.

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Received 9 August 2018
Accepted 25 October 2018