

UDC 595.132.6

MORPHOMETRIC ANALYSIS OF *CAPILLARIA ANATIS* (NEMATODA, CAPILLARIIDAE) FROM *ANAS PLATYRHYNCHOS* *DOMESTICUS*

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Morphometric Analysis of *Capillaria anatis* (Nematoda, Capillariidae) from *Anas platyrhynchos domesticus*. Yevstafieva, V. O., Stybel, V. V., Melnychuk, V. V., Pishchalenko, M. A., Korchan, L. N., Kone, M. S., Nagorna, L. V., Feshchenko, D. V., Antipov, A. A., Bakhur, T. I. — The study presents species-specific morphological traits and metric parameters of mature male and female *Capillaria anatis* (Nematoda, Capillariidae) nematodes. The capillariids were obtained by examinations of caeca of 205 domestic ducks (*Anas platyrhynchos dom.*), reared at poultry farms of Poltava, Sumy and Kyiv Regions of Ukraine. Noted sexual dimorphism was significant by seven morphometric parameters, with measurements of females being larger by 12.40–29.69 % than those of males. In addition to species-specific traits, other diagnostic characters were proposed in male and female *C. anatis*. In males, such taxonomically important features are morphological specifics of pseudobursa, spicule and ornamentation of spicule sheath. In identification of females, morphology of the vulvar area and eggs in uterus should be considered. Eleven metric parameters are suggested for better differential diagnostics of *C. anatis*.

Key words: nematodes, *Capillaria anatis*, domestic duck, differential characters, morphological identification.

Introduction

Nematodes of the family Capillariidae Neveu-Lemaire, 1936 are numerous and widely distributed parasitic helminthes located in almost all organs of their hosts. Various capillariids parasitize humans, apes, wild and domestic carnivores, rodents, marsupials, fish, amphibians, and cattle (De, 1998; Spratt, 2006; Robles Mdel et al., 2012; Iglesias et al., 2013; Khalifa & Othman, 2014). Several of those nematode species are highly pathogenic and are of epizootic and epidemiological importance (El-Dib et al., 2015; Ochi et al., 2017; Kalinkin et al., 2018). Capillariid infections of digestive tracts also are common in wild, synanthropic and domestic birds. By now, more than 16 genera of the family are recognized, mostly due to the publication of Moravec (Moravec, 1982). Seven of those taxa parasitize poultry: *Pseudocapillaria* Freitas, 1959, *Baruscapillaria* Moravec, 1982, *Echinocoleus* Lopez-Neyra, 1947, *Capillaria* Zeder, 1800, *Eucoleus* Dujardin, 1845, *Pterothominx* Freitas, 1959, *Aonchotheca* López-Neyra, 1947 (Madsen, 1945; Skrjabin et al., 1957; Moravec et al., 1987).

A typical representative of capillariids that parasitizes aquatic birds, including poultry, is *Capillaria anatis* (Schränk, 1790) Travassos, 1915 [= *Thominx anatis*]. Additionally, there are reports of the species in wild turkeys (*Meleagris gallopavo*), chickens (*Gallus gallus dom.*), barn swallows (*Hirundo rustica*), and rock pigeons (*Columba livia dom.*) (Ryzhikov, 1967; Permin et al., 1999; Natala et al., 2009; Tamaru et al., 2015; Syrota & Kharchenko, 2015; Fakhar et al., 2018).

The prevalence of *C. anatis* infection in aquatic poultry was reported as up to 50 % in several regions of India, 0.5 % in Eastern Africa, 8.33 % in Japan, and up to 25 % in Poland (Muhairwa et al., 2007; Yoshino et al., 2009; Tanveer et al., 2015; Kornaś et al., 2015). This capillariid species was also found in poultry in Great Britain, Turkey, Iran, South Asia, and New Zealand (Norton, 1964; Wakelin, 1965; Soulsby, 1982; Buluş, 1998; Anisuzzaman et al. 2005; Eslami et al., 2009; McKenna, 2009).

Nematode taxonomy is traditionally based on specific morphological characters. In the case of Capillariidae, there are a lot of differing opinions that treat those traits with various degrees of importance. Capillariids variability introduces significant difficulties to their taxonomic differentiation, in the absence a system of clear-cut diagnostic keys based on assessments of quantitative and qualitative morphological characters. The system of Capillariidae is frequently subject to revision, and identification to the genus level by morphological characters is usually based on specifics of the pseudobursa, spicule and ornamentation of spicule sheath in males (Moravec, 1982; Traversa et al., 2011; Stapf et al., 2013; Tanveer et al., 2015; Yevstafyeva et al., 2017). In some publications, morphometric parameters are suggested to enhance the effectiveness of species identification of capillariids (D'ávila et al., 2012; Dar et al., 2013; Yevstafyeva et al., 2018).

The accumulated reports point to the morphometric variability of *C. anatis*, which can complicate their species identification. Thus, Tamaru et al. (2015) report data on metric parameters of *C. anatis* obtained from domestic chickens. The authors noted the differences in metric parameters of nematodes collected in Japan and Philippines, hence the necessity of a more detailed morphometric study of the nematodes of that species. Hence our work aimed to establish the differential qualitative and metric characters in mature males and females of *C. anatis* from domestic duck in Ukraine.

Material and methods

The specimens were collected in 2017–2019. In total, 205 domestic ducks (*Anas platyrhynchos dom.*) were examined, obtained from farms of Poltava, Sumy and Kyiv regions of Ukraine. Nematodes were collected in helminthological investigation of caeca of dead or killed birds (Skrjabin, 1928). Nematodes were identified using the keys by Skrjabin et al. (1957) and Ryzhikov (1967). Analysis of morphology was conducted on 57 mature nematodes, 21 males and 36 females.

To measure morphometric characteristics of the adult *C. anatis* nematodes, ImageJ for Windows® (version 2.00) software was used in interactive mode using 5×, 10×, 40× objectives and 10× photo eyepiece. To calibrate the image analyzer, ruled scale of ocular micrometer was coincided with the scale of stage micrometer included in MikroMed microscope kit. Microphotographs were taken using a digital camera of MikroMed (China) microscope.

Statistical processing of the experimental results was carried out using Microsoft Excel software. Standard deviation (SD) and average values (\bar{x}) were calculated. Significance of difference between average values in the studied groups of nematodes was established using one-way analysis of variance and F-test for 95 % confidence level.

Results and discussion

Adult males and females of *C. anatis* specimens have the body shape typical of capillariids. The nematodes are thin, slightly translucent, filiform and gradually thickening towards the tail end. The anterior, thinner, body part thickens into the posterior body part at the junction between the esophagus and the intestine. Bacillary bands are almost invisible. The

head end tapers to a blunt cone (fig. 1). The mouth opening is a terminal slit, surrounded by two weakly developed lips. Cuticle of the head end is smooth, otherwise transversally striated. Esophagus consists of a short muscular part and a longer glandular one.

Sexual dimorphism is distinct in the body sizes. By seven morphometric parameters, females are larger than males. The length of body in females is 11.05 ± 0.88 mm (ranging from 9.70 to 12.50 mm). In males, the length of body is shorter by 20.99 % ($P < 0.05$, 8.73 ± 0.95 mm), ranging from 7.20 to 9.80 mm (fig. 2, a). The anterior and posterior body parts in females are correspondingly longer by 12.43 % ($P < 0.05$, 5.47 ± 0.51 mm) and 29.69 % ($P < 0.05$, 5.59 ± 0.48 mm) than those parameters in males (4.79 ± 0.53 and 3.93 ± 0.57 mm) (fig. 2, b, c). At the same time, ratios of those parts are different in males and females. In females, that ratio is 0.98 : 1.0, in males it is 1.23 : 1.0. Width of body at different points is significantly larger in females ($P < 0.05$) than in males (fig. 2, d–g): at middle of head end (by 12.40 %, 36.83 ± 1.10 μ m), at the esophago-intestinal junction (by 15.18 %, 56.65 ± 2.15 μ m), at middle of body (by 16.51 %, 51.12 ± 1.19 μ m), at middle of tail end (by 14.99 %, 53.62 ± 1.73 μ m).

In males of *C. anatis*, specifics of pseudobursa, spicule and spicule sheath (fig. 3, a–d) and their metric parameters (table 1) can be considered as specific morphological traits. The pseudobursa in males of the species has lateral lobes. Its width and length are, correspondingly, 30.61 ± 0.97 and 16.18 ± 0.65 μ m, with a ratio of 1 : 0.53. The distance between the tips of lateral lobes of the pseudobursa (19.82 ± 1.35 μ m) can be considered a differential character. The spicule is single, 1.51 ± 0.06 μ m in length (ranging from 1.40 to 1.64 μ m), relatively wide, trihedral. Distal end of the spicule tapers to a rounded tip, width of that part of the spicule is on average 7.98 ± 0.65 μ m. At the middle, the spicule gradually thickens to 10.94 ± 0.40 μ m. Proximal part of spicule widens to 16.06 ± 0.47 μ m. Spicule sheath is smooth, tight around the spicule, armed with small spikes that can be observed when the spicule is withdrawn. Also to ease the effectiveness of species identification of *C. anatis* males, the following morphometric parameters are suggested: width of the body at the proximal end of the spicule (46.03 ± 0.97 μ m), at the middle of the spicule (45.04 ± 0.71 μ m), at the base of the pseudobursa (28.01 ± 1.89 μ m).



Fig. 1. Head end of *Capillaria anatis*.

Table 1. Metric parameters of *Capillaria anatis* males, n = 15

Parameters	$\bar{x} \pm SD$	Min	Max
Length of spicule, mm	1.51 ± 0.06	1.40	1.64
Width of spicule at, μ m:			
– proximal end	16.06 ± 0.47	15.24	17.11
– the middle	10.94 ± 0.40	10.20	11.62
– distal end	7.98 ± 0.65	7.07	8.92
Width of body at, μ m:			
– proximal end of spicule	46.03 ± 0.97	44.78	47.21
– middle of spicule	45.04 ± 0.71	44.12	46.74
– base of pseudobursa	28.01 ± 1.89	25.27	31.09
Length of pseudobursa, μ m	16.18 ± 0.65	15.08	17.03
Width of pseudobursa, μ m	30.61 ± 0.97	29.10	32.18
Ratio of width to length of pseudobursa	1 : 0.53	1.0 : 0.49	1.0 : 0.56
Distance between tips of lateral lobes of pseudobursa, μ m	19.82 ± 1.35	18.04	21.97

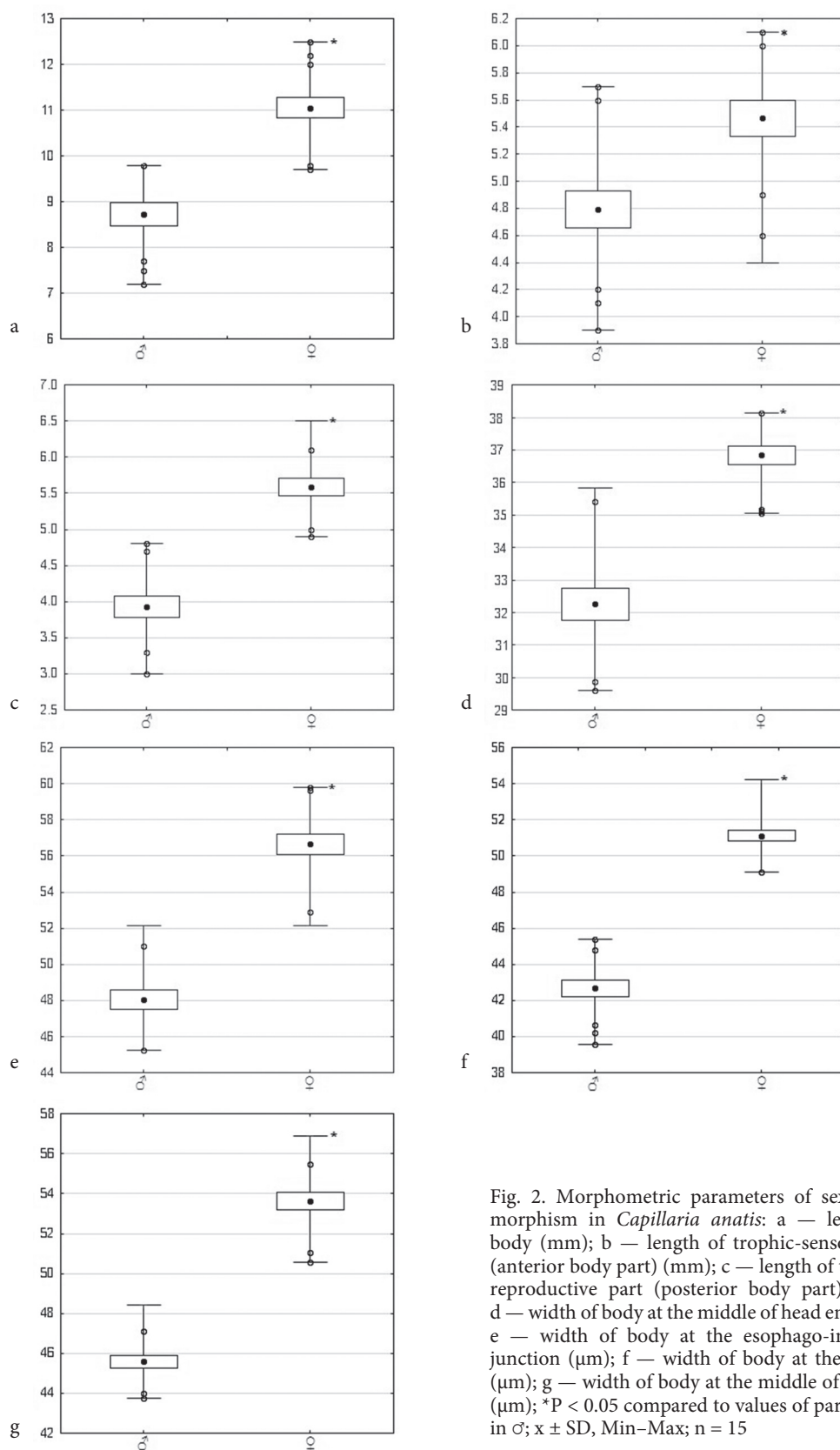


Fig. 2. Morphometric parameters of sexual dimorphism in *Capillaria anatis*: a — length of body (mm); b — length of trophic-sensory part (anterior body part) (mm); c — length of trophic-reproductive part (posterior body part) (mm); d — width of body at the middle of head end (μm); e — width of body at the esophago-intestinal junction (μm); f — width of body at the middle (μm); g — width of body at the middle of tail end (μm); *P < 0.05 compared to values of parameters in ♂; $\bar{x} \pm \text{SD}$, Min–Max; n = 15

As for females of *C. anatis*, such parameters can be considered as species-specific morphological characters: structure of vulva area, and of eggs in uterus (fig. 4, a–d), and their metric characteristics (table 2). Vulva is not prominent, slit-like, without protrusions, processes and valves. Width of body at the vulva area is $59.73 \pm 1.02 \mu\text{m}$. Metric parameters of vulva's location should be considered in species identification too. Hence, distance from vulva to the head end is $5.52 \pm 0.51 \mu\text{m}$, to the posterior end of esophagus $48.97 \pm 2.12 \mu\text{m}$; to anus $5.53 \pm 0.48 \text{ mm}$; to tail end $5.54 \pm 0.48 \text{ mm}$. Vagina does not bend. Tail end in females is bluntly rounded, with a subterminal anus. Width of body at anus level is $21.55 \pm 1.07 \mu\text{m}$. Eggs in uterus have specific shape: elongated, with a thick shell that bears rounded impressions, which resemble a wavy structure. The internal layer of egg shell protrudes as a collar with clearly visible lobes. Egg plugs are large, prominent, slightly indented. Eggs are

Table 2. Metric parameters of *Capillaria anatis* females, n = 15

Parameters	$x \pm \text{SD}$	Min	Max
Width of body at vulva area, μm	59.73 ± 1.02	58.17	61.28
Distance from vulva to head end, mm	5.52 ± 0.51	4.45	6.15
Distance from vulva to end of esophagus, μm	48.97 ± 2.12	45.46	51.47
Distance from vulva to anus, mm	5.53 ± 0.48	4.84	6.44
Distance from vulva to tail end, mm	5.54 ± 0.48	4.85	6.45
Width of body at anus area, μm	21.55 ± 1.07	19.84	23.64
Length of egg with egg plugs, μm	59.12 ± 1.59	56.29	61.25
Length of egg without egg plugs, μm	50.51 ± 1.40	48.22	53.14
Width of egg plugs, μm	13.92 ± 0.86	11.82	14.96
Width of egg, μm	30.92 ± 1.61	29.45	35.41
Thickness of eggshell, μm	3.40 ± 0.44	2.74	4.11

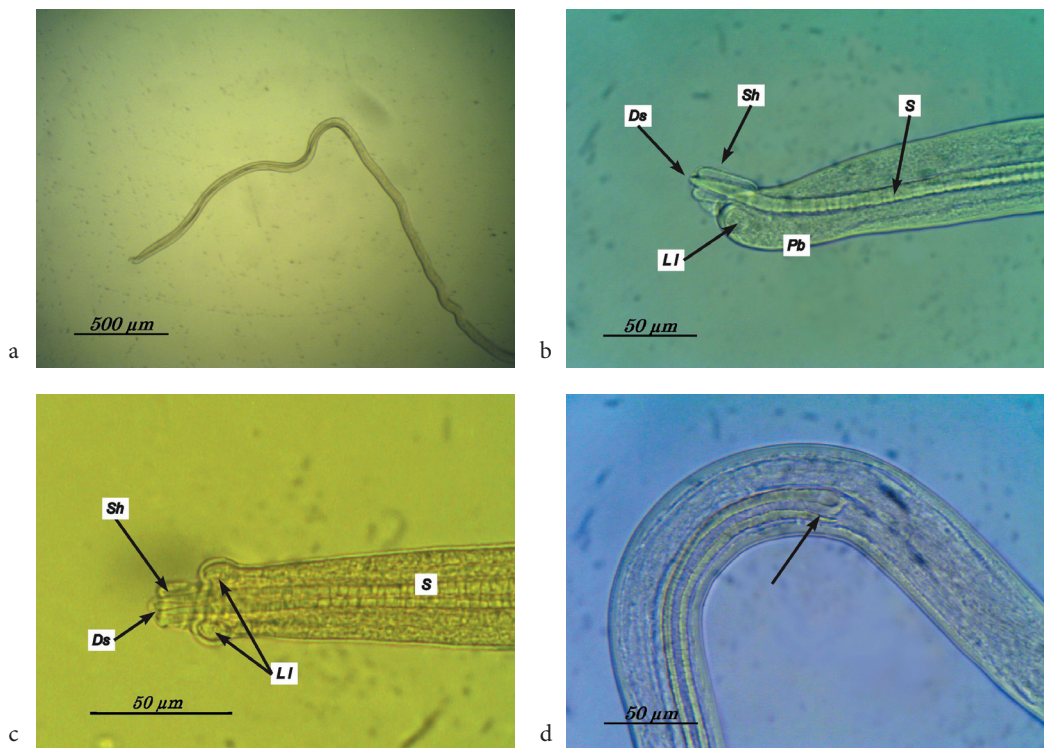


Fig. 3. Tail end of σ *Capillaria anatis*: a — general view; b — laterally; c — dorsally; d — proximal end of spicule; Pb — pseudobursa, Ll — lateral lobes, Sh — spicule sheath with small spikes, S — spicule, Ds — distal end of spicule.

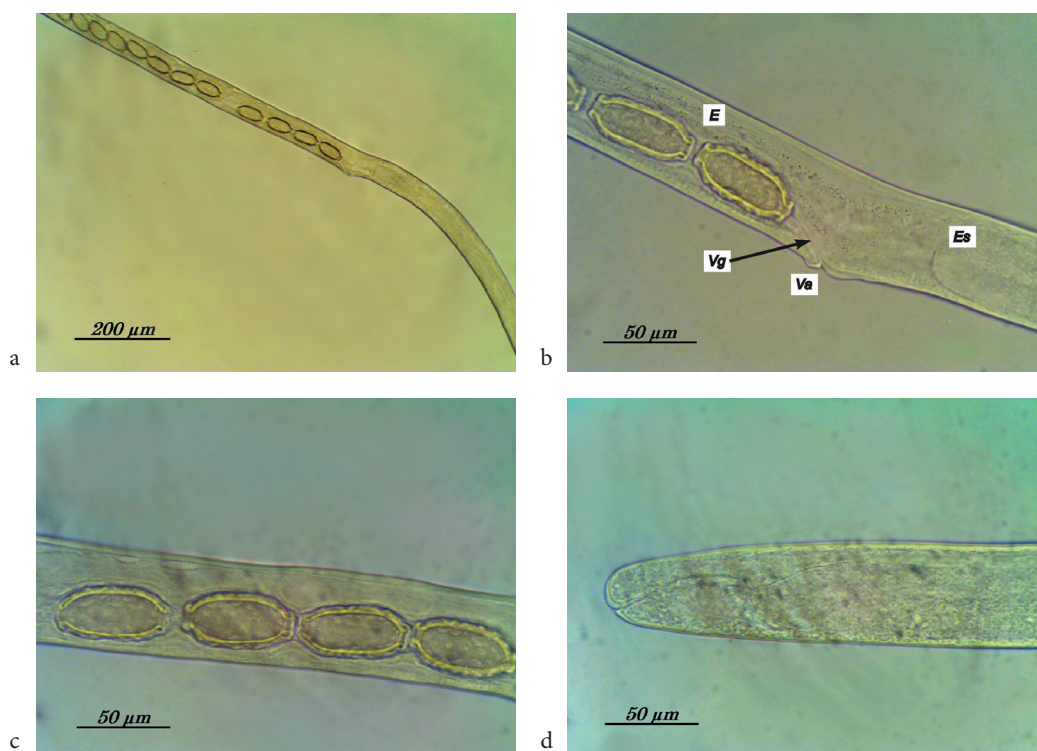


Fig. 4. ♀ *Capillaria anatis*: a — general view; b — body at vulva; c — eggs in uterus; d — tail end; Va — vulva, Vg — vagina, U — uterus, E — eggs, Es — posterior part of esophagus.

50.51 ± 1.40 µm in length (ranging from 56.29 to 61.25 µm) and 30.92 ± 1.61 µm in width (29.45 to 35.41 µm). Egg plugs are 13.92 ± 0.86 µm in width (11.82 to 14.96 µm), eggshell is 3.40 ± 0.44 µm thick (2.74 to 4.11 µm).

Thus, our work presents the results of morphometric study of *Capillaria anatis* (Schrank, 1790) Travassos 1915 specimens, obtained from domestic duck (*Anas platyrhynchos dom.*), which were reared at farms of Poltava, Sumy and Kyiv regions of Ukraine. The nematode species is a common parasite of aquatic poultry in many countries (Soulsby, 1982; Anisuzzaman et al. 2005; Eslami et al., 2009; Tanveer et al., 2015). Despite such wide distribution, there are few studies regarding the species identification of *C. anatis* (Özidal & Ayaz, 2005; Dar et al., 2013; Stapf et al., 2013).

It is established that sexual dimorphism in *C. anatis* is notable in the morphometric characteristics. By seven of those, female nematodes are significantly ($P < 0.05$) larger (by 12.40–29.69 %) than males. Analysis of morphometric data of adult *C. anatis* specimens revealed that for males, species-specific morphological traits include structural features of pseudobursa (presence of two lateral lobes, lack of membrane and wings), spicule (shape), spicule sheath (presence of small spikes in withdrawn position). In females, such traits are: vulva area (vulva slit-like, without cuticular formations) and egg structure (shape, presence of large collar with clearly visible lobes, surface structure). These characters are considered of taxonomic value by other scientists (Skrjabin et al., 1957; Ryzhikov, 1967; Stapf et al., 2013). At the same time, we analyzed 11 metric parameters in *C. anatis* males, including not only the well-recognized (length of body and spicule, width of body and spicule at their widest), but also the dimensions of the pseudobursa (width, length and their ratio; distance between tips of lateral lobes), and width of body at different areas (proximal end of spicule; middle of spicule; base of pseudobursa). For females, 11 metric parameters were also considered (location of vulva in relation to various morphological structures of body;

egg width and length; eggshell thickness and width of egg plugs). At the same time, Tamaru et al. (2015) provided only five metric parameters in male and seven in female *C. anatis*, obtained from chicken. They also noted a significant difference in sizes of nematodes, collected in different countries (Japan and Philippines). The length of body in males ranged from 8.64 ± 1.03 to 12.35 ± 1.36 mm, and that of females from 11.98 ± 1.97 to 17.39 ± 2.52 mm. According to our study, length of body of *C. anatis* obtained from ducks in Ukraine was 8.73 ± 0.95 mm in males and 11.05 ± 0.88 mm in.

Considering that the data of previous reports are presented selectively, our data on the metric parameters of *C. anatis* will help enhancing the species identification of that species by males and females.

Conclusion

Nematodes of the species *Capillaria anatis* (Schrank, 1790) Travassos 1915 are common parasites of domestic duck (*Anas platyrhynchos dom.*) in the central and North-Eastern regions of Ukraine. Morphometric analysis revealed species-specific traits of male and female specimens. Metric sexual dimorphism in this species is significant in seven parameters, by which females are significantly ($P < 0.05$) larger than males. The characteristic morphological features of *C. anatis* males are specifics in structure of the pseudobursa, spicule and spicule sheath. In females, the differential characters to consider are the structure of vulva area, and the shape and structure of eggs in the uterus. We suggest 11 metric parameters in males and 11 in females for the more effective identification of *C. anatis*.

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Received 20 Decwember 2019

Accepted 15 December 2020