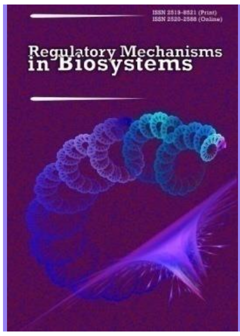

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Differential species traits of *Trichostrongylus tenuis* (Nematoda, Trichostrongylidae)

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Trichostrongylus tenuis Mehlin, 1846 is a helminth species that parasitizes in the gastrointestinal tract of birds and causes trichostrongylosis. Research on the differential features of the pathogen at various stages of development facilitates timely finding the foci of infection and providing recommendations for prevention and control. In the present study, the differential species traits of male and female *Trichostrongylus* nematodes parasitizing in the domestic goose were examined. The nematodes were obtained in helminthological dissection of the intestine of birds kept at farms and private households in Poltava region, Ukraine. It was revealed that the morphological features of *T. tenuis* females that should be considered in species identification include the structural specifics of the reproductive system and the tail end. 22 metric parameters were suggested for species identification, including the female body measurements for different parts, specifics of vulva and anus location, sizes of the ovijector, sphincter, ejector and vulval area. Differences were found in the metric parameters of the lateral crests of the cuticle in female nematodes in relation to the position of crests on the parasite's bodies. The cuticular crests were absent at 0.1 mm from the head and tail ends, and the length of crests was 9.3 mm in total. The longest and widest crests were observed in the middle of the body, the shortest were found in the anus area, and the narrowest crests were from the anterior part of esophagus to its middle. The distance between crests varied 0.2–3.0 µm, the longest at the transition of esophagus into the intestine and the shortest in area between the vulva and the anus. The metric parameters of *T. tenuis* eggs obtained from bird feces and in the nematode uterus differed significantly by 5 characters. The collected data on the morphological and metric parameters of females and eggs of *T. tenuis* can be used in species identification and understanding of the taxonomic position of that species.

Keywords: trichostrongylosis; goose; nematodes; helminth eggs; differential diagnostics.

Introduction

Parasitism is one of the life forms that present multifaceted biological phenomena. The parasitic organisms are an integral part of natural biocoenoses. They also are one of the factors forming biological diversity. Thirdly, they ensure the circulation of matter and energy flows at the level of the parasitic systems (Ewald, 1995; Zelmer, 1998; Araújo et al., 2003). This determines the importance of a scientific and theoretical parasitological study, based on species identification, the study of life cycles, population dynamics and geographical distribution. However, the relevance of the study of parasites is associated, first of all, with the pathogenic significance that they may have for domestic animals (Boyko & Brygadyrenko, 2016, 2019). Indeed, certain parasitic species, including the causative agent of trichostrongylosis, can cause significant damage to the poultry industry (Davidson et al., 1991; Enigk & Dey-Hazra, 1971). This infection causes a delay in the growth and development of young animals, negatively affects the productivity, product quality and functional activity of birds' immune systems, reducing the natural reactivity of their bodies, often to the point of death of birds (Cram & Cuvillier, 1934; Hudson, 1986; Shaw & Moss, 1990).

The avian parasite, *Trichostrongylus tenuis* Mehlin, 1846, according to the taxonomic position belongs to the type Nematoda, class Secernentea, order Strongylida, superfamily Trichostrongyloidea (Cram, 1927), family Trichostrongylidae (Leiper, 1912), genus *Trichostrongylus* (Loos, 1905). Trichostrongyloidea is a nematode group, widespread everywhere and parasitic at imago stage in various organs of amphibians, reptiles, birds and mammals including humans. Among the nematodes of vertebrate parasites, the superfamily Trichostrongyloidea is one of the richest groups in terms of the number of species and genera (Durette-Desset, 1985; Giannetto, 2006; Souza et al., 2013; Andrade et al., 2018). The nematodes of this group descend from the free-living ancestors of the modern rhabditids which still exist today. Trichostrongylids have retained many features in common with their ancestors, related to both morphological and ecological characters. The latter include the feature that Trichostrongyloidea still have a partially free-living lifestyle and some of their life cycle and metamorphoses occur outside the host's organism, namely in the external environment (Connan & Wise, 1993; Sudhaus & Fitch, 2001; O'connor et al., 2006).

The causative agent of trichostrongylosis, *T. tenuis* is localized in the cecum of birds, less often in the small intestine. The infection has a significant distribution and is recorded in Kenya, Nigeria, Mexico, Austria, Germany, Britain, Bulgaria, Estonia, Iceland, the Italian mainland, Kaliningrad region, Latvia, Lithuania, the Norwegian mainland, Poland, the Portuguese mainland, the Spanish mainland, Netherlands, Ukraine (Irungu et al., 2004; Woog et al., 2011; Wascher et al., 2012; De Jong et al., 2014; Afolabi et al., 2016; Cervantes-Rivera et al., 2016). The representatives of the family Trichostrongylidae are often found in domestic and wild birds. Epomidiostomum crami was recorded in *Chen caerulescens caerulescens* (prevalence, 92%; mean intensity, 18.7 ± 13.3 specimens) (Tuggle & Crites, 1984). The same parasite was found in *Anser albifroms* (66.7%; 4.83 ± 7.5 specimens), *Anser fabalis* (100%; 3), *Cygnus cygnus* (13.3%; 2.83 ± 2.79), *C. columbianus* (16.7%; 3.5 ± 3.0) (Yoshino et al., 2009). In ostrich (*Struthio camelus*), *Libyostrongylus* spp. are common: the prevalence can be as high as 100%, and the infection rates are up to 8608 eggs (Mukaratirwa et al., 2004; Bonadiman et al., 2006; Pesenti et al., 2015). Therefore, a comprehensive study of parasitic nematodes of this species is not only of general biological interest, but also of great practical importance in planning and carrying out measures to combat and prevent infection.

Most of the criteria used in the classification of Trichostrongyloidea, and in particular in the identification of *T. tenuis*, are morphological. The main morphological diagnostics is carried out for male nematodes, since they have a greater number of structures in the reproductive apparatus. The structure of the caudal bursa, the features of the interposition of its rays, the structure of the genital cone, the shape and size of the spicules, the presence or absence of a gubernaculum are taken into account. Attention is also paid to the general morphology of the nematode body, namely: the presence or absence of mouth capsule and the nature of its ornamentation, the presence of a vesicle and papillae at the head end and chitinous ornamentation of the cuticle (Gibbons & Khalil, 1982; Durette-Desset, 1992; Durette-Desset et al., 1993). At the same time, the morphology of *T. tenuis* has been described in separate works, which do not fully contain information on the taxonomic characters of *Trichostrongylus* (Ryzhikov, 1967; Atkinson et al., 2008). This, in turn, complicates their differential diagnosis and requires additional study of the morphology of *T. tenuis* at various stages of development.

When classifying Trichostrongyloidea genera and species, attention is now paid principally to the structure of the system of cuticular crests, which are a character of a rather high taxonomic value. Individual crests can be more developed than others to the point of forming lateral wings (Hoberg, 1996; Lichtenfels et al., 1996; Lichtenfels & Pilitt, 2000). It is generally accepted that these characters are reliable and very promising in solving the problems of differential diagnosis of species and genera, improving the system of the superfamily Trichostrongyloidea.

The aim of present study is to research the differential morphometric species characters of the mature females and eggs of *T. tenuis* nematodes, parasitizing in domestic goose.

Materials and methods

The studies were carried out at the laboratory of the Department of Parasitology and Veterinary-Sanitary Examination of the Faculty of Veterinary Medicine of the Poltava State Agrarian Academy in 2018–2020. The nematodes were collected during helminthological investigation of the intestine of 287 domestic geese (*Anser anser* dom.) (Skrjabyn, 1928) on farms in Poltava region (Ukraine). The nematode species was identified by Skriabyn et al. (1954). In total 302 adult female *T. tenuis* nematodes were collected. The prevalence of infection was 31.7% according to the results of helminthological dissection. 2,271 specimens of domestic goose were studied using coproovoscopy, and the prevalence of infection was 22.9%. In Poltava region, *T. tenuis* infection in domestic goose was in the range 17.2–26.7%. Notably, the prevalence of infection was higher in geese kept in private farm plots and households (24.4%) compared to that in geese reared in specialized bird farms (16.1%) (Yevstafieva & Starodub, 2020). The morphological and metric parameters of *T. tenuis* eggs were examined after collection from the feces of infected geese and directly in the uterus of female nematodes. The studied characters included the shape, surface of the shell, length, width of egg, eggshell thickness, as well as the areas of the inner and outer surface of egg.

The morphometric parameters of *T. tenuis* eggs and nematodes were studied using ImageJ for Windows® software (version 2.00) in interactive mode using a ×10, ×40, ×100 objective and a ×10 photo eyepiece. To calibrate the image analyzer, the ruled scale of a ocular micrometer was calibrated with the scale of stage micrometer included in a MikroMed microscope kit. Microphotography was performed using a digital camera attached to a MikroMed 5 Mpix microscope (China).

Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values in the studied *T. tenuis* eggs was established using one-way analysis of variance and F-test for 95% confidence level.

Results

The morphological study of adult *T. tenuis* females revealled the following species traits. The nematodes are small, thin, filiform, somewhat translucent. Length of the roundworm body was 9.5 ± 0.9 mm, slightly tapered to head and tail ends. This is confirmed by metric parameters with width of body being 13.6 µm at mouth opening, 69.7 µm at the middle of body, and 26.4 µm at the anus area (Table 1).

Table 1
Morphometric parameters of ♀ *Trichostrongylus tenuis* (n = 10)

Characters	x ± SD	Min – max
Length of body, mm	9.5 ± 0.9	8.2–11.1
Length of esophagus, mm	1.3 ± 0.1	1.2–1.4
Distance from head end to the excretory pore, µm	123.8 ± 5.6	111.4–132.1
Length of body covered by lateral cuticular crests, mm	9.3 ± 0.9	8.0–10.8
Distance from head end to lateral cuticular crests, mm	0.14 ± 0.01	0.13–0.15
Distance from tail end to lateral cuticular crests, µm	65.9 ± 4.5	60.2–71.1
Distance from anus to tail end, µm	89.0 ± 6.5	80.3–101.6
Distance from vulva to tail end, mm	1.4 ± 0.2	1.0–1.8
Width of body at, µm:		
– mouth opening	13.6 ± 0.6	12.2–14.2
– excretory pore	23.9 ± 2.1	21.9–27.0
– beginning of cuticular striation	25.6 ± 1.7	23.5–28.2
– the middle part of esophagus	29.3 ± 0.9	28.1–31.1
– transition of esophagus to intestine	42.2 ± 2.2	40.2–46.1
– the middle of body	69.7 ± 0.7	68.8–71.0
– vulva	76.9 ± 1.7	73.4–78.9
– anal opening	26.4 ± 1.1	24.1–27.6
Length of slit-like opening of vulva, µm	58.0 ± 3.4	52.3–64.3
Length of ovijector, µm	339.5 ± 15.2	314.4–362.1
Length of sphincter, µm	45.7 ± 2.1	41.1–49.1
Width of sphincter, µm	55.5 ± 2.1	52.1–58.1
Length of ejector, µm	123.9 ± 8.4	109.8–136.5
Width of ejector, µm	60.3 ± 2.7	55.9–65.1

Mouth organs were weakly developed, and the mouth opening was surrounded by three inconspicuous lips. The lips were slightly depressed in the middle. The oral capsule, neck papillae and other cuticular formations were absent at head end. The esophagus was thin and elongated (Fig. 1).

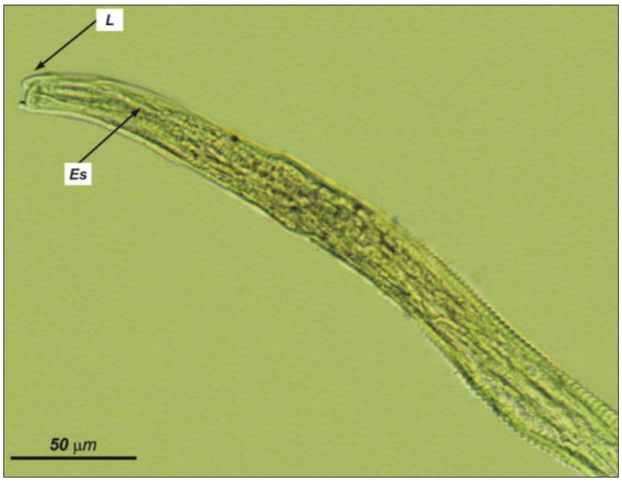
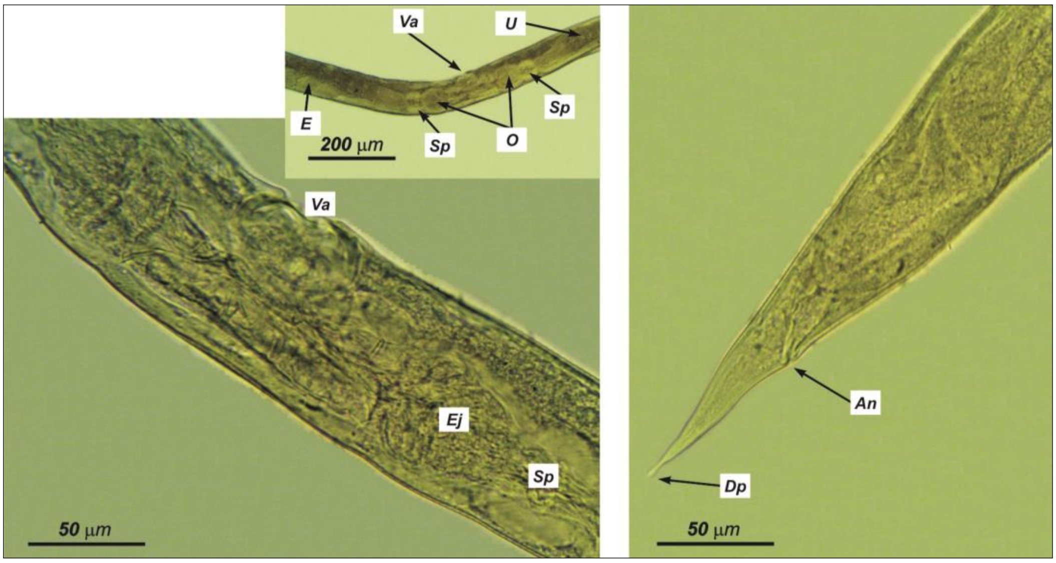


Fig. 1. Head end of ♀ *Trichostrongylus tenuis*: *Es* – esophagus, *L* – lips

Additional differential morphological characters of *T. tenuis* females were found in the present study, including vulva structure, specifics of the copulatory apparatus and tail end. 22 metric parameters are also suggested for use in the species identification of females (Table 1). Thus, the copulatory apparatus of females included vulva, paired ovijector and uterus (Fig. 2a). The vulva had a slit-like opening without protrusions, 58.0 µm in length. It is located in the posterior body part, as confirmed by metric parameters, at 1.4 mm to tail end. The ovijector was well-developed, 339.5 µm long and consisted of a funnel linked to the uterus and preceding the muscle sphincter and the distally located ejector (Fig. 2a). The sphincter was round-oval in shape (45.7 × 55.5 µm), and the ejector was elongated (123.9 × 60.3 µm). The bends of the uterus diverged in opposite directions and were filled with eggs.

Tail end of female nematodes was cone-shaped and ended with a small, digitate process. The anus was well-developed (Fig. 2 b) and located at 89.0 µm to the tail end.



a *b*

Fig. 2. Morphological features of ♀ *Trichostrongylus tenuis*: *a* – copulatory apparatus, *b* – tail end; *Va* – vulva area; *O* – paired ovijector; *Sp* – sphincter, *U* – uterus, *Ej* – ejector, *E* – eggs, *An* – anus, *Dp* – digitate process

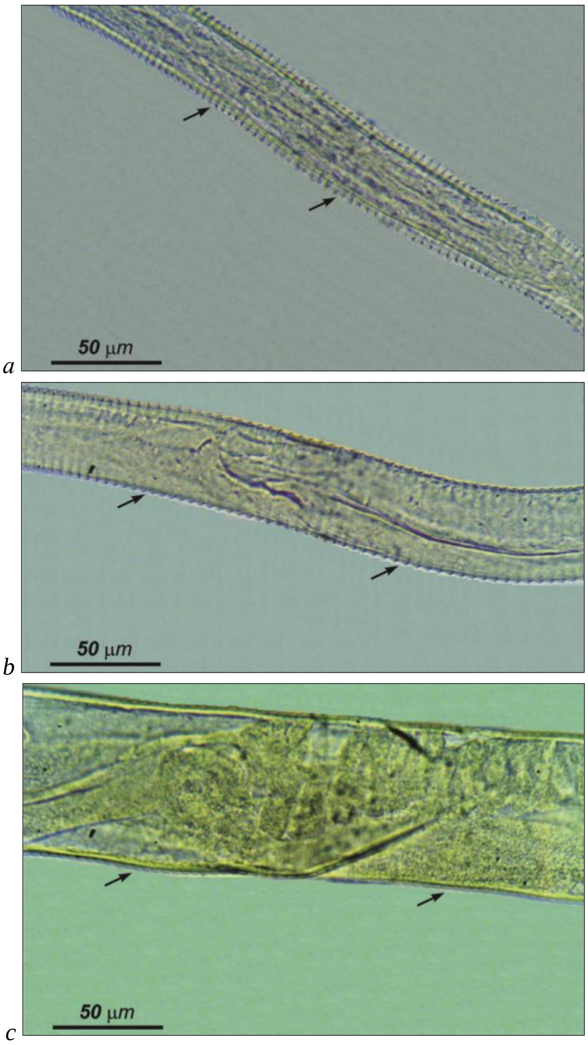


Fig. 3. Morphology of lateral cuticular crests of body of ♀ Trichostrongylus tenuis in: a – middle part of esophagus; b – transition of esophagus to intestine; c – anterior to vulva

We have also found that the lateral crests (wings, growths) of the cuticle were different in height, width and position to each other at all areas of *T. tenuis* females' bodies. This was confirmed in morphological studies (Fig. 3). Thus, in females the lateral crests began and ended at 0.1 mm from head and tail ends (Table 1). The crest's height and width at that body area were 1.3 ± 0.1 and 1.1 ± 0.03 μm, respectively (Fig. 4a, b).

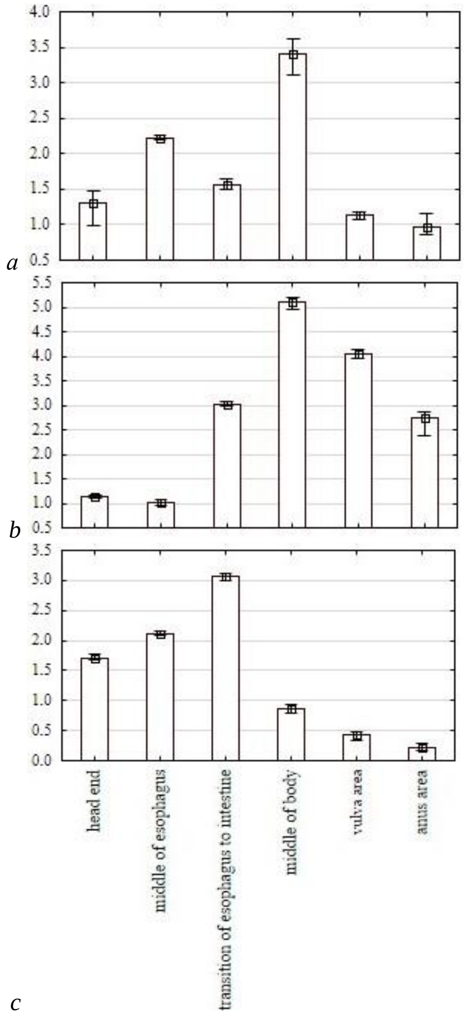


Fig. 4. Metric parameters of lateral cuticular crests of bodies of ♀ *Trichostrongylus tenuis* (n = 10): *a* – height, *b* – width, *c* – distance between crests (μm); small square corresponds to the mean value; the vertical line and segments directed up and down from the small square correspond to the minimum and maximum values

In the middle of the esophagus, the crests became gradually elongated by 40.9% to 2.2 ± 0.02 μm and slightly narrower, by 20.7% to 1.0 ± 0.04 μm. At the transition of the esophagus to intestine, the lateral cuticular crests became shorter, by 28.6% compared to the former parameters to 1.5 ± 0.05 μm, and wider, by 66.0%, to 3.0 ± 0.03 μm. In the middle of the nematode body, the height and width of crests were maximal (3.4 ± 0.13 and 5.1 ± 0.08 μm respectively), higher by 54.0% and 40.5% than the abovementioned values. Nearer to the tail end of female roundworm, the crests gradually became smaller. In the vulva area, the height and width of crests were respectively 1.1 ± 0.04 and 4.0 ± 0.06 μm (by 66.6% and 20.5% compared to the abovementioned values). In the anus area, the crests were shorter and narrower by 14.9% and 32.5% than the other noted values (0.9 ± 0.10 and 2.7 ± 0.14 μm).

The distance between crests was measured and the position of crests to each other was shown to vary in different areas of female *T. tenuis* bodies in a certain way (Fig. 4c). At the head end, the crests were located at a distance of 1.7 ± 0.03 μm between each other. At the middle part of the esophagus, this distance slightly increased by 19.3% (to 2.1 ± 0.03 μm). At the transition of the esophagus to the intestine, the distance between crests was maximal, to 3.0 ± 0.04 μm (larger by 30.7% compared to the abovementioned value). Nearer to the tail end, the distance between crests gradually decreased. In the middle of the body that distance decreased by 71.5% (to 0.8 ± 0.05 μm), in the vulva area by 50.5% (to 0.4 ± 0.05 μm). The minimal distance between crests was in the anus area, 0.2 ± 0.04 μm (smaller by 48.8% compared to the distance in the vulva area).

T. tenuis eggs had an outer structure characteristic for nematodes of the order Strongylida: oval, with thin, smooth, well-developed eggshell. The colour varied from light grey to light brown. The eggs in the nematode uterus and bird feces contained embryos at the stage of cleaving (Fig. 5).

Fig. 5. Eggs of *Trichostrongylus tenuis* nematodes:
a – obtained from feces of infected birds, *b* – in uterus of parasite females

A significant difference was found in comparison of the eggs obtained from the feces of sick geese and the eggs located in the uterus of female nematodes (Table 2). The eggs in the uterus of female nematodes were longer (by 3.4%, P < 0.001) and narrower (by 4.6%, P < 0.01) compared to eggs obtained from feces of birds. At the same time, the eggshell of eggs obtained from the feces was thicker by 17.6% (P < 0.001) compared to eggs in the nematode uterus. Accordingly, the area of inner surface of the eggs in the nematode uterus was larger by 3.7%, P < 0.001, and the area of their outer surface was smaller by 2.8%, P < 0.01 compared to the corresponding parameters of eggs obtained from bird feces.

Table 2
Metric parameters of eggs of *Trichostrongylus tenuis* (n = 10)

Characters		x ± SD	Min–Max
Total length of egg, μm	Eu	72.2 ± 1.46	70.3–74.8
	Ef	69.7 ± 0.87***	68.4–71.1
Width of egg, μm	Eu	35.3 ± 0.95	34.1–36.7
	Ef	37.1 ± 1.53**	35.3–39.4
Eggshell thickness, μm	Eu	0.70 ± 0.03	0.66–0.74
	Ef	0.85 ± 0.05***	0.78–0.92
Area of inner eggshell surface, μm^2	Eu	2076 ± 53	2011–2187
	Ef	1998 ± 32***	1911–2029
Area of outer eggshell surface, μm^2	Eu	2156 ± 44	2096–2208
	Ef	2220 ± 55**	2139–2296

Note: Eu – eggs in the uterus of female nematode, Ef – eggs obtained from feces of infected geese; ** – P < 0.01; *** – P < 0.001 – compared to values of Eu.

The obtained data can be used in species identification of *T. tenuis* nematodes by females and eggs, taking the sampled substrate into account.

Discussion

The nematodes, similarly to insects, are one of the most numerous and widely spread groups of multicellular organisms that have mastered a huge range of habitats and are at the stage of rapid biological progress, many of which lead a parasitic lifestyle. The reason for this lies in the peculiarities of their morphology and biology among other things. This is also the basis for the taxonomic characterization of parasites (Kennedy & Harnett, 2013; Carlson et al., 2017). Therefore, the study of the morphological features of parasitic nematodes, the expansion and revision of already existing data are an urgent area of research.

We have conducted a morphometric analysis of adult and embryonic stages of development of *Trichostrongylus tenuis* Mehlin, 1846 nematodes which parasitize in birds. Most of the studies indicate that the species differentiation of nematodes of the order Strongylida is most effective on the basis of the morphological specifics of males. Their tail end is equipped with a characteristic caudal cuticular bursa, the species features of which are described in detail in a number of works. The structure of spicules, gubernaculum and other reproductive structures is also taken into account (Cram & Wehr, 1934; Jacquiet et al., 1997). Thus, the ratio of the distance between individual rays of the genital bursa in male *T. tenuis* is a differential species parameter. Moreover, this feature can vary depending on the type of host in which the *Trichostrongylus* roundworms parasitize. The ratio of the distance between third and fourth and second and third rays of the caudal bursa is 1:2.7 (in the range of 1:2.2 – 1:3.5) in *T. tenuis* isolated from the red grouse (*Lagopus scoticus*), 1:3.1 (in the range of 1:2.3 – 1:3.8) in roundworms obtained from the grey partridge (*Perdix perdix*) and 1:2.9 (in the range of 1:2.2 – 1:3.9) in those collected from the common peacock (*Pavo cristatus*) (Durette-Desset et al., 1993). However, it is not always possible to identify male nematodes, hence the necessity to differentiate the females or eggs of parasites.

It was shown that the morphology of *T. tenuis* females has a similar structure as in other strongylids. The mouth opening is surrounded by three weakly expressed lips, there are no oral capsule or other specific formations at the head end. Therefore, we suggest taking into account 22 morphometric parameters that characterize the size of the esophagus and body in its various parts (10 parameters), the location of the anus and excretory pore (2 parameters), the size of the ovijector, sphincter, ejector, vulva and its location on the body of the parasite (7 parameters). Scientific works have already pointed to the copulatory apparatus of females, since its morphological and metric parameters are of great importance in species identification (Ederli & Oliveira, 2014; Sukee et al., 2018). According to the data of Skriabyn et al. (1954), it was proposed to take into account 7 parameters that characterize the total length, width at the vulva area, the location of the nerve ring, vulva, and anus, the size of the vulva slit, as well as the ovijector in the metric identification of *T. tenuis* females. And Ryzhikov (1967) suggests using only three of metric parameters, which also characterize the total length of body, its width in the vulva area and the location of the vulva. Other taxonomic features proposed to be used in the identification of Trichostrongyloidea are the specifics of the morphological structure and location of the lateral cuticular crests (wings, outgrowths) on the body of the parasite. Based on these features, keys have been proposed that increase the efficiency of differentiation of this group of nematodes (Lee, 1965; Hoberg & Lichtenfels, 1994; Lichtenfels et al., 1996; Yevstafieva et al., 2019). Studies have shown that the lateral cuticular crests on the entire body in *T. tenuis* females have different sizes in terms of height, width, and location relative to each other. Thus, lateral crests appear and end at a distance of 0.1 mm from the head and tail ends, respectively; their total length is 9.3 mm. The height and width of the crests gradually increase from the head end to the middle of the body, where they reach their maximum values. Towards the tail end, the crests gradually become shorter and narrower. When studying the location of crests relative to each other, it can be noted that the distance between crests increases in the direction from the head end to the tail, and the crests are rarest in the area of transition of esophagus to the intestine. From the middle of the body posteriorly, the distance between crests decreases sharply, almost 4 times, and they are located most densely in the anus area. Studies by other authors have shown that the lateral wings on the anterior part of the body of *T. tenuis* nematode are flattened, and closer to the posterior part of the body they become larger and change from a morphological point of view. The authors found that the morphology of lateral wings varies in the shapes of crests, hooks and spines. In their opinion, such sharp differences in the structure of cuticular processes characterize the adaptation of the parasite to fixation in the thick mucous membrane of the bird's intestine (Rzayev et al., 2020).

We also propose to identify *T. tenuis* by eggs isolated from various substrates. Notably, there are significant differences by 5 parameters (length and width of egg, eggshell thickness, and area of the outer and inner egg surfaces). Thus, the eggs located in the uterine cavity of the female nematode turned out to be longer ($P < 0.001$), narrower ($P < 0.01$), and with a thinner shell ($P < 0.001$) as compared to the eggs isolated from the bird feces. At the same time, area of the inner surface was larger ($P < 0.001$) in the eggs located in the uterine cavity, and the outer surface area was smaller ($P < 0.01$) in comparison with the analogous parameters in eggs obtained from the bird feces. We did not find such comparative data of the morphometric parameters of *T. tenuis* eggs depending on the method of isolation in the previous publications.

The obtained data on the morphological and metric parameters of females and eggs of *T. tenuis* nematodes can be used in species identification and clarifying the taxonomic position of this species.

Conclusion

The parasitic female nematodes of the species *Trichostrongylus tenuis* Mehlin, 1846, isolated from domestic goose, have a morphological structure characteristic of helminths of the superfamily Trichostrongyloidea. Typical species characters include features in the structure of the copulatory apparatus and tail end. To facilitate the differentiation of *T. tenuis* females, it is proposed to use additional morphometric parameters, 10 of which characterize the size of the esophagus and body at various areas, 2 characterize the location of the anus and excretory pores, and 7 features describe the size of the ovijector, sphincter, ejector, vulva and its location on the parasite's body. The specifics of location and metric parameters of the lateral cuticular crests at bodies of *T. tenuis* females can be used for their identification. Three parameters were identified that characterize the location of the crests, namely: the distance from the head and tail ends to crests, the total length of the nematode body bearing crests. It is proposed to take into account the height and width of the lateral crests, and the distance between them in 6 different parts of body (head end, the middle of esophagus, the transition of the esophagus to the intestine, the middle of the body, and areas of vulva and anus). The highest and widest crests are observed at the middle part of body, the shortest crests are near the anus, and the narrowest crests are at the anterior part of the parasite's body. The distance between crests reaches its maximum in the area of transition of the esophagus to the intestine, and the parameter is reduced towards the caudal end. It was found that *T. tenuis* eggs have 5 differential morphometric parameters, which differ significantly in eggs isolated from the feces of infected birds and those located in the uterine cavity of female roundworms.

References

Afolabi, O. J., Simon-Oke, I. A., & Olasunkanmi, A. O. (2016). Intestinal parasites of domestic chicken (*Gallus gallus domesticus*) in Akure, Nigeria. Journal of Biomedical Science, 1(4), e9771.

Andrade, J. G., Kumsa, B., Ayana, D., Vieira, R., Santos, C. P., Iñiguez, A. M., & DaMatta, R. A. (2018). First record of the nematode *Libyostrongylus dentatus* Hoberg, Lloyd & Omar, 1995 (Trichostrongylidae) in ostriches (*Struthio camelus* Linnaeus, 1758) (Struthionidae) outside the Americas. Parasites and Vectors, 11(1), 243.

Araújo, A., Jansen, A. M., Bouchet, F., Reinhard, K., & Ferreira, L. F. (2003). Parasitism, the diversity of life, and paleoparasitology. Memorias do Instituto Oswaldo Cruz, 98(1), 5–11.

Atkinson, C. T., Thomas, N. J., & Hunter, D. B. (2008). Parasitic diseases of wild birds. Wiley-Blackwell, Ames, Iowa.

Bonadiman, S. F., Ederli, N. B., Soares, A. K., de Moraes Neto, A. H., Santos, C., & DaMatta, R. A. (2006). Occurrence of *Libyostrongylus* sp. (Nematoda) in ostriches (*Struthio camelus* Linnaeus, 1758) from the north region of the state of Rio de Janeiro. Brazilian Journal of Veterinary Parasitology, 137, 175–179.

Boyko, A. A., & Brygadyrenko, V. V. (2016). Influence of water infusion of medicinal plants on larvae of *Strongyloides papillosus* (Nematoda, Strongyloididae). Visnyk of Dnipropetrovsk University, Biology, Ecology, 24(2), 519–525.

Boyko, O. O., & Brygadyrenko, V. V. (2019). The impact of acids approved for use in foods on the vitality of Haemonchus contortus and Strongyloides papillosus (Nematoda) larvae. Helminthologia, 56(3), 202–210.

Carlson, C. J., Burgio, K. R., Dougherty, E. R., Phillips, A. J., Bueno, V. M., Clements, C. F., Castaldo, G., Dallas, T. A., Cizauskas, C. A., Cumming, G. S., Doña, J., Harris, N. C., Jovani, R., Mironov, S., Muellerklein, O. C., Proctor, H. C., & Getz, W. M. (2017). Parasite biodiversity faces extinction and redistribution in a changing climate. Science Advances, 3(9), e1602422.

Cervantes-Rivera, K., Villagómez-Cortés, J. A., Arroyo-Lara, A., & Landín-Grandvallet, L. A. (2016). A diagnostic survey of gastroenteric helminths in backyard poultry of a rural village in Mexican tropics. Journal of Agricultural and Biological Science, 11(12), 463–469.

Connan, R. M., & Wise, D. R. (1993). Development and survival at low temperature of the free living stages of *Trichostrongylus tenuis*. Research in Veterinary Science, 55(1), 20–24.

Cram, E., & Cuvillier, E. (1934). Observations on *Trichostrongylus tenuis* infestation in domestic and game birds in the United States. Parasitology, 26(3), 340–345.

Cram, E., & Wehr, E. (1934). The status of species of *Trichostrongylus* of birds. Parasitology, 26(3), 335–339.

Davidson, W. R., Kellogg, F. E., Doster, G. L., & Moore, C. T. (1991). Ecology of helminth parasitism in bobwhites from northern Florida. Journal of Wildlife Diseases, 27(2), 185–205.

De Jong, Y., Verbeek, M., Michelsen, V., Bjørn, P., de P., Los, W., Steeman, F., Bailly, N., Basire, C., Chylarecki, P., Stloukal, E., Hagedorn, G., Wetzel, F. T., Glöckler, F., Kroupa, A., Korb, G., Hoffmann, A., Häuser, C., Kohlbecker, A., Müller, A., Güntsch, A., Stoev, P., & Penev, L. (2014) Fauna Europaea – all European animal species on the web. Biodiversity Data Journal, 2, e4034.

Durette-Desset, M. C. (1985). Trichostrongyloid nematodes and their vertebrate hosts: Reconstruction of the phylogeny of a parasitic group. Advances in Parasitology, 24, 239–306.

Durette-Desset, M. C. (1992). Phylogeny of Trichostrongyloidea nematodes as seen through some of their vertebrate hosts. Parassitologia, 34, 1–16.

Durette-Desset, M. C., Chabaud, A. G., & Moore, J. (1993). *Trichostrongylus cramae* n. sp. (Nematoda), a parasite of bob-white quail (*Colinus virginianus*). Annales de Parasitologie, 68(1), 43–48.

Ederli, N. B., & Oliveira, F. C. (2014). Comparative morphology of the species of *Libyostrongylus* and *Codiostomum*, parasites from ostriches, *Struthio camelus*, with a identification key to the species. Revista Brasileira de Parasitologia Veterinaria, 23(3), 291–300.

Enigk, K., & Dey-Hazra, A. (1971). Propagation and treatment of the *Trichostrongylus tenuis* infestations. Berliner und Munchener tierarztliche Wochenschrift, 84(1), 11–14.

Ewald, P. W. (1995). The evolution of virulence: A unifying link between parasitology and ecology. Journal of Parasitology, 81(5), 659–669.

Giannetto, S. (2006). Biomorphology of gastrointestinal nematodes of small ruminants. Parassitologia, 48(3), 391–395.

Gibbons, L. M., & Khalil, L. F. (1982). A key for the identification of genera of the nematode family Trichostrongylidae Leiper, 1912. Journal of Helminthology, 56(3), 185–233.

Hoberg, E. P. (1996). Emended description of *Mazamastrongylus peruvianus* (Nematoda: Trichostrongylidae), with comments on the relationships of the genera *Mazamastrongylus* and *Spiculopteragia*. Journal of Parasitology, 82(3), 470–477.

Hoberg, E. P., & Lichtenfels, J. R. (1994). Phylogenetic systematic analysis of the Trichostrongylidae (Nematoda), with an initial assessment of coevolution and biogeography. Journal of Parasitology, 80(6), 976–996.

Hudson, P. J. (1986). The effect of a parasite nematode on the breeding production of red grouse. Journal of Animal Ecology, 55, 85–94.

Irungu, L. W., Kimani, R. N., & Kisia, S. M. (2004). Helminth parasites in the intestinal tract of indigenous poultry in parts of Kenya. Journal of the South African Veterinary Association, 75(1), 58–59.

Jacquiet, P., Cabaret, J., Cheikh, D., & Thiam, E. (1997). Identification of *Haemonchus* species in domestic ruminants based on morphometrics of spicules. Parasitology Research, 83(1), 82–86.

Kennedy, M. M., & Harnett, W. (2013). Parasitic nematodes: Molecular biology, biochemistry and immunology. CABI, United Kingdom.

Lee, D. L. (1965). The cuticle of adult *Nippostrongylus brasiliensis*. Parasitology, 55, 173–181.

Lichtenfels, J. R., & Pilitt, P. A. (2000). Synlophe patterns of the Haemonchinae of ruminants (Nematoda: Trichostrongyloidea). Journal of Parasitology, 86(5), 1093–1098.

Lichtenfels, J. R., Hoberg, E. P., Pilitt, P. A., & Craig, T. M. (1996). The synlophe and other structural characteristics of *Sarwaria bubalis* (Nematoda: Trichostrongyloidea) from cattle in Guyana. Journal of Parasitology, 82(1), 146–154.

Mukaratirwa, S., Cindzi, Z. M., & Maononga, D. B. (2004). Prevalence of *Libyostrongylus douglassii* in commercially reared ostriches in the highveld region of Zimbabwe. Journal of Helminthology, 78(4), 333–336.

O'connor, L. J., Walkden-Brown, S. W., & Kahn, L. P. (2006). Ecology of the free-living stages of major trichostrongylid parasites of sheep. Veterinary Parasitology, 142, 1–15.

Pesenti, T. C., Gallina, T., Langone, P. Q., Silva, M. A., Suárez, A., Silva, D. S., & Müller, G. (2015). Infecção por nematóides em *Struthio camelus* (Linnaeus, 1758) (avestruz) no sul do Brasil. Science of Animal Health, 3(1), 103–112.

Ryzhikov, K. M. (1967). Opredelitel' gel'mintov domashnih vodoplavajushhih ptic [Key to the helminths of domestic water birds]. Nauka, Moscow (in Russian).

Rzayev, F. H., Seyidbeyli, M. I., Maharramov, S. H., & Gasimov, E. K. (2020). Formy i ul'trastrukturnye osobennosti lateral'nyh kryl'ev gel'minta *Trichostrongylus tenuis* Mehlis, 1846 (Nematoda: Trichostrongylidae) [Forms and ultrastructural features of the lateral alae of the helminth *Trichostrongylus tenuis* Mehlis, 1846 (Nematoda: Trichostrongylidae)]. Journal of V. N. Karazin Kharkiv National University, 34, 112–119 (in Russian).

Shaw, J. L., & Moss, R. (1990). Effects of the caecal nematode *Trichostrongylus tenuis* on egg-laying by captive red grouse. Research in Veterinary Science, 48(1), 59–63.

Skrjabin, K. I. (1928). Metod polnyh gel'mintologicheskih vskrytij pozvonochnyh, vkljuchaja cheloveka [The method of complete helminthological autopsy of vertebrates, including humans]. Moscow State University, Moscow (in Russian).

Skrjabin, K. I., Shikhobalova, N. P., & Shults, R. S. (1954). Osnovy nematodologii. Trihostrongilidy zhivotnyh i cheloveka [Essentials of nematology. Trichostrongylids of animals and man]. Nauka, Moscow (in Russian).

Souza, R. P., Souza, J. N., Menezes, J. F., Alcântara, L. M., Soares, N. M., & Aquino Teixeira, M. C. (2013). Human infection by *Trichostrongylus* spp. in residents of urban areas of Salvador city, Bahia, Brazil. Biomedica, 33(3), 439–445.

Sudhaus, W., & Fitch, D. (2001). Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). Journal of Nematology, 33(1), 1–70.

Sukee, T., Beveridge, I., & Jabbar, A. (2018). Molecular and morphological characterisation of *Pharyngostrongylus kappa* Mawson, 1965 (Nematoda: Strongylida) from Australian macropodid marsupials with the description of a new species, *P. patriciae* n. sp. Parasites and Vectors, 11(1), 271.

Tuggle, B. N., & Crites, J. L. (1984). The prevalence and pathogenicity of gizzard nematodes of the genera *Amidostomum* and *Epomidiostomum* (Trichostrongylidae) in the lesser snow goose (*Chen caerulescens caerulescens*). Canadian Journal of Zoology, 62(9), 1849–1852.

Wascher, C. A. F., Bauer, A. C., Holtmann, A. R., & Kotrschal, K. (2012). Environmental and social factors affecting the excretion of intestinal parasite eggs in graylag geese. Behavioral Ecology, 23(6), 1276–1283.

Woog, F., Maierhofer, J., & Haag, H. (2011). Endoparasites in the annual cycle of feral greylags *Anser anser*. Wildfowl, 61, 164–179.

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. (2019). Morphological and biological characteristics of *Amidostomum anseris* (Nematoda, Amidostomatidae) from *Anser anser domesticus*. Vestnik Zoologii, 53(1), 65–74.

Yevstafieva, V., & Starodub, Y. (2020). Poshyrennja tryhostrongil'ozu gusej na terytoriji Poltavs'koji oblasti [Distribution of trichostrongylosis of geese on the territory of Poltava region]. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 97, 125–129 (in Ukrainian).

Yoshino, T., Uemura, J., Endoh, D., Kaneko, M., Osa, Y., & Asakawa, M. (2009). Parasitic nematodes of anseriform birds in Hokkaido, Japan. Helminthologia, 46(2), 117–122.

Zelmer, D. A. (1998). An evolutionary definition of parasitism. International Journal for Parasitology, 28(3), 531–533.