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Identification of nematodes *Trichostrongylus colubriformis* (Nematoda, Trichostrongylidae) using morphological and metric characters

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The genus *Trichostrongylus* Looss, 1905 (Nematoda: Trichostrongylidae) includes gastrointestinal parasitic nematodes with a direct life cycle, which are recorded worldwide. Helminths affect animals and also have zoonotic potential. *Trichostrongylus colubriformis* (Giles, 1892) Ransom, 1911 can infect humans. Therefore, accurate identification of trichostrongylid species is one of the most important areas of research and is based on a detailed description of morphological characters and metric parameters of adult nematodes. The objective of the research was to describe the morphometric identification characters of male and female nematodes of the species *T. colubriformis* isolated from sheep in Ukraine, and to compare other published data with our own results. Differential morphological keys for male *T. colubriformis* include the structural features of the caudal bursa, the presence of boat-shaped, unequal-sized spicules with a root and triangular process at the ends and wings, as well as the wavy shape of the gubernaculum. In females, the specific features include the structural features of the tail end, and the vulva and its location. Eighteen morphometric parameters were determined in males, and 13 in females, of which seven and nine parameters, respectively, characterize the general structure of the body such as length and width in different areas, the location of the excretory opening and caudal bursa in males, and the anal opening and the length of the tail end in females. Eleven metric parameters of the male reproductive system were described, including the length of the caudal bursa, the size of the spicules and their morphological components, and the gubernaculum. In females, four metric parameters were described, which characterize the length of the vulva slit, its location, and the length of the ovejector with sphincters. In addition, the sizes of the eggs located in the uterine cavity were determined. The obtained results expand and deepen the existing knowledge about the taxonomic features of both males and females of *T. colubriformis* nematode.

Keywords: Trichostrongyloidea; zoonotic parasites; species differentiation; diagnostics; sheep.

Introduction

Parasitic nematodes of ruminants are among the commonest pathogens of invasive diseases in various parts of the world. Some—for instance—representatives of the family Trichostrongylidae, parasitize not only ruminants but can also infect humans, especially in rural and tribal communities with poor hygiene and limited access to health services. One of such harmful species is *Trichostrongylus colubriformis* (Lattes et al., 2011; Wall et al., 2011; Bhat et al., 2023).

In particular, our meta-analysis of the prevalence of *Trichostrongylus* among ruminants and humans revealed the average infection rates of 5% in humans, 25% in sheep, and 18% in cattle. Moreover, according to the continental location of the territories, the highest rates of human infection were observed in Asia (9.19%), and the lowest in North America (0.19%). Peak values of sheep and cattle infection rates were observed in Oceania (74.27% and 80.89%, respectively), and the lowest in Asia (19.20%) and North America (4.75%), respectively. At the country level, the infection rates were the highest in humans in Korea (16.96%), in sheep in the Czech Republic (87.50%), and in cattle in Austria (100%) (Wei et al., 2026). Other studies have found the prevalence of *Trichostrongylus* to be 44.8% in sheep, 31.2% in goats, 27.4% in cattle, and 10% in humans (Abbas & Hildreth, 2022; Rahimi-Esboei et al., 2022). In Ukraine, on farms of Kyiv, Poltava, and Zaporizhia regions, *T. colubriformis* was detected in 57.4% of sheep (Melnychuk et al., 2020).

One of the factors affecting the reliability of trichostrongylid detection is the widespread use of intravital traditional laboratory methods of coproovoscopy, which are not always highly sensitive, especially at low values of infection intensity and the presence of immature parasites in the body of definitive hosts (Bott et al., 2009; Roeber et al., 2012; Pandi et al., 2021). However, the detection of strongylid-type eggs by intravital methods does not allow for reliable distinction between individual genera and species, since their morphological structure is uniform and similar among species (except for nematodes of the genus *Nematodirus*) (Georgi & McCulloch, 1989; Höglund et al., 2013).

Also, for the genus and species identification of gastrointestinal strongylids, eggs isolated by coproovoscopic examination can be cultivated to third-stage larvae (L_3), which are differentiated by morphometric characteristics (McMurtry et al., 2000; von Samson-Himmestjerna et al., 2002). However, this process is long, laborious, has certain limitations associated with the creation of natural conditions for hatching and development of larvae of certain strongylids, as well as ambiguous taxonomic delimitation (van Wyk et al., 2004; Reslova et al., 2021).

More accurate species identification of *Trichostrongylus* in animals and humans is based on morphometric study of adult nematodes, which are found in the place of their localization (Gasser, 2001; Phosuk et al., 2013). Most researchers report that the main indicators by which trichostrongylids are identified are the general body structure of males and females (size, color, shape); features of the structure of the head and tail ends; and morphometric characteristics of the gubernaculum and spicules in males, which take into account aspects such as shape, size, and symmetry (Rojas et al., 2011; Shahbazi et al., 2012). There are reports of identification of *T. colubriformis* nematodes isolated from humans according to the morphological features of the genital bursa, spicules, and gubernaculum in males. In particular, the spicules in males of this species are of unequal length, have a

boat-shaped structure with a thick process covering the proximal end, and the gubernaculum is wavy with two bends when viewed laterally (Ashrafi et al., 2020). Other scientists suggest using data on the location of synlophe at the anterior, middle, and posterior levels of the body in male and female parasites, namely: the number and shape of the ridges and their location at different parts of the nematode body. Moreover, these features can be used for species identification of both males and females (Durette-Desset, 1978; Falcón-Ordaz & Sanabria, 1996; Falcón-Ordaz & García-Prieto, 2014). Therefore, a more detailed study of the differential traits of the nematode *T. colubriformis* is relevant, taking into account the features of their morphological structure and metric indicators.

The objective of our research was to describe the morphometric identification features of male and female *T. colubriformis* nematodes isolated from sheep in Ukraine, and to compare our findings with the data published by other scientists.

Materials and methods

The work was carried out in the Laboratory of Parasitology and Veterinary and Sanitary Examination of the Poltava State Agrarian University (Ukraine) during 2023–2026.

Helminths were collected during the necropsy from small intestines of 58 sheep from slaughterhouses in Poltava region (Ukraine). After the helminths were isolated from the intestines, they were washed in distilled water, then fixed with 70% ethyl alcohol. Species identification of male and female *T. colubriformis* nematodes was carried out using the taxonomic keys (Skrjabin et al., 1952; Skrjabin et al., 1954; Ivashkin et al., 1998).

Morphometric parameters of *T. colubriformis* nematodes (n = 10) were studied using the ToupView program version × 64, 4.10.17015. 20200426 (Hangzhou ToupTek Photonics Co., Ltd, China). Microphotography was performed using a Sigeta M3Cmos 14000 14.0 MP digital camera (China).

Statistical processing of the experimental results was carried out using Statistica 10 (StatSoft Inc., USA) software. Standard deviation (SD) and average values (x) were calculated.

Results

Morphologically, *T. colubriformis* male (Fig. 1a) and female (Fig. 1b) nematodes are small, thin, and translucent. The average length of males is 7.1 ± 0.5 mm, and that of females is 7.5 ± 0.9 mm. The head end is thin, the mouth opening is weakly expressed, surrounded by small rudimentary lips. The head vesicle and cervical papillae are absent, and the excretory opening is well expressed (Fig. 2).

In males, the caudal bursa is characterized by a thin (compared with the other rays) anteroventral ray, and the posterolateral ray is smaller than the other rays and covered by the external dorsal ray. The end of the posterolateral ray is curved. The dorsal ray is split near the end, and each of the split branches bifurcates (Fig. 3).

The spicules have a specific, characteristic structure. They are of unequal length: The left one is longer (155.7 ± 8.1 μm) than the right one (149.3 ± 9.4 μm). They have the shape of a curved boat with a root process at the proximal end and a triangular process at the distal end, which is clearly visible in the lateral position (Fig. 4a). When the spicules are removed, transparent wings are clearly visible (Fig. 4b). There is a gubernaculum, which has a wavy shape with two bends (Fig. 4a).

When studying the metric parameters of *T. colubriformis* males, 18 indicators were determined, of which seven describe the general structure of the body. The other 11 indicators characterize the metric parameters and structure of the reproductive system, such as the length of the caudal bursa, the size of the spicules and their morphological components of the gubernaculum (Table 1).

Other researchers who studied this parasite determined from one to six indicators characterizing the general structure of the nematodes, namely the length of the body, the width of the body at the base of the caudal bursa, as well as metric parameters of the male genital organs (the length of the genital bursa, the size of the spicules, their morphological structures, and the length of the gubernaculum).

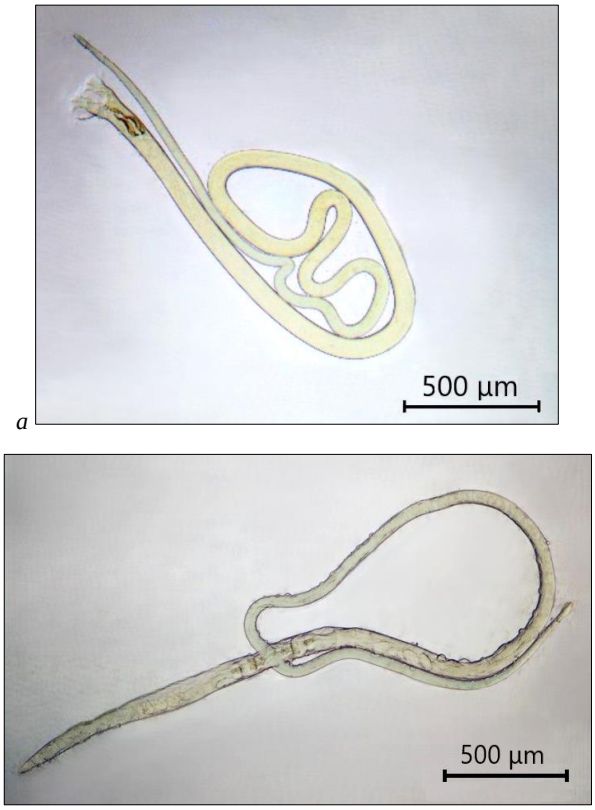

Fig. 1. *Trichostrongylus colubriformis*: *a* – male, *b* – female

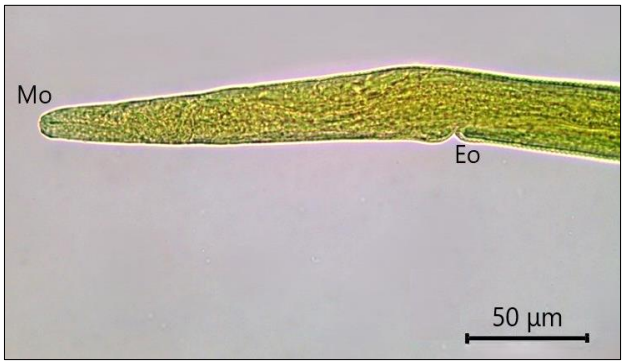

Fig. 2. Head end of *Trichostrongylus colubriformis*: mouth opening (*Mo*), excretory opening (*Eo*)

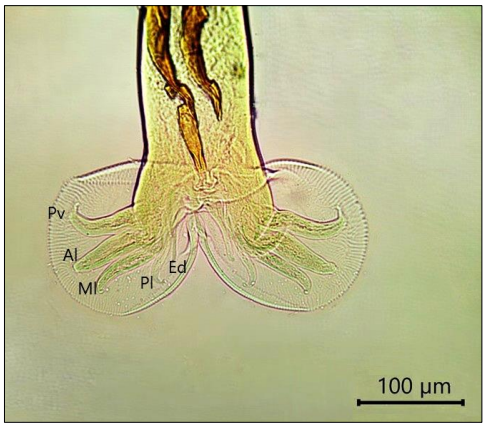

Fig. 3. Caudal bursa of male *Trichostrongylus colubriformis*: posteroventral ray (*Pv*), anterolateral ray (*Al*), mediolateral ray (*Ml*), posterolateral ray (*Pl*), external dorsal ray (*Ed*)

a *b*

Fig. 4. Male *Trichostrongylus colubriformis*: *a* – spicules and gubernaculum; *b* – spicule, lateral view; right spicule (*Sr*), left spicule (*Sl*), root process (*Pr*), triangular process (*Pt*), gubernaculum (*G*), wings (*W*)

Table 1
Morphometrical measurements of the males *Trichostrongylus colubriformis* (n = 10, x ± SD, min – max)

Parameters	Present specimens	Giles, 1892	Ransom, 1911*	Lane, 1913	Hall, 1916**	Travassos, 1921	Skrjabin et al., 1954	Levine, 1980	Ivashkin et al., 1989	Ghasemikhah et al., 2011	Rojas et al., 2011	Sato et al., 2011	González Garduño et al., 2014	Ahmed et al., 2023	Dini et al., 2023	Arbabi et al., 2023
Length of body, mm	7.1 ± 0.5 5.8–7.6	6	4–5.5	7 1–7.5	4–4.6	4–5.5	4–6	4.3–7.7	4–6	4.6–5.2		3.6 2.8–5	5.3	5.8	4.6 3.6–5.2	–
Width of body at the excretory opening, µm	28.7 ± 3.6 20.1–33.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of body at the middle, µm	94.7 ± 18.0 69.2–121.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of body at the base of tail bursa, µm	111.6 ± 12.4 89.1–127.8	113	80	–	105	80	78–95	–	80–95	89–112	–	–	–	1060.61	–	–
Distance from head end to the excretory opening, µm	162.8 ± 12.6 141.6–182.7	–	–	–	140	–	–	–	–	163–172	–	–	–	–	–	–
Distance from head end to the base of tail bursa, mm	6.9 ± 0.5 5.7–7.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Distance from the excretory opening to the base of tail bursa, mm	6.7 ± 0.5 5.5–7.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length of tail bursa, µm	153.4 ± 26.0 112.0–194.5	–	–	–	–	–	–	–	–	–	–	–	–	1970.21	–	–
Length of right spicule, µm	149.3 ± 9.4 140.3–171.4	–	135–140●	–	140–155●	135–145●	118–145●	123–154	120–145●	120–148●	151●	123.5	140.41	1410.06●	134 123–140	127.5●
Length of root process of the right spicule, µm	18.2 ± 2.9 12.9–22.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of right spicule in the middle, µm	22.6 ± 2.3 19.9–27.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length of triangular process of the right spicule, µm	38.3 ± 2.4 35.5–41.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length of left spicule, µm	155.7 ± 8.1 145.1–167.4		135–140●		140–155●	135–145●	118–145●	136–170	120–145●	120–148●	151●	132	144.45	1410.06●	131.7 123–141	127.5●
Length of root process of the left spicule, µm	16.3 ± 3.0 11.1–20.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of left spicule in the middle, µm	21.3 ± 0.8 20.1–22.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Length of triangular process of the left spicule, µm	39.5 ± 3.1 36.1–46.0	–	–	–	–	–	33–44	–	30–40	–	–	–	–	–	–	–
Length of gubernaculum, µm	81.6 ± 2.2 78.6–85.5	–	70	–	70–80	70	65–78		65–80	64–76	–	–	–	810.32	66.5 56–73	81.0
Width of gubernaculum in the middle in the ventral position, µm	17.2 ± 1.7 14.2–20.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Note: * – the species was described as *Trichostrongylus instabilis*, synonym to *T. colubriformis*; ** the species was described as *Trichostrongylus delicatus*, synonym to *T. colubriformis*; ● the length of spicule is given without differentiation of the left and the right one; "–" parameters were not defined.

Most authors indicate the general size of the spicules in males without differentiating them into right and left. In addition to these indicators, we established the following parameters: the width of the male body in the area of the excretory opening (28.7 ± 3.6 µm) and in its middle part (94.7 ± 18.0 µm), the distance from the head end and excretory opening to the base of the caudal bursa (6.9 ± 0.5 and 6.7 ± 0.5 µm, respectively), the width of the right and left spicules in their middle part (22.6 ± 2.3 and 21.3 ± 0.8 µm, respectively), the length of

the root process of the right and left spicules (18.2 ± 2.9 and 16.3 ± 3.0 μm, respectively), the length of the triangular process of the right spicule (38.3 ± 2.4 μm), and the width of the gubernaculum in the middle part (17.2 ± 1.7 μm).

Morphologically, in females of *T. colubriformis*, the body is slightly tapered near the anus, and closer to the tail end it tapers even more to a thin end. The tail end is slightly oriented dorsally (Fig. 5). The vulva is located in the posterior part of the body, has the appearance of a slit with two convex lips. There are weakly expressed cuticular papillae along its edges (Fig. 6a, 6b). The vagina is oval in shape. The nematode eggs that were in the uterine cavity of the female *T. colubriformis* had the shape of a strongylid type (Fig. 7).

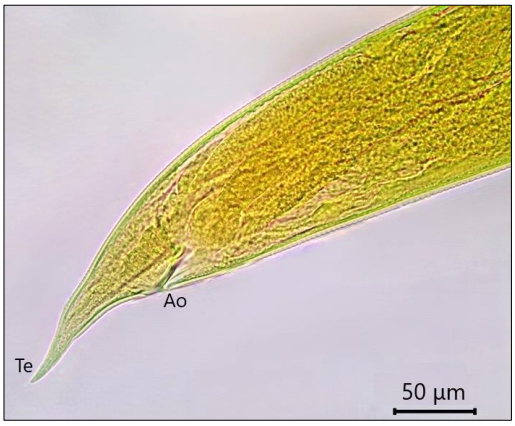

Fig. 5. Tail end of *Trichostrongylus colubriformis* female: anal opening (*Ao*), tail end (*Te*)

When studying the metric parameters of *T. colubriformis* females, 13 indicators were determined. Nine of those describe the general structure of the body, namely the total body length and width in different parts of the body, the location of the excretory opening, the anal opening, and the length of the tail end. The other four indicators characterize the metric parameters, the structure and location of the organs of the reproductive system – the length of the vulva slit, its location, and the length of the ovejector with sphincters (Table 2).

Other researchers who studied female nematodes of this species determined from one to six features that characterize the general structure of the nematodes, including the length of body, the width of the body in the vulva area, the length of the tail end, as well as the metric parameters of the genital organs and their location, the distance from the tail end to the vulva, the length of the slit of the vulva, and the length of the ovejector with sphincters. In addition to these parameters, we established the following indicators: the width of the body in the area of the excretory opening (8.8 ± 1.2 μm), width in the middle of the body (55.6 ± 4.4 μm) and at the anal opening (29.4 ± 6.7 μm), the distance from the head end to the vulva (6.0 ± 0.7 μm) and the anal opening (7.4 ± 0.9 μm), and the distance from the excretory opening to the anal opening (7.2 ± 0.9 μm). The length and width of eggs found in female nematodes accounted for 81.2 ± 4.8 μm and 41.7 ± 3.5 μm, respectively.

Discussion

The family Trichostrongylidae consists of a significant number of gastrointestinal nematodes distributed worldwide, including in Ukraine (Melnychuk et al., 2020; Rahimi-Esboei et al., 2022; Latukhin & Kruchynenko, 2025). The interest of scientists in this group of parasites is also due to the fact that some species of trichostrongylids, such as *T. colubriformis*, have zoonotic potential (Wall et al., 2011; Bhat et al., 2023). At the same time, these nematodes are among the most difficult species for morphological differentiation, since individual species are similar to each other, and females have a certain variability in morphological structures (Skrjabin et al., 1954; Arbabi et al., 2023). Therefore, for the first time in Ukraine, we conducted a thorough study of the morphometric characteristics of male and female *T. colubriformis* nematodes isolated from sheep, and compared the published data with our own.

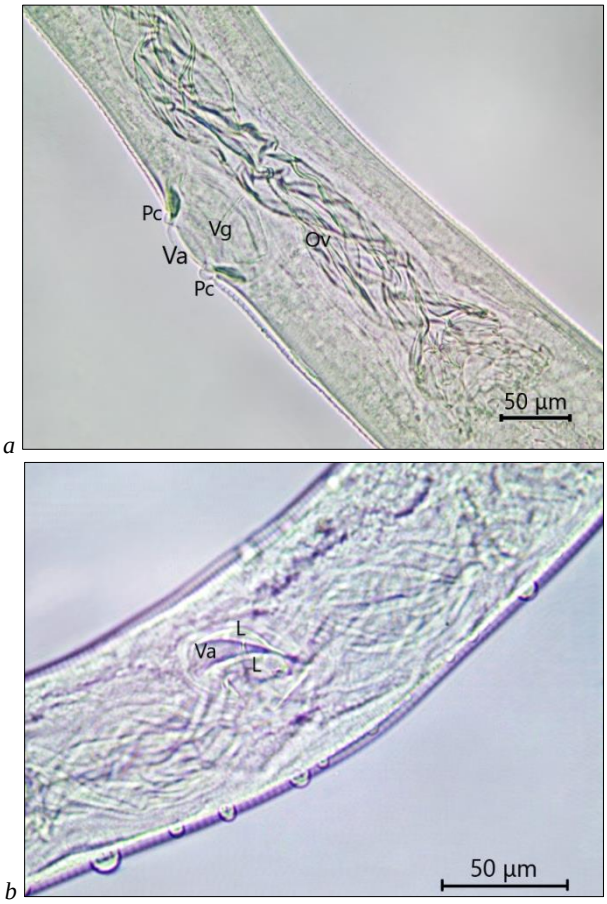

Fig. 6. *Trichostrongylus colubriformis* female: *a* – lateral view; *b* – dorsal view; area of vulva (*Va*), cuticular papillae (*Pc*), lips (*L*), ovejector (*Ov*), vagina (*Vg*)

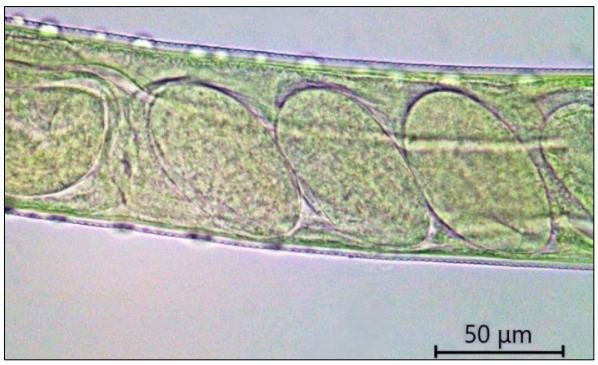

Fig. 7. Eggs in the uterine cavity of *Trichostrongylus colubriformis* female

Morphological studies of *T. colubriformis* males have revealed species-specific characters, including the structure of the spicules, which have the shape of a curved boat with a root process at the proximal end and a triangular process at the distal end. We are the first in Ukraine to discover the presence of transparent wings located along the edges of the spicules, which become noticeable when they are removed from the male body. Also, during identification, it is necessary to take into account the structure of the gubernaculum, which has a wavy shape with two bends, as well as the structure of the genital bursa. Most researchers also recommend taking into account the structure of the spicules, gubernaculum, and genital bursa during morphological identification of *T. colubriformis* (Skrjabin et al., 1952; Skrjabin et al., 1954; Ivashkin et al., 1998; Anderson, 2000; Ghasemikhah et al., 2011).

Table 2
Morphometrical measurements of the females *Trichostrongylus colubriformis* (n = 10, x ± SD, min – max)

Parameters	Present specimens	Giles, 1892	Ransom, 1911*	Lane, 1913	Hall, 1916**	Travassos, 1921	Skrjabin et al., 1954	Levine, 1980	Ivashkin et al., 1989	Sato et al., 2011	*Lattes* et al., 2011	Phosuk et al., 2013	González Garduño et al., 2014	Mahmood et al., 2019	Dini et al., 2023
Length of body, mm	7.5 ± 0.9 6.2–9.0	8	5–6	7.2 6.2–8.4	6	5–6	5–6	5–8.6	5–6	3.7 2.7–4.5	–	–	5.4	–	5.6 4.9–6.2
Width of body at the excretory opening, µm	8.8 ± 1.2 7.2–10.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of body in the middle, µm	55.6 ± 4.4 49.3–62.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width of body at vulva area, µm	102.7 ± 7.2 94.4–118.7	–	–	–	103	80	80–100	–	80–100	–	–	–	–	–	–
Width of body at anal opening, µm	29.4 ± 6.7 15.3–36.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Distance from head end to the excretory opening, µm	60.6 ± 9.4 45.8–73.7	–	–	–	95–125	–	92–129	–	–	–	–	–	–	–	–
Distance from head end to vulva, mm	6.0 ± 0.7 4.8–7.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Distance from head end to the anal opening, mm	7.4 ± 0.9 6.1–8.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Distance from the excretory opening to anal opening, mm	7.2 ± 0.9 6.0–8.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Distance from the anal opening to vulva, mm	1.5 ± 0.2 1.1–1.7	–	1.05–1.2	–	1.22–1.245	–	–	–	–	–	–	–	1.25	–	–
Length of ovejector with sphincters, µm	495.1 ± 65.1 418.2–589.9	–	400–500	–	490	500–600	373–500	–	–	–	–	–	–	–	–
Length of slit of the vulva, µm	51.3 ± 5.4 43.1–60.5	–	50–55	–	85	50–55	50–55	–	–	–	–	–	–	–	–
Length of tail end, µm	78.4 ± 10.7 60.3–94.9	–	55–70	75–100	–	55–85	–	–	–	–	–	–	83.2	–	–
Egg length, µm	81.2 ± 4.8 69.5–87.1	90	73–80	–	50	73–80	73–80	–	70–80	83.3	89	79.36	–	84.1	86 81–89
Egg width, µm	41.7 ± 3.5 37.3–47.5	46	40–43	–	–	40–43	40–43	–	40	49.6	–	42.30	–	44.2	47 45–48

Note: see Table 1.

In our study, we used 18 metric indicators of *T. colubriformis* males, which characterize the total body length and width in different areas, the location of the excretory opening and caudal bursa, the length of the caudal bursa, the size of the spicules and their morphological components, and the gubernaculum. Other researchers determined from one to six indicators in *T. colubriformis* males, which also characterize the length of the nematodes, the width of the body in the area of the base of the caudal bursa, the length of the genital bursa, the size of the spicules and their morphological structures, and the length of the gubernaculum. Most authors indicate the total size of the spicules in males without differentiating them into right and left, although according to our studies, the spicules are of unequal length. Specifically, the left one is longer than the right one (Table 1), which is important for species identification. Our data differed somewhat from those of other authors, who noted that the size of *Trichostrongylus* nematodes may vary depending on the definitive host from which they were isolated. In particular, the researchers found that the morphometric parameters of the nematodes isolated from sheep were higher compared with the nematodes isolated from goats (Ghasemikhah et al., 2011).

Morphological study of *T. colubriformis* females revealed specific features of the tail end and the vulva, which has the appearance of a slit with two convex lips, with weakly expressed cuticular papillae along the edges. Similar morphological characteristics have been noted by other authors (Skrjabin et al., 1954; Ivashkin et al., 1998; Dini al., 2023).

We determined 13 metric indicators of *T. colubriformis* females, which characterize the total body length and width in different parts of the body, the location of the excretory opening, the anal opening, the vulva area, the length of the tail end, the length of the slit of the vulva, and the length of the ovejector with sphincters. Other researchers also determined from one to six indicators in *T. colubriformis* females, which characterize the length of the nematodes, the width of the body in the vulva area, the length of the tail end, the location of the vulva, the length of its slit, and the length of the ovejector with sphincters (Table 2). Our data also have certain differences from the data obtained by other authors, which, in our opinion, may also be related to the description of nematodes isolated from different animal species. Researchers note the uninformativeness of studies of *Trichostrongylus* females due to their similarity in different species (Arbabi et al., 2023). However, the data we obtained can be used to facilitate the differentiation of *T. colubriformis* females.

We measured *T. colubriformis* eggs located in the uterine cavity of females as an additional species-specific trait. Although most eggs of nematodes of the family Trichostrongylidae are morphologically similar, accurate measurements and calculations can play an important role in their differentiation, as evidenced by the results of other scientists (Indre et al., 2010).

Therefore, the results of our study expand and deepen the existing knowledge regarding the taxonomic characteristics of both male and female *T. colubriformis* nematodes.

Conclusion

The parasitic nematodes of the species *T. colubriformis* (Nematoda: Trichostrongylidae), isolated from sheep in Ukraine, were studied morphologically and metrically. Altogether, 18 metric indicators in males and 13 indicators in females that characterize the structure and dimensions of the body in its various parts, and the location of the excretory opening were described and determined. In males, the structure and dimensions of the spicules, their specific morphological structures, the gubernaculum, and the location of the genital bursa were likewise studied. In females, the structure of the vulva region was described, the dimensions and location of the anus, vulva, ovejector with sphincters were determined, and the parameters of the eggs located in the uterine cavity were measured.

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