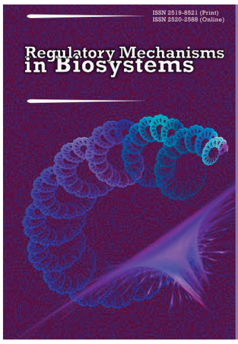

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2021, 12(1), 121–127
doi: 10.15421/022119

Morphological identification of *Nematodirus spathiger* nematodes (Nematoda, Molineidae) obtained from the small intestine of sheep

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Article info

Received 08.02.2021
Received in revised form 02.03.2021
Accepted 03.03.2021

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Melnychuk, V., Yevstafieva, V., Pishchalenko, M., Reshetylo, O., & Antipov, A. (2021). Morphological identification of Nematodirus spathiger nematodes (Nematoda, Molineidae) obtained from the small intestine of sheep. Regulatory Mechanisms in Biosystems, 12(1), 121–127. doi:10.15421/022119

Strongyloidiases are caused by nematodes of the suborder Strongylida and are the most widely prevalent group of gastrointestinal helminthiases of sheep in many regions of the world. Among gastrointestinal strongylids, the helminths of the genus *Nematodirus* are represented by the largest number of species and highest infection rates in sheep. Nematodirosis causes significant economic losses in the sheep industry through decreased sheep productivity, delayed growth and development of young animals, and a reduced resistance to other diseases. Timely and accurate diagnosis of nematodirosis and identification of the pathogen will effectively prevent the disease and help to carry out treatment and prevention measures. Therefore, the aim of the work was to study the definitive morphometric characters of mature males and females of *Nematodirus spathiger* Railliet, 1896, obtained from the small intestine of domestic sheep. The results of experimental studies showed that nematodes of this species morphologically are characterized by a thin filiform body, a vesicle at the head end and a chitinous tooth in a short oral capsule. The differential morphological features of male nematodes of *N. spathiger* include specifics of the structure of spicules, their distal end and the shape and location of the rays of the caudal bursa; in females, those are the features of the structure of the vulva and tail end. In identification of male nematodes of *N. spathiger*, it is proposed to use 40 metric parameters, of which 11 characterize the overall size of the body, esophagus and vesicles, 24 refer to the size of the tail bursa, 5 to the size of the spicules and the enveloping membrane. To help identify the females of *N. spathiger*, 25 parameters are chosen, of which 14 also characterize the overall size of the body, esophagus and head vesicle, 6 refer to the size of the cuticular formations of the vulva and its location, and 5 to the size of the tail end, the location of the anus and the size of the tail spike.

Keywords: helminth; gastrointestinal strongylids; Trichostrongyloidea; morphometry; species characters; differential diagnostics.

Introduction

Helminth pathogens have shown a remarkable ability to adapt to anthropogenic changes in the environment, including technological factors. Virtually every species of helminth, every faunal group of major pathogens in the taxonomy of parasitic worms and every ecosystem is influenced by and must adapt to human activity (Mas-Coma et al., 2008; Cole & Viney, 2018; Ibragimova, 2018; Aleuy & Kutz, 2020). At the same time, modern technologies of sheep breeding induce the spread of various parasitic diseases and, first of all, gastrointestinal strongyloidiases, the causative agents of which belong to a large number of different species of nematodes. The common features of that group of parasitoses are the same localization in the gastrointestinal tract, developmental cycle of pathogens, pathogenetic and clinical manifestations of diseases (Taylor & Thomas, 1986; Zajac, 2006; Charlier et al., 2014; Poddar et al., 2017). The timely and accurate diagnosis of pathogens, identification of their morphological and biological traits in specific natural conditions are important for the success of control and prevention measures against gastrointestinal strongyloidoses of sheep (Amarante, 2011; Roeber et al., 2013).

Among gastrointestinal strongylids, the helminths of the genus *Nematodirus* (Nematoda, Molineidae) are represented by the largest number of species and infection rates. Nematodirosis causes significant economic losses in the sheep industry through decreased sheep productivity, delayed growth and development of young animals, and a reduced resistance to other diseases (van Dijk & Morgan, 2008; Nadler et al., 2000; Oliver et al., 2014; McMahon et al., 2017). In particular, 6 species of *Nematodirus* nematodes were identified from sheep in the USA, and *N. spathiger* (P – 80.0%) and *N. abnormalis* (53.0%) were dominant. Less common were *N. filicollis* (P – 22.0%), *N. lanceolatus* (9.0%), *N. helvetianus* (4.0%) and *N. davtiani* (2.0%) (Becklund & Walker, 1967). In Iran, 6 other species of the same genus were recorded in sheep. The most common were *N. abnormalis* (P – 98.0%), *N. mugosaricus* (73.0%), and *N. oirathianus* (57.0%). The less frequent species were *N. filicolis*, *N. battus*, and *N. spathiger* (P – 12.0%, 27.0%, and 30.0%, respectively) (Shamsi & Naem, 2016). In northern Serbia, nematodirosis was found in 71.2% of examined sheep (Pavlovic, 2017). In some regions of North India, South Asia, and Northeastern Brazil, the prevalence of *Nematodirus* spp. in sheep did not exceed 60.0% (Lone et al., 2012; Raza et al., 2014; Tramboo et al., 2015). In Western, Eastern and Northern Africa, the fauna of *Nematodirus* nematodes is represented by three species, namely *N. spathiger*, *N. filicollis*, *N. battus*, with the average prevalence of 52.8% (Ghada et al., 2011; Akkari et al., 2012; Kelemework et al., 2016; Squire et al., 2018).

Studying the structure and morphological features of the causative agents of the gastrointestinal strongyloidiases of sheep is of great importance when considering the issues of their taxonomy, biology and species. Research in this area has focused mainly on the study of morphological and bioecological specifics, as well as on selected metric parameters of nematodes (Alikhan et al., 1985; Bert et al., 2003; Blaxter & Koutsovoulos, 2015). The representatives of the gastrointestinal strongylids are known to have specific morphological characters at the genus and species level, which are more pronounced in males than in females. These include the body size, structure of the head end, tail end, oral capsule, the presence of various formations on the cuticle and so on. In males, the characteristic differential species traits are the peculiarities in the structure of the caudal bursa, which is supported by symmetrical ray-shaped papillae. The shape and size of the spicules, the presence or absence of the gubernaculum, its shape and structure are also of interest. Females of strongylids are very similar, the differential characters include the morphological features of the ovijector and the vulva, and the parameters of its location relative to the tail and head ends. Also, the individual representatives of the gastrointestinal strongylids are morphologically similar to each other, which complicates the species identification. At the same time, it is argued that the differential morphological characters of helminths can change due to their adaptation to new living conditions (Hoberg et al., 1993; Duggal & Kaur, 2006; Khanmohammadi et al., 2013; Tak et al., 2014). The specific features of strongylids are the degree of viability of their larval stages of development in the environment, their optimal temperature regime, and the morphometric features of eggs and larvae, especially the infectious larvae (Hoberg, 2005; Kumsa et al., 2008; Morgan & van Dijk, 2012; Rodriguez-Vivas et al., 2017).

The aim of the research was to study the differential morphometric characteristics of adult male and female nematodes of the species *Nematodirus spathiger* Railliet, 1896, obtained from the small intestine of domestic sheep.

Materials and methods

The nematodes were collected in the complete helminthological dissection of the small intestine of 710 sheep obtained from slaughterhouses in Poltava, Kyiv and Zaporizhzhia regions (Skriabyn, 1928). The studies were conducted at the Laboratory of Parasitology of Poltava State Agrarian Academy in 2015–2020. After collection from the intestine, the helminths were washed in 0.9% NaCl and fixed in 70.0% ethanol according to standard method (Ivashkin et al., 1971). *Nematodirus* nematodes were identified to species by their morphological and metric parameters with keys (Skrjabin et al., 1954; Ivashkin et al., 1998). In total, 6,585 adult male and female nematodes of the species were collected, 1,906 males and 4,679 females.

Metric parameters of the adult male and female *N. spathiger* nematodes were analyzed using ImageJ for Windows® (version 2.00) in interactive mode using ×5, ×10, ×40 objectives and ×10 photo eyepiece. Photomicrographs were taken using a digital camera mounted on the Sigeta M3CMOS 14000 14.0 MP (China) microscope.

Standard deviation (SD) and average values (x) were calculated. Reliability of the differences in mean values for the studied groups of nematodes was determined by the method of one-way analysis of variance and F-test for 95.0% confidence level.

Results

The nematodes of the species *N. spathiger* have an elongated, filiform, fragile body typical of Trichostrongyloidea, with the anterior end thinner than the tail end. The cuticle is transversely striated. Cervical papillae are absent. The mouth is located terminally, the lips are not pronounced. The oral cavity is short, with a single chitinous tooth on its dorsal wall. The cuticle of the head end is expanded into a well-defined vesicle. The esophagus is elongated, cylindrical, slightly dilated posteriorly, club-shaped. There is also a well-defined excretory pore at the point of transition of the esophagus into the intestine (Fig. 1).

Males of *N. spathiger* are morphologically characterized by the presence of two long, filamentous spicules. The gubernaculum is absent. Characteristically, the distal parts of spicules are connected to each other by a membrane, which seemingly envelops the spicules and welds them together. The proximal ends of the spicules are tubular, with small terminal fringes (Fig. 2). The distal end of the connected spicules has the shape of a spatula (Fig. 3).

The caudal bursa consists of one small dorsal and two large lateral lobes. The dorsal lobe is divided by the median deep notch into two parts and is supported by dorsal rays. The ventral rays are parallel, and the lateral rays begin on a common stem. The middle and posterolateral rays are located in parallel and reach the edge of the bursa. The external dorsal ray is thin and slightly curved. Dorsal rays are strong, distally slightly narrowed to terminal bifurcations, with strongly bent outward lateral branchs (Fig. 4). According to the results of metric studies of *N. spathiger* males, 40 parameters are proposed to use in their identification. The parameters characterize the size of body, tail bursa and spicules (Table 1).

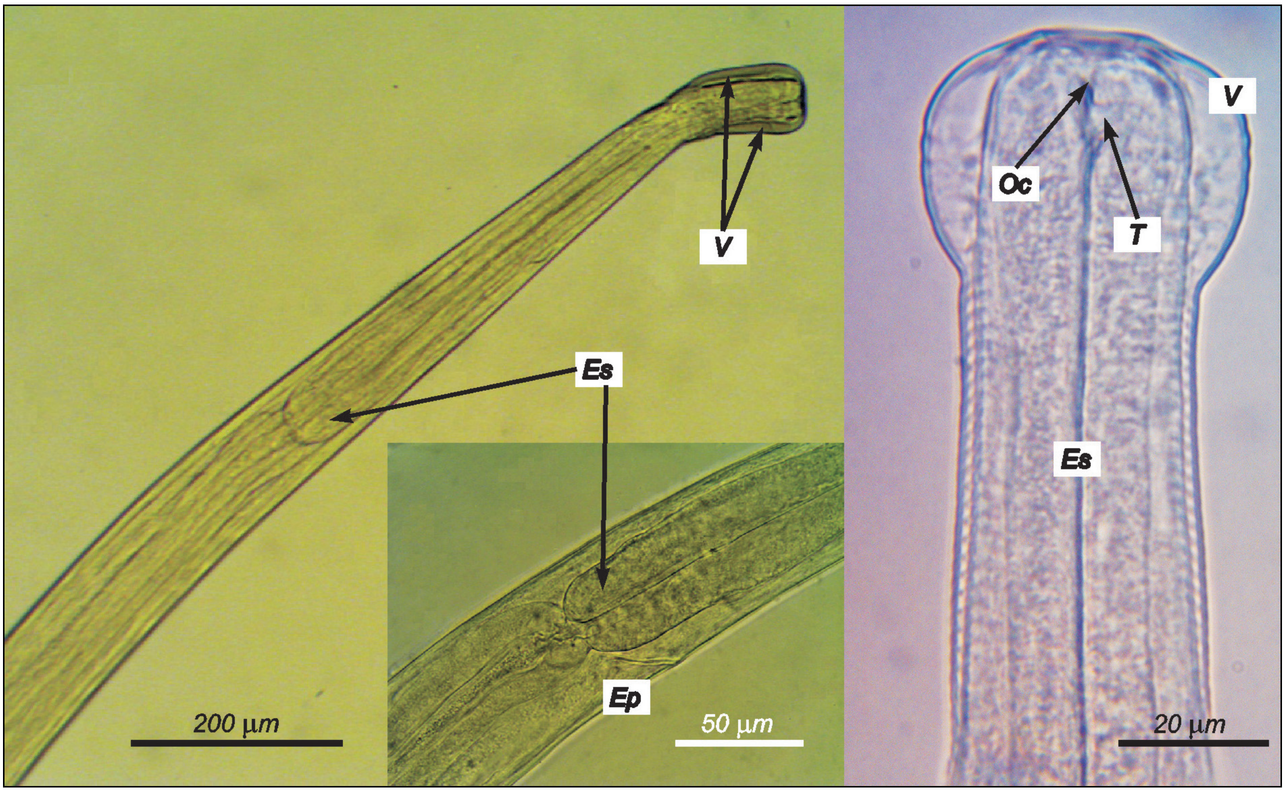


Fig. 1. Head end of *Nematodirus spathiger*. *V* – vesicle, *Oc* – oral cavity, *Es* – esophagus, *Ep* – excretory pore, *T* – tooth

Table 1
Metric parameters of ♂ *Nematodirus spathiger* (n = 15, x ± SD, min–max)

Parameters	Present specimens	May (1920)	Skrjabin et al. (1954)	Trach (1986)	Ivashkin et al. (1989)
Body of nematode					
Length of body, mm	17.8 ± 0.9 (16.0–19.5)	10.0–15.0	8.0–19.0	10.2–18.3	8.0–19.0
Length of esophagus, µm	512.0 ± 16.6 (489.5–539.3)	–	–	–	–
Width of esophagus in the middle, µm	29.9 ± 1.8 (26.3–33.1)	–	–	–	–
Width of esophagus in the widest part, µm	49.5 ± 2.0 (45.9–53.2)	–	–	–	–
Width of esophagus at the transition of esophagus to intestine, µm	36.5 ± 2.2 (32.5–39.1)	–	–	–	–
Length of area with vesicular cuticle, µm	121.1 ± 8.0 (106.6–134.8)	–	–	–	–
Width of proximal part of vesicle, µm	47.5 ± 2.9 (42.7–53.2)	–	–	–	–
Length of widened area of proximal part of vesicle, µm	38.6 ± 3.3 (33.2–43.8)	–	–	–	–
Width of body at the transition of esophagus to intestine, µm	89.3 ± 3.0 (81.5–92.2)	–	–	–	–
Width of body at the widest part, µm	141.1 ± 7.8 (130.2–153.0)	125.0–175.0	116.0–149.0	–	120.0–150.0
Width of body at the base of caudal bursa, µm	119.0 ± 3.9 (110.2–123.5)	–	–	–	–
Caudal bursa, µm					
Length of lateral lobe of tail bursa	194.4 ± 11.8 (180.3–227.8)	–	–	–	–
Width of lateral lobe of tail bursa	176.9 ± 10.0 (156.3–189.2)	–	–	–	–
Antero-ventral ray:					
– length	80.2 ± 3.2 (74.3–85.6)	–	–	–	–
– width in the middle	9.9 ± 0.6 (9.1–10.7)	–	–	–	–
Postero-ventral ray:					
– length	90.1 ± 3.5 (82.6–97.4)	–	–	–	–
– width in the middle	11.3 ± 0.4 (10.7–11.9)	–	–	–	–
Antero-lateral ray:					
– length	123.9 ± 3.7 (118.2–131.0)	–	–	–	–
– width in the middle	17.0 ± 0.6 (16.1–18.2)	–	–	–	–
Media-lateral ray:					
– length	153.0 ± 6.7 (140.7–163.5)	–	–	–	–
– width in the middle	14.9 ± 0.6 (14.0–16.0)	–	–	–	–
Postero-lateral ray:					
– length	155.5 ± 7.1 (143.5–169.3)	–	–	–	–
– width in the middle	18.3 ± 0.8 (17.1–19.5)	–	–	–	–
External dorsal ray:					
– length	125.6 ± 5.6 (118.7–137.2)	–	–	–	–
– width in the middle	6.9 ± 0.8 (5.1–8.2)	–	–	–	–
Length of dorsal ray	60.1 ± 3.7 (55.2–66.9)	–	–	–	–
Length of dorsal ray from stem to the bifurcation point	44.5 ± 3.5 (40.1–50.8)	–	–	–	–
Width of dorsal ray in the middle	15.3 ± 2.7 (8.0–18.8)	–	–	–	–
Width of dorsal ray at the bifurcation point	10.3 ± 1.2 (8.3–12.7)	–	–	–	–
Length of lateral branch of the dorsal ray	15.6 ± 0.9 (14.0–16.9)	–	–	–	–
Width of lateral branch of the dorsal ray at the bifurcation point	5.5 ± 0.4 (4.9–6.1)	–	–	–	–
Width of lateral branch of the dorsal ray in the middle	3.9 ± 0.3 (3.5–4.7)	–	–	–	–
Length of median branch of dorsal ray	7.4 ± 0.5 (6.7–8.3)	–	–	–	–
Width of median branch of dorsal ray at the bifurcation point	5.3 ± 0.4 (4.5–6.0)	–	–	–	–
Width of median branch of dorsal ray in the middle	4.3 ± 0.3 (3.9–5.0)	–	–	–	–
Spicules					
Length of spicules, mm	1.0 ± 0.1 (0.9–1.1)	0.7–1.1	0.9–1.2	0.9–1.2	0.9–1.2
Width of proximal end of spicule, µm	12.2 ± 0.6 (11.1–12.9)	–	–	–	–
Width of spicule in the middle, µm	5.4 ± 0.5 (4.7–6.1)	–	–	–	–
Length of membranous area around the distal end of spicule, µm	66.6 ± 4.4 (60.2–76.9)	–	–	–	–
Width of membranous area around the distal end of spicule, µm	22.0 ± 1.2 (20.2–24.6)	–	–	–	–

Note: "–" – parameters were not defined.

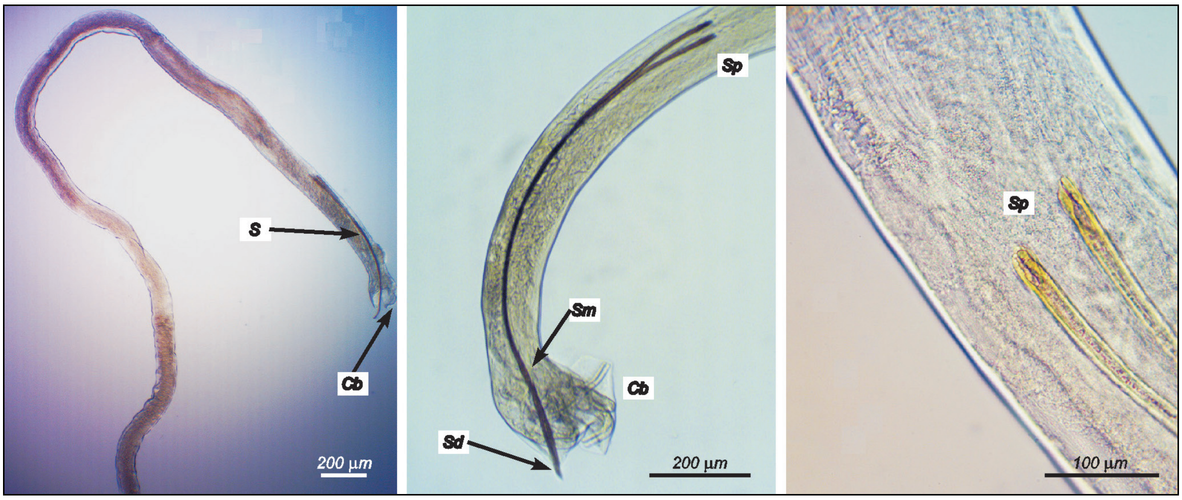
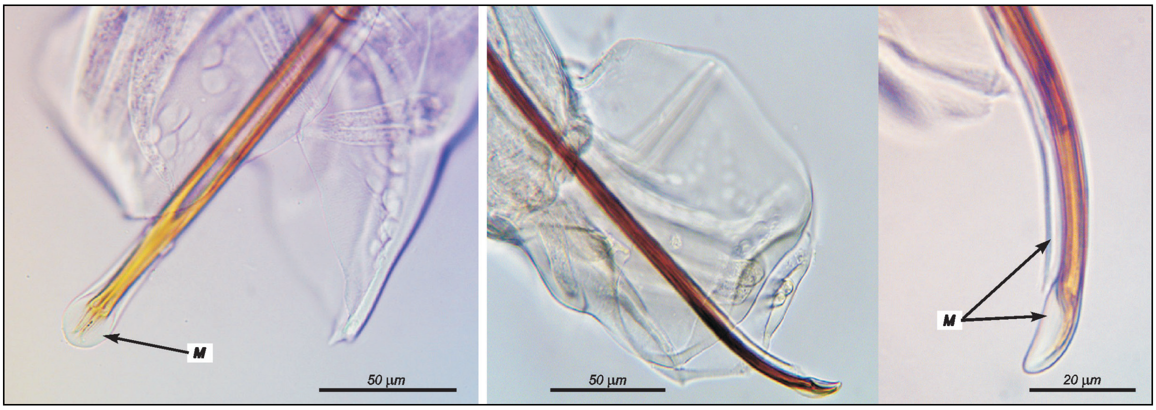


Fig. 2. Tail end of ♂ *Nematodirus spathiger*: *Cb* – caudal bursa, *S* – spicule, *Sp* – proximal end of spicule, *Sd* – distal end of spicule, *Sm* – membranous joining of spicules



a *b*

Fig. 3. Distal end of spicules of ♂ *Nematodirus spathiger*: *M* – membrane shaped as a rounded spatula; *a* – ventral view, *b* – lateral view

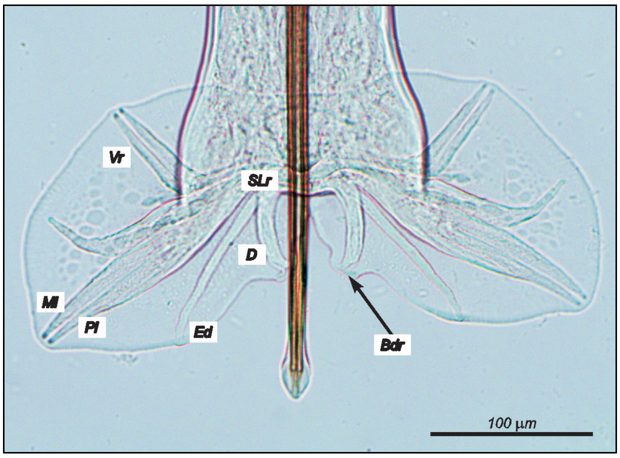
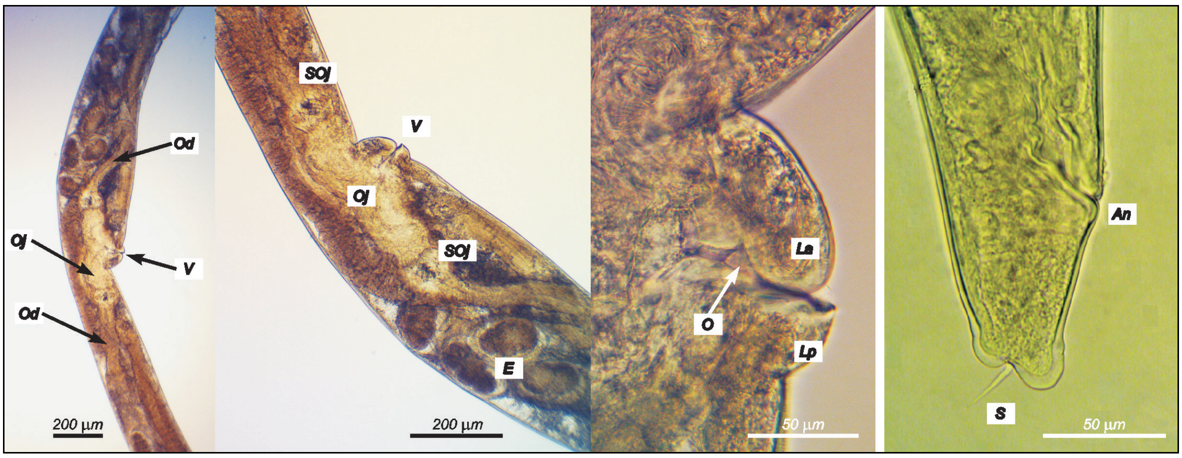


Fig. 4. Caudal bursa of ♂ *Nematodirus spathiger*: *Vr* – ventral rays, *Ml* – media-lateral ray, *Pl* – postero-lateral ray, *Ed* – external dorsal ray, *D* – dorsal ray, *SLr* – common stem of lateral rays, *BDr* – bifurcation of distal end of dorsal ray

Thus, there are 11 general measurements of male nematodes of this species: total body length; width of the body in its various parts (the transition of the esophagus into the intestine, in the widest part, and in the location of the tail bursa); length of the esophagus; width of the esophagus in its various parts (middle, in the widest part, the transition of the esophagus into the intestine); the measurements of the head vesicle (total length, length of the widest area, width in the proximal area). For the tail bursa, in addition to morphological features, it is proposed to use 24 metric parameters that characterize the length and width of the lateral lobe; length and width (in the middle) of the ventral and lateral rays; the length of the dorsal ray; features of the bifurcation location on the dorsal ray; length and width of the median and lateral branches of the dorsal ray. As for spicules, it is proposed to use 5 metric parameters that characterize their length, width in different areas (proximal end, in the middle), and the length and width of the membrane enveloping the distal end of the spicule.

The vulva of *N. spathiger* females morphologically is located in the posterior part of body. Its opening is shaped as a transverse slit between two lips. Moreover, the posterior lip is shorter, more pointed, slightly protrudes beyond the anterior lip. The latter has a rounded end and rests slightly on the posterior lip. In contrast to males, the head part of the body of a female nematode is significantly thinner than the posterior one, especially in the area of the vulva. There are two uteri, their trunks turn into a well-defined ovijector, which leads to the vagina, which opens outside as vulva. There are strong sphincters at the transition of the uterus into the ovijector (Fig. 5a). The anus is located near the tail end, the tail ends bluntly and has a small notch. The tail end has a well-defined spike (Fig. 5b).

For easier identification of females of *N. spathiger*, it is proposed to use 25 parameters that characterize the size of the body, the location of the vulva and anus, the measurements of cuticular formations in the vulva and the of the tail end (Table 2). The overall measurements of females include 14 parameters that characterize total body length, the length of the anterior and posterior parts of the body, width of body at various areas (transition of esophagus to intestine, transition of anterior part of body to posterior, at area of vulva, and in the widest part), length of esophagus; width of esophagus in various areas (in the middle, in the widest part, the transition of esophagus into intestine), the measurements of head vesicle (length, length of the widest area, width of the proximal area). Six metric parameters are suggested to characterize the vulva: length of the vulva, height and width of the cuticular lips and the location of the vulva relative to the anus. For the tail end in females, 5 metric parameters are suggested that characterize the width of the tail end in the anus area, the width of the tail end, the length and width of the tail spike, and the location of the anus relative to the tail end.



a *b*

Fig. 5. Morphological structure of ♀ *Nematodirus spathiger*: *a* – area of vulva, *b* – tail end; *V* – vulva, *La* – anterior lip, *Lp* – posterior lip, *Oj* – ovijector, *SOj* – sphincter of ovijector, *O* – slit-shaped opening, *Od* – oviduct, *E* – eggs, *An* – anus, *S* – spike

Table 2
Metric parameters of ♀ *Nematodirus spathiger* (n = 15, x ± SD, min–max)

Parameters	Present specimens	May (1920)	Skrjabin et al. (1954)	Lichtenfels & Pillit (1983)	Trach (1986)	Ivashkin et al. (1989)
Body of nematode						
Length of body, mm	21.2 ± 1.3 (18.5–22.5)	15.0–23.0	12.0–20.0	12.8–19.0	11.2–30.0	12.0–20.0
Length of anterior thinner part of body, mm	15.0 ± 1.4 (11.6–16.8)	–	–	–	–	–
Length of posterior thicker part of body, mm	6.2 ± 0.4 (5.5–6.9)	–	–	–	–	–
Length of esophagus, µm	599.1 ± 10.6 (581.7–621.1)	–	–	486.0–513.0	–	–
Width of esophagus in the middle, µm	35.3 ± 2.2 (30.6–37.2)	–	–	–	–	–
Width of esophagus in the widest part, µm	57.6 ± 4.0 (50.4–63.2)	–	–	–	–	–
Width of esophagus at the transition to intestine, µm	31.3 ± 3.3 (24.9–38.1)	–	–	–	–	–
Length of area with cuticular vesicle, µm	117.2 ± 5.6 (109.0–125.7)	–	–	–	–	–
Width of proximal part of vesicle, µm	56.5 ± 4.2 (50.2–63.3)	–	–	–	–	–
Length of widened area in the proximal part of vesicle, µm	38.9 ± 5.0 (30.7–49.8)	–	–	–	–	–
Width of body in the area of transition of esophagus to intestine, µm	88.2 ± 3.0 (80.9–92.6)	–	–	–	–	–
Width of body in the area of transition of the thinner anterior part to the thicker posterior part, µm	221.9 ± 11.4 (197.6–240.4)	–	–	–	–	–
Width of body at the area of vulva, µm	288.5 ± 17.7 (251.9–319.0)	–	200.0–360.0	–	–	200.0–360.0
Width of body at its thickest area, µm	335.3 ± 26.4 (295.5–375.9)	–	–	–	–	–
Area of vulva						
Length of the area of vulva, µm	132.9 ± 6.5 (122.1–142.6)	–	–	–	–	–
Length of anterior lip, µm	76.8 ± 4.8 (70.4–85.3)	–	–	–	–	–
Length of posterior lip, µm	56.1 ± 7.2 (46.7–69.5)	–	–	–	–	–
Height of anterior lip, µm	33.5 ± 2.4 (30.5–37.1)	–	–	–	–	–
Height of posterior lip, µm	26.7 ± 1.7 (22.0–28.4)	–	–	–	–	–
Distance from vulva to anus, mm	6.12 ± 0.40 (5.42–6.83)	–	–	–	–	–
Tail end						
Width of body at anus area, µm	70.0 ± 4.7 (60.5–76.3)	–	66.0	–	–	70.0
Distance from anus to tail end, µm	90.9 ± 10.8 (76.3–110.9)	–	70.0–99.0	–	–	70.0–100.0
Width of tail end, µm	30.9 ± 2.4 (27.4–35.1)	–	–	–	–	–
Length of spike, µm	26.3 ± 2.5 (22.3–30.1)	–	–	–	23.0–45.0	–
Width of spike at the base, µm	2.69 ± 0.32 (2.11–3.05)	–	–	–	–	–

Note: see Table 1.

Table 3
Metric parameters of *Nematodirus spathiger* eggs (n = 15, x ± SD, min–max)

Publications	Egg length, µm	Egg width, µm	Eggshell thickness, µm
Present specimens	202 ± 9 (187–219)	112 ± 4 (104–117)	2.38 ± 0.19 (2.14–2.66)
May (1920)	(150–220)	(80–100)	–
Tetley (1935)	(181–210)	(90–105)	–
Shore (1939)	202 (181–230)	98 (91–107)	(3.3–3.8)
Tetley (1941)	195 (179–210)	97 (88–107)	–
Skrjabin et al. (1954)	(221–238)	(119–136)	–
Thomas (1957)	(183–214)	(87–99)	–
Soulsby (1968)	(175–260)	(106–110)	–
Viljeon (1972)	218 (173–238)	103 (97–119)	–
Lichtenfels & Pillit (1983)	(172–217)	(95–114)	–
Ivashkin et al. (1989)	(220–240)	(120–140)	–

Note: see Table 1.

The eggs of *N. spathiger* are located in the uterine cavity and have a structure, typical for the gastrointestinal strongylids: oval, transparent, with a smooth, thin shell, and containing an embryo (Fig. 5a). The parameters of egg length, which amounted to 202.2 ± 9.3 µm on average in the present study, egg width (112.0 ± 3.6 µm on average), and shell thickness (2.38 ± 0.19 µm on average) are suggested for use in the metric studies of eggs (Table 3). A comparative analysis of the results of metric studies of *N. spathiger* eggs with literature data showed that most researchers have used two parameters for identification, the length and width of eggs. We propose to additionally use the parameter of shell thickness of nematode eggs of this species, to facilitate species differentiation.

Discussion

According to the literature, nematodirosis is a very common infection of sheep in many countries, where the *Nematodirus* fauna can be represented by several species (*N. spathiger*, *N. abnormalis*, *N. filicollis*, *N. lanceolatus*, *N. helvetianus*, *N. davtiani*, *N. battus*, *N. mugosaricus*, *N. oirathianus*), and infection rates range 12–98%. Moreover, *N. spathiger* in some regions is dominant and its prevalence in sheep can reach 80.0% (Becklund & Walker, 1967; Shamsi & Naem, 2016; Pavlovic et al., 2017). We have previously found nematodirosis in sheep caused by parasitism of *N. spathiger* to be a common disease on farms in Poltava, Kyiv and Zaporizhzhia regions of Ukraine, the prevalence of infection was 49.0%, the intensity of infection 18.9 ± 0.7 ind./host, and the abundance index did not exceed 9.3 ind./host (Melnychuk et al., 2020).

The genus *Nematodirus* has a significant species diversity, about 18 species are described that parasitize in both domestic and wild ruminants. Several species are morphologically very similar, which leads to difficulties in species identification (Skrjabin et al., 1954; Ivashkin et al., 1998). Therefore, we performed a morphometric analysis of nematodes of the species *N. spathiger* in order to determine their main identification characters. The nematodes are small, the anterior part of their body is thinner than the posterior one. There is a well-defined vesicle at the head end and a chitinous tooth in the oral cavity. However, these features are common to other genera and species of Trichostrongyloidea (Skrjabin et al., 1954; Trach, 1986). The males morphologically were characterized by the specific structure of the caudal bursa: the location of lateral and ventral rays, features in the structure of dorsal rays, and the structure of spicules, especially their distal end which resembles a spatula and is clearly visible from the ventral side. Such species-specific morphological features have been confirmed by other researchers (Skrjabin et al., 1954; Lichtenfels & Pillit, 1983). We suggest 40 parameters to be used in identification of males of *N. spathiger*: 11 of those characterize the measurements of body, esophagus and vesicles; 24 refer to the measurements of the tail bursa; 5 to the measurements of spicules and the membrane that envelops them. The analysis of literature data has revealed that only three metric parameters had been suggested for that purpose previously, namely the length of body, the width of body in its widest part, and the length of spicules. Moreover, these parameters differed significantly (May, 1920; Skrjabin et al., 1954; Trach, 1986; Ivashkin et al., 1989). Thus, according to our studies, the average body length of male nematodes of the species *N. spathiger* was 17.8 ± 0.9 mm with fluctuations from 16.0 to 19.5 mm, and the dimensions given by the authors (Table 1) ranged from 8.0 to 19.0 mm. The width of body of males in its widest part was 141.1 ± 7.8 µm according to our studies (130.2–153.0 µm), and the length of the spicule was 1.0 ± 0.1 mm (0.9–1.1 mm). At the same time, the published data give the range of 116.0 to 175.0 µm for the width of body in its widest part and 0.7 to 1.2 µm for the length of spicule (Table 1).

Females of *N. spathiger* nematodes are morphologically characterized by the presence of thinner anterior and thicker posterior parts of the body. The opening of the vulva is slit-shaped and surrounded by two cuticular lips, which are slightly different from each other. There are two uteri. The tail end bears a well-defined spike. The vulva is located in the posterior part of body. These features are also noted in other publications (Skrjabin et al., 1954; Ivashkin et al., 1989). Therefore, we suggested 25 parameters to use for the identification of *N. spathiger* females: 14 indicators characterize the measurements of the body, esophagus and head vesicle; 6 describe the cuticular formations in the vulva and its location relative to the anus; 5 describe the measurements of the tail end and the tail spike, and the location of the anus. According to publications, 6 parameters were suggested for the species identification of *N. spathiger* females: lengths of body and esophagus, width of body in the vulva and anus area, distance from anus to tail end, and tail end length (May, 1920; Skrjabin et al., 1954; Lichtenfels & Pillit, 1983; Trach, 1986; Ivashkin et al., 1989). Analyzing our data and literature data, we note the differing values of parameters (Table 2). Thus, the length of the body, according to our studies, was 21.2 ± 1.3 mm (18.5–22.5 mm), and according to other studies it ranged from 11.2 to 30.0 mm. The length of the esophagus in *N. spathiger* females was 599.1 ± 10.6 µm (581.7–621.1 µm), and according to Lichtenfels & Pillit (1983) it ranged from 486.0 to 513.0 µm. The width of the body in the vulva and anus areas was 288.5 ± 17.7 µm (251.9–319.0 µm) and 70.0 ± 4.7 µm (60.5–76.3 µm), respectively, and according to the published data it was 200.0–360.0 and 66.0–70.0 µm. The distance from the anus to the tail end was 90.9 ± 10.8 µm (76.3 – 110.9 µm), and the length of the spike was 26.3 ± 2.5 µm (22.3–30.1 µm). According to other authors, these parameters ranged from 70.0 to 100.0 µm and from 23.0 to 45.0 µm, respectively (Table 2).

Also, we established the metric parameters of *N. spathiger* nematode eggs in the uterine cavity, namely the length, width of the egg and thickness of the egg shell. Most authors when conducting metric studies of nematode eggs of this species suggest using only two indicators, the width and length (Table 2). According to our study, the length and width of the egg and thickness of the egg shell were 202.2 ± 9.3 µm (187.3–218.8 µm), 112.0 ± 3.6 µm (103.9–116.8 µm) and 2.38 ± 0.19 µm (2.14–2.66 µm), respectively. According to other studies, the egg parameters ranged from 150.0 to 260.0 µm (length) and from 80.0 to 140.0 µm (width). According to Shore, 1939 the shell thickness of *N. spathiger* eggs ranged from 3.3 to 3.8 µm. Therefore, the proposed additional metric parameters of male and female nematodes of the species *N. spathiger* will increase the efficiency of species identification.

Conclusion

The male and female nematodes of the species *Nematodirus spathiger* Railliet, 1896 have specific differential morphological features and metrical parameters. Morphologically, both male and female nematodes of this species have a vesicle at the head end and a chitinous tooth in the oral cavity. Males are characterized by morphological features in the structure of the caudal bursa and spicules, and females by features in the structure of the vulva and tail end. In order to increase the efficiency of identification of male *N. spathiger* nematodes, 40 metric parameters are suggested that characterize the measurements of helminth body, esophagus, head vesicle, rays of the tail bursa, lateral lobes, as well as spicules and the membrane that envelops them. To differentiate female *N. spathiger* nematodes, 25 metric parameters that characterize the measurements of the body, esophagus, head vesicle, width of the anterior and posterior parts of the body, distance from the vulva to the anus and from the anus to the tail end, cuticular lips in the vulva and the tail spike, are suggested to be considered in addition to morphological features. Metric parameters of nematode eggs in the uterus were determined, three parameters, namely the length and width of the egg, and thickness of the egg shell, are suggested for the differential diagnosis of *N. spathiger*.

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