

Morphological characteristic of *Skrjabinema ovis* (Nematoda, Oxyuridae) obtained from domestic sheep

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Skrjabinemosis is a widely prevalent helminthiasis of sheep, goats and wild ruminants. It is caused by *Skrjabinema ovis* Skrjabin, 1915. Parasitization by *S. ovis* induces inflammatory processes of the large intestine, in skin around the anus and tail base, resulting in reduced productivity and weight loss. The measures of prevention and control of skrjabinemosis on sheep farms depend on the timely and accurate diagnosis, based on the reliable identification of nematodes. We studied the identification morphometric characters of this species. The nematodes were obtained from the large intestine of 710 pasture-kept sheep (*Ovis aries*) on farms of Kyiv, Poltava and Zaporizhzhia regions (Central and South-Eastern Ukraine). In total, 5723 adult *S. ovis* nematodes were collected, 1981 of which were males and 3742 were females. Sexual dimorphism was found in *S. ovis* both in metric and morphological parameters. The size dimorphism is characterized by values larger by 43.9–64.6% of 11 parameters in females compared to males. The differential characters of mature *S. ovis* males and females are morphological specifics of the mouth organ complex and the distribution of cuticular formations on the head end of the parasite. The lips of the female nematodes are anchor-shaped and adorned with tooth-like lamellae, and the lips of the males are oval and lack the tooth-like lamellae. Cuticular nodes on the head ends of the females are considerably longer and wider than in the males. The species-specific morphological characters of the males are the features of morphology and sizes of the pseudobursa, the shape and length of the spicule and gubernaculum, and their width parameters in different areas. In the identification of females, the morphology of the vulva and tail end, and the metric parameters of the location of the vulva, anus, and sizes of eggs in the uterus should be considered. Additional metric and morphological parameters are presented for use in the differentiation of males and females for better identification.

Keywords: skrjabinemosis; helminths; differential characters; metric parameters.

Introduction

The main aim of sheep farming is a full utilization of the biological potential of meat, wool and dairy productivity of sheep breeds (Rasali et al., 2006; Banerjee et al., 2009; Anteneh & Yadav, 2017). One of the approaches used is careful monitoring and prediction of the parasitological situation, the implementation of measures for the prevention and treatment of invasive diseases, especially nematodes of the gastrointestinal tract of sheep. It is well-known that the biotic, abiotic and technological factors directly affect the activity of epizootic processes of animal helminthiasis, including those of sheep (Vlassoff et al., 2001; Tariq et al., 2008; Roerber et al., 2013; Ibrahim et al., 2014; Boyko et al., 2016; Zazharska et al., 2018).

Sheep helminths are widely prevalent and form parasitic systems with multiple levels of protection. They cause infectious diseases that are stable and enzootic in various countries in most of the natural climatic zones. There are reports of significant prevalence of the parasitic *Skrjabinema ovis* (Skrjabin, 1915) nematodes in sheep. Thus, the prevalence of *S. ovis* in sheep ranged 2.2–12.0%. The maximum infection rates were found in the 1.0–1.5 year old age group (Bahadori et al., 2007; Naem & Gorgani, 2011). In the south-east of Spain, 10.28% of the examined sheep were infected with this species, the infection intensity reaching 46.3 individuals per host (Ruiz de Ybáñez et al., 1999). In a number of regions of Pakistan, skrjabinemosis was diagnosed only in 1.2% sheep (Farooq et al., 2012). In Sudan, the prevalence of skrjabinemosis in sheep was 6.7%, and the intensity ranged 1–74 individuals per host (Abdelnabi et al., 2005). Skrja-

binemosis is also found on sheep farms in Italy, Brazil, Africa, and Macedonia (Balbo et al., 1977; Theodoridis et al., 2000; Belem et al., 2005; de Souza et al., 2012). This prevalence of skrjabinemosis is likely related to the biological features of the parasite. Its life cycle lacks an intermediate host. It has been observed that mature *Skrjabinema* females passively crawl from the rectum to the perianal skin of the host, and lay eggs, in which the larvae develop and molt twice. After that, the egg becomes infectious, and is released into the environment where the alimentary infection of hosts occurs (Schad, 1957; Anderson, 2000).

Post-mortem examinations are most significant methods of diagnostics for species identification of helminth pathogens and assessment of their numbers. In veterinary practice this kind of study is the final link in the medical and diagnostic process. It allows one to verify the life-time diagnosis and the adequacy of the prescribed treatment. The post-mortem diagnostics of helminths is the most reliable method. It is based on the results of parasitological and anatomical dissections of animals and their organs, and in the process of post-mortem inspection of bodies. The results of morphological and metric studies of the observed helminths are highly important for their differentiation, and for the questions of systematics, biology, and species identification, including for the pathogen of sheep skrjabinemosis (Shahlapoor, 1965; Ivashkin et al., 1998; Popiolek et al., 2009; Li et al., 2019).

The taxonomic differentiation of mature nematodes is based on the morphological specifics of their body, head end, position of lips in the mouth capsule, and the tail end, shape and size of spicule, bursa and gubernaculum in males. In females, the morphology of copulatory apparatus,

vulva location and egg size while in the uterus are taken in consideration. There are also suggestions for using the length and width of the body, bulbous, esophagus, and wing-like formations on the head end. However, the reported data on the metric parameters of *S. ovis* are scarce and rather variable (Abdussalam, 1938; Skrjabin et al., 1960; Andrews, 1969). Thus, a morphometric study of *S. ovis*, obtained from sheep, would have scientific and practical value, because of the new data on the species identification of these parasites, and enhanced diagnostics of skrjabinemosis.

The aim of the present work was to study the differential morphological and metric parameters of the adult males and females of *S. ovis* nematodes, obtained from sheep (*Ovis aries*).

Materials and methods

Parasitological analysis of *S. ovis* nematodes was conducted in 2015–2020. The helminths were obtained during helminthological investigation of the large intestine of 710 domestic sheep (Skrjabin, 1928) from sheep farms in Kyiv, Poltava and Zaporizhzhia regions (Central and South-Eastern Ukraine). After collection from the intestine, the nematodes were washed in 0.9% solution of NaCl and fixed according to standard technique in 70% ethanol (Ivashkin et al., 1971). Roundworm species were identified by morphological features according to (Skrjabin et al., 1960; Ivashkin et al., 1998). In total, 5723 adult *S. ovis* nematodes were collected, 1981 of which were males and 3742 were females.

The metric parameters of adult *S. ovis* roundworms were measured using ImageJ for Windows® (version 2.00) in interactive mode using $\times 5$, $\times 10$, $\times 40$, $\times 100$ objectives and $\times 10$ photo eyepiece. To calibrate the image analyzer, the ruled scale of an ocular micrometer was coincided with the scale of the stage micrometer included in a MikroMed microscope kit. Photomicrographs were taken using a 5 Mpix digital camera mounted on the MikroMed (China) microscope.

Standard deviation (SD) and mean values (\bar{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 using the Fisher test, with $P < 0.05$ level of significance.

Results

The nematodes of the species *S. ovis* (Skrjabin, 1915) are characterized by significant morphological sexual dimorphism. The body of the female nematode is white, filiform, slightly tapered to the head end and more tapered to the tail end. The male roundworms have hook-shaped bodies, with a straight anterior part and bent posterior part (Fig. 1).

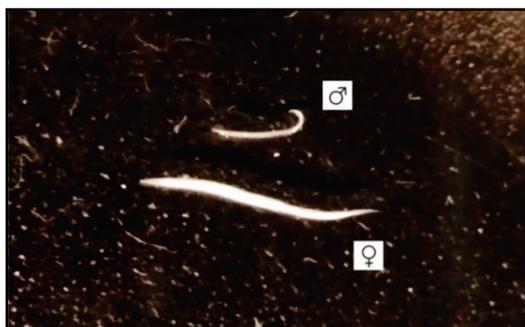


Fig. 1. General view of *Skrjabinema ovis*

The head end of male and female *Skrjabinema* nematodes has two lateral wing-shaped cuticular nodes. Their bases are located at the lip bases, and their ends at the esophagus area. More narrow cuticular wings are located posterior to them. Those run along the lateral lines of the body and end in the area between the anus and the tail end. The mouth opens into the esophagus, which is divided into two clearly distinct parts: cylindrical anterior and behind that the spherical bulbous. We have observed that the wing-shaped nodes are significantly narrower and shorter in males than in females (Fig. 2).

The head end of *S. ovis* is adorned with a complex formation of mouth organs. The mouth opening is surrounded by three symmetrical large lips. Each lip consists of the median and two lateral parts. The me-

dian part of each lip extrudes above the mouth ring and branches into anterior and posterior wedges. These wedges are divided by a deep indentation, hence the illusion of six lips. The anterior wedge of each lip consists of two parts which make up a pyramid, pointed anteriorly. The narrow sides of pyramids face outwards, and the wider sides are positioned to the inside and laterally to mouth opening. The lips of males and females are morphologically different. In females, lips are anchor-shaped, and there are single pairs of tooth-like lamellae, pointed to the centre of the mouth opening, on their inner sides. The lips of males are oval and lack the tooth-like lamellae (Fig. 3).

Aside from the morphological specifics, there are significant metric differences in the sexual dimorphism of *S. ovis*. By 11 parameters, the females were significantly ($P < 0.001$) larger than males (Table 1).

Table 1

Morphometric parameters of sexual dimorphism in *Skrjabinema ovis* ($\bar{x} \pm SD$; $n = 15$)

Parameters	♂	♀
Length of body, mm	3.1 ± 0.2	6.3 ± 0.5***
Length of the lateral cuticular nodes, μm	100.1 ± 7.1	282.9 ± 12.6***
Width of body in the area of, μm :		
– lips	55.2 ± 4.9	104.0 ± 7.6***
– cuticular nodes	103.8 ± 4.0	220.5 ± 23.9***
– middle of the body	218.0 ± 7.9	323.2 ± 34.3***
Total length of esophagus, μm	379.0 ± 10.5	731.1 ± 22.6***
Length of anterior cylindrical part of esophagus, μm	279.1 ± 7.5	552.6 ± 35.2***
Width of anterior cylindrical part of esophagus in the middle, μm	36.6 ± 2.8	71.1 ± 3.4***
Width of esophagus at the transition from the anterior cylindrical part to the posterior bulbous part, μm	23.9 ± 1.9	54.9 ± 4.1***
Length of bulbous, μm	99.8 ± 10.4	178.4 ± 29.4***
Width of bulbous, μm	86.5 ± 2.9	154.2 ± 5.8***
Ratio of the lengths of the anterior cylindrical part of esophagus to the posterior bulbous part	2.8 : 1	3.2 : 1

Note: *** – $P < 0.001$ – compared to values of ♂ *S. ovis*.

Thus, the body length of females was 6.3 ± 0.5 mm, which was larger by 50.7% than in males (6.3 ± 0.5 mm). Accordingly, the females were wider in the lip area, at the cuticular node area and in the middle of body by 52.9–57.4% than males. The parameters of length of lateral cuticular nodes at the head end of the nematodes were larger in females by 64.6%, which is confirmed by morphological studies, too. The esophagus was significantly larger in females than in males. It was longer by 48.1%. The length of the anterior cylindrical part of the esophagus in females was 552.6 ± 35.2 μm (longer by 49.4% than in males), and the length of the posterior bulbous part of the esophagus in females was 178.4 ± 29.4 μm (longer by 44.0% than in males). The width parameters of esophagus in females were also higher than in males, in the middle of the anterior cylindrical part by 48.5%, at the transition of that part into the bulbous part by 56.4%, and in the middle of the bulbous by 43.9%. Thus, the ratio of the length of anterior part of esophagus to the posterior one was 3.2 : 1 in males, and 2.8 : 1 in females.

The specific morphological characters of *S. ovis* males include the morphological features of pseudobursa, spicule, gubemaculum (Fig. 4), and their metric parameters (Table 2).

The tail end of *Skrjabinema* males ends with the pseudobursa, which consists of membrane, terminal tail protrusion, and one pair of pre-anal and one pair of post-anal ribbed papillae. Their apices are shaped like coronas with three conic processes. The parameters of length and width of pseudobursa were 185.6 ± 15.5 and 120.7 ± 8.0 μm , respectively, their ratio was 1.5 : 1, i.e. pseudobursa is elongated. The morphological features of males also include the presence of one pair of elongated, stalk-shaped post-anal papillae between the tail protrusion and the anus. There are small papillae in the cloacal area, which support pseudobursa. There is one spicule 94.4 ± 4.6 μm in length, arrow-shaped. The spicule lies in the groove of the gubemaculum, the mean length of which was 24.4 ± 2.1 μm . In the identification of this species, we also suggest using the parameters of width at the proximal end of the spicule (5.7 ± 0.2 μm) and in the middle of the spicule (4.4 ± 0.2 μm), and the parameters of width of the proximal end of the gubemaculum (5.3 ± 0.1 μm) and in its middle (4.0 ± 0.4 μm).

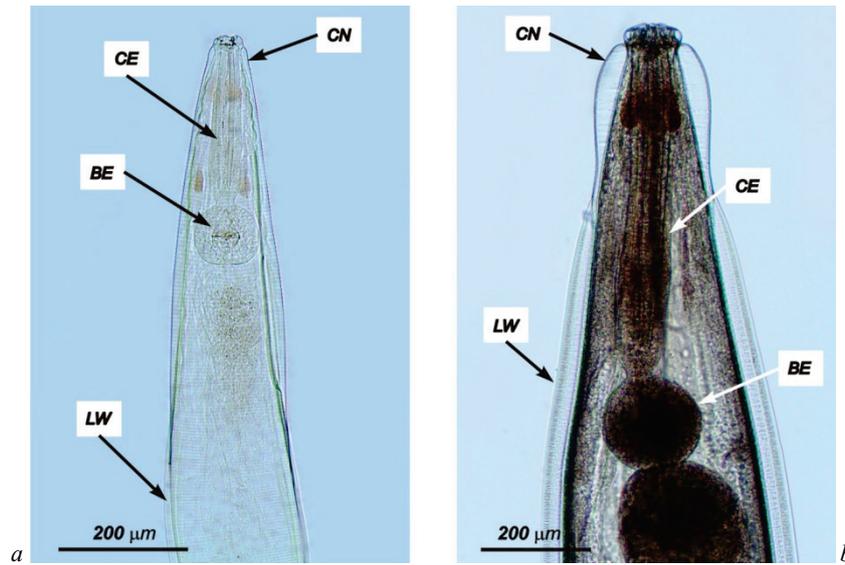


Fig. 2. Head end of *Skrajabinema ovis*: *a* – ♂, *b* – ♀; *CN* – cuticular nodes, *LW* – lateral wings, *CE* – cylindrical part of esophagus, *BE* – posterior part of esophagus, spherical bulbus

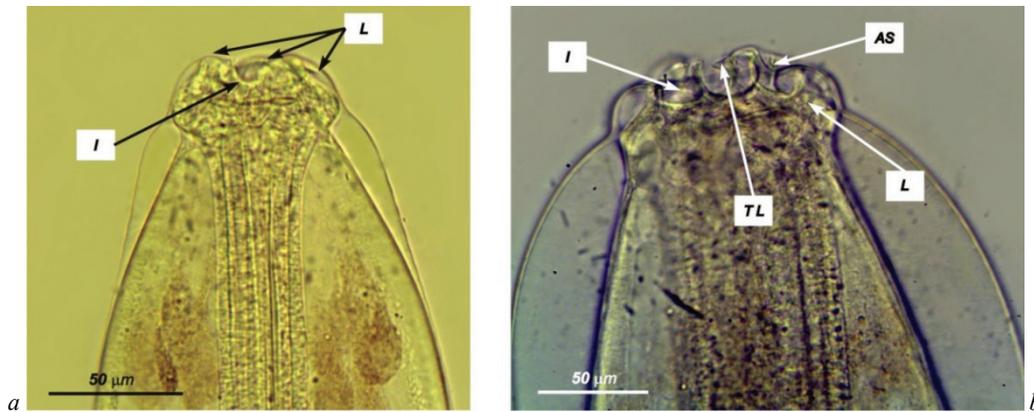


Fig. 3. Mouth organs of *Skrajabinema ovis*: *a* – ♂, *b* – ♀; *L* – lips, *AS* – anchor-shaped lips, *I* – indentation between the anterior and posterior lip wedges, *TL* – tooth-like lamella

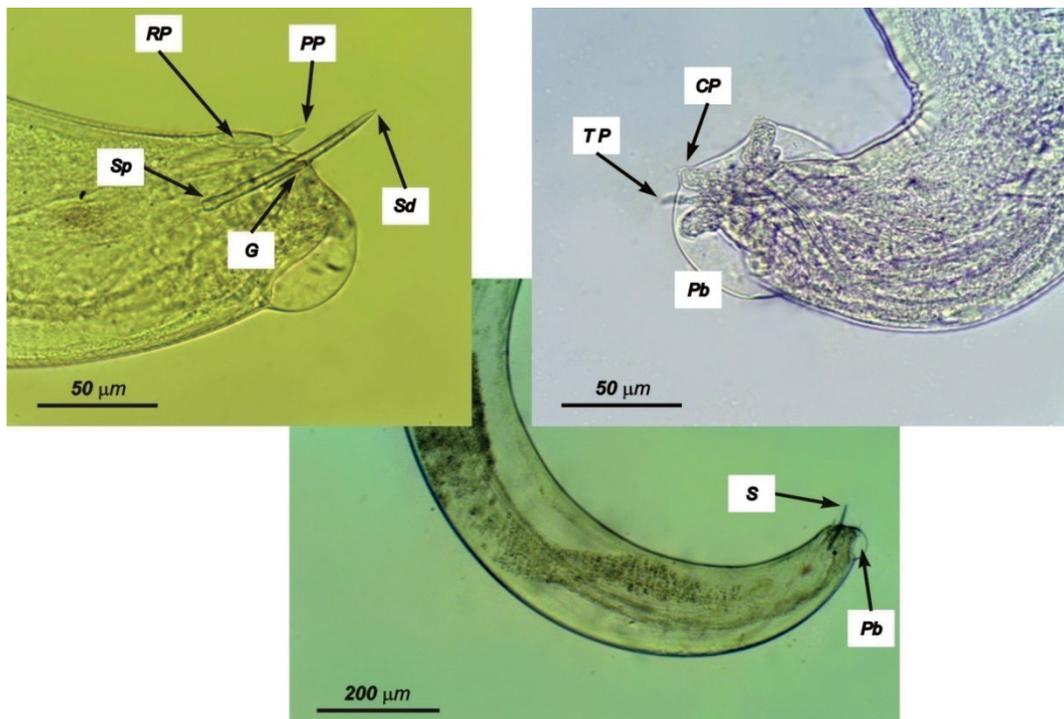


Fig. 4. Head end of ♂ *Skrajabinema ovis*: *Pb* – pseudobursa, *RP* – ribbed papilla, *S* – spicule, *Sp* – proximal end of spicule, *Sd* – distal end of spicule, *G* – gubernaculum, *PP* – post-anal papilla, *CP* – conic protrusions on the ribbed papilla, *TP* – tail protrusion

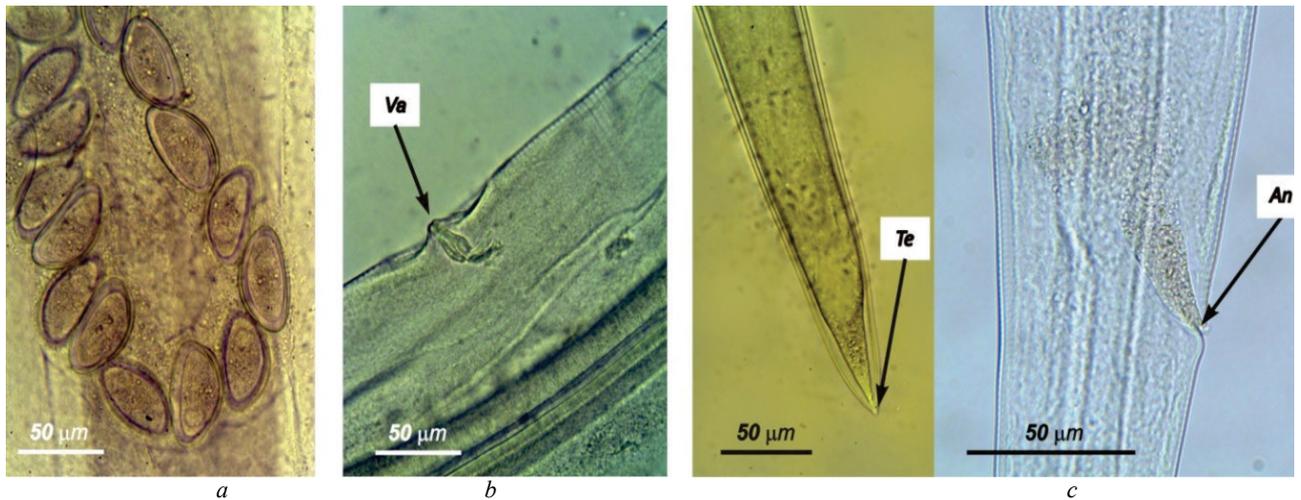


Fig. 5. ♀ *Skrjabinema ovis*: a – uterus with eggs, b – vulva area, c – tail end; Va – vulva area, An – anus, Te – tail end

Table 2

Metric parameters of ♂ *Skrjabinema ovis* nematodes (n = 15, x ± SD, min – max)

Parameters	Present study	Skrjabin et al., 1960	Ivashkin et al., 1998
Length of pseudobursa, µm	185.6 ± 15.5 (159.6–208.0)	–	–
Width of pseudobursa, µm	120.7 ± 8.0 (103.6–133.0)	–	–
Ratio of length to width of pseudobursa	1.5 : 1 (1.2 : 1 – 1.9 : 1)	–	–
Length of spicule, µm	94.4 ± 4.6 (86.5–102.3)	90–120	90–120
Width of proximal end of spicule, µm	5.7 ± 0.2 (5.1–6.0)	–	–
Width of spicule in the middle, µm	4.4 ± 0.2 (4.1–4.8)	–	–
Length of gubemaculum, µm	24.4 ± 2.1 (20.1–27.1)	19–24	–
Width of proximal end of gubemaculum, µm	5.3 ± 0.1 (5.0–5.6)	–	–
Width of gubemaculum in the middle, µm	4.0 ± 0.4 (3.2–4.7)	–	–

Note: “–” parameters were not defined.

Table 3

Metric parameters of ♀ *Skrjabinema ovis* nematodes (n = 15, x ± SD, min – max)

Parameters	Present study	Skrjabin et al., 1960	Ivashkin et al., 1998
Width of body in vulva area, µm	422.4 ± 10.4 (408.2–449.3)	–	–
Distance from vulva to head end, mm	2.1 ± 0.1 (1.9–2.3)	–	–
Distance from vulva to anus, mm	3.7 ± 0.4 (2.8–4.8)	–	–
Distance from vulva to tail end, mm	4.1 ± 0.4 (3.2–5.2)	–	–
Distance from anus to tail end, µm	338.3 ± 26.9 (290.9–392.1)	900	–
Width of body in anus area, µm	63.6 ± 3.5 (60.3–74.0)	150	–
Ratio of distance from head end to vulva to distance from vulva to tail end	0.5 : 1 (0.4 : 1 – 0.6 : 1)	3 : 5	–
Egg length, µm	57.5 ± 2.2 (52.6–60.2)	55–63	50–60
Egg width, µm	28.7 ± 1.9 (24.6–32.2)	34	30
Eggshell thickness, µm	2.0 ± 0.1 (1.8–2.3)	–	–

Note: “–” parameters were not defined.

The specific morphological characters of *S. ovis* females include the specifics of morphology of the vulva area, tail end, and shape of eggs in the uterus (Fig. 5), and their metric parameters (Table 3).

The tails of females are pointed and conical. Their copulatory apparatus is unpaired, and consists of one ovary, uterus, vagina and vulva. The vulva is slit-like, positioned as a pyramid on a slight elevation. The width of body at the vulva area was $422.4 \pm 10.4 \mu\text{m}$. In contrast, the width of body at anus was $63.6 \pm 3.5 \mu\text{m}$. The vulva is located in the anterior part of body, which is clear from the metric indices of its position. The distance from vulva to head end was smaller by 43.2%, than the distance from vulva to tail end. The ratio of the distance from the head end to vulva to the distance from vulva to tail end was 0.5 : 1. The position of the anus on the bodies of *Skrjabinema* females should also be considered. The distance from anus to tail end was $338.3 \pm 26.9 \mu\text{m}$, and the distance from vulva to anus was $3.7 \pm 0.4 \text{ mm}$.

Eggs in the uterus are morphologically distinct. They are elliptic, asymmetrical, with one flatter side and one more convex. At one egg pole, there is a slight pointed process, distinct in well-developed eggs. Metrically, the eggs were $57.5 \pm 2.2 \mu\text{m}$ long (ranging 52.6–60.2 µm), and $28.7 \pm 1.9 \mu\text{m}$ wide (24.6–32.2 µm), with eggshell thickness of $2.0 \pm 0.1 \mu\text{m}$ (1.8–2.3 µm).

Discussion

Nematodes are one of the most diverse types within the animal kingdom. One of the directions of their evolutionary development was entering other living organisms, and creating various relationships from phoresy to true parasitism. As a result, a number of nematode taxa include both free-living and parasitic species, and the transitional forms. That is why nematodes are especially interesting for parasitology, allowing the study of the adaptations to parasitism in progress (Castagnone-Sereno & Danchin, 2014; Lok, 2016; Viney, 2017). The nematodes of the order Oxyurida (Weinland, 1858) are parasitic. They can inhabit the intestines of arthropods, vertebrates, wild and domestic animals, and humans (Hugot et al., 1996; Ariyathenam et al., 2010; Kim et al., 2013; Carreno et al., 2014).

Skrjabinema ovis (Skrjabin, 1915) is a representative of the under-researched Oxyurida parasites of ruminants. Reports on distribution of this nematode species in domestic sheep give the infection rates up to 12% (Bahadori et al., 2007; Naem & Gorgani, 2011; de Souza et al., 2012). Thus, the more detailed study of differential specifics of *S. ovis* will help the timely and accurate diagnosis, and correspondingly, the understanding of the real epizootic situation in a given region. That will allow timely measures of prevention of skrjabinemosis infection in animals.

In our study, additional parameters were found that will help better identification of *S. ovis* by males and females. The sexual dimorphism in *S. ovis* nematodes is significant, and the females are significantly ($P < 0.001$) larger by 11 parameters than males. We also described the morphological differences between the male and female roundworms. They are found in the structure of mouth organs: lips of females are anchor-shaped and have one pair of tooth-like lamellae on their inner side. The lips of males are oval and lack the tooth-like lamellae. Similar morphological differences of the lips have been described in another study (Skjabin et al., 1960). We also suggest taking into account the shape and size of cuticular nodes at the head end: they are notably wider and longer in females than in males. We have not found any reports of such differences in *S. ovis* in previous publications.

We also suggest taking into account the metric parameters of *S. ovis*. Nine parameters are of interest in males, namely: length and width of the pseudobursa, ratio of these parameters, length of the spicule, width of the spicule at the proximal end and in the middle, length of the gubernaculum, width of the gubernaculum at the proximal part and in the middle. In females, seven parameters are notable: width of the body in the vulva area and anus area, distances from vulva to head end and from vulva to tail end and the ratio of these distances, distance from anus to tail end, and distance from vulva to anus. Scientific publications mention only a few scarce parameters, including the morphometric specifics of males and females. Those include length and width of body, length of esophagus, diameter of bulbous, distance from vulva to head and tail ends, lengths of spicule and gubernaculum (Shahlapoor, 1965; Ivashkin et al., 1998). We suggest also measuring the egg length and width, and eggshell thickness. The specific morphology of eggs is given in the identification keys, which mention the elliptical asymmetric shape of eggs (Andrews, 1969; Anderson, 2000), confirmed in our studies too.

The conducted morphological studies show that the morphological specifics of the pseudobursa, the presence and number of papillae that support the membrane, the position and shape of spicule and gubernaculum can be considered as differential characters of the species. In females, the morphology of the vulva and shape of the tail end should be considered. The importance of such parameters in identification of *S. ovis* nematodes has been pointed out in several publications where a number of differential features of the copulatory apparatus have been described for male and female roundworms (Schad, 1957; Skjabin et al., 1960; Ivashkin et al., 1998).

The additional suggested morphometric parameters that can be used in differentiation of male and female *S. ovis* will enhance the efficiency of their identification.

Conclusion

It is established that the morphological and metric parameters of males and females should be considered in the species identification of *Skjabinema ovis* Skjabin, 1915 nematodes. The sexual dimorphism in *S. ovis* is significant and distinct, the females are larger by 11 parameters than males. We also found differences in the morphology of the mouth organs and cuticular nodes at the head ends of the male and female parasites. Nine metric parameters are suggested in males, characterizing the structure of the pseudobursa, spicule, gubernaculum and the body sizes in the area of the copulatory apparatus. The morphology of the pseudobursa, which includes a complex structure of papillae as its base, is typical. In females, seven parameters are suggested relating to the position of vulva, anus, and body sizes at those areas. The morphological criterion of differentiation of females is the specifics of vulva and tail end shapes. Additional characters for the taxonomical evaluation of *S. ovis* may include the metric and morphological parameters of their eggs, such as shape, structure, length, width and eggshell thickness.

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