

UDC 595.132.6

NEMATODES OF THE GENUS *TRICHURIS* (NEMATODA, TRICHURIDAE), PARASITIZING SHEEP IN CENTRAL AND SOUTH-EASTERN REGIONS OF UKRAINE

V. A. Yevstafieva¹, I. D. Yuskiv², V. V. Melnychuk¹, I. O. Yasnolob¹, V. A. Kovalenko¹, K. O. Horb¹

¹Poltava State Agrarian Academy,
Skovorody st., 1/3, Poltava, 36003 Ukraine

²S. Z. Gzhytskiy National Veterinary and Biotech University of Lviv,
Pekarska st., 50, Lviv, 79010 Ukraine
E-mail: evstva@ukr.net

Nematodes of the Genus *Trichuris* (Nematoda, Trichuridae) Parasitizing Sheep in Central and South-Eastern Regions of Ukraine. Yevstafieva, V. A., Yuskiv, I. D., Melnychuk, V. V., Yasnolob, I. O., Kovalenko, V. A., Horb, K. O. — Abundance and distribution of nematodes of the genus *Trichuris* Schrank, 1788 parasitizing domestic sheep (*Ovis aries* Linnaeus, 1758) were studied in Poltava, Kyiv and Zaporizhzhia Regions of Ukraine. Three species of *Trichuris* were found, *Trichuris skrjabini* Baskakov, 1924, *Trichuris ovis* Abildgaard, 1795 and *Trichuris globulosa* Linstow, 1901. *Trichuris ovis* and *T. skrjabini* were more common (54.9 and 35.7 %), whereas *T. globulosa* was relatively rare (9.4 %) in the studied material. New species-specific and sex-related morphological characters and metric indices were reviewed as useful in better identification of *T. skrjabini*, *T. ovis* and *T. globulosa* parasitizing sheep.

Key words: *Trichuris*, sheep, fauna, abundance, morphological characters, metric indices.

Introduction

Parasitic nematodes are one of most diverse and widely distributed group of parasitic worms. They include the economically important family Trichuridae Baird, 1853 with the monotypic genus *Trichuris* Schrank, 1788. The latter comprises more than 70 nematode species parasitic in marsupials, insectivores, rodents, carnivores, lagomorphs, even-toed ungulates, and primate mammals (Fahmy, 1964; Feliu et al., 2000; Lim et al., 2008; Ravasi et al., 2012; Callejón et al., 2015; Hillman et al., 2017). A few *Trichuris* species infect humans and domestic animals (Levecke et al., 2015; Yevstafieva et al., 2015; Asmareta et al., 2016).

In many countries, *Trichuris* nematodes are common in ruminant animals, particularly in sheep (Leguia, 1991; Souza et al., 2013; Gul, Tak, 2016; Vejl et al., 2017). According to previous reports, trichurid nematodes in Europe are presented by three species, *T. skrjabini* Baskakov, 1924, *T. ovis* Abildgaard, 1795 and *T. globulosa* Linstow, 1901 (Balbo, 1977; Špakulová, 1994; Oliveros et al., 2000; Cutillas et al., 2004). *T. ovis* was also found in India, Nigeria and South America (Morales et al., 2001; Kuchai et al., 2011; Jegede et al., 2015). At the same time, *T. globulosa* was reported in Iran (Bahrami et al., 2016).

In sheep from different areas of the Chechnya republic and Moscow Oblast of Russia, two *Trichuris* species were reported, *T. skrjabini* and *T. ovis*, while only *T. ovis* was found in sheep of Ivanovo Oblast of Russia (Krjuchkova, 1993; Baysarova, 2014; Pasechnik, 2015).

The morphological studies are significant for the systematics, biology and species identification of *Trichuris* nematodes, including those parasitic in sheep. These studies were mainly focused on morphological and metric characteristics of nematodes. While discussing the taxonomic value of species-specific morphological features, various authors indicate the importance of the following characters in Trichuridae: length of the body, male tale, female tale, ornamentation of spicule sheath, shape and size of eggs, female vulval area, and biometric parameters of different body parts (Knight, 1971; Oliveros, Cutillas Barrios, 2003; Salaba et al., 2013; Kuchai et al., 2013).

In Ukraine, the fauna and distribution of *Trichuris* nematodes in domestic sheep were studied irregularly. Two species, *T. skrjabini* and *T. ovis* were reported in Kharkiv Region, identified by eggs isolated from sheep feces (Byrka et al., 2013). However, identification of *Trichuris* eggs is at times dubious. Their morphological and metric parameters are also not enough for further faunistic studies of *Trichuris* nematodes in sheep of different climate and geographic zones of the world. Hence, studying the fauna and abundance of separate taxonomic *Trichuris* entities in sheep of Ukraine, and the morphometric characters of observed roundworms will allow to add new data on population specifics and species identification of these helminthes.

Material and methods

The studies were conducted in 2016–2017 at the Laboratory of Parasitology and Veterinary-Sanitary Expertise of the Department of Veterinary Medicine of Poltava State Agrarian Academy.

The abundance indices of *Trichuris* populations were studied at sheep farms of Poltava, Kyiv and Zaporizhzhia Regions of Ukraine. The main indicators were the abundance index and the intensity of infection (Ripolovskyi and Yuskiv, 2010). Nematodes were collected during helminthological investigation of the large intestine of dead or killed sheep (Skrjabin, 1928). Roundworms were identified according to Skrjabin (1957) and Ivashkin et al. (1989). Altogether 917 specimens of adult nematodes of the genus *Trichuris* were studied (327 *T. skrjabini*, 504 *T. ovis*, and 86 *T. globulosa*).

Morphometric parameters of the nematodes were analyzed 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. Photomicrographs were taken using a 5Mpix digital camera mounted on the MikroMed (China) microscope. Statistical processing of the experimental results was carried out using the Student t-test (Lapach et al., 2001).

Results and discussion

Nematodes of the genus *Trichuris* spp. were found to be very common in sheep in the central and south-eastern regions of Ukraine. Prevalence was 65.9 %, abundance was 6.2, and intensity of infection was 1 to 35 specimens per sheep.

Trichuris in sheep were represented by three species: *T. ovis*, *T. skrjabini* and *T. globulosa*. The majority of nematodes belonged to the species *T. ovis* (54.9 % of collected nematodes, abundance 3.4) and *T. skrjabini* (35.7 %, 2.2), *T. globulosa* was rarer (9.4 %, 0.6) (fig. 1).

Collected nematodes of the genus *Trichuris* had typical differential morphological and metric species characteristics in both adult males and females (size, shape and structure of the body).

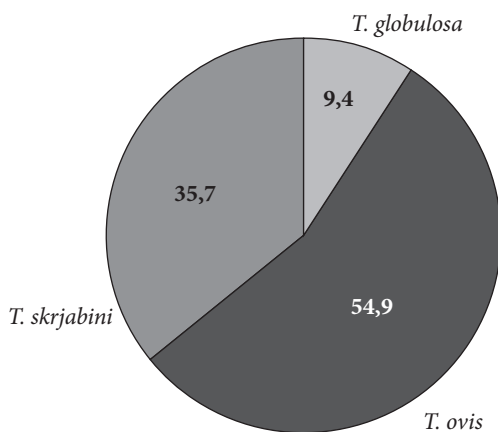


Fig. 1. The species ratio of nematodes of the genus *Trichuris*, isolated from sheep in central and south-eastern regions of Ukraine.



Fig. 2. Anterior ends of ♂ and ♀ in *T. globulosa* (a); *T. ovis* (b); *T. skrjabini* (c); ×100, ×400.

Table 1. Morphometric parameters of ♀ and ♂ *T. globulosa*, *T. ovis* and *T. skrjabini* from sheep (*Ovis aries*), $M \pm m$, $n = 15$

| Characters | ♀ | | | ♂ | | |
|---------------------------------------|---------------------|-------------------|---------------------|---------------------|----------------|---------------------|
| | <i>T. globulosa</i> | <i>T. ovis</i> | <i>T. skrjabini</i> | <i>T. globulosa</i> | <i>T. ovis</i> | <i>T. skrjabini</i> |
| Length of body, mm | 54.10 ± 0.76** | 57.77 ± 1.51 *** | 69.78 ± 0.67*** | 48.83 ± 1.03 | 49.73 ± 1.35 | 62.60 ± 1.11 |
| Anterior to posterior body parts atio | 2.9 : 1 | 3.0 : 1 | 2.3 : 1 | 2.9 : 1 | 2.2 : 1 | 1.8 : 1 |
| Width of body at anterior end, µm | 159.98 ± 3.48** | 198.52 ± 6.19 | 212.01 ± 6.35 | 146.92 ± 2.38 | 186.98 ± 5.60 | 200.74 ± 3.99 |
| Width of body at posterior end, µm | 790.67 ± 5.93*** | 912.06 ± 17.42*** | 826.59 ± 23.74*** | 667.44± 7.42 | 720.90 ± 7.37 | 560.70 ± 4.56 |

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with data for ♂.



Fig. 3. Tail end of ♀ in *T. globulosa* (a); *T. ovis* (b); *T. skrjabini* (c); × 400.

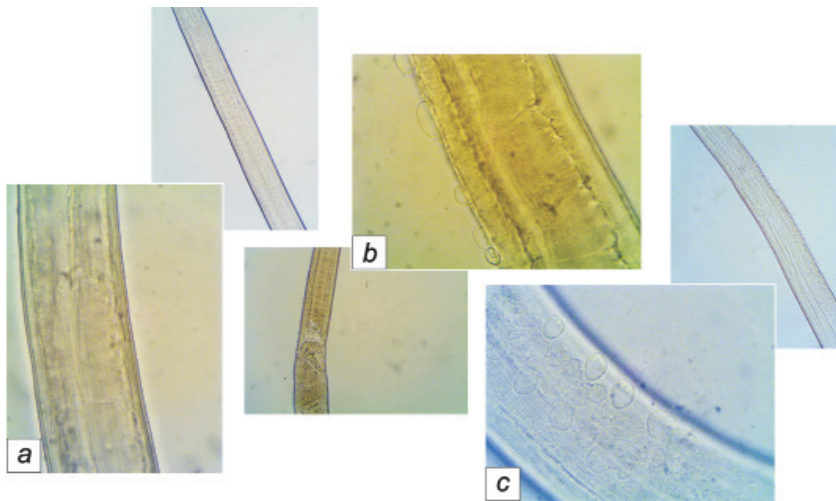


Fig. 4. Cuticle surface of ♂ and ♀ in *T. globulosa* (a); *T. ovis* (b); *T. skrjabini* (c); × 100, × 400.

The specific morphology of *Trichuris* nematodes includes thin, filiform, long, translucent anterior body part in which the esophagus is located. The posterior part of *Trichuris* body is thick, short, and usually white. The mouth is small, oval, surrounded by weakly expressed lips (fig. 2, *a-c*). The head end of *T. ovis* had two lateral, wing-shaped nodes (fig. 2, *b*), which is the diagnostic character of the species.

Sexual dimorphism is very distinct in body size (table 1), tale end structure, and the sexual apparatus. Females are significantly longer than males: by 9.7 % in *T. globulosa* ($p < 0.01$); by 13.9 % in *T. ovis* ($p < 0.001$); by 10.2 % in *T. skrjabini* ($p < 0.001$).

The ratio of anterior to posterior body parts varied significantly from 2.3 : 1 to 3.0 : 1 in females and from 1.8 : 1 to 2.9 : 1 in males. The female body at anterior end was significantly wider, by 8.1 %, than male body only in *T. globulosa* ($p < 0.01$). At the same time female body at the posterior end was significantly wider (by 15.5 — 32.1 %, $p < 0.001$) than male bodies (560.70 ± 4.56 – $720.90 \pm 7.37 \mu\text{m}$).

Tail end of females is slightly curved, in males it is a spiral. The tail ends of *T. ovis*, *T. skrjabini* and *T. globulosa* females are similar, however there are insignificant differences: in *T. ovis* it is slightly bent, while in *T. skrjabini* and *T. globulosa* it is bluntly rounded; in *T. ovis* and *T. skrjabini*, anus is subterminal, in *T. globulosa* it is almost terminal (fig. 3, *a-c*).

The structure of the cuticle surface in the collected nematodes of the genus *Trichuris* can be regarded as specific-specific. For example, the cuticle of male and female *T. globulosa* nematodes is even, with a slight gentle transverse striation, without any formations on its surface (fig. 4, *a*). At the same time, *T. ovis* and *T. skrjabini* have bubble-shaped transparent cuticle protrusions that are clearly visible on their body surface (fig. 4, *b, c*).

The species can be differentiated by metric indices. In *T. ovis* females the distance of head end to the area with bubble-shaped cuticle formations is longer by 7.9 % ($p < 0.05$) than in *T. skrjabini* (0.58 ± 0.02 mm), although the males do not significantly differ by this character (0.58 ± 0.02 and 0.55 ± 0.01 mm respectively). The length of body area with cuticle protrusions and the height of protrusions in both female and male *T. skrjabini* is greater by 28.7–31.4 % and 42.0–45.5 % ($p < 0.001$) than in *T. ovis* (1.61 ± 0.06 – 1.72 ± 0.03 mm and 8.95 ± 0.20 – $9.57 \pm 0.29 \mu\text{m}$, respectively) (table 2, 3).

To identify *T. globulosa*, *T. ovis* and *T. skrjabini* nematodes, we took into account the following morphological features: in females, structure of the vulval and vaginal region; in males, structure of the tail end, namely the spicule and the spicule sheath, as well as some metric parameters, given in tables 2 and 3.

In *T. globulosa* females, the characteristic morphological character is the presence of a slit-shaped vulva with rounded margins without any cuticular protrusions and formations.

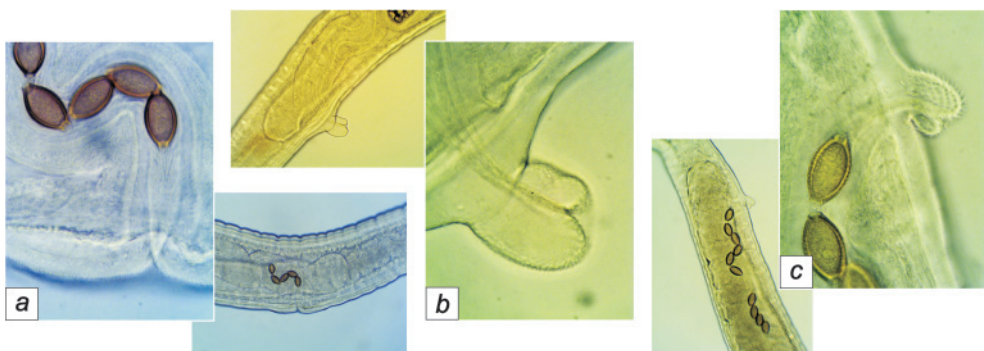


Fig. 5. Vulval area of ♀ *T. globulosa* (*a*); *T. ovis* (*b*); *T. skrjabini* (*c*); $\times 100$, $\times 400$.

Table 2. Morphometric parameters of ♀ *T. globulosa*, *T. ovis* and *T. skrjabini*. M ± m (min–max), n = 15

| Characters | <i>Trichuris globulosa</i> | <i>Trichuris ovis</i> | <i>Trichuris skrjabini</i> |
|---|---|------------------------------------|--------------------------------------|
| Length of body, mm | 54.10 ± 0.76 *■■■ (49.50–59.00) | 57.77 ± 1.51 (47.00–68.00) | 69.78 ± 0.67 *** (64.00–74.50) |
| Length of anterior body part, mm | 40.27 ± 0.71 ■■■ (35.00–44.00) | 42.37 ± 1.09 (35.00–50.00) | 48.50 ± 0.67 *** (42.00–52.00) |
| Length of posterior body part, mm | 13.83 ± 0.50 ■■■ (10.50–17.50) | 15.40 ± 1.13 (6.00–25.00) | 21.28 ± 0.67 *** (15.00–25.00) |
| Width of body at the anterior end, µm | 159.98 ± 3.48 ***■■■ (140.22–189.33) | 198.52 ± 6.19 (165.20–243.89) | 212.01 ± 6.35 (179.60–246.14) |
| Width of body at the posterior end, µm | 790.67 ± 5.93 *** (731.08–804.43) | 912.06 ± 17.42 (818.32–1043.31) | 826.59 ± 23.74 ** (612.38–973.47) |
| Distance from the anterior end to the area with bubble-like protrusions, mm | – | 0.63 ± 0.02 (0.49–0.71) | 0.58 ± 0.02 * (0.44–0.67) |
| Length of area with bubble-like protrusions, mm | – | 1.72 ± 0.03 (1.52–1.91) | 2.51 ± 0.05 *** (2.12–2.75) |
| Height of bubble-like protrusions, µm | – | 9.57 ± 0.29 (8.11–11.67) | 17.59 ± 0.57 *** (12.11–20.04) |
| Width of body at the vulval area, µm | 335.36 ± 4.04 **■ (311.87–361.97) | 359.87 ± 7.71 (306.22–412.35) | 314.40 ± 7.74 *** (219.45–345.06) |
| Height of cuticle protrusion at the middle of vulval area, µm | – | 82.72 ± 1.84 (72.85–98.93) | 52.27±0.42 *** (49.32–54.69) |
| Height of anterior lip of the vulval cuticle protrusion, µm | – | 96.78 ± 2.88 (80.50–126.40) | 56.33 ± 0.99 *** (49.55–62.40) |
| Width of anterior lip of the vulval cuticle protrusion, µm | – | 77.00 ± 2.79 (58.97–99.33) | 31.86 ± 0.47 *** (29.10–35.14) |
| Height of posterior lip of the vulval cuticle protrusion, µm | – | 78.72 ± 1.79 (65.90–88.22) | 38.54 ± 0.48 *** (35.44–41.22) |
| Width of posterior lip of the vulval cuticle protrusion, µm | – | 44.69 ± 1.50 (34.33–55.67) | 23.46 ± 0.46 *** (19.67–26.01) |
| Width of the vulval cuticle protrusion at the base, µm | – | 170.30 ± 5.24 (137.58–196.67) | 45.46 ± 0.19 *** (40.27–49.36) |
| Height of the vulval cuticle protrusion at the apex, µm | – | 159.82 ± 7.27 (106.39–197.22) | 49.98 ± 0.46 *** (47.36–52.33) |
| Length of spines of the vulva, µm | – | 4.79 ± 0.22 (4.19–7.61) | 8.24 ± 0.22 *** (6.23–9.24) |
| Length of eggs in the gonads, µm | 58.95 ± 0.26 *** (56.61–59.76) | 73.81 ± 0.87 (68.78–80.32) | 72.54 ± 0.42 (69.11–74.64) |
| Width of eggs in the gonads, µm | 29.94 ± 0.16 *** (29.25–30.93) | 38.11 ± 1.01 (30.41–44.69) | 36.95 ± 0.22 (35.61–38.29) |

* p < 0.05; ** p < 0.01; *** p < 0.001 compared with values of *T. ovis*; ■ p < 0.05; ■■■ p < 0.001 compared with values of *T. skrjabini*.

The vagina is short, covered with spines, with well developed muscular walls and angular bends in the distal part. In most studied specimens, eggs were located in the proximal part of vagina, in a single row (fig. 5, *a*). In *T. ovis* and *T. skrjabini* females, vulva region was similar. The vulva itself extrudes as two lips of slightly posteriorly bent cylindrical cuticular protrusion. This protrusion is irregularly covered with spines. The vagina is long, muscular, and curved (fig. 5, *a, b*). In most of *T. skrjabini* females, eggs were located in a single row in the proximal part of the vagina, whereas in *T. ovis* eggs were arranged in several rows or in a pile in the middle or distal part of vagina.

Study of the metric indices of *Trichuris* females isolated from sheep revealed significant difference in most of the values in comparisons of selected species, which indicates their

Table 3. Morphometry of males of *T. globulosa*, *T. ovis* and *T. skrjabini*, $M \pm m$ (min–max), $n = 15$

| Characters | <i>Trichuris globulosa</i> | <i>Trichuris ovis</i> | <i>Trichuris skrjabini</i> |
|---|---|--------------------------------------|--|
| Length of body, mm | 48.83 \pm 1.03 ■■■ (40.00–54.00) | 49.73 \pm 1.35 (42.00–57.50) | 62.60 \pm 1.11 *** (57.00–70.50) |
| Length of anterior body part, mm | 36.13 \pm 0.73 *■ (29.00–40.00) | 33.57 \pm 0.97 (27.50–39.50) | 40.10 \pm 1.06 *** (33.50–46.50) |
| Length of posterior body part, mm | 12.70 \pm 0.59 **■■■ (10.00–16.50) | 16.17 \pm 0.93 (9.00–22.00) | 22.50 \pm 0.79 *** (19.00–29.50) |
| Width of body at anterior end, μ m | 146.92 \pm 2.38 ***■■■ (133.78–168.18) | 186.98 \pm 5.60 (154.14–214.18) | 200.74 \pm 3.99 (174.58–228.67) |
| Width of body at posterior end, μ m | 667.44 \pm 7.42 ***■■■ (640.87–752.65) | 720.90 \pm 7.37 (658.09–766.14) | 560.70 \pm 4.56 *** (531.87–592.01) |
| Distance from anterior end to the area with bubble-like formations, mm | – | 0.58 \pm 0.02 (0.44–0.68) | 0.55 \pm 0.01 (0.41–0.64) |
| Length of body area with bubble-like formations, mm | – | 1.61 \pm 0.06 (1.19–1.89) | 2.26 \pm 0.06 *** (1.79–2.58) |
| Height of bubble-like formations, μ m | – | 8.95 \pm 0.20 (7.69–10.52) | 15.44 \pm 0.59 *** (11.64–19.22) |
| Spicule length, mm | 4.99 \pm 0.07 ***■■■ (4.50–5.41) | 6.82 \pm 0.03 (6.59–6.95) | 0.96 \pm 0.02 *** (0.87–1.19) |
| Width of proximal spicule end, μ m | 98.84 \pm 2.34 ***■■■ (82.11–112.67) | 136.64 \pm 1.10 (129.28–142.68) | 28.60 \pm 0.41 *** (25.67–30.97) |
| Width of spicule at the middle, μ m | 49.70 \pm 0.64 ***■■■ (45.17–53.33) | 38.63 \pm 0.87 (34.23–45.01) | 14.52 \pm 0.43 *** (11.33–16.42) |
| Width of spicule sheath at the middle, μ m | 67.52 \pm 0.90 ■■■ (59.17–70.44) | 64.96 \pm 1.16 (56.83–71.46) | 32.62 \pm 0.86 *** (27.69–39.22) |
| Length of spines at the surface of spicule sheath, μ m | 8.42 \pm 0.28 ***■■■ (6.67–11.00) | 4.29 \pm 0.15 (3.21–5.40) | 3.85 \pm 0.11 * (3.15–4.52) |
| Length of spherical dilation of spicule sheath, μ m | 171.31 \pm 1.63 *** (160.87–182.15) | 123.03 \pm 2.56 (103.43–135.17) | – |
| Width of spherical dilation of spicule sheath, μ m | 191.91 \pm 4.44 (162.85–224.36) | 181.09 \pm 3.46 (153.87–199.67) | – |
| Length of cylindrical protrusion of spherical dilation of spicule sheath, μ m | 81.43 \pm 2.67 (60.98–95.44) | – | – |
| Width of cylindrical protrusion of spherical dilation of spicule sheath, μ m | 61.59 \pm 0.45 (58.09–64.68) | – | – |
| Length of distal end of withdrawn distal end of spicule sheath, μ m | – | – | 158.12 \pm 2.53 (142.93–171.23) |

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to values of *T. ovis*; ■ $p < 0.01$; ■■■ $p < 0.001$ compared to values of *T. skrjabini*.

possible use as diagnostic species characters (table 2). The bodies of *T. ovis* were shorter by 17.2 % ($p < 0.001$) than those of *T. skrjabini* and 6.3 % ($p < 0.05$) longer than those of *T. globulosa*. At the same time, *T. skrjabini* females were longer by 22.4 % ($p < 0.001$) than those of *T. globulosa*. Characteristically, the anterior and posterior parts of body in *T. skrjabini* were longer by 12.6 – 27.6 % ($p < 0.001$) than in *T. ovis* and by 16.9 – 35.0 % ($p < 0.001$) than in *T. globulosa*. The anterior end was significantly wider ($p < 0.001$) in *T. skrjabini* (24.5 %) and *T. ovis* (19.4 %) compared with *T. globulosa*. However, the body at posterior end was wider in *T. ovis* by 9.3 % ($p < 0.01$) and 13.3 % ($p < 0.001$) compared with *T. skrjabini* and *T. globulosa*, respectively. The body widths in the vulval area also were different. It was the least in *T. ovis* females ($359.87 \pm 7.71 \mu$ m), which is by 12.6 % ($p < 0.001$) less than in *T. skrjabini*, and by 6.8 % ($p < 0.01$) than in *T. globulosa*.

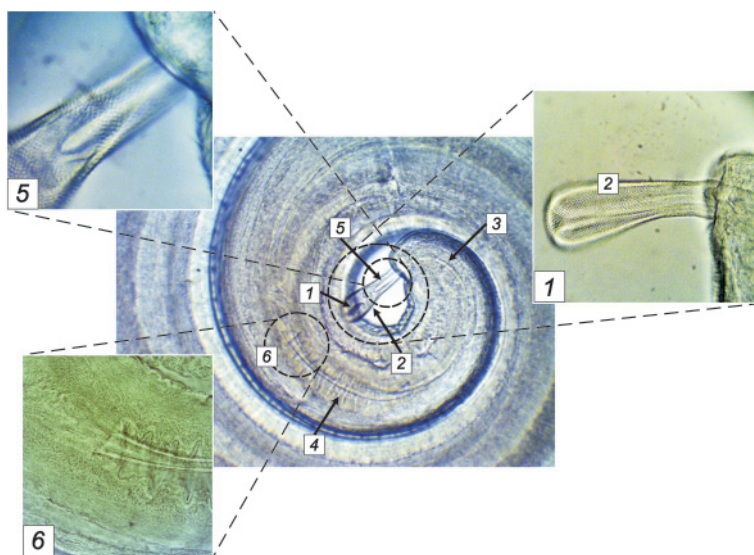


Fig. 6. Tail end of ♂ *T. skrjabini* (×50, ×100, ×400, ×1000): 1 — dilated distal end of spicule sheath; 2 — spines of spicule sheath; 3 — spicule; 4 — spicule sheath; 5 — distal end of spicule; 6 — proximal end of spicule.

Since *T. ovis* and *T. skrjabini* appear to be the most morphologically similar species, most of the morphometric indices were compared for these *Trichurus* species. Significant differences are found in metric indices of vulval cuticular protrusion and spines that cover it. For example, the cuticle protrusion characteristics (total height and width, height and width of anterior and posterior lips) in *T. ovis* are significantly higher (by 36.8–73.3 %, $p < 0.001$) than in *T. skrjabini*. At the same time, the length of spines covering the vulval area in *T. ovis* females is in contrast lesser (by 41.8 %, $p < 0.001$).

The size of eggs in the uterine cavity or vagina did not significantly differ in *T. ovis* and *T. skrjabini* ($73.81 \pm 0.87 \times 38.11 \pm 1.01$ and $72.54 \pm 0.42 \times 36.95 \pm 0.22$ μm). At the same time, the eggs of *T. globulosa* were smaller in length and width by 20.1 and 21.4 % ($p <$

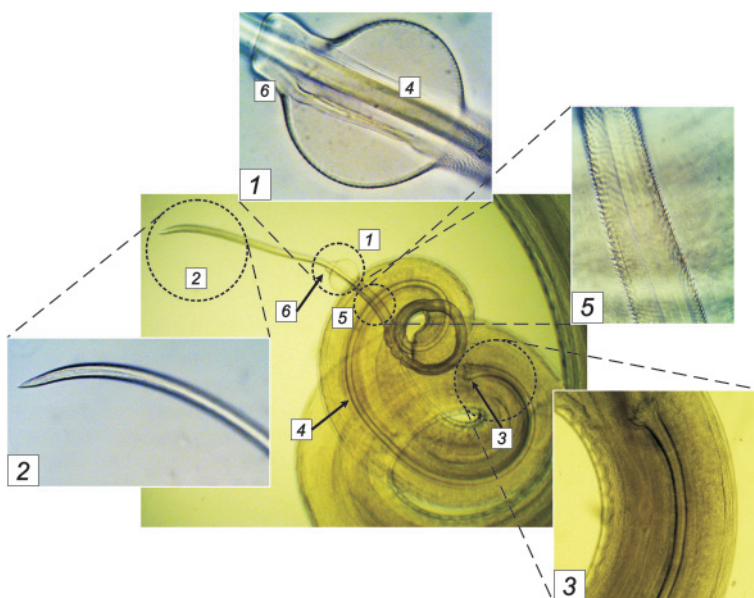


Fig. 7. Tail end of ♂ *T. globulosa* (×50, ×100, ×400, ×1000): 1 — spherical dilation of distal end of spicule sheath; 2 — distal end of spicule; 3 — proximal end of spicule; 4 — spicule; 5 — spines at spicule sheath; 6 — cylindrical protrusion at the apex of the spherical dilation of spicule sheath.

0.001) than those of *T. ovis*, and by 18.7 and 18.9 % ($p < 0.001$) than those of *T. skrjabini*. It also can be considered a differential feature.

Males of studied *Trichuris* spp. also had characteristic morphological features. In *T. skrjabini*, the spicule sheath in the withdrawn state is rather small and has a small dilation, is covered with numerous spines and always covers the distal end of the spicule. The spicule is short, has a rounded, narrowed distal end and dilated proximal end in the form of a handle (fig. 6).

Males of *T. globulosa* and *T. ovis* were morphologically similar, as also noted by other authors (Skrjabyn, 1928; Barus et al., 1977; Špakulová, 1994). In *T. globulosa*, the withdrawn pouch is long, at the distal end it has a globular dilation. The cylindrical protrusion at the apex of spherical dilation of spicule sheath is characteristic and distinctive of *T. ovis*. The spicule pouch is covered with spines, arranged in rows. The spicule is long, distally pointed, the proximal end is expanded in a funnel (fig. 7).

Male *T. ovis* also has a significant dilation at the distal end of the spicule sheath when it is withdrawn. This dilation is clearly limited and visible. The spicule sheath is covered with spines. The spicule is quite long and, as in *T. globulosa*, has a pointed distal end and a dilated proximal end with uneven margins (fig. 8).

The metric characteristics of males of studied *Trichurus* species are significantly different and can be used in species identification (table 3).

Thus, *T. skrjabini* males were longer by 20.5 and 21.9 % ($p < 0.001$) than those of *T. ovis* and *T. globulosa*, respectively. The length of the anterior and posterior body parts in *T. skrjabini* were greater by 16.2 and 28.1 % ($p < 0.001$) than in *T. ovis*, and by 9.9 and 43.5 % ($p < 0.01 \dots p < 0.001$) than in *T. globulosa*. At the same time, the anterior body

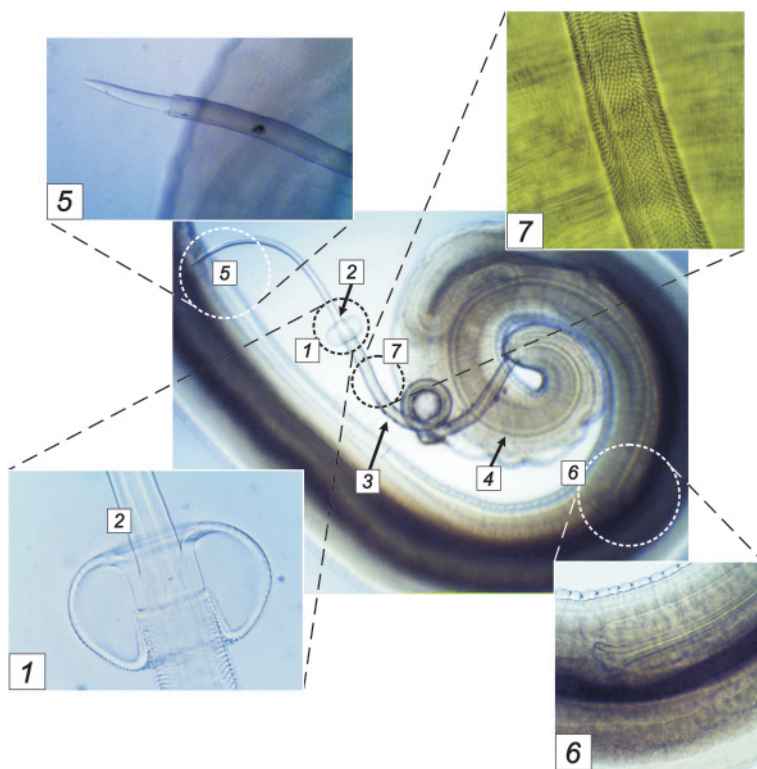


Fig. 8. Tail end of ♂ *T. ovis* ($\times 50$, $\times 100$, $\times 400$, $\times 1000$): 1 — spherical dilation of the distal end of spicule sheath; 2 — apex of spherical dilation of spicule sheath; 3 — spicule sheath; 4 — spicule; 5 — distal end of spicule; 6 — proximal end of spicule; 7 — spines of spicule sheath.

part of *T. globulosa* in comparison with *T. ovis* was longer by 7.0 % ($p < 0.05$), and its posterior part was shorter by 21.4 % ($p < 0.01$). Width indices of different body parts of the examined nematode species also differed. The head end of the *T. skrjabini* body was wider (by 6.8 %, $p < 0.001$), and the tail end was more narrow (by 15.9 %, $p < 0.001$) than those of *T. globulosa*. In *T. ovis*, the anterior body part was wider by 21.4 % ($p < 0.001$) than in *T. globulosa*, and the posterior body part was wider than in *T. skrjabini* and *T. globulosa* by 22.2 and 7.4 % ($p < 0.001$).

The size of spicule can be considered a differential metric character of *Trichuris* males. In *T. skrjabini*, it was the shortest (0.96 ± 0.02 mm). It is less than that in *T. ovis* (6.82 ± 0.03 mm), in which it was the longest, and in *T. globulosa* (4.99 ± 0.07 mm), by 85.9 and 80.7 % respectively ($p < 0.001$). Moreover, although the spicules in *T. ovis* and *T. globulosa* seemed identical morphologically and visually by estimating its length, according to metric indices there are significant differences between these species. The spicule of male *T. ovis* is significantly longer (by 26.8%, $p < 0.001$) than in *T. globulosa*.

Other features, distinguishing the morphologically similar males of *T. globulosa* and *T. ovis* are the length of spines that cover the spicule sheath, and the length of the spherical dilation of withdrawn spicule sheath. Spines and spherical dilation of the spicule sheath of *T. ovis* were shorter in two (4.29 ± 0.15 μ m) and 1.4 times (123.03 ± 2.56 μ m) respectively than in *T. globulosa* ($p < 0.001$).

Differential metric indicators also include the width of spicule and the spicule sheath in the middle. In *T. skrjabini*, these values were the smallest (14.52 ± 0.43 and 32.62 ± 0.86 μ m) as compared to *T. ovis* (by 62.4 and 49.7 %, $p < 0.001$) and *T. globulosa* (by 70.7 and 51.6 %, $p < 0.001$). For *T. ovis* and *T. globulosa*, the indices were higher by 22.2 and 3.7 % ($p < 0.001$) in *T. globulosa*.

We found one more character specific of *T. globulosa* males. It was not previously described in the literature, and, in our opinion, can facilitate its identification. It is the cylindrical protrusion at the apex of the spherical dilation of withdrawn spicule sheath (fig. 7). This character is absent in *T. ovis* males that are morphologically very similar to those of *T. globulosa*. The length of cylindrical protrusion was 81.43 ± 2.67 μ m and its width was 61.59 ± 0.45 μ m.

Summarizing the results, it can be noted that nematodes of the genus *Trichuris* are common in domestic sheep (*Ovis aries*) in the central and southeastern regions of Ukraine (Kyiv, Poltava and Zaporizhzhia Regions). *Trichuris* nematodes of sheep here are represented by three species, *T. skrjabini*, *T. ovis* and *T. globulosa*, of which *T. ovis* and *T. skrjabini* are more prevalent.

Most authors, however, indicate *T. ovis* as the most prevalent species, explaining it with the faster development cycle of this parasite (Hinks, Thomas, 1974; Morales et al., 2001; Kuchai et al., 2011; Jegede et al., 2015). In our opinion, the local dominance of *T. ovis* and *T. skrjabini* can be explained by favorable environmental conditions for the development of embryonic stages of parasites.

Most of morphological parameters (size and shape of body; length and shape of spicule, spicule sheath, and the spines that cover it; shape and structure of vulva and several metric parameters of vulvar formations), mentioned by other scientists (Barus et al., 1977; Barus et al., 1978; Cutillas et al., 1995; Oliveros, Cutillas, 2003; Callejón, 2015), were used in species identification. However, the morphological similarity of *T. ovis* and *T. skrjabini* females, and that of *T. globulosa* and *T. ovis* males, and also the fact that it is not always possible to isolate both males and females of one species inspired us to obtain new data on the significantly different morphometric characteristics of males and females of studied *Trichurus* species. We established that, in addition to sex-related characters, it is necessary to take into account the morphological features of the nematode anterior end, and the metric indices of cuticle formations on their body surface.

Conclusion

The nematodes of the genus *Trichuris* (Schrunk, 1788), which parasitize domestic sheep (*Ovis aries* Linnaeus, 1758), are common in the central and southeastern regions of Ukraine. The prevalence reached 65.9 %, and the abundance index was 6.2 specimens. We found three *Trichuris* species, of which *T. ovis* (abundance index 3.4 specimens) and *T. skrjabini* (2.2 specimens) are prevalent, while *T. globulosa* (0.6 specimens) is less common. Differential species characters of adult roundworms of the genus *Trichuris* are morphometric. *Trichuris ovis* is characterized by the presence of two lateral, wing-like nodes at the anterior end. In contrast, *T. ovis* and *T. skrjabini* species are characterized by the bubble-like cuticle protrusions on their anterior body parts, which in these species significantly differ in metric values. Due to the morphological similarity between the females of *T. ovis* and *T. skrjabini*, and between the males of *T. ovis* and *T. globulosa*, we propose to determine the metric parameters of vulval area structures in females, and spicules and spicule sheath in males for more efficient identification of *Trichuris* nematodes parasitizing sheep in the study region.

References

- Asmarea, K., Sheferawa, D., Aragawa, K., Aberaa, M., Sibhatb, B., Hailec, A., Kiarad, H., Szonyie, B., Skjervef, E., Wieland, B. 2016. Gastrointestinal nematode infection in small ruminants in Ethiopia: A systematic review and meta-analysis. *Acta Tropica*, **160**, 68–77. doi: 10.1016/j.actatropica.2016.04.016.
- Bahrami, A. M., Hosseini, E., Baran, A. I. 2016. A study on histopathological changes due to zoonotic nematodes in sheep in Ilam province, Iran. *Journal of Zoonotic Diseases*, **1** (1), 47–53.
- Balbo, T., Costantini, R., Gallo, M. G., Lanfranchi, P. 1977. Distribution of nematode parasites of the digestive system in sheep (*Ovis aries*) and goats (*Capra hircus*) of the Piedmontese and Valdostano Alpine arc. *Parasitologia*, **19** (1–2), 59–61.
- Barus, V., Kotrla, B., Tenora, F. 1977. A scanning electron microscopy study of spicular sheath of some trichurids (Nematoda). *Folia Parasitology*, **20**, 107–110.
- Barus, V., Kotrla, B., Tenora, F. 1978. Scanning elektron microscopic study of the vulva of some trichurids (Nematoda). *Folia Parasitology*, **25**, 31–34.
- Baysarova, Z. T. 2014. Distribution of trichocephalosis in sheep in the Chechen Republic. *Russian Journal of Parasitology*, **3**, 20–23 [In Russian].
- Byrka, V. I., Prykhodko, Yu. O., Mazannyi, O. V., Hiliieva, M. I. 2013. Peculiarities of epizootology, diagnosis and control of trichurosis and concomitant invasions of cattle at mixed keeping. *Scientific works of Separated subdivision NULESU "Crimean Agroindustrial College"*, **151**, 136–143 [In Ukrainian].
- Callejón, R., Cutillas, C., Nadler, S. A. 2015. Nuclear and mitochondrial genes for inferring *Trichuris* phylogeny. *Parasitology Research*, **114** (12), 4591–4599. doi: 10.1007/s00436-015-4705-7.
- Cutillas, C., German, P., Arias, P., Guevara, D. C. 1995. *Trichuris ovis* and *Trichuris globulosa*: morphological, biometrical and genetic studies. *Experimental Parasitology*, **81**, 621–625.
- Cutillas, C., Oliveros, R., de Rojas, M., Guevara, D. C. 2004. Determination of *Trichuris skrjabini* by sequencing of the TS1–5.8S–ITS2 segment of the ribosomal DNA: comparative molecular study of different species of trichurids. *Journal of Parasitology*, **90**, 648–652.
- Fahmy, M. A. M. 1964. Studies on some helminth parasites of small mammals (carnivores and rodents). *Zeitschrift für Parasitenkunde*, **25** (2), 135–147.
- Feliu, C., Spakulova, M., Casanova, J. C., Renaud, F., Morand, S., Hugot, J. P., Santalla, F., Durand, P. 2000. Genetic and morphological heterogeneity in small rodent whipworms in southwestern Europe: characterization of *Trichuris muris* and description of *Trichuris arvicolae* n. sp. (Nematoda: Trichuridae). *Journal of Parasitology*, **3** (86), 442–449.
- Gul, N., Tak, H. 2016. Prevalence of *Trichuris* spp. in small ruminants slaughtered in Srinagar District (J&K). *Journal of Parasitic Diseases*, **40** (3), 741–744. doi: 10.1007/s12639-014-0570-z.
- Hillman, A. E., Ash, A. L., Kristancic, A. R., Elliot, A. D., Lymbery, A. J., Robertson, I. D., Thompson, R. C. 2017. Validation of various parasite detection tests for use in the Australian marsupials quenda (*Isodon obesulus*) and brushtail possums (*Trichosurus vulpecula*). *Journal of Vet-*

- erinary Diagnostic Investigation. *Journal of Veterinary Diagnostic Investigation*, **29** (1), 64–75. doi: 10.1177/1040638716674509.
- Hinks, M. I., Thomas, R. J. 1974. A new record of the occurrence of *Trichuris skrjabini* Baskakov, 1924 in sheep in Britain. *Journal of Helminthology*, **48** (1), 33–38. doi: 10.1017/S0022149X00022574.
- Ivashkin, V. M., Oripov, A. O., Sonin, M. D. 1989. Key to the helminthes of caprine cattle. Nauka, Moscow, 52–58 [In Russian].
- Jegede, O. C., Adejoh, A. A., Obeta, S. S., Olayemi, O. D. 2015. Gastrointestinal Parasites of Sheep and Goats in Gwagwalada Area Council, Federal Capital Territory, Abuja, Nigeria; with a special reference to sex, breed and age. *Alexandria Journal of Veterinary Science*, **46** (1), 170–176. doi: 10.5455/ajvs.177135.
- Knight, R. A. 1971. Redescription of *Trichuris discolor* (von Linstow, 1906) and *T. skrjabini* (Baskakov, 1924) from domestic ruminants in the United States and comparisons with *T. ovis* (Abildgaard, 1795). *Journal of Parasitology*, **57**, 302–310.
- Krjuchkova, Ye. N. 1993. Peculiarities of sheep trichocephalosis in farms of Ivanovo Oblast. *Proceedings of scientific studies of Moscow veterinary academy*, 106–108 [In Russian].
- Kuchai, J. A., Ahmad, F., Chishti, M. Z., Dar, J. A., Tak, H. 2013. On Morphology and Morphometry of *Trichuris ovis* Abildgaard, 1795 Recovered from Ruminants of Ladakh, India. *Journal of Buffalo Science*, **2**, 49–52.
- Kuchai, J. A., Chishti, M. Z., Zaki, M. M., Ahmad, J., Rasool, M., Dar, S. A., Tak, H. 2011. Prevalence of nematode parasites in Sheep of Ladakh-India. *Journal of Agricultural Extension and Rural Development*, **3** (13), 229–231. doi: 10.5897/JAERD11.099.
- Lapach, S. N., Chubenko, A. V., Babich, P. N. 2001. *Statistical methods in medical and biological research using Excel*. Morion, Kyiv, 11–35 [In Russian].
- Leguia, G. 1991. The epidemiology and economic impact of llama parasites. *Parasitology Today*, **7**, 54–56.
- Levecke, B., Dorny, P., Vercammen, F., Visser, L. G., Van Esbroeck, M., Vercruysse, J., Verweij, J. J. 2015. Transmission of *Entamoeba nuttalli* and *Trichuris trichiura* from Nonhuman Primates to Humans. *Emerging Infectious Diseases*, **21** (10), 1871–1872. doi: 10.3201/eid2110.141456.
- Lim, Y. A., Ngui, R., Shukri, J., Rohela, M., Mat Naim, H. R. 2008. Intestinal parasites in various animals at a zoo in Malaysia. *Veterinary Parasitology*, **157** (1–2), 154–159. doi: 10.1016/j.vetpar.2008.07.015.
- Morales, G., Pino, L. A., Sandoval, E., De Moreno, L. 2001. Gastrointestinal nematode infection in ewes raised in an arid zone of Venezuela. *Parasitología al día*, **25** (1–2), 36–39. doi: 10.4067/S0716-07202001000100007.
- Oliveros, R., Cutillas Barrios, C. 2003. Redescrípción de *Trichuris ovis* (Nematoda) (Abildgaard, 1795) parásito de *Ovis aries* (Linné, 1758) y *Capra hircus* (Linné, 1758). *Revista ibérica de parasitología*, **63** (3–4), 77–83.
- Oliveros, R., Cutillas, B. C. 2003. Redescrípción de *Trichuris ovis* (Nematoda) (Abildgaard, 1795) parásito de *Ovis aries* (Linné, 1758) y *Capra hircus* (Linné, 1758). *Revista ibérica de parasitología*, **63** (3–4), 77–83.
- Oliveros, R., Cutillas, B. C., de Rojas, M., Arias, P. 2000. Characterization of four species of *Trichuris* (Nematoda: Enoplida) by their second internal transcribed spacer ribosomal DNA sequence. *Parasitology Research*, **86**, 1008–1013.
- Pasechnik, V. E. 2015. To the epizootology to *Trichuris* spp. infection and specific composition of *Trichuris* spp. in small ruminants (sheep, goats) and wild ruminants in the Moscow Zoo and Circuses of the Moscow Region. *Theory and Practice of struggle against parasitic disease*, **16**, 330–332 [In Russian].
- Ravasi, D. F., O’Riain, M. J., Davids, F., Illing, N. 2012. Phylogenetic Evidence That Two Distinct *Trichuris* Genotypes Infect both Humans and Non-Human Primates. *PLoS One*, **7** (8), e44187. doi: 10.1371/journal.pone.0044187.
- Ripolovskiy, O. I., Yuskiv, I. D. 2010. Diplostomoses of carp in the ponds of the north-western prykarpathia. *Scientific Bulletin of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj*, **12** (3), 197–205 [In Ukrainian].
- Salaba, O., Rylková, K., Vadlejch, J., Petrtyl, M., Schánková, S., Brožová, A., Jankovská, I., Jebavý, L., Langrová, I. 2013. The first determination of *Trichuris* sp. from roe deer by amplification and sequenation of the ITS1-5.8S-ITS2 segment of ribosomal DNA. *Parasitology Research*, **112** (3), 955–960. doi: 10.1007/s00436-012-3215-0.
- Skrjabin, K. I. 1928. *The method of complete helminthological autopsy of vertebrates, including humans*. Moscow State University, Moscow, 1–43 [In Russian].
- Skrjabin, K. I., Shikhobalova, N. P., Orlov, I. V. 1957. *Trichocephalids and capillariids of animals and man and the diseases caused by them. The essentials of nematodology*. Russian Academy of Sciences, Moscow, 35–232 [In Russian].
- Souza, M. de F., Pimentel-Neto, M., Pinho, A. L., Silva, R. M., Farias, A. C., Guimarães, M. P. 2013. Seasonal distribution of gastrointestinal nematode infections in sheep in a semiarid region, north-eastern Brazil. *Brazilian Journal of Veterinary Parasitology*, **22** (3), 351–359. doi: 10.1590/S1984-29612013000300006.

- Špakulová, M. 1994. Discriminant analysis as a method for the numerical evaluation of taxonomic characters in male trichurid nematodes. *Systematic Parasitology*, **29**, 113–119.
- Vejl, P., Nechybová, S., Peřínková, P., Melounová, M., Sedláková, V., Vašek, J., Čílová, D., Rylková, K., Jankovská, I., Vadlejch, J., Langrová, I. 2017. Reliable molecular differentiation of *Trichuris ovis* and *Trichuris discolor* from sheep (*Ovis orientalis aries*) and roe deer (*Capreolus capreolus*) and morphological characterisation of their females: morphology does not work sufficiently. *Parasitology Research*, **116** (8), 2199–2210. doi: 10.1007/s00436-017-5524-9.
- Yevstafieva, V. A., Yuskiv, I. D., Melnychuk, V. V. 2015. An Investigation of Embryo and Eggshell Development in *Trichuris suis* (Nematoda, Trichuridae) under Laboratory Conditions. *Vestnik Zoologii*, **50** (2), 173–178.

Received 29 January 2018

Accepted 7 May 2018