Zoodiversity, **56**(3): 233–242, 2022
DOI 10.15407/zoo2022.03.233

Morphology

UDC 595.132.6

MORPHOLOGICAL CHARACTERISTICS OF PARASITIC NEMATODES *TRICHURIS SYLVILAGI* (NEMATODA, TRICHURIDAE)

V. Yevstafieva[1], V. Stybel[2], V. Melnychuk[1], L. Nagorna[3], N. Dmitrenko[1], O. Titarenko[1], O. Dubova[4], S. Makarets[1], S. Filonenko[1], M. Pishchalenko[1], M. Kone[1], A. Ilchenko[1]

[1]*Poltava State Agrarian University, Skovorody st., 1/3, Poltava, 36003 Ukraine*
[2]*Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj, Pekarska st., 50, Lviv, 7900 Ukraine*
[3]*Sumy National Agrarian University, G. Kondrateva st., 160, Sumy, 40021 Ukraine*
[4]*Polissia National University, Staryi Blvd st., 7, Zhytomyr, 10008 Ukraine*
E-mail: evstva@ukr.net

V. Yevstafieva (https://orcid.org/0000-0003-4809-2584)
V. Stybel (https://orcid.org/0000-0002-0285-6182)
V. Melnychuk (https://orcid.org/0000-0003-1927-1065)
L. Nagorna (https://orcid.org/0000-0001-8307-183X)
N. Dmitrenko (https://orcid.org/0000-0001-5336-2361)
O. Titarenko (https://orcid.org/0000-0002-7370-8523)
O. Dubova (https://orcid.org/0000-0002-8303-573X)
S. Makarets (https://orcid.org/0000-0002-6844-261X)
S. Filonenko (https://orcid.org/0000-0001-8360-8852)
M. Pishchalenko (https://orcid.org/0000-0001-8954-8256)
M. Kone (https://orcid.org/0000-0002-0828-6619)
A. Ilchenko (https://orcid.org/0000-0003-1862-3639)

Morphological Characteristics of Parasitic Nematodes *Trichuris sylvilagi* (Nematoda, Trichuridae). Yevstafieva, V., Stybel, V., Melnychuk, V., Nagorna, L., Dmitrenko, N., Titarenko, O., Dubova, O., Makarets, S., Filonenko, S., Pishchalenko, M., Kone, M., Ilchenko, A. — Morphometric parameters are analyzed and systematized of parasitic nematodes of the species *Trichuris sylvilagi* Tiner, 1950, obtained from *Lepus europaeus* (Pallas, 1778). The following parameters are suggested for the exact identification of female *T. sylvilagi* nematodes: the sizes of body, its anterior and posterior parts, width of body at the connection of esophagus and intestine, specific location of vesicular cuticular protrusions and vulva. Five metric parameters are established for the nematode eggs. The egg shell thickness, length and width of egg plug are suggested for use in addition to the length and width of eggs. Males of *T. sylvilagi* had strongly curved tail end, long spicule, and transparent spicule sheath which bears spines, and a bulbous inflation in its proximal part. The thirteen metric parameters, which we found to be of identification value for males, include sizes of body, length and width of its anterior and posterior parts, width of body at the connection of esophagus and intestine, sizes of spicule at various areas, and location of the vesicular cuticular protrusions. Obtained data on the metric studies of *T. sylvilagi* nematodes are compared with the published data.
Key words: *Trichuris sylvilagi*, *Lepus europaeus*, parasitic nematodes, morphological features, metric parameters.

Introduction

Parasitic nematodes are one of the most prevalent and rich in species groups of helminths. Among them, trichurids (Trichuridae Railliet, 1915) are an important group of pathogens which cause trichuroses. The pathogens have a wide variety of hosts, are species-specific and diverse (Nechybová et al., 2018; Yevstafieva et al., 2018, 2019; Yamamoto & Kalepouris, 2020; Montalbano Di Filippo et al., 2019). Scientific interest in parasitic nematodes of the genus *Trichuris* Röderer, 1761 is confirmed by the discovery and description of new species, which is possible by studying the peculiarities of their morphological structure, metric parameters, and genetic research (Robles et al., 2014; Hasegawa & Dewi, 2017; Eberhardt et al., 2019; Falcón-Ordaz et al., 2020; Zhou et al., 2021).

The taxonomy of nematodes of the genus *Trichuris* is based mainly on morphological characters. A significant amount of research has focused mainly on the study of individual organs and the reproductive system of males, rarely females (Skrjabin et al., 1957; Cutillas et al., 2009; Torres et al., 2011). Externally, all nematodes of the genus *Trichuris* share the characteristic general body structure, with the much thinner and longer anterior part of the body containing the esophagus, which is why trichurids are called whipworms (Skrjabin et al., 1957). At the same time, such features as the structure of the spicule and spicular sheath, the nature of its surface, features in the structure of the cloaca in males, the structure and location of the vulva in females are specific and serve as identification criteria in species differentiation of *Trichuris* (Mahmoud, 2002; Robles, 2011). Determination of the metric parameters of the morphological structures of nematodes should also be noted in species identification (Panti-May & Robles, 2016; Xu et al., 2016).

Scientists have found that nematodes of the genus *Trichuris* are among the most common helminths among wild species of lagomorphs. The authors note parasitism of the species *Trichuris sylvilagi* Tiner, 1950 in hares of the genus *Lepus* Linnaeus, 1758 (Czaplińska et al., 1965; Keith et al., 1986). To date, only some works are devoted to the study of morphological features of nematodes of this species. In particular, a study was conducted comparing males *T. ovis* Abildgaard, 1795, *T. skrjabini* Baskakov, 1924, *T. sylvilagi*, *T. suis* Schrank, 1788 and *T. trichiura* Linnaeus, 1771 on the basis of 15 morphometric features. When assessing the morphological similarity of the studied species, it was found that the length and width of the spicule and the length of the body were the most characteristic diagnostic features for the studied male nematodes (Špakulová, 1994). According to the identification keys, the main characteristics of morphological differentiation of *T. sylvilagi* were the length of body, the ratio of the length of anterior part of body to the length of its posterior part, the width of the anterior and posterior parts of body, the structure of the bacillary band, the structure and size of spicule and spicule sheath in males and vulva structure in females (Skrjabin et al., 1957; Gvozdev et al., 1970). According to other studies, *T. sylvilagi* is synonymous with *T. leporis* Zeder, 1803. The description of the species *T. leporis* given by Tiner (1905) was shown to refer to another species, for which the proposed name is *T. tineri* (Kutzer, 1978).

The study of morphometric species characters of the parasitic nematodes of the genus *Trichuris* is an urgent task, because they parasitize a wide range of hosts and individual species are very similar, which leads to difficulties in species differentiation. Therefore, the aim of our work was to investigate the morphological characteristics and metric parameters of *T. sylvilagi* nematodes and to conduct a comparative analysis of the obtained and the published data.

Material and methods

During 2020–2021, 15 gray hares (*Lepus europaeus* Pallas, 1778) (Skriabyn, 1928) were examined by complete helminthological dissection of the large intestine. Wild animals were obtained by hunters during the hunting season in Sumy and Poltava regions. The type of isolated *Trichuris* was determined following the identification key (Gvozdev et al., 1970). In total, 51 specimens of the species *T. sylvilagi* were collected, of which 17 were males and 34 females.

Determination of morphometric parameters of adult males and females of *T. sylvilagi* nematodes was performed using ToupView software version × 64, 4.10.17015.20200426 (Hangzhou ToupTek Photonics Co., Ltd, China) and AxioVision, Release SPS 4.8.2 (Carl Zeiss MicroImaging GmbH, Germany). Microphotography was performed using a digital camera attached to a MICROmed 5 Mpix microscope (China) and SIGETA M3CMOS 14000 14.0 MP (China). Standard deviation (SD) and average values (M) were calculated.

Results and discussion

Morphologically, *T. sylvilagi* have a morphological structure of the body characteristic of all trichurids. In particular, the anterior part of body contains the esophagus and is much thinner and longer than the posterior. Also, the posterior end of the male nematode is spirally curved dorsally. In contrast, the posterior end of the female whipworm is slightly inclined laterally, yet has a straight shape (fig. 1).

The mouth is small, surrounded by weak lips. The esophagus goes through the entire anterior part of the body (fig. 2, a) and has the muscular anterior part and the glandular

posterior one. On the surface of the cuticle of the anterior part of body there are vesicular protrusions of different sizes, visible both dorsally and laterally (fig. 2, b).

In female *T. sylvilagi* nematodes, vulva is located directly behind the connection of the esophagus and the intestine. The opening of vulva is barely visible, without protrusions and cuticular formations (fig. 3, a). The uterus is located in the posterior, thicker part of the body. It has a sac-like part filled with eggs and a thinner looping part (fig. 3, b). The tail end has the form of a blunt cone. The anus is located terminally (fig. 3, c).

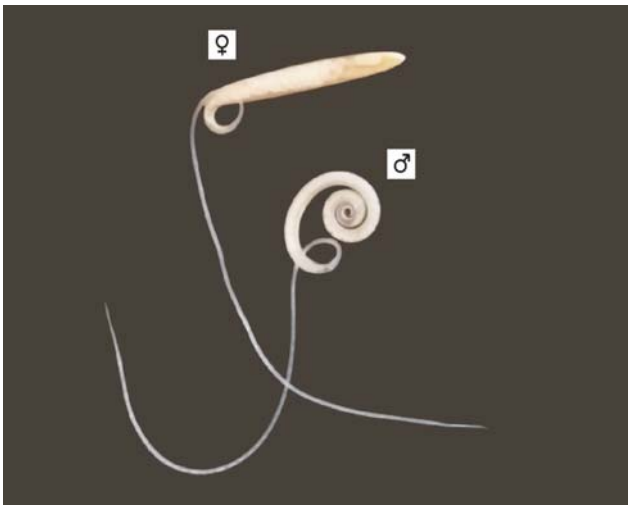

Fig. 1. External view of mature life stages of *Trichuris sylvilagi* nematodes.

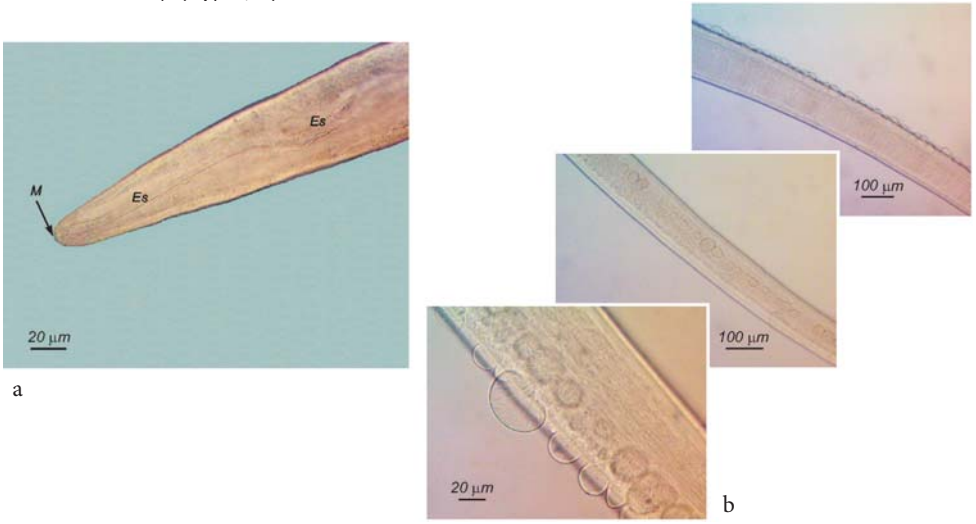

Fig. 2. Morphological characters of *Trichuris sylvilagi* nematodes: a — anterior end, mouth cavity (M), esophagus (Es); b — cuticular protrusions.

Ten of analyzed metric parameters of *T. sylvilagi* females are valuable for identification. At the same time, the number of parameters proposed by other authors for the identification of females of this species ranged from three to eight (table 1).

Thus, it has been proposed to determine the total body length of females, the length and width of the anterior and posterior parts of the body, and their ratio. We propose to use additional metric parameters to identify females of *T. sylvilagi*, which characterize the location of vesicular cuticular protrusions (2 parameters), the width of the body in the connection of esophagus and intestine, the location of the vulva relative to the head end. Metric parameters of eggs in the uterus of females of *T. sylvilagi* were also determined. Five features were identified, two of which have been previously proposed for species identification, namely: the length and width of eggs. We propose to additionally use the indicators of egg shell thickness, and the length and width of plugs.

In males of *T. sylvilagi*, the tail end is strongly curved. The front of the cloaca has a well-defined zigzag-shaped bend. Spicule one, long and thin. The spicule sheath is covered with spines, which are denser in the proximal part and somewhat less often in the distal

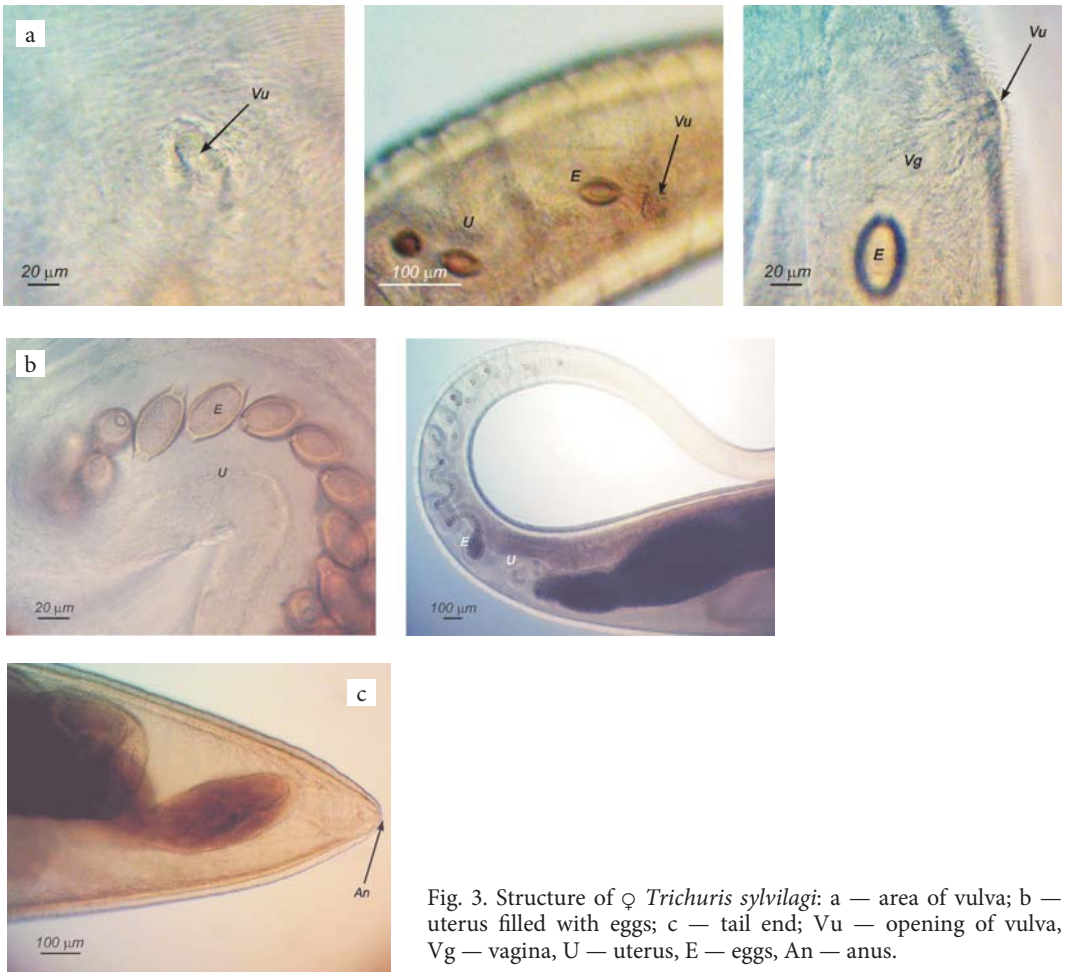

Fig. 3. Structure of ♀ *Trichuris sylvilagi*: a — area of vulva; b — uterus filled with eggs; c — tail end; Vu — opening of vulva, Vg — vagina, U — uterus, E — eggs, An — anus.

Table 1. Metric parameters of ♀ *Trichuris sylvilagi*, n = 15 (x ± SD, min–max)

Parameter	Present specimens	Skrjabin et al. (1957)	Gvozdev et al. (1970)	Hofing & Kraus (1994)	Schoeb et al. (2007)
Length of body, mm	43.41 ± 2.13 (40.0–48.0)	30.5–38.7	30.5–38.7	–	–
Length of the anterior part of body, mm	32.08 ± 2.36 (28.50–37.00)	19.3–28.7	–	17.4–20.9	17.4–20.9
Distance from the head end to the vesicular cuticular protrusions, mm	0.72 ± 0.11 (0.53–0.93)	–	–	–	–
Length of body in the area with the vesicular cuticular protrusions, mm	1.78 ± 0.12 (1.57–1.93)	–	–	–	–
Maximum width of the anterior part of body, mm	0.25 ± 0.02 (0.22–0.29)	0.144–0.170	–	–	–
Width of body at the connection of esophagus and intestine, mm	0.41 ± 0.06 (0.29–0.49)	–	–	–	–
Length of the posterior part of body, mm	11.33 ± 0.59 (10.50–12.50)	8.7–12.5	–	4.2–5.5	–

Maximum length of the posterior part of body, mm	1.60 ± 0.13 (1.44–1.89)	0.75–1.0	–	–	–
Distance from the head end to vulva, mm	34.05 ± 2.04 (30.19–38.18)	–	–	–	–
Anterior to posterior body parts ratio	2.84 : 1 (2.42 : 1–3.36 : 1)	1.8 : 1–3.3 : 1	1.8 : 1–3.3 : 1	–	–
Length of eggs in the gonads, μm	72.70 ± 2.83 (69.15–77.62)	0.068–0.073	0.068–0.073	60.0–65.0	60.0–65.0
Width of eggs in the gonads, μm	32.88 ± 1.83 (30.00–36.52)	0.035–0.037	0.035–0.037	29.0	29.0
Egg shell thickness, μm	1.22 ± 0.07 (1.10–1.33)	–	–	–	–
Egg plugl ength, μm	9.76 ± 0.71 (8.58–11.05)	–	–	–	–
Egg plug width, μm	11.90 ± 0.60 (11.08–12.99)	–	–	–	–

Note. Parameters were not defined.

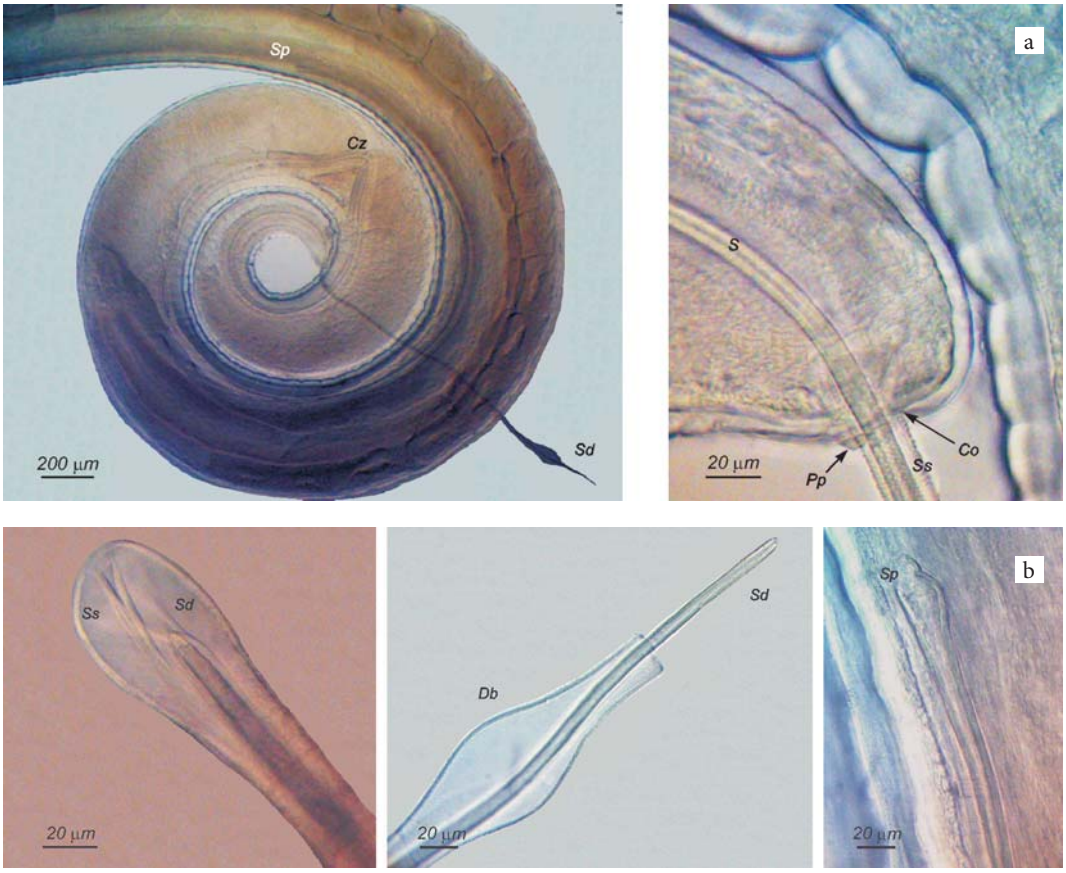

Fig. 4. Structure of ♂ *Trichuris sylvilagi*: a — tail end; b — location of spicule and spicule sheath; Co — opening of cloaca, Pp — pericloacal papilla, Cz — zigzag-shaped opening of cloaca, S — spicule, Sp — proximal end of the spicule, Sd — distal end of the spicule, Ss — spicule sheath, Db — bulbous dilation.

Table 2. Metric parameters of ♂ *Trichuris sylvilagi*, n = 15 (x ± SD, min–max)

Parameter	Present specimens	Skrjabin et al. (1957)	Czaplińska et al. (1965)	Gvozdev et al. (1970)	Hofing & Kraus (1994)	Špakulová (1994)	Schoeb et al. (2007)
Length of body, mm	40.23 ± 1.72 (37.45–42.63)	27.1–35.6	–	27.1–35.6	–	37.70 ± 3.85 (25.9–44.2)	–
Length of the anterior part of body, mm	25.84 ± 1.37 (23.24–28.22)	13.7–20.6	–	–	19.0–21.0	22.44 ± 2.54 (13.3–25.8)	19.0–21.0
Length of the posterior part of body, mm	14.39 ± 1.46 (12.19–16.69)	12.5–17.0	–	–	7.4–8.7	–	–
Anterior to posterior body parts ratio	1.81 : 1 (1.39 : 1– 2.13 : 1)	1 : 1–1 : 1.6	–	1 : 1–1 : 1.6	–	–	–
Maximum width in the anterior part of body, mm	0.20 ± 0.01 (0.18–0.22)	0.16–0.22	–	0.16–0.22	–	0.18 ± 0.01 (0.15–0.21)	–
Maximum width in the posterior part of body, mm	0.54 ± 0.03 (0.49–0.59)	0.25–0.53	–	0.25–0.53	–	0.70 ± 0.05 (0.57–0.85)	–
Distance from the head end to the vesicular cuticular protrusions, mm	0.76 ± 0.06 (0.65–0.83)	–	–	–	–	–	–
Length of body in the area with the vesicular cuticular protrusions, mm	1.97 ± 0.14 (1.77–2.18)	–	–	–	–	–	–
Width of body at the connection of esophagus and intestine, mm	0.33 ± 0.03 (0.29–0.40)	–	–	–	–	0.30 ± 0.04 (0.23 – 0.26)	–
Length of the spicule, mm	7.65 ± 0.48 (7.03–8.26)	6.5–8.8	5.6–8.2	6.5–8.8	6.0–8.0	7.17 ± 0.92 (3.96–9.12)	6.0–8.0
Width of the proximal end of spicule, μm	32.45 ± 2.77 (27.55–36.71)	–	–	–	–	30 (10–40)	–
Width of spicule in the middle, μm	10.95 ± 0.98 (9.14–12.80)	7.2–11.4	–	–	–	–	–
Width of the distal end of spicule, μm	7.80 ± 2.01 (0.69–9.12)	–	–	–	–	–	–

Note. Parameters were not defined.

part. The cloaca opens terminally. The periclocal papilla is also located in this area (fig. 4, a). The distal end of spicule is narrow and pointed, the proximal end is slightly widened with uneven edges. The spicule sheath is long, transparent, and contains a bulbous dilation, which is clearly visible when the spicule is extended (fig. 4, b).

In males of *T. sylvilagi*, 13 features can be considered as identification features. The number of parameters proposed by other authors for the differential diagnosis of nematodes of this species ranged from 1 to 8 (table 2).

Thus, it has been proposed to determine the length of body, the length and width of the anterior and posterior parts of body and their ratio, the width of body at the connection of esophagus and intestine, the length of spicule and width in the middle of spicule and of its proximal end in males. We additionally propose to use metric parameters to identify *T. sylvilagi* males, which characterize the distance from the head end to the vesicular cuticular protrusions, the length of body at their location, and the width of the distal end of the spicule.

A significant number of studies indicate the widespread prevalence of trichurosis caused by *T. sylvilagi* among the population of hares of the genus *Lepus*. Prevalence of this species range in those hosts ranged from 3.2 to 85 % (Czaplińska et al., 1965; Keith et al., 1986; Clemons et al., 2000; Tizzani et al., 2020). Therefore, the issue of correct identification of the pathogen, improving approaches to the differentiation of *T. sylvilagi* is an important area of research. This relevance is due to the fact that there are reports that indicate difficulties in identifying species of *T. sylvilagi* and *T. leporis* (Skrjabin et al., 1957) or their relationship (Kutzer, 1978).

The results of morphological studies of isolated nematodes of *T. sylvilagi* indicate that the general differential characters include the overall structure of the body (the presence of long thin anterior and short thick posterior parts, simple mouth opening, and the presence of vesicular cuticular protrusions in the anterior part of body). In males, the tail end is dorsally spirally curved, there is one long and thin spicule, wrapped in a transparent spicule sheath and covered in spines. Moreover, the spicule in the distal part forms a bulbous dilation, which is clearly visible when the spicule is extended. In females, the opening of the vulva is barely visible, the area around the opening of the vulva is smooth, without any cuticular formations. The data obtained on the important differential features of *T. sylvilagi* are consistent with most published studies (Skrjabin et al., 1957; Gvozdev et al., 1970; Hofing & Kraus, 1994).

Metric studies of adult males and females of *T. sylvilagi* proposed the definition of parameters that will provide increasing amount of data for the effective identification of these parasitic nematodes. The obtained data were also compared with those data proposed by the authors for the identification of *T. sylvilagi*. In males, we proposed to use 13 morphometric parameters for their identification. The number of parameters proposed by other authors ranged from one to eight. Thus, scientists propose to determine the total length of body of males, the length and width of the anterior and posterior parts of the body, their ratio, the width of body at the connection of esophagus and intestine, the length of the spicule and width in the middle part, the width of the proximal end of the spicule. Moreover, the data obtained by us and the results of other authors have some differences (Skrjabin et al., 1957; Czaplińska et al., 1965; Gvozdev et al., 1970; Hofing & Kraus, 1994; Špakulová, 1994; Schoeb et al., 2007). Such differences have been explained by the influence of different hosts and different geographical distribution (Skrjabin et al., 1957). Therefore, we additionally proposed to use metric parameters to identify males of *T. sylvilagi*, which characterize the distance from the main end to the vesicular cuticular protrusions and the length of body at their location, the width of the distal end of the spicule. Some researchers also note that although the determination of metric parameters of spicules is the best diagnostic feature for male *Trichuris*, these characteristics alone are not sufficient to distinguish between species (Špakulová, 1994).

To effectively identify females of *T. sylvilagi*, we propose to use 10 morphometric parameters. At the same time, other authors for this purpose indicate from three to eight parameters. Thus, scientists propose to determine the length of body, the length and width of anterior and posterior parts of body, and their ratio in female nematodes. Their data on some features differ from the results obtained by other authors (Skrjabin et al., 1957; Gvozdev et al., 1970; Hofing & Kraus, 1994; Schoeb, et al., 2007).

Therefore, we propose to use additional metric parameters to identify females of *T. sylvilagi*, which characterize the location of the vesicular cuticular protrusions, the width of the body in the connection of the esophagus and the intestine, the distance from the vulva to the head end.

Thus, the authors propose to determine the total body length of females, the length and width of the anterior and posterior parts of the body, their ratio. We additionally propose to use metric parameters to identify females of *T. sylvilagi*, which characterize the location of vesicular cuticular protrusions (two parameters), the width of the body in the connection of esophagus and intestine, the location of the vulva relative to the head end. Metric parameters of eggs in the uterus of females of *T. sylvilagi* were also determined. Five parameters of eggs located in the uterine cavity have been identified, of which scientists have previously proposed two (length and width of eggs) for species identification. In addition, we proposed to use the indicators of shell thickness, length and width of egg plugs.

Therefore, the use of the proposed morphometric indicators for the identification of *T. sylvilagi* will expand the existing data and facilitate the differential species diagnosis of these parasites.

Conclusion

In order to morphologically identify parasitic nematodes of the species *Trichuris sylvilagi* Tiner, 1950, isolated from the colon of *Lepus europaeus* Pallas, 1778, it is proposed to use additional morphometric parameters that will increase the efficiency of differential diagnosis. Altogether 13 parameters were determined in male nematodes, and 10 in female nematodes, which characterize the length of body, the length and width of the anterior and posterior parts of body, body width in the connection of esophagus and intestine, location of vesicular cuticular protrusions. Parameters, specific for male nematodes, characterize the size of the spicule in different parts. In females, the proposed indicators determine the location of the vulva. Metric parameters of uterine eggs of *Trichuris* were determined. It is proposed to use five of those: length, width of eggs and egg plugs, and thickness of the egg shell. The obtained data will allow to improve the differential diagnosis of *T. sylvilagi*.

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Received 26 April 2022
Accepted 5 June 2022