# Molecular and morphological characterisation of *Syphacia stroma* (Linstow, 1884) (Nematoda, Oxyurida) from the Lipetsk region, Russia

**Daria I. Gorelysheva[1] and Ivan M. Ermakov[2]**

**[1]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii Prospect 33, 119071, Moscow, Russia**
**[2]Faculty of Biology, Moscow State University, Leninskie Gory 1-12, 119991, Moscow, Russia**
**e-mail: gorelysh.di@gmail.com**

Accepted for publication 21 March 2022

**Summary.** Morphological data and nucleotide sequences are provided for specimens of the pinworm, *Syphacia stroma*, collected from two specimens of yellow-necked mouse, *Apodemus flavicollis*, in the Lipetsk region, Russian Federation. Unlike other pinworms, *S. stroma* inhabits the small intestine of the host. The obtained nucleotide data are the first sequence data for *S. stroma* from Russia. The phylogeny of the genus *Syphacia* is discussed.

**Key words:** coevolution, host specificity, pinworm, rodents, Syphaciini, yellow-necked mouse.

The nematodes of the genus *Syphacia* parasitise rodents, mainly Muridae. Unlike the majority of congeners common in the caecum, *Syphacia stroma* inhabit the small intestine. The species was described from wood mice, *Apodemus sylvaticus*, by Linstow (Linstow, 1884) as *Oxyuris stroma*. Then Seurat (1915) synonymised *Oxyuris stroma* with *Syphacia obvelata* Rudolphi, 1802. Later Morgan concluded that *S. stroma* is a separate species from *S. obvelata* (Morgan, 1932).

After comprehensive revision, the genus *Syphacia* Seurat, 1916 was divided into ten groups according to morphological features (Quentin, 1971). *Syphacia stroma* was included into the 'group IX' together with sister species *Syphacia emileromani* Chabaud, Rausch & Desset, 1963. Smaller size of egg-shells was proposed as a distinguishing feature of *S. emileromani*. Hugot (1988) suggested splitting the genus *Syphacia* into three subgenera, and *S. stroma* was included into the type subgenus *Syphacia* (*Syphacia sensu stricto*).

An application of molecular methods to pinworm phylogeny elucidated the relationships between *Syphacia* species. Phylogenetic analysis of the genus is based on two loci of nuclear ribosomal repeats: partial 28S rDNA and complete ITS1-5.8S-ITS2 and a portion of mitochondrial *cytochrome c oxidase* gene subunit 1 (Stewart *et al.*, 2018; Behnke *et al.*, 2022). Topology of the trees inferred from these sequences was mainly concordant with the phylogenetic hypothesis based on morphology: *S. stroma* is a sister species to *S. emileromani* and together with *S. agraria* these belong to the separate clade in the subgenus *Syphacia* (Gorelysheva *et al.*, 2021).

*Syphacia stroma* is considered to be a specific parasite of *Apodemus* mice: *A. sylvaticus* and *A. flavicollis* (Quentin, 1971; Tenora & Meszaros, 1975; Genov, 1984; Stewart *et al.*, 2018; Behnke *et al.*, 2022). In some cases, *S. stroma* was reported as a parasite of other rodent hosts, *e.g.*, *Mesocricetus auratus* (Hasegawa *et al.*, 2008), but such identifications were not supported by molecular data. Remarkably, the co-invasion of the same host with *S. stroma* and *S. frederici* was reported in the UK (Stewart *et al.*, 2018). Identification of *Syphacia* under light microscope is complicated due to the scarcity of males and similarity of female morphology throughout the genus; *S. stroma* and *S. frederici* are quite similar in appearance. Stewart *et al.* (2018) suggested to discriminate these species under low magnification microscopy by the shape of the tails. The tail of *S. frederici* was thinner and usually bent, but in some cases, it was straight. While the tail was straighter, the point of flexion is still clearly visible. This feature should be carefully studied for accurate identification.

In Russia, *S. stroma* was reported in Voronezh reserve in Voronezh region parasitising *A. flavicollis*, *A. uralensis* and *Mus musculus* (Romashova & Romashov, 2019), and in

 doi: 10.24412/0869-6918-2022-1-69-78
Published online 27 June, 2022

'Belogorye' reserve in Belgorod region parasitising *A. flavicollis* (Kononova & Prisniy, 2020). Referring to the book of Ryzhikov *et al*. (1979), *S. stroma* is a parasite of mice: *M. musculus*, *A. flavicollis*, *A. uralensis*, *Micromys minutus* in Europe and *Apodemus speciosus* in Asia. Also, it was reported for a wide range of hosts, but these findings seem to be accidental (Ryzhikov *et al.*, 1979).

All the identifications of *S. stroma* in Russia were based only on morphology. The significance of morphological features for the identification of species of *Syphacia* is quite obscure and the composition of local populations is not clear. Thus, the aim of this research is to provide new morphological data for *S. stroma* and to base further identification of the genus *Syphacia* on nucleotide analysis.

## MATERIAL AND METHODS

**Trapping mammals.** The fieldwork was carried out in August 2019 in Podgornoe, Lipetsk region (52°32′ N, 39°30′ E). Mammals were collected using Shchipanov's traps (Shchipanov, 1987). Two specimens of *A. flavicollis* were examined.

**Nematode sampling.** In the laboratory, rodents were euthanised by cervical dislocation and then dissected. Their intestinal tracts were placed in saline and examined in separate Petri dishes. Worms recovered from the intestinal tract of each animal were then divided into two groups: some nematodes were transferred to 1.5 ml glass tubes containing 96% ethanol, while the remaining specimens were fixed in similar tubes using 4% formalin in saline heated to 60-80°C until further processing for DNA extraction and microscopy, respectively.

**Morphological observation.** Nematodes from the samples were transferred to a glycerin solution, and after evaporation of the water, were mounted in glycerin drops embedded into paraffin rings. Eleven females and seven males were studied under light microscopy and measured using a Leica DFC 425 C (Leica Microsystems, Germany) microscope.

For scanning electron microscopy (SEM) analysis, five female and three male nematodes of *Syphacia* were selected. The nematodes were dehydrated in ethanol series and acetone, underwent critical point drying, mounted onto aluminium stubs with double-face tape, and studied using a CamScan MV 2300 (TESCAN, Czech Republic).

**Molecular profiles.** Two loci of the *Syphacia* genome were selected for the study: the partial sequence of cytochrome c oxidase 1 (CO1 mtDNA) gene of the mitochondrial genome, the partial sequence of the large subunit (LSU). For DNA isolation, we used QIAmp DNA Mini Kit® (Qiagen, Germany). For DNA amplification, the Encyclo Plus PCR kit® (Evrogen, Russia) was used in accordance with the manufacturer's protocol. After screening CO1 mtDNA primers, we found the most

**Table 1.** Accession numbers and additional information for CO1 mtDNA sequences of *Syphacia* species from NCBI GenBank.

| ***Syphacia* species** | **Accession number** | **Host** | **Locality** | **Reference** |
|---|---|---|---|---|
| *Syphacia agraria* | AB282589 | *Apodemus speciosus* | Japan: Hokkaido, Hidaka | Okamoto *et al.*, 2007 |
| *Syphacia agraria* | MN641848 | *Microtus arvalis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia agraria* | MN641850 | *Apodemus agrarius* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia agraria* | MN641844 | *Apodemus agrarius* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia emileromani* | AB282590 | *Apodemus argenteus* | Japan: Ehime, Saijyo | Okamoto *et al.*, 2007 |
| *Syphacia frederici* | AB282586 | *Apodemus speciosus* | Japan: Okayama, Hiruzen | Okamoto *et al.*, 2007 |
| *Syphacia frederici* | AB282587 | *Apodemus speciosus* | Japan: Iwate, Morioka | Okamoto *et al.*, 2007 |
| *Syphacia frederici* | AB282588 | *Apodemus speciosus* | Japan: Hokkaido, Hidaka | Okamoto *et al.*, 2007 |
| *Syphacia frederici* | AB282593 | *Apodemus speciosus* | Japan: Oita, Oita | Okamoto *et al.*, 2007 |
| *Syphacia frederici* | MN641868 | *Apodemus uralensis* | Russia | Gorelysheva *et al.*, 2021 |

**Table 1 (continued).** Accession numbers and additional information for CO1 mtDNA sequences of *Syphacia* species from NCBI GenBank.

| | | | | |
|---|---|---|---|---|
| *Syphacia frederici* | MF142425 | *Apodemus sylvaticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia frederici* | MF142426 | *Apodemus sylvaticus* | Portugal | Stewart *et al.*, 2018 |
| *Syphacia frederici* | MF142429 | *Apodemus sylvaticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia nigeriana* | AB282581 | *Eothenomus smihii* | Japan: Ehime, Saijyo | Okamoto *et al.*, 2007 |
| *Syphacia nigeriana* | MN641860 | *Microtus obscurus* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641851 | *Microtus arvalis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641856 | *Microtus arvalis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641853 | *Microtus arvalis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641866 | *Microtus arvalis/ obscurus* hybrid | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641859 | *Microtus arvalis/ obscurus* hybrid | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | MN641865 | *Microtus obscurus* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia nigeriana* | AB282583 | *Clethrionomys rufocanus* | Japan: Hokkaido, Niseko | Okamoto *et al.*, 2007 |
| *Syphacia nigeriana* | AB282584 | *Clethrionomys rufocanus* | Japan: Hokkaido, Tobetsu | Okamoto *et al.*, 2007 |
| *Syphacia nigeriana* | AB282585 | *Clethrionomys rufocanus* | Japan: Hokkaido, Rishiri Is. | Okamoto *et al.*, 2007 |
| *Syphacia obvelata* | MF142430 | *Mus domesticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia obvelata* | MF142432 | *Mus musculus* | Poland | Stewart *et al.*, 2018 |
| *Syphacia obvelata* | MH427273 | *Mus musculus* | Czech Republic | Goüy de Bellocq *et al.*, 2018 |
| *Syphacia obvelata* | MF142433 | *Mus musculus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia obvelata* | NC029239 | – | China | Wang *et al.*, 2016 |
| *Syphacia ohtaorum* | AB282592 | *Mus caroli* | Japan: Okinawa | Okamoto *et al.*, 2007 |
| *Syphacia stroma* | MF142427 | *Apodemus sylvaticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia stroma* | MF142422 | *Apodemus sylvaticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Syphacia stroma* | MF142419 | *Apodemus sylvaticus* | Ireland | Stewart *et al.*, 2018 |
| *Syphacia stroma* | MF142428 | *Apodemus sylvaticus* | United Kingdom | Stewart *et al.*, 2018 |
| *Enterobius vermicularis* – outgroup | NC011300 | – | Republic of Korea | Kang *et al.*, 2009 |

effective primer mix to be ScyphCO1F (5'-TGG TCT GGT TTT GTT GGT AGT T-3') and ScyphCO1R (5'-AAC CAC CCA ACG TAA ACA TAA A-3'), as proposed by Okamoto *et al.* (2007). The thermal cycling protocol used for these primers was 94°C for 5 min and then 35 cycles of 94°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, followed by a final extension at 72°C for 5 min. To amplify LSU rDNA, the primers C1 (5'-ACC CGC TGA ATT TAA GCA T-3') and D2 (5'-TCC GTG TTT CAA GAC GG-3') proposed by Okamoto *et al.* (2009) were used. PCR cycling parameters for the amplification of LSU rDNA included primary denaturation at 94°C for 1 min, followed by 35

cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by post-amplification extension at 72°C for 7 min. DNA sequencing was performed at the Genome Centre for Collective Use 'Genotech'. Sequences were obtained for several specimens from two different hosts, but all of them were identical, so we deposited only one sequence for each locus. Accession number of the obtained *S. stroma* LSU rDNA (28S rDNA) sequence is ON510071. Accession number of the obtained *S. stroma* CO1 mtDNA sequence is ON479655.

Sequences retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov) for 28S rDNA (LSU) are given in Table 1; for CO1 mtDNA they are given in Table 2. Sequences were aligned with MUSCLE method (Edgar, 2004). Models of molecular evolution were defined with jModeltest (Posada, 2008). Phylogenetic analyses were performed using two ML method. ML tree was reconstructed in IQtree program in webserver http://iqtree.cibiv.univie.ac.at/ with 100,000 iterations.

**Table 2.** Accession numbers and additional information for 28S rDNA (LSU) sequences of *Syphacia* and *Syphabulea* species from NCBI GenBank.

| ***Syphacia* species** | **Accession number** | **Host** | **Locality** | **Reference** |
|---|---|---|---|---|
| *Syphabulea tjanschani* | MH443065 | *Sciurotamias davidianus* | China: Zhangjiakou, Hebei Province | Li *et al.*, 2019 |
| *Syphacia agraria* | AB500167 | *Apodemus speciosus* | Japan: Hokkaido, Hidaka | Okamoto *et al.*, 2009 |
| *Syphacia agraria* | MT929757 | *Apodemus agrarius* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia emileromani* | AB500169 | *Apodemus argenteus* | Japan: Hokkaido, Apoi | Okamoto *et al.*, 2009 |
| *Syphacia frederici* | MT929764 | *Apodemus uralensis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia frederici* | AB500173 | *Apodemus speciosus* | Japan: Hokkaido, Hidaka | Okamoto *et al.*, 2009 |
| *Syphacia montana* | AB500165 | *Clethrionomys rex* | Japan: Hokkaido, Rishiri Is. | Okamoto *et al.*, 2009 |
| *Syphacia montana* | MT929762 | *Microtus arvalis* | Russia | Gorelysheva *et al.*, 2021 |
| *Syphacia muris* | EF464553 | – | USA | Feldman *et al.*, 2007 |
| *Syphacia obvelata* | AB500176 | *Mus musculus* | Japan: Honshu, Osaka, Suita | Okamoto *et al.*, 2009 |
| *Syphacia ohtaorum* | AB500177 | *Mus caroli* | Japan: Okinawa, Okinawa Is. | Okamoto *et al.*, 2009 |
| *Syphacia petrusewiczi* | AB500166 | *Clethrionomys rutilus* | Japan: Hokkaido, Notsuke | Okamoto *et al.*, 2009 |
| *Syphacia rifaii* | LC038095 | *Bunomys penitus* | Indonesia: Southeast Sulawesi, Mekongga | Dewi, 2015 |
| *Syphacia stroma* | LC038098 | *Mesocricetus auratus* | Czech Republic | Dewi, 2015 |
| *Syphacia vandenbrueli* | AB500178 | *Micromys minutus* | Japan: Honshu, Hiroshima, Hiroshima | Okamoto *et al.*, 2009 |
| *Syphatineria* sp. | LC038099 | *Lariscus hosei* | Indonesia: Kalimantan | Dewi, 2015 |

## RESULTS
## *Syphacia stroma* (Linstow, 1884)

All the measurements are given in μm in the form of mean ± standard deviation with size range in brackets, for example: 3275 ± 65 (3200-3350).

**Morphology.** Cephalic vesicle present. The cuticle is transversely striated. Cervical alae are absent.

**Male (n = 7).** Body 1649 ± 76 (1552-1737) long; 98 ± 4 (94-103) wide. Pharynx 262 ± 18 (237-281) long with 197 ± 11 (184-210) long procorpus and 60 ± 10 (56-63) diameter bulb. Nerve ring at the level

111 ± 5 (106-118) from apex. Excretory pore at the level 418 ± 25 (383-442). Three cuticular 59 ± 11 (49-73), 67 ± 17 (55-91), 81 ± 15 (53-84) long mamelons present on the ventral side of the body at 663 ± 16 (646-685) from apex. Second mamelon at 475 ± 25 (449-506), third 229 ± 24 (200-259) from anus. The tail is truncated ventrally, ends with a conical process with sharp tip. Process length 166 ± 12 (159-184). Spicule length 74 ± 3 (71-78), gubernaculum spindle-shaped length 43 ± 5 (36-49).

**Female (n = 11).** Body 3816 ± 327 (3395-4305) long; 272 ± 21 (237-300) wide. The pharynx 361 ± 23 (337-394) long with 261 ± 17 (234-284) long procorpus and 84 ± 6 (74-90) diameter bulb. Nerve ring at the level 134 ± 4 (131-142). Excretory pore and vulva at 580 ± 55 (519-662) and 780 ± 109 (614-942) from the anterior end, respectively. Eggs 131 ± 4 (125-137) long and 43 ± 1 (42-44) wide. Conical tail elongated and narrow 509 ± 34 (451-539) long.

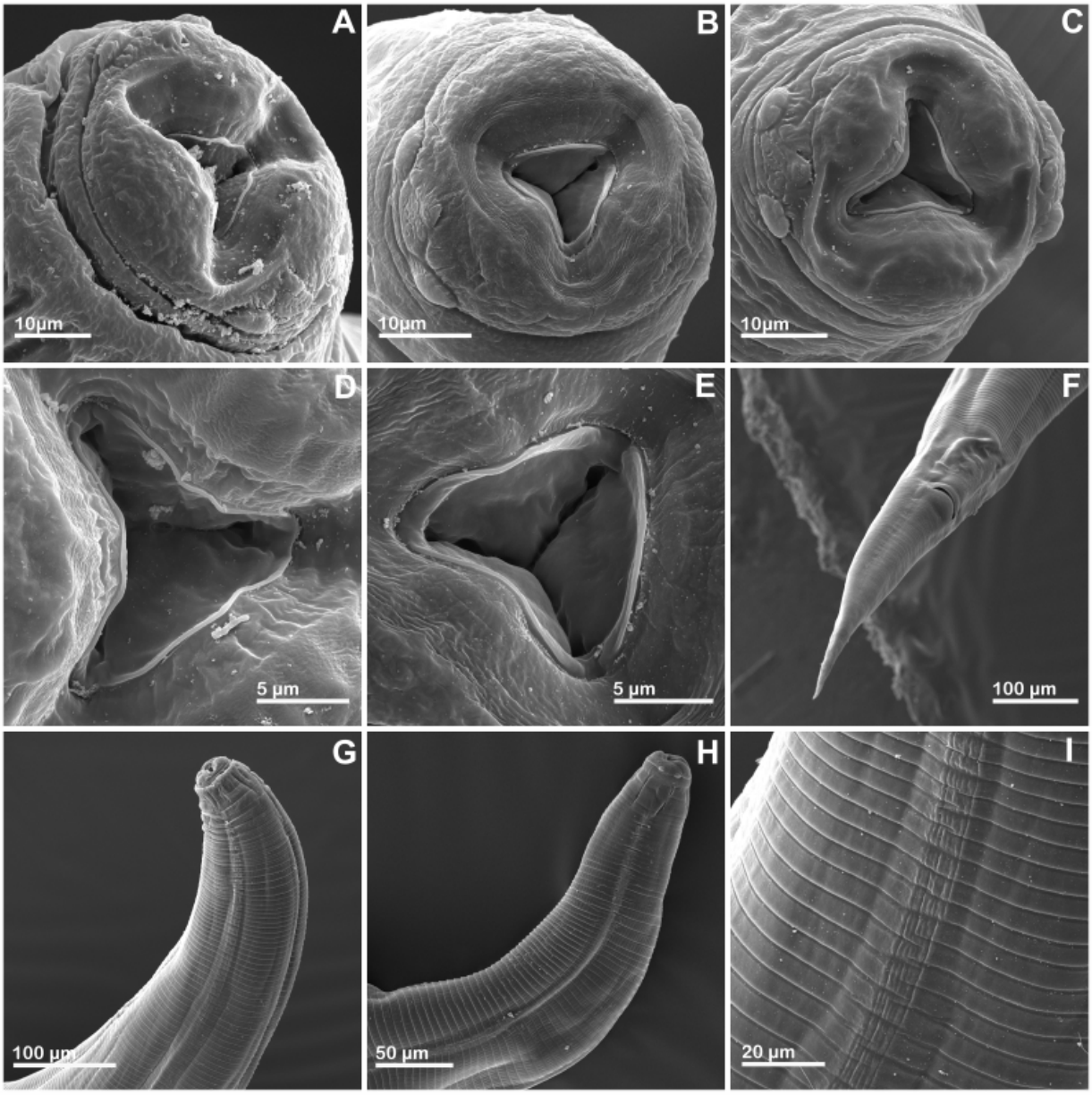


**Fig. 1.** Morphological features of the studied *Syphacia stroma*, SEM: A – female apical view, B – male apical view, C – fourth-stage larvae (L4) apical view, D – female cuticular teeth, E – male cuticular teeth, F – female tail, G – female anterior end of the body, H – male anterior end of the body, I – female cuticle.

**Locality.** Podgornoe, Lipetsk region (52°32′ N, 39°30′ E).

**Localisation.** Small intestine.

**SEM.** The facial mask is slightly laterally elongated, with two hemispherical submedian papillae and one amphid on each side, cephalic plate is rounded (Fig. 1A-C). Three cuticular teeth are present (Fig. 1D & E). The longitudinal media thickening of each tooth is not developed. A transverse round thickening is present at the edge of each tooth. Transverse ridges of denticles near the anterior margin are absent. Cuticular collar is not well developed and present on females only. An annulated cuticle without longitudinal ridges on each cuticular ring (Fig. 1I). Lateral alae are thin (Fig. 1I); cervical alae are absent (Fig. 1G & H). Lateral alae are at 30 μm from the apex (Fig. 1G & H). Tail is conical (Fig. 1F).

**Phylogenetic relationships.** Phylogenetic relationships of the studied *Syphacia* inferred from analysis of partial LSU rDNA (28S rDNA) are shown in Figure 2. The obtained sequence and the most similar sequences found on the NCBI GenBank enabled us to construct the 685 bp long alignment. The *S. stroma* sequences obtained in this study and sequences retrieved from the NCBI GenBank form a clade with 99% support. Monophyly of *Syphacia* including *S. petrusewiczi* (subgenus *Seuratoxyuris*) is strongly supported (100%). *Syphacia stroma* was in one clade with sister species *S. emileromani* from Japan and with *S. agraria* from Europe. The clade that included *Syphacia* parasitising rats, *S. muris*, *S. rifaii* and *S. ohtaorum*, was not supported.

A phylogenetic tree inferred from analysis of the partial mitochodrial CO1 mtDNA is shown in Figure 3. Its topology and composition are similar to that inferred from LSU rDNA data. The total length of the analysed matrix for CO1 mtDNA region was 660 bp.

In this cladogram, there was 100% posterior probability support of monophyly *S. stroma* populations. The isolate from Russia is identical to the isolates from the UK. *Syphacia stroma* is a sister species to *S. emileromani* from Japan; these two species form a clade with 87% support.

The genus *Syphacia* splits into two branches. The first one consists of *S. stroma*, *S. emileromani*, *S. agraria* and *S. ohtaorum* (100% support). The second one includes *S. nigeriana*, *S. frederici* and *S. obvelata*.

## DISCUSSION

All the studied *Syphacia* specimens recovered from the small intestine of *A. flavicollis* showed uniform morphology that corresponds to the descriptions of *S. stroma* (Morgan, 1932; Ryzhikov *et al.*, 1979). Morphological identification was supported by sequences of the nuclear 28S rDNA (Fig. 2) and CO1 mtDNA (Fig. 3). The co-invasion of this host with both *S. stroma* and *S. frederici* was previously reported in the UK (Stewart *et al.*, 2018), but uniform morphology of the specimens from this Russian population indicates the presence of a single *Syphacia* species. Also, the localisation of the discovered nematodes in the small intestine supports their identification as *S. stroma*, as *S. frederici* was mainly discovered in the caecum of *A. flavicollis*.

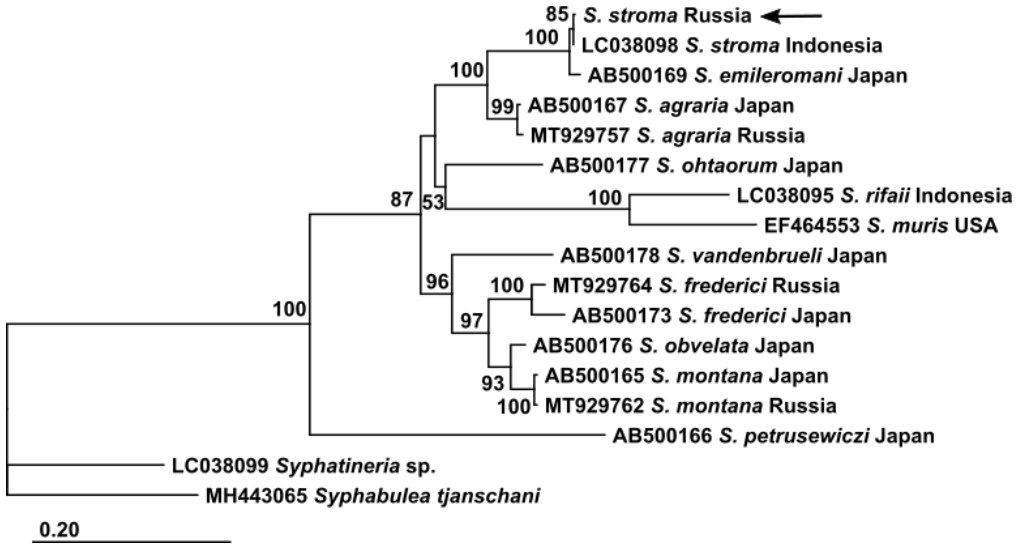


**Fig. 2.** ML tree of *Syphacia* species inferred from the analysis of the LSU (28S rDNA) data with bootstrap values near the nodes. The scale bar represents the number of nucleotide substitutions per site. Nucleotide substitution model – TN93.

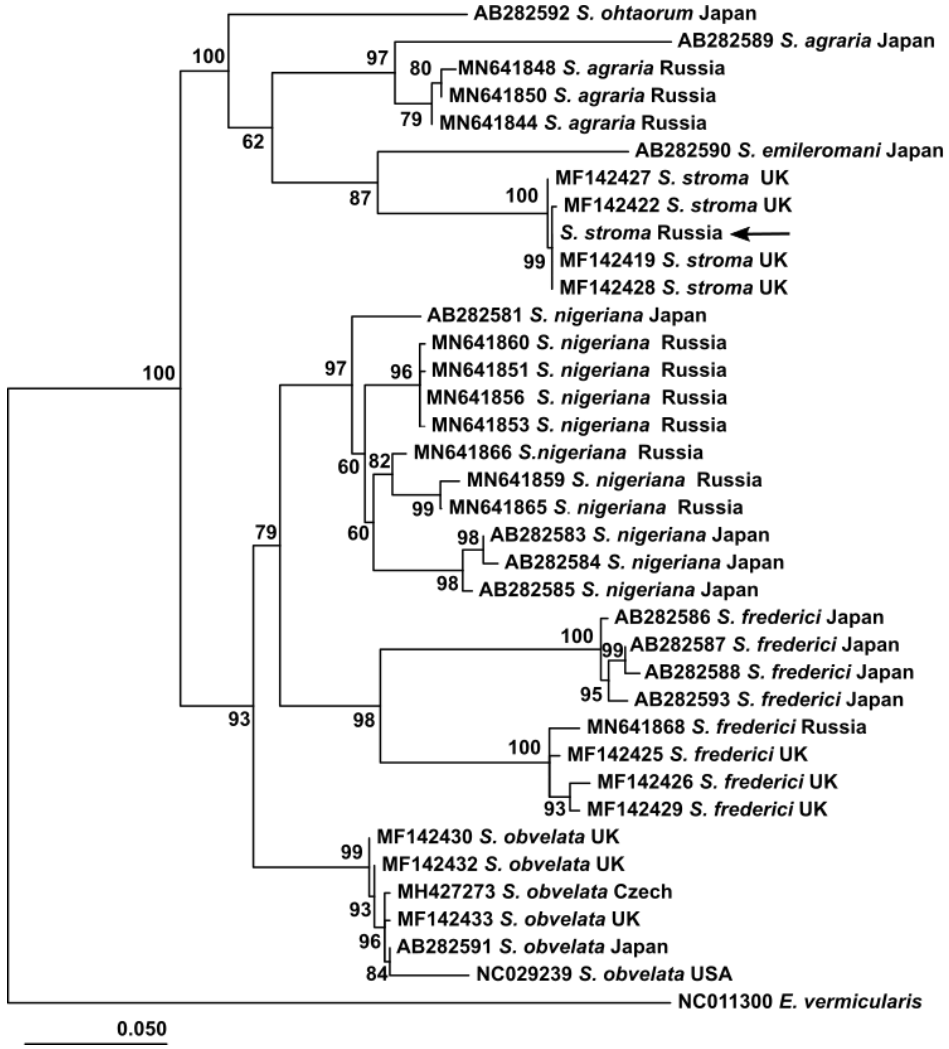


**Fig. 3.** ML tree of *Syphacia* species inferred from the analysis of the CO1 mtDNA data with bootstrap values near the nodes. The scale bar represents the number of nucleotide substitutions per site. Nucleotide substitution model – TIM3 + F + G4.

The body sizes of the studied male and female specimens correspond to those described for *S. stroma* in the literature (Morgan, 1932; Ryzhikov *et al.*, 1979), with only the range for some features differing, *e.g.*, the minimal length values of the pharynx for both sexes were smaller than previously described. The same goes for the minimal value of pharyngeal bulb size of females, egg-shell length, and distance from anterior end to vulval opening. The size of the male spicules is the same as in original and other descriptions, but the gubernaculum (36-49) is larger than described by Morgan (30). At the same time, the minimal value for gubernaculum length is smaller than reported by Ryzhikov (40-50).

The cuticular surface morphology of *Syphacia* studied under SEM proved to be a rich source of diagnostic features for this genus (Wiger *et al.*, 1978; Baruš *et al.*, 1979). The surface structures of *S. stroma* were considered by Wiger *et al.* (1978) as the most primitive between congeners. The surface structures of the Russian specimens (Fig. 1) were similar to those reported by Wiger *et al.* (1978), but there were still some differences. In Russian specimens we showed that the collar is weakly developed and can be found only in females (Fig. 1G). The collar of *S. stroma* is an invagination of a thinner cuticle, and it differs from that of *S. frederici*. Moreover, the annulated cuticle of our specimens lacks longitudinal depressions on each cuticular ring (Fig. 1I), which were reported previously (Wiger *et al.*, 1978: P. 27, Fig. 4). However, such differences might be an artefact of the dehydration/drying of nematodes for SEM study.

We analysed the structure of the anterior end, cuticular teeth and surface of the body cuticle for

females, males and fourth-stage larvae (L4). No difference between them was observed except the size and slight variation in the shape of the facial mask and cephalic plate (Fig. 1A-C). Cephalic plate (area around lips) and facial mask (term proposed by Wiger *et al.* (1978) for an area around a plate – usually ornamented with tiled pattern, surrounding cephalic papillae and amphids) are slightly elongated in females of the Russian population along the transverse axis (Fig. 1A) but more round in males (Fig. 1B). In our opinion the round shape of plate and facial mask are basal features for the genus *Syphacia* as in males and larvae it is always round (Fig. 1C).

As mentioned above, morphological identification of *S. stroma* was confirmed by nucleotide sequences of the nuclear 28S rDNA (Fig. 2) and CO1 mtDNA (Fig. 3). In both phylogenetic trees (Figs 2 & 3) *S. stroma* forms a clade with its sister species *S. emileromani* from Japan and *S. agraria* from Europe. Common features can be found in *S. stroma* and *S. agraria* morphology (Gorelysheva *et al.*, 2021): the absence of prominent longitudinal ornamentation of cuticuar rings and the lack of cervical alae (Fig. 1G-I). Representatives of another clade of *Syphacia*, which includes *S. nigeriana*, *S. frederici* and *S. obvelata*, had an annulated cuticle with longitudinal ornamentation on each cuticular ring (Dick & Wright, 1974; Wiger *et al.*, 1978; Baruš *et al.*, 1979; Gorelysheva *et al.*, 2021). Thus, at least some features of the cuticular surface correspond to the *Syphacia* clades inferred from analysis of nucleotide sequences.

In conclusion, our study provides the first report of *S. stroma* in Russia supported by molecular data. We confirmed that *S. stroma* haplotype from the European part of Russia is identical to *S. stroma* from the UK and obtained new morphological data, extending the information of body size variability, and providing SEM images. Further studies on additional samples collected from various hosts and localities would be required to obtain the full picture of morphological and genetic variability.

## ACKNOWLEDGMENTS

We thankfully acknowledge the practical and logistic help of our colleagues, namely: Mr B.D. Efeykin, Drs E.S. Ivanova and S.E. Spiridonov from the Centre of Parasitology of Severtsov's Institute of Ecology and Evolution of the Russian Academy of Sciences. All the SEM studies were carried out at the Joint Usage Centre 'Instrumental Methods in Ecology' at the Severtsov's Institute of Ecology and Evolution of the Russian Academy of Sciences, and we would like to thank Dr A.N. Neretina from this Centre. The study was supported by RSF (grant no. 19-74-20147).

All procedures in the present study were approved by the Animal Care and Use Committee of the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences and complied with the Federal Law of the Russian Federation no. 498-FZ from 19 December 2018 (on responsible treatment of animals). The collection of rodent material was possible through the general permit issued by the government for the scientific institutions of the Russian Academy of Sciences (including A.N. Severtsov Institute of Ecology and Evolution) to study non-endangered species of animals throughout the territory of the Russian Federation.

## REFERENCES

BARUŠ, V., TENORA, F. & WIGER, R. 1979. Scanning electron microscopy of European species of the genus *Syphacia* Seurat, 1916 (Nematoda). *Folia Parasitologica* 26: 21-26.

BEHNKE, J.M., STEWART, A., SMALES, L., COOPER, G., LOWE, A., KINSELLA, J.M., BAJER, A., DWUŻNIK-SZAREK, D., HERMAN, J., FENN, J., CATALANO, S., DIAGNE, A.C. & WEBSTER, J.P. 2022. Parasitic nematodes of the genus *Syphacia* Seurat, 1916 infecting Cricetidae in the British Isles: the enigmatic status of *Syphacia nigeriana*. *Parasitology* 149: 76-94.

DEWI, K. 2015. *Taxonomical and faunistic studies on the nematode parasites from Indonesian murines (Rodentia; Muridae; Murinae) with special reference to Syphacia spp. and their biogeography*. Ph.D. Dissertation, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, 195 pp.

DICK, T.A. & WRIGHT, K.A. 1973. The ultrastructure of the cuticle of the nematode *Syphacia obvelata* (Rudolphi, 1802). II. Modifications of the cuticle in the head end. *Canadian Journal of Zoology* 51: 197-202. DOI: 10.1139/z73-028

EDGAR, R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 113. DOI: 10.1186/1471-2105-5-113

FELDMAN, S.H. & BOWMAN, S.G. 2007. Molecular phylogeny of the pinworms of mice, rats and rabbits, and its use to develop molecular beacon assays for the detection of pinworms in mice. *Laboratory Animals* 36: 43-50. DOI: 10.1038/laban1007-43

GENOV, T. 1984. *Khelminti na Nasekomoyadnite Bozaĭnitsi i Grizachite v B''lgariya* – [*Helminths of Insectivorous Mammals and Rodents in Bulgaria*]. Bulgaria, Publishing House of the Bulgarian Academy of Sciences. 348 pp. (in Bulgarian).

GORELYSHEVA, D.I., GROMOV, A.R., LAVRENCHENKO, L.A. & SPIRIDONOV, S.E. 2021. Genetic diversity of *Syphacia* Seurat, 1916 (Nematoda: Oxyuridae) across the hybrid zone of their rodent hosts in Russia. *Parasitology Research* 120: 2017-2030. DOI: 10.1007/s00436-021-07134-7

GOÜY DE BELLOCQ, J., RIBAS, A., BRYJA, J., PIÁLEK, J. & BAIRD, S.J. 2018. Holobiont suture zones: parasite evidence across the European house mouse hybrid zone. *Molecular Ecology* 27: 5214-5227. DOI: 10.1111/mec.14938

HASEGAWA, H., SATO, H., IWAKIRI, E., IKEDA, Y. & UNE, Y. 2008. Helminths collected from imported pet murids, with special reference to concomitant infection of the golden hamster with three pinworm species of the genus *Syphacia* (Nematoda: Oxyuridae). *Journal of Parasitology* 94: 752-754. DOI: 10.1645/GE-13471.1

HUGOT, J.-P. 1988. *Les nématodes Syphaciinae, parasites de Rongeurs et de Lagomorphes. Taxonomie. Zoogéographie. Évolution*. In: *Mémoires du Muséum National d'Histoire Naturelle. Série A, Zoologie, Tome 141* (P. Bouchet, A. Dubois & C. Erard Eds). France, Editions du Museum national d'Histoire naturelle. 148 pp.

KANG, S., SULTANA, T., EOM, K.S., PARK, Y.C., SOONTHORNPONG, N., NADLER, S.A. & PARK, J.-K. 2009. The mitochondrial genome sequence of *Enterobius vermicularis* (Nematoda: Oxyurida) – an idiosyncratic gene order and phylogenetic information for chromadorean nematodes. *Gene* 429: 87-97. DOI: 10.1016/j.gene.2008.09.011

KONONOVA, M.I. & PRISNIY, YU.A. 2020. Helminthes of mouse-like rodents in the Belogorye State Nature Reserve (Russia). *Nature Conservation Research* 5 (Supplementary 2): 11-18. DOI: 10.24189/ncr.2020.036

LI, Y., CHEN, H.X., YANG, X.L. & LI, L. 2019. Morphological and genetic characterization of *Syphabulea tjanschani* (Nematoda: Oxyuridae), with phylogenetic position of *Syphabulea* in Oxyuridae. *Infection, Genetics and Evolution* 67: 159-166. DOI: 10.1016/j.meegid.2018.11.016

LINSTOW, O. 1884. Helminthologische Studien. *Archiv für Naturgeschichte* 48: 1-25.

MORGAN, D.O. 1932. *Oxyuris stroma* Linstow, 1884. *Journal of Helminthology* 10: 15-20. DOI: 10.1017/S0022149X00001255

OKAMOTO, M., URUSHIMA, H., IWASA, M. & HASEGAWA, H. 2007. Phylogenetic relationships of rodent pinworms (genus *Syphacia*) in Japan inferred from mitochondrial CO1 gene sequences. *Journal of Veterinary Medical Science* 69: 545-547. DOI: 10.1292/jvms.69.545

OKAMOTO, M., URUSHIMA, H. & HASEGAWA, H. 2009. Phylogenetic relationships of rodent pinworms (genus *Syphacia*) in Japan inferred from 28S rDNA sequences. *Parasitology International* 58: 330-333. DOI: 10.1016/j.parint.2009.07.001

POSADA, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256. DOI: 10.1093/molbev/msn083

QUENTIN, J.C. 1971. Morphologie comparée des structures céphaliques et génitales de oxyures du genre *Syphacia*. *Annales de Parasitologie Humaine et Comparée* 46: 15-60.

ROMASHOVA, N.B. & ROMASHOV, B.V. 2019. [Modern distribution of *Syphacia* (Nematoda, Oxyuridae) in populations of murid rodents in the Voronezh Reserve]. In: *Teoriya i Praktika Borby s Parazitarnymi Boleznyami (Sbornik Nauchnykh Statei), Vypusk 20*. pp. 505-510. Moscow, Russia, Nauka (in Russian).

RYZHIKOV, K.M., GVOZDEV, E.V., TOKOBAEV, M.M., SCHALDYBIN, L.S., MACABERIDZE, G.V., MERKUSCHEVA, I.V., NADTOCHIJ, E.V., CHOCHLOVA, I.G. & SHARPILO, L.D. 1979. [*Key to the Helminth Fauna of Rodents in the U.S.S.R. Nematodes and Acanthocephales*]. USSR, Nauka. 278 pp. (in Russian).

SEURAT, L.G. 1915. Sur deux nouveaux oxyures du Maroc. *Bulletin de la Societé d'Histoire Naturelle d'Afrique du Nord* 6: 24-31.

SEURAT, L.G. 1916. Sur les Oxyures des Mammiferes. *Comptes Rendus Des Séances de La Société de Biologie Et de Ses Filiales* 69: 64-68.

SHCHIPANOV, N.A. 1987. [Universal live trap for small mammals]. *Zoologicheskii Zhurnal* 66: 759-76 (in Russian).

STEWART, A., LOWE, A., SMALES, L., BAJER, A., BRADLEY, J., DWUŻNIK, D., FRANSSEN, F., GRIFFITH, J., STUART, P., TURNER, C., ZALEŚNY, G. & BEHNKE, J.M. 2018. Parasitic nematodes of the genus *Syphacia* Seurat, 1916 infecting Muridae in the British Isles, and the peculiar case of *Syphacia frederici*. *Parasitology* 145: 269-280. DOI: 10.1017/S0031182017001470

TENORA, F. & MESZAROS, F. 1975. Nematodes of the genus *Syphacia* Seurat, 1916 (Nematoda) – parasites of rodents (Rodentia) in Czechoslovakia and Hungary. *Acta Universtatis Agriculturae* 23: 537-554.

WANG, C.R., LOU, Y., GAO, J.F., QIU, J.H., ZHANG, Y., GAO, Y. & CHANG, Q.C. 2016. Comparative analyses of the complete mitochondrial genomes of the two murine pinworms *Aspiculuris tetraptera* and *Syphacia obvelata*. *Gene* 585: 71-75. DOI: 10.1016/j.gene.2016.03.037

WIGER, R., BARUŠ, V. & TENORA, F. 1978. Scanning electron microscopic studies on four species of the genus *Syphacia* (Nematoda, Oxyuridae). *Zoologica Scripta* 7: 25-31. DOI: 10.1111/j.1463-6409.1978.tb00586.x

URL: http://www.ncbi.nlm.nih.gov (accessed: March 25, 2022).

URL: http://iqtree.cibiv.univie.ac.at/ (accessed: April 5, 2022).

**Д.И. Горелышева и И.М. Ермаков.** Молекулярная и морфологическая характеристика *Syphacia stroma* (Linstow, 1884) (Nematoda, Oxyurida) из России, Липецкая область.

**Резюме.** Предоставлены новые молекулярные и морфологические данные об острицах *Syphacia stroma* из Российской Федерации, собранных от двух особей желтогорлой мыши *Apodemus flavicollis* в Липецкой области. В отличие от других остриц *S. stroma* обитает в тонком кишечнике грызунов. Полученные нуклеотидные данные по *S. stroma* из России публикуются впервые. Также обсуждается филогения рода *Syphacia* на основе молекулярных данных и сканирующей электронной микроскопии.