Molecular and morphological characterisation of *Syphacia petrusewiczi* Bernard, 1966 (Nematoda, Oxyurida) from Russia

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Summary. This study provides new molecular and morphological data on *Syphacia petrusewiczi* – a pinworm of rodents. The representatives of populations parasitising different host species in two distant regions of Russia are studied: the European North of Russia and the Northern part of Central Siberia. In the latter region, the specificity of *S. petrusewiczi* was not strict: rodents of different families served as hosts. Obtained nucleotide data are the first sequence data for *S. petrusewiczi* from Russia.

Key words: *Clethrionomys*, phylogeny, pinworms, rodent hosts, specificity.

Syphacia petrusewiczi Bernard, 1966 is a common pinworm parasite of *Clethrionomys glareolus* Schreber, 1780 and *Clethrionomys rutilus* Pallas, 1779 in the Palearctic region (Quentin, 1971; Tenora & Mészáros, 1975; Behnke *et al.*, 2001; Hasegawa *et al.*, 2008; Milazzo *et al.*, 2003). The females of this species are easily recognisable by the laterally elongated cephalic plate and wide ornamented cervical alae. Males are significantly smaller than females. Three cuticular mamelons are present on the ventral side of the body. The tail is truncated ventrally and ends with a very short, wide, conical process and rounded tip.

In the comprehensive revision of the genus *Syphacia* Seurat, 1916 it was divided into ten groups according to morphological features (Quentin, 1971). Group VI includes *Syphacia petrusewiczi* together with *S. frederici*, *S. vandenbrueli* and *S.alata*. Quentin and Gran (1977) reported the *S. petrusewiczi* parasitising *Clethrionomys rutilus* in Alaska and proposed considering these Nearctic forms as a separate subspecies – *Syphacia petrusewiczi rauschi* Quentin, 1969. In his opinion (Quentin, 1971), these Nearctic taxa are an intermediate link in the evolution of *Syphacia* of Palearctic Microtidae (voles) towards Nearctic Cricetidae (hamsters). Since these findings, no *Syphacia* nematodes were reported or described from North American voles.

Hugot (1988) proposed dividing the genus *Syphacia* into several subgenera. In his classification, *S. petrusewiczi* belongs to the subgenus *Seuratoxyurus*, while *S. frederici* is a member of the type subgenus *Syphacia*. An introduction of molecular methods brought new tools to *Syphacia* studies. Okamoto *et al.* (2009) based on sequence analysis and SEM data confirmed that *S. frederici* and *S. petrusewiczi* are quite different and do not belong to the same group inside the genus. Their data partially confirmed the views of Hugot (1988) and did not reveal the partition of the genus into the groups established by Quentin (1971). Unfortunately, the nucleotide sequences were studied for comparatively few representatives of *Syphacia* (Okamoto *et al.*, 2007, 2009). These were mainly species of the type subgenus *Syphacia*, which were sampled when the subgenus *Seuratoxyurus* was only represented by *S. petrusewiczi.* Remarkably, in the phylogenetic tree inferred from the analysis of 28S rDNA, *S. petrusewiczi* is in a basal position, which corresponds to the hypothesis of Hugot (1988) about the early divergence of this species from *Syphacia*'s main evolutionary line. The peculiar morphology and systematic position of *S. petrusewiczi* was also demonstrated *via* SEM study of several species of this genus (Wiger *et al.*, 1978).

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Recently *S. petrusewiczi* was reported in Russia in the Republic of Karelia, which is North of the European Russia (Bugmyrin *et al.*, 2015), and in Voronezh Region, which is in the central part of the European Russia (Romashova & Romashov, 2019). In both locations, *S. petrusewiczi* was only found in *Clethrionomys glareolus* voles. Identification in both cases was based solely on morphological features.

Parasitic nematodes of rodents constitute a very inviting model to study host-parasite co-evolution and phylogeography. Still the significance of morphological features for the species identification of *Syphacia* is quite obscure and the composition of local faunas is not clear. Thus, the aim of this research is to put the species identification in the genus *Syphacia* on the solid basis of nucleotide analysis.

MATERIAL AND METHODS

Trapping mammals. Mammals were trapped using 20 m long ditches of 15-20 cm depth and with two large pitfalls placed 5 m from the two ends of the ditch. In the habitats with a high level of groundwater, the ditches were replaced by polythene fences of the same length with two pitfalls. The pitfalls were provided with some water to kill the small mammals. In addition, live traps were used in the catches.

Nematode sampling. The fieldwork was carried out from August to September 2019 and 2020 in the 'Mirnoye' Yenisei Ecological Station of the A.N. Severtsov Institute of Ecology and Evolution (Russian Academy of Sciences) in the Middle Yenisei Taiga (62°17' N, 89°02' E); in august 2019 in the Gomselga field station of the Institute of Biology KarRC RAS (62°7' N, 34°0' E) in Republic of Karelia. 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, with particular attention paid to the caecum and colon. 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 light 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. Ten females from each location were studied under light microscopy and measured using a Leica DFC 425 C microscope. All the measurements are given in μm. For female in the form of mean ± standard deviation with min and max sizes in brackets, for example: 3275 ± 65 (3200-3350). As we have only two males from Gomselga, we give measurements for one of the specimens in brackets and for the other without brackets.

Molecular phylogeny. DNA was extracted from specimens fixed in 95% ethanol with QiAmp DNA Mini Kit (Qiagen). Two fragments of two genes were selected for phylogenetic analysis based on the availability of sequenced markers for *Syphacia* in GenBank. ITS1-5.8S-ITS2 region and the LSU gene (28S rDNA) were used. All sequences obtained were compared to the GenBank database using the BLAST algorithm (Altschul *et al.*, 1990). Polymerase chain reaction (PCR) products were visualised in gel, cut out, and cleaned using SV Gel and PCR CleanUp System kit (Evrogen, Russia). DNA sequencing was performed at the Genome Centre for Collective Use 'Genotech'. Sequences were aligned with MUSCLE method (Edgar, 2004). Models of molecular evolution were defined with jModeltest (Posada, 2008). Phylogenetic analyses were performed using two diffirent methods: MrBayes 3.2.7 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), run for 10 million generations, and sampled every 1000 generations. The first 25% of trees from each run were discarded as burn-in. The remaining trees were used to create a 50% majority consensus tree and calculate posterior probabilities. ML tree was reconstructed in IQtree program in web-server http://iqtree.cibiv.univie.ac.at/ with 100,000 iterations.

RESULTS

Syphacia petrusewiczi Bernard, 1966 from mid-Yenisei valley, Krasnoyarsk Region, Siberia

Morphology. Cephalic vesicle present. Well-developed cervical alae with transverse striation arise behind the cephalic plate. One pair of cervical papillae at the level of nerve ring.

Male (n = 1). Body 870 long; 80 wide. Pharynx 135 long with 80 long procorpus and 40 diameter bulb. Nerve ring at the level 70 from apex. Excretory pore at the level 190. Three cuticular 50, 48, 50 long mamelons present on the ventral side of the body at 240, 340, 640 from apex and length 50, 48, 50, respectively. The tail is truncated ventrally, ends with a very short, wide conical process with a

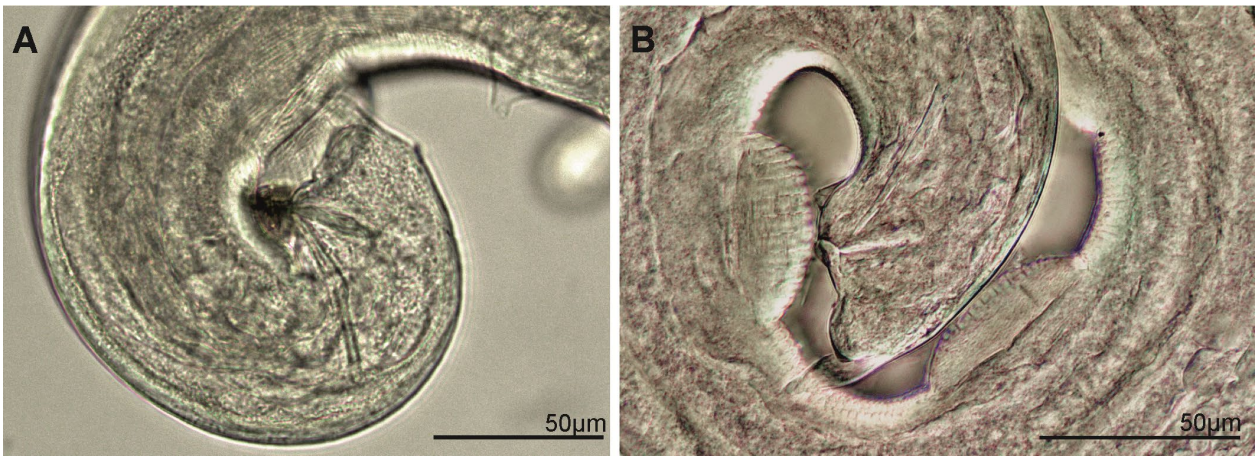

Fig. 1. Caudal part of the male body of *Syphacia petrusewiczi* with spicules and gubernaculum. A – specimen from 'Mirnoye' Yenisei Ecological Station of the A.N. Severtsov Institute of Ecology and Evolution, RAS; B – specimen from Gomselga, Republic of Karelia.

rounded tip (Fig. 1A). Process length 25. Spicule length 52, gubernaculum length 32. Gubernaculum fused with a tooth-like hook on the posterior lip of the cloaca (Fig. 1A).

Female (n = 10). Body 3233 ± 247 (2950-3400) long; 220 ± 44 (170-250) wide. The pharynx 256 ± 13 (245-270) long with 172 ± 13 (157-180) long procorpus and 67 ± 3 (65-70) diameter bulb. Nerve ring at the level 123 ± 6 (120-130). Excretory pore and vulvar opening at 256 ± 13 (245-270) and 550 from the anterior end, respectively. Eggs 100 long and 34 ± 1 (33-35) wide. Tail elongated and narrow.

Locality. 'Mirnoye' Yenisei Ecological Station of the A.N. Severtsov Institute Ecology and Evolution RAS; (62°17' N, 89°02' E).

Host. *Clethrionomys rutilus* Pallas, 1779.

Location. Caecum.

Syphacia petrusewiczi Bernard, 1966 from the Russian north, Republic of Karelia

Morphology. Cephalic vesicle present. Well-developed cervical alae with transverse striation arise behind cephalic plate. One pair of cervical papillae at the level of nerve ring.

Male (n = 2). Body 910 (820) long; 80 wide. Pharynx 145 long with 95 long procorpus and 40 diameter bulb. Nerve ring at the level 75 (70) from apex. Excretory pore at the level 210 (220). Three cuticular 40 (26), 48 (56), 50 (55) long mamelons present on the ventral side of the body at 280 (260), 410 (350), 640 (600) from apex, respectively. The tail truncated ventrally, ends with a very short, wide, conical process with a rounded tip. Process length 30 (27). Spicule length 52, gubernaculum length 20 (Fig. 1B). Gubernaculum fused with a tooth-like hook on the posterior lip of the cloaca (Fig. 1B).

Female (n = 10). Body 3275 ± 65 (3200-3350) long; 258 ± 24 (240-290) wide. Pharynx 263 ± 9 (255-270) long with 185 ± 6 (180-190) long procorpus and 74 ± 12 (73-75) diameter bulb. Nerve ring at the level 125 ± 6 (120-130). Excretory pore and vulvar opening at 351 ± 5 (345-355) and 550 from anterior end, respectively. Eggs 100 long and 34 ± 1 (33-35) wide. Tail elongated and narrow.

Locality. Republic of Karelia, Gomselga field station of the Institute of Biology, KarRC RAS (62°7' N, 34°0' E).

Host. *Clethrionomys glareolus* Schreber, 1780.

Location. Caecum.

Phylogenetic relationships. Phylogenetic relationships of the studied *Syphacia* inferred from analysis of partial LSU rDNA (28S rDNA) are shown in Figure 2. The obtained sequences and the most similar sequences found on the NCBI GenBank allowed us to construct the 693 b.p. long alignment. The *S. petrusewiczi* sequences obtained in this study and one sequence retrieved from the NCBI GenBank form a clade with 100% support. This latter sequence refers to *S. petrusewiczi* from *Clethrionomys rutilus* studied in Japan (Okamoto *et al.*, 2009). There is a subdivision in the clade of *S. petrusewiczi*. Sequences of *S. petrusewiczi* from *C. glareolus* differ from that parasitising *C. rutilus* by 3 b.p. *Syphacia* from *C. rutilus*, Japanese and Russian ones, form subclades with 95% posterior probability support. Nucleotide differences between specimens from Japan and Siberia were not observed.

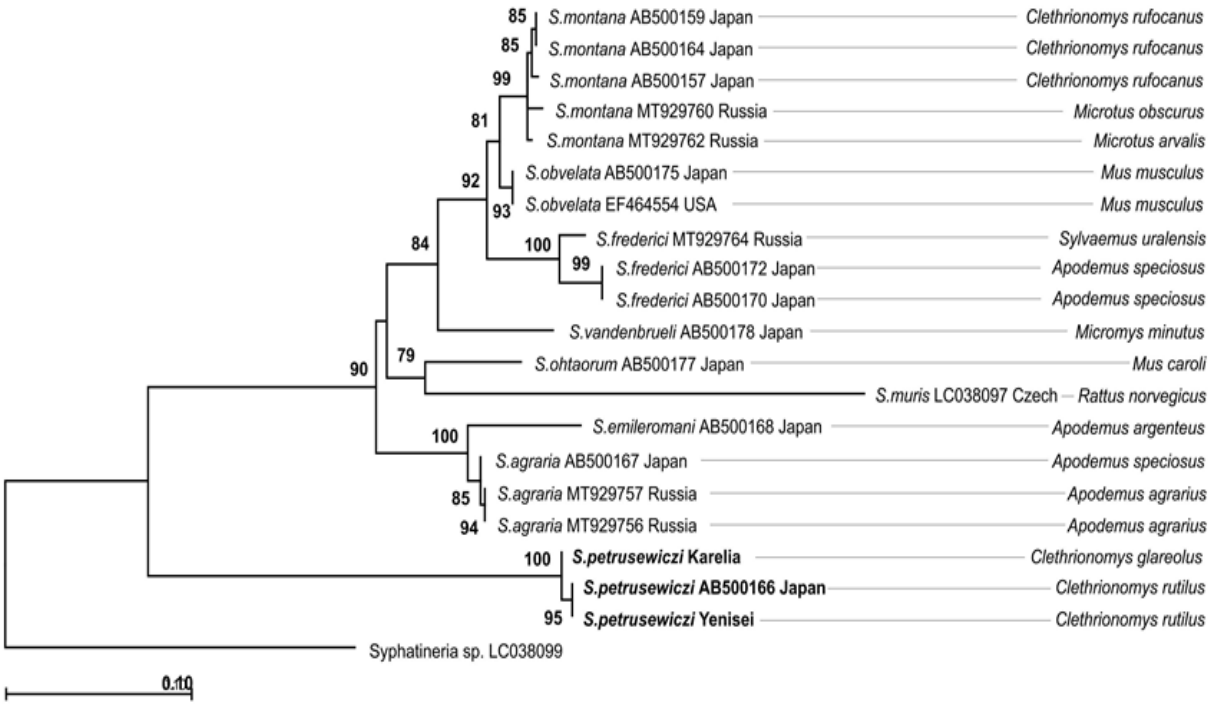

Fig. 2. ML tree of *Syphacia* species inferred from the analysis of the LSU (28S rDNA) data with bootstrap values (over 70%) near the nodes. The scale bar represents the number of nucleotide substitutions per site. Nucleotide substitution model – TN93.

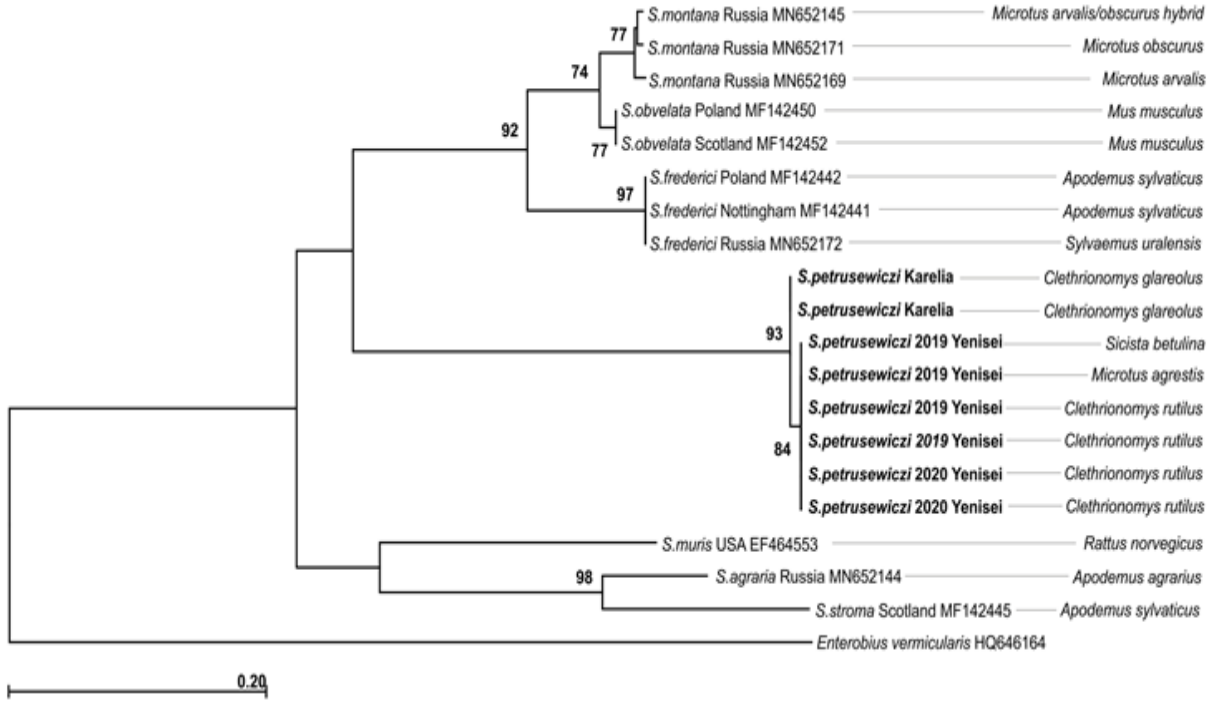

Fig. 3. Bayesian tree of *Syphacia* species inferred from the analysis of the partial ITS1+5.8S+ITS2 rDNA region of ribosomal nuclear repeats data with posterior probabilities values (over 70%) near the nodes. The scale bar represents the number of nucleotide substitutions per site. Nucleotide substitution model – GTR+I+G.

A phylogenetic tree inferred from analysis of the partial ITS1+5.8S+ITS2 rDNA region of ribosomal nuclear repeats 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 ITS1+5.8S+ITS2 rDNA region was 695 b.p. In this cladogram, there was 93% posterior probability support of monophyly for the species of *S. petrusewiczi*. Isolates from Yenisei form subclades with 95% posterior probability support. Nucleotide differences between isolates from Karelia and Yenisey were 4 b.p.

DISCUSSION

The morphology of both studied *Syphacia* populations correspond to the descriptions of *S. petrusewiczi* (Ryzhikov *et al.*, 1979; Tenora *et al.*, 1983): laterally elongated cephalic plate; developed cervical alae with ornamentation; and the appearance of the male caudal end and copulatory system (shape of the tail, and the size and shape of spicules and gubernaculum (Fig. 1). The body size of female specimens was the same as described in the literature, but males are smaller (Ryzhikov *et al.*, 1979; Tenora *et al.*, 1983). The morphology of males is similar to that described for *Syphacia petrusewiczi rauschi* (Quentin & Gran, 1977) from *Clethrionomys rutilus dawsoni* Merriam, 1888 caught at Anchorage, Alaska, and *S. petrusewiczi* from *C. glareolus* and *C. rutilus* from the Palearctic region (Ryzhikov *et al.*, 1979). The ratio of pharynx to body length is the same (0.16-0.18) as previously described (Quentin & Gran, 1977; Ryzhikov *et al.*, 1979; Tenora *et al.*, 1983). The mean size of spicules is 52 for both populations, which is equal to published data for *S. petrusewiczi* (Ryzhikov *et al.*, 1979). The size of gubernaculum for the Yenisey population is 32 (*i.e.*, corresponds to the species description) and the size of gubernaculum in the specimen from Karelia is 2, which is smaller than the 25-35 reported in the literature (Quentin & Gran, 1977; Ryzhikov *et al.*, 1979). Combined evidence (molecular data, morphology, hosts) led us to conclude that pinworms of both Karelian and Yenisey populations belong to the species *S. petrusewiczi*.

Syphacia petrusewiczi is considered to be a common pinworm parasite of *C. glareolus* and *C. rutilus* in the Palearctic region (Quentin, 1971; Tenora & Mészáros, 1975; Behnke *et al.*, 2001; Hasegawa *et al.*, 2008; Milazzo *et al.*, 2003). It was also found in *C. rufocanus* (Ryzhikov *et al.*, 1979). In our study, *S. petrusewiczi* in Yenisey valley was found not only in *C. rutilus* but also in the field vole *Microtus agrestis* and the northern birch mouse *Sicista betulina*. *Microtus agrestis* is a species from the genus *Microtus* that is a common host of *S. nigeriana* or *S. montana* (Ryzhikov *et al.*, 1979; Hugot, 1988), but was never reported as a host for *S. petrusewiczi*. Another reported host, Northern birch mouse *Sicista betulina*, belongs to another family, Dipodidae, and to our knowledge has never been reported as a host for this nematode.

An absence of well-defined correspondence between evolutionary lines of *Syphacia* and their hosts persuaded Okamoto *et al.* (2009) to reject ideas about strict host-parasite co-evolution in this group of nematodes. Still, in our opinion, some elements of co-evolution can be traced. For example, there are two close but well-defined haplotypes of *S. petrusewiczi* (Figs 2 & 3) from *C. glareolus* and *C. rutilus*, which are sister species (Kohli *et al.*, 2015). Moreover, the Russian haplotype of *S. petrusewiczi* from *C. rutilus* of Siberia is identical to the haplotype of *S. petrusewiczi* from the same host in Japan (Okamoto *et al.*, 2009). Thus, co-evolution can be traced, but only in connection with recent evolutionary events.

Further study on additional samples collected from various hosts and localities would be required to discover a range of hosts and to obtain the full picture of morphological and genetical variability.

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Д.И. Горелышева, Б.Д. Ефейкин, В.Д. Якушов и Б.И. Шефтель. Молекулярная и морфологическая характеристика *Syphacia petrusewiczi* Bernard, 1966 (Nematoda, Oxyurida) из России.

Резюме. Это исследование предоставляет новые молекулярные и морфологические данные о *Syphacia petrusewiczi* – острице грызунов. Изучены представители популяций, заражающих разные виды хозяев, в двух отдаленных регионах: на Европейском Севере России и в Северной части Средней Сибири. В последнем регионе специфичность *S. petrusewiczi* не была строгой: хозяевами служили грызуны разных семейств. Полученные нуклеотидные данные являются первыми для *S. petrusewiczi* из России.