# Molecular characterisation of lungworms from the genus *Parafilaroides* (Strongylida: Filaroididae) parasitising Caspian and Baikal seals

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**Summary.** For the first time, a molecular characterisation study was carried out on nematodes of the genus *Parafilaroides* parasitising the respiratory tract of Caspian (*Pusa caspica*) and Baikal (*P. sibirica*) seals. These seals live in isolation in the Caspian Sea and Lake Baikal respectively; water bodies which have no connection with the ocean. The lungworms were originally described as *P. caspicus* (Caspian seals) and *P. krascheninnikovi* (Baikal seals), but currently, do not have the status of valid species. The sequences of the D2-D3 of 28S and ITS2 rRNA gene and partial mitochondrial *COI* gene were obtained from the two forms of lungworms. The analysis of genetic distances showed that these two forms, despite their long-term isolation, are genetically close to each other and differ from *P. gymnurus*, which parasitises seals of the Phocinae living in seas of the northern hemisphere. Currently, *P. krascheninnikovi* is considered a synonym for *P. gymnurus*, but our initial findings dispute this statement. However, the clarification of taxonomic status of *Parafilaroides* parasitising the Caspian and Baikal seals needs further investigation using morphological methods and a larger sampling for the molecular analyses.

**Key words:** *COI* gene, ITS2 rRNA gene, marine mammal parasites, *Parafilaroides caspicus*, *Parafilaroides krascheninnikovi*, 28S rRNA gene.

Lungworms of the genus *Parafilaroides* (Strongylida: Filaroididae) are nematodes parasitising the respiratory tract of pinnipeds. The life cycles of these nematodes are not well known, but it has been shown that fish (Dailey, 1970; Lehnert *et al.*, 2010) and, possibly, nereid polychaetes (Kontrimavichus & Delyamure, 1979) may serve as intermediate hosts. Currently, seven (Elson-Riggins *et al.*, 2020, based on Dailey, 2009) or nine (WoRMS, 2021) species of *Parafilaroides* are considered valid. Either two or four – *P. decorus* (Dougherty & Herman, 1947), *P. nanus* (Dougherty & Herman, 1947), *P. normani* (Dailey, 2009), and *P. prolificus* (Dougherty & Herman, 1947) – species have eared seal (Otariidae) definitive hosts and five species (*P. gymnurus* (Railliet, 1899), *P. hydrurgae* (Mawson, 1953), *P. hispidus* (Kennedy, 1986), *P. gullandae* (Dailey, 2006), *P. measuresae* (Dailey, 2006)) have true seal (Phocidae) definitive hosts.

Three other species have been described – *P. arcticus* (Delyamure & Alekseev, 1966) of ringed seals (*Pusa hispida*) in the Chukchi Sea, *P. krascheninnikovi* (Yurakhno & Skrjabin, 1971) of ringed seals in the Chukchi and Bering seas and *P. caspicus* (Kurochkin & Zablotsky, 1958) of Caspian seals (*Pusa caspica*), which is the Caspian Sea endemic species. In 1979, Kontrimavichus & Delyamure identified *P. krascheninnikovi* in ringed seals of the Okhotsk Sea, in spotted (*Phoca largha*) and bearded (*Erignathus barbatus*) seals of the Bering and Okhotsk seas, and in Baikal seals (*Pusa sibirica*), which is the Lake Baikal endemic species. However, Gosselin & Measures (1997) in their revision of *Filaroides* (*Parafilaroides*), which was based on analysis of original descriptions and measurements of museum specimens, concluded that *P. arcticus* and *P. krascheninnikovi* should be considered synonyms of *P. gymnurus*. They also concluded that *P. caspicus* was *species inquirenda* as the original description was insufficiently informative and the measurements reported in the original description were within the range known for *P. gymnurus*. We are not aware of any later works that would return to this question, and, as mentioned above, *P. arcticus*, *P. krascheninnikovi* and *P. caspicus* are currently absent from the list of valid species of this genus (Dailey, 2009; Elson-Riggins *et al.*, 2020; WoRMS, 2021).

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Caspian and Baikal seals are unique in their landlocked isolation, without connection to the ocean. Additionally, Baikal seals live in fresh water. The Caspian Sea and Lake Baikal are bodies of water with a long geological history. The manner and timing of the seals' arrival into these isolated reservoirs and their subsequent evolution into separate species is under discussion. It could have happened in the Middle Pleistocene, 200-300 thousand years ago, the period of the maximum continental glaciations. Arctic ancestors of Caspian and Baikal seals were presumably trapped in large ice-dammed lake at the foot of the Central Siberian ice sheet into which both the Ob and Yenesei Rivers flowed. This lake was connected *via* Yenisei River with the Lake Baikal Basin and, presumably, with the Aral and Caspian Seas through the Tobol River (Davies, 1958). According to other hypotheses only the Baikal seal invaded Baikal from Arctic in the Middle Pleistocene (Repenning *et al.*, 1979) or much earlier, about 3 million years ago (Koretsky & Barnes, 2006), whereas the Caspian seal is proposed to be a direct descendant of Paratethyan phocines. Pliocene time of the species divergence is in agreement with the estimated timing of *Pusa* species separation (2-3 Mya) based on molecular genetic data (Palo & Väinölä, 2006; Nyakatura & Bininda-Emonds, 2012). There were also assumptions that both Caspian and Baikal seals may originate from Paratethys and inhabit inland basins more than 5 Mya, since the late Miocene (McLaren, 1960; Ray, 1976).

Clearly, pinniped-specific parasites entered the Caspian Sea and Lake Baikal with their hosts. Over a long isolation period with a single host population these nematodes could evolve into forms that are noticeably different from their ancestors. This study represents the first assessment of these differences based on molecular methods.

## MATERIAL AND METHODS

**Nematode populations.** Two-four mm long fragments of three individual nematodes were collected from the lungs of three different Baikal seals. Visual checks ensured that fragments from each nematode came from the same individual. Seals were caught in Chivyrkuyskiy bay (about 53°43' N and 109°07' E) during the October 2020 scientific catch of the Baikal branch of the Russian Federal Research Institute of Fisheries and Oceanography ("BaikalNIRO"). On the coast of the Caspian Sea fragments of three nematodes were obtained from two Caspian seal carcasses (two lungworms were taken from different lobes of the lung of one seal), which were found on the seashore in the Makhachkala area (Daghestan) in December 2020. Fragments of each individual nematode were preserved in 96% alcohol in separate individual tubes. In the current paper, we use the designation '*P. krascheninnikovi*' and '*P. caspicus*' for the nematodes found in the lungs of the Baikal and Caspian seals, respectively. This usage does not, however, purport to confirm the taxonomic status of these designations.

**DNA extraction, PCR and sequencing.** For the DNA extraction, we used a Diatom DNA Prep reagent kit (Isogene Lab. Ltd, Russia) according to the manufacturer's protocol. Prior to lysing, samples were homogenised in 100 µl of the lysis reagent using a stainless-steel bead in a MM400 mixer mill (Retsch GmbH, Germany) at 30 oscillations $s^{-1}$ for 30 s.

The molecular markers most represented in GenBank for lungworms of *Parafilaroides* were chosen for analysis: *i*) nuclear ITS2 rRNA gene (amplified with primers specified in Lehnert *et al.*, 2010); *ii*) D2-D3 region of the 28S rRNA gene (amplified using primers 500/501 specified in Nadler *et al.* (2000) for Ancylostomatidae and used for *Parafilaroides* by Rengifo-Herrera *et al.* (2014); and *iii*) first half (about 700 bp) of the cytochrome c oxidase subunit 1 (*COI*) gene of mitochondrial DNA (mtDNA). The latter was amplified at a 42°C annealing temperature using *cox* 1 forward primer (Dailey, 2009) paired with the originally designed COI_v2_R (5'-GGR TGA CCA AAA AAY CAA A-3'). The COI_v2_R design was based on the alignment of more than 50 complete mitochondrial genome and/or *COI* sequences of representatives of 16 genera of the order Strongylida. All PCR products were visualised on 1.5% agarose gels and purified using a cleanup S-Cap purification kit (Evrogen, Russia). The purified products were used as template for sequencing using the BigDye Terminator v.3.1 kit (Thermo Fisher Scientific, USA) with both forward and reverse primer for each product. Sequencing was performed on a Genetic Analyzer 3500 (Thermo Fisher Scientific, USA). The resulted sequences were aligned using the BioEdit v.7 software (Hall, 1999) and deposited in GenBank under accepted numbers: OK318457-OK318466.

**Sequence and phylogenetic analysis.** GenBank sequences with accession numbers LT984651-655, MT041759-761, MT041763-764, FJ787304, KP402084-085 (ITS2), AM039757, AY292802, LT984656-657, KC013593, KC013601, MG584860 (28S rRNA), and LT591890-893 (*COI*) were used for comparative analysis. We estimated the distance between sequences (p-distance) by pairwise alignment in BioEdit or by pairwise distance and means of within and between groups distance estimation in MEGA v.10 (Kumar *et al.*, 2018). Indels of more than 1 bp

length were counted using two methods: *i*) each gapped position in the alignment was treated as an independent single mutation (all indel positions distance, AIPD); and *ii*) each indel was counted as a sole mutational event despite the number of deleted/inserted bases (single indel position distance, SIPD). Neighbour Joining cladograms (based on 10,000 bootstrap replications) reconstruction and translation of *COI* sequences (invertebrate mitochondrial code table) were done in MEGA.

## RESULTS

**The D2-D3 of 28S rRNA gene.** We obtained an identical 605 bp sequence for this marker for both *P. krascheninnikovi* (OK318457) and *P. caspicus* (OK318458). For the three 28S sequences of similar length available on GenBank for *P. decorus* (AM039757, Chilton *et al.*, 2006; MG584860, Seguel *et al.*, 2018; AY292802, Carreno & Nadler, 2003), two were identical to each other, but the third differed from them by 0.83%. The sequence of an unidentified *Parafilaroides* found in the Antarctic fur seal (*Arctocephalus gazella*, Otariidae) (KC013601 Rengifo-Herrera *et al.*, 2014) differed from *P. decorus* by a similar smaller value: 0.50-0.83%. The difference between these sequences and the sequence of *P. krascheninnikovi* / *P. caspicus* was bigger: 5.82-6.16% AIPD or 5.16-5.53% SIPD. The sequence of another specimen of unidentified *Parafilaroides* (KC013593) found in the southern elephant seal (*Mirounga leonina*, true seals, Phocidae) differed from both *P. decorus* (6.40-6.73% AIPD or 5.00-5.33% SIPD) and *P. krascheninnikovi* / *P. caspicus* (4.47% AIPD or 3.83% SIPD).

*Parafilaroides gymnurus*, a species suggested by Gosselin and Measures (1997) to be the same as *P. krascheninnikovi*, is represented in GenBank by two 28S sequences of nematodes found in the harbour seal (*Phoca vitulina*, Phocidae), one from the North Sea and one from the Pacific Coast of the USA (GenBank LT984656 and LT984657 Elson-Riggins *et al.*, 2020). Regarding the latter, the authors (Elson-Riggins *et al.*, 2020) noted that the species identification was presumptive, since morphological control of these parasites was not carried out. It is a short sequence (310 bp corresponding to 320-629 positions of our OK318457/58 sequences), a part of 28S rRNA gene which seems to be conservative. These sequences were identical both to each other and to the *P. krascheninnikovi* / *P. caspicus* sequence. At the same time, all three sequences of *P. decorus* and the sequence of lungworm from the Antarctic fur seal were also identical to each other in this region of 28S rRNA gene. The sequence of *Parafilaroides* from the elephant seal had 3.18% AIPD or 1.79% SIPD differences from *P. decorus* and 1.80% differences from *P. gymnurus* / *P. krascheninnikovi* / *P. caspicus* sequence in this region.

**ITS2 rRNA gene region.** We obtained three identical sequences of 621 bp for the three specimens of *P. krascheninnikovi* (OK318459) and three identical sequences of 626 bp for the three specimens of *P. caspicus* (OK318460). These two variants differed by eight single nucleotide substitutions and one 5 bp indel, resulting in 2.08% AIPD or 1.45% SIPD differences from each other. There were five ITS2 sequences (454-649 bp) available on GenBank for comparison obtained from *P. gymnurus* in harbour seals of the Baltic and North Seas and the Pacific coast of the USA (FJ787304 Lehnert *et al.*, 2010 and LT984651-653 Elson-Riggins *et al.*, 2020) and one from parasites in Pacific harbor seal for which the species identification has not been confirmed morphologically (LT984654 Elson-Riggins *et al.*, 2020). For the 453-481 bp fragment overlapping with our OK318459/60d, the five sequences differed from each other by single substitutions within 0.21-1.04%.

Comparing our ITS2 sequences to those of *P. gymnurus*, *P. krascheninnikovi* had 2.71% to 3.54% differences, and *P. caspicus* had 4.54-5.36% AIPD or 3.74-4.57% SIPD differences. The distances between these sequences are shown in Figure 1.

One known sequence of *P. decorus* from the Guadalupe fur seal (*Arctocephalus townsendi*) and five sequences from the California sea lion (*Zalophus californianus*, Otariidae) from the Pacific coast of the USA (MT041759-761, MT041763-764 Williams *et al.*, 2020; LT984655 Elson-Riggins *et al.*, 2020) differed from each other by 0.25-1.52% for the common fragment of 394 bp.

Two sequences from nematode eggs found in faeces of *Arctocephalus australis* from Brazil (KP402084-085, Jacobus *et al.*, 2016), differed from each other by 0.51% for the same 394 bp fragment, and had 5.63-7.16% SIPD differences from the *P. decorus* sequences.

Sequences from the Phocidae and Otariidae lungworms differed by a large number of mismatches and long indels. For instance, the similarity of sequences LT984655 (*P. decorus*) and LT984651 (*P. gymnurus*) for a 400 bp fragment is only 68.0%. This low similarity of the ITS2 region of *Parafilaroides* parasitising the eared seals to that of *P. gymnurus* was also reported earlier (Jacobus *et al.*, 2016). In our study, it is possible that the MT041759-761, MT041763-764, LT984655, and KP402084-085 sequences were properly aligned with each other but not with the *P. gymnurus* / *P. krascheninnikovi* / *P. caspicus* sequences.

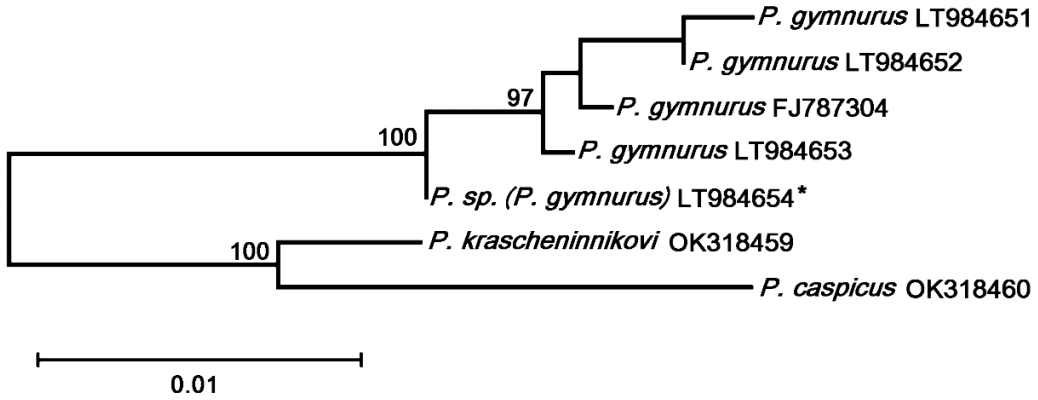


**Fig. 1.** Neighbour Joining cladogram of a 481 bp ITS2 fragment of *Parafilaroides kraschenninnikovi*, *P. caspicus*, and four *P. gymnurus* sequences. A 5-bp long indel in the *P. caspicus* sequence was replaced by a single substitution and the pairwise-deletion option was applied for sequences of different length. * – species identification was noted as presumptive.

To evaluate the span of distances within and between these groups we implemented the ClustalW multiple alignment algorithm and chose a fragment corresponding to the 195-442 positions of OK318460 (*P. caspicus* sequence), which seemed to be of good similarity in all sequences. After replacing all indels (regardless of length) by single nucleotide substitutions, we obtained an alignment of 253 positions. Based on this, we found the mean distance between the two large groups (*P. kraschenninnikovi* + *P. caspicus* + *P. gymnurus*) and (*P. decorus* + unidentified species from otariid host) was 18.98%. The interspecific differences between representatives of these groups were within similar ranges (Table 1; Fig. 2).

**Table 1.** Means of intra- (upper row, in brackets) and interspecific (above the diagonal) SIPD distances (p-distance, %%) evaluated for a 253 bp sequence within the ITS2 region of *Parafilaroides*.
For GenBank accession numbers of used sequences see Fig. 2.

| **Species** | ***P. kraschenninnikovi*** **(0.00)** | ***P. caspicus*** **(0.00)** | ***P. gymnurus**** **(0.79)** | ***P. decorus*** **(1.32)** | ***Parafilaroides*** **sp. (0.40)** |
|---|---|---|---|---|---|
| *P. kraschenninnikovi* | *** | 1.98 | 3.24 | 18.31 | 19.57 |
| *P. caspicus* | | *** | 4.43 | 18.71 | 19.96 |
| *P. gymnurus* | | | *** | 18.63 | 19.49 |
| *P. decorus* | | | | *** | 6.26 |

* – including *Parafilaroides* sp., LT984654.

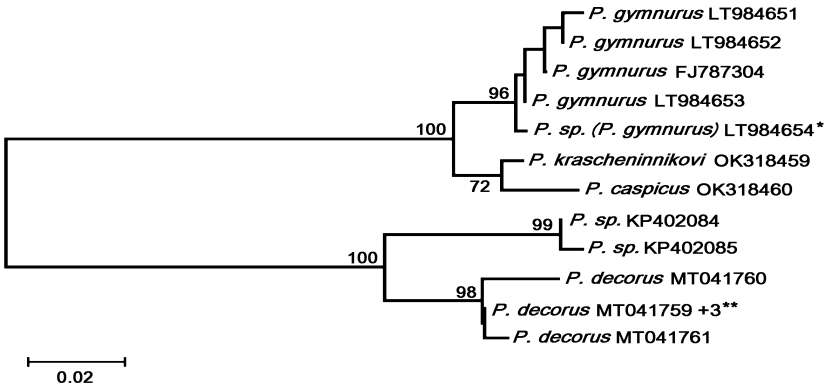


**Fig. 2.** Neighbour Joining cladogram of the most similar fragment (253 bp) of all known *Parafilaroides* ITS2 sequences. All indels, regardless of length, were replaced by single nucleotide substitutions. * – species identification was noted as presumptive. ** – LT984655, MT041759, MT041763 and MT041764 which were identical for this fragment.

**Table 2.** Means of intra- (upper row, in brackets) and interspecific (above the diagonal) distances (p-distance, %%, pairwise-deletion option) between *Parafilaroides COI* sequences.
For GenBank accession numbers of used sequences see Fig. 3.

| Species | *P. krascheninnikovi* (0.00) | *P. caspicus* (0.43) | *P. gymnurus** (0.95) | *P. decorus* (0.00) | *P. normani* (0.00) |
|---|---|---|---|---|---|
| *P. krascheninnikovi* | *** | 4.56 | 6.49 | 11.33 | 9.23 |
| *P. caspicus* | | *** | 7.62 | 10.25 | 9.12 |
| *P. gymnurus* | | | *** | 12.54 | 10.43 |
| *P. decorus* | | | | *** | 8.60 |

* – including *Parafilaroides* sp., LT591893.

**Partial mitochondrial *COI* gene.** Our initial attempts to sequence the first half of the *COI* mitochondrial gene of *P. krascheninnikovi* and *P. caspicus* using the *cox* 1 forward and revers primers proposed for *Parafilaroides* by Dailey (2009) were not successful. We obtained amplicons of the anticipated length, but a lot of sequence positions were unrecognisable and/or represented by double peaks. Only the mix of *cox* 1 forward and par_COI_v2_R resulted in sequences of acceptable quality. These sequences corresponded with the 67$^{th}$-699$^{th}$ positions of the complete *COI* gene of *P. normani* (KJ801815). However, despite using two independent PCR runs followed by bidirectional sequencing these sequences also contained some double peaks (GenBank OK318461-66). Therefore, we used the pairwise-deletion option in MEGA to estimate the pairwise distances.

After excluding unrecognised positions, all three sequences of *P. krasheninnikovi* were identical. For *P. caspicus*, the sequences of two specimens were identical, but that of the third specimen differed from the other two by four unambiguously confirmed substitutions, all in the third codon position. The distances between the *P. krasheninnikovi* sequence and the two variants of *P. caspicus* were 3.61-4.35%.

There were five *Parafilaroides COI* sequences available for analysis: two *P. gymnurus* sequences from harbour seals of the North Sea (LT591890-891, Elson-Riggins *et al.*, 2020), one sequence of unidentified *Parafilaroides* larvae from Pacific harbour seals (*Parafilaroides* sp., LT591893), one *P. decorus* sequence (LT591892) and one *P. normani* sequence (KJ801815). The distance between the two sequences of *P. gymnurus* from the North Sea was 0.16%, and the sequence of *Parafilaroides* sp. from Pacific harbour seal had 1.26 and 1.42% differences from them. Since the Neighbour Joining method unambiguously combined all three sequences into a common clade, we accepted the LT591893 sequence as a sequence of *P. gymnurus* in the interspecies distances estimation. The results of this estimation are shown in Table 2 and the Neighbour Joining cladogram of all known sequences is shown in Figure 3.

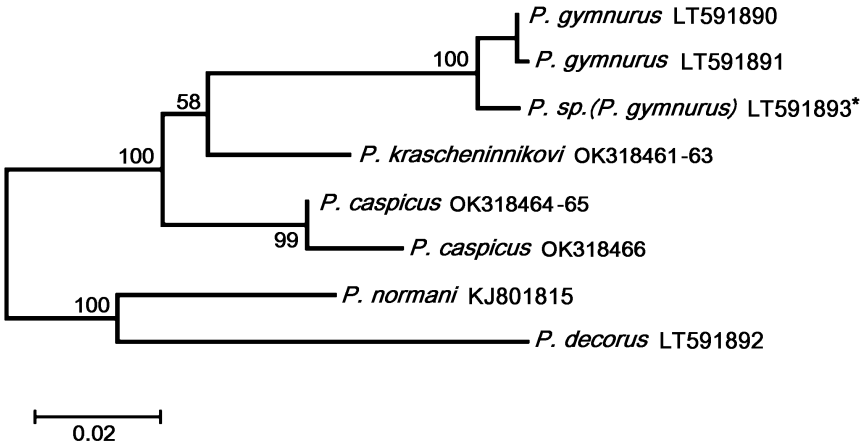


**Fig. 3.** Neighbour Joining cladogram of the *Parafilaroides COI* sequences (67$^{th}$-699$^{th}$ positions of complete gene sequence KJ801815) after excluding unrecognised positions (pairwise-deletion option). * – species identification was noted as presumptive.

**Table 3.** Amino acids found in six variable positions of *Parafilaroides COI* protein sequences. For GenBank accession numbers of used sequences see Fig. 3.

| Species | Position of complete protein sequence (AIG23797) | | | | | |
|---|---|---|---|---|---|---|
| | **35** | **42** | **63** | **118** | **141** | **160** |
| *P. decorus* | Leu | Val | Ile | Ile | Ser | Met |
| *P. normani* | Leu | Val | Val | Ile | Leu | Met |
| *P. gymnurus*[*] | Val | Leu | Ile | Val | Val or Ala | Leu |
| *P. krascheninnikovi* | Leu | Leu | Ile | Val | Val | Leu |
| *P. caspicus* | Ile | Leu | Ile | Val | [Val/Ala][**] | Leu |

[*] – including *Parafilaroides* sp., LT591893 nucleotide sequence.
[**] – double C/T peak in GCT/GTT codon in all three samples.

After translation of all five previously known *Parafilaroides COI* gene sequences into amino acid sequences, six variable positions that may differ in these species could be seen. These are the 35, 42, 63, 118, 141 and 160 positions of the complete *P. normani* protein sequence (AIG23797). Due to some unrecognised nucleotide positions, not all positions of the amino acid sequences of the lungworms could be properly determined. However, amino acids in the six variable sites were determined unambiguously in most cases (Table 3). For the 42$^{nd}$, 63$^{rd}$, 118$^{th}$ and 160$^{th}$ positions of the complete protein sequence, the substitutions found in the true seal parasites *P. krascheninnikovi* and *P. caspicus* corresponded to the substitutions that differed between the true seal parasite *P. gymnurus* and the eared seal parasites *P. normani* and *P. decorus*. The amino acid in the 141$^{st}$ position could not be determined for *P. caspicus* because of a double C/T peak in the second codon position found in all three samples. But as the GCT codon corresponds to alanine and GTT to valine, only these two amino acids could occur in this position. Notably, valine was found in this position for both *P. gymnurus* sequences from harbour seals of the North Sea, whereas alanine was found for the sequence of the harbour seal parasite from the North West Pacific.

## DISCUSSION

Based on the data presented here, we conclude that, despite originally being described as different species and living in complete isolation, the lungworms of Baikal and Caspian seals are genetically close to each other, and the parasite of the northern sea seals of the subfamily Phocinae, *P. gymnurus*, is closest to them. However, the knowledge of the ranges of intra- and interspecific variability inherent to this genus is necessary to make an adequate assessment of taxonomic status for these three forms. The limited number of genetically studied *Parafilaroides* species and individuals did not allow us to evaluate these ranges properly. For the ITS2 rRNA gene, we analysed 253 bp fragment and the distance between *P. caspicus* and *P. krasheninnikovi* (1.98%) was considerably greater than the intraspecific distance estimated for *P. gymnurus* (0.79% for the five sequences, LT984651-654 and FJ787304). However, the difference between values of intra- and interspecific distance was not so pronounced if the value of 1.98% estimated for *P. caspicus* / *P. krasheninnikovi* was compared to a distance of 1.32% calculated for three haplotypes (LT984655, MT041760-61) known for this fragment in *P. decorus*. The other problem is essential difference between *Parafilaroides* parasitising on eared and on true seals. The difference between phocid host species was estimated at 3.24% for *P. krasheninnikovi* / *P. gymnurus* and at 4.43% for *P. caspicus* / *P. gymnurus*. At the same time, sequences of phocid parasite *P. gymnurus* and otariid parasite *P. decorus* differed at 18.6%. Phocidae and Otariidae are two pinniped families with different morphologies, physiologies and life styles and it is possible that differences in the genome of their parasites would exceed interspecific level, appropriate for each group apart and direct comparison between them is incorrect.

Based on sequences of several strongylids deposited in GenBank, Blouin (2002) found that for the congeneric species, interspecific distances in *COI* gene sequences were in the range of 6.9-13.0%. The distances between *P. gymnurus* / *P. decorus* (12.54%) and *P. gymnurus* / *P. normani* (10.43%) are greater than between two otariid host species *P. decorus* / *P. normani* (8.60%) but all the values indeed are within the range of interspecific distances. The same is true for *P. gymnurus* and *P. caspicus* (7.62%), and the difference between *P. gymnurus* and *P. krasheninnikovi* (6.49%) is only slightly less. At the same time, the difference between *P. caspicus* and *P. krasheninnikovi* (4.56%)

is less than presumably species level but greater than the 0.95% value of intraspecific differences estimated for *P. gymnurus* (Table 2).

The reason for the double peak phenomenon in our *COI* study is not clear. It may be explained by: *i*) heteroplasmy; *ii*) co-amplification of a pseudogene; and *iii*) unknown methodical or technical problems. Heteroplasmy is known for nematodes (Wernick *et al.*, 2016; Kim *et al.*, 2018), but it has not been reported previously for *Parafilaroides*. Co-amplification of a pseudogene is possible, but our sequences did not include stop codons and the amino acid substitutions found resulted in peptide sequences typical for the pattern of difference between otariid and phocid parasites. This allows us to suggest that after excluding unrecognised positions, the sequences obtained in our study are acceptable for reliable analysis.

Summarising the above, despite the *Parafilaroides* from Caspian and Baikal seals being genetically close to *P. gymnurus*, their differences were great enough to discuss their possible distinct taxonomic status. Clearly, additional sampling and a detailed morphological study is required to make final conclusions. It is also possible that after revision based on both genetic and morphological data, the worms described previously as *P. caspicus* and *P. krasheninnikovi* may actually be described as subspecies of a single species.

It is important to note that Gosselin & Measures (1997) synonymised *P. krasheninnikovi* with *P. gymnurus* based on the morphology (data on original description and their own measurements of holotype and allotype) of *P. krasheninnikovi* specimens collected from seals in the Chukchi Sea. The identification of specimens from the Okhotsk Sea and Lake Baikal as *P. krasheninnikovi* was postulated by Kontrimavichus & Delyamure (1979). However, they did not provide morphological comparisons of the worms from different regions, or other arguments for the identity of these nematodes. Thus, it is possible that the morphological identification of *Parafilaroides* from Lake Baikal as the same species as *Parafilaroides* from the Chukchi Sea could be erroneous.

The genetic similarity of Baikal and Caspian *Parafilaroides* suggests their origin from a common ancestor. Was this the ancient form of *P. gymnurus*? This assumption corresponds to the scenario in which Baikal and Caspian seals independently reached their destinations from Arctic seas in the Pleistocene or Late Pliocene, as proposed by Davies (1958) and Palo & Väinölä (2006). Or are the Baikal and Caspian *Parafilaroides* on the one hand and *P. gymnurus* on the other, sister taxa evolved from an ancient common ancestor, but not from one another? This scenario assumes a Pliocene or late Miocene origin of the Caspian and Baikal seals as descendants of phocines inhabited the inland Paratethys basin (McLaren, 1960; Ray, 1976). This question remains open. Genetic data on other *Parafilaroides* parasitising true seals of the Northern hemisphere (*P. hispidus*, *P. gullandae*, *P. measuresae*) are required for reconstruction of the phylogenetics of these species and evaluation of their divergence times.

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**И.Г. Мещерский и И.В. Суворова.** Молекулярное исследование легочных паразитов рода *Parafilaroides* (Strongylida: Filaroididae), паразитирующих на каспийском и байкальском тюленях.

**Резюме.** Впервые проведено молекулярно-генетическое исследование нематод рода *Parafilaroides*, паразитирующих в дыхательных путях каспийского (*Pusa caspica*) и байкальского (*P. sibirica*) тюленей – эндемиков Каспийского моря и озера Байкал, водоемов, не имеющих связи с мировым океаном. Эти нематоды были изначально описаны как *P. caspicus* и *P. krascheninnikovi* соответственно, однако в настоящее время они не имеют статуса валидных видов. Нами для двух данных форм были определены последовательности D2-D3 региона гена 28S рРНК, ITS2 рРНК и частичная последовательность митохондриального гена *COI*. Анализ генетических дистанций показал, что эти два представителя рода, несмотря на длительную изоляцию, генетически близки друг другу и отличаются от вида *P. gymnurus*, паразитирующего на тюленях трибы Phocinae, обитающих в морях северного полушария. В настоящее время *P. krascheninnikovi* считается синонимом *P. gymnurus*, однако наши данные дают основание оспаривать это. Для дальнейшего уточнения таксономического статуса представителей *Parafilaroides*, паразитирующих на каспийском и байкальском тюленях, необходимо использование морфологических методов и увеличения выборки для молекулярного анализа.