


## New records of Pauropoda (Myriapoda) from the European part of Russia Новые находки Паулоподы (Мурилоподы) из европейской части России


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**Abstract.** Two species of Pauropoda, *Allopauropus danicus* (Hansen, 1902) and *Decapauropus gracilis* (Hansen, 1902), are recorded from Russia (Syktyvkar, Komi Republic) for the first time. The morphological characters and habitat of these species are briefly described. The COI sequences of mitochondrial DNA of *D. gracilis* are obtained and analysed for the first time. The previous records of Pauropoda in Russia, with identifications to species and genera, are briefly reviewed.

**Резюме.** Впервые для России (Республика Коми, Сыктывкар) зарегистрированы два вида Паулоподы – *Allopauropus danicus* (Hansen, 1902) и *Decapauropus gracilis* (Hansen, 1902). Кратко описаны морфологические признаки и биотоп этих видов. Для *D. gracilis* впервые получены и проанализированы нуклеотидные последовательности митохондриального гена COI. Дан краткий обзор предыдущих находок Паулоподы на территории России, сделанных с указанием родов и видов.

**Key words:** soil microarthropods, DNA barcode, bark-wood waste warehouse, Komi Republic Pauropoda, Pauropodidae, new records

**Ключевые слова:** почвенные микроартроподы, ДНК-штрихкодирование, склад коро-древесных отходов, Республика Коми, паулоподы, Pauropodidae, новые находки

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## Introduction

Pauropoda are a class within Myriapoda. They are the smallest myriapods, with body length not exceeding two mm, usually of whitish-brownish colour (Scheller, 2008, 2011). Externally, pauropods resemble springtails, but unlike them, they

are able to fold their body in the shape of a U (Scheller et al., 2004). Compared to vast numbers of studies dealing with Chilopoda and Diplopoda, Pauropoda are mostly neglected in ecological and faunistic studies, as they are generally very small and are difficult to identify (Voigtländer et al., 2016).

The data on the Pauropoda in the European part of Russia are almost absent. There are published records of pauropods from the Ulyanovsk Province (*Stylopauropus* Cook, 1896 and *Pauropus* Lubbock, 1867) (Yudin & Volkova, 2015) and the Komi Republic [*Allopaupopus helveticus* (Hansen, 1902) and *Amphipauropus rhenanus* (Hüther, 1971)] (Kolesnikova, 2022). In earlier publications, *Pauropus huxleyi* Lubbock, 1867 was recorded from the Leningrad Province (Rimsky-Korsakov, 1921) and the Republic of Crimea (Lignau, 1905, cited by Scheller, 1981). From the Asian part of Russia, four species of pauropods were recorded: *Stylopauropus golovatchi* Sheller, 1981 from Ussuriysk in the Primorskiy Territory (Sheller, 1981), *Allopaupopus frigidipatiens* Sheller, 1986, *Stylopauropus siberiae* Scheller, 1986 and *S. californianus* Remy, 1958 from Magadan (Sheller, 1986) and *S. californianus* from the Chukotka peninsula (Scheller, 1986). In total, four genera and seven species of Pauropoda were recorded from Russia in the publications known to us.

One of the useful tools for rapid species identification is the DNA barcoding method, usually based on COI sequences of mitochondrial DNA. The use of this method may be especially important when the morphological identification of an organism is problematic or impossible. However, this method has a number of significant limitations, for example, the lack of a complete reference base of sequences for all related species (Shadrin, 2021). In our study, both classical zoological and genetic techniques of species diagnostics were used to identify Pauropoda.

## Material and methods

The material was collected in 2019–2020 in a bark-wood waste warehouse located on the territory of a sawmill and woodworking plant in Syktyvkar at the left bank of the Sysola River (61°39'29"N 50°53'38"E).

The territory of the bark-wood waste warehouse is an artificial hill with an area of about ten hectares, up to 25 m high. Between 1950 and 2010, bark, sawdust, wood chips, cuttings of boards, as well as the remains of leaves, branches, and stones collected from city streets were brought here. Long-term storage of such waste in the open air in

large volumes periodically leads to a temperature increase, methane accumulation, and spontaneous combustion inside the artificial hill. Between 2000 and 2010, storage of warehouse was stopped, and natural restorative succession began expressed in peat accumulation, colonisation of the substrate by invertebrates, formation of the litter horizon, and plant associations. During the sampling period, the collected substrate was partially decomposed tree bark. The plant cover was represented by herbaceous vegetation and shrubs.

Sampling was carried out in the summer and autumn. Fifty-five soil samples for microarthropods (10×10×10 cm) were taken. In the laboratory, they were extracted to 96% ethanol using Berlese-Tullgren funnels for 7–10 days (the period sufficient for achieving the air-dry state of the soil). Fifty-five specimens of Pauropoda from seven samples were found. Forty-nine specimens were mounted on slides in Phoea liquid.

Pauropods were identified to species according to the keys by Scheller (1976) and Andersson et al. (2005) under a Leica DM 4000B microscope. All specimens were classified as adults, subadults, and juveniles according to the number of leg pairs. The sex of adults was determined following Rodríguez (2015). All specimens are kept at the Department of Animal Ecology, Institute of Biology, Komi Science Centre (Syktyvkar). Microphotographs were taken with a Nikon Digital Sight DS-2Mv camera using a Nikon Eclipse80i microscope equipped with a system of differential interference contrast (DIC) and video recording of images at 1000× magnification.

For DNA extraction, amplification, and sequencing, six specimens of Pauropoda were taken. The samples were fixed with purified 96% ethanol and stored at -20°C until use. The DNA was isolated with a 6% Chelex-100 solution in bidistilled water. A cut-up worm fragment was placed in 10 µL of the solution, 1 µL of proteinase-K was added, and then it was heated in a thermostat to 55 °C for 30 min and to 99 °C for 15 min. PCR amplification was performed in a 50-µl reaction mixture containing 10 µl of ScreenMix (Evrogen, Russia), 10 µl of each primer (final concentration 0.3 µM) (Evrogen, Russia), 18 µl of ddH<sub>2</sub>O (Paneco, Russia), and 2 µl of DNA (1÷100 ng). The COI gene was amplified by PCR and sequenced with the primers COIL 1490 and COIH 2198 (Folmer et

al., 1994). The PCR amplification conditions were similar to those recommended by the referred authors. Sequencing was carried out using the GenSeq set on the Nanofor 05 (Sintol, Russia). As a result of sequencing in both directions, twelve COI sequences were obtained. The alignments and phylogenetic trees were constructed using Mega X and NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The nucleotide sequences obtained in this study were submitted to the GenBank database and are available under accession numbers from OQ058996 to OQ059001.

## Taxonomic part

Two species discussed below were identified in the study material. Previous records of two other species, *Amphipauropus rhenanus* and *Allopauropus helveticus*, made by Kolesnikova (2022), are misidentifications; they are based on the material examined here.

### Order Tetramerocerata

#### Family Pauropodidae

#### Subfamily Pauropodinae

#### Genus *Allopauropus* Silvestri, 1902

#### *Allopauropus danicus* (Hansen, 1902) (Figs 1–3)

**Material examined.** **Russia, Komi Republic,** Syktyvkar, 61°39'29"N 50°53'38"E, bark-wood waste warehouse, partially decomposed tree bark, 7.VII.2020, 8 adults 9 (5 males, 3 females), 3 subadults 8, 4 juveniles 6, 2 juveniles 3 (A.A. Ditts & T.N. Konakova leg., A.A. Taskaeva det.).

**Diagnostic characters.** Body white, length about one mm (Fig. 1). Anterior margin of lower antennal branch shorter than posterior margin. Antennal globulus very short. Sternal part of anal segment with three pairs of setae, *b1*, *b2* and *b3*. Setae of posterior part of body long, directed posteriorly. Coxa and trochanter of last pair of legs with setae each consisting of two equally long, almost cylindrical branches (Fig. 2). Anal plate short, subsquare with rounded posterior corners and two short clavate appendages (Fig. 3). Adult stage with nine pairs of legs.

**Distribution.** Subcosmopolite. The species is widely distributed in Europe, Africa, and the

North and South Americas (Scheller, 1990), but it is rare in the Nordic countries, known only from five localities (Andersson et al., 2019). Here, it is recorded for the first time from Russia.

**Bionomics.** This species is eurytopic; it inhabits gardens, greenhouses, dry leaves, deadwood, and litter in coniferous forests at a depth of up to ten cm. It co-occurs with *Decapauropus gracilis* (Hansen, 1902) (Scheller, 1998; Scheller et al., 2004).

#### Genus *Decapauropus* Remy, 1957

#### *Decapauropus gracilis* (Hansen, 1902) (Figs 4–5)

**Material examined.** **Russia, Komi Republic,** Syktyvkar, 61°39'29"N 50°53'38"E, bark-wood waste warehouse, partially decomposed tree bark, 29.VIII.2019, 12 adults 9 (9 males, 3 females), 1 subadult 8, 3 juveniles 5; same data but 7.VII.2020, 11 adults 9 (8 males, 3 females), 2 subadults 8, 1 juvenile 6, 2 juveniles 5 (A.A. Ditts & T.N. Konakova leg., A.A. Taskaeva det.).

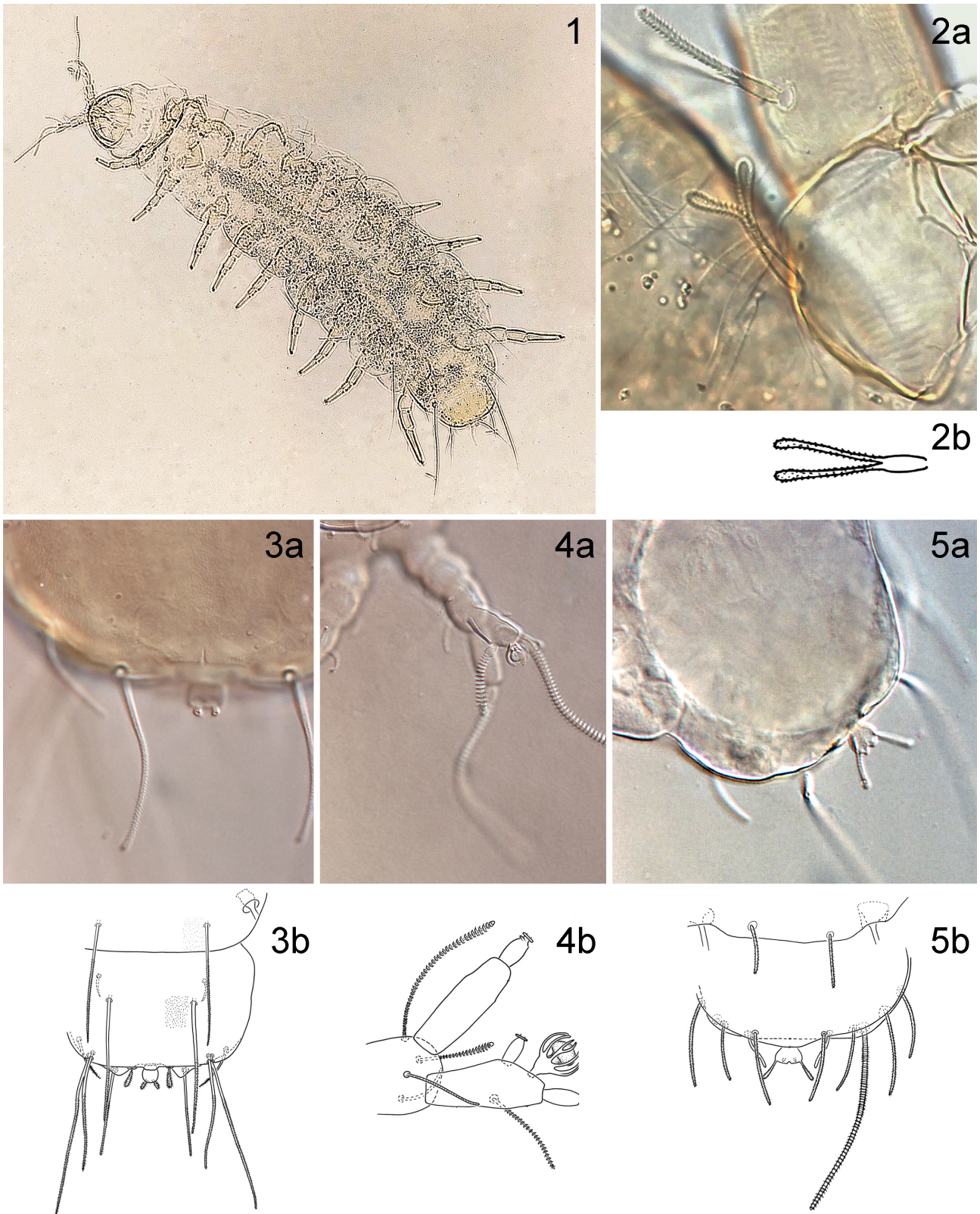
**Diagnostic characters.** Length about one mm, width 0.2 mm. As shown for other species of *Decapauropus*, anterior margin of lower antennal branch shorter than posterior margin (Fig. 4). Antennal globulus proportionately large. Setae *b3* on pygidium absent. Anal plate relatively rounded with two small lobes at posterior end and a pair of long divergent appendages located laterally (Fig. 5). Adult stage with ten pairs of legs.

**Distribution.** Subcosmopolite. The species is widely distributed throughout the world (Scheller, 1990). Here, it is recorded for the first time from Russia.

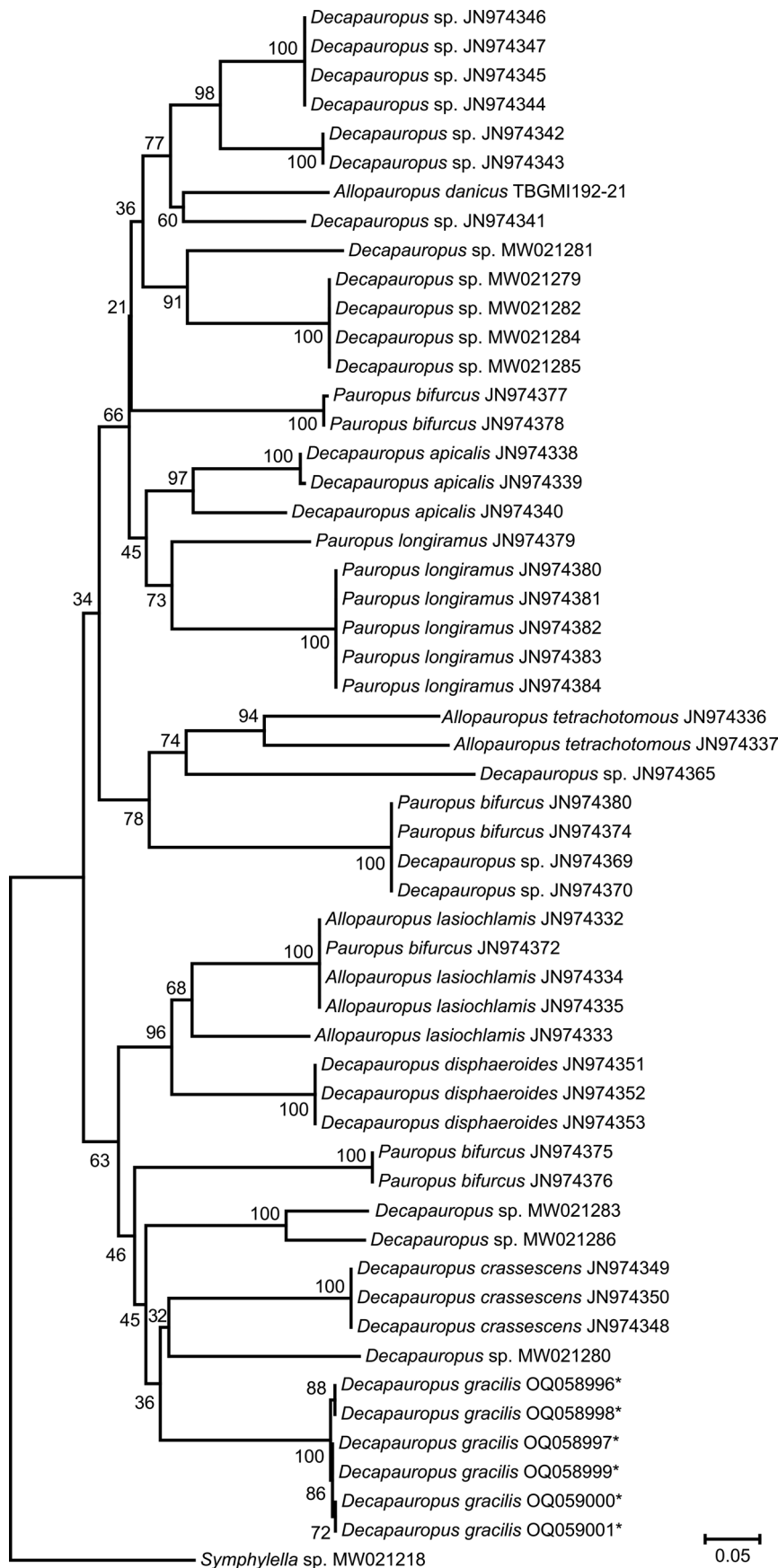
**Bionomics.** Eurytopic species; it occurs in forests (both deciduous and coniferous), meadows, gardens, greenhouses, floodplains, dunes, deadwood, under the bark of trees and stones, etc. It is also recorded from caves. This abundant species is often the only one present in samples. According to the published data, it can be found down to 60 cm, but 70% of specimens prefer a litter layer of zero to five cm (Scheller et al., 2004; Voigtländer et al., 2016).

## DNA analysis

The COI sequences of the specimens identified to species and taken from databases, includ-



**Figs 1–5.** Total view and morphological details (DIC) of *Allopauropus danicus* (Hansen, 1902) (1–3) and *Decapauropus gracilis* (Hansen, 1902) (4–5) from Syktyvkar (Komi Republic). **1**, ventral view; **2a, b**, seta on trochanter of leg 9; **3a, b**, **5a, b**, anal plate; **4a, b**, lower antennal branch. Drawings (2b, 3b, 4b, 5b) are from Andersson et al. (2005), with modifications.



ing our original specimens, were clustered on a phylogenetic tree (Fig. 6). At present, the barcode library (BOLD Systems, 2023) contains COI sequences of three species of *Allopauropus* (*A. danicus*, *A. lasiochlamis* Guo, 2010, and *A. tetrachotomous* Guo, 2010), three species of *Decapauropus* (*D. apicalis* Gai et Scheller, 2007, *D. crassescens* Shen et Song, 2007, and *D. disphaeroides* Shen et Song, 2007) and *D. gracilis* we added. All of these species are represented on the phylogenetic tree (Fig. 6). We suggest that the data on other species is missing from the BOLD Systems due to the poor knowledge of pauropods, their small size, the difficulty of identification under a stereoscopic microscope, the lack of qualified experts, as well as the high level of DNA sequence polymorphism and difficulties in extracting DNA from small animals.

According to the morphological features, we identified the six sequenced specimens from our material as *Decapauropus gracilis*. According to the results of COI sequencing, these specimens formed a single clade with high bootstrap support (100%). Unfortunately, there are no COI sequences as-

**Fig. 6.** Phylogenetic tree of Pauropoda based on COI mtDNA sequences. The tree was built by the maximum likelihood (ML) method; bootstrap support values are shown at the nodes. Specimens collected by the authors are asterisked. *Symphylella* sp. is taken as an outgroup.

signed to *D. gracilis* in the databases (BOLD Systems, 2023; NCBI, 2023). Among the specimens of *D. gracilis*, three haplotypes were identified, each represented by two specimens (Fig. 6: asterisked). The first haplotype includes two specimens (OQ058996, OQ058998) collected in 2020. The second haplotype with two positions included four specimens, of which two specimens (OQ058997, OQ058999) were collected in 2020 and two specimens (OQ059000, OQ059001), in 2019.

The samples from genetic databases identified to species are clustered according to taxa as well (Fig. 6). As distinct from other species, seven samples of *Pauropus bifurcus* Zhang et Chen, 1988 by the same authors taken from the NCBI database (NCBI, 2013) did not group into a separate cluster but diverged, clustering with other samples. A repeated alignment analysis showed that the sequences of *P. bifurcus* samples are polymorphic within the species and are more similar to the samples of other species than to each other. Currently, the taxonomic status of *P. bifurcus* is questioned. Quan et al. (2013), based on morphological characters and their own unpublished DNA barcoding data, suggested that this species belongs to another genus. In addition to possible errors in species identifications in genetic databases, it is likely that some genera of pauropods are artificial. The latter is indicated by the absence of monophyletic genera on the molecular phylogenetic tree. These facts leave open the question of species identification of samples from databases. Thus, despite the rapid development of genetic methods, the identification of organisms to species is now highly dependent on taxonomists (Abramson, 2009), and only the combined use of classical and modern methods will achieve a good result.

## Conclusions

Two species of Pauropoda, *Allopauropus danicus* and *Decapauropus gracilis*, are recorded here for the first time from Russia. These two species are subcosmopolites, so their records were expected. The obtained results extend the northern limit of the distribution of Pauropoda in northeastern Europe. The list of Pauropoda recorded from European Russia now includes five taxa (four genera); the list of Pauropoda of Russia includes four genera and seven species.

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