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Genetic lineages of *Parisotoma notabilis* sensu lato (Collembola) in Eastern Europe and the Caucasus

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Abstract

Molecular genetic studies of springtails (Collembola) reveal hidden diversity in many common species. Detailed studies of their distribution and ecology are necessary to clarify the taxonomic status of genetic lineages. This study contributes to the solution of these problems using the widespread polytypic species *Parisotoma notabilis* (Schäffer, 1896) sensu lato as a model object. The material was collected in 11 locations of Eastern Europe and 10 locations of the Caucasus. Genotyping was performed on a fragment of the CO1 marker gene and additionally on the D3-D5 region of the 28S gene. The phylogenetic tree and Automatic Barcode Gap Discovery (ABGD) and PTP analyses allowed to fine 10 lineages, of which 4 were new: L-Crimea, L-Cheget, L-Georgia, and L-Khosta. A lineage recently described from Iran has been found in Azerbaijan. Geographic distribution of lineages has been mapped. In Eastern Europe, the L1, L2, and L4-Hebert lineages are fairly evenly distributed. There are 10 lineages found in the Caucasus: L1 and L4-Hebert are the most common; various locally widespread lineages comprise some probably endemic to this region. In approximately half locations, 2—3 lineages are found together, but 4 lineages are the maximum co-inhabiting ones. Most lineages of *P. notabilis* s. l. are more or less confined to certain habitats, except the L4-Hebert lineage, which is recorded in most of the studied habitats. The other lineages of springtails living in the same area usually prefer different habitats. We suppose that genetic heterogeneity of *P. notabilis* s. l. combined with the ecological divergence of the most common lineages may partly explain the biological progress of this species in Eastern Europe.

Keywords COI | 28S | sympatry | phylogeny | polytypic species

1. Introduction

Molecular genetic methods have revolutionized the systematics of soil organisms and have allowed to estimate the approximate share of latent diversity in various taxa (Porco et al. 2012a). Taking into account integrative approaches in taxonomy (Goulding & Dayrat 2016) and the lack of knowledge on fauna for many areas worldwide, the share of known species of various taxa of soil meso- and microfauna varies from 2 % in nematodes up to 55 % in mites. It is considered that more than 80 % of Collembola species have not yet been described (Orgiazzi et al. 2016). Cryptic diversity has proven to be common in many species. For example, six out of 16 Collembola species investigated by molecular genetic methods turned out to be polytypic (Porco et al. 2012a): widespread

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Lepidocyrtus lanuginosus (Gmelin, 1788), *L. cyaneus* Tullberg, 1871 (Zhang et al. 2018a, 2019), *Folsomia manolachei* Bagnall, 1939 (Raschmanova et al. 2016), *Ceratophysella denticulata* (Bagnall, 1941), *Podura aquatica* Linnaeus, 1758, *Heteromurus major* (Moniez, 1889) (Porco et al. 2012a), and even the species with local distribution *Tomocerus problematicus* Cassagnau, 1964, *Deutonura monticola* (Cassagnau, 1954), and *Bilobella aurantiaca* Cassagnau, 1968 (Porco et al. 2012a). Latent diversity is found in both parthenogenetic species *Isotomiella minor* (Schaeffer, 1896), and bisexual ones, *Folsomia quadrioculata* (Tullberg, 1871) and *Ceratophysella denticulata* (Bagnall, 1941) (von Saltzwedel et al. 2016).

In Collembola, the genetic distances between the detected lineages (16—25% by COI) are close to interspecific distances for morphologically defined springtails species (Sun et al. 2018). Surely, this raises the question of the status of these lineages. We use the term "lineage" following Porco et al. (2012b). The lineage means a clade of the highest level, which is not always determined morphologically although determined genetically. Many morphological species consist of intraspecific lineages differing by genetic distances similar to distances between species. It was shown for *D. monticola* (17%), *C. denticulata* (19%), *H. major* (21.5%) (Porco et al. 2012a), *Desoria alaskensis* (Fjellberg, 1978) (22%), *Folsomia fimetaria* (Linnæus, 1758) (25%), *Isotomurus plumosus* Bagnall, 1940 (27%), *Sminthurides malmgreni* (Tullberg, 1877) (29%), *Entomobrya marginata* (Tullberg, 1871) (30%) (Porco et al. 2014), etc. When the genetic differences between the lineages were supported by morphological features, the authors gave them species status. In particular, the color variations of *Isotomurus* turned out to be quite different genetically, which made it possible to substantiate their species status and to distinguish six species in this genus (Carapelli et al. 1995). Morphological descriptions of species new to science are increasingly confirmed by genotyping data (Sun et al. 2017, Zhang et al. 2018b, Cipola & Aron 2021, etc.).

Cryptic diversity issues turn out to be more complicated, when genetic differences are not accompanied by noticeable morphological differences of the lineages. Sometimes, authors describe new species, as for the genus *Coecobrya* from caves in Thailand (Zhang et al. 2018c). However, in most cases, experts are in no hurry to give a species status to cryptic forms, since they realize technical complications in studying the fauna and, further on, community ecology based on the doubtful species definition. Nevertheless, the problem of the status of cryptic forms requires a solution, and this can be facilitated by a detailed study of a particular species as a model object.

Widespread springtail *Parisotoma notabilis* (Schäffer, 1896) sensu lato is a polytypical species. It is a ubiquitous parthenogenetic species, especially numerous in natural and anthropogenic habitats of Europe (Potapov 2001) and North America (Porco et al. 2013, Rousseau et al. 2018, Chagnon et al. 2000). Recently, six genetic lineages of this species have been described, differing by 15—18% in the nucleotide set of the standard mtDNA barcode 658bp in the COI-5P gene (von Saltzwedel et al. 2017). *P. notabilis* s. l. has been genetically studied in various regions of Western and Southern Europe, and in Canada. The status of *P. notabilis* sensu stricto was assigned to individuals sampled from the type locality (Germany, Hamburg, flower pot). Subsequent genetic analysis of these specimens grouped them into a separate cluster and it was named L0 (Porco et al. 2012b).

Western Europe and Canada are characterized mainly by a parapatric distribution of lineages (Porco et al. 2012b, von Saltzwedel et al. 2017). Information on Eastern Europe is scarce to date: only 10 specimens from the Moscow Oblast and Karelia have been included into one of the latest analyses (von Saltzwedel et al. 2017). Our recent work added genetic information for COI gene fragment (12 ind.) and 28S rDNA gene fragment (87 ind.) for the central Russian Plain (Moscow city and Moscow Oblast) (Struchkova et al. 2022). Interestingly, these studies evidence on the sympatries of the lineages found at least in the central part of this region, in contrast to the data obtained for Western Europe. To better understand this shift in genetic structure of the population between Western and Eastern Europe, a broader study is required.

To improve the lineages sampling, we paid special attention to the Caucasus region, where no data was obtained until now. This region belongs to Western Asia, although it is adjacent to Eastern Europe from the south. We assume that, on the one hand, the L0, L3, and L4-Saltzwedel lineages common in southern and western Europe can be found in the Caucasus (Porco et al. 2012b, Salzwedel et al. 2017). On the other hand, Asian lineages may occur in the Caucasus, such as the recently described one from Iran (Lafooraki et al. 2022). In addition, the Caucasus region may be the habitat of yet unknown *P. notabilis* s. l. lineages, since this region is one of the 25 biodiversity hotspot ecoregions in the world known for the endemism of many groups of organisms (Myers et al. 2000).

The study aims to describe the distribution of various genetic lineages of *P. notabilis* s. l. in Eastern Europe and the Caucasus region. This data helps to get a general idea of the lineages' ranges in these poorly studied regions. In addition, we focus on assessing the degree of lineage sympatries, noted earlier for the Moscow Oblast. These results are important for understanding the genetic structure of *P. notabilis* s. l. populations, since this species is the most common representative of polytypical parthenogenetic Collembola species in European and North American Holarctic. Information about habitats allow us to conclude on the presence/absence of a divergence of genetic lineages by habitat in other areas, as found earlier in the Moscow Oblast.

2. Materials and methods

Study locations. The springtails were collected in the lowland and mountainous regions of Eastern Europe and the Caucasus, in various remote locations. Plain regions were sampled in: boreal forests of Karelia, mixed forests of Republic of Belarus, Kaliningrad Oblast, Ryazan Oblast, Vladimir Oblast, Nizhny Novgorod city, and Kazan city, forest-steppe of Voronezh city, and in semi-desert zone of Astrakhan Oblast). Previously published data on the Moscow Oblast have been also included into analysis (Striuchkova et al. 2022). The springtails were sampled also in the forests and coastal subtropical habitats of the Crimea. In the Caucasus region, we collected materials in the steppe locations of Rostov Oblast, Stavropol Krai, and Dagestan, in the coastal locations of Krasnodar Krai, and in the mountainous locations of Kabardino-Balkaria, North Ossetia, Georgia, Armenia, and Azerbaijan. Sampling sites with exact geographical coordinates are given in Appendix 1. Our collections cover natural locations from taiga to deserts within 62.49–38.49′ N and from 20.5–49.15′ E. Most of the collections were carried out in 2019–2022 (apart from Armenian material).

Habitats. Samples were mostly taken in forests. Even in the zones of steppes and deserts, there were mountain or floodplain forests. Since genetic lineages prefer differently disturbed habitats (Striuchkova et al. 2023), in some locations (Karelia, Moscow Oblast, Stavropol Krai), the material was collected both in forest and urban green spaces: forest parks and/or lawns. In some locations, sampling was performed in cities only (Nizhny Novgorod, Voronezh, Kazan). In the Crimea and the Caucasus, other habitats were also covered (coastal thickets, bushes, alpine and nival belts of mountains).

The analysis also included an additional material from Greece (lineage L3, 2 ind. from our collection), one sequence from GenBank for a new lineage from Iran (MW477776; Lafooraki et al. 2022), and the sequence L4-Saltzwedel from Croatia (KJ792230; von Saltzwedel et al. 2017).

Sampling. In total 58 samples were taken from 21 locations. In each sampling plot, one mixed 1–2 liters sample of decaying plant debris and topsoil was taken.

Laboratory processing of samples. Springtails were extracted into 96% alcohol using Tullgren funnels. Generally, 7 to 10 predominantly adult individuals of *P. notabilis* s. l. from each sample were selected under a microscope for molecular analysis.

Genotyping. The standard mtDNA barcode (658bp in the COI-5P gene), and the D3-D5 region of the 28S rDNA gene were used for analysis. DNA extraction was performed from a single specimen using the Phire Tissue Direct PCR Master Mix (Thermo Scientific). According to the Dilution & Storage manufacturer protocol, the specimen was placed in 20 μL DNA Dilution Buffer, added 0.5 μL DNARelease Additive and incubated at 98°C for 2 min. This technique allowed DNA extraction with relatively little damage to the specimen, enabling vouchers to be retrieved and preserved. Each PCR reaction had a total volume of 20 μL: 10 μL of Phire Tissue Direct PCR Master Mix, 7 μL of nuclease-free Water, 1 μL of each primer (4 pmol/μL), and 1 μL of specimen's DNA. The primers ColFol-for 5'-TTT CAA CAA ATC ATA ARG AYA TYG G-3' and ColFol-rev 5'-TAA ACT TCN GGR TGN CCA AAA AAT CA-3' were used to amplify the COI-5P loci (Ramirez-Gonzalez et al. 2013). Amplification of the D3–D5 region of the 28S rDNA was performed using primers 28Sa 5′-GAC CCG TCT TGA AGC ACG-3′and 28Sbout 5′-CCC ACA GCG CCA GTT CTG CTT ACC-3′ (Whiting 2002, Prendini et al. 2005). Amplification protocol: one initial activation step at 98°C for 5 min, then 30 amplification cycles: denaturation at 98°C for 5 s, primer annealing at 52°C (CO1) or 57°C (28S) for 5 s, elongation at 72°C for 20 s, and a final elongation at 72°C for 1 min. The PCR products were evaluated on gel electrophoresis in 1% agarose with ethidium bromide. PCR products were purified using an enzyme mix of 0.5 μL of exonuclease I (Exo I) and 1 μL of recombinant alkaline phosphatase (rSAP) per 5 μL of PCR product, according to the protocol: 37°C for 15 min and 80°C for 15 min. After assessing the DNA amount, the samples were dried and sent for sequencing to SYNTOL LLC (Moscow, Russia).

Data analysis. The sequencing chromatograms were edited in Chromas Lite software (v. 2.6.6) and were aligned using BioEdit software (v. 7.2.5). Maximum likelihood tree construction and calculation of average genetic distances within and between lineages (K2P-pairwise distance and p-distance) were performed using the MEGA-11 program. Uniform rates among sites were selected and pairwise deletion was applied for calculation of distances. Tree calculation was performed with the Maximum Likelihood method with Tamura 3-parameter + Gamma Distributed + Inverse-gamma Distributed parametric model proposed for the COI gene and the Jukes-Cantor method + Gamma Distributed parametric model for the 28S gene. Automatic Barcode Gap Discovery (ABGD) analyses were performed online (bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with three distance metrics (Jukes-Cantor, K2P, p-distance) and minimum genetic divergence threshold of 8 % based on previous research (Porco et al. 2012a). Defaults were employed for all other parameter values. The Poisson tree processes (PTP) model to infer putative lineages boundaries was calculated online (species.h-its.org/ptp/). MCMC generations number was 300 000. Maps with sample locations were prepared using QGIS (v.3.30.0) (qgis.org/ru/site/).

The lineages were identified and named after Porco et al. (2012b) and Striuchkova et al. (2022). We have designated the recently described lineage from Iran (Lafooraki et al. 2022) as L-Hyrcan.

Localities of the Caucasus region were divided into four groups: 1 - the foothills of the Caucasus (Stavropol Krai, Rostov Oblast, northern Dagestan), 2 - the slopes of the Main Caucasian Ridge: northern (Kabardino-Balkaria, North Ossetia) and southern (Georgia), 3 - the Black Sea coast (Krasnodar Krai) and 4 - Transcaucasia (Dagestan, Armenia, Azerbaijan). Maps with the locations of genetic lineages in Eastern Europe and the Caucasus were based on our new data (129 ind.) and the materials (12 ind.) obtained earlier (Striuchkova et al. 2022). Data was uploaded to GenBank (accession numbers provided in Appendix 1).

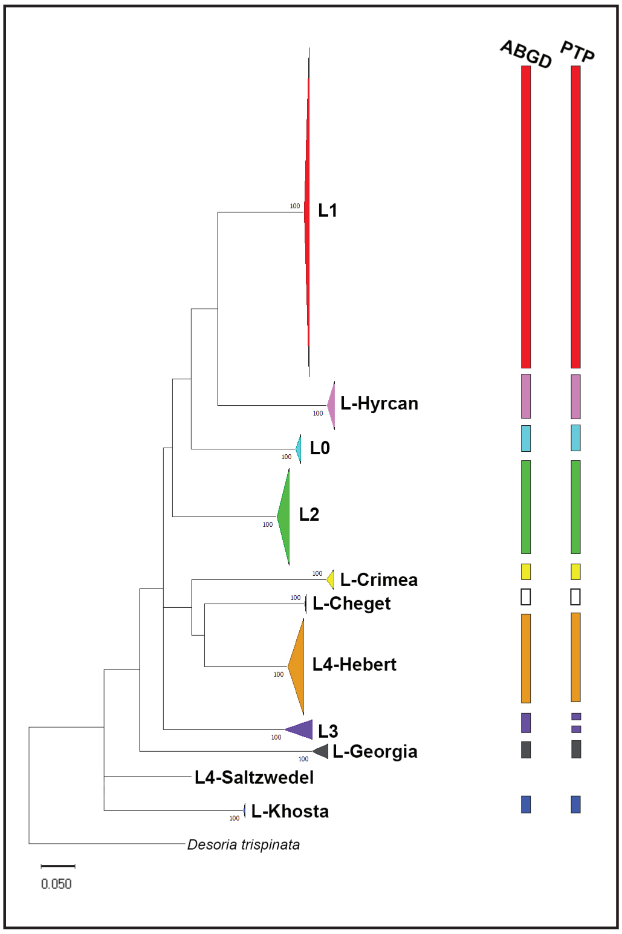

Figure 1. Maximum Likelihood genetic tree of 10 lineages of *P. notabilis* based on the COI gene fragment.

3. Results

3.1 Phylogeny of lineages

We obtained the genetic data on COI for 141 individuals of *P. notabilis* s. l., from 21 locations of Eastern Europe and the Caucasus region.

Three distance metrics (JC, K2P, and p-distance) with ABGD each supported the same 10 groups, matching the previously recognized lineages. The PTP model for Maximum likelihood and Highest Bayesian supported solution brought 11 lineages in both cases. The lineages found by the ABGD and PTP models were similar in all cases, at the exception of lineage L3, for which the PTP model suggested a further split into two lineages (Fig. 1). The mean intralineage K2P-pairwise distances according to COI gene vary as 0.20–4.09 %, p-distance, 0.20–3.83 %, interlineage, 15.77–23.35 % and 14.18–19.82 % respectively (Tab. 1). The sequence of lineage L4-Saltzwedel from GenBank (KJ792230) has been added to calculate interlineage distances.

Three distance metrics (JC, K2P, and p-distance) with ABGD produced an equal number (10) of groups matching recognized lineages. PTP model for Maximum likelihood and Highest Bayesian supported solution brought 11 lineages in both cases. The number of lineages using the ABGD and PTP models was similar in all cases, except

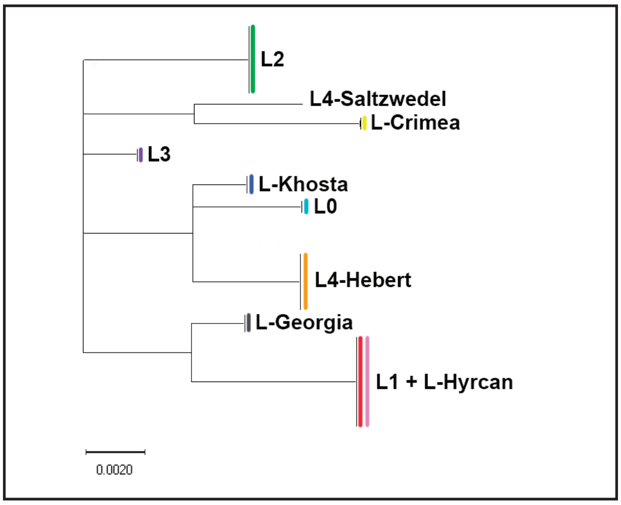

Figure 2. Maximum Likelihood genetic tree of *P. notabilis* s. l. based on the D3–D5 region of 28S gene.

for lineage L3, when the PTP model suggested dividing it into two lineages (Fig. 1).

Representing all the lineages was recognized using COI except for the L-Cheget lineage. For the rest of the specimens, the 28S tree is congruent with the COI gene tree. However, the L1 and L-Hyrcan lineages could not be distinguished, all specimens sharing an identical 28S rDNA gene.

3.2 The locations of findings of *P. notabilis* s. l.

The L1 genetic lineage is the most widespread in both studied regions (Fig. 2). It probably occupies the entire territory of Eastern Europe and the Caucasus. It is registered on the plain from the northernmost location (Karelia) to the southern territories (Crimea, Astrakhan Oblast). It is found everywhere in the Caucasus region also: in the foothills (Rostov Oblast, Stavropol Krai), on the northern and southern macroslopes of the Main Caucasian Ridge, on the Black Sea coast, and in Transcaucasia (Armenia, Azerbaijan). A total of 68 ind. at 14 sites have been found.

The L2 lineage is common in the central and northern part of Eastern Europe (Fig. 2). There have been only 20 ind. in 6 locations found.

The L4-Hebert lineage has been recorded in the forest zone of Eastern Europe from Karelia to the Voronezh city, as well as at the foothills of the Caucasus (Stavropol Krai) and on the northern macroslope of the Main Caucasian Ridge (Kabardino-Balkaria). A total of 20 ind. in 7 locations have been found (Fig. 3).

Previously known lineages (L0, L1, L2, L3, L4-Hebert, and L-Hyrcan), as well as new lineages (L-Crimea, L-Cheget, L-Georgia and L-Khosta) were found in the Crimea and in the Caucasus region (Fig. 4).

The L0 lineage is common in the Caucasus region at the northern macroslope of the Main Caucasian Ridge (North Ossetia), in Transcaucasia (Armenia), and at the foothills of the Caucasus (Stavropol Krai), but only 10 ind. have been found in these three locations in total.

The L3 lineage was registered on the Black Sea coast in Crimea (2 ind.).

The L-Hyrcan lineage was found in the Caucasus region in the central and southern parts of Azerbaijan; in total, 5 ind. in two locations. This lineage was recently described from Iran (Lafooraki et al. 2022).

The new lineage (designated as L-Crimea) was found in the southern Crimea in the Yalta Mountain Forest Reserve at Cape Martian (4 ind.).

The new L-Cheget lineage was found on Mount Cheget, located in the central part of the Main Caucasian Ridge at an altitude of 3460 m a.s.l. (Kabardino-Balkaria), 4 ind. in total.

The new L-Georgia lineage (3 ind.) was found at the foothills of the eastern part of Georgia in the Lagodekhi National Park.

A new L-Khosta lineage (3 ind.) was discovered in the Krasnodar Krai on the Black Sea coast in the area of the protected Khosta yew-boxwood grove.

3.3 Sympatries of the genetic lineages of *P. notabilis* s. l.

The joint occurrence of lineages was found in eleven out of the 21 studied locations: 4 lineages were noted in the Crimea; 3 lineages, in the Moscow Oblast, Karelia, Kabardino-Balkaria, and Stavropol Krai; 2 lineages, in Armenia, Georgia, Kaliningrad Oblast, Krasnodar Krai (Novorossiysk and Sochi environs), Vladimir Oblast and Voronezh city. In ten locations only one lineage has been found: 5 locations in Eastern Europe (Republic of Belarus and Russia: Kazan city, Nizhny Novgorod, Vladimir and Astrakhan Oblasts) and 4 locations in the Caucasus region (North Ossetia, Dagestan, Azerbaijan, and Rostov Oblast) (Tab. 2).

3.4 Habitats of various lineages

The L0 and L2 lineages inhabit mainly forests, as well as poorly disturbed habitats (meadows, forest parks). At the same time, L0 is found in the southern Eastern Europe and in the Pre-Caucasus, L2 is common in the central and northern Eastern Europe.

The L1 and L3 lineages are found in disturbed habitats: urban green spaces, wastelands, agrocoenoses, deposits, roadsides, meadows, and may be also found in forests. L1 is usually numerous in the disturbed habitats of the studied regions, but L3 is found only in the southernmost coastal locations.

The L4-Hebert is the most eurytopic lineage, found in forests, meadows, deposits, forest parks, and urban lawns throughout Eastern Europe and in the Caucasus region.

The L-Hyrcan lineage prefers mountain forests of the south of Transcaucasia.

The L-Crimea lineage is found in a subtropical Mediterranean–type seaside forest in Crimea.

The L-Cheget lineage is a high–altitude lineage found in the nival belt of mountains.

The L-Georgia lineage inhabits the forests of the foothills of eastern Georgia.

The L-Khosta lineage prefers coastal forests of the Caucasus.

Table 1. Intralineage and interlineage average pairwise distances (%) according to COI gene. Values below the diagonal indicate K2P-distance (K2P-d), above the diagonal, p-distance (P-d).

Lineage	Intralineage		Interlineage										
	K2P-d	P-d	L0	L1	L2	L3	L4-Hebert	L4-Saltzwedel	L-Hyrcan	L-Crimea	L-Cheget	L-Georgia	L-Khosta
L0	1.29	1.26	–	17.12	17.90	19.62	17.66	18.69	17.52	18.26	16.94	18.57	17.94
L1	1.73	1.68	19.66	–	17.20	18.84	18.37	18.95	16.24	18.74	17.72	19.09	19.82
L2	1.62	1.59	20.86	19.73	–	17.69	15.99	16.05	17.89	16.64	14.18	19.11	18.34
L3	4.09	3.83	23.03	21.90	20.44	–	17.56	17.79	17.74	17.76	16.66	18.17	18.68
L4-Hebert	2.13	2.07	20.36	21.30	18.17	20.11	–	16.86	17.32	17.19	14.62	19.75	18.17
L4-Saltzwedel	–	–	21.80	22.10	18.25	20.47	19.25	–	15.84	18.50	15.61	17.64	16.40
L-Hyrcan	0.20	0.20	20.27	18.49	20.67	20.33	19.84	17.94	–	17.67	16.33	18.19	19.22
L-Crimea	0.55	0.55	21.12	21.84	18.89	20.49	19.78	21.53	20.36	–	15.63	18.37	17.77
L-Cheget	0.42	0.42	19.37	20.47	15.77	19.02	16.37	17.59	18.57	17.69	–	17.70	17.49
L-Georgia	1.17	1.14	21.60	22.25	22.28	21.94	23.16	20.30	21.00	21.28	19.43	–	19.47
L-Khosta	0.21	0.21	20.96	23.35	21.30	21.60	21.15	18.67	22.65	20.52	20.11	22.79	–

Table 2. Genetic lineages of *P. notabilis* s. l. found in the studied location.

Location	Total number of lineages	Lineages	Habitat
Eastern Europe			
Republic of Belarus	1	L2	Forest
Astrakhan Oblast	1	L1	Floodplain forest, shrubs, reeds
Crimea	4	L1, L2, L3, L-Crimea	Reed (L1), mountain forest (L1, L2), coastal vegetation (L3), subtropical forest (L-Crimea)
Kaliningrad Oblast	2	L2, L4-Hebert	Forest (L2), pine forest planting (L4-Hebert)
Karelia	3	L1, L2, L4-Hebert	Forest (L2), forest park (L2), city lawn (L1, L4-Hebert)
Moscow Oblast	3	L1, L2, L4-Hebert	Forest (L2, L4-Hebert), forest parks (L1, L2, L4-Hebert), urban lawn (L1)
Nizhny Novgorod city	1	L1	City lawn
Ryazan Oblast	1	L4-Hebert	Forest
Kazan city	1	L1	City lawn
Vladimir Oblast	2	L2, L4-Hebert	Forest
Voronezh city	2	L1, L4-Hebert	City lawn
The Caucasus region			
Dagestan	1	L1	Forest, shrubbery in the meadow, littoral
Georgia	2	L1, L-Georgia	Forest
Kabardino-Balkaria	3	L1, L4-Hebert, L-Cheget	Nival belt (L-Cheget), Alpine belt (L1, L4-Hebert)
Krasnodar Krai (city of Novorossiysk)	1	L1	Forest park
Krasnodar Krai (Sochi environs)	1	L-Khosta	Forest
North Ossetia	1	L0	Forest
Republic of Armenia	2	L0, L1	Forest park
Republic of Azerbaijan	2	L-Hyrcan	Forest
Rostov Oblast	1	L1	Forest
Stavropol Krai	3	L0, L1, L4-Hebert	Forest (L0, L1, L4-Hebert), forest park (L4-Hebert), city lawn (L1)

4. Discussion

Five previously described lineages of *P. notabilis* s. l. are registered in our samples (L1–L3, L4-Hebert, L-Hyrcan, except L4-Saltzwedel), and four lineages are newly found (L-Crimea, L-Cheget, L-Georgia, L-Khosta). Both ABGD and PTP models, which detect the barcode gaps, distinguish these clades as distinct molecular lineages. It is interesting that L1 and L-Hyrcan lineages based on the 28S gene were included in one clade, although according to the main analysis of the COI gene, they belonged to different clades. Thus, the D3-D5 region of the 28S gene cannot always correctly determine the genetic lineage of *Parisotoma notabilis*. Besides, the PTP model, unlike ABGD, allocates two separate clades from Greece and Crimea for 4 ind. within the L3 lineage. Indeed, the L3 lineage has large values of intralineage K2P-pairwise distances (4.09 %) and p-distance (3.83 %), compared to other lineages, where this indicator does not exceed 2.13 and 2.07 %, averaging 1.04 and 1.01 % respectively. Nevertheless, we do not yet distinguish these sub-lineages as lineages due to a small amount of material and the mismatch of the results of models applied.

Parisotoma notabilis s. l. is one of the few species of the genus *Parisotoma* found in Eastern Europe and the Caucasus, along with the small *P. ekmani* (Fjellberg, 1977), rare *P. agrelli* (Delamare, 1950), locally entering from Siberia *P. reducta* (Rusek, 1984), and invasive *P. trichaetosa* Martynova, 1977 (Potapov 2001, Potapov et al. 2021). This is one of the most numerous species among all the springtails of the forest belt of Europe and North America. Genetic studies can partly explain this phenomenon by a high degree of polymorphism, from which one can expect increased adaptability to various environmental conditions. Interestingly, another species of this genus, *P. ekmani*, has a lower level (13 %) of intraspecific divergence of lineages (Porco et al. 2012b) comparing to *P. notabilis* (15–18 %) (von Saltzwedel et al. 2017). In Europe, *P. ekmani* is a rare species and is associated mainly with wet habitats (sphagnum swamps) and cold sites in the mountains (Potapov 2001). We assume that the lower divergence of lineages in *P. ekmani* is explained by its narrowed ecological niche. When studying *P. notabilis* lineages that inhabit everywhere on the plain, ecological differentiation of the lineages is a more likely explanation. Here, we also

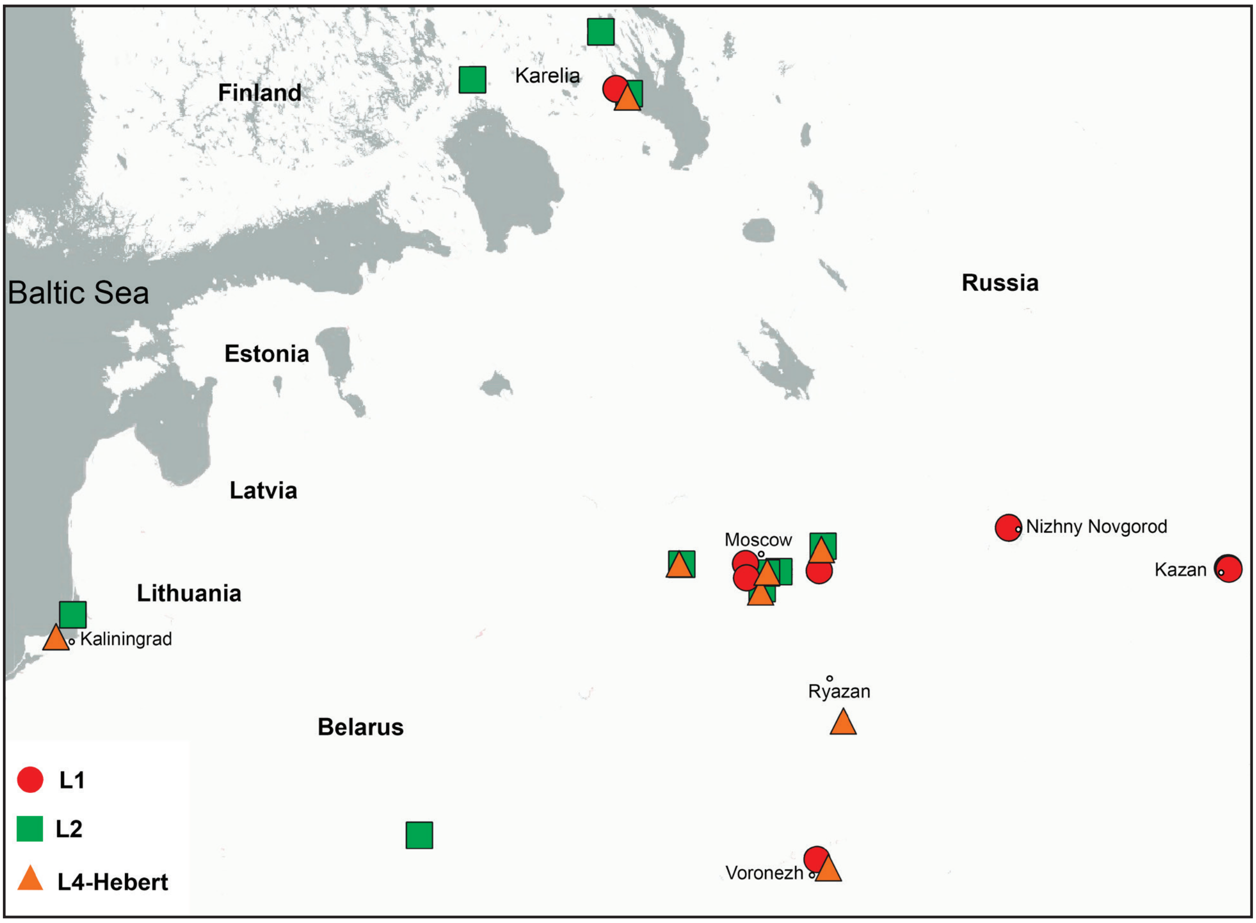

Figure 3. L1, L2, and L4-Hebert lineage finds in Eastern Europe.

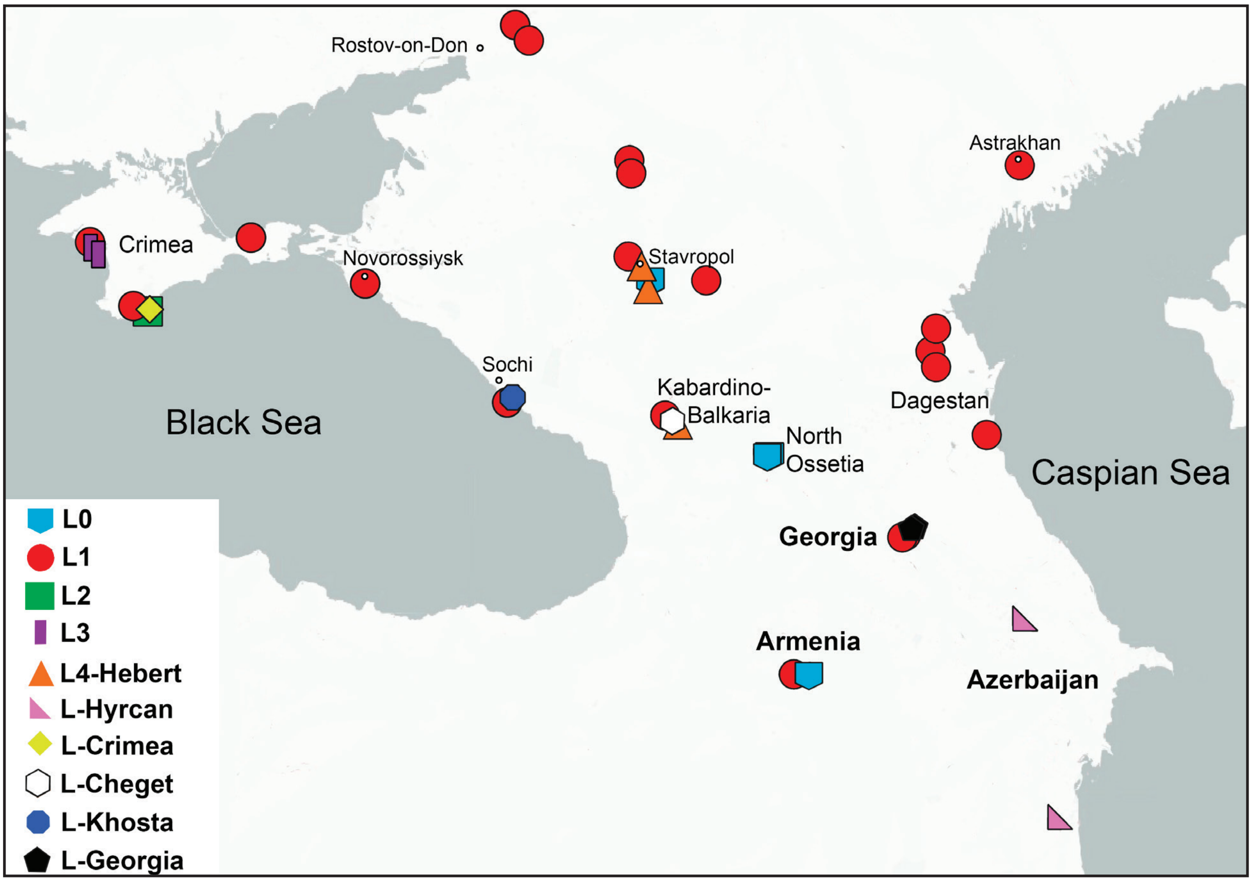

Figure 4. Sites of lineage findings in the Caucasus region and in southern Eastern Europe (L0, L1, L2, L3, L4-Hebert, L-Hyrcan, L-Crimea, L-Cheget, L-Georgia, L-Khosta).

point to the preference of the most common lineages in Eastern Europe for different disturbed habitats. In natural forests and at meadows, the most common lineage is L2, at urban lawns and in agrocenoses, L1. The L4-Hebert lineage is even more cosmopolitan, since it inhabits the entire range of habitats, especially slightly disturbed forest parks and deposits (Striuchkova 2023). At least, the L1 and L2 lineages represent ecotypes, i.e., the groups of individuals within a species with hereditarily fixed adaptations to certain environmental factors (Turesson 1922). The unexpected finding of *P. notabilis* in the nival belt of the mountains suggests the presence of adaptations of the new L-Cheget lineage to low temperature, i.e., may be also considered as ecotype.

Quite expected results have been obtained for L1 and L2 lineages when comparing their distribution in Eastern Europe and the Caucasus with more western and southern locations (Porco et al. 2012b, von Saltzwedel et al. 2017). The L1 lineage, associated with human-developed territories, is widely found throughout Europe, as well as in Asia Minor. Throughout Europe, the L2 lineage is widespread and confined to forests (at least in Eastern Europe). The L0 lineage, common in the Caucasus, is reported for Western Europe only in Germany. Among the rarely observed lineages, L3 (known from Greece) is found in Crimea. The L4-Saltzwedel lineage, found in Croatia, has not yet been found further east in the locations we have studied. Interestingly, the L4-Hebert lineage, widespread in Eastern Europe, including Estonia (Anslan & Tedersoo 2015) and the Caucasus, has not yet been found in Western Europe. In North America (Canada) there are many findings of the L1 and L2 lineages; single specimens of the L0 and L4-Hebert lineages are found very rarely (once). According to our data, *P. notabilis* s. l. is small in number in the Asian part of the Palaearctic (Potapov 2001); but genetic analysis of these lineages has not been yet performed. The L-Hyrcan lineage was found in ancient Caspian Hyrcanian mixed forests in Mazandaran Province of Iran (Lafooraki et al. 2022). Our finds of the L-Hyrcan lineage in Azerbaijan refer to the northern part of the same forest area. New lineages discovered in Crimea and the Caucasus are found locally and are associated mainly with protected areas: Yalta Nature Reserve in Crimea (L-Crimea), Lagodekhi National Park in Georgia (L-Georgia), and relict yew-boxwood grove in Khosta (L-Khosta). The most surprising is the discovery of a new lineage (L-Cheget) in the highlands, namely, on Mount Cheget of the Main Caucasus Range. *P. notabilis* is numerous in the forests of temperate climate; but in high latitudes, this species is found rarely, so it would be difficult to expect it in the nival belt of mountains. Overall, the discovery of a large number of lineages of *P. notabilis* s. l. in the Caucasus region (9 lineages out of 11 known, including new ones), confirms the classification of this territory as a biodiversity hotspot (Myers et al. 2000). Due to the southern location and complex topography of the Caucasus Mountains, this territory could serve as a refugium for various lineages of *P. notabilis* s. l. during the Pleistocene glaciations.

The sympatry of genetic lineages is confirmed for half of the studied regions, both for the plains (Eastern Europe) and for the mountainous region (Caucasus). Given the relatively small number of specimens genotyped, we expect an increase in the number of lineage co-occurrences in the future studies. In particular, the large number of cases of lineage sympatry observed in our material may be due to the study of disturbed habitats along with natural biotopes, when the L1 lineage has been found almost always and everywhere. In addition, L4-Hebert is common in Eastern Europe, but rare in Western Europe. Apparently, there are no lineages specific only to Western Europe and widespread there. Locally registered lineages in southern Europe and the Caucasus are found together with widespread ones in half the cases. Overall, the high diversity of lineages in the Caucasus region and their common sympatry in Eastern Europe and the Caucasus appear to reflect the dispersal vector of *P. notabilis* s. l. from east to west.

The ecological divergence of lineages may be preliminarily assessed by their preference for certain habitats. We have discovered such differentiation for the L1 and L2 lineages in regard to the degree of habitat disturbance for the Moscow Oblast (Struchkova et al., 2022, Struchkova, 2023), as well as for Karelia and the Stavropol Krai (Struchkova, in press). In all these locations, the L1 lineage is common in urban green areas, and the L2 and L0 lineages, in natural forests of Eastern Europe and the Caucasus region, respectively.

In Crimea, where the most lineages are listed (4), only the L1 and L2 lineages are recorded together in the mountain forest. The L1 lineage has been also found in reed thickets near the village, L3, under coastal herbaceous plants, and L-Crimea, in a protected subtropical forest. The last two lineages may have greater tolerance to high temperatures compared to other *P. notabilis* lineages found in the Crimea and the Caucasus, mainly in forests.

In Kabardino-Balkaria, on Mount Cheget, three lineages are found; however, only the L-Cheget lineage is recorded in the nival belt, the two others (L1 and L4-Hebert), in the alpine belt. The L-Cheget lineage, found in the nival mountain belt, should be characterized by high frost resistance. In general, if we consider *P. notabilis* as one polytypic species, then at least five lineages can tentatively be considered as ecotypes (L1, L2, L3, L-Crimea, and L-Cheget) according to (Turesson 1922).

In the future, recognition of the lineages as separate species is possible due to the large genetic distances between them (18–28 % along the marker region of the COI gene). However, such species still remain cryptic with the exception of one case involving the two most common lineages. The characteristic of *P. notabilis* as a species that ‘prefers moderately disturbed biotopes’ (Potapov 2001) is apparently ensured by the properties of the L1 lineage. This lineage differs from the others by its high ability to colonize disturbed habitats (city lawns, fields, and the outskirts of villages) in various regions.

P. notabilis s. l. is characterized by wide range, high abundance, and genetic polymorphism. Interestingly, this is combined with the parthenogenetic reproduction. A generalization of the data obtained on the geography and ecology of genetic lineages evidences that the *P. notabilis* s. l. the complex can be considered as a polytypic species under the active process of ecological (mainly in the plains) and geographical (mainly in the mountains) speciation.

5. Conclusions

1. Wide distribution of *P. notabilis* s. l. is provided primarily by genetic lineages L1 and L2. In Eastern Europe and the Caucasus, the L1 and L4-Hebert lineages are most common.
2. The Caucasus is a biodiversity hotspot of genetic lineages of *P. notabilis* s. l.
3. Sympatry of genetic lineages is noted in approximately half of the studied geographical locations.
4. Most lineages of *P. notabilis* s. l. are more or less confined to certain habitats, except the L4-Hebert lineage, which is recorded in most of the studied habitats.
5. Genetic heterogeneity of *P. notabilis* s. l. combined with the ecological divergence of the most common lineages may partly explain the biological progress of this species in Eastern Europe.
6. The most common genetic lineages L1 and L2 have morphological differences.

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Appendix 1

Table S1. Material volume and its characteristics. N is the number of individuals.

Label	Latitude	Longitude	Location	Region	N	Lineage	Accession number COI	Accession number 28S
Armenia-2016-7(Ef5)	40.1962	44.5202	Armenia	Caucasus	1	L0	PP235108	
					4	L1	PP235104 – PP235107	
Athens-2022-1	37.9690	23.7417	Athens, Greece	Southeast Europe	1	L3	PP235224	PP231846
Athens-2022-2	37.9685	23.7394	Athens, Greece	Southeast Europe	1	L3	PP235225	PP231847
Azerbaijan-2019-10	38.4854	48.6230	Azerbaijan	Caucasus	2	L-Hyrcan	PP235109, PP235110	PP231835, PP231836
Azerbaijan-2019-20	40.8918	48.0694	Azerbaijan	Caucasus	3	L-Hyrcan	PP235111 – PP235113	PP231837, PP231838
Balash-2021-6	55.8317	37.9674	Moscow Oblast	Eastern Europe	3	L2	PP235208 – PP235210	OR892315 – OR892317
Belarus-2022-1	52.0916	29.2841	Republic of Belarus	Eastern Europe	4	L2	PP235114, PP235116 – PP235118	
Bitsa-2021-3	55.6022	37.5634	Moscow city	Eastern Europe	1	L4-Hebert	PP235219	OP643634
					1	L2	PP235216	OP643653
Centr.Cauc-2021-3	43.2396	42.4911	Kabardino-Balkaria	Caucasus	4	L-Cheget	PP235120 – PP235123	
Centr.Cauc-2021-6	43.2396	42.491	Kabardino-Balkaria	Caucasus	1	L4-Hebert	PP235125	
					1	L1	PP235124	
Crimea-2021-11	44.485	34.101	Crimea	Eastern Europe	1	L2	PP235126	
Crimea-2021-16	45.198	33.417	Crimea	Eastern Europe	2	L1	PP235115	
						L3	PP235222	PP231842
Crimea-2021-18	45.122	33.538	Crimea	Eastern Europe	1	L3	PP235223	
Crimea-2021-2	45.249	35.956	Crimea	Eastern Europe	4	L1	PP235127 – PP235130	
Crimea-2021-4	44.508	34.245	Crimea	Eastern Europe	4	L-Crimea	PP235131 – PP235134	PP231844, PP231845
Crimea-2021-9	44.485	34.101	Crimea	Eastern Europe	2	L1	PP235135, PP235136	
Georgia-2019-1(26)	41.8444	46.2850	Georgia	Caucasus	2	L-Georgia	PP235138, PP235139	PP231843
					2	L1	PP235137, PP235140	
Georgia-2019-3(28)	41.8254	46.2302	Georgia	Caucasus	1	L-Georgia	PP235141	
					3	L1	PP235142 – PP235144	
Hovrino-2020-1	55.8736	37.4784	Moscow city	Eastern Europe	5	L1	OP861639 – OP861643	OM728286 – OM728290
Kaliningrad-2022-1	54.9857	20.5519	Kaliningrad Oblast	Eastern Europe	1	L4-Hebert	PP235213	
Kaliningrad-2022-3	55.2433	20.9177	Kaliningrad Oblast	Eastern Europe	2	L2	PP235119, PP235212	
Karelia-2022-1	61.7880	34.3501	Karelia	Eastern Europe	1	L1	PP235145	OR885443
					1	L4-Hebert	PP235146	OR885425
Karelia-2022-2	61.7773	34.3138	Karelia	Eastern Europe	1	L2	PP235147	OR885448
Karelia-2022-3	62.4852	33.6703	Karelia	Eastern Europe	2	L2	PP235148, PP235149	OR885452, OR885457
Karelia-2022-6	61.9482	30.5692	Karelia	Eastern Europe	2	L2	PP235150, PP235151	OR885460, OR885461
Kazan-2022-1	55.8156	49.1095	Tatarstan, Kazan city	Eastern Europe	2	L1	PP235152, PP235153	
Kazan-2022-2	55.8165	49.1430	Tatarstan, Kazan city	Eastern Europe	2	L1	PP235154, PP235155	

Label	Latitude	Longitude	Location	Region	N	Lineage	Accession number COI	Accession number 28S
Kazan-2022-3	55.7984	49.1497	Tatarstan, Kazan city	Eastern Europe	1	L1	PP235156	
Losin-Ostr.2021-1	55.8703	37.7233	Moscow city	Eastern Europe	1	L4-Hebert	PP235218	OP643655
NiNo-2022-2	56.3567	43.8286	Nizhny Novgorod city	Eastern Europe	4	L1	PP235229 – PP235232	
OrehZuev-2020-2	55.7793	39.2559	Moscow Oblast	Eastern Europe	3	L1	PP235202 – PP235204	PP231848 – PP231850
ProspVernad-2020-4	55.6815	37.4999	Moscow city	Eastern Europe	1	L1	OP866972	OM746082
Ryazan-2022-1	53.8036	39.5619	Ryazan Oblast	Eastern Europe	3	L4-Hebert	PP235197 – PP235199	
Shakh-2021-2	55.9322	35.6193	Moscow Oblast	Eastern Europe	1	L2	OP861657	OM778170
Shakh-2021-3	55.9334	35.6225	Moscow Oblast	Eastern Europe	1	L2	OP861658	OM778166
Shakh-2021-6	55.9840	35.5973	Moscow Oblast	Eastern Europe	3	L4-Hebert	OP861662 – OP861664	OM757828 – OM757830
Sochi-2022-1	43.3902	39.9978	Krasnodar Krai	Caucasus	3	L1	PP235205 – PP235207	
Sochi-2022-4	43.5099	39.8792	Krasnodar Krai	Caucasus	3	L-Khosta	PP235226 – PP235228	PP231839 – PP231841
S-Russia-2022-12	46.0262	48.1189	Astrakhan Oblast	Eastern Europe	3	L1	PP235160 – PP235162	
S-Russia-2022-13	46.0469	48.0826	Astrakhan Oblast	Eastern Europe	1	L1	PP235163	
S-Russia-2022-2	44.7723	43.1361	Stavropol Krai	Caucasus	3	L1	PP235157 – PP235159	
S-Russia-2022-22	44.254	46.7230	Dagestan	Caucasus	3	L1	PP235164 – PP235166	
S-Russia-2022-23	44.0177	46.5841	Dagestan	Caucasus	2	L1	PP235167, PP235168	
S-Russia-2022-26	43.823	46.6754	Dagestan	Caucasus	3	L1	PP235169 – PP235171	
S-Russia-2022-27	43.0284	47.4619	Dagestan	Caucasus	2	L1	PP235172 – PP235173	
SW-Russia (AK) 2022-2	47.5586	40.1242	Rostov Oblast	Caucasus	3	L1	PP235174 – PP235176	
SW-Russia (AK) 2022-3	47.3937	40.34016	Rostov Oblast	Caucasus	3	L1	PP235177 – PP235179	
SW-Russia (AK) 2022-4	46.1001	41.9230	Stavropol Krai	Caucasus	1	L1	PP235180	
SW-Russia (AK) 2022-6	44.7367	37.7575	Krasnodar Krai	Caucasus	3	L1	PP235181 – PP235183	
Tsey-2021-5	42.7782	43.8632	North Ossetia	Caucasus	2	L0	PP235184, PP235185	
Tsey-Acer-2021	42.7869	43.8893	North Ossetia	Caucasus	3	L0	PP235186 – PP235188	
Tsey-Fagus-2021	42.7890	43.9064	North Ossetia	Caucasus	2	L0	PP235189, PP235190	
VDNH-2020-2	55.8135	37.6654	Moscow city	Eastern Europe	1	L2	OP861659	OM778143
Vlad.obl-2022-1	56.2998	39.0018	Vladimir Oblast	Eastern Europe	3	L4-Hebert	PP235214, PP235215, PP235217	
					1	L2	PP235211	
Voronezh-2021-1	51.6594	39.2020	Voronezh city	Eastern Europe	2	L4-Hebert	PP235220, PP235221	OM778158, OM778160
					1	L1	PP235201	OM778154
W Caucasus-2022-1	44.7761	42.0222	Stavropol Krai	Caucasus	2	L0	PP235191, PP235192	OR886829, OR886831
					2	L4-Hebert	PP235193, PP235194	OR886813, OR886825
W Caucasus-2022-3	45.0306	41.9186	Stavropol Krai	Caucasus	2	L4-Hebert	PP235195, PP235196	OR886818, OR886823
W Caucasus-2022-4	45.0389	41.9111	Stavropol Krai	Caucasus	1	L1	PP235200	OR886834

Appendix 2

Locations of new genetic lineages of *Parisotoma notabilis* sensu lato. Lineages L-Hyrcan – L-Crimea.

L-Hyrcan (Azerbaijan, Talish, older beech forest with oak, 670 m a.s.l.)

L-Crimea (Crimea, Reserve at Cape Martian, oak forest (*Quércus pubéscens*) with *Arbutus unedo*, southern slope, 150 m a.s.l.)

L-Cheget (Caucasus, Mount Cheget, northern slope, nival mountain belt, 3460 m a.s.l.)

L-Georgia (Georgia, Lagodekhi National Park, beech forest, 540 m a.s.l.)