



Article

Nearctic Species in the Palearctic: Trans-Beringian Range, Phylogeny and Phylogeography of *Pterostichus (Cryobius) mandibularoides* (Coleoptera, Carabidae)

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Simple Summary: Among insects of the soil megafauna in the Arctic and Subarctic, the species of subgenus *Cryobius* have demonstrated a high abundance and wide distribution. The fossil remains of *Cryobius* are used for paleoenvironmental reconstructions of the Arctic and Subarctic in the Pleistocene and Holocene. However, the taxonomy, ecology, and distribution of *Cryobius* are insufficiently studied. Here, we report that *Pterostichus (Cryobius) mandibularoides* Ball, 1966, previously known from the Nearctic, was found in Chukotka and on Wrangel Island. Thus, it is a trans-Beringian species with a wide distribution in the western part of North America and a disjunctive range within the northeastern margin of Asia. The phylogeny of *P. mandibularoides* determined using a genetic approach revealed inconformity with previous conclusions regarding its differentiation within the *brevicornis* group stock of subgenus *Cryobius*. Considering the taxonomic complexity of subgenus *Cryobius*, the fossil samples of *brevicornis* group species with radiocarbon dating may not correspond to the real time of species differentiation; consequently, additional revision using genetic tools is required.

Abstract: Subgenus *Cryobius* is one of the most numerous among the megafauna of tundra soils, but studies on its species distribution, taxonomy, and ecology are lacking. Phylogeny and phylogeography reconstructions of insects with taxonomic complexity have become possible using an integrative approach. Here, we report that specimens of *Pterostichus (Cryobius) mandibularoides*, described from North America, were detected in Eurasia. Thus, this species has a trans-Beringian range with high distributions in North America, as well as a disjunctive part of the range on the northeastern edge of Asia within Chukotka and Wrangel Island. Eight COI haplotypes with closed relationships (1–2 mutation steps) were detected within the whole range, and one 28S rRNA haplotype was detected for Eurasia. Bayesian phylogeny revealed that *P. mandibularoides* had the most recent common ancestor with sister species *P. brevicornis* and *P. nivalis*. Mean genetic distances of both markers were similar and higher between *P. mandibularoides* and both *P. brevicornis* and *P. nivalis* ($>5\% \pm 1.0\%$) than between the latter species ($<4\% \pm 1.0\%$). The obtained results change the previous view about *brevicornis* group stock differentiation within *Cryobius* in the Arctic and require a revision of the phylogeny and phylogeography of *brevicornis* group species and *Cryobius* altogether.

Keywords: ground beetles; trans-Beringia range; Wrangel Island; Chukotka; phylogeny; phylogeography



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1. Introduction

The Bering Strait was the last bridge connecting the tundra fauna of Eurasia and North America during the last Ice Age [1–4]. A recent study based on time-calibrated phylogenies of a broad range of specific taxon groups (plants, fungi, arthropods, amphibians, reptiles, and mammals) showed that the biotic exchange was asymmetric with a higher degree of dispersal from Eurasia to North America [4]. However, a number of species were restricted to only one side of Western or Eastern Beringia [2]. Range reconstructions and the phylogeny of some arctic species with taxonomic complexity have become possible via the integrative approach. A disjunctive range on Novaya Zemlya and Wrangel Island with close affinities was detected for *Bombus glacialis* (Friese, 1902) [5]. It was shown that the widespread Arctic tiger moth *Arctia tundra* (Tshistjakov, 1990) has a low genetic divergence among distanced populations from Kolguev Island (north Europe) to Chukotka [6]. Widely distributed specimens of North American moth *Udea itysalis* (Walker, 1859) were registered on Kolguev Island (north Europe) [7]. Moreover, specimens of *Gnorimoschema vastificum* (Braun, 1929) to date known only from North America were detected on Yugorsky Peninsula (north Europe) [8]. Some insect taxa with Nearctic distribution were found on Wrangel Island, which were not registered in other regions of Eurasia. They are represented in various taxonomic groups: Aphididae [9], Erebididae [10], and the dipteran from families Empididae [11], Syrphidae [12], Agromyzidae [13], and Muscidae [14,15].

Among insects of the soil megafauna in the Arctic and Subarctic, the species of subgenus *Cryobius* demonstrate high frequency and distribution [16]. Nevertheless, there are only a few studies on the phylogeny and ecology of *Cryobius* in the North Holarctic [17–22]. The most studied subgenus group is *brevicornis*, with the last revision in 1998 by Erjomin [22]. Five species were detected in the group: *P. brevicornis* (Kirby, 1837) with circumpolar distribution, *P. empetrikola* (Dejean, 1828) and *P. nivalis* F. (Sahlberg, 1844) with trans-Beringian range, *P. kolymensis* (Erjomin, 1998) with a restricted range in East Siberia, and *P. mandibularoides* (Ball, 1966) widely distributed in North America [19,22]. Three species, *P. brevicornis*, *P. kolymensis*, and *P. mandibularoides*, have a close morphology with similar body size, pronotum shape, and male genitalia. Furthermore, for species *P. brevicornis*, three subspecies were detected: *P. brevicornis brevicornis* (Kirby, 1837), *Pterostichus brevicornis delicatus* (Casey, 1918), and *Pterostichus brevicornis yasudai* (Morita, 2002). Only *P. b. brevicornis* has a Holarctic range, while the other two subspecies are distributed on restricted territories of the islands of the Bering Sea and Chukotka for *Pterostichus b. delicatus*, and Hokkaido Island for *Pterostichus b. yasudai* [19,23–25]. In our samples of soil arthropods from the northeast edge of Russia, several *Cryobius* species were found and examined using the integrative approach with genetic tools. The specimens of *P. mandibularoides* were detected in samples from Chukotka and Wrangel Island. Thus, the aims of this study were to (1) clarify the range distribution of *P. mandibularoides*, and (2) focus on its phylogeography and phylogeny within the *brevicornis* group using the integrative approach.

2. Materials and Methods

2.1. Data Collection

Specimens of *P. mandibularoides* were collected on Chukotka (Alkatvaam and Meynipilgino settlements, subzone of southern tundra) and Wrangel Island (Somnitelnye Mountain and Somnitelnaya Bay, subzone of Arctic tundra) (Figure 1, Table 1). The specimens were sampled using pitfall traps and hand collection (with an exhaustor).



Figure 1. Map of collection localities of *Pterostichus mandibularoides* in Chukotka and on Wrangel Island (red points). The list of sequences is presented in Table S1.

Table 1. Collecting localities and samples of *Pterostichus mandibularoides* from the northeastern edge of Russia.

Locality	GPS Code	Number of Specimens	Site
Chukotka, suburbs of Alkatvaam settlement, 2019	63°8′9.6″ N, 179°2′9.6″ E	49	Well-drained slope with mossy–shrub–rhododendron tundra
Chukotka, suburbs of Meynipilgino settlement, 2020	62°33′3.6″ N, 177°4′4.2″ E	9	Damp depression with mossy– <i>Salix</i> vegetation
Wrangel Island, Somnitelnye Mountain, 2015	70°58′1.2″ N, 179°34′58.8″ E	2	Wet loam soil at the foot of the hill with <i>Salix</i> –dryad–sedge and mossy spotted vegetation
	70°59′11.4″ N, 179°35′14.2″ E	2	Dry loam–rubble soil on a raised terrace of the southern expose with <i>Salix</i> and grass–mossy vegetation
Wrangel Island, Somnitelnaya Bay, 2015	70°57′7.6″ N, 179°32′26.5″ E	3	Dry loam–rubble soil on plain with grassy–dryad–sedge spotted vegetation

Ground beetles were preserved in 96% ethanol or dried after collection at room temperature (21–25 °C) for subsequent DNA and morphological analyses. The *P. mandibularoides* specimens were studied morphologically using a stereomicroscope (Leica M165C, Germany) in accordance with the approaches of Ball [19] and Lindroth [20,21]. Furthermore, we explored the morphological structures of the taxonomically closest species to *P. mandibularoides* from the *brevicornis* group: *P. nivalis* and *P. brevicornis* (subspecies *P. brevicornis brevicornis*) (Table S1). Images of morphological details were taken using a stereomicro-

scope Axio Zoom V16 (Carl Zeiss, Berlin, Germany) and Canon EOS 80D Digital Camera (Canon Inc., Japan, Tokyo) (Figure 2). The full morphological descriptions with dichotomic keys for these species are given in the checklists of Ball and Erjiomin [19,22]. According to imago habitus, *P. mandibularoides* is most like *P. brevicornis*, and both species can overlap in all characteristics [19]. Hence, the main distinctions between these species are in the form of the male genitalia [19,22]. The median lobe of *P. mandibularoides* in the lateral aspect without curving of the shaft features an apex with a slight ventral lip (Figure 2F) [19]. For the male of *P. brevicornis*, the median lobe has a truncate or narrowly rounded apex, and sides of the shaft in ventral aspect were almost parallel (Figure 2D) [19]. According to the combination of morphology similarities, Ball concluded that *P. mandibularoides* and *P. brevicornis* are more closely related to one another than they are to *P. nivalis* [19].

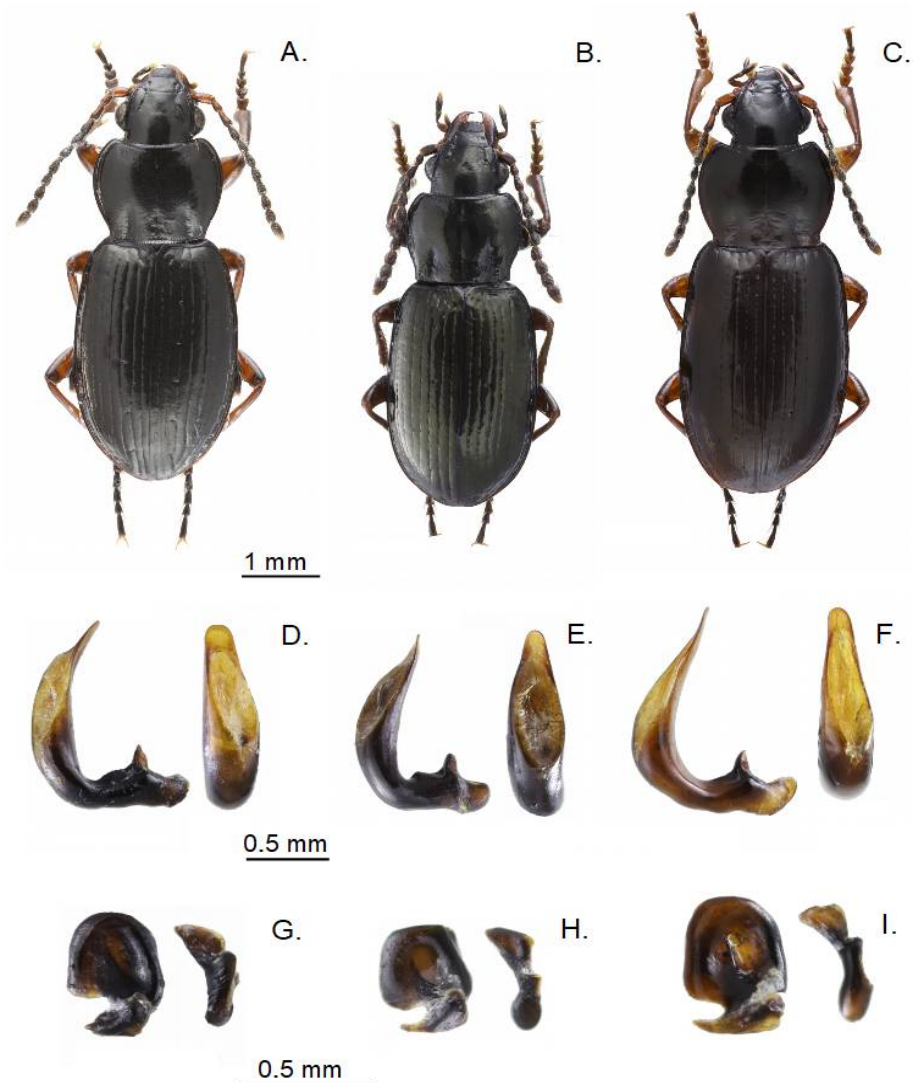


Figure 2. Habitus and male genitalia of *P. b. brevicornis* (A,D,G), *P. nivalis* (B,E,H), and *P. mandibularoides* (C,F,I). (A–C) Habitus; (D–F) median lobe in left lateral and ventral aspect, apical portion; (G–I) left and right parameres.

Overall, 65 specimens of *P. mandibularoides* were revealed in our samples from Chukotka and Wrangel Island. *Cryobius* samples from Chukotka specimens of *P. mandibularoides* were not found in the suburbs of Pevek settlement (closest mainland locality to Wrangel Island) and in the suburbs of Hatyrka settlement (about 100 km from Meynipilgino settlement near the coast of Bering Sea). We also examined the collected species of *brevicornis* group in samples from West and Central Siberia, as well as North Europe, and we did not detect

P. mandibularoides. Unfortunately, we had no samples of species of the *brevicornis* group from localities adjacent to the Chukotka territory of East Siberia. The specimens were deposited in the Zoological Institute of the Russian Academy of Sciences (Saint Petersburg, Russia) from East Siberia, belonging to *P. brevicornis* or *P. kolymensis* (without ID numbers). One paratype of *P. mandibularoides* is deposited in the coleoptera collection of the aforementioned Zoological Institute (Figure S1). A female specimen was explored by Ball, and it was sampled in Alaska on Delta Islands in the period of 13–15 June 1962 by Andersen (without ID number). The pinned and dried specimens of *P. mandibularoides* are deposited in the collection of the Russian Museum of Biodiversity Hotspots (RMBH), Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Northern (Arctic) Federal University named after M.V. Lomonosov (Arkhangelsk), and the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (Moscow), Russian Federation.

2.2. DNA Extraction, PCR, and Sequencing

For molecular analysis, 11 specimens of *P. mandibularoides* were used (Table S1). In the present study, we obtained the *cytochrome c oxidase subunit I* (COI) gene and the *large subunit ribosomal RNA 28S* (28S rRNA) gene sequences. The sequence dataset involved 22 COI and eight 28S rRNA sequences combined with our materials (11 COI and eight 28S rRNA sequences) and published GenBank (NCBI and (BOLD) IDS) data (11 COI sequences) of *P. mandibularoides* (Table S1). Moreover, we used datasets of sequences from the *brevicornis* group, *P. nivalis* and *P. brevicornis*, which are taxonomically closely related species to *P. mandibularoides*. For the *P. nivalis* dataset, we used 79 COI and four 28S rRNA sequences (GenBank: 70 COI sequences; our materials: nine COI and four 28S rRNA sequences). For the *P. b. brevicornis* dataset, we used 170 COI and 33 28S rRNA sequences (GenBank data: 93 COI sequences; our materials: 77 COI and 33 28S rRNA sequences). All datasets of the aforementioned three species from GenBank belong to North American (Alaska, Canada) specimens, and our materials were sampled in North Eurasia (Table S1).

DNA was extracted from dissected abdominal muscle tissue and legs using a standard phenol/chloroform procedure [26]. The mitochondrial gene COI was amplified using two primer pairs: C1-J-1718 with C1-N-2329 [27], and LCO 1490 [28] with LepR [29]. Amplified COI barcode fragments were 612 bp and 660 bp in length, respectively. The primers D23F [30] and D2 [31] were used for the amplification of 528 bp long fragments of the 28S rRNA gene. The PCR mix contained approximately 200 ng of total cellular DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µL of PCR buffer (with 10×2 mmol $MgCl_2$), 0.8 units of Taq DNA polymerase (SibEnzyme Ltd., Novosibirsk, Russia), and H_2O added to a final volume of 25 µL. Temperature cycling was as follows: 95 °C (4 min); 32–37 cycles at 95 °C (50 s), 42 °C (50 s), and 72 °C (50 s) for COI or 25 cycles at 95 °C (50 s), 60 °C (50 s), and 72 °C (50 s) for 28S; a final extension at 72 °C (5 min). Forward and reverse sequencing was conducted on an ABI PRISM® 3730 DNA (Thermo Fisher Scientific Inc., Waltham, MA, USA) using the ABI PRISM® Big-Dye Terminator v.3.1 reagent kit. The results of the 19 new sequences (11 COI and eight 28S rRNA sequences) were deposited in the GenBank sequence database at NCBI (Table S1). All sequences were analyzed using BioEdit v. 7.2.5 [32]. The alignment of the COI and 28S rRNA sequences was performed using the Muscle and the ClustalW algorithms in MEGA X [33]. Poorly aligned positions and divergent regions from the alignment of the 28S rRNA gene were eliminated using the online GBlocks server v0.9b [34,35].

2.3. Phylogenetic and Phylogeographic Analyses

Both genes were analyzed, combined and separately (Table S2). The resulting aligned sequences of the two gene fragments (COI, 28S rRNA) were joined to obtain a combined nucleotide sequence alignment (total length of 1130 bp). This combined dataset was collapsed into a set of unique haplotypes (including haplotypes of outgroup taxa *Pterostichus* (*Cryobius*) *ventricosus* (Eschscholtz, 1823) and *Pterostichus* (*Phonias*) *strenuus* (Panzer, 1797)) using an online FASTA sequence toolbox [36]. The best evolution models were selected

for each gene separately on the basis of the corrected Akaike information criterion (AICC) of jModelTest [37] in MEGA X [33]. The GTR + G model of sequence evolution was best for both studied genes: COI gene ($G = 0.15$) and 28S rRNA ($G = 0.16$). Phylogenetic relationships were reconstructed on the basis of Bayesian inference performed in MrBayes v.3.2.6 [38] through the CIPRES Science Gateway [39]. The following parameters were used for analyses: nchains = 4, nruns = 4, samplefreq = 1000, temp = 0.2. The first 15% of trees were discarded as burn-in (pre-convergence part), and the majority rule consensus tree was calculated from the remaining trees. Convergence of the MCMC chains to a stationary distribution was checked using Tracer v. 1.7 [40]. The effective sample size (ESS) value for each parameter sampled from the MCMC analysis was always recorded as >600. Trees were viewed using FigTree v. 1.4.4 [41]. Genetic differences (p-distances) between analyzed haplotypes were calculated using MEGA X [32].

The phylogeographic analyses were performed on the basis of a median-joining network approach using Network v. 5.0.0.1 software with default settings [42]. Additionally, 264 COI sequences from 28 localities of Northern Europe and North America were used (Table S1). The sequence length leaving the dataset was 571 bp long due to the removal of missing sites and cuts of different lengths of available sequences.

3. Results

The sequenced specimens of *P. mandibularoides* consisted of eight unique COI and one 28S rRNA haplotypes. An equal number of unique COI haplotypes (four haplotypes) were detected for North America and northeast Russia. The mtDNA haplotypes were closely related to each other with 1–2 mutation steps but did not share a common haplotype within samples of subdivided continents and Wrangel Island (Figure 3). The mean uncorrected pairwise COI p-distance (\pm SEM) between North American and Far Eastern samples of *P. mandibularoides* was $0.49\% \pm 0.17\%$. According to both median-joining network analysis and pairwise p-distance, *P. mandibularoides* had equidistant relations with *P. brevicornis* and *P. nivalis*. The pairwise COI p-distance for complete haplotype dataset and pairwise 28S rRNA p-distance for Eurasian samples did not exceed $5\% \pm 1\%$ between *P. mandibularoides* and the other two species (Table 2). Haplotypes of *P. brevicornis* and *P. nivalis* were more closely related to each other and did not exceed $4\% \pm 1\%$ mean p-distance for both genetic markers (Table 2).

Bayesian phylogeny revealed the same patterns as network analysis, with *P. mandibularoides* representing the most distant lineage among three studied species of *brevicornis* group with a fully supported clade (Figure 4). *P. b. brevicornis* appeared to be a sister species to *P. nivalis*, and their clades had quite high support (Figure 4). The subclade of Canadian sequences of *P. mandibularoides* with a high probability value had more distance with samples from America and Chukotka.

Table 2. Genetic divergences (mean uncorrected p-distances \pm standard error estimations, %) between *P. mandibularoides* and the two other related species of the *brevicornis* group on the mitochondrial COI gene for the full dataset (below diagonal) and the nuclear 28S rDNA gene for Eurasian samples (above diagonal) sequences.

Species	<i>P. mandibularoides</i>	<i>P. b. brevicornis</i>	<i>P. nivalis</i>
<i>Pterostichus mandibularoides</i>		5.45 \pm 1.11	5.42 \pm 1.12
<i>Pterostichus b. brevicornis</i>	5.25 \pm 0.87		3.98 \pm 0.88
<i>Pterostichus nivalis</i>	5.60 \pm 0.98	3.37 \pm 0.71	

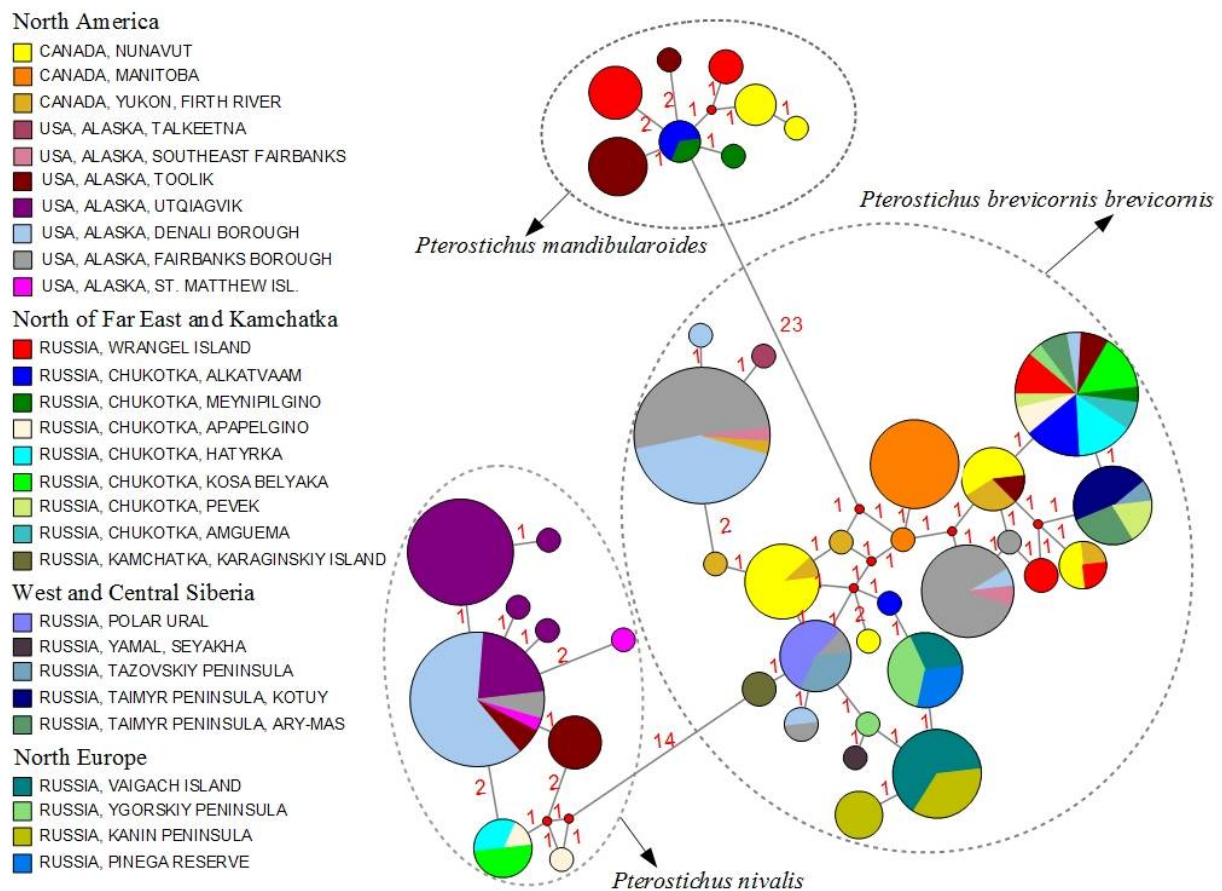


Figure 3. Phylogeography of *P. mandibularoides*, *P. b. brevicornis*, and *P. nivalis*. Median-joining network of COI sequences (see Table S1).

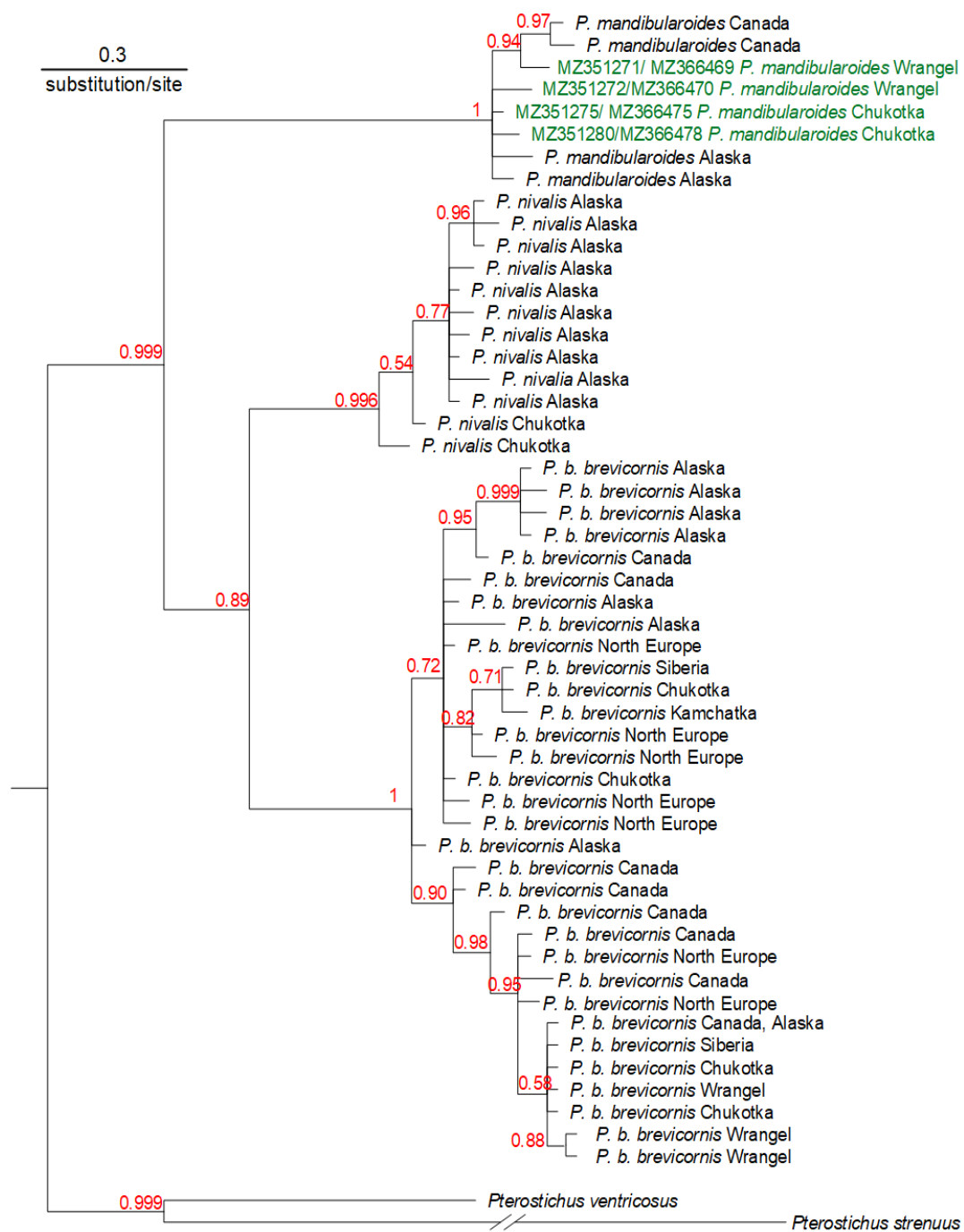


Figure 4. The majority-rule consensus phylogenetic tree of *P. mandibularoides*, *P. b. brevicornis*, and *P. nivalis* recovered from Bayesian inference analysis, based on the combined mitochondrial and nuclear sequence dataset (COI, 28S rRNA). Sequences of *P. mandibularoides* from Chukotka and Wrangel Island are highlighted in green with accession numbers to NCBI. New haplotype codes are as indicated in Table S1. Numbers near branches indicate the Bayesian posterior probability (BPP). The scale bar indicates the branch length.

4. Discussion

This study showed that the previous Nearctic range of *P. mandibularoides* must be extended into the Palearctic along the northeastern edge of Asia (Chukotka and Wrangel Island). The observed disjunctive range with a wide marine barrier between the mainland of Chukotka and Wrangel Island (140 km) makes it possible to assume a wider distribution of *P. mandibularoides* at the Last Glacial Age in Eurasia before sea transgression, at least on

trans-Beringian territory. A similar result was detected using time-calibrated phylogeny for some species of bumblebees from the *lapponicus* group [5]. Our result is another species with a range wider than the Nearctic region, which continues the list of recent detections of Nearctic species in the Holarctic for the taxa of different insects [7–15].

P. mandibularoides is widely distributed in North America but has low occurrences within ground beetle compositions of the northeastern part of the range. In the mountainous part of Wrangel Island and in both localities in Chukotka, it was found together with *P. brevicornis*. At the same time, in a much more significant number of Chukotka localities, where *P. b. brevicornis* was collected (Table S1), *P. mandibularoides* was absent. This is especially interesting, since the species was collected in the northern and southern parts of the tundra zone, in biotopes with different ecological characteristics, which suggests that it is quite polytopic.

Phylogeny by both genetic markers did not correspond with the previous conclusion on the two species (*P. mandibularoides* and *P. brevicornis*), which did not share common ancestry within the *brevicornis* group in the late Pleistocene [19]. The result indicates that *P. mandibularoides* had the most recent common ancestor with sister species *P. brevicornis* and *P. nivalis*. Bayesian phylogeny was also confirmed by the genetic distances of both mtDNA and 28 rRNA, with lower p-distance measures between *P. b. brevicornis* and *P. nivalis*, and higher p-distance measures between both species and *P. mandibularoides*. The number of unique COI haplotypes for *P. mandibularoides* samples (eight haplotypes) was comparable with another species from the *brevicornis* group—*P. nivalis* (nine unique COI haplotypes); however, the number of species samples was three times higher for *P. nivalis*. The ranges of both species overlap in Alaska and Chukotka, but *P. nivalis* is found principally in coastal areas [19]. In comparison, *P. b. brevicornis* has a circumpolar range with 23 COI haplotypes in northern territories, overlapping with the ranges of the other two species. *P. b. brevicornis* is distributed in different subzones of tundra, north taiga, and mountain [16,19,21,43].

According to the latest taxonomy revision of the *brevicornis* group in the Palearctic, a new species, *P. kolymensis* (Erjiomin, 1998) was described in specimens from west Chukotka and the boundary territory with Yakutia [22]. *P. kolymensis* has similar male genitalia to *P. mandibularoides* and morphologically differs from *P. mandibularoides* in terms of width of body and shape of pronotum angles. Furthermore, there is no reliable information about the biology, ecology, and range of this species [22]. Considering the complexity of species identification, with high variability of morphological structures between closely related species of subgenus *Cryobius*, the specimens of *P. kolymensis* have to be distinguished using the integrative approach with genetic analysis. In particular, this methodology was required to confirm new data on *P. mandibularoides* distribution in the Palearctic.

Three morphologically close species with overlapping ranges from the *brevicornis* group (*P. brevicornis*, *P. mandibularoides*, and *P. kolymensis*) have been registered for the northeastern territories in the Palearctic. Thus, paleontological data are hardly useful for time-calibrated phylogeny of this group. Some body structures such as elytra or pronotum are not strong morphological features for *Cryobius* species taxonomy, and male genitalia are rarely found among fossil ground beetles. Fossil insects are being used as ecology indicators for Arctic paleoenvironmental reconstructions in the Pleistocene and the Holocene [3,44–48]. Carabidae and particularly *Cryobius* frequently predominate in samples from different stratigraphic layers of Arctic territories, and they are associated with mesic to moist tundra habitats or meadows and bogs in boreal forest [47,48]. For instance, in the taiga and tundra of North Europe, specimens of *P. b. brevicornis* have been sampled in broader environmental conditions [49–53]. Specimens of *P. nivalis* are usually found on loamy soil in rather dry tundra [19]. North American specimens of *P. mandibularoides* were sampled on bare, damp, sandy soil under the dead leaves of *Salix* and *Alnus* [19]. However, in the extreme northeast of Asia, on Wrangel Island and in Chukotka, this species was collected in both wet and dry biotopes. It is interesting to note that specimens with different haplotypes were associated with these different types of biotopes on Wrangel Island.

5. Conclusions

Specimens of *P. mandibularoides* were detected in the trans-Beringian range with high distributions in North America, and the disjunctive part of the range was on the north-eastern edge of Asia, within Chukotka and on Wrangel Island, where it was found in areas with different environmental conditions. Bayesian phylogeny for *P. mandibularoides* revealed a recent common ancestor with sister species *P. b. brevicornis* and *P. nivalis*. We also conclude that the integrative approach in general would be required for further studies of *Cryobius* species and the *brevicornis* group. Overall, the lack of studies on *Cryobius* taxonomy, distribution, and ecology makes the species the weak link in paleoenvironmental reconstructions of the Arctic and the Subarctic in the Pleistocene and Holocene, which could lead to biased conclusions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14060415/s1>, Figure S1: Paratype of *P. mandibularoides* (female specimen) is deposited in the coleoptera collection of the Zoological Institute of the Russian Academy of Sciences (Saint Petersburg, Russia); Table S1: List of sequenced specimens of *Pterostichus mandibularoides*, *Pterostichus brevicornis* and *Pterostichus nivalis*, including the location with BOLD and NCBI's GenBank accession numbers; Table S2: The 28S rRNA gene polymorphism in three species of the *brevicornis* group.

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