Molecular characteristics of Bombus (Alpinobombus) polaris from North Greenland with comments on its general biology and phylogeography

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Abstract
The bumble bee Bombus polaris (Curtis 1835) is known from the northernmost region of Greenland. But how it can survive there, where in terms of geographic origin it came from, and which species in addition to B. pyrrhopygus (Friese 1902) genetically it is most closely related to are insufficiently answered questions that have motivated us to carry out this study. On the basis of a molecular analysis of the cytochrome oxidase I gene of a B. (Alpinobombus) polaris from North Greenland (82° 48′ N; 42° 14′ W), we conclude that the female specimen we analysed was most closely related to the Canadian populations of B. polaris. Geographic proximity, occurrence of B. polaris on Ellesmere Island and wind direction are likely factors that have aided B. polaris to establish itself in northern and eastern Greenland. The presence of five haplotypes in the studied sequences from Greenland indicates a moderately high level of genetic diversity of B. polaris in Greenland, reflecting the successful adaptation of B. polaris populations. In the broader context of entomological life in the high Arctic, our results on B. polaris allow us to conclude that the survival of pollinating species in the high Arctic under the changing climate scenario depends not only on the weather but also on an individual’s opportunity to continue to locate suitable food sources, i.e. pollen and nectar in the case of B. polaris. This aspect, briefly touched upon in this study, is of relevance not just to B. polaris, but the Arctic entomofauna generally.

Keywords Arctic pollinator · Polar insects · DNA extraction and sequencing · Haplotypes · Population relationships

Introduction
Bumble bees are important pollinators (Wahengam et al. 2019; Parrey et al. 2021) and famous for their cold hardiness (Couvillon et al. 2010). Thus, they have to be considered a crucial factor in the pollination ecology of flowering plants in the polar region. Alpinobombus is a monophyletic group of bumble bees distributed in the Arctic, alpine tundra and sub-Arctic area (Potapov et al. 2014; Williams et al. 2019). Recently, the classification of Alpinobombus has been studied in detail by different authors (e.g. Williams et al. 2015, 2019; Potapov et al. 2019). Based on morphological and molecular characterizations, the presence of at least eight species in this subgenus, namely Bombus alpinus (Linnaeus 1758), B. pyrrhopygus (Friese 1902), B. polaris (Curtis 1835), B. balteatus (Dahlbom 1832), B. kirbiellus (Curtis 1835), B. neoboreus (Sladen 1919), B. kluanensis (Williams and Cannings 2016) and B. hyperboreus (Schönherr 1809), has been suggested. However, there is some disagreement regarding the taxonomic position of B. hyperboreus natvigi having been raised to species level by Williams et al. (2015,
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est amount of biological, distributional and molecular data

2018b), it is the true Arctic species for which the small-

northern hemisphere (Kolosova et al. 2016; Martinet et al.

occurring in sub-Arctic and temperate environments of the

is not completely clear (Hatfield et al. 2016) due to low population size, shortage of sampling season in the

Arctic and small number of specialists focusing on the Arctic species. Therefore, observations on even a single speci-

from a remote locality have to be seen as a welcome piece of information in the puzzle of the genetic diversity, phylogeography and evolution of this northernmost group of bumble bees.

Owing to their relatively large size and deep buzz during flight, Arctic bumble bees are spectacular insects. However, despite their huge geographic ranges over which they are known to occur and their undoubtedly important role as pol-

inators and members of the Arctic ecosystem (Kevan 1972), bumble bees are frequently ignored or, if mentioned at all, referred to only in passing in ecological texts and treatises of the Arctic (Born and Böcher 2001; Thomas 2021). Part of the reason is almost certainly the fact that nowhere in the Arctic, these hymenopteran insects are very common and abundant and thorough searches often yield no more than one or two specimens per location (e.g. Table 2: Potapov et al. 2019).

In regard to Arctic regions north of latitude 80° N, only three species have ever been recorded: B. polaris, B. kirbiel-
lus and B. hyperboreus natvigi. To what extent the molecular
data of a female specimen of B. polaris from North Green-

dland that we had access to would match those of specimens from other localities in the Arctic was one of the objectives of this study and it was hoped that the results would also allow us to suggest from where the population of B. polaris in the north of Greenland could have originated. Other aspects were to examine if our data could help to shed light on the genetic variation and phylogenetic relationships between haplotypes of B. polaris to add information on how such bees survive in the high Arctic only 800 km south of the North Pole (equivalent to the distances between Paris and Marseille or Helsinki and Rovaniemi).

Materials and methods

The single female specimen we had a chance to work with stemmed from North Greenland (82° 48′ N; 42° 14′ W). It was caught on 19 July 2018 at 18.00 h by Dr Won Young Lee with a hand net in the vicinity of the Korean–Danish campsite at a location known as "Sirius Passet" whilst he was collecting wolf faeces for subsequent scat analyses. The land was covered by green plants, such as Saxifraga oppositifolia (Linnæus 1758) and Dryas octopetala (Linnæus 1758), and was part of a valley where strong wind can blow away the snow and expose the vegetation.

Although the highest temperature of the day of sampling was only 9.1 °C, summer season temperatures at Sirius Passet frequently reach highs close to 16 °C (Fig. 1). The specimen was carefully air-dried, taken to the “Korea Polar Research Institute” in Incheon (Republic of Korea) and subjected to molecular analysis at Andong National University (Republic of Korea). The dry specimen had a total body length of approximately 15 mm and according to the photographs (Fig. 2) given in Vilhelmsen (2015) and taken by Gert Brovad, it was identified as a B. polaris (Curtis 1835) queen (workers are never that large and males have a different body shape: Fig. 2). Although B. hyperboreus and B. polaris can be confused with each other (Vilhelmsen 2015), like many bumble bee species can, morphological characters using keys given in Williams et al. (2019) allowed us to identify the specimen quite reliably. Our identification was confirmed by comparing the COI sequence with the other published sequences of B. polaris available in GenBank (NCBI, https://www.ncbi.nih.gov/).

DNA extraction, primer, PCR and sequencing

Total DNA was extracted using DNeasy Blood and Tis-

tissue kit (Qiagen, Seoul, Korea). A right front leg of the specimen was used for DNA extraction. A DNA barcoding region of the mitochondrial COI gene was amplified by the polymerase chain reaction (PCR) using AccuPower
PCR PreMix (Bioneer, Daejeon, Korea) with the primer set LCO-1490 (5-GGT CAA CAA ATC ATA AAG ATA TTG G-3) and HCO2198 (5-TAA ACT TCA GGG TGA CCA AAA AAT CA-3) (Folmer et al. 1994). Amplifications were conducted with 5 min initial denaturation at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 52 °C, a 1 min extension at 72 °C and a final extension for 5 min at 72 °C. Sequencing was performed in both directions by Macrogen (Seoul, South Korea). The COI sequence data obtained in this study were deposited in GenBank databases under accession MN022986 and the voucher specimen is deposited in the insect collection of Andong National University, Republic of Korea.

Phylogenetic analysis and network construction

Sequence reads were edited and assembled, using the Bioedit v7.0.5.2 (Hall 1999). Fifty-four previously reported B. polaris COI sequences were retrieved from NCBI database and were incorporated in the analysis (Online Resource No 1). The sequences were aligned, using Clustal X version 1.8 (Thompson et al. 1994). The jModelTest (version 2.1.3) programme was used to select the best nucleotide substitution model using the default parameters (Darriba et al. 2012). Bayesian analyses were conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2011), using the HKY mutation model. The analyses were performed for 2 × 10⁶ generations at a temperature of 0.1 °C, with four chains each. Trees were sampled every 500 generations with 20% burn-in. Analyses were considered convergent when the average standard deviation of split frequencies was < 0.01. B. pyrrhopygus, a sister taxon of B. polaris (Williams et al. 2015, 2019), was used as an outgroup. The number of haplotypes was defined using DNAsp v5 (Librado and Rozas 2009) and a haplotype network was constructed using the median-joining method (Bandelt et al. 1999) in Network software version 10 to infer the relationships amongst haplotypes and their geographical distribution.
Results

Genetic distance of the populations of *B. polaris*

A total of 55 COI sequences of *B. polaris* from three countries (Canada, Greenland and USA) were analysed. In total, 14 haplotypes were obtained from 654-bp part of COI gene. Amongst countries, the pairwise genetic distance of *B. polaris* in the USA (0.31–0.61%) was higher than that for Canada and Greenland where the genetic distances of haplotypes were 0.15–0.46% and 0.15–0.31, respectively. On the other hand, the genetic distance between sequences from Canada and the USA was 0–0.6%, whilst the genetic distances between haplotypes from Greenland with Canada and the USA were 0–0.8% and 0.15–1.07%, respectively. The haplotype analyses revealed five haplotypes in Greenland (*N* = 17), eight haplotypes in Canada (*N* = 29) and three haplotypes in the USA (*N* = 8). Haplotype H1 was shared between Canada and the USA, whereas H5 was shared between Canada and Greenland. Distribution of four haplotypes was restricted to Greenland. Six haplotypes were only encountered in Canada, whereas the number of haplotypes restricted to the USA samples was two.

Phylogenetic analysis and network construction

A phylogenetic analysis was conducted to determine the relationships between available haplotypes of *B. polaris* from different regions. The results confirmed the monophyly of *B. polaris* with high support (PP = 1). All Greenland haplotypes except H5 (shared between Canada and Greenland) clustered together in one subclade (PP = 0.68) and two Canadian haplotypes clustered together with high posterior probability (PP = 0.98), but the relationship between other haplotypes from Canada and the USA remained unresolved (Fig. 3).

The unrooted haplotype network demonstrated that Canadian and Greenland populations of *B. polaris* were genetically close to each other and the COI sequence of the newly collected sample from North Greenland was identical to H5 from Nunavut, Canada. According to the wide distribution of *B. polaris* in Canada and availability of representative sequences from different localities, the frequencies of sequences in different territories were also studied. Only a single haplotype was found from the Canadian Northwest (H12) and Yukon territories (H1) due to the lack of studied sequences. Most of the Canadian haplotypes exhibited a star-like topology with short branch lengths showing that the Canadian haplotypes were closely related to the common central haplotype (H1). Similarly, all haplotypes from

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**Fig. 3** Bayesian haplotype phylogenetic tree of COI gene of *Bombus polaris*. Numbers on branches are Bayesian posterior probabilities. *B. pyrrophagus* is used as an outgroup. CA Canada, GL Greenland, Ma Manitoba, Nu Nunavut, NW Northwest, Yu Yukon
Greenland produced a star-shape structure with H3 in the centre; however, this haplotype was not the most abundant haplotype in Greenland (Fig. 4).

Discussion

Relationships to other B. polaris populations

The phylogenetic tree and haplotype network both indicate a close relationship between the Canadian and Greenland populations. The newly collected specimen from North Greenland shared the same haplotype (H5) with a sequence from North Canada (Nunavut) and was closely related to the H1, a common haplotype in Canada (Nunavut, Manitoba and Yukon), connecting the more divergent haplotypes from even Eastern Greenland to the Canadian populations. In spite of the presence of some geographical barriers such as Baffin Bay and the Labrador Sea, Greenland is quite close to Canada geographically and part of the Nearctic biogeographic realm. Furthermore, B. polaris has earlier been reported from Ellesmere Island (Kevan 1972) 500 km to the west of the place in North Greenland where our specimen was collected. Therefore, our finding of a close genetic relationship between Canadian and Greenland B. polaris individuals does not come as a total surprise.

It is well known that migratory birds flying from northern Canada to north-east Greenland and western Iceland are aided by weather conditions and often fly across North Greenland. Although we cannot say since when B. polaris and B. hyperboreus have been occurring in North Greenland and we can probably rule out passive transport as hitchhikers on, for example, migratory Brent Geese (Branta bernicla hrota), it does seem possible that winds from Canada could have aided bumble bees in colonizing northern and north-eastern Greenland from the West. Mass migrations of bumble bees have indeed been reported by Fijen (2021), who even suggested that the recent colonization of Iceland by several continental European bumble bee species (Potapov et al. 2018b) could have been the consequence of migrating queens from continental Europe, aided by wind and warmer climatic conditions. On the other hand, an anthropogenic introduction (another suggestion for the presence of European mainland Bombus species in Iceland: Prys-Jones et al.

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Fig. 4  a Geographical distribution of haplotype frequencies of the COI gene in Bombus polaris. Pie charts indicate the frequency of haplotypes within each population. Each circle corresponds to a locality. Haplotypes found are indicated by different colours. b Median-joining haplotype network for 654-bp fragment of the COI gene of the B. polaris. Each circle represents one haplotype. The numbers under each circle indicate total haplotypes. The size of the haplotypes is drawn proportionately to their frequencies and the new haplotype 5 is marked with an asterisk next to it.
distribution and survival of Arctic bumble bees

Bumble bee survival and occurrence depends on the availability of pollen and nectar sources and summer as well as winter temperature extremes (Potapov et al. 2019). In this regard, Arctic species, generally, show similarities to cold-adapted, high-altitude species of the Himalayas (Streinzer et al. 2019). The causality of the species abundance in such extreme habitats, however, was not studied in connection with the Himalayan species, but it was the subject of a study in connection with Arctic species. Williams et al. (2015) pointed out that speciation is likely to have cut across widespread ancestral polymorphisms and was affected by allopatrically separate populations prevented from interbreeding by barriers like, e.g. oceans and mountain ridges. An important environmental parameter, however, is also the much greater UV-irradiation at a high-altitude, low-latitude environment than the UV, which prevails at a high latitude and low altitude, the latter a situation influenced additionally by the amount of cloud cover that organisms would encounter in North Greenland (Blumthaler et al. 1997; Meyer-Rochow 2000).

The dearth of UV-radiation in the far north could possibly affect the way the bumble bees under debate detect their pollen sources. Bumble bees, like bees generally and like the sub-polar northern Finnish species of B. hortorum (Linnaeus 1761), studied in detail by Meyer-Rochow (1981), possess specific photoreceptive cells in their eyes with maximum sensitivity to light of around 360 nm wavelength, i.e. UV-A radiation. The bees respond to the UV component of the light that is reflected by the inflorescences that they seek to obtain pollen and nectar from and, as shown by Kevan (1972), B. polaris plays a major role as a pollinator of high-Arctic flowers, especially those of the abundant Pedicularis spp., Salix arctica (Pallas 1788) and Ericaceae of the region. Other plants with a northern distribution like Stylophorum and crowberries can be considered pollen and nectar providers, respectively, and are likely to be also visited by B. polaris, although direct observations have not been reported.

Whether the northernmost stands of these flowering plants possess stronger UV-signals on their petals to compensate for the weaker UV-radiation at high latitudes (as has been reported for the wings of sexually dimorphic sub-Arctic Pieris napi (Linnaeus 1758) butterflies by Meyer-Rochow and Järviilehto 1997 as well as Stella et al. 2018) or high-Arctic bumble bees possess a superior sensitivity to the available lower UV remains to be investigated. Given that differences in photoreceptor spectral sensitivities have been recorded in island and mainland populations of the Italian B. terrestris by Skorupski et al. (2007), it would not be surprising to find similar differences in different populations of Arctic bumble bee species.

And in the future…

To explore the extent that global warming might have in affecting the distribution of bumble bees adapted to the cold environments of Arctic and alpine biomes, Lee et al. (2019) have recently carried out predictive analyses based on five climate variables. According to their results, the Arctic bumble bees are in greater danger of being adversely affected by the climate change than the alpine species and the former would be “required to disperse across larger distances than [the] alpine species” to avoid becoming extinct. A great deal, however, depends on how the pollen and nectar sources of the high Arctic would respond to global warming and whether the bumble bees of the high Arctic would accept other pollen and nectar sources than those that they are currently accustomed to. However, this is just one more aspect in connection with high-Arctic bumble bees that still needs to be investigated.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00300-021-02952-y.

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Author contributions SMN performed the molecular analyses, interpreted the data and located some key publications. VBM-R suggested the study, wrote the first draft of the manuscript and its last version, located key publications and contributed information on biology and ecology of Arctic bumble bees. TYP was responsible for the expedition to North Greenland and provided weather data and CJ made laboratory facilities and analytical equipment available.

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Declarations

Competing interests The authors declare they have no competing interests.

Ethical approval The research has been conducted under permission from the Greenland government, and the permits include consideration and approval (permission no. G16-074, C-17-4). The local ethics committee (Ministry of Industry and Mineral Resources, the Government of Greenland) specifically reviewed and approved the application for survey license on the use of biological resources for commercial and research purposes (in the: “Act on Biological Resources”).

Informed consent All four authors agreed to participate.

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