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Integrative taxonomy of an arctic bumblebee species complex highlights a new cryptic species (Apidae: *Bombus*)

BAPTISTE MARTINET[1*], THOMAS LECOCQ[1,2], NICOLAS BRASERO[1], MAXENCE GERARD[1], KLÁRA URBANOVÁ[4], IRENA VALTEROVÁ[3,4], JAN OVE GJERSHAUG[5], DENIS MICHEZ[1] and PIERRE RASMONT[1]

[1]*University of Mons, Research Institute of Biosciences, Laboratory of Zoology, Place du Parc 20, 7000 Mons, Belgium*
[2]*Université de Lorraine, INRA, URAFPA, F-54000 Nancy, France*
[3]*Academy of Sciences of the Czech Republic, Institute of Organic Chemistry and Biochemistry, Flemingovo nám 2, CZ-166 10 Prague, Czech Republic*
[4]*Czech University of Life Sciences, Faculty of Tropical AgriSciences, Department of Sustainable Technologies, Kamýcká 129, CZ-165 21 Prague, Czech Republic*
[5]*Norwegian Institute for Nature Research, PO Box 5685 Sluppen, NO-7485 Trondheim, Norway*

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Bumblebees have been the focus of much research, but the taxonomy of many species groups is still unclear, especially for circumpolar species. Delimiting species based on multisource datasets provides a solution to overcome current systematic issues of closely related populations. Here, we use an integrative taxonomic approach based on new genetic and eco-chemical datasets to resolve the taxonomic status of *Bombus lapponicus* and *Bombus sylvicola*. Our results support the conspecific status of *B. lapponicus* and *B. sylvicola* and that the low gradual divergence around the Arctic Circle between Fennoscandia and Alaska does not imply speciation in this species complex. Therefore, based on our molecular and morphological analyses, we propose to assign them subspecific status: ***Bombus lapponicus lapponicus*** from Fennoscandia and West Siberia and ***Bombus lapponicus sylvicola*** **comb. nov.** from Alaska and Yukon. In addition, our analyses reveal a cryptic species in the *B. lapponicus* complex from Alaska, which we describe here as new: ***Bombus* (*Pyrobombus*) *interacti* sp. nov**.

ADDITIONAL KEYWORDS: circumpolar species – subspecies.

INTRODUCTION

Most biodiversity hotspots are found in the tropics, with a pattern of increasing biodiversity from the poles to the equator (Brown, 2014). However, the highest latitudes have many conspicuous and endemic species living in some of the most extreme conditions on Earth (Lomolino *et al.*, 2010; Botero *et al.*, 2014). This arctic and boreal biodiversity has been shaped by speciation processes driven by the cold climate and local adaptations to environmental harshness linked to arctic ecology and by spatial and temporal geographical patterns; specifically, the heterogeneity of resource patches as landscape focal points (Chapin & Körner, 1995; Willig *et al.*, 2003).

Potential speciation processes between allopatric populations inhabiting different continents around the Arctic Circle have been the focus of much research and the subject of long-standing debates (Reinig, 1937; Irwin *et al.*, 2001b, 2005; Päckert *et al.*, 2005; Monahan *et al.*, 2012; Alcaide *et al.*, 2014). Indeed, the topography of the continents around the North Pole could lead to the formation of a chain of intergrading populations (e.g. across Eurasia) connecting two reproductively isolated taxa (e.g. across the Atlantic region); the

*Corresponding author. E-mail: baptiste.martinet@umons.ac.be
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so-called 'ring species' (Stresemann & Timofeeff-Ressovsky, 1947; Irwin *et al.*, 2001b) or *Artenkreis* speciation process (*sensu* Rensch, 1933). In this process, each intergrading population is able to reproduce with immediately adjacent populations, but not with the more remote populations, through a set of parapatric speciation processes (Irwin *et al.*, 2001a). This could be explained, for instance, by small interpopulational variations of the species mate-recognition system that prevent the specific recognition between individuals of distant populations or by ecological differentiation (Rensch, 1933; Stresemann & Timofeeff-Ressovsky, 1947). Several cases of ring speciation processes were emphasized in birds, such as for *Phylloscopus trochiloides* (Sundevall, 1837) in Siberia (Alström, 2006) or *Melospiza melodia* (Wilson, 1810) in the Sierra Nevada, USA (Patten & Pruett, 2009).

Bumblebees (Hymenoptera: Apidae) are cold-adapted species, an adaptation that enables them to live in some of the highest latitude and elevation ecosystems and reach high diversity in the arctic and boreal regions (Shamurin, 1966; Kevan, 1973; Williams, 1998; Michener, 2007; Biella *et al.*, 2017). As a result, bumblebees are an excellent model group in which to explore speciation processes in circumpolar areas with disjunct distributions (Williams *et al.*, 2015). There is some evidence that a circumpolar speciation process could have shaped the *Bombus lapponicus* (Fabricius, 1793)–*Bombus sylvicola* Kirby, 1837 complex. In the eastern Palaearctic, Skorikov (1922) described a multitude of forms across the circumboreal region. These taxa are connected by a long set of potential interbreeding populations around the Arctic Circle (Skorikov, 1922). When comparing these forms with American taxa, Pittioni (1942) pointed out a possible ring speciation process by highlighting the variability of *B. lapponicus*, with an increased melanization process in the east (Skorikov, 1937). These different forms could be attributable to the fragmentation of the arctic habitat. More recently, several authors have questioned the taxonomic relationship that connects *B. sylvicola* and *B. lapponicus* (Thorp *et al.*, 1983; Savard, 2009; Williams *et al.*, 2014). Among these circumpolar populations, only *B. lapponicus*, *B. sylvicola*, *Bombus glacialis* Friese, 1902 (Novaya Zemlya, Wrangel Island) and *Bombus karaginus* (Skorikov, 1912) (Kamchatka) are currently recognized as valid species (Proshchalykin & Kupianskaya, 2005; Williams *et al.*, 2014; Potapov *et al.*, 2017). Although it has been suggested that *B. lapponicus* and *B. sylvicola* could be conspecific (Sladen, 1919; Skorikov, 1922, 1937; Pittioni, 1942, 1943; Thorp, 1962; Thorp *et al.*, 1983), data from 16S and cytochrome *c* oxidase I (*COI*) gene fragments supported two divergent taxa in phylogenetic analyses (Hines *et al.*, 2006; Cameron *et al.*, 2007). However, a comparison of all available data leaves the taxonomic status of this group uncertain.

The systematics of bumblebees remains challenging (Bertsch & Schweer, 2012; Lecocq *et al.*, 2015a; Williams *et al.*, 2012) because of the limitations of morphological traits as diagnostic characters (Bickford *et al.*, 2007; Batalha-Filho *et al.*, 2010; Carolan *et al.*, 2012). The development of integrative taxonomy, involving a consensus between several independent alternative traits (e.g. molecular, eco-chemical traits), provides a solution to help resolve bumblebee systematics at the species level (Estoup *et al.*, 1996; Ings *et al.*, 2010; Leaché & Fujita, 2011; Engel, 2011; Lecocq *et al.*, 2015c). Here, we propose to investigate the ring speciation process, focusing on the most common circumarctic bumblebee taxa complex: *B. (Pyrobombus) lapponicus* (northern Scandinavia, western Siberia)–*B. (Pyrobombus) sylvicola* (North America). We address the taxonomic uncertainties that exist between these distant populations (Williams, 1998; Cameron, 2007; Williams *et al.*, 2014) and we present new morphometrical, genetic and eco-chemical evidence to resolve the taxonomic status of *B. lapponicus* and *B. sylvicola* using an integrative taxonomic approach.

MATERIAL AND METHODS

Sampling and morphological identification

Bombus lapponicus is a common Euro-Siberian boreo-alpine species (Fig. 1A). Its geographical distribution extends from the north of the taiga to the tundra (except in the Taymyr Peninsula, northern Siberia) between the 65th and 70th parallels in Europe and between the 60th and 72nd parallels in Siberia (Løken, 1973; Pekkarinen, *et al.* 1981; Pekkarinen, 1982). *Bombus sylvicola* is a widespread species from the northern and western mountains of North America (Fig. 1B). This Nearctic taxon is morphologically similar to *B. lapponicus* (Williams *et al.*, 2014). In North America, two forms of *B. sylvicola* have been described: one with the metasomal tergite (T)2–T3 red, from the Rocky Mountains, and the second with T2–T3 mainly black, from the Sierra Mountains. DNA barcoding supports the two principal colour forms of *B. sylvicola* in North America as conspecific, including the doubtful taxon named *Bombus gelidus* Cresson, 1878 from Alaska, which has black hairs on the face and on the sides of the thorax (Williams *et al.*, 2014).

We were able to sample females and males (Appendix S1) of *B. lapponicus* from north Scandinavia ($N = 12$) and Siberia ($N = 10$), *B. sylvicola* from Northern Alaska ($N = 29$) and Yukon ($N = 4$) (Supporting Information, Fig. S1). For comparison, we used the phylogenetically closely related species *Bombus*

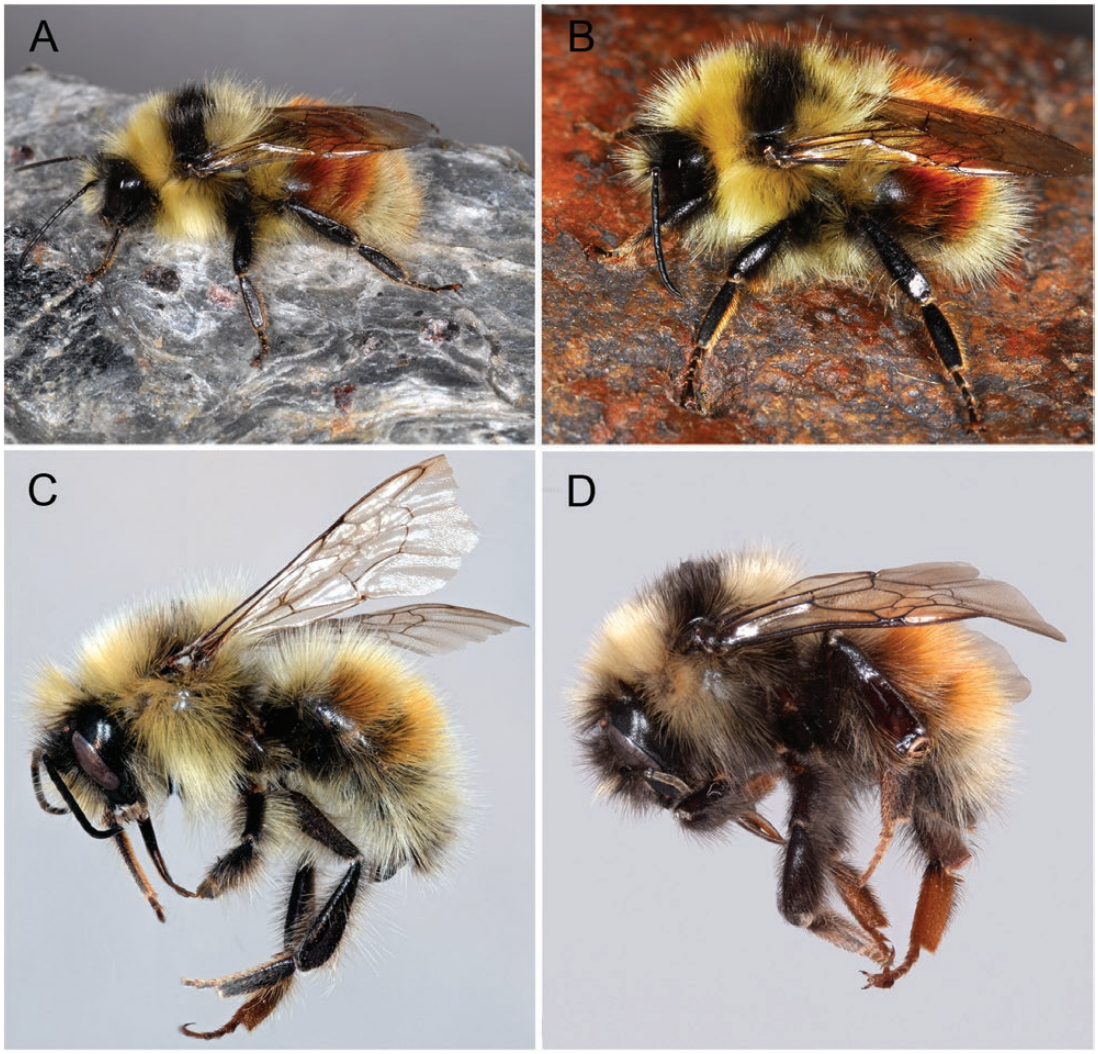

Figure 1. Photographs of three studied bumblebees: A, *Bombus lapponicus* male. B, *Bombus sylvicola* male. C, ***Bombus interacti* sp. nov.** male (holotype). D, ***B. interacti* sp. nov.** female (paratype). (Photographs by P. Rasmont.)

(Pyrobombus) monticola scandinavicus Friese, 1912 (*N* = 9) from North Scandinavia (Cameron *et al.* 2007), *Bombus* (*Pyrobombus*) *bimaculatus* Cresson, 1863 (*N* = 10) from Canada, *Bombus* (*Pyrobombus*) *ephippiatus* Say, 1837 (*N* = 6) from Biobest NA (Chiapas), *Bombus* (*Pyrobombus*) *konradini* Reinig, 1965 (*N* = 5) from Italy, *Bombus* (*Pyrobombus*) *glacialis* (*N* = 1) from Novaya Zemlya, *Bombus* (*Pyrobombus*) *melanopygus* Nylander, 1848 (*N* = 2) from California and, as an outgroup, *Bombus* (*Bombus*) *terrestris* (Linnaeus, 1758) (*N* = 14) from Italy, France, Sweden, Belgium and Scotland. The individual bumblebee specimens were killed by freezing at –20 °C.

Specimens were identified based on their morphology, with identification keys from Løken (1973) and Williams *et al.* (2014). A total of 147 bumblebees, collected between 2013 and 2018 in Europe, Siberia and North America, were analysed (Supporting Information, Table S1). For initial identification, we performed a comparative table (male and female), gathering diagnostic characters and colour patterns for the studied specimens to compare morphological characters within the *B. lapponicus–B. sylvicola* complex.

Genetic differentiation

In this study, we sequenced two genes commonly used to assess the specific status in bumblebees (Pedersen, 2002; Hines *et al.*, 2006; Cameron *et al.*, 2007; Lecocq *et al.*, 2013a, b): the mitochondrial cytochrome *c* oxidase I (*COI*) gene and the nuclear phosphoenolpyruvate carboxykinase (*PEPCK*) gene. The DNA extraction protocol, polymerase chain reaction, amplification reactions, sequencing

procedures and alignment of DNA sequences were performed according to the methods described by Lecocq *et al.* (2015a, c). *COI* and *PEPCK* sequences were deposited in GenBank (Supporting Information, Table S1). For each gene, we carried out phylogenetic analyses to investigate genetic differentiations between *B. lapponicus* and *B. sylvicola*. We performed maximum likelihood (ML) and Bayesian (MB) analyses. For all methods, the *PEPCK* gene was partitioned into two exons and two introns to explore the best substitution model. The *COI* fragment and each nuclear exon were partitioned by base positions (first, second and third nucleotide). For each dataset, we used JModelTest Server v.2.0 (Posada, 2008) with the corrected Akaike information criterion (AICc) to find the best-fitting substitution models. The models chosen were as follows: (1) for *COI*, GTR+I (first position), TIM2+I (second position) and TrN+G (third position); (2) for *PEPCK* first intron, TPM1 uf +I; (3) for *PEPCK* exon 1, HKY+I (first position), JC (second position) and TrN+I (third position); (4) for *PEPCK* second intron, TrN+I; and (5) for *PEPCK* exon 2, JC (first, second and third positions). Selected models that are not implemented in MrBayes were substituted by the closest over-parameterized model. For ML analyses, we performed ten independent runs in GARLI v.2.0 for both genes (Zwickl, 2006); the topology and −lnL were the same among replicates. Only the run with the highest likelihood was saved. We assessed statistical significance of nodes with 10 000 non-parametric bootstrap replicates. We considered a topology well supported (high confidence) when the bootstrap value (branch supports) was > 85% (Hillis & Bull, 1993). We carried out MB analyses with MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). We achieved ten independent analyses for each gene (100 million generations, four chains with mixed models, default priors, saving trees every 100 generations). Then we removed the first ten million generations as a burn-in procedure. A majority-rule 50% consensus tree was constructed. Only branch supports (topologies) with high posterior probabilities (≥ 0.95) were considered statistically significant (Wilcox *et al.*, 2002). Trees were rooted on *B. terrestris* (outgroup species). For genetic analyses, we used clustering computers provided by the Consortium des Équipements de Calcul Intensif [CÉCI, Fonds de la Recherche Scientifique-Fonds national de la recherche scientifique (F.R.S.-FNRS)].

To recognize a species threshold, we used a Bayesian implementation of the general mixed Yule-coalescent model (bGMYC) based on the *COI* tree (Reid & Carstens, 2012; see Lecocq *et al.*, 2015c). These analyses were performed with 'bGMYC' R packages (Reid & Carstens, 2012). A range of probabilities > 0.95 was considered as strong evidence that taxa were conspecific, whereas a range of probabilities < 0.05 suggested that taxa were heterospecific (Reid & Carstens, 2012). We performed a phylogenetic analysis with BEAST v.1.7.4 (Drummond & Rambaut, 2007) to generate ultrametric trees using a phylogenetic clock model to generate a posterior distribution of trees (length of the Markov chain Monte Carlo chain: 100 million generations). The first million sampled trees were treated as burn-in, using the maximum clade credibility method and setting the posterior probability limit to zero. We based the bGMYC analysis on 1000 trees sampled every 10 000 generations. For each of these 1000 trees, the Markov chain Monte Carlo was made of 100 000 generations, discarding the first 90 000 as burn-in and sampling every 100 generations. Posterior probability distributions have been applied against the first sample tree.

Reproductive trait differentiation

In the genus *Bombus*, conspecific individuals share the same recognition signals to recognize each other as sexual partners (Calam, 1969). We focused on the most studied reproductive trait involved in the bumblebee pre-mating recognition (Svensson, 1980; Baer, 2003; Ayasse & Jarau, 2014): the cephalic labial gland secretions (CLGSs). The CLGSs are commonly used for species discrimination in bumblebees (Rasmont *et al.*, 2005; Terzo *et al.*, 2005; Bertsch & Schweer, 2012). The CLGSs are synthesized *de novo* by cephalic labial glands (Žáček *et al.*, 2013) in the head of bumblebee males and are known to be species specific (Lecocq *et al.*, 2015c). The CLGSs consist of a complex mixture of (mainly aliphatic or isoprenoid) compounds, with variable main compounds (Coppée *et al.*, 2008; Lecocq *et al.*, 2011). By main compounds, we mean compounds that have the highest relative proportion (RA) among all compounds of CLGSs, at least in one individual of the taxon.

We extracted CLGS in 400 µL of *n*-heptane, according to the method described by De Meulemeester *et al.* (2011). Samples were stored at −40 °C before the analyses. For *B. lapponicus*, *B. monticola* and *B. bimaculatus*, the data of CLGS compositions are the same as those described by Martinet *et al.* (2018) (Supporting Information, Table S2). For *B. terrestris*, we used the CLGS dataset described by Lecocq *et al.* (2016).

The qualitative composition of the CLGS was determined by gas chromatography–mass spectrometry (GC/MS) using a Finigan GCQ quadrupole system with a non-polar DB 5 ms capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m; inner diameter 0.25 mm; film thickness 0.25 µm]. All samples of CLGS were quantified with a gas chromatograph Shimadzu GC-2010 system with

flame ionization detector (GC-FID) equipped with a non-polar SLB-5 ms capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m; inner diameter 0.25 mm; film thickness 0.25 μm] and a flame ionization detector. The composition of CLGSs was analysed according to the protocol described by Lecocq *et al.* (2015c). All compounds for which the relative abundance was recorded as < 0.1% for all specimens were excluded from the analysis (De Meulemeester *et al.*, 2011). The data matrix for each taxon (Supporting Information, Table S2) was based on the alignment of each relative proportion of compound between all samples performed with GCAligner v.1.0 (Dellicour & Lecocq, 2013a, b). To facilitate the alignment of compounds and the identification, before each sample injection, a standard mixture of alkenes (Kovats) from C10 (decane) to C40 (tetracontane) was injected. We calculated Kovats indices with GCKovats v.1.0 according to the method described by Dellicour & Lecocq (2013a, b).

Statistical analyses

We performed statistical comparative analyses of the CLGSs using R v.3.3.2 (R Development Core Team, 2016) to detect CLGS differentiations. We transformed data [$\log(x + 1)$] to reduce the great difference of abundance between highly and lowly concentrated compounds. We used a principal components analysis (PCA; R package MASS; Venables & Ripley, 2002) based on correlation distance matrices and a clustering method computed with the unweighted pair-group method with average linkage (UPGMA) based on Canberra distance matrices (RA of each compound) (R package ape; Paradis *et al.*, 2004). We assessed the uncertainty in hierarchical cluster analysis using *P*-values calculated by multiscale bootstrap resampling, with 100 000 bootstrap replications (significant branch supports > 0.85) (R package pvclust; Suzuki & Shimodaira, 2011). We also assessed CLGS differentiations between taxa by performing a multiple response permutation procedure (MRPP; R package vegan; Oksanen *et al.*, 2014) based on groups identified by hierarchical cluster analysis. When a significant difference was detected, pairwise multiple comparisons were performed with an adjustment of *P*-values (Bonferroni correction) to avoid type I errors. To determine specific compounds of each taxon (i.e. indicator compounds), the indicator-value (IndVal) method was used (Claudet *et al.*, 2006; Dufrêne & Legendre, 1997). This value is the product of relative concentration and relative occurrence frequency of a compound within a group. The statistical significance of an indicator compound (> 0.7) was evaluated with a randomization procedure (Dufrêne & Legendre, 1997).

Data integration and decision framework

We based our species delimitation hypothesis on the method performed by Lecocq *et al.* (2015a), derived from the integrative approach established by Schlick-Steiner *et al.* (2010) according to the unified species concept (De Queiroz, 2007). With our approach, criteria are not balanced, and the assignment of species status is allocated by unanimity of all criteria to avoid species overestimation (Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010). The specific status was assigned if this taxon: (1) was genetically differentiated in all genetic markers (i.e. potential unique haplotypes); (2) constituted a monophyletic group with high branch support; and (3) was significantly differentiated in CLGS compositions (including IndVal indicator compounds, MRPP test and bootstrap values > 0.85). This conservative approach could lead to underestimation of the species differentiation, but reduces the taxonomic inflation (Lecocq *et al.*, 2015a; Williams *et al.*, 2015). To highlight taxa with infraspecific-level differentiation, we assigned the subspecies status to phenotypically distinct allopatric populations with differentiations in some traits to highlight these populations displaying such a differentiation. This approach reduces the risk of underestimating taxonomic diversity (Hawlitschek *et al.*, 2012; Ennen *et al.*, 2014; Lecocq *et al.*, 2015a, c).

Geometric morphometrics

Given that fresh material of *B. gelidus* was not available for molecular and chemical analyses, we ran an additional study to test the similarity between *B. gelidus* type material and other *B. lapponicus* group taxa. The right forewings of 44 queens were photographed using an Olympus SZH10 microscope, an AF-S NIKKOR 18–105 mm lens (Shinjuku, Japan) and GWH10X-CD oculars coupled with a Nikon D200 camera: 39 queens of *B. lapponicus* [including specimens of *B. lapponicus lapponicus* (N = 14) and *B. lapponicus sylvicola* (N = 29)] and one queen of *B. gelidus* (holotype).

For *B. gelidus*, in the Smithsonian National Museum of Natural History, Massachusetts Agricultural College and United States National Museum, there are one queen, 14 workers and one male labelled 'Cotype' by Franklin (1912). All these specimens should not be part of the typical series and have been labelled erroneously. These specimens were collected later and in other areas than the only holotype described by Cresson (1878). After examination, we consider that these specimens belong to *B. lapponicus sylvicola*.

All easily available material has been evaluated, including specimens from the Aleutian Islands. We have revised the type series, including the 'false type anachronic inclusion'.

Wing shapes were captured by digitizing two-dimensional Cartesian coordinates of 18 landmarks (Supporting Information, Fig. S2) on wing veins with tps-DIG v.2.17 (Rohlf, 2013a, b). The landmark configurations were scaled, translated and rotated against the consensus configuration using the generalized least-squares Procrustes superimposition method to remove all non-shape differences and to separate the size from shape components of the form (Rohlf & Slice, 1990; Bookstein, 1991). The superimposition was performed using R functions of the package geomorph (Adams & Otárola-Castillo, 2013). Each wing was digitized twice by the same experimenter (M.G.), to account for measurement error. The aligned landmark configurations were projected into the Euclidean space tangent to the curved Kendall's shape space to aid further statistical analyses. The correlation coefficient between the Procrustes distances in the shape space and the Euclidean distances in the linear tangent space equalled 1.00. This indicates that the curvature of the shape space around our data was negligible (Rohlf, 1999). The least-squares regression slope through the origin (0.999) and the correlation coefficient between the two distances were calculated with tps-SMALL v.1.25 (Rohlf, 2013c).

After checking of application assumptions, perMANOVA (permutational analysis of variance) analyses were performed to assess differences in wing size and wing shape between groups. A PCA was performed to assess the variation in shape among the different groups, using the geomorph function 'plotTangentSpace', and to visualize potential differentiation between taxa.

Before the assignment of the holotype queen *B. gelidus*, shape variation in the reference dataset and discrimination of the different taxa was assessed by linear discriminant analyses (LDA) of the projected aligned configuration of landmarks. These analyses were performed at species level as a priori grouping by using the software R v.3.0.2. The effectiveness of the LDA for discriminating taxa was assessed by the percentages of individuals correctly classified to their original taxon (hit ratio, HR) in a leave-one-out cross-validation procedure based on the posterior probabilities of assignment. Given the observed scores of an 'unknown', the posterior probability (PP) equals the probability of the unit belonging to one group compared with all others. The unit is consequently assigned to the group for which the posterior probability is the highest (Huberty & Olejnik, 2006). Taxonomic affinities of the holotype queen *B. gelidus* were first assessed based on their score in the predictive discriminant space of shapes. After superimposition of the landmark configurations, aligned coordinates of the specimens from the reference dataset were used to calculate the LDA. A unique superimposition of both the reference dataset and the assigned specimens is sometimes disregarded, although it is of primary importance because generalized least-squares Procrustes superimposition is sampling dependent. We included a posteriori the holotype queen *B. gelidus* in the computed LDA space as 'unknown' specimen and calculated their score. Assignments of the holotype *B. gelidus* were estimated by calculating the Mahalanobis distance between 'unknown' and the group mean of each taxon. We also calculated posterior probabilities of assignment to confirm the assignment to one taxon.

RESULTS

Genetic analyses

A total of 938 bp from the *COI* gene and 925 bp from *PEPCK* were obtained. All phylogenetic analyses (ML and MB) on each genetic dataset showed a similar topology and identical phylogenetic differentiation (Fig. 2). As expected, we found a less structured tree in the *PEPCK* gene for the *B. lapponicus–B. sylvicola* group. For the two gene fragments, the *B. lapponicus–B. sylvicola* group resulted in two lineages: (1) one comprising all *B. lapponicus* and some of the *B. sylvicola* specimens (group A, 1.92% of divergence between *COI* sequences); and (2) a second lineage comprising the remaining specimens of *B. sylvicola* (group B) (Fig. 2). Genetic analyses based on the mitochondrial gene *COI* showed a slightly supported divergence between *B. lapponicus* and *B. sylvicola* group A (Fig. 2B), but there was no differentiation between these two taxa for the nuclear marker, *PEPCK* (Fig. 2A). In *COI* sequences, 18 of 938 (1.92%) phylogenetically informative nucleotide sites were uniquely diagnostic to separate *B. sylvicola* group A and *B. lapponicus*. These divergence estimations between *B. sylvicola* group A and *B. lapponicus* were performed excluding specimens of *B. sylvicola* group B forming a separate clade (Fig. 2). Other species-specific branches were supported by high bootstrap values (bootstrap > 90%).

Our genetic analyses revealed a new cryptic taxon from Alaska from our *B. sylvicola* samples (group B), which are closely similar and co-occurring with *B. sylvicola* in Alaska. This new taxon was strongly supported as a monophyletic group by both *COI* (8.74% of sequence divergence from *B. sylvicola* group A and 9.80% from *B. monticola*) and *PEPCK* (> 1% of divergence from *B. monticola* and *B. sylvicola*) analyses. We describe this taxon below as *B. interacti* sp. nov.

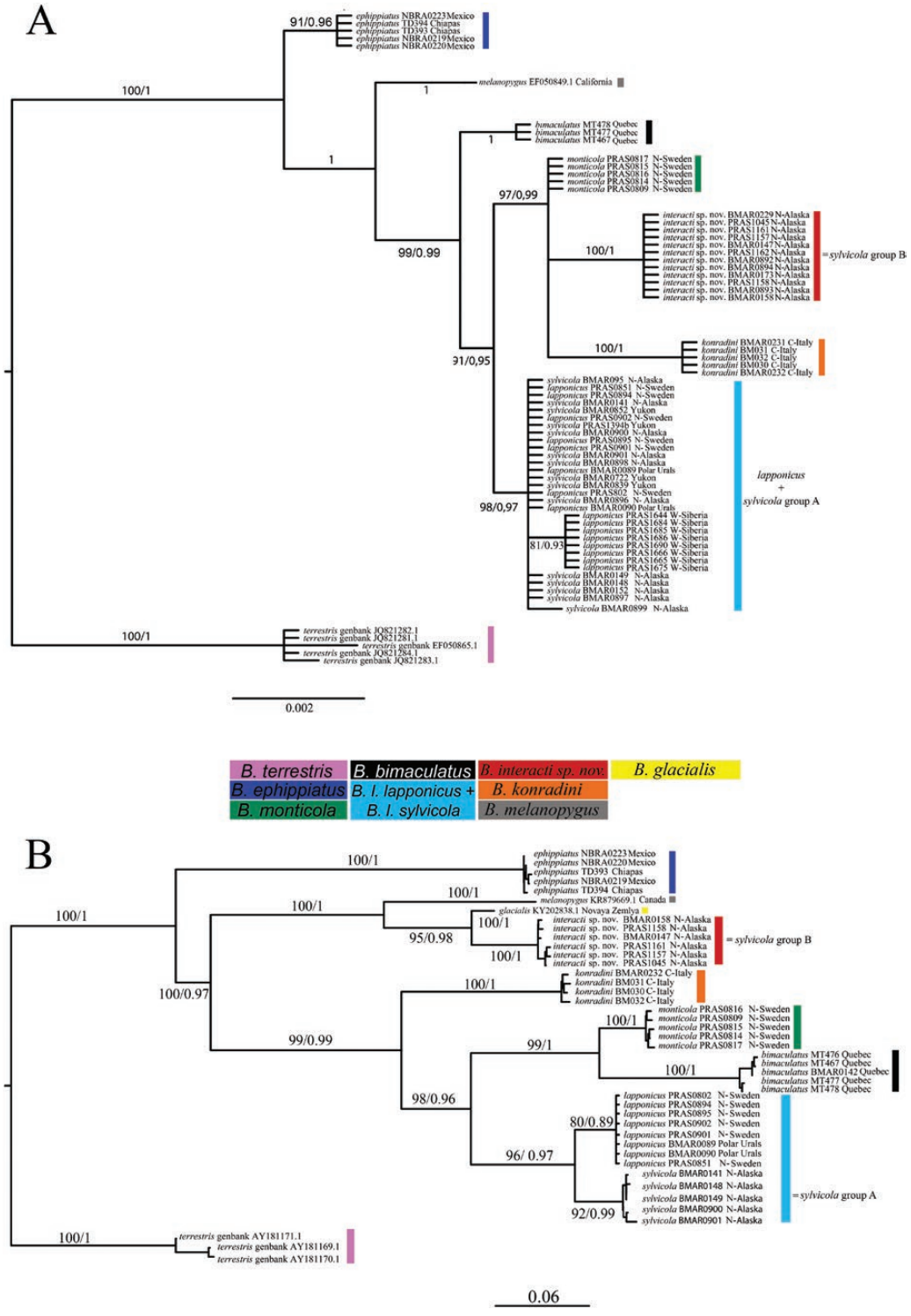

Figure 2. A, majority rule (50%) consensus tree based on maximum likelihood analyses of nuclear *PEPCK* marker. B, majority rule (50%) consensus tree based on maximum likelihood analyses of mitochondrial *COI* marker. Values above branches are maximum likelihood bootstrap values/Bayesian posterior probabilities.

Contrary to the *COI* marker, the phylogenetic affinities inside the group including *B. interacti*, *B. monticola* and *B. konradini* were not resolved with the nuclear *PEPCK* fragment (Fig. 2A). *PEPCK* and *COI* sequences of *B. interacti* have been blasted to the National Center for Biotechnology Information GenBank database. Sequences matched most closely to the studied species complex *B. lapponicus–B. sylvicola–B. monticola* but with no complete identity (99% of identity and 100% of query cover for *PEPCK*, 96% of identity and 97% of query cover for *COI* from *B. monticola* in GenBank). In our phylogenetic analyses, *B. interacti* differed significantly from *B. melanopygus* and *B. glacialis* (high branch supports and posterior probabilities; Fig. 2A, B).

The bGMYC analysis (Fig. 3) highlighted nine entities with low probabilities (< 0.05) to be conspecific with the other ones. These results matched with results from the phylogenetic analyses of *COI* gene (ML and MB analyses). Overall, the bGMYC suggested the delimitation of nine prospective species ($P < 0.05$):

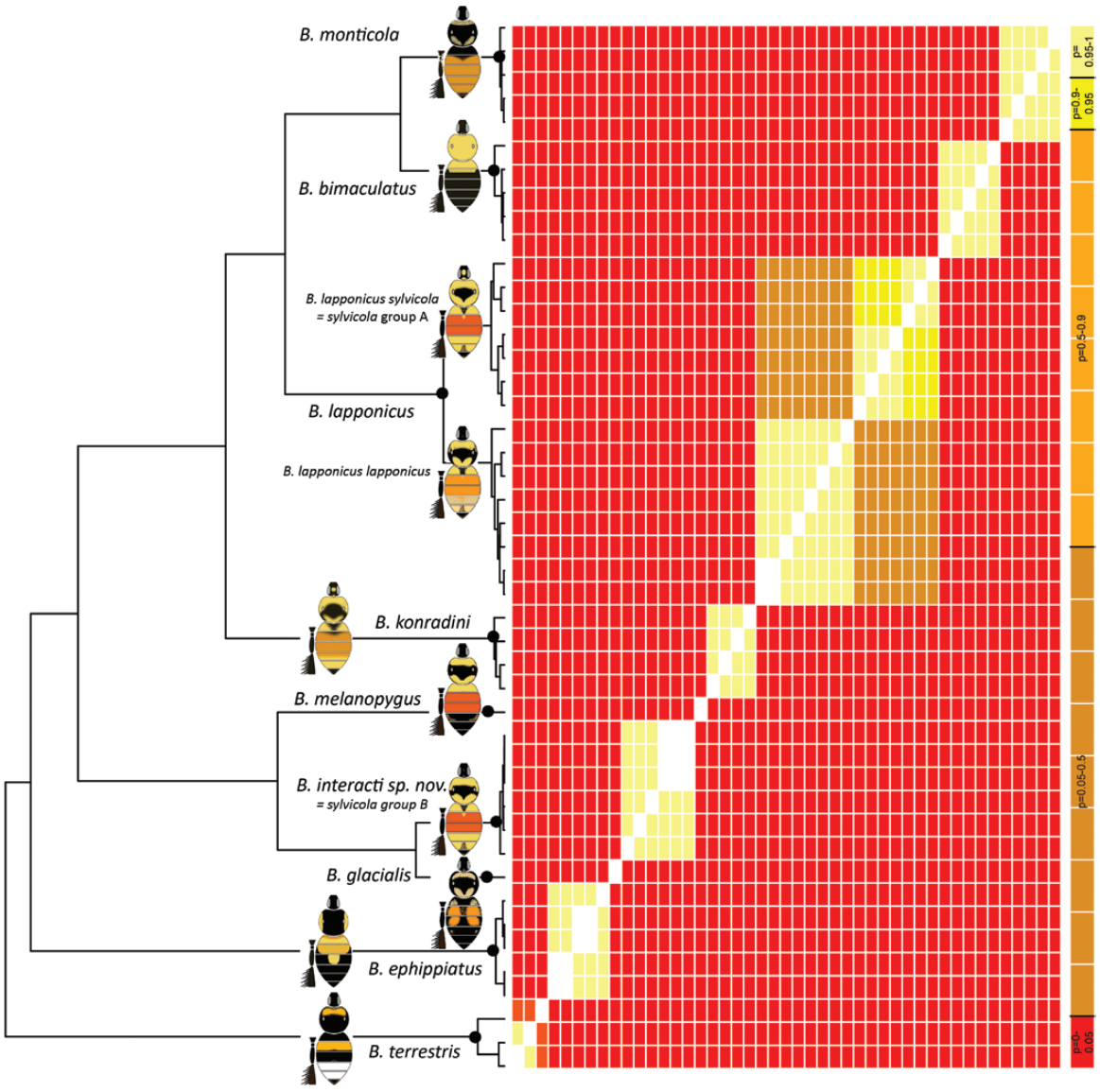

Figure 3. Species recognition pairwise matrix based on ultrametric tree of cytochrome *c* oxidase I (*COI*) sequences with a Bayesian implementation of the general mixed Yule-coalescent model (bGMYC) pairwise probability of conspecificity plotted on a sample tree from BEAST. The coloured matrix corresponds to the pairwise probabilities of conspecificity returned by the bGMYC method (colour scale on the right of the figure). Black spots show the coalescent node for each species. The larger bees represent the typical colour patterns of queens.

(1) *B. terrestris* (bGMYC conspecificity probabilities between individuals included in the group, $P > 0.12$–1); (2) *B. ephippiatus* ($P > 0.97$–1); (3) *B. interacti* ($P > 0.98$–1); (4) *B. monticola* ($P > 0.47$–1); (5) *B. bimaculatus* ($P > 0.89$–1); (6) *B. konradini* ($P > 0.99$–1); (7) a group with *B. sylvicola* and *B. lapponicus* ($P > 0.07$–1); (8) a group with *B. glacialis*; and (9) a group with *B. melanopygus*. The pairwise matrix (Fig. 3) shows a non-significant heterospecificity threshold between *B. lapponicus* and *B. sylvicola* ($P > 0.05$).

Chemical analysis

A total of 134 compounds were detected in the CLGS of the different studied species: 60 identical compounds were detected and shared by *B. sylvicola* (group A) and *B. lapponicus*, 57 compounds for *B. monticola*, 39 compounds for *B. bimaculatus*, 50 compounds for *B. konradini*, 25 for *B. terrestris*, 45 compounds for *B. ephippiatus*, and 64 compounds for *B. interacti* (= *B. sylvicola* group B). Our chemical analyses showed qualitative and quantitative differentiations between all taxa including specific main compounds, except between *B. lapponicus* and *B. sylvicola* group A, where the CLGS composition was statistically identical (Supporting Information, Appendix S2). Chemical analyses supported the presence of a new taxon in the *B. sylvicola* samples from Alaska (described below as *B. interacti* sp. nov.).

The main compounds detected were as follows: (1) geranylcitronellol (55.28–77.30%) shared by *B. sylvicola* and *B. lapponicus*; (2) hexadec-9-enyl acetate (45.91–61.74%) from *B. monticola* and *B. konradini* (48.36–54.71%); (3) ethyl octadec-9-enoate from *B. konradini* (7.43–9.14%); (4) hexadec-9-enyl acetate (20.55–40.63%) and geranylgeranyl acetate (25.98–39.42%) from *B. bimaculatus*; (5) dihydrofarnesol (19.08–40.45%) from *B. terrestris*; (6) hexadecanoic acid (19.03–31.63%) from *B. ephippiatus*; and (7) citronellyl hexadec-9-enoate (12.37–23.57%) from *B. interacti* (Table 1; Supporting Information, Table S2). Statistical analyses supported the differentiation (MRPP, $A = 0.6973$, $T = 0.1759$, all $P < 0.001$) of seven groups also supported by high multiscale bootstrap resampling values (Cluster and ACP; Fig. 4): (1) *B. monticola* (pairwise test, $P < 0.01$); (2) *B. konradini* (pairwise test, $P < 0.01$); (3) *B. bimaculatus* (pairwise test, $P < 0.01$); (4) *B. ephippiatus* (pairwise test, $P < 0.01$); (5) *B. terrestris* (pairwise test, $P < 0.01$); (6) *B. sylvicola* group A + *B. lapponicus* (pairwise test, $P < 0.01$); and (7) *B. interacti* (pairwise test, $P < 0.01$). No statistical differentiation was found in the statistical hypothesis test and in hierarchical clustering between *B. lapponicus* and *B. sylvicola* group A (Fig. 4; MRPP, $A = 0.004641$, $T = 0.1577$, $P = 0.30$). Several significant

Table 1. List of main compounds identified for *Bombus lapponicus*, *Bombus sylvicola*, *Bombus monticola*, ***Bombus interacti* sp. nov.**, *Bombus konradini*, *Bombus bimaculatus*, *Bombus ephippiatus* and *Bombus terrestris* from cephalic labial gland secretions

Compound		*B. monticola* (*N* = 9)	*B. sylvicola* (*N* = 14)	*B. lapponicus* (*N* = 20)	***B. interacti* sp. nov.**(*N* = 9)	*B. konradini* (*N* = 2)	*B. bimaculatus* (*N* = 10)	*B. terrestris* (*N* = 6)	*B. ephippiatus* (*N* = 3)
	MW	Median (%)	Median (%)	Median (%)	Median (%)	Median (%)	Median (%)	Median (%)	Median (%)
Dihydrofarnesol	224	0.00	0.00	0.00	0.00	0.00	0.00	31.72	0.00
Hexadecanoic acid	256	0.95	1.45	0.30	0.00	0.00	0.00	0.00	22.06
Hexadec-9-enyl acetate	282	52.60	0.23	0.08	0.15	51.53	34.67	0.00	0.20
Geranylcitronellol	292	0.00	64.00	71.15	0.00	0.00	0.00	0.00	0.00
Ethyl octadec-9-enoate	310	0.46	0.00	0.00	1.80	8.28	0.00	0.00	1.44
Citronellyl hexadec-9-enoate	332	0.00	0.00	0.00	15.18	0.00	0.00	0.00	0.00
Geranylgeranyl acetate	392	0.00	0.00	0.00	0.00	0.00	31.22	0.00	0.00

Complete information is available in the Supporting Information (Appendix S2).
Abbreviations: Median, median of relative concentration of compound (as a percentage); MW, molecular weight; *N*, number of specimens analysed.

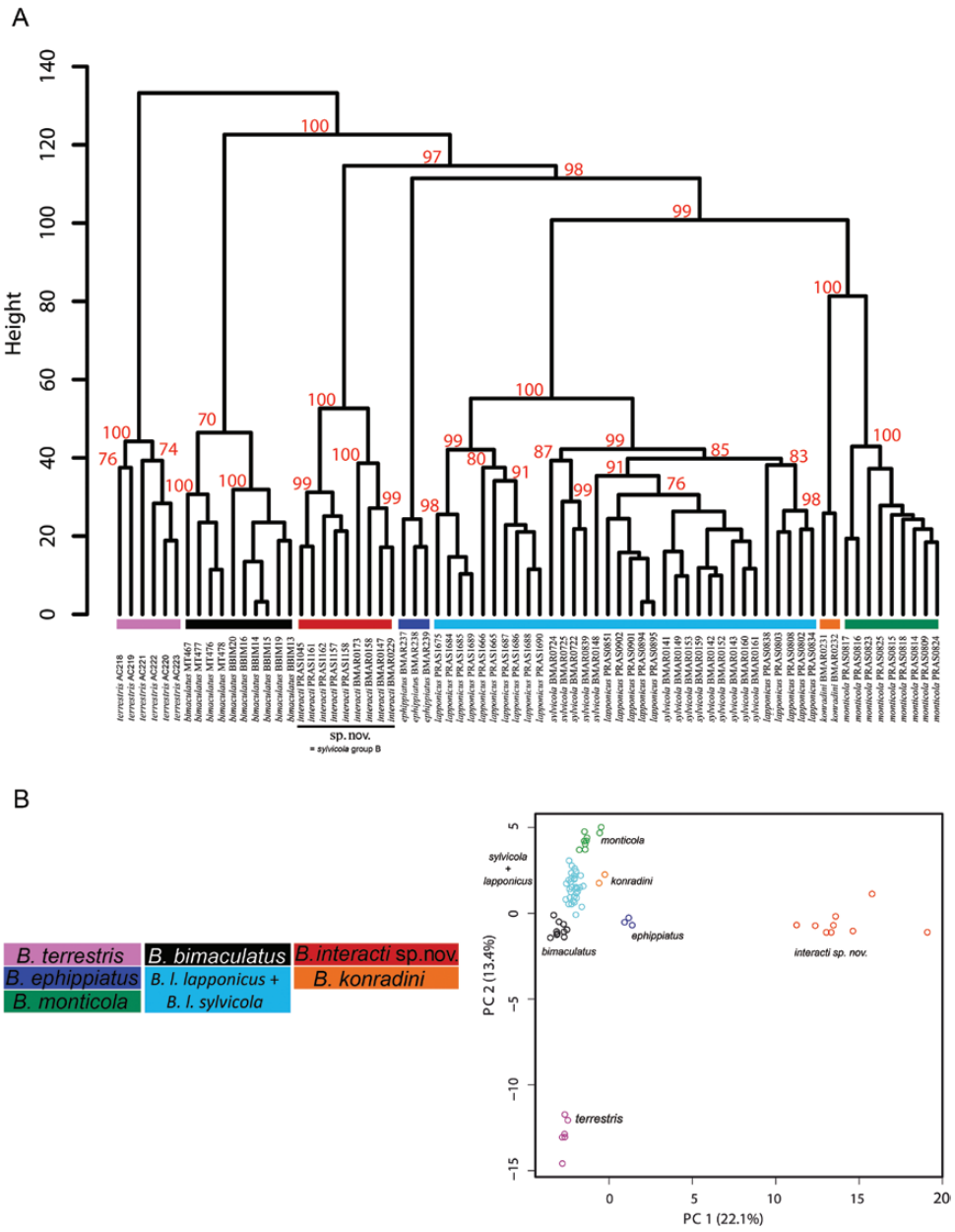

Figure 4. A, dendrogram based on cephalic labial gland secretions in *Bombus lapponicus* + *Bombus sylvicola* (light blue), *Bombus monticola* (green), *Bombus bimaculatus* (black), *Bombus interacti* (red), *Bombus terrestris* (pink), *Bombus ephippiatus* (dark blue) and *Bombus konradini* (orange). This cluster was obtained by hierarchical clustering using an unweighted pair-group method with arithmetic mean (UPGMA) based on a Canberra matrix calculated from the cephalic labial gland secretion matrix. The values near nodes represent multiscale bootstrap resampling values. B, principal components analysis of cephalic labial gland secretion differentiation in the *B. lapponicus–B. sylvicola* complex: *B. lapponicus* + *B. sylvicola* (light blue), *B. monticola* (green), *B. bimaculatus* (black), *B. interacti* (red), *B. terrestris* (pink), *B. ephippiatus* (dark blue) and *B. konradini* (orange). Abbreviations: PC1 and PC2 are the first and second principal component axes.

and specific indicator compounds were revealed by the IndVal method (IndVal > 0.70), but no compound was identified to discriminate *B. lapponicus* from *B. sylvicola* (Supporting Information, Table S2).

WING SIZE AND SHAPE ANALYSES

No significant difference in centroid size was found among the different taxa (F = 2.73; P = 0.08). However, significant differences in wing shape

were present (perMANOVA, F = 1.83; P = 0.006). Pairwise perMANOVA tests showed a significant difference between *B. lapponicus* and *B. interacti* (F = 2.44; P = 0.004) and between *B. interacti* and *B. gelidus* (F = 1.96; P = 0.035), whereas no difference was detected between *B. lapponicus* and *B. gelidus* (F = 1.21; P = 0.32). A PCA plot highlights two distinct groups (Fig. 5): one cluster gathering specimens of *B. lapponicus*, *B. sylvicola* and *B. gelidus*; and a second cluster with specimens of *B. interacti*. The two groups *B. lapponicus* and *B. sylvicola* were not discriminated in the PCA and LDA, whereas the *B. interacti* group was strongly differentiated. In the morphometric space defined by the PCA, the specimen of *B. gelidus* was undoubtedly clustered with the group of the *B. lapponicus* (Fig. 5). A posteriori assignment of the holotype of *B. gelidus* in the discriminant shape space (LDA) allowed a reliable species attribution. This analysis revealed that this specimen was assigned to *B. lapponicus* species (Mahalanobis distance to *B. sylvicola* group = 1.44; PP = 1).

Morphological diagnosis

For this morphological comparison, we assessed only males and queens (minimum of 15 individuals per taxon according to the availability of specimens). Except for the coloration of the face, which is black in *B. lapponicus* and yellow in *B. sylvicola*, no diagnostic character was found to discriminate these species based on our morphological examinations (Table 2).

Concerning *B. sylvicola*, we found two discrete morphotypes among our sampling from Alaska [corresponding to *B. sylvicola* group A and *B. sylvicola* group B (= *B. interacti*) in our molecular analyses] that could be separated by several diagnostic characters. *Bombus interacti* males differed from *B. sylvicola* in the pubescence of the tibia, which is hairier in *B. sylvicola* (Fig. 6). No difference in the structures of the genitalia was detected. Females of *B. interacti* differed from *B. sylvicola* in the face clypeus coloration: black with intermixed dark yellow hair in *B. interacti* and yellow in *B. sylvicola*. Besides, the density of pubescence of tergite 5 is higher in *B. interacti* and the yellow coloration of the collar does not reach the bases of the legs (Fig. 6). Moreover, the morphological character 'shape and pubescence of basitarsus', used by Gjershaug *et al.* (2013) to distinguish *B. lapponicus* from *B. monticola*, does not allow distinction of *B. interacti* from *B. monticola*, and this character is also similar between *B. lapponicus* and *B. sylvicola*. From *B. glacialis*, females of *B. interacti* differ in several characters: (1) labral furrow (narrow for *B. interacti*, broad for *B. glacialis*); (2) punctuations into the labral furrow (very few in *B. glacialis*); and (3) dorsal furrow of gena, which is weakly developed in *B. glacialis*. Males of *B. interacti* differ from *B. glacialis* in: (1) the colour of the vertex and the clypeus (yellow for *B. glacialis*); (2) hind basitarsus (gradually narrowing towards basal part in *B. glacialis*); and (3) punctuations into the labral furrow (very dense in *B. interacti*, and labrum covered with reddish bristles at the front part).

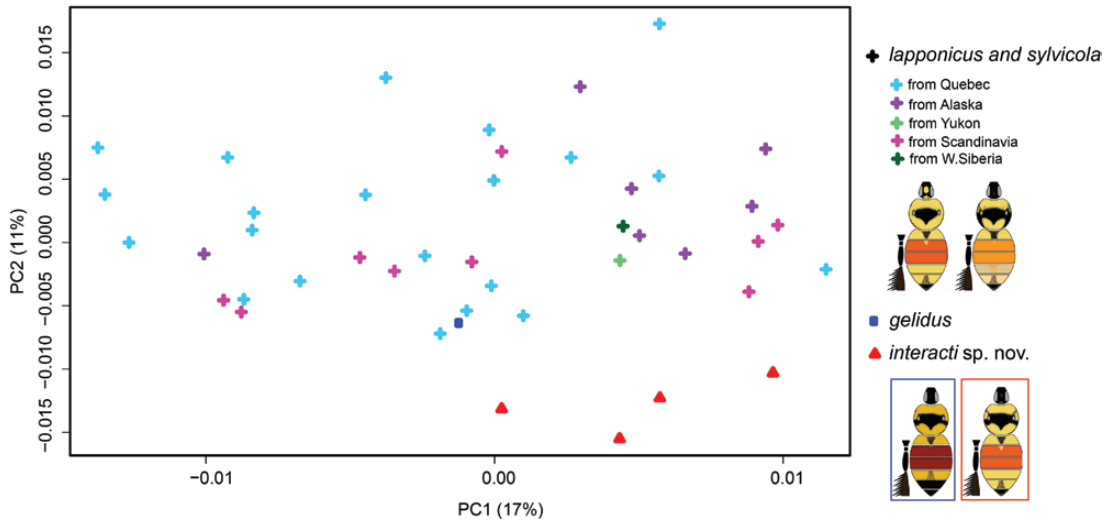

Figure 5. Ordination of wing morphometry of *Bombus lapponicus lapponicus*, *B. lapponicus sylvicola*, *B. lapponicus sylvicola* f. *gelidus* and *Bombus interacti* along the first two axes of the principal components analysis (PCA). The two first axes of the PCA (PC1 and PC2) explain 28% of the total variance. Group means are represented by different symbols: red triangle for *B. interacti* queens; a light blue cross for *B. lapponicus lapponicus* and *B. lapponicus sylvicola*; and a dark blue rectangle for *B. lapponicus sylvicola* f. *gelidus*.

Table 2. Main distribution range and morphological and colour pattern differences (male and female) according to Franklin (1912), Løken (1973), Williams (2014) and personal observations (B.M., P.R.)

	B. interacti	*B. lapponicus sylvicola*	*B. lapponicus lapponicus*	*B. monticola scandinavicus*	*B. konradini*	*B. lapponicus sylvicola* f. *gelidus*
Range	North Alaska	Widespread in most northern North America and Californian mountains	Fennoscandia, N. Russia	Fennoscandia	Central Apennines	Aleutian Islands
Female						
Morphology						
Coat colour variation	Light and colourful	Light and colourful	Varies from very light and colourful in northern Fennoscandia to rather dark in southern Fennoscandia (southern Norway)	Dark	Large and light	Dark
Body size (mm)	15–18	15–17	15–19	14–19	15–18	16–18
Colour pattern						
Face	Black, with few yellow hairs	Yellow	Black, with few yellow hairs	Black	Yellow	Black, with few yellow hairs
Collar and scutellar	Yellow, but collar does not go down to leg insertion	Large and yellow, goes down to leg insertion	Yellow, but less wide; the coloration stops at the tegulae	Small, dark yellow	Wide yellow band to the tegulae/yellow	Yellow, but collar does not go down to leg insertion
Hind meta-basitarsus	Slight pubescence, and the maximal width of the basitarsus is high, as in *B. monticola* (*sensu* Gjershaug *et al.*, 2013)	Strong pubescence, and the maximal width of the basitarsus is low (*sensu* Gjershaug *et al.*, 2013), as in *B. lapponicus*	Strong pubescence, and the maximal width of the basitarsus is low (*sensu* Gjershaug *et al.*, 2013)	Slight pubescence, and the maximal width of the basitarsus is high (*sensu* Gjershaug *et al.*, 2013)	Strong pubescence, and the maximal width of the basitarsus is low (*sensu* Gjershaug *et al.*, 2013), as in *B. lapponicus*	Strong pubescence, and the maximal width of the basitarsus is low (*sensu* Gjershaug *et al.*, 2013), as in *B. lapponicus*
Tergite 1	Yellow, with some red and black hairs	Yellow, with some red and black hairs	Yellow, with some red and black hairs	Black/red	Yellow/red/black	Yellow, with some red and black hairs
Tergite 4	Yellow	Yellow	Yellow (pinkish)	Dark red	Yellow	Yellow
Tergite 5	Yellow (higher density)	Yellow	Yellow (pinkish)	Dark red	Yellow	Yellow

Table 2. Continued

	B. interacti	*B. lapponicus sylvicola*	*B. lapponicus lapponicus*	*B. monticola scandinavicus*	*B. konradini*	*B. lapponicus sylvicola* f. *gelidus*
Male						
Body size (mm)	11–13	11–14	11–14	11–14	11–14	12
Tibia	Hairy	Very hairy	Hairy	Hairy	Hairy	Very hairy
Colour pattern						
Face	Yellow	Yellow	Yellow	Dark yellow	Yellow	Yellow
Collar/scutellar	Yellow/large, yellow	Yellow/large, yellow	Yellow/large, yellow	Dark yellow/no	Yellow/large, yellow	Yellow/large, yellow
Tergite 1	Yellow	Yellow	Yellow	Black and red	Yellow	Yellow
Tergite 4	Yellow/red	Yellow/red	Yellow/red	Dark red	Red/yellow	Yellow/red
Tergite 5	Yellow/red	Yellow/red	Yellow/red	Dark red	Red/yellow	Yellow/red

TAXONOMY

Here, we describe the new species, *B. interacti* sp. nov., and provide synonymy with *B. lapponicus* and *B. sylvicola*.

FAMILY APIDAE LATREILLE, 1802

GENUS *BOMBUS* LATREILLE, 1802

***BOMBUS* (*PYROBOMBUS* DALLA TORRE, 1880) *INTERACTI* MARTINET, BRASERO & RASMONT SP. NOV.**

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Diagnosis

Bombus interacti males differ slightly from *B. lapponicus* subsp. *sylvicola* in the pubescence of the tibia (very hairy for *B. lapponicus sylvicola*). No difference in the structure of the genitalia was found. Female *B. interacti* differ from *B. lapponicus* subsp. *sylvicola* in the face coloration: black with a few intermixed yellow hairs in *B. interacti* and yellow with a few intermixed black hairs in *B. lapponicus* subsp. *sylvicola*. The density of pubescence of tergite 5 is greater in *B. interacti*, and the yellow coloration of the collar does not extend down to the level of the front leg. Description of males and females is reported in Table 2.

Holotype: One pinned male (Fig. 1C). Labels: (1) white, printed with 'USA, Alaska, Toolik field station, 725 m, 28.VII.2015, 68°37′32.9″N 149°35′48.8″W, *Epilobium angustifolium*, leg. Martinet/Rasmont St88, PRAS1045'; (2) red, printed with 'HOLOTYPE'; and (3) white, printed with 'det. Martinet & Rasmont 2016, *Bombus interacti* Martinet, Brasero & Rasmont'. The left anterior leg is missing because it was removed for genetic analysis. The type specimen has been deposited in the Royal Belgian Institute of Natural Sciences in Brussels. GenBank accession numbers: MG280603 (*COI*), MG280606 (*PEPCK*).

Paratypes: Nine males and four queens pinned (Fig. 1D) and labelled 'Paratype'.

Description

Females: Length 15–18 mm.

Coat colour: Face and vertex densely pubescent, with black hairs intermixed with a few yellowish–greyish hairs. Thorax with a collare as large as one-third of the thorax length, with a few intermixed black hairs at front of tegulae; scutellare as large as one-quarter of the thorax length. The hairs of the scutellare are

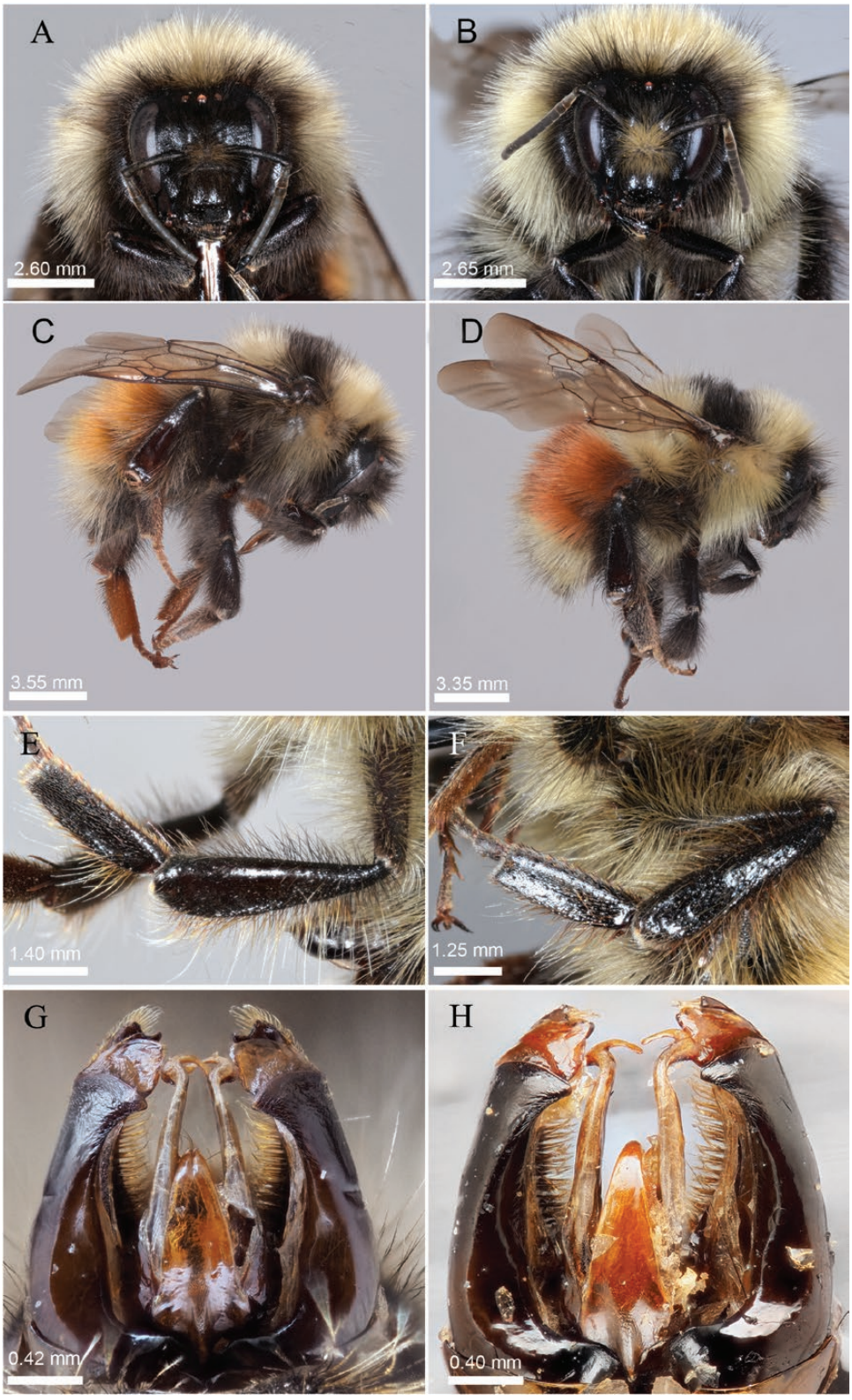

Figure 6. Photographs of the different morphological diagnostic characters between *Bombus sylvicola* and *Bombus interacti* A, face of *B. interacti* female BMAR0892. B, face of *B. sylvicola* female BMAR0900. C, right profile of *B. interacti*

shaped in two oblique tufts. Pleura covered with greyish hairs on the anterior third, intermixed with black in front of the tegulae; mesopleura with intermixed grey and black hairs; metapleura are mostly black. Wings are not particularly dark (contrary to *B. gelidus*). T1 mostly covered with greyish hairs, intermixed with black hairs in the middle. T2 covered with red hairs, intermixed with a few black ones on the sides and with numerous greyish ones at the middle of the anterior margin. T3 all red, with a few greyish hairs at the middle of the posterior margin. T4 mostly covered with greyish hairs, with very few red hairs at the anterior margin and black ones in the middle. T5 with greyish hairs, with numerous black ones in the middle. T6 mostly with black hairs and some greyish ones on the sides. Coxae and femurs with black hairs intermixed with a few greyish ones. Mesotibias with black hairs, some of them with a red tip. Metatibias with corbiculae surrounded by decumbent bristles slightly longer that the width of the organ, mostly reddish with light blonde tip and some completely black at the base of the anterior margin; meso- and metabasitarsi with short reddish bristles. Distal tarsi red. Otherwise black.

Labrum with a narrow labral furrow as wide as 0.23 times its total width, V-shaped. The labral tubercles are well defined. There is an imbricated microsculpture in front of the tubercles and into the labral furrow. Punctuations are dense into the labral furrow and more spaced back to the tubercles. The front part of the labrum is covered with plumose reddish bristles.

Basis of mandibulae with numerous punctuations, dense at the base between the condyle.

Clypeus slightly bombed, densely covered with black plumose bristles at the distal part, and short reddish plumose ones in the middle, along the anterior edge. There is a narrow glabrous area in the middle of the anterior third. This area is covered with deep and broad punctuations. These punctuations are joining at the side of the frontal part and are more spaced in the middle. There is a thin band of microsculptures along the transverse furrow at the distal part of the clypeus.

Ocellar field is covered with large spaced punctuations along the inner margin of the compound eyes, covering half the distance between the ocelli and compound eyes. Between the distal margin of the compound eyes, there is a poorly defined supra-orbital line, defined only near the eyes.

Antennae: $L(A5) = 0.67 \times L(A3)$; $L(A4) = 0.51 \times L(A3)$ (not different from *B. lapponicus*) (*L*, length; *A*, antennal segment).

Metabasitarsus: Maximal width situated apically of the diverging transversely directed hair, at 0.27 of the basitarsus length (0.19 in *B. lapponicus*). The glabrous area at the base of the metabasitarsus with slightly imbricated micro-sculptured surface (this area is much smaller in *B. lapponicus* and without imbricated surface).

Males: Length 11–13 mm.

Coat colour: Males are greyish and shaggy. Face and vertex largely covered by yellow hairs, and a slight mixture of black and yellow hairs on vertex. Thorax with a collare as large as one-third of the thorax length, with yellow hairs; scutellare with yellow hairs as large as one-quarter of the thorax length. The hairs of the scutellare are shaped in two oblique tufts. Pleura covered with yellow hairs; mesopleura with yellow hairs; metapleura are mostly yellow. Inter-alar band is yellow, with some intermixed black hairs. In some specimens, the inter-alar band is attenuated, with a mixture of black and yellow hairs. T1 is mostly covered with yellow hairs. T2 is covered with red hairs, intermixed with some yellow ones at the middle of the anterior margin. T3 and T4 are all red, with a few greyish hairs at the middle of the posterior margin. T5 is mostly covered with yellow hairs, with few red hairs at the anterior margin and black ones in the middle. T6 has greyish hairs, with numerous black ones in the middle. T7 mostly with black hairs and some greyish ones on the sides. Coxae and femurs with mostly yellow hairs intermixed with a few black ones. Mesotibias with yellow hairs, some of them with reddish base with few intermixed black hairs. Metatibias with corbiculae surrounded by decumbent bristles slightly longer than the width of the organ, mostly reddish with light blonde tip and some completely black at the base of the anterior margin; meso- and metabasitarsi with short reddish bristles. Distal tarsi red. Otherwise black.

Labrum with a narrow labral furrow as wide as 0.21 times its total width, V-shaped. The labral tubercles are well defined. Punctuations are dense into the labral furrow and more spaced back to the tubercles. The front part of the labrum is covered with plumose reddish bristles.

Basis of mandibulae with numerous punctuations, very dense at the base between the condyle.

female BMAR0892, with the yellow coloration of the collar that does not go down to the leg insertion. D, right profile of *B. sylvicola* female BMAR0900, with the yellow coloration of the collar that goes down to the leg insertion. E, posterior legs of *B. interacti* male PRAS1045, with hairy tibia. F, posterior legs of *B. sylvicola* male BMAR 0141, with very hairy tibia. G, genitalia of *B. interacti* male PRAS1045. H, genitalia of *B. sylvicola* male BMAR 0141. (Photographs by P. Rasmont.)

Clypeus slightly bombed, densely covered with short black bristles in the middle and longer reddish ones at the distal part. The anterior third is covered with deep and broad punctuations. There is a thin band of microsculptures along the transversal furrow at the distal part of the clypeus.

Ocellar field is covered with large spaced punctuations along the inner margin of the compound eyes, covering half the distance between ocelli and compound eyes. Between the distal margin of the compound eyes, there is a poorly defined supra-orbital line, defined only near the eyes.

Antennae: $L(A5) = 0.63 \times L(A3)$; $L(A4) = 0.52 \times L(A3)$ (not different from *B. lapponicus*).

Metabasitarsus: in the middle, the external side of the posterior is characterized by an area with short black bristles (contrary to *B. lapponicus*, which has long and numerous bristles).

Type locality: Toolik field station, AK, USA (68°38′N, 149°36′W).

Distribution: *Bombus interacti* was found at higher latitudes in the arctic tundra habitat near Toolik field station in Alaska, USA (68°37′–68°46′N, 149°35′–149°56′W). The available data are not sufficient to draw up a distribution map.

Etymology: The specific name was chosen in reference to the International Network for Terrestrial Research and Monitoring in the Arctic (INTERACT) project, which funded most of our sampling costs, allowing us to discover this taxon.

Remarks: Considering the morphology, genetic and the semio-chemical secretions, there is no available name to describe our new taxon. However, there are some uncertainties about the taxon *B. gelidus* (Fig. 7) described by Cresson (1878) and re-described by Franklin (1912), which was considered as a subspecies of *B. lapponicus sylvicola* by Pittioni (1943). The morphological description by Cresson (1878) is poor and not sufficient to compare with our specimens. That description was based on a single queen from the Aleutian Islands (Henry Edwards). This specimen is described as black, with a long and loose pubescence; with a slight admixture on face and vertex. The sides of the thorax, scutellum and first and fourth segments of the abdomen are described as pale yellow and the second and third segments mostly fulvo-ferruginous, mixed with black on the middle and sides. The clypeus is sparsely punctured, labrum with fulvous hair, and wings are dark and stained. In the re-description by Franklin (1912), *B. gelidus* is described as closely allied to *B. lapponicus sylvicola*. However, the face of the queen is mostly dark, and the mesopleura is largely covered with yellow pile, but the yellow does not reach the bases of the legs in *B. gelidus*. In males, coxae, trochanters and femora are characterized by a large amount of pale yellow pile.

According to Franklin (1912), no difference in structure between *B. gelidus* and *B. lapponicus sylvicola* could be found except for slight differences in coloration, and these taxa should be considered as conspecific. The queen holotype from the Academy of Natural Sciences (Philadelphia) and three co-type workers (from the Smithsonian National Museum of Natural History) have been examined for the present study. However, for these old specimens only morphological traits are available to compare with our specimens. The morphological characters distinguishing *B. gelidus*, *B. sylvicola* and *B. interacti* females are mainly based on coat colour

Figure 7. Photographs of the holotype of *B. lapponicus sylvicola* f. *gelidus* (female). Head of *B. lapponicus sylvicola* f. *gelidus* female (left). Habitus of *B. lapponicus sylvicola* f. *gelidus* female (right) (Photographs by P. Rasmont.)

variation. The type series of *B. gelidus* is different from *B. interacti* based on wing coloration (*B. gelidus* has darker wing colour than *B. interacti*), labrum punctuation (large punctuations in the middle and on the sides), the shape of the basitarsus (*sensu* Gjershaug *et al.*, 2013) (Table 2), the density of hairs on the collar for females and the pubescence of the tibia for males. The workers of the type series that we examined are not different from *B. lapponicus sylvicola*. There is no indication that *B. gelidus* would be anything other than a dark form of *B. lapponicus sylvicola*. However, given that the type series is older (i.e. 1878), we cannot make this rational decision concerning their taxonomic assignment. In the light of our wing morphometric analysis and the lack of strong taxonomic evidence (genetic, semio-chemical) and the fact that *B. gelidus* has been described only from the Aleutian Islands, we hypothesize that *B. gelidus* is different from *B. interacti*, and we describe this latter taxon as a new species. Our wing morphometric geometric analysis shows that the holotype of *B. gelidus* is assigned to *B. lapponicus*. Even if the definitive status of *B. gelidus* remains unsettled, as far as we can understand now, after revision of the holotype, the taxon described here as *B. interacti* is unlikely to be conspecific with *B. gelidus*. Nine males and three queens, based on chemical, genetic and morphological analyses, and seven other males based only on morphological characters, are considered to belong to *B. interacti*. No variation in colour pattern has been observed in the taxon except for the density of the yellow inter-alar band for males in our sampling. However, our specimens have been collected from only one site (Toolik and surroundings), and the colour variation could be underestimated. Its recorded host plants are *Epilobium angustifolium* L., *Senecio lugens* Richardson and *Solidago multiradiata* Aiton. *Bombus interacti* is similar to *B. sylvicola* and was discovered using: (1) analysis of a mitochondrial gene (*COI*) and a nuclear gene (*PEPCK*); (2) analysis of the cephalic labial gland secretions; and (3) complete morphological examination.

Given the slight genetic divergence obtained by the *COI* analysis, the colour pattern and the geographical distribution, we propose to assign a subspecific status to the north population of *B. sylvicola*: *Bombus (Pyrobombus) lapponicus* subsp. *lapponicus* in Fennoscandia and *Bombus (Pyrobombus) lapponicus* subsp. *sylvicola* (Kirby, 1837) comb. nov. in Alaska and Yukon.

BOMBUS LAPPONICUS SUBSP. *SYLVICOLA* F. *GELIDUS* (CRESSON, 1878) COMB. NOV.

Holotype: One queen pinned (Fig. 7). Labels: (1) red, printed with 'HoloTYPE 2638'; (2) white, written with 'aleutian Islds Dau'; (3) red, printed with 'HOLOTYPE'; and (4) white, printed with 'Rasmont & Martinet 2018, *Bombus (Pyrobombus) lapponicus sylvicola* f. *gelidus* Cresson, 1878'. The type specimen is conserved in the Academy of Natural Sciences in Philadelphia (PA, USA).

Bombus gelidus appears as a very dark form of *B. lapponicus sylvicola* and should be considered as a form of that subspecies.

Further material: In the Smithsonian National Museum of Natural History, Massachusetts Agricultural College and United States National Museum, there are one queen, 14 workers and one male labelled 'Cotype' by Franklin (1912). All these specimens should not be part of the typical series and have been labelled erroneously. These specimens were collected later and in other areas than the only holotype described by Cresson (1878). After examination, we consider that these specimens have the typical colour form of *B. lapponicus* subsp. *sylvicola*.

DISCUSSION

TAXONOMIC STATUS OF *B. INTERACTI*, *B. LAPPONICUS* SUBSP. *LAPPONICUS*, *B. LAPPONICUS* SUBSP. *SYLVICOLA* AND *B. LAPPONICUS* SUBSP. *SYLVICOLA* F. *GELIDUS*

The phylogenetic trees built by Hines *et al.* (2006) and Cameron *et al.* (2007) showed low bootstrap values between *B. lapponicus* subsp. *lapponicus* and *B. lapponicus* subsp. *sylvicola*, and the two taxa displayed some genetic divergences in *ArgK*, 16S and *Ef-1α*. However, the only specimen of *B. lapponicus* subsp. *sylvicola* used by Cameron *et al.* (2007) and Hines *et al.* (2006) had been collected in New Mexico (USA), where the taxon displays a particular colour form, with tergites 2 and 3 predominantly black (T2–T3 red in Alaska), although these two forms of *B. lapponicus* subsp. *sylvicola* are considered conspecific (Williams *et al.*, 2015). Without a complete taxonomic revision, we cannot exclude the possibility that northern and southern populations are two different lineages, considering the taxonomic ambiguities present in this group. Koch *et al.* (2017) showed that in its distribution, *B. lapponicus* subsp. *sylvicola* displays different allelic diversity and emphasizes different genetic clusters (population genetic structure differentiation). As suggested by Cameron *et al.* (2007), the *PEPCK* gene fragment showed no differentiation between *B. lapponicus* subsp. *lapponicus* and the northern population of *B. lapponicus* subsp. *sylvicola*, whereas the *COI* fragment showed a low divergence (Fig. 2). This could reflect geographical intraspecific variability (Andriollo *et al.*, 2015; Mutanen *et al.*, 2016) between two isolated and geographically distant

Table 3. Taxonomic decision table, with all criteria used for species delimitation

Former taxonomic status	Morphology (diagnostic character)	Wing shape/size	CLGS	*COI* gene/ bGMYC	*PEPCK* gene	Proposed taxonomic status
B. lapponicus, Sweden, W. Siberia	– (A)	– (A) /–	– (A)	+/–	– (A)	*B. lapponicus lapponicus*
B. sylvicola, Alaska, Yukon	– (A)	– (A) /–	– (A)	+/–	– (A)	*B. lapponicus sylvicola*
B. gelidus, Aleutian Islands	– (A)	– (A)/–	NA	NA	NA	*B. lapponicus sylvicola* f. *gelidus*
Unnamed species, Alaska	+	+/–	+	+/+	+	*B. interacti*
B. bimaculatus	+	NA	+	+/+	+	*B. bimaculatus*
B. monticola	+	NA	+	+/+	+	*B. monticola*
B. konradini	+	NA	+	+/+	+	*B. konradini*
B. terrestris	+	NA	+	+/+	+	*B. terrestris*
B. ephippiatus	+	NA	+	+/+	+	*B. ephippiatus*
B. melanopygus	+	NA	NA	+/+	+	*B. melanopygus*
B. glacialis	+	NA	NA	+/+	NA	*B. glacialis*

Morphology indicates whether a taxon has a diagnostic morphological character (+/– means that morphology is/is not diagnostic). Wing shape and size indicate whether a taxon has a diagnostic wing shape and size (+/– means that wing measures are/are not diagnostic). Cephalic labial gland secretions indicate whether the taxon has/does not have diagnostic composition of CLGSs with different main compounds (+/– means that the taxon has/does not have a specific CLGS composition). When the taxon shares CLGS composition with other ones, the letters group together taxa that share similar CLGS. Phylogenetic analyses indicate whether a taxon forms a strongly supported monophyletic group (+/– means that the taxon is/is not a monophyletic group). When the taxon is not a distinct monophyletic group, the letters group together taxa included in the same monophyletic group (A).
Abbreviations: bGMYC, the general mixed Yule-coalescent model; CLGS, cephalic labial gland secretions; *COI*, cytochrome *c* oxydase I; LS, low supported differentiation; NA, not assessed; *PEPCK*, phosphoenolpyruvate carboxykinase.

populations. The genetic results are in line with CLGS analyses, which support a lack of divergence between *B. lapponicus* subsp. *lapponicus* and *B. lapponicus* subsp. *sylvicola* (intraspecific variability) and suggest that these taxa are conspecific according to the species recognition concept (Paterson, 1993) (Fig. 3B) and our taxonomic integrative approach (Table 3). There could be no chemical reproductive barrier (Ayasse & Jarau, 2014) between *B. lapponicus* from Scandinavia and western Siberia and *B. sylvicola* from Alaska and Yukon. However, the reinforcement of a reproductive barrier process could not be exerted on the CLGS between allopatric species. Overall, given that there is no divergence in the nuclear gene, in morphology (structure of the genitalia) and in CLGS, we can expect that there is no reproductive barrier between the populations. Poor quantitative differences observed could reflect a 'dialect divergence' owing to the geographical gap between these two sampled populations (Lecocq *et al.*, 2013b) or chemical background noise.

Moreover, our integrative framework (Table 3) highlights an unknown species from Alaska in the *B. lapponicus* group supported by all our independent criteria: *B. interacti* (Table 3). Morphological, genetic and semio-chemical datasets support the presence of two biologically distinct taxa within *B. sylvicola* sampling from Alaska. The CLGS results also provide strong support for the new species, *B. interacti*, with different major and indicator compounds from *B. monticola*, *B. konradini*, *B. lapponicus lapponicus* and *B. lapponicus sylvicola*. Although we have a restricted sampling and different tree topologies between the two genetic markers, nuclear gene analysis suggests that *B. interacti* is closer to *B. monticola* (also consistent with the morphology; see Table 2), a strictly European taxon. This could call into question the distribution of their potential common ancestor around the Arctic Circle. One hypothesis could be that the speciation of *B. interacti* occurred from successive waves of range expansion and contraction following glaciations and the dynamics of the Bering Strait (Abbott & Brochmann, 2003; Elias & Brigham-Grette, 2013; Pringle, 2014). In the case of our new species, *B. interacti*, we emphasize that we have especially strong and straightforward evidence of the differentiation of this taxon, given that all data support this divergence. Further interpopulation genetic analyses are needed to explore this hypothesis.

For the taxon *B. gelidus*, considering morphological criteria and wing geometric morphometric analyses (Fig. 6), we propose that this taxon should be considered as a dark-coloured form of *B. lapponicus* subsp. *sylvicola*, as forma *gelidus*.

BOMBUS LAPPONICUS: A CIRCUMPOLAR TAXON

Based on morphological characters and the coat coloration, Skorikov (1922) and Pittioni (1942) had already hypothesized that there is a set of conspecific

taxa related to the *B. lapponicus* complex all around the Arctic Circle (i.e. *B. glacialis* from Novaya Zemlya and Wrangel Island, *B. lapponicus karaginus* Skorikov, 1912 from Chukotka and *B. lapponicus zaitzevi* Skorikov, 1913 in the northern Urals). However, most taxa of this *B. lapponicus* complex have never been investigated using genetic or chemical data. Formerly, only the Scandinavian population of the *B. lapponicus* group was sampled by Svensson & Bergström (1977) to study the cephalic labial gland secretions. Our results based on the sampling of five distant populations (northern Sweden, western Siberia, northern Alaska, Yukon and northern Quebec for wing shape analysis), therefore, seem to confirm the hypothesis of Skorikov (1922) and Pittioni (1942), presenting *B. lapponicus s.s.* as a northern Holarctic species, with different isolated allopatric subspecies in the polar portion of its distribution. Moreover, Potapov *et al.* (2017) have shown the conspecificity of specimens of *B. lapponicus* from Norway, Kamchatka, Yamal and Chukotka based on *COI* analysis. These results confirm our hypothesis: *B. lapponicus* is found across northern Holarctic regions, including a circumpolar distribution, and exhibits subspecific differentiation across at least the polar section of its distribution. The absence of differentiation in CLGS and genetic analyses across the Holarctic region suggest that there is no isolation mechanism between any *B. lapponicus* populations. These taxa do not seem to be involved in an *Artenkreis* speciation process *sensu* Rensch (1933).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Circumarctic sampling map (azimuthal equidistant projection, after Uwe Dedering, licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license), on which the red dots indicate the areas where we collected specimens of *Bombus lapponicus lapponicus*, *Bombus lapponicus sylvicola* and *Bombus interacti,* and the red square indicates the *locus typicus* of *Bombus lapponicus sylvicola* f. *gelidus*.
Figure S2. Right forewing of *Bombus lapponicus sylvicola*, with the 18 landmarks indicated to describe the shape.
Table S1. List of all specimens analysed. Sample code refers to the sample labels used in different analyses. *COI* and *PEPCK* are the GenBank accession numbers for each sample.
Table S2. Summary of data matrix of cephalic labial gland secretions (with minimum, median and maximum of relative concentration of each compound), list of the identified compounds and indicator-value (IndVal) analysis with species-specific compounds. Unknown x are undetermined compounds.