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

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

Running title: Systematics of an arctic bumblebee species complex

Abstract

While bumblebees have been the focus of much research, the taxonomy of many species groups is still unclear, especially within circum-polar 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 novel genetic and eco-chemical datasets to resolve the taxonomic status of *Bombus lapponicus* and *B. 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 the sub-specific status: *B. lapponicus lapponicus* **comb. nov.** from Fennoscandia and W-Siberia and *B. lapponicus sylvicola* **comb. nov.** from Alaska and Yukon. Moreover, our analyses reveal a new cryptic arctic species in the *B. lapponicus* complex: *B. (Pyrobombus) interacti* **sp. nov.** Martinet, Brasero & Rasmont from Alaska.

Keywords: *Bombus* - Circum-polar species - Cryptic species - Integrative taxonomic approach

Introduction

Most biodiversity hotspots are found in 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 under 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 (i) the cold climate and local adaptations to environmental harshness linked to arctic ecology and by (ii) spatial and temporal geographic 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 has been the subject of long-standing debates (Reinig 1937; Irwin *et al.* 2001a, 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 so called ring species (Stresemann & Timofeeff-Ressovsky 1947; Irwin *et al.* 2001a) 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.* 2001b). This could be explained for instance by small interpopulational variations of the species mate recognition system which prevent the specific recognition between individuals of distant populations or by ecological differentiation (Rensch 1933; Stresemann & Timofeeff-Ressovsky 1947). Several cases of ring speciation process were emphasized in birds, such as for *Phylloscopus trochiloides* in Siberia (Alström 2006) or *Melospiza melodia* in Sierra Nevada (Patten & Pruett 2009).

Bumblebees (Hymenoptera: Apidae) are cold-adapted species, an adaptation that enable them to live in some of the highest latitude and altitude 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 to explore speciation processes in circum-polar areas with disjunct distributions (Williams *et al.* 2015). There is some evidence that a circum-polar speciation process could have shaped the *Bombus lapponicus* - *sylvicola* complex. In the East-Palaearctic, Skorikov (1922) described a multitude of forms across the circum-boreal region. These taxa are connected by a long set of potential interbreeding populations around the Arctic Circle (Skorikov 1922). When comparing these

forms to American taxa, Pittioni (1942) pointed out a possible ring speciation process by highlighting the variability of *B. lapponicus* with increased melanization process in the East (Skorikov 1937). These different forms could be due to the fragmentation of the arctic habitat. More recently, several authors have questioned the taxonomic relationship which connects *sylvicola* and *lapponicus* (Thorp *et al.* 1983; Savard 2009; Williams *et al.* 2014). Among these circum-polar populations, only *B. lapponicus*, *B. sylvicola*, *B. glacialis* (Novaya Zemlya, Wrangel Island), and *B. karaginus* (Kamchatka) are currently recognized as valid species (Proshchalykin & Kupianskaya 2005; Williams *et al.* 2014; Potapov *et al.* 2017). Though, 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 oxidase I (COI) gene fragments support 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) due to 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 circum arctic bumblebee taxa complex: *Bombus* (*Pyrobombus*) *lapponicus* (Fabricius, 1793) (North Scandinavia, W-Siberia) - *B. (Pyrobombus) sylvicola* Kirby, 1837 (North America). We address the taxonomic uncertainties that exist between these distant populations (Williams 1998; Williams *et al.* 2014; Cameron 2007) and we present new morphometric, 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 geographic distribution extends from the north of the taiga to the tundra (except in Taymyr peninsula, N-Siberia) between the 65th and 70th parallels in Europe and 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 very similar to *B. lapponicus* (Williams *et al.* 2014). In

North America, two forms of *sylvicola* have been described: one with the metasomal T2-3 red from the Rocky Mountains and the second with T2-3 mainly black from the Sierra Mountains. DNA barcoding supports the two principal color forms of *sylvicola* in North America as conspecific including the doubtful taxon named *gelidus* from Alaska (Cresson 1878) 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 of *B. lapponicus* from North Scandinavia ($n=12$) and Siberia ($n=10$), *B. sylvicola* from Northern Alaska ($n=29$) and Yukon ($n=4$) (Fig. S1). For comparison, we used the phylogenetically closely related species *B. (Pyrobombus) monticola scandinavicus* ($n=9$) from North Scandinavia (Cameron *et al.* 2007), *Bombus (Pyrobombus) bimaculatus* ($n=10$) from Canada, *Bombus (Pyrobombus) ephippiatus* ($n=6$) from Biobest NA (Chiapas), *Bombus (Pyrobombus) konradini* ($n=5$) from Italy, *Bombus (Pyrobombus) glacialis* ($n=1$) from Novaya Zemlya, *Bombus (Pyrobombus) melanopygus* ($n=2$) from California and as an outgroup *Bombus (Bombus) terrestris* ($n=14$) 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 such as Løken (1973); Williams *et al.* (2014). A total of 147 bumblebees, collected between 2013 and 2018 in Europe, Siberia and Northern America, were analyzed (Table S1). For a first identification, we performed a comparative table (male and female) gathering diagnostic characters and color patterns for the studied specimens to compare morphological characters within the *lapponicus-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 oxidase 1 gene (COI), and the nuclear phosphoenolpyruvate carboxykinase gene (PEPCK). DNA extraction protocol, polymerase chain reaction, amplification reactions, sequencing procedures and alignment of DNA sequences were performed according to the method described in Lecocq *et al.* (2015a, c). COI and PEPCK sequences were deposited on GenBank (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. COI fragment and each nuclear exon were partitioned by base positions (first, second, and third nucleotide). For each dataset, we used JModelTest Server 2.0 (Posada 2008) with the

corrected Akaike information criterion (AICc) to find the best-fitting substitution models. The models chosen were: (i) for COI : GTR+I (first position), TIM2+I (second position), TrN+G (third position) ; (ii) for PEPCK first intron: TPM1 uf +I ; (iii) for PEPCK exon 1 : HKY+I (first position), JC (second position), TrN+I (third position) ; (iv) for PEPCK second intron : TrN+I ; (v) for PEPCK exon 2: JC (first, second, and third positions). Selected models which are not implemented in MrBayes were substituted by the closest over-parameterized model. For ML analyses, we performed ten independent runs in GARLI 2.0 for both genes (Zwickl 2006); the topology and $-\ln L$ were the same among replicates. Only the run with the highest likelihood was saved. We assessed statistical significance of nodes with 10,000 nonparametric bootstrap replicates. We considered a topology well supported (high confidence) when the bootstrap value (branch supports) was higher than 85% (Hillis & Bull 1993). We carried out MB analyses with MRBAYES 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 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, F.R.S.-FNRS).

To recognize a species threshold, we used a Bayesian implementation of the general mixed Yule-coalescent model (bGMYC) based on 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 while a range of probabilities < 0.05 suggested that taxa were heterospecific (Reid & Carstens 2012). We performed a phylogenetic analysis with BEAST 1.7.4 (Drummond *et al.* 2012) to generate ultrametric trees using a phylogenetic clock model to generate a posterior distribution of trees (length of the MCMC 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 0. We based the bGMYC analysis on 1000 trees sampled every 10,000 generations. For each of these 1000 trees, the MCMC 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; Ayasse & Jarau 2014; Baer 2003): the cephalic labial gland secretions (CLGSs). CLGSs are commonly used for species discrimination in bumblebees (Rasmont *et al.* 2005; Terzo *et al.* 2005; Bertsch & Schweer 2012). 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). 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 prior to 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.* (2017) (Table S2). For *B. terrestris*, we used the CLGS dataset described in Lecocq *et al.* (2016).

The qualitative composition of the CLGS was determined by gas chromatography-mass spectrometry using a Finigan GCQ quadrupole system (GC/MS) 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 (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 CLGS was analyzed according to the protocol described in Lecocq *et al.* (2015c). All compounds for which the relative abundance was recorded as less than 0.1% for all specimens were excluded from the analysis (De Meulemeester *et al.* 2011). The data matrix for each taxon was based (Table S2) on the alignment of each relative proportion of compound between all samples performed with GCAAligner 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 1.0 according to the method described by Dellicour & Lecocq (2013a, b).

Statistical analyses

We performed statistical comparative analyses of the CLGSs using R 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 component 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.* 2011) 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 (Clausen *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 Quieroz 2007). With our approach, criteria are not balanced, and the assignment of species status is allocated by unanimity of all criteria to avoid species over-estimation (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). The specific status was assigned if this taxon: (i) was genetically differentiated in all genetic markers (i.e. potential unique haplotypes), (ii) constituted a monophyletic group with high branch support, and (iii) was significantly differentiated in CLGS compositions (including IndVal indicator compounds, MRPP test, 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 infra-specific-level differentiation, we assigned the subspecies status to phenotypically distinct allopatric populations with differentiations in some traits to highlight these populations displaying of 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

As fresh material of *B. gelidus* was not available for molecular and chemical analyses, we run an additional study to test the similarity between *gelidus* type material and other *lapponicus* group taxa. The right forewings of 44 queens were photographed using an Olympus SZH10 microscope an AF-S NIKKOR 18-105 millimetres (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 1 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 1 queen, 14 workers and 1 male labelled “Cotype” by Franklin (1912). All these specimens should not be part of the typical series and have been erroneously labeled. 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 (Fig. S2) on wing veins with tps-DIG v2.17 (Rohlf 2013a, b). The landmark configurations were scaled, translated, and rotated against the consensus configuration using the GLS 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 (MG), 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 v1.25 (Rohlf 2013c).

After checking of application assumptions, PerMANOVA (Permutational analysis of variance) analyses were performed to assess wing size and wing shape differences between

groups. A PCA was performed to assess shape variation among the different groups, using the geomorph function “plotTangentSpace” and to visualize potential differentiation between taxa.

Prior to the assignment of the holotype queen *B. gelidus*, shape variation within 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 version 3.0.2. The effectiveness of the LDA for discriminating taxon 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 to belong to one group compared to 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 while it is of primary importance because GLS 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 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 *lapponicus-sylvicola* group. For the two gene fragments, the *lapponicus-sylvicola* group resulted in two lineages: (a) one comprising all *lapponicus* and a part of *sylvicola* specimens (group A, 1.92% of divergence between COI sequences) and (b) a second lineage comprising the second part of *sylvicola* (group B) (Fig. 2). While genetic analyses based on the mitochondrial gene COI showed a slightly supported divergence between *B. lapponicus* and *B. sylvicola* group A (Fig. 2B), there was no differentiation between these two taxa for the nuclear marker PEPCK (Fig. 2A). In COI sequences, 18/938 (1.92%) of

phylogenetically informative nucleotide sites are uniquely diagnostic to separate *sylvicola* group A and *lapponicus*. These divergence estimations between *sylvicola* part A and *lapponicus* were performed excluding specimens of *sylvicola* group B forming a separate clade (see below). Other species-specific branches were supported by high bootstrap values (bootstrap > 90%).

Our genetic analyses revealed a new cryptic taxon from Alaska in our *sylvicola* samples (part B): *B. interacti* sp. nov. **Martinet, Brasero & Rasmont** closely similar and co-occurring with *sylvicola* in Alaska. This new taxon was strongly supported as a monophyletic group with both COI (8.74 % of sequence divergence from *sylvicola* part A and 9.80% from *monticola*) and PEPCK (> 1% of divergence from *monticola* and *sylvicola*) analyses. Contrary to the COI marker, the phylogenetic affinities inside the group including *interacti* sp. nov., *monticola*, and *konradini* were not resolved with the nuclear PEPCK fragment (Fig. 2A). PEPCK and COI sequences of *B. interacti* sp. nov. have been blasted to NCBI Genbank database. Sequences matched most closely to the studied species complex *lapponicus-sylvicola-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* sp. nov. differs significantly from *B. melanopygus* and *B. glacialis* (high branch supports and posterior probabilities, Fig. 2A, 2B).

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, MB analyses). Overall, the bGMYC suggested the delimitation of seven prospective species ($p < 0.05$): (i) *B. terrestris* (bGMYC conspecificity probabilities between individuals included in the group, $p > 0.12-1$), (ii) *B. ehippiatus* ($p > 0.97-1$), (iii) *B. interacti* sp. nov. ($p > 0.98-1$), (iv) *B. monticola* ($p > 0.47-1$), (v) *B. bimaculatus* ($p > 0.89-1$), (vi) *B. konradini* ($p > 0.99-1$), (vii) a group with *sylvicola* and *lapponicus* ($p > 0.07-1$), (viii) a group with *B. glacialis* and (ix) a group with *B. melanopygus*. The pairwise matrix (Fig. 3) showed a non-significant heterospecificity threshold between *lapponicus* and *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. ehippiatus*, 64 compounds for *B. interacti* sp. nov. (= *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 (Appendix S2). Chemical analyses supported the presence of another taxon in the *sylvicola* samples from Alaska (*B. interacti* sp. nov.).

The main compounds detected were (a) the geranyl citronellol (55.28% - 77.30%) shared by *B. sylvicola* and *B. lapponicus*; (b) the hexadec-9-enyl acetate (45.91% - 61.74%) for *B. monticola* and *B. konradini* (48.36%-54.71%); (c) Ethyl octadec-9-enoate for *B. konradini* (7.43%-9.14%); (d) the hexadec-9-enyl acetate (20.55% - 40.63%) and the geranyl geranyl acetate (25.98% - 39.42%) for *B. bimaculatus*, (e) the dihydrofarnesol (19.08% - 40.45%) for *B. terrestris*, (f) the hexadecanoic acid (19.03%-31.63%) for *B. ehippiatus*, and (g) the citronellyl hexadec-9-enoate (12.37% - 23.57%) for *B. interacti* sp. nov. (Tables 1 and 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): (a) *B. monticola* (pairwise test, $p < 0.01$), (b) *B. konradini* (pairwise test, $p < 0.01$) (c) *B. bimaculatus* (pairwise test, $p < 0.01$), (d) *B. ehippiatus* (pairwise test, $p < 0.01$), (e) *B. terrestris* (pairwise test, $p < 0.01$), (f) *B. sylvicola* group A + *B. lapponicus* (pairwise test, $p < 0.01$), and (g) *B. interacti* sp. nov. (pairwise test, $p < 0.01$). No statistical differentiation was found in the statistical hypothesis test and in hierarchical clustering between *lapponicus* and *sylvicola* group A (Fig. 4; MRPP, A= 0.004641, T= 0.1577, $p = 0.30$). Several significant and specific indicator compounds were revealed by the IndVal method (IndVal > 0.70), but no compound has been identified to discriminate *B. lapponicus* to *B. sylvicola* (Table S2).

Wing size and shape analyses

No significant difference in centroid size was found among the different taxa ($F = 2.73$; $p\text{-value} = 0.08$). However, significant differences in wing shape were found among the different taxa (perMANOVA, $F = 1.83$; $p\text{-value} = 0.006$). Pairwise perMANOVA tests have shown a significant difference between *B. lapponicus* and *B. interacti* sp. nov. ($F = 2.44$; $p\text{-value} = 0.004$) and between *B. interacti* sp. nov. and *B. gelidus* ($F = 1.96$; $p\text{-value} = 0.035$) while no difference was detected between *B. lapponicus* and *B. gelidus* ($F = 1.21$; $p\text{-value} = 0.32$). PCA plot highlights two distinct groups (Fig. 5): (i) one cluster gathering specimens of *B. lapponicus*, *B. sylvicola* and *B. gelidus*, (ii) a second cluster with specimens of *B. interacti* sp. nov. The two groups *B. lapponicus* and *B. sylvicola* were not discriminated in the PCA and LDA while *B. interacti* group is strongly differentiated. In the morphometric space defined by the PCA, the specimen of *gelidus* is undoubtedly clustered with the group of the *B. lapponicus* (Fig. 5). A-posteriori assignment of the holotype *B. gelidus* in the discriminant shape space (LDA) allows

a reliable specific attribution. This analysis reveals that this specimen is assigned to *B. lapponicus* species (Mahalanobis Distance to *B. sylvicola* group = 1.44; pp = 1).

Morphological diagnosis

For this morphological comparison, we assess only males and queens (minimum 15 individuals per taxon according to the availability of specimens). Except for the coloration of the face which is black for *lapponicus* and yellow for *sylvicola*, no diagnostic character was found to discriminate *lapponicus* from *sylvicola* based on our morphological examinations (Table 2). Concerning *sylvicola*, we found two discrete morphotypes among our sampling from Alaska (corresponding to *sylvicola* part A and *sylvicola* part B (= *Bombus interacti* sp. nov.) in our molecular analyses) that could be separated by several diagnostic characters. *Bombus interacti* sp. nov. males differed from *sylvicola* in the pubescence of the tibia which is hairier for *sylvicola* (Fig. 6). No difference in the genitalia's structures was detected. For female, *B. interacti* sp. nov. differed from *sylvicola* in the face clypeus coloration: black with intermixed dark yellow hair for *interacti* sp. nov. and yellow for *sylvicola*. Besides, the density of pubescence of the tergite 5 is higher for *B. interacti* sp. nov. and the yellow coloration of collar not reaching 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* sp. nov. from *B. monticola* and this character is similar between *lapponicus* and *sylvicola*. From *B. glacialis*, females of *B. interacti* sp. nov. differ for several characters: (i) labral furrow (narrow for *B. interacti* sp. nov., broad for *B. glacialis*); (ii) punctuations into the labral furrow (very few in *B. glacialis*); (iii) dorsal furrow of gena which is weakly developed in *B. glacialis*. Males of *B. interacti* sp. nov. differ from *B. glacialis* for: (i) the color of the vertex and the clypeus (yellow for *B. glacialis*); (ii) hind basitarsus (gradually narrowing towards basal part for *B. glacialis*); (iii) punctuations into the labral furrow (very dense for *B. interacti* and labrum covered with reddish bristles at the front part).

Taxonomic status: Description of the new species B. interacti Martinet, Brasero & Rasmont sp. nov. and synonymy of *lapponicus* and *sylvicola*

Family APIDAE Latreille, 1802

GENUS *BOMBUS* Latreille, 1802

Bombus (*Pyrobombus* Dalla Torre 1880) *interacti* sp. nov. Martinet, Brasero & Rasmont,

2018

Synonyms: None

Type species: Bombus interacti sp. nov. Martinet, Brasero & Rasmont, 2018. By subsequent diagnosis.

Description:

Females. Length 15-18 mm.

Coat color. Face and vertex densely pubescent with black hairs intermixed with few yellowish-greyish hairs. Thorax with a collare as large as 1/3 of the thorax length, with few intermixed black hairs at front of tegulae; scutellare as large as 1/4 of the thorax length. The hairs of the scutellare are 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 grayish hairs, intermixed with black hairs in the middle. T2 covered with red hairs, intermixed with few black ones on the sides and with numerous grayish ones at the middle of anterior margin. T3 all red with few grayish hairs at the middle of posterior margin. T4 mostly covered with grayish 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 grayish ones on the sides. Coxae and femurs with black hairs intermixed with very few greyish ones. Mesotibias with black hairs some of them with red tip. Metatibias with corbiculae surrounded by decumbent bristles slightly longer than the width of the organ, mostly reddish with light blond tip and some whole black at the base of anterior margin, meso- and metabasitarsus 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 designed. There is an imbricated microsculpture in front of the tubercles and into the labral furrow. Punctuations are very 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. Clypeus slightly bombed, very 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 more spaced in the middle. There is a thin band of microsculptures along the transversal furrow at the distal part of clypeus. Ocellar field covered with large spaced punctuations along the inner margin of the compound eyes, covering 1/2 of the distance between ocelli and compound eyes.

Between the distal margin of compound eyes, there is a poorly designed supra-orbital line only defined near the eyes.

Antennae. $L(A5) = 0,67 * L(A3)$; $L(A4) = 0,51 * L(A3)$ (not different from *B. lapponicus*).

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 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 color. 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 1/3 of the thorax length, with yellow hairs; scutellare with yellow hairs as large as 1/4 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 interalar band is attenuated with a mixture of black and yellow hairs. T1 mostly covered with yellow hairs. T2 covered with red hairs, intermixed with some yellow ones on the at the middle of anterior margin. T3 and T4 all red with few grayish hairs at the middle of posterior margin. T5 mostly covered with yellow hairs, with very few red hairs at the anterior margin and black ones in the middle. T6 with greyish hairs with numerous black ones in the middle. T7 mostly with black hairs and some grayish ones on the sides. Coxae and femurs with mostly yellow hairs intermixed with very 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 blond tip and some whole black at the base of anterior margin, meso- and metabasitarsus 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 designed. Punctuations are very 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. Clypeus slightly bombed, very covered with short black bristles in the middle and longer reddish ones at the distal part. The first 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 clypeus.

Ocellar field covered with large spaced punctuations along the inner margin of the compound eyes, covering 1/2 of the distance between ocelli and compound eyes. Between the distal margin of compound eyes, there is a poorly designed supra-orbital line only defined near the eyes.

Antennae. $L(A5) = 0,63 * L(A3)$; $L(A4) = 0,52 * L(A3)$ (not different from *B. lapponicus*).

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

Diagnosis

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

Holotype: One pinned male (Fig. 1C). Labels: (1) white, printed with 'USA, Alaska, Toolik field station, 725m, 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'; (3) white, printed with "det. Martinet & Rasmont 2016, *Bombus interacti* Martinet, Brasero & Rasmont". The left anterior leg is missing as it was removed for genetic analysis. The type has been deposited in the *Royal Belgian Institute of Natural Sciences* in Brussels. Genbank accession numbers: MG280603 (COI), MG280606 (PEPCK).

Paratypes: 9 males and 4 queens pinned (Fig. 1D) and labelled "Paratype".

Remarks: Considering 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 Franklin (1912), considered as a subspecies of *B. lapponicus sylvicola* by Pittioni (1943). The morphological description of Cresson (1878) is very poor and not sufficient to compare with our specimens. This description is based on one queen from Aleutian Islands (Henry Edwards). This specimen is described as black, with a long and loose pubescence; with a slight admixture on face and vertex. Sides of thorax, scutellum, and first and fourth segments of abdomen are described as pale yellow and the second and third segments mostly fulvo-ferruginous, mixed with black on middle and sides. The clypeus is sparsely punctured, labrum with fulvous hair and wings are dark and stained. In the re-description of Franklin (1912), *B. gelidus* is described as very closely allied to *B. lapponicus sylvicola*. However, the face of queen is mostly dark and the mesopleura is largely

covered with yellow pile but the yellow not reaching the bases of the legs in *B. gelidus*. For males, coxae, trochanters and femora are characterized by a large amount of pale yellow pile.

According Franklin (1912), no difference in structure between *B. gelidus* and *B. lapponicus sylvicola* could be found except 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-types workers (from the Smithsonian National Museum of Natural History) have been examined for this study. However, for these old specimens only morphological traits are available to compare with our specimens. The morphological characters distinguishing *gelidus/sylvicola/interacti* females are mainly based on coat color variation. The type series is different from *B. interacti* sp. nov. based on wings coloration (*gelidus* has darker wing color than *interacti*), labrum punctuation (large punctuations in the middle and on the sides), the shape of 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, as the type series is older (i.e. 1878), we cannot take a 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 that *B. gelidus* has been described only from the Aleutian Islands, we hypothesize that *B. gelidus* is different from *B. interacti* sp. nov. and we describe this latter taxon as new species. Our wing morphometric geometric analysis show that the holotype of *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 the holotype, the taxon described here as *B. interacti* sp. nov. is unlikely to be conspecific with *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 *interacti* sp. nov. No variation in color pattern has been observed within the taxon except the density of the yellow interalar band for males in our sampling. However, our specimens have been collected from only one site (Toolik and surroundings) and the color variation could be underestimated. Its recorded host plants are *Epilobium angustifolium*, *Senecio lugens* and *Solidago multiradiata*. *Bombus interacti* sp. nov. is similar to *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, (3) complete morphological examination.

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

Distribution: *B. interacti* sp. nov. was found higher latitudes in the arctic tundra habitat near Toolik field stations 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 INTERACT project which funded most of our sampling costs allowing us to discover this taxon.

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

Bombus lapponicus 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”; (4) white, printed with “Rasmont & Martinet 2018, *Bombus (Pyrobombus) lapponicus sylvicola* f. *gelidus* Cresson, 1878”. The type is conserved in the *Academy of Natural Sciences* in Philadelphia (USA).

B. gelidus appears as a very dark form of *B. lapponicus sylvicola* and should be considered as *B. lapponicus sylvicola* f. *gelidus* **comb. nov.**

Further material. In the *Smithsonian National Museum of Natural History, Massachusetts Agricultural College* and *United States National Museum*, there are 1 queen, 14 workers and 1 male labelled “Cotype” by Franklin (1912). All these specimens should not be part of the typical series and have been erroneously labelled. These specimens were collected later and in other areas than the only holotype described by Cresson (1878). After examination, we consider that these specimens as typical color form of *B. lapponicus sylvicola*.

Discussion

Taxonomic status of B. lapponicus sylvicola f. *gelidus* comb. nov. , *B. interacti* sp. nov., *B. lapponicus* and *B. lapponicus sylvicola* comb. nov.

The phylogenetic trees performed by Hines *et al.* (2006) and Cameron *et al.* (2007) show low bootstrap values between *B. lapponicus lapponicus* and *B. lapponicus sylvicola* while the two taxa displayed some genetic divergences in ArgK, 16S and Ef-1 α . However, the only specimen of *sylvicola* used in Cameron *et al.* (2007) and Hines *et al.* (2006) had been collected in New Mexico (USA) where the taxon displays a particular color form with tergite 2 and 3 predominantly black (T2-3 red in Alaska), although these two forms of *B. lapponicus sylvicola* are considered as conspecific (Williams *et al.* 2015). Without a complete taxonomic revision,

we cannot exclude 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, *sylvicola* display different allelic diversity and they emphasize different genetic cluster (population genetic structure differentiation). As suggested in Cameron *et al.* (2007), the PEPCK gene fragment showed no differentiation between *B. lapponicus lapponicus* and the north population of *B. lapponicus sylvicola* while COI fragment showed a low divergence (Fig. 2). This could reflect a geographic intraspecific variability (Andriollo *et al.* 2015; Mutanen *et al.* 2016) between two isolated and geographically distant populations. Genetic results are in line with CLGS analyses which support a lack of divergence between *B. lapponicus lapponicus* and *B. lapponicus sylvicola* (intra-specific 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, W-Siberia and *B. sylvicola* from Alaska and Yukon. However, the reinforcement of reproductive barriers process could not be exerted on the CLGS between allopatric species. Overall, as there is no divergence in 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 just reflect a “dialect divergence” due 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 *lapponicus*-group supported by all our independent criteria: *B. interacti* sp. nov. (Table 3). Morphological, genetic and semio-chemical datasets support the presence of two biologically distinct taxa within *sylvicola* sampling from Alaska. CLGS results strongly support also the new species *B. interacti* sp. nov. with different major and indicator compounds from *monticola*, *konradini*, *lapponicus lapponicus* and *lapponicus sylvicola*. Although we have a restricted sampling and different tree topologies between the two genetic markers, nuclear gene analysis suggests that *B. interacti* sp. nov. is closer to *B. monticola* (also consistent with the morphology, see Table 2), a strictly European taxon. This could question the distribution of their potential common ancestor around the Arctic Circle. One hypothesis could be that the speciation of *B. interacti* sp. nov. occurred from successive waves of range expansion and contraction following glaciations and the dynamic of Bering Strait (Abbott and Brochmann 2003; Elias and 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 inter-population genetic analyses are needed to explore this hypothesis.

For the taxon *gelidus*, considering morphological criteria and wing geometric morphometric analyses (Fig. 6), we propose to consider this taxon as a “dark” form of *B. lapponicus sylvicola*: *B. lapponicus sylvicola* f. *gelidus* comb. nov.

Bombus lapponicus: a circum polar 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 *B. lapponicus*-complex all around the Arctic Circle (i.e. *B. lapponicus zaitzevi* Skorikov, 1913 in the northern Urals; *B. lapponicus karaginus* Skorikov, 1912 from Chukotka; *B. glacialis* Friese, 1902 from Novaya Zemlya, and Wrangel Island). However, most taxa of this *B. lapponicus*-complex have never been investigated by genetic or chemical data. Formerly, only the Scandinavian population of the *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 (North Sweden, West Siberia, North Alaska, Yukon and North Quebec for wing shape analysis) seem therefore to confirm the hypothesis of Skorikov (1922) and Pittioni (1942) presenting *B. lapponicus sensu stricto* 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 *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 exhibit 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 *lapponicus* populations. These taxa do not seem to be involved in an *Artenkreis* speciation process *sensu* Rensch 1933.

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

Figure 2. A) Majority rule (50%) consensus tree based on maximum likelihood analyses of nuclear PEPCCK 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.

Figure 3. Species recognition pairwise matrix. Species recognition pairwise matrix based on ultrametric tree of cytochrome oxidase 1 (COI) sequences with bGMYC pairwise probability of conspecificity plotted on a sample tree from BEAST. The colored matrix corresponds to the pairwise probabilities of conspecificity returned by the bGMYC method (color scale on the right of the figure). Black spots show the coalescent node for each species. The larger bees represent the typical color patterns of queens.

Figure 4. A) Dendrogram based on cephalic labial gland secretions within *Bombus lapponicus* + *B. sylvicola* (light blue), *B. monticola* (green), *B. bimaculatus* (black), *B. interdicti* sp. nov. (red), *B. terrestris* (pink), *B. ephippiatus* (dark blue) and *B. 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 component analysis (PCA) of cephalic labial gland secretion differentiation within *lapponicus-sylvicola* complex: *Bombus lapponicus* + *B. sylvicola* (light blue), *B. monticola* (green), *B. bimaculatus* (black), *B. interdicti* sp. nov. (red), *B. terrestris* (pink), *B. ephippiatus* (dark blue) and *B. konradini* (orange). PC1 and PC2 are the first and second principal component axes.

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

Figure 6. Photos of the different morphological diagnostic characters between *sylvicola* and *interdicti* sp. nov.: A. Face of *B. interdicti* female BMAR0892, B. Face of *sylvicola* female BMAR0900, C. Right profile of *B. interdicti* female BMAR0892 with the yellow coloration of collar that does not go down until leg insertion, D. Right profile of *B. sylvicola* female BMAR0900 with the yellow coloration of collar that goes down until leg insertion, E. Posterior

legs of *B. interacti* male with hairy tibia PRAS1045, F. Posterior legs of *sylvicola* male BMAR 0141 with very hairy tibia, G. Genitalia of *B. interacti* male PRAS1045, H. Genitalia of *B. sylvicola* male BMAR 0141 (Photos P. Rasmont).

Figure 7. Pictures of the holotype *B. lapponicus sylvicola* f. *gelidus* (female): A. head of *B. lapponicus sylvicola* f. *gelidus* female, right *habitus* of *B. lapponicus sylvicola* f. *gelidus* female (Photos P. Rasmont).

Figure S1. Circum arctic sampling map (Azimuthal equidistant projection, after Uwe Dederer, 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*, *B. lapponicus sylvicola* and *B. interacti* sp. nov., the red square indicates the *locus typicus* of *B. lapponicus sylvicola* f. *gelidus*.

Figure S2. Right forewing of *Bombus lapponicus sylvicola* with the 18 landmarks indicated to describe the shape.

Table 1. List of main compounds identified for *Bombus lapponicus*, *B. sylvicola*, *B. monticola*, *B. interdicti* sp. nov., *B. konradini*, *B. bimaculatus*, *B. ephippiatus*, and *B. terrestris* within cephalic labial gland secretions. MW= the molecular weight, M= median of relative concentration of compound (%), n= number of specimens analyzed. Complete information is available in Appendix S2.

Compounds	MW	<i>B. monticola</i> (n=9)	<i>B. sylvicola</i> (n=14)	<i>B. lapponicus</i> (n=20)	<i>B. interdicti</i> sp. nov. (n=9)	<i>B. konradini</i> (n=2)	<i>B. bimaculatus</i> (n=10)	<i>B. terrestris</i> (n=6)	<i>B. ephippiatus</i> (n=3)
		M	M	M	M	M	M	M	M
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%
Geranyl citronellol	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%
Geranyl geranyl acetate	392	0.00%	0.00%	0.00%	0.00%	0.00%	31.22%	0.00%	0.00%

964 Table 2. Main morphological and color pattern differences (male and female) between *B.*
965 *lapponicus sylvicola*, *B. lapponicus lapponicus*, *B. monticola scandinavicus*, and *B. interacti*
966 sp. nov., *B. konradini* and *B. lapponicus sylvicola* f. *gelidus* comb. nov. according to Franklin
967 (1912), Løken (1973), Williams (2014) and personal observations.

	<i>interacti</i> sp. nov.	<i>lapponicus sylvicola</i>	<i>lapponicus lapponicus</i>	<i>monticola scandinavicus</i>	<i>konradini</i>	<i>lapponicus sylvicola</i> f. <i>gelidus</i> comb. nov.
Range	North Alaska	Widespread in most northern North America and in Californian mountains	Fennoscandia, N. Russia	Fennoscandia	Central Apennines	Aleutian Islands
Female						
Morphology						
Coat color variation	Light and colorful	Light and colorful	Varies from very light and colorful in Northern Fennoscandia, to rather dark in Southern Fennoscandia (Southern Norway)	Dark	Large and light	Dark
Body size	15-18 mm	15-17 mm	15-19 mm	14-19 mm	15-18 mm	16-18mm
Color 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 until leg insertion	Large and yellow goes down until 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 until leg insertion
Hind meta-basitarsus	Slight pubescence and the maximal width of the basitarsus is high as <i>B. monticola</i> (<i>sensu</i> Gjershaug <i>et al.</i> , 2013).	Strong pubescence and the maximal width of the basitarsus is low (<i>sensu</i> Gjershaug <i>et al.</i> , 2013) as in <i>lapponicus</i> .	Strong pubescence and the maximal width of the basitarsus is low (<i>sensu</i> Gjershaug <i>et al.</i> , 2013).	Slight pubescence and the maximal width of the basitarsus is high (<i>sensu</i> Gjershaug <i>et al.</i> , 2013).	Strong pubescence and the maximal width of the basitarsus is low (<i>sensu</i> Gjershaug <i>et al.</i> , 2013) as in <i>lapponicus</i> .	Strong pubescence and the maximal width of the basitarsus is low (<i>sensu</i> Gjershaug <i>et al.</i> , 2013) as in <i>lapponicus</i> .
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

Male						
Body size	11-13 mm	11-14 mm	11-14 mm	11-14 mm	11- 14 mm	12mm
Tibia	Hairy	Very hairy	Hairy	Hairy	Hairy	Very hairy
Color 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

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Table 3. Taxonomic decision table with all criteria used for species delimitation. CLGS, cephalic labial gland secretions; COI, cytochrome oxydase 1; bGMYC, the general mixed Yule-coalescent model, PEPCK, phosphoenolpyruvate carboxykinase. Morphology indicates if a taxon has a diagnostic morphological character (+/- means that morphology is/is not diagnostic). Wing shape and size indicate if a taxon has a diagnostic wing shape and size (+/- means that wing measures are/are not diagnostic). CLGS indicates if the taxon has/has not diagnostic composition of CLGSs with different main compounds (+/- means that the taxon has/ has not 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 if 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). LS= low supported differentiation. NA = not assessed.

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

Supporting information

Table S1. List of all specimens analyzed. 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 IndVal analysis with species-specific compounds. Unknown x are undetermined compounds.

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