

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

Baptiste Martinet, Thomas Lecocq, Nicolas Brasero, Maxence Gérard, Klara Urbanova, Irena Valterová, Jan Ove Gjershaug, Denis Michez, Pierre Rasmont

▶ To cite this version:

Baptiste Martinet, Thomas Lecocq, Nicolas Brasero, Maxence Gérard, Klara Urbanova, et al.. Integrative taxonomy of an arctic bumblebee species complex highlights a new cryptic species (Apidae: Bombus). Zoological Journal of the Linnean Society, 2019, 187 (3), pp.599-621. 10.1093/zoolinnean/zlz041. hal-02501083

HAL Id: hal-02501083 https://hal.univ-lorraine.fr/hal-02501083

Submitted on 8 Dec 2020 $\,$

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3

Running title: Systematics of an arctic bumblebee species complex

5

4

6 Abstract

While bumblebees have been the focus of much research, the taxonomy of many species groups 7 is still unclear, especially within circum-polar species. Delimiting species based on multisource 8 datasets provides a solution to overcome current systematic issues of closely related 9 populations. Here, we use an integrative taxonomic approach based on novel genetic and eco-10 11 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 12 divergence around the Arctic Circle between Fennoscandia and Alaska does not imply 13 speciation in this species complex. Therefore, based on our molecular and morphological 14 15 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 16 17 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. 18 19

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

22 Introduction

Most biodiversity hotspots are found in tropics with a pattern of increasing biodiversity 23 from the poles to the equator (Brown 2014). However, the highest latitudes have many 24 conspicuous and endemic species living under some of the most extreme conditions on Earth 25 (Lomolino et al. 2010; Botero et al. 2014). This arctic and boreal biodiversity has been shaped 26 by speciation processes driven by (i) the cold climate and local adaptations to environmental 27 harshness linked to arctic ecology and by (ii) spatial and temporal geographic patterns 28 specifically the heterogeneity of resource patches as landscape focal points (Chapin & Körner 29 1995; Willig et al. 2003). 30

Potential speciation processes between allopatric populations inhabiting different 31 32 continents around the Arctic Circle have been the focus of much research, and has been the 33 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 34 35 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 36 37 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 38 39 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 40 for instance by small interpopulational variations of the species mate recognition system which 41 prevent the specific recognition between individuals of distant populations or by ecological 42 differentiation (Rensch 1933; Stresemann & Timofeeff-Ressovsky 1947). Several cases of ring 43 speciation process were emphasized in birds, such as for *Phylloscopus trochiloides* in Siberia 44 (Alström 2006) or Melospiza melodia in Sierra Nevada (Patten & Pruett 2009). 45

Bumblebees (Hymenoptera: Apidae) are cold-adapted species, an adaptation that enable 46 them to live in some of the highest latitude and altitude ecosystems and reach high diversity in 47 the arctic and boreal regions (Shamurin 1966; Kevan 1973; Williams 1998; Michener 2007; 48 Biella et al. 2017). As a result, bumblebees are an excellent model group to explore speciation 49 processes in circum-polar areas with disjunct distributions (Williams et al. 2015). There is some 50 evidence that a circum-polar speciation process could have shaped the Bombus lapponicus -51 52 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 53 interbreeding populations around the Arctic Circle (Skorikov 1922). When comparing these 54

forms to American taxa, Pittioni (1942) pointed out a possible ring speciation process by 55 highlighting the variability of *B. lapponicus* with increased melanization process in the East 56 (Skorikov 1937). These different forms could be due to the fragmentation of the arctic habitat. 57 More recently, several authors have questioned the taxonomic relationship which connects 58 sylvicola and lapponicus (Thorp et al. 1983; Savard 2009; Williams et al. 2014). Among these 59 circum-polar populations, only B. lapponicus, B. sylvicola, B. glacialis (Novaya Zemlya, 60 61 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 62 has been suggested that *B. lapponicus* and *B. sylvicola* could be conspecific (Sladen 1919; 63 Skorikov 1922, 1937; Pittioni 1942, 1943; Thorp 1962; Thorp et al. 1983), data from 16S and 64 cytochrome oxidase I (COI) gene fragments support two divergent taxa in phylogenetic 65 analyses (Hines et al. 2006; Cameron et al. 2007). However, a comparison of all available data 66 leaves the taxonomic status of this group uncertain. 67

The systematics of bumblebees remains challenging (Bertsch & Schweer 2012; Lecocq 68 et al. 2015a; Williams et al. 2012) due to the limitations of morphological traits as diagnostic 69 characters (Bickford et al. 2007; Batalha-Filho et al. 2010; Carolan et al. 2012). The 70 71 development of integrative taxonomy involving a consensus between several independent alternative traits (e.g. molecular, eco-chemical traits) provides a solution to help resolve 72 73 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 74 75 process focusing on the most common circum arctic bumblebee taxa complex: Bombus (Pyrobombus) lapponicus (Fabricius, 1793) (North Scandinavia, W-Siberia) - B. (Pyrobombus) 76 77 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 78 79 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. 80

81 Material and Methods

82 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 94 (n=12) and Siberia (n=10), B. sylvicola from Northern Alaska (n=29) and Yukon (n=4) (Fig. 95 96 S1). For comparison, we used the phylogenetically closely related species B. (Pyrobombus) monticola scandinavicus (n=9) from North Scandinavia (Cameron et al. 2007), Bombus 97 (Pyrobombus) bimaculatus (n=10) from Canada, Bombus (Pyrobombus) ephippiatus (n=6) 98 from Biobest NA (Chiapas), Bombus (Pyrobombus) konradini (n=5) from Italy, Bombus 99 (Pyrobombus) glacialis (n=1) from Novaya Zemlya, Bombus (Pyrobombus) melanopygus 100 101 (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 102 103 freezing at -20°C.

104 Specimens were identified based on their morphology with identification keys such as 105 Løken (1973); Williams *et al.* (2014). A total of 147 bumblebees, collected between 2013 and 106 2018 in Europe, Siberia and Northern America, were analyzed (Table S1). For a first 107 identification, we performed a comparative table (male and female) gathering diagnostic 108 characters and color patterns for the studied specimens to compare morphological characters 109 within the *lapponicus-sylvicola* complex.

110 *Genetic differentiation*

In this study, we sequenced two genes commonly used to assess the specific status in 111 bumblebees (Pedersen 2002; Hines et al. 2006; Cameron et al. 2007; Lecocq et al. 2013a,b): 112 the mitochondrial cytochrome oxidase 1 gene (COI), and the nuclear phosphoenolpyruvate 113 carboxykinase gene (PEPCK). DNA extraction protocol, polymerase chain reaction, 114 115 amplification reactions, sequencing procedures and alignment of DNA sequences were 116 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 117 analyses to investigate genetic differentiations between B. lapponicus and B. sylvicola. We 118 performed maximum likelihood (ML) and Bayesian (MB) analyses. For all methods, the 119 120 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, 121 122 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 123 models chosen were: (i) for COI: GTR+I (first position), TIM2+I (second position), TrN+G (third 124 position); (ii) for PEPCK first intron: TPM1 uf +I; (iii) for PEPCK exon 1 : HKY+I (first position), 125 126 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 127 128 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 129 and-ln L were the same among replicates. Only the run with the highest likelihood was saved. 130 We assessed statistical significance of nodes with 10,000 nonparametric bootstrap replicates. 131 132 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 133 134 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 135 136 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 137 138 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 139 clustering computers provided by the Consortium des Équipements de Calcul Intensif (CÉCI, 140 F.R.S.-FNRS). 141

To recognize a species threshold, we used a Bayesian implementation of the general 142 mixed Yule-coalescent model (bGMYC) based on COI tree (Reid & Carstens 2012, see Lecocq 143 et al. 2015c). These analyses were performed with "bGMYC" R packages (Reid & Carstens 144 2012). A range of probabilities > 0.95 was considered as strong evidence that taxa were 145 conspecific while a range of probabilities < 0.05 suggested that taxa were heterospecific (Reid 146 147 & 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 148 149 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 150 151 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, 152 153 discarding the first 90,000 as burn-in and sampling every 100 generations. Posterior probability distributions have been applied against the first sample tree. 154

155 *Reproductive trait differentiation*

In the genus Bombus, conspecific individuals share the same recognition signals to 156 recognize each other as sexual partners (Calam 1969). We focused on the most studied 157 reproductive trait involved in the bumblebee pre-mating recognition (Svensson 1980; Ayasse 158 & Jarau 2014; Baer 2003): the cephalic labial gland secretions (CLGSs). CLGSs are commonly 159 used for species discrimination in bumblebees (Rasmont et al. 2005; Terzo et al. 2005; Bertsch 160 & Schweer 2012). CLGSs are synthesized *de novo* by cephalic labial glands (Žácek *et al.* 2013) 161 in the head of bumblebee males and are known to be species-specific (Lecocq et al. 2015c). 162 CLGSs consist of a complex mixture of (mainly aliphatic or isoprenoid) compounds, with 163 variable main compounds (Coppée et al. 2008; Lecocq et al. 2011). By main compounds, we 164 mean compounds that have the highest relative proportion (RA) among all compounds of 165 166 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 172 spectrometry using a Finigan GCQ quadrupole system (GC/MS) with a non-polar DB-5 ms 173 capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m; 174 inner diameter 0.25 mm; film thickness 0.25 µm]. All samples of CLGS were quantified with a 175 gas chromatograph Shimadzu GC-2010 system (GC-FID) equipped with a non-polar SLB-5 ms 176 capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m; 177 inner diameter 0.25 mm; film thickness 0.25 µm] and a flame ionization detector. The 178 composition of CLGS was analyzed according to the protocol described in Lecocq et al. 179 (2015c). All compounds for which the relative abundance was recorded as less than 0.1% for 180 all specimens were excluded from the analysis (De Meulemeester et al. 2011). The data matrix 181 182 for each taxon was based (Table S2) on the alignment of each relative proportion of compound 183 between all samples performed with GCAligner 1.0 (Dellicour & Lecocq 2013a,b). To facilitate the alignment of compounds and the identification, before each sample injection, a standard 184 185 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 186 187 & Lecocq (2013a, b).

188 Statistical analyses

We performed statistical comparative analyses of the CLGSs using R 3.3.2 (R 189 Development Core Team, 2016) to detect CLGS differentiations. We transformed data [log (x 190 + 1)] to reduce the great difference of abundance between highly and lowly concentrated 191 compounds. We used a principal component analysis (PCA, R-package MASS, Venables & 192 Ripley 2002) based on correlation distance matrices, and a clustering method computed with 193 the unweighted pair-group method with average linkage (UPGMA) based on Canberra distance 194 matrices (RA of each compound) (R package ape; Paradis et al. 2004). We assessed the 195 uncertainty in hierarchical cluster analysis using p-values calculated by multiscale bootstrap 196 197 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 198 199 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 200 201 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 202 203 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 204 relative occurrence frequency of a compound within a group. The statistical significance of an 205 indicator compound (>0.7) was evaluated with a randomization procedure (Dufrêne & 206 Legendre 1997). 207

208 Data integration and decision framework

We based our species delimitation hypothesis on the method performed by Lecocq et 209 al. (2015a), derived from the integrative approach established by Schlick-Steiner et al. (2010) 210 according to the unified species concept (De Quieroz 2007). With our approach, criteria are not 211 balanced, and the assignment of species status is allocated by unanimity of all criteria to avoid 212 213 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 214 unique haplotypes), (ii) constituted a monophyletic group with high branch support, and (iii) 215 216 was significantly differentiated in CLGS compositions (including IndVal indicator compounds, MRPP test, bootstrap values > 0.85). This conservative approach could lead to underestimation 217 218 of the species differentiation but reduces the taxonomic inflation (Lecocq et al. 2015a; Williams 219 et al., 2015). To highlight taxa with infra-specific-level differentiation, we assigned the 220 subspecies status to phenotypically distinct allopatric populations with differentiations in some 221 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;

223 Lecocq *et al.* 2015a, c).

224 Geometric morphometrics

As fresh material of *B. gelidus* was not available for molecular and chemical analyses, 225 we run an additional study to test the similarity between gelidus type material and other 226 *lapponicus* group taxa. The right forewings of 44 queens were photographed using an Olympus 227 SZH10 microscope an AF-S NIKKOR 18-105 millimetres (Shinjuku, Japan) and GWH10X-228 CD oculars coupled with a Nikon D200 camera: 39 queens of B. lapponicus (including 229 specimens of *B. lapponicus lapponicus* (n = 14) and *B. lapponicus sylvicola* (n = 29) and 1 230 queen of B. gelidus (holotype). For B. gelidus, in the Smithsonian National Museum of Natural 231 232 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 233 234 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 235 236 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 237 revised the type series, including the "false type anachronic inclusion". Wing shapes were 238 captured by digitizing two-dimensional Cartesian coordinates of 18 landmarks (Fig. S2) on 239 240 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 241 242 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 243 244 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. 245 The aligned landmark configurations were projected into the Euclidean space tangent to the 246 curved Kendall's shape space to aid further statistical analyses. The correlation coefficient 247 248 between the Procrustes distances in the shape space and the Euclidean distances in the linear 249 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 250 251 the correlation coefficient between the two distances were calculated with tps-SMALL v1.25 252 (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 257 reference dataset and discrimination of the different taxa was assessed by Linear Discriminant 258 Analyses (LDA) of the projected aligned configuration of landmarks. These analyses were 259 performed at species level as a priori grouping by using the software R version 3.0.2. The 260 effectiveness of the LDA for discriminating taxon was assessed by the percentages of 261 262 individuals correctly classified to their original taxon (hit-ratio, HR) in a leave-one-out crossvalidation procedure based on the posterior probabilities of assignment. Given the observed 263 scores of an "unknown", the posterior probability (pp) equals the probability of the unit to 264 265 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 266 267 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 268 269 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 270 271 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 272 LDA space as "unknown" specimen and calculated their score. Assignments of the holotype 273 B. gelidus were estimated by calculating the Mahalanobis Distance between "unknown" and 274 275 group mean of each taxon. We also calculated posterior probabilities of assignment to confirm 276 the assignment to one taxon.

277 **Results**

278 *Genetic analyses*

A total of 938 bp from the COI gene and 925 bp from PEPCK were obtained. All 279 phylogenetic analyses (ML and MB) on each genetic dataset showed a similar topology and 280 281 identical phylogenetic differentiation (Fig. 2). As expected, we found a less structured tree in 282 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 283 284 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 285 286 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 287 288 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 293 294 (part B): B. interacti sp. nov. Martinet, Brasero & Rasmont closely similar and co-occurring 295 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) 296 297 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 298 299 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 300 301 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 302 303 and 97% of query cover for COI from *B. monticola* in Genbank). In our phylogenetic analyses, 304 B. interacti sp. nov. differs significantly from B. melanopygus and B. glacialis (high branch supports and posterior probabilities, Fig. 2A, 2B). 305

The bGMYC analysis (Fig. 3) highlighted nine entities with low probabilities (<0.05) 306 307 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 308 seven prospective species (p < 0.05): (i) *B. terrestris* (bGMYC conspecificity probabilities 309 between individuals included in the group, p > 0.12-1), (ii) *B. ephippiatus* (p > 0.97-1), (iii) *B.* 310 *interacti* sp. nov. (p >0.98-1), (iv) *B. monticola* (p > 0.47-1), (v) *B. bimaculatus* (p > 0.89-1), 311 (vi) *B. konradini* (p > 0.99-1), (vii) a group with sylvicola and lapponicus (p > 0.07-1), (viii) a 312 group with *B. glacialis* and (ix) a group with *B. melanopygus*. The pairwise matrix (Fig. 3) 313 showed a non-significant heterospecificity threshold between lapponicus and sylvicola (p > 314 0.05). 315

316 *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*, 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%) 326 shared by B. sylvicola and B. lapponicus; (b) the hexadec-9-enyl acetate (45.91% - 61.74%) for 327 B. monticola and B. konradini (48.36%-54.71%); (c) Ethyl octadec-9-enoate for B. konradini 328 (7.43%-9.14%); (d) the hexadec-9-enyl acetate (20.55% - 40.63%) and the geranyl geranyl 329 acetate (25.98% - 39.42%) for *B. bimaculatus*, (e) the dihydrofarnesol (19.08% - 40.45%) for 330 B. terrestris, (f) the hexadecanoic acid (19.03%-31.63%) for B. ephippiatus, and (g) the 331 citronellyl hexadec-9-enoate (12.37% - 23.57%) for B. interacti sp. nov. (Tables 1 and S2). 332 Statistical analyses supported the differentiation (MRPP, A = 0.6973, T = 0.1759, all p < 0.001) 333 of seven groups also supported by high multiscale bootstrap resampling values (Cluster and 334 ACP Fig. 4): (a) *B. monticola* (pairwise test, p < 0.01), (b) *B. konradini* (pairwise test, p < 0.01) 335 (c) *B. bimaculatus* (pairwise test, p < 0.01), (d) *B. ephippiatus* (pairwise test, p < 0.01), (e) *B.* 336 337 *terrestris* (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (pairwise test, p < 0.01), (f) B. sylvicola group A + B. lapponicus (p 0.01), and (g) B. interacti sp. nov. (pairwise test, p < 0.01). No statistical differentiation was 338 found in the statistical hypothesis test and in hierarchical clustering between lapponicus and 339 sylvicola group A (Fig. 4; MRPP, A= 0.004641, T= 0.1577, p = 0.30). Several significant and 340 specific indicator compounds were revealed by the IndVal method (IndVal > 0.70), but no 341 compound has been identified to discriminate *B. lapponicus* to *B. sylvicola* (Table S2). 342

343 Wing size and shape analyses

No significant difference in centroid size was found among the different taxa (F = 2.73; 344 345 p-value = 0.08). However, significant differences in wing shape were found among the different taxa (perMANOVA, F = 1.83; p-value = 0.006). Pairwise perMANOVA tests have shown a 346 significant difference between B. lapponicus and B. interacti sp. nov. (F = 2.44; p-value = 347 0.004) and between B. interacti sp. nov. and B. gelidus (F = 1.96; p-value = 0.035) while no 348 difference was detected between *B. lapponicus* and *B. gelidus* (F = 1.21; p-value = 0.32). PCA 349 350 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 351 352 groups B. lapponicus and B. sylvicola were not discriminated in the PCA and LDA while B. 353 interacti group is strongly differentiated. In the morphometric space defined by the PCA, the 354 specimen of gelidus is undoubtedly clustered with the group of the B. lapponicus (Fig. 5). Aposteriori assignment of the holotype *B. gelidus* in the discriminant shape space (LDA) allows 355

a reliable specific attribution. This analysis reveals that this specimen is assigned to B.

357 *lapponicus* species (Mahalanobis Distance to *B. sylvicola* group = 1.44; pp = 1).

358 Morphological diagnosis

For this morphological comparison, we assess only males and queens (minimum 15 359 individuals per taxon according to the availability of specimens). Except for the coloration of 360 the face which is black for *lapponicus* and yellow for *sylvicola*, no diagnostic character was 361 362 found to discriminate lapponicus from sylvicola based on our morphological examinations 363 (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 364 our molecular analyses) that could be separated by several diagnostic characters. Bombus 365 366 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. 367 interacti sp. nov. differed from sylvicola in the face clypeus coloration: black with intermixed 368 dark yellow hair for interacti sp. nov. and yellow for sylvicola. Besides, the density of 369 370 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 371 pubescence of basitarsus", used by Gjershaug et al. (2013) to distinguish B. lapponicus from B. 372 monticola, does not allow distinction of B. interacti sp. nov. from B. monticola and this 373 374 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 375 376 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 377 378 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 379 380 the labral furrow (very dense for *B. interacti* and labrum covered with reddish bristles at the front part). 381

382

386

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

GENUS BOMBUS Latreille, 1802

2018

388

389 *Synonyms*: None

<sup>Taxonomic status: Description of the new species B. interacti Martinet, Brasero & Rasmont sp.
nov. and synonymy of lapponicus and sylvicola
Family APIDAE Latreille, 1802</sup>

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

392

393 *Description*:

Females. Length 15-18 mm.

Coat color. Face and vertex densely pubescent with black hairs intermixed with few yellowish-395 greyish hairs. Thorax with a collare as large as 1/3 of the thorax length, with few intermixed 396 black hairs at front of tegulae; scutellare as large as 1/4 of the thorax length. The hairs of the 397 scutellare are shaped in two oblique tufts. Pleura covered with greyish hairs on the anterior 398 third, intermixed with black in front of the tegulae; mesopleura with intermixed grey and black 399 400 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 401 402 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. 403 404 T4 mostly covered with grayish hairs, with very few red hairs at the anterior margin and black 405 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 406 intermixed with very few greyish ones. Mesotibias with black hairs some of them with red tip. 407 Metatibias with corbiculae surrounded by decumbent bristles slightly longer that the width of 408 the organ, mostly reddish with light blond tip and some whole black at the base of anterior 409 margin, meso- and metabasitarsus with short reddish bristles. Distal tarsa red. Otherwise black. 410 Labrum with a narrow labral furrow as wide as 0.23 times its total width, V-shaped. The labral 411 412 tubercles are well designed. There is an imbricated microsculptures in front of the tubercles and into the labral furrow. Punctuations are very dense into the labral furrow and more spaced 413 back to the tubercles. The front part of the labrum is covered with plumose reddish bristles. 414

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.

421 Ocellar field covered with large spaced punctuations along the inner margin of the compound
422 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 onlydefined near the eyes.

425 Antennae. L(A5) = 0.67 * L(A3); L(A4) = 0.51*L(A3) (not different from *B. lapponicus*).

426 Metabasitarsus. maximal width situated apically of the diverging transversely directed hair, at 427 0.27 of the basitarsus length (0.19 in *B. lapponicus*). The glabrous area at the base of

428 metabasitarsus with slightly imbricated micro-sculptured surface (this area is much smaller in

- 429 *B. lapponicus* and without imbricated surface).
- 430 431

432 Males. Length 11-13 mm.

Coat color. Males are greyish and shaggy. Face and vertex largely covered by yellow hairs and 433 a slight mixture of black and yellow hairs on vertex. Thorax with a collare as large as 1/3 of the 434 435 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; 436 437 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 438 439 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 440 441 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 442 hairs with numerous black ones in the middle. T7 mostly with black hairs and some grayish 443 ones on the sides. Coxae and femurs with mostly yellow hairs intermixed with very few black 444 ones. Mesotibias with yellow hairs some of them with reddish base with few intermixed black 445 hairs. Metatibias with corbiculae surrounded by decumbent bristles slightly longer that the 446 width of the organ, mostly reddish with light blond tip and some whole black at the base of 447 anterior margin, meso- and metabasitarsus with short reddish bristles. Distal tarsa red. 448 Otherwise black. 449

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.

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

454 Clypeus slightly bombed, very covered with short black bristles in the middle and longer 455 reddish ones at the distal part. The first anterior third is covered with deep and broad 456 punctuations. There is a thin band of microsculptures along the transversal furrow at the distal 457 part of clypeus.

- 458 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
- 460 of compound eyes, there is a poorly designed supra-orbital line only defined near the eyes.
- 461 Antennae. L(A5) = 0.63 * L(A3); L(A4) = 0.52*L(A3) (not different from *B. lapponicus*).
- 462 Metabasitarsus. In the middle, the external side of the posterior is characterized by an area with
- 463 very short black bristles (contrary to *B. lapponicus* with long and numerous bristles).
- 464 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.
- 472 *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.
- 474 Martinet/Rasmont St88, PRAS1045; (2) red, printed with 'HOLOTYPE'; (3) white, printed
- 475 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
- 477 the Royal Belgian Institute of Natural Sciences in Brussels. Genbank accession numbers:
- 478 MG280603 (COI), MG280606 (PEPCK).
- 479 *Paratypes:* 9 males and 4 queens pinned (Fig. 1D) and labelled "Paratype".
- Remarks: Considering morphology, genetic and the semio-chemical secretions, there is no 480 available name to describe our new taxon. However, there are some uncertainties about the 481 482 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 483 Cresson (1878) is very poor and not sufficient to compare with our specimens. This description 484 485 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 486 487 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. 488 489 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. 490 491 *lapponicus sylvicola*. However, the face of queen is mostly dark and the mesopleura is largely

492 covered with yellow pile but the yellow not reaching the bases of the legs in *B. gelidus*. For493 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. 494 lapponicus sylvicola could be found except slight differences in coloration and these taxa 495 should be considered as conspecific. The queen holotype from the Academy of Natural Sciences 496 (Philadelphia) and three co-types workers (from the Smithsonian National Museum of Natural 497 History) have been examined for this study. However, for these old specimens only 498 morphological traits are available to compare with our specimens. The morphological 499 500 characters distinguishing *gelidus/sylvicola/interacti* females are mainly based on coat color 501 variation. The type series is different from B. interacti sp. nov. based on wings coloration 502 (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 503 504 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 505 506 indication that *B. gelidus* would be anything other than a dark form of *B. lapponicus sylvicola*. 507 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 508 strong taxonomic evidence (genetic, semio-chemical) and that B. gelidus has been described 509 only from the Aleutian Islands, we hypothesize that *B. gelidus* is different from *B. interacti* sp. 510 nov. and we describe this latter taxon as new species. Our wing morphometric geometric 511 analysis show that the holotype of gelidus is assigned to *B. lapponicus*. Even if the definitive 512 status of B. gelidus remains unsettled, as far as we can understand now, after revision the 513 514 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, 515 516 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 517 518 density of the yellow interalar band for males in our sampling. However, our specimens have 519 been collected from only one site (Toolik and surroundings) and the color variation could be 520 underestimated. Its recorded host plants are Epilobium angustifolium, Senecio lugens and 521 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 522 523 the cephalic labial gland secretions, (3) complete morphological examination.

524 *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
- 533 *Bombus (Pyrobombus) lapponicus sylvicola* **comb. nov.** in Alaska and Yukon.
- 534 Bombus lapponicus sylicola f. gelidus Cresson 1878 comb. nov.
- 535 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
- 538 Cresson, 1878". The type is conserved in the *Academy of Natural Sciences* in Philadelphia
- 539 (USA).
- 540 *B. gelidus* appears as a very dark form of *B. lapponicus sylvicola* and should be considered as
 541 *B. lapponicus sylvicola* f. *gelidus* comb. nov.
- 542 Further material. In the Smithsonian National Museum of Natural History, Massachusetts 543 Agricultural College and United States National Museum, there are 1 queen, 14 workers and 1 544 male labelled "Cotype" by Franklin (1912). All these specimens should not be part of the typical 545 series and have been erroneously labelled. These specimens were collected later and in other 546 areas than the only holotype described by Cresson (1878). After examination, we consider that 547 these specimens as typical color form of *B. lapponicus sylvicola*.
- 548

549 **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 559 the taxonomic ambiguities present in this group. Koch et al. (2017), showed that in its 560 distribution, sylvicola display different allelic diversity and they emphasize different genetic 561 cluster (population genetic structure differentiation). As suggested in Cameron et al. (2007), 562 the PEPCK gene fragment showed no differentiation between B. lapponicus lapponicus and the 563 north population of *B. lapponicus sylvicola* while COI fragment showed a low divergence (Fig. 564 2). This could reflect a geographic intraspecific variability (Andriollo et al. 2015; Mutanen et 565 566 al. 2016) between two isolated and geographically distant populations. Genetic results are in 567 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 568 569 conspecific according to the species recognition concept (Paterson 1993) (Fig. 3B) and our 570 taxonomic integrative approach (Table 3). There could be no chemical reproductive barrier 571 (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 572 573 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 574 reproductive barrier between the populations. Poor quantitative differences observed could just 575 reflect a "dialect divergence" due to the geographical gap between these two sampled 576 populations (Lecocq et al. 2013b) or chemical background noise. 577

Moreover, our integrative framework (Table 3) highlights an unknown species from 578 Alaska in the *lapponicus*-group supported by all our independent criteria: *B. interacti* sp. nov. 579 (Table 3). Morphological, genetic and semio-chemical datasets support the presence of two 580 581 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 582 583 monticola, konradini, lapponicus lapponicus and lapponicus sylvicola. Although we have a restricted sampling and different tree topologies between the two genetic markers, nuclear gene 584 analysis suggests that B. interacti sp. nov. is closer to B. monticola (also consistent with the 585 586 morphology, see Table 2), a strictly European taxon. This could question the distribution of 587 their potential common ancestor around the Arctic Circle. One hypothesis could be that the 588 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 589 590 2003; Elias and Brigham-Grette 2013; Pringle 2014). In the case of our new species B. interacti, 591 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 geneticanalyses are needed to explore this hypothesis.

594 For the taxon *gelidus*, considering morphological criteria and wing geometric 595 morphometric analyses (Fig. 6), we propose to consider this taxon as a "dark" form of *B*. 596 *lapponicus sylvicola*: *B. lapponicus sylvicola* f. *gelidus* comb. nov.

597

Bombus lapponicus: a circum polar taxon

Based on morphological characters and the coat coloration, Skorikov (1922) and Pittioni 598 (1942) had already hypothesized that there is a set of conspecific taxa related to B. lapponicus-599 600 complex all around the Arctic Circle (i.e. B. lapponicus zaitzevi Skorikov, 1913 in the northern 601 Urals; B. lapponicus karaginus Skorikov, 1912 from Chukotka; B. glacialis Friese, 1902 from 602 Novaya Zemlya, and Wrangel Island). However, most taxa of this B. lapponicus-complex have 603 never been investigated by genetic or chemical data. Formerly, only the Scandinavian 604 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 605 606 (North Sweden, West Siberia, North Alaska, Yukon and North Quebec for wing shape analysis) 607 seem therefore to confirm the hypothesis of Skorikov (1922) and Pittioni (1942) presenting B. 608 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 609 the conspecificity of specimens of *lapponicus* from Norway, Kamchatka, Yamal and Chukotka 610 based on COI analysis. These results confirm our hypothesis: B. lapponicus is found across 611 northern Holarctic regions, including a circumpolar distribution and exhibit subspecific 612 differentiation across at least the polar section of its distribution. The absence of differentiation 613 614 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 615 616 Artenkreis speciation process sensu Rensch 1933.

- 618 **References**
- Abbott RJ, Brochmann C. 2003. History and evolution of the arctic flora: in the footsteps of
 Eric Hultén. *Moleular Ecolology* 12: 299–313.
- Adams DC & Otárola-Castillo E. 2013. Geomorph: An R package for the collection and
 analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*4(4): 393–399.
- Alcaide M, Scordato ESC, Price TD, Irwin DE. 2014. Genomic divergence in a ring
 species complex. *Nature* 51: 83–85.
- Alström P. 2006. Species concepts and their application: Insights from the genera *Seicercus* and *Phylloscopus*. *Acta Zoologica Sinica* 52: 429-434.
- Andriollo T, Naciri Y, Ruedi M. 2015. Two mitochondrial barcodes for one biological
 species: the case of European Kuhl's Pipistrelles Chiroptera. *PlosOne* 10:
 e0134881.
- Ayasse M, Jarau S. 2014. Chemical ecology of bumble bees. *Annual Review of Entomology*59: 299–319.
- Baer B. 2003. Bumblebees as model organisms to study male sexual selection in social insects.
 Behavioral Ecology and Sociobiology 54: 521–533.
- Batalha-Filho H, Waldschmidt A, Campos LAO, Tavares MG, Fernandes-Salomao T.
 2010. Phylogeography and historical demography of the Neotropical stingless bee
 Melipona quadrifasciata (Hymenoptera, Apidae): incongruence between
 morphology and mitochondrial DNA. *Apidologie* 41: 534–547.
- Bertsch A, Schweer H. 2012. Cephalic labial gland secretions of males as species recognition
 signals in bumblebees: are there really geographical variations in the secretions of
 the *Bombus terrestris* subspecies? *Beiträge zur Entomologie* 62: 103–124.
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I.
 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22: 148–155.
- Biella P, Bogliani G, Cornalba M, Manino A, Neumayer J, Porporato M, Rasmont P,
 Milanesi P. 2017. Distribution patterns of the cold adapted bumblebee Bombus
 alpinus in the Alps and hints of an uphill shift (Insecta: Hymenoptera: Apidae). *Journal of Insect Conservation* 21 : 357.
- Bookstein FL. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge
 University Press, Cambridge, 435 p.

651	Botero CA, Dor R, McCain CM, Safran RJ. 2014. Environmental harshness is positively
652	correlated with intraspecific divergence in mammals and birds Molecular Ecology
653	23 : 259-268.
654	Brown JH. 2014. Why are there so many species in the tropics? Journal of Biogeography 41
655	(1): 8-22.
656	Calam, DH. 1969. Species and sex-specific compounds from the heads of male bumblebees
657	(Bombus spp). Nature 221: 856–857.
658	Cameron SA, Hines HM, Williams PH. 2007. A comprehensive phylogeny of the bumble
659	bees (Bombus). Biological Journal of the Linnean Society 91: 161–188.
660	Carolan JC, Murray TE, Fitzpatrick U, Crossley J, Schmidt H, Cederberg B, McNally L,
661	Paxton RJ, Williams PH, Brown MJF. 2012. Colour patterns do not diagnose
662	species: quantitative evaluation of a DNA barcoded cryptic bumblebee complex.
663	<i>PLoS ONE</i> 7 : e29251.
664	Chapin FS, Körner C. 1995. Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem
665	consequences. Ecological studies 113, Berlin Heidelberg, p.336.
666	Claudet J, Pelletier D, Jouvenel JY, Bachet F, Galzin R. 2006. Assessing the effects of
667	Marine Protected Area (MPA) on a reef fish assemblage in a northwestern
668	Mediterranean marine reserve: identifying community-based indicators. Biological
669	<i>Conservation</i> 130 : 346-369.
670	Coppée A, Terzo M, Valterova I, Rasmont P. 2008. Intraspecific variation of the cephalic
671	labial gland secretions in Bombus terrestris (L.) (Hymenoptera: Apidae). Chemistry
672	& Biodiversity 5: 2654–2661.
673	Cresson ET.1878. Descriptions of new species of North American bees. Proceedings of the
674	Academy of natural Sciences of Philadelphia 1878: 181-221.
675	Dellicour S, Lecocq T. 2013a. GCALIGNER 1.0 and GCKOVATS 1.0 – Manual of a Software
676	Suite to Compute a Multiple Sample Comparison Data Matrix from Eco-chemical
677	Datasets Obtained by Gas Chromatography. University of Mons, Mons.
678	Dellicour S, Lecocq T. 2013b. GCALIGNER 1.0: an alignment program to compute a multiple
679	sample comparison data matrix from large eco-chemical datasets obtained by GC.
680	Journal of Separation Science 36 : 3206–3209.
681	De Meulemeester T, Gerbaux P, Boulvin M, Coppee A, Rasmont P. 2011. A simplified
682	protocol for bumble bee species identification by cephalic secretion analysis.
683	Insectes Sociaux 58: 227–236.

- De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–
 886.
- **Drummond AJ & Rambaut A. 2007.** BEAST: bayesian evolutionary analysis bysampling
 trees. *BMC Evolutionary Biology* 7: 214.
- Dufrene M, Legendre P. 1997. Species assemblages and indicator species: The need for a
 flexible asymmetrical approach. *Ecological Monographs* 67: 345–366.
- Elias SA & Brigham-Grette J. 2013. Glaciations Late Pleistocene Glacial Events in
 Beringia, in: Encyclopedia of Quaternary Science (Second Edition), Elsevier,
 Amsterdam.
- Engel MS. 2011. Systematic melittology: where to from here? *Systematic Entomology* 36: 2–
 15.
- Ennen JR, Kalis ME, Patterson AL, Kreiser BR, Lovich JE, Godwin J, Qualls CP. 2014.
 Clinal variation or validation of a subspecies? A case study of the *Graptemys nigrinoda* complex (Testudines: Emydidae). *Biological Journal of the Linnean Society* 111: 810–822.
- Estoup A, Solignac M, Cornuet JM, Goudet JS. 1996. Genetic differentiation of continental
 and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe.
 Molecular Ecology 5: 19–31.
- Franklin HJ. 1912. The Bombidae of the New World. Transactions of the American
 Entomological Society 38 (3/4): 177-486.
- Gjershaug J, Staverløkk A, Kleven O, Ødegaard F. 2013. Species status of *Bombus monticola* Smith (Hymenoptera: Apidae) supported by DNA barcoding. *Zootaxa* 3716: 431–440.
- Hawlitschek O, Nagy ZT, Glaw F. 2012. Island evolution and systematic revision of Comoran
 snakes: why and when subspecies still make sense. *PLoS ONE* 7: e42970.
- Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing
 confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
- Hines HM, Camero SA, Williams PH. 2006. Molecular phylogeny of the bumble bee
 subgenus Pyrobombus (Hymenoptera : Apidae : *Bombus*) with insights into gene
 utility for lower-level analysis. *Invertebrate Systematics* 20: 289–303.
- Huberty CJ & Olejnik S. 2006. Applied MANOVA and discriminant analysis. Second
 Edition. New Jersey, 488 p.

- Ings TC, Ings NL, Chittka L, Rasmont P. 2010. A failed invasion? Commercially introduced
 pollinators in Southern France. *Apidologie* 41: 1–13.
- 718 Irwin DE, Irwin HJ, Price TD. 2001a. Ring species as bridges between microevolution and
 719 speciation. *Genetica* 112/113: 223–243.
- 720 Irwin DE, Bensch S, Price TD. 2001b. Speciation in a ring. *Nature* 409: 333–337.
- Irwin DE, Bensch S, Irwin JH, Price TD. 2005. Speciation by distance in a ring species.
 Science 307: 414–416.
- Kevan PG. 1973. Flowers, insects, and pollination ecology in the Canadian high Arctic. *Polar Record* 16: 667–674.
- Koch JB, Looney C, Sheppart WS, Strange JP. 2017. Patterns of population genetic structure
 and diversity across bumble bee communities in the Pacific Northwest. *Conservation Genetics* 18 (3): 507-520.
- Leaché AD, Fujita MK. 2011. Bayesian species delimitation in West African forest geckos
 (*Hemidactylus fasciatus*). *Proceeding of the Royal Society B* 278: 493–495.
- Lecocq T, Lhomme P, Michez D, Dellicour S, Valterova I, Rasmont P. 2011. Molecular and
 chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *Bombus maxillosus* (Hymenoptera, Apidae, Bombini). *Systematic Entomology* 36: 453–469.
- Lecocq T, Dellicour S, Michez D, Lhomme P, Vanderplanck M, Valterova I, Rasplus JY,
 Rasmont P. 2013a. Scent of a break-up: phylogeography and reproductive trait
 divergences in the red-tailed bumblebee (*Bombus lapidarius*). *BMC Evolutionary Biology* 13: 263.
- Lecocq T, Vereecken NJ, Michez D, Dellicour S, Lhomme P, Valterova I, Rasplus JY,
 Rasmont P. 2013b. Patterns of genetic and reproductive traits differentiation in
 mainland vs. Corsican populations of bumblebees. *PLoS ONE* 8: e65642.
- Lecocq T, Brasero N, De Meulemeester T, Michez D, Dellicour S, Lhomme P, De Jonghe
 R, Valterova I, Urbanova K, Rasmont P. 2015a. An integrative taxonomic
 approach to assess the status of Corsican bumblebees: implications for
 conservation. *Animal Conservation* 18: 236–248.
- Lecocq T, Coppee A, Mathy T, Lhomme P, Cammaerts-Tricot MC, Urbanova K,
 Valterova I, Rasmont P. 2015b. Subspecific differentiation in male reproductive
 traits and virgin queen preferences, in *Bombus terrestris*. *Apidologie* 46 (5): 595605.

Lecocq T, Dellicour S, Michez D, Dehon M, Dewulf A, De Meulemeester T, Brasero N,
 Valterova I, Rasplus JY, Rasmont P. 2015c. Methods for species delimitation in
 bumblebees (Hymenoptera, Apidae, *Bombus*): towards an integrative approach.
 Zoologica Scripta 44: 281–297.

- Lecocq T, Coppée A, Michez D, Brasero N, Rasplus JY, Valterová I, & Rasmont P. 2016.
 The alien's taxonomic identity: consequences of taxonomic status for the
 international bumble bee trade regulation. *Biological Conservation* 195 : 169-176.
- Løken A. 1973. Studies on Scandinavian bumble bees Hymenoptera, Apidae. Norsk
 Entomologisk Tidsskrift 20: 1–218.
- Lomolino MV, Riddle BR, Whittaker RJ, Brown JH. 2010. Biogeography, 4th edn.
 Sinauer, Sunderland MA.
- Martinet B, Lecocq T, Brasero N, Biella P, Urbanová K, Valterová I, Cornalba M,
 Gjershaug JO, Michez D, Rasmont P. 2017. Following the Cold: Geographic
 Differentiation between Interglacial Refugia and Speciation in Arcto-Alpine
 Species Complex *Bombus monticola* (Hymenoptera: Apidae). *Systematic Entomology*, doi 10.1111/syen.12268.
- Mayr E. 1942. Systematics and the Origin of Species. Columbia University Press, New York,
 New York.
- 767 Mayr E. 1963. Animal Species and Evolution. Harvard University Press, Cambridge,
 768 Massachusetts.
- Michener CD. 2007. The Bees of the World. Second Edition. Johns Hopkins University,
 Baltimore, 1016 p.
- Monahan WB, Pereira RJ, Wake DB. 2012. Ring distributions leading to species formation:
 a global topographic analysis of geographic barriers associated with ring species.
 BMC Biology 10: 20.

Mutanen M, Kivelä SM, Vos RA, Doorenweerd C, Ratnasingham S, Hausmann A,
Huemer P, Dincă V, van Nieukerken EJ, Lopez-Vaamonde C, Vila R, Aarvik
L, Decaëns T, Efetov KA, Hebert PD, Johnsen A, Karsholt O, Pentinsaari M,
Rougerie R, Segerer A, Tarmann G, Zahiri R, Godfray HC. 2016. SpeciesLevel Para- and Polyphyly in DNA Barcode Gene Trees: Strong Operational Bias
in European Lepidoptera. *Systematic Biology* 65: 1025-1040.

Oksanen FJ, Blanchet G, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, 780 Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2011. Tertiary 781 Vegan: Community Ecology Package. 782 Päckert M, Martens J, Eck S, Nazarenko AA, Valchuk OP, Petri B, Veith M. 2005. The 783 great tit (Parus major) - A misclassified ring species. Biological Journal of the 784 Linnean Society 86: 153–174. 785 Padial JM, Miralles A, De la Riva I & Vences M. 2010. The integrative future of 786 787 taxonomy. Frontiers in zoology 7: 16. Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R 788 language. Bioinformatics 20: 289–290. 789 790 Patten MA, Pruett CL. 2009. The Song Sparrow, Melospiza melodia, as a ring species: patterns of geographic variation, a revision of subspecies, and implications for 791 792 speciation. Systematics and Biodiversity 7 (1): 33-62. Paterson HEH. 1993. Evolution and the Recognition Concept of Species. The Johns Hopkins 793 794 University Press, Baltimore, Maryland. Pedersen BV. 2002. European bumblebees Hymenoptera: Bombini – Phylogenetic 795 796 relationships inferred from DNA sequences. Insect Systematics and Evolution, **33**:361–386. 797 Pekkarinen A. 1982. Morphology and specific status of *Bombus lapponicus* Fabricius and *B*. 798 monticola Smith Hymenoptera: Apidae. Entomologica Scandinavia 13: 41-46. 799 Pekkarinen A, Teräs I, Viramo J, Paatela J. 1981. Distribution of bumblebees 800 (Hymenoptera, Apidae: Bombus and Psithyrus) in eastern Fennoscandia. Notulae 801 802 *Entomologicae* **61**: 71-89. 803 Pittioni B. 1942. Die boreoalpinen Hummeln und Schmarotzerhummeln (Hymen., Apidae, Bombinae). I. Teil. Mitteilungen aus den Köninglichen Naturwissenschaftlicaen 804 805 Instituten in Sofia 15: 155-218. Pittioni B. 1943. Die boreoalpinen Hummeln und Schmarotzerhummeln (Hymen., Apidae, 806 Bombinae). II. Teil. Mitteilungen aus den Köninglichen Naturwissenschaftlicaen 807 Instituten in Sofia 16: 1-77. 808 Posada D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 809 **25**: 1253–1256. 810

- Potapov GS, Kondakov AV, Spitsyn VM, Filippov B YU, Kolosova Yu S, Zubrii NA,
 Bolotov IN. 2017. An integrative taxonomic approach confirms the valid status of
 Bombus glacialis, an endemic bumblebee species of the High Arctic. *Polar Biology* 41: 629-642.
- 815 **Pringle H. 2014.** Welcome to Beringia. *Science* **343**: 961-963.
- Proshchalykin MY, Kupianskaya AN. 2005. The bees (Hymeoptera, Apoidea) of the
 Northern part of the Russian far east. *Far Eastern Entomologist* 153:1-39.
- Rasmont P, Terzo M, Aytekin AM, Hines H, Urbanova K, Cahlikova L, Valterova I. 2005.
 Cephalic secretions of the bumblebee subgenus Sibiricobombus Vogt suggest *Bombus niveatus* Kriechbaumer and *Bombus vorticosus* Gerstaecker are
 conspecific (Hymenoptera, Apidae, Bombus). *Apidologie* 36: 571–584.
- R Development Core Team. 2016. R: A Language and Environment for Statistical Computing.
 R Foundation for Statistical Computing, Vienna. [WWW document]. URL
 http://www.R-project.org/ [accessed on 17 October 2017].
- Reid NM. & Carstens BC. 2012. Phylogenetic estimation error can decrease the accuracy of
 species delimitation: a Bayesian implementation of the general mixed Yule coalescent model. *BMC Evolutionary Biology* 12: 196.
- Reinig WF. 1937. Die Holarktis. Ein Beitrag zur diluvialen und alluvialen Geschichte der
 Cirkumpolaren Faunen- und Florengebiete. Gustav Fischer, Jena, 124 p.
- Rensch B. 1933. Zoologische Systematik und Artbildungsprobleme. Verhandlungen der Dtsch.
 Zool. *Geselschaft Zoologischer Anzeiger* 6: 19–83.
- Rohlf FJ. 1999. Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification* 16: 197–223.
- Rohlf FJ. 2013a. tpsSMALL Version 1.25. Department of Ecology and Evolution, State
 University of New York at Stony Brook, New-York.
- Rohlf FJ. 2013b. tpsUTIL Version 1.56. Department of Ecology and Evolution, State
 University of New York at Stony Brook, New-York.
- Rohlf FJ. 2013c. tpsDIG Version 2.17. Department of Ecology and Evolution, State University
 of New York at Stony Brook, New-York.
- Rohlf FJ & Slice D. 1990. Extensions of the Procrustes method for the optimal superimposition
 of landmarks. *Systematic Zoology* 39: 40–59.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: bayesian phylogenetic inference under mixed
 models. *Bioinformatics* 19: 1572–1574.

- Savard M. 2009. Aperçu sur la diversité des bourdons de la Minganie, Québec (Hymenoptera :
 Apidæ : Bombus). *Le Naturaliste canadien* 133 (2): 31–36.
- Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier R.H. 2010.
 Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421–438.
- Shamurin VF. 1966. Rol' nasekomikh-opilitelei v tundrovikh soobshchestvakh. [The role of insects in tundra communities]. Organizmi iprirodnayasreda. Voprosi geographii
 69: 98–117 (in Russian).
- Sladen FWL. 1919. The wasps and bees collected by the Canadian Arctic Expedition, 191318. In: Report of the Canadian Arctic Expedition, 1913-18. *Insects* 3: 25G-35G.
- Skorikov AS. 1922. Shmeli paleartiki. Chast I. Obshchaya biologia (so vklyucheniem zoogeografi i). [Les bourdons de la faune palearctique. Partie 1. Biologie générale
 (la zoogéographie y compris)]. Izvestiya Severnoi Oblastnoi Stantsii Zashchity *Rastenii ot Vreditelei* 4: 1-160.
- 858 Skorikov AS. 1937. Die grönländischen Hummeln im Aspekte der
 859 Zirkumpolarfauna. *Entomologiske Meddelelser* 20: 37–64.
- 860 Stresemann E, Timofeeff-Ressovsky NW. 1947. Artentstehung in geographischen
 861 Formenkreisen. I. Der Formenkreis Larus argentatus-cachinnans-fucus.
 862 *Biologisches Zentralblatt* 66: 57–76.
- Suzuki R, Shimodaira H. 2011. Pvclust: Hierarchical Clustering with P-values via Multiscale
 Bootstrap Resampling. Contributed package. Version 1-1.10. R Foundation for
 Statistical Computing, Vienna. [WWW document]. URL <u>http://www.R-project.org</u>
 [accessed on 17 October 2017].
- 867 Svensson BG. 1980. Species-isolating mechanisms in male bumblebees (Hymenoptera,
 868 Apidae). *Acta Universitatis Upsaliensis* 549: 1-42.
- Svensson BG, Bergström G. 1977. Volatile marking secretions from the labial gland of north
 European Pyrobombus D. T. males (Hymenoptera, Apidae). *Insectes Sociaux* 24:
 213–224.
- Terzo M, Urbanova K, Valterova I. Rasmont P. 2005. Intra and interspecific variability of
 the cephalic labial glands' secretions in male bumblebees: the case of *Bombus*(Thoracobombus) *ruderarius* and *B*. (Thoracobombus) *sylvarum* Hymenoptera,
 Apidae. *Apidologie* 36: 85–96.

- Thorp RW. 1962. Notes on the distributions of some bumblebees of western North America
 (Hymenoptera: Apidae). *Pan-Pacific Entomologist* 38: 21-28.
- Thorp RW, Horning DS, Dunning LL. 1983. Bumble bees and Cuckoo Bumble bees of
 California (Hymenoptera: Apidae. *Bulletin of the California insect Survey* 23: viii
 + 79 p.
- Wilcox TP, Zwickl DJ, Heath TA, Hillis DM. 2002. Phylogenetic relationships of the dwarf
 boas and a comparison of Bayesian and bootstrap measures of phylogenetic
 support. *Molecular Phylogenetics and Evolution* 25: 361–371.
- Williams PH. 1998. An annotated checklist of bumble bees with an analysis of patterns of
 description (Hymenoptera: Apidae, Bombini). *Bulletin of the Natural History Museum (Entomology)* 67: 79–152.
- Williams PH, Brown MJF, Carolan JC, An J, Goulson D, Aytekin AM, Best LR, Byvaltsev
 AM, Cederberg B, Dawson R, Huang J, Ito M, Monfared A, Raina RH,
 Schmid-Hempel P, Sheffield CS, Sima P, Xie Z. 2012. Unveiling cryptic species
 of the bumblebee subgenus *Bombus s. str.* worldwide with COI barcodes
 (Hymenoptera: Apidae). *Systematics and Biodiversity* 10(1): 21–56.
- Williams PH, Thorp R, Richardson L, Colla S. 2014. Bumble Bees of North America. New
 Jersey: Princeton University Press, 208 p.
- Williams PH, Byvaltsev AM, Cederberg B, Berezin MV, Ødegaard F, Rasmussen C,
 Richardson LL, Huang J, Sheffield CS, Williams ST. 2015. Genes suggest
 ancestral colour polymorphisms are shared across morphologically cryptic species
 in arctic bumblebees. *PLOS One* 10(2): e0144544.
- Willig MR, Kaufman DM, Stevens RD. 2003. Latitudinal gradients of biodiversity: pattern,
 process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics* 34: 273–309.
- Žacek P, Prchalova-Hornakova D, Tykva R, Kindl J, Vogel H, Svatoš A, Pichová I,
 Valterová I. 2013. De novo biosynthesis of sexual pheromone in the labial gland
 of bumblebee males. *ChemBioChem* 14: 361–371.
- 2wickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large
 biological sequence datasets under the maximum likelihood criteria. PhD
 Dissertation, The University of Texas, Austin, Texas.
- 907

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

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.

- **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* 921 922 + B. sylvicola (light blue), B. monticola (green), B. bimaculatus (black), B. interacti sp. nov. (red), B. terrestris (pink), B. ephippiatus (dark blue) and B. konradini (orange). This cluster 923 was obtained by hierarchical clustering using an unweighted pair-group method with arithmetic 924 mean (UPGMA) based on a Canberra matrix calculated from the cephalic labial gland secretion 925 matrix. The values near nodes represent multiscale bootstrap resampling values. B) Principal 926 component analysis (PCA) of cephalic labial gland secretion differentiation within lapponicus-927 sylvicola complex: Bombus lapponicus + B. sylvicola (light blue), B. monticola (green), B. 928 bimaculatus (black), B. interacti sp. nov. (red), B. terrestris (pink), B. ephippiatus (dark blue) 929
- 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. interacti* 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. interacti* 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 *interacti* sp. nov.: A. Face of *B. interacti* female BMAR0892, B. Face of *sylvicola* female
BMAR0900, C. Right profile of *B. interacti* 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

- 942 legs of *B. interacti* male with hairy tibia PRAS1045, F. Posterior legs of *sylvicola* male BMAR
- 943 0141 with very hairy tibia, G. Genitalia of *B. interacti* male PRAS1045, H. Genitalia of *B. sylvicola* male BMAR 0141 (Photos P. Rasmont).
- 945 **Figure 7.** Pictures of the holotype *B. lapponicus sylvicola* f. *gelidus* (female): A. head of *B.*
- 946 *lapponicus sylvicola* f. *gelidus* female, right *habitus* of *B. lapponicus sylvicola* f. *gelidus* female
- 947 (Photos P. Rasmont).
- 948 Figure S1. Circum arctic sampling map (Azimuthal equidistant projection, after Uwe Dedering,
- 949 licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license) on which
- 950 the red dots indicate the areas where we collected specimens of *Bombus lapponicus lapponicus*,
- 951 *B. lapponicus sylvicola* and *B. interacti* sp. nov., the red square indicates the *locus typicus* of *B.*
- 952 *lapponicus sylvicola* f. *gelidus*.
- 953 Figure S2. Right forewing of *Bombus lapponicus sylvicola* with the 18 landmarks indicated to
- 954 describe the shape.
- 955

956 Table 1. List of main compounds identified for *Bombus lapponicus*, *B. sylvicola*, *B. monticola*,

957 *B. interacti* sp. nov., *B. konradini*, *B. bimaculatus*, *B. ephippiatus*, and *B. terrestris* within 958 cephalic labial gland secretions. MW= the molecular weight, M= median of relative

959 concentration of compound (%), n= number of specimens analyzed. Complete information is

available in Appendix S2.

961

Compounda	MW	B. monticola	B. sylvicola B. lapponicus B. interacti		B. interacti sp.	B. konradini	B. bimaculatus	B. terrestris	B. ephippiatus
Compounds		(n=9)	(n=14)	(n=20)	nov. (n=9)	(n=2)	(n=10)	(n=6)	(n=3)
		М	М	М	М	М	М	М	М
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%

962

- 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.

	interacti sp. nov.	lapponicus	lapponicus	monticola	konradini	lapponicus
		sylvicola	lapponicus	scandinavicus		sylvicola f.
						gelidus comb.
						nov.
Range	North Alaska	Widespread in most	Fennoscandia, N.	Fennoscandia	Central	Aleutian
		northern North	Russia		Apennines	Islands
		America and in				
		Californian				
		mountains				
Female	I	I	L			
Morphology						
Coat color	Light and	Light and colorful	Varies from very	Dark	Large and light	Dark
variation	colorful		light and colorful			
			in Northern			
			Fennoscandia, to			
			rather dark in			
			Southern			
			Fennoscandia			
			(Southern			
			Norway)			
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	Black with few	Black	Yellow	Black with few
	yellow hairs		yellow hairs			yellow hairs
Collar and	Yellow but collar	Large and yellow	Yellow but less	Small dark yellow	Wide yellow	Yellow but
scutellar	does not go down	goes down until leg	wide, the		band to the	collar does not
	until leg insertion	insertion	coloration stops at		tegulae/ yellow	go down until
			the tegulae			leg insertion
Hind meta-	Slight	Strong pubescence	Strong pubescence	Slight pubescence	Strong	Strong
basitarsus	pubescence and	and the maximal	and the maximal	and the maximal	pubescence and	pubescence
	the maximal	width of the	width of the	width of the	the maximal	and the
	width of the	basitarsus is low	basitarsus is low	basitarsus is high	width of the	maximal width
	basitarsus is high	(sensu Gjershaug et	(sensu Gjershaug	(sensu Gjershaug	basitarsus is low	of the
	as B. monticola	al., 2013) as in	et al., 2013).	et al., 2013).	(sensu	basitarsus is
	(sensu Gjershaug	lapponicus.			Gjershaug et al.,	low (sensu
	et al., 2013).				2013) as in	Gjershaug et
					lapponicus.	al., 2013) as in
						lapponicus.
Tergite 1	Yellow with	Yellow with some	Yellow with some	Black/red	Yellow/	Yellow with
	some red and	red and black hairs	red and black hairs		red/black	some red and
	black hairs					black hairs
Tergite 4	Yellow	Yellow	Yellow (pinkish)	Dark red	Yellow	Yellow
Tergite 5	Yellow (higher	Yellow	Yellow (pinkish)	Dark red	Yellow	Yellow
	density)					

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/	Yellow / large	Yellow / large	Yellow / large	Dark yellow /no	Yellow / large	Yellow / large
scutellar	yellow	yellow	yellow		yellow	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

Table 3. Taxonomic decision table with all criteria used for species delimitation. CLGS, 970 cephalic labial gland secretions; COI, cytochrome oxydase 1; bGMYC, the general mixed Yule-971 coalescent model, PEPCK, phosphoenolpyruvate carboxykinase. Morphology indicates if a 972 taxon has a diagnostic morphological character (+/- means that morphology is/is not 973 diagnostic). Wing shape and size indicate if a taxon has a diagnostic wing shape and size (+/-974 means that wing measures are/are not diagnostic). CLGS indicates if the taxon has/has not 975 diagnostic composition of CLGSs with different main compounds (+/- means that the taxon 976 has/ has not a specific CLGS composition. When the taxon shares CLGS composition with 977 978 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 979 980 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 981 982 differentiation. NA = not assessed.

Former taxonomic status	Morphology	Wing	CLGS	COI	PEPCK gene	Proposed taxonomic status
	(diagnostic character)	shape /		gene/bGMYC		
		size				
B. lapponicus Sweden, W.	- (A)	- (A) /-	- (A)	+/-	- (A)	B. lapponicus lapponicus
Siberia						
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 comb. nov.
Unnamed species Alaska	+	+/-	+	+/+	+	<i>B. interacti</i> sp. nov.
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

983

985 Supporting information

Table S1. List of all specimens analyzed. Sample code refers to the sample labels used indifferent 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

990 and IndVal analysis with species-specific compounds. Unknown x are undetermined

991 compounds.

















