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

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

3

4 Running title: Systematics of an arctic bumblebee species complex

5

6 **Abstract**

7 While bumblebees have been the focus of much research, the taxonomy of many species groups
8 is still unclear, especially within circum-polar species. Delimiting species based on multisource
9 datasets provides a solution to overcome current systematic issues of closely related
10 populations. Here, we use an integrative taxonomic approach based on novel genetic and eco-
11 chemical datasets to resolve the taxonomic status of *Bombus lapponicus* and *B. sylvicola*. Our
12 results support the conspecific status of *B. lapponicus* and *B. sylvicola* and that the low gradual
13 divergence around the Arctic Circle between Fennoscandia and Alaska does not imply
14 speciation in this species complex. Therefore, based on our molecular and morphological
15 analyses, we propose to assign the sub-specific status: *B. lapponicus lapponicus* **comb. nov.**
16 from Fennoscandia and W-Siberia and *B. lapponicus sylvicola* **comb. nov.** from Alaska and
17 Yukon. Moreover, our analyses reveal a new cryptic arctic species in the *B. lapponicus*
18 complex: *B. (Pyrobombus) interacti* **sp. nov.** Martinet, Brasero & Rasmont from Alaska.

19

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

21

22 Introduction

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

31 Potential speciation processes between allopatric populations inhabiting different
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;
34 Monahan *et al.* 2012; Alcaide *et al.* 2014). Indeed, the topography of the continents around the
35 North Pole could lead to the formation of a chain of intergrading populations (e.g. across
36 Eurasia) connecting two reproductively isolated taxa (e.g. across the Atlantic region); the so
37 called ring species (Stresemann & Timofeeff-Ressovsky 1947; Irwin *et al.* 2001a) or *Artenkreis*
38 speciation process (*sensu* Rensch 1933). In this process, each intergrading population is able to
39 reproduce with immediately adjacent populations, but not with the more remote populations
40 through a set of parapatric speciation processes (Irwin *et al.* 2001b). This could be explained
41 for instance by small interpopulational variations of the species mate recognition system which
42 prevent the specific recognition between individuals of distant populations or by ecological
43 differentiation (Rensch 1933; Stresemann & Timofeeff-Ressovsky 1947). Several cases of ring
44 speciation process were emphasized in birds, such as for *Phylloscopus trochiloides* in Siberia
45 (Alström 2006) or *Melospiza melodia* in Sierra Nevada (Patten & Pruett 2009).

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

55 forms to American taxa, Pittioni (1942) pointed out a possible ring speciation process by
56 highlighting the variability of *B. lapponicus* with increased melanization process in the East
57 (Skorikov 1937). These different forms could be due to the fragmentation of the arctic habitat.
58 More recently, several authors have questioned the taxonomic relationship which connects
59 *sylvicola* and *lapponicus* (Thorp *et al.* 1983; Savard 2009; Williams *et al.* 2014). Among these
60 circum-polar populations, only *B. lapponicus*, *B. sylvicola*, *B. glacialis* (Novaya Zemlya,
61 Wrangel Island), and *B. karaginus* (Kamchatka) are currently recognized as valid species
62 (Proshchalykin & Kupianskaya 2005; Williams *et al.* 2014; Potapov *et al.* 2017). Though, it
63 has been suggested that *B. lapponicus* and *B. sylvicola* could be conspecific (Sladen 1919;
64 Skorikov 1922, 1937; Pittioni 1942, 1943; Thorp 1962; Thorp *et al.* 1983), data from 16S and
65 cytochrome oxidase I (COI) gene fragments support two divergent taxa in phylogenetic
66 analyses (Hines *et al.* 2006; Cameron *et al.* 2007). However, a comparison of all available data
67 leaves the taxonomic status of this group uncertain.

68 The systematics of bumblebees remains challenging (Bertsch & Schweer 2012; Lecocq
69 *et al.* 2015a; Williams *et al.* 2012) due to the limitations of morphological traits as diagnostic
70 characters (Bickford *et al.* 2007; Batalha-Filho *et al.* 2010; Carolan *et al.* 2012). The
71 development of integrative taxonomy involving a consensus between several independent
72 alternative traits (e.g. molecular, eco-chemical traits) provides a solution to help resolve
73 bumblebee systematics at the species level (Estoup *et al.* 1996; Ings *et al.* 2010; Leaché & Fujita
74 2011; Engel 2011; Lecocq *et al.* 2015c). Here, we propose to investigate the ring speciation
75 process focusing on the most common circum arctic bumblebee taxa complex: *Bombus*
76 (*Pyrobombus*) *lapponicus* (Fabricius, 1793) (North Scandinavia, W-Siberia) - *B. (Pyrobombus)*
77 *sylvicola* Kirby, 1837 (North America). We address the taxonomic uncertainties that exist
78 between these distant populations (Williams 1998; Williams *et al.* 2014; Cameron 2007) and
79 we present new morphometric, genetic and eco-chemical evidence to resolve the taxonomic
80 status of *B. lapponicus* and *B. sylvicola* using an integrative taxonomic approach.

81 **Material and Methods**

82 *Sampling and morphological identification*

83 *Bombus lapponicus* is a common Euro-Siberian boreo-alpine species (Fig. 1A). Its
84 geographic distribution extends from the north of the taiga to the tundra (except in Taymyr
85 peninsula, N-Siberia) between the 65th and 70th parallels in Europe and the 60th and 72nd
86 parallels in Siberia (Løken 1973; Pekkarinen *et al.* 1981; Pekkarinen 1982). *Bombus sylvicola*
87 is a widespread species from the northern and western mountains of North America (Fig. 1B).
88 This Nearctic taxon is morphologically very similar to *B. lapponicus* (Williams *et al.* 2014). In

89 North America, two forms of *sylvicola* have been described: one with the metasomal T2-3 red
90 from the Rocky Mountains and the second with T2-3 mainly black from the Sierra Mountains.
91 DNA barcoding supports the two principal color forms of *sylvicola* in North America as
92 conspecific including the doubtful taxon named *gelidus* from Alaska (Cresson 1878) which has
93 black hairs on the face and on the sides of the thorax (Williams *et al.* 2014).

94 We were able to sample females and males of *B. lapponicus* from North Scandinavia
95 ($n=12$) and Siberia ($n=10$), *B. sylvicola* from Northern Alaska ($n=29$) and Yukon ($n=4$) (Fig.
96 S1). For comparison, we used the phylogenetically closely related species *B. (Pyrobombus)*
97 *monticola scandinavicus* ($n=9$) from North Scandinavia (Cameron *et al.* 2007), *Bombus*
98 (*Pyrobombus*) *bimaculatus* ($n=10$) from Canada, *Bombus (Pyrobombus) ephippiatus* ($n=6$)
99 from Biobest NA (Chiapas), *Bombus (Pyrobombus) konradini* ($n=5$) from Italy, *Bombus*
100 (*Pyrobombus*) *glacialis* ($n=1$) from Novaya Zemlya, *Bombus (Pyrobombus) melanopygus*
101 ($n=2$) from California and as an outgroup *Bombus (Bombus) terrestris* ($n=14$) from Italy,
102 France, Sweden, Belgium and Scotland. The individual bumblebee specimens were killed by
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*

111 In this study, we sequenced two genes commonly used to assess the specific status in
112 bumblebees (Pedersen 2002; Hines *et al.* 2006; Cameron *et al.* 2007; Lecocq *et al.* 2013a,b):
113 the mitochondrial cytochrome oxidase 1 gene (COI), and the nuclear phosphoenolpyruvate
114 carboxykinase gene (PEPCK). DNA extraction protocol, polymerase chain reaction,
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
117 sequences were deposited on GenBank (Table S1). For each gene, we carried out phylogenetic
118 analyses to investigate genetic differentiations between *B. lapponicus* and *B. sylvicola*. We
119 performed maximum likelihood (ML) and Bayesian (MB) analyses. For all methods, the
120 PEPCK gene was partitioned into two exons and two introns to explore the best substitution
121 model. COI fragment and each nuclear exon were partitioned by base positions (first, second,
122 and third nucleotide). For each dataset, we used JModelTest Server 2.0 (Posada 2008) with the

123 corrected Akaike information criterion (AICc) to find the best-fitting substitution models. The
124 models chosen were: (i) for COI : GTR+I (first position), TIM2+I (second position), TrN+G (third
125 position) ; (ii) for PEPCK first intron: TPM1 uf +I ; (iii) for PEPCK exon 1 : HKY+I (first position),
126 JC (second position), TrN+I (third position) ; (iv) for PEPCK second intron : TrN+I ; (v) for PEPCK
127 exon 2: JC (first, second, and third positions). Selected models which are not implemented in
128 MrBayes were substituted by the closest over-parameterized model. For ML analyses, we
129 performed ten independent runs in GARLI 2.0 for both genes (Zwickl 2006); the topology
130 and $-\ln L$ were the same among replicates. Only the run with the highest likelihood was saved.
131 We assessed statistical significance of nodes with 10,000 nonparametric bootstrap replicates.
132 We considered a topology well supported (high confidence) when the bootstrap value (branch
133 supports) was higher than 85% (Hillis & Bull 1993). We carried out MB analyses with
134 MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). We achieved ten independent analyses for
135 each gene (100 million generations, four chains with mixed models, default priors, saving trees
136 every 100 generations). Then we removed the first ten million generations as burn-in procedure.
137 A majority-rule 50% consensus tree was constructed. Only branch supports (topologies) with
138 high posterior probabilities (≥ 0.95) were considered statistically significant (Wilcox *et al.*
139 2002). Trees were rooted on *B. terrestris* (outgroup species). For genetic analyses, we used
140 clustering computers provided by the *Consortium des Équipements de Calcul Intensif* (CÉCI,
141 F.R.S.-FNRS).

142 To recognize a species threshold, we used a Bayesian implementation of the general
143 mixed Yule-coalescent model (bGMYC) based on COI tree (Reid & Carstens 2012, see Lecocq
144 *et al.* 2015c). These analyses were performed with "bGMYC" R packages (Reid & Carstens
145 2012). A range of probabilities > 0.95 was considered as strong evidence that taxa were
146 conspecific while a range of probabilities < 0.05 suggested that taxa were heterospecific (Reid
147 & Carstens 2012). We performed a phylogenetic analysis with BEAST 1.7.4 (Drummond *et al.*
148 2012) to generate ultrametric trees using a phylogenetic clock model to generate a posterior
149 distribution of trees (length of the MCMC chain: 100 million generations). The first million
150 sampled trees were treated as burn-in, using the maximum clade credibility method and setting
151 the posterior probability limit to 0. We based the bGMYC analysis on 1000 trees sampled every
152 10,000 generations. For each of these 1000 trees, the MCMC was made of 100,000 generations,
153 discarding the first 90,000 as burn-in and sampling every 100 generations. Posterior probability
154 distributions have been applied against the first sample tree.

155 *Reproductive trait differentiation*

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

167 We extracted CLGS in 400 µl of n-heptane, according to the method described by De
168 Meulemeester *et al.* (2011). Samples were stored at -40° C prior to the analyses. For *B.*
169 *lapponicus*, *B. monticola*, and *B. bimaculatus*, the data of CLGS compositions are the same as
170 those described by Martinet *et al.* (2017) (Table S2). For *B. terrestris*, we used the CLGS dataset
171 described in Lecocq *et al.* (2016).

172 The qualitative composition of the CLGS was determined by gas chromatography-mass
173 spectrometry using a Finigan GCQ quadrupole system (GC/MS) with a non-polar DB-5 ms
174 capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m;
175 inner diameter 0.25 mm; film thickness 0.25 µm]. All samples of CLGS were quantified with a
176 gas chromatograph Shimadzu GC-2010 system (GC-FID) equipped with a non-polar SLB-5 ms
177 capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30 m;
178 inner diameter 0.25 mm; film thickness 0.25 µm] and a flame ionization detector. The
179 composition of CLGS was analyzed according to the protocol described in Lecocq *et al.*
180 (2015c). All compounds for which the relative abundance was recorded as less than 0.1% for
181 all specimens were excluded from the analysis (De Meulemeester *et al.* 2011). The data matrix
182 for each taxon was based (Table S2) on the alignment of each relative proportion of compound
183 between all samples performed with GCAAligner 1.0 (Dellicour & Lecocq 2013a,b). To facilitate
184 the alignment of compounds and the identification, before each sample injection, a standard
185 mixture of alkenes (Kovats) from C10 (decane) to C40 (tetracontane) was injected. We
186 calculated Kovats indices with GCKovats 1.0 according to the method described by Dellicour
187 & Lecocq (2013a, b).

188 *Statistical analyses*

189 We performed statistical comparative analyses of the CLGSs using R 3.3.2 (R
190 Development Core Team, 2016) to detect CLGS differentiations. We transformed data [$\log(x$
191 $+ 1)$] to reduce the great difference of abundance between highly and lowly concentrated
192 compounds. We used a principal component analysis (PCA, R-package MASS, Venables &
193 Ripley 2002) based on correlation distance matrices, and a clustering method computed with
194 the unweighted pair-group method with average linkage (UPGMA) based on Canberra distance
195 matrices (RA of each compound) (R package ape; Paradis *et al.* 2004). We assessed the
196 uncertainty in hierarchical cluster analysis using p-values calculated by multiscale bootstrap
197 resampling with 100,000 bootstrap replications (significant branch supports >0.85) (R package
198 pvclust, Suzuki & Shimodaira 2011). We also assessed CLGS differentiations between taxa by
199 performing a multiple response permutation procedure (MRPP, R package vegan, Oksanen *et*
200 *al.* 2011) based on groups identified by hierarchical cluster analysis. When a significant
201 difference was detected, pairwise multiple comparisons were performed with an adjustment of
202 p-values (Bonferroni correction) to avoid type I errors. To determine specific compounds of
203 each taxon (i.e. indicator compounds), the indicator-value (IndVal) method was used (Clausen
204 *et al.* 2006; Dufrêne & Legendre 1997). This value is the product of relative concentration and
205 relative occurrence frequency of a compound within a group. The statistical significance of an
206 indicator compound (>0.7) was evaluated with a randomization procedure (Dufrêne &
207 Legendre 1997).

208 *Data integration and decision framework*

209 We based our species delimitation hypothesis on the method performed by Lecocq *et*
210 *al.* (2015a), derived from the integrative approach established by Schlick-Steiner *et al.* (2010)
211 according to the unified species concept (De Quieroz 2007). With our approach, criteria are not
212 balanced, and the assignment of species status is allocated by unanimity of all criteria to avoid
213 species over-estimation (Padial *et al.* 2010; Schlick-Steiner *et al.* 2010). The specific status was
214 assigned if this taxon: (i) was genetically differentiated in all genetic markers (i.e. potential
215 unique haplotypes), (ii) constituted a monophyletic group with high branch support, and (iii)
216 was significantly differentiated in CLGS compositions (including IndVal indicator compounds,
217 MRPP test, bootstrap values > 0.85). This conservative approach could lead to underestimation
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

222 the risk of underestimating taxonomic diversity (Hawlitschek *et al.* 2012; Ennen *et al.* 2014;
223 Lecocq *et al.* 2015a, c).

224 *Geometric morphometrics*

225 As fresh material of *B. gelidus* was not available for molecular and chemical analyses,
226 we run an additional study to test the similarity between *gelidus* type material and other
227 *lapponicus* group taxa. The right forewings of 44 queens were photographed using an Olympus
228 SZH10 microscope an AF-S NIKKOR 18-105 millimetres (Shinjuku, Japan) and GWH10X-
229 CD oculars coupled with a Nikon D200 camera: 39 queens of *B. lapponicus* (including
230 specimens of *B. lapponicus lapponicus* (n = 14) and *B. lapponicus sylvicola* (n = 29)) and 1
231 queen of *B. gelidus* (holotype). For *B. gelidus*, in the *Smithsonian National Museum of Natural*
232 *History, Massachusetts Agricultural College and United States National Museum*, there are 1
233 queen, 14 workers and 1 male labelled “Cotype” by Franklin (1912). All these specimens should
234 not be part of the typical series and have been erroneously labeled. These specimens were
235 collected later and in other areas than the only holotype described by Cresson (1878). After
236 examination, we consider that these specimens belong to *B. lapponicus sylvicola*. All easily
237 available material has been evaluated, including specimens from the Aleutian Islands. We have
238 revised the type series, including the "false type anachronic inclusion". Wing shapes were
239 captured by digitizing two-dimensional Cartesian coordinates of 18 landmarks (Fig. S2) on
240 wing veins with tps-DIG v2.17 (Rohlf 2013a, b). The landmark configurations were scaled,
241 translated, and rotated against the consensus configuration using the GLS Procrustes
242 superimposition method to remove all non-shape differences and to separate the size from shape
243 components of the form (Rohlf & Slice 1990; Bookstein 1991). The superimposition was
244 performed using R functions of the package geomorph (Adams & Otárola-Castillo 2013). Each
245 wing was digitized twice by the same experimenter (MG), to account for measurement error.
246 The aligned landmark configurations were projected into the Euclidean space tangent to the
247 curved Kendall's shape space to aid further statistical analyses. The correlation coefficient
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
250 was negligible (Rohlf 1999). The least-squares regression slope through the origin (0.999) and
251 the correlation coefficient between the two distances were calculated with tps-SMALL v1.25
252 (Rohlf 2013c).

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

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

257 Prior to the assignment of the holotype queen *B. gelidus*, shape variation within the
258 reference dataset and discrimination of the different taxa was assessed by Linear Discriminant
259 Analyses (LDA) of the projected aligned configuration of landmarks. These analyses were
260 performed at species level as a priori grouping by using the software R version 3.0.2. The
261 effectiveness of the LDA for discriminating taxon was assessed by the percentages of
262 individuals correctly classified to their original taxon (hit-ratio, HR) in a leave-one-out cross-
263 validation procedure based on the posterior probabilities of assignment. Given the observed
264 scores of an “unknown”, the posterior probability (pp) equals the probability of the unit to
265 belong to one group compared to all others. The unit is consequently assigned to the group for
266 which the posterior probability is the highest (Huberty & Olejnik 2006). Taxonomic affinities
267 of the holotype queen *B. gelidus* were first assessed based on their score in the predictive
268 discriminant space of shapes. After superimposition of the landmark configurations, aligned
269 coordinates of the specimens from the reference dataset were used to calculate the LDA. A
270 unique superimposition of both the reference dataset and the assigned specimens is sometimes
271 disregarded while it is of primary importance because GLS Procrustes superimposition is
272 sampling dependent. We included *a posteriori* the holotype queen *B. gelidus* in the computed
273 LDA space as “unknown” specimen and calculated their score. Assignments of the holotype
274 *B. gelidus* were estimated by calculating the Mahalanobis Distance between “unknown” and
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*

279 A total of 938 bp from the COI gene and 925 bp from PEPCK were obtained. All
280 phylogenetic analyses (ML and MB) on each genetic dataset showed a similar topology and
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
283 *lapponicus-sylvicola* group resulted in two lineages: (a) one comprising all *lapponicus* and a
284 part of *sylvicola* specimens (group A, 1.92% of divergence between COI sequences) and (b) a
285 second lineage comprising the second part of *sylvicola* (group B) (Fig. 2). While genetic
286 analyses based on the mitochondrial gene COI showed a slightly supported divergence between
287 *B. lapponicus* and *B. sylvicola* group A (Fig. 2B), there was no differentiation between these
288 two taxa for the nuclear marker PEPCK (Fig. 2A). In COI sequences, 18/938 (1.92%) of

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

293 Our genetic analyses revealed a new cryptic taxon from Alaska in our *sylvicola* samples
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
296 both COI (8.74 % of sequence divergence from *sylvicola* part A and 9.80% from *monticola*)
297 and PEPCK (> 1% of divergence from *monticola* and *sylvicola*) analyses. Contrary to the COI
298 marker, the phylogenetic affinities inside the group including *interacti* sp. nov., *monticola*, and
299 *konradini* were not resolved with the nuclear PEPCK fragment (Fig. 2A). PEPCK and COI
300 sequences of *B. interacti* sp. nov. have been blasted to NCBI Genbank database. Sequences
301 matched most closely to the studied species complex *lapponicus-sylvicola-monticola* but with
302 no complete identity (99% of identity and 100% of query cover for PEPCK, 96% of identity
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
305 supports and posterior probabilities, Fig. 2A, 2B).

306 The bGMYC analysis (Fig. 3) highlighted nine entities with low probabilities (<0.05)
307 to be conspecific with the other ones. These results matched with results from the phylogenetic
308 analyses of COI gene (ML, MB analyses). Overall, the bGMYC suggested the delimitation of
309 seven prospective species ($p < 0.05$): (i) *B. terrestris* (bGMYC conspecificity probabilities
310 between individuals included in the group, $p > 0.12-1$), (ii) *B. ehippiatus* ($p > 0.97-1$), (iii) *B.*
311 *interacti* sp. nov. ($p > 0.98-1$), (iv) *B. monticola* ($p > 0.47-1$), (v) *B. bimaculatus* ($p > 0.89-1$),
312 (vi) *B. konradini* ($p > 0.99-1$), (vii) a group with *sylvicola* and *lapponicus* ($p > 0.07-1$), (viii) a
313 group with *B. glacialis* and (ix) a group with *B. melanopygus*. The pairwise matrix (Fig. 3)
314 showed a non-significant heterospecificity threshold between *lapponicus* and *sylvicola* ($p >$
315 0.05).

316 *Chemical analysis*

317 A total of 134 compounds were detected in the CLGS of the different studied species:
318 60 identical compounds were detected and shared by *B. sylvicola* (group A) and *B. lapponicus*,
319 57 compounds for *B. monticola*, 39 compounds for *B. bimaculatus*, 50 compounds for *B.*
320 *konradini*, 25 for *B. terrestris*, 45 compounds for *B. ehippiatus*, 64 compounds for *B. interacti*
321 sp. nov. (= *B. sylvicola* group B). Our chemical analyses showed qualitative and quantitative
322 differentiations between all taxa including specific main compounds except between *B.*

323 *lapponicus* and *B. sylvicola* group A where the CLGS composition was statistically identical
324 (Appendix S2). Chemical analyses supported the presence of another taxon in the *sylvicola*
325 samples from Alaska (*B. interacti* sp. nov.).

326 The main compounds detected were (a) the geranyl citronellol (55.28% - 77.30%)
327 shared by *B. sylvicola* and *B. lapponicus*; (b) the hexadec-9-enyl acetate (45.91% - 61.74%) for
328 *B. monticola* and *B. konradini* (48.36%-54.71%); (c) Ethyl octadec-9-enoate for *B. konradini*
329 (7.43%-9.14%); (d) the hexadec-9-enyl acetate (20.55% - 40.63%) and the geranyl geranyl
330 acetate (25.98% - 39.42%) for *B. bimaculatus*, (e) the dihydrofarnesol (19.08% - 40.45%) for
331 *B. terrestris*, (f) the hexadecanoic acid (19.03%-31.63%) for *B. ehippiatus*, and (g) the
332 citronellyl hexadec-9-enoate (12.37% - 23.57%) for *B. interacti* sp. nov. (Tables 1 and S2).
333 Statistical analyses supported the differentiation (MRPP, A= 0.6973, T= 0.1759, all $p < 0.001$)
334 of seven groups also supported by high multiscale bootstrap resampling values (Cluster and
335 ACP Fig. 4): (a) *B. monticola* (pairwise test, $p < 0.01$), (b) *B. konradini* (pairwise test, $p < 0.01$)
336 (c) *B. bimaculatus* (pairwise test, $p < 0.01$), (d) *B. ehippiatus* (pairwise test, $p < 0.01$), (e) *B.*
337 *terrestris* (pairwise test, $p < 0.01$), (f) *B. sylvicola* group A + *B. lapponicus* (pairwise test, $p <$
338 0.01), and (g) *B. interacti* sp. nov. (pairwise test, $p < 0.01$). No statistical differentiation was
339 found in the statistical hypothesis test and in hierarchical clustering between *lapponicus* and
340 *sylvicola* group A (Fig. 4; MRPP, A= 0.004641, T= 0.1577, $p = 0.30$). Several significant and
341 specific indicator compounds were revealed by the IndVal method (IndVal > 0.70), but no
342 compound has been identified to discriminate *B. lapponicus* to *B. sylvicola* (Table S2).

343 *Wing size and shape analyses*

344 No significant difference in centroid size was found among the different taxa ($F = 2.73$;
345 p -value = 0.08). However, significant differences in wing shape were found among the different
346 taxa (perMANOVA, $F = 1.83$; p -value = 0.006). Pairwise perMANOVA tests have shown a
347 significant difference between *B. lapponicus* and *B. interacti* sp. nov. ($F = 2.44$; p -value =
348 0.004) and between *B. interacti* sp. nov. and *B. gelidus* ($F = 1.96$; p -value = 0.035) while no
349 difference was detected between *B. lapponicus* and *B. gelidus* ($F = 1.21$; p -value = 0.32). PCA
350 plot highlights two distinct groups (Fig. 5): (i) one cluster gathering specimens of *B. lapponicus*,
351 *B. sylvicola* and *B. gelidus*, (ii) a second cluster with specimens of *B. interacti* sp. nov. The two
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). A-
355 posteriori assignment of the holotype *B. gelidus* in the discriminant shape space (LDA) allows

356 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*

359 For this morphological comparison, we assess only males and queens (minimum 15
360 individuals per taxon according to the availability of specimens). Except for the coloration of
361 the face which is black for *lapponicus* and yellow for *sylvicola*, no diagnostic character was
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
364 Alaska (corresponding to *sylvicola* part A and *sylvicola* part B (= *Bombus interacti* sp. nov.) in
365 our molecular analyses) that could be separated by several diagnostic characters. *Bombus*
366 *interacti* sp. nov. males differed from *sylvicola* in the pubescence of the tibia which is hairier
367 for *sylvicola* (Fig. 6). No difference in the genitalia's structures was detected. For female, *B.*
368 *interacti* sp. nov. differed from *sylvicola* in the face clypeus coloration: black with intermixed
369 dark yellow hair for *interacti* sp. nov. and yellow for *sylvicola*. Besides, the density of
370 pubescence of the tergite 5 is higher for *B. interacti* sp. nov. and the yellow coloration of collar
371 not reaching the bases of the legs. (Fig. 6). Moreover, the morphological character "shape and
372 pubescence of basitarsus", used by Gjershaug *et al.* (2013) to distinguish *B. lapponicus* from *B.*
373 *monticola*, does not allow distinction of *B. interacti* sp. nov. from *B. monticola* and this
374 character is similar between *lapponicus* and *sylvicola*. From *B. glacialis*, females of *B. interacti*
375 sp. nov. differ for several characters: (i) labral furrow (narrow for *B. interacti* sp. nov., broad
376 for *B. glacialis*); (ii) punctuations into the labral furrow (very few in *B. glacialis*); (iii) dorsal
377 furrow of gena which is weakly developed in *B. glacialis*. Males of *B. interacti* sp. nov. differ
378 from *B. glacialis* for: (i) the color of the vertex and the clypeus (yellow for *B. glacialis*); (ii)
379 hind basitarsus (gradually narrowing towards basal part for *B. glacialis*); (iii) punctuations into
380 the labral furrow (very dense for *B. interacti* and labrum covered with reddish bristles at the
381 front part).

382

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

385 Family APIDAE Latreille, 1802

386 GENUS *BOMBUS* Latreille, 1802

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

388 2018

389 *Synonyms:* None

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

392

393 *Description:*

394 Females. Length 15-18 mm.

395 Coat color. Face and vertex densely pubescent with black hairs intermixed with few yellowish-
396 greyish hairs. Thorax with a collare as large as 1/3 of the thorax length, with few intermixed
397 black hairs at front of tegulae; scutellare as large as 1/4 of the thorax length. The hairs of the
398 scutellare are shaped in two oblique tufts. Pleura covered with greyish hairs on the anterior
399 third, intermixed with black in front of the tegulae; mesopleura with intermixed grey and black
400 hairs; metapleura are mostly black. Wings are not particularly dark (contrary to *B. gelidus*). T1
401 mostly covered with grayish hairs, intermixed with black hairs in the middle. T2 covered with
402 red hairs, intermixed with few black ones on the sides and with numerous grayish ones at the
403 middle of anterior margin. T3 all red with few grayish hairs at the middle of posterior margin.
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
406 with black hairs and some grayish ones on the sides. Coxae and femurs with black hairs
407 intermixed with very few greyish ones. Mesotibias with black hairs some of them with red tip.
408 Metatibias with corbiculae surrounded by decumbent bristles slightly longer than the width of
409 the organ, mostly reddish with light blond tip and some whole black at the base of anterior
410 margin, meso- and metabasitarsus with short reddish bristles. Distal tarsi red. Otherwise black.
411 Labrum with a narrow labral furrow as wide as 0.23 times its total width, V-shaped. The labral
412 tubercles are well designed. There is an imbricated microsculpture in front of the tubercles
413 and into the labral furrow. Punctuations are very dense into the labral furrow and more spaced
414 back to the tubercles. The front part of the labrum is covered with plumose reddish bristles.
415 Basis of mandibulae with numerous punctuations, very dense at the base between the condyle.
416 Clypeus slightly bombed, very covered with black plumose bristles at the distal part, and short
417 reddish plumose ones in the middle, along the anterior edge. There is a narrow glabrous area in
418 the middle of the anterior third. This area is covered with deep and broad punctuations. These
419 punctuations are joining at the side of the frontal part and more spaced in the middle. There is
420 a thin band of microsculpture 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.

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

433 Coat color. Males are greyish and shaggy. Face and vertex largely covered by yellow hairs and
434 a slight mixture of black and yellow hairs on vertex. Thorax with a collare as large as 1/3 of the
435 thorax length, with yellow hairs; scutellare with yellow hairs as large as 1/4 of the thorax length.
436 The hairs of the scutellare are shaped in two oblique tufts. Pleura covered with yellow hairs;
437 mesopleura with yellow hairs; metapleura are mostly yellow. Inter-alar band is yellow with
438 some intermixed black hairs. In some specimens, the interalar band is attenuated with a mixture
439 of black and yellow hairs. T1 mostly covered with yellow hairs. T2 covered with red hairs,
440 intermixed with some yellow ones on the at the middle of anterior margin. T3 and T4 all red
441 with few grayish hairs at the middle of posterior margin. T5 mostly covered with yellow hairs,
442 with very few red hairs at the anterior margin and black ones in the middle. T6 with greyish
443 hairs with numerous black ones in the middle. T7 mostly with black hairs and some grayish
444 ones on the sides. Coxae and femurs with mostly yellow hairs intermixed with very few black
445 ones. Mesotibias with yellow hairs some of them with reddish base with few intermixed black
446 hairs. Metatibias with corbiculae surrounded by decumbent bristles slightly longer than the
447 width of the organ, mostly reddish with light blond tip and some whole black at the base of
448 anterior margin, meso- and metabasitarsus with short reddish bristles. Distal tarsi red.
449 Otherwise black.

450 Labrum with a narrow labral furrow as wide as 0.21 times its total width, V-shaped. The labral
451 tubercles are well designed. Punctuations are very dense into the labral furrow and more spaced
452 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
459 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*

465 *Bombus interacti* sp. nov. males differed from *B. lapponicus sylvicola* slightly in the pubescence
466 of the tibia (very hairy for *B. lapponicus sylvicola*). No difference in the structure of the
467 genitalia was found. For female, *B. interacti* sp. nov. differed from *B. lapponicus sylvicola* in
468 the face coloration: black with a few intermixed yellow hairs for *B. interacti* sp. nov. and yellow
469 with few intermixed black hairs for *B. lapponicus sylvicola*. The density of pubescence of
470 tergite 5 is greater for *B. interacti* sp. nov. and the yellow coloration of collar does not extend
471 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
473 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
476 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".

480 *Remarks*: Considering morphology, genetic and the semio-chemical secretions, there is no
481 available name to describe our new taxon. However, there are some uncertainties about the
482 taxon *B. gelidus* (Fig. 7) described by Cresson (1878) and Franklin (1912), considered as a
483 subspecies of *B. lapponicus sylvicola* by Pittioni (1943). The morphological description of
484 Cresson (1878) is very poor and not sufficient to compare with our specimens. This description
485 is based on one queen from Aleutian Islands (Henry Edwards). This specimen is described as
486 black, with a long and loose pubescence; with a slight admixture on face and vertex. Sides of
487 thorax, scutellum, and first and fourth segments of abdomen are described as pale yellow and
488 the second and third segments mostly fulvo-ferruginous, mixed with black on middle and sides.
489 The clypeus is sparsely punctured, labrum with fulvous hair and wings are dark and stained. In
490 the re-description of Franklin (1912), *B. gelidus* is described as very closely allied to *B.*
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*. For
493 males, coxae, trochanters and femora are characterized by a large amount of pale yellow pile.

494 According Franklin (1912), no difference in structure between *B. gelidus* and *B.*
495 *lapponicus sylvicola* could be found except slight differences in coloration and these taxa
496 should be considered as conspecific. The queen holotype from the Academy of Natural Sciences
497 (Philadelphia) and three co-types workers (from the Smithsonian National Museum of Natural
498 History) have been examined for this study. However, for these old specimens only
499 morphological traits are available to compare with our specimens. The morphological
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
503 middle and on the sides), the shape of basitarsus (*sensu* Gjershaug et al. 2013) (Table 2), the
504 density of hairs on the collar for females and the pubescence of the tibia for males. The workers
505 of the type series that we examined are not different from *B. lapponicus sylvicola*. There is no
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
508 their taxonomic assignment. In the light of our wing morphometric analysis and the lack of
509 strong taxonomic evidence (genetic, semio-chemical) and that *B. gelidus* has been described
510 only from the Aleutian Islands, we hypothesize that *B. gelidus* is different from *B. interacti* sp.
511 nov. and we describe this latter taxon as new species. Our wing morphometric geometric
512 analysis show that the holotype of *gelidus* is assigned to *B. lapponicus*. Even if the definitive
513 status of *B. gelidus* remains unsettled, as far as we can understand now, after revision the
514 holotype, the taxon described here as *B. interacti* sp. nov. is unlikely to be conspecific with
515 *gelidus*. Nine males and three queens, based on chemical, genetic and morphological analyses,
516 and seven other males based only on morphological characters, are considered to belong to
517 *interacti* sp. nov. No variation in color pattern has been observed within the taxon except the
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
522 using (1) analysis of a mitochondrial gene (COI) and a nuclear gene (PEPCK), (2) analysis of
523 the cephalic labial gland secretions, (3) complete morphological examination.

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

525 *Distribution:* *B. interacti* sp. nov. was found higher latitudes in the arctic tundra habitat near
526 Toolik field stations in Alaska (USA) (68°37' – 68°46'N 149°35' – 149°56' W). The available
527 data are not sufficient to draw up a distribution map.

528 *Etymology:* The specific name was chosen in reference to the INTERACT project which funded
529 most of our sampling costs allowing us to discover this taxon.

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

534 *Bombus lapponicus sylvicola* f. *gelidus* Cresson 1878 **comb. nov.**

535 *Holotype.* One queen pinned (Fig. 7). Labels: (1) red, printed with “HoloTYPE 2638”; (2)
536 white, written with ‘aleutian Islds Dau; (3) red, printed with “HOLOTYPE”; (4) white, printed
537 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**

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

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

559 we cannot exclude that northern and southern populations are two different lineages considering
560 the taxonomic ambiguities present in this group. Koch *et al.* (2017), showed that in its
561 distribution, *sylvicola* display different allelic diversity and they emphasize different genetic
562 cluster (population genetic structure differentiation). As suggested in Cameron *et al.* (2007),
563 the PEPCK gene fragment showed no differentiation between *B. lapponicus lapponicus* and the
564 north population of *B. lapponicus sylvicola* while COI fragment showed a low divergence (Fig.
565 2). This could reflect a geographic intraspecific variability (Andriollo *et al.* 2015; Mutanen *et*
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*
568 and *B. lapponicus sylvicola* (intra-specific variability) and suggest that these taxa are
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*
572 from Alaska and Yukon. However, the reinforcement of reproductive barriers process could not
573 be exerted on the CLGS between allopatric species. Overall, as there is no divergence in nuclear
574 gene, in morphology (structure of the genitalia) and in CLGS, we can expect that there is no
575 reproductive barrier between the populations. Poor quantitative differences observed could just
576 reflect a “dialect divergence” due to the geographical gap between these two sampled
577 populations (Lecocq *et al.* 2013b) or chemical background noise.

578 Moreover, our integrative framework (Table 3) highlights an unknown species from
579 Alaska in the *lapponicus*-group supported by all our independent criteria: *B. interacti* sp. nov.
580 (Table 3). Morphological, genetic and semio-chemical datasets support the presence of two
581 biologically distinct taxa within *sylvicola* sampling from Alaska. CLGS results strongly support
582 also the new species *B. interacti* sp. nov. with different major and indicator compounds from
583 *monticola*, *konradini*, *lapponicus lapponicus* and *lapponicus sylvicola*. Although we have a
584 restricted sampling and different tree topologies between the two genetic markers, nuclear gene
585 analysis suggests that *B. interacti* sp. nov. is closer to *B. monticola* (also consistent with the
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
589 contraction following glaciations and the dynamic of Bering Strait (Abbott and Brochmann
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

592 of this taxon given that all data support this divergence. Further inter-population genetic
593 analyses 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

598 Based on morphological characters and the coat coloration, Skorikov (1922) and Pittioni
599 (1942) had already hypothesized that there is a set of conspecific taxa related to *B. lapponicus*-
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
605 cephalic labial gland secretions. Our results based on the sampling of five distant populations
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
609 subspecies in the polar portion of its distribution. Moreover, Potapov et al. (2017) have shown
610 the conspecificity of specimens of *lapponicus* from Norway, Kamchatka, Yamal and Chukotka
611 based on COI analysis. These results confirm our hypothesis: *B. lapponicus* is found across
612 northern Holarctic regions, including a circumpolar distribution and exhibit subspecific
613 differentiation across at least the polar section of its distribution. The absence of differentiation
614 in CLGS and genetic analyses across the Holarctic region suggest that there is no isolation
615 mechanism between any *lapponicus* populations. These taxa do not seem to be involved in an
616 *Artenkreis* speciation process *sensu* Rensch 1933.

617

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

911 **Figure 2.** A) Majority rule (50%) consensus tree based on maximum likelihood analyses of
912 nuclear PEPCCK marker. B) Majority rule (50%) consensus tree based on maximum likelihood
913 analyses of mitochondrial COI marker Values above branches are maximum likelihood
914 bootstrap values/Bayesian posterior probabilities.

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

921 **Figure 4.** A) Dendrogram based on cephalic labial gland secretions within *Bombus lapponicus*
922 + *B. sylvicola* (light blue), *B. monticola* (green), *B. bimaculatus* (black), *B. interacti* sp. nov.
923 (red), *B. terrestris* (pink), *B. ephippiatus* (dark blue) and *B. konradini* (orange). This cluster
924 was obtained by hierarchical clustering using an unweighted pair-group method with arithmetic
925 mean (UPGMA) based on a Canberra matrix calculated from the cephalic labial gland secretion
926 matrix. The values near nodes represent multiscale bootstrap resampling values. B) Principal
927 component analysis (PCA) of cephalic labial gland secretion differentiation within *lapponicus-*
928 *sylvicola* complex: *Bombus lapponicus* + *B. sylvicola* (light blue), *B. monticola* (green), *B.*
929 *bimaculatus* (black), *B. interacti* sp. nov. (red), *B. terrestris* (pink), *B. ephippiatus* (dark blue)
930 and *B. konradini* (orange). PC1 and PC2 are the first and second principal component axes.

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

937 **Figure 6.** Photos of the different morphological diagnostic characters between *sylvicola* and
938 *interacti* sp. nov.: A. Face of *B. interacti* female BMAR0892, B. Face of *sylvicola* female
939 BMAR0900, C. Right profile of *B. interacti* female BMAR0892 with the yellow coloration of
940 collar that does not go down until leg insertion, D. Right profile of *B. sylvicola* female
941 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.*
944 *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 Dederling,
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. interdicti* 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
 960 available in Appendix S2.

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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%

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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 sp. nov.</i>	<i>lapponicus sylvicola</i>	<i>lapponicus lapponicus</i>	<i>monticola scandinavicus</i>	<i>konradini</i>	<i>lapponicus sylvicola f. gelidus comb. nov.</i>
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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970 Table 3. Taxonomic decision table with all criteria used for species delimitation. CLGS,
 971 cephalic labial gland secretions; COI, cytochrome oxydase 1; bGMYC, the general mixed Yule-
 972 coalescent model, PEPCK, phosphoenolpyruvate carboxykinase. Morphology indicates if a
 973 taxon has a diagnostic morphological character (+/- means that morphology is/is not
 974 diagnostic). Wing shape and size indicate if a taxon has a diagnostic wing shape and size (+/-
 975 means that wing measures are/are not diagnostic). CLGS indicates if the taxon has/has not
 976 diagnostic composition of CLGSs with different main compounds (+/- means that the taxon
 977 has/ has not a specific CLGS composition. When the taxon shares CLGS composition with
 978 other ones, the letters group together taxa that share similar CLGS. Phylogenetic analyses
 979 indicate if a taxon forms a strongly supported monophyletic group (+/- means that the taxon
 980 is/is not a monophyletic group). When the taxon is not a distinct monophyletic group, the letters
 981 group together taxa included in the same monophyletic group (A). LS= low supported
 982 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.
Unnamed species 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>

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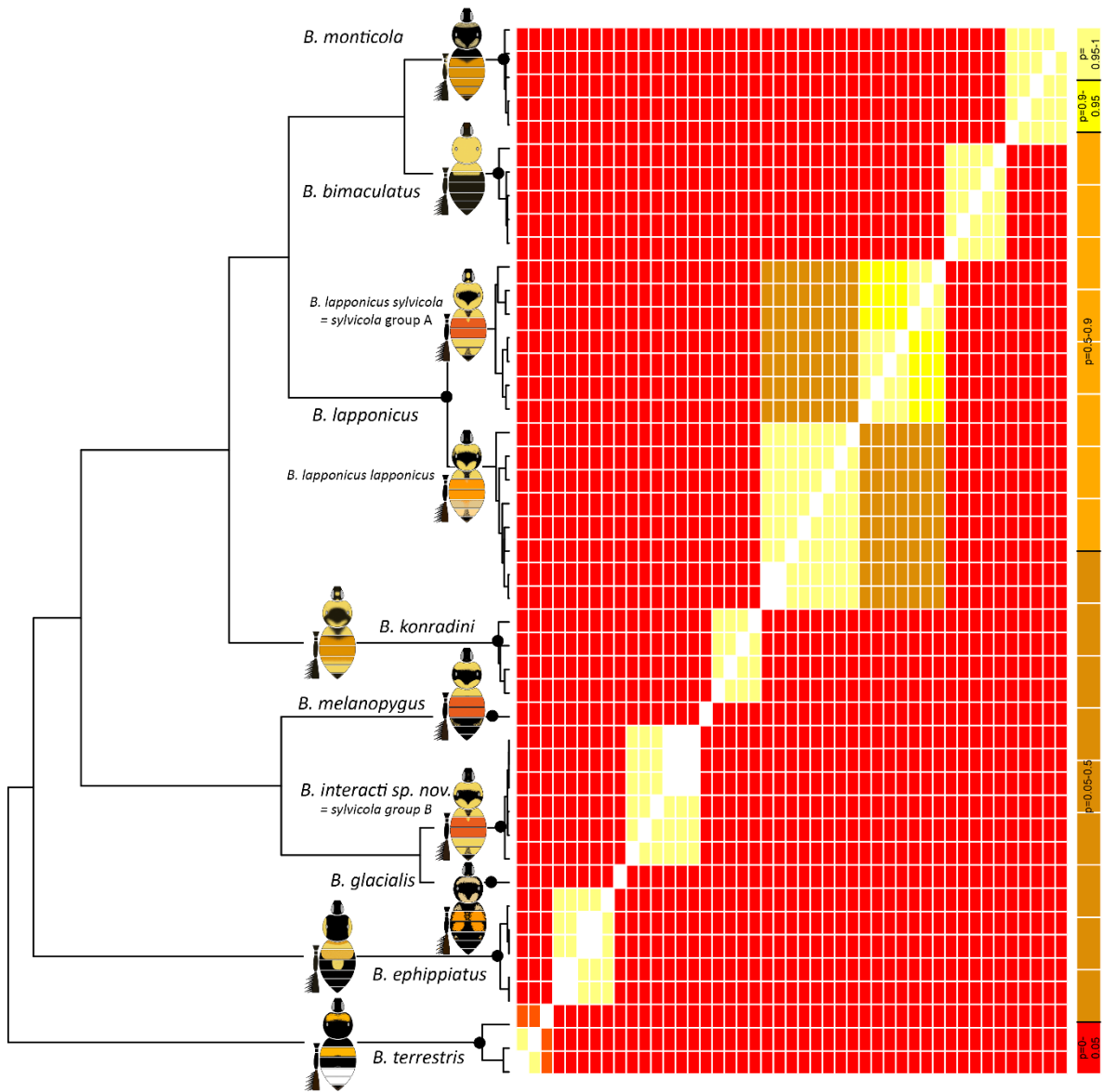
984

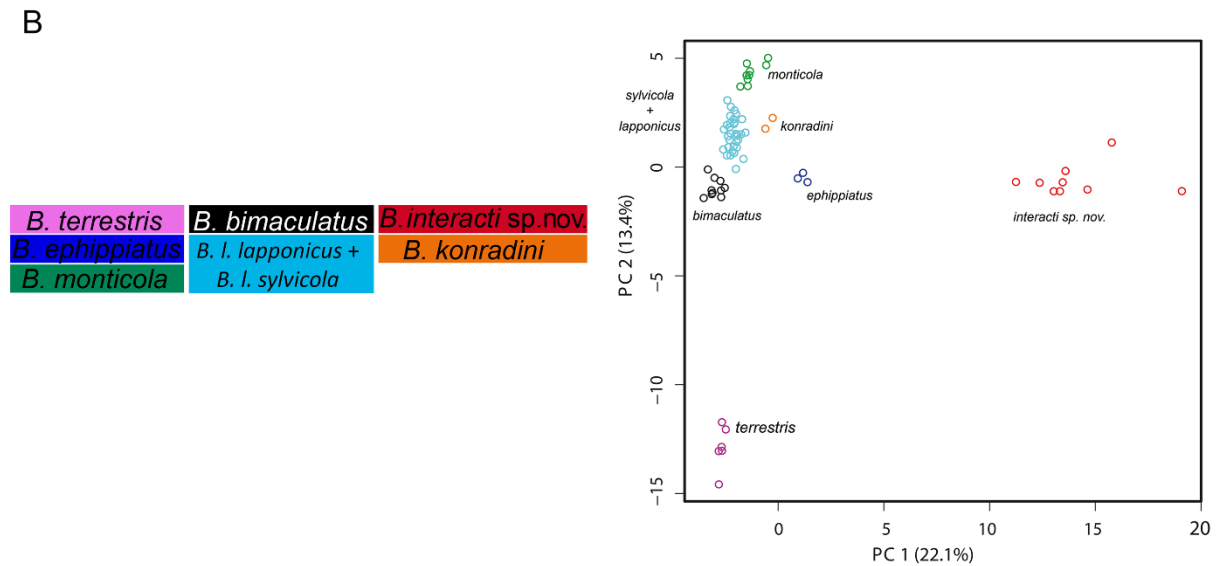
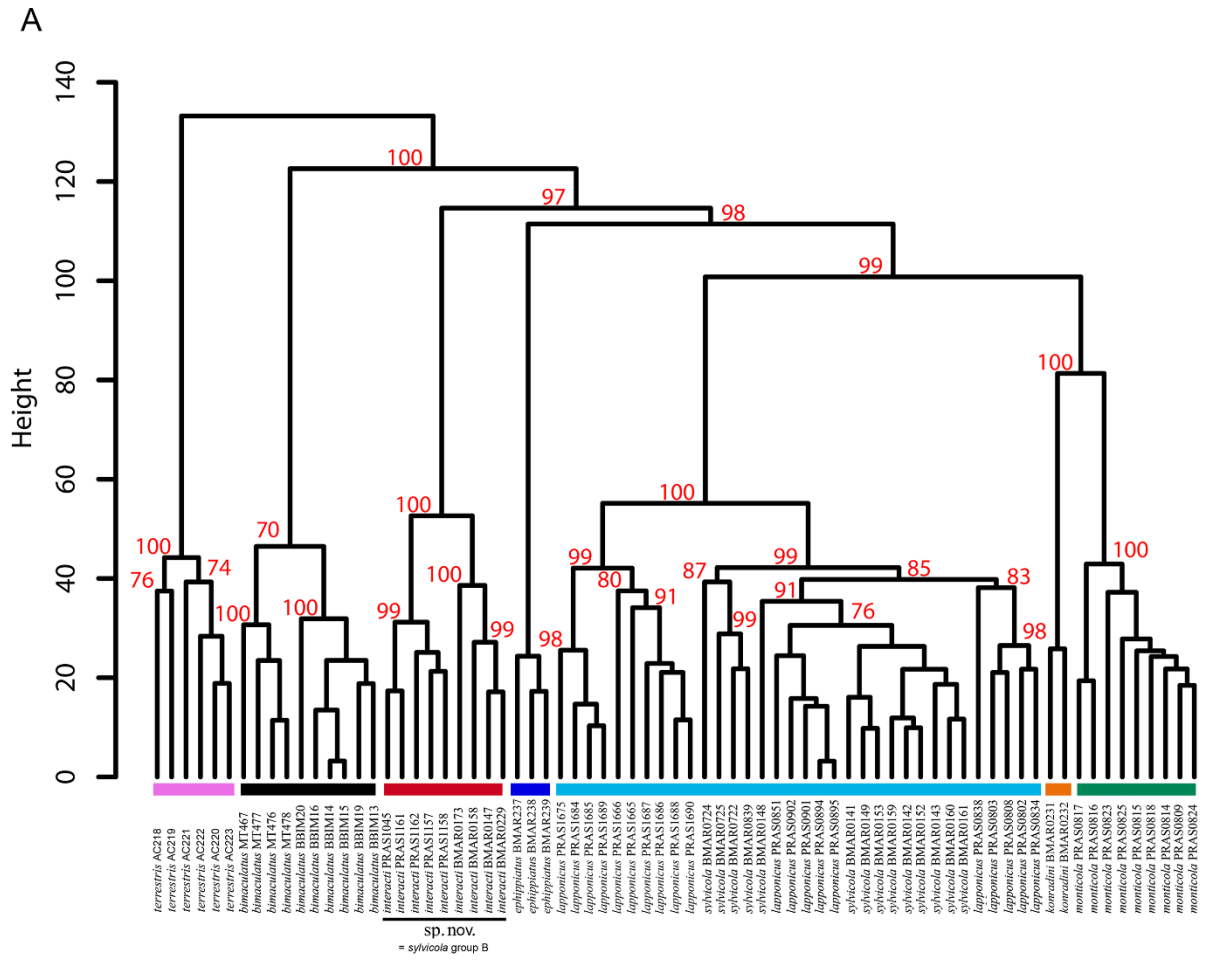
985 **Supporting information**

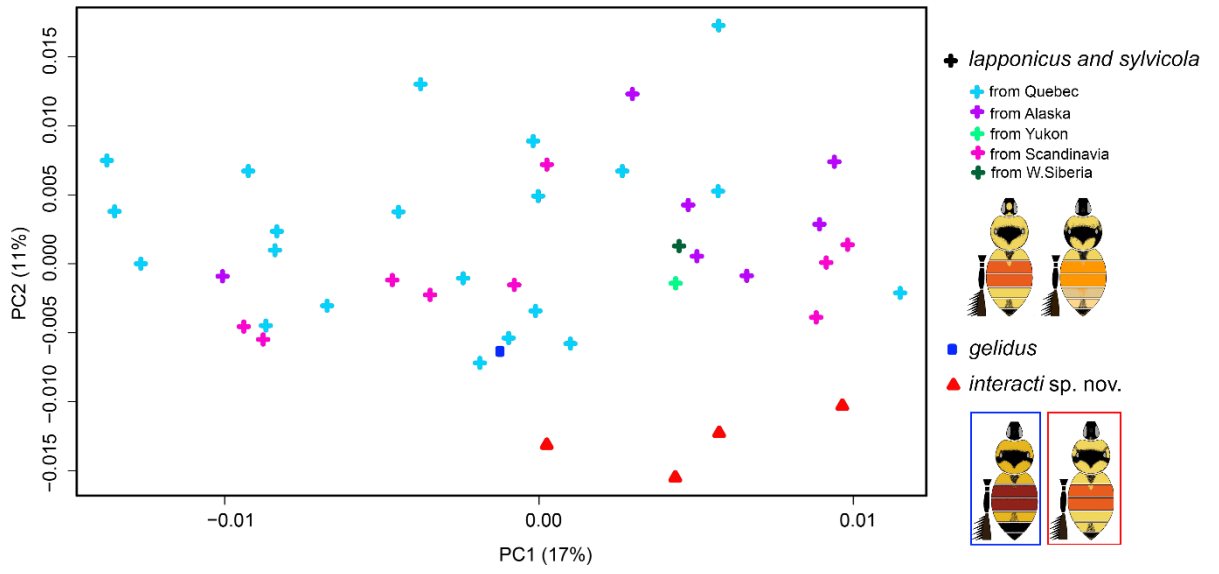
986 Table S1. List of all specimens analyzed. Sample code refers to the sample labels used in
987 different analyses. COI and PEPCK are the GenBank accession numbers for each sample.

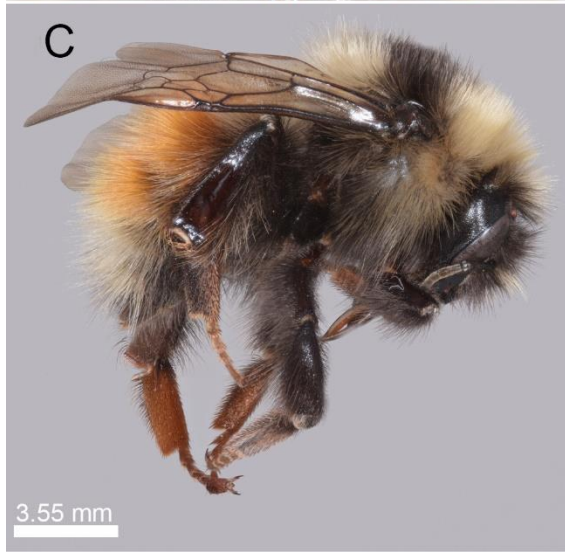
988 Table S2. Summary of data matrix of cephalic labial gland secretions (with minimum, median
989 and maximum of relative concentration of each compound), list of the identified compounds
990 and IndVal analysis with species-specific compounds. Unknown x are undetermined
991 compounds.

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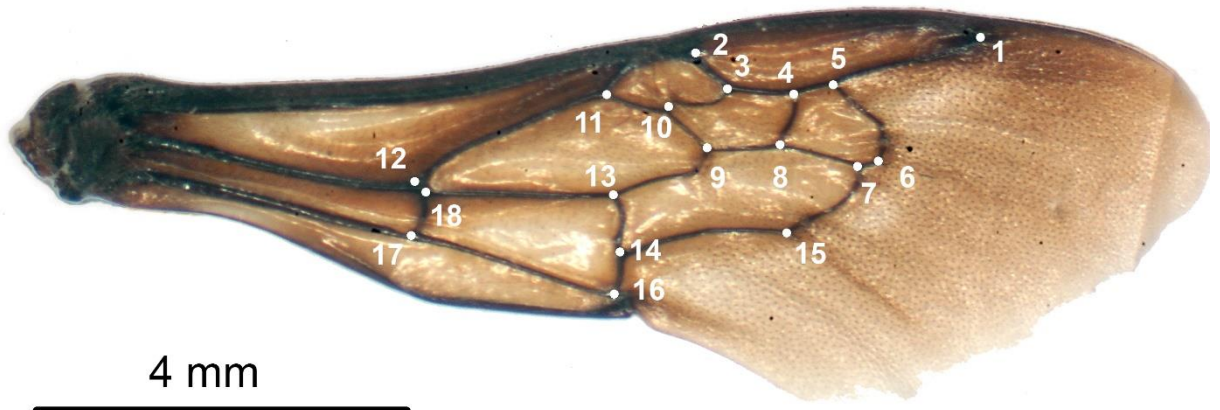


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