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1 The alien's identity: consequences of taxonomic status for the 2 international bumblebee trade regulations

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13 **Short running title:** The alien's identity

14 **Highlights**

15 The international trade fosters non-native taxon invasions

16 Several commonly traded species are taxon complexes

17 Preserving endemic taxa and differentiated populations require trade regulation

18 Such regulations need a resolved taxonomy and population structure

19 Integrative taxonomy provides guidelines for regulation of population importations

20 **Abstract**

21 The species international trade leads to multiple non-native invasions. Beside species invasions, commercial
22 exchanges may also contribute to translocation between closely related taxa or allopatric populations.
23 Consequently, preserving endemic taxa and specificity of local populations require to regulate commercial
24 translocations of species or populations. To be efficient such regulation needs a resolved taxonomy and a
25 thorough analysis of the population structure of native taxa/populations. To provide guidelines for an efficient
26 regulation of the trade of *Bombus terrestris* within its natural range, we analyzed its taxonomy and its population
27 structure using an integrative taxonomic approach. Our results show that *B. terrestris* translocations involve two
28 species, three subspecies, and several populations with weak differentiation. These different levels of
29 differentiation imply specific and appropriate regulations of translocations with different levels of prioritization.
30 We ultimately assess the relevance of current policies and propose potentially efficient regulations for policy-
31 makers. Such integrative taxonomic approach should be used in other traded polytypic species.

32 **Keywords**

33 alien taxa; evolutionary significant units; integrative taxonomy; species international trade; bumblebee

34 **1.Introduction**

35 Invasions by non-native organisms is one of the main causes of the species endangerment throughout the world
36 (Mack et al., 2000). These invasions are facilitated by the globalized trade of many species as food, pets, or
37 biological control agents (Lowry et al., 2012; Perrings et al., 2010). While the invasions by non-native species
38 have been the focus of abundant research (Courchamp et al., 2003; Mooney and Cleland, 2001; Simberloff et al.,
39 2009), anthropogenic translocations between closely related taxa or allopatric populations have received much
40 less attention (but see Rhymer and Simberloff, 1996). Yet, preserving endemic taxa and evolutionarily
41 significant units (ESUs; i.e. allopatric or parapatric populations differentiated in neutral genetic markers and/or
42 displaying locally adapted phenotypic traits) is a key step to preserve biodiversity and to ensure its long-term
43 persistence (Conner and Hartl, 2004; Crowhurst et al., 2011; Frankham et al., 2010; Sgrò et al., 2011). The study
44 of biological invasions resulting from the international trade of *Bombus terrestris* exemplifies this relatively low
45 interest. Since the 1980s, *B. terrestris* hives have been massively produced to improve pollination of many crops
46 (Velthuis and van Doorn, 2006). Each year, more than two million colonies are shipped throughout the world
47 leading to deliberate releases or accidental escapes that foster *B. terrestris* establishments (Goulson, 2010; Inari
48 et al., 2005; Murray et al., 2013; Velthuis and van Doorn, 2006). Most studies have focused on the invasions of
49 *B. terrestris* out of its natural range (e.g. Inoue et al., 2008; Kanbe et al., 2008; Meeus et al., 2011) but not within
50 (but see Ings et al., 2010; Murray et al., 2013). Yet, several *B. terrestris* subspecies (Fig. 1) have been reared and
51 introduced in areas (Velthuis and van Doorn, 2006) where related populations or subspecies already occurred
52 (e.g. *B. terrestris audax* in contact with *B. terrestris dalmatinus* in UK).

53 Preserving endemic taxa and specificity of differentiated populations requires a resolved taxonomy and
54 population structure of native taxa/populations. This implies also to regulate commercial translocations and to
55 forecast the potential consequences of translocations (i.e. the extent of invasion consequences partly depends on
56 the phylogenetic relatedness between invasive and native organisms: non-native species introductions may lead
57 to interspecific conflicts such as displacement of native species while invasions by non-native conspecific
58 individuals could foster dilution of characters; Mooney and Cleland, 2001). However, determining (i) the most
59 adapted species definition and, therefore, the most efficient species diagnostic traits or, even, (ii) the most
60 efficient traits reflecting inter-population differentiation is challenging for most organisms (De Queiroz, 2007)
61 including bumblebees (Lecocq et al., 2015d). Since bumblebees can display a large intraspecific or a low
62 interspecific variability in morphology (Michener, 1990), alternative features (i.e. wing shape, DNA, or eco-

63 chemical traits) have been used to discriminate entities leading however to controversial results (e.g. Aytekin et
64 al., 2007; Brasero et al., 2015; Ellis et al., 2006). The recent integrative taxonomy based on unified species
65 concept (De Queiroz, 2007) that considers multiple independent lines of evidence to evaluate taxonomic status
66 (Schlick-Steiner et al., 2010) provides a solution to overcome such taxonomic issues as well as to identify inter-
67 population differentiation (Lecocq et al., 2015b, 2015d). This approach was applied to a partial sampling of *B.*
68 *terrestris* populations suggesting that *B. terrestris* is a species complex with several differentiated subspecies
69 (Lecocq et al., 2015b). These results question the relevance of current regulations of *B. terrestris* trade that
70 mainly disregard the intra-*B. terrestris* differentiation or aim to protect populations (e.g. *B. terrestris audax* in
71 UK) differentiated in character that potentially poorly reflect differentiation processes (i.e. hair body color
72 pattern; Bertsch and Schweer, 2012).

73 In this paper, we aim to provide guidelines for efficient regulations of *B. terrestris* translocation within its
74 natural range according to a resolved taxonomy and population structure within *B. terrestris*.

75 **2. Materials and methods**

76 *2.1. Studied species and sampling*

77 *Bombus terrestris* has been considered by some taxonomists as a species complex while others recognized only
78 one species (Rasmont et al., 2008). Beside, the first studies based on a unsuitable diagnostic character (i.e. color
79 pattern; Carolan et al., 2012), barcoding studies suggested that all *B. terrestris* taxa are conspecific (Bertsch,
80 2010; Williams et al., 2012b). In contrast, studies of the cephalic labial gland secretions (CLGS), a reproductive
81 trait involved in the bumblebee pre-mating recognition reported significant differentiations between some
82 subspecies that question their conspecificity (Coppée et al., 2008; Lecocq et al., 2015c, 2013). Recently, an
83 integrative approach based on differentiation in multiple genetic markers and CLGS composition suggested that
84 *B. terrestris* included two species (*B. terrestris* and *B. xanthopus*) but only six taxa of the complex were
85 analyzed (Lecocq et al., 2015b).

86 Here, we extended the integrative taxonomic studies of Lecocq et al. (2015b) to the nine *B. terrestris* taxa
87 (recognized as subspecies by Rasmont et al., 2008 according to their diagnostic hair body color pattern).
88 Between 2004 and 2013, we sampled (i) 147 specimens of *B. terrestris africanus* from Morocco, *B. terrestris*
89 *audax* from England and Ireland, *B. terrestris calabricus* from Sicily, *B. terrestris canariensis* from Canary
90 Islands, *B. terrestris dalmatinus* from SE France, Aegean islands and Turkey, *B. terrestris lusitanicus* from SW

91 Europe and Madeira, *B. terrestris sassaricus* from Sardinia, *B. terrestris terrestris* from W and N Europe, and *B.*
92 *terrestris xanthopus* from Corsica, and (ii) six specimens of *B. terrestris dalmatinus* from two colonies produced
93 by Biobest bvba (Westerlo, Belgium) in 2007 and 2008 (Appendix Table A1). We considered all taxa without *a*
94 *priori* taxonomic status and referred to them as *africanus*, *audax*, *calabricus*, *canariensis*, *dalmatinus*,
95 *lusitanicus*, *sassaricus*, *terrestris*, and *xanthopus*. Among these taxa, five are or were used in the international
96 trade: *canariensis*, *dalmatinus*, *sassaricus*, *terrestris*, and *xanthopus*. Taxa with a large-range distribution were
97 sampled in a higher number of locations (Appendix Table A1). Moreover, we tried to sample at different
98 locations (distant by more than 10 km corresponding to the maximum male and queen flight distances; Kraus et
99 al., 2009; Lepais et al., 2010) to avoid sampling family members but we failed for *africanus*, *calabricus*, and
100 *canariensis* (for the last taxa, its restricted geographic range does not allow sampling at distant places). We
101 considered the phylogenetically related species *B. ignitus* as outgroup. We killed specimens by freezing them at -
102 20°C.

103 2.2. COI sequence-based species delimitation for the DNA taxonomic analyses

104 We sequenced the mitochondrial cytochrome oxidase 1 (COI) gene commonly used to study the interspecific
105 relationship in bumblebees (Williams et al., 2012b). We followed the total DNA extraction protocol, polymerase
106 chain reaction amplification reactions, sequencing procedures, and alignment method described in Lecocq et al.
107 (2015b). Sequences were deposited in GenBank (Appendix Table A1). The final molecular dataset spanned
108 849bp.

109 We delimited species based on the COI sequences using the Bayesian implementation of the general mixed
110 Yule-coalescent (bGMYC) method integrating the uncertainty related to phylogenetic inference (Reid and
111 Carstens, 2012), recently underlined as an efficient species delimitation method compared to alternative single
112 molecular- based methods (i.e. barcode, GMYC; see Dellicour and Flot, 2015; Lecocq et al., 2015d). For each
113 pair of DNA sequences, this method estimates the posterior probability that specimens are conspecific. The
114 probability that a lineage was conspecific with other lineages was here estimated by reporting ranges of posterior
115 probabilities among sequences from different lineages. The bGMYC method relies on the prediction that
116 independent evolution leads to the appearance of distinct genetic clusters (i.e. monophyly), separated by longer
117 internal branches. A range of probabilities >0.90 was considered as strong evidence that the groups compared
118 were conspecific while a range of probabilities <0.05 strongly suggested that the groups compared were not
119 conspecific (Reid and Carstens, 2012). Intermediate probabilities were interpreted as indicating non-significance;

120 in these cases the method was not able to confirm if the groups compared were conspecific or were not
121 conspecific (Reid and Carstens, 2012). Since the bGMYC algorithm requires several phylogenetic ultrametric
122 trees, we used BEAST 1.7.4 (using a GTR+I+G; number of gamma categories = 4; partition into codon base
123 positions, unlink substitution rate parameters across codon positions and unlink base frequencies across codon
124 positions; a random starting tree; four chains with mixed-models; under Yule process model) (Drummond et al.,
125 2012) with a phylogenetic clock model to generate a posterior distribution of trees (length of the MCMC chain: 1
126 billion generations). We used Tracer version 1.5 (Rambaut and Drummond, 2007) to assess convergence
127 between the chains. The bGMYC analysis was based on 1000 trees sampled every 10 000 generations from the
128 BEAST analysis. For each of these 1000 trees, the MCMC was made of 100 000 generations, discarding the first
129 90 000 as burn-in and sampling every 100 generations.

130 *2.3. Male chemical reproductive traits for chemo-taxonomical analyses*

131 We analyzed the CLGS commonly used as chemical markers for resolving species status (Lecocq et al., 2015d).
132 CLGS are a complex mixture of compounds, with several main components (i.e. compounds that have the
133 highest relative proportion among all compounds of CLGS at least in one individual of the taxa) (Lecocq et al.,
134 2015d). We analyzed CLGS (extracted by hexane) by (i) gas chromatography-flame ionization detector (GC/FID
135 for quantification), (ii) gas chromatography-mass spectrometry (GC/MS for qualification) and (iii) dimethyl
136 disulfide derivatization (DMDS for the double bond position determination) following protocol and GC/FID,
137 GC/MS, and DMDS conditions described in Lecocq et al. (2015b). We used quantification procedure, relative
138 amount calculation, and alignment method described in Lecocq et al. (2015b). The final chemical dataset spanned
139 68 compounds (Appendix Table A2).

140 We performed statistical analyses in R (R Development Core Team, 2013) to detect CLGS differentiations
141 among taxa. We transformed data ($\log(x+1)$) to reduce the great difference of abundance between compounds in
142 highly and low concentration. We compared the CLGS composition between individuals with a clustering
143 analysis performed with UPGMA (unweighted pair group method with arithmetic mean) clustering method on
144 Pearson r Correlation distances matrix (R-package ape, Paradis et al., 2004). We assessed the uncertainty in
145 hierarchical cluster analysis with p-values calculated via multiscale bootstrap resampling with 100 000 number
146 of bootstrap sample size (R-package pvelust, Suzuki and Shimodaira 2011). We assessed CLGS differentiations
147 between groups detected in cluster by performing multiple response permutation procedure (MRPP) (R-package
148 vegan, Oksanen et al., 2011). The MRPP is a nonparametric, multivariate procedure that tests the null hypothesis

149 of no difference between groups. MRPP has the advantage of not requiring distributional assumptions (such as
150 multivariate normality and homogeneity of variances). To determine compounds specific and regular to each
151 group defined by the clustering analyses and the MRPP (indicator compounds), we used the indicator value
152 (IndVal) method (Dufrene and Legendre, 1997). We evaluated the statistical significance of a compound as an
153 indicator at the 0.01 level with a randomization procedure.

154 Reproductive trait differentiation can result in low regional variation with minor behavioral consequences (e.g.
155 Vereecken, Mant, & Schiestl, 2007) or in the rise of reproductive isolation barrier (e.g. Martens, 1996). The
156 CLGS differentiation consequences remain poorly predictable without field observations or ethological tests
157 (most of the time unavailable; Lecocq et al., 2013). Nevertheless, the CLGS comparison between closely related
158 bumblebee taxa with a recognized species status support that the interspecific differentiation involves changes in
159 main compounds (e.g. Bertsch & Schweer 2012; Calam 1969). Therefore, we considered a significant (assessed
160 by MRPP) CLGS differentiation along with a main compound divergence (assessed by IndVal) as a strong
161 indicator of potential ethological consequences for pre-mating recognition.

162 *2.4.Data integration and decision framework of taxonomic status*

163 We used the decision framework developed by Lecocq et al. (2015b,d). We considered that a taxon deserved a
164 species status with a high degree of certainty if the taxon (i) was genetically differentiated (COI original
165 haplotypes), (ii) was not significantly conspecificity with other taxa in bGMYC analyses (probability < 0.9 to be
166 conspecific), (iii) was significantly differentiated in CLGS compositions, and (iv) was differentiated in the main
167 CLGS compounds (see argumentation in Lecocq et al., 2015b). We assigned subspecies status if there were
168 divergences in some but not all operational criteria according to the subspecies definition proposed by
169 Hawlitschek et al. (2012). We considered color pattern specificity as a further evidence of differentiation when
170 there is differentiation in at least one operational criterion but we did not consider divergence observed only
171 color pattern as reflecting a differentiation process because of limitations of this trait as diagnostic criterion
172 (Bertsch and Schweer, 2012a; Carolan et al., 2012).

173 **3.Results**

174 *3.1.COI sequence-based species delimitation for the DNA taxonomic analyses*

175 COI phylogenetic analysis detected five monophyletic groups that were strongly supported (Fig. 2A; see
176 sequence divergences between taxa in Appendix Table A3): (i) *B. ignitus*, (ii) *xanthopus*, (iii) one clade
177 including *audax*, *calabricus*, several *dalmatinus*, few *lusitanicus*, and most of *terrestris* specimens, (iv) one
178 clade with *africanus* and *canariensis* (in two different lineages), (v) one clade with other *dalmatinus* from
179 bumblebee breeders, most of *lusitanicus*, *sassaricus* (in one distinct clade), and other *dalmatinus*, *terrestris* (Fig.
180 2A). *Xanthopus* was resolved as the sister taxa of all *B. terrestris* taxa (Fig. 2A). The bGMYC analysis showed
181 probabilities of conspecificity of 0 between outgroup and ingroup (Appendix Table A4). Within the ingroup, the
182 bGMYC analysis showed two entities with low probabilities (<0.05) to be conspecific: *xanthopus* versus all
183 other *B. terrestris* taxa (Appendix Table A4). At least the bGMYC analysis revealed that all other *B. terrestris*
184 taxa have a non-significant probability (from 0.9 to 0.05 but most of probabilities were near the hetero-
185 specificity threshold; i.e. 0.06) or a high probability (>0.95) to be conspecific (Appendix Table A4).

186 3.2. Male chemical reproductive traits for chemo-taxonomical analyses

187 The cluster analysis of our CLGS data matrix (*B. ignitus* and all *B. terrestris* subspecies) revealed four strongly
188 supported (bootstrap > 95) groups (Fig. 2B): G1 (*xanthopus*), G2 (*B. ignitus*), G3 (including three subgroups:
189 two groups of *dalmatinus* and one group of *sassaricus*), G4 (all other *dalmatinus* and all other *B. terrestris* taxa)
190 that included two distinct sub-groups (all *africanus* and *canariensis* specimens respectively formed two
191 differentiated sub-groups). Five specimens did not cluster with other specimens (ungrouped specimens). The
192 examination of the wing condition of the ungrouped individuals indicated that these specimens were obviously
193 old specimens (Lecocq et al., 2011) that are known to show different CLGS composition and to be lesser
194 attractive to females (Coppée et al., 2011; Žáček et al., 2009).

195 Our global MRPP confirmed these intergroup differentiations (T [the overall weighted mean of group mean
196 distances]= 0.33, A [chance-corrected estimate of the proportion of the distances explained by group
197 identity]=0.28, *p-value* <0.01). Pairwise MRPP supported divergences between these groups (G1 versus G2: T=
198 0.17, A=0.58, *p-value* <0.01; G1 versus G3: T= 0.26, A=0.35, *p-value* <0.01; G1 versus G4: T= 0.36, A=0.11, *p-*
199 *value* <0.01; G2 versus G3: T= 0.21, A=0.41, *p-value* <0.01; G2 versus G4: T= 0.35, A=0.16, *p-value* <0.01; G3
200 versus G4: T= 0.35, A=0.16, *p-value* <0.01). We also checked that the two distinct groups included in G4 that
201 correspond to *africanus* and *canariensis* with Pairwise MRPP (*africanus* versus G4 expected *africanus* and
202 *canariensis*: T= 0.35, A=0.03, *p-value* <0.01; *canariensis* versus G4 expected *africanus* and *canariensis*: T=
203 0.35, A=0.08, *p-value* <0.01; *africanus* versus *canariensis*: T= 0.21, A=0.34, *p-value* <0.01). For each group

204 (G1, G2, G3, and G4 divided between *africanus*, *canariensis*, and other taxa), the main compounds are (i) G1:
205 dihydrofarnesol, hexadecenyl hexadecenoate, icos-17-enal, tricosane, (ii) G2: dihydrofarnesol and octadec-13-
206 en-1-ol, (iii) G3: dihydrofarnesol, (iv) G4 without *canariensis* and *africanus*: dihydrofarnesol, dihydrofarnesyl
207 dodecanoate and ethyl dodecanoate, (v) *africanus*: dihydrofarnesol, dihydrofarnesyl dodecanoate, ethyl
208 dodecanoate, and geranylcitronellol, (vi) *canariensis*: dihydrofarnesol. For each group, the IndVal method
209 revealed several significantly and strongly indicator compounds (IndVal value >0.90) including main
210 compounds only in *xanthopus* and *B. ignitus* (Appendix Table A2).

211 3.3. Taxonomic status

212 Species status is assigned to *B. ignitus* (outgroup) and to *B. xanthopus* according to their genetic differentiation,
213 their non-significant conspecificity with other taxa in bGMYC analyses (probability < 0.9 to be conspecific) and
214 their specific CLGS composition (including main compounds) (Table 1). All other taxa are included in one
215 species (Table 1). *Africanus*, *canariensis*, and *sassaricus* were differentiated in several operational criteria
216 (specific CLGS composition, without main compound changes, and private haplotypes). Consequently, the
217 subspecies status is confirmed for these taxa. In contrast, *audax*, *calabricus*, *dalmatinus* (wild and commercial),
218 *lusitanicus*, and *terrestris* were differentiated only in color pattern (Table 1).

219 4. Discussion

220 4.1. Solving the taxonomic status and the population structure

221 The integrative taxonomy approach applied previously to a subset of *B. terrestris* taxa (Lecocq et al., 2015b) and
222 extended here to all *B. terrestris* entities strongly suggests the existence of two species: *B. xanthopus* and *B.*
223 *terrestris*. Consequently, the species status of *B. xanthopus* proposed by Lecocq et al. (2015b) is confirmed
224 according to COI and CLGS differentiations (Tab. 1). While previous studies based on single criterion (Bertsch
225 and Schweer, 2012a; Williams et al., 2012b) do not regard these divergences as deserving a species status
226 (Lecocq et al., 2015b), we assume that the concordance of differentiation in multiple diagnostic traits reflects a
227 speciation process. The conspecificity of all other *B. terrestris* entities (including new taxa compared to Lecocq
228 et al., 2015b) assessed through our multi-criterion approach agrees with all recent single criterion-based
229 taxonomic studies (Bertsch and Schweer, 2012a; Rasmont et al., 2008; Williams et al., 2012b).

230 At the intraspecific level, a geographic differentiation process is detected for some allopatric *B. terrestris* taxa
231 (Fig. 2, Tab. 1). As observed in several organisms such as amphibians (Bisconti et al., 2011), insects (Damgaard,
232 2005), or mammals (Evin et al., 2011), the South West islands and North Africa host highly differentiated
233 populations. These populations are genetically (in the studied mitochondrial DNA marker) (Fig. 2; previously
234 observed by Estoup et al., 1996) and ecologically (Rasmont et al., 2008) differentiated and deserve a subspecies
235 status (*sensu* Hawlitschek et al., 2012): *B. terrestris africanus*, *B. terrestris canariensis*, and *B. terrestris*
236 *sassaricus*. In contrast, other insular populations (*audax* and Madeira *lusitanicus*) do not display such level of
237 differentiations (Fig. 2; also previously observed by Widmer et al., 1998). A persistent gene flow between
238 European mainland and British Islands as suggested by bumblebee channel crossings (Goulson et al., 2011;
239 Mikkola, 1978) could explain the observed pattern for *audax*. Further studies on a putative gene flow between
240 UK and Europe are needed to assess this hypothesis. For the Madeira *lusitanicus*, the lack of phenotypic and
241 genetic differentiation with continental *lusitanicus* contradicts the strong geographic isolation of the island and
242 suggests an ancient human-related colonization of the island from the Iberian Peninsula (as far as we know there
243 are no bumblebee traded for pollination in Madeira has not been imported for commercial purpose in Madeira).

244 Considering continental taxa, *calabricus*, wild *dalmatinus*, *lusitanicus*, and *terrestris* do not display
245 differentiation in COI or CLG (similar results in Bertsch and Schweer, 2012; Estoup et al., 1996). This may
246 reflect that (i) *a priori* classification based on color patterns poorly reflects the genetic differentiation (Lecocq et
247 al., 2015a), (ii) there is no geographic structure across the mainland (Bertsch and Schweer, 2012a). Although
248 classification based on color patterns has been criticized (Bertsch and Schweer, 2012a; Lecocq et al., 2015a), the
249 second hypothesis appears more likely since there is no alternative coherent population structure in our analyses
250 (Fig. 2) regardless of the *a priori* taxonomic classification. Indeed, our analyses detects (i) two types of CLGS
251 within wild sympatric *dalmatinus* populations (Fig.2b) and (ii) two genetically distinct and broadly sympatric
252 groups each with wild *dalmatinus*, *lusitanicus*, and *terrestris* specimens (i.e. the two CLGS groups observed in
253 *dalmatinus* do not match with the two genetic groups observed in this taxon) but these groups do not correspond
254 to a geographic structure. Moreover, since these distinct groups are not related to other phenotypic or ecologic
255 differentiations, this observation is most likely a consequence of a smaller sampling to evaluate the whole
256 genetic/CLGS variability of these widespread taxa (Lecocq et al., 2015d) and/or, for the CLGS, a result from an
257 age differentiation that impacts the chemical composition (Žáček et al., 2009). This hypothesis is further
258 supported by several inter-taxa geographic clines observed on mainland (Bertsch and Schweer, 2012a; Rasmont
259 et al., 2008). Alternatively, the lack of differentiation between most of *B. terrestris* entities (including *audax*)

260 could result from the genetic homogenization linked to the importation of commercial *B. terrestris* during the
261 last 20 years and that may have erased the past population structure (Velthuis and van Doorn, 2006). Indeed, all
262 samples analyzed (and those used in previous works; e.g. Bertsch and Schweer, 2012; Estoup et al., 1996) were
263 sampled long after the start of the commercialization of *B. terrestris*. Pre-commercialization samples (i.e.
264 museum specimens) need to be analyzed to test this hypothesis. At least, we cannot exclude that diagnostic
265 characters chosen could be relatively invariant/inconsistently differentiated traits within *B. terrestris* (could be
266 likely for CLGS; i.e. Bertsch and Schweer, 2012a; Lecocq et al., 2015a; but the COI marker has been
267 successfully used to solve the population structure of many bumblebee species, e.g. Dellicour et al., 2015;
268 Duennes et al., 2012). This could hide some local adaptations and differentiation process since the accuracy of
269 the integrative approach is depending on selected characters (Lecocq et al., 2015d). This may likely happen for
270 *dalmatinus* and *lusitanicus* that display ecological, ethological, and phenological specificity (e.g. larger colony
271 size, shorter setae, aestivation; Peat et al., 2005; Rasmont et al., 2008; Velthuis and van Doorn, 2006) suggesting
272 a differentiation process while other undifferentiated taxa are similar for these features. Taking into account
273 these specificity and the fact that *dalmatinus* is the most used taxa for international trade, we consider them as an
274 ESU within *B. terrestris*. These taxa and other poorly differentiated taxa should be analyzed through alternative
275 traits and further analyses (e.g. microsatellite analyses, Duennes et al., 2012) to detected potential population
276 structure.

277 Considering commercial *dalmatinus* strain, the lack of differences in studied characters suggest that the breeding
278 process did not lead to differentiation process but further potential differentiation of traded strain should be
279 investigated.

280 4.2. Toward an improved regulation of *B. terrestris* trade within the species range

281 Given the important advantages of traded bumblebee for crop pollinations (Velthuis and van Doorn, 2006), a
282 complete ban of international *B. terrestris* trade is not feasible. Therefore, efficient strategies to manage the trade
283 of *B. terrestris* are needed to reconcile biological conservation and economic reality. This implies mainly
284 regulations of translocations/importations of *B. terrestris* taxa (Velthuis and van Doorn, 2006).

285 Nowadays, such regulation mostly concerns importation of *B. terrestris* out of its natural range (e.g. Australia;
286 North America; Velthuis and van Doorn, 2006) in order to avoid interspecific competitions and the highly
287 problematic pathogen spillover due to importations of exotic pollinators (Murray et al., 2013; Schmid-Hempel et

288 al., 2014). Given the new species status of *B. xanthopus* and to protect this endemic species, importations of *B.*
289 *terrestris* should be prohibited in Corsica. Moreover the high percentage of endemism in the Corsican fauna of
290 bumblebees (several endemic species and subspecies; Lecocq et al., 2015b) reinforces the necessity of such a
291 prohibition.

292 Within the natural distribution of *B. terrestris*, measures to regulate its translocation have been taken in Canary
293 Islands, Israel, Norway, Turkey, and UK only (Velthuis and van Doorn, 2006). For the here assessed *B. terrestris*
294 subspecies (*B. terrestris africanus*, *B. terrestris canariensis*, and *B. terrestris sassaricus*), regulations promoted
295 by Canary Islands authorities provide an example of adapted regulations that should be extended to Sardinia and
296 North Africa. These regulations only allow using the local subspecies (*B. terrestris canariensis*) for crop
297 pollination (Velthuis and van Doorn, 2006). However, current legislation within Canary Islands allow the
298 breeding of the local subspecies out of its natural range and the subsequent importation of colonies (Velthuis and
299 van Doorn, 2006) that could lead to pathogen spillovers (Murray et al., 2013). Prohibition of all bumblebee
300 importations to isolated lands could hamper such potential issues. Consequently, local crop pollination would
301 require productions of local populations (Williams et al., 2012a) but such productions can only become
302 economically viable if the size of the local market for pollination is substantial (e.g. see the decision of
303 bumblebee breeders to leave the Norwegian market after importation prohibition; Velthuis and van Doorn,
304 2006). Since Canary Islands, North Africa, and Sardinia export a huge amount of crops commercially pollinated
305 by bumblebees (Velthuis and van Doorn, 2006), complete interdiction of bumblebee importation should foster
306 development of local production leading to beneficial effect for biological conservation and local economy.

307 Considering other *B. terrestris* populations, one could argue that the importation of allopatric bumblebee
308 populations within British Islands, Europe, and West Asia is not an issue according to the lack of differentiations
309 in our integrative taxonomic approach. However, since this lack of differentiation could be a consequence of an
310 undergoing genetic homogenization of *B. terrestris* or could be linked to the limitation of our integrative
311 approach (see before), a precautionary approach should be promoted pending further analyses on the *B. terrestris*
312 intraspecific structure. While the Israeli, Norwegian, Turkish, UK laws prohibiting importation and use of non-
313 native strains remain the safest approach, its economic viability definitively depends on the size of local market
314 for pollination (Velthuis and van Doorn, 2006). Minimum regulation should concern *dalmatinus* and *lusitanicus*
315 ESU since they (i) display the most conspicuous specificity within "undifferentiated taxa" and (ii) are the most
316 used strains in international trade. Their importation should be restricted to its natural range (Rasmont et al.,

317 2008) pending further analyses on its ecological, genetic, and phenotypic specificity. Considering our results but
318 also the few evidence of differentiations previously recorded and the observed clines *in natura* (Bertsch and
319 Schweer, 2012a; Estoup et al., 1996; Rasmont et al., 2008; Widmer et al., 1998; Williams et al., 2012b),
320 importations between *audax*, *calabricus*, and *terrestris* ranges could be alternatively prohibited or authorized but
321 not encouraged taking into account the risks of pathogen spillover.

322 Beside this first recommendation for regulation of *B. terrestris* trade, a major uncertainty remains in the identity
323 of the commercial strains. Our analyses show the lack of specificity for a commercial strain compared to wild
324 *dalmatinus* but similar analyses should be conduct on other commercial strains. Indeed, commercial imports can
325 involve individuals (i) bred for several generations in non-natural conditions to generate strains with specific
326 ecological requirements (different from natural populations) or (ii) resulting from
327 interspecific/interpopulation hybridization during the breeding process depending on the breeding company
328 (Velthuis and van Doorn, 2006). In this case, commercial strains would be considered as aliens everywhere.

329 *4.3. Further applications*

330 Here applied to the *B. terrestris* trade, the definition of guidelines for the regulation of species trade through
331 integrative taxonomy assessments should be extended to other traded organisms exhibiting intraspecific
332 polymorphism (e.g. Australian parrots or rice; Song & Carter 1996; Low 2014). Such assessment of taxonomic
333 and population structure (including ESUs highlighting) can provide pragmatic guidelines to legislate for smart
334 regulation that reconciles biological conservation and economic reality. Nevertheless, the efficiency of such
335 regulations requires that the taxonomic identity or the geographic origin of traded strains should be assessed and
336 provided by trade companies.

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345 **Appendix A. Supplementary material**

346 Supplementary data associated with this article can be found in the online version.

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514

515 **Table**

516 **Table 1** Current taxonomic status according to Rasmont et al. 2008 and Williams et al. 2012b, and proposed
 517 taxonomic status. Color pattern is indicated only when a taxon has an original color pattern (+/-means that the
 518 taxon has/ has not a specific color pattern; ++ means a very conspicuous original color pattern). Private
 519 haplotypes indicates if a taxon has an original haplotype (+/-means that the taxon has/ has not only private
 520 haplotype(s)). bGMYC indicates if a taxon has a low probability to be conspecific with other taxa according to
 521 bGMYC analyses (+/-means that the taxon has a probability <0.05/>0.05 to be conspecific with other taxa).
 522 When the taxon is not regarded as a prospective species, the number in square brackets indicates the number of
 523 taxa recognized as conspecific according to bGMYC methods. CLGS indicates if the taxon has/has not specific
 524 composition of cephalic labial gland secretions (+/-means that the taxon has/ has not a specific CLGS
 525 composition according to multiple response permutation procedure analyses. ++ means that the specific
 526 composition involved main compounds. When the taxon shares CLGS composition with other ones, the number
 527 in square brackets indicates the number of taxa that share similar CLGS) while the letter in square brackets
 528 indicates the letter of taxa that share same main compounds.
 529

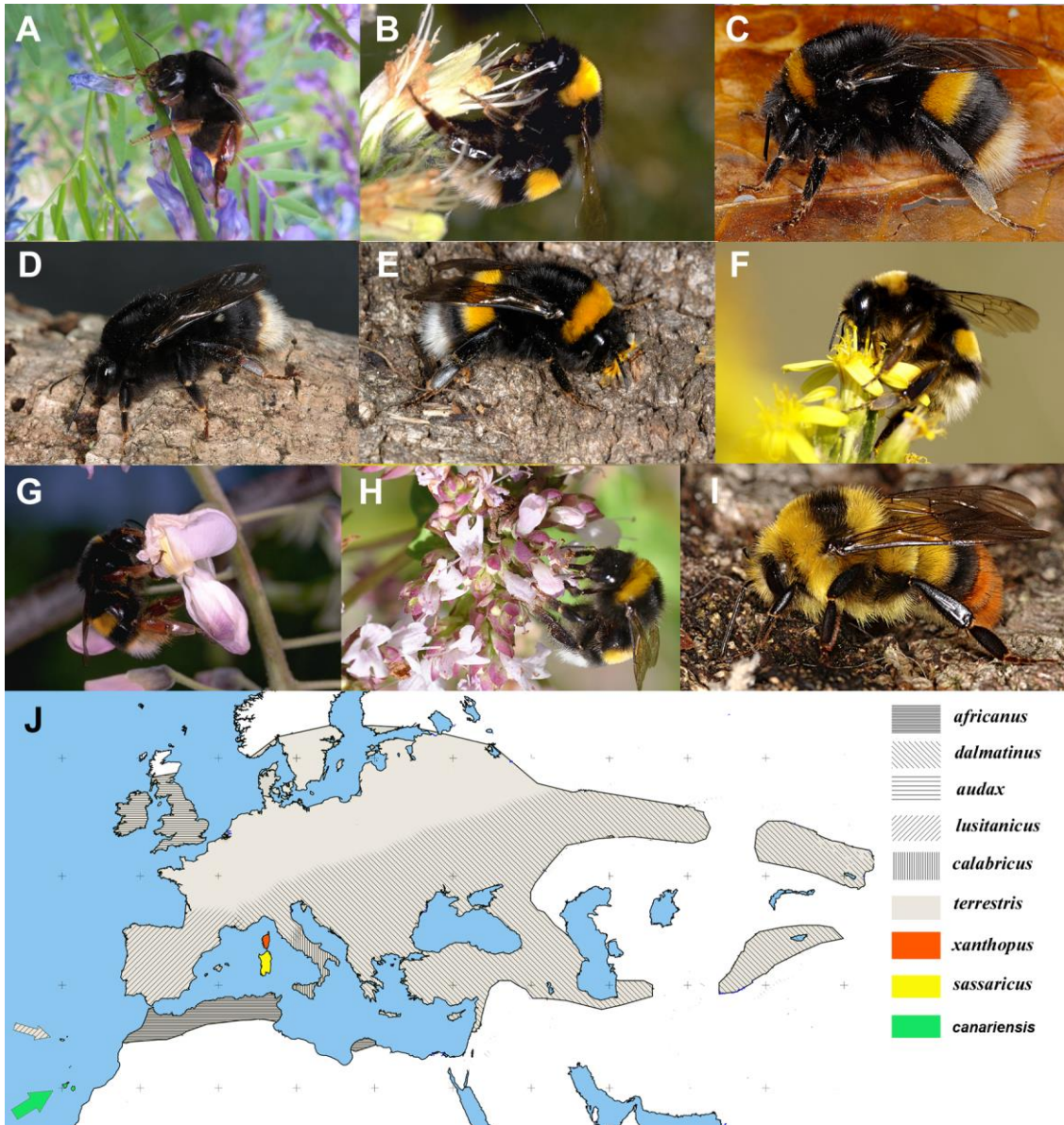
Current taxonomic status	Color pattern	Private haplotypes	bGMYC	CLGS	Proposed taxonomic status
<i>B. terrestris africanus</i>	+	+	- [1]	+[A]	<i>B. terrestris africanus</i>
<i>B. terrestris audax</i>	+	- [1]	- [1]	- [1] [A]	<i>B. terrestris audax</i>
<i>B. terrestris calabricus</i>	+	- [1]	- [1]	- [1] [A]	<i>B. terrestris calabricus</i>
<i>B. terrestris canariensis</i>	++	+	- [1]	+[A]	<i>B. terrestris canariensis</i>
<i>B. terrestris dalmatinus</i>	+	- [1]	- [1]	- [1] [A/B]	<i>B. terrestris dalmatinus</i>
<i>B. terrestris lusitanicus</i>	++	- [1]	- [1]	- [1] [A]	<i>B. terrestris lusitanicus</i>
<i>B. terrestris sassaricus</i>	++	+	- [1]	+[B]	<i>B. terrestris sassaricus</i>
<i>B. terrestris terrestris</i>	+	- [1]	- [1]	- [1] [A]	<i>B. terrestris terrestris</i>
<i>B. terrestris xanthopus</i> / <i>B. xanthopus</i>	++	+	+	++	<i>B. xanthopus</i>
<i>B. ignitus</i>	++	+	+	++	<i>B. ignitus</i>

530

531

532 **Figure Captions**

533 **Fig. 1** *Bombus terrestris* taxa, their geographic distribution (around 1985), and the closely related species
 534 *Bombus ignitus*. A. *Bombus terrestris xanthopus*. B. *Bombus terrestris africanus*. C. *Bombus terrestris audax*. D.
 535 *Bombus terrestris canariensis*. E. *Bombus terrestris dalmatinus*. F. *Bombus terrestris lusitanicus*. G. *Bombus*
 536 *terrestris sassaricus*. H. *Bombus terrestris terrestris*. I. *Bombus ignitus*. J. Distribution of *Bombus terrestris* taxa
 537 (modified from Rasmont et al., 2008). All photos by P. Rasmont to the exception of A. and H. by T. Lecocq.



538

539

540 **Fig. 2** Phylogenetic, DNA sequence-based species delimitation, and cephalic labial gland secretion analysis of
541 *Bombus terrestris* taxa and *Bombus ignitus*. A. Bayesian ultrametric tree of *Bombus terrestris* taxa and *B. ignitus*
542 based on COI sequences with bGMYC (Bayesian implementation of the general mixed Yule-coalescent)
543 pairwise probability of conspecificity. Values above tree branches are Bayesian posterior probabilities values.
544 Only posterior probabilities >0.95 are showed. The colored matrix corresponds to the pairwise probabilities of
545 conspecificity returned by the bGMYC method (see also the related color scale on the right). B. Unweighted pair
546 group method with arithmetic mean (UPGMA) cluster based on a correlation matrix calculated from the cephalic
547 labial gland secretions matrix of *Bombus terrestris* taxa and *B. ignitus*. The values near nodes are multiscale
548 bootstrap resampling (only values > 90 of main groups are shown).

551 **Appendix A**

552 **Appendix A Table A1** Table of sampling of *Bombus terrestris* and *Bombus ignitus*. Sample Code is the sample
553 labels used in analyses. Taxa column is the name of taxa. COI is the Genbank accession numbers for each
554 sample (when consubspecific samples display the same gene sequence, only one of them has been submitted to
555 Genbank).

556 **Appendix A Table A2** Cephalic labial gland matrix. Data matrix of cephalic labial gland secretions (relative
557 amounts of each compound) and list of the identified compounds in *Bombus terrestris* and *Bombus ignitus*.
558 Unknown x are undetermined compounds. IndVal results are indicator value obtained in IndVal method.

559 **Appendix A Table A3.** Sequence divergences between *Bombus terrestris* taxa.

560 **Appendix A Table A4** Results of the bGMYC (Bayesian implementation of the general mixed Yule-coalescent)
561 analysis (pairwise table) in *Bombus terrestris* and *Bombus ignitus*. Values are probability to be conspecific (1 =
562 100% of chance to be conspecific).