

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

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Thomas Lecocq, Audrey Coppee, Denis Michez, Nicolas Brasero, Jean-Yves Rasplus, et al.. The alien's identity: consequences of taxonomic status for the international bumblebee trade regulations. Biological Conservation, 2016, 195, pp.169-176. 10.1016/j.biocon.2016.01.004 . hal-01575596

## HAL Id: hal-01575596 https://hal.univ-lorraine.fr/hal-01575596

Submitted on 4 Dec 2020

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

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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 eco63 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).

In this paper, we aim to provide guidelines for efficient regulations of *B. terrestris* translocation within its
 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).

Here, we extended the integrative taxonomic studies of Lecocq et al. (2015b) to the nine *B. terrestris* taxa
(recognized as subspecies by Rasmont et al., 2008 according to their diagnostic hair body color pattern).
Between 2004 and 2013, we sampled (i) 147 specimens of *B. terrestris africanus* from Morocco, *B. terrestris audax* from England and Ireland, *B. terrestris calabricus* from Sicily, *B. terrestris canariensis* from Canary
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 byba (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

We sequenced the mitochondrial cytochrome oxidase 1 (COI) gene commonly used to study the interspecific relationship in bumblebees (Williams et al., 2012b). We followed the total DNA extraction protocol, polymerase chain reaction amplification reactions, sequencing procedures, and alignment method described in Lecocq et al. (2015b). Sequences were deposited in GenBank (Appendix Table A1). The final molecular dataset spanned 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 bound 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 pvclust, 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 of no difference between groups. MRPP has the advantage of not requiring distributional assumptions (such as multivariate normality and homogeneity of variances). To determine compounds specific and regular to each group defined by the clustering analyses and the MRPP (indicator compounds), we used the indicator value (IndVal) method (Dufrêne and Legendre, 1997). We evaluated the statistical significance of a compound as an 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 (G1, G2, G3, and G4 divided between *africanus*, *canariensis*, and other taxa), the main compounds are (i) G1: dihydrofarnesol, hexadecenyl hexadecenoate, icos-17-enal, tricosane, (ii) G2: dihydrofarnesol and octadec-13en-1-ol, (iii) G3: dihydrofarnesol, (iv) G4 without *canariensis* and *africanus*: dihydrofarnesol, dihydrofarnesyl dodecanoate and ethyl dodecanoate, (v) *africanus*: dihydrofarnesol, dihydrofarnesyl dodecanoate, ethyl dodecanoate, and geranylcitronellol, (vi) *canariensis*: dihydrofarnesol. For each group, the IndVal method revealed several significantly and strongly indicator compounds (IndVal value >0.90) including main compounds only in *xanthopus* and *B. ignitus* (Appendix Table A2).

#### 211 3.3.Taxonomic status

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

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

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

al., 2014). Given the new species status of *B. xanthopus* and to protect this endemic species, importations of *B. terrestris* should be prohibited in Corsica. Moreover the high percentage of endemism in the Corsican fauna of
 bumblebees (several endemic species and subspecies; Lecocq et al., 2015b) reinforces the necessity of such a
 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 (different (ii) ecological requirements from natural populations) resulting from or 327 intersubspecific/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*

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

#### 337 Acknowledgements

We thank R. De Jonghe, N. E. Raine, L. Chittka, and Biobest byba for providing the biological material. TL was post-doc funded by the Belgian Scientific Politic (BELSPO, BR/132/A1/BELBEES). Financial support was provided by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no244090, STEP Project (Status and Trends of European Pollinators, www.step-project.net) and the Czech Science Foundation (grant No. 14-04291S). This project was supported by the network *Bibliothèque du Vivant* funded by the CNRS, the *Muséum National d'Histoire Naturelle* and the *Institut National de la Recherche Agronomique*.

#### 345 Appendix A. Supplementary material

- 346 Supplementary data associated with this article can be found in the online version.
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### 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
B. terrestris africanus	+	+	- [1]	+[A]	B. terrestris africanus
B. terrestris audax	+	- [1]	- [1]	- [1] [A]	B. terrestris audax
B. terrestris calabricus	+	- [1]	- [1]	- [1] [A]	B. terrestris calabricus
B. terrestris canariensis	++	+	- [1]	+[A]	B. terrestris canariensis
B. terrestris dalmatinus	+	- [1]	- [1]	- [1] [A/B]	B. terrestris dalmatinus
B. terrestris lusitanicus	++	- [1]	- [1]	- [1] [A]	B. terrestris lusitanicus
B. terrestris sassaricus	++	+	- [1]	+[B]	B. terrestris sassaricus
B. terrestris terrestris	+	- [1]	- [1]	- [1] [A]	B. terrestris terrestris
B. terrestris xanthopus / B. xanthopus	++	+	+	++	B. xanthopus
B. ignitus	++	+	+	++	B. ignitus

530

### 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.



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).



africanus + audax + calabricus + canariensis + commercial dalmatinus + wild dalmatinus + lusitanicus + terrestris



#### 551 Appendix A

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

- 556 Appendix A Table A2 Cephalic labial gland matrix. Data matrix of cephalic labial gland secretions (relative
- 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).