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

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ABSTRACT

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

1. Introduction

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

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

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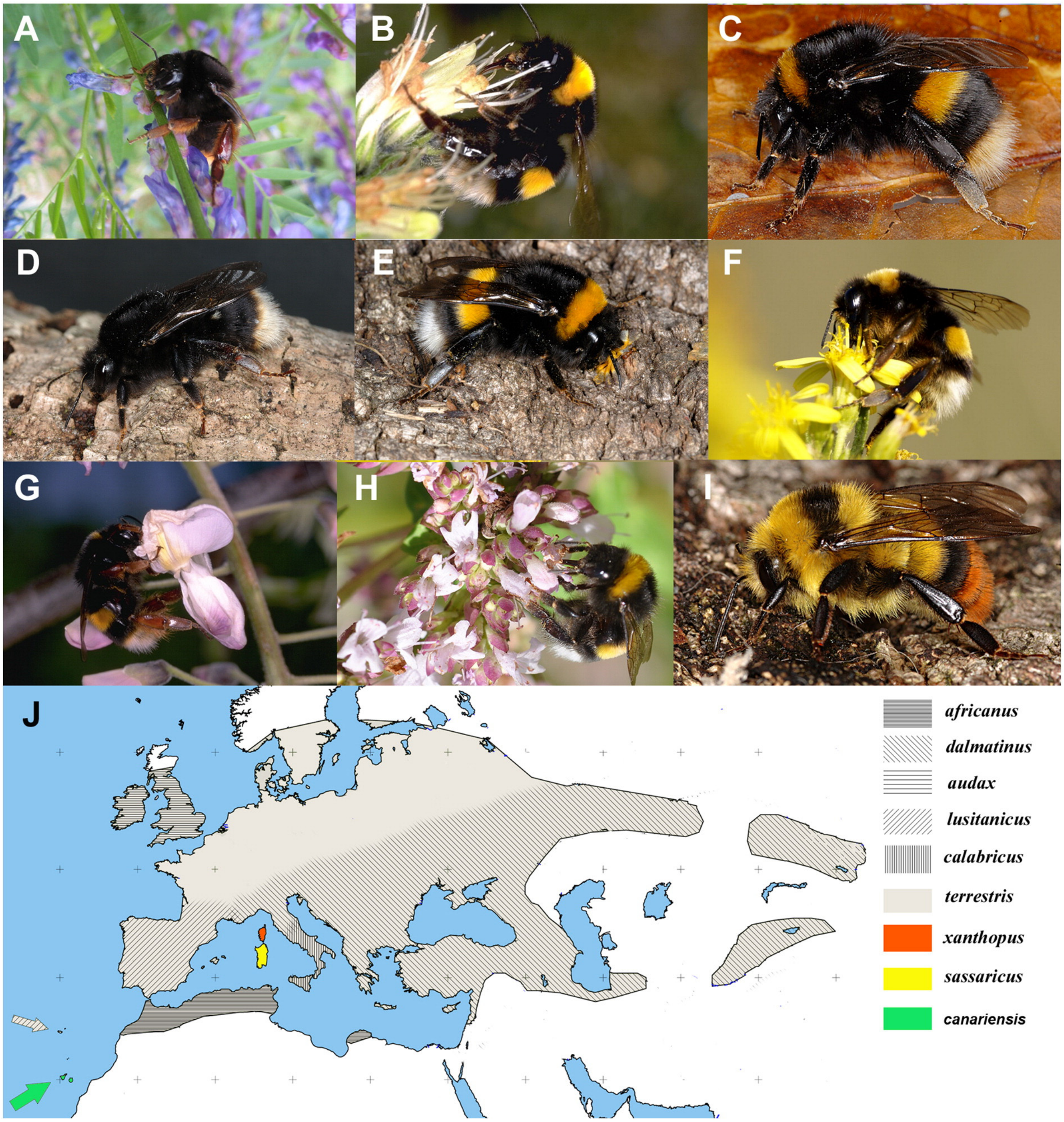

Fig. 1. *Bombus terrestris* taxa, their geographic distribution (around 1985), and the closely related species *Bombus ignitus*. A. *Bombus terrestris xanthopus*. B. *Bombus terrestris africanus*. C. *Bombus terrestris audax*. D. *Bombus terrestris canariensis*. E. *Bombus terrestris dalmatinus*. F.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

low interspecific variability in morphology (Michener, 1990), alternative features (i.e. wing shape, DNA, or eco-chemical traits) have been used to discriminate entities leading however to controversial results (e.g. Aytekin et al., 2007; Brasero et al., 2015; Ellis et al., 2006). The recent integrative taxonomy based on unified species concept (De Queiroz, 2007) that considers multiple independent lines of evidence to evaluate taxonomic status (Schlick-Steiner et al., 2010) provides a solution to overcome such taxonomic issues as well as to identify inter-population differentiation (Lecocq et al., 2015b, 2015d). This approach was applied to a partial sampling of *B. terrestris* populations suggesting that *B. terrestris* is a species complex with several differentiated subspecies (Lecocq et al., 2015b). These results question the relevance of current regulations of *B. terrestris* trade that mainly disregard the intra-*B. terrestris* differentiation or aim to protect populations (e.g. *B. terrestris* audax in UK) differentiated in character that potentially poorly reflects differentiation processes (i.e. hair body color 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*.

2. Materials and methods

2.1. Studied species and sampling

Bombus terrestris has been considered by some taxonomists as a species complex while others recognized only one species (Rasmont et al., 2008). Besides, the first studies based on an unsuitable diagnostic character (i.e. color pattern; Carolan et al., 2012), barcoding studies suggested that all *B. terrestris* taxa are conspecific (Bertsch, 2010; Williams et al., 2012b). In contrast, studies of the cephalic labial gland secretions (CLGS), a reproductive trait involved in the bumblebee pre-mating recognition reported significant differentiations between some subspecies that question their conspecificity (Coppée et al., 2008; Lecocq et al., 2013, 2015c). Recently, an integrative approach based on differentiation in multiple genetic markers and CLGS composition suggested that *B. terrestris* included two species (*B. terrestris* and *B. xanthopus*) but only six taxa of the complex were 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 Europe and Madeira, *B. terrestris sassaricus* from Sardinia, *B. terrestris terrestris* from W and N Europe, and *B. terrestris xanthopus* from Corsica, and (ii) six specimens of *B. terrestris dalmatinus* from two colonies produced by Biobest bvba (Westerlo, Belgium) in 2007 and 2008 (Appendix A Table A1). We considered all taxa without a priori taxonomic status and referred to them as *africanus*, *audax*, *calabricus*, *canariensis*, *dalmatinus*, *lusitanicus*, *sassaricus*, *terrestris*, and *xanthopus*. Among these taxa, five are or were used in the international trade: *canariensis*, *dalmatinus*, *sassaricus*, *terrestris*, and *xanthopus*. Taxa with a large-range distribution were sampled in a higher number of locations (Appendix A Table A1). Moreover, we tried to sample at different locations (distant by more than 10 km corresponding to the maximum male and queen flight distances; Kraus et al., 2009; Lepais et al., 2010) to avoid sampling family members but we failed for *africanus*, *calabricus*, and *canariensis* (for the last taxa, its restricted geographic range does not allow sampling at distant places). We considered the phylogenetically related species *B. ignitus* as outgroup. We killed specimens by freezing them at −20 °C.

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 A Table A1). The final molecular dataset spanned 849 bp.

We delimited species based on the COI sequences using the Bayesian implementation of the general mixed Yule-coalescent (bGMYC) method integrating the uncertainty related to phylogenetic inference (Reid and Carstens, 2012), recently underlined as an efficient species delimitation method compared to alternative single molecular-based methods (i.e. barcode, GMYC; see Dellicour and Flot, 2015; Lecocq et al., 2015d). For each pair of DNA sequences, this method estimates the posterior probability that specimens are conspecific. The probability that a lineage was conspecific with other lineages was here estimated by reporting ranges of posterior probabilities among sequences from different lineages. The bGMYC method relies on the prediction that independent evolution leads to the appearance of distinct genetic clusters (i.e. monophyly), separated by longer internal branches. A range of probabilities >0.90 was considered as strong evidence that the groups compared were conspecific while a range of probabilities <0.05 strongly suggested that the groups compared were not conspecific (Reid and Carstens, 2012). Intermediate probabilities were interpreted as indicating non-significance; in these cases the method was not able to confirm if the groups compared were conspecific or were not conspecific (Reid and Carstens, 2012). Since the bGMYC algorithm requires several phylogenetic ultrametric trees, we used BEAST 1.7.4 (using a GTR + I + G; number of gamma categories = 4; partition into codon base positions, unlink substitution rate parameters across codon positions and unlink base frequencies across codon positions; a random starting tree; four chains with mixed-models; under Yule process model) (Drummond et al., 2012) with a phylogenetic clock model to generate a posterior distribution of trees (length of the MCMC chain: 1 billion generations). We used Tracer version 1.5 (Rambaut and Drummond, 2007) to assess convergence between the chains. The bGMYC analysis was based on 1000 trees sampled every 10,000 generations from the BEAST analysis. For each of these 1000 trees, the MCMC was made of 100,000 generations, discarding the first 90,000 as burn-in and sampling every 100 generations.

2.3. Male chemical reproductive traits for chemo-taxonomical analyses

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

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

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

2.4. Data integration and decision framework of taxonomic status

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

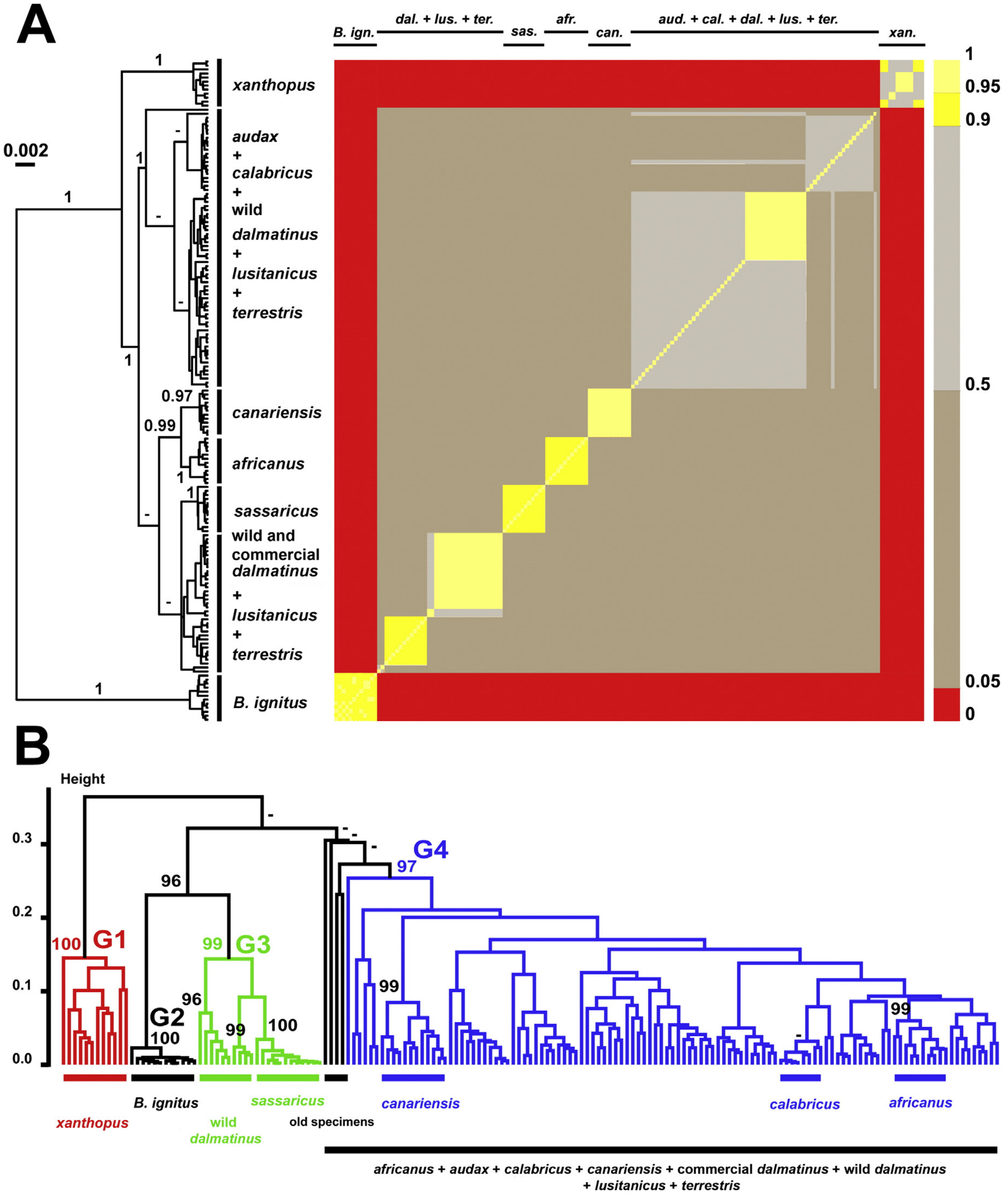

Fig. 2. Phylogenetic, DNA sequence-based species delimitation, and cephalic labial gland secretion analysis of *Bombus terrestris* taxa and *Bombus ignitus*. A. Bayesian ultrametric tree of *Bombus terrestris* taxa and *B. ignitus* based on COI sequences with bGMYC (Bayesian implementation of the general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

3. Results

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

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

3.2. Male chemical reproductive traits for chemo-taxonomical analyses

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

Our global MRPP confirmed these intergroup differentiations (T [the overall weighted mean of group mean distances] = 0.33, A [chance-corrected estimate of the proportion of the distances explained by group identity] = 0.28, *p-value* < 0.01). Pairwise MRPP supported divergences between these groups (G1 versus G2: T = 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-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 versus G4: T = 0.35, A = 0.16, *p-value* < 0.01). We also checked that the two distinct groups included in G4 that correspond to *africanus* and *canariensis* with Pairwise MRPP (*africanus* versus G4 expected *africanus* and *canariensis*: T = 0.35, A = 0.03, *p-value* < 0.01; *canariensis* versus G4 expected *africanus* and *canariensis*: T = 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-13-en-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 A Table A2).

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).

4. Discussion

4.1. Solving the taxonomic status and the population structure

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

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

Considering continental taxa, *calabricus*, wild *dalmatinus*, *lusitanicus*, and *terrestris* do not display differentiation in COI or CLG (similar results in Bertsch and Schweer, 2012; Estoup et al., 1996). This may reflect that (i) a priori classification based on color patterns poorly reflects the genetic differentiation (Lecocq et al., 2015a), and (ii) there is no geographic structure across the mainland (Bertsch and Schweer, 2012). Although classification based on color patterns has been criticized (Bertsch and Schweer, 2012; Lecocq et al., 2015a), the second hypothesis appears more likely since there is no alternative coherent population structure in our analyses (Fig. 2) regardless of the a priori taxonomic classification. Indeed, our analyses detects (i) two types of CLGS within wild sympatric *dalmatinus* populations (Fig. 2b) and (ii) two genetically distinct and broadly sympatric

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

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*

groups each with wild *dalmatinus*, *lusitanicus*, and *terrestris* specimens (i.e. the two CLGS groups observed in *dalmatinus* do not match with the two genetic groups observed in this taxon) but these groups do not correspond to a geographic structure. Moreover, since these distinct groups are not related to other phenotypic or ecologic differentiations, this observation is most likely a consequence of a smaller sampling to evaluate the whole genetic/CLGS variability of these widespread taxa (Lecocq et al., 2015d) and/or, for the CLGS, a result from an age differentiation that impacts the chemical composition (Žáček et al., 2009). This hypothesis is further supported by several inter-taxa geographic clines observed on mainland (Bertsch and Schweer, 2012; Rasmont et al., 2008). Alternatively, the lack of differentiation between most of *B. terrestris* entities (including *audax*) could result from the genetic homogenization linked to the importation of commercial *B. terrestris* during the last 20 years and that may have erased the past population structure (Velthuis and van Doorn, 2006). Indeed, all samples analyzed (and those used in previous works; e.g. Bertsch and Schweer, 2012; Estoup et al., 1996) were sampled long after the start of the commercialization of *B. terrestris*. Pre-commercialization samples (i.e. museum specimens) need to be analyzed to test this hypothesis. At least, we cannot exclude that diagnostic characters chosen could be relatively invariant/inconsistently differentiated traits within *B. terrestris* (could be likely for CLGS; i.e. Bertsch and Schweer, 2012; Lecocq et al., 2015a; but the COI marker has been successfully used to solve the population structure of many bumblebee species, e.g. Dellicour et al., 2015; Duennes et al., 2012). This could hide some local adaptations and differentiation process since the accuracy of the integrative approach is depending on selected characters (Lecocq et al., 2015d). This may likely happen for *dalmatinus* and *lusitanicus* that display ecological, ethological, and phenological specificity (e.g. larger colony size, shorter setae, aestivation; Peat et al., 2005; Rasmont et al., 2008; Velthuis and van Doorn, 2006) suggesting a differentiation process while other undifferentiated taxa are similar for these features. Taking into account these specificity and the fact that *dalmatinus* is the most used taxa for international trade, we consider them as an ESU within *B. terrestris*. These taxa and other poorly differentiated taxa should be analyzed through alternative traits and further analyses (e.g. microsatellite analyses, Duennes et al., 2012) to detected potential population structure.

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

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.

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

Considering other *B. terrestris* populations, one could argue that the importation of allopatric bumblebee populations within British Islands, Europe, and West Asia is not an issue according to the lack of differentiations in our integrative taxonomic approach. However, since this lack of differentiation could be a consequence of an undergoing genetic homogenization of *B. terrestris* or could be linked to the limitation of our integrative approach (see before), a precautionary approach should be promoted pending further analyses on the *B. terrestris* intraspecific structure. While the Israeli, Norwegian, Turkish, UK laws prohibiting importation and use of non-native strains remain the safest approach, its economic viability definitively depends on the size of local market for pollination (Velthuis and van Doorn, 2006). Minimum regulation should concern *dalmatinus* and *lusitanicus* ESU since they (i) display the most conspicuous specificity within "undifferentiated taxa" and (ii) are the most used strains in international trade. Their importation should be restricted to its natural range (Rasmont et al., 2008) pending further analyses on its ecological, genetic, and phenotypic specificity. Considering our results but also the few evidence of differentiations previously recorded and the observed clines *in natura* (Bertsch and Schweer, 2012; Estoup et al., 1996; Rasmont et al., 2008; Widmer et al., 1998; Williams et al., 2012b), importations between *audax*, *calabricus*, and *terrestris* ranges could be alternatively prohibited or authorized but not encouraged taking into account the risks of pathogen spillover.

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

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 and 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.

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