Naturalis Repository

An integrative taxonomic approach to assess the status of Corsican bumblebees: implications for conservation

T. Lecocq, N. Brasero, T. De Meulemeester, D. Michez, S. Dellicour, P. Lhomme, R. de Jonghe, I. Valterová, K. Urbanová, P. Rasmont

Downloaded from:
https://doi.org/10.1111/acv.12164

Article 25fa Dutch Copyright Act (DCA) - End User Rights

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with consent from the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available following a reasonable period after the work was first published, provided that reference is made to the source of the first publication of the work.

This publication is distributed under the Naturalis Biodiversity Center 'Taverne implementation' programme. In this programme, research output of Naturalis researchers and collection managers that complies with the legal requirements of Article 25fa of the Dutch Copyright Act is distributed online and free of barriers in the Naturalis institutional repository. Research output is distributed six months after its first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and copyrights owner(s) of this work. Any use of the publication other than authorized under this license or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the department of Collection Information know, stating your reasons. In case of a legitimate complaint, Collection Information will make the material inaccessible. Please contact us through email: collectie.informatie@naturalis.nl. We will contact you as soon as possible.

Animal Conservation

Animal Conservation. Print ISSN 1367-9430

An integrative taxonomic approach to assess the status of Corsican bumblebees: implications for conservation

T. Lecocq[1], N. Brasero[1], T. De Meulemeester[2], D. Michez[1], S. Dellicour[3], P. Lhomme[1], R. de Jonghe[1], I. Valterová[4], K. Urbanová[4] & P. Rasmont[1]

1 Research Institute of Biosciences, Laboratory of Zoology, University of Mons, Mons, Belgium
2 Naturalis Biodiversity Center, Leiden, The Netherlands
3 Evolutionary Biology and Ecology, Université Libre de Bruxelles, Brussels, Belgium
4 Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Keywords
Bayesian; bumblebees; endemic; evolutionarily significant unit; genetic marker; insular; integrative taxonomy; phylogenetic analysis.

Correspondence
Thomas Lecocq, Research Institute of Biosciences, Laboratory of Zoology, University of Mons, Place du Parc 20, Mons, B-7000, Belgium. Tel: +003265373405
Email: thomas.lecocq@umons.ac.be

Editor: Jeff Johnson
Associate Editor: Mariana Morando

Received: 6 January 2014; accepted 23 July 2014

doi:10.1111/acv.12164

Abstract

Many islands are biodiversity hotspots that host numerous endemic species. Unfortunately, insular faunas suffer from high rates of extinction and endangerment, and numerous conservation plans have been developed for their protection. These conservation plans are often assessed on the basis of occurrence and proportion of endemic taxa. However, delimitations of species and subspecies are still confusing and controversial. From a practical point of view, these disagreements make it difficult for government agencies and non-governmental organizations to initiate conservation measures. The present study develops a pragmatic integrative taxonomic approach on the basis of molecular and eco-chemical criteria. This method is applied to the insular bumblebee fauna of Corsica. For each taxon, the differentiation of Corsican taxa from the nearest related allopatric parents is characterized using genetic markers and the chemical composition of cephalic labial gland secretions. Phylogenetic analyses, Bayesian implementation of the general mixed Yule-coalescent approach, and comparative chemical studies show that two Corsican taxa can be considered as endemic species while five others can be considered as subspecies. Regardless of the taxonomic assessment the method facilitates diagnosis of evolutionarily significant units and rank taxa according to their distinctiveness. International Union for Conservation of Nature red lists are reconsidered according to the new taxonomic hypothesis for Corsican bumblebees. Modifications in species assessments are proposed. The present approach provides useful data sets for policy-makers and conservation organizations.

Introduction

More than any other geographical feature, many islands are well-known biodiversity hotspots that host numerous endemic taxa (Kier *et al*., 2009). Unfortunately, insular taxa are among the most threatened organisms (Collar, 1994). Therefore, well-planned conservation actions are needed to safeguard threatened insular taxa.

In conservation biology, faunal inventories are the basic data used to compile IUCN (International Union for Conservation of Nature) red lists (www.iucnredlist.org) and identify biodiversity hotspots and areas of endemism (Whittaker & Fernández-Palacios, 2007). Conservation plans are often assessed on the basis of occurrence and proportion of endemic species (Whittaker & Fernández-Palacios, 2007) and subspecies (Phillimore & Owens, 2006). This places a premium on consistently delimiting appropriate taxonomic status among insular faunas. However, the criteria for delimiting species and subspecies are still confusing and controversial (e.g. De Queiroz, 2007). The large number of species delimitation approaches based on reproductive isolation, on recognition, on phylogenetic relationships or on ecological criteria (Mayden, 1997) exemplify these disagreements. From a practical point of view, the disagreements on criteria for defining the taxonomic status of organisms make it difficult for government agencies and non-governmental organizations to evaluate the validity of taxonomic status in the listing process for conservation (Haig *et al*., 2006).

The recent development of integrative taxonomy based on the unified species concept (USC) provides an improved method for the taxonomic characterizations (De Queiroz, 2007; Schlick-Steiner *et al*., 2010). The USC argues that all species concepts acknowledge species as separately evolving fragments of metapopulation lineages but diverge in their criterion for delimiting species. The USC proposes that the numerous delimiting species criteria are maintained as operational criteria that can identify the split from one into two species at some step of the speciation process. Therefore, metapopulation lineage separation could be inferred from different lines of evidence for reproductive isolation, phylogenetic divergence or ecological differentiation. Integrative taxonomy considers these multiple independent kinds of evidence as separate criteria when assigning species status (Fisher & Smith, 2008), although species diagnosis is more likely when indicated by multiple lines of evidence. Moreover, by considering subspecies as a step in the process of allopatric speciation (Mayr, 1942), assigning subspecies rank to lineages in ambiguous allopatric cases (i.e. disagreement in selected criteria) has been proposed as a solution (see argumentation in Hawlitschek, Nagy & Glaw, 2012). This solution helps to attribute particular taxonomic status to populations with at least one conspicuous specific operational criterion. In the context of new trends in conservation biology to preserve evolutionarily significant units below the species level (e.g. Crowhurst *et al*., 2011), this subspecies definition provides an effective short cut for estimating patterns of intraspecific diversity (Phillimore & Owens, 2006) and allows assignment of a legal taxonomic status for conservation plans to theses differentiation stages. The present paper applies an integrative taxonomy approach on the basis of USC and the above defined subspecies definition to the bumblebee fauna of Corsica.

Bumblebees (genus *Bombus*) are the most important pollinators for ecosystem services in the North Temperate Zone (Free, 1993). Unfortunately, many bumblebee species are experiencing a strong decline (Williams & Osborne, 2009; Rasmont & Iserbyt, 2012). IUCN red lists include many bumblebee species (Cederberg *et al*., 2013). Moreover, bumblebee species like *B. terrestris* are important commercially for crop pollination and are exported outside their natural range (Velthuis & van Doorn, 2006). This has resulted in multiple invasions and competition with native species (Williams *et al*., 2012a). In the context of decline and commercial movement, an efficient and practical taxonomic approach is needed prior to planning conservation management strategies. Here, we apply such an approach to the Corsican bumblebee fauna that includes two taxa also distributed on the European mainland (*B. barbutellus* and *B. pascuorum*) and six endemics to Corsica (*B. ruderatus corsicola*, *B. hortorum jonghei*, *B. perezi*, *B. pereziellus*, *B. lucorum renardi* and *B. terrestris xanthopus*) (Rasmont & Adamski, 1996). Most Corsican endemic taxa have been previously considered as species by their original descriptor mainly because of specific Corsican colour patterns (black hairs and a red-brownish tail) despite the unsuitability of colour pattern as a diagnostic character for bumblebee species (Carolan *et al*., 2012). More recently, based on the same colour pattern, taxonomists have regarded the Corsican endemics as subspecies of continental species (Table 1, review in Rasmont & Adamski, 1996), but the suitability of colour pattern in discriminating some subspecies has been also criticized (Bertsch & Schweer, 2012). Additional features were found to diverge in Corsican endemics; this includes ecology of *B. perezi* by Rasmont & Adamski (1996), behaviour of *B. terrestris xanthopus* by De Jonghe (1986) and molecular and chemo-ecological characters of species-specific secretions involved in the pre-mating behaviour in *B. perezi*, *B. lucorum renardi* and *B. terrestris xanthopus* (Lecocq *et al*., 2013b). However, bioassays on *B. terrestris xanthopus* show that Corsican taxa interbreed with continental *B. terrestris* in experimental conditions

Table 1 Corsican taxa/population and their nearest continental parents

Corsican taxa	*n*	Nearest continental parents	*n*
B. (*Psithyrus*) *barbutellus* (Kirby, 1802)	3	*B*. (*Psithyrus*) *barbutellus* (Kirby, 1802)	6
B. (*Megabombus*) *ruderatus corsicola* Strand, 1917	19	*B*. (*Megabombus*) *ruderatus* (Fabricius, 1775)	
Alternative taxonomic status: *B. corsicola*		*B. ruderatus autumnalis* (Fabricius, 1793)	5
		B. ruderatus ruderatus (Fabricius, 1775)	20
B. (*Megabombus*) *hortorum jonghei* Rasmont, 1996	14	*Bombus* (*Megabombus*) *hortorum* (L. 1761)	
		B. hortorum hortorum (L. 1761)	27
		B. hortorum asturiensis (Tkalců, 1974)	1
B. (Thoracobombus) pascuorum melleofacies Vogt, 1909	5	*B. (Thoracobombus) pascuorum melleofacies* Vogt, 1909	10
B. (*Psithyrus*) *perezi* (Schulthess-Rechberg, 1886)	19	*B*. (*Psithyrus*) *vestalis* (Fourcroy, 1785)	
Alternative taxonomic status: *B. vestalis perezi*		*B. vestalis vestalis* (Fourcroy, 1785)	29
B. (*Thoracobombus*) *pereziellus* (Skorikov, 1922)	10	*B*. (*Thoracobombus*) *muscorum* (L. 1758)	
Alternative taxonomic status: *B. muscorum pereziellus*		*B. muscorum muscorum* (L. 1758)	6
		B. muscorum allenelus Stelfox, 1933	1
		B. muscorum liepetterseni Löken, 1973	6
B. (*Bombus*) *lucorum renard*i Radoszkowski,1884	18	*B*. (*Bombus*) *lucorum* (L. 1761)	
Alternative taxonomic status: *B. renard*i		*B. lucorum lucorum* (L. 1761)	24
B. (*Bombus*) *terrestris xanthopus* Kriechbaumer, 1870		*B*. (*Bombus*) *terrestris* (L. 1758)	
Alternative taxonomic status: *B. xanthopus*	19	*B. terrestris dalmatinus* Dalla Torre, 1882	11
		B. terrestris lusitanicus Krüger, 1956	9
		B. terrestris terrestris (L. 1758)	10

The subspecies of continental species refer to taxa used in the present study. *n* is the number of specimens collected.

(De Jonghe, 1986) and other recent taxonomic studies suggest that *B. terrestris xanthopus* is a *B. terrestris* subspecies (Rasmont *et al.*, 2008; Bertsch & Schweer, 2012; Williams *et al.*, 2012b).

In this paper, we develop an integrative and pragmatic taxonomic approach to assess the species and subspecies status, an essential first step in conservation biology programs. We apply this approach to the Corsican bumblebees by using two genetic markers and one eco-chemical trait. We also show the consequences of relevant taxonomic statuses on the conservation status of the focal taxa.

Materials and methods

Sampling

We sampled all Corsican taxa and their nearest continental relatives (Corsican–mainland pairs) (Table 1; Supporting Information Table S1). Several samples were described in Lecocq *et al.* (2011, 2013b). In order to perform taxonomic analyses on the broadest possible monophyletic group that includes the Corsican taxa, we also tried to sample all the closely related species of each Corsican–mainland pair. Except for subspecies of *B. terrestris*, the monophyly of groups formed by each Corsican–mainland pair and its closely related species were established in previous phylogenetic studies (Pedersen, 2002; Cameron, Hines & Williams, 2007). Bees were killed by freezing at −20°C.

Here, we considered Corsican bumblebees without *a priori* taxonomic status and referred to them as *corsicola*, *jonghei*, *perezi*, *pereziellus*, *renardi*, *xanthopus*, Corsican *barbutellus* and Corsican *pascuorum*.

Genetic divergence

We sequenced two genes commonly used in bumblebees phylogenetic studies (e.g. Pedersen, 2002): mitochondrial cytochrome oxidase 1 (COI) and nuclear protein-coding gene elongation factor-1 alpha, F2 copy (EF-1α). We extracted total DNA and carried out polymerase chain reaction (PCR) amplifications (Supporting Information Appendix S1). We sequenced both strands of each PCR product and then computed the consensus of both strands with CodonCode Aligner 3.0.1 (Supporting Information Appendix S1). Sequences were aligned with MAFFT ver.6 (Katoh *et al.*, 2002). The final molecular datasets spanned 849 bp from COI [250 parsimony informative sites (PIS)] and 773 bp from EF-1α F2 copy containing a ~200 bp intron (118 PIS; GenBank numbers in Supporting Information Table S1).

We performed phylogenetic analyses to investigate the genetic differentiation of Corsican bumblebees. We analyzed each gene independently with maximum likelihood (ML) and Bayesian (MB) methods. For both methods, we partitioned each gene to choose the best fitting substitution models with jModeltest (Posada, 2008; Supporting Information Appendix S1).

We conducted ML analyses with Garli 2.0 (Zwickl, 2006; Supporting Information Appendix S1). We performed 10 independent runs in Garli for each gene; the topology and –ln L were identical among replicates. The run with the highest likelihood was retained. We evaluated statistical confidence in nodes with 10 000 non-parametric bootstrap replicates. Topologies with bootstrap values ≥ 70% were considered well supported (Hillis & Bull, 1993).

We performed MB analyses with Mr.Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003). We carried out five independent analyses for each gene (100 million generations, four chains with mixed-models, default priors, saving trees every 100 generations; Supporting Information Appendix S1). Then, we discarded the first ten million generations as burn-in. The phylogeny and posterior probabilities were then estimated from the remaining trees and a majority-rule 50% consensus tree was constructed. Topologies with posterior probabilities ≥ 0.95 were considered as well supported (Wilcox *et al.*, 2002).

The extent of genetic differentiation

In order to characterize the extent of the genetic divergence of Corsican taxa, we used the bGMYC method (Reid & Carstens, 2012), a Bayesian implementation of the general mixed Yule-coalescent (GMYC; Pons *et al.*, 2006) integrating the uncertainty related to phylogenetic inference (Reid & Carstens, 2012). 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 (Barraclough, Birky & Burt, 2003). We applied this method on loci where divergences of Corsican taxa were detected by MB and ML phylogenetic analyses (here we detected only divergences of Corsican taxa in COI see MB and ML results). The bGMYC analyses were performed on each broadest available monophyletic group that included each Corsican taxon. 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 was not conspecific (Reid & Carstens, 2012). Other 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 & Carstens, 2012). The bGMYC algorithm requires several ultrametric trees (i.e. trees whose tips are all equidistant from the root). We then used BEAST 1.7.4 (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 based the bGMYC analysis on 1000 trees sampled every 10 000 generations. For each of these 1000 trees, the MCMC was made of 100 000 generations, discarding the first 90 000 as burn-in and sampling every 100 generations.

Eco-chemical trait divergence

We focused on the most studied reproductive trait involved in the bumblebee pre-mating recognition (Ayasse, Paxton & Tengö, 2001; Baer, 2003): the cephalic labial gland secretions (CLGS) used in resolving species status (e.g. Bertsch *et al.*, 2005). CGLS are a species-specific mixture of (mainly aliphatic) compounds, with several main components (e.g. Calam, 1969; Lecocq *et al.*, 2013b), synthesized *de novo* (Žáček *et al.*, 2013). By main compounds, we mean those that have the highest relative amount (RA) in at least one individual of the taxon.

We extracted the CLGS in 400 μL *n*-hexane following De Meulemeester *et al.* (2011). We determined the composition of CLGS by gas chromatography-mass spectrometry (GC/MS, Supporting Information Appendix S1). We analyzed all samples with a gas chromatograph-flame ionization detector with the same chromatographic conditions as in GC/MS (Supporting Information Appendix S1). We calculated RA of compounds in each sample (Supporting Information Appendix S1). We elaborated the data matrix as the alignment of each compound between all samples performed with GCAligner 1.0 (Dellicour & Lecocq, 2013).

We performed statistical comparative analyses of the CLGS of each species groups in R (R Development Core Team, 2013) to detect differentiations of Corsican taxa. We transformed data [$\log (x - 1)$] to reduce the great difference of abundance between highly and lowly concentrated compounds, and then standardized (mean = 0, standard deviation = 1) to reduce the sample concentration effect (De Meulemeester *et al.*, 2011). We compared Corsican taxa and their nearest parents with principal component analyses (PCA; R-package MASS, Venables & Ripley, 2002). We assessed CLGS differentiations of Corsican taxa by performing multiple response permutation procedure (MRPP; R-package vegan, Oksanen *et al.*, 2011). To determine compounds specific to and regular to Corsican taxa, we used the indicator value (IndVal) method (Dufrêne & Legendre, 1997; see Supporting Information Appendix S1). We evaluated the statistical significance of a compound as an indicator at the 0.01 level with a randomization procedure.

Results

Phylogenetic analyses

Phylogenetic analyses on the same genetic markers led to identical relationships between Corsican taxa and their nearest parents (supplementary trees at TreeBase TB2:S14553). EF-1α phylogenetic analyses recovered all deep hierarchical-level relationships among subgenera but failed to discriminate closely related species (i.e. haplotype shared between *B. lucorum* and *B. terrestris*; Fig. 1a). All Corsican taxa/populations were not differentiated in EF-1α but *renardi* and *perezi* had some specific haplotypes not shared with their mainland counterparts (Fig. 1a). All Corsican taxa except Corsican *pascuorum* were differentiated by specific haplotypes using COI. COI phylogenetic analyses resolved the relationships between *corsicola*, *jonghei*, *perezi*, *renardi* and *xanthopus* and their nearest parents in two well-supported clades (Fig. 1b). The *pereziellus-muscorum* and *barbutellus* groups split in three main clades (including one Corsican clade; Fig. 1b).

bGMYC analyses

The bGMYC analyses on COI showed probabilities of conspecificity ranging from 0 to 0.30 between out-groups and in-groups (Fig. 2; Supporting Information Table S2). Comparisons between Corsican taxa and their nearest parents displayed bGMYC conspecificity probabilities of 0.9–1 (Corsican *pascuorum* vs. *B. pascuorum*), 0.85 (*corsicola* vs. *B. ruderatus*), 0.81 (*jonghei* vs. *B. hortorum*), 0.7 (*perezi* vs. *B. vestalis*), 0.54 (*renardi* vs. *B. lucorum*), 0.39 (*pereziellus* vs. *B. muscorum*), 0.2 (*xanthopus* vs. *B. terrestris*) and 0.09–0.23 (Corsican *barbutellus* vs. *B. barbutellus*; Fig. 2; Supporting Information Table S2). The bGMYC results were not significant except for some Corsican *barbutellus* and some Corsican *pascuorum*.

CLGS analyses

We detected several compounds in the CLGS (*corsicola* group: 38; *jonghei* group: 50; *pereziellus* group: 35; *pascuorum* group: 50; Supporting Information Table S3). The CLGS of continental taxa were similar to previous studies (Kullenberg *et al.*, 1973; Appelgren *et al.*, 1991; Urbanová *et al.*, 2004). CLGS results of the four other groups were reported by Lecocq *et al.* (2011, 2013b) with the same GC methods.

PCA indicated CLGS differentiation of *corsicola*, *perezi*, *renardi* and *xanthopus* (Fig. 3). MRPP confirmed these differentiations (all P values < 0.01): *corsicola* versus *B. ruderatus* ($T = 0.2317$, $A = 0.2535$); *perezi* versus *B. vestalis* ($T = 0.3782$, $A = 0.1543$); *renardi* versus *B. lucorum* ($T = 0.2869$, $A = 0.2405$); and *xanthopus* versus *B. terrestris* ssp. ($T = 0.3023$, $A = 0.51$). The IndVal method revealed several indicator compounds with strong significance (IndVal > 0.70) for these four Corsican taxa (Supporting Information Table S3): *corsicola*: 13 indictor compounds (IC); *perezi*: three IC; *renardi*: 13 IC (including one main compound); *xanthopus*: 14 IC (including three main compounds).

Discussion

Integrative decision framework

The development of an integrative taxonomic approach aims to overcome the specific limitations of genetic and reproductive trait analyses in order to draw a strongly

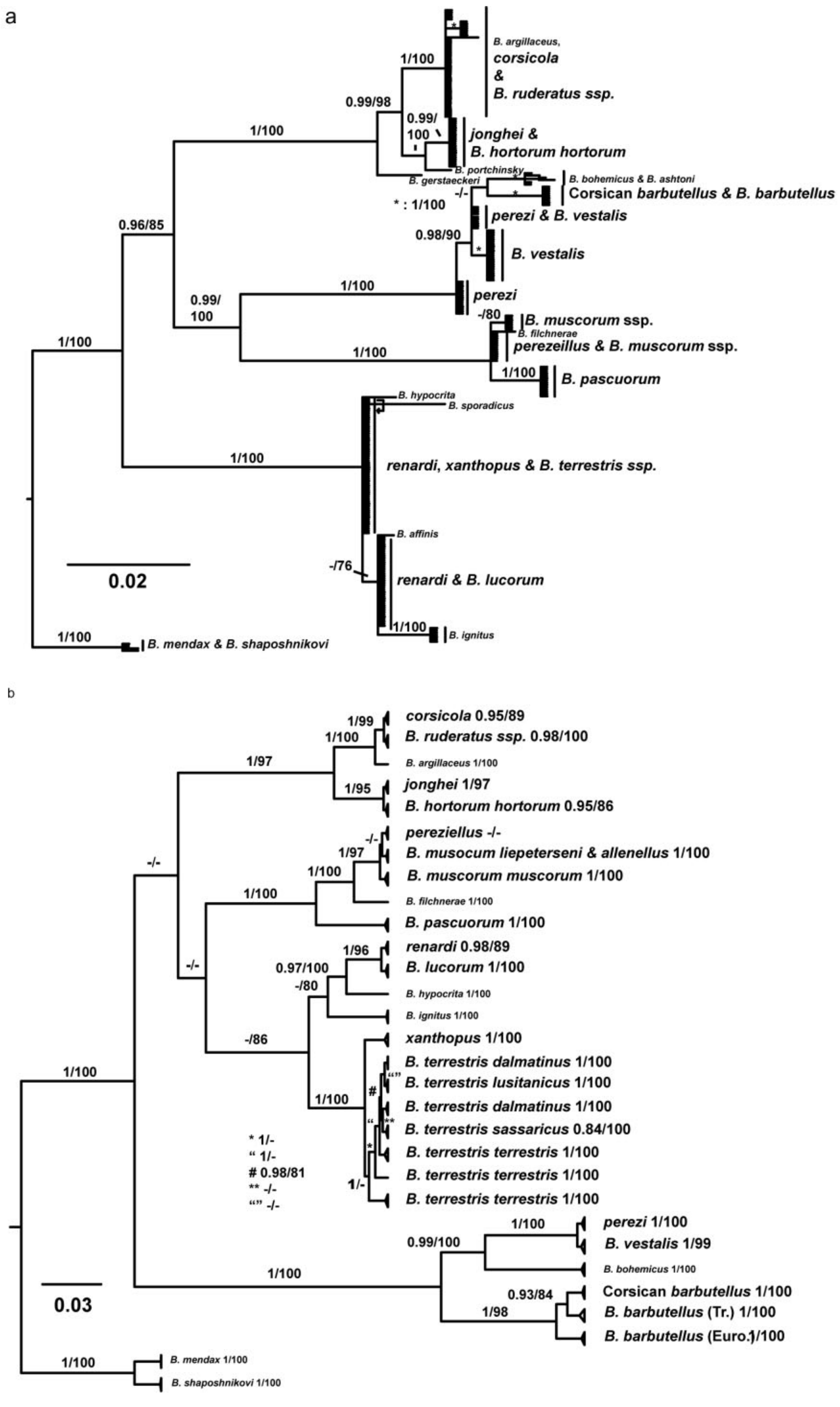

Figure 1 Majority rule consensus of Bayesian analyses of the EF-1α and COI marker. (a) Majority rule (50%) consensus tree of Bayesian analyses of the EF-1α marker. (b) Majority rule (50%) consensus tree of Bayesian analyses of the COI marker. For both trees, the values above branches and after names (1B) are Bayesian posterior probabilities/maximum likelihood bootstrap values of this group. Taxa names in small are out-groups; taxa names in large are Corsican taxa and their continental nearest relatives. Only posterior probabilities > 0.95 and maximum likelihood bootstrap values > 70% are showed.

supported taxonomic hypothesis. Here, we consider that partial genetic differentiation along with reproductive trait differentiation can attest to speciation processes among taxa, with a high degree of certainty. Therefore, we assign species status if the taxon (1) is genetically differentiated (COI or/and EF-1α specific haplotypes); (2) is not significantly conspecific with its continental nearest parents in bGMYC analyses (probability < 0.9 to be conspecific); (3) is significantly differentiated in CLGS compositions; and (4) is differentiated in the main CLGS compounds. We assign subspecies status if there are divergences in some but not all operational criteria according to the subspecies definition proposed by Hawlitschek *et al.* (2012). Since we consider that the speciation between island and mainland populations is the result of a continuous differentiation process, subspecies status allows capturing this process at various stages between the initial conspecificity and the complete speciation.

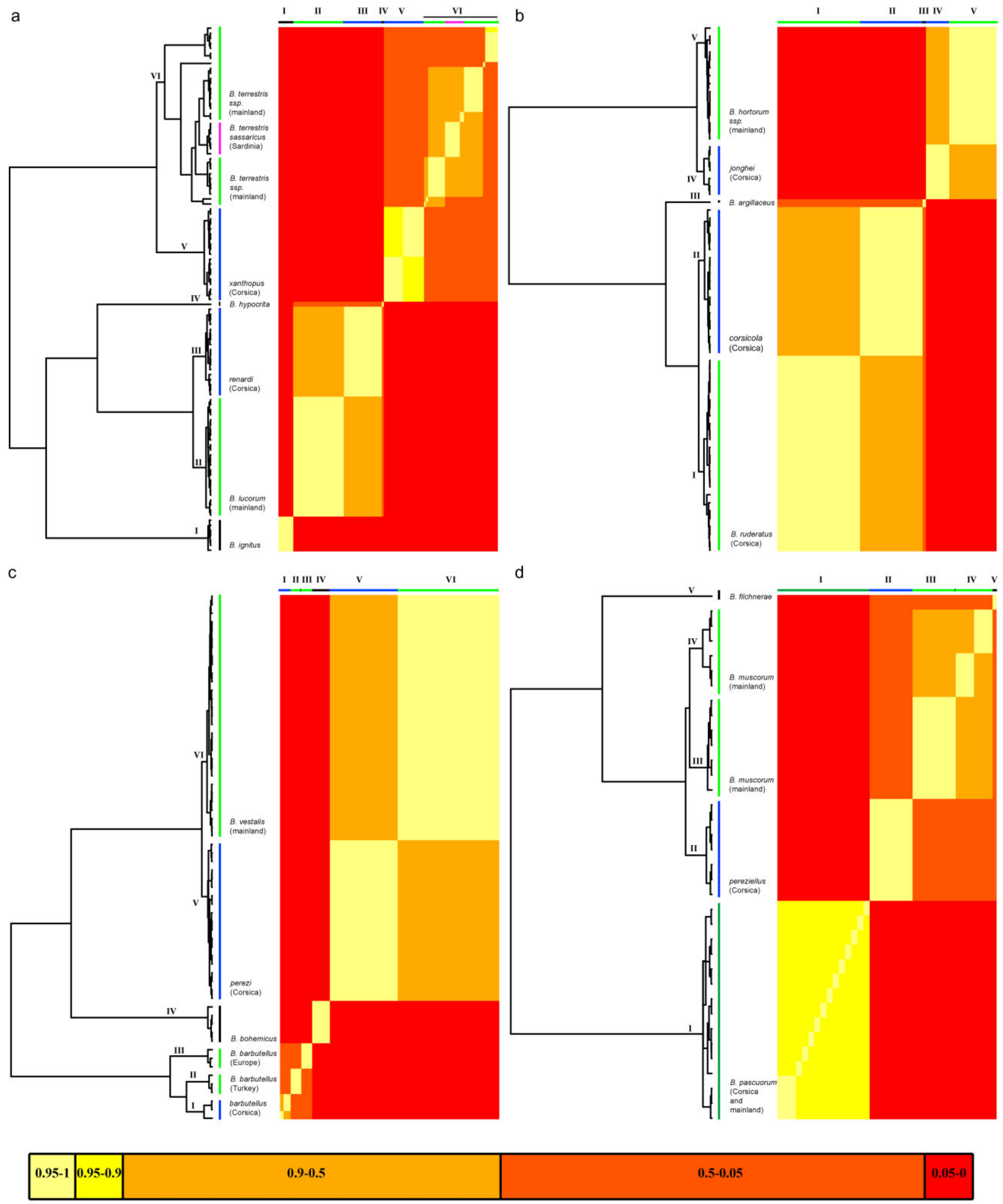

Figure 2 bGMYC results based on COI phylogenetic trees. (a) *renardi* and *xanthopus* (blue vertical lines) and their allopatric nearest parents (green vertical lines). (b) *corsicola* and *jonghei* (blue vertical lines) and their allopatric nearest parents (green vertical lines). (c) *perezi* and Corsican *barbutellus* (blue vertical lines) and their allopatric nearest parents (green vertical lines). (d) *pereziellus* and Corsican *pascuorum* (blue vertical lines) and their allopatric nearest parents (green vertical lines). The black vertical lines are out-groups. The colour scale is the probability scale of conspecificity.

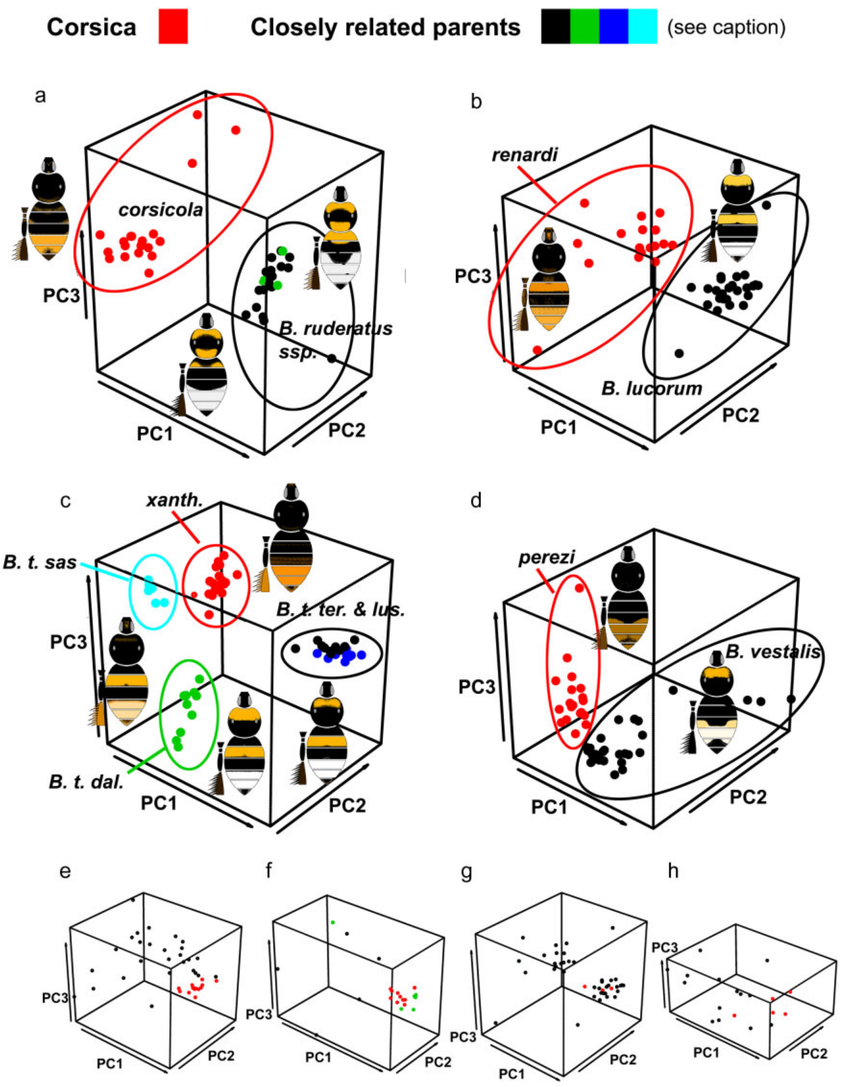

Figure 3 Principle components analyses (PCA) of CLGS of Corsican bumblebees and their continental nearest relatives. PC1, PC2 and PC3 are first, second and third axes of the PCA. Ellipses represent significant groups detected in MRPP. (a) *corsicola* (red), *B. ruderatus ruderatus* (black) and *B. ruderatus autumnalis* (green). (b) *renardi* (red) and *B. lucorum* (black). (c) *xanthopus* (*B. xan.*) (red), *B. terrestris terrestris* (*B. t. ter.*) (black), *B. terrestris dalmatinus* (*B. t. dal.*) (green), *B. terrestris lusitanicus* (*B. t. lus.*) (dark blue) and *B. terrestris sassaricus* (*B. t. sas.*) (light blue). (d) *perezi* (red) and *B. vestalis* (black). (e) *jonghei* (red) and *B. hortorum hortorum* (black). (f) *pereziellus* (red), *B. muscorum muscorum* (black), *B. muscorum liepeterseni* and *B. muscorum allenellus* (green). (g) Corsican *B. barbutellus* (red) and continentals (black). (h) Corsican *B. pascuorum* (red) and mainland population (black).

The accuracy of the proposed integrative method is depending on selected features (see discussion below) and sampling. All modern taxonomic methods based on intra- and interspecific variability comparisons are expected to consider monophyletic groups. Not considering all members of a monophyletic group is especially likely to affect the bGMYC results because the method compares branching patterns within and among subgroups (Fujisawa & Barraclough, 2013). Similarly, limited sampling of a group of taxa makes it impossible to estimate the CLGS diversity among the group. Here, we managed to sample most of the taxa included in all Corsican-mainland clades except for *B. terrestris* (Rasmont *et al*., 2008), *B. barbutellus* (Lecocq *et al*., 2011) and *B. muscorum* groups (the sampling included only distant/isolated populations that could overvalue the bGMYC results). We speculate that limited sampling did not significantly affect our results.

Taxonomic statuses

Species status is assigned to *renardi* and *xanthopus* according to their genetic differentiation and their main compound CLGS differentiations (Table 2). The resulting nomenclature is *B. renardi* Radoszkowski, 1884 and *B. xanthopus* Kriechbaumer, 1870 (nomenclature review in Rasmont & Adamski, 1996). The species status of *B. xanthopus* and *B. renardi* are congruent with their ecological and ethological divergences (review in Rasmont & Adamski, 1996). This result for *B. xanthopus* is conflicting with previous works on *B. terrestris* subspecies that underline divergences in morphology (Rasmont *et al*., 2008), CLGS (Bertsch & Schweer, 2012) or COI (Williams *et al*., 2012b) but do not regard these divergences as deserving a species status. Comprehensive revision is needed to re-evaluate other *B. terrestris* subspecies.

Table 2 Decision-taking table

Corsican bumblebees	COI/ EF-1α Orig. Haplo.	bGMYC	CLGS	Main comp. CLGS	New taxonomic status
corsicola	++/–	*	+	–	*B. ruderatus corsicola*
jonghei	++/–	*	–	–	*B. hortorum jonghei*
perezi	++/+	*	+	–	*B. vestalis perezi*
pereziellus	++/–	*	–	–	*B. muscorum pereziellus*
renardi	++/+	*	+	+	*B. renardi*
xanthopus	++/–	*	+	+	*B. xanthopus*
Corsican *barbutellus*	++/–	*	–	–	*B. barbutellus* ssp. (Corsica)
Corsican *pascuorum*	–/–	–	–	–	*B. pascuorum*

COI/ EF-1α Orig. Haplo. indicate whether Corsican COI/ EF-1α haplotypes are shared with closely related allopatric parents (++ means that all Corsican haplotypes are not shared with allopatric parents, + means that some Corsican haplotypes are shared with allopatric parents, – means that all Corsican haplotypes are shared with allopatric parents). bGMYC indicates the probability of Corsican taxa to be conspecific with their continental nearest parents in bGMYC analyses [– means that the taxa/population have a mean probability > 0.9 to be conspecific with their continental parents, * indicates the taxa/population have not a significant probability (< 0.9) to be conspecific with their continental parents]. CLGS indicates whether the Corsican taxa/population is significantly differentiated (+) in their cephalic labial gland secretions. Main comp. CLGS indicates whether the CLGS differentiation involves main compounds.

Subspecies status is assigned to *corsicola*, *jonghei*, *pereziellus*, and *perezi*, and is confirmed for Corsican *B. barbutellus* (see Lecocq *et al*., 2011) according to divergence in one or some operational criteria (Table 2). The new nomenclatures are *B. ruderatus corsicola* Strand, 1917, *B. hortorum jonghei* Rasmont, 1996, *B. muscorum pereziellus* (Skorikov, 1922), *B. vestalis perezi* (Schulthess-Rechberg, 1886) and Corsican *B. barbutellus* spp. (nomenclature review in Rasmont & Adamski, 1996). For Corsican *B. barbutellus*, we currently lack of Italian *B. barbutellus* (previously considered as consubspecific with Corsican population; see Lecocq *et al*., 2011) to assess the status of endemic subspecies. All taxonomic statuses of these taxa with conspicuous specific characters should be reconsidered if future genetic analyses or bioassays point out higher differentiation degree from their continental parents. Corsican *B. pascuorum* is considered as similar to its continental parents according to a lack of divergence (Table 2). These results agree with most of previous studies (see Rasmont & Adamski, 1996; Lecocq *et al*. 2011, 2013b).

Limitation of studied characters

Monophyly based on molecular data or at least original haplotypes can provide evidence of speciation between taxa (Avise, 2000, 2004). However, the detection of this genetic evidence depends on the targeted markers that could lead to different tree topologies and thus to conflicting taxonomic statuses. For example, the usefulness of nuclear gene sequences in discriminating closely related taxa appears generally limited in many animal groups compared with mitochondrial markers for similar taxonomic levels as observed in the present study (Fig. 1). This is presumably a consequence of the substantially greater coalescence time of nuclear genes (Boursot & Bonhomme, 1986). Further studies on nuclear markers with higher mutation rates (e.g. phosphoenolpyruvate carboxykinase) could provide a more efficient tool in discriminating closely related taxa (e.g. Leys, Cooper & Schwarz, 2002; Lecocq *et al*., 2013a). However, determining objective markers for species delimitation is difficult because a variety of factors can cause the genealogy from a particular locus to be discordant with the true history of speciation (Maddison, 1997; Reid & Carstens, 2012). An alternative solution is to develop a multilocus approach such as restriction site-associated DNA sequencing to avoid taxonomic conclusions based on few loci whose power of recognizing species may be limited by the total amount of variation (Cruaud *et al*., 2014). However, such approaches are not within an easy reach for all taxonomists.

Taxonomic assessment methods exclusively based on genetic distance (e.g. methods of cut-off rule, Brower, 1994, or pairwise distance thresholds, Tang *et al*., 2012) generally leads to higher splitting (Agapow *et al*., 2004), especially in island taxa because of reduced gene flow with other populations, founder events and genetic drift (Peterson & Navarro-Sigüenza, 1999). Moreover, those methods based on genetic distance suffer (1) from a weak connection to evolutionary theory; (2) from variation in typical levels of intraspecific and interspecific variation among clades; and (3) from substitution rate variation among lineages (Barraclough *et al*., 2009). Delimiting species approaches based on phylogenetic inferences such as bGMYC aims to avoid these limitations and allow taking in account the species lineages as well as other conspicuous evolutionary units below the species level. However, our bGMYC analyses failed to detect several out-group species as significantly not conspecific with other species despite their recognized species status (e.g. Williams *et al*., 2012b). This is presumably a consequence of GMYC methods, which assume that species are distinct genetic clusters (i.e. monophyly) separated by longer internal branches (Barraclough *et al*., 2003) that could be not observed between closely related species (Esselstyn *et al*., 2012; Zhang *et al*., 2013). Indeed, all genetic-based approaches for species delimitation (e.g. cut-off rule, pairwise distance thresholds, bGMYC) can be contested because (1) speciation processes are not always characterized by accumulation of many genetic differences (e.g. Ferguson, 2002; Kuhlmann *et al*., 2007);

(2) differentiation between two species does not always result in two monophyletic groups (e.g. paraphyletic species; Kruckenhauser *et al.*, 2014); (3) conspecific populations can display high genetic divergence (e.g. Salvato *et al.*, 2002); and (4) mating isolation can happen faster than differentiation of genetic markers (Trewick, 2008; Symonds, Moussalli & Elgar, 2009; Bauer *et al.*, 2011). One alternative approach is to base species delimitations on reproductive traits involved in species mating recognition (Paterson, 1993).

Divergence in reproductive traits provides useful criteria to detect pre-mating isolation between individuals (Paterson, 1993). However, consequences of divergences in reproductive traits can range from simple regional variation (i.e. 'dialects' consisting of different relative amounts of the same key compounds; e.g. Vereecken, Mant & Schiestl, 2007) to the establishment of a reproductive isolation barrier (Martens, 1996). In the case of Corsican bumblebees, four taxa are significantly differentiated in CLGS from their closest parents. The *xanthopus* CLGS differentiation (also detected by Bertsch & Schweer, 2012) as well as the *renardi* CLGS differentiation involve main compounds. We regard these main compound differentiations as a strong indicator of potential ethological consequences for pre-mating recognition because most bumblebee species diverge in CLGS main compounds (e.g. Bertsch *et al.*, 2005). In contrast, CLGS divergence of *corsicola* and *perezi* does not involve main compounds and suggests only a 'Corsican dialect' that presumably does not lead to establishment of a reproductive isolation barrier (e.g. Vereecken *et al.*, 2007), even if few changes in chemical reproductive traits can lead to such a consequence. Further bioassays are needed, but this requires species-specific year-round rearing methods (Lhomme *et al.*, 2012, 2013) that are not available for all species (Hasselrot, 1960).

Conservation implications

In conservation, erroneous decisions may be made if taxonomic status is incorrectly assigned. It could lead to ignorance of an endangered species that prevents conservation plans, legal protection of different populations of a common species erroneously considered as distinct species or hybridization issues in conservation management (review in Frankham, Ballou & Briscoe, 2010). Regardless of taxonomic status (species or subspecies), our integrative approach brings to attention the relevance of geographically isolated conspicuous groups differentiated in genetic and reproductive traits, corresponding to Evolutionarily Significant Units (ESUs; Conner, 2004). This allows the definition of management units important for conservation. For example, crossing between species or between genetically differentiated conspecific populations (outbreeding) can result in reduced fitness (e.g. outbreeding depression); awareness of ESUs can prevent this issue. Moreover, these ESU might reflect adaptive variation (Crandall *et al.*, 2000). For example, conservation of subspecies with different CLGS dialects preserves the diversity of communication signals. This diversity could increase the adaptive potential of such taxa (Fisher, 1930) in the context of anthropogenic disturbances of animal communication (Rosenthal & Stuart-Fox, 2012).

The assignment of suitable taxonomic statuses to ESUs is crucial in conservation (Frankham *et al.*, 2010). Indeed, elevation of all ESUs to the species level to focus management plans on these units leads to a taxonomic inflation making it increasingly difficult to provide funding for conservation (e.g. Isaac, Mallet & Mace, 2004). The possibility to assign several taxonomic statuses (conspecific, subspecies and species) and to quantify the number of differentiated characters (genetic markers, CLGS) and the strength of these divergences (probability in bGMYC, divergence in CLGS main compounds) in our integrative methods can provide a ranking of distinctiveness for all studied ESU. This can provide a decision framework for policy-makers and conservation organizations to allocate funding and management efforts.

The taxa of the insular Corsican bumblebee fauna can be ranked as follow: (1) endemic species: *B. xanthopus* and *B. renardi*; (2) endemic subspecies with conspicuous divergences: *B. ruderatus corsicola*, *B. hortorum jonghei*, *B. muscorum pereziellus*, *B. vestalis perezi* and Corsican *B. barbutellus*; and (3) non-endemics: Corsican *B. pascuorum*. These new taxonomical hypotheses have implications for the red list assessments of some European bumblebees according to IUCN criteria (IUCN Species Survival Commission, 2012). Cederberg *et al.* (2013) considered two taxa as endemic in Corsica *B. perezi* and *B. pereziellus* (both are assessed least concern). Now populations of *B. muscorum pereziellus* should be evaluated with other continental populations of *B. muscorum*. As this species was considered as vulnerable following criteria A (category and criteria A2c), populations of *B. muscorum pereziellus* should be now considered as red listed and protected. Moreover, the two 'new' species *B. renardi* and *B. xanthopus* should have a new original assessment. As *B. terrestris* is not present now on Corsica, its trade should be ceased to avoid competition with its close relative *B. xanthopus* (Williams *et al.*, 2012a).

Further applications of the present method

Our integrative taxonomic approach allows assignment of taxonomic status (consubspecific, subspecies or species) to ESUs defined by their specificity (divergence) in genetic and reproductive traits as well as the strength of this specificity for endemic taxa by comparison with allopatric ones. This provides a decision framework for policy-makers and conservation organizations. Our integrative approach and taxonomic decision framework could be applied to other species that use chemical reproductive traits.

Acknowledgements

This research was supported by the *Office de l'Environnement de la Corse*, the *Direction Régionale de l'Environnement de Corse* and the Academy of Sciences of the Czech Republic (RVO61388963). The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 244090, STEP Project (Status and Trends of European Pollinators, www.step-project.net). T.L. and S.D. are grant holders of the *Fonds pour la Recherche dans l'Industrie et l'Agriculture*. We thank B.N. Danforth (Ithaca, USA) for help for the molecular analysis and S. Lambert (Mons, Belgium) for her helpful comments. We thank A. Coppée (Mons, Belgium) for her help on this project. We acknowledge W.B. Walker (Swedish University of Agricultural Sciences) and David G. Notton (Natural History Museum, London) for correcting the English.

References

Agapow, P.-M., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C. & Purvis, A. (2004). The impact of species concept on biodiversity studies. *Q. Rev. Biol.* **79**, 161–179.

Appelgren, M., Bergström, G., Svensson, B.G. & Cederberg, B. (1991). Marking pheromones of *Megabombus* bumble bee males. *Acta Chem. Scand.* **45**, 972–974.

Avise, J.C. (2000). *Phylogeography: the history and formation of species*: Cambridge: Harvard University Press.

Avise, J.C. (2004). What is the field of biogeography, and where is it going? *Taxon* **53**, 893–898.

Ayasse, M., Paxton, R.J. & Tengö, J. (2001). Mating behavior and chemical communication in the order Hymenoptera. *Annu. Rev. Entomol.* **59**, 299–319.

Baer, B. (2003). Bumblebees as model organisms to study male sexual selection in social insects. *Behav. Ecol. Sociobiol.* **54**, 521–533.

Barraclough, T.G., Birky, C.W. Jr. & Burt, A. (2003). Diversification in sexual and asexual organisms. *Evolution (N. Y.)* **57**, 2166–2172.

Barraclough, T.G., Hughes, M., Ashford-Hodges, N. & Fujisawa, T. (2009). Inferring evolutionarily significant units of bacterial diversity from broad environmental surveys of single-locus data. *Biol. Lett.* **5**, 425–428.

Bauer, A.M., Parham, J.F., Brown, R.M., Stuart, B.L., Grismer, L., Papenfuss, T.J., Böhme, W., Savage, J.M., Carranza, S., Grismer, J.L., Wagner, P., Schmitz, A., Ananjeva, N.B. & Inger, R.F. (2011). Availability of new Bayesian-delimited gecko names and the importance of character-based species descriptions. *Proc. Roy. Soc. Lond. Ser. B.* **278**, 490–493.

Bertsch, A. & Schweer, H. (2012). Cephalic labial gland secretions of males as species recognition signals in bumblebees: are there really geographical variations in the secretions of the *Bombus terrestris* subspecies? *Beitr. Ent.* **62**, 103–124.

Bertsch, A., Schweer, H., Titze, A. & Tanaka, H. (2005). Male labial gland secretions and mitochondrial DNA markers support, species status of *Bombus cryptarum* and *B. magnus* (Hymenoptera, Apidae). *Insectes Soc.* **52**, 45–54.

Boursot, P. & Bonhomme, F. (1986). Génétique et évolution du génome mitochondrial des Métazoaires. *Genet. Sel. Evol.* **18**, 73–78.

Brower, A.V.Z. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. USA* **91**, 6491–6495.

Calam, D.H. (1969). Species and sex-specific compounds from the heads of male bumblebees (*Bombus* spp. *Nature* **221**, 856–857.

Cameron, S.A., Hines, H.M. & Williams, P.H. (2007). A comprehensive phylogeny of the bumble bees (*Bombus*). *Biol. J. Linn. Soc.* **91**, 161–188.

Carolan, J.C., Murray, T.E., Fitzpatrick, U., Crossley, J., Schmidt, H., Cederberg, B., McNally, L., Paxton, R.J., Williams, P.H. & Brown, M.J.F. (2012). Colour patterns do not diagnose species: quantitative evaluation of a DNA barcoded cryptic bumblebee complex. *PLoS ONE* **7**, e29251.

Cederberg, B., Michez, D., Nieto, A., Radchenko, V., Rasmont, P. & Roberts, S. (2013). Bombus In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Downloaded on 01 Januray 2014. http://www.iucnredlist.org (accessed 2 January 2014).

Collar, N. (1994). Extinction, endangerment and everything. *World Birdwatch* **16**, 6–9.

Conner, J. (2004). *A primer of ecological genetics*. Sunderland: Sinauer Associates.

Crandall, K.A., Bininda-Emonds, O.R.R., Mace, G.M. & Wayne, R.K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**, 290–295.

Crowhurst, R.S., Faries, K.M., Collantes, J., Briggler, J.T., Koppelman, J.B. & Eggert, L.S. (2011). Genetic relationships of hellbenders in the Ozark highlands of Missouri and conservation implications for the Ozark subspecies (*Cryptobranchus alleganiensis bishopi*). *Conserv. Genet.* **12**, 637–646.

Cruaud, A., Gautier, M., Galan, M., Foucaud, J., Sauné, L., Genson, G., Dubois, E., Nidelet, S., Deuve, T. & Rasplus, J.-Y. (2014). Empirical assessment of RAD sequencing for interspecific phylogeny. *Mol. Biol. Evol.* **31**, 1272–1274.

De Jonghe, R. (1986). Crossing experiments with *Bombus terrestris terrestris* (Linnaeus, 1758) and *Bombus terrestris xanthopus* Kricehbaumer, 1870 and some notes on diapause and nosemose (Hymenoptera: Apoidea). *Phegea* **14**, 19–23.

De Meulemeester, T., Gerbaux, P., Boulvin, M., Coppée, A. & Rasmont, P. (2011). A simplified protocol for bumble bee species identification by cephalic secretion analysis. *Insectes Soc.* **58**, 227–236.

De Queiroz, K. (2007). Species concepts and species delimitation. *Syst. Biol.* **56**, 879–886.

Dellicour, S. & Lecocq, T. (2013). GCALIGNER 1.0: an alignment program to compute a multiple sample comparison data matrix from large eco-chemical datasets obtained by gas chromatography. *J. Sep. Sci.* **36**, 3206–3209.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973.

Dufrêne, M. & Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* **67**, 345–366.

Esselstyn, J.A., Evans, B.J., Sedlock, J.L., Khan, F.A.A. & Heaney, L.R. (2012). Single-locus species delimitation: a test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. *Proc. Roy. Soc. Lond. Ser. B.* **279**, 3678–3686.

Ferguson, J.W.H. (2002). On the use of genetic divergence for identifying species. *Biol. J. Linn. Soc.* **75**, 509–516.

Fisher, B.L. & Smith, M.A. (2008). A revision of Malagasy species of *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae). *PLoS ONE* **3**, e1787.

Fisher, R. (1930). *The genetical theory of natural selection*. Oxford: Clarendon Press.

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2010). *Introduction to conservation genetics*. 2nd edn. Cambridge: Cambridge University Press.

Free, J.B. (1993). *Insect pollination of crops*. 2nd edn. London: Academic Press.

Fujisawa, T. & Barraclough, T.G. (2013). Delimiting species using single-locus data and the generalized mixed yule coalescent approach: a revised method and evaluation on simulated data sets. *Syst. Biol.* **62**, 707–724.

Haig, S.M., Beever, E.A., Chambers, S.M., Draheim, H.M., Dugger, B.D., Dunham, S., Elliott-Smith, E., Fontaine, J.B., Kesler, D.C., Knaus, B.J., Lopes, I.F., Loschl, P., Mullins, T.D. & Sheffield, L.M. (2006). Taxonomic considerations in listing subspecies under the U.S. Endangered Species Act. *Conserv. Biol.* **20**, 1584–1594.

Hasselrot, T.B. (1960). Studies on Swedish bumblebees (genus *Bombus* Latr.), their domestication and biology. *Opusc. Entomol.* (Suppl.) **17**, 1–192.

Hawlitschek, O., Nagy, Z.T. & Glaw, F. (2012). Island evolution and systematic revision of comoran snakes: why and when subspecies still make sense. *PLoS ONE* **7**, e42970.

Hillis, D.M. & Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **42**, 182–192.

Isaac, N.J.B., Mallet, J. & Mace, G.M. (2004). Taxonomic inflation: its influence on macroecology and conservation. *Trends Ecol. Evol.* **19**, 464–469.

IUCN Species Survival Commission (2012). *Guidelines for application of IUCN red list criteria at regional and national levels version* 4.0. Gland: IUCN.

Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066.

Kier, G., Kreft, H., Tien, M.L., Jetz, W., Ibisch, P.L., Nowicki, C., Mutke, J. & Barthlott, W. (2009). A global assessment of endemism and species richness across island and mainland regions. *Proc. Natl. Acad. Sci. USA* **106**, 9322–9327.

Kruckenhauser, L., Duda, M., Bartel, D., Sattmann, H., Harl, J., Kirchner, S. & Haring, E. (2014). Paraphyly and budding speciation in the hairy snail (Pulmonata, Hygromiidae). *Zool. Scr.* **43**, 273–288.

Kuhlmann, M., Else, G.R., Dawson, A. & Quicke, D.L.J. (2007). Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae). *Org. Divers. Evol.* **7**, 155–165.

Kullenberg, B., Bergström, G., Bringer, B., Carlberg, B. & Cederberg, B. (1973). Observations on scent marking by *Bombus* Latr. and *Psithyrus* Lep. males (Hym., Apidae) and localization of site of production of the secretion. *Zoon* (Suppl.) **1**, 23–30.

Lecocq, T., Lhomme, P., Michez, D., Dellicour, S., Valterová, I. & Rasmont, P. (2011). Molecular and chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *Bombus maxillosus* (Hymenoptera, Apidae, Bombini). *Syst. Entomol.* **36**, 453–469.

Lecocq, T., Dellicour, S., Michez, D., Lhomme, P., Vanderplanck, M., Valterová, I., Rasplus, J.-Y. & Rasmont, P. (2013a). Scent of a break-up: phylogeography and reproductive trait divergences in the red-tailed bumblebee (*Bombus lapidarius*). *BMC Evol. Biol.* **13**, 263.

Lecocq, T., Vereecken, N.J., Michez, D., Dellicour, S., Lhomme, P., Valterová, I., Rasplus, J.-Y. & Rasmont, P. (2013b). Patterns of genetic and reproductive traits differentiation in mainland vs. Corsican populations of bumblebees. *PLoS ONE* **8**, e65642.

Leys, R., Cooper, S.J.B. & Schwarz, M.P. (2002). Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). *Biol. J. Linn. Soc.* **77**, 249–266.

Lhomme, P., Ayasse, M., Valterová, I., Lecocq, T. & Rasmont, P. (2012). Born in an alien nest: how do social parasite male offspring escape from host aggression? *PLoS ONE* **7**, e43053.

Lhomme, P., Sramkova, A., Kreuter, K., Lecocq, T., Rasmont, P. & Ayasse, M. (2013). A method for year-round rearing of cuckoo bumblebees (Hymenoptera:

Apoidea: *Bombus* subgenus *Psithyrus*). *Ann. Soc. Entomol. Fr.* **49**, 37–41.

Maddison, W.P. (1997). Gene trees in species trees. *Syst. Biol.* **46**, 523–536.

Martens, J. (1996). Vocalizations and speciation of Palearctic birds. In *Ecology and evolution of acoustic communication in birds*: 221–240. Kroodsma, D.E. & Miller, E.H. (Eds). Ithaca: Comstock Publishing.

Mayden, R. (1997). A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: the units of biodiversity*: 381–424. Claridge, M.F., Dawah, H.A. & Wilson, M.R. (Eds). London: Chapman and Hall Ltd.

Mayr, E. (1942). *Systematics and the origin of species*. New York: Columbia University Press.

Oksanen, F.J., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2011). Vegan: community ecology package. Tertiary Vegan: Community Ecology Package.

Paterson, H.E.H. (1993). *Evolution and the recognition concept of species*. Baltimore: The Johns Hopkins University Press.

Pedersen, B.V. (2002). European bumblebees (Hymenoptera: Bombini) – phylogenetic relationships inferred from DNA sequences. *Insect Syst. Evol.* **33**, 361–386.

Peterson, A.T. & Navarro-Sigüenza, A.G. (1999). Alternate species concepts as bases for determining priority conservation areas. *Conserv. Biol.* **13**, 427–431.

Phillimore, A.B. & Owens, I.P.F. (2006). Are subspecies useful in evolutionary and conservation biology? *Proc. R. Soc. B Biol. Sci.* **273**, 1049–1053.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* **55**, 595–609.

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253–1256.

R Development Core Team (2013). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. ISBN 3-900051-07-0. http://www.R-project.org (accessed 2 January 2014).

Rasmont, P. & Adamski, A. (1996). Les Bourdons de la Corse (Hymenoptera, Apoidea, Bombinae). *Notes fauniques de Gembloux* **31**, 1–87.

Rasmont, P. & Iserbyt, S. (2012). Atlas of the European bees: genus *Bombus*. STEP Project. Status Trends Eur. Pollinators, Tertiary Atlas of the European Bees: genus Bombus. STEP Project. http://www.zoologie.umh.ac.be//hymenoptera/page.asp?ID=169 (accessed 2 January 2014).

Rasmont, P., Coppée, A., Michez, D. & De Meulemeester, T. (2008). An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). *Ann. Soc. Entomol. Fr.* **44**, 243–250.

Reid, N.M. & Carstens, B.C. (2012). Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evol. Biol.* **12**, 196.

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Rosenthal, G.G. & Stuart-Fox, D. (2012). Environmental disturbance and animal communication. In *Behavioural responses to a changing world*: 17–31. Candolin, U. & Wong, B.B.M. (Eds). Oxford: Oxford University Press.

Salvato, P., Battisti, A., Concato, S., Masutti, L., Patarnello, T. & Zane, L. (2002). Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa – wilkinsoni* complex), inferred by AFLP and mitochondrial DNA markers. *Mol. Ecol.* **11**, 2435–2444.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annu. Rev. Entomol.* **55**, 421–438.

Symonds, M.R.E., Moussalli, A. & Elgar, M.A. (2009). The evolution of sex pheromones in an ecologically diverse genus of flies. *Biol. J. Linn. Soc.* **97**, 594–603.

Tang, C.Q., Leasi, F., Obertegger, U., Kieneke, A., Barraclough, T.G. & Fontaneto, D. (2012). The widely used small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of the meiofauna. *Proc. Natl. Acad. Sci. USA* **109**, 16208–16212.

Trewick, S.A. (2008). DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics* **24**, 240–254.

Urbanová, K., Halák, J., Hovorka, O., Kindl, J. & Valterová, I. (2004). Marking pheromones of the cuckoo bumblebee males (Hymenoptera, Apoidea, *Bombus* Latreille): compositions of labial gland secretions of six species found in the Czech Republic. *Biochem. Syst. Ecol.* **32**, 1025–1045.

Velthuis, H.H.W. & van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37**, 421–451.

Venables, W.N. & Ripley, B.D. (2002). *Modern applied statistics with S*. 4th edn. New York: Springer.

Vereecken, N.J., Mant, J. & Schiestl, F.P. (2007). Population differentiation in female sex pheromone and male preferences in a solitary bee. *Behav. Ecol. Sociobiol.* **61**, 811–821.

Whittaker, R.J. & Fernández-Palacios, J.M. (2007). *Island biogeography – ecology, evolution, and conservation*. 2nd edn. Oxford: Oxford University Press.

Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. (2002). Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Mol. Phylogenet. Evol.* **25**, 361–371.

Williams, P.H. & Osborne, J.L. (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie* **40**, 367–387.

Williams, P.H., An, J., Brown, M.J.F., Carolan, J.C., Goulson, D., Huang, J. & Ito, M. (2012a). Cryptic bumblebee species: consequences for conservation and the trade in greenhouse pollinators. *PLoS ONE* **7**, e32992.

Williams, P.H., Brown, M.J.F., Carolan, J.C., An, J., Goulson, D., Aytekin, A.M., Best, L.R., Byvaltsev, A.M., Cederberg, B., Dawson, R., Huang, J., Ito, M., Monfared, A., Raina, R.H., Schmid-Hempel, P., Sheffield, C.S., Šima, P. & Xie, Z. (2012b). Unveiling cryptic species of the bumblebee subgenus *Bombus s. str.* worldwide with COI barcodes (Hymenoptera: Apidae). *Syst. Biodivers.* **10**, 21–56.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**, 2869–2876.

Zwickl, D.J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criteria*. D. Phil. Thesis, The University of Texas at AustinEditor, Austin, Texas.

Žáček, P., Prchalová-Hornákov, D., Tykva, R., Kindl, J., Vogel, H., Svatoš, A., Pichová, I. & Valterová, I. (2013). De novo biosynthesis of sexual pheromone in the labial gland of bumblebee males. *Chembiochem* **14**, 361–371.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Table of sampling. Taxa, name of taxa; sample codes, sample labels used in analyses and supplementary tree; groups: groups of individuals (Corsica, mainland or out-group), COI and EF-1α, are the GenBank accession numbers for each sample.

Table S2. Results of the bGMYC analysis (pairwise table). Values are probability to be conspecific (1 = 100% of chance to be conspecific). When there is only one value, all individuals from the same taxa/populations have the same probability otherwise the probability range is provided.

Table S3. List of the identified compounds in cephalic labial glands secretion (CLGS) and CLGS data matrix (relative amounts of each compound) in *corsicola* in *jonghei*, in *perezi*, in *pereziellus*, in *renardi*, in *xanthopus*, in Corsican *barbutellus*, in Corsican *pascuorum* and their closely related allopatric parents. Unknown x, undetermined compounds; MW, molecular weight of compounds; IndVal results, indicator value of each compounds revealed by IndVal methods.

Appendix S1. Methodology: detailed genetic/chemical material and methods.