Molecular and chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *Bombus maxillosus* (Hymenoptera, Apidae, Bombini)

Thomas Lecocq, Patrick Lhomme, Denis Michez, Simon Dellicour, Irena Valterová, Pierre Rasmont

► To cite this version:

Thomas Lecocq, Patrick Lhomme, Denis Michez, Simon Dellicour, Irena Valterová, et al.. Molecular and chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *Bombus maxillosus* (Hymenoptera, Apidae, Bombini). *Systematic Entomology*, 2011, 36 (3), pp.453-469. 10.1111/j.1365-3113.2011.00576.x . hal-01972193

HAL Id: hal-01972193

<https://hal.univ-lorraine.fr/hal-01972193>

Submitted on 4 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Molecular and chemical characters to evaluate species status of two cuckoo bumblebees: *Bombus barbutellus* and *B. maxillosus* (Hymenoptera, Apidae, Bombini)

THOMAS LECOCQ¹, PATRICK LHOMME¹, DENIS MICHEZ¹, SIMON DELLICOUR², IRENA VALTEROVÁ³ and PIERRE RASMONT¹

¹Laboratoire de Zoologie, University of Mons, Place du Parc 20, 7000 Mons, Belgium, ²Evolutionary Biology and Ecology, *Université Libre de Bruxelles*, av. FD Roosevelt 50, 1050 Brussels, Belgium, ³Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flamingovo nám 2, CZ-166 10 Prague, Czech Republic

Corresponding authors:

Thomas Lecocq, *Laboratoire de Zoologie*, University of Mons, B-7000, Mons, Belgium. Phone : 003265373405. E-mail : thomas.lecocq@umons.ac.be

Irena Valterova, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flamingovo nám 2, CZ-166 10 Prague, Czech Republic. E-mail: irena@uochb.cas.cz

Short Title

Species discrimination by molecular and chemical evidences

Abstract

Many methods, based on morphological, molecular or chemical characters, have been used to address the question of species taxonomic status. Integrative taxonomy aims to define stronger supported taxonomic hypotheses by considering complementary datasets from different characters. By following an integrative approach, the present study includes molecular, chemical, and morphological criteria to establish the taxonomic status of two rare and doubtful cuckoo bumblebee taxa, *Bombus (Psithyrus) barbutellus* and *Bombus (Psithyrus) maxillosus*. These two sympatric taxa are discriminated by few morphological criteria (mainly wing darkness and hair length). We used these morphological character diagnoses to establish an *a priori* status of our samples (23 specimens). We developed a combined molecular dataset from one nuclear gene (EF-1 α) and one mitochondrial gene (COI) spanning 1623 bp and a chemical dataset of sexual marking pheromones (73 compounds). The molecular data were subjected to maximum likelihood and Bayesian phylogenetic inference under partitioned model and maximum parsimony. The chemical data were analysed by clustering and the 2-group k-means method to test divergences between the two species. The resulting phylogenetic trees show no consistent divergence between the two taxa. Moreover, we found no divergence in the sexual marking pheromones in the clustering and 2-group k-means analyses. These converging results support the conspecificity of both taxa. Nonetheless, our determinations using the traditional morphological criteria separated our samples into two taxa. We conclude that the morphological criteria seem to relate to intraspecific variations. *B. maxillosus* is regarded as a **syn.n.** of *B. barbutellus*.

Keyword

Bee taxonomy, Sexual marking pheromones, Phylogeny, *Bombus barbutellus*, *Bombus maxillosus*

Introduction

The species notion is one of the most confusing and controversial concepts in biology (Mayr, 1942; Templeton, 1989; Baum, 1992; De Queiroz, 2007; Gourbière & Mallet, 2009). However, the species is a fundamental biological unit and its definition is of major importance to biology. Numerous ways have been explored to determine the species status. In hymenopteran systematics (review for bees in Engel, 2011), authors have tried to define taxonomic status using morphology (i.e. Michener, 1951; Pekkarinen, 1982; Williams, 1998), geometric morphometry (i.e. Aytekin *et al.*, 2007), allozyme based models (i.e. Pamilo *et al.*, 1997), genetic (i.e. Koulianous & Schmid-Hempel, 2000; Hines *et al.*, 2006; Bernasconi *et al.*, 2010), or chemoecology (i.e. Svensson, 1979; Bertsch *et al.*, 2005; Rasmont *et al.*, 2005; Martin *et al.*, 2008).

The morphological approach is the earliest, the easiest and the most common method used to define species (i.e. Løken, 1983; Bolton, 1995; Michez & Eardley, 2007). However, determining objective morphological characters which accurately reflect biological species is difficult (Bickford *et al.*, 2007). Results of morphological analyses have conflicted with other kind of evidences (i.e. Svensson, 1979; Batalha-Filho *et al.*, 2010).

Genetic characters are taken more and more into consideration in order to assess species status (i.e. Leaché & Fujita, 2010). Development of DNA barcoding studies (i.e. Hebert *et al.*, 2003) to describe cryptic species (i.e. Yassin *et al.*, 2007), or included into integrative taxonomic framework (i.e. Gibbs, 2009) exemplify this trend. A phylogenetic species concept (Baum, 1992) has been developed and defined a species as a monophyletic group (Papadopoulou *et al.*, 2008), perhaps based on multiple gene genealogies (Baum & Shaw, 1995). Nevertheless, the genetic approach remains controversial in defining species limits

because DNA sequences analyzed are chosen arbitrarily or because mating isolation can happen faster than differentiation of the genetic markers used (Avisé, 2000; Trewick, 2008; Symonds *et al.*, 2009; Bauer *et al.*, 2010).

Chemical features, mainly species specific secretions (i.e. Lockety, 1991), can provide useful tools in separating species. Among bumblebees, Sexual Marking Pheromones (SMP), synthesized by male cephalic labial glands (Kullenberg *et al.*, 1973), play a major role in species specific pre-mating behavior (Bergman, 1997) and are highly species specific (Calam, 1969; Valterova & Urbanova, 1997). Therefore, authors used these chemical markers in resolving species status (Bergström *et al.*, 1981; Svensson & Bergström, 1977; Rasmont *et al.*, 2005; Coppée *et al.*, 2008; Lecocq *et al.*, 2009a, 2009b) according to the species recognition concept (Paterson, 1993). In this concept, conspecific individuals sharing the same recognition signals are supposed to recognize each other as sexual partners (review in Terzo *et al.*, 2003). However it is difficult to determine a threshold of species level differentiation, even with ethological test (i.e. Ings *et al.*, 2010). Only *B. terrestris* has been studied enough to determine a well supported threshold thanks to numerous chemo-ethological tests (review in Coppée 2010).

The current trend in species limitation studies is to follow a multiple evidence approach (Burns *et al.*, 2008; Fisher & Smith, 2008; Smith *et al.*, 2008) to differentiate species (i.e. morphological, chemical, molecular, and ecological).

The present study deals with taxonomic problems in the monophyletic subgenus *Psithyrus* included in the genus *Bombus* Latreille, 1802. 30 *Psithyrus* species described worldwide live in the Northern hemisphere (Williams, 1998 update at NHN). *Psithyrus* species are social parasites (cuckoo bumblebees) of other bumblebees. Cuckoo bumblebees lack pollen-collecting corbiculae and a worker caste. They are therefore completely dependent on hosts to rear their offspring. They parasitize hosts from across the bumblebee phylogeny with very

little cophylogenetic pattern (Williams, 2008). Typically, *Psithyrus* species parasitize just one host or a few host species of the same subgenus (review in: Alford 1975; Løken, 1984; Lhomme, 2009), but some species are considered to be generalists, with the extent of their host range often unclear. The ecology of host-parasite interactions of most of the cuckoo bumblebees is poorly known or unknown for almost half of *Psithyrus*.

This study focuses on two sister taxa, *Bombus (Psithyrus) barbutellus* (Kirby, 1802) and *Bombus (Psithyrus) maxillosus* Klug in Germar, 1817 of the former subgenus *Allopsithyrus* Popov, 1931, or *barbutellus*-group (Fig. 1) (i.e. Williams, 1998). The taxonomic status of these two taxa is doubtful (Grütte, 1940; Tkalců, 1969; Rasmont, 1988; Williams, 1998). Most authors consider these taxa as separate species (Popov, 1931; Pittioni, 1939; May, 1942, 1943; Tkalců, 1969; Løken, 1984) while very few argue for their conspecificity (Grütte, 1940). Most of these studies refer to morphology, highlighting slight differences between the two taxa (Table1), mainly from hair length and wing color (e.g. Tkalců, 1969). With such weak morphological criteria a large number of individuals remain unidentified (Rasmont, 1988; Rasmont & Adamski, 1996; Urbanová *et al.*, 2004) especially in geographical areas shared by both taxa. *B. barbutellus* has a large palaearctic distribution while *B. maxillosus* is restricted around the Mediterranean Sea (Fig. 1) (Rasmont, 1988; Pagliano, 1993; Rasmont & Flagothier, 1996; Williams, 1998 update at NHM). Many overlapping areas and exceptions to these trends are known (Vogrin, 1955; Schwartz *et al.*, 1996; Schwartz *et al.*, 2005; Straka *et al.*, 2007; Pawlikowski, 2008; Iserbyt, 2009). The splitting into subspecies differs for many authors (Pittioni, 1939; Grütte, 1940; Rasmont 1988). In this study, as a preliminary approach, we use the subspecies classification of Rasmont (1988): *Bombus barbutellus* no subspecies, *B. maxillosus maxillosus* Klug, 1817 (France, S-E Europe, Anatolia, Transcaucassia, Caucasus, and N Iran) and *B. maxillosus italicus* (Grütte, 1940) (Iberian Peninsulas, Italian Peninsulas, Corsica, and Sardinia).

Fig. 1. Distribution area of *Bombus maxillosus* and *Bombus barbutellus* in the Palaearctic region according to the literature (Maidl, 1922; Pittioni, 1939; Haas, 1949, 1967; Vogrin, 1955; M'ocz'ar, 1957; Dylewska, 1966; Elfving, 1968; Reinig, 1971; Ressel, 1974; Alford, 1975; Tum'ss, 1975; Løken, 1984; Ornos, 1984; Intoppa *et al.*, 1995; Monsevicius, 1995; Rasmont *et al.*, 1995; Amiet, 1996; Baker, 1996; Rasmont & Flagothier, 1996; Rasmont & Adamski, 1996; Peeters *et al.*, 1999; Dathe *et al.*, 2001; Yefremova, 2001; Anagnostopoulos, 2005; Fitzpatrick *et al.*, 2006; Byvaltsev, 2008, 2009; Pawlikowski, 2008; W. F. Reinig, personal data).

The sparsity of *B. barbutellus* and *B. maxillosus* makes information about their ecology very scarce. According to Pittioni & Schmidt (1942) and Rasmont (1988), *B. barbutellus* and *B. maxillosus* have different habitats. *B. barbutellus* is a forest edge dweller. It is a plains species in the north of its range and a mountain species in the south. *B. maxillosus* seems to be more of an open field species (Pittioni & Schmidt, 1942) or a wood edge species (Rasmont, 1988). They also seem to have different hosts. The main host of *B. barbutellus* is *Bombus (Megabombus) hortorum* (L., 1761) (Sladen, 1912; Ball, 1914; Müller, 1936; Cumber, 1949; Tkalců, 1969; Løken, 1984) whereas the main hosts of *B. maxillosus* seem to be two sister species *Bombus (Megabombus) argillaceus* (Scopoli, 1805) and *Bombus (Megabombus) ruderatus* (Fabricius, 1793) (Reinig, 1935; Rasmont, 1988; Rasmont & Adamski, 1996) close related to *B. hortorum* inside the *Megabombus* subgenus. However, other observations (Reinig, 1935; Pittioni & Schmidt, 1942; May, 1943; Pouvreau, 1973; Ornos, 1984; Rasmont & Adamski, 1996; Westrich, 1989; Peeters *et al.*, 1999) show that both taxa have no strict host-parasite relationship (Table 2). In this case, there is no definitive evidence to separate both taxa according to their host choice contrary to Tkalců's (1969) observations.

Chemical data are scarce too. The SMP of a few *B. barbutellus* specimens from Sweden have been described by Kullenberg *et al.* (1970) and Cederberg *et al.* (1984). The SMP of four specimens of *B. maxillosus* from Czech Republic have been described by Urbanová *et al.* (2004). These last authors compared their data with *B. barbutellus* of Kullenberg *et al.* (1970) and Cederberg *et al.* (1984). They found similarities in the blend of the SMP. However, due to the improvement in analysis techniques, data published before 1996 need to be re-examined (Terzo *et al.*, 2003). Comparison between such old data and new data is difficult. Very few genetic studies including both taxa have been undertaken and none have dealt with species status. In recent estimates of bumblebee phylogeny, the two taxa are treated as different species (Cameron *et al.*, 2007; Hines 2008).

Morphological, phylogenetic, ecological, and ethological data from literature about *B. barbutellus* and *B. maxillosus* are conflicting. A comparison of all available data fails to determine a strongly supported taxonomic hypothesis. We need more evidences in order to evaluate the status of both taxa (Williams, 1998 update at NHN). This paper aims to solve the taxonomic status of *B. barbutellus* and *B. maxillosus* using new molecular and chemical data from larger samples than previous studies.

Material and Methods

Sampling and Morphological identification

We compared morphological, molecular, and chemical (SMP) features from the same sampling of males (Table 3). Specimens were caught in the wild and killed by freezing. Identification keys which discriminate *B. barbutellus* and *B. maxillosus* as two species were used to determine individuals (Popov 1931; May 1943; Pittioni 1939; Rasmont & Adamski, 1996). The SMP were sampled by dissection of cephalic labial glands which were placed in

200 µl hexane (Terzo *et al.*, 2005). The rest of the male bodies were conserved in ethanol (99%) for molecular analyses. All samples were stored at -40°C until analysis.

Scarcity of the species in the field restricts collection of a large quantity of specimens. We have analyzed 9 specimens of *B. barbutellus*, 10 of *B. maxillosus maxillosus*, and 3 of *B. maxillosus italicus* for pheromonal dataset. We added 4 specimens of *B. barbutellus* females to the molecular sampling. One male of *B. maxillosus maxillosus* from SMP sample was too deteriorated for DNA extraction. Sampled populations are mainly European, corresponding to the sympatric area of the two taxa (Table3; Fig.1).

In order to root phylogenetic trees, we choose basal *Psithyrus* species and one other bumblebee species as outgroup. We used *Bombus (Psithyrus) campestris* (Panzer, 1801) from the *Metapsithyrus* group (Pedersen, 1996; Pedersen, 2002; Cameron *et al.*, 2007), *Bombus (Psithyrus) rupestris* (Fabricius, 1793) from the *Psithyrus s.s.* group (Cameron *et al.*, 2007; Hines, 2008) and *Bombus (Kallobombus) soroeensis* (Fabricius 1777) as outgroup (Table 3).

DNA extraction, PCR, sequencing and sequence analyses

Total DNA was extracted using a QIAGEN DNeasy® Tissue Kit (Quiagen Inc., Valencia, CA). Legs were removed from the specimen and crushed using liquid nitrogen and digested (4h in proteinase K at 56°C). Voucher specimens and PCR products used in the molecular investigation were deposited at the University of Mons (Belgium). Genes were sequenced with ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA). Both strands of each PCR product were sequenced. Consensus sequences were computed with CodonCode Aligner 3.0.1. (Table 3). The bumblebee origin of each sequence was checked with BLAST 2.2.20 (Zhang *et al.*, 2000). The alignment was performed by MAFFT ver.6. (using FFT-NS-2 algorithms, default parameters) (Katoh *et al.*, 2002). The translation in proteins (using the drosophila mt DNA genetic code) for verification and the data matrix was realized on

Mesquite 2.6. (build 486) (Maddison & Maddison, 2007). Sequences were deposited in Genbank (Table 3).

We generated sequences for two genes: Mitochondrial Cytochrome Oxidase 1 (COI) and nuclear protein-encoding gene Elongation Factor-1 alpha (EF-1 α). COI show a high mutation rate and it is commonly used as a species and subspecies level marker for phylogenetic analyses (e.g. Boursot & Bonhomme, 1986; Estoup *et al.*, 1996; Danforth, 1999; Koulianos & Schmid-Hempel, 2000). EF-1 α has been used to infer relationships at multiple taxonomic levels in many insect taxa including bumblebees (Danforth, 1999; Carapelli, 2000; Kjer, 2001; Jordal, 2002; Cameron *et al.*, 2007; Hines, 2008). Both genes have already been used to infer the phylogenetic relationship among European bumblebees (Pedersen, 2002). Primers used for all individuals were Jerry (5'-CCA CAT TTA TTT TGA TTT TTT GG-3') and Pat (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') (Danforth 1999) for COI and F2-ForH (5'-GGR CAY AGA GAT TTC ATC AAG AAC-3') and F2-RevH2 (5'-TTG CAA AGC TTC RKG ATG CAT TT-3') (Hines *et al.*, 2006) for EF-1 α . PCR amplification was carried out (initial denaturing for 4min at 94°C, 35 cycles of 1min denaturing at 94°C, 1min annealing at 54°C (EF-1 α) -51°C (COI), 1min elongation at 72°C and a final extension for 1min 15sec (EF-1 α) -1min 30sec (COI) at 72°C). Gene fragments of the following sizes were amplified: 849 nucleotides of COI and 786 nucleotides of EF-1 α F2 copy, containing a ~200 bp intron.

Phylogenetic analyses

We analyzed each gene (COI and EF-1 α) independently and in combination using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Methods (MB). A test of saturation was applied to each fragment in PAUP* 4.0b 10 (Swofford, 2001). The Incongruence Length Difference test (ILD test) (Farris *et al.*, 1994) was used to test for incongruence between the two genes. The test was implemented in PAUP* (Swofford, 2001).

All trees were rooted with the basal taxon *B. soroensis* (Williams, 1985; Cameron *et al.*, 2007).

Heuristic searches were performed in MP using 1000 random additions and TBR branch swapping, keeping the best trees only. Gaps were regarded as 5th state. Majority rule 50% consensus (MJ50) trees were constructed from analyses of individual genes and from all genes combined using parsimony criteria in Paup* 4.0b 10 (Swofford, 2001) for equally-weighted MP analyses. Clades support values were estimated using nonparametric bootstrapping (Felsenstein, 1985) in PAUP* (10000 replicates, 1000 random additions, 500 trees saved per replicate).

ML analyses were conducted in GARLI-PART Version 0.97 (Zwickl, 2006). The dataset was partitioned as followed to explore the best submission model: (1) EF-1 α into two exons and one intron, (2) COI and each EF-1 α exon by base position. The best fitting substitution models were chosen with jModeltest (Posada, 2008) using Akaike information criteria (Akaike 1974) for each dataset. The models chosen are: GTR+G (COI 1st position), GTR (COI 2nd position), GTR+I+G (COI 3rd position), F81 (EF-1 α exon 1 1st position), F81 (EF-1 α exon 1 2nd position), HKY (EF-1 α exon 1 3rd position), GTR+G (EF-1 α intron), HKY (EF-1 α exon 2 1st position), JC (EF-1 α exon 2 2nd position) and GTR+G (EF-1 α exon 2 3rd position). A random starting tree and the automated stopping criteria (stop when the ln score remained constant for 20000 consecutive generations) were used. Ten independent runs in GARLI were carried out for each gene and for the combined data; the topology and $-\ln L$ were nearly identical among replicates. The highest likelihood of those runs was retained. Statistical confidence in nodes was evaluated using 100 non-parametric bootstrap replicates (Felsenstein, 1985) using the automated stopping criteria set at 10000 generations. More bootstrap replicates could not be performed because it would have required impractical computing

times. Topology with $\geq 70\%$ of the bootstrap trees were considered well supported (Hillis & Bull, 1993).

Bayesian analyses (MB) were carried out using Mr. Bayes 3.1.2 (Ronquist & Huelsenbeeck, 2003). The model selection process was the same as that for ML analysis. Moreover, genes were analyzed individually and collectively. Five independent analyses were carried out for each gene and for the combined data (10 millions generations, four chains with mixed-models, default priors, saving trees every 100 generations). The analyses were stopped after checking convergence between runs using the average standard deviation of split frequencies and by plotting likelihood values across generations using Tracer 1.2 (Rambaut & Drummond, 2003). The first one million generations were discarded 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 well supported (Wilcox *et al.*, 2002).

Chemical Analyses

The samples were analyzed using a gas chromatograph Shimadzu GC-2010 with a SLB-5ms non-polar capillary column (5% diphenyl/95% dimethyl siloxane; 30 m X 0.25 mm X 0.25 μ m) and a flame ionization detector. A split less injector mode (220°C) and He carrier gas (50cm/s) were used. The temperature program of the column was 70°C for 2min; 10°C/min to 320°C; 5min. Relative proportions in percentage of each compound were calculated by summing up the absolute amounts of all compounds using GCsolution Postrun. The data matrix was elaborated with the relative proportion of each compound for each individual. The composition of SMP was determined using a gas chromatograph - mass spectrometer (GC-MS) Finigan GCQ with a DB-5ms non-polar capillary column (5% phenyl (methyl) polysiloxane stationary phase; 30 m X 0.25 mm X 0.25 mm) and an ion trap in electron impact mode “full scan (300-600)”. A split less injector mode (220°C) and He carrier

gas (50cm/s) were used. The temperature program of the column was 70°C for 2min; 10°C/min to 320°C; 5 min. Compounds were identified in XcaliburTM using their mass spectra compared to those at National Institute of Standards and Technology library (NIST, U.S.A) using Nist MS Search 2.0.

Statistical analyses were performed in R (Quinn & Keough, 2006). Data were transformed and standardized. Clustering methods were used to detect divergence between taxa (Rasmont *et al.*, 2005). Four different association matrixes based on pheromonal data matrix (relative abundance of each compound) were computed: Euclidian, Pearson Phi Correlation, Chi-Square, and Manhattan. Three clustering methods were used for each association matrix: single, complete and UPGMA. Only one of these clusters is shown in the results (UPGMA cluster based on Phi Correlation matrix). In addition, a 2-group k-means method (MacQueen, 1967) was performed (repeated 50.000 times) (De Meulemeester *et al.*, 2011) to test a putative split into two groups: *B. barbutellus* versus *B. maxillosus*.

Correlation analyses

The spatial structuring of the chemical and genetic data set and a potential correlation between these two data sets were investigated by performing three Mantel tests (9999 random permutations) (Mantel, 1967) in R (Quinn & Keough, 2006): (1) geographical distance among population *versus* individual by individual correlation distance in relative amounts of SMP compounds, (2) geographical distance *versus* individual by individual genetic distance and (3) correlation distance of the SMP *versus* Genetic distance. The correlation distance matrix of SMP compounds was performed in R (Quinn & Keough, 2006). Genetic distances were calculated in Mega 4.0.1 (Tamura *et al.*, 2007) using the maximum composite likelihood (MCL). The MCL calculations used data for both transitions and transversions and assumed a heterogeneous pattern of sequence evolution with a gamma distribution of among-site rate variation (gamma parameter = 1.0). Gaps, missing data and individuals without all data set

were deleted from the analysis and standard errors were calculated with 1000 bootstrap replicates.

Results

Phylogenetic analyses

A total of 850 bp from the COI gene (145 parsimony informative sites), 773 nucleotides of EF-1 α F2 copy (24 parsimony informative sites), containing a ~200 bp intron and the combined sequences were compared to estimate the phylogenetic relationships. All analyses (MP, ML and MB) on each single gene and combined dataset present the same topology (Figs 2, 3, 4) but higher branch support values were found in MB. Phylogenetic analyses based on EF-1 α partially recover all deep relationships among *Psithyrus* subgroups but failed to provide much sequence variation within and between *B. barbutellus* and *B. maxillosus*. Moreover there is not any divergence between *B. barbutellus* and *B. maxillosus* while a clear divergence appeared in the *Ashtonipsithyrus* species group (*B. bohemicus* and *B. vestalis*). COI tree topology failed to resolve deep relationships among *Psithyrus* subgroups but provided considerable sequence variation of *B. barbutellus* and *B. maxillosus*. As in the EF-1 α analyses, *B. barbutellus* and *B. maxillosus* were not split into two groups. Nevertheless geographic groups appeared: A West-Central European group, a Southern French group, two Turkish groups and a Corsican group (Fig. 3). However branch support values are low in posterior probability but well supported in ML and MP. Combined genes analyses summarize the differing contributions of the two molecules at the two ends of the tree. These trees presented a similar topology and similar branch supports to EF-1 α for deep relationship between *Psithyrus* subgroups and to COI for the tips of the phylogeny (including COI geographic groups). In each of the analyses, therefore, there was an unambiguous support for monophyly of the *B. barbutellus* + *B. maxillosus* group.

Fig. 2. Majority-rule (50%) consensus tree based on Bayesian analyses of elongation factor 1 α (*EF-1 α*) sequences; values above branches are Bayesian posterior probabilities; values in bold below branches are maximum likelihood bootstrap values; values in italic below branches are maximum parsimony bootstrap values. *Bombus soroeensis* is used as the out-group. The triangles represent clades with shared haplotypes. The sample codes are defined in Table 3.

Fig. 3. Majority rule (50%) consensus tree based on Bayesian analyses of cytochrome *c* oxidase subunit I (*COI*) sequences; values above branches are Bayesian posterior probabilities; values in bold below branches are maximum likelihood bootstrap values; values in italics below branches are maximum parsimony bootstrap values. *Bombus soroeensis* is used as the out-group. The triangles represent clades with shared haplotypes. The sample codes are defined in Table 3.

Fig. 4. Majority rule (50%) consensus tree based on Bayesian analyses of the combined molecular data matrix (*EF-1α* and *COI*); values above branches are Bayesian posterior probabilities; values in bold below branches are maximum likelihood bootstrap values; values in italic below branches are maximum parsimony bootstrap values. *Bombus soroeensis* is used as the out-group. The triangles represent clades with shared haplotypes. The sample codes are defined in Table 3.

Chemical analyses

73 compounds were detected in SMP of *B. barbutellus* and *B. maxillosus* (Table 4). *B. barbutellus* and *B. maxillosus* shared all compounds. No discrimination between *B. barbutellus* and *B. maxillosus* was found in the statistical analyses (Figs 5, 6). PCA analysis did not separate any group. However, all the clusters separated the samples into two main groups; a Turkey cluster with nearly all the Turkish individuals and a European cluster with all European samples and one Turkish specimen (Fig. 6). The two group k-means method detected the same separation. Only slight quantitative and qualitative divergences in the minor compounds separated the two groups. Examination of the wing condition (Tkalcić, 1969) of the Turkish individual grouped with the European group indicated that it is obviously an old specimen. In some bumblebee species, old individuals, scarce in the wild, have a pheromonal composition modification and are probably no longer attractive to females (Žaček *et al.*, 2009;

Coppée, 2010). In the Turkey group, *B. maxillosus* and *B. barbutellus* are separated (Fig. 6).
 A comparison between Turkish *B. barbutellus* and Turkish *B. maxillosus* compounds showed
 only slight differences in relative abundance.

Fig. 5. Three first axes of the principle components analysis (PCA) based on 20 compounds in 21 specimens of *Bombus barbutellus*, *Bombus maxillosus maxillosus* and *Bombus maxillosus italicus*; , *Bombus barbutellus*; , *Bombus maxillosus italicus*; , *Bombus maxillosus maxillosus*.

Fig. 6. Unweighted pair group method with arithmetic mean (UPGMA) cluster based on a correlation matrix calculated from the sexual marking pheromone (SMP) matrix of *Bombus barbutellus*, *Bombus maxillosus maxillosus* and *Bombus maxillosus italicus* (73 compounds in 21 specimens). The sample codes are defined in Table 3.

Fig. 7. Mantel's correlogram of (A) genetic distance among genetic samples plotted by spatial distance among samples sites (Mantel's $r = 0.293$; $P < 0.05$); (B) correlation distance among chemical samples plotted by spatial distance among samples sites (Mantel's $r = 0.298$; $P < 0.05$); (C) genetic distance among genetic samples plotted by correlation distance among chemical samples (Mantel's $r = 0.089$; $P > 0.05$).

Correlation analyses

The Mantel tests show a significant positive correlation between SMP dissimilarity and geographical distance (Mantel's $r=0.298$, $p\text{-value}<0.05$) and between genetic distance and geographical distance (Mantel's $r=0.293$; $p\text{-value}<0.05$) (Figs 7A, B). There is no correlation between SMP dissimilarity and genetic distance (Mantel's $r=0.089$; $p\text{-value}>0.05$) (Fig. 7C).

Discussion

Species status

The phylogenetic trees showed conflict with Cameron *et al.* (2007) and Hines (2008). Though these studies were not designed to deal with species problems, they showed genetic differences in EF-1 α sequence between *B. barbutellus* and *B. maxillosus*. However, these studies used only one Swedish *B. barbutellus* specimen and one Turkish *B. maxillosus* specimen. By performing a wider specimen sampling taking into consideration the intraspecific variability, we show that *B. barbutellus* or *B. maxillosus* respectively do not constitute monophyletic groups. Therefore they are not different species according to the Phylogenetic Species Concept (Baum, 1992).

Our results in SMP composition are similar to Urbanová *et al.* (2004). 58 compounds detected in GC-MS analysis were identical to the former study; 15 components with a relative abundance below 0.1% and four components with a relative abundance higher than 0.1% differed. Among these compounds only three were present in all individuals. However the differences observed with Urbanová *et al.* (2004) are of the same order of magnitude as the inter-population variation observed in our data set for *B. barbutellus*. Pheromonal analyses also support a lack of divergence between *B. barbutellus* and *B. maxillosus*. The comparison of SMP between the two taxa showed only some slight quantitative modifications and no qualitative modifications and supports their conspecificity according to the species recognition concept (Paterson, 1993). Indeed, the variation observed between *B. barbutellus* and *B. maxillosus* is lower than the intraspecific variation observed in *Bombus terrestris* (Coppée *et al.*, 2008) and even more so the variation between other bumblebees species (Bertch, 1997; Bertsch *et al.*, 2005; Rasmont *et al.*, 2005; Terzo *et al.*, 2005).

Our chemical and molecular analyses point towards conspecific status for *B. barbutellus* and *B. maxillosus* whatever the species concept considered. Moreover, these analyses do not support any separation of alternative species inside the *B. barbutellus* + *maxillosus* group. This result agrees with Grütte (1940). The taxonomic status proposed by Grütte (1940) was

criticized by Tkalců (1969) according to wing darkness variations and host choice. Tkalců (1969) considered wing darkness of all bees as a constant specific criterion unrelated to individual or geographic variations. However, according to our results, wing darkness is not a diagnostic species character of *B. barbutellus*. The relevance of the wing darkness as a species diagnostic character among bees, never demonstrated statistically from a large sample, therefore still needs to be proven. To summarize, according to the conspecificity shown in our results, the main morphological criteria (the hair length and the wings darkness) used to distinguish both taxa (Popov, 1931; Pittioni, 1939; May, 1942; May, 1943) is probably due to intraspecific variability. This could explain the numerous intermediate forms observed by Grütte (1940) and the large number of undeterminable individuals (Rasmont, 1988).

Intraspecific variability

We found three colour patterns corresponding to the following subspecies (*sensus* Rasmont, 1988): *barbutellus*, *italicus*, and *maxillosus*. However colour patterns do not fit with any particular pheromonal clustering or phylogenetic tree. This could show that there is no clear subspecific subdivision with the Rasmont (1988) subspecies status but only two extreme forms: (1) a form with long hairs and hyaline wings (*barbutellus*) living in the North of Europe and in the Mediterranean mountains; (2) another form with short darker hairs and darkened wings (*maxillosus*) living mainly in the South of Europe and (3) between these two, there are intermediate forms; sometimes described as subspecies (Grütte, 1940). Similar patterns of variations have been observed in other bumblebees. On the one hand, hairs insulate the bumblebee bodies and decrease heat loss (Heinrich, 1975). Peat *et al.* (2005) showed that bumblebee species from hot regions exhibit shorter hairs than species from cold regions. They found the same geographic pattern at an intraspecific level for *B. terrestris*. On the other hand, melanism is also due to thermoregulation (Gloger, 1833). Hot region bumblebees are darker and temperate region bumblebees are paler (Williams, 2007). In

Scandinavia, Løken (1984) observed a slight tendency to a more pronounced melanism in the south of Sweden than in the north among *B. barbutellus*.

The genetic distance observed in *B. barbutellus* is correlated to geographic distances according to our Mantel test like amongst other bumblebees (Estoup *et al.*, 1996; Widmer *et al.*, 1998). However, any groups inside the *barbutellus* phylogenetic tree match with any distinct coloration group. It could support population variations but not a clear subspecific taxonomy.

The slight SMP geographic variations mainly between Turkey and Europe are explained by the geographic distance. Mantel test shows that SMP distance is correlated to geographic distance like in other bees (Vereecken *et al.*, 2007; Coppée *et al.*, 2008; Lecocq *et al.*, 2009b). Among the Turkish group, the splitting into two forms (*barbutellus* forms and *maxillosus* forms) may be due to an interpopulational variation as observed in *B. terrestris* (Coppée 2010) or more likely reflects sampling bias. Indeed, Turkish *B. barbutellus* samples were collected at the same time and from the same place. The likelihood that these individuals were related and thus have a nearly identical SMP composition is high. In this case, the clustering method separated them from other samples with no other biological reason than the kin relationship.

Even if we observed variations between *B. barbutellus* populations, currently, we do not know if these variations have an impact on the intraspecific sexual mating recognition among *B. barbutellus*. Ethological studies, difficult to perform among scarce species, would resolve this problem.

Moreover, phylogenetic and pheromonal geographic groups never match in our analysis and are not correlated according to the Mantel test. Therefore, we are unable to discover any subspecies differentiation.

Host relationship

As a consequence of our present taxonomic definition, *B. barbutellus* seems to be an opportunistic cuckoo bumblebee with preferences for host species belonging to the subgenus *Megabombus*. The differences in the main host choice of the form *barbutellus* (*B. hortorum*) and the form *maxillosus* (*B. argillaceus* and *B. ruderatus*) do not reflect a species differentiation. The definition of the species status in inquiline bumblebees should not be based on host choice differences. Indeed, in few cases where information is available (review in Williams, 2008) bumblebee socially parasitic lineages tend not to be strict specialists, several *Psithyrus* species are known to parasitize multiple species in their communities

Impact on zoological nomenclature

Bombus barbutellus (Kirby) is the only species belonging to the group formerly known as the subgenus *Allopsithyrus* Popov. *Bombus maxillosus* should be regarded as a simple synonym. The oldest available names for the species are the following:

? *Apis brachyptera* Villers in L., 1789: 331, *nomen oblitum*

? *Apis monarcha* Christ, 1791: 131, *nomen oblitum*

? *Apis cincta* Preyssler, 1793: 232, nec Fabricius, 1781, *nomen oblitum*

? *Apis saltuum* Panzer, 1800-1801, part 75, Table 21, *nomen oblitum*.

Apis barbutella Kirby, 1802: 343, *nomen protectum*; locus typicus: England E.Suffolk Barham; lectotype ♀ Natural History Museum, London, designated by Yarrow, 1968. Revised by P. Rasmont 2010. Labels 1) handwritten "93♀"; 2) round blue pale label with glued right protarsus; 3) round with a black circle printed "Lectotype"; 4) handwritten "Apis barbutella Kirby 1802 LECTOTYPE I.H.H. Yarrow 1968; 5) printed and handwritten "B.M. TYPE HYM 17a 2996". The right fore tarsis is broken and glued to the second label. The 3 distal segments of the hind tarsus are missing. The coat colour pattern is typical to the northern form of *Bombus barbutellus*, but the wings are more infuscated than usual.

Bombus maxillosus Klug, 1817:269; locus typicus: "Süd Europa"; Holotype ♀ Zoologisches Museum, Berlin, see Tkalců, 1969. Syn. N.

The oldest names, *Apis brachyptera* Villers, *Apis monarcha* Christ, *Apis cincta* Preysslér and *Apis saltuum* Panzer have a description that does not allow us to identify the taxa with certainty, while no types are presently known. These names have not been used since their initial description. They are therefore all *nomina oblita*.

The oldest available name is *Apis barbutellus* Kirby; *Bombus maxillosus* Klug is therefore a subjective junior synonym. The valid name should be *Bombus (Psithyrus) barbutellus* (Kirby, 1802). Many names have been used for infraspecific colour forms, mainly by Kriechbaumer (1870), Popov (1931), Quilis-Pérez (1927), Müller (1936), Pittioni (1939) and May (1944). They are all considered here as synonyms of *Bombus barbutellus* (Kirby).

Acknowledgments

We are very grateful to B. N. Danforth and M. M. López-Urbe for the hosting in Cornell University (Ithaca, U.S.A.) and to P. Mardulyn for the hosting in the ULB (Bruxelles, Belgium) for the molecular analysis. We gratefully acknowledge M. Terzo (Mons, Belgium), A. Roelandts (Mons, Belgium), E. Stolle (Halle, Germany), and G. Mahé (Saint-Nazaire, France) for providing *Psithyrus* samples. Thanks also to P. H. Williams (London, United Kingdom) for help and comment on the taxonomic nomenclature. We are also grateful to T. De Meulemeester (Mons, Belgium) for statistical analysis. The authors acknowledge the *Fonds pour la recherche dans l'industrie et l'agriculture* (FRIA), *Fonds pour la Formation à la Recherche Fondamentale et Collective* (FNRS), the *Office de l'Environnement de la Corse*, the *Direction Régionale de l'Environnement de Corse* for the financial support. Special thanks are also due to Dr. A. Coppée (Mons, Belgium), Prof. A. M. Aytekin (Ankara, Turkey), Dr. S. Patiny (Gembloux, Belgium), Dr. D. Lanterberq (Mons, Belgium) and S. Lambert for helpful

487 comments and discussions on earlier versions of this manuscript and B. Lhomme for the
488 English.

- Akaike, H. (1974) A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control*, **19**, 716-723.
- Alford, D. V. (1975) *Bumblebees*. London.
- Amiet, F. (1996) Hymenoptera Apidae, 1. Teil. Allgemeiner Teil, Gattungsschlüssel, die Gattungen Apis, *Bombus* und *Psithyrus*. *Insecta Helvetica (Fauna)*, **12**, 98 pp.
- Anagnostopoulos, I. T. (2005) The bumblebee fauna of Greece: An annotated species list including new records for Greece (Hymenoptera: Apidae, Bombini). *Linzer biol. Beitr.*, **37**, 1013-1026.
- Avice, J. (2000) *Phylogeography: the history and formation of Species*. Harvard University Press, Cambridge, MA. 447 pp.
- Aytekin, A. M., Terzo, M., Rasmont, P. & Cagatay, N. (2007) Landmark based geometric morphometric analysis of wing shape in *Sibiricobombus* Vogt (Hymenoptera : Apidae : *Bombus* Latreille). *Annales De La Societe Entomologique De France (n.s.)*, **43**, 95-102.
- Baker, D. B. (1996) On a collection of humble-bees from northern Iran (Hymenoptera: Apoidea, Bombinae). *Beiträge zur Entomologie*, **46**, 109-132.
- Ball, F. J. (1914) Les bourdons de la Belgique. *Annales de la Société entomologique de Belgique*, **58**, 77-108.
- Batalha-Filho, H., Waldschmidt, A., Campos, L. A. O., Tavares, M. G. & Fernandes-Salomão, T. (2010) Phylogeography and historical demography of the Neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera, Apidae): incongruence between morphology and mitochondrial DNA. *Apidologie*, 6-14.
- 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. (2010) Availability of new Bayesian-delimited gecko names and the importance of character-based species descriptions. *Proceeding of the royal society B*, (doi: 10.1098/rspb.2010.1330)
- Baum, D. (1992) Phylogenetic species concepts. *Trends in Ecology and Evolution*, **7**, 1-3.
- Baum, D. A. & Shaw, K. L. (1995) Genealogical perspectives on the species problem. In *Experimental and molecular approaches to plant biosystematics* (ed. P. C. Hoch), pp. 289-303. St. Louis, MI: Missouri Botanical Garden.
- Bergman, P. (1997) *Chemical communication in bumblebee premating behaviour*. Ph-D thesis. Göteborg University. Göteborg.
- Bergström, G., Svensson, B. G., Appelgren, M. & Groth, I. (1981) Complexity of bumble bee marking pheromones: biochemical, ecological and systematical interpretations. *Systematics Association special volume*, **19**, 175-183.
- Bernasconi, C., Pamilo, P. & Cherix, D. (2010) Molecular markers allow sibling species identification in red wood ants (*Formica rufa* group). *Systematic Entomology*, **35**, 243-249.
- Bertsch, A. (1997) Wieviele Arten der Untergattung *Terrestribombus* (Hymenoptera, Apidae) gibt es in Nordhessen; die Abgrenzung von *Bombus cryptarum* und *B. lucorum* mittels männlicher Labialdrüsen-Sekrete und morphologischer Merkmale. *Marburger Entomologische Publikationen*, **2**, 1-28.
- 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 Sociaux*, **52**, 45-54.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in ecology and evolution*, **22**, 3, 148-155.
- Bolton, B. (1995) *A new general catalogue of the ants of the world*. Harvard University Press, Cambridge, MA. 504 pp.
- Boursot, P. & Bonhomme, F. (1986) *Génétique et évolution du génome mitochondrial des Métazoaires*. Versailles, France.
- Burns, J., M., Janzen, D. H., Hajibabaei, M., Hallwachs, W. & Hebert, P. D. (2008) DNA barcode and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *Proceeding of the National Academy of Sciences of the United States of America*, **105** (17), 6350-6355.
- Byvaltsev, A. M. (2008) Bumblebee (Hymenoptera : Apidae, Bombini) fauna of the forest steppes and steppe zones of the West Siberian Plain. *Eurasian entomological journal*, **7**, 141-147.
- Byvaltsev, A. M. (2009) The community of Bumble Bees (Hymenoptera : Apidae, Bombini) in the city of Novosibirsk and its environs. *Contemporary Problems of Ecology*, **2**, 216-223.
- Calam, D. H. (1969) Species and sex-specific compounds from the head of male bumblebees (*Bombus* spp). *Nature*, **21**, 856-857.
- Cameron, S. A., Hines, H. M. & Williams, P. H. (2007) A comprehensive phylogeny of the bumble bees (*Bombus*). *Biological Journal of the Linnean Society*, **91**, 161-188.
- Carapelli, A., Frati, F., Nardi, F., Dallai, R. & Simon, C. (2000) Molecular phylogeny of the apterygotan insects based on nuclear and mitochondrial genes. *Pedobiologia*, **44**, 361-373.
- Cederberg, B., Svensson, B. G., Bergström, G., Appelgren, M. & Groth, I. (1984) Male Marking Pheromones in the North European Cuckoo Bumble Bees, *Psithyrus* (Hymenoptera, Apidae). *Nova Acta Regiae Societatis Scientiarum Upsaliensis.*, **3**, 161-166.
- Coppée, A. (2010) *Bombus terrestris* : a complex species or a species complexe. Université de Mons. Mons (Belgium).
- Coppée, A., Terzo, M., Valterová, I. & Rasmont, P. (2008) Intraspecific Variation of the Cephalic Labial Gland Secretions in *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Chemistry & Biodiversity*, **5**, 2654-2661.
- Cumber, R. A. (1949) The biology of the humble-bees, with special reference to the production of the worker caste. *Transactions of the Royal Entomological Society of London*, **100**, 1-45.
- Danforth, B. N. (1999) Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data. *Systematic*

- 618 *Entomology*, **24**, 377-393.
- 619 Dathe, H. H., Taeger, A. & Blank, S. M. (2001)
- 620 Verzeichnis der Hautflügler Deutschlands (Fauna
- 621 Germanica 4). *Entomologische Nachrichten und*
- 622 *Berichte*, **7**, 143-155.
- 623 De Meulemeester, T., Gerbaux, P., Boulvin, M., Coppée,
- 624 A. & Rasmont, P. (2011) A simplified protocol for
- 625 bumble bee species identification by cephalic
- 626 secretion analysis. *Insectes Sociaux*,
- 627 De Queiroz, K. (2007) Species Concepts and Species
- 628 Delimitation. *Systematic Biology*, **56**, 879-886.
- 629 Dylewska, M. (1966) The Apoidea of the Babia Gora
- 630 Mountain. *Acta Zoologica Cracoviensia*, **XI**, 111-
- 631 175.
- 632 Elfving, R. (1968) Die Bienen Finnlands. *Fauna Fennica*,
- 633 **21**, 1-69.
- 634 Engel, M. S. (2011) Systematic melittology: where to
- 635 from here? *Systematic Entomology*, **36**, 2-15
- 636 Estoup, A., Solignac, M., Cornuet, J. M., Goudet, J. &
- 637 Scholl, A. (1996) Genetic differentiation of
- 638 continental and island populations of *Bombus*
- 639 *terrestris* (Hymenoptera: Apidae) in Europe.
- 640 *Molecular Ecology*, **5**, 19-31.
- 641 Farris, J.M., Källersjö, M., Kulge, A. G., Bult, A. C.
- 642 (1994) Testing significance of incongruence.
- 643 *Cladistics*, **10**, 315-319.
- 644 Felsenstein, J. (1985) Phylogenies and the Comparative
- 645 Method. *The American Naturalist*, **125**, 1.
- 646 Fisher, B. L. & Smith, M. A. (2008) A Revision of
- 647 Malagasy Species of *Anochetus* Mayr and
- 648 *Odontomachus* Latreille (Hymenoptera: Formicidae).
- 649 *PLoS ONE*, **3**(5), e1787. doi:10.1371/journal.pone.
- 650 0001787.
- 651 Fitzpatrick, U., Murray, T. E., Paxton, R. J. & Brown, M.
- 652 J. F. (2006) Regional red data list for Irish Bees.
- 653 *Report for The National Parks and Wildlife Service*
- 654 *(Ireland) and Environment and Heritage Service (N.*
- 655 *Ireland)*. 38pp.
- 656 Gibbs, J. (2009) Integrative taxonomy identifies new
- 657 (and old) species in the *Lasioglossum* (Dialictus)
- 658 *tegulare* (Robertson) species group (Hymenoptera,
- 659 Halictidae). *Zootaxa*, **2032**, 1-38.
- 660 Gloger, C. L. (1833) *Das Abändern der Vögel durch*
- 661 *Einfluss des Klimas*. Breslau, Germany.
- 662 Gourbière, S. & Mallet, J. (2009) Are species real? The
- 663 shape of the species boundary with exponential
- 664 failure, reinforcement, and the "missing snowball".
- 665 *Evolution*, **64**, 1-24.
- 666 Grütte, E. (1940) Beitrag zur Kenntnis des Subgenus
- 667 *Allopsithyrus* Popov (Hym. Apid.). *Deutsche*
- 668 *Entomologischen Zeitschrift*, **4**, 204-223.
- 669 Haas, A. (1949) Arttypische Flugbahnen von
- 670 Hummelmännchen. *Zeitschrift für vergleichende*
- 671 *Physiologie*, **31**, 281-307.
- 672 Haas, A. (1949) Arttypische Flugbahnen von
- 673 Hummelmännchen. *Zeitschrift für vergleichende*
- 674 *Physiologie*, **31**, 281-307.
- 675 Haas, A. (1967) Vergleichende Verhaltensstudien zum
- 676 Paarungsschwarm der Hummeln (*Bombus*) und
- 677 Schmarotzerhummeln (*Psithyrus*). *Zeitschrift für*
- 678 *Tierpsychologie*, **24**, 257-277.
- 679 Hebert, P. D. N., Ratnasingham, S. & deWaard, J. R.
- 680 (2003) Barcoding animal life: cytochrome c oxidase
- 681 subunit 1 divergences among closely related species.
- 682 *Proceedings of the Royal Society of London Series B-*
- 683 *Biological Sciences*, **270**, S96-S99.
- 684 Heinrich, B. (1975) Thermoregulation in bumblebees II.
- 685 Energetics of warm-up and free flight. *Journal of*
- 686 *Comparative Physiology*, **96**, 155-166.
- 687 Hillis, D. M. & Bull, J. J. (1993) An Empirical-Test of
- 688 Bootstrapping as a Method for Assessing Confidence
- 689 in Phylogenetic Analysis. *Systematic Biology*, **42**,
- 690 182-192.
- 691 Hines, H. M. (2008) Historical biogeography, divergence
- 692 times, and diversification patterns of bumble bees
- 693 (Hymenoptera : Apidae : *Bombus*). *Systematic*
- 694 *Biology*, **57**, 58-75.
- 695 Hines, H. M., Cameron, S. A. & Williams, P. H. (2006)
- 696 Molecular phylogeny of the bumble bee subgenus
- 697 *Pyrobombus* (Hymenoptera : Apidae : *Bombus*) with
- 698 insights into gene utility for lower-level analysis.
- 699 *Invertebrate Systematics*, **20**, 289-303.
- 700 Hoffer, E. (1889) Die Schmarotzerhummeln Steiermarks.
- 701 Lebensgeschichte und Beschreibung derselben.
- 702 *Mitteilungen Naturwissenschaftlicher Verein*
- 703 *Steiermark*, **25**, 82-158.
- 704 Ings, T. C., Ings, N.L., Chittka, L. & Rasmont, P. (2010)
- 705 A failed invasion? Commercially introduced
- 706 pollinators in Southern France. *Apidologie*, **41**, 1-13.
- 707 Intoppa, F., Piazza, M. G. & Riccardelli D'Albore, G. C.
- 708 (1995) Catalogo bibliografico delle specie di
- 709 Bombidae (Hymenoptera Apidae) segnalate per
- 710 l'Italia. *Apicoltura*, **10**, 1-132
- 711 Iserbyt, S. (2009) La faune des bourdons (Hymenoptera :
- 712 Apidae) du Parc National des Pyrénées occidentales
- 713 et des zones adjacentes. *Annales de la Société*
- 714 *Entomologique de France (n.s.)*, **45**, 217-244.
- 715 Jordal, B. H. (2002) Elongation Factor 1 alpha resolves
- 716 the monophyly of the haplodiploid ambrosia beetles
- 717 Xyleborini (Coleoptera : Curculionidae). *Insect*
- 718 *Molecular Biology*, **11**, 453-465.
- 719 Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002)
- 720 MAFFT: a novel method for rapid multiple sequence
- 721 alignment based on fast Fourier transform. *Nucleic*
- 722 *Acids Research*, **30**, 3059-3066.
- 723 Kjer, K. M., Blahnik, R. J. & Holzenthal, R. W. (2001)
- 724 Phylogeny of Trichoptera (caddisflies):
- 725 Characterization of signal and noise within multiple
- 726 datasets. *Systematic Biology*, **50**, 781-816.
- 727 Knechtel, W. K. (1955) Fauna Republicy Populare
- 728 Roumîne. Insecta. Hymenoptera, Subfamilia Apinae.
- 729 *Academia Republicy Populare Roumîne*, **9**, 1-111.
- 730 Koulianos, S. & Schmid-Hempel, P. (2000) Phylogenetic
- 731 relationships among bumble bees (*Bombus*, latreillei)
- 732 inferred from mitochondrial cytochrome b and
- 733 cytochrome oxidase I sequences. *Mol. Phylogenet.*
- 734 *Evol.*, **14**, 335-341.
- 735 Kullenberg, B., Bergström, G. & Stållberg-Stenhagen, S.
- 736 (1970) Volatile components of the cephalic marking
- 737 secretion of male bumblebees. *Acta Chem. Scand.*,
- 738 **24**, 1481-1483.
- 739 Kullenberg, B., Bergström, G., Bringer, B., Carlberg, B.
- 740 & Cederberg, B. (1973) Observations on Scent
- 741 Marking by *Bombus* Latr. and *Psithyrus* Lep. Males
- 742 (Hym., Apidae) and Localization of Site of
- 743 Production of the Secretion. *Zoon*, **Suppl. 1**, 23-30.
- 744 Leaché, A. D. & Fujita, M. K. (2010) Bayesian species
- 745 delimitation in West African forest geckos
- 746 (*Hemidactylus fasciatus*). *Proceeding of the royal*
- 747 *society B*, (doi:10.1098/rspb.2010.0662)
- 748 Lecocq, T. (2009a) *Génétique et phéromones sexuelles*
- 749 *des bourdons de la Corse*. Master thesis. University
- 750 of Mons. Mons (Belgium).
- 751 Lecocq, T., Coppée, A., Michez, D. & Rasmont, P.

- 752 (2009b) Phylogeny and sexual pheromones of
753 Corsican bumblebees, in Systematics, First meeting
754 of BioSyst EU 2009, Seventh Biennial Conference of
755 the Systematics Association. 14/08/2009, Leiden,
756 Netherlands.
- 757 Lhomme, P. (2009) L'inquilinisme chez les bourdons.
758 *Osmia*, **3**, 17-22.
- 759 Lockey, K. (1991) Insect hydrocarbon classes –
760 implications for chemotaxonomy. *Insect*
761 *Biochemistry*, **21**, 91-97.
- 762 Løken, A. (1984) Scandinavian species of the genus
763 *Psithyrus* Lepeletier (Hymenoptera: Apidae).
764 *Entomologica Scandinavia (supplement)*, **23**, 45 pp.
- 765 MacQueen, J. B. (1967) "Some Methods for
766 classification and Analysis of Multivariate
767 Observations, *Proceedings of 5-th Berkeley*
768 *Symposium on Mathematical Statistics and*
769 *Probability*", Berkeley, University of California
770 Press, **1**, 281-297.
- 771 Maddison, W. & Maddison, D. (2007) *Mesquite: a*
772 *modular system for evolutionary analysis. Version*
773 *2.6 (buid 486)*.
- 774 Maidl, F. (1922) Beiträge zur Hymenopterenfauna
775 Dalmatiens Montenegros und Albaniens. I. Teil:
776 Aculeata und Chrysididae. *Ann. Naturhist. Mus.*, **35**,
777 36-106.
- 778 Mantel, N. (1967) The Detection of Disease Clustering
779 and a Generalized Regression Approach. *Cancer*
780 *Research*, **27**, 209-220.
- 781 Martin, S. J., Helanterä, H. & Drijfhout, F. P. (2008)
782 Evolution of species-specific cuticular hydrocarbon
783 patterns in Formica ants. *Biological Journal of the*
784 *Linnean Society*, **95**, 131-140.
- 785 May, J. (1942) Subgenus *Allopsithyrus* Popov in Böhmen
786 und Mähren. (Hym. Apid.). *Sbornik entom. odd. Zem.*
787 *Musea v Praze*, **XX**, 223-231.
- 788 May, J. (1943) Beitrag zur Systematik einiger arten der
789 schmarotzerhummeln - *Psithyrus* Lep. (Hymen.
790 Apoid.). *Sbornik entom. odd. Zem. Musea v Praze*,
791 **XXI-XXII**, 231-275 + 2 pl. Mayr, E. (1942)
792 *Systematics and the Origin of Species*. Columbia
793 University Press, New York.
- 794 Mayr, E. (1942) *Systematics and the Origin of Species*.
795 Columbia University Press, New York.
- 796 Michener, C. (1951) Subgeneric groups of Hemisia
797 (Hymenoptera, Apoidea). *The journal of the kansas*
798 *entomological society*, **24**, 1-11
- 799 Michez, D. & Eardley, C. (2007) Monographic revision
800 of the bee genus *Melitta* Kirby 1802
801 (Hymenoptera:Apoidea: Melittidae). *Annales De La*
802 *Societe Entomologique De France (n.s.)*, **43**, 379-
803 440.
- 804 Móczár, M. (1957) *Méhfélék, Magyarország állatvilága*.
805 Budapest.
- 806 Monsevicus, V. (1995) A check-list of wild bee species
807 (Hymenoptera: Apoidea) of Lithuania with data to
808 their distribution and bionomics. in *New and Rare for*
809 *Lithuania insect species. Records and descriptions of*
810 *1994-1995*. Vilnius.
- 811 Müller, M. (1936) *Psithyrus barbutellus* Kirby und P.
812 *maxillosus* Klug (Apid., Hym.). *Mitteilungen der*
813 *Deutschen Entomologischen Gesellschaft*, **6**, 73-76.
- 814 Ornos, G. (1984) *La subfamilia Bombinae* (Hym.,
815 *Apidae*) *de la fauna espanola*. PH-D thesis.
816 Universidad Complutense de Madrid. Madrid.
- 817 Pagliano, G. (1993) *Catalogo Degli Imenotteri Italiani*.
818 IV. (Apoidea: Colletidae, Andrenidae, Megachilidae,
819 Anthophoridae, Apidae). *Memorie della Società*
820 *Entomologica Italiana*, **72**, 331-467.
- 821 Pamilo, P., Tengö, J., Rasmont, P., Pirhonen, K.,
822 Pekkarinen, A. & Kaarnam, E. (1997) Pheromonal
823 and enzyme genetic characteristics of the *Bombus*
824 *lucorum* species complex in northern Europe.
825 *Entomol.Fenn*, **7**, 187-194.
- 826 Papadopoulou, A., Bergsten, J., Fujisawa, T., Monaghan,
827 M. T., Barraclough, T. G. & Vogler, A. P. (2008)
828 Speciation and DNA barcodes: testing the effects of
829 dispersal on the formation of discrete sequence
830 clusters. *Philosophical Transaction of the Royal*
831 *Society B*, **363**, 2987-2996.
- 832 Paterson, H. E. H. (1993) *Evolution and the recognition*
833 *concept of species*, The Johns Hopkins University
834 Press, 234 pp.
- 835 Pawlikowski, T. (2008) *A distribution atlas of*
836 *bumblebees of Poland*. 104pp
- 837 Peat, J., Darvill, B., Ellis, J. & Goulson, D. (2005) Effects
838 of climate on intra- and interspecific size variation in
839 bumble-bees. *Functional Ecology*, **19**, 145-151.
- 840 Pedersen, B. V. (1996) A Phylogenetic Analysis of
841 Cuckoo Bumblebees (*Psithyrus*, Lepeletier) and
842 Bumblebees (*Bombus*, Latreille) Inferred from
843 Sequences of the Mitochondrial Gene Cytochrome
844 Oxidase I. *Molecular Phylogenetics and Evolution*, **5**,
845 289-297.
- 846 Pedersen, V. (2002) European bumblebees
847 (Hymenoptera: Bombini)- phylogenetic relationships
848 inferred from DNA sequences. *Insect Systematics &*
849 *Evolution*, **33**, 361-386.
- 850 Peeters, T. M. J., Raemakers, I. P. & Smit, J. (1999)
851 *Voorlopige atlas van de Nederlandse bijen (Apidae)*.
852 European Invertebrate Survey, Leiden (Netherlands).
- 853 Pekkarinen, A. (1982) Morphology and specific status of
854 *Bombus lapponicus* (Fabricius) and *B. monticola*
855 *Smith* (Hymenoptera: Apidae). *Entomologica*
856 *Scandinavia*, **13**, 41-46.
- 857 Pittioni, B. & Schmidt, R. (1942) Die Bienen des
858 fudöftlichen Niederdonau. 1. Apidae, Podriidae,
859 Xylocopidae und Certinidae. *Niederdonau/Natur und*
860 *Kultur*, **19**, 1-69.
- 861 Pittioni, B. (1939) Die Hummeln und
862 Schwarotzerhummeln der Balkan-Halbinsel.
863 *Mitteilungen aus den Königl.*
864 *Naturwissenschaftlichen Instituten*, **XII**, 49-115 + VI
865 pl.
- 866 Popov, V. B. (1931) Zur Kenntnis der paläarktischen
867 Schmarotzerhummeln (*Psithyrus* Lep.). *Eos*, **7**, 131-
868 209.
- 869 Posada, D. (2008) jModelTest: Phylogenetic model
870 averaging. *Molecular Biology and Evolution*, **25**,
871 1253-1256.
- 872 Postner, M. (1952) Biologisch-ökologische untersuchen an
873 Hummeln und ihre Nestern. *Veröffentlichungen*
874 *museum für Naturkunde*, **2**, 45-86.
- 875 Pouvreau, A. (1973) Les ennemis des bourdons. I.- Etude
876 d'une zoocénose : le nid de bourdons. *Apidologie*, **4**,
877 103-148.
- 878 Quinn, G. P. & Keough, M. J. (2006) *Experimental*
879 *Design and Data analysis for Biologists*. Cambridge.
- 880 Rambaut, A. & Drummond, A. (2007) *Tracer. v1.5.0*.
- 881 Rasmont, P. & Adamski, A. (1996) Les Bourdons de la
882 Corse (Hymenoptera, Apoidea, Bombinae). *Notes*
883 *fauniques de Gembloux*, **31**, 1-87.
- 884 Rasmont, P. & Flagodier, D. (1996) Biogéographie et
885 choix floraux des bourdons (Hymenoptera, Apidae)

- de la Turquie. N.A.T.O. - O.T.A.N. Tu-Pollination project, rapport préliminaire. *Report for Université de Mons Hainaut, Çukurova Üniversitesi*. Mons, Andana
- Rasmont, P. (1988) *Monographie écologique et zoogéographique des bourdons de France et de Belgique (Hymenoptera, Apidae, Bombinae)*. PH-D thesis. Faculté des Sciences agronomiques de l'Etat. Gembloux (Belgium).
- Rasmont, P., Ebmer, P. A., Banaszak, J. & Zanden, G. v. d. (1995) Hymenoptera Apoidea Gallica. Liste taxonomique des abeilles de France, de Belgique, de Suisse et du Grand-Duché de Luxembourg. *Bulletin de la Société de France*, **100**, 1-98.
- Rasmont, P., Terzo, M., Aytekin, A. M., Hines, H., Urbanová, K., Cahliková, L. & Valterová, I. (2005) Cephalic secretions of the bumblebee subgenus *Sibiricobombus* Vogt suggest *Bombus niveatus* Kriechbaumer and *Bombus vorticosus* Gerstaecker are conspecific (Hymenoptera, Apidae, *Bombus*). *Apidologie*, **36**, 571-584.
- Reinig, W. F. (1935) On the variation of *Bombus lapidarius* L. and its cuckoo, *Psithyrus rupestris* Fabr., with notes on mimetic similarity. *Journal of genetics*, **XXX**, 321-356.
- Reinig, W. F. (1971) Zur Faunistik und Zoogeographie des Vorderen Orients. 3. Beitrag zur Kenntnis der Hummeln und Schmarotzerhummeln Anatoliens. *Veröffentlichungen der zoologischen Staatssammlung München*, **15**, 139-165.
- Ressel, F. (1974) Beitrag zur Kenntnis der Hummelfauna des südwestlichen Niederösterreichs (Hym., Apidae). *Beitr. Ent.*, **24**, 67-71.
- Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572-1574.
- Schenck, A. (1859) Die nassauischen Bienen. Revision und Ergänzung der früheren Bearbeitungen. *Jahrbuch des Nassauischen Vereins für Naturkunde*, **14**, 1-414.
- Schmiedeknecht, H. L. O. (1883) *Apidae Europaeae (Die Bienen Europa's) per genera, species et varietates. Dispositae atque descriptae*. Volume I fascicule 6, pp. 9(393)-29(413), 1(415)-56(470). Berlin.
- Schwarz, M., Gusenleitner, F. & Kopf T. (2005) Weitere Angaben zur Bienenfauna Österreichs sowie Beschreibung einer neuen *Osmia*-Art. Vorstudie zu einer Gesamtbearbeitung der Bienen Österreichs VIII. *Entomofauna*, **26**, 117-164.
- Schwarz, M., Gusenleitner, F., Westrich, P., & Dathe, H. (1996) Katalog der Bienen Österreichs, Deutschlands und der Schweiz (Hymenoptera, Apidae). *Entomofauna Supplement*, **8**, 1-398.
- Skorikov, A.S., (1922) Faunae Petropolitanae catalogus. *Petrogradskii agronomicheskii institut*, **2**, 51 pp.
- Sladen, F. W. L. (1912) *The humble-bee, its life history and how to domesticate it, with descriptions of all the British species of Bombus and Psithyrus*. London.
- Smith, A., Rodriguez, J. J., Whitfield, J. B., Deans, A. R., Janzen, D. H., Hallwachs, W. & Hebert, P. D. N. (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology and collections. *Proceeding of the National Academy of Sciences of the United States of America*, **105** (35), 12359-12364.
- Straka, J., Bogusch, P. & Pridal, A. (2007) Apoidea: Apiformes (včely). Pp. 241-299. In: Bogusch, P., Straka, J. & Kment, P. (eds.): Annotated checklist of the Aculeata (Hymenoptera) of the Czech Republic and Slovakia. Komentovaný seznam žahadlových blanokřídlých (Hymenoptera: Aculeata) České republiky a Slovenska. *Acta Entomologica Musei Nationalis Pragae, Supplementum*, **11**, 1-300.
- Svensson, B. G. & Bergström, G. (1977) Volatile marking secretions from the labial gland of north European *Pyrobombus* D.T. males (Hymenoptera, Apidae). *Insect Socialia*, **24**, 213-224.
- Svensson, B. G. (1979) *Pyrobombus lapponicus* auct. In Europe recognized as two species: *P. lapponicus* (Fabricius, 1793) and *P. monticola* (Smith, 1849) (Hymenoptera, Apoidea, Bombinae). *Entomological Scandinavia*, **10**, 275-296.
- Swofford, D. L. (2001) *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods)*. 4.0b10. Sinauer Associates, Sunderland, MA.
- Symonds, M. R. E., Moussalli, A. & Elgar, M. A. (2009) The evolution of sex pheromones in an ecologically diverse genus of flies. *Biological Journal of the Linnean Society*, **97**, 594-603.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.
- Templeton, A. R. (1989) The meaning of species and speciation, a genetic perspective. in *Speciation and its consequences*. (eds Otte D, Endler JA), pp. 3-27. Sinauer, Sunderland, Massachusetts.
- Terzo, M., Urbanová, K., Valterová, I. & Rasmont, P. (2005) Intra and interspecific variability of the cephalic labial glands' secretions in male bumblebees: the case of *Bombus (Thoracobombus) ruderarius* (Müller) and *B. (Thoracobombus) sylvarum* (L.) [Hymenoptera, Apidae]. *Apidologie*, **36**, 85-96.
- Terzo, M., Valterová, I., Urbanová, K. & Rasmont, P. (2003) De la nécessité de redécrire les phéromones sexuelles des mâles de bourdons [Hymenoptera, Apidae, Bombini] publiées avant 1996 pour leur utilisation en analyse phylogénétique. *Phytoprotection*, **84**, 39-49.
- Tkalčů, B. (1969) Ergebnisse der Albanien-Expedition 1961 des Deutschen Entomologischen Institutes. 78. Beitrag. Hymenoptera: Apidae IV (Bombinae). *Beiträge zur Entomologie*, **19**, 887-916.
- Trewick, S. A. (2008) DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics*, **24**, 240-254.
- Tumšs, V. (1975) Materiāli Latvijas bišu (Hymenoptera, Apoidea) fauna III. *Zoologijas Muzeja Raksti*, **13**, 5-24.
- Urbanová, K., Halik, 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. *Biochemical Systematics and Ecology*, **32**, 1025-1045.
- Valterová, I. & Urbanová, K. (1997) Chemical signals of bumblebees. *Chemicke Listy*, **91**, 846-857.
- Vereecken, N., Mant, J. & Schiestl, F. P. (2007) Population differentiation in female sex pheromone and male preferences in a solitary bee. *Behavioral Ecology and Sociobiology*, **61** (4), 811-821.
- Vogrin, V. (1955) Prilog fauni Hymenoptera Aculeata Jugoslavije. *Zaštita bilja, Beograd*, **31**, 15-21.
- Vogt, O. (1909) Studien über das Artproblem. 1.

- 1020 Mitteilung. Über das variieren der Hummeln, 1. Teil. 1052
 1021 *Naturforschender Freunde*, **1**, 27- 84. 1053
 1022 Voveikov, G.S. (1953) Estestvennaya smena samok vo 1054
 1023 cem'ya Schmelej (Hym. Bomb.). *Entomologzrheskoe* 1055
 1024 *Obozrenie*, **33**, 174-181. 1056
 1025 Westrich, P. (1989) *Die Wildbienen Baden-* 1057
 1026 *Württembergs. Spezieller Teil: Die Gattungen und* 1058
 1027 *Arten*. Verlag Eugen Ulmer. Stuttgart, 437-972 1059
 1028 pp.Widmer, A., Schmid-Hempel, P., Estoup, A. & 1060
 1029 Scholl, A. (1998) Population genetic structure and 1061
 1030 colonization history of *Bombus terrestris* s.l. 1062
 1031 (Hymenoptera: Apidar) from the Canary islands and 1063
 1032 Madeira. *Heredity*, **81**, 563-572. 1064
 1033 Widmer, A., Schmid-Hempel, P., Estoup, A. & Scholl, 1065
 1034 A. (1998) Population genetic structure and 1066
 1035 colonization history of *Bombus terrestris* s.l. 1067
 1036 (Hymenoptera : Apidae) from the Canary Islands and 1068
 1037 Madeira. *Heredity*, **81**, 563-572. 1069
 1038 Wilcox, T. P., Zwickl, D. J., Heath, T. A. & Hillis, D. M. 1070
 1039 (2002) Phylogenetic relationships of the dwarf boas 1071
 1040 and a comparison of Bayesian and bootstrap 1072
 1041 measures of phylogenetic support. *Molecular* 1073
 1042 *Phylogenetics and Evolution*, **25**, 361-371. 1074
 1043 Williams, P. H. (1985) A preliminary cladistic 1075
 1044 investigation of relationships among the bumble bees 1076
 1045 (Hymenoptera, Apidae). *Systematic Entomology*, **10**, 1077
 1046 239- 255. 1078
 1047 Williams, P. H. (1998) An annotated checklist of bumble 1079
 1048 bees with an analysis of patterns of description 1080
 1049 (Hymenoptera: Apidae, Bombini). *Bulletin of The* 1081
 1050 *Natural History Museum (Entomology)*, **67**, 79-152. 1082
 1051 Williams, P. H. (2007) The distribution of bumblebee
 colour patterns worldwilde: possible significance for
 thermoregulation, crypsis, and warming mimicry.
Biological Journal of the Linnean Society, **92**, 97-
 118.
 Williams, P. H. (2008) Do the parasitic *Psithyrus*
 resemble their host bumblebees in colour pattern?
Apidologie, **39**, 637-649.
 Yassin, A., Cappy, P., Madi-Ravazzi, L., Ogereau, D. &
 David, J. R. (2007) DNA barcode discovers two
 cryptic species and two geographical radiations in the
 invasive drosophilid *Zaprionus indianus*. *Molecular*
Ecology Notes, **8**, 491-501.
 Yefremova, Z. A. (2001) Faunal and ecological notes on
 species of *Bombus* Latreille, 1802, and *Psithyrus*
 Lepeletier, 1832 (Hymenoptera: Apidae) from the
 Tarbagatai and Saur mountains ranges in eastern
 Kazakhstan. *Entomologist's Gazette*, **52**, 269-275.
 Žáček, P., Kalinová, B., Šobotník, J., Hovorka, O.,
 Ptáček, V., Coppée, A., Verheggen, J. F. &
 Valterová, I. (2009) Comparison of Age-dependent
 Quantitative Changes in the Male Labial Gland
 Secretion of *Bombus terrestris* and *Bombus lucorum*.
Journal of Chemical Ecology, **35**, 698-705.
 Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000)
 A greedy algorithm for aligning DNA sequences.
Journal of Computational Biology, **7**, 203-214.
 Zwickl, D. J. (2006) *Genetic algorithm approaches for*
the phylogenetic analysis of large biological
sequence datasets under the maximum likelihood
criteria. Ph.D. thesis. The University of Texas at
 Austin. Austin.

1084
1085

Table 1. Main morphological and colour pattern differences between *B. maxillosus* and *B. barbutellus* males and females according Popov, (1931), Pittioni (1939), May (1943) and Løken (1984).

Female	<i>B. maxillosus</i>	<i>B. barbutellus</i>
Morphology		
Longest hairs of the posterior fringe of the hind tibia	Shorter than half the largest width of the segment	Longer than half the largest width of the segment.
Longest hairs of the hind basitarsus	Shorter than the width of the segment; clearly narrower at the apex than at the base	Longer than the width of the segment; Its anterior and posterior edges almost parallel
Coat	Short	Long and shaggy
Colour pattern		
Wings	Darkened, almost opaque	Hyaline
Collare and scutellare	Narrower, mixed with black hairs	Very wide, yellow
T1	Black	Yellow mixed with black
T4	Off-white	With white sides
T5	With off-white sides	White

Male		
Morphology		
Longest hairs of the anterior fringe on the hind basitarsus	Shorter than the width of the segment	As long as, or slightly longer than the width of the segment
Coat	Short	Shaggy
Colour pattern		
Wings	Darkened	Hyaline
Vertex	With a few yellowish hairs	With a few yellow hairs
Collare and scutellare	Narrower, yellowish, often mixed with many black hairs	Wide, yellow
T1	Either black or yellowish, mixed with black hairs	Yellow
T3	With off-white sides	With white posterior margins
T5	With off-white sides	With white posterior margins
T6	With small off-white tuft on its side	With white sides

1086

1087
1088

Table 2. Host/parasite relations in the *Allopsithyrus* group. Main hosts are in bold.(*): Colonies in which *Psithyrus* offspring have been observed

<i>Allopsithyrus</i>	<i>Bombus</i> Host species	Main references
<i>B. barbutellus</i>	<i>B. (Megabombus) hortorum</i> *	Sladen 1912; Ball 1914; Müller 1936; Cumber 1949; Postner 1952
	<i>B. (Megabombus) ruderatus</i>	Westrich 1989; Peeters <i>et al.</i> , 1999
	<i>B. (Megabombus) portschinsky</i>	Rasmont unpublished data
	<i>B. (Pyrobombus) hypnorum</i> *	Voveikov 1953; Pouvreau 1973
	<i>B. (Pyrobombus) jonellus</i>	Schenck 1859; Schmiedekneht 1883; Skorikov 1922
	<i>B. (Pyrobombus) pratorum</i>	Hoffer 1889; Skorikov 1922
	<i>B. (Thoracobombus) pascuorum</i>	Hoffer 1889; Skorikov 1922; Popov 1931
	<i>B. (Thoracobombus) humilis</i>	Hoffer 1889; Skorikov 1922
	<i>B. (Thoracobombus) ruderarius</i>	Skorikov 1922
	<i>B.(Subterraneobombus) distinguendus</i>	Popov 1931
<i>B. maxillosus</i>	<i>B.(Subterraneobombus) subterraneus</i>	Knechtel 1955
	<i>B. (Megabombus) ruderatus</i>	Vogt 1909 ; Popov 1931; Pittioni & Schmidt 1942; Rasmont 1988
	<i>B. (Megabombus) argillaceus</i>	Popov 1931; Skorikov 1922; Pittioni & Schmidt 1942; Pouvreau 1973
	<i>B.(Subterraneobombus) subterraneus</i>	Tkalců 1969; Pouvreau 1973

1089

1090

1091

1092

1093
1094

Table 3. *Allopsithyrus* and outgroup taxa examined, their collection locality, collector, vouchers numbers and GenBank accession numbers. # = number of samples; V.# = voucher numbers and sample codes used in trees and clustering; Ef-1 α = GenBank accession numbers for EF-1 α sequences; COI = GenBank accession numbers for COI sequences.

	Species	Subspecies	#	Locality	Lat	Long	Recorder	V.#	EF-1 α	COI
Ingroup	<i>barbutellus</i>	-	7	Turkey, Rize	40°56'01''N	41°08'26''E	M. Terzo & P. Rasmont	BAR1, BAR2, BAR4, BAR5, BAR6, BAR8, BAR9	JF699169	JF699183
	<i>barbutellus</i>	-	1	Sweden, Uppsala	59°51'15''N	17°39'14''E	P. Rasmont	BAR3	JF699171	JF699185
	<i>barbutellus</i>	-	1	Germany, Rottleberode	51°31'43''N	10°57'08''E	E. Stolle	BAR7	JF699172	JF699186
	<i>barbutellus</i>	-	4	France, Ablon	49°23'38''N	0°17'43''E	P. Lhomme	BAR10, BAR11, BAR12, BAR13	JF699170	JF699184
	<i>barbutellus</i>	-	1	Denmark, Ekkodalen	-	-	Pedersen (2002)	BAR14	-	AY181177.1
	<i>barbutellus</i>	-	1	Germany, Tüchersfeld	-	-	Pedersen (2002)	BAR15	-	AY181176.1
	<i>barbutellus</i>	-	1	Denmark	-	-	Pedersen (1996)	BAR17	-	L26574.1
	<i>barbutellus</i>	-	1	Russia, Primorsky	-	-	Tanaka <i>et al.</i> (unpublished)	BAR16	-	AF279576.1
	<i>maxillosus</i>	<i>maxillosus</i>	4	Turkey, Kayseri	38°28'36''N	35°30'06''E	M. Terzo	MAX1, MAX3, MAX5, MAX6	JF699176	JF699190
	<i>maxillosus</i>	<i>maxillosus</i>	3	Turkey, Erzincan	39°52'06''N	39°33'56''E	T. De Meulemeester & P. Rasmont	MAX7, MAX8, MAX9	JF699179	JF699193
	<i>maxillosus</i>	<i>maxillosus</i>	2	France, Gonfaron	43°18'28''N	06°18'33''E	P. Rasmont	MAX2, MAX4	JF699178	JF699192
	<i>maxillosus</i>	<i>maxillosus</i>	1	France, Bargemon	43°38'22''N	06°34'07''E	P. Rasmont	MAX10, MAX11	JF699177	JF699191
	<i>maxillosus</i>	<i>italicus</i>	3	France, Evisa	42°17'25''N	08°52'40''E	T. Lecocq	ITA1, ITA2, IT3	JF699175	JF699189
Outgroup	<i>campestris</i>	<i>campestris</i>	2	France, Saint-Lyphard	47°23'52"N	02°18'24"W	G. Mahé	CAM1, CAM2	JF699174	JF699188
	<i>bohemicus</i>	<i>bohemicus</i>	2	France, Nyer	42°30'06"N	02°19'00"E	T. Lecocq	BOH1, BOH2	JF699173	JF699187
	<i>rupestris</i>	<i>rupestris</i>	2	Germany, Geiseltal	51°18'00''N	11°52'00''E	E. Stolle	RUP1, RUP2	JF699180	JF699194
	<i>vestalis</i>	<i>vestalis</i>	2	France, Paris	48°50'37"N	02°21'35"E	P. Rasmont	VES1, VES2	JF699182	JF699196
	<i>soroensis</i>	<i>lectitatus</i>	1	France, Nyer	42°30'09"N	02°19'12"E	T. Lecocq	SOR1	JF699181	JF699195

1095

1096

1097

1098
1099
1100

Table 4. List of the identified compounds. Molecular weight (MW [m/z]), Median (Med [%]), first and fourth quartiles (Q1 [%] and Q2 [%]), minimum and maximum (Min [%] and Max [%]) of the 73 identified compounds in *B. barbutellus*, *B. maxillosus maxillosus* and *B. maxillosus italicus*. The undetermined compounds are indicated as Ux.

Compounds	MW	<i>B. barbutellus</i>					<i>B. maxillosus</i>				
		Min	Q1	Med	Q3	Max	Min	Q1	Med	Q3	Max
Decanoic acid	172	0,01	0,02	0,02	0,02	0,15	0	0,02	0,02	0,05	0,15
Geranyl acetone	194	0,01	0,02	0,02	0,15	0,2	0	0,02	0,03	0,05	0,2
Tetradecane	198	0,27	0,36	0,75	1,58	3,49	0	1,43	2,3	2,85	4,12
Dodecanoic acid	200	0,01	0,02	0,03	0,16	2,72	0,01	0,05	0,3	1,83	5,15
Farnesal, isomer I	220	0,04	0,12	0,18	0,21	0,31	0	0,05	0,16	0,33	0,61
Farnesal, isomer II	220	0,14	0,29	0,36	0,45	0,59	0	0,1	0,23	0,63	0,92
Farnesol	222	0,03	0,05	0,1	0,2	0,37	0	0	0,21	0,47	1,54
Dihydrofarnesol	224	0,13	0,2	0,38	0,42	0,53	0	0,08	0,15	0,24	0,53
Ethyl dodecanoate	228	0	0,02	0,02	0,07	0,15	0	0	0,01	0,02	0,15
Tetradecenoic acid	228	0	0	0,02	0,03	0,04	0	0,01	0,04	0,1	0,16
Heptadecane	240	0	0,01	0,02	0,02	0,06	0	0,01	0,02	0,05	0,15
Ethyl tetradecenoate	254	0	0	0	0,01	0,07	0	0,02	0,1	0,25	19
Hexadecenoic acid	254	0	0,03	0,16	0,21	0,76	0	0,2	0,35	0,44	1,69
Ethyl tetradecanoate	256	0	0	0	0,01	0,01	0	0	0,02	0,11	0,79
Farnesyl acetate	264	10,21	11,35	16,58	20,98	37,89	0,19	20,84	25,53	28,37	45,4
Octadecenal	266	0,02	0,03	0,04	0,06	0,6	0	0,08	0,32	0,55	1,93
Dihydrofarnesyl acetate	266	0,09	0,29	0,49	0,64	0,82	0	0,1	0,31	0,89	1,7
Octadecanal	268	0	0	0,01	0,01	0,13	0	0,02	0,07	0,13	0,32
Octadecenol	268	11,81	13,23	16,17	20,68	24,28	0,02	2,25	9,58	13,94	24,28
Ethyl hexadecenoate	282	0	0	0	0,01	0,02	0	0,03	0,04	0,18	0,47
Octadecenoic acid	282	0	0,02	0,07	0,15	0,61	0	0,01	0,13	0,22	1,95
Ethyl hexadecanoate	284	0,01	0,03	0,06	0,08	0,22	0	0,04	0,15	0,36	0,79
Geranylgeranial	288	0,14	0,33	0,46	0,48	1,04	0	0,12	0,19	0,23	1,04
Farnesyl butyrate	292	0	0	0,18	0,5	0,9	0	0,26	0,63	0,83	2,33
Geranylcitronellol	292	1,54	2,6	3,25	3,69	6,34	0,12	1,49	2,04	2,4	6,61
Heneicosene	294	0	0	0,03	0,66	4,85	0	0,12	0,33	0,81	4,72
Heneicosane	296	0,3	0,77	0,97	1,54	1,68	0,02	0,11	0,15	0,17	1,68
Eicosenol	296	0	0	0	0	9,03	0	0	4,22	4,73	9,03
Octadecenoic acid ethyl ester	310	0	0	0	0,08	0,08	0	0	0,03	0,09	0,44
Octadecenyl acetate	310	0,75	0,85	0,9	1,05	2,12	0,4	0,93	1,04	1,1	2,04
Docosane	310	0	0,14	0,16	0,18	0,22	0	0,11	0,15	0,21	0,25
Tricosene	322	0,08	0,1	0,19	0,21	0,33	0	0,02	0,18	0,23	0,54
Tricosane	324	0,04	0,18	6,19	7,51	9,91	0,02	0,07	0,11	0,14	10,91
1,3-Diacetyl-2-decanoylglycerol	330	0	0,17	0,68	7,72	14,32	0	0,3	0,47	1,14	15,6
Geranylcitronellyl acetate	334	0,12	0,46	0,51	0,98	1,47	0,2	0,57	0,67	0,82	2,87
Eicosenyl acetate	338	0,03	0,17	0,31	0,35	0,8	0,04	0,07	0,1	0,19	2,61
Tetracosane	338	0,04	0,04	0,07	0,08	0,49	0	0,04	0,06	0,08	0,49
Eicosyl acetate	340	0	0	0	0,01	0,18	0	0,01	0,02	0,03	0,22
Pentacosene I	350	0,02	0,04	0,07	0,1	0,23	0,01	0,03	0,05	0,09	3,62
Pentacosene II	350	0,04	0,08	0,11	0,17	0,28	0,02	0,05	0,08	0,11	0,28
Pentacosane	352	1,17	1,7	1,96	2,15	4,66	0,76	1,18	1,6	1,85	4,66
1,3-Diacetyl-2-dodecanoylglycerol	358	8,72	16,83	25,11	26,86	31,11	0,96	10,85	19,02	24,08	31,11

Hexacosene	364	0,02	0,02	0,04	0,08	0,12	0,01	0,01	0,02	0,04	0,12
Farnesyl decanoate	376	0,04	0,07	0,08	0,13	0,2	0,05	0,06	0,08	0,22	0,38
Heptacosene, isomer I	378	0	0,03	0,04	0,1	0,22	0,01	0,03	0,05	0,09	3,01
Heptacosene, isomer II	378	0,05	0,27	0,37	0,59	1,08	0,07	0,13	0,27	0,45	1,08
Heptacosane	380	0,52	0,83	1,06	1,63	3,34	0,61	0,8	0,94	1,49	4,11
Hexacosenol	380	0,04	0,09	0,12	0,17	0,35	0	0,04	0,12	0,23	0,35
Hexacosanol	382	0,02	0,05	0,05	0,13	0,32	0,01	0,02	0,06	0,12	1,55
1,3-Diacetyl-2-tetradecanoylglycerol	384	0	0,23	0,37	0,45	0,53	0,04	0,22	0,91	1,74	2,58
1,3-Diacetyl-2-tetradecanoylglycerol	386	0,04	0,08	0,13	0,15	0,19	0	0,05	0,18	0,35	1,12
Octadecenyl octanoate	394	0,02	0,03	0,05	0,07	0,09	0,01	0,03	0,05	0,08	0,19
Hexadecyl decanoate	396	0,03	0,04	0,05	0,07	0,33	0	0,01	0,02	0,07	0,33
Farnesyl dodecanoate	404	0,07	0,08	0,1	0,24	0,69	0,02	0,15	0,38	1,36	4,41
Nonacosene	406	0,04	0,07	0,12	0,16	0,21	0,03	0,04	0,08	0,14	0,27
Nonacosane	408	0,1	0,16	0,2	0,25	0,72	0,11	0,15	0,18	0,26	0,76
Squalene	410	0	0	0,01	0,01	0,02	0	0,01	0,01	0,03	0,09
1,3-Diacetyl-2-hexadecanoylglycerol	412	0	0	0	0	0,13	0	0	0	0	0,13
Octadecenyl decanoate	422	0,09	0,19	0,28	0,38	1,79	0,05	0,13	0,42	0,95	3,35
Hexadecyl dodecanoate	424	0,01	0,03	0,04	0,09	0,16	0	0,02	0,08	0,13	0,24
Hentriacontene	434	0,02	0,02	0,03	0,06	0,08	0,01	0,02	0,04	0,07	0,16
Geranylcitronellyl decanoate	446	0	0,01	0,02	0,04	0,25	0	0,01	0,02	0,07	0,61
Octadecenyl dodecanoate	450	1,53	2,61	3,64	7,04	10,08	0,58	2,51	7,4	13,93	20,94
Farnesyl hexadecanoate	460	0	0,01	0,01	0,12	0,46	0	0	0,09	0,3	0,93
Geranylcitronellyl dodecanoate	474	0,14	0,16	0,19	1,21	1,76	0,02	0,14	0,33	1,75	3,93
Eicosenyl dodecanoate	478	0,74	0,88	1,3	2,03	3,64	0,3	0,72	1,61	4,72	12,06
Farnesyl octadecenoate	486	0	0	0	0,06	0,12	0	0,04	0,06	0,08	0,5
U1	MW?	0,26	0,4	3,51	7,37	9,87	0	2,67	3,61	3,88	9,87
U2	MW?	0,01	0,07	0,09	0,18	0,35	0	0,09	0,15	0,21	2,68
U3	MW?	0	0,02	0,03	0,04	2,89	0	0	0,01	0,03	2,89
U4	MW?	0,03	0,05	0,06	0,1	0,18	0	0,03	0,08	0,1	0,21
U5	MW?	0,01	0,02	0,02	0,03	0,04	0,01	0,03	0,07	0,14	0,59
U6	MW?	0,01	0,01	0,01	0,02	0,11	0	0,01	0,03	0,05	0,41