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Distribution and flower visitation records of bumblebees in Lebanon (Hymenoptera: Apidae)

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Summary. West Palearctic bumblebees are common wildflowers and crop pollinators that are well studied in their central and northern distribution ranges, but fewer information is available on their southern distribution areas. Lebanon falls on the southern limit of their distribution and no published information is available on the local bumblebees. Our study aims to produce a data baseline of the local bumblebee species. In order to do so we grouped available old records of bumblebees in Lebanon with recent author collections and produced preliminary distribution maps. We listed four species: *Bombus terrestris, B. argillaceus, B. niveatus vorticosus* and *B. melanurus*. Preliminary distribution shows that *Bombus terrestris* and *B. argillaceus* are widespread and have a large foraging range, whereas *B. niveatus vorticosus* and *B. melanurus* have a restricted distribution to altitudes above 1800 m with a smaller foraging range. The male cephalic labial gland secretions analysis of local *Bombus terrestris* specimens provides preliminary evidence that the local subspecies could be *Bombus terrestris calabricus*. Therefore, we highlight the importance of regulating foreign *Bombus terrestris* subspecies importation for agriculture purposes, as well as monitoring *B. niveatus vorticosus* and *B. melanurus* that are rendered vulnerable by their isolated populations.

Résumé. Distribution et enregistrement des visites de fleurs par les bourdons au Liban (Hymenoptera : Apidae). Les bourdons du Paléarctique occidental sont des pollinisateurs communs de fleurs sauvages et de cultures, qui sont bien étudiés dans leurs aires de répartition centrale et septentrionale, mais moins d'informations sont disponibles pour leurs aires de distribution méridionales. Le Liban se trouve à la limite sud de leur répartition et aucune information publiée n'est disponible sur les bourdons locaux. Notre étude vise à produire une base de données sur les espèces locales de bourdons. Pour ce faire, nous avons regroupé les anciens signalements disponibles de bourdons au Liban avec les collections récentes des auteurs, et produit des cartes de distribution préliminaires. Nous avons répertorié quatre espèces : *Bombus terrestris, B. argillaceus, B. niveatus vorticosus* et *B. melanurus*. La distribution préliminaire montre que *Bombus terrestris* et *B. argillaceus* sont répandus et ont une grande aire de butinage, tandis que *B. niveatus vorticosus* et *B. melanurus* ont une distribution restreinte à des altitudes supérieures à 1800 m, avec une aire de butinage plus petite. L'analyse des sécrétions des glandes labiales céphaliques mâles des spécimens locaux de *Bombus terrestris* fournit une preuve préliminaire que la sous-espèce locale pourrait être *B. terrestris calabricus*. Par conséquent, nous soulignons l'importance de réglementer l'importation de sous-espèces étrangères de *Bombus terrestris* à des fins agricoles, ainsi que de surveiller *B. niveatus vorticosus* et *B. melanurus*, rendus vulnérables par l'isolement de leurs populations.

Keywords: bio diversity; distribution; pollination; faunistics; Palaearctic; Near East

Bumblebees (*Bombus* Latreille, 1802) form a genus of wild bees that includes about 260 species worldwide (Williams 1998; Cameron & Sadd 2020). They are pollinators of many wild plants and cultivated crops (Mänd et al. 2002; Goulson 2010; Ballantyne et al. 2015). Well adapted to colder regions, they are found all over Europe and Asia to the Arctic. They are also present throughout the Americas but are absent from Africa south of the Sahara and from Oceania (Williams 1998; Hines 2008; Rasmont & Iserbyt 2010-2014), except New Zealand and Tasmania where they have been imported for pollination (Goulson & Hanley 2004). The West Palearctic distribution and status of bumblebees are relatively well documented (Rasmont et al. 2015), but less information is available on species found in the south of this region, including Lebanon. The topography of this country presents several mountainous areas that are potentially favorable to bumblebees (Özbek 2002; Rasmont & Iserbyt 2010-2014; Saini et al. 2012). However, the Lebanese wild bee fauna remains poorly documented. The main references are from Mavromoustakis (1955, 1956, 1962) that produced an inventory of wild bees belonging mainly to the tribes Anthidiini and Osminii based on his own collection trips, as well as describing several new species. His findings were included in the review compiled by Grace (2010). Aside from these

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records, to our knowledge there have been no documented findings. On the other hand, records from neighboring countries report 45 species from Turkey (Rasmont et al. 2009), two from Syria (Solaiman-Khaled et al. 2012) and Israel (Rasmont & Iserbyt 2010-2014).

In order to fill this gap of information on the presence and diversity of Lebanese bumblebees, our objectives for this paper are to present our observations from several habitats across Lebanon, and secondly to assemble unpublished records and museum collection specimens in order to produce a biogeographic data baseline.

Materials and methods

Study area

Our collections were carried out in the governorates of North Lebanon, Mount Lebanon and Beqaa. Following the vegetation levels classification of Abi-Saleh & Safi (1988), the collection areas fall into the Eumediterranean (500–1000 m), Supramediterranean (1000–1500 m), Montane Mediterranean (1500–2000 m) and Oromediterranean (>2000 m) on the western slopes of Mount Lebanon (Typical Mediterranean); and into the Mediterranean (1000–1500 m), Supramediterranean (1400–1800 m), Montane Mediterranean (1800–2400 m) and Oromediterranean (>2400 m) on the Eastern slopes (Presteppic Mediterranean). It has to be noted when validating old data that the Lebanese border has undergone changes from Ottoman Empire, the French mandate and independence (Barnett et al. 2019). In this paper, we only consider the present Lebanese borders (from 1943).

New specimen collection protocols

Collections were carried out individually by M. Boustani, W. Yammine and P. Rasmont between April 2016 and September 2017 in the following regions separately: Ammiq (973 m), Baskinta (1430 m), Beit Chabab (740 m), Dahr El Qabib (2500 m) and Qornael (1234 m). Collections were also carried on a transect between Tannourine El Tahta (900 m) and Arz Tannourine (1800 m), and between Arz Bcharré (1800 m) and Qornet El Sawda (3000 m).

All collections were made using hand nets. Bee specimens were killed using ethyl acetate or by freezing, then pinned and identified to species level, using reference specimens from the University of Mons collection. Flowers visited by bumblebees were sampled by collecting specimens for herbarium, they were then identified to species level using Mouterde (1966, 1970, 1984).

Databasing and mapping

Bombus specimens from entomological student collections of the American University of Beirut (AUB) and the Holy Spirit University of Kaslik (USEK) collections were examined, identified, and integrated into our database in addition to the new specimens collected. We also integrated the available original data from Lebanon included in the *Banque de Données Fauniques de Gembloux et Mons*.

Specimens without labels were eliminated from the counts. Specimens labeled only with country name were included in the counts but are not displayed on the map.

All data were digitized using Data Fauna Flora 5.1.2 (Barbier et al. 2000) and mapped with Quantum GIS 2.18.27.

Subspecies identification of Bombus terrestris *samples*

The diagnostic character of cephalic labial gland secretions (CLGS) is often used as a tool to delimitate bumblebee species (e.g. Lecocq et al. 2015b; Martinet et al. 2018) and has also been used to differentiate subspecies in *Bombus terrestris* (Lecocq et al. 2016). We cross-checked the morphological subspecies identifications of *B. terrestris* with a quantitative GC-FID analysis of CLGS according the protocol established by Demeulemeester et al. (2011) using five Lebanese specimens collected as follows: 1 male from Tannourine Reserve Gate Area (34°12′27.9″N 35°55′56.9″E, 1796 m) and 4 males from Tannourine Reserve Trail 4 (35°55′56.9″N 35°55′56.0″E, 1781 m). All details of the methodology are presented in supporting information (Appendix S1). We assessed CLGS differentiations of the 5 Lebanese specimens to the 9 Sicilian *Bombus terrestris calabricus* of the same cluster using a multiple response permutation procedure (MRPP) (R-package vegan, Oksanen et al. 2011).

Results

A total of 269 specimens and field observations were assembled from the following sources: 11 from *Base de données Gembloux et Mons*, 12 from AUB, and 7 from USEK collections, 116 from the Mira Boustani collection, 83 from the Pierre Rasmont collection, and 39 from the Wael Yammine individual collections. Details of the specimens examined from the AUB and USEK collections are listed in Table 1.

Four species of *Bombus* were identified: *B. niveatus vorticosus* (Kriechbaumer, 1870) (55 specimens, Figure 1a), *B. terrestris* (Linnaeus, 1758) (164 specimens, Figure 1b), *B. argillaceus* (Scopoli, 1763) (28 specimens, Figure 1c) and *B. melanurus* (Lepeletier, 1836) (21 specimens, Figure 1d).

Bombus terrestris (Linnaeus, 1758)

Species distribution. Centered on the Mediterranean (except Egypt) stretching north to Stockholm and east to Altai (Rasmont et al. 2008, 2015; Rasmont & Iserbyt 2010-2014).

Local distribution. Figure 2b. 27♀, 90☿, 46♂, 2 sex not specified: Ehden (5♀, 37☿, 5♂), Fatri (1☿), Arsoun (2☿, 1♂), Qartaba (1☿), Arz Bcharre (5♀, 5☿, 7♂), Qannoubine (1♂), Dahr El Adib (2☿), Qornet El Sawda (1♀, 3☿), Arz Tannourine (1♀, 2☿, 12♂), Harissa (1☿), Batroun (1♀), Falougha (2☿), Aley (1♂), Maameltein (1♀), Ammiq (3☿, 1♂), Qnat (5☿), Qornayel (1♂), Jounieh (1♂), Baskinta (3☿, 1♂), Beit Chabab (4☿), Hboub (1♀), Halate (1♀), Bawarij (1☿), Sawfar (2☿), Hadath Al Jebbeh (11♀, 15☿, 16♂, 2 unknown).

Altitude range in Lebanon. From sea level (Maameltein) to 3000 m (Qornet El Sawda).

Flowers visited in Lebanon. Apiaceae: *Prangos asperula* Boiss. (1☿). Asteraceae: *Cousinia libanotica* D.C.

Table 1. Details of specimens of *Bombus* examined from American University of Beirut (AUB) and Holy Spirit University of Kaslik (USEK).

Taxon	Sex	N	Collector	Collection	Date	Locality	Governorate	Altitude
B. argillaceus	♀	1	Boustani M., Kyrk S.	USEK	2.V.2016	Arsoun	Mount Lebanon	640
B. argillaceus	♀	1	Boustani M.	USEK	20.VI.2016	Bcharre Forest	North Lebanon	1928
B. argillaceus	♀	1	Boustani M.	USEK	19.VI.2016	Ehden	North Lebanon	1567
B. argillaceus	☿	1	Usayran W.	AUB	8.VI.1985	Zahrani	South Lebanon	500
B. melanurus	♀	2	Boustani M.	USEK	24.VI.2016	Bcharre	North Lebanon	2723
B. melanurus	☿	1	Boustani M.	USEK	20.VI.2016	Bcharre	North Lebanon	2611
B. niveatus	♀	1	Yammine W.	USEK	24.VI.2016	Bcharre	North Lebanon	2723
B. niveatus	♀	1	Boustani M.	USEK	20.VI.2016	Bcharre	North Lebanon	2611
B. terrestris	☿	1		AUB	10.V.1975	Bawarij	Beqaa	1300
B. terrestris	♂	1		AUB	25.VIII.1975	Aley	Mount Lebanon	700
B. terrestris	☿	2	Nemer N.	USEK	X.2015	Arsoun	Mount Lebanon	640
B. terrestris	♂	1	Nemer N.	USEK	X.2015	Arsoun	Mount Lebanon	640
B. terrestris	☿	1	Boustani M., Kyrk S.	USEK	2.V.2016	Arsoun	Mount Lebanon	640
B. terrestris	☿	1		AUB	24.VI.1975	Falougha	Mount Lebanon	1250
B. terrestris	☿	1		AUB	24.VI.1975	Falougha	Mount Lebanon	1250
B. terrestris	☿	1	Daou R.	USEK	12.IV.2016	Fatri	Mount Lebanon	500
B. terrestris	♀	1	Kazan A., Moussa R.	USEK	3.12.2014	Halate	Mount Lebanon	100
B. terrestris	♀	1	Makdissi R.	USEK	2.XII.2013	Hboub	Mount Lebanon	375
B. terrestris	♀	1	Shwayri	AUB	21.III.1981	Jounieh	Mount Lebanon	0
B. terrestris	☿	1		AUB	21.VI.1973	Sawfar	Mount Lebanon	1320
B. terrestris	☿	1		AUB	21.VI.1973	Sawfar	Mount Lebanon	1320
B. terrestris	♀	1	Najem N., Saade C.	USEK	XI.2014	Batroun	North Lebanon	900
B. terrestris	☿	1	Boustani M.	USEK	20.VI.2016	Bcharre	North Lebanon	1928
B. terrestris	♀	1	Yammine W.	USEK	24.VI.2016	Bcharre	North Lebanon	2723
B. terrestris	♀	2	Yammine W., Kotan A.	USEK	22.VI.2016	Bcharre	North Lebanon	1926
B. terrestris	♂	2	Boustani M.	USEK	19.VI.2016	Ehden	North Lebanon	1567
B. terrestris	☿	2	Boustani M., Kyrk S.	USEK	19.VI.2016	Ehden	North Lebanon	1567
B. terrestris	♂	1	Boustani M.	USEK	22.VI.2016	Wadi Quannoubine	North Lebanon	1129

(1♀, 1☿), *Echinops* sp. (1♂, 4☿), *Echinops viscosus* D. C. (1♂, 1☿). Berberidaceae: *Berberis libanotica* Ehr. (1♀). Boraginaceae: *Cynoglossum nebrodense* Guss. (1☿), *Echium italicum* L. (1☿), *Solenanthus stamineus* (Desf.) Wettst. (1♀, 13♂). Caprifoliaceae: *Lonicera nummulariifolia* Jaub. & Spach (2♂). Dipsacaceae: *Cephalaria setosa* Boiss. & Hohen. (4♂). Fabaceae: *Colutea cilicica* Boiss. & Bal. (23☿), *Genista libanotica* Boiss. (1☿), *Ononis natrix* L. (3☿), *Trifolium* sp. (1☿), *Vicia canescens* Labill. (2☿), *Vicia tenuifolia* Roth (10♀, 13☿, 15♂), *Vicia villosa* Roth (5♀, 8☿). Lamiaceae: *Marrubium* sp. (1☿), *Marrubium libanoticum* Boiss. (2 W), *Mentha* sp. (1♂, 3☿), *Origanum ehrenbergii* Boiss. (1☿), *Phlomis* sp. (1☿). Malvaceae: *Alcea apterocarpa* (Fenzl) Boiss. (1☿). Ranunculaceae: *Ranunculus demissus* D.G. (1☿). Rosaceae: *Rubus* sp. (1♂).

Bombus argillaceus (Scopoli, 1763)

Species distribution. Eastern Mediterranean distribution, stretching North to Czech Republic (Rasmont & Iserbyt 2010-2014) East to Russia, and South to Israel (Ascher & Pickering 2018).

Local distribution. Figure 2c. 17♀, 9☿, 1♂, 1 sex not specified: Ainata Al Arz (3☿), Aintoura (1♀), Arsoun (1♀), Arz Tannourine (1♂), Baskinta (1☿), Bcharre (2♀, 1☿), Bhamdoun (1♀), Dahr El Baidar (1☿), Ehden (9♀), Hadath El Jebbe (2♀), Harissa (Tannourine) (1☿), Hlaliye (1♀), Qornael (1☿), Zahrani (1☿).

Altitude range in Lebanon. From 230 m (Aintoura) to 2332 m (Dahr el Adib).

Flowers visited in Lebanon. Asteraceae: *Echinops* sp. (1☿). Dipsacaceae: *Cephalaria setosa* Boiss. & Hohen. (1♂). Fabaceae: *Vicia tenuifolia* Roth (2♀), *Vicia villosa* Roth (5♀). Lamiceae: *Lamium* sp. (1♀), *Phlomis chrysophylla* Boiss. (1☿), *Salvia microstegia* Boiss. & Bal. (1☿), *Stachys ehrenbergii* Boiss. (2☿). Plantaginaceae: *Linaria aucheri* Boiss. (1☿). Rosaceae: *Rubus* sp. (1☿).

Bombus niveatus vorticosus (Kriechbaumer, 1870)

Species distribution. Centered on the Aegean Sea, reaching Eastern European Russia to the North, Spain to the West, Iran to the East, and Israel to the South (Rasmont et al. 2015).

Local distribution. Figure 2a. 10♀, 32☿, 10♂, 4 not specified: Ainata Al Arz (5♀, 27☿, 6♂), Arz Bcharre

Figure 1. The four *Bombus* species from Lebanon. **A**, *B. niveatus vorticosus* queen foraging on *Astragalus angustifolia*, Bcharre, 30.VI.2017 (Photo M. Boustani); **B**, *B. terrestris calabricus* male foraging on *Solenanthus stamineus*, Arz Tannourine, 6.V.2017 (Photo M. Boustani); **C**, *B. argillaceus* queen, Horch Ehden, 31.V.2017 (Photo P. Rasmont); **D**, *B. melanurus* queen foraging on *Vicia canescens*, Plateau Qornet El Sawda, 24.VI.2016 (Photo M. Boustani).

(1♀, 4☿, 2♂, 1 unknown), Duhor Barnasa (4♀), Ehden (1☿), Fehta (1♂), Mount Hermon (1♂), Qartaba (1☿).

Altitude range in Lebanon. From 1648 m (Qartaba) to 3000 m (Qornet El Sawda).

Flowers visited in Lebanon. Asteraceae: *Cousinia libanotica* D.C. (4♂). Fabaceae: *Astragalus angustifolius* D. C. (3♀, 6☿), *Vicia canescens* Lab. (1♀, 6☿, 2♂). Lamiaceae: *Marrubium libanoticum* Boiss. (6☿, 1♂), *Stachys ehrenbergii* Boiss. (13☿, 1♂).

Bombus melanurus (Lepeletier, 1836)

Species distribution. Central Asia, reaching the Caucasus and Eastern Turkey to the East (Rasmont et al. 2015).

Local distribution. Figure 2d. 5♀, 10☿, 6♂: Qadisha (1♀), Arz Bcharre (1♀, 1☿, 1♂), Ainata Al Arz (3♀, 9☿, 5♂).

Altitude range in Lebanon. From 1815 m (Bcharre Cedars) to 3000 m (Qornet El Sawda).

Flowers visited in Lebanon. Asteraceae: *Cousinia libanotica* D.C. (3♀, 2♂). Fabaceae: *Vicia canescens* Labill. (3♀, 3☿, 2♂), *Vicia tenuifolia* Roth (1☿). Lamiaceae: *Marrubium libanoticum* Boiss. (2♂), *Stachys ehrenbergii* Boiss. (3☿).

Distribution by governorate

Bumblebee species presence or absence in the Lebanese governorates is displayed in Table 2 from left to right. The governorates are shown on every map from west to

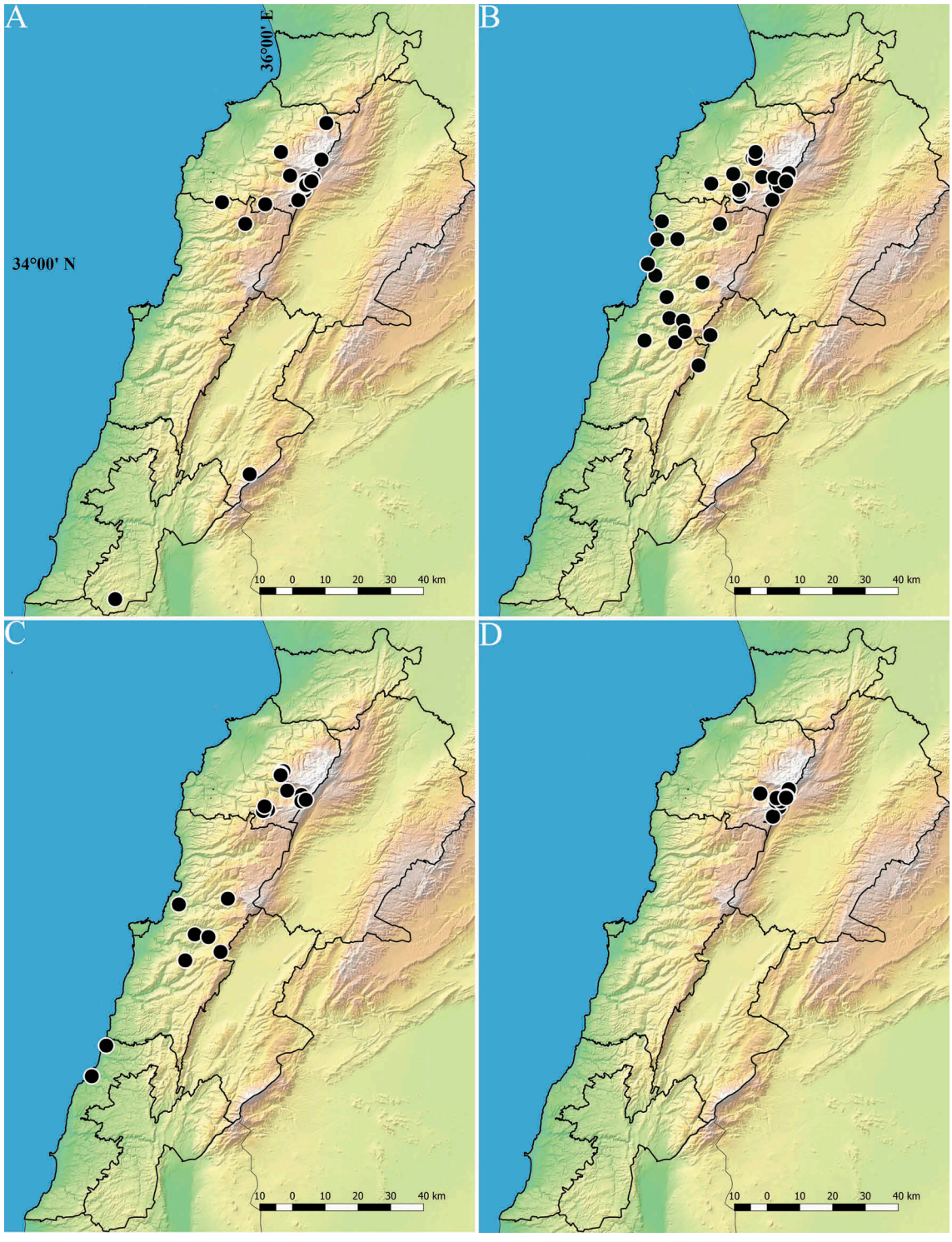

Figure 2. Distribution maps of the four *Bombus* species from Lebanon. **A**, *B. niveatus vorticosus*; **B**, *B. terrestris calabricus*; **C**, *B. argillaceus*; **D**, *B. melanurus.*

east and from north to south (Table 1) in order to highlight sampled areas and the ones that are still data deficient.

Bombus terrestris *subspecies*

The morphological criteria of the examined *Bombus terrestris* material suggest the subspecies *Bombus terrestris*

Table 2. Bumblebee species by governorate in Lebanon: recorded (+), not recorded (–).

	Beirut	Akkar	North Lebanon	Mount Lebanon	Nabatieh	Baalbek-Hermel	Beqaa	South Lebanon
Bombus argillaceus	–	–	+	+	–	–	+	+
B. melanurus	–	–	+	–	–	–	–	–
B. niveatus vorticosus	–	–	+	+	+	–	+	–
B. terrestris	–	–	+	+	–	–	+	–

calabricus Krüger, 1958 as the yellow band of the thorax extends under the tegulae in addition to the color pattern (Rasmont et al. 2008). Furthermore, the cephalic labial gland secretions analysis provides preliminary evidence that the local subspecies may be *B. t. calabricus*. Indeed, the Lebanese specimens fall in the only cluster of the individuals identified as this subspecies when compared to the database of Lecocq et al. (2016) (Figure 3), the *p*-value of the MRPP analysis being >0.05; therefore, the difference is not significant, and the chemicals profiles match the Sicilian *B. t. calabricus* specimens.

Discussion

Distribution

Bombus terrestris is newly recorded in Lebanon despite it being locally widespread. This species is one of the most common in the West Palearctic (Williams 2011), and is a common species in Mediterranean ecosystems (Potts et al. 2006). Regional data report its presence in Syria coastal area (Solaiman-Khaled et al. 2012), and in Israel and Jordan (Rasmont & Iserbyt 2010-2014). Therefore, its absence from any published records is possibly due to under-sampling of the region. We can also eliminate the possibility of its recent incursion due to introduction of colonies for agricultural use as our oldest specimens in the database date back to 1973 (Sawfar) from the AUB collection, well before any introduction of non-native *B. terrestris* in 2001 (FAO Representation in Lebanon 2011). *Bombus argillaceus* on the other hand has already been recorded from some localities; older records include Reinig (1939) and another from 1977, both collected in Bcharre (*Base de données fauniques Gembloux et Mons*). It has also been collected in a variety of different habitats locally including woodlands, pastures, grasslands and ruderal areas, with a local distribution spanning sea level to 2500 m, although Solaiman-Khaled et al. (2012) found it significantly less abundant than *B. terrestris* in Syrian coastal areas. Indeed, it can live in several habitat types and is a common forager in cultivated and natural

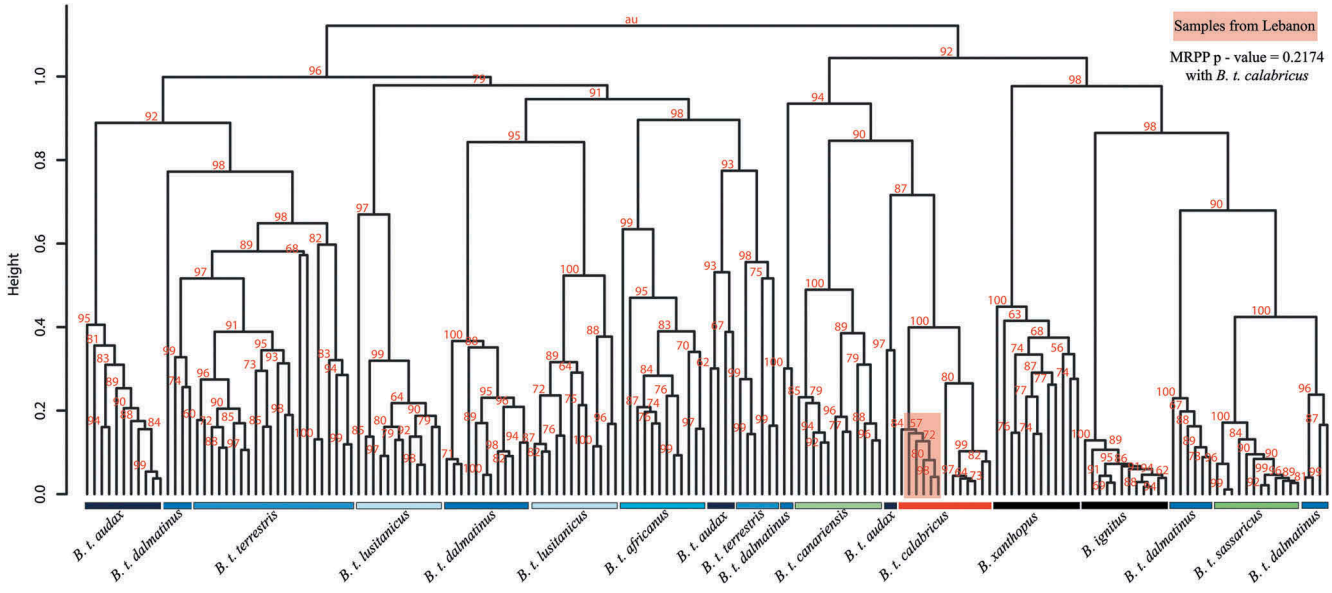

Figure 3. Unweighted pair group method with arithmetic mean (UPGMA) cluster based on a correlation matrix calculated from the cephalic labial gland secretions matrix of *Bombus terrestris* taxa, *B. ignitus* and *B. xanthopus* data from Lecocq et al. (2016). The values near nodes are multiscale bootstrap resampling (only values N90 of main groups are shown). The Lebanese specimens are highlighted in red.

landscapes on an altitudinal range reaching 3500 m in Turkey (Özbek 2002). *Bombus terrestris* and *B. argillaceus* have broader distributions in Lebanon, contrasting with *B. niveatus vorticosus* and *B. melanurus* that seem to be restricted in the higher altitudes. *Bombus niveatus vorticosus* has also been reported from several locations including Rmeich from 1870 (Rasmont, original data), Jabal Al Shaikh in 2010 (*Base de données fauniques Gembloux et Mons*), and Bcharre in 1931 collected by Zerny (GBIF 2019). Despite these records from lower altitude villages, in our collections we only found specimens from altitudes above 1800 m. *Bombus niveatus vorticosus* is indeed typical to steppe areas (Rasmont et al. 2009) as in the alpine steppe regions of Mount Lebanon chain, where most of our specimens were found. This species is also one of the most abundant species in its distribution range (Özbek 2002; Rasmont et al. 2015). *Bombus melanurus* also has some older records and has been reported through one collected specimen by Monty (2004), and two from the BMNH collection with one specimen of unknown date from Qadisha valley and the other from 1931 from Bcharre. In Lebanon it is at the southernmost point of its distribution (Rasmont et al. 2009) and is found mainly in altitudes higher than 1500 m (Aslan 2003; Rasmont et al. 2009; An et al. 2011). Its presence is somewhat unexpected as the closest known population is in the Taurus Mountains in Turkey (Rasmont et al. 2009).

The GBIF dataset (2019) reports other species: *Bombus fragrans, B. muscorum, B. sylvarum, B. maxillosus, B. subterraneus* and *B. zonatus* from Jezzine in 1905, identified by Vogt and preserved in UiT, the Arctic University of Norway. The examination of the original labels of the material (Kjærandsen J., pers. comm. 2019) showed that this is a misinterpretation of the original label "Jassian, Asia Minor" [currently Yasyan in Turkey (Konya)], and the locality is not Jezzine in Lebanon.

Bombus terrestris *subspecies*

The cephalic labial gland secretions analysis suggests that the *Bombus terrestris* subspecies found in Lebanon is *Bombus terrestris calabricus*, as suggested by the morphological criteria from Rasmont et al. (2008). The morphological criteria method for differentiating species level for *Bombus* has been criticized as insufficient, such as in the case of *Bombus lucorum* complex (Carolan et al. 2012), and in certain subspecies, e.g. the difference between *Bombus terrestris calabricus* and *B. terrestris dalmatinus* (Bertsch & Schweer, 2012). The CLGS on the other hand has allowed bumblebee species to be separated in certain cases (e.g. Lecocq et al. 2015b; Martinet et al. 2018), and has also been used to differentiate subspecies in *B. terrestris* (Lecocq et al. 2016). However, it remains difficult to rely solely on this method as individuals may present variability among age (Žáček et al. 2009), and for certain species such as *Bombus montanus* and *B. ruderarius*, for example, the differences are not conclusive (Terzo et al. 2005). Furthermore, Bertsch & Schweer (2012) add that differentiation is difficult through CLGS for the different *B. terrestris* subspecies. Therefore, it has often been used in an integrative taxonomy framework, alongside the sequencing method of the mitochondrial gene cytochrome C oxidase I (COI) region, COI (e.g. Williams et al. 2012; Lecocq et al. 2016; Williams et al. 2019), and in certain cases where the DNA evidence is not enough, it is necessary to find molecular markers in order to differentiate the subspecies (Cejas et al. 2018). On the other hand, the known distribution of the *Bombus terrestris calabricus* subspecies is Sicily and south Italy (Rasmont et al. 2008; Coppée 2010), and the identified subspecies closest to the area is *Bombus terrestris dalmatinus* from Turkey (Rasmont et al. 2008), and the records from Israel and Turkey (Rasmont & Iserbyt 2010-2014) do not provide any information on the subspecies. This suggests that the local *Bombus terrestris* subspecies may be an isolated population. Therefore, and with the current lack of information of the biogeography of *Bombus terrestris* subspecies in the East Mediterranean, the evidence in our case remains very circumstantial and a larger sampling over the whole altitudinal range in Lebanon and neighboring countries, in an integrative taxonomy framework, are necessary to confirm the subspecies identification.

Flower resources

Bombus terrestris remains the most polyphagous species, foraging on 26 flowering species based on our observations. Indeed, it is a species known to forage from a broad spectrum of flowers (Rasmont 1988; Aslan 2003; Monfared et al. 2007; Williams 2011), highlighting its importance as a wild pollinator and potential interest for agricultural rearing in Lebanon. *Bombus argillaceus* is equally polyphagous (Rasmont 1988; Rasmont & Flagothier 1996; Monfared et al. 2007; Rasmont et al. 2015), with a preference for flowers with long corolla like Fabaceae (Rasmont et al. 2015). *Bombus niveatus vorticosus* is also a forager that visits a wide range of flowers but focuses mainly on Lamiaceae and Fabaceae (Rasmont & Flagothier 1996; Monfared et al. 2007). This concurs with our results for the plant family preference, but the number of species foraged locally from our records is only five. Similarly, *Bombus melanurus* seems to have restricted preferences with five foraged plants recorded locally, this can be due to the distribution of *B. niveatus vorticosus* and *B. melanurus* above 2000 m in the Oro-Mediterranean strip where fewer seasonal foraging plants are available.

Primary climatic risk assessment and recommendations

From a conservation perspective, the four species are of interest as they visit and are potential pollinators of a wide range of wild plants, of which several are endemic such as *Vicia canescens* Labill., *Stachys ehrenbergii* Boiss. and *Cousinia libanotica* D.C. This is especially relevant for *Bombus niveatus vorticosus* and *B. melanurus* that are found in areas of high plant endemism in Lebanon (Bou Dagher-Kharrat et al. 2018). *Bombus terrestris* does not appear to be at risk due to climate change as the current conditions are causing its expansion towards the north (Martinet et al. 2015); therefore, we can expect it to preserve its current wide distribution locally. Similarly, the future distribution predictions for *Bombus argillaceus* suggest an expansion and no risk (Rasmont et al. 2015), although it is legally protected in Hungary (Kosior et al. 2007). *Bombus niveatus vorticosus* predictions also suggest that it could expand beyond its distribution range (Rasmont et al. 2015); in Lebanon, however, it seems restricted to altitudes above 1800 m from our collected specimens. Given its current local distribution, this species may yet be locally vulnerable as it is close to its southernmost distribution limit and does not seem to be expanding towards coastal areas. *Bombus melanurus* on the other hand has the most restricted local distribution mostly in the Oro-Mediterranean strip, rendering it vulnerable to extreme climatic events. This is emphasized by its total isolation from its closest known population in Turkey (Rasmont et al. 2009). The isolated local distribution of *Bombus niveatus vorticosus* and *B. melanurus* could be the result of a climatic induced shift towards higher altitudes (Biella et al. 2017), especially in the case of *B. niveatus vorticosus* for which literature records come from lower altitudes than the author's collections. All four species should be included in any conservation plan as these pollinators potentially play an important role in the local flower–pollinator network, with emphasis on *B. melanurus* that must be closely monitored through observation as it is easily recognizable. Furthermore, current *Bombus* rearing efforts for agricultural purposes should focus on local *B. terrestris* as to avoid foreign strain invasions and protect the local subspecies (Lecocq et al. 2015a; Cejas et al. 2018).

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Supplementary material

Supplemental data for this article can be accessed here.

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Supporting Information Appendix S1

Male cephalic labial gland secretions analysis

In order to verify the morphological subspecies identification for *Bombus terrestris*, we used CLGS, an eco-chemical trait involved in bumblebee pre-mating recognition (Baer, 2003; Ayasse & Jarau, 2014). These secretions are complex mixtures of mainly aliphatic compounds synthesized by male cephalic labial glands (Coppée et al., 2008; Lecocq et al., 2011; Žacek et al., 2013) and are commonly used for resolving species differentiation issues, and have been successful in differentiating *B. terrestris* subspecies (Lecocq et al., 2015; Lecoq et al, 2016; Martinet et al, 2018).

We identified the main component as the compound that had the highest relative area (RA) among all compounds of CLGSs at least in one specimen of the taxon. We extracted the CLGS with 400 μL of n-hexane, according to De Meulemeester et al. (2011). Samples were stored at−20∘C prior to the analyses. We quantified the CLGS compounds with a gas chromatograph Shimadzu GC-2010 system (GC-FID) equipped with a nonpolar SLB-5ms capillary column [5% phenyl (methyl) polysiloxane stationary phase; column length 30m; inner diameter0.25 mm; film thickness 0.25 μm] and a flame ionization detector. We quantified the peak areas of compounds in GC solution postrun (Shimadzu Corporation) with automatic peak detection and noise measurement. The relative areas (RAs, expressed in %) of compounds in each sample were calculated by dividing the peak areas of compounds by the total area of all compounds. We excluded compounds for which RA were less than 0.1% for all specimens (De Meulemeester et al., 2011). The data matrix for each taxon was based (Appendix S2) on the alignment ofeach relative proportion of compound between all samples performed with GCA ligner 1.0 (Dellicour & Lecocq, 2013a,b).For GC-FID analyses, we injected 1 μL, using a splitless injection mode (injector temperature of 220∘C) and helium as carrier gas (1mL/min, constant velocity of 50 cm/s).The oven temperature (of the column) was programmed isothermally, starting at 70∘C for 2min and then rising from 70 to320∘C at a rate of 10∘C/min. The temperature was then held at320∘C for 5min.

In order to facilitate the alignment of compounds and their identification, before each sample injection, a standard (Kovats) was injected containing a mix of hydrocarbons (alkanes) fromC10 (decane) to C40 (tetracontane). Kovats indices were calculated with GC Kovats 1.0 according to the method described by Dellicour & Lecocq (2013 a,b).

We performed statistical comparative analyses of the CLGSs using R environment (R Development Core Team, 2013) to detect CLGS differentiations including the Lebanese *Bombus terrestris* specimens. We used a clustering method, computed with the unweighted pair-group method with average linkage (UPGMA) based on correlation distance matrices (RA of each compound) (R package ape; Legendre & Legendre, 2004; Paradis et al., 2004), to detect the divergence between taxa in the CLGS composition. We assessed CLGS differentiations of the 5 Lebanese specimens to the 9 Sicilian *Bombus terretris calabricus* of the same cluster using a multiple response permutation procedure (MRPP) (R-package vegan, Oksanen et al., 2011).

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