# 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 $\mu$L 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 $\mu$m] 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 $\mu$L, 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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