

Cryptic bumblebee species of the *Bombus lucorum*-complex in the Austrian Alps

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Abstract

Bumblebees are among the most important pollinators in the high mountain regions. Within the *Bombus lucorum*-complex, at least two cryptic species (*Bombus lucorum*, *B. cryptarum*) inhabit the Austrian Alps, a third one (*B. magnus*) cannot be excluded to occur.

Recent molecular studies indicate that colour-patterns used for identification do not correspond with distinct molecular operational taxonomic units, each of which represents one of the species of the *Bombus lucorum*-complex. Furthermore, no characteristic colour pattern for one of these species was found and some traits show a gradual variation among the species. A reliable identification of females seems to be impossible based on morphological characters.

DNA-barcoding represents an appropriate method for the determination of species and provides a basis for studying altitudinal preferences as well as ecological and morphological characteristics within the *Bombus lucorum*-complex. The present study focuses on the following research questions: Are there differences in the occurrence of the species in various geographical expositions and elevations in the Austrian Alps? Do the species show different flower preferences? How reliable are morphological characters when re-evaluating DNA-barcoded specimens of the *Bombus lucorum*-complex?

Keywords

Bombus lucorum-complex, bumblebees, cryptic species, Austrian Alps, DNA-barcoding

Introduction

Bumblebees (*Bombus* spp.) are important pollinators in various habitats, especially above the treeline of European alpine regions (NEUMAYER & PAULUS 1999). Around 250 bumblebee-species are described worldwide, that predominantly prefer the temperate and northern parts of the world. In Austria, 47 species of the genus *Bombus* were reported representing one of the “hot spots” of biodiversity of these insects.

One group of very common bumblebees form a cluster of species, the so called *Bombus lucorum*-complex. The taxonomic status of these closely related species belonging to the complex of *Bombus lucorum* s. l. has been intensively discussed in the last decades. Three distinct species, i.e., *Bombus lucorum* L., *Bombus cryptarum* Fabr., *Bombus magnus* Vogt are strongly suggested to be widespread in various habitats across Central Europe. Studies on morphological/morphometric characters, enzyme electrophoresis, male labial gland compounds, and in particular nucleotide sequences improved the current understanding however did not settle the discussion about the species composition within this group. It turned out to be impossible to clearly separate the males and workers using morphological characters (MURRAY et al. 2008) as they are used in the common literature for the determination of bumblebees (MAUSS 1994, AMIET 1996, GOKCEZADE et al. 2010, NEUMAYER & MAUSS in prep). A number of cryptic species was revealed that are nearly identical in morphology (MURRAY et al. 2008, WILLIAMS et al. 2012). Although diagnostic characters frequently overlap, queens of the *Bombus lucorum*-complex can reasonably be determined by experts (CAROLAN et al. 2012).

Especially in field work, discrimination methods are not trustworthy and, therefore, reliable information about niche differentiation of the cryptic species is very limited. DNA-barcoding represents an appropriate method for determination of these bumblebee species and will be used in the ongoing investigation to study niche differentiation of the cryptic species within the *Bombus lucorum*-complex in the Austrian Alps.

Our study will focus on the following research questions:

Which of the cryptic species of the *Bombus lucorum*-complex occur in the Austrian Alps and where do they occur?

Do the species show different habitat preferences regarding exposition and elevation of their habitats?

Do they prefer different flower species and do they differ in flower visiting behaviour?

Studying the distribution of the cryptic species in Austria will provide the basis for future monitoring programs and long-term studies. The investigation of the habitat requirements of the bumblebees of the *Bombus lucorum*-complex is fundamental for a more detailed knowledge of pollination networks in alpine ecosystems.

Methods

Field study

Bumblebees were collected in seven different regions of the Austrian Alps (Silvretta, Kaunertal, Karwendel, Glocknergroup/north, Glocknergroup/south, Gesäuse, Schneeberg) in July and August 2012. We aimed to collect ten bumblebees of the *B. lucorum*-complex in each of the following altitudinal ranges (if present) in the various mountains: 1000 – 1100 m, 1300 – 1400 m, 1600 – 1700 m, 1900 – 2000 m, 2200 – 2300 m, 2500 – 2600 m, and 2800 – 2900 m a.s.l. From all collected individuals GPS-data of sampling site (geographic position, exposition, altitude), habitat type, flower species visited and activity on the plant (pollen collecting, nectar feeding, nectar robbing) were recorded. All collected bumblebees were stored in 99% ethanol in the field.

DNA barcoding procedure

DNA was extracted from three legs of each individual using DNeasy Blood & Tissue extraction Kit (Qiagen); the CO1 region was amplified and sequenced using standard techniques. Primers used for PCR and sequencing were LCO1490 and HCO2198 (FOLMER et al. 1994). Sequences were assembled and aligned using BioEdit v7.1.7; neighbour joining trees were constructed using Mega v5.1 for phylogenetic analysis.

Results and Discussion

The year 2012 showed exceptionally low numbers of bumblebees of the *B. lucorum*-complex in comparison to other years (NEUMAYER unpublished). In total, 77 individuals of the *B. lucorum*-complex were collected (plus three individuals of *B. terrestris*).

The DNA barcodes revealed 52 individuals of *B. lucorum* and 25 individuals of *B. cryptarum*. No *B. magnus* was found in the investigated areas (Fig. 1). Up to now, it seems that *B. magnus* does not occur in the Austrian Alps above 1000 m a. sl., although *Calluna vulgaris* rich habitats were sampled that are known to be preferred by *B. magnus* in Scotland (WATERS et al. 2011). Syntopic occurrence of *B. lucorum* and *B. cryptarum* was recorded in different sampling areas and indicates species separation. The results suggest that *B. lucorum* was more abundant than *B. cryptarum* in 2012 except in the southern slopes of the Glockner Group. Furthermore, 71% of *B. cryptarum* were found between 1900 m and 2000 m. The altitudinal distribution suggests that *B. cryptarum* prefers habitats located around 2000 m a.s.l. However, due to the incomplete sampling in the last year these results must be regarded as preliminary.

First analysis of the flower visiting preferences revealed that both studied species of the *B. lucorum*-complex were collected mainly on *Calluna vulgaris* (*B. lucorum*: 34% vs. *B. cryptarum*: 38% of visited flowers) and on flowers of *Trifolium*-species (*B. lucorum*: 28% vs. *B. cryptarum*: 19% of visited flowers).

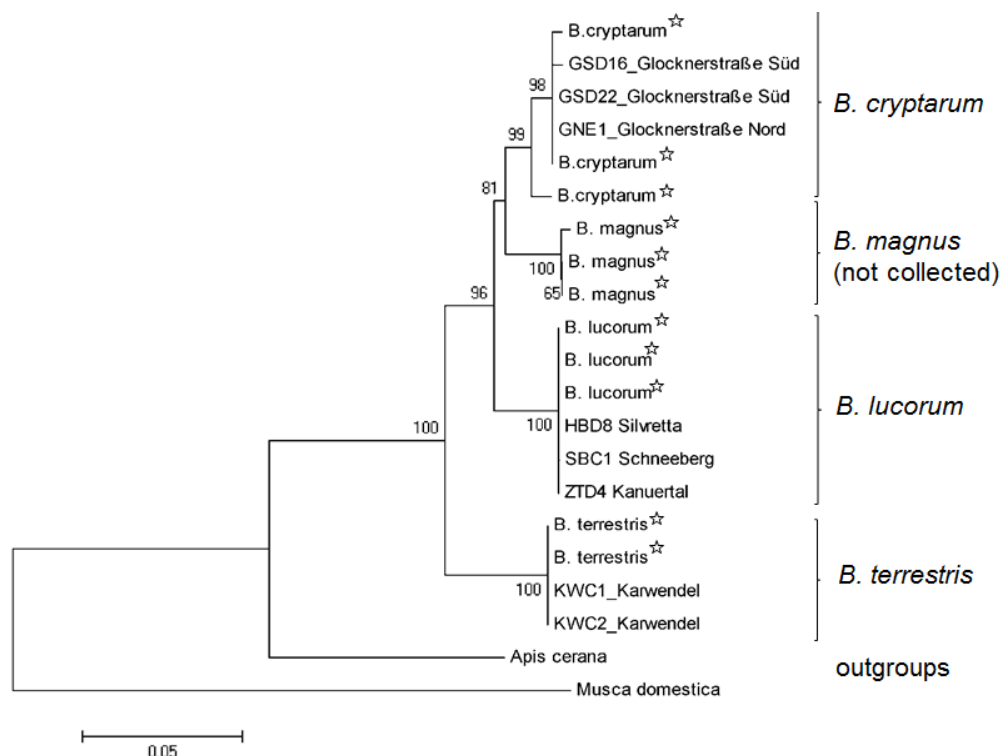


Figure 1: The closely related bumblebees *Bombus lucorum*, *B. cryptarum* and *B. magnus* represent clear operational taxonomic units as shown in the phylogenetic tree. A K2P based Neighbour-Joining Tree (CO1) of selected specimens from different sampling areas in Austria; node support values from 1000 replicates; * sequences from GeneBank.

Conclusions and outlook

Based on previous studies in the Austrian Alps, we conclude that only 5-15% of the usual numbers were present in the study areas in the summer 2012 (NEUMAYER unpublished). The sampling in the study areas will be completed in 2013. Based on the experiences of the last year we decided to expand the sampling transects to lower parts of the respective region. Additionally, bumblebees will be collected also in northern regions of Austria and in eastern parts of the Austrian Alps according to the established sampling protocol. The recently started investigation of morphological features of the DNA-barcoded specimens will give us the opportunity to re-evaluate the diagnostic characters of the various taxonomic entities in the *B. lucorum*-complex. This includes the study of well-established diagnostic characters of the species but it is also aimed to find new characters for reliable determination. Expected results of the study will allow analyses of ecological differentiation of closely related insects concerning geographic and altitudinal distribution, habitat preferences and foraging behaviour.

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Literature

- AMIET, F. 1996. Hymenoptera Apidae, 1. Teil: Allgemeiner Teil, Gattungsschlüssel, die Gattungen *Apis*, *Bombus* und *Psithyrus*. Insecta Helvetica Fauna 12: 1-98. Neuchâtel.
- CAROLAN, J.C., MURRAY, T.E., FITZPATRICK, U., CROSSLEY, J., SCHMIDT, H., CEDERBERG, B., MCNALLY, L., PAXTON, R.J., WILLIAMS, P.H. & M. J. F. BROWN 2012. Colour patterns do not diagnose species: quantitative evaluation of a DNA barcoded cryptic bumblebee complex. PLoS one 7: e29251.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R., & R. VRIJENHOEKET 1994. DNA Primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology & Biotechnology 3: 294-299.
- GOKCEZADE, J. F., GEREKEN-KRENN, B.-A., NEUMAYER, J. & H.W. KRENN 2010. Feldbestimmungsschlüssel für die Hummeln Österreichs, Deutschland und der Schweiz. Linzer Biologische Beiträge 42: 5-42. Linz.
- MAUSS, V. 1994. Bestimmungsschlüssel für Hummeln. 6. Aufl. DJN (ed.), 1-50. Hamburg.
- MURRAY, T.E., FITZPATRICK, U., BROWN, M.J.F. & R.J. PAXTON 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conservation Genetics 9: 653-666.
- NEUMAYER, J. & V. MAUSS, in prep. Illustrierte Bestimmungstabellen der Hummeln Deutschlands, Österreichs und der Schweiz.
- NEUMAYER, J. & H. PAULUS 1999. Ökologie alpine Hummelgemeinschaften: Blütenbesuch, Ressourcenaufteilung und Energiehaushalt. Untersuchungen in den Ostalpen Österreichs. Staphia 67: 5-246. Linz.
- WATERS, J., DARVILL, B. GILLIAN, C.L. & D. GOULSON 2011. Niche differentiation of a cryptic bumblebee complex in the Western Isles of Scotland. Insect Conservation and Diversity 4: 46-52.
- WILLIAMS, P.H., BROWN, M.J.F., CAROLAN, J.C., AN, J., GOULSON, D., AYTEKIN, A.N., BEST, L.R., BYVALTSEV, A.M., CEDERBERG, B., DAWSON, R., HUANG, J., ITO, N., MONFARÉ, A., RAINA, R.H., SCHMID-HEMPEL, P., SHEFFIELD, C.S., SIMA, P. & Z. XIE 2012. Unveiling cryptic species of the bumblebee subgenus *Bombus* s. str. Worldwide with COI barcodes (Hymenoptera: Apidae). Systematics and Biodiversity 10: 21-56.

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