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

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Introduction

The taxonomic status of the bumblebees belonging to the complex of *Bombus lucorum* s. l. has been intensively discussed in the last decades. Studies on morphological/morphometric characters, enzyme electrophoresis, male labial gland compounds, and in particular nucleotide sequences have improved our understanding of the species composition within this group. Three distinct species, i.e., *Bombus lucorum* L., *Bombus cryptarum* Fabr., *Bombus magnus* Vogt are strongly suggested to be widespread across Europe. Our current understanding implies that the species are closely similar or identical in morphology [1]. It is not possible to separate the males or workers of the species using morphological characters [2]. However, queens can reasonably be determined by experts, even though the identifications remain questionable since diagnostic characters frequently overlap [3]. Discrimination methods used thus far are not trustworthy, and reliable information about niche differentiation of the single species is not available. DNA-barcoding represents an appropriate method for determination of bumblebee species and provides a basis to study niche differentiation of the cryptic species within the Bombus lucorum-complex (Fig. 1).

Our study has just begun. It will focus on the following research questions:
- Which of the cryptic species in the *Bombus lucorum* - complex occurs in the Austrian Alps?
- Do the species show different habitat preferences regarding exposition and elevation?
- Do they have different flower preferences?

Material and Methods

Field study

Bumblebees were collected in seven different regions of the Austrian Alps (Fig. 2) 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. GPS-data (geographic position, exposition, altitude), habitat type, flowers visited and activity on the plant (pollen collecting, nectar feeding, nectar robbing) of each collected bumblebee were recorded; specimens were stored in 99% ethanol.

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 [5]. 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

- In total 80 individuals of the *B. lucorum* - complex and *B. terrestris* were collected. The year 2012 showed exceptionally low numbers of bumblebees of the *B. lucorum* - complex. 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 summer 2012 (Neumayer, unpublished).
- DNA barcoding technique revealed 52 individuals of *B. lucorum*, 25 individuals of *B. cryptarum* and 3 individuals of *B. terrestris*.
- No *B. magnus* was found (Fig. 1). Up to now, it seems that *B. magnus* does not occur in the Alps above 1000 m a. sl., although *Calluna vulgaris* rich habitats were sampled that are preferred by *B. magnus* in Scotland [4].
- 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 (Fig. 2). Furthermore, the altitudinal distribution suggests that *B. cryptarum* prefers habitats located about 2000 m a.sl. (Fig. 3). However, due to the incomplete sampling in 2012 these results must be regarded as preliminary!
- First analysis of the flower visiting behaviour 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 different *Trifolium*-species (*B. lucorum*: 28% vs. *B. cryptarum*: 19% of visited flowers).

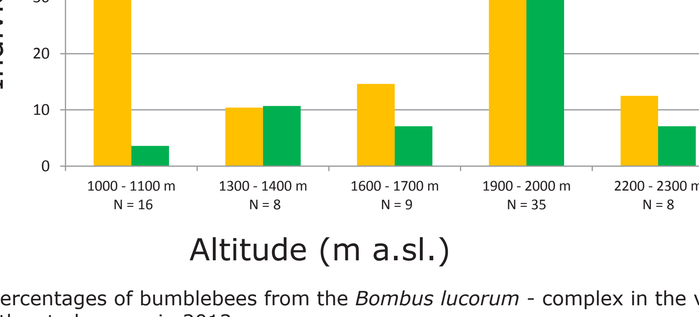

Fig. 1: K2P based Neighbor-Joining Tree (CO1) of selected specimens from different sampling areas; node support values from 1000 replicates. ★ Sequences from GeneBank

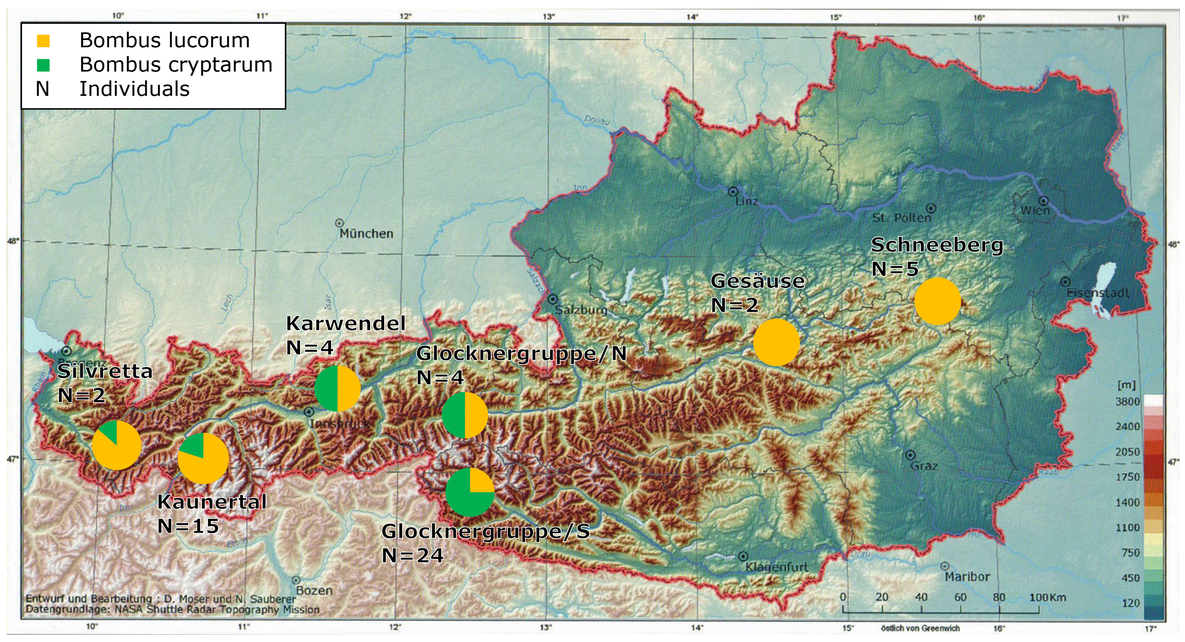

Fig. 2: Occurrence of *Bombus lucorum* and *B. cryptarum* in the study areas in the Austrian Alps 2012

Fig. 3: Percentages of bumblebees from the *Bombus lucorum* - complex in the various altitudes of the study areas in 2012

Outlook

- The sampling in the study areas will be completed in 2013. Bumblebees will be collected in further areas in the Austrian Alps. Additionally, sampling will be expanded to the lower regions, including northern and eastern Austria.
- Investigation of morphological characters by re-evaluating DNA-barcoded specimens of the *B. lucorum* - complex
- Expected results will allow analyses in species differentiation concerning geographic and altitudinal distribution, habitat preferences and foraging behaviour

Literature

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