

***Bombus subterraneus* (L., 1758) (Hymenoptera, Apidae) rediscovered in Norway**

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The bumble bee *Bombus subterraneus* (L., 1758) has been regarded as a regional extinct species in Norway. We here present the first confirmed record of the species in more than 60 years. A queen was recorded from Østfold, Hobøl: Tomter 3 June 2010. The specimen was identified based on comparison of morphological characters and confirmed with DNA-sequencing methods.

Key words: *Bombus subterraneus*, bumble bees, determinant traits, DNA-sequencing, extinct, identification, Norway

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Introduction

Bumble bees have a northerly skewed geographical range and Norway has a rather large proportion (13,7%) of the 249 bumble bee species known worldwide. A total of 34 species are recorded from Norway (Ødegaard et al. 2009). There are growing evidence that the bumble bee diversity is declining, especially in developed regions of the World, such as Western Europe and North America (Williams 1982, Goulson 2003, Goulson et al. 2005, Kosior et al. 2007). Late-emerging long tongued bumble bee species associated with deep perennial flowers are thought to be most dramatically affected (Goulson et al. 2005). Mapping and monitoring of bumble bees in the last 10 years have revealed recent records of all species known from Norway except for the long tongued species *Bombus subterraneus* (L., 1758).

The lack of confirmed records during the last 60 years (Løken 1973) together with evidence for a general decline in bumble bees made Ødegaard et al. (2009) presume that *B. subterraneus* had gone extinct in Norway.

In the present paper we report a rediscovery of a queen of *B. subterraneus* in Norway. The identification is based on comparison of morphological characters and confirmed with DNA-sequencing methods. Due to difficulties with unambiguous traits and character states limited to our region the most determinant traits are described and illustrated.

The species

B. subterraneus is a long tongued bumble bee first described from Uppland in Sweden by

Linnaeus in 1758. The species belong to the subgenus *Subterraneobombus* Vogt, 1911, which in Scandinavia is represented by *B. subterraneus* and *B. distinguendus* (Morawitz, 1869). Sladen (1912) described the species as large, with an elongated head and a short coat. The head is black, thorax black with a yellow band in front and a very narrow yellow one behind. Abdomen is black, with 4th and 5th segments white and “with a fringe of yellowish or dingy white on the 3rd segment, a narrower and fainter one on the edge of the 2nd segment”. Løken (1973) emphasizes, though, that the melanic dark female and worker is the nominate form in Norway. The dark form is lacking the yellow bands on thorax, and T4 and T5 is not white, but grey.

The first Norwegian record dates back to 1880 (Siebke) from the location Oslo confirmed by Løken (1973). All confirmed specimens are summarized in Table 1. The presented specimen is a dark *B. subterraneus* queen, collected by the first author on 3 June 2010 in Hobøl Municipality, Østfold County (EIS 29).



FIGURE 1. *Bombus subterraneus* (L., 1758), the new specimen. Photo: Karsten Sund, NHM Oslo

Identification

The description of the species' color pattern, according to Løken (1973), fits very well with our specimen through the brownish black appearance, and T4 + T5 with greyish white fuscous hairs (Fig. 1). On the other hand, malar space for the specimen was measured to be 0.93mm and radial length 4.25mm, and these results are not consistent with Løken's measurements, which is 1.05 (SD ± 0.05 , ME ± 0.01) and 4.38 (SD ± 0.11 , ME ± 0.02), respectively. However, Løken's measurements are based on 15 specimens from Skåne in Sweden. It is shown from other species and bumble bees that the measures may vary from region to region.

One of the most telling characters is likely to be the very sparse punctures on the clypeus (Løken 1973) (Fig. 2). In addition, clypeus is bending towards the base. The specimen has a long face, $a > b$ (Fig. 3), but $a < 1,5 \times b$. The compound eyes are directed in front of posterior mandibular condyle. Distal margin of mid-basitarsus posteriorly produces an acute angled spine (Fig. 4), as described in Løken (1973, 1985). The 6th sternite has a distinct keel (Fig. 5), as described in Løken (1973) and Alford (1975) for the subgenus *Subterraneobombus*.

The sting sheet was dissected (Fig. 6) and compared with drawings from Prys-Jones & Corbet (1991) and Alford (1975). Although we found additional characters not described anywhere, the inner projection of the sting sheet resembled the sting sheet described for subgenus *Subterraneobombus*.

We compared the specimen with specimens from the entomological collections of Natural History Museum, University of Oslo, and found that

TABLE 1. Confirmed Norwegian records of *Bombus subterraneus* (L., 1758). Abbreviations: F=female, W=worker, M=male.

County	Municipality	Location	Gender/Caste	Date	Year	Leg
Akershus/Oslo	Oslo	Oslo	F	27 July	1844	Dahlblom
	Oslo	Oslo	F, W		ca. 1850	Esmark
	Oslo	Oslo	3F		ca. 1870	Siebke
	Oslo	Tøyen	4W		ca. 1870	Siebke
	Oslo	V. aker	2W		ca. 1870	Siebke
	Oslo	Slemdal	F	30 May	1910	Schøyen
	Ås	Vollebekk	F	July	1949	?
Østfold	Moss	Jeløy	F	24 May	1908	Barca
	Hobøl	Tomter	F	3 June	2010	Aase
Vestfold	Nøtterøy	Teie	W	25 August	1934	Backe
	Nøtterøy	Teie	W	2 August	1935	Backe
Hordaland	Fana	Skipanes	F	28 July	1907	Barca
	Etne	Etne	F	21 June	1923	Meidell
Buskerud	Hurum	Filtvedt	3W, M	8 August	1910	Schøyen

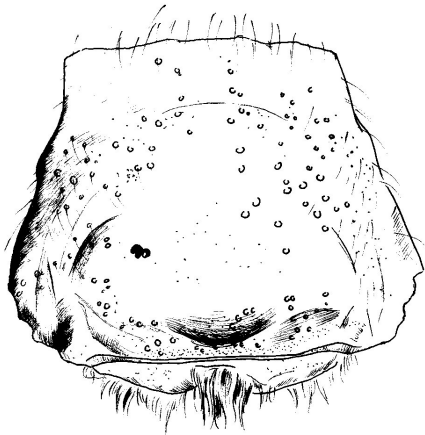


FIGURE 2. *Bombus subterraneus* (L., 1758). Head. Face front.



FIGURE 3. *Bombus subterraneus* (L., 1758). Head. Face profile.

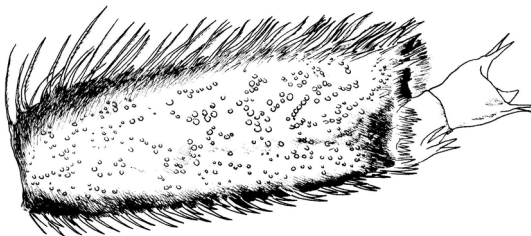


FIGURE 4. *Bombus subterraneus* (L., 1758). Leg: mid basitarsus.



FIGURE 5. *Bombus subterraneus* (L., 1758). 6th sternite: keel. Photo: Karsten Sund, NHM Oslo

in general the new specimen was very similar to the museum-specimens of the species *B. subterraneus*. Some characters differed, though, like the distinctness and transparency of the keel and the amount of punctuation on clypeus: The museum specimens had a more distinct keel, and it was transparent, but this trait may be caused by fading in old specimens. In addition, the museum specimens exposed an almost unpunctuated clypeus, compared to the sparsely punctuated clypeus of the new specimen.

DNA was extracted from internal fragments collected from the dissected genitals, using the E.Z.N.A.[®] MicroElute[®] Genomic DNA Kit (Omega Bio-Tek Inc., Norcross, GA, U.S.A). A partial CO1 fragment from the mitochondrial genome was amplified by PCR using primers originally developed for *Apis mellifera* (Tanaka et al. 2001). Primer sequences were: 5'-ATAATTTTTTTTATAGTTATA-3' (forward) and 5'-GATATTAATCCTAAAAAATGTTGAGG-3' (reverse). PCR amplification was performed in a 30 µl reaction containing 2.25 mM MgCl₂, 0.2 mM of each dNTP, 9.6 pmol of each primer, 1.5 µg of Bovine Serum Albumine (BSA), 0.9 units of HotStar DNA polymerase (Qiagen, GmbH,



FIGURE 6. Sting sheet of *Bombus subterraneus* (L., 1758). Photo: Karsten Sund, NHM Oslo.

Hilden, Germany) and 5 µl of template. A 15min pre-denaturation step at 95°C was followed by 45 cycles of amplification with 30s at 94°C, 30s at 45°C and 1 min at 72°C. A 10 min extension step was added at the end. PCR products were purified with the E.Z.N.A.[®] Cycle Pure Kit (Omega Bio-Tek) and sequenced using BigDye terminator cycle sequencing chemistry on an ABI 3130 instrument (Applied Biosystems, Foster City, CA, USA), following the protocol provided by the manufacturer.

The resulting 1011 bp DNA sequence (Genbank accession number HQ446456) was matched against previously published sequences in Genbank. A perfect match (100%) was found with accession number AY181165, representing a Danish strain of *B. Subterraneus* (Pedersen 2002).

Discussion

Løken (1973) reviewed the Norwegian collections of bumble bees and found the most recent specimen of *B. subterraneus* to be sampled in Ås, 1949, leg uncertain. In their recent overview of the status for Norwegian bumble bee species,

Ødegaard et al. (2009) presumed *B. subterraneus* to be extinct, which is also the present status for this species in UK (Goulson 2003) and Middle Europe (Holmström 2007). This new record of *B. subterraneus* is therefore quite surprising. Especially, as long tongued bumble bee species associated with deep perennial flowers are thought to be dramatically affected by habitat loss (Goulson et al. 2005). *B. subterraneus* is a late-emerging long tongued species highly specialized on gathering pollen from flowers with deep corollas, like Fabaceae and Lamiaceae, thus depending on diminishing habitats such as unimproved, flower-rich grassland (Goulson et al. 2005).

In Sweden, however, *B. subterraneus* has a broad southern distribution, with concentrations of recent findings in Uppsala, Skåne and Gotland (Holmström 2007). It seems like the species has shown a weak expansion towards the north during the last decades in Sweden (pers. comm. B. Cederberg). It is therefore unclear whether the present Norwegian record represents an old relict population or a recent immigration from Sweden. The recent finding was based on one single queen, and frequent revisits to the locality during the summer of 2010, did not reveal further specimens. It is therefore questioned whether this record indicates a present population in Southeastern Norway. If subsequent investigations confirm the existence of a viable population, it is of great importance to understand the local ecological demands of the species, in order to ensure the future survival of this species in Norway.

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