

Nest architecture and species status of the bumble bee *Bombus (Mendacibombus) shaposhnikovi* (Hymenoptera: Apidae: Bombini)

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Abstract – The nesting behaviour of the subgenus *Mendacibombus* is known only from *Bombus mendax*. Here, we describe the nest of a second species of *Mendacibombus*, that of *Bombus shaposhnikovi*. The nest was discovered in an abandoned rodent nest at 2,295 m near Artvin (Turkey) on August 12, 2007. Except for the absence of a canopy and the non-hexagonal shape of the honey and pollen pots, the architecture of the *B. shaposhnikovi* nest is consistent with that of the previously described *B. mendax*: eggs are oviposited individually in independent egg cell, post-emergence pupal cells are not reused for food storage and males return to the parental colony overnight. Observation of intranidal colour polymorphism provides evidence that the yellowish *B. shaposhnikovi* and the greyish *Bombus handlirschianus* might be conspecific. This conspecific status had been suggested by a recent comprehensive molecular study.

bumblebee / *Bombus (Mendacibombus) shaposhnikovi* / nest / description

1. INTRODUCTION

Bombus shaposhnikovi Skorikov 1910 is one of 12 species comprising the subgenus *Mendacibombus* Skorikov 1914, which appears as the sister clade to the remaining bumble bees (Williams 1985; Cameron et al. 2007). The distribution of *Mendacibombus* extends from the Pyrenees in the west to Kamtchatka in the east and is restricted to higher altitudes, from 1,900 m to 4,800 m (Skorikov 1931; Williams 1991, 1998). The nesting behaviour of *Mendacibombus* species, aside from a short remark on a nest of *Bombus makarjini* Skorikov 1910

(Reinig 1930; Bischoff 1931), is known only from observations of *Bombus mendax* Gerstaecker 1869, a species restricted to the highest altitudes of the Alps and the Pyrenees (Aichhorn 1976; Haas 1976). From what little is known, *B. mendax* nesting behaviour is unusual among bumble bees. As distinctive characteristics described thus far, the queen oviposits a single egg in each egg cell, and the larvae are reared individually in independent cells. In most other bumble bee species, the queen deposits multiple eggs into a single egg cell, and the larvae are reared in common cells initially, later becoming separated into single larval cells. Also, characteristic of *B. mendax*, the cocoons are torn down soon after the emergence of adults so that pollen and honey pots are built de novo, separate from the brood comb. The food pots are arranged in hexagonal

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cells of pure wax and store large quantities of food reserves. Other bumble bee species typically re-use empty pupal cocoons for food storage, thus, the honey pots and pollen pots are commonly intermixed with the brood cells, although separate food pots have been observed in some species of the *Fervidobombus* species group (Cameron et al. 1999). Lastly, during the first weeks of their life, the *B. mendax* males overwinter in the parental colony (Haas 1976). This behaviour is unique among bumble bees. The males also exhibit courtship that involves racing behaviour associated with enlarged eyes (Haas 1949; Williams 1991). The male perches in one small area and pursues potential mates. This perching behaviour is clearly different from the commonly observed patrolling behaviour, in which males fly along a circuit in search of females. Nevertheless, males performing perching behaviour use oral secretion to scent-mark their perching site (Williams 1991; Kindl et al. 1999). As mentioned by Williams (1991), the courtship could be associated with likely territorial behaviour.

The independent rearing of larvae and the separation between brood cells and storage cells recall the Meliponini features (Aichhorn 1976; Haas 1976; Sakagami 1976). Interestingly, the most updated phylogeny of corbiculate bee tribes strongly support Bombini and Meliponini as sister tribes (Kawakita et al. 2008). This is congruent with the plesiomorphic morphology of *Mendacibombus* (Williams 1985). Two familiar categories are ascribed to bumble bees' nest architecture. Pollen-storers category refers to bumble bees that intermixed pollen and honey pots with brood cells, as *Bombus terrestris* (L.). Pocket-makers category refers to bumble bees that lay a pocket of pollen at the base of the brood cell, as *Bombus pascuorum* (Scopoli). Because the nest architecture of *B. mendax* falls outside of these familiar categories, Haas (1976) proposed a new nesting category for *B. mendax*: “*Wabenbauer*” (=“*honeycomb builder*”). Here, we describe the nest of a second species of *Mendacibombus*, that of *B. shaposhnikovi*.

Bombus shaposhnikovi is known from mountain meadows of east Anatolia, Caucasus and northern Iran (Rasmont 1983). Its “sister species”, *Bombus handlirschianus* Vogt 1909, is recognised by a variant female colour pattern, with yellowish bands in *B. shaposhnikovi* and grey white bands in *B. handlirschianus*. A recent molecular phylogeny of *Bombus* based on nucleotide sequences from five genes suggests that these taxa may be conspecific (Cameron et al. 2007). Here, we report behavioural observations of the nesting biology of *B. shaposhnikovi* and provide new evidence for the conspecific status of *B. shaposhnikovi* and *B. handlirschianus* in our discovery of a nest containing both colour forms.

2. MATERIALS

The nest was discovered near a spring at 2,295 m above the sea level by following a track to Eğrisu Yaylası in Gül Dağı (41°07'37"N, 41°31'03" E), near Murgul (Turkey, Artvin province) on August 12, 2007 (Figure 1). The summer climate of this region is extremely wet, sometimes hot in the afternoon but cold in the evening, with frost on some summer nights. The area is under deep snow cover in winter. The grassland was dominated by several species of Poaceae, *Alchemilla tiryalensis* B. Pawl., *Cirsium obvallatum* (Bieb.) Fischer, *Gentiana septemfida* Pallas, *Prunella vulgaris* L., *Swertia iberica* Fisch ex C. A. Meyer, *Thymus transcausicus* Ronniger; less abundant were *Veratrum album* L., *Campanula* sp., *Myosotis* sp., *Epilobium* sp., Umbelliferae and yellow Compositae.

3. RESULTS

3.1 Nest description

The nest was established in rankers soil (thin soil covering siliceous material), in an abandoned rodent nest. Dried *Festuca* sp. and flowering *T. transcausicus* hid the small opening of the nest ($\varnothing=1.5$ cm). The entrance tunnel (35 cm long) reached a small spheroid

Nest architecture of *Bombus shaposhnikovi*



Figure 1. Gül Dağı, on the way to Eğrisu Yaylası where the nest was found (Murgul, Artvin province).

Figure 2. Front view of the entire structure of the nesting cavity closely crowded with hay: *a* three entrance honey pots, *b* honey pots, *c* pollen pots, *d* pupal cocoons.

Figure 3. Two individual egg cells both containing a single egg.

Figure 4. Yellowish (*left*) and grey white (*right*) workers of *B. shaposhnikovi*.

cavity ($\text{Ø}=10\text{--}12$ cm) at 12 cm below the soil surface. The nest was located in this cavity. There was no wax involucrum (nest envelope). In the cavity, the brood comb was crowded with dried grass (Figure 2).

On the boundary of the cavity, in front of the entrance tunnel, three wax *honey pots* contained a clear and very liquid honey (height= 14.5 ± 0.4 mm [mean \pm SD]; $\text{Ø}=9.2\pm 0.6$ mm; size was measurable for two pots). The remaining wax honey pots were located beside the brood ($N=8$; height= 13.5 ± 4.2 mm; $\text{Ø}=10.3\pm 1.5$ mm), and wax *pollen pots* ($N=2$; height= 14.0 ± 0.7 mm; $\text{Ø}=10.7\pm 0.6$ mm). No visible post-emergence pupal cells were found to have been reused for honey or pollen storage. The *pupal cocoons* were organized into three clusters of 2, 3 and 4 cocoons, respectively; there were also two single cocoons (height= 13.3 ± 1.5 mm; $\text{Ø}=8.4\pm 0.8$ mm). Two *egg clusters* were both constructed on an isolated pupal cocoon. The first cluster included three individual cells, each with a single egg (Figure 3). The second cluster contained two cells, also containing a single egg each. There were no larval clusters and no gynes cocoons in the nest.

3.2 Individuals inside the nest

While excavating the nest, individuals escaped from the cavity with no defensive behaviour. All individuals, including returning foragers and males, were caught with aerial nets, and the nest components were transferred to the laboratory. Eighteen workers and six males inhabited the nest. No queen or adult gynes were found, as determined by the length of the radial cell (RL, distance from the first submarginal cell venule to the radial cell apex), which was used as a proxy for total body length (Medler 1962). (Approximate total body length is also given as additional information: worker total length= 12.1 ± 0.9 mm, RL= 2.85 ± 0.21 mm; male total length= 13.50 ± 0.4 mm, RL= 4.26 ± 0.11 mm). Females and males had yellowish bands, except one worker with grey white bands (Figure 4). The worker with grey white bands was not a callow; if so, the regions

displaying black pile would have appeared silvery, as is the case when an adult first emerges from its cocoon. The dark bands on this worker were truly black.

Most males were captured when they were returning to the nest in the evening. This suggests that the flying males of *B. shaposhnikovi* do not sleep on thistles as most male bumble bees do (Alford 1975) but return to the nest overnight, as was observed with *B. mendax* (Haas 1976). Even after excavating the nest, males continued to land on the bottom of the nest cavity. Individuals appeared to orient to the nest on return flights by flying along the nearby creek.

3.3 Brood parasitism

The nest was heavily parasitized. Two of the recorded 11 pupal cocoons contained a *B. shaposhnikovi* male imago; each of the remaining nine contained a parasitic *Mutilla saltensis* pupa. It was impossible for us to differentiate a priori if cocoons contained parasites or bumble bee brood. Many undetermined mites were also found in the nest, both inside and outside of the cocoons. Some of them were observed within the egg cells. One bumble bee male imago had four mites, one of which was located on the head and another between the thorax and abdomen. Pupae belonging to the Cyclorrhapha (Diptera: Muscomorpha), probably Syrphidae (J. Kindl, unpublished data), were found within the nest mass, ($N=4$; length= 7.4 ± 0.4 mm; $\text{Ø}=3.1\pm 0.2$ mm; size measurable for three pupae).

4. DISCUSSION

With the exception of the absence of a protective canopy and the non-hexagonal shape of the honey and pollen pots, the architecture of the examined *B. shaposhnikovi* nest closely recalls that of *B. mendax*. According to Sakagami (1976), a wax envelope has homeostatic functions, and its construction is facultative in bumble bees, depending on environmental and colony conditions. The absence of the queen and larvae, the presence of adult

males, the small amount of stored food and the high level of parasitism in the *B. shaposhnikovi* nest were probably the result of its being in a late post-reproductive phase. It is likely, therefore, that the eggs observed were oviposited by workers instead of by the foundress queen.

Our observation of the worker with grey white bands in the nest is consistent with a previous observation made in 2002 at a site in northeast Turkey (Yalnızçam), where workers of both the white and yellow colour forms were observed to share a common nest entrance (observations by Hines reported in Cameron et al. 2007). Moreover, sequencing of the genes mt 16S, EF-1 α , Opsin, ArgK and PEPCK, comprising ~3500 bp, revealed that *B. shaposhnikovi* and *B. handlirschianus* differ by only two bases (1 bp 16S, 1 bp PEPCK; Cameron et al. 2007). Based upon the two independent observations of intranidal colour polymorphism and the genetic similarity of the two taxa, we suggest that *B. shaposhnikovi* and *B. handlirschianus* comprise a single species, exhibiting a simple colour dimorphism. There appear to be no clear morphological differences separating the females of these two forms, except the colour pattern. The hair colour inheritance might be controlled by a single-locus polymorphism with two alleles, as Owen and Plowright (1980) discovered for the inheritance of red and black colour forms of *Bombus melanopygus*, red being dominant to black. Males of both *B. shaposhnikovi* and *B. handlirschianus* have yellowish bands and are therefore more difficult to distinguish than workers. However, Skorikov (1931) pointed out a slight difference in the gonostylus and volsella shape of their genitalia. Genitalia differences were also noted by Williams (1991) from an unusual male with white pubescence rather than yellow. To be certain of the conspecific status across the entire range of both forms will require additional study of the population genetic structure.

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Architecture du nid et statut d'espèce du bourdon *Bombus (Mendacibombus) shaposhnikovi* (Hymenoptera: Apidae: Bombini).

bourdon / nid / description / Turquie

Zusammenfassung – Nestarchitektur und Art-Status der Hummel *Bombus (Mendacibombus) shaposhnikovi* (Hymenoptera: Apidae: Bombini) Die Untergattung *Mendacibombus* zeigt ein unverkennbares Nistverhalten, das hauptsächlich aufgrund von Beobachtungen an *B. mendax* beschrieben wurde. Hier beschreiben wir das Nest von *B. shaposhnikovi*, einer zweiten Art von *Mendacibombus*. Das Nest wurde in der östlichen Türkei auf einer Höhe von 2295 m in der verlassenen Nisthöhle eines Nagetiers entdeckt (Abb. 1 und 2). Die an diesem Nest beobachtete Bauweise bestätigt die von *B. mendax* bekannten Eigenschaften: Eier werden einzeln in individuellen Zellen abgelegt, und Pollen- und Honigtöpfe werden getrennt von den Brutzellen neu gebildet. Das Nest befand sich in einem späten Stadium jenseits der reproduktiven Phase und war stark parasitiert: von den 11 noch vorhandenen Puppenkokons der Hummeln enthielten neun Puppen von *M. saltensis*. Der bei den Arbeiterinnen in dem einzelnen Nest beobachtete Pigmentdimorphismus liefert einen weiteren Hinweis darauf, dass es sich bei der gelblichen *B. shaposhnikovi* und der grauen *B. handlirschianus* um dieselbe Art handelt.

Hummel / *Bombus Mendacibombus shaposhnikovi* / Nest / Beschreibung

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