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Nidicolous mites associated with *Bombus niveatus* Kriechbaumer (Hymenoptera, Apidae) in Iran

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Each spring, bumblebee queens establish their new nests in abandoned nests of birds, reptiles, rodents and other burrows in the soil. These bees, which are known as eusocial pollinators collect pollen and nectar from flowering plants, and form close relationships with various commensal, phoretic, or parasitic animals, as well as viral or protist pathogens (Ruiz-González and Brown 2006; Revainera *et al.* 2020). Mites are among important associates (parasites or commensals) frequently recorded from wild and commercial colonies of bumblebees (Husband and Husband 1996; Husband and Sinha 1970; Rozej *et al.* 2012). For example, the tracheal mite *Locustacarus buchneri* Stammer (Acari: Podapolipidae), is the most important and widespread parasitic mite of *Bombus* spp., having harmful effects on its hosts' fitness and behavior (Husband and Shina 1970; Otterstatter and Whidden 2004). Invasive mite haplotypes have been detected in commercial bumblebee colonies exported from Europe to Japan and in the Japanese native bee, *Bombus hypocrita* (Goka *et al.* 2006; Yoneda *et al.* 2008; Rozej *et al.* 2012). Although this endoparasite is known from at least 25 wild bumblebee species in the Holarctic (Husband and Husband 1996), there is no record in Iran yet.

Most phoretic mites associated with bumblebees are commensals having little or no negative effects on their hosts (Revainera *et al.* 2020). Recently, there are some reports that pathogen spillover from commercial and managed colonies could be transported to wild bee populations and have potential negative consequences for both crop pollination and conservation of native bees (Potts *et al.* 2010; Cameron *et al.* 2011; Blitzer *et al.* 2012). Phoretic mites associated with bumblebees attach to foragers to transport into their nest to reach to adequate nutritional supply such as pollen and nectar persevered in pollen pots and honey pots and comb material, nematodes, and microorganisms based on their feeding regimes (Cordeiro *et al.* 2011; Revainera *et al.* 2020). Nidicolous mites have a limited mobility and, naturally, their life cycle should be synchronized with their hosts in order to disperse (Binns 1982).

Bumblebee has received a lot of attention in Iran recently. The colonies of these bees have been imported to Iran from Biobest Company in recent years and are used in pollination of greenhouses. There are reports that along with these colonies, pathogens and associated mites have been transmitted around the world. It seems that the species of these bees in natural colonies also have extensive

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connections with other types of organisms. In this study, colony-associated mites in the nature of Iran were investigated.



Figure 1. Excavating a nest of *Bombus niveatus* around Meshkinshahr, Ardabil Province, Iran – **A.** Enclosing the colony area, we saw the location of the nest when we saw a bee enter the hole; **B.** The entrance hole of the colony is shown in the center of the arranged stones; **C.** Begin digging carefully with a small shovel to access the bee colony; **D.** Digging deeper into the soil and approaching to find bumblebee colony; **E.** Exposure to the highest layer of the colony and the appearance of the first colony larvae; **F.** The complete emergence of bumblebee colony, bees had used waste materials left by bird nest for making their nest colony; **G.** A photo from a distance to show the depth at which the colony was located; **H.** Complete transfer of bee colony to bucket.

In this survey we worked on mites associated to a bumblebee nest comb and the soil around it. While surveying bumblebees in the Shabil area (38° 30' 43.43" N, 47° 43' 48.59" E, 2730 m a.s.l.), Meshkinshahr county, Ardabil Province, Iran, we observed worker bees entering their subterranean nest through a small hole in the ground. This hole was marked (Fig. 1.) and then 50 to 60 cm of soil was dug using a small spade. As soon as the bee comb was observed around the nest, the nest materials (including bee pupae and larvae) was carefully transferred to a plastic bucket and covered with a lid. Adult bumblebees were collected using a hand net and then pinned and stored in a collection box. Soil and nest debris were mixed with 70% alcohol and then the sediment was inspected for mites. We also checked adults for mites. Phoretic mites were removed from bumblebee hosts using an entomological pin. Mite specimens were cleared in lactic acid solution and mounted in Hoyer's medium (Walter and Krantz 2009). Bumblebees (Fig. 2.) were identified as *Bombus niveatus* Kriechbaumer, 1870 by AM with help from Paul H. Williams (Natural History Museum of London). Identification of mites was done by PK (key used; Klimov *et al.* 2016).

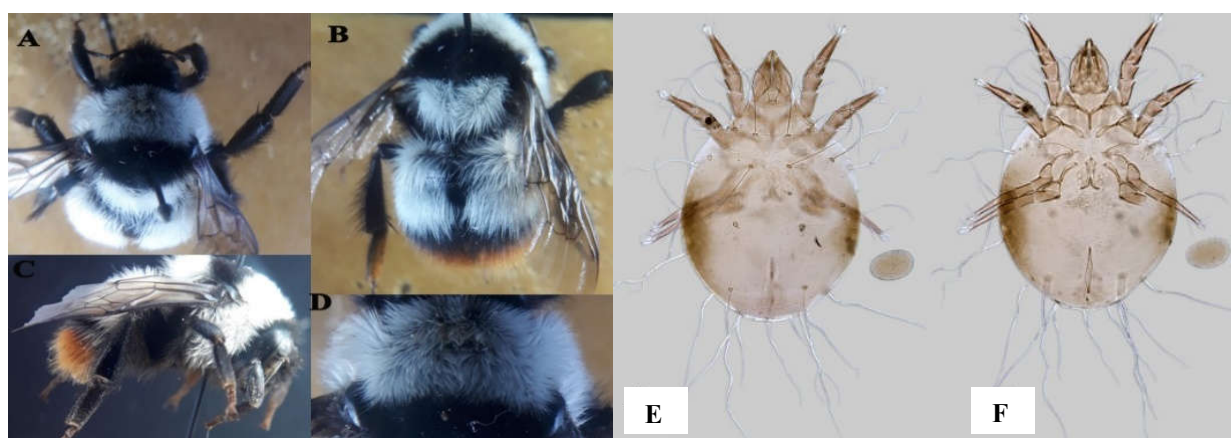


Figure 2. A–D: *Bombus niveatus* diagnostic photographs (photo by AM) – A. The frontal view of the queen; the forehead is covered with dark hair with no white hair; B. Full dorsal view of the queen, tail with red hair; C. Lateral view of the queen, new queen without collected pollen; D. Collar area of queen with white hair; E–F: *Kuzinia laevis* (Dujardin, 1849) – E. Dorsal view of body; F. Ventral view of body (Photo by P. Klimov).

RESULTS AND DISCUSSION

Among the mites collected from the extracted comb and soil attached to nest of the bumblebees, we found five species of mites associated with the subterranean nest of the bumblebee *Bombus niveatus* Kriechbaumer (Table 1).

Table 1. Nidicolous mite species associated with *Bombus niveatus* Kriechbaumer nest excavated from Ardabil Province.

Species	stage/sex	Number of specimens
<i>Parasitellus fucorum</i> (De Geer, 1778)	8 deutonymphs	8
<i>Kuzinia laevis</i> (Dujardin, 1849)	1 female	1
<i>Proctolaelaps longisetosus</i> (Postner, 1951)	2 females	2
<i>Pneumolaelaps colomboi</i> (Evans & Till, 1966)	1 female	1
<i>Pneumolaelaps hyatti</i> (Evans & Till, 1966)	20 females (+ 1 probably male)	21

The species *P. fucorum* belongs to the order Mesostigmata (Parasitidae). These mites are cleptoparasitic or neutral to beneficial (depending on life stage); females and deutonymphs feed on

provisioned pollen, while other stages are predators of small arthropods (Klimov *et al.* 2016). Mites hibernate in the deutonymphal stage. Deutonymphs of this species have the opisthogastric region with more than 40 pairs of setae, while in all other species of Parasitidae, the opisthogastric region has fewer than 30 pairs of setae. All species of the genus *Parasitellus* are obligatory associates of bumblebees (*Bombus*). There are several occasional records from honey bee (*Apis*) hives and burrows of small mammals, which are preferred sites for bumble bee nests (Huck *et al.* 1998).

Kuzinia laevis belongs to the order Sarcoptiformes (Acaridae) and is distributed in the Nearctic, Palearctic, Neotropical, and Oriental regions. It has been introduced to the Australian region (Australia, Tasmania, and New Zealand) with commercial bumblebee colonies used for crop pollination. *Kuzinia laevis* is primarily associated with bumblebees (*Bombus*), but can occasionally be found on other bees (Klimov *et al.* 2016).

Proctolaelaps longisetosus belongs to the order Mesostigmata (Melicharidae). Many species belonging to genus *Proctolaelaps* have broad, intercontinental distributions. *Proctolaelaps sibiriensis* is among bumblebee-specialist species like *P. bombophilus*, *P. longanalis*, *P. ornatus*, and *P. sibiriensis*. This is a cosmopolitan species, but specimens associated with bumblebees have been recorded in the Palearctic and Neotropical regions. Mite females disperse and overwinter on adult queen bees (Klimov *et al.* 2016).

Pneumolaelaps colomboi belongs to the order Mesostigmata (Laelapidae). It is distributed in the Holarctic, Oriental and Neotropical regions and has been introduced with bumblebees to the Australia and New Zealand (Klimov *et al.* 2016). Here we found one male specimen probably belonging to this species, but its identity is not certain since males have not been described for *P. colomboi* yet. All stages of these mites live in nests of bumblebees, feeding on pollen. Females disperse and overwinter on adult queen bees (Klimov *et al.* 2016).

Pneumolaelaps hyatti also belongs to the order Mesostigmata (Laelapidae), we found 21 mite specimens. One male was found, which probably belongs to *P. hyatti*, but this is not certain because males have not been described for *P. hyatti*, either. Furthermore, four female specimens were examined having additional setae between the genital anal shield as compared to the typical *P. hyatti*; but their number was less than that in *P. marginipilosa* (syn.: *P. marginalis*). *Pneumolaelaps hyatti* is known from Great Britain from *Bombus muscorum* L. This mite species is widely distributed in Asia, Europe and Russia in association with bumblebees or their nests (Davydova and Nikolsky 1986; Joharchi *et al.* 2019; Trach *et al.* 2019). Mite genera associated with bumblebees around the world are shown in Table 2. List of Iranian bumblebee-associated mite species is subject of subsequent study and under preparation to be published.

Table 2. Mite genera associated with bumblebees around the world (Klimov 2021).

No.	Mite Genera	No.	Mite Genera	No.	Mite Genera
1	<i>Acarus</i>	10	<i>Imparipes</i>	19	<i>Proctolaelaps</i>
2	<i>Aeroglyphus</i>	11	<i>Kuzinia</i>	20	<i>Scutacarus</i>
3	<i>Ameroseius</i>	12	<i>Lasioseius</i>	21	<i>Tarsonemus</i>
4	<i>Androlaelaps</i>	13	<i>Locustacarus</i>	22	<i>Thyreophagus</i>
5	<i>Cerophagus</i>	14	<i>Macrocheles</i>	23	<i>Tortonia</i>
6	<i>Cosmolaelaps</i>	15	<i>Parascutacarus</i>	24	<i>Tydeus</i>
7	<i>Glycyphagus</i>	16	<i>Parasitellus</i>	25	<i>Tyrophagus</i>
8	<i>Horstia</i>	17	<i>Parasitus</i>	26	<i>Vidia</i>
9	<i>Hypoaspis</i>	18	<i>Pneumolaelaps</i>	27	Uropodina

The distribution of mites associated with bumblebees depends on the frequency of some instars such as *deutonymphs*. This stage is a specialized phoretic instar, which attaches to adult bumblebees

(Huck *et al.* 1998). Studies have shown that stage of deutonymphs prefers attachment to queens, but may be transported by other castes of males or workers (Huck *et al.* 1998). It has been shown that all developmental stages of the mite *Parasitellus fucorum* live in the nests of *Bombus* species (Huck *et al.* 1998). In turn, some other species of phoretic mites may not all live in the bumblebee nests, for example, in *Proctolaelaps*; the genus includes about 140 described species, but only five species are associated with bumblebees (Trach *et al.* 2019). Some of the bumblebee-associated mite species may feed on other mites that live in this bee's nest. For example, there is a report that *P. hyatti* was observed to feed on *K. laevis* (Macfarlane 1975; Fan *et al.* 2016), which lives in the nest of bumblebees. However, overall our knowledge on the bumblebee-associated mites and their ecology is rather limited and more studies are needed.

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