

New Balkan records of *Bombus subterraneus* (Linnaeus 1758) and *Bombus cryptarum* (Fabricius 1775) from Greece

IOANNIS TH. ANAGNOSTOPOULOS*

Technological Educational Institute of Western Macedonia (Florina), School of Agricultural Technology, Laboratory of Apiculture, 53100 Florina, Greece

ABSTRACT

From the study of the Greek bumblebee fauna (Hymenoptera: Apidae, Bombini), species lists have been published based on both literature records and original data from collected bees. Since 1995 a special effort to confirm with newly collected bees all bumblebee species reported in literature records for Greece has been in progress. Although numerous specimens have been collected and examined and in some instances yielding new *Bombus* species for the Greek insect fauna, some species, mainly those reported in older references, have not yet been found. Recently, identification of bumblebees collected in the Florina Prefecture - Northwest Macedonia, during the years 2006 and 2007 yielded information for two "literature cited" species, *Bombus subterraneus* (Linnaeus 1758) and *Bombus cryptarum* (Fabricius 1775). A *B. subterraneus* queen (collected at 40°47'38N, 21°26'10E on *Vicia cracca*) was distinguished by morphological characteristics and a worker *B. cryptarum* (collected at 40°41'58,7N, 21°28'18,5E on *Echium* spp) was revealed using mitochondrial DNA RFLP analysis of the CO1 gene. These new records from Florina are provided with comments, confirming the species presence in Greece for the first time after approximately 40 years.

KEYWORDS: Hymenoptera, Apidae, *Bombus*, Florina, distribution, ecology, taxonomy, new records, Greek insect fauna

Introduction

Bumblebees are a group of the corbicular bees, all in the genus *Bombus* (Latreille) with approximately 250 species recognized worldwide that occupy a wide diversity of habitats (Williams 1998). The Greek bumblebee fauna has been studied in recent years and species lists have been published (Anagnostopoulos 1996, 1999, 2005, 2009). Since 1995 a special effort to confirm, with newly collected bees, all bumblebee species reported in literature records has been in progress. Although a fair number of

bumblebee specimens were caught and examined, *B. subterraneus* (Linnaeus 1758) and *B. cryptarum* (Fabricius 1775) were included in the latest bumblebee fauna list (Anagnostopoulos 2005) only as "literature records", referencing the studies of Reinig (1966) and Rasmont (1984), respectively.

Recently, identification of bumblebees collected in the Florina Prefecture during the years 2006 and 2007 yielded information, for the first time after approximately 40 years, on the *B. subterraneus* and *B. cryptarum* status in Greece. This paper presents the conformation of the occurrence of *B.*

*Corresponding author, e-mail: yiannisalfa@excite.com

subterraneus and *B. cryptarum* in Greece, with new records, along with comments for each species.

Materials and Methods

Bumblebees were collected during field trips in the Northwestern mountainous region of Greece. Information for each specimen caught, such as location and altitude of the collection site, were recorded with a GPS device (Garmin Etrex). The insects were pinned and stored until their identification to species level and then they were sent to international research institutes for confirmation. These institutes were: The Institute of Technology Carlow, Ireland and Hacettepe University, Ankara, Turkey. The *B. subterraneus* specimen was distinguished by morphological characteristics and was returned to be included in the author's private reference collection in Florina. A worker bee belonging to the *B. lucorum* complex (Williams 1998), suspected to be *B. cryptarum*, was revealed using mitochondrial DNA RFLP analysis of the CO1 gene (Murray et al. 2007). The *B. cryptarum* specimen, along with the DNA extracts, was retained in Ireland. The findings presented in this paper are by species, updating the literature with new field record.

Results

Subgenus *Subterraneobombus* Vogt 1911 *Bombus subterraneus* (Linnaeus 1758)

Original literature record:

Author: Reinig, W.F. (1966 pg. 84)

Locality: Mt. Olympos

Species name listed: *B. subterraneus* L.

Collected material examined:

Greece, Florina Prefecture: 1 queen, Florina, uncultivated field, 40°47'38N,

21°26'10E, 605m, on *Vicia cracca*, 24.5.2006. Species identification confirmed by Dr. A. M. Aytekin and Prof. P. Rasmont.

Comments:

B. subterraneus distribution is that of the Palaearctic region and although considered by some authors rare and in decline, it is found throughout most of Europe (Rasmont et al. 1993, Williams 1998, Walther-Hellwig and Frankl 2000, Goulson et al. 2005, Kosior 2007, Iserbyt et al. 2008). Compared to the bumble bee species known for Greece (Anagnostopoulos 2005, 2009) *B. subterraneus* is difficult to misidentify since it is the only bumble bee of the subgenus *Subterraneobombus*. It is a relatively large, long-tongued, late emerging species (Alford 1975, Goulson et al. 2005) that prefers open terrain (Reinig 1972, Svensson et al. 2000) and low altitude (Iserbyt et al. 2008). The females (queens and workers) have very short even hair, hence the common name "Short Haired Bumblebee", have the head longer than broad and have a spine on the mid basitarsus (Alford 1975, Prys-Jones and Corbet 1991). As food plants, *B. subterraneus* prefers visiting deep flowers with long corolla tubes such as Labiatae and Leguminosae. It nests underground and is a "pocket-maker" bumblebee if behavioral classification is considered (Sladen 1912, Alford 1975, Williams 1988). The specimen examined in this study was collected in open landscape of very low agricultural activity and was probably in the initial stages of founding a colony since there were pollen pellets at its corbicula. If taken in consideration a paper by Friese (1923) who at page 199 reports the species (three workers at 1000m altitude) at Tetovo - with Florina situated in-between Tetovo and Mt. Olympos - *B. subterraneus* may be considered as a bee that although is not abundant, if looked for will be found in the Balkan highlands.

Subgenus *Bombus* Latreille 1802
***Bombus cryptarum* (Fabricius 1775)**

Original literature record:

Author: Rasmont, P. (1984 pg. 154)

Locality: Pisodéri (Mt. Vigla) – Florina, 2000-2100m

Collector: Reinig, W.F. at 3.11.1965

Species name listed: *B. cryptarum reinigianus* Rasmont

Collected material examined:

Greece, Florina Prefecture: 1worker, Ano Idroúsa, mountain slope, 40°41'58,7N, 21°28'18,5E, 796m, on *Echium* spp, 23.11.2007.

Comments:

In some bumblebee studies, the authors have treated *B. cryptarum* as conspecific with *B. lucorum*, allowing *B. lucorum* in the broader sense to include a complex of similar taxa (Williams 1998). Although these taxa require more critical work to clarify this complex issue, recent studies (Bertsch et al. 2004, 2005, Cameron et al. 2007, Murray et al. 2007) lend weight to the view that *B. cryptarum* and *B. lucorum* are distinct species that can be distinguished reliably through genetic methods. In a previous study on Greek bumblebees (Anagnostopoulos 2005) the identification of the collected material was based on treating *B. lucorum* s. l., thus *B. cryptarum* specimens that may have been collected were not identified as such. Little is known about the ecology of *B. cryptarum*. If the species is considered sympatric to *B. lucorum* then, with some caution, it would apply a wide distribution throughout most of Eurasia and the north-western Nearctic, which is that of *B. lucorum* (Williams 1998). In Europe *B. cryptarum* is reported well distributed although in order to have a better understanding there are areas where field studies are still needed (Banaszak and Rasmont 1994, Bertsch et al. 2004).

Bertsch (1997) and Bertsch et al. (2004) consider *B. cryptarum* to be an early spring species, preceding *B. lucorum*, with strong flight activity in the early morning. It is stated to be more associated with forest areas (Banaszak and Rasmont 1994) and uplands (Murray et al. 2007). The specimen examined in this study was collected on a mountain slope, outside the Florina plain, before the lower range of the Mt. Vitsi beech forest. This worker bumble bee was observed to be considerably “shy” and nervous in its foraging activity, in contrast to many other *B. terrestris* workers observed - and for those collected identified as such - which acted quite keen to human presence. This deviation in the bees foraging activity, along with the fact that it was the only “terrestrisform” observed in the altitude range between 680m to 850m were the bases of the suspicion that it may be in fact *B. cryptarum* and not *B. terrestris*.

The recent records of *B. subterraneus* and *B. cryptarum* in the Northwestern mountainous region of Greece may be considered valuable faunistic information. Within this mountainous region lies Florina Prefecture, of 1924 km², which with recent records of at least 29 valid bumblebee species that combine data from the present and past studies (Rasmont 1984, Reinig and Rasmont 1988, Anagnostopoulos 1999, 2005, 2009), scores high in bumblebee biodiversity. Ongoing changes in rural Greece, such as for example intensification of agricultural practices may have resulted in the degrading of some suitable habitats for bees with a parallel decline of wild bees. This case scenario, although possible is difficult to justify since to the author's knowledge there is no published study on this matter. As for bumblebees in Greece, there are very few studies with ecological information (Anagnostopoulos 2005, 2009) and none with data on their abundance. Thus very little is known concerning their overall

distribution and ecology and some scientist may consider the reported records to provide more information on collection points and less on the habitats used by the bees. On bumblebee biodiversity for Greece, it is the author's opinion that possible limiting factors may consist of parameters other than human activity such as for example climatic conditions and territory landscape and if a suitable - within logic - habitat for a species is located inside its distribution zone, through field work insistence it is most probable that the species will be found. Thus further systematic field studies and collection of sample specimens throughout Greece is expected to reveal data on bumblebee distribution and ecology and hopefully new records for the remaining Greek "literature cited" bumblebees *B. ruderarius* (Müller 1776), *B. pomorum* (Panzer 1805), *B. monticola* Smith 1849 and *B. pyrenaicus* Pérez 1880 will arise.

Acknowledgements

The author would like to thank Dr A. Murat Aytekin of Hacettepe University (Turkey) and Prof. Pierre Rasmont of Université de Mons-Hainaut (Mons, Belgium) who examined part of the collected material and confirmed the identification of *B. subterraneus*, Dr. Thomais Kakouli-Duarte and her colleagues at the Institute of Technology Carlow and Teagasc Oak Park (Ireland) who contributed with their genetic work to the identification of *B. cryptarum* and Dr. Andreas Bertsch, Philipps-University Marburg (Germany) for his discussion and encouragement to engage with the writing of this paper and his many helpful comments on the manuscript.

References

Alford, D.V. 1975. *Bumblebees*. Davis-Poynter, London, U.K.. 352 pp.

- Anagnostopoulos, I.T. 1996. The bumble bees of Greece, first results of a biogeographical study. *Melissokomiki Epitheorisi* 10: 122-123 (in Greek).
- Anagnostopoulos, I.T. 1999. A first species list of the bumblebee fauna recorded in Greece, (Hymenoptera: Apidae). In *Proceedings of the 7th Panhellenic Entomological Congress*, Kavala, Greece 1997, pp 117-124 (in Greek).
- Anagnostopoulos, I.T. 2005. The bumblebee fauna of Greece: An annotated species list including new records for Greece (Hymenoptera: Apidae, Bombini). *Linzer biol. Beitr.* 37: 1013-1026.
- Anagnostopoulos, I.T. 2009. New records of bumble bees from the Northwestern mountainous region of Greece (Hymenoptera, Apidae). *Entomofauna* 30: 445-449.
- Banaszak, J. and P. Rasmont. 1994. Occurrence and distribution of the subgenus *Bombus* Latreille sensu stricto in Poland (Hymenoptera, Apoidea). *Pol. Pismo. Ent.* 63: 337-356.
- Bertsch, A. 1997. Wieviele Arten der Untergattung *Terrestribombus* (Hymenoptera, Apidae) gibt es in Nordhessen; die Abgrenzung von *Bombus cryptarum* und *B. lucorum* mittels männlicher Labial-drüsen-Sekrete und morphologischer Merkmale. *Marburger Ent. Pub.* 2: 1-28.
- Bertsch, A., H. Schweer and A. Titze. 2004. Discrimination of the bumblebee species *Bombus lucorum*, *B. cryptarum* and *B. magnus* by morphological characters and male labial gland secretions (Hymenoptera: Apidae). *Beitr. Ent.* 54: 365-386.
- Bertsch, A., H. Schweer, A. Titze and H. Tanaka. 2005. Male labial gland secretions and mitochondrial DNA markers support species status of *Bombus cryptarum* and *B. magnus* (Hymenoptera, Apidae). *Insect. Soc.* 52: 45-54.
- Cameron, S.A., H.M. Hines and P.H. Williams. 2007. A comprehensive

- phylogeny of the bumble bees (*Bombus*). Biol. J. Linn. Soc. 91: 161–188.
- Friese, H. 1923. Eine Kriegausbeute an Apiden (Bienen) aus Makedonien. Zool. Jahrb. Syst. 46: 175–216.
- Goulson, D., M.E. Hanley, B. Darvill, J.S. Ellis and M.E. Knight. 2005. Causes of rarity in bumblebees. Biol. Conserv. 122: 1–8.
- Iserbyt, S., E.-A. Durieux and P. Rasmont. 2008. The remarkable diversity of bumblebees (Hymenoptera: Apidae: *Bombus*) in the Eyne Valley (France, Pyrénées-Orientales). Ann. soc. entomol. Fr. (n.s.) 44: 211–241.
- Kosior, A., W. Celary, P. Olejniczak, J. Fijał, W. Król, W. Solarz and P. Płonka. 2007. The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. Oryx 41: 79–88.
- Murray, T.E., U. Fitzpatrick, M.J.F. Brown and R.J. Paxton. 2007. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conserv. Genet. 9: 653–666.
- Prys-Jones, O.E. and S.A. Corbet. 1991. *Bumblebees*. 2nd Ed. Richmond Publishing Co. Ltd., Slough, U.K.. 92 pp.
- Rasmont, P. 1984. Les bourdons du genre *Bombus* LATREILLE sensu stricto en Europe Occidentale et Centrale (Hymenoptera, Apidae). Spixiana 7: 135–160.
- Rasmont, P., J. Leclercq, A. Jacob-Remacle, A. Pauly and C. Gaspar. 1993. The faunistic drift of Apoidea in Belgium. In *Bees for pollination*. Bruneau, E. (Ed) Commission of the European Communities, Brussels, pp 65–87.
- Reinig, W.F. 1966. *Bombus lapponicus* (FABRICIUS 1793) ein für den Olymp neues Eiszeitrelikt (Hym. Apidae). Nachrichtenbl. Bayer. Ent. 15: 81–85.
- Reinig, W.F. 1972. Ökologische Studien an mittel- und südost-europäischen Hummeln (*Bombus* Latr., 1802) (Hym., Apidae). Mitt. Münchner Ent. Ges. 60: 1–56.
- Reinig, W.F. and P. Rasmont. 1988. Beitrag zur Kenntnis der Bergwaldhummel *Alpigenobombus wurfleini* (Radoszkowski, 1859) (Hymenoptera, Apidae, Bombinae). Spixiana 11: 37–67.
- Sladen, F.W.L. 1912. *The humble-bee. Its life history and how to domesticate it, with descriptions of all the British species of Bombus and Psithyrus*. MacMillan and Co. Ltd., London, U.K.. 283 pp.
- Svensson, B., J. Lagerlöf and B.G. Svensson. 2000. Habitat preferences of nesting-seeking bumble bees (Hymenoptera: Apidae) in a agricultural landscape. Agric. Ecosyst. Environ. 77: 247–255.
- Walther-Hellwig, K. and R. Frankl. 2000. Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. J. Appl. Ent. 124: 299–306.
- Williams, P.H. 1988. Habitat use by bumble bees (*Bombus* spp.). Ecol. Entomol. 13: 223–237.
- Williams, P.H. 1998. An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini). Bull. Br. Mus. (Nat. Hist.) Entomol. 67: 79–152.

Νέες Βαλκανικές καταγραφές των *Bombus subterraneus* (Linnaeus 1758) και *Bombus cryptarum* (Fabricius 1775) από την Ελλάδα

ΙΩΑΝΝΗΣ ΘΕΜ. ΑΝΑΓΝΩΣΤΟΠΟΥΛΟΣ

Τεχνολογικό Εκπαιδευτικό Ίδρυμα Δυτικής Μακεδονίας (Παράρτημα Φλώρινας), Σχολή Τεχνολογίας Γεωπονίας, Εργαστήριο Μελισσοκομίας, 53100 Φλώρινα, Ελλάδα

ΠΕΡΙΛΗΨΗ

Από τη μελέτη των βομβίνων της Ελλάδος, έχουν δημοσιευθεί από το 1995 και μετά καταστάσεις των ειδών οι οποίες είχαν βασιστεί για τη σύνταξη τους σε δημοσιευμένες καταγραφές των εντόμων αλλά και σε πρωτογενή συλλεγμένο υλικό. Η καταγραφή πληροφοριών σχετικά με τους βομβίνους στην Ελλάδα, εκτός των άλλων, εστιάζεται και στον εντοπισμό των καταγραμμένων μοναχά στη βιβλιογραφία ειδών για την επιβεβαίωση της ύπαρξης τους με νέες πλέον καταγραφές. Στην προσπάθεια αυτή, πολλοί βομβίνοι συλλέχθηκαν και εξετάστηκαν και σε ορισμένες περιπτώσεις καταγράφηκαν νέα για την Ελλάδα είδη *Bombus*. Υπάρχουν όμως είδη, κυρίως από παλιές βιβλιογραφικές πηγές, που δεν έχουν ξαναβρεθεί γεγονός που καθιστά την ύπαρξη τους ή μη στην σύγχρονη Ελλάδα ένα υπό έρευνα αντικείμενο. Με την εξέταση βομβίνων που συλλέχθηκαν κατά τα έτη 2006 και 2007 στον νομό της Φλώρινας προέκυψαν πρόσφατα πρωτογενής πληροφορίες για δύο είδη «βιβλιογραφικών» βομβίνων, τα *Bombus subterraneus* (Linnaeus 1758) και *Bombus cryptarum* (Fabricius 1775). Βασίλισσα του *B. subterraneus* (που συλλέχθηκε 40°47'38N, 21°26'10E επί *Vicia cracca*) αναγνωρίστηκε χρησιμοποιώντας μορφολογικά χαρακτηριστικά και εργάτρια του *B. cryptarum* (που συλλέχθηκε 40°41'58,7N, 21°28'18,5E επί *Echium* spp) καθορίστηκε με μιτοχονδριακό DNA RFLP ανάλυση του CO1 γονιδίου. Οι νέες αυτές καταγραφές βομβίνων από την περιοχή του νομού Φλώρινας επιβεβαιώνουν τη παρουσία των δύο ειδών *Bombus* στην Ελλάδα μετά από περίπου 40 χρόνια και παρουσιάζονται στην παρούσα εργασία με σχετικά σχόλια.