



Hacettepe University  
Faculty of Science  
Department of Biology  
06800 Beytepe  
Ankara TURKEY



Harvard  
University

Department of Organismic  
and Evolutionary Biology  
Harvard University  
26 Oxford Street  
Cambridge, Massachusetts  
02138, USA

## Scientific Project Protocol

**Determination of Population Structure and Distribution of *Evylaeus calceatus* (Scopoli 1763) and *Evylaeus albipes* (Fabricius 1781) with Molecular and Geometric Morphometrics Analysis**

**Prof. Dr. Naomi Pierce (Harvard University-USA)**

**Assoc. Prof. Dr. A. Murat Aytekin (Hacettepe University-Turkey)**

Dr. Sarah Kocher, Dr. Julien Ayroles (Harvard University-USA), PhD<sub>std.</sub> Seçil Aytekin PhD<sub>std.</sub>, Fatih Dikmen (Hacettepe University-Turkey)

### Introduction

Bees (Apoidea: Hymenoptera) are the most important plant pollinators in nature. This service makes them beneficial to agriculture to improve fruit set and yield, and to ecosystem management. While there have been a small number of studies examining the bee fauna of Turkey, we still lack a clear understanding of the current status of many of Turkey's native pollinators.

Anatolia (Asian part of Turkey) has a very interesting biogeographical history due to the glaciation periods that occurred in Pleistocene era. Extreme climatic changes during these periods pushed many northern populations to the south, especially the ones found in the Balkans, Caucasia, and in northern Anatolia. Because there are many high mountains creating geographical barriers, many of these populations

remained isolated at higher altitudes in the southern regions, This process led them to be isolated and speciation occurred. As a result, many insect groups have been examined in these regions in order to clarify their latitudinal shifts and distributions through Anatolia and especially throughout the Anatolian Diagonal.

*Evyllaëus calceatus* (Scopoli 1763) and *Evyllaëus albipes* (Fabricius 1781) can be found throughout the entire Palearctic and they are appropriate candidates for phylogeographical studies. However, data on the distribution of these species in Turkey are quite limited. According to our inspections and observations, their distribution patterns also fits with the other relict species from other insect groups, suggesting that they may be one of the glacial relict groups in Anatolia. Determining their current distributions and population structure would be very helpful in exposing their phylogeographic history within Anatolia. The proposed project will lay the foundation for examining these types of questions.

Field studies will be conducted in Northern Anatolia and Thrace regions of Turkey between August-September at 2012 and 2013. Once collected, the bees will be sorted and species identification will be determined at the Morphometry Laboratory of Biology Department in Hacettepe University. Following these identifications, the molecular studies will continue in Department of Organismic and Evolutionary Biology at Harvard University. Exploring the distributions and the population structures of the relevant species within these regions will be one of our main goals; this will greatly contribute to the database of biodiversity of Turkey. Furthermore geometric morphometric analysis will be applied on collected specimens. The phylogeographic studies that are used to determine the evolutionary history and the gene flows among populations require well preserved material and good sampling efforts that display general patterns of distributions of populations. In this respect, specimens will be preserved in alcohol for further DNA sequence analysis. Population structures and the distributions of these species from Northern Anatolia will be designated in a way that all the distribution areas are represented. By this means, the material and preliminary work that are needed for the forthcoming phylogeographic studies about this genus will be provided.

### **Material and method**

---

Field studies will be conducted in Northern Anatolia and Thrace regions of Turkey, which are the main genetic reservoirs for European and Caucasian fauna for these two species. The field studies will be performed in summer seasons between 2012 and 2013 with all researchers. It will take 15 days of excursions for each year between August and September, but specific dates will be determined according to the possible changes in the climatic conditions. Bees will be collected using suitable equipment, and will be determined, sorted and packaged in the Morphometry Laboratory of Department of Biology in Hacettepe University.

## **1. Geometric Morphometrics Analysis**

Phylogenetic and morphometric data will be compared using geometric morphometrics. This analysis will be done in the lab of Assoc. Prof. Dr. A. Murat Aytekin and PhD student Seçil Aytekin.

## **2. DNA analysis**

The collected material will be preserved in alcohol for further DNA analysis. These analyzes will be performed in the lab of Prof. Dr. Naomi Pierce (Harvard University-USA) by Dr. Sarah Kocher (Harvard University-USA). For each species extracting, amplifying, and sequencing of the mitochondrial (16S rRNA, COI) and nuclear (EF-1 $\alpha$  and opsin) DNA will be performed for confirmation of species determinations and phylogenetic analysis. These genes together have proven useful for analyses of bee relationships at both higher and lower levels. For the phylogeographic study, genome-wide molecular markers will be developed using next-generation sequencing methods, and the molecular data will be analyzed both separately, and in combination with morphological data.

## **3. Plant observations**

The association with the plants is vitally important for the bees. This association also gives some information about their niches. Monitoring the richness and abundances of pollinators and their plant preferences would provide crucial information. Furthermore, the accumulation of this kind of data will be helpful in reaching out the exact explanations of diversification of bees and related plants. For these reasons we will also record the plants visited by relevant bees. The determinations of the plants will be handled in Hacettepe University Department of Biology by PhD student Fatih Dikmen.

## **4. Biogeographical analysis**

Biogeographical data analysis will be done by PhD student Fatih Dikmen and Dr. Sarah Kocher. The current distributions of the species within Turkey and the distributions of the collected material will be summarized in CFF (Carto Fauna-Flora) maps.

This project is a part of the collaboration protocol (initiated in 2011) between Hacettepe University and Harvard University.

### **Researchers in the project:**

Assoc. Prof. Dr. A. Murat Aytekin, Dr. Sarah Kocher, Dr. Julien Ayroles, PhD<sup>std.</sup> Seçil Aytekin , PhD<sup>std.</sup> Fatih Dikmen.

## Timing of the Study

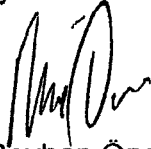
25 August – 10 September 2012: Field observations

October 2012 – February 2013: Identification of plants, insects, laboratory studies, meetings

25 August – 10 September 2013: Field observations

October 2013 – February 2014: Identification of plants, insects, laboratory studies

May 2014: Preparing manuscripts.



Prof. Dr. Reyhan Öner

Head of  
**Hacettepe University**  
Faculty of Science  
Department of Biology



Prof. Dr. Naomi Pierce

Principal Investigator  
Hessel Professor of Biology  
Curator of Lepidoptera  
**Museum of Comparative Zoology**  
**Harvard University**



Asst. Prof. Dr. A. Murat Aytekin

---

**Morphometry Laboratory of Biology, Department**



Mons, le 17 mai 2013

Prof. Dr. Ir. P. Rasmont  
Laboratoire de zoologie  
Université de Mons  
Place du parc, 20  
B-7000 Mons  
Belgique  
Tél : (32) 65 37 34 36  
FAX : (32) 65 37 34 38  
E-mail : [pierre.rasmont@umons.ac.be](mailto:pierre.rasmont@umons.ac.be)

Assc. Prof. Dr. A. Murat Aytekin  
Hacettepe University  
Faculty of Sciences  
Dept. Of Biology  
06800 Beytepe / Ankara  
[ama@hacettepe.edu.tr](mailto:ama@hacettepe.edu.tr)  
Tél : (90)312 297 71 58

### **Integrative taxonomy of the *Thoracobombus* group**

**Research project in collaboration between Assc. Prof. Dr. A. Murat Aytekin (Hacettepe University) and Prof. Dr. Ir. Pierre Rasmont (University of Mons)**

**Aim of the project:** to capture bumblebees of *Thoracobombus* subgenus to raise them and obtain males.

**Abstract.** Since their descriptions, there is much confusion in the taxonomy of the *Thoracobombus* group and this one has always raised discussion within the scientific community. The subgenus *Thoracobombus* count approximately 50 species distributed in Palaeartic, Nearctic and Neotropical regions. Within this group, some species show a very similar morphology.

Integrative taxonomy provides a major approximation to species delimitation based on integration of different perspectives. The aim of this work is to shed some light on the confusion existing in *Thoracobombus* group. Three different perspectives will be used; pheromonal composition of the labial cephalic glands of the males, geometric morphometrics of the wing and by genetic analyses.

**Literature summary.** Turkish Bumblebee fauna have been studied by Fahringer & Friese (1921), Fahringer (1922), Radoszkowski (1890), Özbek (1978, 1983, 1987, 1990), Rasmont (1984), Reing (1967, 1968, 1971, 1973, 1974), Reing & Rasmont (1983, 1988), Rasmont & Flagother (1996), Aytekin (2002, 2006).

The largest part of this literature concerns taxonomy.

Fähringer, J., 1921 Eine Hymenopteren-Ausbeute aus dem Amanusgebirge (Kleinasien und Nord-Syrien, südl. Armenien) *Archiv Naturgeschichte*, 87 A: 150-176; Fähringer, J., 1922 Hymenopterologische Ergebnisse einer wissenschaftlichen Studienreise nach der Türkei und Kleinasien (mit Ausschluss des Amanusgebirges) *Archiv Naturgeschichte*, 88 A: 149-222; Özbek, H., 1978 Dogu Anadolu'nun bazı yörelerinde elma ağaçlarında tozlaşma yapan arılar (Hymenoptera: Apoidea) *Atatürk Üniversitesi Ziraat Fakültesi Z. Dergisi Cilt*, 1978: 73-83; Özbek, H., 1987 Türkiye'nin *Psithyrus* Lepeletier (Hym.: Apidae) türleri. *Türkiye I. Entomoloji Kongresi, İzmir*, pp. 661-673; Özbek, H., 1983 Dogu Anadolu'nun Bazı Yörelerinde Bombinae (Hymenoptera: Apoidea, Bombidae) Türleri Üzerinde Taksonomik ve Bazı Biyolojik Çalışmalar. *Atatürk Üniversitesi Yayınları*, 621; *Ziraat Fakültesi Yayınları*, 128; Arastirmalar Serisi, 188: 1-70; Özbek, H., 1990 A new bumblebee species of *Pyrobombus* Dalla Torre in East-Anatolia. *Türk. Entomol. Derg.*, 14(4): 207-214; Radoszkowski, O., 1890 Hyménoptères récoltés sur le mont Ararat. *Trudy russk. ent. Obsch.*, 24: 502-510; Rasmont, P., 1984 Les Bourdons du genre *Bombus* Latreille sensu stricto en Europe Occidentale et Centrale (Hymenoptera, Apidae). *Spixiana, München*, 7: 135-160; Rasmont, P. & D. Flagothier, 1996 *Bitytographie et choix floraux des bourdons (Hymenoptera, Apidae) de la Turquie*. N.A.T.O.-O.T.A.N. T11-Pollination project, rapport préliminaire, Université de Mons-Hainaut, Adana Çukurova Üniversitesi, 69 + 3 pp; Reinig, W.F., 1968 Über die Hummeln und Schmarotzerhummeln Nordwest-Anatoliens (Hym., Apidae). *Nachrichtenblatt der Bayerischen Entomologen*, 17: 101-112; Reinig, W.F., 1971 Zur faunistik und Zoogeographie des vorderen Orients. 3. Beitrag zur Kenntnis der Hummeln und Schmarotzerhummeln Anatoliens (Hym., Apidae). *Veröffentlichungen der Zoologischen Staatssammlung München*, 15: 139-165; Reinig, W.F., 1973 Faunistische und zoogeographische Studien in Kleinasien. 4. Beitrag zur Kenntnis der anatolischen Hummeln (*Bombus* Latr., 1802) und Schmarotzerhummeln (*Psithyrus* Lep., 1832). (Hym., Apidae). *Mitteilungen der Münchner Entomologischen Gesellschaft*, 69: 110-133; Reinig, W.F., 1974 Faunistische und zoogeographische Studien in Kleinasien. 5. Auf Hummenfang in Taurus (*Bombus* Latr., 1802 et *Psithyrus* Lep., 1832; Hym., Apidae). *Nachrichtenblatt der Bayerischen Entomologen*, 23: 67-80; Reinig, W.F. & Rasmont, P., 1983 Über den anatolischen *Megabombus* (*Thoracobombus*) *pascuorum* (Scopoli, 1763). (Hymenoptera, Apidae). *Spixiana, München* 6: 153-165; Reinig, W.F. & Rasmont, P., 1988 Beitrag zur Kenntnis der Bergwaldhummeln *Alpigenobombus wurfleini* (Radoszkowsky, 1859). (Hymenoptera, Apidae, Bombinae). *Spixiana, München*, 11: 37-68;

#### Researchers in the project

Prof. Dr. Ir. Pierre Rasmont  
Assoc. Prof. Dr. A. Murat Aytekin  
Dr. Fatih Dikmenn  
PhD. Student Pinar Barkan  
PhD. Student Nicolas BRASERO ET MARTIN

#### Timing of the study

June 2013: field collections

Dean of UMONS Science Faculty  
Prof. Dr. Christian Vachaux



Dean of Hacettepe Faculty of Sciences  
Prof. Dr. Kadir Pekmez

Director for Belgian team:  
Head of zoology Service  
Prof. Dr. Ir. Pierre Rasmont

laboratoire de zoologie  
UNIVERSITÉ DE MONS  
av. du champ de mars, 6  
7000 MONS - BELGIQUE  
tel 065 373437 • fax 065 3734...

Director for Turkish Team  
Assc. Prof. Dr. A. Murat Aytekin

Prof. Dr. Kadir PEKMEZ  
Fen Fakültesi  
Dekan Vekili