

Deliverable No 4.2.1.: Defining appropriate bat population for genetic research

In the frame of project:

**"Sustainable bats conservation in the
cross border area"**

1846 BatsConserve

Department of Forestry and Natural Environment

Aristotle University of Thessaloniki

Thessaloniki 2017

The Western Rhodopes is an area particularly rich in bat species, hosting a very high diversity with a total of 30 species in of which 27 appear in the Greek part (Mertzanis et al. 2015, Youlatos 2015, Petrov & Helversen 2011), including 7 species listed under Annex II of the Habitats Directive, and 11 listed in the Greek Red Data Book of Threatened Animals (1 Endangered, 2 Vulnerable and 8 Near Threatened). Although significant progress has been made on the study of the batfauna of the area, researchers point at various gaps that need to be filled in order to acquire a more precise view of species composition, distribution and specific habitat requirements as well as genetic variation. New data will evidently lead to drafting effective conservation measures for both cave and tree-dwelling species, especially for those that are the most threatened ones.

The study area comprises the Nestos River catchment from the Greek-Bulgarian border to its estuary at the Aegean Sea. Although the major colonies of cave-dwelling bats have been investigated in the upper part of Nestos river (Mertzanis et al. 2015, Petrov & Helversen 2011), there is need for further research of potentially suitable underground sites, buildings and galleries at the middle and lower parts of the catchment. Concerning the tree-dwelling species, information is more limited and consequently forest-dwelling bats are underestimated both in the Greek and the Bulgarian part of Rhodope Mts (Petrov & Helversen 2011). Research should focus on bats in forests of all types and altitudes, including the upper tree line zone.

Furthermore, genetic analysis of samples from selected species will contribute to ongoing research on phylogenetics and the assessment of population genetic status for these species. Such assessments will provide information on genetic diversity and population connectivity and increase our understanding of the need for management and conservation of the selected species. For example accumulation of genetic and morphologic data is needed to justify the variations and allow practical species identification, especially for the Whiskered bats, or the *Myotis mystacinus* morphogroup, which is a group of morphologically very similar bats, including the smallest representatives of the genus *Myotis* (Benda et al. 2016). On the Balkan Peninsula, *M. aurascens* is considered the most common species from the whole group (Benda & Tsytsulina 2000), while

Myotis mystacinus is reported from the Bulgarian part of Rhodope Mts. (Petrov & Helversen 2011). At present, firm identification of these species in field conditions is not possible.

At first, in order to address the above-mentioned research necessities, we selected 6 focal species based on their conservation status, distribution in the study area, representative habitat and population status in order to ensure adequate genetic sampling. Three cave/crevice-dwelling species and three tree-dwelling species were selected:

1. ***Myotis aurascens***: crevice-dwelling, Data Deficient (GRB), wide distribution, forest and scrub habitats (including Mediterranean-type scrub) particularly near water, sedentary.
2. ***Rhinolophus euryale***: status Near Threatened (NT, IUCN & GRDB) cave dwelling.
3. ***Miniopterus schreibersii***: cave-dwelling, Near Threatened (GRB), wide distribution, regional movements. In Bulgaria, over 95% of the wintering population is in three caves: Parnitsite, Devetashkata, Dyavolsko garlo Smolyan.
4. ***Rhinolophus blasii***: cave-dwelling, Near Threatened (GRB), restricted distribution in the Balkans, shrubland and woodland habitat, sedentary. It is a species of interest due to its limited distribution in the Balkans. In more southerly situated Greece, *R. blasii* is the dominant species among the medium-sized horseshoe bats (Hanák et al. 2001), and this phenomenon is also evident in southern regions of Bulgaria (Popov & Ivanova 2002).
5. ***Barbastella barbastellus***: tree-dwelling, Endangered (GRB), mature forests, sedentary. Special design is needed to capture this species. It has been recorded in the birch forest. It requires special technique in nets and long length.
6. ***Pipistrellus pygmaeus***: tree-dwelling, Data Deficient (GRB), wide distribution, migratory.
7. ***Nyctalus leisleri***: tree-dwelling, Least Concern (GRB), wide distribution, woodland (both Eurosiberian and Mediterranean), pasture, and river valleys, feeds on flies including mosquitos, migratory.

During the program, as sampling, meetings and cross-border communication between the researchers who participated in the program, took place, and as new literature and scientific

research came to our knowledge, the initial estimates were enriched and adapted to the new data. Additional parameters, that were taken into account, were the ease or difficulty of sampling in relation to the particularities of each species. Thus, more species were added to the initial estimates.

As common species were selected *Rhinolophus hipposideros* and *Rhinolophus ferrumequinum*, which are widely distributed in the study area, while they can also be captured during the day in shelters (they are usually found in caves and man-made structures).

Pipistrellus group (P. pipistrellus, P. pygmaeus / mediterraneus): Distinctive call frequency and variability in genetic markers reveal the sympatric coexistence of two separate species.

Myotis spp. : M. emarginatus (status IUCN: Least Concern, GRDB Near Threatened), ***M. bechsteinii*** (status Near Threatened IUCN & GRDB), ***M. capaccinii***, ***M. daubentonii***, ***M. myotis*** (status IUCN Least Concern, GRDB Near Threatened) (at the points Potamon bridge, Volaka mines, Maronia cave). ***Myotis myotis***: Genetic markers: mitochondrial DNA (mtDNA) control region polymorphism and variation at 10 nuclear microsatellite loci. Rodopi appears as a major barrier to gene flow (Ruedi & Castella, 2003).

In this project, we focused on the monitoring of known sites as well as the research for new areas. The final definitions of the species and the sampling performed are presented in the [deliverable 4.2.2.](#)

Roost site condition, habitat characteristics and quality assessed at suitable extend around each major colony, and at all acoustic sampling and mist netting sites. The associations between bat roosting or foraging locations and landscape structure were examined.

For the genetic analyses we used mitochondrial DNA control region sequences and suitable polymorphic microsatellite loci to infer genetic relationships among subpopulations, estimate levels of genetic diversity, and potentially examine population connectivity among colonies. From each individual we took three wing membrane samples with a 3-mm-diameter biopsy punch. Bats were captured using mist nets or a harp trap at cave entrances and at their feeding grounds during periods of peak foraging activity in forest, riverine and agricultural habitats. In our laboratories, with the first genetic samples that arrived, preliminary analyses were performed using the method of genetic

analysis of microsatellite DNA (SSRs), to determine if our material was in good condition for the analyzes and to select the appropriate SSR markers. Total DNA was extracted using the standard Phenol:Chloroform:Isoamyl alcohol protocol. The DNA extraction process was successfully carried out in all samples that were collected. The mitochondrial COI gene was amplified in all the samples. Most of these amplified COI samples, have been sequenced. The samples with good quality of DNA sequencing were used for identification. A total of 18 microsatellite markers were selected that are capable to amplify *Miniopterus schreibersii*, *Myotis* and *Rhinolophus* species.

After an open competition according to the program's technical bulletin, the company ACCESS TO GENOME (ATG) was selected to carry out the genetic analysis.



Litterature

- Appleton B.R., McKenzie J.A., Christidis L. 2004, Molecular systematics and biogeography of the bent-wing bat complex *Miniopterus schreibersii* (Kuhl 1817) *Molecular Phylogenetics and Evolution*, 31, 431-439.
- Battersby, J. (comp.) (2010): *Guidelines for Surveillance and Monitoring of European Bats*. EUROBATS Publication Series No. 5. UNEP / EUROBATS Secretariat, Bonn, Germany, 95 pp.
- Benda, P, S. Gazaryan, P. Vallo, 2016. On the distribution and taxonomy of bats of the *Myotis mystacinus* morphogroup from the Caucasus region (Chiroptera: Vespertilionidae). *Turk J Zool* (2016) 40: 842-863.
- Benda, P., K.A. Tsytsulina, 2000. Taxonomic revision of *Myotis mystacinus* group (Mammalia: Chiroptera) in the western Palearctic. *Acta Soc Zool Bohem* 64: 331-398.
- Clare E.L. et al, 2006. DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular notes* 2006.
- Kruskop S.V. 2012. Genetic diversity of northeastern Palaeartic bats as revealed by DNA barcodes. *Acta Chiropterologica*, 14(1): 1–14.
- Loureiro O.L., Lim B.K., Engstrom M.D., 2018, Molecular data on the CO1 and beta fibrinogen gene in the evolutionary relationships of the mastiff bat (Chiroptera, Molossidae, Molossus). *Data in brief*, 18, 1609-1613
- Petrov, B., von Helversen, O., 2011. Bats (Mammalia: Chiroptera) of the Western Rhodopes mountain (Bulgaria & Greece). — In: Beron, P. (ed.). *Biodiversity of Bulgaria*, 4. *Biodiversity of Western Rhodopes (Bulgaria and Greece)*. Pensoft & Nat. Mus. Natur. Hist., Sofia, 525—581.
- Ruedi, M., Castella, V., 2003. Genetic consequences of the ice ages on nurseries of the bat *Myotis*

myotis: A mitochondrial and nuclear survey. *Molecular Ecology*. doi:10.1046/j.1365-294X.2003.01828.x.

Ruedi M. et al, 2015, Molecular phylogeny and morphological revision of *Myotis* bats (Chiroptera: Vespertilionidae) from Taiwan and adjacent China. *Zootaxa*, 3920, 301-342

Γιουλάτος, Δ., 2015. Επιστημονικός Υπεύθυνος «Πρόγραμμα Παρακολούθησης Θηλαστικών στην περιοχή του ΕΠΑΜΑΘ (2012-2015)».

Μερτζάνης, Γ., Γαλανάκη, Α., Γεωργιακάκης, Π., Ηλιόπουλος, Γ., Θεοδωρόπουλος, Ι. Κομηνός, Θ., Κοντσιώτης, Β., Παπαϊωάννου, Χ., Παλάσκας, Δ., Τράγος, Α., Τσιάρας, Δ. και Νούσκα, Π. 2015. Φάση Γ': Ολοκλήρωση της αξιολόγησης της κατάστασης διατήρησης – Συγκρότηση πρότασης προγράμματος μελλοντικής συστηματικής παρακολούθησης. Έργο: «Εποπτεία και αξιολόγηση της κατάστασης διατήρησης ειδών θηλαστικών κοινοτικού ενδιαφέροντος του Εθνικού Πάρκου Οροσειράς Ροδόπης». Ανάδοχοι Δ. Τσιάρας - Α. Τσιάρας. Σελ. 453.