

## Blood Serum Proteins of *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii* (Tomes, 1857) (Chiroptera: Vespertilionidae)

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### Abstract

This study is based on the globulin and albumin proteins, determined using SDS-PAGE, of two sibling bat species *Myotis myotis* (Borkhausen, 1797) (Greater Mouse-eared Myotis) and *M. blythii* (Tomes, 1857) (Lesser Mouse-eared Myotis) distributed in Turkey. No difference is found in the globulin and albumin protein bands. It was concluded that blood serum proteins could not be enough diagnostic character for separating *Myotis myotis* from *M. blythii*.

**Key words:** *Myotis myotis*, *Myotis blythii*, SDS-PAGE, Turkey

### INTRODUCTION

The genus *Myotis* is represented by 103 species in the Holoarctic Region [1, 2]. The taxonomic status of two taxa of this genus, *Myotis myotis* (Borkhausen, 1797) (Greater Mouse-eared bat) and *Myotis blythii* (Tomes, 1857) (Lesser Mouse-eared bat), are still controversial.

*Myotis myotis* (Borkhausen, 1797) is represented by *M. myotis myotis* distributed in Turkish Thrace, and *M. myotis macrocephalicus* distributed in Mediterranean Region. In addition, *M. blythii* (Tomes, 1857) is represented by *M. blythii oxygnathus* and *M. blythii omari* [3].

Although taxonomic, karyologic or biologic studies of two taxa were achieved from Turkey [4-7] no studies have been achieved on the SDS-PAGE patterns of blood serum proteins.

The objective of this paper is to describe the blood serum proteins, using SDS-PAGE, of both sibling species.

### MATERIAL AND METHODS

Study area: Edirne (41° 40' N 26° 33' E), Kırklareli (41° 44' N 27° 13' E), Bolu (40° 47' N 32° 11' E), Kırıkkale (39° 40' N 33° 36' E), Tokat (40° 16' N 36° 17' E), Artvin (41° 07' N 42° 03' E), Şanlıurfa (37° 01' N 37° 59' E), Diyarbakır (38° 16' N 39° 45' E), Batman (37° 42' N 41° 26' E), Mersin (36° 38' N 33° 26' E), Kilis (36° 43' N 37° 06' E), Gaziantep (37° 03' N 37° 22' E).

This study is based on 34 (29 ♂♂ and 5 ♀♀) *Myotis myotis* and 9 (6 ♂♂ and 3 ♀♀) *M. blythii* specimens collected from different localities of Turkey between the years 2003 and 2006 (Fig. 1).



**Fig. 1.** Two sibling species *Myotis myotis* (A) and *Myotis blythii* (B)

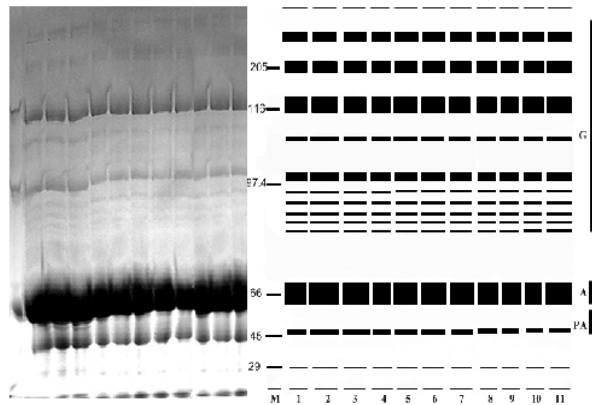
Blood was taken by cardiac puncture with a dispersal 2 ml syringe, from anesthetized specimen. After blood clotting the sera, mixed with 0.0625 M Tris Cl, pH 6.8, 2 % SDS, 10 % Glycerol, 5% 2-Mercaptoethanol and 0.01% bromphenol blue, were boiled for 3-3.5 minutes and preserved at -80°C until electrophoresis. SDS-PAGE was performed according to the method of Laemmli [8]. Separating (7.5 %) and stacking (4 %) gels were prepared according to the method of Sambrook et al. [9]. SIGMA Protein Molecular Weight Marker (MW-SDS-200) was applied. Samples and the marker of 30 µl were applied to the gel. Electrophoresis was carried out using a MBT80EL model double vertical slab gel electrophoresis apparatus. 10 V/cm and 20 V/cm were applied to the stacking and separating gel, respectively. The gel was run about 4.5 hours and then stained with 0.25 % Coomassie Brilliant Blue R-250 over the night. After destaining in a mixed washing solution of 45 ml methanol: 10 ml acetic acid: 45 ml dH<sub>2</sub>O, gel was photographed. Blood serum proteins were evaluated as globulin and albumin regions.

The albumin region is subdivided into prealbumin (PA), albumin (A) and postalbumin (PsA) zones.

The stuffed and skinned specimens as well as karyotype preparations are deposited in the zoology museum of Department of Biology, University of Kırıkkale.

## RESULTS

Blood serum proteins of *Myotis myotis* and *M. blythii* using SDS-PAGE were first examined by this study. Female and male specimens are evaluated together, no differences were found between the sexes (Fig. 2).



**Fig. 2.** Blood serum proteins of *Myotis myotis* (6-11) and *Myotis blythii* (1-5). G= Globulin, A= Albumin, PA= Prealbumin. 1. Mersin (KKU04003 ♂), 2. Diyarbakır (KKU05008 ♂), 3. Gaziantep (KKU06012 ♂), 4. Kırıkkale (KKU04019 ♂), 5. Diyarbakır (KKU05009 ♀), 6. Edirne (KKU04032 ♂), 7. Kilis (KKU06016 ♂), 8. Kırıkkale (KKU04017♂), 9. Tokat (KKU05030 ♂), 10. Kırıkkale (KKU04018 ♀), 11. Artvin (KKU04040 ♂), M=Marker

There were 10 bands in the globulin region, one band in albumin and three bands in the prealbumin zones. In contrast, no band was found in the postalbumin zone. In both species, the bands in the line of marker protein 205.000 D and 66.000 D were stained very weakly. The bands between the line of 97.400 D and 66.000 D of the specimens 8-11 were more detectable than the others.

## DISCUSSION

Morphology, chromosomal banding, protein electrophoresis, protein sequencing, immunology, DNA hybridization and DNA sequencing techniques are used for determining the relationship levels between the taxa [10].

In Turkey, many electrophoretic studies on the blood serum proteins of various rodents, were achieved [11-17]. To date, no electrophoretic study on blood serum proteins of bats has not been done yet. This study represents the first records of blood serum proteins of two sibling vespertilionid bats.

The electrophoretic patterns expected to be a useful diagnostic character for separating the two taxa with this

study. In conclusion, similarities in the electrophoretic band patterns of blood serum proteins supported the claim; in addition to identical karyotype and morphology, electrophoretic aspects of blood serum proteins also could not be a diagnostic character for separating greater mouse-eared bat from lesser mouse-eared bat.

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