



Taxonomic separation of the red-cheeked ground squirrel from South-East Kazakhstan



www.bioacoustica.org

Volodina Elena, Matrosova V., Volodin I., Ivanova A., Alexandrov D., Sibiryakova O. & Ermakov O.

Moscow Zoo,
Engelhardt Institute of Molecular Biology RAS
Lomonosov Moscow State University,
Severtsov Institute of Ecology and Evolution RAS
Penza State University

INTRODUCTION

At least three species of ground squirrels are known from South-East Kazakhstan. These species are often considered as one wide-range polymorphic species complex, the red-cheeked ground squirrel *Spermophilus erythrogenys* sensu lato. From another point of view, *S. erythrogenys* s.l. includes a few morphologically different forms considered as separate species. The distribution area and taxonomic borders of this combined species remain questionable. We used **molecular & bioacoustic tools** for taxonomic separation of ground squirrels inhabiting the Dzungarian Alatau region of South-East Kazakhstan and adjacent territories.

METHODS & RESULTS

Alarm call and DNA samples have been collected in the South-East Kazakhstan in June of 2015 in three natural populations (Tara, Altyn-Emel and Balkhash), 10 individuals per population.



ACOUSTICS (*N=30 alive individuals*)

Ground squirrels have species-specific alarm calls and bioacoustic analysis have proved to be useful taxonomic tool for identifying ground squirrel species, subspecies and interspecies hybrids. We examined acoustic structure of alarm calls produced by individually marked animals toward a human from a live-traps. Spectrographic analysis of calls was made with Avisoft SASLab Pro 4.3.

The alarm call variables were **very similar** between all the three study populations and **distinctive** from those of *S. erythrogenys* from the Kurgan region of Russia by their maximum fundamental frequencies (8.46 ± 0.75 kHz vs. 5.62 ± 0.06 kHz) and by the note *duration* (167 ± 25 ms vs. 119.8 ± 73.98 ms) (Zhilin 2002; Nikol'skii & Rumyantsev 2004). Our results fully confirmed the early evidence of large acoustic differences of the probable *S. intermedius* from the South-East Kazakhstan and of the probable *S. erythrogenys* from Russia.



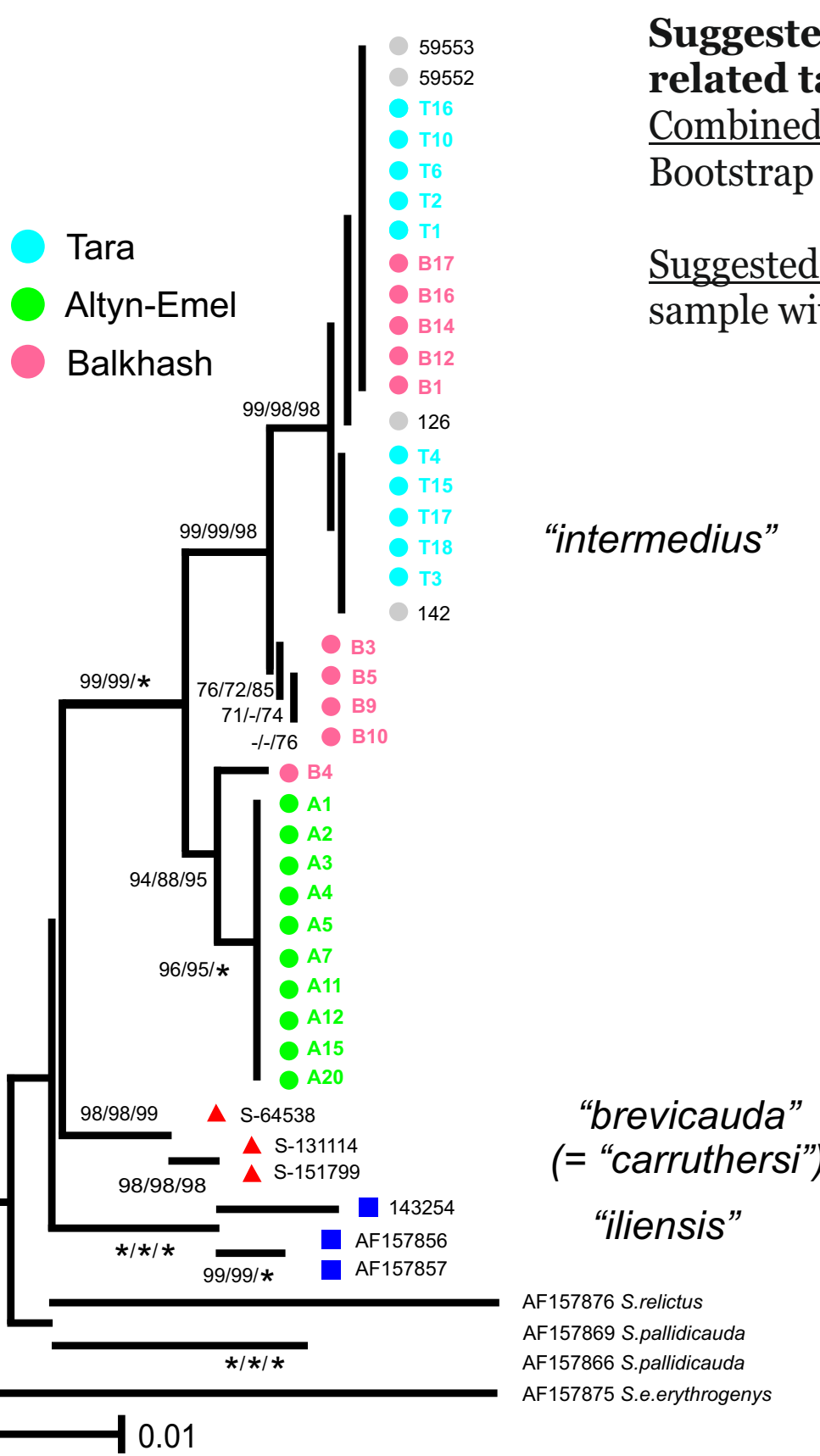
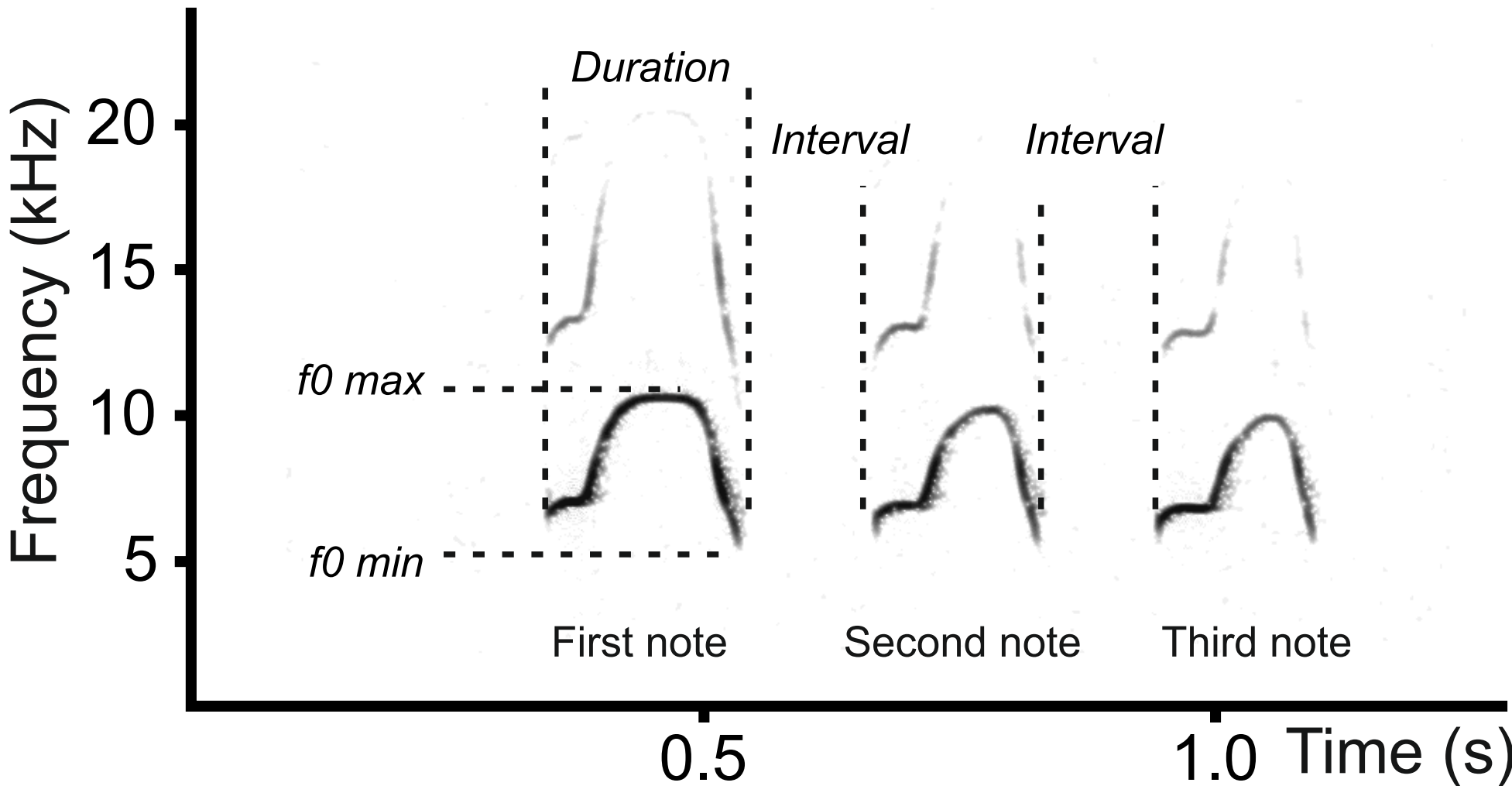
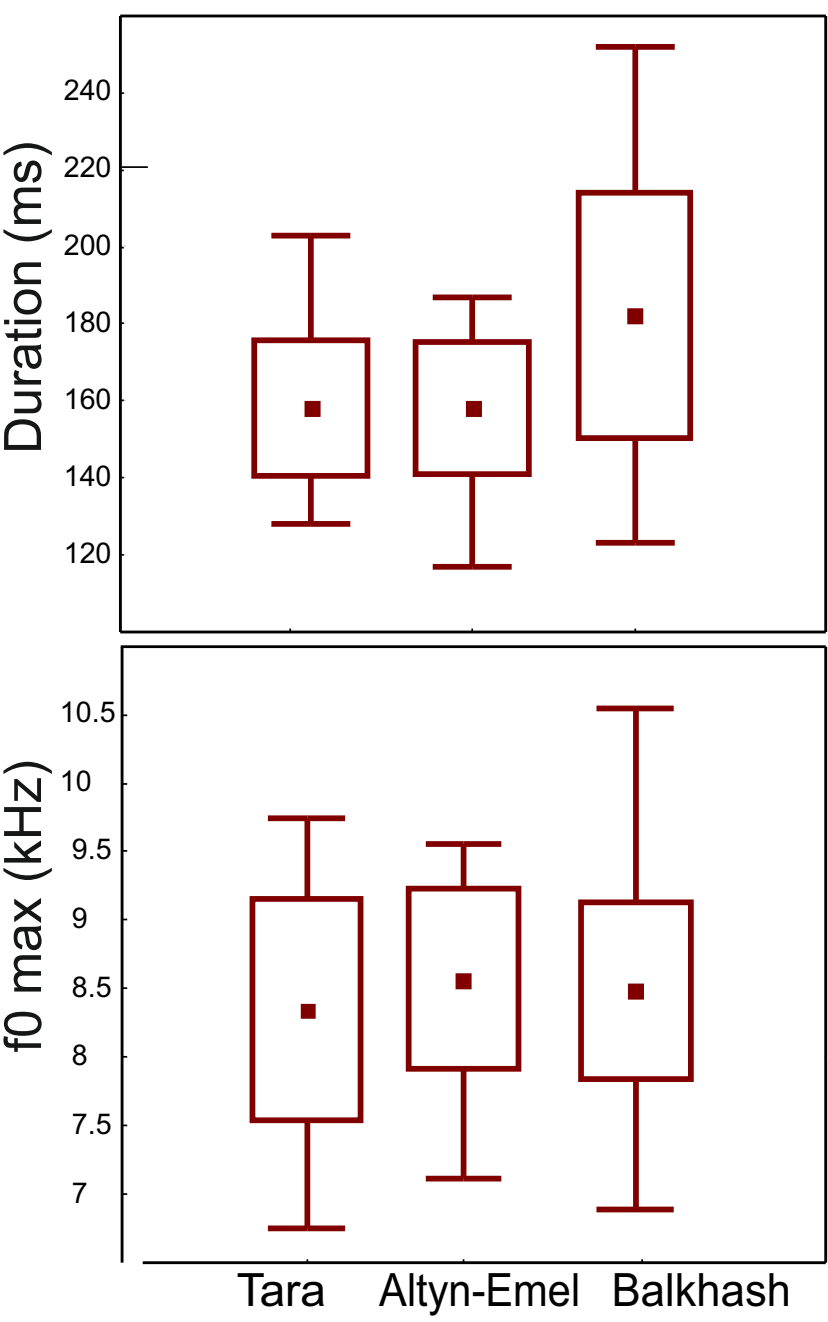
GENETICS (*N=30 alive individuals, 8 museum samples & 6 sequences from Genbank*)

We examined individuals for nucleotide polymorphism of the **mtDNA Control region** (1005-1006 bp) and **cyt b gene** (1140 bp). We also examined DNA of eight museum specimens of *S. brevicauda*, *S. intermedius*, *S. carruthersi* and *S. iliensis* originating from the species complex distribution area. Additionally, six *cytb* sequences from Genbank were used.

The study animals were quite conservative in the structure of mtDNA (variation in Control region 3% , in *cyt b* - 2.5%). On the phylogenetic tree based on the *cyt b* polymorphism, the ground squirrels from South-East Kazakhstan and adjacent territories **divided on three clades** with high (98–100%) bootstrap support:
1) *S. iliensis* (west of species area);
2) *S. intermedius* (center);
3) *S. brevicauda* (= *S. carruthersi*) (south-east).

Inter-population differences of the alarm call *duration* and maximum fundamental frequency (*f0 max*). Central points show means, boxes show \pm SD, whiskers show min-max values.

Measured alarm call variables:
f0 max – the maximum fundamental frequency;
f0 min – the minimum fundamental frequency;
duration – duration of a note;
interval – between-note interval.



Suggested phylogenetic relationships of red-cheeked ground squirrels and the closely related taxa. Combined phylogenetic tree (NJ/ML/ME) based on complete *cyt b* gene sequences. Bootstrap support is shown only for the values exceeding 70%. Stars designate 100% bootstrap support. Suggested distribution areas for the given forms. Figures near a sample name designate clustering of the sample with a particular taxon accordingly to the *cyt b* polymorphism. Stars designate type locality.



CONCLUSIONS:

Study populations of *S. intermedius* probably deserve the **species-level taxonomic rank**. These data suggest that *S. erythrogenys* represents a paraphyletic taxon on **both genetic (mtDNA) and phenotypic (alarm call) traits**. A new taxonomic revision is needed.



Financial Support: RFBR (grant 18-04-00400).