Zoologischer Anzeiger 270 (2017) 9–18

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Zoologischer Anzeiger

Research paper

Craniometrics are not outdated: Interspecific morphological divergence in cryptic arvicoline rodents from Iran

Ahmad Mahmoudi[a,*], Boris Kryštufek[b], Jamshid Darvish[a,c], Mansour Aliabadian[a,d], Fatemeh Tabatabaei Yazdi[e], Faezeh Yazdani Moghaddam[a,d], Franc Janžekovič[f]

[a] Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[b] Slovenian Museum of Natural History, Prešernova 20, SI-1000 Ljubljana, Slovenia
[c] Rodentology Research Department, Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[d] Zoological Innovations Research Department, Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[e] Department of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran
[f] University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia

ARTICLE INFO

Article history:
Received 19 February 2017
Received in revised form 12 August 2017
Accepted 30 August 2017
Available online 6 September 2017
Corresponding Editor: Alexander Kupfer

Keywords:
Traditional morphometrics
Morphometric evolution
Multivariate statistics
Phenotypical plasticity
Phylogenetic signal
Microtus
Sumeriomys

ABSTRACT

We studied interspecific variability in external, cranial, and dental traits in seven species belonging to two closely related arvicoline (Arvicolinae) subgenera, the social voles (*Sumeriomys*) and the grey voles (*Microtus*). These voles were for long regarded as morphologically cryptic and the species complexity was fully appreciated only after chromosomal and molecular markers were utilized. Specimens we examined displayed clear external differences between the subgenera. Social voles had five plantar pads, relatively shorter tail (22–27% of head and body length), lighter dorsal pelage, whitish grey belly, and monochromatic or indistinctly bicolored tail. Grey voles showed six plantar pads, relatively longer tail (33–44%), dark brown back, grey belly, and sharply bicolored tail. Our results retrieved considerable heterogeneity in cranial and dental morphology. Major cranial differences between the subgenera associated with the interorbital region, the braincase and the tympanic bullae. Genetic (Kimura 2-parameter) distances, which presumably provided a priori correct estimate of the true phylogenetic divergences, explained 42% of morphometric distances between species. Below the level of a subgenus the phylogenetic signal was conserved in grey voles but it dissolved in social voles. The molecular and morphological rates of evolution were obviously decoupled in the latter possibly by selective pressures for a particular phenotype. Climatic variables however explained only 11% of interspecific heterogeneity in cranial shape. The most distinct in terms of morphology and the climatic properties of its habitat was *Microtus irani* which also occupies the very edge of *Microtus* distribution. High species richness of social and grey voles combined with heterogeneity of environmental conditions makes the Middle East an ideal region for studying diversity of developmental trajectories in voles at the levels of species and populations.

1. Introduction

Molecular studies are profoundly reshaping our understanding of organic evolution in general and of speciation in particular. We are gaining an entirely new insight into the evolutionary structuring of species and on the geographic pattern of infraspecific phylogenetic lineages. Interrelationships among species and species groups are getting clarity which could not be deduced in the past from non-molecular markers. New and powerful molecular techniques are cracking "good old species" into a bunch of (frequently allopatric) species (Zachos et al., 2013). These new species are often regarded as morphologically cryptic, but phylogenetic studies usually restrict themselves to genes (Baker and Bradley, 2006). Morphology is no longer at the edge of taxonomic studies and is lagging behind the rapidly expanding molecular assessments. As a result we are frequently left ignorant of the morphology of the newly recognized lineages and species. There are, however, good reasons for analyzing morphometric variation and combining molecular trees with dendrograms based on morphometric divergences (Padial et al., 2010). Morphometrics incorporates ele-

* Corresponding author at: Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, P.O. Box: 9157948974, Mashhad, Iran.
E-mail addresses: a.mahmoudi.bio@gmail.com (A. Mahmoudi), bkrystufek@pms-lj.si (B. Kryštufek), darvishj2001@yahoo.com (J. Darvish), aliabadian@yahoo.com (M. Aliabadian), fatemeh.tabata@gmail.com (F. Tabatabaei Yazdi), faezeh.um@gmail.com (F. Yazdani Moghaddam), franc.janzekovic@um.si (F. Janžekovič).

http://dx.doi.org/10.1016/j.jcz.2017.08.008
0044-5231/

ments of quantification and hypothesis testing and is therefore not outdated as a tool in evolutionary, developmental and systematic biology (Henderson, 2006; Laffont et al., 2011). All this makes morphometric analysis a vital part of any approach towards integral taxonomy (Schlick-Steiner et al., 2010).

We addressed craniometric variability in *Microtus* Schrank, 1798, voles from Iran. Specifically, we studied seven species belonging to two sister groups which are classified either as subgenera (Gromov and Polyakov, 1977; Shenbrot and Krasnov, 2005) or species groups of the genus *Microtus* (Jaarola et al., 2004; Martínková and Moravec, 2012). These are the social voles (subgenus *Sumeriomys* Argyropulo, 1933, or *socialis* species group) and grey voles (subgenus *Microtus* s.str. or *arvalis* species group). The first comprehensive assessment of the Iranian mammal fauna, which was based entirely on the morphological examination of museum vouchers (Lay, 1967) recognized only one species in each group, i.e. *M. socialis* (Pallas, 1773) and *M. arvalis* (Pallas, 1778). Following the application of chromosomal (Mahmoudi et al., 2014b) and molecular markers (Mahmoudi et al., 2017) the number of species raised up to the current eight.

In this paper we report on cranial variability in Iranian representatives of grey and social voles. Quite remarkably but this issue was never addressed beyond a simple comparison of linear measurements (e.g. Lay, 1967). By performing a postcladistic morphological analysis of monophyletic lineages (cf. Cardini and O'Higgins, 2004) in an animal group with a reasonably known evolutionary history, we first explored whether there are significant morphological differences between closely related and presumably cryptic species? Next we were interested on the underlying drivers of morphometric divergence. Diverse morphologies may be the consequence of different genomes which reflect idiosyncratic phylogenetic history of each species. This would produce concordance divergence estimates between the two datasets, namely between the nucleotide sequences and the morphometric structures (Ahrens and Ribera, 2011).

In reality selection pressures frequently favor a particular phenotype and therefore decouple the molecular and morphological rates of evolution (Ahrens and Ribera, 2011). Under such circumstance a response in the phenotype is due to phenotypic plasticity to environmental change rather than to a genetic process (Teplitsky et al., 2008). Such responses can be swift. In arvicolines they are known to develop even in genetically uniform populations (Pankakoski and Nurmi, 1986; Yoccoz et al., 1993).

We investigated in this study whether craniodental structures in seven Iranian voles produce phenetic trees of compatible topology to the accepted taxonomic hierarchy (Musser and Carleton, 2005) and to phylogenetic trees (Mahmoudi et al., 2017). If this would be the case than the grey voles would be morphologically more similar among themselves than to social voles, and vice versa. Alternatively the phylogenetic signal in craniodental structures would be diluted by adaptive plasticity which would result in discordance between morphometric and genetic distances.

2. Material and methods

2.1. Samples

We studied 192 *Microtus* specimens collected from 27 localities in Iran (Fig. 1, Table 1). Specimens are deposited in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM). Analyses were conducted on complete skulls belonging to seven species: *Microtus paradoxus* (Ognev and Heptner, 1928) (14 females/15 males), *M. socialis* (11/10), *M. qazvinensis* (Golenishchev, 2003) (24/17), *M. irani* (Thomas, 1921) (11/13), *M. obscurus* (Evermsann, 1841) (11/6), *M. transcaspicus* (Satunin, 1905) (15/15), and *M. mystacinus* (de Filippi 1865) (14/16). Mitochondrial and/or chromosomal profile of museum vouchers has been retrieved in earlier studies (Mahmoudi et al., 2014a,b, 2015, 2017). To minimize the effect of ontogenetic growth, only adult voles were used in all analyses. Following age criteria set by Kryštufek and Vohralik (2005), we defined adults as having well-developed temporal ridges and prominent postorbital processes.

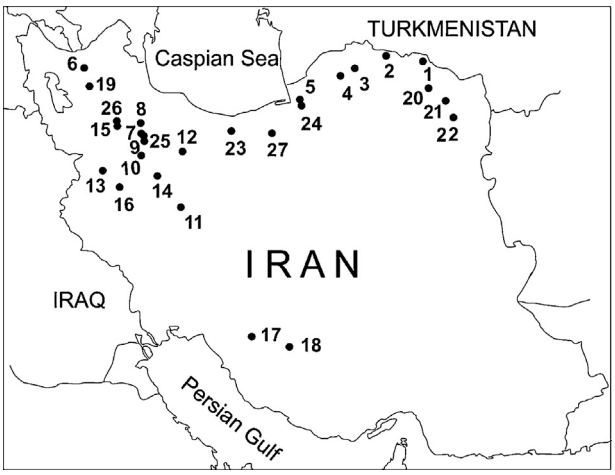

Fig. 1. Map showing sampling localities of *Microtus* species used in this study. For sample identities see Table 1.

2.2. Morphometric characters and analyses

A total of 22 craniodental measurements were scored under a dissecting microscope fitted with an eyepiece graticule (dental measurements) or using a digital caliper accurate to the nearest 0.1 mm (all other measurements). See Fig. 2 for definition of measurements and their abbreviations. All measurements were transformed to logarithms in order to decrease differences in variance between variables. The normality of data distribution and the homogeneity of the variances were tested by Shapiro-Wilk and Levence' tests, respectively. Because no substantial departures from normality and/or homoscedasticity were found within the samples (all $p > 0.05$), a Two-way multivariate analysis of variance (MANOVA) was performed to evaluate variation across the variables in relation to species and sex. The significance level was set at $p < 0.05$. To summarize the data and the distributions of the variables per species, descriptive statistics (mean, standard deviation, minimum and maximum) were applied for all measurements (see Appendix A).

We used principal components analysis (PCA) to characterize the morphological variation among species and to find patterns in our high-dimensional data (Lemen, 1983). PCA was performed on a correlation matrix of $\log_{10}$-transformed craniodental variables. Because the resulting principal components (PCs) are mutually uncorrelated, each of them measures different dimensions of the original data set. The first morphological PC (MPC1) is responsible for the largest proportion of variance and is interpreted in morphometrics as size vector while the remaining components account for size-out (shape) variance (cf. Kryštufek et al., 2015). Discriminant analysis (DA) was carried out on the first few MPCs to estimate the percentage of correctly classified specimens into each species. The Jackknife procedure was used to avoid the risk of overfitting the data.

An unweighted pair group method with arithmetic mean (UPGMA), a fast and simple approach to make distance based trees, was used to visualize phenetic relationships using distance matrix derived from morphological measurements (Sokal and Michener,