

Skull Non-Metrical Characters of European Water Vole (*Arvicola amphibius*, L.) from Hungary: Cranial Evidence for its Geographic Detachment

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Abstract: The epigenetic cranial polymorphism and population epigenetic variation of the European water vole (*Arvicola amphibius* Linnaeus, 1758) from Hungary based on the frequencies of 13 non-metric cranial traits was studied. The comparative analysis of epigenetic distances of Hungarian water vole from other populations of *A. amphibius* and from southern water vole (*Arvicola sapidus* Miller, 1908) from Europe revealed a sharp distinction of the Hungarian population from the others. Registered considerable epigenetic uniqueness of *A. amphibius* in the central part of its European area proved the polytypical cranial epigenetic character of the water vole in Europe.

Key words: non-metric variability, European water vole, *Arvicola amphibious*

Introduction

According to contemporary taxonomic concepts (BATSAIKHAN *et al.* 2012), the Linnaeus' species names *amphibius* and *terrestris* are considered as conspecific species of European water vole; CORBET (1978) pointed that the name *amphibius* has a priority over *terrestris*, based on BLASIUS (1857) as the first reviser; thus *A. amphibius* is the valid name of the European water vole (MUSSEY AND CARLETON 2005). The species range in Europe is large, extending from United Kingdom and France in the west, through much of continental Europe, in the central part of which is located Hungary, through European Russia and eastwards as far as the Lena Basin and Lake Baikal in Siberia (SHENBROT AND KRASNOV 2005).

Because of its high ecological plasticity, European water vole is able to inhabit various biotopes in the wetlands (HARRISON AND BATES 1991).

In Hungary, it occupies a range of habitats around rivers, streams and marshes and irrigation ditches in burrows excavated within the banks of rivers, ditches, ponds, and streams. Burrows are normally located adjacent to slow moving calm water.

The results of the analyses on sequences from 800 to 1, 200 bp of the mitochondrial cytochrome b gene, revealing the mitochondrial phylogeny of European water vole, represented the intraspecific taxonomic structure of *A. amphibius* as a complex of three main groups: the first group consists of strictly fossorial water voles and is distributed in the mountain regions of Europe; the second group consists of aquatic and transitional populations and occurs south of the Alps, and the third one is heterogeneous and covers the remaining aquatic populations (WUST SAUCY 1998).

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The heterogeneity of intraspecies taxonomic structure of *A. amphibius* was also confirmed by the rate of epigenetic cranial divergence of water vole in Bulgaria, which revealed its considerable epigenetic uniqueness and proves the polytypical epigenetic character of the water vole in Europe (MARKOV 2004).

The lack of information about skull non-metrical characters of *A. amphibius* from Hungary, which can reveal the degree of similarity of its cranial epigenetic characteristics with other populations of European water vole and to prove its geographic differentiation among coexisting population in Europe, determined the goals of the present study to: (i) elaborate the population epigenetic characteristics of *A. amphibius* from Hungary; (ii) carry out comparative analysis of the epigenetic distances between the water vole from Hungary and other populations from Europe as well as between Hungarian specimens and specimens of the Southern water vole (*A. sapidus* Miller, 1908) from Europe; and (iii) find out the rate of epigenetic divergence of water vole in Hungary and its relative epigenetic differentiation within the studied populations of genus *Arvicola* in Europe.

Material and Methods

This study is based on epigenetic comparison of the skull non-metrical features of 64 adult specimens of water vole *A. amphibius*. The studied skulls have been kept in the mammals' collection of Hungarian Natural History Museum in Budapest. The examined specimens were obtained from Southern Great Plain, from typical for the species habitats in Hungary; one of the sample locations is situated in Bács-Kiskun county, in the centre of the region between Danube and Tisza rivers, in the sandy territory of Kiskunság, and the other is situated in Somogy county, which lies in South-western Hungary and stretches between the river Drave and the southern shore of Lake Balaton.

The non-metric cranial characteristics of the specimens were revealed based on 13 characters used by CORBET *et al.* (1970). All the traits were scored as discrete variables. In accordance with the adopted scheme their status were scored as follows: N 1. *Fenestra flocculi* present; N 2. *Preorbital foramen* double; N 3. *Anterior frontal foramen* present; N 4. *Posterior frontal foramen* double; N 5. *Maxillary foramen I* present; N 6. *Maxillary foramen II* (lat-

eral) present; N 7. *Infra maxillary crest* medium or large; N 8. *Foramen sphenoidale* medium present; N 9. *Processus pterygoideus* present; N 10. *Foramen ovale* double; N 11. *Foramen hipoglossi* double; N 12 *Basioccipital foramen* present. N 13. *Mental foramen* double.

The characters were scored at 12x magnification, on the left side of the cranium. The age of the specimens individuals were determined using the criteria proposed by CORBET *et al.* (1970).

The sexual dependence of the studied characters was tested by Chi-square method.

The comparative analysis of the epigenetic distances of the water vole population from Hungary [Group *A. a.* ₍₁₀₎] with other populations of *A. amphibius* and with the southern water vole (*A. sapidus*) from Europe was carried out using the frequency distribution of 13 non-metric cranial traits determine by CORBET *et al.* (1970) in 8 samples of genus *Arvicola* from Europe – five samples belonging to *A. amphibius* (former *A. terrestris*) from the Alps - France, Italy and Switzerland [Group *A. a.* ₍₂₎]; former Yugoslavia [Group *A. a.* ₍₃₎]; Germany [Group *A. a.* ₍₄₎]; Norway [Group *A. a.* ₍₅₎]; Pyrenees I [Group *A. a.* ₍₆₎] and three samples of *A. sapidus* (from South Spain [Group *A. s.* ₍₇₎]; Pyrenees II [Group *A. s.* ₍₈₎]; North Spain [Group *A. s.* ₍₉₎]) used in a study of a taxonomic status of British water voles and 1 sample belonging to *A. amphibius* (former *A. terrestris*) from South-eastern Europe [Bulgaria [Group *A. a.* ₍₁₎], the epigenetic cranial characteristics of which was described by MARKOV (2004). The epigenetic differentiation among the compared populations was statistically evaluated through the methods proposed by SJØVOLD (1973) and SMITH (1981). Epigenetic Variation (Vi), Mean Measure of Divergence (MMD) and Measure of Uniqueness (MU) were calculated on the base of distribution frequency of examined characters. Based on a matrix of MMD values the dendrogram of epigenetic similarity was constructed (cluster analysis, Amalgamation rule: Unweighted pair-group centroid; Distance measure: Euclidian distances). All calculations were performed using the statistical package STATISTICA 2008 version 8.0 (StatSoft, Inc. 2008).

Results and Discussion

The absence of sexual dependence in the studied characters proved by Chi-square method ($\chi^2_0 = 0.3135 < \chi^2_{0.95, 12} = 21.026$) allowed to pool to-

gether the specimens of both sexes. These pooled population samples from Hungary were subject of the study.

The frequencies of 13 non-metric traits of the water vole from Hungary are given in Fig. 1.

Perceptible differences in the appearance of particular traits were established between the characters *Infra maxillary crest* medium or large (N 7) and *Foramen ovale* double (N 10), which were not found in the studied sample and the trait *Maxillary foramen II (lateral)* present (N 6), which had the highest appearance (0.8). The rest of the traits exhibited variation within 0.05 to 0.55.

The index of epigenetic variability (V_i) of Hungarian sample of *A. amphibius* was 0.1173. Among the compared populations of genus *Arvicola* from Europe the values of epigenetic variability index (Table 1) varied from 0.1173 in *A. amphibius* from Hungary to 0.146 to Southern water vole (*A. sapidus*) from South Spain.

Among the populations of *A. amphibius* the sample from Norway had the maximum value of the same index and the sample from Bulgaria had the minimum value, and the mean value of the population epigenetic variation of this group was $V_i = 0.1292$. In the group of populations of *A. sapidus* the mean value of the population epigenetic variation was $V_i = 0.1324$ with maximum values in the populations from South Spain and minimum in the populations from Pyrenees II. Among all the studied populations of both species, the sample of *A. amphibius* from Hungary has the second lowest population epigenetic variation within *A. amphibius*, which is by 9% lower than the mean group population variation of this species and by 12% lower than the mean of *A. sapidus*.

Epigenetic distances (MMD) between water vole from Hungary and other populations of *A. amphibius* and *A. sapidus* from Europe are shown in Table 2. The values are accepted as statistically significant at 0.05 level if the value was twice higher than the standard deviation of MMD (S_{JØVOLD}

1973). Thirty seven (82.22%) of all 45 pair wise comparisons of MMD between population showed significant differences. There were five statistically insignificant MMD within *A. amphibius* populations mainly between the groups *A. a.* ⁽²⁾ (Alps - France, Italy and Switzerland, *A. a.* ⁽⁴⁾ (Germany) and *A. a.* ⁽⁵⁾ (Norway) and 3 within *A. sapidus* populations between the groups *A. s.* ⁽⁸⁾ (Pyrenees II), *A. s.* ⁽⁹⁾ (North Spain) and *A. s.* ⁽⁷⁾ (South Spain).

The Hungarian sample displays statistically significant values of epigenetic interpopulation distances to all studied populations of both species. The mean MMD of Hungarian population of *A. amphibius* to the rest of the populations was 1.228 or by 5% lower than its mean MMD to the populations of *A. sapidus*, which was 1.293. The highest individual MMD of Hungarian population of *A. amphibius* was to the population from South-eastern part of its range – 1.879. The lowest value of MMD of Hungarian population within *A. amphibius* was realized with the geographically nearest population from former Yugoslavia, and it showed the highest relative degree of its epigenetic dissimilarity with *A. sapidus* population *A. s.* ⁽⁸⁾ (Pyrenees II).

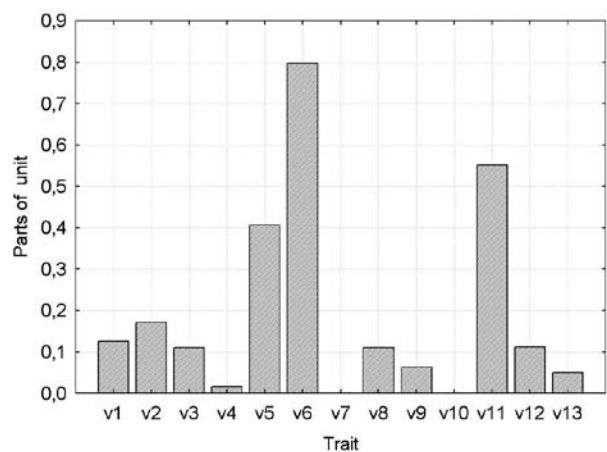


Fig. 1. Frequency distribution of the epigenetic non-metric cranial traits in the samples of *A. amphibius* from Hungary. (for traits descriptions see “Material and Methods”)

Table. 1. Epigenetic variation (V_i), among population of genus *Arvicola* in Europe Populations of *A. amphibius*: *A. a.* ⁽¹⁾ - Bulgaria; *A. a.* ⁽²⁾ -Alps-France, Italy and Switzerland; *A. a.* ⁽³⁾ - former Yugoslavia; *A. a.* ⁽⁴⁾ - Germany; *A. a.* ⁽⁵⁾ - Norway; *A. a.* ⁽⁶⁾ -Pyrenees I; *A. a.* ⁽¹⁰⁾ – Hungary. Populations of *A. sapidus*: *A. s.* ⁽⁷⁾ -South Spain; *A. s.* ⁽⁸⁾ -Pyrenees II; *A. s.* ⁽⁹⁾ - North Spain;

Pop.	<i>A. a.</i> (1)	<i>A. a.</i> (2)	<i>A. a.</i> (3)	<i>A. a.</i> (4)	<i>A. a.</i> (5)	<i>A. a.</i> (6)	<i>A. s.</i> (7)	<i>A. s.</i> (8)	<i>A. s.</i> (9)	<i>A. a.</i> (10)
V_i	0,1114	0,139	0,1357	0,1366	0,1442	0,1205	0,1461	0,1205	0,1307	0,1173

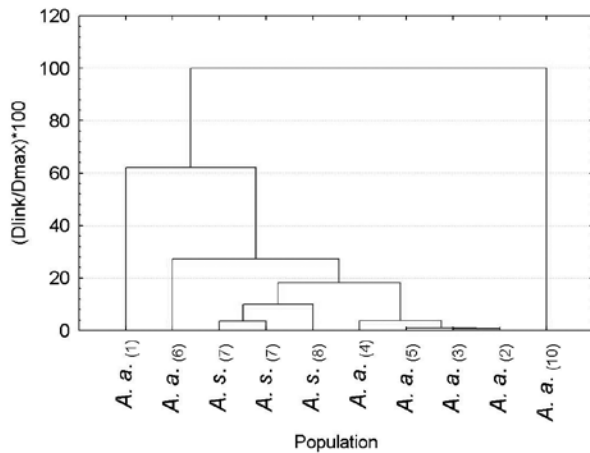


Fig. 2. Dendrogram (Tree Clustering), based on dissimilarities from matrix of MMD-values of population of epigenetic relationships within genus *Arvicola* in Europe: Populations of *A. amphibius*: *A. a.* (1) - Bulgaria; *A. a.* (2) - Alps-France, Italy and Switzerland; *A. a.* (3) - former Yugoslavia; *A. a.* (4) - Germany; *A. a.* (5) - Norway; *A. a.* (6) - Pyrenees I; *A. a.* (10) - Hungary. Populations of *A. sapidus*: *A. s.* (7) - South Spain; *A. s.* (8) - Pyrenees II; *A. s.* (7) - North Spain.

The relative degree of epigenetic relationships among *Arvicola* populations, revealed graphically on the base of the distance matrix of the mean measure of divergence (Tree Clustering), showed two groups of closely connected populations (Fig. 2). The first group consisted of *A. sapidus* populations from Pyrenees II (*A. s.* (8)), North Spain (*A. s.* (9)), South Spain (*A. s.* (7)) and the second one included populations from Alps - France, Italy and Switzerland (*A. a.* (2)), former Yugoslavia (*A. a.* (3)), Germany (*A. a.* (4)) and Norway (*A. a.* (5)) of *A. amphibius*. The position of Hungarian population (*A. a.* (10)) was clearly defined among the studied group of populations from genus *Arvicola* in Europe and showed some similarity with the population (*A. a.* (1)) of *A. amphibius* from south-eastern part of its European range. Nevertheless, this degree of similarity was weaker than all the others obtained in comparisons between populations.

Among the studied populations of genus *Arvicola* from Europe, the maximum value of epige-

Table, 2. Mean measure of divergence (MMD) and standard deviation of MMD (in italics) between examined samples; significant values of MMD in bold. MU - Measure of uniqueness; Populations of *A. amphibius*: *A. a.* (1) - Bulgaria; *A. a.* (2) - Alps-France, Italy and Switzerland; *A. a.* (3) - former Yugoslavia; *A. a.* (4) - Germany; *A. a.* (5) - Norway; *A. a.* (6) - Pyrenees I; *A. a.* (10) - Hungary. Populations of *A. sapidus*: *A. s.* (7) - South Spain; *A. s.* (8) - Pyrenees II; *A. s.* (9) - North Spain;

Pop.	<i>A. a.</i> (2)	<i>A. a.</i> (3)	<i>A. a.</i> (4)	<i>A. a.</i> (5)	<i>A. a.</i> (6)	<i>A. s.</i> (8)	<i>A. s.</i> (9)	<i>A. s.</i> (7)	<i>A. a.</i> (1)	MU
<i>A. a.</i> (10)	1.1900 <i>0.1586</i>	1.0480 <i>0.1614</i>	1.2339 <i>0.1894</i>	1.2938 <i>0.1754</i>	1.4813 <i>0.1966</i>	1.5656 <i>0.2360</i>	1.1954 <i>0.1590</i>	1.1304 <i>0.1494</i>	1.8739 <i>0.1266</i>	12,0123
<i>A. a.</i> (2)		0.0066 <i>NON</i>	0.0391 <i>0.0394</i>	0.0082 <i>0.0168</i>	0.2882 <i>0.1028</i>	0.5187 <i>0.1548</i>	0.4113 <i>0.1143</i>	0.4028 <i>0.1106</i>	0.7977 <i>0.1236</i>	3,6087
<i>A. a.</i> (3)			0.0520 <i>0.0475</i>	0.1563 <i>0.0772</i>	0.3685 <i>0.1221</i>	0.6797 <i>0.1842</i>	0.6147 <i>0.1477</i>	0.5128 <i>0.1322</i>	0.6515 <i>0.1221</i>	4,0315
<i>A. a.</i> (4)				0.0869 <i>0.0606</i>	0.2851 <i>0.1128</i>	0.4217 <i>0.1511</i>	0.2119 <i>0.0917</i>	0.1904 <i>0.0854</i>	0.8010 <i>0.1473</i>	3,1440
<i>A. a.</i> (5)					0.4032 <i>0.126</i>	0.5168 <i>0.1588</i>	0.3482 <i>0.1094</i>	0.2997 <i>0.0995</i>	0.9880 <i>0.1468</i>	4,0060
<i>A. a.</i> (6)						0.7441 <i>0.1951</i>	0.4600 <i>0.1299</i>	0.5146 <i>0.1348</i>	1.3614 <i>0.1812</i>	5,9064
<i>A. s.</i> (8)							0.1029 <i>0.0689</i>	0.1047 <i>0.0685</i>	1.8933 <i>0.2522</i>	6,3399
<i>A. s.</i> (9)								0.0378 <i>NON</i>	1.5342 <i>0.1714</i>	4,7757
<i>A. s.</i> (7)									1.4016 <i>0.1579</i>	4,4523
<i>A. a.</i> (1)										11,3026

netic uniqueness (MU = 12.0123) revealed the sample from Hungary, but it was too close to MU of *A. amphibius* (*A. a.*₍₁₎) from the south-eastern part of its European area (Table 2).

Discussion

The comparative analysis of the relative epigenetic inter-population distances of genus *Arvicola* confirms the presence of well-differentiated groups of populations of *A. sapidus* and *A. amphibius* inhabiting the European continent. The values of the relative inter-population distances in the group of *A. sapidus* are not very high, some of them are even insignificant and this result confirms that the group is homogenous. These values are higher in the group of *A. amphibious*, so it is considerably more heterogeneous. The epigenetic similarity between some of the populations of this species (*A. a.*₍₂₎, *A. a.*₍₃₎, *A. a.*₍₄₎ and *A. a.*₍₅₎) was almost the same, so they were well differentiated from the rest of the studied populations from Europe. The similarities among the populations are largely comparable to the heterogeneity of natural geographic pattern of their habitats – varied geographic position, extremely diversified altitudes and local climate peculiarities. Probably the rate of the relative population epigenetic variation is determined by the relatively wide range of climatic factors, which influenced the ecological characteristics of these population habitats, and exactly these local specifics of the ecological conditions are expressed in epigenetic variation of the populations inhabiting them.

The comparative analysis of epigenetic distances between water vole from Hungary and other populations of *A. amphibius* and *A. sapidus* from Europe presents the sharp distinctness of Hungarian population among the others. Its strong differentiation by epigenetic characteristics is well expressed. Although its epigenetic distinction exceeds the value, describing the inter-species differences between both main groups of populations of *A. amphibius* and *A. sapidus* in Europe, it remains attached to the group of *A. amphibius*. Thereby this population highly increases the heterogeneity of the inter-population epigenetic variation of the species in its European area.

Registered considerable epigenetic uniqueness of *A. amphibius* in the central and south-eastern part of its European area proves the polytypical epigenetic character of water vole in Europe. The epigenetic differentiation of it from Southern Great Plain in Hungary, south-eastern part of Balkan Peninsula and the presence of unified group of populations from Central and Western Europe, together with the results obtained by WUST SAUCY (1998) on the mitochondrial cytochrome b gene, showing the heterogeneous intraspecies structure of *A. amphibius* and demonstrates the need of taxonomic review of intraspecies structure of *A. amphibius* in European area. The future detailed population analysis of *A. amphibius*, based on morphological, epigenetical and biochemical description, would reveal explicitly the population biological diversity of this species, and would outline the geographic areas, inhabited by similar in their biological characteristics population groups, and would establish their taxonomic rank.

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