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Morphometric differentiation of the moor frog (*Rana arvalis* Nilss.) in Central Europe

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Abstract

Morphometric variation of 11 characters was studied in 14 samples of *Rana arvalis* from allopatric population groups in Central Europe, representing two subspecies formerly recognised: *R. a. arvalis* and *R. a. wolterstorffi*. All samples from Poland (nine) were collected from the area, which is believed to be populated by the nominal form. In addition, the isolated population of *R. arvalis* from the Eastern Carpathians of Romania was classified to that form. All the Hungarian samples fall into the range of *R. a. wolterstorffi*. No significant differences in body size between sexes and among the populations from the three geographic regions (Poland, Hungary and Romanian Eastern Carpathians) were found. Multivariate analysis of the differences in body shape among groups showed that the populations from Hungary differed from the nominal form mainly in the relative hindlimb length. However, substantial overlap in overall shape differences resulted in a relatively low percentage of correct classifications to the respective geographic groups in the Discriminant Analysis. Large intrapopulation variation in the values of the two ratios (SVL/TL and TL/IMT) formerly used for differentiate the two European subspecies of *R. arvalis* makes them unreliable characters for distinguishing the two forms. The pattern of morphometric variation does not match the overall genetic divergence of the *R. arvalis* populations, which suggests that the body shape differences in this species result from the phenotypic plasticity correlated with local climatic factors.

Key words: *Rana arvalis wolterstorffi* – differentiation – moor frog – morphometrics – subspecies

Introduction

The moor frog, *Rana arvalis* Nilsson, 1842 is a widely distributed Eurasian species ranging from the Eastern France in the west to the Baikal Lake in the east and from Kola Peninsula in the north to the southern part of the Pannonian Basin (Bannikov et al. 1977; Ishchenko 1997). In Europe the distribution of the moor frog can be divided into two main areas. The larger one extends across the lowlands north of the Alps and north of the Carpathians and reaches the Urals in the East, the second, smaller one covers the lowlands of the Pannonian Basin. An isolated small group of populations inhabits the plateau in the Eastern Carpathians in Romania (Fig. 1). Frogs from the Pannonian Basin were described in 1919 as belonging to the separate subspecies, *R. a. wolterstorffi* (Fejérváry 1919). In contrast, the whole of the northern area is thought to be inhabited by the nominal subspecies *R. a. arvalis* (Stugren 1966). Frogs from the Pannonian Basin were described as a separate subspecies on the basis of the relatively longer legs, larger body size and 'slender habit' (Fejérváry 1919). Yet a small, isolated group of populations from the vicinity of Reci (Romanian Eastern Carpathians) was assigned to the nominal form (Dely 1953; Fuhn 1962) because they had body proportions similar to the specimens from the area north to the Carpathians.

Some authors question the validity of *R. a. wolterstorffi* distinctiveness, claiming that the body proportions is a phenotypically plastic trait in the moor frog which shows clinal variation parallel to the variation of climatic conditions (Kauri 1959; Stugren 1966). Indeed, populations with body proportions intermediate between *R. a. wolterstorffi* and the nominal form were reported from southern Slovakia (Lác 1956). Large intrapopulation variability in body proportions was the most probable reason for repeated reports of *R. a. arvalis* and *R. a. wolterstorffi* sympatry in southern Poland (Fejérváry 1923; Dely 1964; Nöllert and Nöllert 1992). The presence of *R. a. wolterstorffi* in southern Poland is reported also in the *European Atlas of Amphibians and Reptiles* (Ishchenko 1997), in spite of the fact, that subspecies being defined as morphologically distinguishable groups of allopatric populations cannot be sympatric by definition. On the other hand, a sample of frogs from the surroundings of Hatvan in Hungary, from within the range of *R. a. wolterstorffi*, was described as having body proportions not different from the nominal form (Fejérváry 1921). Thus the taxonomic status of the *R. a. wolterstorffi* remains unclear.

Morphological differentiation of amphibian taxa of subspecific and even specific rank is often very small and involves mainly differences in body proportions. Taxa of subspecific rank were described in *Triturus cristatus* on the basis of only subtle differences in body size (Wolterstorff 1923; Kalezić et al. 1990). Later, when molecular techniques were used to asses the degree of genetic differentiation among these subspecies, they were elevated to the specific rank (Wallis and Arntzen 1989; Arntzen and Wallis 1994). There is no reason, however, for the general genetic divergence, as measured by molecular techniques, to be coupled with the morphological differentiation. The morphological quantitative traits are usually polygenically inherited and show much plasticity in relation to environmental factors. Because of that both studies on morphological differentiation and genetic divergence should be combined to assess the taxonomic status of the taxa of uncertain rank. Here we present data on the morphological differentiation and relationships in Central European moor frog populations complementing an earlier study of the genetic divergence of this species (Rafiński and Babik 2000.

All earlier descriptions of the morphological differentiation of Central European populations of *R. arvalis* were based on univariate statistics and ratios obtained from relatively small samples from few populations (Fejérváry 1919; Dely 1953, 1964; Fuhn 1960, 1962). The aim of our study is to reanalyse the extent of morphological variation in populations of *R. arvalis* both from across Poland and from the southern parts

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of the species' distribution in Central Europe, including the isolated populations from the Romanian Carpathians. Body size differences were analysed by means of univariate statistics and body proportions by means of multivariate methods; i.e. principal component analysis (PCA), canonical variate analysis (CVA), and discriminant analysis (DA). In order to allow comparisons of our results with those of earlier studies and to establish utility of the methods formerly widely used, we investigated the variation of morphometric ratios which are believed to be diagnostic for the two subspecies. The genetic differentiation of the same samples was also studied by means of the allozyme electrophoresis (Rafiński and Babik 2000), so the patterns of morphometric and genetic differentiation among the populations could be compared.

Materials and methods

A total of 319 individuals of *R. arvalis* were collected from three geographic regions (Poland, Hungary and the Romanian Carpathians). The collection sites and sample sizes are given in Fig. 1 and Table 1. The frogs were anaesthetized in MS 222 and measured with callipers with accuracy of 0.1 mm (J.R.). The same specimens were later used for electrophoresis. The details of the electrophoretic study were given elsewhere (Rafiński and Babik 2000). Eleven morphometric measurements were recorded for each specimen: internarial distance (IND), anterior interorbital distance (AID), posterior interorbital distance (PID), eye–naris distance (END), tympanum diameter (TD), snout–vent length (SVL), femur length (FL), tibia length (TL), foot length (F), length of first toe (T1), length of internal metatarsal tubercle (IMT) (Fig. 2). We performed a two-way ANOVA on untransformed values of SVL to verify whether the samples from three allopatric population groups and sexes differed in the overall size. In this analysis we used specimens larger than 36 mm SVL only, since smaller individuals are not sexually mature (Ishchenko 1977; Berger and Rybacki 1994). Since the analysis did not reveal significant difference between sexes (Table 3), we pooled males and females in subsequent analyses.

As there was a significant heterogeneity in body size among samples (data not shown) we corrected the measurements for size differences using the allometric method of Thorpe (1976) prior to the analysis of shape differences. In a comparative study of size-correction methods this method proved to remove the size variation efficiently without distorting shape information (Reist 1985). As a size adjusted, log-transformed value we computed:

$$K = \log[\log Y - \beta(\log X - \log \bar{X})]$$

where Y is the observed measurement; β is the common within-region regression slope of log(Y) on log(X), X is the SVL of the specimen, $\bar{X}$ is the grand mean SVL of all the individuals studied. Using the common within-groups regression slope requires parallelism of the regression lines between groups for each variable, therefore the parallelism of each log(var) on log(SVL) between groups was tested. As variables END and TD did not satisfy this condition, we excluded them from further analyses.

The significance of differences among groups for size-corrected values of measurements was tested by means of one-way ANOVAs complemented with Spjøtvoll–Stoline *a posteriori* test (Sokal and Rohlf 1995). Size-corrected measurements were also subjected to PCA, with subsequent ANOVA on the PC 1 and PC 2 scores.

Morphometric differentiation of the samples studied was also assessed by means of CVA in which samples were taken as groups. Correlations between adjusted measurements and canonical variates were computed to establish the contribution of the given variable for

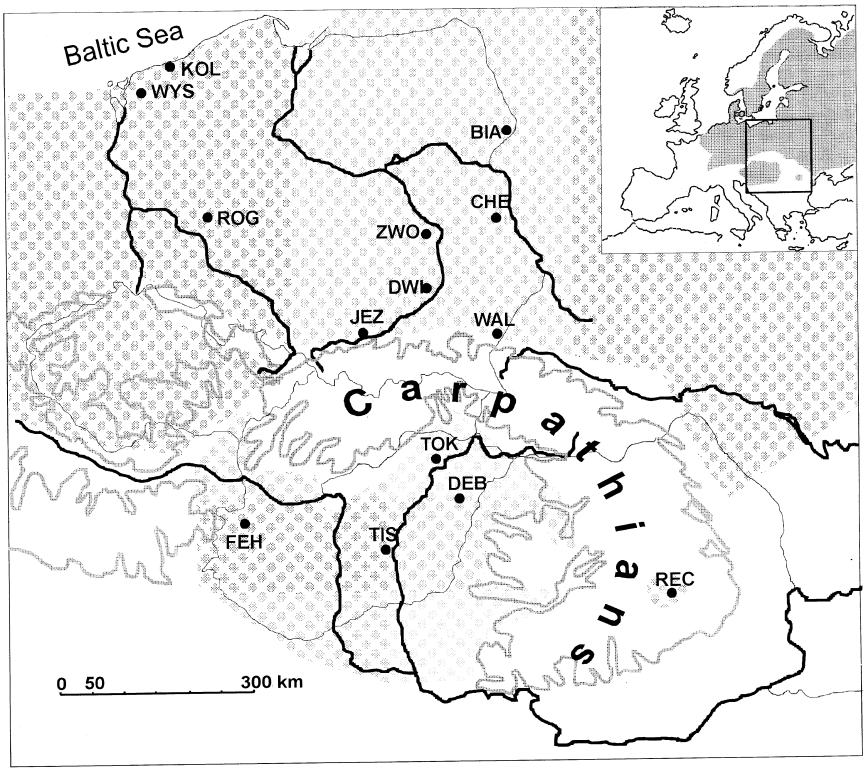

Fig. 1. Distribution of *R. arvalis* in Central Europe (Tiedeman 1979; Moravec 1994; Ishchenko 1997). Abbreviations – see Table 1. A contour line 500 m a.s.l. is shown. Inset – distribution of *R. arvalis* in Europe (Ishchenko 1997)

Table 1. Localities and the sample sizes (*n*)

Locality	Country	Abbreviation	Geographical coordinates	*n*
Kołobrzeg	Poland	KOL	15°33′ E 54°11′ N	16
Wysoka Kamieńska	Poland	WYS	14°50′ E 53°50′ N	15
Białowieża	Poland	BIA	23°49′ E 52°43′ N	19
Rogaczewo Wielkie	Poland	ROG	16°50′ E 52°03′ N	21
Zwoleń	Poland	ZWO	21°35′ E 51°21′ N	30
Chełm	Poland	CHE	23°48′ E 51°08′ N	21
Dwikozy	Poland	DWI	21°46′ E 50°44′ N	22
Jeziorzany	Poland	JEZ	19°68′ E 50°00′ N	28
Walawa	Poland	WAL	22°55′ E 49°54′ N	19
Tokaj	Hungary	TOK	21°24′ E 48°08′ N	24
Fehertó	Hungary	FEH	17°24′ E 47°44′ N	23
Debrecen	Hungary	DEB	21°39′ E 47°34′ N	26
Tiszaálpár	Hungary	TIS	20°01′ E 46°48′ N	26
Reci	Romania	REC	25°45′ E 45°30′ N	29

the total among-group variation explained by a given canonical variate.

We used DA to create the functions which maximized the probability of correct classification of specimens to their original population. Subsequently, percentage of the proper classifications to the geographical region was computed.

A two-dimensional scaling (MDS) of Mahalanobis distances squared between samples was used to visualize the overall pattern of morphometric differentiation among populations from different geographical regions.

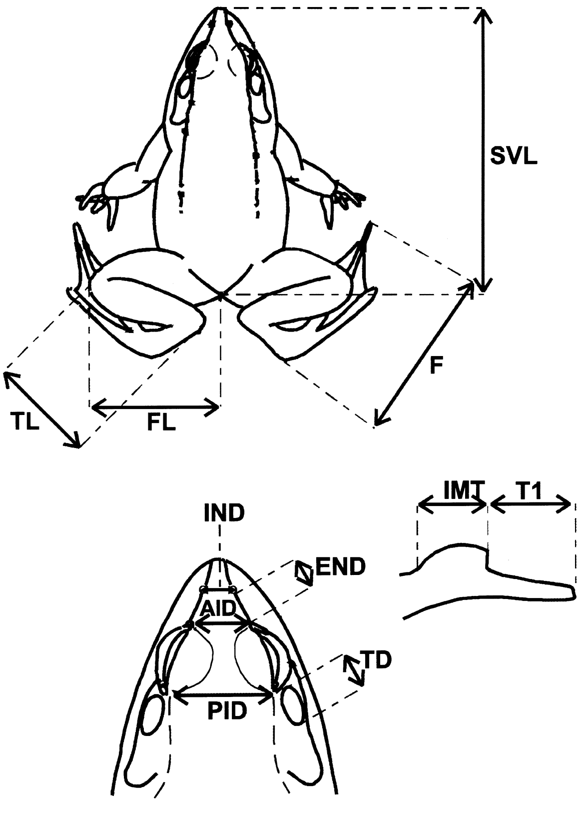

Fig. 2. Morphological measurements

The two-dimensional scaling of the matrix Nei's (1978) unbiased genetic distances based on Rafiński and Babik (2000) was used to elucidate the patterns of genetic differentiation of the samples studied.

We performed hierarchical ANOVAs on natural logarithm-transformed values of diagnostic indices SVL/TL and TL/IMT for each sex separately to establish the extent of variation in values of the indices. They were previously used for distinguishing *R. a. arvalis and R. a. wolterstorffi* (Fejérváry 1919; Fuhn 1960; Dely 1964). Natural logarithm transformation has been applied as after the transformation the distributions of the indices approached normality more closely (Shapiro–Wilk test, data not shown). Two levels of hierarchy were distinguished: geographical region (northern and southern) and population from which we obtained our samples. A sample from Reci should be treated as representing a separate geographic region, but since it came from one population only, estimating all levels of variation for this region was not possible, so it was excluded from the analysis.

Results

No significant differences in adult body size were found among the three geographical regions: Poland, Hungary, and Romanian Carpathians (Tables 2, 3).

Analysis of variance of size-adjusted measurements showed

Table 2. Mean body length of the adult (above 36 mm) individuals, and 95% confidence intervals

Country	Mean (mm)	95% confidence intervals (mm)
Poland	46.19	44.92 — 47.45
Hungary	47.62	46.35 — 48.89
Romania	44.52	43.34 — 45.71

Table 3. Two-way ANOVA of body length of adult individuals

Factor	*F*	d.f.	p
Sex	3.494	1, 248	0.063
Country	2.377	2, 248	0.095
Sex × Country	0.205	2, 248	0.815

Table 4. Results of one-way anovas for differences in means of size-adjusted variables among countries. Significant p-values in bold

Variable	$F_{2,316}$	p
FL	21.966	**< 0.0001**
TL	88.577	**< 0.0001**
F	64.040	**< 0.0001**
IMT	15.791	**< 0.0001**
T1	22.164	**< 0.0001**
IND	12.757	**< 0.0001**
AID	6.234	**< 0.005**
PID	2.529	0.081

IND, internarial distance; AID, anterior interorbital distance; PID, posterior interorbital distance; FL, femur length; TL, tibia length; F, foot length; T1, length of first toe; IMT length of internal metatarsal tubercle.

that all variables but PID differed significantly between the regions (Table 4). For all these variables an *a posteriori* test of Spjøtvoll–Stoline showed a significant difference between the Polish and Hungarian samples. The Romanian sample did not differ significantly from Polish samples for any variable. In contrast, the Romanian sample differed significantly from the Hungarian ones for some variables (TL, F, IMT, T1).

Shape differences among groups were analysed by PCA of size-adjusted measurements. The first principal component (PC 1) explained 36.7% of the total variance; 94.5% of the total variance was explained by as many as six principal components; PCs 2–6 explained similar amounts of variance (Table 5). Leg measurements (FL, TL, F, T1) showed the highest positive loadings for PC 1. Thus, it can be concluded that this component represents differences in leg proportions. The first principal component (PC) 1 differentiated frogs from the three geographic groups most efficiently. This is shown by highly significant result of anova on PC 1 scores ($F_{2;316} = 72.952$, $p < 0.0001$), both for the differences between the Polish and Hungarian samples ($p < 0.0001$), and between those from Romania and Hungary ($p < 0.0002$). The head measurements showed high positive loadings for PC 2, whereas all the leg measurements had negative loadings; thus, PC 2 emphasized differences in the head and leg shape. However, PC 2 scores did not differentiate the geographic regions significantly (anova $F_{2;316} = 2.631$, $p = 0.0736$). The Polish and Hungarian populations were separated along the PC 1 axis only, although the overlap was considerable. The sample from Romania took an intermediate position (Fig. 3).

We computed the correlations between the variables and the canonical variates (Table 6) to find out, which variables

Table 5. Principal component loadings for PC 1 — PC 6

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
FL	0.778	−0.144	−0.091	−0.343	0.164	−0.273
TL	0.921	−0.060	−0.010	−0.159	−0.058	−0.046
F	0.924	−0.070	−0.034	−0.066	−0.060	−0.013
IMT	0.072	−0.299	−0.757	0.510	0.147	−0.224
T1	0.644	−0.212	0.092	0.374	−0.131	0.582
IND	0.296	0.692	0.003	0.131	0.630	0.136
AID	0.338	0.666	0.094	0.347	−0.463	−0.301
PID	−0.101	0.395	−0.728	−0.389	−0.244	0.304
Eigenvalue (λ)	**2.939**	**1.242**	**1.129**	**0.837**	**0.743**	**0.667**
% of variance explained	36.7	15.5	14.1	10.5	9.3	8.3
Cumulative percentage explained	**36.7**	**52.2**	**66.3**	**76.8**	**86.1**	**94.4**

IND, internarial distance; AID, anterior interorbital distance; PID, posterior interorbital distance; FL, femur length; TL, tibia length; F, foot length; T1, length of first toe; IMT length of internal metatarsal tubercle.

Table 6. Correlations of the variables with the canonical variates 1–5 and cumulative proportion of the variance explained

Variable	CV 1	CV 2	CV 3	CV 4	CV 5
FL	0.418	−0.625	−0.016	−0.063	−0.314
TL	0.858	−0.271	−0.038	−0.388	−0.072
F	0.723	−0.411	−0.103	0.159	−0.089
IMT	−0.128	−0.181	−0.514	−0.348	−0.617
T1	0.457	0.453	−0.240	0.085	−0.360
IND	0.134	−0.043	0.720	0.043	−0.597
AID	0.157	−0.232	0.083	−0.200	0.090
PID	−0.177	−0.123	0.356	−0.558	0.176
% variance explained	**62.8**	**75.6**	**86.4**	**90.6**	**94.2**

IND, internarial distance; AID, anterior interorbital distance; PID, posterior interorbital distance; FL, femur length; TL, tibia length; F, foot length; T1, length of first toe; IMT length of internal metatarsal tubercle.

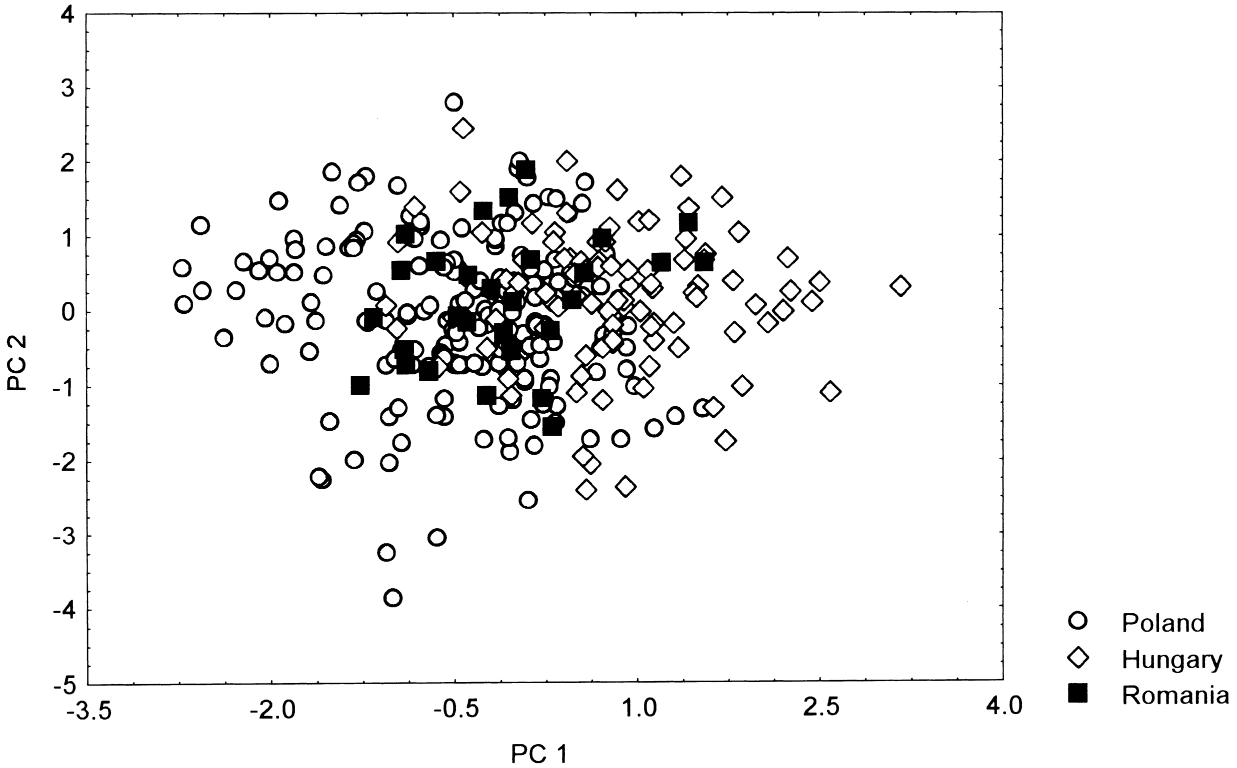

Fig. 3. Scatterplot of principal component scores PC 2 (PC 1)

contributed most to the overall among-groups differences. CV 1 accounted for 62.8% of the total among-groups variance and was highly correlated with positive values of TL, F, T1, and FL. T1 was positively correlated with the CV 2 (which explained 12.8% of variance), whereas the other leg measurements were negatively correlated. In total, 94.2% of the total variance was explained by the first five canonical variates.

Discriminant functions developed for the samples correctly classified 42.3% cases to the corresponding samples. However, the percentage of correct classifications of individuals to a geographical region based on the same discriminant functions was much higher, being 75.9%.

Two-dimensional scaling of the generalized multivariate distances (Mahalanobis distances squared) showed a clear separation of the samples from Hungary and Poland. The

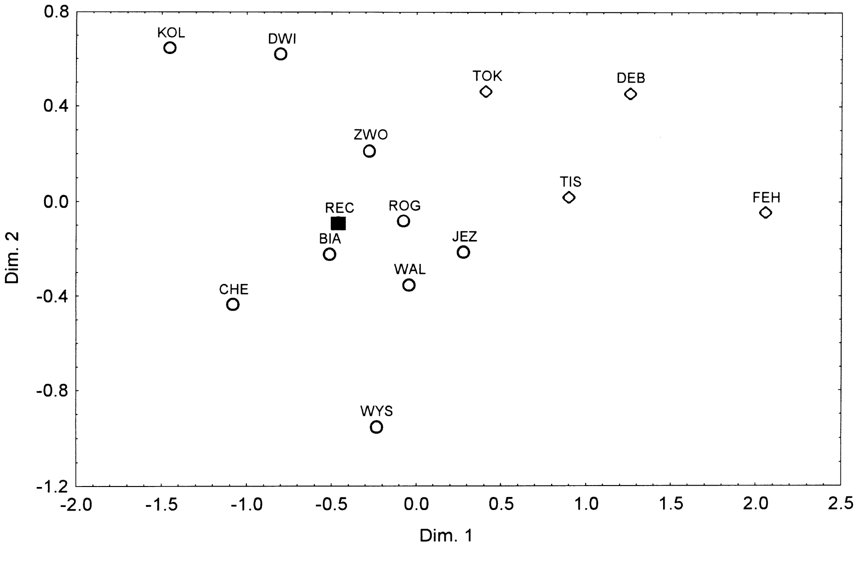

Fig. 4. Two-dimensional scaling of Mahalanobis distances squared between populations for size adjusted data. Stress = 0.0849. Abbreviations – see Table 1

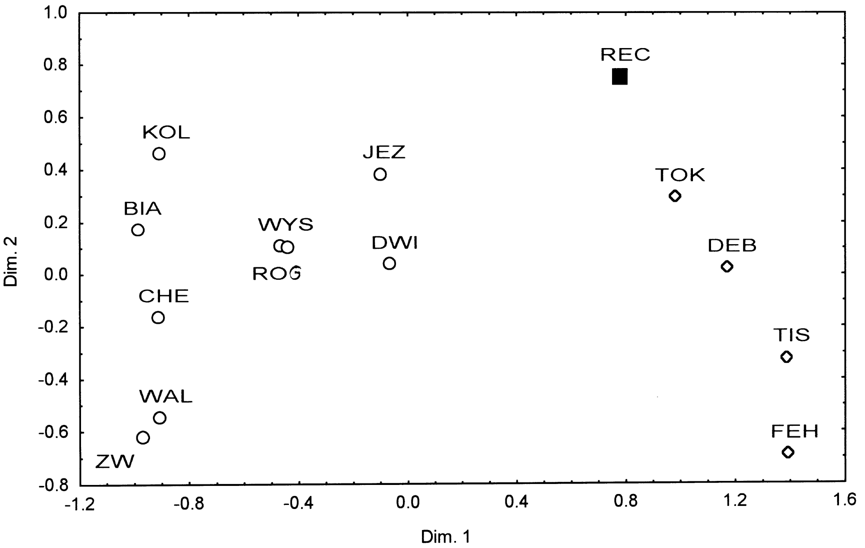

Fig. 5. Two-dimensional scaling of Nei's (1978) genetic distances between the populations studied (stress = 0.0418). Abbreviations – see Table 1

sample from Romania was placed in the middle of the Polish group (Fig. 4). Genetic data gave different groupings of populations. The population from Romania clustered together with the Hungarian samples after the MDS analysis (Fig. 5).

Nested ANOVA of the values of the SVL/TL index showed that the variation at all levels, i.e. among regions, among populations within a region and within populations, was considerable (Table 7). For both males and females more than half of the total variance was explained by the differences between regions (Poland versus Hungary), but as could be seen from Fig. 6(a), the overlap of values of the SVL/TL index among geographic regions was substantial. The values of the Reci sample were intermediate, but closer to those of the Polish populations.

A different pattern emerged from the analysis of the values of the TL/IMT index. There was no significant variance component added by the interpopulational level within regions neither for males nor for females. Regional differentiation was significant but slightly lower than for SVL/TL; it was noticeable that the intrapopulational variation was very high, especially for males (Table 8). As with the former index, the sample from Reci took a position closer to the Polish samples (Fig. 6b).

Discussion

Our analysis of the body length variation in *R. arvalis* populations studied did not confirm earlier descriptions, which stated that the samples from Danube plains, attributed to *R. a. wolterstorffi* showed larger body size than samples classified as the nominal form (Fejérváry 1919; Fuhn 1960, 1962; Stugren 1966). Most probably the few and small samples, which were at the disposal of the earlier authors were not representative of the total variation in body size of *R. arvalis* present in Central Europe. A sample of frogs collected north of the Carpathians was much larger and showed that the interpopulational differences in body size were considerable in this region. The mean body size in the Polish samples ranged from 39.8 to 50.2 mm, whereas the Hungarian samples ranged from 42.2 to 50.1 mm. Body size of *R. arvalis* individuals from the extensive areas in Russia also showed much variation (Ishchenko 1977). The only geographic pattern, which emerged from the extensive data of Ishchenko (1977) was that the frogs from the most northern localities grew larger.

Principal component analysis on size-adjusted data revealed that the main difference in body shape among the three groups of populations resulted from the differences in

Table 7. Nested ANOVA of the SVL/TL index for adult individuals. Significant p in bold. Error degrees of freedom (d.f.) was computed using the Satterthwaite method

Level	*F*	df	p	% of variance attributed to a given level
Males				
Between regions	14.425	1, 9.904	**< 0.005**	51.7
Among populations within region	5.250	11, 102	**< 0.0001**	16.2
Within populations (error)				32.1
Females				
Between regions	10.909	1, 10.53	**< 0.01**	51.5
Among populations within region	9.010	11, 97	**< 0.0001**	24.0
Within populations (error)				24.5

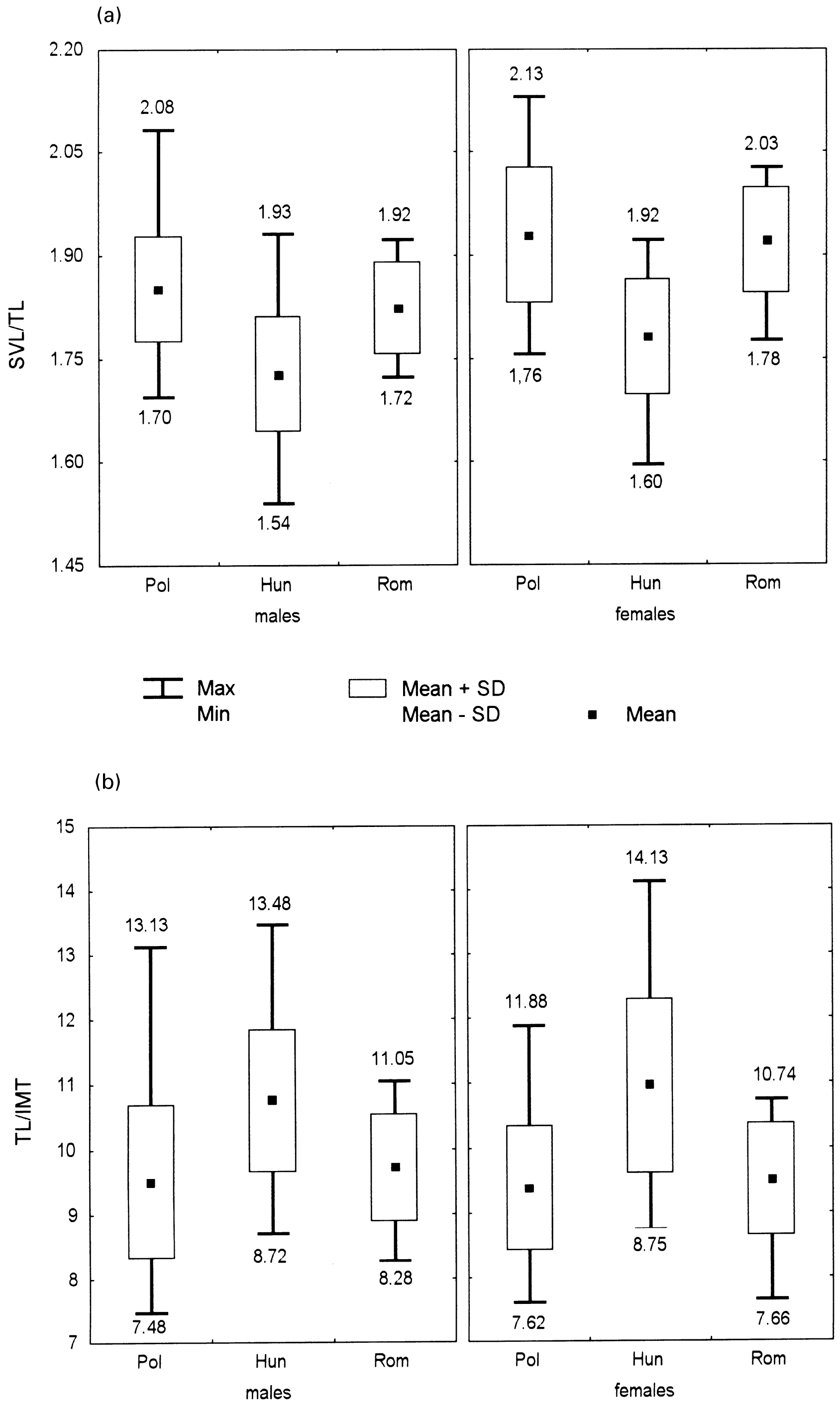

Fig. 6. Box and whiskers plots of the values of the SVL/TL (a) and TL/IMT (b) index for the individuals above 36 mm SVL. Minimal and maximal values are given

the relative hindlimb length. Differences in measurements describing the head shape did not contribute significantly to the among-group variation. Our analysis showed that in spite of much variation in the relative leg length and a substantial overlap with the samples from other regions, frogs from the Hungarian populations had on average relatively longer legs than individuals from the Polish or Romanian samples. This result confirms earlier descriptions of the morphological differences between northern and southern populations of *R. arvalis* (Fejérváry 1919; Dely 1953, 1964; Fuhn 1960, 1962). Furthermore, our discriminant analysis showed that despite some overlap, the frogs from the Polish and Hungarian populations were classified in most cases to the proper geographic region. Different results were obtained from the analysis of the diagnostic indices. In spite of substantial regional differences, there was a broad overlap in the ranges of these indices

Table 8. Nested ANOVA of the TL/IMT index for adult individuals. Significant p in bold

Level	*F*	df	p	% of variance attributed to a given level
Males				
Between regions	65.566	1, 3.76	**< 0.002**	37.4
Among populations within region	0.652	11, 102	0.6523	0.0
Within populations (error)				62.6
Females				
Between regions	35.260	1, 8.24	**< 0.001**	49.4
Among populations within region	1.442	11, 97	< 0.167	2.6
Within populations (error)				48.0

among the three geographic regions, which was mostly due to large within-population variation. This questions the validity of the indices as diagnostic characters.

A functional interpretation of differentiation in the relative hindlimb length found for the *R. arvalis* populations studied is not easy. One can expect that the relative leg length may influence the locomotive performance of frogs. However, experimental studies showed that only relatively large differences (> 10%) can affect the jumping performance (Stokely and Berbarian 1953; Emerson 1978). On the other hand, differences in the relative hindlimb length may be the result of the unequal growth and developmental rate during the larval period (Emerson 1986; Emerson et al. 1988). A negative correlation between the relative length of legs and the duration of the larval period was found for both *Rana sphenocephala* and *Hyla crucifer* (Emerson 1986; Emerson et al. 1988). Shorter larval period resulted in a smaller body size at metamorphosis but relatively longer legs. The experimental data for *H. crucifer* indicate that differences in relative hindlimb length were largely due to phenotypic plasticity. However the influence of the duration of the larval period on the relative hindlimb length was studied for newly metamorphosed froglets only, so it is not known if it persists later in life (Emerson 1986). No relationship between length of the larval period and relative leg length was found for *H. cinerea* (Blouin and Loeb 1991).

One of the main factors influencing the length of the amphibian larval period is temperature (Wilbur and Collins 1973). Experiments on larval development of the ranid frogs demonstrated that growth and differentiation were separable developmental processes with different temperature sensitivities (Smith-Gill and Berven 1979). Low temperature depresses rates of differentiation to a greater degree than growth rate so we can expect that in lower temperatures frogs develop slower and metamorphose at a larger size. That differences in water temperature during larval period may be responsible for the variation in the hindlimb length in Central European *R. arvalis* is indicated by the observation that shorter legs of frogs from the Polish samples correlate with the generally much cooler climate of the area north of the Carpathians than in the Pannonian Basin. Frogs from the Romanian Carpathians also have relatively short legs. Presumably the cooler climate of this locality (Stugren 1966) also influences the body proportions by lengthening the duration of the larval period. The data from the Reci population strengthen the view that the leg length differences are the result of phenotypic plasticity. Genetically, the population from Reci clusters close with the Hungarian samples (compare Figs 4 and 5). Thus, similar body proportions in Polish *R. arvalis* and the sample from the Eastern Carpathians of Romania probably developed independently. There are, as far as we know, no comparative data on the mean size at metamorphosis for *R. arvalis* from the northern and southern part of the species distribution in Central Europe, but we expect it to be lower in the long-legged populations.

The differences in body proportions of *R. arvalis* from different parts of the species distribution were already attributed to the phenotypic plasticity by Kauri (1959). In fact, a general tendency for *R. arvalis* having relatively longer legs in the south was found for the samples collected along the north–south transect in the eastern part of the species distribution (Ishchenko 1977). The notion that relative hindlimb length is influenced by climatic factors, most probably temperature, is also supported by the observation that long-legged forms of *R. temporaria* (Schuster 1950; Dubois 1982), and *R. macrocnemis* (Eiselt and Schmidtler 1971) are found close to the southern borders of these species' distribution. A clinal variation in the relative leg length along the north–south axis was reported for the North American species *R. sylvatica* (Schmidt 1938). The altitudinal cline for body proportions found in *R. macrocnemis*, frogs from higher altitudes being relatively short-legged (Ishchenko 1977), also indicates that the temperature may be the most important factor influencing the relative hindlimb length in ranid frogs.

Our former study of genetic differentiation in *R. arvalis* showed that populations from Hungary and Romania were very close and clearly different from the Polish samples (Rafiński and Babik 2000). Results of this study indicate that morphometric differentiation is not concordant with genetic differentiation and is most likely a result, at least to some extent, of phenotypic plasticity. In view of the above results distinguishing the subspecies *R. a. wolterstorffi* as a separate taxon solely on the basis of the body size and body proportions should be abandoned and the name should be treated as a synonym of *R. arvalis* Nilsson, 1842.

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Zusammenfassung

Morphometrische Differenzierung beim Moorfrosch (Rana arvalis Nilss.) in Mitteleuropa

Die morphometrische Variation von 11 Merkmalen von *Rana arvalis* wurde in 14 Stichproben aus allopatrischen Populationsgruppen Mitteleuropas, die früher als zwei Unterarten (*R. a. arvalis* und *R. a. wolterstorffi*) angesehen wurden, untersucht. Alle Stichproben aus Polen (9) waren in einem Gebiet gesammelt worden, von dem angenommen wurde, daß es von der Nominalform bewohnt wird. Auch die isolierte Population von *R. arvalis* aus den Ostkarpaten Rumäniens wurde dieser Form zugeordnet. Alle ungarischen Stichproben fallen in das Verbreitungsgebiet von *R. a. wolterstorffi*. Es wurden jedoch keine Unterschiede in der Körpergröße in beiden Geschlechtern und zwischen den drei geographischen Regionen (Polen, Ungarn und rumänische Ostkarpaten) gefunden. Eine Multivariatanalyse der Unterschiede in der Körperform zeigte vielmehr, daß sich die Populationen aus Ungarn hauptsächlich durch die relative Länge des Hinterbeins von der Nominalform unterscheiden. Bei einer Diskriminantenanalyse der Unterschiede in den Gesamtmerkmalen der Körperform ergab sich eine wesentliche Überlappung mit den Klassifikationskriterien der entsprechenden geographischen Gruppe nur relativ selten. Eine starke Differenzierung zwischen den Populationen bezüglich von zwei Verhältniswerten (SVL/TL und TL/IMT), die früher zur Unterscheidung der beiden europäischen Unterarten von *R. arvalis* benutzt wurden, macht diese für eine Zuordnung zu den beiden Formen unzulänglich. Das Muster der morphometrischen Variation paßt nicht zu einer genetischen Divergenz zwischen den Populationen von *R. arvalis* und weist darauf hin, daß die Unterschiede in den Körperformen in dieser Art das Ergebnis einer phänotypischen Plastizität sind, die mit den lokalen klimatischen Faktoren in Korrelation steht.

References

Arntzen, J. W.; Wallis, G. P., 1994: The "Wolterstorff Index" and its value to the taxonomy of the crested newt superspecies. Abh. Ber. Naturk. Vorgesch., Magdeburg. **17,** 57–66.

Bannikov, A. G.; Darevsky, I. S.; Ishchenko, V. G.; Rustamov, A. K.; Shcherbak, N. N., 1977: Opredelitel' zemnovodnykh i presemykhajushchesja fauny SSSR. Moskva: Prosveshchenije.

Berger, L.; Rybacki, M., 1994: Growth and maturity of brown frogs, *Rana arvalis* and *Rana temporaria*, in central Poland. Alytes **11,** 11–24.

Blouin, M. S.; Loeb, M. L. G., 1991: Effects of environmentally induced development-rate variation on head and limb morphology in the green tree frog, *Hyla cinerea*. Am. Nat. **138,** 717–728.

Dely, O. G., 1953: Examen systématique et ostéo-biométrique de la *Rana arvalis* Nilss. et de la var. *wolterstorffi* Fejérváry. Ann. Mus. Nat. Hung. **3,** 231–243.

Dely, O. G., 1964: Contribution à l'étude systématique, zoogéographique et génétique de *Rana arvalis* Nilss. et *Rana arvalis wolterstorffi* Fejérv. Acta Zool. Acad. Sci. Hung. **10,** 309–361.

Dubois, A., 1982: Notes sur les grenouilles brunes (groupe de *Rana temporaria*) I. Introduction. Alytes **1,** 56–70.

Eiselt, J.; Schmidtler, J. T., 1971: Vorläufige Mitteilung über zwei neue Subspecies von Amphibia Salientia aus dem Iran. Ann. Naturhist. Mus. Wien **75,** 383–385.

Emerson, S. B., 1978: Allometry and jumping in frogs: helping the twain to meet. Evolution **32,** 551–564.

Emerson, S. B., 1986: Heterochrony and frogs: the relationship of a life history trait to morphological form. Am. Nat. **127,** 67–183.

Emerson, S. B.; Travis, J.; Blouin, M., 1988: Evaluating a hypothesis about heterochrony: larval life history traits and juvenile hind-limb morphology in *Hyla crucifer*. Evolution **42,** 68–78.

Fejérváry, G. J., 1919: On two south-eastern varieties of *Rana arvalis* Nilss. Ann. Mus. Nat. Hung. **17,** 178–183.

Fejérváry, G. J., 1921: Beiträge zur Kenntnis der Raniden-Fauna Ungarns. Arch. Naturg. **87A,** 18–21.

Fejérváry, G. J., 1923: Über die von Dr. A. Pongrácz in Polen gesammelten Amphibien und Reptilien. Arch. Naturg. **89A,** 1–35.

Fuhn, I., 1960: Fauna Republicii Populare Romîne — Amphibia. Bucuresti: Editura Academiei Republicii Populare Romîne.

Fuhn, I., 1962: Étude éidonomique et taxonomique de la grenouille oxyrhine (*Rana arvalis* Nilss.) de Roumanie. Acta Soc. Zool. Bohemosl. **26,** 352–364.

Ishchenko, V. G., 1977: Dinamicheski polimorfizm burych lyagushek fauny SSSR. Moskva: Nauka.

Ishchenko, V., 1997: *Rana arvalis* Nilsson, 1842. In: Gasc, J.-P. (ed.), Atlas of Amphibians and Reptiles in Europe. Paris: Muséum National D'Histoire Naturelle, pp. 128–129.

Kalezić, M. L.; Džukić, G.; Stamenković, S.; Crnobrnja, J.; 1990: Morphometrics of the crested newt (*Triturus cristatus* complex) from Yugoslavia: relevance for taxonomy. Arh. Biol. Nauka **42,** 17–37.

Kauri, H., 1959: Die Rassenbildung bei europäischen Rana-Arten und die Gültigkeit der Klimaregeln. Ann. Soc. Tart. Nat. Inv. Const., Ser. Nova **2,** 1–172.

Lác, J., 1956: K vyskytu skokana rašelinoveho pánonskeho *Rana arvalis wolterstorffi* Fejérváry na Slovensku. Biológia (Bratislava) **11,** 102–106.

Moravec, J., (ed.), 1994: Atlas of Czech Amphibians. Praha: Národni Muzeum.

Nei, M., 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89,** 583–590.

Nöllert, A.; Nöllert, C., 1992: Die Amphibien Europas. Stuttgart: Franckh-Kosmos.

Rafiński, J.; Babik, W., 2000: Genetic differentiation among northern and southern populations of the moor frog *Rana arvalis* Nilsson in Central Europe. Heredity **84,** 610–618.

Reist, J. D., 1985: An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can. J. Zool. **63,** 1429–1439.

Schmidt, K. P., 1938: A geographic variation gradient in frogs. Zool. Ser. Field Mus. Nat. Hist. Chicago **20,** 377–382.

Schuster, O., 1950: Die klimaparallele Ausbildung der Körperproportion bei Poikilothermen. Abh. Senckenberg. Naturforsch. Ges. **482,** 1–89.

Smith-Gill, S. J.; Berven, K. A., 1979: Predicting amphibian metamorphosis. Am. Nat. **13,** 563–585.

Sokal, R. R.; Rohlf, F. J., 1995: Biometry, 3rd edn. New York: W.H. Freeman.

Stokely, P.; Berbarian, J., 1953: On the jumping ability of frogs. Copeia **1953,** 187.

Stugren, B., 1966: Geographic variation and distribution of the moor frog, *Rana arvalis* Nilss. Ann. Zool. Fen. **3,** 29–39.

Thorpe, R. S., 1976: Biometric analysis of geographic variation and racial affinities. Biol. Rev. **51,** 407–452.

Tiedemann, F., 1979: Erstnachweis von *Rana a. arvalis* in Österreich (Amphibia. Salientia: Ranidae). Salamandra **15,** 180–184.

Wallis, G. P.; Arntzen, J. W., 1989: Mitochondrial-DNA variation in the crested newt superspecies: limited cytoplasmic gene flow among species. Evolution **43,** 88–104.

Wilbur, H. M.; Collins, J. P., 1973: Ecological aspects of amphibian metamorphosis. Science **182,** 1305–1314.

Wolterstorff, W., 1923: Übersicht der Unterarten und Formen des *Triton cristatus* Laur. Blätter Aquar. Terrar. Kde. **34,** 120–126.

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