Amphibia-Reptilia 13 (1992): 235-243, E. J. Brill, Leiden

Morphological analysis of two Hungarian water frog (*Rana lessonae-esculenta*) populations

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Abstract. Two water frog populations, *Rana lessonae-esculenta* systems, have been studied in Hungary from the morphological point of view. Multivariate analyses of selected morphological measurements based on biochemical identification of the species provide two ratios as possible key characters for determination of the two forms.

Introduction

The Central-European water frogs are usually divided into three forms, which were originally described as *Rana esculenta* Linnaeus, 1758, *Rana ridibunda* Pallas, 1771 and *Rana esculenta* var. *lessonae* Camerano, 1882. There has been a long-standing controversy among herpetologists regarding the taxonomic status of these forms (for further reviews see e.g. Juszczyk, 1971; Dely and Stohl, 1972; Wijnands and van Gelder, 1976). Biochemical investigations (Tunner, 1970; Uzzel and Berger, 1975; Günther and Hannel, 1976; Vogel and Chen, 1976; Eikhorst, 1984) and crossing experiments (Berger, 1967, 1968, 1970, 1971; Blankenhorn et al., 1971; Günther, 1973, 1978; Tunner, 1973; Uzzel et al., 1975; Graf and Müller, 1979) of the three types indicate that the marsh frog (*Rana ridibunda*) and the pool frog (*Rana lessonae*) are good biological species, whereas the edible frog (*Rana esculenta*) represents their hybrid, usually coexisting with one of the parental species (see e.g. Graf and Pelaz, 1989 for review). Considering this fact, Dubois and Günther (1982) proposed a new evolutionary systematic category, the klepton (e.g., *Rana* kl. *esculenta*), as a species-level taxon, which represents a third taxonomic category differing from both species and subspecies.

As a result of its hybrid origin (Berger, 1973; Uzzel et al., 1977; Dubois and Günther, 1982), *Rana esculenta* is genetically heterogenous and has a morphological character set intermediate between the two parental species. Consequently, morphological ratios are generally used to distinguish the three forms (Berger, 1966; Günther, 1973, 1975; Borkin et al., 1979; Tunner and Dobrowsky, 1976; Hotz and Uzzel, 1982).

The few recent karyological, biochemical and morphological investigations (Mészáros and Bartos, 1978; Gubányi, 1988, 1990; Löw et al., 1989) have not yet provided a detailed picture of the Hungarian water frog populations. The aim of this study was to clarify the morphological composition of some *R. lessonae*—*R. esculenta* (L-E) population systems, and to try to provide a few external key characters to facilitate identification.

Material and methods

For the morphological and biochemical analysis, 276 frogs were collected in the following localities:
1. Bátorliget Nature Reserve (L-E population) at the eastern edge of the Great Hungarian Plain,
2. Kis-Balaton Nature Reserve (L-E population) joint to the Lake Balaton.
In order to get a better comparison among the three hardly distinguishable forms, data of a pure *R. ridibunda* population (from Dömsöd, south of Budapest, near the River Danube), were also included into the analyses (R population).

Fourteen morphometric characters were measured: body length (BL), head width (HW), internarial distance (IND), inter-eyelid distance (IED), upper eyelid width (UEW), eye diameter (ED), maximum diameter of tympanum (MDT), nostril to anterior eyelid commissure (NAEC), snout to nostril (SN), intercanthal distance (ICD), tibia length (TL), femur length (FL), first toe length (FTL) and inner metatarsal tubercle length (IMTL). From these measurements, 12 additional ratios were computed for morphological analysis (BL/TL, BL/FL, BL/IND, BL/IMTL, BL/FTL, FL/TL, TL/IMTL, FTL/IMTL, IND/IED, ICD/NAEC, ICD/SN, ICD/IND).

Water frog forms were identified based on the electrophoretic phenotype of LDH-B izozyme (Gubányi, 1991a). Significance levels of the differences between sexes were evaluated by using Mann-Whitney U-tests (Hotz and Uzzel, 1982). Finally, the morphometric ratios were used in a stepwise discriminant analysis (SPSS PC+ in MTA-SZTAKI).

Results

Means and standard deviations for the 14 morphometric characters for pooled samples of males and females of the three water frog forms are summarized in table 1. A sexual dimorphism was found between male and female frogs according to their morphological characteristics. However, it was only significant in the case of *Rana lessonae* and *Rana esculenta* specimens. Between male and female pool frogs (*Rana lessonae*) the difference was highly significant in all measurements. We have not found, however, sexual dimorphism in the case of marsh frog (*Rana ridibunda*) specimens.

When comparing the pool frog specimens from two different populations (Bátorliget and Kis-Balaton), the morphological differences between sexes appeared mostly in the

Table 1. Summary of variation in 14 measurements for adult female and male frogs. Upper line: means and standard deviations; lower line: significance of the difference between males and females (Mann-Whitney U test). * = difference significant at 95% confidence level. ** = difference significant at 99% confidence level. Sample sizes given in parentheses.

	Rana lessonae		Rana ridibunda		Rana esculenta	
	male (61)	female (66)	male (16)	female (11)	male (14)	female (108)
BL	50.81±5.87	57.02±7.16	69.42±6.47	73.89±7.31	62.71±5.08	68.67±10.95
	0.0000**		0.1034		0.0119*	
HW	16.96±1.85	18.80±2.55	24.29±2.37	25.01±2.59	20.79±1.81	22.51± 3.72
	0.0001**		0.4592		0.0301*	
HW	16.96±1.85	18.80±2.55	24.29±2.37	25.01±2.59	20.79±1.81	22.51± 3.72
	0.0001**		0.4592		0.0301*	
IMD	4.00±0.37	4.32±0.54	4.73±0.49	4.79±0.58	4.37±0.25	4.81± 0.65
	0.0002**		0.6024		0.0011**	
IED	2.22±0.34	2.66±0.47	2.61±0.40	2.83±0.36	2.64±0.26	2.89± 0.48
	0.0000**		0.1703		0.0285*	
UEW	4.41±0.51	4.65±0.53	5.59±0.64	5.82±0.55	4.69±0.47	5.10± 0.63
	0.0092**		0.3213		0.0118*	
ED	6.33±0.68	6.77±0.70	8.29±0.83	8.74±0.92	6.88±0.50	7.59± 0.93
	0.0006**		0.1662		0.0119**	
MDT	3.51±0.57	4.04±0.65	4.94±0.69	5.15±0.62	4.54±0.49	4.87± 0.75
	0.0000**		0.3845		0.0697	
ICD	6.90±0.69	7.49±0.91	8.72±1.07	9.54±0.96	8.13±0.87	8.60± 1.22
	0.0003**		0.0883		0.0876	
NAEC	3.76±0.43	4.16±0.55	5.58±0.48	5.78±0.41	4.64±0.28	5.14± 0.75
	0.0000**		0.4405		0.0118**	
SN	4.43±0.49	4.83±0.66	6.26±0.67	6.42±0.49	5.35±0.39	5.69± 0.92
	0.0005**		0.5512		0.0831	
FL	24.33±3.10	26.74±3.35	37.39±3.91	38.93±4.20	30.88±2.75	33.00± 5.33
	0.0002**		0.3235		0.0421*	
TL	22.91±2.77	25.34±3.38	38.06±3.56	39.40±3.54	30.45±2.74	32.78± 5.29
	0.0001**		0.3001		0.0326*	
IMTL	3.65±0.45	3.96±0.51	3.53±0.40	3.58±0.59	3.85±0.33	4.16± 0.66
	0.0003**		0.8622		0.0286*	
FTL	6.29±0.90	7.05±1.03	11.21±1.32	11.70±1.29	8.84±1.12	9.38± 1.58
	0.0001**		0.2167		0.1020	

BL = body length, HW = head with, IND = internarial distance, IED = inter eyelid distance, UEW = upper eyelid with, ED = eye diameter, MDT = maximum diameter of tympanum, NARC = nostril to anterior eyelid commisure, SN = snout to nostril, ICD = intercanthal distance, TL = tibia length, FL = femur length, FTL = first toe length, IMTL = inner metatarsal tubercle length.

sample of the Kis-Balaton. This result is discussed in detail elsewhere (Gubányi, 1991a, 1991b).

To avoid misinterpretations, caused by sexual differences, in the discriminant analysis we selected morphological indices which were not sexually dimorphic (table 2). Of the 12 character ratios calculated in our study, only seven were included into the discriminant analysis. Two indices (body length/first toe length, tibia length/inner metatarsal tubercle length) were found to be the most important discriminating features, between the three species. Three of the five remaining ratios contained also some discriminating

Table 2. Summary of variation in twelve ratios for adult female and male frogs. Upper lines: means and standard deviations; lower line: signficance of the difference between males and females (Mann-Whitney U text). * = difference significant at 95% confidence level. ** = difference significant at 99% confidence level. Sample sizes given in parentheses.

	Rana lessonae		Rana ridibunda		Rana esculenta	
	male (61)	female (66)	male (16)	female (11)	male (14)	female (108)
BL/TL	2.22±0.10	2.25±0.11	1.82±0.06	1.87±0.06	2.06±0.09	2.10±0.10
	0.0375 *		0.0679		0.2927	
BL/FL	2.09±0.09	2.13±0.09	1.86±0.09	1.90±0.11	2.03±0.07	2.08±0.10
	0.0076**		0.4298		0.0575	
BL/IND	12.70±1.01	13.25±1.07	14.73±1.15	15.52±1.30	14.39±1.41	14.27±1.01
	0.0201*		0.1143		0.7177	
BL/FTL	8.13±0.59	8.14±0.71	6.22±0.38	6.33±0.27	7.15±0.53	7.35±0.46
	0.6466		0.4298		0.0752	
BL/IMTL	13.99±1.04	14.49±1.35	19.81±1.86	20.88±2.03	16.35±1.38	16.55±1.39
	0.0281*		0.0757		0.8503	
FL/TL	1.06±0.04	1.06±0.05	0.98±0.03	0.99±0.04	1.01±0.01	1.01±0.02
	0.9442		0.2523		0.0843	
TL/IMTL	6.31±0.57	6.45±0.79	10.86±0.95	11.15±1.17	7.94±0.77	7.90±0.73
	0.9231		0.3237		0.5576	
FTL/IMTL	1.73±0.17	1.80±0.29	3.20±0.34	3.30±0.30	2.30±0.30	2.26±0.22
	0.4259		0.2776		0.3350	
IND/IED	1.83±0.24	1.66±0.28	1.84±0.26	1.71±0.27	1.67±0.16	1.68±0.22
	0.0004**		0.5052		0.9073	
ICD/NAEC	1.85±0.16	1.81±0.19	1.56±0.16	1.65±0.11	1.75±0.16	1.68±0.17
	0.0024		0.1825		0.1864	
ICD/SN	1.56±0.11	1.56±0.12	1.40±0.20	1.49±0.11	1.52±0.14	1.52±0.14
	0.7925		0.1993		0.9552	
ICD/IND	1.73±0.11	1.74±0.15	1.84±0.12	2.01±0.21	1.86±0.22	1.79±0.16
	0.6141		0.02631*		0.3769	

BL/TL = body length / tibia length, BL/FL = body length / femur length, BL/IND = body length / internarial distance, BL/TFL = body length / first toe length, BL/IMTL = body length / inner m. t. length, FL/TL = femur length / tibia length, TL/IMTL = tibia length / inner m. t. length, FTL/IMTL = first toe length / inner m. t. length, IND/IED = internarial distance / inter eyelid distance, ICD/NAEC = intercanthal distance / nostril to anterior eyelid commisure, ICD/SN = intercanthal distance / snout to nostril, ICD/IND = intercanthal distance / internarial distance.

Table 3. Classification results on the basis of discriminant analysis applied to ratios body length/first toe length and tibia length/inner metatarsal tubercle length

Actual Group	No. of Cases	Predicted Group R. l.	R. r.	R. e.
Rana lessonae	127	115	0	12
		90.6%	0.0%	9.4%
Rana ridibunda	27	0	26	1
		0.0%	96.3%	3.7%
Rana esculenta	122	9	0	113
		7.4%	0.0%	92.6%
Percent of frogs correctly classified:				92.3%

Rana ridibunda = R. r., Rana lessonae = R. l., Rana esculenta = R. e.

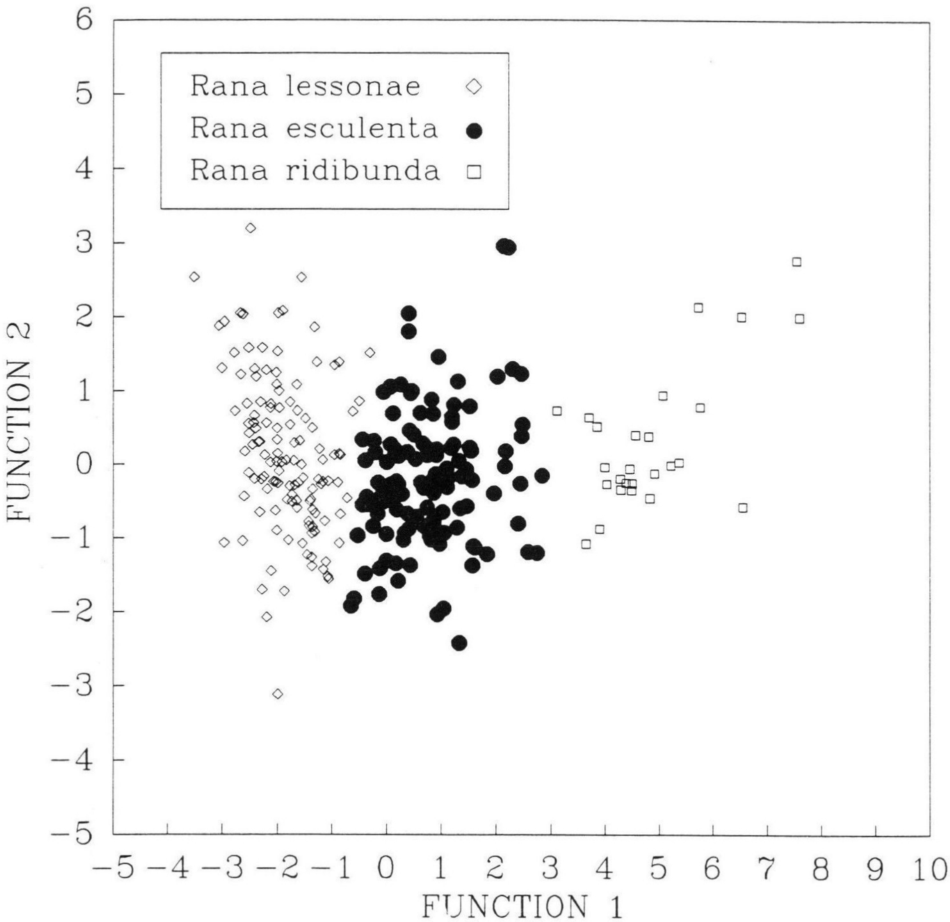

Figure 1. Distribution of three types of frogs along the first two canonical discriminant functions calculated in a stepwise discriminant analysis, using two morphometric ratios (BL/FTL, TL/IMTL) not significantly sexually dimorphic.

information, but they did not contribute to any great extent. Consequently, applying discriminant analysis only to body length/first toe length (BL/FTL) and tibia length/inner metatarsal tubercle length (TL/IMTL) indices gave correct classification percentage of the frogs which were high enough (92.03%, see table 3). Misclassifications mostly occurred among *Rana lessonae* and *Rana esculenta* specimens (12 and 9 individuals, respectively). All specimens, including these intermediate forms, were diploid, as shown by a parallel analysis (Gubányi, 1991a).

Distribution of water frog specimens according to the first two discriminant functions is shown in fig. 1. The three forms are clearly separated from each other. *Rana esculenta* specimens are found in an intermediate position between *Rana lessonae* and *Rana ridibunda*

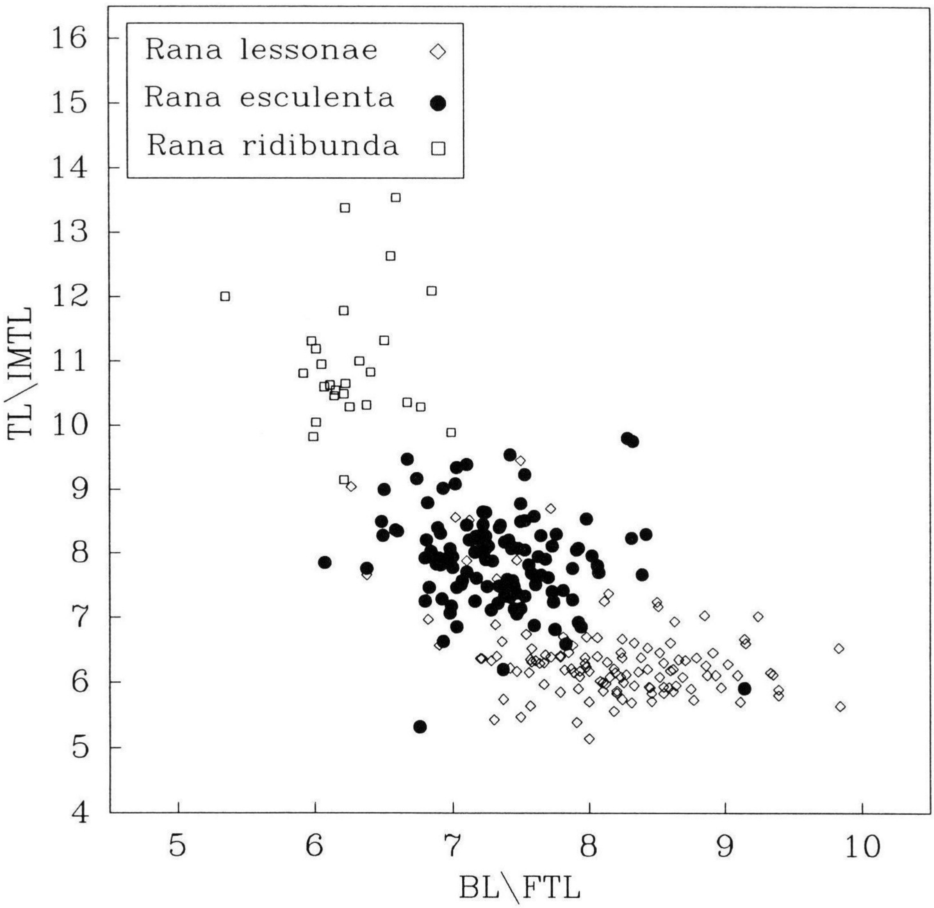

Figure 2. Distribution of three types of green frogs along the axes of BL/FTL and TL/IMTL chosen by a stepwise discriminant analysis.

samples, the latter being perhaps the most separated group. Morphological differences of the three forms, based on the two ratios mentioned above, are illustrated in fig. 2. *Rana ridibunda* is again quite distinct from the two other groups. *Rana esculenta* shows here, too, intermediate morphological features between their parental species.

Discussion

The following indices: tibia length/inner metatarsal tubercle length, first toe length/ inner metatarsal tubercle length, body length/first toe length are mentioned in the literature as the best morphological characters to be used for identification of the Central European water frog forms (e.g. Berger, 1966; Günther, 1973; Tunner and Dobrowsky, 1976; Borkin et al., 1979).

Discriminant analyses have also been considered as suitable methods for distinguishing the taxa of water frogs (Wijnands and van Gelder, 1976; Wijnands, 1979; Hotz and Uzzell, 1979, 1982). According to the studies of Wijnands and van Gelder (1976) and Wijnands (1979), the most useful indices for separating water frog species in the Netherlands are: tibia length/inner metatarsal tubercle length and tibia length/inner metatarsal tubercle height.

In our case, based on two L-E population systems and an R population, body length/ first toe length and tibia length/inner metatarsal tubercle length were found to be the most important discriminators. The classification result of the discriminant analysis with these ratios was correct at a generally accepted high level (92.03 and 92.3%, respectively, see above). Tibia length/inner metatarsal tubercle length seems to be a generally useful index for discrimination, since it was also selected by Wijnands and van Gelder (1976). Height of inner metatarsal tubercle was not measured in our study for practical reasons (our original aim was to provide a character set easy to measure in the field) and hence its ratio with the tibia length was not introduced in the discriminant analysis either. We also wanted to avoid using a measurement twice in the same analysis. Taking all these into consideration, application of the body length/first toe length index as a second ratio, seems to be theoretically more reasonable and proved in addition to be better in the identification process.

Acknowledgements. We are indebted to Dr. I. Matskási for providing opportunities to carry out the biochemical investigations on the frogs.

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Received: May 31, 1991. Accepted: August 21, 1991.