THE VARIABILITY OF SOME MORPHOLOGICAL CHARACTERS IN *RANA RIDIBUNDA* (PALL.)

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Abstract: For the biometric analysis at *Rana ridibunda* (Pall.) we studied the 12 morphological features and we calculated the report between some analyzed biometric indexes. The interpretation of the obtained results was done using the comparative statistic tests. The analysis of the variability shows that most morphological features have a low variability. Only the interpalpebral distance and the length of the internal metatarsal tubercle presented an average variability. Another observation would be that, generally, male individuals had a slightly higher variability of the investigated parameters.

INTRODUCTION

Rana ridibunda species Pallas 1771 is part of the green frog complex together with the species *Rana esculenta* Linnaeus 1758 and *Rana lessonae* Camerano 1882. The general opinion regarding the three species emphasizes the hybrid nature of the *Rana esculenta* (L.) by expressing some morphological features intermediary to the parental species of the complex. It is the reason for which the hybrid identification methods for such features are used in the identification of the 3 species.

Besides, the global decline of biodiversity, in this case the amphibian – about 200 species are let down and 32 species are about to disappear (Blaustein and Wake, 1990, Alford and Richards, 1999, Houlahan et all., 2000) – impose the launching of some monitoring programs involving the use of some investigation methods and techniques as varied as possible to form a clear image upon the adaptation potential of these species. Thus, the variability may be considered a result of the interactions between the genotype and the environment.

This paper was meant to bring some contributions to the knowledge of biodiversity, by assessing the variability of some morphological features at *Rana ridibunda* (Pall.), using adult individuals collected from Balții pool in Dorohoi (Botoșani county).

MATERIAL AND METHODS

To comply with the above mentioned study, 49 individuals from the *Rana ridibunda* (Pall.) species were collected (32 males and 17 females) from Balții pool located in a swampy area (with groundwater located in little depths) from the east of Dorohoi.

Capturing individuals to be used in our study was done by means of a fish landing. We observed that, by submersion of the fish landing ring and attracting individuals by means of a vivid colored cork, fixed at the end of a fishing rod, the efficiency of the capturing action obviously increased. After the completion of biometric measurements, the individuals were released in their natural environment. To avoid a possible investigation of the same individuals in case of a re-capture, before their release, the individuals were marked. As marking technique, we used the band method (Elmberg, 1989; Rice and Taylor, 1993, quoted by Cogălniceanu, 1997). For the procurement of biometric data, we used a digital caliper, Mannesmann brand (Brüder Mannesmann Werkzenge GmbH, Germany), with 0.01 mm precision.

To complete the samples, all the data should be characteristic to the adult population. Therefore, we used as a criterion in the sample formation the length of the body of the collected individuals, according to the mentions from Fauna R.P.R. Amphibia, vol. XIV, 1(1960). Thus, the sample from *Rana ridibunda* (Pall.) contains individuals with the length between 62 - (76, 4) - 94 mm.

For the biometric analysis we studied the morphological features below: interpalpebral distance (Sp.p.), the eye length (L.o.), the head width (Lt.c.), the eardrum length (L.tymp.), the length of the head (L.c.), the length of the body (L.), the length of the anterior limb (L.m.a.), the length of the femur (F.), the length of the tibia (T.), the length of the shin articulation (L.tars.), the length of the metatarsal tubercle (C.int.), the length of the first finger (D.p.).

Based on the obtained results, we calculated the report between some analyzed biometric indexes. Among these, the most used in the specialty literature (Wijnands și Van Gelder, 1976; Gubányi și Kórsós, 1992; Csata, 1998; Zamfirescu, 2002; Krizmanić, 2008) are:

- The report between the length of the tibia and the length of the internal metatarsal length (T/C.int.);
- The report between the first finger and the length of the internal metatarsal tubercle (D.p./C.int.);
- The report between the length of the body and the length of the first finger (L/D.p.).

For each morphological feature analyzed, the minimum and maximum value were determined, as well as the arithmetic average, standard error and the variation coefficient. The interpretation of the obtained results was done using the comparative statistic tests. To determine the dispersion, we used the Fisher-Snedecor test and according to it, the Student homoscedastisc or heteroscedastisc.

The obtained results are presented in tables 1 and 2 and figure 1.

RESULTS AND DISCUSSIONS

After the completion of the biometric measurements, the registered data were centralized on each researched parameter and 2 sexes, setting the values of the statistic indicators for each of the morphometric features (tab. 1). In figure 1 the arithmetic averages of the researched morphological parameters are graphically represented at the 2 sexes.

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Biometric	Statistic parameter	R. ridibunda	R. ridibunda	R. ridibunda
parameter			4	8
	Minimum	2.8	3.0	2.8
Interpalpebral	Maximum	4.5	4.5	4.2
distance	Average	3.414	3.511	3.362
(Sp.p.)	Standard error	0.052	0.100	0.059
	Variation coefficient	10.72	11.81	9.90
	Minimum	7.4	7.8	7.4
Length of the eye	Maximum	11.0	10.2	11.0
(L.o.)	Average	8.955	8.935	8.965
	Standard error	0.110	0.174	0.142
	Variation coefficient	8.59	8.03	9.00
	Minimum	23.8	24.0	23.8
Width of the head	Maximum	35.4	31.7	35.4
(Lt.c.)	Average	28.518	27.482	29.086
	Standard error	0.387	0.492	0.510
	Variation coefficient	9.49	7.38	9.92
Length of the	Minimum	4.5	5.0	4.5
	Maximum	6.6	6.4	6.6
eardrum	Average	5.628	5.594	5.646
(L.tymp.)	Standard error	0.049	0.079	0.085
	Variation coefficient	7.62	5.86	8.55
Length of the head (L.c.)	Minimum	23.6	24.1	23.6
	Maximum	37.0	32.3	37.0
	Average	28.891	28.464	29.118
	Standard error	0.399	0.571	0.533
	Variation coefficient	9.68	8.27	10.37
	Minimum	62.6	62.6	67.6
Length of the	Maximum	91.7	83.7	91.7
body (L.)	Average	75.932	74.317	76.79
	Standard error	0.872	1.529	1.046

Tab. 1 – Values of some morphological parameters (in mm) at Rana ridibunda (Pall.)

Biometric parameter	Statistic parameter	R. ridibunda	<i>R. ridibunda</i>	R. ridibunda
	Variation coefficient	8.04	8.48	7.70
Length of the	Minimum	16.5	16.5	18.0
anterior member	Maximum	24.2	23.4	24.2
(L.ma.)	Average	21.030	20.470	21.328
	Standard error	0.259	0.496	0.289
	Variation coefficient	8.63	10.00	7.66
	Minimum	31.0	31.0	31.3
Length of the	Maximum	47.3	40.2	47.3
femur	Average	37.102	36.288	37.534
(F.)	Standard error	0.488	0.702	0.643
	Variation coefficient	9.22	7.97	9.69
	Minimum	30.8	31.0	30.8
Length of the tibia	Maximum	43.7	40.1	43.7
(T.)	Average	36.320	35.517	36.746
	Standard error	0.434	0.610	0.573
	Variation coefficient	8.37	7.08	8.82
	Minimum	14.9	15.5	14.9
Tars length	Maximum	21.7	21.1	21.7
(L.tars.)	Average	18.426	18.24	18.525
	Standard error	0.251	0.406	0.321
	Variation coefficient	9.54	9.17	9.81
	Minimum	3.0	3.1	3.0
Length of the	Maximum	4.7	4.7	4.7
metatarsal	Average	3.971	3.952	3.981
tubercle	Standard error	0.070	0.131	0.084
(C.int.)	Variation coefficient	12.46	13.66	12.00
	Minimum	12.0	12.0	12.7
Length of the first	Maximum	16.9	16.5	16.9
finger (D.p.)	Average	14.620	14.364	14.756
	Standard error	0.167	0.334	0.183
	Variation coefficient	7.99	9.59	7.04





Fig. 1 Graphical representation of the average values of some biometric parameters at the *Rana ridibunda* Pall species, according to the sexes of the individuals

Interpalpebral distance (Sp.p.), measured between the ocular globes, at the middle of the eyelids, at the investigated population of *Rana ridibunda* (Pall.), is between 2.8 - 4.5 mm with an average of 3.41 ± 0.05 mm, the variability of the individuals from that population being average to reduced (CV = 10.72), being a little bit increased to females as compared to males. Even with the calculated mean of the interpalpebral distance at the 2 sexes (3.51 ± 0.10 mm to female and 3.36 ± 0.06 mm to males) we cannot talk about the possibility of differentiation between the 2 sexes using as a criterion this biometric parameter, since the T test applied with the level of signification ($\alpha = 0.05$) has confirmed the null hypothesis (H₀) of the equality between the 2 averages.

The length of the eye (L.o.), measured at the ends of the ocular longitudinal diameter, varies at the sample of the *Rana ridibunda* (Pall.) population between 7.4 mm and 11 mm, with an average of 8.96 ± 0.11 mm, the parameter variability being a reduced one both to males and females, according to the values of the variation coefficient (CV = 8.59). Comparative tests (F test and T test) confirm the existence of a non-significant difference between the averages, with an error of 0.05.

The width of the head (Lt.c.), measured between the mouth corners, varies between 23.8 mm and 35.4 mm. The average value of this feature is of 28.51 ± 0.39 mm, and the variation coefficient (CV = 9.49) indicates a reduced variability of the parameter for both sexes. The head width can be used as a discriminator parameter of the sexes in the *Rana ridibunda* (Pall.) population, males having bigger average as compared to females with an error of 0.05.

The length of the eardrum (L.tymp.), represented by the horizontal diameter of the eardrum, varies between 4.5 si 6.6 mm. The average length of the drum ear, calculated for the sample, was of 5.63 ± 0.05 mm, and the variability of the feature is reduced (CV = 7.62). The averages of the eardrum length do not significantly differ according to the sexes of the individuals.

The length of the head (L.c.), measured from the top of the muzzles to one of the corners of the mouth, is a biometric parameter whose registered valued in the studied population of *Rana ridibunda* (Pall.) were between 23.6 mm and 37 mm. The dispersion interval of this parameter has an average of 28.89 ± 0.4 mm, and the variability of the feature is reduced (CV = 9.68). If we consider the sex of the individuals, there are no important changes regarding the dispersion of the biometric data and the comparative analysis of the averages.

The length of the body (L.), measured from the top of the muzzle to the anus, at the population of *Rana ridibunda* (Pall.), had as variability limits between 62.6 and 91.7 mm, the average length of the body being of 75.93 ± 0.87 mm. The variability of this feature was reduced

(CV = 8.04). In case of females, the length of the body varied between 62.6 and 83.7 mm, with an average of 74.32 ± 1.53 mm. As for the males, the limits of the analyzed feature were between 67.6 and 91.7 mm, with a mean of 76.79 ± 1.05 mm. Comparative analysis of the samples differentiated by sexes indicate an equal dispersion of the values between the 2 data rows (F testnon important – H₀) and the comparative analysis of the means with the help of the homoscedastic T-test present a greater possibility than the level of significance (0.05), which leads to the acceptance of the null hypothesis (H₀).

The length of the anterior member (L.ma.), measured from the wrist to the top of the longest finger (3^{rd} finger), within the studied *Rana ridibunda* (Pall.) population, varied between 16.5 and 24.2 mm, with an average mean of 21.03±0.26 mm, the feature variability being reduced (CV = 8.63). Although the arithmetic means differ according to sex, the statistic applied tests do not indicate significant differences.

The length of the femur (L.), measured from the anus to the middle of the knee articulation, represents an often used parameter in the specialty literature from the biometry works at *Ranidae*. In the analyzed *Rana ridibunda* (Pall.) population, this parameter oscillated between 31 and 47.3 mm, having a sample average of 37.10 ± 0.49 mm. The feature variability is reduced (CV = 8.37), including in the samples differentiated by sexes. Moreover, we support the equality of the averages of the 2 samples, with an error of 0.05.

The length of the tibia (T.), measured from the middle of the knee articulation to the middle of the tibia tarsal articulation, varies between 30.8 and 43.7 mm, having a sample average of 36.32 ± 0.43 mm. The variability of the feature is reduced (CV = 8.37). Although the arithmetic averages of the tibia length are a bit different at the 2 sexes, the difference is not significant statistically.

The legth of the tars (L.tars.), measured from the middle of the tibia tarsal articulation to the middle of the tars metatarsal articulation, varies between 14.9 mm and 21.7 mm at the *Rana ridibunda* (Pall.) population. The average length of the tars is of 18.43 ± 0.25 mm, and the variability of the feature is reduced (CV = 9.54). Statistic tests applied for the 2 differentiated samples according to sexes indicate equal dispersions of the data, and the average values of the tars length are statistically equal to the 2 sexes, with an error of 0.05.

The length of the internal metatarsal tubercle (C.int.) represents an important biometric parameter for the determination and characterization of the species of green frogs. Limit values for this parameter were of 3.0 and 4.7 mm, with an average calculated of 3.97 ± 0.07 mm. It is a feature showing intermediate variability (CV = 12.46). As for the possibility of differentiating the 2 sexes using as discriminatory criterion the length of the internal metatarsal tubercle, the applied calculation are not important.

The length of the first finger (digittus primus – D.p.), measured from the top to the distal edge of the internal metatarsal tubercle, represent another morphological parameter extremely important in the biometric research of green frogs. It is a parameter oscillating between 12.0 and 16.9 mm, arithmetic average being of 14.62 ± 0.17 mm. The feature variability is low (CV = 7.99), being similar to females and males. There are no statistic significant differences between the averages registered by this parameter to the 2 sexes.

The analysis of the variability of the 12 morphological features studied at *Rana ridibunda* (Pall.) show that most of them have a low variability, the variation coefficient displaying values under 10. Only 2 of the investigated parameters presented an average variability: the interpalpebral distance and the length of the internal metatarsal tubercle. Another observation would be that, generally, male individuals (although they outnumber the females in

the analyzed sample) had a slightly higher variability of the investigated parameters, but for the interpalpebral distance, length of the femur, length of the anterior limb, length of the internal metatarsal tubercle, and length of the first finger, with a much increased variability in females.

As for the report between one of the analyzed indicators (tab. 2), the calculated values are included in the intervals stated by the specialty literature (Wijnands and Van Gelder, 1976, Gubányi and Korsós, 1992, Zamfirescu, 2002, Krizmanić, 2008). The most extended of their variability interval was in the case of the T/C.int report. The minimal value of this report is of 6.57, maximum value of 12.09, and the average of 9.26 ± 0.16 . The situation does not change in case of the analysis of this report on sexes. The other studied reports vary in lower limits. Thus, the D.p./C.int. is somewhere between 3.02 and 4.91, with an average of 3.72 ± 0.06 in the whole collected sample, (between 3.11 and 4.10 – with an average of 3.67 ± 0.08 to females; between 3.02 and 4.91 – with an average of 3.75 ± 0.08 to males. The L./D.p. report has values between 4.64 and 5.73 – with an average of 5.20 ± 0.04 in the analyzed *Rana ridibunda* (Pall.) population (limit values of 4.64 and 5.62 – with a mean of 5.19 ± 0.07 to females; limit values of 4.83 and 5.73 – with an average of 5.21 ± 0.04 to males). Comparative analysis of these reports, according to the sex of the individuals, shows non important differences between the averages of the reports, with an error of 0.05.

Number of		Report		
individuals (<i>Rana ridibunda</i>)	Statistic parameter	T/C.int.	D.p./C.int.	L./D.p.
Total individuals (49)	Minimum	6.574	3.021	4.636
	Maximum	12.093	4.906	5.731
	Average	9.255	3.720	5.200
	Standard error	0.163	0.058	0.037
	Variation coefficient	12.32	10.99	5.03
	Minimum	7.782	3.108	4.636
Females (17 individuals)	Maximum	10.636	4.102	5.619
	Average	9.092	3.666	5.187
	Standard error	0.229	0.082	0.072
	Variation coefficient	10.40	9.24	5.78
	Minimum	6.574	3.021	4.828
Males (32 individuals)	Maximum	12.093	4.906	5.731
	Average	9.342	3.749	5.207
	Standard error	0.218	0.078	0.043
	Variation coefficient	13.25	11.87	4.69

Tab. 2 - Report value between some of the analyzed biometric indicators

CONCLUSIONS

The study of variability of 12 morphological features of individuals belonging to a *Rana ridibunda* (Pall.) population from Balții pool (Botoșani county), showed that, except the width of the head, all the other investigated parameters register average values approximately similar, according to sex.

The variability of the researched features is reduced both at the level of the analyzed sample, and according to sex. Yet male individuals generally present slightly increased values of the variation coefficient than females for most of the studied parameters.

Analysis of the report of some of the parameters investigated at *Rana ridibunda* (Pall.) shows a low variability of the report between the length of the body and the length of the first finger (L./D.p.) to both sexes, which makes this report reliable in the characterization of *Rana ridibunda* (Pall.) species.

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