Arch. Biol. Sci., Belgrade, 60 (3), 449-457, 2008 DOI:10.2298/ABS0803449K

WATER FROGS (*RANA ESCULENTA* COMPLEX) IN SERBIA - MORPHOLOGICAL DATA

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Abstract —The main purpose of this paper was to estimate morphometric variability of the water frog (*Rana* synklepton *esculenta* complex) population in Serbia. Altogether, 396 water frogs were collected at 15 localities in Serbia and analyzed using principal components for 18 selected indices, in addition to which correspondent analyses were conducted for 30 qualitative external morphological traits. The results showed that the population samples were heterogeneous and included three separate forms (*Rana ridibunda*, *Rana lessonae* and *Rana* kl. *esculenta*). Significant interspecific differences were found between *R. ridibunda* and *R. lessonae*, which are clearly distinct from each other. *Rana* kl. *esculenta* specimens were in an intermediate position between *R. ridibunda* and *R. lessonae*, with values more similar to the *R. ridibunda* parent species.

Key words: Rana ridibunda, Rana lessonae, Rana kl. *esculenta,* morphometry, Serbia

UDC 597.851(497.11):591.5

INTRODUCTION

The presence of water frogs in the Serbian part of the Balkan Peninsula and adjacent territory was recorded over a hundred years ago (Đorđević, 1900 a, 1900 b). It is known that the water frog group in Serbia is composed of three species (*Rana ridibunda* Pall., 1771; *Rana lessonae* Camerano, 1882; and their hybrid *R.* kl. *esculenta* Linnaeus, 1758).

In addition, the southern boundary of the distribution range of *R. lessonae* runs through part of the range of the *Rana* synklepton *esculenta* complex in Serbia.

However, analyses of their morphological attributes in this area are conspicuously scarce (Gavrilović et al., 1999; Spasić-Bošković et al., 1999; Džukić et al., 2001, 2003).

Moreover, in those analyses, the morphological characteristics considered were referable to a few elementary attributes only. The significance of morphological indices and qualitative characteristics was not considered.

Although doubts exist about the usefulness of morphological characteristics in the identification of water frogs (Pagano and Joly, 1999), many authors still use these characters (Gubányi and Korsós, 1992; Борисовский et al., 2000; Budak et al., 2000; Lode and Pagano, 2000; Sinsch et al. 2002; Nekrasova et al., 2003; Želev and Mollov, 2004).

Overlap between values for elementary morphometric attributes is partly corrected with indices, but although the main factor in their applicability is sample size.

As for different methodological approaches, qualitative characteristics have rarely been used for taxonomical analyses of water frogs (Karaman, 1948; Dely, 1967; Berger, 1968; Berger, 1976; Nevo and Young, 1982; Schneider et al., 1984; Günther et al., 1991, Disner et al., 1997; Arikan et al., 1998; Clark, 1998; Tosunoglu, 2005).

Their significance in the identification of water frogs can be estimated through the mutual influence of morphological characteristics (Günther et al., 1991; Schröer, 1997).

It is possible to recognize parent species from the variation range of morphological indices and specific qualitative traits. However, the values of these characters in hybrid forms cover major parts of the ranges of both parent species (Uzzell et al., 1977; Günther et al., 1991; Joly et al., 1994; Spasić Bošković et al., 1999, Nekrasova et al., 2003).

Moreover, morphological identification is important from the practical aspect, especially for protection of water frogs.

The aim of the present study was to examine the structure and pattern of morphometric variability among water frogs in the investigated area. Do populations from the contact zone follow similar morphometric variation patterns? Are there some diagnostic indices that could be used for taxonomical determination in the analyzed population systems?

MATERIALS AND METHODS

Altogether 396 specimens (179 males and 217 females), including members of all three taxa of the *Rana* synklepton *esculenta* complex were collected at 15 localities (Appendix 1) (Fig. 1).

Morphometrical analyses (two-way ANOVA and principal component analysis) were performed on the following 18 selected indices of external morphological characteristics:

L/F – body length/femur length; L/T – body length/tibia length; L/Spi – body length/internasal distance; L/Ltc – body length/maximum head width; L/DpPp – body length/length of the first toe of hind leg; L/Cint – body length/metatarsal tubercle length; L/Spp – body length/minimal interorbital distance; L/Dro – body length/snout-eye distance; L/Ltym – body length/diameter of the tympanic membrane; F/T – femur length/tibia length; F/DpPp – femur length/length of the first hind leg toe; F/Cint – femur length /metatarsal tubercle length; T/DpPp – tibia length /length of the first hind leg toe; T/Cint – tibia length/metatarsal tubercle length; DpPp/Cint – length of the first hind leg toe/metatarsal tubercle length; SPI/Spp – internasal distance/minimal interorbital distance; Ltc/Dro – maximum head width/snout-eye distance; and Ltc/Lc – maximum head width/head length.

Correspondent analysis was performed on 10 qualitative characteristics with 30 selected traits: I Vocal sacs: 1. white, 2. dark gray, 3. light gray, 4. absent; II Main color of external surface of hind legs: 1. no coloration, 2. olive and green, 3. completely yellow, 4. partly yellow, 5. yellow in traces; III Yellow coloration on flanks: 1. present, 2. absent; IV Dark stripes on hind legs: 1. present, 2. absent, 3. stripes differentiated into spots; V Coloration of the internal surface of hind legs: 1. yellow (sulfur), 2. yellowish–green; VI Dorsal stripe: 1. present, 2. absent; VII Ventral side of torso and throat : 1. white, 2. marmorated, 3. weakly marmorated; VIII Metatarsal tubercle: 1. large, 2. medium-sized, 3. slightly raised and elongated; IX Shape of metatarsal tubercle: 1. symmetrically semicircular, 2. asymmetrical, highest point directed to the first toe, 3. asymmetrical, highest point directed to the metatarsal joint, 4. low and flat; X Front side of head: 1. elliptically obtuse, 2. sharpened.

The Statsoft statistical software package (Statsoft Inc., 1997) was used for all statistical analyses.

RESULTS

Morphometric analysis

Two-factor ANOVA with taxa and sex as the factors confirmed that several indices differed between the taxa. The most significant effects of taxa were observed for the following morphometrical indices: T/Cint ($F= 52.8$, $p< 0.000$), DpPp/Cint ($F= 47.1$, $p< 0.000$), L/T ($F= 40.1$, $p< 0.000$), F/T ($F= 30.1$, $p< 0.000$), F/Cint ($F= 21.5$, $p< 0.000$), and L/Cint ($F= 20.5$, $p< 0.000$).

The first two components of the principal component analysis were supported by 73.78% of the total variance. The first component (45.67% of total variance) mainly described variation in the relative ratio of body lengths and head measure. The second (28,11% of total variance) mainly consisted of relative variation of hind leg size, especially for relative length of the *callus internus*.

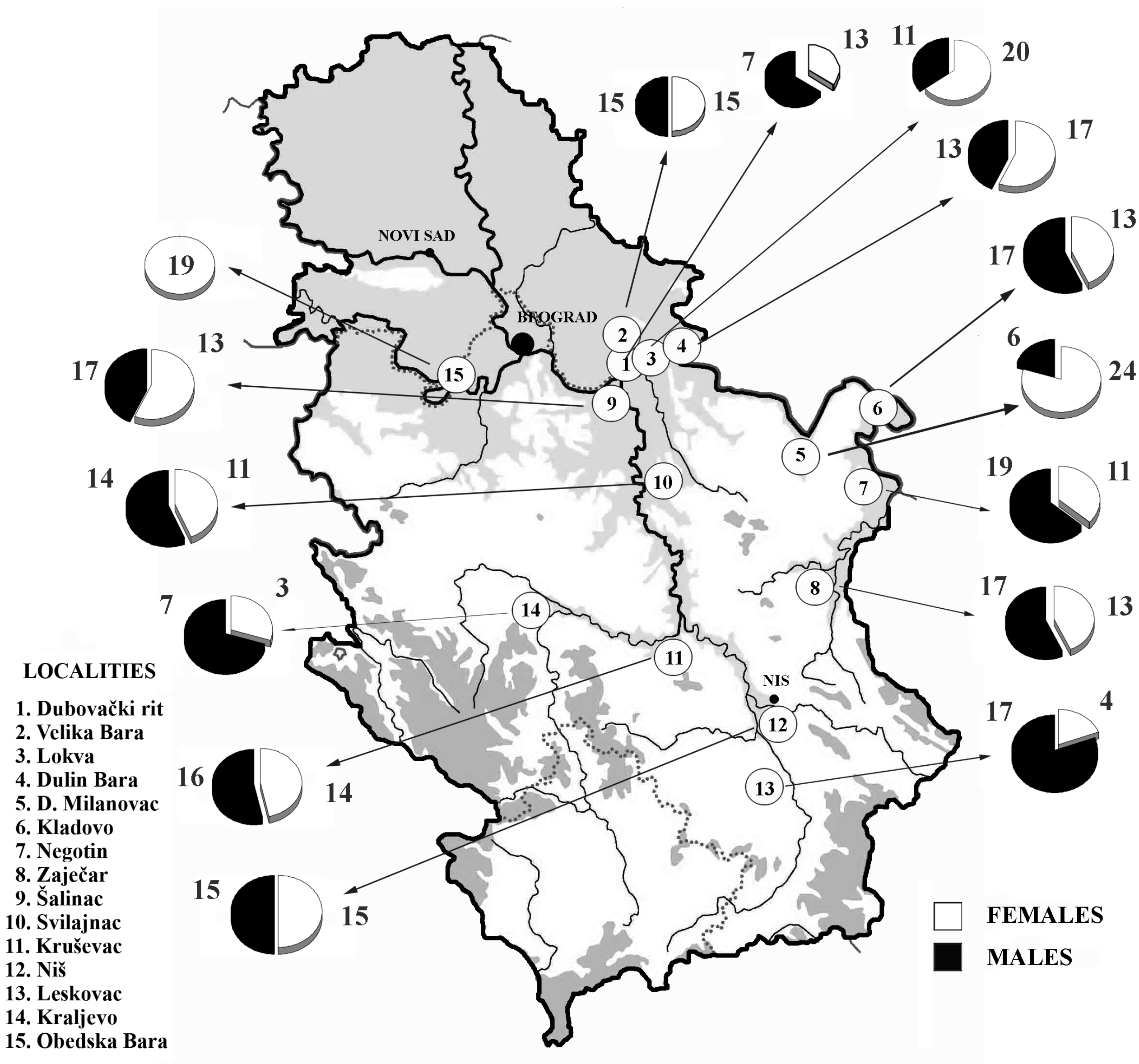

Fig. 1. Map of localities and populations with number of females and males.

The contributions of these indices to the first two components are given in Table 1.

Table 2 and Fig. 2 show the distribution of each taxa on the factorial map C1-C2. Both components separated *R. lessonae* from the other species, mainly due to shorter body and lower dimensions of hind leg.

Both components indicated that variation between the *ridibunda* and *esculenta* population samples was low.

Analysis of the ratios DpPp/Cint and T/Cint, DpPp/Cint and L/T, and T/Cint and L/T converged with the PCA in separating population samples of *R. lessonae* (Figs. 3, 4, and 5). The ranges of variation of this index are given in Table 3. The cleanest separation was for relations with *callus internus* (T/Cint, DpPp/Cint).

Table 1. Contribution of significant indices to the first two components of PCA.

Indices	Component 1	Component 2
L_SPI	1.624	0.032
L_CINT	0.369	2.482
L_SPP	2.750	0.191
L_LTYM	0.960	0.265
F_CINT	0.175	1.049
T_CINT	0.143	1.320

Table 2. Mean coordinates of each taxon on the first two components of morphometrical indices.

Taxon	PC1	PC2
R. ridibunda	-0.018	0.273
R. lessonae	-0.510	-1.476
R. kl. esculenta	0.146	-0.218

Table 3. Ranges of variation of three main morphometrical indices.

Taxon	T/CINT	DPPP/CINT	L/T
R. ridibunda	7.6 - 15.7	2.1 - 3.8	0.9 - 2.2
R. lessonae	4.3 – 7.7	1.2 - 1.9	1.9 - 3.0
R. kl. esculenta	6.4 - 11.8	1.6 - 3.0	1.7 - 2.5

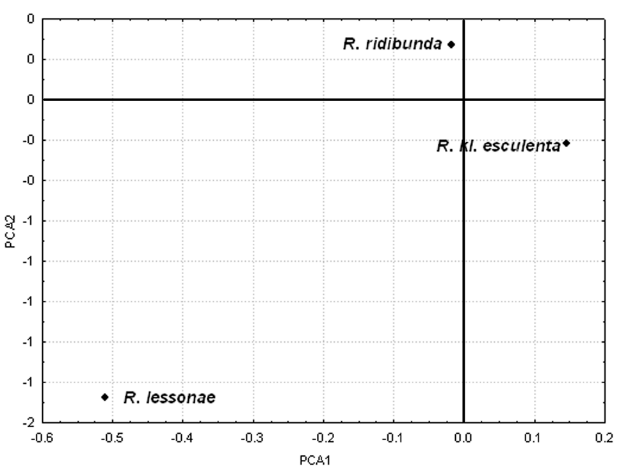

Fig. 2. Principal component analysis: factorial map PCA 1-PCA 2. Dots represent the mean coordinates of each taxon.

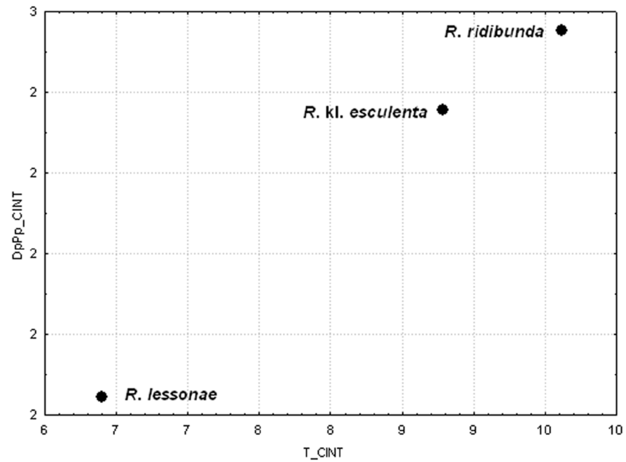

Fig. 3. Distribution of *R. ridibunda, R. lessonae*, and *R.* kl. *esculenta* in relation to DpPp/Cint and T/Cint.

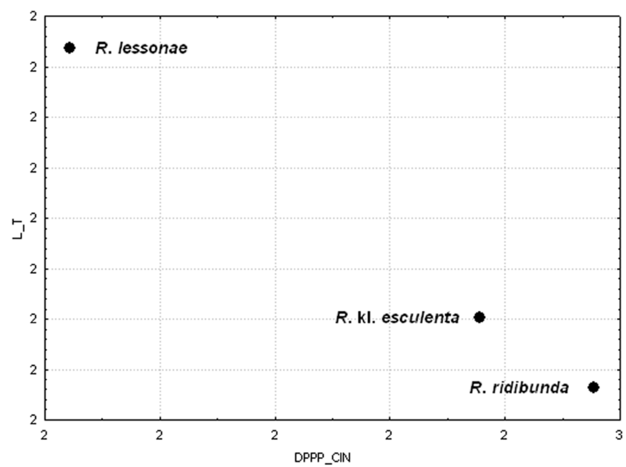

Fig. 4. Distribution of *R. ridibunda, R. lessonae*, and *R.* kl. *esculenta* in relation to DpPp/Cint and L/T.

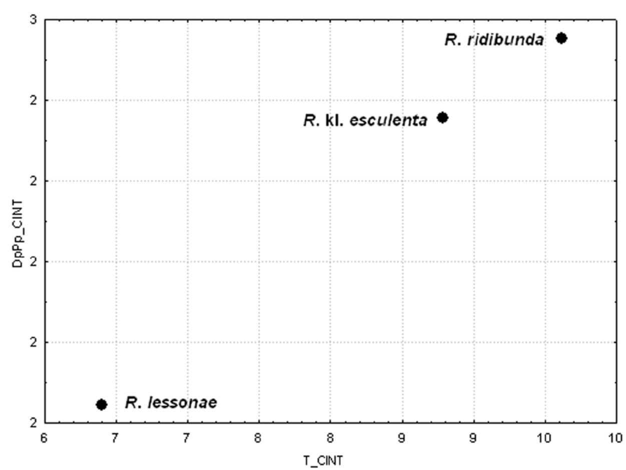

Fig. 5. Distribution of *R. ridibunda, R. lessonae*, and *R.* kl. *esculenta* in relation to T/Cint and L/T.

Table 4. Frequencies of qualitative character states of males.

Character states	*R. ridibunda*	*R. lessonae*	*R.* kl. *esculenta*
I:1	25.0	50.0	25.0
I:2	80.1	0.0	19.9
I:3	20.0	2.2	77.8
II:2	65.6	0.0	34.4
II:3	0.0	100.0	0.0
II:4	0.0	100.0	0.0
II:5	0.0	100.0	0.0
III:1	0.0	100.0	0.0
III:2	65.2	0.5	34.2
IV:1	67.3	1.0	31.6
IV:2	75.0	12.5	12.5
IV:3	60.2	1.2	38.6
V:1	25.0	75.0	0.0
V:2	65.4	0.0	34.6
VI:1	69.7	3.0	27.3
VI:2	63.5	1.3	35.3
VII:1	33.3	20.0	46.7
VII:2	78.3	0.0	21.7
VII:3	45.8	0.0	54.2
VIII:1	0.0	66.7	33.3
VIII:2	25.8	1.6	72.6
VIII:3	85.5	0.0	14.5
IX:1	0.0	75.0	25.0
IX:2	22.1	0.0	77.9
IX:3	100.0	0.0	0.0
IX:4	91.4	0.0	8.6
X:1	67.6	0.9	31.5
X:2	60.5	2.5	37.0

Table 5. Frequencies of qualitative character states of females.

Character states	*R. ridibunda*	*R. lessonae*	*R.* kl. *esculenta*
II:2	68.0	0.6	31.5
II:3	0.0	87.5	12.5
II:4	0.0	100.0	0.0
II:5	0.0	50.0	50.0
III:1	0.0	91.3	8.7
III:2	66.8	1.6	31.5
IV:1	60.5	12.9	26.6
IV:2	44.4	11.1	44.4
IV:3	59.5	9.5	31.1
V:1	0.0	92.0	8.0
V:2	67.6	0.5	31.9
VI:1	37.5	29.7	32.8
VI:2	69.2	3.5	27.3
VII:1	23.1	34.6	42.3
VII:2	73.0	6.1	20.9
VII:3	50.0	12.1	37.9
VIII:1	3.8	76.9	19.2
VIII:2	16.9	6.8	76.3
VIII:3	91.8	0.0	8.2
IX:1	0.0	88.0	12.0
IX:2	21.9	2.7	75.3
IX:3	100.0	0.0	0.0
IX:4	98.1	0.0	1.9
X:1	69.4	3.5	27.1
X:2	52.5	17.2	30.3

Qualitative traits

With respect to dependence on sex, the analyzed qualitative traits showed similar differentiation patterns for each group (Figs. 6 and 7). The samples were differentiated into three groups defined by the specific frequencies of characters and states (Tables 4 and 5).

As for the results of morphometrical analyses, these traits enabled identification of three forms. *R. lessonae* was obviously different from the other two taxa (in both sexes). Significant characters were II, III, V, VIII, and IX.

Separation of the second parent species from the hybrid was less distinct and limited primarily to the shape of the *callus internus*.

DISCUSSION

The presence of a contact zone between *R. lessonae* and the other two taxa of the complex, the specific composition and arrangement of population systems, and considerable anthropogenic influence are factors that make detailed analysis of their morphological characteristics necessary.

The identification of water frog taxa in Serbia mainly relies on variation in the relative size of hind

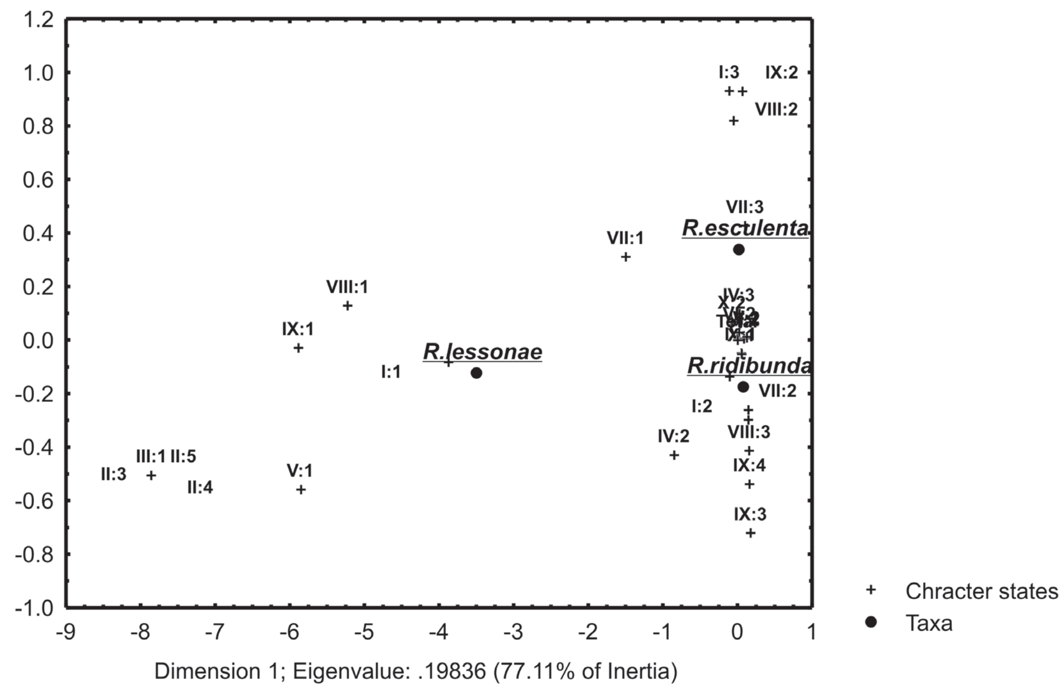

Fig. 6. Scatter-plot of coordinates of columns (taxa) and rows (character states) on the first and second correspondence axes (qualitative data) for males.

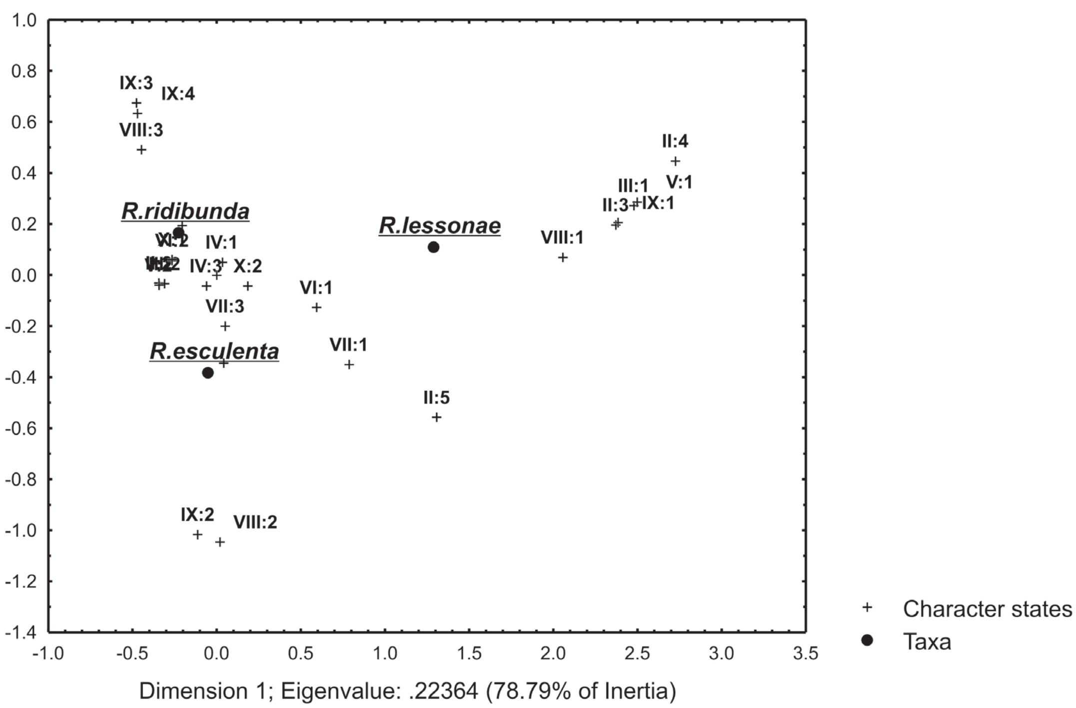

Fig. 7. Scatter-plot of coordinates of columns (taxa) and rows (character states) on the first and second correspondence axes (qualitative data) for females.

leg elements, shape of the *callus internus*, and coloration of the body and vocal sacs (male). The morphometric divergence found in this study is a result of size effects in the principal component analysis.

According to other authors (Berger, 1966; Wijnands and Van Gelder, 1976; Uzzell et al., 1977; Günther et al., 1991; Joly et al., 1994), the taxonomic type defined in the present study by a short *callus internus* and long tibia corresponds to *Rana ridibunda*. A second taxonomic type, defined by a large *callus internus* and short tibia length corresponds to *Rana lessonae*.

The range of variation of the ratios T/Cint, DpPp/Cint, and L/T in *Rana* kl. *esculenta* populations is within the range of variation of both parent species. T/Cint and DpPp/Cint ranges were most similar to *ridibunda* ranges, but the L/T range in the hybrid taxon overlapped with the range of the second parent (*lessonae*). Low variation among the *ridibunda* and *esculenta* population samples (Fig. 1) denotes a possible major influence of *ridibunda* genomes on the development of some morphometric traits. Moreover, higher morphometric variability in the *Rana* kl. *esculenta* population sample than in the parental species indicates a greater degree of polymorphism in the hybrids (Plötner, 1994).

As far as the specific frequencies of qualitative characters and their states are concerned, the separation of water frog species in Serbia seems possible, to judge from studies on other water frogs (Günther et al., 1991; Schröer, 1997).

The identification of water frogs in Serbia from their qualitative traits depends on shape of the metatarsal tubercle and specific coloration of the flanks, internal surface of the hind legs, and the male vocal sacs.

Shape of the *callus internus* (according to Günther et al., 1991) and body coloration were the most valuable characters, especially for separation of the parental species. The phenotypic pattern of *R. ridibunda* included low and flat metatarsal tubercles (VIII-3, IX-4) and absence of yellow coloration on the flanks (II-2, III-2, V-2), while *R. lessonae* possessed large semicircular convex metatarsal tubercles (VIII-1, IX-1) with yellow flanks and hind legs (II-3,4,5, III-1, V-1).

Differences from this pattern of metatarsal tubercle shape were present in a small number of individuals (7% of *R. ridibunda* and less than 4% of *R. lessonae*).

For *R.* kl. e*sculenta*, no diagnostic traits were established. The most important characteristics — olive and green coloration of the external surface of the hind legs and absence or rare yellow coloration of the flanks — were present in all males. A moderately raised, asymmetrical metatarsal tubercle, with its highest point directed toward the first toe of the hind legs, was characteristic of the hybrid form.

Different forms of metatarsal tubercles in the population samples from Serbia confirm the identification patterns found elsewhere (Günther et al., 1991; Berger, 1976).

The results presented here indicate the necessity of dealing with combinations of many characters when defining the taxonomic status of populations. Using isolated characters does not give a correct interpretation. In addition, phenetic traits of the hybrid form are intermediate between *R. ridibunda* and *R. lessonae* (Gubányi and Korsós, 1992), so several traits may be closer to one of the parental species. This phenomenon also occurs in other parts of the distribution range of the given complex (Günther et al., 1991; Kotlik and Šulova, 1994; Nekrasova et al., 2003).

External coloration is highly variable, and this character alone would probably not justify their independent status.

The identification of water frogs in Serbia by morphometrical and qualitative traits is possible on the basis of relative length of the metatarsal tubercle, first toe of the hind legs, and tibia; the snout-vent distance; shape of the metatarsal tubercle; coloration of parts of the body and legs; and (in males particularly) coloration of the vocal sacs.

In general, the analyses indicate that *R.* kl *esculenta* is morphologically closer to *R. ridibunda* than to *R. lessonae*.

Acknowledgments — Collection of specimens from the analyzed localities was approved by licence for collection No. 02-868/2 issued to order by the Nature Protection Institute of Serbia. This study was financed by the Serbian Ministry of Natural Resources and Environmental Protection (Grant No. 401-00-00468/2003-01).

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ВОДЕНЕ ЖАБЕ (*RANA ESCULENTA* COMPLEX) У СРБИЈИ - МОРФОЛОШКИ КАРАКТЕРИ

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Анализа спољашњих морфолошких карактеристика комплекса водених жаба (*Rana* synklepton *esculenta*), извршена је на узорку од 396 јединки са 15 локалитета на територији Републике Србије. Резултати анализе главних компоненти (PCA) као и кореспондентне анализе (CA) указују на присуство три различите форме (*Rana ridibunda, Rana lessonae* i *Rana* kl. *esculenta*). Статистички значајне разлике су установљене између родитељских врста (*R. ridibunda* и *R. lessonae*). Хибридогенетска форма *R.* kl. *esculenta* заузима интермедијерни положај између родитељских врста, са израженијом сличношћу са врстом *R. ridibunda*.