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A procedure for taxon assessment based on morphological variation in European water frogs (*Pelophylax esculentus* complex)

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Abstract: Methods for morphological identification of water frogs from the *Pelophylax esculentus* complex have changed over the decades. Still, without resort to genetic analyses, water frogs remain among the most difficult European amphibians to distinguish due to their hybrid nature. The aim of this study was to identify a reliable set of quantitative and qualitative morphological characters that objectively resolve species' identification in the *Pelophylax esculentus* complex and provide a rapid and robust procedure for taxon identification. Our results showed a substantial difference in morphological characteristics among taxa. The shape and size of the metatarsal tubercle along with the coloration of the hind limbs and flanks appear to be the most important diagnostic traits. Linear discriminant analysis with both quantitative and qualitative traits as a rapid method for the assessment of water frog taxa showed satisfactory accuracy and prediction classification for all 3 members of the complex.

Key words: Green frogs, *Pelophylax ridibundus, Pelophylax lessonae, Pelophylax esculentus,* taxon identification

1. Introduction

Morphological variability, as a general biological phenomenon, is still at the forefront of research in many subject areas of biology (McCarthy and Mason-Gamer, 2019; Stanchak and Santana, 2019; Whelan et al., 2019). Apart from investigating the complex mechanisms through which variability arises and is maintained in natural populations, morphological variability can confound the clarity of zoological classification, introducing variable degrees of uncertainty in all other studies relying on clear identification of taxa.

European water frogs, *Pelophylax esculentus* complex (Linnaeus, 1758) represent a unique and challenging model system in studies of morphological variation and taxon delimitation due to high morphological polymorphism and interspecific hybridisation. The complex consists of 2 parental species: the marsh frog *P. ridibundus* (Pallas, 1771) and the pool frog *P. lessonae* (Camerano, 1882) and a hybrid form-the edible frog *P. esculentus* (Linnaeus, 1758). *P. esculentus* is genetically heterogeneous, either diploid or triploid with various proportions of parental genomes and has a morphological character set intermediate between the 2 parental species (Uzzell and Berger, 1975; Uzzell et al., 1975; Dubois and Gunther, 1982; Ogielska et al., 2004).

Recent studies of frogs from the *Pelophylax esculentus* complex have been mainly focused on the identification and characterization of genetic variability, DNA contents, and genomic composition (Ragghianti et al., 1995, 2007; Bucci et al., 2000; Ogielska et al., 2004; Marracci et al., 2011; Dedukh and Krasikova, 2017). The focus of these studies, however, did not provide advances in efficient and fast identification of frogs in field conditions or the laboratory (Günther 1990; Plöther 2005), although both parental species, as well as the hybrid, are seemingly well-differentiated genetically (Uzzell and Berger, 1975; Uzzell et al., 1975; Gunther et al., 199; Spasić Bošković et al., 1999; Krizmanić and Ivanović, 2010; Kierzkowski et al., 2011, 2013). Further, genetic identification requires well-equipped laboratories, sufficient funding, and competent research staff to efficiently perform the complex analyses necessary for identification.

Before the development of molecular and genetic techniques, the most common method for the identification of water frogs was using morphological data and ratios. Even today, ratios are used to distinguish the 3 taxa (Günther et al., 1991; Gubányi and Korsós, 1992; Mayer et al., 2013), although published research papers show overlap among the analysed taxa (Polls–Pelaz, 1989; Günther et al., 1991; Rybacki, 1995; Pagano and Joly, 1999;

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Krizmanić, 2008a). Thus, published research shows that morphological identification could show discordance with genetic identification (Polls–Pelaz, 1989; Günther et al., 1991).

Qualitative characteristics by themselves have been rarely used for the identification of water frogs, and then only in addition to other methods of discrimination. Besides the shape of inner metatarsal tubercle, the most commonly used qualitative characters are the level of ventral maculation, presence/absence of the dorsal stripe, presence/absence of stripes or spots on the dorsal side of the frog. However, these characters do not provide satisfactory taxa discrimination and are used frequently as accessory characters with morphological ratios (Nekrasova et al., 2003; Tosunoğlu et al., 2005; Krizmanić, 2008a; Mayer et al., 2013).

Serbia has all tree taxa with different population structures of taxa in analysed habitats (Spasić Bošković et al., 1999; Krizmanić and Ivanović, 2010). *P. ridibundus* is a widely distributed species occupying the entire Serbia. *P. esculentus* inhabits more than 50% of Serbia and *P. lessonae* can be considered a rare amphibian species, occupying less than 50% of the country (Vukov et. al., 2013). Both *P. esculentus and P. lessonae* in Serbia are within the marginal zone of their European range (Vukov et al., 2013). Scarce data on the distribution of *P. lessonae* are from the region north of the Danube and Sava Rivers (Karaman, 1948). *Pelophylax esculentus* inhabits mostly northern Pannonian and peri-Pannonian parts of Serbia (Spasić Bošković et al., 1999; Džukić et al., 2001; Krizmanić, 2008a) but it is also found southwards from the Western Morava River and in eastern Serbia (the town of Zaječar, Krizmanić and Ivanović, 2010).

The most common population system in eastern Europe, as well as Serbia is the *P. ridibundus- P. esculentus* system (RE system) where the hybrid *P. esculentus* occurs in sympatry with *P. ridibundus* (Krizmanić and Ivanović, 2010). Although the most common in central and western Europe (Blain et al. 2015), *P. lessonae–P. esculentus* system (LE system) is recorded in Serbia only at 3 localities along the River Danube (Krizmanić and Ivanović, 2010; Spasić Bošković et al., 1999). The *P. ridibundus –P. esculentus–P. lessonae* population system (REL system) is recorded only in a few localities in South Banat along the river Danube (Krizmanić and Ivanović, 2010). As South Banat represents the southern geographic range limit of *P. lessonae,* these peripheral populations are often less abundant and more vulnerable to extinction than populations at the centre of the species' range (Curnutt et al., 1996). Fringe populations may also be of greater conservation priority because of their potentially unique genetic characteristics and/or because they are highly vulnerable to loss of genetic diversity (Vucetich and Waite, 2003). Although

listed as least concern (LC) by the International Union for Conservation of Nature on a global scale, *P. lessonae* could be potentially considered threatened on a regional level since it is the most susceptible to environmental threats (Vukov et al. 2015) and is listed as data deficient (DD) species for Serbia (Krizmanić, 2015). The uneconomical and time-consuming process of identification using genetic methods along with data scarcity on distribution and ecology makes it difficult to determine the conservation status of the taxa. This makes rapid identification in the field imperative in future studies especially when it comes to sites that change under anthropogenic pressure or when conservation actions need to be implemented quickly e.g., after ecological accidents.

Therefore, the aim of this study was to identify a reliable set of quantitative and qualitative morphological characters that objectively resolve species identification in the *Pelophylax esculentus* complex.

2. Materials and methods

A total of 317 adult individuals from the *Pelophylax esculentus* complex (*P. ridibundus*, *P.* kl. *esculentus*, and *P. lessonae*) were collected from 3 localities in South Banat, Serbia: 1) Stevanove ravnice, within the Special Nature Reserve "Deliblatska peščara", 2) Canal Banatska Palanka – Novi Becej, 3) Canal Jaruga in the peripheral zone of the protected natural landscape "Karaš-Nera" (Figure 1). Sampling localities were selected according to the confirmed existence of the REL system (Krizmanić and Ivanović, 2010). Animals were collected after the onset of reproduction from May to October, at night, using flash lamps and landing nets. Animals were transferred to a field laboratory within 3 h of capture. All body measurements (mm) were performed by the same person (I.K.) using a digital Vernier calliper with appropriate precision for this study. Individuals with snout-vent length less than 55 mm (*P. ridibundus*) and 45 mm (*P. esculentus, P. lessonae*), respectively, were considered juveniles and were not included in further analyses (Mikulíček et al. 2014). All captured individuals were released within 12 h at the capture sites.

The following morphological data were recorded: (1) 8 morphometric characters for each specimen according to Hotz and Uzzel (1982), Gubányi and Korsós (1992), and Günther and Plötner (1994): body length (L); tibia length (T); femur length (F); length of first toe of hind leg (DPPP); length of inner metatarsal tubercle basis (CINT); maximum head width (LTC); internasal distance (SPI), and snout–eye distance (DNO); (2) 6 qualitative characters with a total of 19 character states according to Krizmanić (2008a): I - Main colour of external surface of hind legs (1. no coloration, 2. olive and green, 3. completely yellow, 4. partly yellow, 5. yellow in traces); II - Yellow coloration on
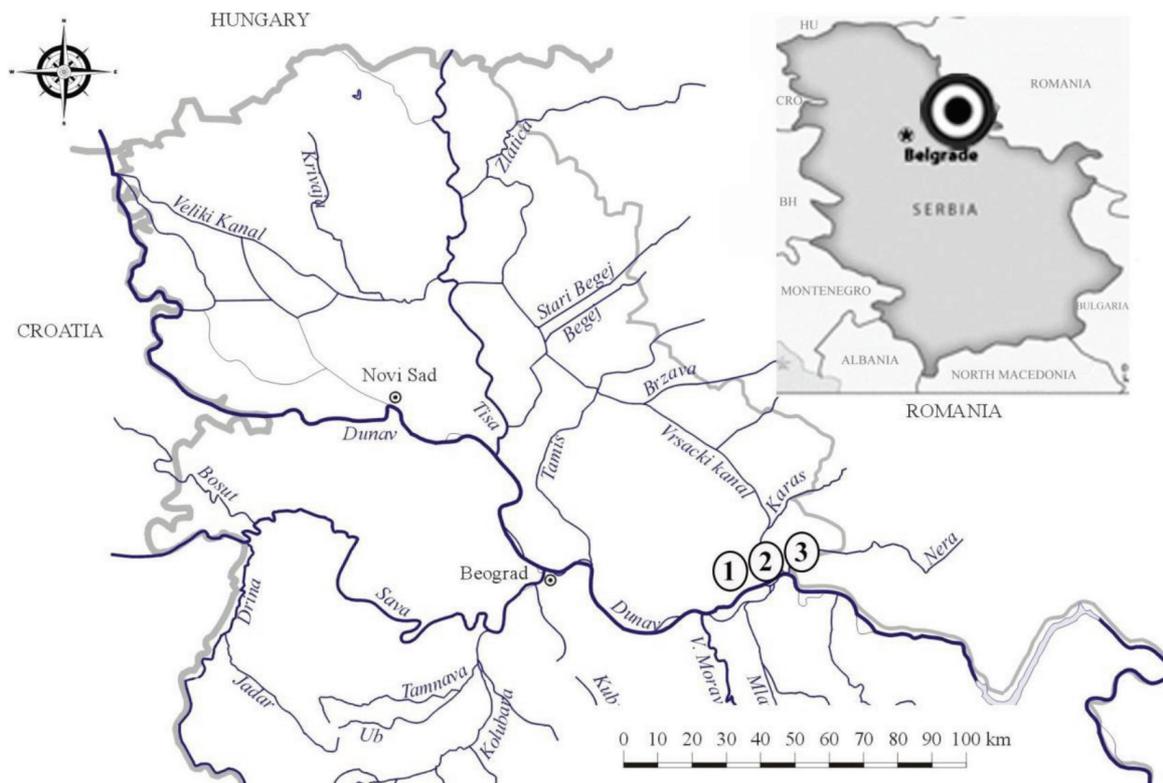
Figure 1. Frogs were collected from 3 different localities in South Banat, Serbia: 1) Stevanove ravnice, within the Special Nature Reserve "Deliblatska peščara" (44°49′57.8″N 21°18′33.1″E, 44°50′14.3″N 21°18′14.0″E), 2) Canal Banatska Palanka – Novi Becej (44°51′14.4″N 21°18′17.8″E).; 3) Canal Jaruga in the peripheral zone of the protected natural landscape "Karaš-Nera" (44°52′30.8″N 21°28′16.0″E).

flanks (1. present, 2. absent); III- Coloration of the internal surface of hind legs (1. sulphur yellow, 2. yellowish-green); IV- Ventral mottling (1. white/no mottling, 2. strongly mottled, 3. weakly mottled); V- Cɪɴᴛ prominence (1. large and prominent, 2. medium prominence, 3. lows, laterally compressed); VI- Cɪɴᴛ shape (1. symmetrically semicircular, 2. asymmetrical, highest point directed towards the first toe, 3. asymmetrical, highest point directed towards the metatarsal joint, 4. flat).

Prior to analysis, the Mosimann correction usual in morphometric studies was applied to reduce the impact of differences in the overall size of the 3 water frog taxa (Darroch and Mosimann, 1985, Jungers et al. 1995). This adjustment removes isometric size but not size-related (allometric) shape. We calculated the geometric mean of 8 selected quantitative characters (L, T, F, Ltc, Spi, Dno, DpPp, and Cint) for each individual, these measurements were divided by the obtained means, and used in further analyses.

A step-by step outline of the further analyses is as follows: a) a preliminary FAMD to explore the overall variability of the dataset; b) a HCPC (hierarchical clustering on principal components) on scores of the

FAMD to identify natural clusters in the dataset if present; c) identification and interpretation of FAMD and HCPC results to assign clusters to taxa based on morphological measurements from previous studies; d) an LDA (linear discriminant analysis) to verify our taxa assignment including validation with an independently derived dataset based on genetically identified frogs.

To explore morphological variability within the dataset comprising both continuous and categorical variables, FAMD was used as it balances the influence of 2 types of variables in the analysis (Pagès and Camiz, 2008). FAMD reduces dimensionality in a multivariate dataset by constructing principal components that are linear combinations of analysed variables, and which account for the majority of the variation in the dataset (Dillon and Goldstein, 1984). It also determines which characters describe the greatest variance in the dataset.

Hierarchical clustering on principal components (HCPC), was performed on the scores from FAMD to identify the presence of "natural groups" within the dataset (Husson et al., 2010). Hierarchical clustering initially treats each individual as an individual group and then aims to combine individuals into larger clusters. While

nonhierarchical cluster analysis groups individuals based on their shared similarity, hierarchical clusters themselves are defined by inter-cluster similarities, with smaller variances within a cluster than between clusters (Dillon and Goldstein, 1984). The number of clusters present in the dataset is determined by a measure of the within-group variance through analysis of inertia gain. The greatest jump in inertia gain (i.e. the greatest decrease in within-group variance) is taken as the best node that divides clusters (Husson et al., 2010). This method also determines which characters contribute the most to the separation of clusters by the largest explained variance (eta²) and v-test (Escofier and Pagès, 2008).

If morphological characters of the analysed water frogs can be used in a taxonomically meaningful manner, we expected that any cluster structure (in our case the expectation was 3) in our dataset recognised by multivariate methods (FAMD and HCPC) can be assigned to 3 water frog taxa. Assignment of clusters to taxa was based on identifying the most important morphological characters on which the ordination and classification were based, and ascertained according to previously published studies (e.g., Ribacky, 1995; Pagano and Joly, 1999; Krizmanić, 2008a, 2008b), specifically length and shape of the metatarsal tubercle, length of the tibia and the first toe of the hind limbs, specific coloration of the certain parts of the body and limbs.

The reliability of our morphology-associated identification was assessed by performing a linear discriminant analysis (LDA) on the original 8 Mossiman-corrected characters along with 6 qualitative characters, in total 14 variables in analysis. First, we identified the "best" subset of characters that will discriminate the 3 a priori defined groups (taxon assignment according to the HCPC analysis), and predict taxon membership. LDA is an effective method for this purpose as it provides an unambiguous and unique estimate of the variability of the outcome uniquely attributed to each variable, regardless of the number of variables analysed, and minimizes the effect of redundancy in morphometric and meristic measurements. LDA uses the logic of general linear modelling (GLM) and applies it to discrimination analysis, thus, coping well with both continuous and ordinal characters. Our initial data set (316 specimens identified according to the morphological characteristics: 64 *P. ridibundus*, 216 *P.* kl. *esculentus*, and 36 *P. lessonae*) was used to estimate the classification functions for a 'best' subset of predictor variables. Next, the misclassification rates for an independent cross-validation data set that was not included in the initial model were calculated. For the cross-validation data set we used 91 specimens from the same localities as the initial sample. Specimens from the cross-validation sample were measured by the same

person as the initial sample (I.K.), but identified according to their allozyme polymorphism (57 *P. ridibundus*, 30 *P.* kl. *esculentus*, and 4 *P. lessonae*) (Krizmanić and Ivanović, 2010). Assessment of classification error by using a cross-validation data set validates the model's ability to predict correctly group membership and evaluates the performance of the classification functions (Moder et al., 2007). We assumed equal prior probabilities for predicting the identity of new cases because expected class sizes in natural populations are unknown and a correct answer has no associated cost.

All statistical tests were performed in R v3.6.1 (R Development Core Team 2015), FAMD and HCPC by using the FactoMineR package (Lê et al., 2008), and LDA by using MASS package (Venables and Ripley, 2002). In all analyses P < 0.05 was set as the level of significance.

3. Results

The first 2 principal components of the FAMD accounted for 28.5% of the total variance, with all 14 components explaining 91.8% of the total variation. The qualitative characters III, V, and VI were strongly correlated with the first component. Quantitative characters correlated to a lesser extent, with C$_{INT}$ having the highest correlation (Figure 2a). The quantitative characters, S$_{PI}$, D$_{NO}$, L, and T (Figure 2a) were highly correlated with the second component. HCPC on all 14 components suggested the presence of 3 clusters (Figure 2b) in the dataset. All qualitative characters had statistically significant links with the clusters' partition (P < 0.0001). Four out of 8 morphometric characters had statistically significant links with clusters' partition with the largest explained variance for C$_{INT}$ (C$_{INT}$: eta^2 = 0.28, P < 0.000; L$_{TC}$: eta^2 = 0.07, P < 0.000; D$_P$P$_P$: eta^2 = 0.04, P < 0.001; T: eta² = 0.03, P < 0.05). The individuals from the first cluster had flat (VI4) and low (V3) C$_{INT}$, where 100% individuals with flat C$_{INT}$ and 83.3% of individuals with low C$_{INT}$ belong to the first cluster, with 96.9% and 93.8% individuals in the first cluster had character states described as flat and low C$_{INT}$, respectively (Table). Regarding morphometric characters, the individuals from the first cluster had the widest heads (L$_{TC}$: *v.test* = 4.47), the longest first toe of hind leg (D$_P$P$_P$: *v.test* = 2.56), and the shortest basis of inner metatarsal tubercle (C$_{INT}$: *v.test* = –6.78). The individuals from the second cluster had medium prominence (V2) and asymmetrical C$_{INT}$ with the highest point directed towards the first toe (VI2), where 94.3% individuals with medium prominent C$_{INT}$ and 93.3% of individuals with asymmetrical C$_{INT}$ belong to the second cluster, with 92.6% and 97.2% individuals in the second cluster had medium prominent and asymmetrical C$_{INT}$ with the highest point directed towards the first toe, respectively (Table). Regarding morphometric characters, the individuals from
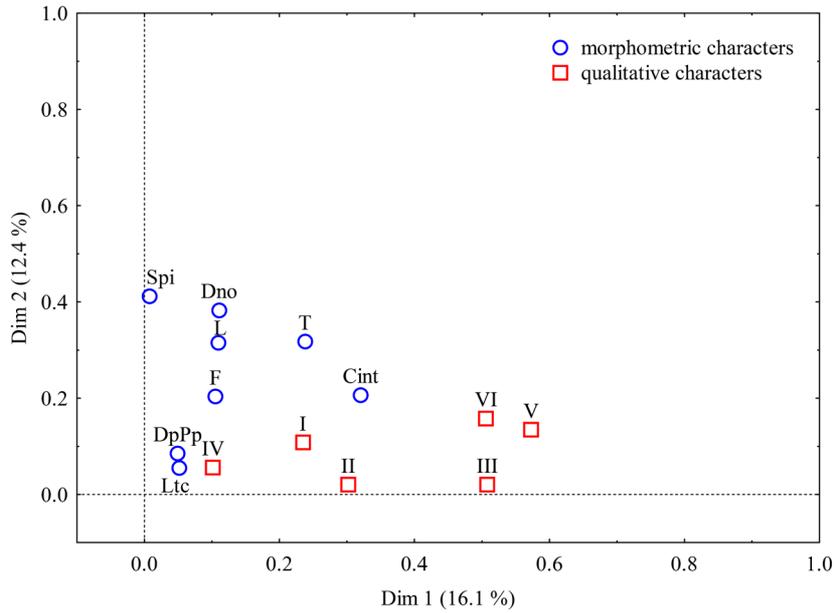
Figure 2a. Correlations of each morphological character with the first 2 dimensions of the FAMD.
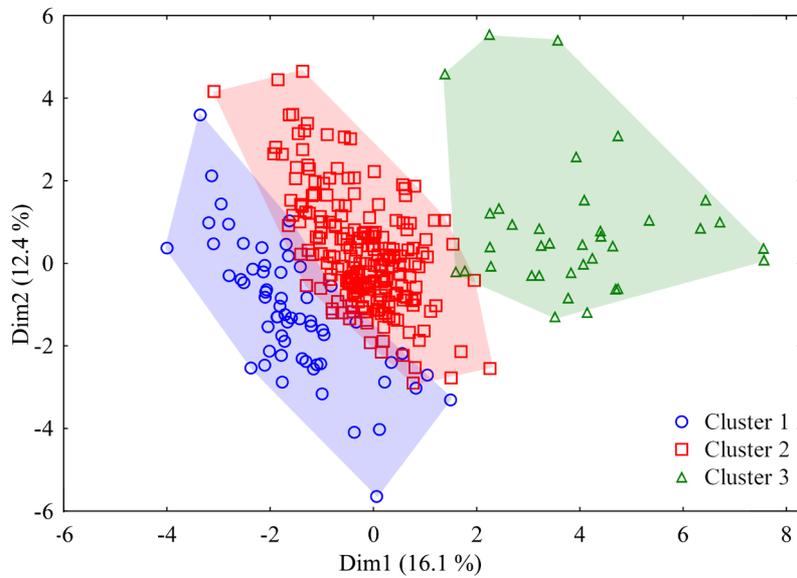

Figure 2b. Individual scores resulting from FAMD plotted on the first 2 dimensions. Clusters derived from the hierarchical clustering on principle components (HCPC) are superimposed onto the ordination.

the second cluster had narrower heads than individuals in the first cluster (Ltc: *v.test* = −2.48). The individuals from the third cluster had large and prominent Cint (V1) and yellow colouration of the internal surface of hind legs (III1), where 87.5% individuals with large and prominent Cint and 69.0% of individuals with yellow colouration of the internal surface of hind legs belong to the third cluster, and 77.8% and 80.6% of the individuals in the third cluster

were with large and prominent Cint and yellow colouration of the internal surface of hind legs, respectively (Table). Regarding morphometric characters, the individuals from the third cluster had the longest basis of inner metatarsal tubercle (Cint: *v.test* = 7.64), the narrowest heads (Ltc: *v.test* = −2.01), the shortest tibia (T: *v.test* = −2.76), and the shortest first toe of hind leg (DpPp: *v.test* = −3.15). Based on results previously published (Ribacky, 1995; Pagano

Table. Description of each cluster by the qualitative character categories. Cla/Mod: proportion (expressed as percentages) of individuals with specific qualitative character category in the cluster; Mod/Cla: proportion (expressed as percentages) of individuals within the cluster with the specific qualitative character category.

First cluster	Cla/Mod	Mod/Cla	v.test	Second cluster	Cla/Mod	Mod/Cla	v.test	Third cluster	Cla/Mod	Mod/Cla	v.test
VI = VI4	100.0	96.9	16.91	VI = VI2	93.3	97.2	15.37	V = V1	87.5	77.8	11.12
V = V3	83.3	93.8	14.44	V = V2	94.3	92.6	14.48	III = III1	69.0	80.6	10.22
IV = IV2	43.3	40.6	4.58	II = II2	73.3	99.1	6.31	VI = VI1	79.3	63.9	9.29
III = III2	23.0	98.4	3.48	III = III2	74.5	94.4	5.66	II = II1	75.0	50.0	7.75
I = I2	21.5	98.4	2.08	IV = IV3	76.1	47.2	2.55	I = I4	100.0	11.1	3.80
I = I1	0.0	0.0	−2.06	I = I1	92.9	6.0	2.08	I = I3	100.0	8.3	3.20
IV = IV1	11.5	21.9	−3.13	I = I3	0.0	0.0	−2.16	IV = IV1	18.0	61.1	2.86
VI = VI1	0.0	0.0	−3.29	I = I4	0.0	0.0	−2.59	IV = IV3	6.0	22.2	−2.63
III = III1	2.4	1.6	−3.48	IV = IV2	46.7	13.0	−3.86	I = I2	9.2	75.0	−3.57
V = V1	0.0	0.0	−3.50	VI = VI1	20.7	2.8	−5.48	VI = VI4	0.0	0.0	−3.69
V = V2	1.9	6.3	−11.58	III = III1	28.6	5.6	−5.66	V = V3	0.0	0.0	−4.07
VI = VI2	0.9	3.1	−13.33	II = II1	8.3	0.9	−6.31	VI = VI2	5.8	36.1	−4.62
				V = V1	12.5	1.9	−6.87	V = V2	3.8	22.2	−5.82
				V = V3	16.7	5.6	−10.49	II = II2	6.2	50.0	−7.75
				VI = VI4	0.0	0.0	−13.18	III = III2	2.6	19.4	−10.22

and Joly, 1999; Krizmanić, 2008a, 2008b) the first cluster obtained in our analyses corresponds to *P. ridibundus*, the second cluster to *P.* kl. *esculentus*, and the third cluster to *P. lessonae*.

According to LDA, the best "morphological model" that discriminates the 3 a priori defined groups (taxa) includes the length of the basis of inner metatarsal tubercle - Cint (*Wilks' lambda* 0.8935, $F_{2,311}$ = 18.53, P < 0.0001) and the shape of metatarsal tubercles - V (*Wilks' lambda* 0.1230, $F_{4,622}$ = 194.49, P < 0.0001), with 93.8%, 92.6%, and 77.8% of *P. ridibundus*, *P.* kl. *esculentus*, and *P. lessonae* specimens correctly classified, respectively. The length of the basis of inner metatarsal tubercle (Cint) statistically differs between all 3 taxa (Duncan's test, P <0.05). The length of the basis of metatarsal tubercle (Mean ± SD, mm) was the shortest in *P. ridibundus* (3.9 mm ± 0.8), intermediate in *P. esculentus* (4.2 mm ± 0.7), and the longest in *P. lessonae* (4.9 mm ± 0.8). *P. ridibundus* had low, laterally compressed metatarsal tubercle, *P. esculentus* had metatarsal tubercle with medium prominence, and *P. lessonae* had large and prominent metatarsal tubercle. When our "morphological model" was cross-validated with the dataset comprised of individuals identified by allozyme polymorphism, the misclassification rate was low, only 3.3% (correct classification of *P. ridibundus* 94.7 %, *P.* kl. *esculentus* 100 %, *and P. lessonae* 100 %).

4. Discussion

In this study, carried out in a region where the sympatry of all 3 water frog taxa occurs, we found substantial differences in morphological characteristics among taxa. According to the results of HCPC and LDA, we identified the phenotypic patterns for parental taxa. Longer legs (higher values for T, F), flat and low Cint (VI4, V3), absence of yellow coloration on the flanks (I2, II2, and III2), stubby snouts (lower values in Dno, Spi), and the widest heads (higher values for Ltc) among the complex were typical for *P. ridibundus*. Shorter limbs (lower values in T, F), large, prominent, symmetrical and semicircular Cint (V1, VI1), yellow flanks and hind legs (I3, 4, 5, II1, III1), pointed snouts (higher values in Dno, Spi) and the narrowest heads among the complex (lower values for Ltc) were typical for the second parental species, *P. lessonae*. Hybrid genotypes are reported to be morphologically closer to *P. lessonae* than to *P. ridibundus* (Kierzkowski et al., 2011). However, some authors (Krizmanić, 2008a) reported that *P. esculentus* is morphologically closer to *P. ridibundus* than to *P. lessonae*. Our results indicated that *P. esculentus* was more similar to *P. ridibundus* in Cint shape (arched but never semicircular with its highest point situated close to the tip of the first toe) but in body and hind limbs size, *P. esculentus* resembled *P. lessonae*. The only established diagnostic traits for *P. esculentus* were medium prominent

and asymmetrical Cɪɴᴛ with the highest point directed towards the first toe (V2, VI2). Our results imply that the length of the basis of inner metatarsal tubercle-CINT and the shape of metatarsal tubercles-VI appear to be the most important diagnostic traits for water frogs in the study area. LDA including qualitative traits showed satisfactory accuracy and prediction classification for all 3 taxa of water frogs which was cross-validated by including individuals with identified enzyme loci in the discrimination. To our knowledge, only 2 studies using morphological features and morphometric measurements reported misclassification rates: Mayer et al. (2013) for R-E and L-E population systems as well as for pure populations of all 3 taxa in Bavaria, and Tognarelli et al (2014) for an L-E population system in Italy. Both studies provided misclassification rates in the range of 13% (Mayer et al., overall sample, ANOVA of morphometric indices) to 21% (Tognarelli et al., hybrid frogs, discrimination of residuals). Thus, we emphasise that our combination of qualitative and quantitative characters unambiguously discriminates all 3 taxa (overall misclassification rate 7%–11%, linear discrimination of morphometric traits, 97% correct genetic corroboration) in the complex which was not possible using only quantitative characters. Although suggested to be the most reliable, genetic methods are not the most cost-effective approach and usually require a lot of time to perform. Often, assessment of the species' conservation status and implementation of conservation actions in both protected areas and/or areas under anthropogenic pressure (including pollution, habitat alteration, and exploitation) requires very rapid taxa identification and maximum efficiency with limited resources. A nonexhaustive list of possible events requiring such rapid assessment could include:

- natural and anthropogenic ecological accidents (e.g., physical and chemical accidents, fertilization drains, pesticide spills, fires, etc.);

- legal and illegal cross-border animal trade including poaching for commercial and noncommercial human consumption;

- impact assessment studies related to hydro-engineering of water bodies: construction of small hydropower plants; development of sewage systems; development of canal systems for drainage, melioration, and other purposes;

- pathogen spread, infection incidence

Apart from rapid assessment for mitigation and conservation actions, we stress that numerous other researches related to other subject areas in biology (comparative life-history studies, trophic and behavioural ecology, eco-evo-devo studies, phylogeographic studies, biogeographical analyses, etc.) can greatly benefit from rapid and robust morphological taxon identification.

The importance of rapid identification is especially pronounced in the case of endangered species such as *P. lessonae.* This species has a globally declining population trend (Kuzmin et al., 2019) and its range in Serbia is associated with areas under high and long-lasting anthropogenic influences. It is also highly vulnerable to constant and growing environmental threats (Vukov et al., 2015).

Therefore, for such situations, we suggest using qualitative measures in combination with the selected quantitative measure (Cɪɴᴛ) for a rapid assessment of water frogs from the *Pelophylax esculentus* complex. We also suggest that further research on European water frogs in different population systems and areas is necessary to test the applicability of using both quantitative and qualitative characters in rapid taxon identification.

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