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Comparative morphology and ecology of the *Pelophylax esculentus* complex in Croatia

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Abstract. The Eurasian *Pelophylax esculentus* hybridogenetic complex, including *Pelophylax ridibundus* (R), *P. lessonae* (L), and their hybrid *P.* kl. *esculentus* (E), is considered to be one of the most complicated animal hemiclonal systems. It has been attracting the attention of evolutionary and molecular biologists as well as ecologists for decades. Despite the considerable number of studies describing population structure, reproductive modes, and distribution of the species complex, data on the rarely observed REL scenario, i.e., the presence of all three species at one site, are lacking. This study compared the morphological (morphometry, external morphology) and ecological (diet) profiles of all three species from a REL complex population located in the northwestern part of Croatia (Bjelovar-Bilogora County). In terms of external morphology, the species varied interspecifically, but some features, for example, the yellow or green femoral coloration found in *P. ridibundus*, had not been expected. All three species exhibited a strong predilection for terrestrial prey, but interspecific differences were noticed. This paper aims to provide a better and more comprehensible insight into the ecological relationships of the REL complex, allowing comparisons with other study areas across Europe.

Key words. *Pelophylax esculentus* complex, morphometry, morphology, diet, niche breadth.

Introduction

The *Pelophylax esculentus* complex is one of the most peculiar hybridogenetic complexes of the entire animal kingdom and has attracted the attention of evolutionary and molecular biologists, and ecologists alike for several decades. It is widespread throughout Europe and consists of three green frog species: *Pelophylax* kl. *esculentus* (Linnaeus, 1758) (edible frog) is derived by the primary hybridisation of *Pelophylax ridibundus* (Pallas, 1771) (marsh frog) and *Pelophylax lessonae* (Camerano, 1882) (pool frog). In comparison with other vertebrate hybrid complexes, which are usually unisexual, the *P. esculentus* complex is specific for the presence of both female and male fertile hybrid individuals with the capability of producing gametes with hybridogenetic characters (Schultz 1969, Tunner 1973). This complex is also considered one of the generally most complicated hemiclonal systems, due to pronounced diversities in population structures and reproduction modes (Graf & Polls-Pelaz 1989). *Pelophylax esculentus* population complexes at various localities differ in structure and several types have been reported: the LE complex (the most widespread population type, comprising *P.* kl. *esculentus* and *P. lessonae*); the RE complex (comprising *P. ridibundus* and *P. esculentus*); the REL complex (very rarely observed; all three species are present); and all-hybrid populations (consisting only of *P.* kl. *esculentus*) (Bergen et al. 1997, Graf & Polls-Pelaz 1989, Holenweg et al. 2002, Pruvost et al. 2013). Although the population structure, reproductive modes, and population type distribution of the *P. esculentus* complex have been investigated, data on morphological and ecological differences between the three species involved are still scarce. This study aimed to compare morphological (morphometry, external morphology) and ecological (diet) characters of all three species comprising this complex in order to gain better insights into their ecological requirements and niche overlaps. *Pelophylax* kl. *esculentus* usually lives in syntopy with one or both of its parental species (Holenweg et al. 2002) since it is reproductively dependent on at least one (with the exception of pure hybrid populations, known only from the northern parts of the complex' distribution range, that have overcome this reproductional dependence by the mechanism of meiotic hybridogenesis;

Pruvost et al. 2013). The hybridogenetic *P.* kl. *esculentus* biotype can arise in situ via the primary hybridisation of two parental species in the REL population type, but also by the more widespread mechanism of premeiotic exclusion of one parental genome, transmission of a clonal copy of the remaining genome to gametes, and restoration of the excluded genome by mating with one parental species (Graf & Müller 1979, Pruvost et al. 2013). The morphological and/or ecological characters of green frog species have already been compared by several authors for species inside either LE or RE population types, but only rarely in cases when all three species are present in the same population. Several papers report differences between *P. lessonae* and *P.* kl. e*sculentus* in average size, certain allometric characteristics, and allozyme patterns (Abt & Reyer 1993, Berger 1966, Kotlík & Šůlová 1994, Krizmanić & Ivanović 2010, Spasić-Bošković et al. 1999). Morphological differences among species in the RE, LE, and REL complexes were investigated by Krizmanić & Ivanović (2010) in the southern part of the complex' distribution range. Socha & Ogielska (2010) investigated differences between *P. ridibundus* and *P.* kl. *esculentus*, but only regarding body size. Our investigation is based on the genetically verified REL population complex located in continental Croatia. Identification of the genetic constitution and taxonomic position of each individual was conducted through gel electrophoresis for three diagnostic allozyme loci (Čavlović 2007). The simultaneous presence of all three species enabled us to compare their morphological and ecological profiles and examine the ecological relationships inside this extraordinary complex.

Material and methods

Specimen collection

Specimens belonging to three species (*P. ridibundus*, *P. lessonae*, and *P.* kl. *esculentus*) of the *P. esculentus* complex were collected randomly at five localities in the northwestern part of Croatia (Bjelovar-Bilogora County) (Table 1, Fig. 1) during May 2007. The frogs were captured during day and night time by hand, with small fishing nets, or fish hooks with a pink rubber band bait. In situ preliminary species identification was based on morphological traits such as colour, spot pattern, size, and shape of the metatarsal tubercle. Identification was subsequently verified by allozyme analyses, using immediately frozen tissue samples; however, this paper does not go into detail on this,

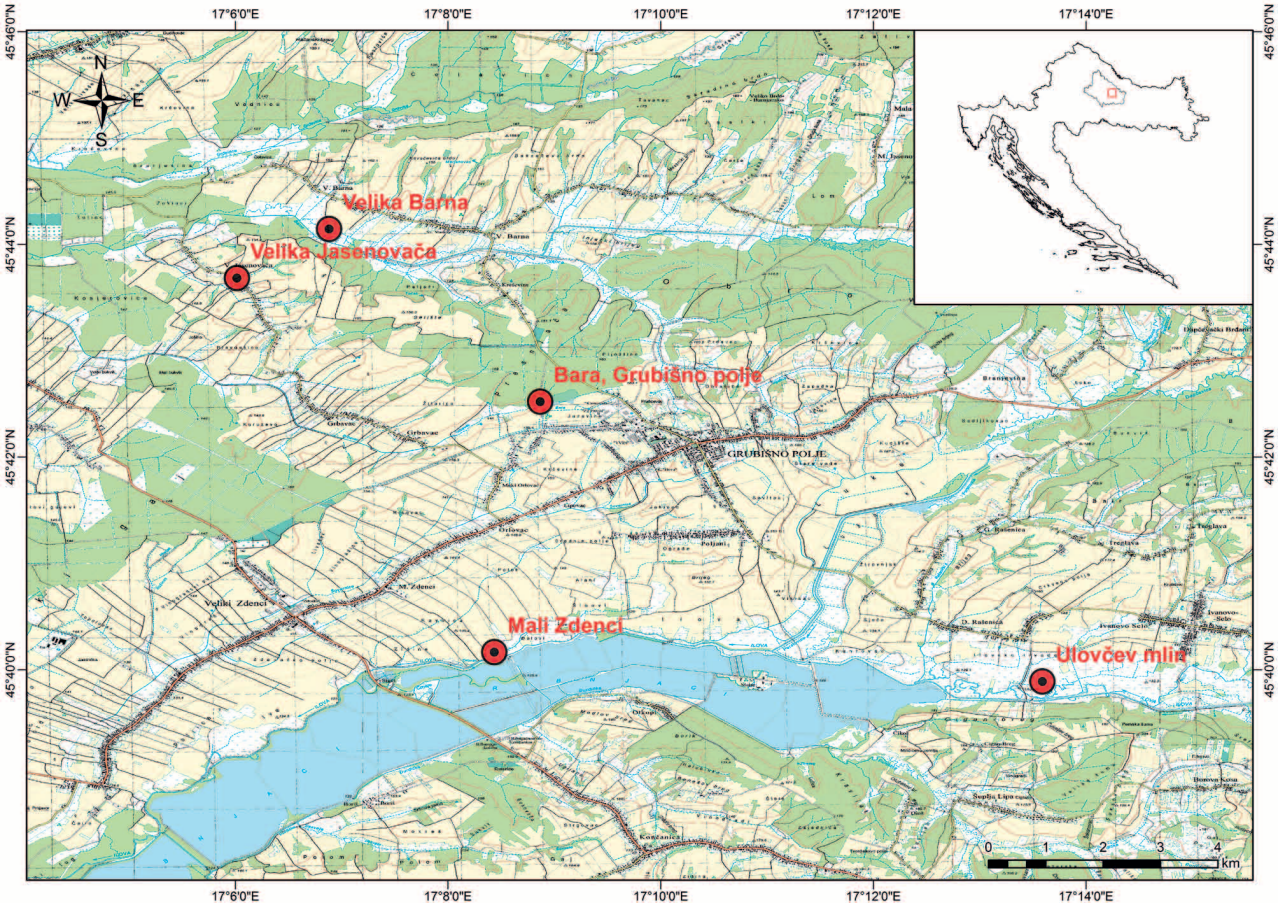

Figure 1. Map of the study area with sampling localities marked as red circles with a black dot.

Table 1. Number of green frog individuals caught at a certain locality included in the study, as well as description of main ecological characters for each locality. PE – *P.* kl. *esculentus*; PL – *P. lessonae*; PR – *P. ridibundus*; F – female; M – male; Juv – juvenile.

Locality	Coordinates	Locality characters	Number of individuals				Total number per locality
			Species	M	F	Juv	
Grubišno polje	45°42'31"N 17°08'52"E	marsh near fish pond (hatchery)	PE	–	9	–	29
				14	–	–	
				–	–	6	
			PL	–	2	–	6
				4	–	–	
				–	–	–	
			PR	3	–	–	3
Ulovčev mlin	45°23'39"N 17°05'31"E	around Ilova river; near the dam, with overgrown pools, meadows and channels	PE	–	7	–	25
				3	–	–	
				–	–	15	
			PL	–	3	–	16
				5	–	–	
				–	–	8	
Mali Zdenci	45°23'09"N 17°03'15"E	high-grass meadow along Ilova river	PE	–	1	–	7
				6	–	–	
			PL	–	2	–	2
			PR	–	–	2	2
Velika Jasenovača	45°25'59"N 17°03'40"E	low-grass meadow along stream close to the road	PR	–	2	–	2
Velika Barna	45°26'27"N 17°04'49"E	low-grass meadow near small stream	PE	–	–	1	1

as allozyme variations are the subject of our as yet unpublished study (K. Čavlović unpubl. data). Individuals were preserved in 70% alcohol. Identification of sex was based on the presence or absence of vocal sacs and thumb pads. Juveniles were identified as such by the absence of gonads.

The described collection of samples was approved and carried out with the permission of the Directorate for Nature Protection, Ministry of Culture of the Republic of Croatia (see Acknowledgements). The exact geographical coordinates of the localities were taken using a Garmin eTrex GPS 60CSx. All georeferences were recorded in WGS 1984 (World Geodetic System 1984) datum format and the map of the study area was created using ArcGIS (ESRI 2009).

Morphology analysis

Five morphometric characters were measured in all of the collected individuals with a slide calliper to the nearest 0.5 mm: body length (L, measured from tip of the snout to the vent), femur length (F), tibia length (T), first toe length (DpPp), and metatarsal tubercle length (Cint). Measurements were taken three times on the right side of an individual's body and used for subsequent calculation of morphometric indices. Morphometric indices already used for *Pelophylax* species discrimination (Berger 1973, Krizmanić & Ivanović 2010), as well as new ones, were employed: T/L, F/L, DpPp/L, Cint/DpPp, F/T, DpPp/T, and Cint/T. Comparisons of indices among species and sexes were made using analysis of variance (ANOVA) and principal component analysis (PCA). All analyses were conducted using Statsoft© Statistica Version 7. The coloration pattern investigated in each individual included overall colouring, colour of the femur, and the spotting pattern on the ventral and dorsal body sides. The length of the hind legs was also analysed by stretching the leg cranially. Furthermore, the shape of the metatarsal tubercle was described for each individual.

Diet

The stomach contents of 81 *Pelophylax* individuals were extracted during the dissection of their gastrointestinal tracts. Recovered material was stored in 70% alcohol for subsequent identification to the lowest possible taxonomic level, and afterwards adjusted for the analysis purposes. Taxa were identified according to Chinery (2007), Móra et al. (2004), Nilsson (1996, 1997), Sansoni (1988), Sauers (1994), Schmidt (1970), Tachet et al. (2000), Tolman & Lewington (2009), and Wiggins (1977). Plant matter, such as seeds, small leaves, and twigs, was also isolated from the stomach content, but due to its small ratio in the overall content, it was regarded as material accidentally ingested during foraging.

The collected data were analysed quantitatively and qualitatively in order to identify possible dietary differences between the three complex members. Three different indices were calculated to estimate the dietary niche breadth and niche overlap of each species. A modified (standardized) Levins formula or Hurlbert's index (Hurlbert 1978) was calculated and used as a parameter of food niche breadth for each green frog species. The value of this index ranges from 0, which indicates a high frequency of ingestion of one or a few prey categories found for each species, to 1, which indicates an equal proportion of ingestion of all prey categories by a particular green frog species.

The Hurlbert's index (Hurlbert 1978) was calculated using the following equation:

$$B_A = \frac{\left[\left(\frac{1}{\Sigma p_i^2}\right) - 1\right]}{n - 1}$$

p_i – proportion of individuals using resource i
n – total number of prey species in a frog species' diet.

A MacArthur and Levins index (MacArthur & Levins 1967) was calculated as asymmetrical, while Pianka's index (Pianka 1986) was calculated as a symmetrical measure of food niche overlap between green frog species belonging to the investigated complex population.

The MacArthur and Levins index (MacArthur & Levins 1967) was calculated using the following formula:

$$M_{jk} = \frac{\Sigma p_{ij} p_{ik}}{\Sigma p_{ij}^2}$$

p_{ij} – proportion that prey species i is of the total prey species that species j utilizes
p_{ik} – proportion that prey species i is of the total prey species that species k utilizes
M_{jk}– MacArthur and Levins measure of food niche overlap of species k on species j

The Pianka's index (Pianka 1986) was based on the following equation:

$$O_{jk} = \frac{\Sigma p_{ij} p_{ik}}{\sqrt{\Sigma p_{ij}^2 p_{ik}^2}}$$

p_{ij} – proportion that prey species i is of the total prey species that species j utilizes
p_{ik} – proportion that prey species i is of the total prey species that species k utilizes
O_{jk} – Pianka's measure of food niche overlap between species j and k

The MacArthur and Levins index is a measure that indicates the estimated extent to which the dietary niche of one species (j) is overlapped by the dietary niche of the other species (k), whereas a symmetrical Pianka's index indicates the extent to which dietary niches of two species overlap with each other (0 = no overlap, 1 = complete overlap).

Results

Comparison of morphometric characters

The numbers of specimens from the investigated species belonging to a certain size category are presented in Figure 2, whereas the mean, minimal, and maximal values of morphometric characteristics measured for species of the *P. esculentus* complex are presented in Table 2. Although there was a certain amount of overlap in the measures, *P. lessonae* individuals were the smallest, while *P. ridibundus* individuals represented the physically largest members of this complex. In contrast, the *P.* kl. *esculentus* biotype was the most diverse regarding its morphometry: there were adult individuals smaller than *P. lessonae*, whereas the largest values correspond to those recorded for *P. ridibundus* individuals. The mean, minimal, and maximal values of the calculated morphometric ratios are summarized in Table 3. The comparison of morphometric ratios with ANOVA (Table 4) on the three *P. esculentus* complex species showed significant differences ($p < 0.05$) in one ratio in females (Cint/T) and three ratios in males (T/L, Cint/T, Cint/DpPp). When compared using a Fisher LSD post-hoc test, the Cint/T ratio was found to differ between females of *P. lessonae* and *P.* kl. *esculentus*, and between all three species in males. Regarding T/L and Cint/DpPp in males, individuals of *P. ridibundus* were different from those of *P. lessonae*, whereas individuals of *P.* kl. *esculentus* and *P. lessonae* were similar. The results of our PCA analysis (Fig. 3) based on all morphometric ratios also showed a differentiation of *P. lessonae* from *P. ridibundus* individuals. Individuals of *P.* kl. *esculentus* occupied the greatest area in the plot and overlapped with both parental species, indicating that this species has the greatest morphological diversity. Factor 1, which comprises 45.87% of the total variance, was mostly determined by T/L and DpPp/L ratios. The second factor, comprising 27.26% of the total variance, was mostly correlated with Cint/T and Cint/DpPp.

External morphology

Table 5 summarizes the results of our external morphology analysis: colour pattern, length of hind legs, and shape of metatarsal tubercle.

Food composition

We extracted a total of 418 prey items from the stomachs of the 81 *Pelophylax* individuals present in our sample; 276 from *P.* kl. *esculentus*, 118 from *P. lessonae*, and 24 from *P. ridibundus*. Only seven individuals had empty stomachs (five *P.* kl. *esculentus* and two *P. lessonae*). The highest number of different prey categories (50) was found in *P.* kl. *esculentus*, 34 in *P. lessonae*, and 13 in *P. ridibundus*. The majority of the consumed prey items originate from terrestrial environments (75.5, 77.6, and 60% for *P.* kl. *esculentus*, *P. lessonae*, and *P. ridibundus*, respectively), some are

found in both terrestrial and aquatic environments (16.1, 13.8, and 33.3%, respectively), while the smallest amount of prey items is limited to aquatic environments (8.4, 8.6, and 6.7%, respectively). The orders Coleoptera, Diptera, Hymenoptera, and Collembola (Table 6) contributed the most to the dietary content of the studied green frog population.

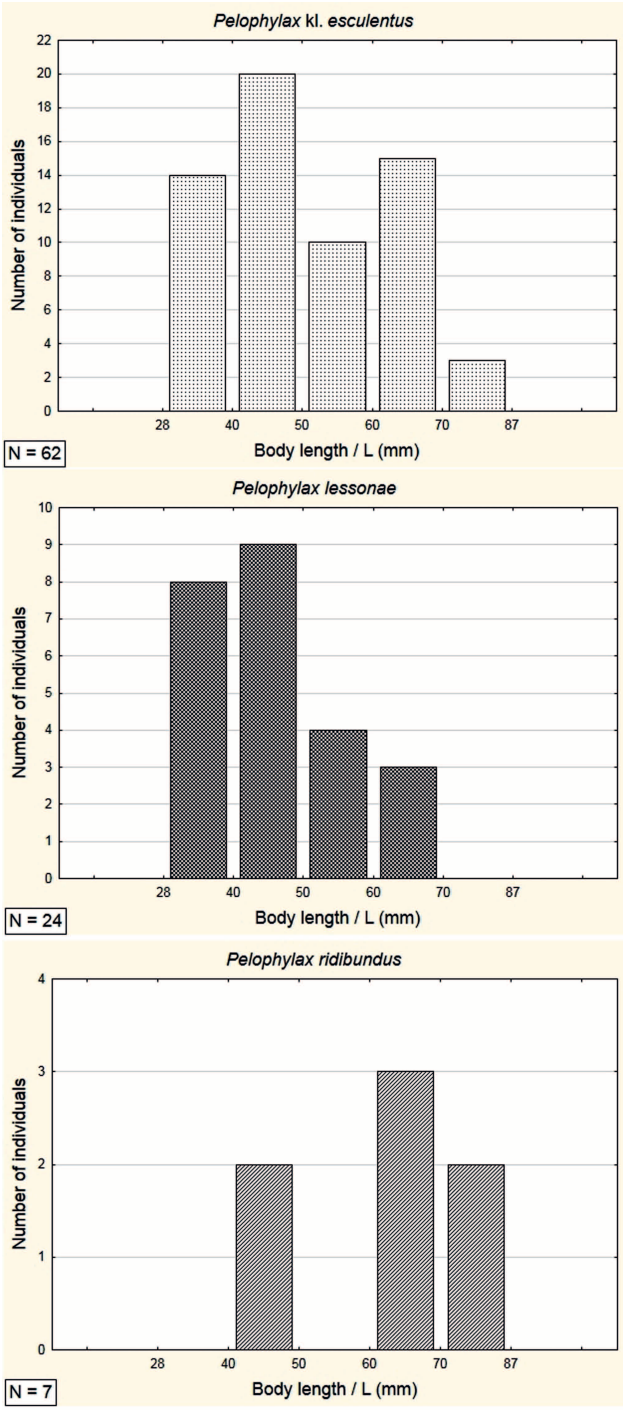

Figure 2. Number of specimens belonging to each *Pelophylax esculentus* complex species and its size category. L – body length; N – number of individuals.

Interestingly, gastropods, as well as remains of Lepidoptera, were found only in *P.* kl. *esculentus* and *P. lessonae*, but not in *P. ridibundus*. Plant material (four items; twigs and seeds) was found only in four *P.* kl. *esculentus* individuals. Because of our assumption that the material was accidentally ingested during feeding, we did not include it in our statistical analysis.

Dietary niche breadth and overlap

Hurlbert's measure of niche breadth was calculated for each of the studied species. The highest value was obtained for *P. ridibundus* (0.6909), a considerably lower one for *P. lessonae* (0.4078), and the lowest for *P.* kl. *esculentus* (0.3690). MacArthur and Levins niche overlap indices showed that the *P.* kl. *esculentus* dietary niche largely overlaps the niches of *P. lessonae* and *P. ridibundus*. In contrast, the dietary niche overlap indices of *P.* kl. *esculentus*, as well as of *P. lessonae* on the *P. ridibundus* dietary niche, showed the lowest value (Table 7). Pianka's niche overlap index showed that the diet composition of *P.* kl. *esculentus* parental species overlapped to the lowest extent, while the highest overlap was calculated between *P. ridibundus* and *P.* kl. *esculentus* (Table 7).

Discussion

Our investigation extrapolated differences in all of the investigated morphological characteristics (morphometry as well as external morphology) among the three species of the *P. esculentus* complex. Although most morphological traits characteristic for each species have already been described (Arnold & Ovenden 2004), several features found in our study have never before been reported. For example, all of the sampled *P. ridibundus* individuals had yellow or green coloured femurs, which in the literature is described as rare (Arnold & Ovenden 2004). The comparison of morphometric characters showed that *P.* kl. *esculentus* is more similar to *P. lessonae* than to *P. ridibundus*, whereas its morphometric ratios suggests an intermediate stage between its parental species. Very long hind legs with a small and usually low metatarsal tubercle were typical for *P. ridibundus*. The ventral side was densely spotted, and the colour of the femur was yellow to green. All *P. ridibundus* individuals had a spotted dorsal side. For the smallest frog in the complex, *P. lessonae*, a short hind leg (reaching the snout when stretched out or shorter) with large, almost semicircular metatarsal tubercle was typical, even though some individuals had longer hind legs. The ventral side was most often spotless and white, but individuals with spotted bellies were also found. The colour of the femur was yellow or yellowish green. All these variations in appearances are possible in hybrid forms, and they usually show intermediate characteristics of their parental species. Hybrid individuals are similar to *P. ridibundus* regarding the shape of the metatarsal tubercle (obliquely positioned and moderately

Table 2. Mean, minimum, and maximum values of morphometric characters measured in *Pelophylax esculentus* complex species from Croatia. L – body length (measured from the tip of the snout to the vent); F – femur length; T – tibia length; DpPp – first toe length; Cint – metatarsal tubercle length; N – number of individuals.

Species		Parameter	L (mm)	T (mm)	F (mm)	DpPp (mm)	Cint (mm)
P. kl. *esculentus*	females	mean	59.51	31.16	31.96	12.74	3.88
(N=62)	(N=17)	min	29.44	23.67	22.17	9.45	2.72
		max	80.86	41.27	43.15	22.33	5.26
		SD	12.00	5.38	6.43	3.18	0.69
	males	mean	53.34	26.33	27.40	10.60	3.46
	(N=23)	min	33.84	14.63	17.11	6.30	1.57
		max	70.11	33.33	35.22	14.03	4.90
		SD	9.70	5.54	5.49	2.07	0.83
	juveniles	mean	40.12	20.21	20.90	8.11	2.73
	(N=22)	min	32.19	15.39	15.39	5.88	1.66
		max	48.14	25.49	25.70	10.48	3.42
		SD	4.60	2.78	2.84	1.28	0.44
P. lessonae	females	mean	53.70	25.64	27.50	11.03	3.65
(N=24)	(N=7)	min	43.85	20.85	21.09	8.84	2.75
		max	61.88	31.79	35.03	14.14	4.33
		SD	7.27	4.22	4.84	2.17	0.65
	males	mean	46.44	21.57	23.60	9.11	3.30
	(N=9)	min	36.90	16.64	17.70	6.83	2.21
		max	66.35	27.37	30.03	13.42	4.18
		SD	9.43	3.88	4.75	2.03	0.68
	juveniles	mean	38.10	18.48	19.98	8.12	3.01
	(N=8)	min	34.18	16.68	16.55	6.59	2.59
		max	46.05	21.32	22.30	13.67	3.45
		SD	4.16	1.61	2.30	2.31	0.26
P. ridibundus	females	mean	64.23	29.84	33.19	11.34	3.61
(N=7)	(N=2)	min	63.45	29.67	32.68	10.44	2.53
		max	65.01	30.01	33.70	12.24	4.69
		SD	1.10	0.24	0.72	1.27	1.53
	males	mean	73.97	41.50	42.01	15.36	4.08
	(N=3)	min	60.52	35.27	38.82	12.66	3.66
		max	86.16	45.15	44.41	17.75	4.45
		SD	12.87	5.42	2.88	2.56	0.40
	juveniles	mean	47.13	24.31	23.41	9.26	2.68
	(N=2)	min	45.03	23.99	20.55	9.17	2.30
		max	49.23	24.62	26.27	9.35	3.06
		SD	2.97	0.45	4.04	0.13	0.54

raised) and ventral coloration (usually spotted), whereas the hind legs resemble those of *P. lessonae* in length. At our study site, we found the grey-brownish colour of the femur to be unique to *P*. kl. *esculentus*, which is evidence that hybrids are capable of producing new, unique features in addition to those inherited from their parental species. For a quick identification of species in the field, the results of our morphometric analyses imply that the length of the hind leg, as well as the shape and size of the metatarsal tubercle, appear to be the most valuable diagnostic traits. Representatives of *P. lessonae* are the smallest and *P. ridibundus* individuals have the largest morphometric characters within this complex, whereas *P.* kl. *esculentus* is the most diverse regarding morphometry. A principal component analysis based on morphometric indices shows no overlapping of parental species, while *P.* kl. *esculentus* occupies the greatest area of the plot. Nevertheless, the highest concentration of *P.* kl. *esculentus* individuals was in the middle of

Table 3. Mean, minimum, and maximum values of morphometric ratios calculated in the *Pelophylax esculentus* complex. L – body length; F – femur length; T – tibia length; DpPp – first toe length; Cint – metatarsal tubercle length.

Morphometric ratio \ Species	*P.* kl. *esculentus*	*P. lessonae*	*P. ridibundus*
	N=62 min–max mean SD	N=24 min–max mean SD	N=7 min–max mean SD
T/L	0.35–0.58 0.51 0.10	0.41–0.55 0.48 0.03	0.46–0.59 0.52 0.05
F/L	0.41–1.18 0.53 0.09	0.45–0.61 0.52 0.05	0.42–0.64 0.54 0.07
F/T	0.87–1.33 1.04 0.09	0.95–1.19 1.08 0.07	0.86–1.14 1.03 0.10
DpPp/L	0.15–0.76 0.21 0.07	0.17–0.30 0.20 0.03	0.16–0.21 0.20 0.02
DpPp/T	0.36–0.63 0.40 0.04	0.37–0.64 0.43 0.05	0.35–0.41 0.38 0.02
Cint/T	0.09–0.18 0.13 0.02	0.12–0.20 0.15 0.02	0.08–0.16 0.11 0.03
Cint/DpPp	0.20–0.47 0.33 0.05	0.23–0.47 0.36 0.06	0.23–0.38 0.29 0.05

Table 4. Results of ANOVA statistical test for each morphometric ratio between females and males of the *Pelophylax esculentus* complex. Statistically significant values ($p < 0.05$) are shown in bold.

Morphometric ratio	Males (p value)	Females (p value)
T/L	**0.0257**	0.52
F/L	0.09	0.78
F/T	0.36	0.23
DpPp/L	0.55	0.76
DpPp/T	0.06	0.48
Cint/T	**0.0025**	**0.0283**
Cint/DpPp	**0.0245**	0.50

the diagram, probably due to their expression of intermediate characteristics. Some hybrid individuals can be found to be closer to *P. lessonae* or *P. ridibundus* individuals, due to the greater similarity of their morphometric profiles to one parental species. Our ANOVA pointed out that metatarsal tubercle length was the characteristic with the greatest diagnostic value and the only characteristic (in relation to tibia length) that is significantly different in all of the species, both in females and males. Furthermore, two interesting conclusions arise from our ANOVA results: a greater number of significant differences were noticed among males than among females; *P.* kl. *esculentus* males are more similar to *P. lessonae* males than to *P. ridibundus* males, which are, in three morphometric indices, significantly different from the two remaining species. Such results have not been obtained before, but it is important to mention that in many of the previous studies (e.g., Kotlík & Šůlová 1994, Spasić Bošković et al. 1999, Krizmanić & Ivanović 2010, Mayer et al. 2013) males and females

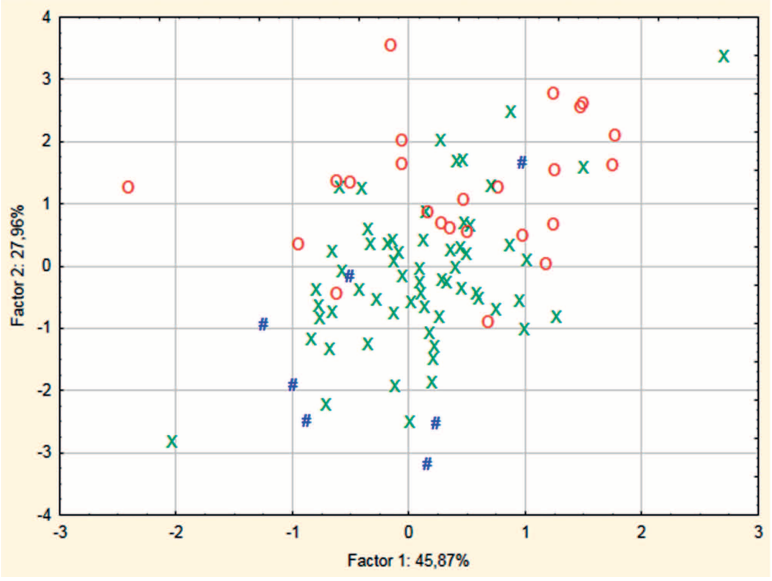

Figure 3. Plot of factor scores for factors 1 and 2 based on morphometric ratios of all investigated individuals. X – *Pelophylax* kl. *esculentus*; o – *Pelophylax lessonae*; # – *Pelophylax ridibundus*.

Table 5. External morphology characters of the three *Pelophylax esculentus* complex species from Croatia.

	P. kl. *esculentus*	*P. lessonae*	*P. ridibundus*
Coloration of femur	Grey to brown in most juveniles, but all colours present. In adults, all colours are present, with the exception of red.	Most often yellow, sometimes reddish, and rarely yellow to green	Yellow or green
Ventral coloration	All stages from white with few spots to densely spotted	Most often with no or few spots, but individuals with scant or dense spotting are also present.	Densely spotted in all of the individuals examined
Dorsal spots	Very large and few in number in most specimens; large and dense in some specimens (particularly juveniles); only rarely absent	Sometimes large and dense; sometimes scant and sometimes absent	Sometimes large and dense; sometimes scant and sometimes absent
Hind leg length	Most often reaching only to beneath snout, but sometimes reaching the snout or beyond when stretched out	Reaching the snout or a point beneath the snout when stretched out	Reaching to a point beneath the snout, the snout, or beyond
Metatarsal tubercle shape	Oblique, small or moderate in size; tall and vertical in position, in only one individual	Most often tall and vertical, but sometimes oblique in shape and small to moderate in size	Small and oblique

Table 6. Numbers and percentages (in parentheses) of the most important prey categories exploited by the *Pelophylax esculentus* complex species in Croatia. N – total number of prey items for each species.

Prey category	*P.* kl. *esculentus* (N=276)	*P. lessonae* (N=118)	*P. ridibundus* (N=24)
Insecta			
Coleoptera	91 (33%)	31 (26.3%)	7 (29.2%)
Megaloptera	1 (0.4%)	/	/
Hemiptera	11 (4%)	5 (4.2%)	/
Lepidoptera	8 (2.9%)	9 (7.6%)	/
Trichoptera	1 (0.4%)	1 (0.8%)	/
Diptera	52 (18.8%)	11 (9.3%)	6 (25%)
Hymenoptera	35 (12.7%)	17 (14.4%)	4 (16.7%)
Ephemeroptera	/	/	1 (4.2%)
Collembola	19 (6.9%)	20 (16.9%)	1 (4.2%)
Odonata	1 (0.4%)	1 (0.8%)	/
Mecoptera	4 (1.4%)	/	/
Orthoptera	1 (0.4%)	1 (0.8%)	/
Neuroptera	1 (0.4%)	/	/
Annelida	/	3 (2.5%)	/
Myriapoda	2 (0.7%)	/	/
Crustacea	15 (5.4%)	1 (0.8%)	/
Gastropoda	12 (4.3%)	9 (7.6%)	/
Arachnida	9 (3.3%)	5 (4.2%)	2 (8.3%)
Arthropoda indet.	4 (1.4%)	3 (2.5%)	3 (12.5%)
indet. material	6 (2.2%)	1 (0.8%)	/
Insecta larvae indet.	3 (1.1%)	/	/
Plant material	4	0	0

were not analysed separately. Although our results confirm that *P. lessonae* is the smallest species in the complex, we have found individuals with greater body lengths than ever before recorded (up to 6.5 cm in our study vs. 4.4–5.5 cm in the literature; Arnold & Ovenden 2004). Our diet analysis of *P. esculentus* complex population samples from the northwestern part of Croatia shows a high prey predilection for arthropods, more specifically insects. This is in concordance with most literature data, which indicate insects as a dominant part of the *P. esculentus* complex diet (Çiçek & Mermer 2006, 2007, Sas et al. 2007, Balint et al. 2010, Mollov et al. 2010, Ruchin & Ryzhov 2002, Yilmaz & Kutrup 2006, Dimancea et al. 2010, Paunović et al. 2010). Several studies (Mollov 2008, Dimanacea et al. 2010) report that green frogs are not to be particularly selective regarding their prey, taking size and mobility as cues. Our results corroborate this assessment since most of the frequently found food items for all of the three species were coleopterans, dipterans, and hymenopterans. These animals are larger and more mobile compared to the other animals found to a lesser extent in the stomach content. All three green frog species have a strong predilection for terrestrial prey, as has already been pointed out by other authors (Çiçek & Mermer 2007, Balint et al. 2010, Sas et al. 2007). However, the proportion of terrestrial prey items is still much higher in *P.* kl. *esculentus* and *P. lessonae* (75.5 and 77.6%, respectively) than in *P. ridibundus* (60%). On the other hand, the proportion of prey items living in both terrestrial and aquatic environments is much higher in *P. ridibundus* than in the remaining two species (33.3 vs. 16.1 and 13.8%). Such results indicate that the *P.* kl. *esculentus* hybrid is more similar regarding its dietary preferences to *P. lessonae* than to *P. ridibundus*. It is important to mention that our results thus deviate from observations made in *P. ridibundus* single-species populations in the Sura and Moksha river basins (Russia) during June–July (Ruchin &

Table 7. Calculated MacArthur and Levins indices (asymmetrical measure) and Pianka's indices (symmetrical measure) for *Pelophylax esculentus* complex species in Croatia (species compared by both equations are marked 'j' and 'k').

	Species	*P.* kl. *esculentus* (k)	*P. lessonae* (k)	*P. ridibundus* (k)
Asymmetrical measure	*P.* kl. *esculentus* (j)	–	0.7933	1.2042
	P. ridibundus (j)	0.5863	0.4133	–
	P. lessonae (j)	0.6859	–	0.7340
Symmetrical measure	*P.* kl. *esculentus* (j)	–	0.73765	0.8402
	P. ridibundus (j)	–	–	0.55081

Ryzhov 2002). This may indicate that the dietary preference of a particular green frog species reflects the characteristics of a particular habitat or disposition as well as the abundance of a particular food item in the environment. However, it might also indicate that species have different dietary preferences and niche breadths in single vs. complex populations. This observation requires further investigation into the ecological profiles of green frog species in different population types. Our niche breadth estimations also pointed out a differentiation of *P. lessonae* and *P.* kl. *esculentus* from *P. ridibundus* with regard to prey predilection. The Hurlbert's index indicated a tendency towards concentrating foraging efforts on fewer prey categories in *P.* kl. *esculentus* and *P. lessonae* than in *P. ridibundus*, which appears to utilize a wider range of prey categories to a relatively equal extent. This is contrary to findings by Paunović et al. (2010) from a similar REL population in the Petrovaradin marsh region (Serbia) where *P. lessonae* was found to have the most uniform diet during spring. Our Pianka's niche overlap index showed that the diet composition of *P.* kl. *esculentus* largely overlaps with those of both of its parental species. This is in agreement with the MacArthur and Levins indices and suggests that *P.* kl. *esculentus* exploits mainly the same prey categories as its parent species. According to this index value, the *P. lessonae* and *P. ridibundus* dietary niche overlap is the least extensive, meaning these species differ the most in their food resource predilections. This is in concordance with the findings by Paunović et al. (2010), but also with the results of our morphological analyses.

In conclusion, our study provides new insights into the morphology and ecology (diet) of three green frog species making up the studied *Pelophylax* complex population, which may help shed new light on the species-to-species relationships in this potentially evolutionary important hybridogenetic complex.

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