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# MORPHOMETRIC STUDY OF THE JUVENILES BELONGING TO COMPLEX *RANA ESCULENTA* FROM CIRIC RIVER'S BASIN (IAŞI)

BY

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**Key words:** water green frogs, morphometry, polymorphism, River Ciric

Distribution of morphometric characters (parameters and colour pattern) is given for the identification of water green frogs from Ciric River's basin. Many anuran species exhibit striking colours or dorsal pattern polymorphisms, and also provide an excellent instrument to answer questions related to evolution and maintenance of polymorphisms.

## Introduction

European water green frog complex consists of three species: *Rana ridibunda* Pallas 1771, *Rana lessonae* Camerano 1882 and *Rana esculenta* Linnaeus 1758. Hybrid individuals of *Rana esculenta* usually have intermediate characters between the parental species; for this reason the study used morphometric method to identify the three species [6].

Morphometric measurements were made starting Boulenger (1885, 1891). Later, (1923, 1927) Terentjev run biometrical studies and found correlation (0.5-0.9) between the morphometric characters. Berger (1964, 1966) [2,3], Günther (1968, 1973), Wijnands (1976) [17] and Gubanyi (1992, 1995) [9,10] also run deep biometrical investigations. In 1878 Camerano described the species *Rana esculenta lessonae* while Boulenger (1898) considered it as a variety of *Rana esculenta*. Starting with Berger (1966) [3] for scientists was more and more obvious that *Rana esculenta* is the hybrid of *Rana ridibunda* and *Rana lessonae*[8].

Polymorphism can be defined as simultaneous occurrence of two or more discrete and genetically based phenotypes in a population where frequency of the rarest type is higher than might be maintained by recurrent mutation (Ford, 1975) [11]. Factors that affect movement of the pigment include temperature, light, and humidity. Warm and dry conditions result in contraction of the pigment granules making the frog pale; cold and damp conditions facilitate the dispersal of pigment making the frog appear darker.

The hypothesis that phenotypic plasticity is an adaptation to environmental variation rests on the two assumptions that plasticity improves the performance of

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individuals that possess it, and that it evolved in response to selection imposed in heterogeneous environments [4].

**Material and method**

Ciric River's basin is located in the southern part of Moldavian Plain (Fig. 1) and belongs to geographic unit of Jijia-Bahlui Depression included in Moldavian Platform. It has a surface of approximately 54 km². River Ciric is a left side affluent of River Bahlui. It springs from Popricani and Rusului Hills and after 20 km enters the Iaşi town. Maximal altitude is 205 m [1].

River Ciric has a varied hydrological potential represented by underground and surface waters. The ponds existing on river itinerary have an anthropogenic origin. They were created by river repeated damming aiming at regulation of the water quantity in River Bahlui, contributing to prevention of floods. Ponds cover about 127 ha and contain 2,405,000 m³.

60 juveniles captured in August, September and October were analyzed. Individuals captured by hand or using the sweep net were released after data collection. Measurements were made on living individuals, in the field and in the laboratory, on the right side of the body. A vernier callipers, with an error of 0.1 mm was used.

Dorsal and ventral colour morphs were differentiated according to Ishcenko [5]:

Maculata: can be recognized due to presence of dark spots 2-3 or 6-7 mm diameter, with varied numbers and distribution.

Hemimaculata: similar with maculata but with 2-6 spots only.

Burnsi: spots are missing.

Punctata: many little spots, like points.

Hemipunctata: number of points is smaller than in punctata morph.

Striata: shows an intensely green median line (usually found in combination with other morphs).

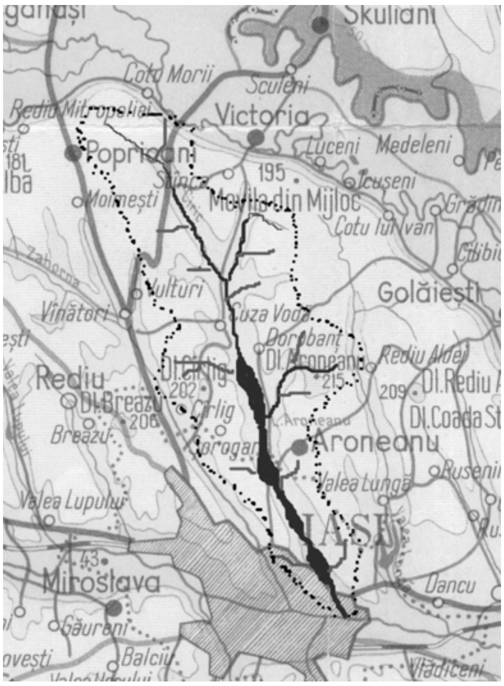


**Fig.1 Physico-geographical location of the River Ciric basin (Iaşi county map, 1993)**

These six fundamental morphs can be combined resulting complex morphs like hemimaculata-hemipunctata, maculata-punctata etc. [5]. These morphs can be used to answer questions about frogs' population structure and distribution.

There are three colour patterns on frog's ventral side: nigricollis-nigriventris (N/N, with pigmented abdominal and orofaringian/oral regions), albicollis-nigriventris (A/N, with orofaringian region depigmented while abdominal region pigmented) and nigricollis-albiventris (N/A; only with orofaringian region pigmented). Ventral side depigmentation is for albicollis-albiventris morph [5].

15 morphometric parameters of juveniles were analyzed: L (body length, distance between tip of the muzzle and cloak orifice); L.c. (head length, distance between tip of the muzzle to the edge of occipital orifice); Lt.c. (head breadth, measured at mandible base), D.r.o. (distance between rostrum and eye); Sp.c.r. (space between rostral edges, measured în dreptul anterior eye region); D.n.o. (distance between nostril and eye); L.o. (eye length); Lt.p. (breadth eyelid); Sp.p. (interorbital space); Sp.n. (space between nostrils); L.tym. (tympanum diameter); F. (femur length); T. (tibia length); D.p. (hindleg first toe length); C.int. (tuberculului tarsial length).

12 morphometric indices were calculated: L/L.c., L.c./Lt.c., D.r.o./D.n.o., L.o./L.tym., Sp.p./Sp.n., Lt.p./Sp.p., F./T., 2T./L., L./T., L/D.p., T./C.int, D.p./C.int.

Both for morphometric parameters and indices were calculated: maximal values (MAX), minimal values (MIN) and mean values (M), standard deviation (DS), standard error (ES), modal value (MOD).

**Results and discussions**

**1. Data regarding the biometry of juveniles from *Rana esculenta* complex**

Most important biometrical variables are: body length (L), tibia length (T), metatarsal tubercle length (*callus internus*, C.int.) and hind legs first toe length (*digitus primus*, D.p.) Table 1 shows the mean, maximal and minimal values, standard deviation and standard error. Body length of analyzed juveniles is between 1.7 and 4.95 cm, having the mean values of 2.61±0.09 cm.

**Tab. 1 Morphometric measurements in juveniles from *Rana esculenta* complex**

| No. | Parameters | MIN | MAX | M | MOD | DS | ES |
|---|---|---|---|---|---|---|---|
| 1 | L | 1.7 | 4.95 | 2.61 | 2.3 | 0.71 | 0.09 |
| 2 | L.c. | 0.77 | 1.78 | 1.04 | 0.88 | 0.20 | 0.02 |
| 3 | Lt.c. | 0.63 | 1.84 | 0.89 | 0.7 | 0.24 | 0.03 |
| 4 | D.r.o. | 0.3 | 0.78 | 0.43 | 0.4 | 0.10 | 0.01 |
| 5 | SP.c.r. | 0.22 | 0.9 | 0.44 | 0.48 | 0.11 | 0.01 |
| 6 | D.n.o. | 0.12 | 0.36 | 0.19 | 0.18 | 0.04 | 0.005 |

| No. | Parameters | MIN | MAX | M | MOD | DS | ES |
|---|---|---|---|---|---|---|---|
| 7 | L.o. | 0.24 | 0.62 | 0.34 | 0.34 | 0.07 | 0.009 |
| 8 | Lt.p. | 0.12 | 0.35 | 0.19 | 0.15 | 0.04 | 0.005 |
| 9 | Sp.p. | 0.22 | 0.5 | 0.32 | 0.32 | 0.07 | 0.009 |
| 10 | Sp.n. | 0.15 | 0.39 | 0.23 | 0.2 | 0.06 | 0.007 |
| 11 | L.tym. | 0.13 | 0.41 | 0.21 | 0.2 | 0.07 | 0.009 |
| 12 | F. | 0.47 | 2.58 | 1.28 | 1.4 | 0.50 | 0.06 |
| 13 | T. | 0.45 | 2.64 | 1.31 | 1.24 | 0.52 | 0.06 |
| 14 | D.p. | 0.22 | 0.71 | 0.37 | 0.31 | 0.13 | 0.01 |
| 15 | C.int. | 0.09 | 0.35 | 0.16 | 0.13 | 0.08 | 0.01 |

3 of the 12 indices calculated in our study are most important in order to identify the three species: T/C.int., D.p./C.int şi L/D.p. (tab. 2) [3, 9, 12, 16, 18].

**Tab. 2 Biometrical indices in juveniles from *Rana esculenta* complex**

| No. | Indices | MIN | MAX | M | DS | ES |
|---|---|---|---|---|---|---|
| 1 | L/L.c. | 1.99 | 3.43 | 2.47 | 0.23 | 0.03 |
| 2 | L.c./Lt.c. | 1.04 | 4.51 | 3.53 | 0.65 | 0.08 |
| 3 | D.r.o./D.n.o | 1.72 | 3 | 2.26 | 0.27 | 0.03 |
| 4 | L.o./L.tym | 1.14 | 2.46 | 1.63 | 0.30 | 0.04 |
| 5 | Sp.p./Sp.n. | 0.95 | 2.29 | 1.39 | 0.19 | 0.02 |
| 6 | Lt.p./Sp.p. | 0.43 | 0.86 | 0.61 | 0.09 | 0.01 |
| 7 | F./T. | 0.76 | 1.14 | 0.98 | 0.06 | 0.009 |
| 8 | 2T/L | 0.30 | 1.21 | 1.003 | 0.12 | 0.01 |
| 9 | L./T. | 1.64 | 6.6 | 2.06 | 0.63 | 0.08 |
| 10 | L/D.p. | 4.83 | 9.7 | 7.15 | 0.94 | 0.12 |
| 11 | T/C.int. | 3.46 | 15 | 8.38 | 1.94 | 0.25 |
| 12 | D.p/C.int. | 1.52 | 4.2 | 2.38 | 0.58 | 0.07 |

Indices most used to distinguish between the three species are tibia length/inner metatarsial tubercle length and tibia length/inner metatarsial tubercle height (Wijnands and Van Gelder, 1976). A. Gubanyi and Z. Korsos (1992) considered body length/first toe length and tibia length/inner metatarsal tubercle length as most relevant indices [9, 17]. In order to avoid misinterpretations during discriminant analysis T/C.int. index was used to make a prediction about the number of individuals of each species, represented in tab. 3.

**Tab. 3 Number of individuals of each species from different discriminating values of T/C.int. index:**

| References | Species | Discriminating values | No. of individuals |
|---|---|---|---|
| Berger (1966) | *Rana lessonae* | < 7 | 13 |
| | *Rana esculenta* | 7-9 | 24 |
| | *Rana ridibunda* | >9.5 | 14 |
| | *Rana lessonae - esculenta* | | 1 |
| | *Rana ridibunda - esculenta* | | 8 |
| Gunther (1975) | *Rana lessonae* | < 7 | 10 |
| | *Rana esculenta* | 6.5-8.6 | 7 |
| | *Rana ridibunda* | - | 26 |
| | *Rana lessonae - esculenta* | | 6 |
| | *Rana ridibunda - esculenta* | | 11 |
| Wijnands & Van Gelder (1976) | *Rana lessonae* | < 6 | 3 |
| | *Rana esculenta* | 6-8.5 | 26 |
| | *Rana ridibunda* | > 8.5 | 25 |
| | *Rana lessonae - esculenta* | | 3 |
| | *Rana ridibunda - esculenta* | | 3 |
| Regnier & Neveu (1986) | *Rana lessonae* | < 9.5 | 36 |
| | *Rana esculenta* | 9-10.4 | 7 |
| | *Rana ridibunda* | - | 6 |
| | *Rana lessonae - esculenta* | | 11 |
| | *Rana ridibunda - esculenta* | | - |
| Polls-Pellaz (1991) | *Rana lessonae* | < 8 | 23 |
| | *Rana esculenta* | 8-9.5 | 18 |
| | *Rana ridibunda* | - | 16 |
| | *Rana lessonae - esculenta* | | 3 |
| | *Rana ridibunda - esculenta* | | - |
| A. Gubanyi, Z. Korsos (1992) | *Rana lessonae* | 6-7 | 14 |
| | *Rana esculenta* | 7.2-8 | 10 |
| | *Rana ridibunda* | 10-11 | 10 |
| | *Rana lessonae - esculenta* | | 2 |

| | | | |
|---|---|---|---|
| | *Rana ridibunda - esculenta* | | 24 |
| Cogalniceanu & Tesio (1993) | *Rana lessonae* | < 7 | 13 |
| | *Rana esculenta* | 7-9.5 | 34 |
| | *Rana ridibunda* | > 9.5 | 13 |
| | *Rana lessonae - esculenta* | | - |
| | *Rana ridibunda - esculenta* | | - |
| Joly, Pagano, Morand (1994) | *Rana lessonae* | < 7 | 11 |
| | *Rana esculenta* | 6.6-9 | 22 |
| | *Rana ridibunda* | >9 | 20 |
| | *Rana lessonae - esculenta* | | 1 |
| | *Rana ridibunda - esculenta* | | 6 |

Tibia length/inner metatarsal tubercle length reported to hind leg first toe length/inner metatarsal tubercle length permitted the identification of 18 specimens of *Rana ridibunda* (–), 26 of *Rana esculenta* (♦) and 16 of *Rana lessonae* (■). Thus, in aquatic habitats from Ciric River's basin the three species were found in the following proportion: 30% *Rana ridibunda*, 43.3% *Rana esculenta* and 26.6% *Rana lessonae* (Fig. 2).

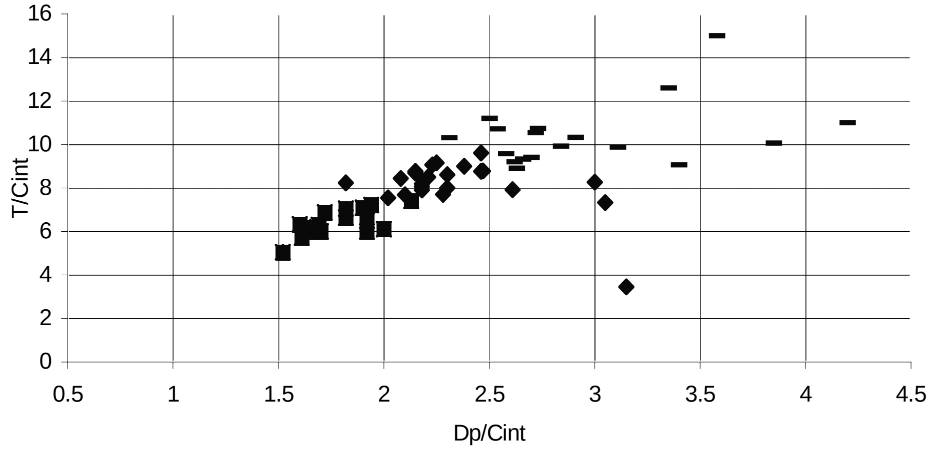


Fig. 2 Distribution of Rana esculenta complex's species against Dp/Cint. and T/Cint. discriminant index

Application of variation for juveniles measured in Ciric River's basin permitted the identification of 15 specimens of *Rana ridibunda* (–) (25%), 34 of *Rana esculenta* (♦) (56.6%) and 11 of *Rana lessonae* (■) (18.3%) based on indices T/cint and L/Dp (Fig. 3).

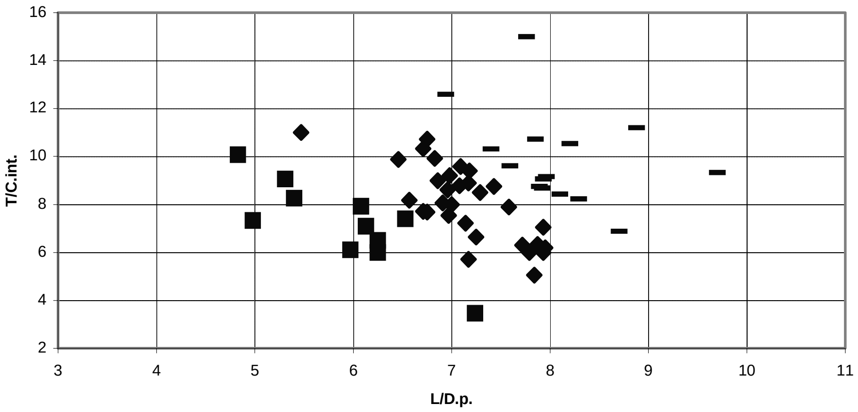


Fig. 3 Distribution of Rana esculenta complex's species against L/Dp and T/Cint. discriminant index

Most abundant specie was *Rana esculenta* hybrid with a percentage of 43-56%. *Rana ridibunda* represented 25-30% of the total population while *Rana lessonae* 18-25%. Vancea et al. (1989) found in Mirceşti (approx. 40 km from Iaşi) small pure populations of *Rana lessonae* and for this reason we think that the species is far for being absent in Iaşi county [7, 15].

**2. Polymorphism of *Rana esculenta* complex juveniles**

The 60 analyzed juveniles from Ciric River's basin presented 3 simple dorsal morphs and 8 combined dorsal morphs. Simple dorsal morphs found were 33.3% - maculata (M), 13.3% - hemipunctata (Hm) and 3.3% - punctata (P). Complex morphs which represent adaptive reserve of population were: maculata-punctata (MP) 21.7%, maculata-striata (MS) 11.7%, maculata-punctata-striata (MPS) 3.3%, hemimaculata-striata (HmS) 5%, hemimaculata-punctata (HmP) 5%, burnsi-striata (BS) 1.7%, hemimaculata-punctata-striata (HmPS) 1.7% (Tab. 4).

**Tab. 4 Dorsal morphs identified of *Rana esculenta* complex's juveniles**

| Morphs | M | Hm | P | MP | MS | MPS | HmS | HmP | HmPS | BS |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of individuals | 20 | 8 | 2 | 13 | 7 | 2 | 3 | 3 | 1 | 1 |
| % | 33.3 | 13.3 | 3.3 | 21.7 | 11.7 | 3.3 | 5 | 5 | 1.7 | 1.7 |

The most frequent ventral chromatic variations were albicollis-albiventris (AA) – 50% and nigricollis-albiventris (NA) – 45%. The other two ventral morphs were found in lower proportion albicollis-nigriventris (AN) 3.3% and nigricollis-nigriventris (NN) –

1.7%. Abdominal colour is very important for aquatic species because influence individuals survival against aquatic predators. Tab. 5 shows that 95% of specimens had a white abdomen, thus heavily observed by natural enemies.

**Tab. 5 Ventral morphs identified of *Rana esculenta* complex's juveniles**

| Morphs | NN | AA | NA | AN |
|---|---|---|---|---|
| No. of individuals | 1 | 30 | 27 | 2 |
| % | 1.7 | 50 | 45 | 3.3 |

Polymorphism gives the species capacity to populate different habitats while the presence of more complex morphs (8 types, observed in 50% of individuals) indicate the fact that *Rana esculenta* complex's species from Ciric River's basin show adaptive plasticity. Polymorphism within a population reflects the adaptive capacity under environmental conditions, especially offers camouflage in different microhabitats. Thus polymorphism is low when environmental factors are uniform, and is high when these factors are varied. The colour is highly determined by substrate [5].

Merrell and Rodell (1968) [11] observed in the field a higher proportion of burnsi morph among *Rana pipiens* survivors than among dead individuals after an episode of winter mortality. That percentage of burnsi higher in spring than in previous autumn is also consistent with higher burnsi overwintering survival.

These data are not the result of planned experiments. Various correlation of colour pattern were tested during different seasons, including susceptibility to desiccation, stress resistance, emergence of adults, diseases, fecundity, mating preference, length of larval period, size at metamorphosis, juvenile size, adult size, tadpole growth and survival rate (*Acris* sp. - Nevo, 1973; Gray, 1977, 1983; *Crinia* sp. - Main, 1961, 1968; Walker, 1966; Bull, 1977; *Hyla regilla* -Jameson & Pequegnat, 1971; *Phelsuma ornata* - Travis & Trexler, 1984; Blouin 1989a; *Pseudacris triseriata* - Mathews, 1971; Hoppe & Pettus, 1984; *Rana arvalis* - Ishchenko & Shchupak, 1974; *Rana pipiens* - Merrell, 1965; Merrell & Rodell, 1967; Gill, 1970; Merrell, 1972; Dapkus, 1976; Corn, 1981) [11]. Most of these studies found no correlation between morphs and specific traits. Hoppe & Pettus (1984) measured nine variables and found two statistically significant correlation but these disappeared when a correction for multiple comparisons was made. Nevertheless, few examples of fitness traits correlated with colour pattern exist. The survival difference between *Rana pipiens* morphs suggested a correlation with some tolerance to physiological stress (Merrell & Rodell, 1968; Dapkus, 1976). Some data (Nevo, 1973) suggested a correlation with resistance to diseases in *Acris crepitans* [11].

Only four studies used an experimental approach to test direct selection on the polymorphism itself: Wendelken (1968), Gray (1978), Tordoff (1980) and Morey

(1990). Two of the four studies found that predators preferentially ate non-matching morphs (Tordoff, 1980; Morey, 1990). The mechanisms behind the selective maintenance of colour and/or pattern polymorphism have been investigated in 19 species. Most of these studies attempted to determine if some fitness-related trait was correlated with morph type, and were mostly inconclusive. Although only four studies investigated direct selection by predators on polymorphism itself, two of these demonstrated differential selection (albeit in the laboratory) [11].

## Conclusions

Morphometric investigations of green frog juveniles permitted the identification of the three species: *Rana ridibunda*, *Rana esculenta* and *Rana lessonae* in Ciric River's basin. Most frequent species was *Rana esculenta*, individuals representing half of population. Morphometric indices are not always valid for taxonomic identification in the field but several biometrical investigations showed an overlap of the morphometric measurement of taxa [3, 9, 17]. Morphology is determined by phylogeny and environmental factors, thus existence of many morphs is a result of an unpredictable and heterogeneous environment [13].

Variety of dorsal morphs found in field could be result of distinct microhabitats (25 types of microhabitats and 10 colour patterns). The most frequent ventral morphs were albicollis-albiventris (50%) while 95% specimens had white abdomen, being more difficult to observe for natural enemies. We believe that the simplest explanation for the maintenance of colour pattern is indeed direct selection by visually oriented predators. Well-designed studies are needed on the effects of predation on morph frequencies in the field. This remains a wide-open and potentially rewarding area of study.

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