Southern distribution limit of *Pelophylax lessonae* and the L-R-E population system in Romania

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Abstract. In this paper we present the most southern distribution record of *Pelophylax lessonae* species in Romania, and of the L-R-E (*lessonae-ridibundus-esculentus*) population system as well. The recorded population is situated near the village Izvoarele, in Mehedinti County, South-Western Romania. We have captured 52 individuals of water frogs during one hour of work in the investigated habitat. In the case of each form of water frogs we have captured both males and females. Most individuals of water frogs were represented by the species *P. ridibundus*, followed by the hybrid *P. esculentus*. The fewest captured individuals belonged to the species *P. lessonae*. The fewest captured individuals belonged to the species *P. lessonae*.

Key words: *Pelophylax lessonae*, *Pelophylax esculentus* complex, L-R-E population system, southern distribution limit, Romania.

The genus *Pelophylax* is well-known for its hybridogenic systems, each system being consisted of two valid species and a hybrid form between them (see in: Plötzner & Ohst 2001). From those complexes of water frogs, in Romania is found solely the hybridogenetic system represented by the *P. esculentus* complex. This system consists of two valid species, *P. lessonae* and *P. ridibundus*, and the hybrid *P. kl. esculentus* respectively. In Romania the *P. ridibundus* species and the hybrid *P. esculentus* are the most widespread, the number of localities with the *P. lessonae* species being significantly lower (see in: Sas 2009), as the latter is one of the rarest amphibian species from Romania’s fauna. One hitch in clarifying the distribution of water frogs in Romania is also represented by the fact that many herpetologists do not try to distinguish among the three forms of water frogs during one hour of work in the investigated habitat. There were caught individuals belonging to all three forms of water frogs occurring in Romania. In the case of each form of water frogs we have captured both males (Fig.3) and females. The caught juveniles were identified as belonging to *ridibundus* and *esculentus* forms. The most individuals of water frogs were represented by the species *P. ridibundus* (5 males, 12 females, 12 juveniles), followed by the hybrid *P. esculentus* (2 males, 10 females, 5 juveniles). The fewest captured individuals belonged to the species *P. lessonae* (3 males, 3 females).

In 17 April 2009, in an aquatic habitat located near the village Izvoarele (Mehedinți County) (44°19'00.20" N, 22°38'46.35"E, 49-51m a.s.l. – Fig.1) we have identified the *P. lessonae* species together with the other two forms of water frogs, *P. ridibundus* and the hybrid *P. esculentus* respectively. The habitat, located at about 2100m from the Danube, is represented by a large pond (length of approximately 135 m, width of 20-35 m, with a maximum depth of 1-1.2m) positioned in close vicinity of a communal road (Fig.2). Frogs were identified based on their morphological and chromatic characters (e.g. Berger 1966, Wijnands & Van Gelder 1976); these determinations being used successfully by many herpetologists, including for identification of individuals for molecular studies (e.g. Neveu 2009, Arioli et al. 2010). We have used those characters which are considered the most important in identifying water frogs: shape of the *callus internus* (e.g. Berger 1966), femur spot pattern (e.g. Fog 1994), and presence or absence of yellow coloration on the flanks (also see in: Krizmnić 2008a).

We have captured 52 individuals of water frogs during one hour of work in the investigated habitat. There were caught individuals belonging to all three forms of water frogs occurring in Romania. In the case of each form of water frogs we have captured both males (Fig.3) and females. The captured juveniles were identified as belonging to *ridibundus* and *esculentus* forms. The most individuals of water frogs were represented by the species *P. ridibundus* (5 males, 12 females, 12 juveniles), followed by the hybrid *P. esculentus* (2 males, 10 females, 5 juveniles). The fewest captured individuals belonged to the species *P. lessonae* (3 males, 3 females).

Generally, the populations of the species *P. lessonae* count a low number of individuals (Günther 1997, also see in: Sas 2009). After a study conducted in Poland, in the composition of water frogs’ populations the individuals of *P. esculentus* prevailed, *P. lessonae* occurred with a slightly lower percentage, while *P. ridibundus*.
Figure 1. The geographical position of the Pleophylax lessonae population from Izvoarele (red dot) and the other populations in the area (grey dots) after Covaci-Marcov et al. 2008a.

Figure 2. The water frogs habitat from Izvoarele, Mehedinti county.

Figure 3. Males of water frogs from Izvoarele: a.) P. ridibundus; b.) P. kl. esculentus; c.) P. lessonae.
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was rarer (Rybacki & Berger 1994). In return, taking account of the only available data on the numerical composition of some populations of water frogs from Romania, in the L-R-E system populations from north-western Romania the species *P. lessonae* or the hybrid *P. esculentus* prevails numerically (Sas 2009); and in these cases also, the species *P. ridibundus* occurs with a lower number of individuals. It is very important that in the identified population from Izvoarele the individuals of the species *P. ridibundus* are prevalent. This can be explained primarily by the fact that in this area, compared with *P. ridibundus*, both *P. lessonae* and the hybrid *P. esculentus* are at the southern limit of distribution (see the distribution maps at IUCN 2010).

Taking account of the wide distribution of the species *P. ridibundus* and the hybrid *P. esculentus* in Romania compared to that of the species *P. lessonae* (see in Sas 2009), the most valuable outcome is the identification of the new pool frog population. In Romania there have been recently identified numerous new populations of *P. lessonae* in different areas (e.g. Covaciu-Marcov et al. 2006, 2007, 2008a,b, 2010, Strugariu et al. 2008a, also see in: Sas 2009). There has also been a record of the pool frog in south-western Romania since 1975 (Fuhn 1975).

Although this record had been considered uncertain for a long time, recently the presence of the species *P. lessonae* in this area was reconfirmed, being identified several new populations (Covaciu-Marcov et al. 2008a, 2009b).

Although in south-western Romania there have been recently identified several populations of *P. lessonae* (see above), the discovery of the population presented in this paper has importance because it is the most southern record of this species in Romania. On the other hand it is very important that the identified population of water frogs is integrated into an L-R-E population system (*lessonae-ridibundus-esculentus*). Within the common distribution area of the water frogs from the *P. esculentus* complex the L-E (*lessonae-esculentus*) and R-E (*ridibundus-esculentus*) systems are considered to be the most common (reviewed by Plötner 2005), the L-R-E population system is regarded as being much rarer). In the literature, data on the distribution of the species *P. lessonae* more south of the record presented in this paper are available from Serbia (e.g. Krizmanić 2008a,b) and Italy (see in Canestrelli & Nascetti 2008). Taking account of the fact that *P. ridibundus* is missing from the Iberian Peninsula, southern L-R-E system populations have been identified only in Serbia (Krizmanić 2008a,b). However, it remains to be seen if there are other populations of *P. lessonae* also, closer to the Danube.

*P. esculentus* is a hybrid between the species *P. lessonae* and *P. ridibundus*. In the common hybridogenesis model, the hybrid *P. esculentus* can produce viable offspring through sexual parasitism (e.g. Uzzell & Berger 1975), its sexual host being represented by the species *P. lessonae* (R-hybridogenesis sensu Polls-Pelaz 1994). On the other hand, the *P. ridibundus* individuals from Balkan populations are already resistant to hybridogenesis (Hotz & Uzzell 1983, Guerrini et al. 1997). It was observed that the *P. ridibundus* individuals from the original stock (south-eastern Europe, Anatolia) in most of the mating cases with *P. lessonae* individuals produce sterile, non-hybridogenetic hybrids (e.g. Berger et al. 1994, Bucci et al. 1990). Thus it remains to be seen how the hybridogenetic system works in the case of the population from Izvoarele: if matings between the parental species produce also hybrids or not. The high number of *P. ridibundus* individuals might indicate that the population operates as an L-E system, respectively as an R-R system (*R*[LE] system) and not as a whole, an L-R-E system.

**Acknowledgments.** We have to express out thanks to Dr. Alfred Ș. Cioică-Lucaciu and to the biology students from the University of Orașea for their help in the fieldwork.

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*Bihorean Biol.* 4, 2010
Published Online: 22 October 2010.