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Description of the larval stages of the Pyrenean frog,
Rana pyrenaica Serra-Cobo, 1993
(Amphibia: Ranidae)

ABSTRACT

The larvae of the recently discovered brown frog *Rana pyrenaica* differ from other European brown frog tadpoles by their dark brown to deep black dorsal colour with silvery white spots on the flanks. Tooth formula of the examined specimens was 1:3+3/1+1/3. Two distinct large and two very small lingual papillae do occur, thus representing an intermediate character state between European *Rana* species with two and with four distinct papillae.

A diagnostic difference to *Rana temporaria* tadpoles from the Pyrenees is the gap between both portions of the second upper tooth row, which is much smaller in *Rana pyrenaica*. Additionally, a multivariate morphometric comparison showed highly significant differences between tadpoles of *Rana pyrenaica* and sympatric *Rana temporaria* regarding several morphometric characters. A rather large interpopulational variability in several oral structures was found in *Rana temporaria* tadpoles from the Pyrenees.

1. INTRODUCTION

Until recently, the taxonomic status of brown frog populations from the Spanish and French Pyrenees was subject to controversial opinions. The presence or absence of *Rana dalmatina* in the Spanish part of the Basque country and in Catalonia was uncertain (Garcia-Paris, 1985), and discussions arose on the supposed - but never evidenced - presence of *Rana iberica* in the Pyrenean mountain range (see Garcia-Paris, 1985; Bea, 1989). The classification

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of Iberian grass frogs (*Rana temporaria*) as different subspecies (*R. t. canigonensis*, *R. t. parvipalmata*) also remained rather obscure. Long-legged pyrenean *temporaria* specimens were preliminary named "Gassers frog", pending further study (see Dubois, 1983; Nöllert & Nöllert, 1992).

Still today several open questions on Iberian brown frog systematics do remain. A general overview, however, can be summarized from recent publications:

- Several rather isolated populations of *Rana dalmatina* occur in the Spanish basque country (Arrayago & Bea, 1985). However, the presence of the species in the Pyrenees as reported in the map of Guyétant (1989) is still unclear. Records from Catalonia have been demonstrated to be erroneous (Llorente et al., 1995).

- *Rana iberica* regularly occurs in the Spanish basque country (Arrayago & Bea, 1985; Bea, 1989), but does not seem to penetrate into the montane range of the Pyrenees, neither into France.

- *Rana temporaria parvipalmata* lives in north-western Spain (Galicia and western Asturias). It is well diagnosed by allozyme genetics (Arano et al., 1993), morphology (Galan, 1989b), extended breeding period (Galan, 1989a; Vences, 1992, 1994) and possibly also bioacoustics (Vences, 1992). This taxon may even merit a rank as distinct species (Arano et al., 1993).

From central Asturias eastwards, but possibly also in mountain ranges of Galicia such as the Serra do Caurel, only grass frogs are known which clearly do not belong to *parvipalmata*. Their status (typical form or differentiated subspecies) is not sufficiently known (Arano et al., 1993; Vences, 1992).

- *Rana temporaria* shows clear interpopulational differences also in the Pyrenees. At high elevations (eg. Mont Canigou in the eastern Pyrenees, Dubois, 1983; but also in the western Pyrenees, own data) many frogs show rather short legs (tibiotarsal articulation reaching the eye). Both in Spanish populations (Vidra, Maranges following Balcells, 1956) and at lower elevations in the French western Pyrenees (eg. the low Vallée d'Aspe, own observations), specimens have much longer hindlimbs, the tibiotarsal articulation reaching between nostrils and snout tip - thus corresponding with the form named Gasser's frog by Dubois (1983). The short-legged population from Mont Canigou has been described as subspecies *canigonensis*, and this name may be applied to the short-legged forms from high altitude Pyrenees in a preliminary way according to Dubois (1983).

- Only two years ago a new species of brown frog was described from the central western Spanish Pyrenees: *Rana pyrenaica* Serra-Cobo, 1993. This species is a well differentiated, rather small, uniformly light beige coloured high mountain frog. Old records of *Rana iberica* in the Pyrenees possibly are due to confusion with this new species. So, the frog from El Pueyo de Jaca pictured by Garcia-Paris (1985) in fig. 156 as *R. iberica*-like *R. tem-*

poraria with long legs is certainly a *R. pyrenaica*.

In the present study we give a first detailed description of the larval stages of *R. pyrenaica*, a species for which we propose the English common name of Pyrenean frog. We especially focus on larval mouthpart structures and on differences to *R. temporaria* tadpoles from the Pyrenees; additionally we summarize some anecdotal observations on habitat and habits of the species, and illustrate adults and larvae in colour.

2. MATERIAL AND METHODS

Field observations were carried out by the two senior authors in the area of the Parque Nacional de Ordesa y Monte Perdido, Huesca province, Spain. Tadpoles were observed along the Rio Arazas, which constitutes the National Park border, and five tadpoles were captured at one locality in the immediate vicinity of Rio Arazas but outside the National Park (about 1500 m above sea level, near Mondicieto mountain).

Detailed measurements, mainly of oral disk structures, were taken from these five *Rana pyrenaica* tadpoles and from two *Rana temporaria* tadpoles from the Lacs d'Ayous (in the vicinity but outside the borders of the Parc National des Pyrénées Occidentales, western central Pyrenees, France) and one *R. temporaria* tadpole from the upper Vallée d'Aspe (Pool along Lescun river, near Lescun, western central Pyrenees, France). Tadpoles were fixed and preserved in the field in 70% ethanol; they were measured with a binocular with measuring device to the nearest 0.1 mm. Many more tadpoles of both species were examined in the field and in other samples (see below) and were found to agree in the main external characters.

Tadpole keratodont rows are described using the standardized formula proposed by Dubois (1995). To enable better comparison, abbreviations of measurements mainly follow Grillitsch et al. (1993). Also the terms and structure of the following description are largely based on Grillitsch et al. (1993) which is the most detailed and complete description of a European brown frog larva so far published.

The following abbreviations are used: SVL Snout-vent-length; TL total length; BH maximum body height; BW maximum body width; TaL tail length; UF height of upper (dorsal) tail fin at the point of maximum tail height; UF1 height of dorsal tail fin at the middle of the tail; UF2 height of dorsal tail fin at the beginning of the tail; LF height of lower (ventral) tail fin at the point of maximum tail height; LF1 height of ventral tail fin at the middle of the tail; LF2 height of ventral tail fin at the beginning of the tail; TM1 height of caudal musculature at the middle of the tail; TM2 height of caudal musculature at the beginning of the tail; HT maximum height of tail (including dorsal and ventral tail fin); TW1 maximum tail width at the middle of the

tail; TW2 maximum tail width at the beginning of the tail; ID interocular distance; SS distance tip of snout-opening of spiracle; VS distance vent-opening of spiracle; OD maximum width of oral disk; HBW approximate width of horny beak; NE distance between nostril and eye; TsE distance between eye and tip of snout; NN distance between nostrils; ED eye diameter; SD mean diameter of white lateral spots; MP number of marginal papillae; IMP number of inframarginal papilla.

Anterior (upper) tooth rows are named UTR, posterior (lower) tooth rows LTR. They are numbered from the margin towards the center of the oral disk.

For general morphometric comparisons, additional 49 *R. pyrenaica* tadpoles from Valle de Bujaruelo (Huesca province, Spain; locality 1) and 45 *R. temporaria* tadpoles from several localities of the eastern Pyrenees were studied by the three junior authors. These localities were (locality numbers as used in fig. 5): Liat (2), Montgarri (3) and Artiga de Lin (4) -Lleida province-, Meranges (5), Ogassa (6), Ull de Ter (7) and Espinavell (8) -Girona province-, Spain). The Gosner stage of each individual was recorded. The following morphometrical variables were measured (with a digital caliper to the nearest 0.1 mm): SVL, TaL, HT, BH, BW, OD, NN, ID, NE, TsE. In order to compare the overall morphometry of both species, a Multivariate Analysis of the Covariance (MANCOVA) was performed keeping SVL as covariate to standardize the restant variables with regard to body size. Since variation throughout larval development could be expected, the sample was divided into two homogeneous groups: individuals belonging to Gosner stages lower (17 *R. pyrenaica*, 33 *R. temporaria*) and higher (32 *R. pyrenaica*, 10 *R. temporaria*) than 30. This subdivision was applied due to the distribution of stage classes in the studied sample (most specimens either in stage < 25 or > 30). After finding significant results, Scheffé's *post hoc* comparisons between both species were carried out for each group and variable.

Furthermore, individuals were plotted in two dimensions using the Principal Component Analysis (PCA) for reducing dimensionality. The factorial axes were conserved unrotated.

3. RESULTS

3.1. ADULT GENERAL APPEARANCE AND HABITAT OF *RANA PYRENAICA*

In general *R. pyrenaica* (pl. I.3 and I.4) is a relatively small-sized *Rana* species, phenetically belonging to the palearctic brown frogs (subgenus *Rana*; Dubois, 1992), with SVL ranges of 33-46 mm in males and 36-51 mm in females (Serra-Cobo, 1993). Dorsal colour typically is uniform and varies from beige to creme brown. Typical V-shaped pattern can be found on the dorsum in some specimens, otherwise no distinct pattern is present. Ventrally

the colour is much lighter and goes into white. Nuptial pads of the males are coloured greyish or brownish. In these traits our observations are much in accordance with the findings of Serra-Cobo (1993).

In open pasture landscape of the Ordesa-valley (pl. I.1) we observed adult frogs during the day in slow running streams, sitting near or in the water. When disturbed they immediately tried to escape by hiding under stones and rocks below water surface. Vertical height was about 1800 m above sea level.

Larvae and about a dozen of recently metamorphosed juveniles were found down the valley at 1600 m (pl. I.2), in a small, slow moving branch of Rio Arazas, which formed numerous large pools with nearly stagnant water. The newt *Euproctus asper* was very common in these pools (1-2 specimens captured per meter of examined brook, without intensive searching). From this observation it can be speculated that *Euproctus* may constitute an important predator of *R. pyrenaica* larvae, although direct observations are lacking.

3.2. THE TADPOLE OF *RANA PYRENAICA*

By first appearance most tadpoles of *Rana pyrenaica* are completely different from all other European brown frog tadpoles. This is mainly caused by the striking black dorsal colour, which makes many larvae closely resembling a *Bufo* tadpole by first sight. This colour pattern is the most distinctive character which generally alone allows an immediate field identification. The following description refers to larvae in Gosner stages 33-38. The larvae in earlier stages (25-29) differ in being darker and showing brighter and smaller spots on tail and body, which are more separated from each other than in the later stages. Some additional measurements of the specimens used for detailed mouthpart descriptions can be found in tab. 1 and tab. 2. Specimens, in the table as well as in the following text, are referred to as RPI to RP5.

The whole dorsal and lateral surface of the body is black (pl. I.6) to dark brown. The dorsum is usually black and gets clearer towards the belly due to the presence of spots which are silvery, white-yellowish or even golden and always with irregular borders. The same spots are also present on dorsal and ventral caudal fins, and caudal musculature. Their diameter is 0.25 to 0.5 mm, and on the body their size increases gradually from dorsum to belly, as does the intensity of the spotting. The belly is greyish transparent, except for the dense speckling with the white spots.

The tail, including the caudal fin, is dark, sometimes black, and shows smaller spots than the rest of the body. When observed through a binocular microscope, the background dark colour of the tail is composed of independent melanocytes whose pigment is arranged in a very ramified shape.

The tail makes up for 54-66% of the total length. The tail is high, with a rounded end. At midlength of the tail, the caudal musculature accounts for

