

# New life for old specimens: studies on yellow-bellied toad collections

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## ABSTRACT

The yellow-bellied toads *Bombina variegata* and *B. pachypus* alcohol preserved specimens from four Italian Natural History museums have been studied. The specimens have been measured and photographed. Morphometric body measures have been taken, with the purpose to analyse sexual size dimorphism characters of the species. Snapshots of the characteristic colour ventral pattern of each specimen have been taken, with a tripod mounted digital camera, in order to detect, by means of computer-aided image analysis, pattern based correlations and differences between populations and species.

For both species, clues of sexual dimorphism, probably related to the mating behaviour, have been detected in fore and hind-limbs size. However, the overall size of the two species did not significantly differ. Concerning the colour pattern, it has been possible to recognize a geographic gradient in the ratio between yellow and dark coloured parts, as well as in the distribution of size and shape of the dark particles.

Key words:

*Bombina variegata*, *Bombina pachypus*, alcohol collection, image analysis, sexual dimorphism.

## RIASSUNTO

Nuova vita a vecchi preparati: studi su esemplari di ululone dal ventre giallo.

Sono stati studiati gli esemplari di ululone dal ventre giallo (*Bombina variegata*) e ululone appenninico (*B. pachypus*) conservati in quattro Musei di Storia Naturale italiani. Su di essi sono state rilevate misure morfometriche, allo scopo di analizzare il dimorfismo sessuale ed acquisite immagini digitali del caratteristico pattern ventrale per studiarne, attraverso tecniche di analisi computerizzata, le caratteristiche e le differenze geografico-popolazionali. Da un punto di vista morfometrico, sono state riscontrate differenze statisticamente significative fra maschi e femmine negli arti anteriori e posteriori, probabilmente legate al comportamento riproduttivo, ma non è stato evidenziato dimorfismo sessuale nelle dimensioni generali. Queste ultime non risultano invece significativamente differenti tra le due specie. Nelle caratteristiche del pattern ventrale è possibile riconoscere un gradiente geografico nella grandezza e distribuzione delle macchie nere e nel rapporto fra le aree delle parti gialle e di quelle nere.

Parole chiave:

*Bombina variegata*, *Bombina pachypus*, collezioni in alcool, analisi immagini, dimorfismo sessuale.

## INTRODUCTION

The Yellow-bellied toad, *Bombina variegata* (Linnaeus, 1758) and the Apennine yellow-bellied toad *B. pachypus* (Bonaparte, 1838), are two anuran species of relevant conservation interest. Being considered a sub-species of *B. variegata* for many years, *B. pachypus* is currently widely acknowledged as a full species, endemic in the Italian Apennines (Fromhage et al., 2004; Guarino et al., 2007). In Italy, on the other hand, *B. variegata* has a discontinuous distribution in the north-eastern Alps (Di Cerbo & Bressi, 2007).

Long term studies on *B. variegata* have been carried out by the Centro Studi Faunistica dei Vertebrati (CSFV), a research group of the Società Italiana di Scienze

Naturali, through field work, for more than 15 years (e.g. Di Cerbo & Ferri, 1996, 2001; Di Cerbo & Biancardi, 2004, 2010a; Di Cerbo et al., 2011). Besides, thanks to the collaboration between CSFV and the section of Vertebrate Zoology of the Natural History Museum of Milano, a branch of research, focused on morphometric differences and geographic variation of colour pattern, has been carried out on preserved specimens of different Italian museum collections (Di Cerbo & Biancardi, 2010b, 2012). In addition to the Italian species, specimens belonging to the balkanic subspecies *B. v. scabra* (Müller, 1940) and to the cogenetic Common fire belly toad *B. bombina* (Linnaeus, 1761) have been analysed for ventral pattern variations (Di Cerbo & Biancardi, 2010b).

MUSEUM CODE	TOWN	N. B. BOMBINA	N. B. PACHYPUS	N. B. VARIEGATA	N. B. V. SCABRA	N. TOT
MSNTO	Turin	17	30	68	19	134
MSNG	Genoa		45	35		80
MZUF	Florence		48	31		79
MSNM	Milan		14	11		25
<b>Total</b>		<b>17</b>	<b>137</b>	<b>145</b>	<b>19</b>	<b>318</b>

Tab. 1. Sample size and source.

## MATERIALS AND METHODS

The analysed sample consists in 318 alcohol preserved specimens from four Italian Natural History museums: Firenze, Genova, Milano and Torino (tab. 1). The selected specimens were individually photographed and body measures were taken (fig. 1). The catalogue code, sex and geographic origin were also recorded for each animal.

As common preservation techniques can cause, in amphibians and snakes, an effective decreasing of some morphometric features (e.g. body length), especially during the first 2-5 years (Reed, 2001; Vervust et al., 2009), only specimens preserved for more than five years have been included in the morphometric study. An assessment of the mean measurement error (MME) of each variable had been previously performed, as well as a preliminary univariate analysis of the sexual size dimorphisms (SSD). The estimated MME of SVL has been used, in this work, to validate the comparison of the overall body size between *B. variegata* and *B. pachypus*. The mean difference of the two species has been divided

by the estimated MME, to give the measurement error index (MEI). When  $MEI > 2$ , the mean differences are likely to be real differences. Otherwise, the results should be taken with caution, because the differences are in the vicinity of the measurement error. The methodology is explained in full details in Di Cerbo & Biancardi (2012).

Yellow-bellied and fire belly toads are characterized by a ventral colour pattern, dark-black spots on a yellowish to orange-reddish background, which is both individual and specie-specific. Snapshots of the pattern of each specimen have been taken, with a tripod mounted digital camera, in order to detect, by means of computer-aided image analysis techniques, pattern based correlations and differences between populations and species according to the methodology described in Biancardi & Di Cerbo (2010).

## RESULT

In figure 2, the distributions of snout-vent length (SVL), as indicators of body size, of amplexus (AMP), a measure that account for both humeri length and the pectoral girdle width, and of hind-limbs (FOL, TIL), as example of dimorphic characters, are shown. In both species, the overall size of females and males (SVL) did not significantly differ. However, clues of sexual dimorphism, probably related to the mating behaviour, have been detected in the fore and hind-limbs size (AMP, FOL, TIL), (Di Cerbo & Biancardi, 2012). In particular, the amplexus was the most significantly different variable, larger in males than in females (fig. 2). Also legs and feet appeared to be significantly longer in males than in females, however these measures were affected by a remarkable measurement error and therefore should be taken with caution (Di Cerbo & Biancardi, 2012).

The sex independent overall size of *B. variegata* (SVL:  $39.7 \pm 4.2$  mm) and *B. pachypus* (SVL:  $43.1 \pm 4.5$  mm) did significantly differ (ANOVA,  $F_{1, 267} = 40.88$ ,  $P < 0.001$ ,  $MEI = 2.51$ ), with the endemic Apennine species that appear of slightly larger size than the Alpine populations of *B. variegata*.

The colour pattern of the belly has been quantitatively analysed, and all the considered

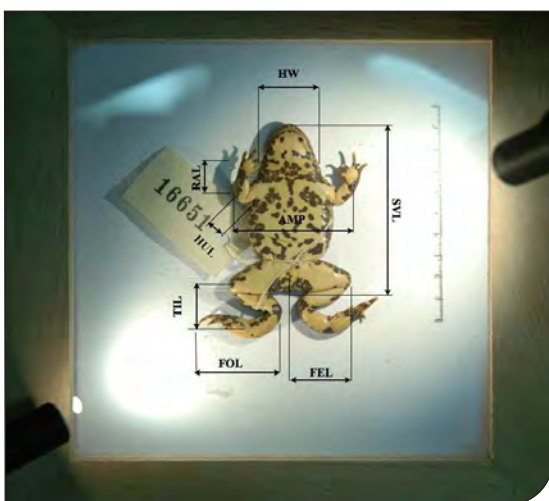


Fig. 1. Measurements on the body of the specimens. SVL: Snout-Vent Length; HL: Head Length; HW: Head Width; FEL: Femur (Thigh) Length; TIL: Tibia (Shank) Length; FOL: Foot Length; AMP: Amplexus width; HUL: Humerus (Arm) length; RAL: Radio (Forearm) length.

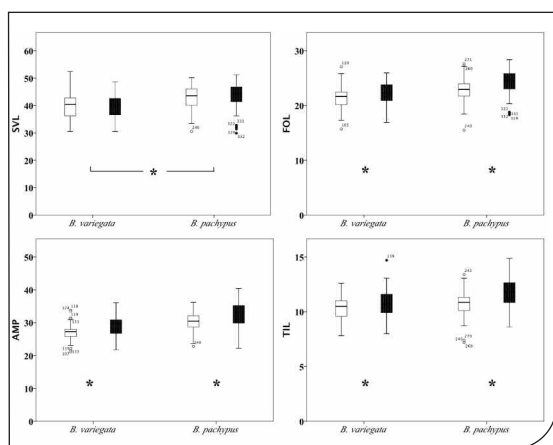


Fig. 2. Box plot of four measures. White box: Females, Black box: Males; Star: statistically significant comparisons. Measures in ordinates are expressed in mm.

parameters (i.e. colour area ratio, number, size and shape of the dark particles on the yellowish background) were statistically significantly different among the four taxa (Di Cerbo & Biancardi, 2010b). In particular, it has been possible to recognize a geographic gradient in the ratio between yellow and dark coloured parts, as well as in the distribution of size and shape of the dark particles. Yellowish background became significantly more extended from the northern *B. bombina* and *B. variegata* to the southern *B. pachypus* and *B. v. scabra* (fig. 3). Applying a canonical discriminant analysis to the pattern variables, the *B. bombina* group resulted significantly split from the others, while a smoother separation interests the populations of the *variegata-pachypus* group ( $P < 0.001$ ).

## DISCUSSION

While taxonomy and phylogeography of the whole *Bombina* genus are still debated (e.g. Hofman et al.,

2007; Zheng et al., 2009), we focused our analyses on morphometric and morphological differences among genders, populations and species.

Sexual dimorphism in *Bombina* is strongly related to the mating behaviour: males could benefit of longer forelimbs during fights, as well as during inguinal amplexus (Halliday & Tejedo, 1995). Further, longer hind limbs could allow males to better perform some mating related actions, like guarding posture (Wells, 1977) and water wave communication (Seidel, 1999; Di Cerbo, 2001).

*B. pachypus* and *B. variegata* showed significant differences in size and ventral colour pattern. Size differences between populations, in animals where the individual size is affected by the age, should be taken with caution. However, the large sample size (Table 1) and the MEI > 2 point to a slight but real size difference. In the Apennine yellow-bellied toad the yellow areas are more extended and the dark particles more regular and circular-like than in *B. variegata*. The ratio between black and yellow areas in the investigated populations shows a decrease gradient north to south, as observed in the eastern part of the distribution area of *Bv* (Stugren & Vancea, 1968). This study underlines the usefulness of museum samples in biogeographic and morphometric researches. The application of new investigative techniques can give new life to these important museum collections.

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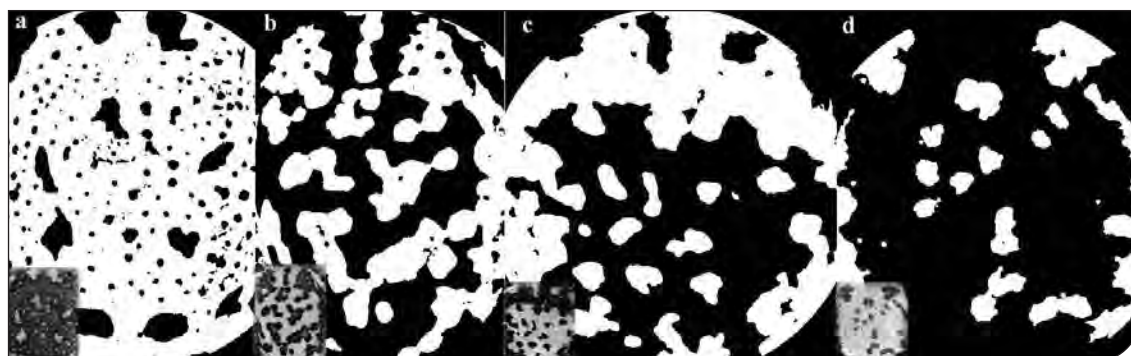


Fig. 3. Example of four analysed bellies. Black: yellowish background; White: dark particles; In the bottom left corner the source picture of each analysis. a) *B. bombina*; b) *B. variegata*; c) *B. pachypus*; d) *B. v. scabra*

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