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The oral apparatus of tadpoles of *Rana dalmatina*, *Bombina variegata*, *Bufo bufo*, and *Bufo viridis* (*Anura*)

Antonella Bonacci*, Elvira Brunelli, Emilio Sperone, Sandro Tripepi

Laboratory of Electron Microscopy "Abele Saita" - Department of Ecology, University of Calabria, Via P. Bucci, I-87036 Rende (Cosenza), Italy

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Abstract

The morphology and development of the larval oral apparatus of *Rana dalmatina*, *Bombina variegata*, *Bufo bufo*, and *Bufo viridis* are described and compared using scanning electron microscopy. The species show different arrangements of the mouthparts. The small oral apparatus of *R. dalmatina* larvae has three labial tooth rows on the upper labium, while there are four tooth rows on the lower labium with a medial gap in row proximal to the mouth. The margins of the oral apparatus are defined by papillae that encircle the lower labium. *B. variegata* tadpoles have two upper labial tooth rows and three lower labial tooth rows that are uninterrupted, unlike the ones of *R. dalmatina*. The mouth is encircled by papillae that are larger than those of *R. dalmatina*. The oral discs of tadpoles of both *B. bufo* and *B. viridis* are similar. They are defined by two upper labial tooth rows (the second of which is interrupted by a medial gap) and by three lower tooth rows that differ in lengths in the two *Bufo* species. Both species develop papillae on the mouth angles and in two rows on the upper labium. Some morphological differences among the oral discs of *R. dalmatina*, *B. variegata*, *B. bufo*, and *B. viridis* tadpoles can be attributed to phylogenetic differences, but most can be related to their varying feeding habits and/or to their dietary specializations.

Keywords: Tadpole; SEM; Oral apparatus; Mouthparts

1. Introduction

Despite their relatively uniform body plan, anuran tadpoles exhibit an amazing morphological diversity, such as the positions of the spiracle opening, the eyes and the mouthparts. This morphological variation reflects the ecological and phylogenetic diversification that Anurans have undergone (Duellman and Trueb 1986). Some remarkable adaptations to the environment are found especially in the arrangement of the mouthparts, and these characters can be used for the identification of species and for inferring ecological adaptations.

Tadpole morphology was first used for the systematics of *Anura* by Lataste (1879) and, since then, larval anatomy has become a useful tool in Anuran classification and phylogeny (Griffiths and De Carvalho 1965; Altig 1970; Ford and Cannatella 1993; Haas 2003). Focusing her attention on the presence or absence of keratinized mouthparts, Orton (1953) described four types of tadpoles; morphological descriptions of mouthparts are now available for various species (Nichols 1937; De Jongh 1968; Nelson and Cuellar 1968; Wassersug 1980).

*Corresponding author.
E-mail address: a.bonacci@unical.it (A. Bonacci).

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The relevant characters of the tadpole oral apparatus are illustrated in Fig. 1; the terminology follows Altig and McDiarmid (1999). The oral apparatus of tadpoles consists of an upper and a lower labium with tooth ridges that bear the embryonic rows of teeth (AR1, AR2… ARn; PR1, PR2… PRn). Rows on the upper labium are numbered from the distal labial margin to the proximal mouth opening (A1, A2… An). Rows on the lower labium are numbered from proximal to distal (P1, P2… Pn). The number and configuration of tooth rows is indicated by the labial tooth row formula (LTRF) that is species-specific. Located in the center of the oral disc are the serrated upper and lower jaw sheaths. They surround the mouth of the tadpole (Nichols 1937). The labia are framed by lateral rows of marginal and submarginal papillae (Altig and McDiarmid 1999; Fig. 1).

The functioning of the mouthparts, however, is still poorly understood, and the morphology of the labial teeth and the functional significance of structural differences among species is still poorly known (Wassersug and Yamashita 2001). Furthermore, most of the available literature regarding the tadpole oral apparatus involves observations by stereomicroscope (Nichols 1937; Wassersug 1976), which cannot provide the structural details needed for comparisons with more recent studies based on scanning electron microscopy (Viertel 1982; Marinelli et al. 1985; Marinelli and Vagnetti 1988; Thibaudeau and Altig 1988; Tubbs et al. 1993).

Our study intends to reduce this gap in knowledge by contributing scanning electron microscopic morphological descriptions of the oral apparatus of the exotrophic tadpoles of the four most common Calabrian species: *Rana dalmatina* Fitzinger in Bonaparte, 1838, *Bombina variegata* (Linnaeus, 1758), *Bufo bufo* (Linnaeus, 1758), and *Bufo viridis* Laurenti, 1768.

2. Materials and methods

Egg clutches of *R. dalmatina*, *B. bufo*, *B. viridis*, and *B. variegata pachypus* (in this study we do not consider *B. v. pachypus* to be a separate species from *B. variegata*, see Razzetti et al. 2001) were collected from semipermanent pools near Cosenza (Southern Italy) between February and July in 1999–2001. The spawns of the four species were brought to the laboratory and kept in separate 20 l aquaria at a temperature of 16–17 °C under a natural light/dark cycle. After hatching, 10 tadpoles were selected from each of the four species studied and were allocated to separate 15 l aquaria, in order to avoid density influence on the mouthparts morphology (Relyea and Auld 2005). Tadpoles were fed with boiled lettuce on alternate days.

The larval and embryonic stages were identified on the basis of the chronological tables of Gosner (1960) and with the help of a stereomicroscope. For the purpose of our study, we have identified and selected two developmental stages: (1) the appearance of the mouthparts; and (2) the completed formation of the oral apparatus.

Specimens for the SEM investigation were anaesthetized by immersion in 2 g/l tricaine–methane–sulphonate (MS 222, Sandoz, Sigma, St. Louis, MO), fixed in 2% phosphate-buffered (pH 7.2) glutaraldehyde for 2 hours, placed in 0.1 M (pH 7.2) phosphate buffer, and post-fixed in 1% osmium tetroxide for 2 hours in the same buffer. The specimens were subsequently dehydrated in increasing concentrations of ethanol, submerged in hexamethyldisilazane, and dried by complete evaporation (Nation 1983). Specimens were gold-coated in an Emitech K550 ion sputter unit and examined under a scanning electron microscope (Zeiss "Stereoscan" DSM 940).

All measurements were taken from SEM images in accordance with the method of Altig and Pace (1974), i.e. the length of each labial tooth from head apex to body basal part, the maximum width of each labial tooth, and the length of each papilla and the length of tooth row (Hall et al. 2002). All measurements were taken at stage 23–25 of Gosner (1960) when the mouthparts are completely developed, because the subsequent development does not affect the length of the tooth rows until their regression (Tubbs et al. 1993), which begins just prior to metamorphosis, which starts

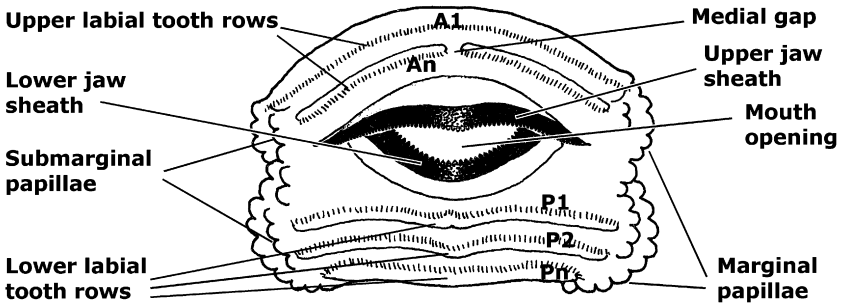

Fig. 1. Diagram of a tadpole oral apparatus (modified from Lanza 1983). A1–An = upper labial tooth rows, P1, P2–Pn = lower labial tooth rows.