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Full Length Research Paper

Application of premetamorphic oral cavity electron micrographs for Egyptian toads' taxonomy

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In the present study, the microanatomy of both the oral disc and buccal cavity of the tadpole of *Bufo regularis* was described. Tadpoles of 32, 38 and 40 stages were dissected and analyzed using scanning electron microscope. In all the stages, the mouth was ventral and the oral disk width was large, that is, equal to about 44% of the greatest width of the body. The disk was provided with a broad gap on the lower lip; the rest of the mouth was bordered by a large number of papillae. The papillae were arranged in a single row on the dorsolateral part of the mouth; the ventrolateral and ventral lip was surrounded by a double row of papillae. The number of papillae increased with larvae growth, from zero in stage 32 to about 150 in stage 40. The tooth row formula is 2(1)/3(2). The upper and lower beaks were pigmented and serrated. While the upper beak was broadly arched and formed a smooth arc, the lower beak had V-shape. Premetamorphic papillae were observed during the early metamorphic stages, and these degenerated rapidly at about late metamorphic stage. Metamorphic atrophy of the oral structures occurred roughly in the reverse order of development, although the procedure was rapid and more haphazard than the development. We suggested that the oral flaps and the roof papillae play a significant role in the capture of food particles by establishing the inflow of "alimentary water", and aggregating food particles and mucus inside the buccopharyngeal cavity, which may reflect ecological and functional constraints that are relative to the morphology of other suspension feeding anuran larvae. Herein, we described the oral features of the tadpoles of *B. regularis*.

Key words: Anuran tadpoles, scanning electron microscope, oral disc, oral papillae, jaws, gap.

INTRODUCTION

The number and arrangement of tooth rows on the oral disc of tadpoles is specific. The labial tooth row formula (LTRF) is a synaptic representation of this arrangement. A number of systems have been devised for numbering labial tooth rows and designating the rows with medial gaps. Accordingly, we used the fractional designation (Altig, 1970) to specify the number and gross morphology of tooth row. This system accommodates all row configuration easily, does not use Roman numerals, has been used for quite a long time, is familiar to many workers and takes less space than formulas that are written in spatial order.

Rows on the anterior labium are numbered from distal (labial margin) to proximal (mouth). The notation "A-1" denotes the first anterior (most distal from mouth) row; also A-n denotes the row adjacent to the mouth. Rows on the posterior labium are numbered proximal to distal. The first row to the mouth is p-1, and more rows are numbered sequentially through P-n. Rows with medial gaps are designated with parentheses, and rows that vary between individuals (gap present or absent) are placed within brackets. A gap in a tooth row is a physical break in the tooth ridge and therefore expressed in the tooth row. The functional and developmental considerations of gaps in tooth rows have not been examined. These gaps allow larger excursions of the jaw sheaths during feeding.

In summary, a labial tooth row formula (LTRF) of 5 (2-5) / 3[1] indicates a tadpole with 5 upper tooth rows with medial gaps in rows A-2 through A-5 and 3 lower rows with or without a gap in P-1. Some tadpoles, particularly of Ranids species and Pelotids species have accessory rows situated in the lateral areas of the oral disc. So the formulation of Altig (1970) was modified by Webb and Korky (1977) by placing the number of accessory rows between solid, 5 (2-5)4/3[1].

Variations in the size, density and shape of the oral disc, the papillae at the margins of the oral disc, the shape of the jaws, the numbers of denticle rows and any gaps in those rows are all important features in identifying tadpoles of different species. Even among closely related taxa and in many cases, they seem to reflect lineage and habitats (Duelman and Trueb, 1983, 1986; Grandison, 1981; Channing, 2001). McDiarmid and Ronald (1999), Nascimento et al. (2005), and Rossa-Feres and Nomura (2006) showed that in Bufonidae, both oral disc and keratinized mouth parts were present and oral disc emerged; teeth formula was 2/2 or 2/3. In Ranidae, the formula was smaller (3/3) or larger (5/3, 2/4, 3/4 or 6-7/6); in Hylidae, Pipidae *"Xenopus laevis"* and Rhinophrynidae *"Rhinophrynus dorsalis*", oral disc did not emerge and the formula was 2/2, 2/3 or 2/4. Tadpoles of *Hylorina sylvatica* have unique characteristics (Echeverría et al., 2001; de Sá and Langone, 2002; Formas and Brieva, 2004; Alcalde and Blotto, 2006; Altig, 2007; Vieira et al., 2007).

The Central American tree frog, *Hyla microcephala*, which feeds on large food particles in ponds, has small jaws set back in an oral tube and has no labial teeth. Tadpoles of a certain tropical stream-dwelling *Hyla*, in contrast, have the highest number of rows: 17 upper and 21 lower rows. The tadpole of *Rhamphophryne proboscidea* is characterized by a small size (17.8 mm), with tooth row formulae of 2(2)/3, oral papillae only on lateral margins and jaw sheath of V-shape (Menin, 2006; Gomes et al., 2010; Zimkus et al., 2010). Albertina (2007) shows that seven papillae of oral disc of *Colostethus marchesianus* tadpole occur on the right side of the disc and six on the left, probably resulting from the fusion of two papillae. A few labial teeth are missing from A-1 in this specimen. Tadpoles of *Eupsophus emiliopugini* have four lingual and four infralabial papillae and third lower labial ridge is absent. Savage (2002) showed that in *Bufo coniferus*, the mouth is moderate and anteroventral and the oral disc emerges with the papillae of the upper labium confined to the corners of the mouth; on the right side, there are 10 to 13 small papillae in the outer row and three to six inner, and on the left side there are 10 to 11 outer and 2 to 4 inner. The lower labium is also free of papillae except at the corners; on each side, there are 9 to 15 papillae. The incidence of oral deformities could be high in natural populations. For instances *Batrachochytrium dendrobatidis* infection exerts a strong influence on the occurrence and type of oral deformities in tadpoles (Dana et al., 2007; Frost, 2009; Matthew et al., 2010; Marion et. al., 2010).

Matthew et al. (2010) suggested that tadpoles with missing teeth compensate for inferior feeding kinematics during mouth closing in each gape cycle by increasing the number of gape cycles per unit time. The ways in which these structures actually function have received little study. However, it is clear that large oral discs with many denticle rows are common among stream-dwelling tadpoles exposed to water currents. The larvae use these structures to hold on to surfaces and resist being swept downstream (Van and McCollum, 2000; Tolledo et al., 2009). Jaw sheaths that have sharp edges are characteristic to many tadpoles that feed on active prey. High-speed video of feeding North American bullfrog (*Rana catesbeiana*) larvae, which have the common pattern of two upper and three lower tooth rows, showed that tadpoles use their labial teeth to anchor the oral disc to surfaces while their jaws bite at the substrate.

Feeding behaviour changes drastically during metamorphosis as larval suction feeders become adult lingual feeders (Sanderson and Sarah, 1999; Relyea, 2001). In order to understand this transition, the general morphological development of the floor of the buccal cavity in Egyptian anurans species *Bufo regularis* larvae was studied up to the completion of metamorphosis by scanning electron microscope.

MATERIALS AND METHODS

Ribbons of fertilized eggs from couples of the available *B. regularis* were collected from breeding sites. After hatching, the larvae were daily fed on a meal of boiled spinach daily, until they reached the desired stages needed for experimental work, according to the normal table of Sedra and Michael (1961). Three larval stages were used for the present study namely, stages number 32, 38 and 40. Specimens were dissected and subsequently fixed in a 2 to 3% glutaraldehyde solution for 3 to 4 h at room temperature, then washed in 0.1 M phosphate buffer for 15 min each. Next, specimens were dehydrated in a graded ethanol series as follows: 35, 50, 70, 80, 95%; three changes at 100% for 15 min each, and a final wash in acetone for 5 min. Specimens were dried in CO_2, mounted on aluminium stubs and sputter coated with gold. Features of dorsal and ventral internal oral anatomy were examined and photographed using a scanning electron microscope (Jeol) attached to a computer. Terms used to describe features of the oral cavity are derived from Wassersug (1997, 1980) and Wassersug and Heyer (1988).

RESULTS

In the early stages, particularly stage 32, it is shown that the oral disc structures were not developed yet in the mouth (Figure 1) while during development of the tadpoles in stage 36, it is observed that the oral structures started to appear gradually. In this, the upper jaw sheath was formed first followed by the 87 appearance of the ventrolateral margins of the lower labium, which were considered the first soft tissues of the oral disc that materialized from the surrounding body surfaces. Nascent marginal papillae subsequently appeared in these areas before they did on the other margins of the disc. After that the lateral emergency

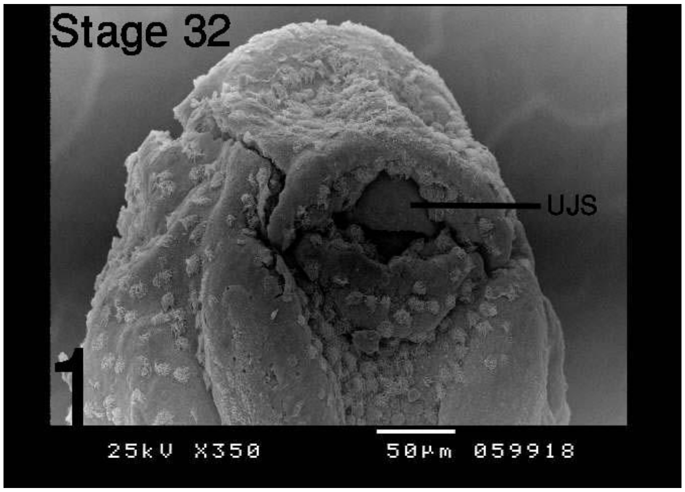

Figure 1. Scanning electron micrograph of oral apparatus of *B. regularis* tadpole (stage 32). UJS- upper jaw sheath.

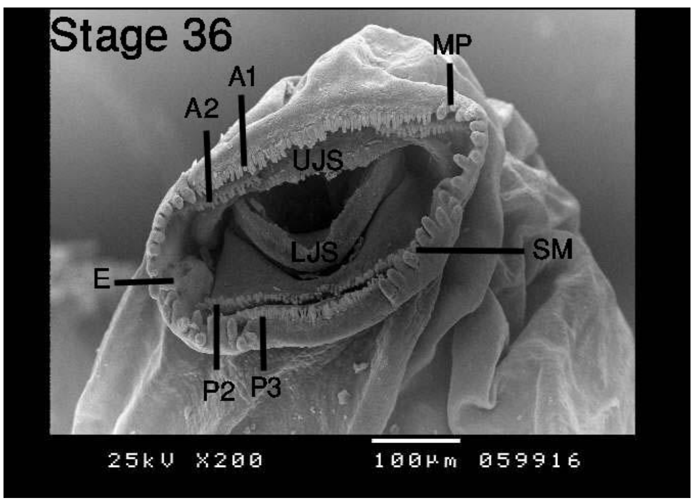

Figure 2. Scanning electron micrograph of oral apparatus of *B. regularis* tadpole (stage 36). A-1 and A-2, First and second anterior tooth rows; E, lateral emargination of oral disc; LJS, lower jaw sheaths; MP, marginal papillae; P-2-3, second and third posterior tooth rows; SM, submarginal papillae; UJS, upper jaw sheath.

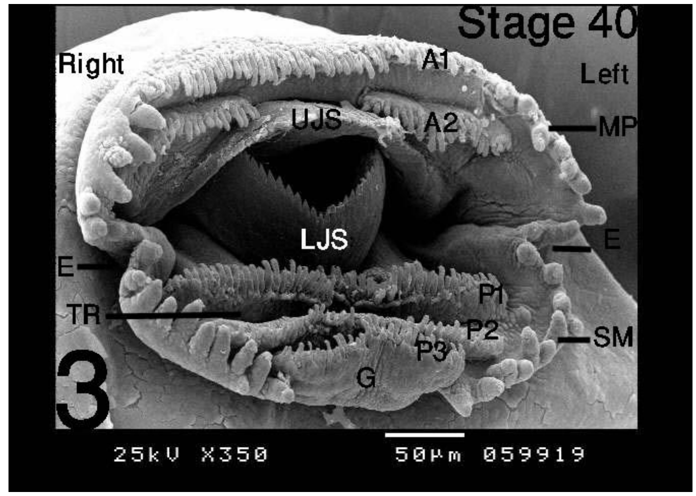

Figure 3. Scanning electron micrograph of oral apparatus of *B. regularis* tadpole (stage 40) with 2(1)/3(2) tooth rows formula. A-1 and A-2, first and second anterior tooth rows; E, lateral emargination of oral disc; LJS, lower jaw sheaths; MP, marginal papillae; P-1-3, first through third posterior tooth rows; SM, submarginal papillae; TR, tooth ridge for tooth row P-2; UJS, upper jaw sheath.

appeared with small notch on one side only. In addition, there were only two rows of oral papillae which appeared in the posterior side, named (p2 and p3) (Figure 2). The fully developed oral disc of the tadpole of *B. regularis* (Figure 3; stage 40) consisted of various structures (although some tadpoles lack all of these), two keratinized jaw sheaths for grasping and shearing food, one above and the other below the mouth; tooth row ridges that bear the keratinized teeth (these existed as rows above, or anterior to the jaw sheaths named the A rows, and below or posterior to the jaw sheaths named the P rows), and one or more rows of papillae along the borders of the oral disc (these can completely surround the oral disc or be interrupted at the anterior or both anterior and posterior sides).

Oral disc located ventrally, emerged on both sides (Figure 3); border of disc was surrounded with 38 marginal papillae: 16 located antero-laterally (eight on right side and eight on left side); and 22 post-laterally papillae (11 on right side and 11 on left side). Also, there was a single dorsal gap in papillae while the submarginal papillae were absent, and all these papillae were the last structures to be atrophied during metamorphosis. Furthermore, it was noted that the ventral (posterior) gap was clearly discernable and there was no dorsal (anterior) gap formed. Concerning the jaw sheaths in the present result, the upper jaw sheath barely had a concave medial shape while the lower jaw sheath took V-shape; both upper and lower jaw sheaths had serrated edges; serrations extended on the entire lengths of sheaths. Labial tooth row formula LTRF of the tadpoles of *B. regularis* was: 2(2)/3(2). The corners of the mouth of a tadpole did not extend backwards to form a toad mouth until tissue atrophies.

DISCUSSION

Our observations indicate that the oral morphology of *B. regularis* could serve as a specialized feeding mode. The functional roles that have been proposed for oral papillae fall into the following basic categories: chemosensory, tactile receptors and structures that control water flow (Van Dijk, 1981), enhance attachment to substrates (Altig and Brodie, 1972), modify the shape of the oral disc during feeding and manipulate food and substrate particles.

The number and prominence of marginal papillae varies concordantly with observed reductions in the size of the oral disc among tadpoles within the same lineage. For example Ranid tadpoles have larger papillae arranged more sparsely than Hylids; and stream Hylids have smaller, more densely arranged papillae than pond Hylids. For these and other reasons, oral structures have been used infrequently in systematic analyses (Grandison, 1981, Duelman and Trueb, 1983, 1986; Rdel, 2000; Anstis, 2002). As Eterovick et al. (2002) suggested that, the tadpoles of the species in the *Hypsiboas polytaenius* clade may be distinguished by their tooth row formula. *H. polytaenius* has LTRF 2(2)/3(1,2) (Heyer et al., 1990); *Hypsiboas cipoensis,* 2(2)/3(1) and *H. goianus,* 2(1,2)/3(1) (Eterovick et al., 2002).

Although the tadpole of *B. regularis* presented the same LTRF as *H. cipoensis*, 2(2)/3(1), the position of the marginal papillae was different. *H. cipoensis* has a single row of marginal papillae on the upper and lower lips, presenting a rostral gap, and two rows laterally, while *H. leptolineatus* has a row of marginal papillae on the upper lip, also with a rostral gap, and two rows of papillae laterally and on the lower lip. Our results on the internal oral anatomy of the tadpole of *B. Regularis* tadpole are in agreement with previous description (Wassersug and Heyer, 1988), but we are adding two new characters that were observed in the present study, viewed by means of a scanning electron microscope. The first character is that, from the roof of the oral cavity there was a posterior gap instead of anterior one as in the other species and the second one is: the oral discs of toad tadpoles showed a caudad double row of papillae.

Tadpoles of these species possibly do not aggregate when predators are present compared to *Boraras maculatus* that does (Channing, 2001). Tadpoles of *B. regularis* differ from other species by having (i) numerous papillae surrounding the oral disc compared to 18 papillae in back-riding tadpoles in stages 25 to 62 papillae; 12 to 16 papillae in *Colostethus marchesianus* and 13 to 16 papillae in *C. caeruleodactylus*, in stages 25 to 42 (Caldwell et al., 2002; Castillo, 2004 Trenn, 2004); (ii) small and numerous papillae arranged in a double row surrounding the oral disc, except for a medial gap in the posterior disc. These characters may be useful in elucidating the phylogeny of *B. regularis* within its genus and even to provide evidence of relationships at the species level. These variables likely reflect an nterplay of evolutionary history and functional demands (for example, stream vs. pond dwellers). Species with rapid developmental times (for example, *Bufo*) seemingly start and complete develop-mental sequences slightly earlier than species with longer developmental times. Species with labial tooth row formula greater than 2/3 start development earlier (Marinelli and Vagnetti, 1988), complete it later and retain mouth parts longer into metamorphosis than species with tooth formula equal to or lesser than 2/3 tooth rows.

Lentic species tend to form the oral structures later and faster and atrophy the oral apparatus earlier and faster than lotic forms. For these reasons, oral development is often discordant with features of limb development used in staging (Tubas et al., 1993; Liu et al., 1997).

The mechanisms that account for the formation of marginal and submarginal papillae are unknown, although apoptosis is surely an important mechanism. As development progresses toward metamorphosis, these structures gradually regress until they disappear.

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