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Comparison study of oral disc morphology of Saudi *Bufo dhufarensis* and *Rana ridibunda* tadpoles and their oral deformities

Gamal A. Bekhet[1,2]

[1]Department of Zoology, Faculty of Science, University of Alexandria, Alexandria 21511, Egypt.
[2]Department of Biology, Faculty of Science, King Faisal University, P.O. Box 1759, Al Hufuf 31982, Al Hassa, Saudi Arabia.

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The microanatomy of the oral discs of the tadpoles of *Bufo dhafarensis and Rana ridibunda* were described. Tadpoles of stage 49 and 41, respectively were examined and analyzed using scanning electron microscope. In *Rana* tadpoles, the mouth was ventral and the oral disk opening had the same vertical and horizontal opening size. While in *Bufo* tadpoles, the mouth was anterio-ventral and the oral disc had its horizontal opening larger than the vertical one. The border of oral disc of tadpoles of *B. dhufarensis* was surrounded with 22 marginal papillae and 8 submarginal papillae and a dorsal gap was observed. The oral disc of *Bufo* had less number of marginal papillae where they were short and had broad smooth ends with no apical parts. On the other hand, the border of the *Rana*'s oral disc was surrounded by 50 marginal papillae and 10 submarginal where the dorsal gap was also observed. Both marginal and the submarginal papillae were long, numerous, closely spaced and ending with 3-7 apical parts in each papilla. The labial tooth row formula (LTRF) of the tadpoles of *B. dhufrenensis* was 2/3, while that of the tadpoles of *R. ridibunda pallas* was 2(1)/3(1-2). In *B. dhufarensis*, the labial teeth were keratinized, short ending with 4-7 terminal cusps and arranged as a single row. On the contrary, the labial teeth of *R. ridibunda pallas* were elongated, closely spaced and numerous. These labial teeth were noticed to be either vertically positioned or curved downwards carrying 3-4 terminal cusps in their free ends. Herein, we described Saudi Arabian Amphibian *B. dhufrenensis* and *R. ridibunda pallas* by utilizing scanning electron micrographs. In the present study, we described deformities in the oral disc of *B. dhufrenensis* and *R. ridibunda pallas* tadpoles in their natural conditions.

Key words: Oral disc, tadpoles, *Rana ridibunda*, *Bufo dhufarensis,* labial tooth row formula, deformities.

INTRODUCTION

The marsh frog, *Rana ridibunda* is the only true frog reported from the oasis of Al-Hassa and Al-Qatif regions in Saudi Arabia. This frog has been subjected to several studies to determine the seasonal changes in population

E-mail: g.bekhet@ kfu.edu.sa. Tel: 00966 3 5800000. Extn: 1858 or 00966 0569323209. Fax: 00966 3 5886437 or 00966 3 5886439.

structure, the breeding behavior, the tadpole development and the individual growth in Saudi climate (Briggs, 1980, 1981; Haas, 2003; Al-Shehri and Al-Saleh, 2005a, b, 2008). Meanwhile, recent research on the amphibian chromosomes of the Arabian Peninsula and *Bufo dhufrenesis* has just started, and few papers have been published (Haas, 2003; Al-Shehri and Al-Saleh 2005a, b, 2008). It is very important to study and protect such creatures because they are under grave threat not only due to general habitat alteration but also to climate change (Fellers et al., 2001). However, more interest has been concentrated on studies of comparative aspects of buccal anatomy related to feeding of Saudi amphibian tadpoles mainly on taxonomy and distribution.

Variations in the size and shape of the oral disc, the papillae at the margins of the oral disc, the shape of the jaws, the numbers of teeth rows and any gaps in those rows are all important features in identifying tadpoles of different species (Duelman and Trueb, 1986). Even among closely related taxa and in many cases, they seem to reflect lineage and habitats (Grandison, 1981; Duelman and Trueb, 1986; Channing, 2001).

Dental formula of a tadpole depicts number and arrangement of tooth rows on its oral disc. The number which is written on the left of "/ "refer to the anterior labium, while that which is written on the right is for posterior labium. Open numbers indicate total number of tooth rows on each labium, number in parenthesis are the number of interrupted rows in order of arrangement on labium. A dental formula of 2(2)/3(1-2) indicates 2 rows on anterior labium where the second one is interrupted with a median gap,while that of the posterior labium has 3 rows where the first and second ones only are interrupted (Channing, 2001).

In *Bufonidae*, both protruded oral disc and keratinized mouth parts were present; the teeth formula was 2/2 or 2/3. In Ranidae, the formula was smaller, 3/3 or more (5/3, 2/4, 3/4 or 6-7/6). In some Hylidae, Pipidae *"Xenopus laevis"* and Rhinophrynidae*"Rhinophrynus dorsalis*" the oral disc were not protruded and the formula was 2/2, 2/3 or 2/4 (Nascimento et al., 2005; Alcalde and Blotto, 2006; Rossa-Feres and Nomura, 2006; Altig, 2007; Vieira et al., 2007; Bekhet, 2012). These keratinized teeth are derived from cells present in the base of the tooth ridge (Gosner, 1959). The function of teeth in frogs is primarily to grasp prey or to position it for swallowing. The oral apparatus of tadpoles of different anuran tadpoles is different and that this difference reflects different feeding habits. The ontogeny of the labial teeth row structure of anuran tadpoles inhabiting temperate regions has been studied by several workers (Bonacci et al., 2008; Tolledo et al., 2009; Erik et al., 2010; Lunaet al., 2012).

Oral deformities may be used as biomonitoring tool, both for detecting contamination and for determining the efficacy of occurrence of deformities in unimpacted and in contaminated wetlands (Cooke, 1981).

Oral deformities have not generated widespread media attention because they are not linked to the declines in amphibian populations, although the pathogens which are believed to cause mortality can also cause deformities in larval mouth parts (Morell, 1999). The developing larvae may remain longer in ponds with longer hydroperiods and thus have a greater incidence of developing oral deformities (Snodgrass et al., 2000). The variability of oral deformities as pigmentation in keratinized cells in tooth rows and jaw sheaths may also be related to either seasonal changes in temperature (Rachowicz, 2002) or infection by *Batrachochytrium dendrobatidis* (Vieira et al., 2013). Deformities in the oral discs have been reported for animals either exposed to the organic pesticide DDT (Osborn et al., 1981) or to coal combustion residues (Rowe et al., 1996, 1998a, b; Peterson et al., 2008). However, Dunson and Travis (1994) stated that the oral deformities have an effect on the feeding ability and growth.

In the current work, the circum oral disc of the Saudi tadpoles was described in details. Here, we complemented these data by taxonomizing the tadpoles of *B. dhufarensis* and *R. ridibunda pallas.* In addition, we investigated oral deformities in the marginal papillae and tooth rows of *B. dhufrenensis* and *R. ridibunda* tadpoles.

MATERIALS AND METHODS

Manipulation

Fertilized eggs from some of the available Saudi Dhufar toad *B. dhufarensis* (Al-Derayya village, Riyadh) and true frog *R. ridibunda* (Al Hassa oasis) were collected from natural ponds with fine mesh hand net .In the laboratory, eggs hatched and tadpoles were reared. After hatching, the larvae were fed on a 20 g meal of boiled spinach daily. The experimental stages were 49 for *Bufo* and 41 for *Rana* were selected according to the normal table of Sedra and Michael (1961), for circumoral studies, since at these stages the tadpoles have typical morphology and the oral disc is fully developed and functional. Both stages are similar in characters and developmental rate of growth.

Experimental design

A minimum of 15 tadpoles for each species were euthanized using 1:10000 MS-222 in distilled water, they were examined using a dissecting microscope to detect the selected stages, then incubated on the surface of moist tissue, in a Petri dish at 25°C.

Scanning electron microscopy

These specimens were fixed in a 2-3% glutaraldehyde solution for 3-4 h at room temperature, followed by three 15 min washes in 0.1 M 50, 70, 80, 95%, three changes at 100%, for 15 min each and a final 5 min wash in acetone 100%. Specimens were critical point dried in CO_2, mounted on aluminum stubs and sputter coated with gold. Structure of oral discs was examined and photographed using a scanning electron microscope attached to acomputer. Terminology used to describe features of the oral cavity follows Wassersug (1997).

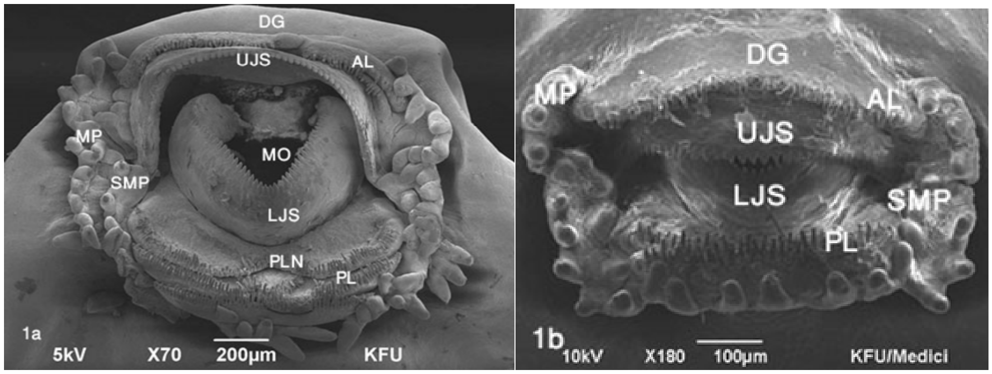

Figure 1. Scanning electron micrographs of the oral disc of: a) *Rana ridibunda* and (b) *Bufo dhufarensis* showing the anterior labium (AL), the dorsal gap (DG), the lower jaw sheath (LJS), the mouth opening (MO), the marginal papillae (MP),the posterior labium (PL), posterior labial notch (PLN), submarginal papillae (SMP) and the upper jaw sheath (UJS).

Table 1. The morphometric measurements (mean ± S.E. (range) of the oral disc components in tadpoles of *B. dhufarensis* and *R. ridibunda* species.

Morphometric measurements	*Rana ridibunda*	*Bufo dhufarensis*
ODW	1.13 ± 0.089 (1.04-1.19)	2.18 ± 0.165 (2.1-2.45)
DGMP	1.05 ± 0.1 (0.98-1.4)	0.50 ± 0.60(0.48-0.52)
LMP	0.23 ± 0.21 (0.12-0.3)	0.11 ± 0.02 (0.09-0.1)
ATRL1	1.55 ± 0.95 (1.3-1.45)	1.01 ± 0.9 (0.9-1.1)
ATRL2	1.65 ± 0.11 (1.0-2.0)	1.25 ± 0.15 (1.16-1.2)
PTRL1	1.45 ± 0.09 (1.42-1.5)	0.95 ± 0.09 (0.84-0.9)
PTRL2	1.30 ± 0.05 (1.3-1.4)	0.80 ± 0.75 (0.7-0.85)
PTRL3	0.85 ± 0.8 (0.65-1.5)	0.45 ± 0.65 (0.35-0.41)

Oral disc width: ODW; dorsal gap of the marginal papillae: DGMP; length of marginal papillae: LMP; anterior tooth row length 1-2: ATRL1-2; posterior tooth row length 1-3: PTRL1-3. Mean ± S.E.(range).

Quantitative data

The measurements taken using a stereomicroscope with measuring device and converted later into mm are: ODW = maximum oral disc width; DGMP = dorsal gap of the marginal papillae. The mouth parts include: MP = number of marginal and SMP = submarginal papillae; LMP = length of the marginal papillae; ATR = number of anterior tooth row; PTR = number of posterior tooth row; ATRL = anterior tooth row length; PTRL = posterior tooth row length.

RESULTS

Ultrastructure, morphometric and numeric measurments of oral disc

In tadpoles of *Rana*, the oral disc structures were present and located ventrally (Figure 1a). The vertical and horizontal length of the opened oral cavity was almost the same (1.13 ± 0.089 mm). The border of oral disc was surrounded by 50 marginal papillae and 10 submarginal papillae (Table 1). There was a dorsal gap (1.05 ± 0.1) that lacked the marginal papillae, their number was 50 and they were 0.23 ± 0.21 mm in width. Sixteen papilla out of the marginal ones were located antero-laterally (eight papillae on each side). And the remaining 34 papillae were located post-laterally (17 papillae on each side). Meanwhile, 5 submarginal papillae were found on each side of the oral disc. It was also found that the marginal and submarginal papillae were the last structures undergoing atrophy during metamorphosis. Concerning the jaw sheaths, it consisted of a curved edge upper jaw sheath and a V-shaped lower one. The edges of both jaw sheaths were serrated along the length of the jaw sheaths. It was obvious that the upper jaw sheath embraced the lateral ends of the lower jaw sheath.

Furthermore, the labial tooth row formula (LTRF) of the tadpoles of *Rana* was 2(1)/3(1-3), indicating that these tadpoles had two anterior tooth row ridges that bear the keratinized teeth named the “A” rows with a median gap on the row A-1, and three posterior tooth row ridges named

Table 2. The numeric measurements of marginal, submarginal papillae and number of teeth per labial tooth row of specimens examined.

Components of oral disc	Species	
	R. ridibunda	*B. dhufarensis*
MP	50	22
SMP	10	8
ATR1	30	25
ATR2	41	23
PTR1	52	34
PTR2	39	29
PTR3	22	16
LTRF	2(1)/3(1-2)	2(0)/3(0)

Marginal papillae: MP; anterior tooth row 1-2: ATR1-2; posterior tooth row 1-3: PTR1-3; SMP: submarginal papillae.

the “P” rows with median gaps observed in the P1 to P3 (Figures 1a and 4a).The length of ATRL1 was 1.55 ± 0.95 mm and that of the ATR2 was 1.65 ± 0.11 mm. The width of the PTR1, 2 and 3 were 1.45 ± 0.09, 1.30 ± 0.05 and 0.85 ± 0.8, respectively (Table 1). Furthermore, the number of teeth was 30 and 41 in ATR1 and 2, respectively. In addition, the number of teeth was 52, 39 and 22 in PTR1, 2 and 3, respectively (Table 2).

On the other hand, in tadpoles of *Bufo,* the oral disc and their keratinized mouth parts were present and extented anteriolly (Figure 1b). The horizontal length (width) of the oral opening was double in length of that of the vertical one, which was 2.18 ± 0.165 mm (Table 1). The border of the oral disc was surrounded by 22 marginal papillae and 8 submarginal papillae, where their length was 0.11 ± 0.02 mm. A dorsal gap was found with 0.50 ± 0.60 mm width. Eight out of twenty two marginal papillae were located antero-laterally (four on each side), and the remaining 14 papillae were located post-laterally (8 on each side). Meanwhile, the 8 submarginal papillae were equally divided on each side.The upper jaw sheath was straight with slight upward curvature, while the lower one was horizontally straight. Both upper and lower sheaths were serrated. The LTRF of the tadpoles of *Bufo* was 2/3 indicating that the tadpoles possessed two anterior and three posterior tooth rows. Both tooth rows lacked the median gap (Figures 1b and 5a). The width of the ATR1 and 2 were 1.01 ± 0.9 and 1.25 ± 0.15 mm, respectively. And the PTR1, 2 and 3 were 0.95 ± 0.09, 0.80 ± 0.75 and 0.45 ± 0.65 mm, respectively (Table 1). Furthermore, ATR had 25 teeth in the first row and 23 in the second one. While PTR had 34 teeth in the first row, 29 in the second one and 16 in the third row (Table 2).

Ultrastructure of marginal and submarginal papillae

Both the marginal and the submarginal papillae of *Rana* were found to be elongated, numerous and closely spaced, in which they ended with 3-7 apical parts (Figure 2a and b). There is individual variation in the size and spacing among these papillae, these marginal papillae border the oral disc, except for a dorsal gap. Two lateral folds are clearly visible so the oral disc laterally emarginated. In *Bufo*, the marginal and submarginal papillae were less in number and shorter than in *Rana*. They were also found to be widely-spaced from each other and their ends were smooth, broad and lack apical parts. the rosteral (dorsal) gap is small and also the oral disc is emarginated (Figure 3a and b).

Labial teeth

In *Rana ridibunda,* the labial teeth were elongated, closely spaced, numerous in number and arranged in one row for each labium. The teeth were noticed to be either vertically positioned or curved downwards ending with three terminal cusps (Figure 4a and b). On the other hand, the tooth rows in *Bufo* were uniserial carrying keratinized labial teeth. Each tooth consisted of three distinct regions; a distal head with 5-7 terminal cusps, an intermediate region known as the neck and the rest of the tooth body known as the base (Figure 5a and b).

Malformed oral structures

It was observed in some cases, that the *B. dhufarensis* had malformed oral structures represented in the marginal and submarginal papillae on both side of the oral disc. In some cases, malformation was found in the presence of teeth on the marginal papillae forming toothed marginal papillae (TMP) and toothed submarginal papillae (TSM) (Figure 6a, b, 7a and b),these teeth had the same structure as that of the labial teeth. Moreover, in some cases, it was shown that two or three marginal papillae were fused into one single marginal papilla

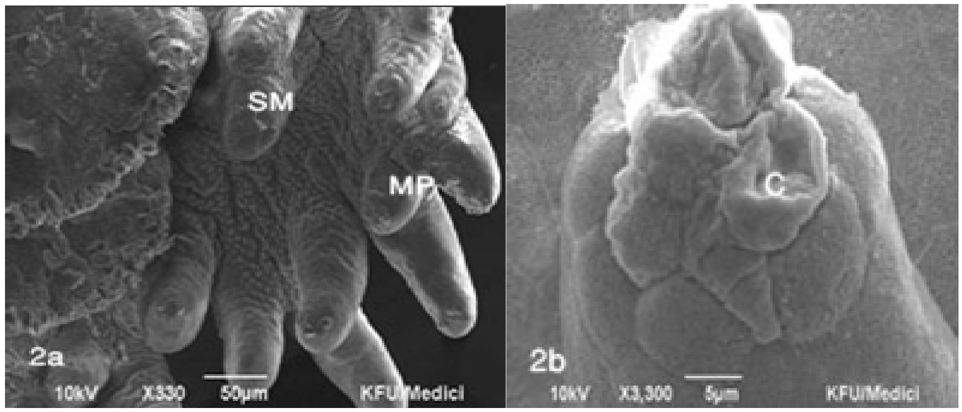

Figure 2. Scanning electron micrographs in *Rana ridibunda* showing a) marginal (MP) and submarginal papillae (SM), b) enlarged end of the marginal papillae with 5 apical parts (C).

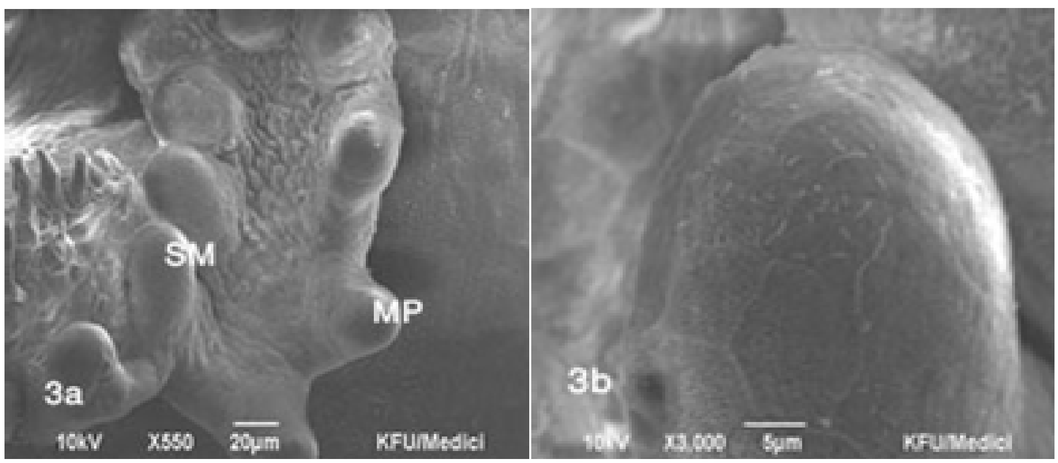

Figure 3. Scanning electron micrographs of *Bufo dhufarensis* showing a) the marginal papillae (MP) and submarginal papillae (SM), b) enlarged marginal papilla of *Bufo* (broad, smooth and lacking of apical parts).

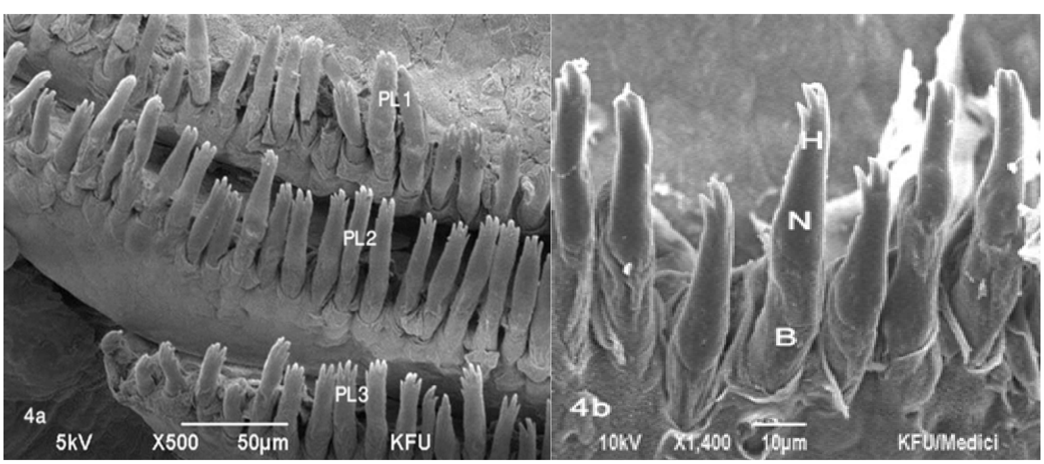

Figure 4. Scanning electron micrographs showing labial teeth in *Rana ridibunda* pallas: a) Vertical positioned posterior labial teeth rows (PL1-3) and their cusps, b) enlarged labial teeth, where the head (H), neck (N) and base (B) were obvious, ending with 3 cusps (C).

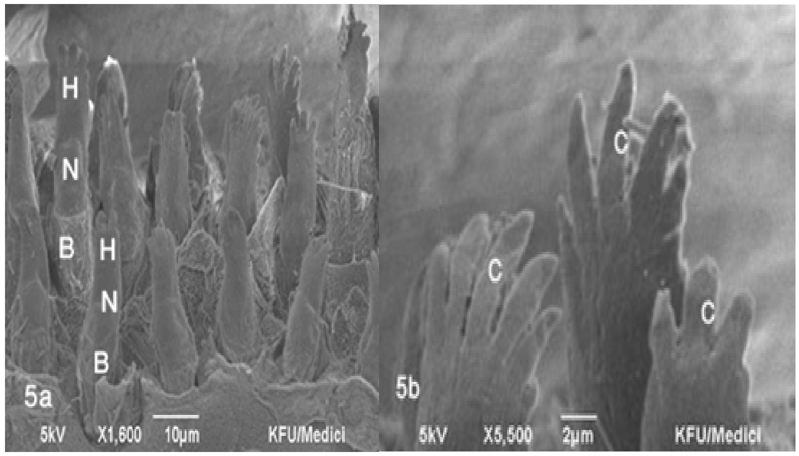

Figure 5. Scanning electron micrographs in *Bufo dhufarensis* showing: a) three rows of labial teeth, where the head (H), neck (N) and base (B) were obvious, b) enlarged labial teeth with 5-7 cusps (C).

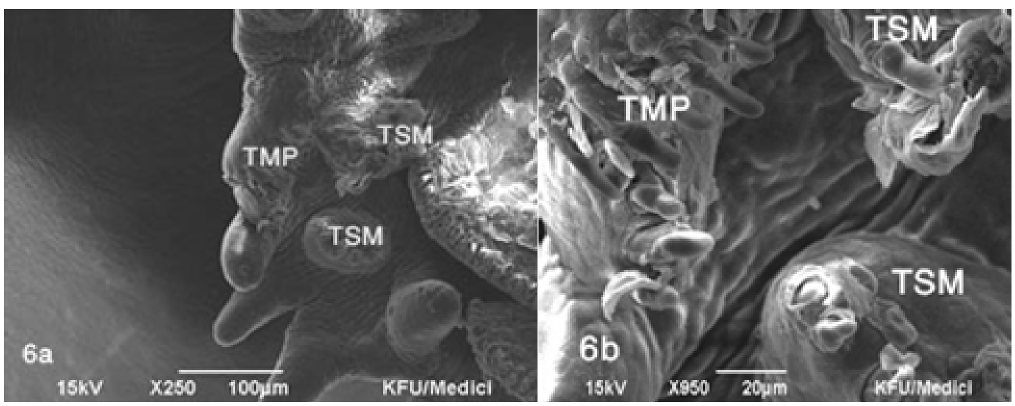

Figure 6. Scanning electron micrographs of *Bufo dhufarensis* showing: a) right side of the mouth with malformed teethed marginal and submarginal papillae, b) enlarged part of teethed submarginal papillae (TSM) note the tooth carrying 3 cuspes.

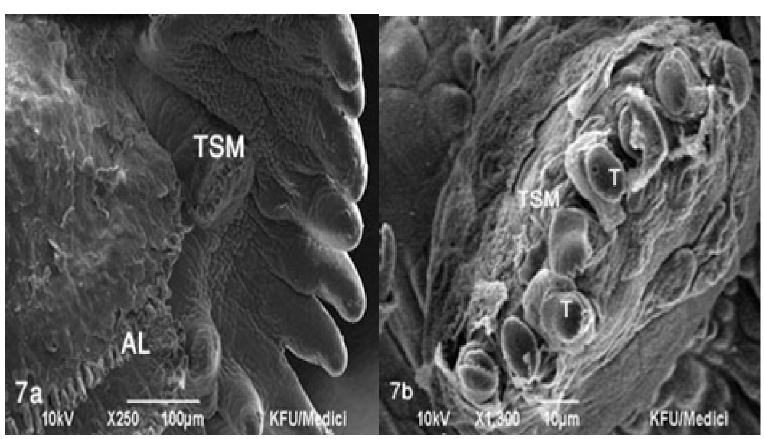

Figure 7. Scanning electron micrographs of *Bufo dhufarensis* showing: a) left side of the mouth with malformed teethed submarginal papillae (TSM); b) magnified part of (TSM).The teeth (T) carrying 5-9 cusps.

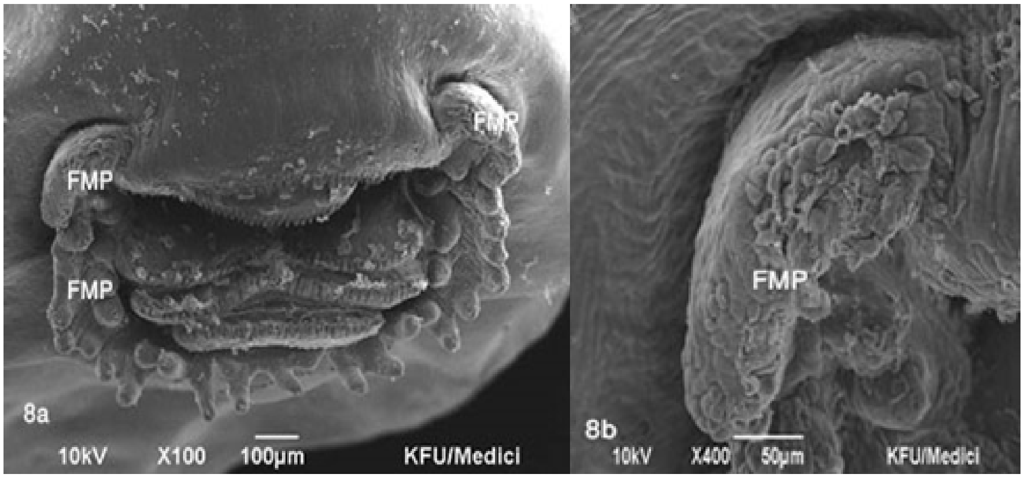

Figure 8. Scanning electron micrographs of *Bufo dhufarensis* showing: a) malformed fused marginal papillae (FMP) on both sides of the mouth; b) magnified part of FMP.

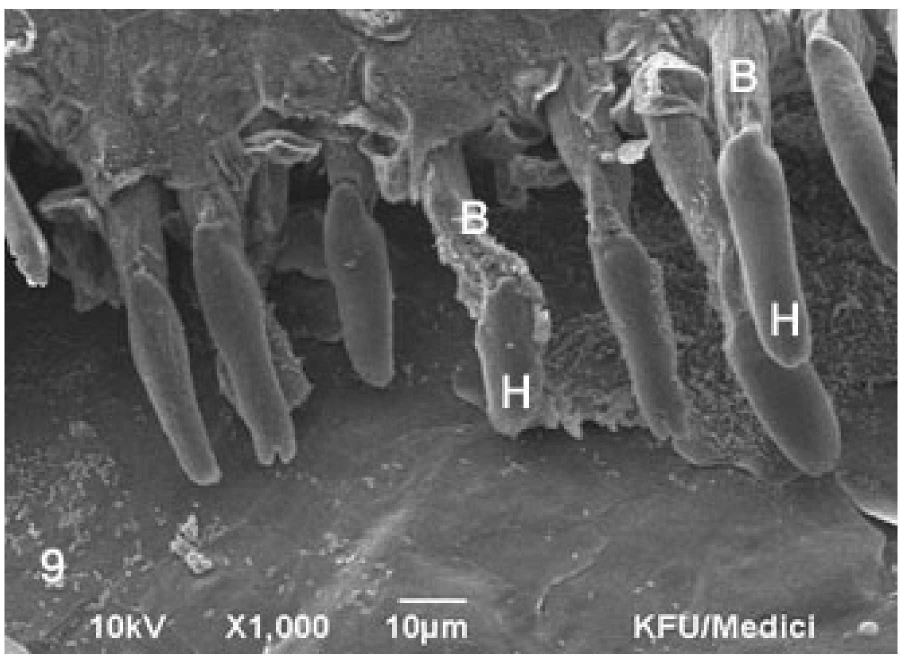

Figure 9. Scanning electron micrographs of *Bufo dhufarensis* showing: a) malformed anterior labial teeth with elongated basal part (B). Note the head (H) ends with 0-2 cusps.

(FMP) at both sides of the oral disc (Figure 8a and b). In other cases, it was noticed that labial teeth appeared with malformation forms such as the appearance of basal part of the teeth and lacked cuspes (Figure 9a) or underdeveloped labial teeth without head and neck or shorted one (Figure 10 a and b).

On the other hand, in some cases, in *Rana* it was shown that the postrior labial teeth changed their position from straight to downwardly curved teeth (Figure 11).

DISCUSSION

The oral disc of the examined tadpoles of *R. ridibunda* and *B. dhufarensis* had the same general organization of keratinized jaw sheaths and with keratinized tooth rows,

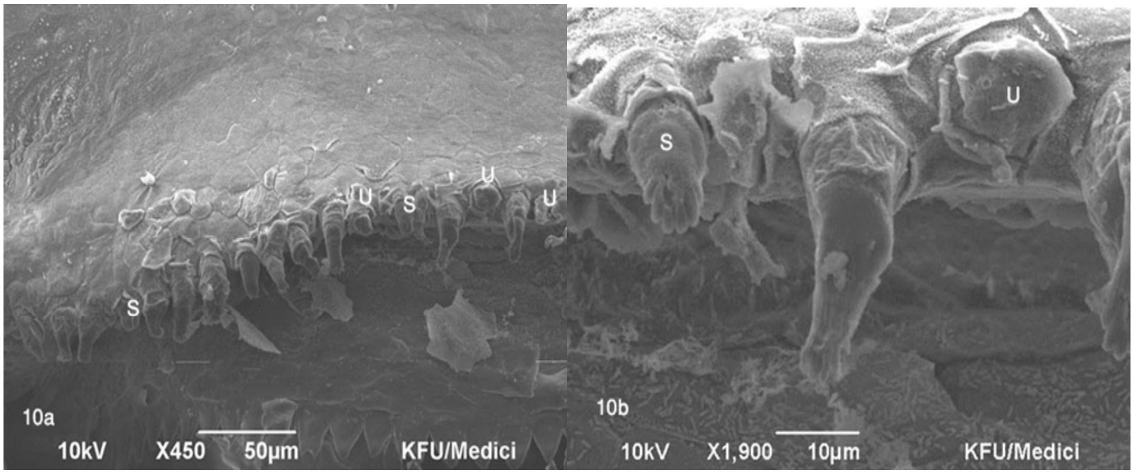

Figure 10. Scanning electron micrographs of anterior labial teeth row in *Bufo dhufarensis* showing: a) malformed short teeth (S) with 7 cuspes or undeveloped labial tooth (U) without cusps; b) Enlarged part of malformed teeth.

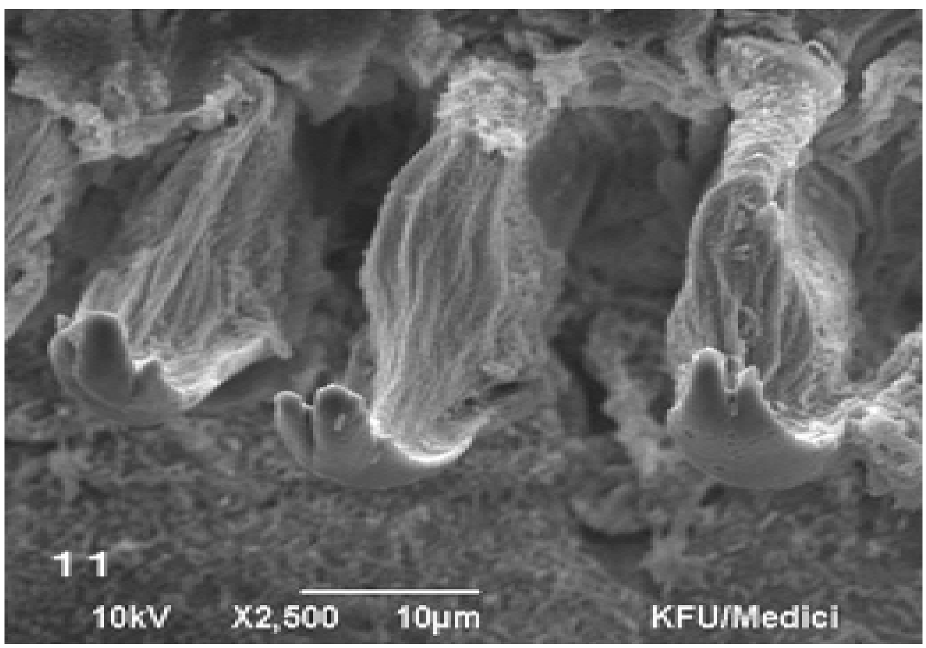

Figure 11. Scanning electron micrographs of posterior labial teeth row in *Rana ridibunda* showing labial teeth changing their position from straight to downwardly curved teeth with 2-3 cusps.

which are the most common feature of the oral discs of most tadpoles (Thibaudeau and Altig, 1988; Altig and Johnston, 1989). However, the oral discs of these two species differed specifically in orientation. It was anterio-ventral in *Rana*, while in *Bufo* it was unique in having anterior disc. The anterio-ventral disposition of oral disc of *Rana* tadpoles indicated their mainly detritus feeding habits and it grazed on algal vegetation and it also filter feeds the planktonic bloom of the pond (Khan and Mofti, 1994a, b).

Nevertheless, some differences in the arrangement and morphology of the mouthparts were observed. First, the arrangement of the upper and lower jaw sheaths varied among the two species. The horizontal width of the oral disc was double its vertical height in *Bufo*, while the width and the vertical height had almost the same size in *Rana.*

The differences among the shape and size of the oral discs could be related to the nature of the food particles ingested by both species. In fact, *Rana* tadpoles feed probably by taking large bites of macrophytes and algae attached on submerged substrates, while *Bufo* tadpoles ingest smaller particles of detritus and algae generated by rasping food (Savage, 2002; Kinne et al., 2004). The present results support their feeding behavior.

The structure, length and arrangement of the marginal and submarginal papillae also varied among the investigated species. The distribution pattern of the marginal papillae of *Rana* was taxonomically and ecologically the most common (Altig and Johnston, 1989) particularly in ranid tadpoles (McDiarmid and Altig, 1999). On the other hand, a dorsal gap among the marginal papillae was found in *Bufo* tadpoles; a result which matches with the configuration that occurred commonly in most bufonids (McDiarmid and Altig, 1999). In fact, Van Dijk (1981) assumed that the presence of a ventral gap in the row of marginal papillae of bufonidae tadpoles could play a role as a “weir-like flow-controlling structure”which acts as a barrier against water flow. The marginal papillae have tactile and chemosensory functions and help to control the water flow conveying food particles towards the mouth (McDiarmid and Altig, 1999), but the functional significance of the differences of this papillary pattern is still not understood. In *B. variegata*, it was found that the marginal papillae surrounded the entire oral disc (Altig and Johnston, 1989). They confirmed that this configuration was found only in some larval types, mainly in stream inhabitants of several families. While in *Rana* tadpoles, the lack of dorsal gap was probably because they are carnivores and live in small ponds. Haas (2003) found that the tadpoles use their complete papillary row as a filter for water flow and for a better adhesion to the irregularities of substrates. The presence of a gap in the row of labial papillae was among the apomorphic characters that defines the ranids, while this character is absent in Bufonidae (Haas, 2003).

Another characteristic was present, which was the presence of multiserial teeth in the LTRF. It was 2(1)/3(1-3) in *Rana* and 2/3in *Bufo*. This finding is in accordance with the observation of McDiarmid and Altig (1999) in Bombinatroids and that of Grillitsch and Grillitsch (1989) and Tubbs et al. (1993) in *Bufo* species. On the contrary, the present observation is in contrast to the previous finding of Bekhet (2012) in *B. regularis* where LTRF was 2(1)/3(2). Our observation supports the uniform morphology of the oral apparatus in the genus *Bufo* as proposed by McDiarmid and Altig (1999). Some authors have noticed that the number of upper labial tooth rows in *R. dalmatina* can vary between 3 and 5 rows (Nikolsky, 1915; BarbadilloEscriva, 1987). In contrast, both Grillitsch and Grillitsch (1989) and Picariello et al. (1996) reported that the LTRF of *R. graeca* and *R. italic* tadpoles were 3(2,3)/4(1). The variation between the present tooth rows in *R. ridibunda* and that of *R. dalmatina* may be related to pond dimensions. It is known that tadpoles in temporary ponds have few labial tooth rows because the progress of development and the metamorphosis are induced before the full development of the oral structures (Vences et al., 2002). They also assumed that the first tooth row on the upper labium is always longer than the lower tooth rows. The lower tooth rows have a similar length in *Bufovariegata*, but in *Rana,* their lengths decrease from the proximal to the distal row (Altig and Johnston, 1989). They suggested that the tooth row lengths are correlated to the microhabitats of the tadpoles. Indeed, the morphologies, which occupy standing water, may have a shorter distal lower tooth row than the proximal row, whereas in species that live in running water, the lower tooth rows are typically long.

In the present work, it was found that the labial teeth had the same pattern in both species, but some differences in their morphology can be recognized. The teeth in *Rana* were long and ended with 3 cusps either straight or curved, whereas that of *Bufo* were wider, short and ended with 5-7 cusps.

Regarding the deformities, extra keratinized structures in the marginal and submarginal papillae in the *Bufo* tadpoles was observed. These results coincided with the same oral deformities that aided in stuffing large pieces of food into oro-pharyngeal passage of the tadpole (Atlig and Johnson 1989; Khan and Mufti 1994a; Hopkins et al., 2000; Drake et al., 2007). In addition, oral anomalies such as eroded jaw sheaths and gaps in tooth rows were also reported in the natural ponds (Altig, 2007). Drake et al. (2007) reported oral deformities (teeth in the marginal papillae, tooth rows and jaw sheaths) of tadpoles from 13 population samples. The frequency of oral deformities can be high in natural population due to the presence of *Batrachochytrium dendrobatidis* infection, which exerts a strong influence on the occurrence and type of oral deformities in tadpoles. The chytrid fungus *B. dendrobatidis* was found to cause oral abnormalities in the species *R. muscosa* (Fellers et al., 2001) and in *Rhinella quechua* (Barrionuevo et al., 2008). *B. dendrobatidis* (chytrid)-induced mouthpart deformitiesin *B. fowleri* and *Hyla chrysoscelis* tadpoles. On the other hand, the incidence of oral deformities increased in the American Bullfrog (*Lithobates catesbeianus*) tadpoles, due to the exposure to coal combustion residues (Rowe et al., 1996, 1998a and b, 2001; Peterson et al., 2008). However, the jaw sheaths had significantly more deformations than labial teeth (Venesky et al., 2010). All or part of the previous reasons could result in the deformities found in the present work. Further environmental studies are required to investigate the present observed malformations.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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