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The larva of *Schismaderma carens* (Smith, 1849) (Anura: Bufonidae) – a redescription

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We describe the outer morphology of *Schismaderma carens* larvae in greater detail than previous studies, with special attention to the histology of the unusual head fold in this taxa. The oral cavity is described for the first time. External morphology, including the oral disc (LTRF 2/3), overall shape, biometrical indices, oral cavity morphology, and the filter apparatus confirm that *Schismaderma carens* is a generalized filter-feeding tadpole, not very different from most toad tadpoles of the world. Internally some papillae around the choanae appear taller and fused than in common toad tadpoles, but their function is unclear. The dorsal horseshoe-shaped head fold is an integumental expansion, rich in blood vessels and surrounded by a thin epithelium consistent with it serving a respiratory function. Its role is interpreted as an additional gas exchange site allowing the utilization of alpha-mesosaprobic and polysaprobic waters.

INTRODUCTION

Schismaderma carens (Smith, 1848), the red toad, is a bufonid distributed in southern Kenya, Tanzania, Democratic Republic of Congo, Malawi, Zambia, Zimbabwe, Botswana, Mozambique and South Africa (Channing & Howell, 2006; Channing *et al*., 2012). Liedtke *et al*. (2016) recognised three lineages within what is currently known as *S. carens*. A review of all available GenBank 16S sequences shows that there are only two lineages with 16S differences of 3 % (the recommended minimum to separate two amphibian species according to Vences *et al*., 2005). One lineage is known from Dodoma in central Tanzania and “Tanzania”, with 0 % difference between the samples. The second lineage is known from Iringa (southern Tanzania), Harare (Zimbabwe), Pemba (Mozambique), Pietermaritzburg and Polokwane (South Africa), with a difference of 0-1 % between the samples. *Schismaderma carens* was described from the “interior of South Africa” (Smith, 1848) from material collected by J.A. Wahlberg. Wahlberg collected only in South Africa before 1848 (Plug, 2014), confirming the type locality as “interior of South Africa” (Frost, 2016). The material described here is without doubt part of the nominal clade from South Africa, Zimbabwe, Mozambique and southern Tanzania.

The following sequences were examined: AY325997 Dodoma, Tanzania (Darst & Cannatella, 2004); FJ882849 “Tanzania” (Van Bocxlaer *et al*., 2009); DQ158424 Harare, Zimbabwe (Pramuk, 2006); U52786 Harare, Zimbabwe (Graybeal, 1997); DQ283425 Iringa, Tanzania (Frost *et al*. 2006); KF665121 30 km S of Pemba, Mozambique (Liedtke); KF664829, KF665363 Polokwane, South Africa (Liedtke); AF220866, AF220913 Pietermaritzburg, South Africa (Cunningham & Cherry, 2004).

Power (1926) illustrated the larva and its oral disc, although with few details. However, he observed a dorsal cutaneous structure, which he termed the head fold. Charter & MacMurray (1939) mentioned a horseshoe-shaped “frill” (head fold) with capillary vessels and larger blood vessels under the skin of variable size. These authors suggested that it had a respiratory function. However, no figures of the larva nor micrographs of the “frill” anatomy were provided. Van Dijk (1966) and Channing *et al*. (2012) further described external

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morphology for diagnostic purposes. The aim of the present study is to present a redescription of the larva, including morphological details not available in the existing literature, such as the head fold histology and the oral cavity.

Red toad larvae are found in a wide range of saprobic levels including alpha-mesosaprobic and polysaprobic waters organically enriched by human activities (Kolkwitz & Marsson, 1909; Channing *et al*., 2012). These waters are defined by low oxygen concentrations as a consequence of decomposition of organic substances by aerobic saprolytic organisms and by high carbon dioxide (CO_2) concentrations (Lampert & Sommer, 1993; Tachibana *et al*., 2009). Swimming near the water surface (Power, 1926) the head fold slightly elevated above the surface (Charter & MacMurray, 1939) is a typical behaviour of the larvae to come into contact with a milieu rich in oxygen. So the dorsal position of the head fold corresponds to the behaviour.

MATERIALS AND METHODS

This published work has been registered in ZooBank. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser. The LSID for this publication is: urn:lsid:zoobank.org:pub:0D5ED4A0-4BA0-4A8E-AD54-CED029E5680E

All samples were collected with the land-owners' permission, under research project ethics clearance UWC 04/4/10. The material has been deposited in the Hessisches Landesmuseum Darmstadt, Friedensplatz 1, D-64283 Darmstadt, Germany, and accessioned under HLMD-RA-3185 to HLMD-RA-3208. Tadpoles were collected from schools in mid-water and at the surface from three ponds in the Skeerpoort area, Gauteng Province, South Africa. These were within 5 km distance of each other; Scott Farm dam (25°48'55"S, 27°41'27"E), Balloon Farm dam (25°48'54"S; 27°44'22"E) and Magalies River pool (25°48'35"S, 27°44'15"E) all at an elevation of 1200 m above sea level. Identification was based on the presence of breeding adults in the ponds earlier, the schooling behaviour of the tadpoles that form rolling balls in mid-water, dropping to the substrate to ingest material, and the characteristic horseshoe-shaped flap on the head (fig. 1.A-C).

After fixation in 5 % formalin and determination of the developmental stages (Gosner, 1960), tadpoles were examined and measured under a Wild M3 stereo microscope with a measuring eyepiece. Macro photographs were taken with a Leica S8APO stereo microscope with digital camera DFC290. The buccal cavity and the keratodonts were illustrated after being photographed using the software CorelDraw, Home & Student Suite X7.

For histology, four specimens of stage 32 were processed following Mallory & Wright (1904). They were embedded in HistoResin, sectioned to 10 µm thickness, staining with toluidine blue and eosin, mounted on slides with Entellan® from Merck Millipore, Due to the similarity of their histology, thin section photos were only taken from specimen HLMD-RA-3208 (fig. 2) with an Olympus BX-50 microscope (Tokyo, Japan). Descriptive terminology follows Altig & McDiarmid (1999).

RESULTS

Measurement of primary landmarks and biometrical ratios of 20 larvae of stage 36 are given in tab. 1 (see also fig. 1.A, B). The body is slightly broader than high (BW and BH). In dorsal view, the body is elongated and is widest at the level of the branchial baskets. In dorsal aspect, the snout is rounded and the anterior labium of the oral disc (OD) is not visible. The ovoid nostrils (IND) are dorsally positioned and are close together (IND). They are nearer to the eyes than to the snout. The interorbital distance (IOD) is the same as the shortest distance from the eye lens to the tip of the snout, but larger than the internarial distance, illustrating the lateral position of the eyes. The diameter of the eye is slightly larger than half of IOD. The tail is higher than the body (BH/MTH). Tail and dorsal fins are highest at the anterior third of the tail length. The dorsal fin extends slightly onto the body and is slightly larger in height than the lower fin (i.e. moderately to well-arched fins *sensu* Anstis 2002). The tail tip is rounded and tail muscle height at the base is about half tail height (TMH/MTH). The ratio of tail muscle width at the base to body width (TMW/BW) demonstrates a moderate to robust tail musculature (*sensu* Anstis, 2002).

Coloration in preservative. dark brown melanophores on the epidermis, the subepidermis and the epithelia envelop the inner organs; spots absent; inner organs of densely or lightly marbled appearance. Melanophores densely concentrated around the nostrils and less densely in the dorsal region around the eyes, on the snout and the region around the oral disc. On the lateral and the ventral sides melanophores are large, but more sparsely distributed than in the other parts of the head-body region; here coloration is dominated by the melanophores of gills, the anlagen of the fore legs and the musculature of the throat (fig. 1.A, B). Tail pigmentation with large dispersed melanophores around the musculature; additionally small and finely reticulated melanophores (*fide* Anstis, 2002) form a light brown uniform area at the level of the musculature; pigmented vessels extending into the dorsal fin (fig. 1.A-C).

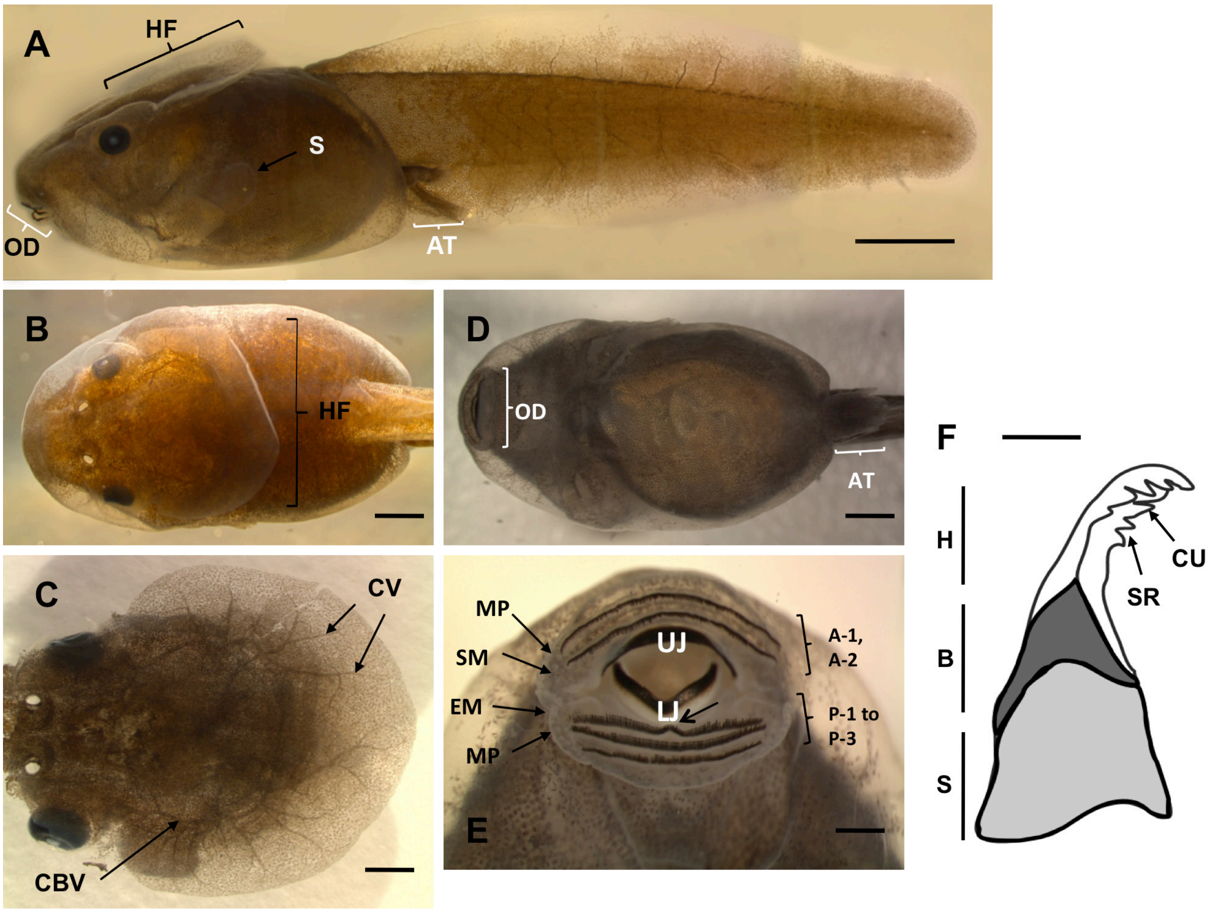

Figure 1. Preserved *Schismaderma carens*, Gosner stage 35 larva (HLMD-RA-3205). The micrographs demonstrate the outer morphology of the tadpole especially the position and size of the head fold and of the oral disc: **A.** Lateral view with the head fold (HF), the sinistral spiracular tube (S) and the oral disc (OD); **B.** Dorsal view representing the position of the head fold; **C.** Head fold isolated from the body with capillary vessels (CV) at the periphery and collecting blood vessels (CBV) at the base; **D.** Ventral view demonstrating the slightly ventral position of the oral disc; **E.** Oral disc with two anterior tooth rows (A-1 and A-2) and three posterior tooth rows (P-1 to P-3), the arrow indicates a wave in P-1; **F.** Reconstruction of a keratodont. Abbreviations: B body, CU cusp, EM emargination, H head, LJ lower jaw sheath, MP marginal papillae, S sheath, SR serration, SM submarginal papillae, UJ upper jaw sheath. Scale bar equals 4 mm in A., 2 mm in B.-D. 0.6 mm in E. and 20 µm in F.

Descriptive notes

The larvae are characterized by a sinistral spiracular tube (larval Type IV of Orton, 1953, 1957) originating more laterally than ventrally from the branchial basket posterior to the level of the eyes at an angle with the horizontal body axis of approximately 35° (fig. 1.B). The rounded spiracle opening is located at the longitudinal body axis and opens caudal to the buccopharynx in a dorsoposterior direction. The vent tube is relatively long, medial, posteriorly directed and with both walls attached directly to the ventral fin (fig. 1.B, D).

The anteroventral oral disc has a single row of marginal papillae (MP) framing the laterals of the anterior and the posterior labia, leaving median gaps in the dorsal and the ventral sides. A few submarginal papillae (SM) occur only at the sides of the anterior labium. Sides of the posterior labium and the lateral transition zones to the anterior labium form pocket-like structures. Emarginations (EM) present between the posterior and the anterior labia (fig. 1.E). Labial teeth (keratodonts) arranged uniserially in rows. Tooth morphology resembling the spoon-shaped type with six to nine mostly short- or medium-sized cusps per labial tooth (fig. 1.F). All larvae have only two undivided anterior tooth rows (A-1 and A-2) and three undivided posterior tooth rows (P-1 to P-3; P-1 with a median fold or wave, fig. 1.E). The LTRF (labial tooth row formula) is 2/3 (fig. 1.E), which is the most common formula in anuran larvae (Altig & McDiarmid, 1999). The anterior jaw sheath is arch-like and slightly broader than the posterior. The posterior jaw sheath appears V-shaped in outline. Both jaw sheaths have fine serrations along the edges. Collective these morphological features are not unique and are often found in bufonid larvae.

The head-body region is dorsally dominated by a horseshoe-shaped head fold (fig. 1.A-C). Transmitted-light microscopy reveals capillary vessels (CV) connected to larger blood vessels (CBV) at

Table 1. Measurement of primary landmarks [mm] and biometrical ratios of *Schismaderma carens* tadpoles, Gosner Stage 36, *n* = 20, vouchers HLMD-RA-3185 to HLMD-RA-3204. Abbreviations: TL total length, BL body length, BW maximum body width, BH maximum body height, IND internarial distance (measured between centers of narial apertures), IOD interorbital distance (measured between centers of pupils), TAL tail length, MTH maximum tail height, TMH tail muscle height at the level of the hind limb buds, TMW tail muscle width at the level of the hind limb buds (all according to Altig & McDiarmid, 1999), SD standard deviation, decimals truncated. Ratios calculated on an individual basis.

	Mean	Range	SD
Distances of landmarks			
TL	34.80	32.1-39.4	2.14
BL	13.78	12.3-15.3	0.76
BW	8.43	7.8-9.5	0.52
BH	7.02	6.4-7.9	0.45
IND	1.53	1.3-1.8	0.11
IOD	3.91	3.7-4.2	0.13
TAL	20.78	19.1-24.1	1.38
MTH	7.65	7.1-8.5	0.43
TMH	3.66	3.1-4.2	0.26
TMW	1.78	1.4-2.1	0.18
Ratios			
BL/TL	0.396	0.342-0.410	0.01
BH/MTH	0.912	0.837-1.054	0.04
TMH/MTH	0.476	0.410-0.520	0.03
TMW/BW	0.211	0.175-0.240	0.01

the base (fig. 1.C). Histological examination demonstrates the cutaneous nature of the fold. The epidermis (EP) surrounds spongy connective tissue (CT) with the blood vessels (CV and CBV) embedded (fig. 2.A). The connecting blood vessel (CBV) (fig. 2.B) is in contact with another bigger vessel (BV), which is in close proximity to a larger blood vessel (LV) (fig. 2.C). The series of histological sections show that the BV leads into LV, which is in close neighbourhood to the caudal part of the chondrocranium (P). In cross section the head fold has the similar morphology of gill filaments in anuran larvae (McIndoe & Smith, 1984; Viertel, 1985).

The study of Kolesova *et al*. (2007) on the metamorphosis of *Pelobates fuscus* (Pelobatidae) sheds light on the position of the blood vessels in the neighbourhood of the aortic arches in anuran larvae. Cutaneous blood supply is facilitated by the *arteria cutanea* which originates from the *ductus* Botalli, a connecting vessel or shunt between the 5th and 6th aortic arches. This suggests that the BV in the *S. carens* larva could be equivalent to the *arteria cutanea* and the LV the *ductus* Botalli.

The details of buccal morphology are given in fig. 3.A and B. They differ in many features from other generalized bufonids, such as the European *Bufo bufo*, *Epidalea calamita* and *Bufotes viridis* (both species previously referred to the genus *Bufo*) (Viertel, 1982). The most diverging trait is the tall bag-like structure on the border of the choanae (CH). It could be the result of fusion of the anterior prenarial papillae (PRP) and the posterior (NVP), which have grown together (in fig. 3.B prenarial-narial fusion, PNF). This seems to be more likely than the formation of a new structure independent of PRP and NVP. However, the origin remains unclear because the bag-like structure does not show morphological properties and criteria allowing a definite conclusion. The role of the bag-like structure remains unclear. For most structures of the oral cavity an olfactory function and an influence on the water flow is not excluded. Literature does not provide a clear answer.

All the features of a filter apparatus are present (Seale & Wassersug, 1979; Viertel, 1985). They comprise the ciliary cushions (CC), the filter plates (FP) and the branchial food traps (not visible in fig. 3.B).

DISCUSSION

Oxygen and carbon dioxide are strong limiting factors for aerobic organisms (Odum, 1971). The number of amphibian species reproducing in alpha-mesosaprobic and polysaprobic waters is limited. *Rana temporaria* larvae actively avoid low ambient oxygen tension (Savage, 1961; Costa, 1967). However, the number of species and abundance of fungi, bacteria and protozoa coping with the hypoxic and hypercarbic milieu are high (Uhlmann, 1975; Lampert & Sommer, 1993; Tachibana *et al*., 2009), offering a nearly unlimited food source for filter-feeding

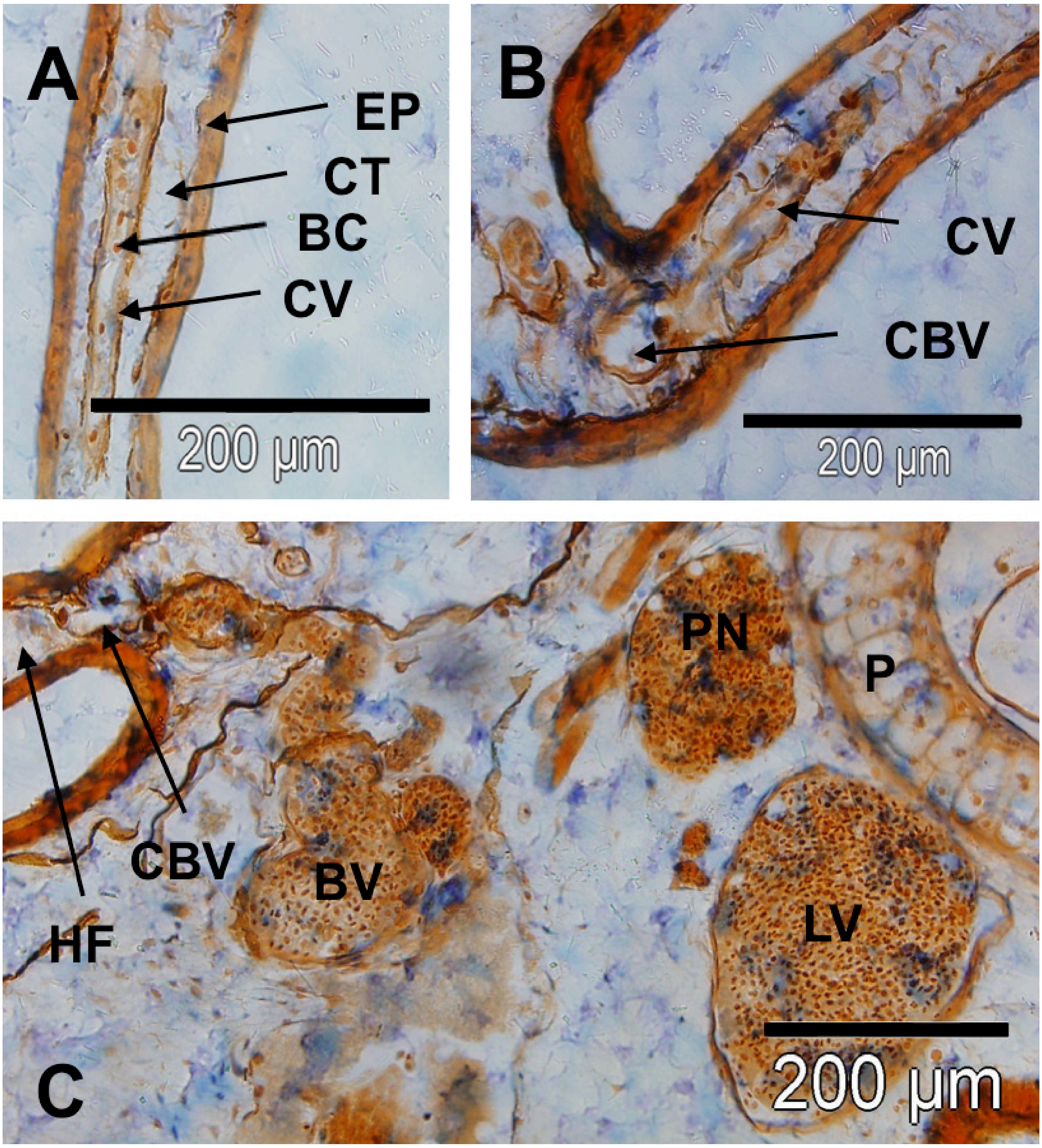

Figure 2. Light-microscopy paramedian section in the region of the head fold of *Schismaderma carens*, Gosner stage 32 larva (HLMD-RA-3208): **A.** Head fold (HF) with capillary vessels (CV) including blood cells (BC) and embedded in connective tissue (CT) and surrounded by the integument (EP, epithelium); **B.** Base of head fold with collecting blood vessel (CBV); **C.** Collecting blood vessel (CBV) in contact with another bigger vessel (BV) in neighbourhood of the large blood vessel LV, P cartilage from the caudal part of the chondrocranium, PN pronephros.

anuran larvae. A strategy to survive and to be in the position to exploit the enormous biomass in the hypoxic environment could be the development of an additional cutaneous respiratory capillary system (Ultsch *et al.*, 1999). The head fold occurs only in *S. carens* larvae developing in hypoxic waters, but not in larvae from a normoxic environment (Charter & MacMurray, 1939), suggesting the respiratory nature of the head fold. The inducibility of the head fold meets the concept of phenotypic plasticity for respiratory surfaces as discussed for amphibian larvae (Rose & James, 2013; see also Wassersug, 1996).

The lung anlagen in *S. carens* are around 0.8 mm long at larval stage 34, which is in agreement with *Bufo bufo* at the same stage (personal observations). It is suggested that their function is not adequate to sufficiently support gas exchange in a hypoxic and hypercarbic milieu as outlined. Bufonid tadpoles worldwide tend to develop their lungs very late and are not capable of air-breathing (Wassersug & Seibert, 1975). *Osteopilus brunneus*, which lives in an extremely anoxic environment, does not develop accessory respiratory organs because functioning lungs are developed early (Lannoo *et al*., 1987).

If the considerations are correct, the head fold would comply with the criteria of a key innovation as discussed by Yoder *et al*. (2010).

A ring of expanded epithelial tissue around the eyes and the nostrils, the crown, is present in the bufonid species *Mertensophryne anotis* (living in small pockets of water in tree holes), *M. taitana* (in temporary ponds) and *M. micranotis* (in water-filled holes, crevices and snail-shells). The crown contains connective tissue and a blood capillary net lining the epithelium. The larvae achieve contact with their crown to the air. Both the capillary net and the behaviour support the conclusion that the crown serves as respiratory organ (Grandison 1980, 1983; Channing 1978, 1993; Müller *et al*., 2005). To cope with low oxygen concentrations *S. carens* and *Mertensophryne* develop the same strategy. They increase the body surface. This is interpreted as the consequence of the restrictions of the anuran bauplan (Hall, 1992; Hanken, 1999; Arthur, 2004). It does not allow other possibilities such as the early development of lungs or the increase of gills to enhance respiration. However, morphology is not restricted concerning the site of body surface extension. This is corroborated by the operculum of *Lithobates catesbeianus* (Ranidae), which is rich in blood capillaries and respiratory active (Gradwell, 1969). Haas *et al*. (2014) concluded from data on the adaptation of *Occidozyga baluensis* (Dicroglossidae) to carnivorous feeding that the morphospace occupied by neobatrachian tadpoles is compact and lacks innovation (except Microhylidae).

Schismaderma carens tadpoles are similar to other common, pond dwelling bufonid larvae. They do not show an arrangement of special morphological features except the head fold. Further research is needed to understand the ecophysiological criteria triggering the development of the head fold. Knowledge of the blood vessel system is crucial to understand the role of its functional anatomy.

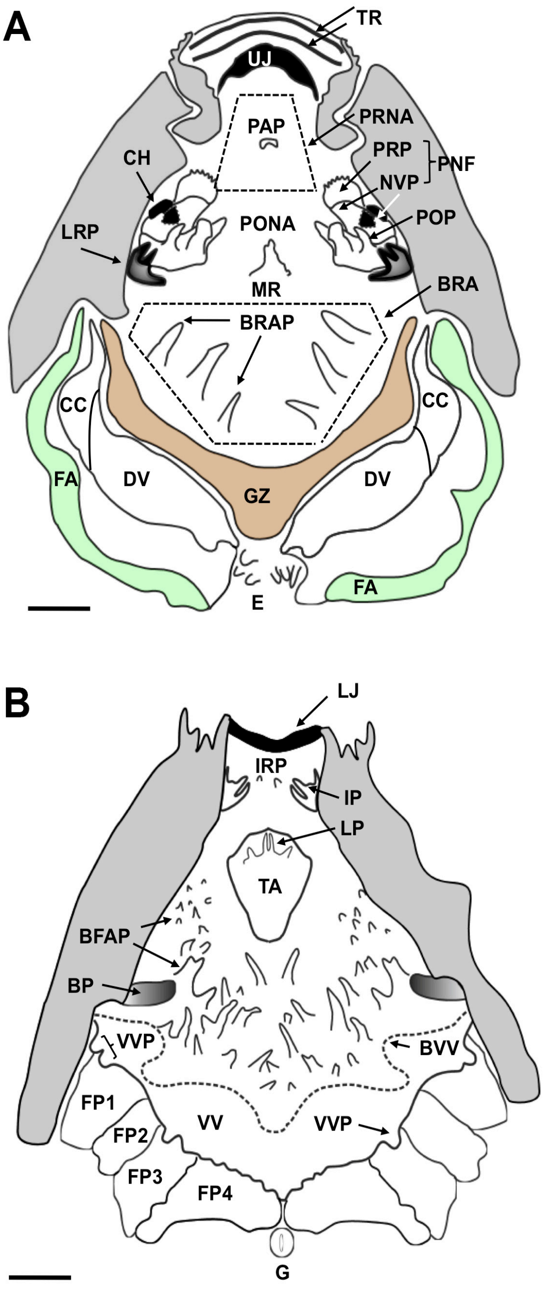

Figure 3. Reconstruction of the oral cavity from preserved *Schismaderma carens*, Gosner stage 35 larva: **A.** Roof of mouth (HLMD-RA-3206); CC ciliary cushions, BRA buccal roof arena, BRAP buccal roof arena papillae, CH choana or inner nose opening, DV dorsal velum, E anterior esophagus, FA filter apparatus sectioned, GZ glandular zone (brown), LRP lateral ridge papillae, MR median ridge, NVP narial valve projection, PAP prenarial arena pustulation, PNF prenarial-narial fusion of prenarial papillae and narial valve projection, PONA postnarial arena, POP postnarial papillae, PRNA prenarial arena, PRP prenarial papillae, TR tooth rows, UJ upper jaw sheath; **B.** Floor of mouth (HLMD-RA-3207); BFAP buccal floor arena papillae, BP buccal pocket, BVV anterior border of ventral velum, dashed line, FP1 to FP4 filter plates 1 to 4 (cut edges green), G glottis, IP infralabial papillae, IRP infrarostral pustulations, LJ lower jaw sheath, LP labial papillae, TA anlage of tongue, VVP velar papilla, VV ventral velum, grey shading indicates cut edges. Scale bars equal 1 mm in A. and B.

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