

Evolutionary significance of oral morphology in the carnivorous tadpoles of tiger frogs, genus *Hoplobatrachus* (Ranidae)

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We describe the larval stages of three species of the Asian-African tiger frogs *Hoplobatrachus chinensis*, *H. occipitalis* and *H. tigrinus*. The tadpoles of all three species are very similar, with peculiar oral features: (1) double rows of needle-like labial teeth, (2) strong emarginations on the large jaw sheaths and (3) keratinized spurs on the buccal floor. Characters 1 and 2 (and perhaps 3) are probably related to the carnivorous habits of these tadpoles. A molecular phylogeny based on 2430 base pairs of two nuclear and four mitochondrial genes corroborated monophyly of Asian and African *Hoplobatrachus*, and identified *Euphlyctis* as their sister group. Tadpoles of the latter genus lack buccal spurs and double labial tooth rows but share large jaw sheaths, the upper with a medial projection. Therefore, the common ancestor of *Euphlyctis* and *Hoplobatrachus* probably was also characterized by this state, and may have been facultatively carnivorous. Further carnivorous specializations in *Hoplobatrachus* could explain why tiger frogs have been so successful in populating arid environments where ponds are at high risk of desiccation. Larval morphology may prove to be the key innovation which enabled them to disperse, in the Late Cenozoic, into their current very wide distribution area in Asia and Africa. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 81, 171–181.

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INTRODUCTION

One of the most important evolutionary innovations of anuran amphibians is the derived larval stage, commonly known as the tadpole. In contrast to the other two recent amphibian orders (salamanders and caecilians), frog larvae are usually filter-feeding omnivores. They are able to feed both on the phytoplanktonic community by means of filtration, and on a large variety of substrates (including algae, macrophytes and carrion) by rasping, scraping and chopping with their jaw sheaths and labial teeth (Seale & Wassersug, 1979; Seale, 1982). Their digestive system is adapted to processing vegetable matter, and the major components of

their mouthparts are not homologous to the jaw apparatus of the vertebrate bauplan (Altig & McDiarmid, 1999).

It is appealing to hypothesize that this conquest of a new adaptive zone in the larval stage was one of the factors causing the enormous diversity of frogs in terms of numbers of species and reproductive modes. Tadpole morphology has been evolutionarily modified into a wealth of special adaptations, and in many tropical groups independent trends towards terrestriality involving reduction of larval stages and direct development are observed (Thibaudeau & Altig, 1999; Marmayou *et al.*, 2000). A few genera have even reversed the main ecophysiological adaptation of tadpoles (filter-feeding) and have become secondary carnivores and/or cannibals (reviewed in Polis & Myers, 1985; McDiarmid & Altig, 1999).

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Such a specialization has also been noted for species of the Asian-African genus *Hoplobatrachus* (Smith, 1917; McCann, 1932; Spieler & Linsenmair, 1997; Rödel, 1998), although the significance of these observations has remained uncertain. Recently, Kosuch *et al.* (2001) provided molecular evidence for the monophyly of *Hoplobatrachus*, and for an origin of its African representative *H. occipitalis* (Günther, 1859) by recent dispersal from Asia. These authors also noted an extraordinary oral morphology present in *Hoplobatrachus* from both continents: keratinized spurs in the oral cavity and double labial tooth rows which otherwise are only known in basal anurans of the families Ascaphidae and Discoglossidae (Noble, 1927). This unique feature was used by Dubois (1992) to define *Hoplobatrachus*, which currently contains one African (*H. occipitalis*) and three Asian species (*H. chinensis* (Osbeck, 1765), *H. crassus* (Jerdon, 1853) and *H. tigrinus* (Daudin, 1802)). Additionally, a further, probably undescribed (tetraploid) species of *Hoplobatrachus* has been identified from Africa (Bogart & Tandy, 1976). These species have been named tiger frogs by Kosuch *et al.* (2001).

In the present paper, we provide further evidence for the monophyly of Asian and African *Hoplobatrachus*, based on analysis of additional nuclear and mitochondrial gene fragments and on detailed morphological study of oral structures in three species. Our aims were: (1) to identify the closest living relative of *Hoplobatrachus* using a molecular data set, and (2) to formulate a hypothesis of the evolution of the species' unusual larval morphology in an ecological and a biogeographical context.

MATERIAL AND METHODS

STUDY AREAS AND FIELD TECHNIQUES

Tadpoles of *H. chinensis* were collected by the first author in the Ben En National Park (19°35'N, 105°28'E), in Thanh Hoa Province, Vietnam, during August 1997. They were collected from their natural habitat and reared in plastic bowls of 24 cm of average diameter and 10 cm depth. They were fed with fish food and living tadpoles of *Fejervarya limnocharis* (Gravenhorst, 1829). Some tadpoles were reared through metamorphosis assuring the identity of our material. Tadpoles in developmental stages ranging from stages 31 to 40 (Gosner, 1960) were collected regularly and preserved in a mixture of equal parts of 4% formalin and 70% ethanol. Tadpoles of *H. tigrinus* were collected at Narayanghat, Nepal, by the third author in August 1973 and preserved in 10% formalin. Tadpoles of *H. occipitalis* were collected at Gagnoa, Ivory Coast, by M. Lamotte in May 1960 and preserved in 10% formalin.

All specimens are deposited in the collections of the Muséum national d'Histoire naturelle, Paris (MNHN). Descriptions of external morphology are based on the following: *H. tigrinus*, two tadpoles at stage 36 (MNHN 1991.3507, 1991.3527) and two at stage 37 (MNHN 1991.3508, 1991.3528); *H. chinensis*, one tadpole at stage 36 (MNHN 1999.0400); *H. occipitalis*, one tadpole at stage 36 (MNHN 2000.2911) and one at stage 37 (MNHN 2000.2917). Descriptions of buccopharyngeal features are based on the following: *H. tigrinus*, one tadpole at stage 36 (MNHN 1991.3526); *H. chinensis*, one tadpole at stage 34/36 (MNHN 1999.0401); *H. occipitalis*, one tadpole at stage 37 (MNHN 2000.2917).

MORPHOLOGICAL TECHNIQUES AND ABBREVIATIONS

Morphological terminology follows Altig & McDiarmid (1999), terminology of the oral disk follows Altig (1970), labial tooth row formula (LTRF) follows Dubois (1995) and developmental stages were determined according to Gosner (1960). Measurements are abbreviated as follows: BH (maximum height of body), BW (maximum width of body), ED (maximum diameter of eye), HT (maximum height of tail), LF (maximum height of lower tail fin), MC (maximum height of caudal muscle), NN (internarial distance), NP (narrow pupular distance), ODW (oral disk width), PP (interpupular distance), RN (rostronarial distance), SS (distance from tip of snout to opening of spiracle), SU (distance from tip of snout to insertion of upper tail fin), SVL (snout-vent length), TL (total length), UF (maximum height of upper tail fin), VT (distance from opening of vent to tip of tail). For exact definitions of measurements see Grosjean (2001: fig. 2). Measurements were taken with a graduated eye-piece attached to a stereomicroscope except TL and VT (and SVL in *H. occipitalis*) which were measured with a hand caliper. Terminology of bucco-pharyngeal features follows Wassersug (1976). Drawings were made with the aid of a camera lucida. Preparation for SEM examination (JEOL JSM 840) comprised dehydration (ethanol), critical-point-drying (liquid carbon dioxide), and gold sputter surface coating.

MOLECULAR TECHNIQUES AND PHYLOGENETIC ANALYSIS

DNA was extracted from muscle tissue samples preserved in ethanol. Sequences of fragments of the following genes were sequenced using primers of Vences *et al.* (2000) and Bossuyt & Milinkovitch (2000), or obtained from GenBank: rhodopsin exon 1, tyrosinase, 16S rRNA, 12S rRNA and tRNA^{Val}, cytochrome *b*. PCR procedures followed Vences *et al.* (2000), except for the addition of final elongation steps of 10 min for

cytochrome *b*, rhodopsin and tyrosinase. For the latter two genes, initial PCR products were reamplified from an agarose gel. Products were directly sequenced on ABI 377 and 3100 automated sequencers. Newly obtained sequences were deposited in GenBank (accession numbers AJ564729–35). GenBank data refer to the works of Bossuyt & Milinkovitch (2000) and Kosuch *et al.* (2001). Taxon sampling included *Euphlyctis cyanophlyctis* (Schneider, 1799), *Fejervarya cf. limnocharis*, *Hoplobatrachus crassus*, *Hyperolius viridiflavus* (Duméril & Bibron, 1841) and *Rana (Sylvirana) temporalis* (Günther, 1864) (used as out-group), *Nannophrys ceylonensis* Günther, 1869, *Limnonectes kuhlii* (Tschudi, 1838), *Sphaerotheca pluvialis* (Jerdon, 1853). These taxa fully represented those lineages which in the comprehensive works of Bossuyt & Milinkovitch (2000), Marmayou *et al.* (2000) and Kosuch *et al.* (2001) were identified as close relatives of *Hoplobatrachus*.

Sequences were aligned using Sequence Navigator (Applied Biosystems). All sections of the rRNA genes which could not be reliably aligned were excluded from the analysis, as were all positions with gaps in one or more sequences. We determined the substitution model best fitting the concatenated sequences by a hierarchical likelihood test using Modeltest (Posada & Crandall, 1998): TrN + I + G (–lnL = 11180.6328), with empirical base frequencies (freqA = 0.3065; freqC = 0.2618; freqG = 0.1627; freqT = 0.2690) and substitution rates ([A–C] = 1.0000; [A–G] = 4.0480; [C–T] = 7.3773; other rates = 1), a proportion of invariable sites of 0.3516, and a gamma distribution shape parameter of 0.4686. Data were submitted to phylogenetic analysis using PAUP* (Swofford, 2001). We used the two generally accepted optimality criteria (e.g. Swofford *et al.*, 1996; De Queiroz & Poe, 2003), Maximum Parsimony (MP) and Maximum Likelihood (ML), in heuristic searches with TBR branch swapping and random addition sequences with ten replicates. For ML, substitution model settings proposed by Modeltest were used. Two thousand MP and 100 ML bootstrap replicates were run to test the robustness of the topologies.

RESULTS

MOLECULAR PHYLOGENY

After exclusion of variable and gapped positions, the data set comprised a total of 2430 base pairs (bp). The two tree-reconstruction methods (MP and ML) recovered largely similar topologies. A single most parsimonious tree was found (Fig. 1) that required 2048 steps and had a consistency index of 0.626 and a retention index of 0.318. *Hoplobatrachus* was monophyletic, corroborating previous results based on a smaller data

set (Kosuch *et al.* 2001: 903 bp of 12S and 16S rRNA). Bootstrap percentages for this grouping were high. *Euphlyctis* (or a lineage comprising *Euphlyctis* and *Nannophrys*) was identified as sister group of *Hoplobatrachus* (Fig. 1). ML differed by placing *Nannophrys* as sister-group of the *Euphlyctis*–*Hoplobatrachus* clade, while this relationship was unresolved in the MP cladogram.

TADPOLE MORPHOLOGY

This section gives a detailed morphological description of tadpoles of *H. tigerinus*; those of *H. chinensis* and *H. occipitalis* were very similar, and their descriptions are therefore abbreviated, mainly mentioning differences to *H. tigerinus*.

HOPLOBATRACHUS TIGERINUS (DAUDIN, 1802)

TL and SVL of a tadpole at stage 36 are, respectively, 43.8 and 17.3 mm. In dorsal view (Fig. 2A), body pear-shaped, widest in the posterior part, constricted at the level of gills, snout rounded; SS 56.1% of SVL; SU 85.0% of SVL. In profile (Fig. 2B), body slightly depressed, BW 110% of BH, snout acuminate. Eyes moderately large; ED 11% of SVL, bulging, not visible in ventral view, positioned almost dorsally but directed anterolaterally. Nares slightly oval, moderately large, rimmed, positioned almost dorsally but directed anterolaterally and horizontally, slightly closer to pupils than to snout, RN 110.5% of NP; NN 49.0% of PP. Spiracle sinistral, tubular, sometimes curved backwards, large, entirely attached to body wall, ventral to horizontal body axis, orientated posterodorsally or at an angle of 30° to the horizontal; spiracular opening from the hindlimbs level to between hindlimbs and apex of myotomes of caudal muscle. Tail musculature moderate to moderately weak, MC 54.9% of BH and 52.9% of HT, gradually tapering or parallel in the proximal half, reaching tail tip. Upper tail fin moderately high, UF 32.9% of HT, not extending onto body, lower tail fin moderate, LF 28.2% of HT, both convex; point of maximum height of tail located at the first third of tail length, HT 103.7% of BH, tail tip fine and pointed to lanceolate. Anal tube short, medial, tubular or bulb-shaped, directed posteriorly, linked to the ventral tail fin, opening medial. Neither lateral line nor glands.

Oral disk (Fig. 3A): position and direction subterminal, emarginated, moderately small, ODW 21.4% of SVL and 42.5% of BW; an important extension of lower labium in medial position occupying greater part of lower labium. Bordered by a continuous row of small, flat and very blunt marginal papillae, almost indistinct on the upper labium. Neither submarginal papillae nor denticulate papillae. Labial tooth row formula

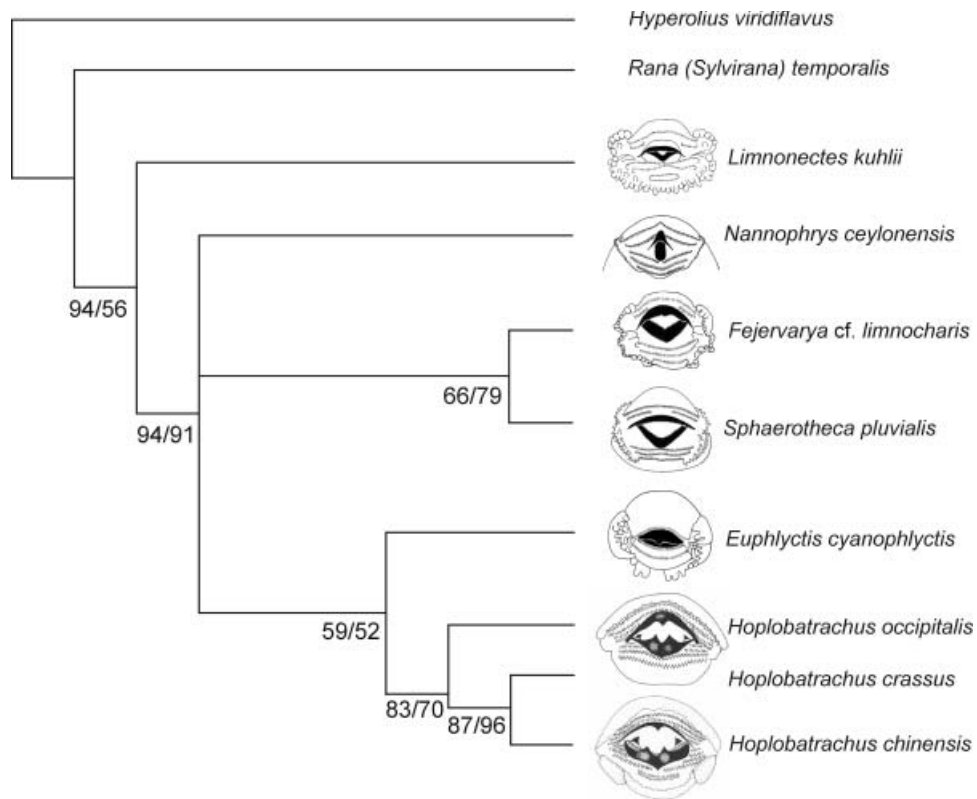


Figure 1. Most parsimonious tree of *Hoplobatrachus* and related taxa, based on on 2430 base pairs of the rhodopsin, tyrosinase, 16S rRNA, 12S rRNA and tRNA^{Val} genes. The drawings are schematical representations of tadpole mouth-parts. Numbers are bootstrap values in per cent for Maximum Parsimony (2000 replicates) and Maximum Likelihood (100 replicates).

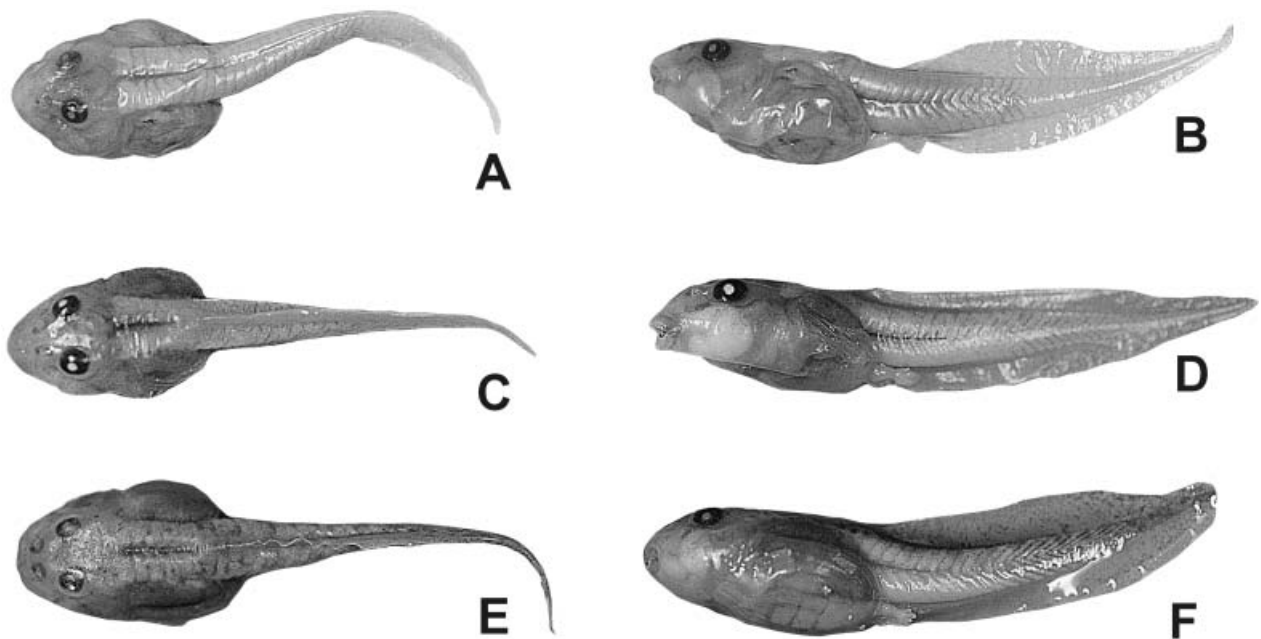


Figure 2. Photographs of tadpoles of *Hoplobatrachus* species: *H. tigerinus* (stage 36, MHNH 1991.3527, TL = 43.8 mm), dorsal (A) and lateral view (B); *H. chinensis* (stage 36, MHNH 1999.0400, TL = 39.1 mm), dorsal (C) and lateral view (D); *H. occipitalis* (stage 36, MHNH 2000.2911, TL = 50.1 mm), dorsal (E) and lateral view (F).

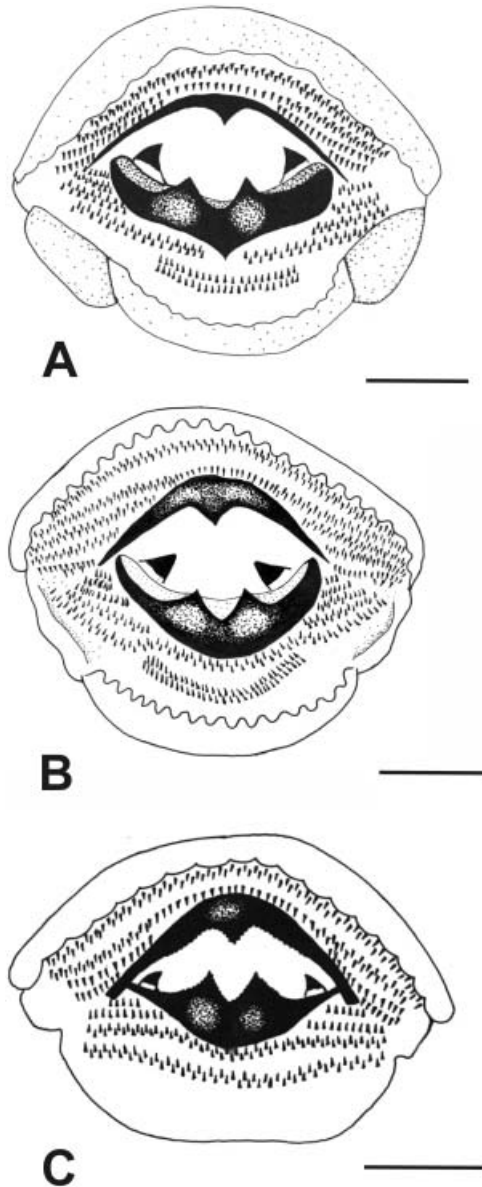


Figure 3. Oral disks of the tadpole of (A) *Hoplobatrachus tigerinus* (MNHN 1991.3529, stage 38) (B) *H. chinensis* (1999.425, stage 40), and (C) *H. occipitalis* (2000.2931, stage 36). Scale bars = 1 mm.

(LTRF) $2^2 : 3^2 + 3^2/4^2 + 4^2 : 2^2$. Rows formed of double series of labial teeth but A_2 becomes simple when it directly borders the upper beak, and both A_5 and P_1 are simple. Upper rows almost equal, lower rows almost equal except P_6 , which is restricted to the medial portion. Labial teeth (Fig. 4A) needle-like. Jaw sheaths very finely serrated, mostly black, white at base with a fine brown halo between the two; upper beak a large arch with a strong medial projection, lower beak flat with two strong projections into which the projection of the upper beak fits. Two lateral kera-

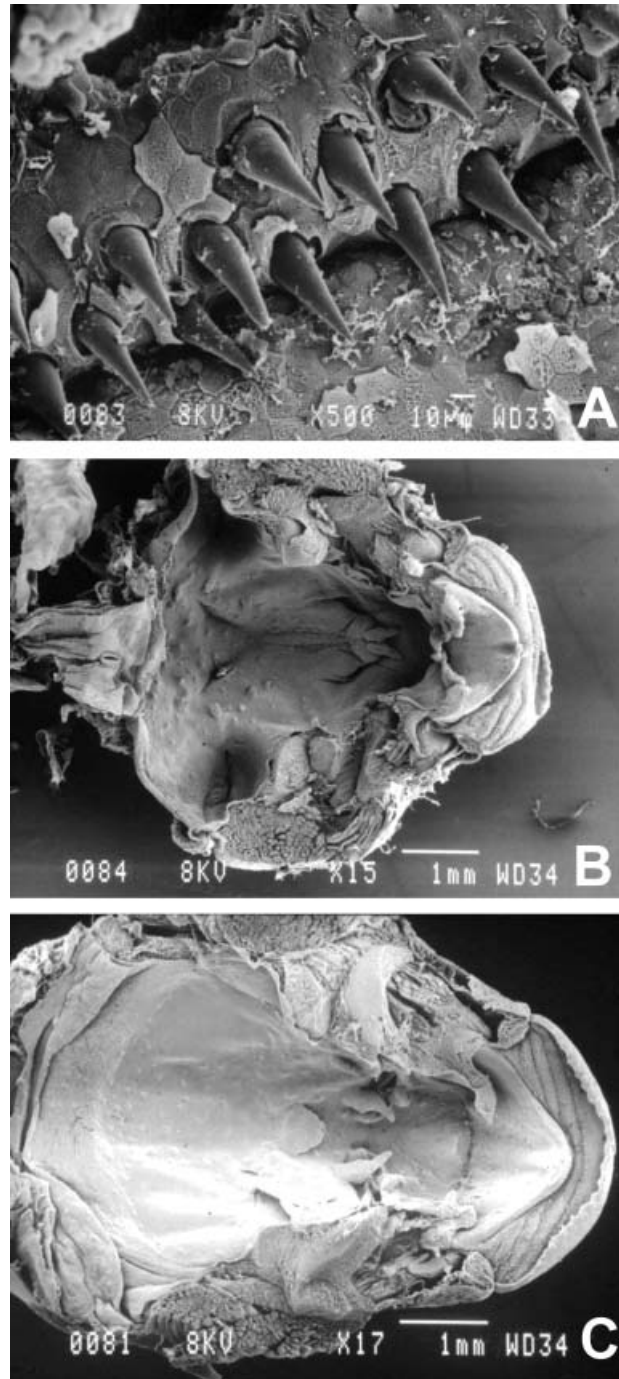


Figure 4. SEMs of characters of the mouthparts of *Hoplobatrachus tigerinus* (MNHN 1991.3526, stage 36). (A) labial teeth of row A_1 ; (B) buccal floor; (C) buccal roof.

tinized spurs in buccal floor at entrance of buccal cavity.

LTRF of 13 tadpoles (MNHN 1991.3507–3515; MNHN 1991.3529–3532) varies from $2^2 : 2^2 + 2^2/3^2 + 2^2 : 2^2$ in stage 29 to $2^2 : 4^2 + 4^2/4^2 + 4^2 : 2^2$ in stage 38.

Colour: back, flanks and ventral surface grey, translucent in preservative, viscera visible through skin; brown-red spots around eyes and nares, on the extensions of caudal muscles on back and upper flanks. Caudal muscles flesh-coloured proximally to light grey distally in gradation, a few brown-red spots in the proximal quarter. Tail fins grey, translucent. Hindlimbs whitish with very slight traces of pigmentation.

Buccal floor (Fig. 4B): prelingual arena diamond-shaped; two pairs of infralabial papillae, first pair of palps at the base of the beak flat anteroposteriorly, edge indented with a deep cleft midway (which could give an impression of two palps), positioned laterally and orientated transversally; second pair fine and long, slightly pustulose, positioned medially, in the same plane as the first pair, orientated upward. A pair of large spurs at the base of the lower beak orientated anteromedially. Tongue anlage oval, depressed anteroposteriorly; two large pustulose papillae in central position, in a transverse row, orientated anteriorly. Buccal floor arena oval, defined by 3–4 stout and short papillae posteriorly on each side, some of them pustulose, increasing in size from front to back, orientated transversally and inward. Interior of arena occupied by a long groove from the posterior end of tongue anlage to halfway, the prolongation of this groove widening in the posterior part of the arena making a triangle; fewer than 40 pustules, uniformly distributed and not very densely arranged, with a depression on the top. Buccal pockets transverse, slightly curved backward, closer to medial end of ventral velum than to tongue anlage; about ten prepocket papillae. Ventral velum continuous, with spicular support, its margin slightly wavy, without projections; medial notch absent, glottis open, exposed; posterior end of buccal floor and margin of ventral velum with secretory pits. Branchial baskets oblique, much wider than long; three gill chambers on each side; filter ruffles with tertiary folds.

Buccal roof (Fig. 4C): prenarial arena wide with a medial cornified knob (the keratinized shield of Khan, 1996), triangle-shaped with rounded angles, anterior part highest. Prenarial papillae present, short and wide, rising on the lateral part of the anterior narial wall. Choanae slightly oblique (posteromedially directed). Posterior narial wall relatively high and smooth. Postnarial papillae long, with a wide base until halfway then suddenly narrowing, directed anteromedially. Postnarial arena with five pustules, one at each angle and a larger one in the centre. Medial ridge triangular, truncate at top with a small cleft medially, slightly wider than high and slightly pustulose. Lateral ridge papillae fine, slightly pustulose on the upper side, orientated anteriorly. Buccal roof arena much longer than wide with right lateral side slightly widening anteriorly, no buccal roof arena

papillae; interior with about 40 pustules, large and not prominent anteriorly, small and well defined posteriorly. Posterolateral ridge fine, comprising a line of small papillae. Glandular zone continues across buccal cavity and is about 15 secretory pits wide. Dorsal velum continuous, curved laterally forward, with glandular pits.

HOPLOBATRACHUS CHINENSIS (OSBECK, 1765)

(FIG. 2C, D)

TL and SVL of a tadpole at stage 36 are, respectively, 38.9 and 15.5 mm. SS 64.5% of SVL; SU 86.5% of SVL; BW 115.5% of BH. Eye diameter 13% of body length. Nares rounded, slightly rimmed, closer to pupils than to snout, RN 129.4% of NP; NN 52% of PP. Spiracle square, short. Tail musculature moderate, MC 50.7% of BH and 48.8% of HT, gradually tapering. UF 36.5% of HT; LF 27.0% of HT; point of maximum height of tail located at the first quarter of tail length, HT 104.2% of BH, tail tip fine and pointed. Anal tube moderate, tubular.

Oral disk (Fig. 3B): moderate, ODW 25.8% of SVL and 48.8% of BW, bordered by a continuous row of blunt and almost indistinct marginal papillae, larger than in *H. tigerinus*. LTRF $2^2 : 2^2 + 2^2/4^2 + 4^2 : 1^2$. A_4 simple but P_1 double. No variation in LTRF was noted in 13 tadpoles ranging from stage 32 to stage 38 (MNHN 1999.402–414).

Colour: in preservative, body grey, melanophores diffuse on back and upper flanks, ventral surface immaculate. Caudal muscles off-white with melanophores underlining the myotomes. Upper tail fin with very few melanophores equally distributed, lower fin with a few minute spots in the distal end. Hindlimbs without pigmentation at this stage.

Buccal floor: prelingual arena triangular. First pair of infralabial papillae more sharply indented and the cleft less sharply marked; second pair smooth but pustulose at tip. Tongue anlage oval, slightly prominent, very elongate and less defined than in *H. tigerinus*; lingual papillae with better defined pustules, pustulose at tip, orientated dorsoposteriorly (although this difference may be due to their position during fixation and must not be taken as a taxonomic character). Buccal floor arena wider than in *H. tigerinus*, defined by 4–5 papillae; the prolongation of the groove widens posteriorly; interior with about 20 pustules without a depression on the top. Buccal pockets narrower than in *H. tigerinus*; 15–20 prepocket papillae. Medial notch present.

Buccal roof: prenarial arena rounded with a small pustulose curved ridge anteriorly and a few small pustules. Prenarial papillae pustulose. Choanae transverse. Postnarial papillae pustulose on anterior margin, their tips crossing. Postnarial arena with five

pustules, the centre one larger than in *H. tigrinus*. Medial ridge pustulose at top and on posterior side. Lateral ridge papillae orientated transversally (same remark as for the lingual papillae). Arena oval, wider than in *H. tigrinus*; interior with about 60 small and well-defined pustules, evenly distributed. Posterolateral ridge ill-defined. Glandular zone with about ten secretory pits wide. Dorsal velum not curved laterally forward.

HOPLOBATRACHUS OCCIPITALIS (GÜNTHER, 1859)

TL and SVL of a tadpole at stage 37 are, respectively, 46.5 and 20.0 mm. In dorsal view (Fig. 2E), body ovoid-elongate, snout semicircular; SS 48.0% of SVL; SU 85.0% of SVL. In profile (Fig. 2F), BW 113.9% of BH, snout rounded. Eyes large, ED 12% of SVL. Nares large, rimmed with a slight projection dorsally, slightly closer to snout than to pupils, RN 66.7% of NP; NN 45% of PP. Spiracle rectangular, attached to body wall except margin; spiracular opening just below apex of myotomes of caudal muscle. MC 43.5% of BH and 61.8% of HT. UF 38.2% of HT; LF 27.6% of HT; point of maximum height of tail at first quarter of tail length, HT 70.4% of BH, tail tip bluntly pointed. Anal tube long, tubular, not linked to the ventral tail fin.

Oral disk (Fig. 3C): ODW 22.5% of SVL and 36.6% of BW, bordered by a continuous row of marginal papillae which are better defined than in *H. tigrinus*. LTRF $2^2 : 2^2 + 2^2/2^2 + 2^2 : 2^2$. Rows A_4 and P_1 simple. Lower labial tooth rows almost equal except P_4 , which is restricted to a medial portion. Labial teeth stronger than in *H. tigrinus*. Lateral line present. LTRF varies from $2^2 : 1^2 + 1^2/2^2 + 2^2 : 2^2$ to $2^2 : 2^2 + 2^2/2^2 + 2^2 : 2^2$ in 13 tadpoles (MNHN 2000.2912–6; MNHN 2000.2918–9; MNHN 2000.2923–8) ranging between stages 28 and 40.

Colour: in preservative similar to *H. tigrinus* but darker. Internal half of nares surrounded with dark grey. Dark brown spots on snout, between eyes, on back, from eyes to ventral side in an anteroventral direction, on caudal muscle, upper fin and distal part of lower fin.

Buccal floor: first pair of palps at the base of the beak bifid (which could give an impression of two palps), each part multilobed, orientated anteromedially. Tongue anlage with one long and fine papilla, orientated upwards. Interior of arena with few prominent, about 40 pustules, without a depression on the top. More than ten prepocket papillae. Margin of ventral velum with four or five secretory pits wide.

Buccal roof: prenarial arena wide with a large rounded protuberance. Prenarial papillae triangular and pustulose. Postnarial arena with some pustules

freely arranged. Medial ridge without a small cleft medially, much wider than high. Lateral ridge papillae long, fine, slightly pustulose on the upper side, orientated transversally. Buccal roof arena very large, defined by one fine buccal roof arena papilla on each side; interior with about 100 prominent pustules widely and uniformly distributed throughout the whole buccal surface. Glandular zone across the roof wide, with about eight secretory pits wide.

DISCUSSION

UNUSUAL MOUTHPART CHARACTERS IN *HOPLOBATRACHUS*

The tadpoles of *Hoplobatrachus* have been the subject of numerous studies (*H. occipitalis*: Scortecchi, 1936; Lamotte & Zuber-Vogeli, 1954; *H. tigrinus*: Annandale, 1917; Annandale & Rao, 1918; Boulenger & Annandale, 1918; McCann, 1932; Khan, 1969, 1982, 1996; Agarwal & Niazi, 1980; Dutta & Mohanty-Hejmadi, 1984; Ray, 1999; *H. chinensis*: Flower, 1899 (as *H. tigrinus*); Smith, 1917; Noble, 1927; Pope, 1931; Chou & Lin, 1997). Various labial tooth row formulas are given by these authors, which in part differ from those observed in our study. However, similar variations in labial tooth row formula are also known from other anurans, e.g. species in the family Pelobatidae (Bresler & Bragg, 1954; Gosner & Black, 1954; Bragg & Hayes, 1963; Bragg, Mathews & Kingsinger, 1963; Hampton & Volpe, 1963), and ontogenetic changes as well as interindividual variation at equivalent stages of development have been demonstrated in *Hoplobatrachus* (Lamotte & Zuber-Vogeli, 1954; Dutta & Mohanty-Hejmadi, 1984).

Little information is available on the tadpole of *H. crassus*, although it seems to possess the same peculiar beak as the species described above (Kirtisinghe, 1957). A later description of this tadpole from Uttar Pradesh Province, India, shows a specimen with a generalized oral disk and is clearly based on another species (Ray, 1999).

Our descriptions of internal buccal features are in agreement with previously obtained albeit less detailed data on *H. chinensis* and *H. tigrinus* (Khan, 1996; Chou & Lin, 1997). Also, the most striking characters of *Hoplobatrachus* larvae have often been noted, namely the keratinized spurs in the anterior part of the buccal floor (Annandale, 1917 (in his drawing); Noble, 1927; Okada, 1931; Lamotte & Zuber-Vogeli, 1954; Khan, 1982; Chou & Lin, 1997) and the double labial tooth rows (Noble, 1927; Pope, 1931; Bourret, 1942; Lamotte & Zuber-Vogeli, 1954; Liu & Hu, 1961; Khan, 1982; Chou & Lin, 1997). The observation of triple labial tooth rows mentioned by Khan (1996) for *H. tigrinus* is almost certainly wrong. The

buccal spurs have also been observed in tadpoles of other anuran families ((*Gastrotheca riobambae* (Fowler, 1913), *Agalychnis callidryas* (Cope, 1862), *Colostethus subpunctatus* (Cope, 1899)) and their function remains uncertain (Wassersug, 1980).

In contrast, the needle-like labial teeth and their arrangement in double rows seem to be unique states among anuran tadpoles (Dubois, 1992) which, in the absence of thorough documentation, have hitherto not been understood as such and not been included in comprehensive treatments of tadpole morphology (Duellman & Trueb, 1985; McDiarmid & Altig, 1999). Multiple labial tooth rows (2–4) are also present in archaic frogs *Ascaphus truei* Stejneger, 1899 (Ascaphidae) and in the family Discoglossidae (Noble, 1927). However, this character state is not found in more advanced frogs. The biserial rows of labial teeth of *Hoplobatrachus* (which are unique among ranids) must certainly be seen as a synapomorphy of the species in this genus and are probably not homologous to the multiserial rows of *Ascaphus truei* and discoglossids.

Lastly, the medial cornified knob present in the pre-narial arena is a peculiar structure, known also in *Scaphiopus bombifrons*, a species in which cannibalistic morphs frequently develop.

ECOLOGICAL SIGNIFICANCE OF MOUTHPART ADAPTATIONS IN *HOPLOBATRACHUS*

Observations by Smith (1917), Spieler & Linsenmair (1997) and Rödel (1998) indicate that tadpoles of *H. occipitalis* and *H. chinensis* regularly prey upon other anuran larvae. *H. tigerinus* has been reported to consume conspecifics (McCann, 1932). This is corroborated by field observations of the first author, where *H. chinensis* tadpoles attacked those of sympatric species such as *Rhacophorus mutus* Smith, 1940, *Fejervarya limnocharis*, and others. In captivity, we reared *H. chinensis* tadpoles with young *F. limnocharis* tadpoles as food; they also preyed readily on younger conspecific individuals (S. Grosjean, pers. observ.).

Clutches of *H. occipitalis* as well as of *H. chinensis* and *H. tigerinus* are deposited in temporary puddles or flooded areas which are often at risk of drying out quickly (Fugler, 1984; Spieler & Linsenmair, 1997; S. Grosjean pers. observ.). In North America, pelobatid frogs of the genera *Scaphiopus* and *Spea*, which live in xeric environments, have developed adaptations similar to those observed in the *Hoplobatrachus* tadpoles. Adults breed from the beginning of rains in temporary puddles or flooded areas. The tadpoles develop very quickly and carnivorous larval morphs with accelerated growth rate arise ontogenetically among 'normal' filter-feeding morphs which are under threat of desiccation (Bragg, 1946), although other factors may be

involved as well (Bragg, 1964; Bragg & Nelson, 1966; Pfennig, 1990, 1992). Unlike *Hoplobatrachus* tadpoles, only some individuals develop into carnivorous morphs which are usually cannibalistic, although they have been observed preying upon other congeneric species as well (Bragg, 1962). In contrast, in *Hoplobatrachus* no different larval morphs are known; their tadpoles seem to be obligate carnivores, feeding equally on conspecifics and other tadpole species.

Inter- and intraspecific competition and growth-inhibiting crowding effects are usual selection pressures on anuran larvae, but become especially severe if the rapid drying of a pond threatens the extirpation of the complete tadpole population. Such strong selective pressures may be favourable for the evolution of predatory larvae because (1) they can exploit a new trophic resource when suspended nutritive matter becomes scarce, (2) the protein-rich animal food allows a faster growth and thus a higher chance of metamorphosis in the drying pond (Bragg, 1962), and (3) they might even reduce the overall tadpole density, thereby lowering the crowding effect. This hypothesis of tadpole carnivory evolving as an adaptation to rapidly desiccating ponds needs further evaluation. Phylogenetic contrast methods will be useful for testing its validity once more instances of carnivorous tadpoles become known and anuran phylogeny is better understood.

Both *Hoplobatrachus* and the *Scaphiopus/Spea* complex have a peculiar structure, the medial cornified knob of the pre-narial arena. Although Wassersug (1980) suggested a function for this structure as a "preparative aid to ingestion by cutting long, firm plant material", its presence in carnivorous larvae of two species of phylogenetically distinct lineages with analogous ecology is a strong indication for a correlation with carnivorous habits.

Carnivorous larvae of other species (*Phrynoglossus magnapustulosus* (Taylor & Elbel, 1958) (S. Grosjean, unpubl. data), *Lepidobatrachus laevis* Budgett, 1899 (Ruibal & Thomas, 1988)) have a very short intestine compared to herbivorous species. Such a specialization is not known in *Hoplobatrachus*, which has an intestine of generalized length (S. Grosjean, pers. observ.). However, it is appealing to hypothesize that the needle-like labial teeth of *Hoplobatrachus* tadpoles, and their arrangement in double rows, may be related to their carnivorous habits. It is easy to imagine that these sharp instruments will be more effective than standardized labial teeth in attacking other tadpoles, penetrating skin to gain access to the underlying soft tissues. The same function can also be attributed to the strong medial projection of the upper jaw sheath, in combination with the two projections of the lower jaw sheath that form a perfect tool for slicing skin and muscle tissue of other tadpoles. Similar modifications

are also observed in the cannibalistic morphs of the tadpole of the genus *Spea* (Smith, 1934; Turner, 1952). The keratinized spurs might also be related to feeding behaviour, but their function is less obvious. To understand their significance, it would be important to study in more detail the natural history of other tadpoles in which they are found; currently, no indications of carnivory in these species (*Gastrotheca riobambae*, *Agalychnis callidryas*, *Colostethus subpunctatus*) have been published (Grenard, 1958; Wassersug, 1980; Warkentin, 1999; but see also Stebbins & Hendrickson, 1959).

PHYLOGENY AND EVOLUTION OF TIGER FROGS

Our phylogenetic analysis identified the genus *Euphlyctis* as the most probable closest relative of *Hoplobatrachus*. This placement is in accordance with previous studies (Bossuyt & Milinkovitch, 2000; Kosuch *et al.*, 2001), although it was supported by low bootstrap values. Of the remaining taxa that were placed close to *Hoplobatrachus*, only *Nannophrys* are highly specialized cascade-dwelling frogs with partially terrestrial larvae (Kirtisinghe, 1958), while three other genera (*Euphlyctis*, *Fejervarya*, *Sphaerotheca*) share its pond-breeding habits. The most basal dicroglossine genus in our analysis, *Limnonectes*, is composed of brook breeders.

Examination of tadpoles of *Euphlyctis cyanophlyctis* (MNHN 1986.3885–8, detailed data to be published elsewhere) confirmed that these do have smooth teeth (i.e. without indentations but scythe-shaped, similar to those of megophryid and pelobatid tadpoles) in single rows, and no keratinized spurs. However, they are reminiscent of *Hoplobatrachus* tadpoles in having large jaw sheaths with a strong medial upper projection. It is parsimonious to hypothesize that these large jaw sheaths evolved only once, and therefore were already present in the common ancestor of *Hoplobatrachus* and *Euphlyctis*, which thereby may have been preadapted to the evolution of carnivorous specializations.

Euphlyctis cyanophlyctis is a common species widely distributed throughout South Asia. Its tadpole is found in a large variety of perennial or temporary water pools, such as paddyfields (even when a great amount of organic pollution or detergent is present), as well as stagnant pools or running streams (Ray, 1999; A. Dubois, pers. observ.). Little information is available about its diet and the cannibalism reported in McCann (1932) and then in Bourret (1942) is obviously to have been based on observation of tadpoles of *H. tigrinus*.

Tiger frogs are one of the few amphibian genera with a distribution that comprises Asia and Africa; their presence in the latter is most probably due to a

recent invasion from the former (Kosuch *et al.*, 2001). They are well adapted to unforested habitats and sometimes found in seasonal or arid environments. Also the other two groups of frogs for which a Mid to Late Cenozoic dispersal from Asia to Africa is likely contain species adapted to seasonal savanna environments: the rhacophorids of the genus *Chiromantis* as well as the *Hylarana* section of the genus *Rana* (e.g. Channing, 2001).

According to the wider molecular phylogenetic survey of Kosuch *et al.* (2001), the genera *Fejervarya*, *Sphaerotheca*, *Nannophrys*, *Euphlyctis* and *Hoplobatrachus* form a well-defined clade. Because this clade contains the type genus of the subfamily Dicroglossinae (*Euphlyctis*, as senior synonym of *Dicroglossus*), its members can be securely classified within this subfamily. Their relationships to other taxa from Asia and Africa (e.g. *Limnonectes*, *Conraua*, *Pyxicephalus*) that are considered to be dicroglossines (Dubois, 1992) remain to be clarified. However, considering this well-defined dicroglossine clade only, it is striking that of an otherwise completely Asian lineage, only *Hoplobatrachus* succeeded in colonizing Africa, while *Euphlyctis* reached as far as the Arabian Peninsula. One explanation for this could be found in the evolution of predatory tadpoles in *Hoplobatrachus*. This may have been a key innovation that facilitated a more efficient survival in extreme environments and a more successful and rapid colonization of seasonal and arid habitats.

Recent studies have emphasized the frequent covariations between larval and adult traits in frogs (Bossuyt & Milinkovitch, 2000). If our hypothesis is further corroborated, i.e. if the evolution of carnivorous tadpoles was the key innovation that enabled *Hoplobatrachus* to disperse rapidly into its present wide distribution area, it would support the proposition that larval characters are sometimes crucial for anurans to conquer new adaptive zones.

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