

Sand-eating tadpoles in Madagascar: morphology and ecology of the unique larvae of the treefrog *Boophis picturatus*

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Abstract. Several anuran larvae of the family Mantellidae endemic to Madagascar and the Comoro islands have remarkable specializations, typically reflected in their derived mouthparts that often are characterised by a strong reduction of keratinised structures. While most of these specialized tadpoles are found in the genus *Mantidactylus*, the treefrogs of the genus *Boophis* typically have rather generalized tadpoles, with some lineages being adapted to strong currents by an enlarged oral disc with an increased number of keratodont rows and papillae. A remarkable exception is *Boophis picturatus*, which has an extremely derived oral disc without any keratodonts and with completely reduced jaw sheaths and is known to ingest sand particles. We here provide a detailed description of the external morphology and buccal anatomy of this tadpole and evidence for constancy of this morphology in various localities in the southern central east and northern central east of Madagascar. We document that sand particles are mixed with organic matter in the anterior but not in the posterior part of the gut, suggesting that the organic matter is largely digested during its passage through the intestine. Ecologically, *B. picturatus* tadpoles were mainly found in streams with a low incline in forest areas with high numbers of trees but fewer shrubs and thick leaf litter. No statistical microhabitat preference could be demonstrated, but in streams where the tadpoles occurred, sand was by far the predominant substrate and most specimens were found on sand. The related *B. majori* tadpole shows derived mouthparts and sand ingestion to a lesser degree.

Key words. Amphibia, Mantellidae, Ranomafana National Park, tadpole morphology, buccal anatomy, clutches, habitat preference.

Introduction

Tadpoles, the mostly aquatic larvae of frogs, are strikingly different from the terrestrial metamorphosed (juvenile as well as adult) frogs (ALTIG & MCDIARMID 1999). Besides their general adaptation to aquatic life, the major differences all are related to their feeding behaviour: while metamorphosed frogs are without exception carnivorous, most tadpoles are omnivorous suspension feeders (ALTIG & MCDIARMID 1999), and have strongly modified mouthparts with numerous elements that are not homologous to any structures of the adult frogs or to the typical vertebrate *bauplan*. Nevertheless, the question of what tadpoles really eat is still largely unclarified and has been highlighted as one of the major questions in herpetological research (ALTIG et al. 2007).

Typically, tadpoles are characterised by an oral disc with keratinised jaw sheaths and equally keratinised labial “teeth” (also called keratodonts), which they use to rasp algae or bacterial films from underwater surfaces for consumption. However, this typical tadpole morphology has been modified in multiple ways, and besides carnivorous tadpoles that usually have reduced keratodonts in fa-

vour of stronger jaw sheaths, there are also tadpoles that have altogether reduced all of the keratinised oral structures during the course of their evolution. In most cases, the natural history, feeding behaviour and food of these species are totally unknown. Three such remarkable tadpoles, all belonging into the endemic Malagasy-Comoran family Mantellidae, have been recently described by ALTIG & MCDIARMID (2006). Most unusual, these authors reported that the tadpole of the treefrog *Boophis picturatus*, without any keratinised oral structures, is specialized to ingest sand.

During an intensive tadpole survey in the Ranomafana National Park in the southern central east of Madagascar, and at several other localities on the island, we were able to collect numerous tadpole specimens of *Boophis picturatus*, and of two species, *Boophis majori* and *B. sp. 35* (named *Boophis sp. aff. majori* “long calls” in GLAW & VENCES 2007 and SCHMIDT et al. 2008), that molecular data place as close relatives of *B. picturatus* (VIEITES et al. 2009) and that also show a trend of reduced keratinised oral disc structures (SCHMIDT et al. 2008). We also systematically obtained data on the preferred microhabitat in the streams of these three species in the context of a wider ecological

tadpole community study (e.g., STRAUSS et al. 2010). In this paper, we complement previous data (ALTIG & MCDIARMID 2006) on the *B. picturatus* tadpole by (1) describing its buccopharyngeal anatomy, (2) assessing genetic diversity and morphological uniformity among populations across its range, (3) provide an analysis of its microhabitat preferences based on data from Ranomafana, and (4) analyse and illustrate more in detail the intestine content of the species, comparing it to that of the tadpole of *B. majori*.

Material and methods

Morphological and anatomical descriptions

Tadpoles were collected with different kinds of nets with mesh sizes ranging from 2 to 5 mm, depending on the size of stream, the strength of the current, and the type of substrate. They were euthanised by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters. From each series, one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. These specimens are here called “DNA vouchers”. All detailed morphological tadpole characterizations and drawings are based on these DNA vouchers, whereas variation is described on the basis of further specimens of the series. After tissue collection, all specimens were preserved in 5% formalin or 70% ethanol. The specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM). A total of 20 tadpoles at stages 25–37 from three localities (Ranomafana National Park, Fierenana, and Vevembe; collection numbers ZSM 808/2004, ZSM 821/2004 and ZSM 833/2004; see Supplementary Table 1 for geographical coordinates, dates and collectors) were examined. Field numbers (ZCMV, Zoological Collection Miguel Vences) are also provided along with collection numbers (ZSM) for each tadpole series.

Morphological terminology follows ALTIG & MCDIARMID (1999) and developmental stages were determined according to GOSNER (1960). Measurements were taken by SG with a graduated ocular attached to a stereomicroscope except for the total length which was measured with a hand calliper, and the tail lengths of the largest specimens (from stage 27 on), and rounded to the nearest 0.1 mm. The landmarks are those shown in ALTIG & MCDIARMID (1999: 26, Fig. 3.1.), for others see GROSJEAN (2006). Abbreviations used in the description are the following: BH, maximum body height; BL, body length; BW, maximum body width; ED, maximum diameter of eye; LF, maximum height of lower tail fin; MTH, maximum tail height; NN, internarial distance; NP, naris–pupilar distance; ODW, oral disc width; PP, interpupilar distance; RN, rostral–narial distance; SS, distance from tip of snout to opening of spiracle; SU, distance from snout to beginning of upper tail fin; TL, total length; TMH, maximum tail muscle height; TMW, maximum tail muscle width; UF, maximum height of upper tail fin.

Preparation for SEM examination (with a JEOL JSM–840A) comprised dehydration in a graded ethanol series, critical-point drying (liquid carbon dioxide) and gold sputter surface coating. Terminology of buccal structures follows WASSERSUG (1976).

In addition to the detailed morphological and anatomical study of the above-mentioned specimens, we provide morphological data for additional specimens from Ranomafana, An'Ala, Ambohitsara-Tsitolaka, and Fierenana; Supplementary Table 1), and for these use the following abbreviations: A_1 , first upper keratodont row; A_2 , second upper keratodont row; A_{2gap} , medial gap in row A_2 ; A_3 , third upper keratodont row; A_4 , fourth upper keratodont row; A_5 , fifth upper keratodont row; $A_{1-5 den}$, density of the keratodonts in row A_{1-5} ; $A_{1-5 len}$, length of the row A_{1-5} ; $A_{1-5 num}$, number of keratodonts in row A_{1-5} ; DG, size of the dorsal gap of marginal papillae; DMTH, distance of maximal tail height from the tail–body junction; EH, eye height (measured from the lower curve of the belly); HAB, height of the point where the axis of the tail myotomes contacts the body (measured from the lower curve of the belly); JW, maximal jaw sheath width; MC, medial convexity of the upper jaw sheath; MCL, length of the medial convexity of the upper jaw sheath; MP, marginal papillae; ND, naris diameter; NH, naris height (measured from the lower curve of the belly); OD, oral disc; ODW, maximum oral disc width; P_1 , first lower keratodont row; P_2 , second lower keratodont row; P_3 , third lower keratodont row; $P_{1-3 den}$, density of the keratodonts in row P_{1-3} ; $P_{1-3 len}$, length of the row P_{1-3} ; $P_{1-3 num}$, number of keratodonts in row P_{1-3} ; (R/L), Right/Left; SBH, distance between snout and the level of maximal body height; SBW, distance between snout and the level of maximal body width; SE, snout–eye distance; SH, spiracle height (measured from the lower curve of the belly); SL, spiracle length; SMP, submarginal papillae; SV, spiracle–vent distance; TAL, tail length; TH, tail height at the beginning of the tail; THM, tail height at mid-tail; Thorn-pap, thorn-shaped papillae; TMH, tail muscle height at the beginning of the tail; TMHM, tail muscle height at mid-tail; LR, number of the lower rows of keratodonts; UR, number of the upper rows of keratodonts; VG, size of the ventral gap of marginal papillae; VL, vent tube length; following RANDRIANAINA et al. (2011). These measurements (Supplementary Table 2) were taken by RDR with a Zeiss StereoDiscovery V12 microscope with integrated digital camera connected to a computer.

Examination of the gut content was done by dissection through the belly skin. The gut was divided into two parts (front and rear) and the contents of each part were examined separately. The gut content of the front part of *Boophis picturatus* was washed to liberate the grains of sand contained therein (Fig. 7b). Three square plots of 9 mm² were photographed and the maximum width of each grain of sand within this area was measured.

Molecular analysis

DNA barcoding of tadpoles and frogs was based on a fragment of the mitochondrial 16S rRNA gene (THOMAS et al. 2005), which is known to be sufficiently variable among species of Malagasy frogs (VENCES et al. 2005). We amplified a fragment of ca. 550 bp, using primers 16Sar–L and 16Sbr–H from PALUMBI et al. (1991), or a shorter fragment of ca. 400 bp, using the newly developed specific mantellid primers 16S–Frog–L1 (CAT AAT CAC TTG TTC TTT AAA) and 16S–Frog–H1 (GAT CCA ACA TCG

AGG TCG). PCR was carried out with standard protocols (VENCES et al. 2005) and sequences resolved on automated sequencers. Sequences were preliminarily identified using BLAST searches against a near-complete database of sequences of adult Malagasy frog species. Results were subsequently verified by manually aligning and comparing sequences to the closest hits in the database. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and clearly less similar to all sequences from other species. DNA sequences were deposited in Genbank (accession numbers GU974671 – GU974709) and JF793633–JF793647.

Ecological field data and analysis

Within a study on stream tadpole communities in Ranomafana National Park (RNP) in the southern central east of Madagascar, species occurrence was recorded in 33 streams. To be first able to evaluate habitat features of importance for breeding stream site choice of frog species, the following habitat parameters of the streams and their surrounding forest were recorded: slope, width, depth, overhanging vegetation, and canopy cover of the stream; the density of shrubs, small trees, large trees, depth of leaf litter, slope of the forest floor, and canopy cover of the forest.

Within the stream, 30 m long sections were exhaustively sampled for tadpoles. The sampling process was separate for all available microhabitats within the section. These microhabitats were predefined as per their bottom substrates (rock, gravel, leaves, sand) as well as the stream velocity categories “fast” (obviously flowing) and “slow” (almost stagnant), resulting in eight different possible types of microhabitat. To be able to test for microhabitat preferences of the tadpoles, the frequency of the microhabitats available was recorded for each stream. Tadpoles sampling was conducted in the wet season of 2008 (January and February) and repeated in a subset of 13 of the streams during the dry season (July) of 2008. The dry season is assumed to be less suitable for frog reproduction in RNP than the wet season (ANDREONE 1996), which might be mainly caused by the low temperatures from June to August.

To analyse the breeding site choice of *Boophis picturatus* frogs, first a principal component analysis (PCA) was performed using all 11 habitat parameters of all 33 streams sampled during the tadpole community study. PCA was run on the correlation matrix in order to standardise for the influence of unequal variance. To evaluate data outliers and the linear interdependence of variables, box-plots and pair-plots (ZUUR et al. 2007) were used. As PCA requires a multinormality of data, box-cox-power-transformations (Box & Cox 1964) were applied when necessary. The significance of the PC loadings was assessed on the basis of the bootstrapped-eigenvalue method as suggested by PERES-NETO et al. (2003). The number of meaningful PCs was estimated by a scree plot (ZUUR et al. 2007). A multiple logistic regression (glm with binomial error structure) with the first three PCs as independent variables and the incidence of *B. picturatus* tadpoles as binary dependent variable was run to extract the key habitat features important for breeding site choice of these species. Independ-

ent terms and interaction terms were deleted sequentially from all full models based on Akaike's Information Criterion (AIC, BURNHAM & ANDERSON 1998) until the minimum adequate models were reached. The same procedure was applied to incidence data of further groups of tadpoles for comparison. These tadpoles belonged to two morphologically different groups of tadpoles: suctorial *Boophis* tadpoles and the funnel-mouthed tadpoles of the *Mantidactylus* subgenus *Chonomantis*, as well as on a third tadpole group that, similar to *B. picturatus*, is characterised by strong reductions of oral disc structures: the *Mantidactylus* subgenus *Ochthomantis*.

To evaluate tadpole distribution across microhabitats in the streams chosen for reproduction by this species, in a first step, plotting of tadpole abundance in the specific microhabitats was used. In order to quantify true preferences for microhabitats, Ivlev's electivity index (E, IVLEV 1961) was calculated. E is defined as $E = (r-p)/(r+p)$, with r being the proportions of the microhabitats used and p the proportion of microhabitats available. If a microhabitat is not used by a species, the respective E value is -1 (strong avoidance). In streams where a species is present with a number of specimens lower than the number of available microhabitats, this strong avoidance value will automatically be assigned to some microhabitats. However, this might not be a true avoidance, but be caused by very low abundance. Therefore, only those streams were chosen for calculations in which at least 8 specimens of *B. picturatus* were found to provide the probability of having one individual in every microhabitat. One-way ANOVA was performed to test for differences in microhabitat use.

Description of the tadpole

External morphology

The external morphological description is based on a DNA voucher specimen at stage 26 (ZSM 821/2004, field number FG/MV 2002.1835, BL 11.7 mm) from Ranomafana National Park. Because a part of the tail was removed for DNA barcoding, information on vent tube, tail fin and tip of tail was taken from another individual at stage 27 (ZSM 833/2004, TL 37.1 mm, BL 14.6 mm), as were measurements for the calculation of ratios and for drawings. Another specimen at stage 25 (ZSM 808/2004, TL 18.3 mm, BL 7.7 mm) was used for the drawing of the oral disc because it was the least closed of all specimens. Measurements of six DNA voucher *B. picturatus* tadpoles from different localities are provided in Supplementary Table 2.

In dorsal view (Fig. 1a), body elliptical, widest in the middle of the coiled intestine, snout nearly rounded. In profile (Fig. 1b), body depressed, BW 138% of BH, snout rounded. Eyes moderately large, ED 14% of BL, bulging, not visible in ventral view, positioned more dorsally than strictly dorsolaterally and directed slightly more laterally than strictly dorsolaterally and anterolaterally. Nares elliptical, large, rimmed with a very slight posteromedial projection, positioned almost dorsally and directed slightly more laterally than strictly anterolaterally and more dorsally than dorsolaterally, closer to snout than to pupils, RN 90% of NP; NN 63% of PP. Spiracle sinistral, slightly conical, small, attached to body wall but its tip free, laterally po-

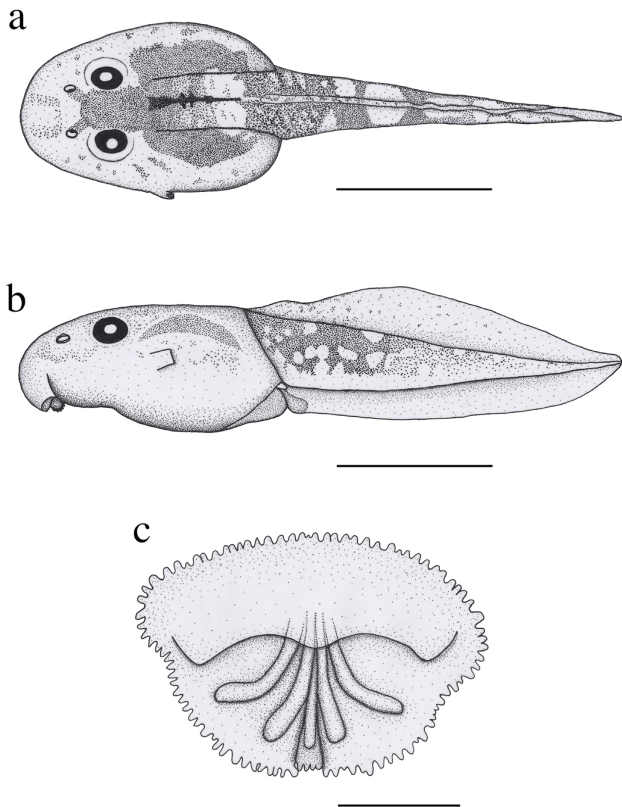


Figure 1. Dorsal (a) and lateral (b) views of the tadpole of *Boophis picturatus* (ZSM 833/2004, stage 27, scale bar 10 mm), and oral disc (c) of *Boophis picturatus* (ZSM 808/2004, stage 25, TL 18.3 mm, BL 7.7 mm, scale bar 1 mm). Note that the upper labium of the oral disc has been artificially lifted to expose the suprarostal.

sitioned, oriented posteriorly, slightly closer to end of body than to tip of snout, SS 67% of BL; spiracular opening at the height of the apex of caudal myotomes. Tail musculature moderate, TMH 66% of BH and 65% of MTH, TMW 46% of BW, gradually tapering, almost reaching tail tip. Upper tail fin of moderate size, UF 32% of MTH, not extending on to body, SU 89% of BL, beginning at the dorsal junction of caudal muscle and body, slightly convex although it increases abruptly at the beginning of the proximal sixth; lower fin moderately shallow, LF 19% of MTH, following the caudal muscle; point of maximum height of tail located just before halfway of tail, MTH 101% of BH, tail tip irregularly rounded. Coiled gut very short (three loops), filled with sand and sediments. Vent tube moderately large, dextral to ventral fin, tubular, directed posteriorly, attached to ventral tail fin except the tip, opening dextral, bevelled, directed posterodorsally. No lateral line organs or glands visible.

Oral disc (Fig. 1c) positioned ventrally, directed posteriorly and slightly ventrally, not emarginated, of moderate size, ODW 30% of BL and 52% of BW. Upper labium bowl-shaped, slightly concave, covering most part of the mouth opening when at rest. Lower labium projecting anteroventrally, the medial part folded longitudinally in a small space forming a depression in which lies a straight low-profile ridge over most of the length of the labium; two other pairs of such ridges lie laterally to the medial one, these ridg-

es are curved towards the sides of the lower labium. An uninterrupted row of small and round marginal papillae around the oral disc. No submarginal papillae. No denticulate papillae. No keratodonts. Upper jaw sheath shallow, convex medially, bearing no serrations, and not keratinised; lower jaw sheath as a short rectangle, slightly concave, ending abruptly laterally, bearing no serrations and very few keratinised.

Colour in preservative: Tegument transparent, unstuck from all underlying organs laterally and anteriorly to the coiled gut. Upper side (coiled gut, dorsalis trunci muscles, areas between the eyes, between the nares and anteriorly to them) coloured by densely arranged dark brown dots contained in underlying tissues. Two unpigmented areas just anterior to the dorsal junction between tail and body. Upper part of flanks very little pigmented with very small dots; lower parts of flanks immaculate. Ventral side immaculate and transparent, the coiled gut, pericardium, gills and hyoidian muscles are very clearly visible. Anterior half of the upper and lower parts of caudal muscle densely covered with relatively large dark brown dots, anterior half of the ventral part of caudal muscle immaculate, producing some very clear white spots; posterior half with the same pattern, but the density and size of the dots decrease quickly. Upper fin with some dots in the anterior third, the rest almost devoid of pigmentation, lower fin immaculate.

In life, all *Boophis picturatus* tadpoles have the same colouration and pattern, and are predominantly yellowish brown (see example T 08/0076 in comparison to *B. majori* ZSM 37/2007 – ZCMV 1369; Fig. 3). Dark brown blotches distributed irregularly, condensed over the brain and on the dorsal part of the tail muscle. Silvery patches scattered over the skin, mainly on the dorsum. Ventrally, oral disc and gular regions transparent; the branchial region reddish, rendering the beating heart and gills visible. The abdominal surface translucent with few silvery patches, and the regular spiral-shaped intestinal coils well visible. Tail musculature transparent with a yellowish zone and scarce light brown dots that are consolidated to form patches. Fins transparent. The dorsal fin has a patched pattern whereas the ventral fin is free of pigmentation. The lateral space under the skin is well visible. In preservative, tadpoles lose the silvery iridiophoric pigment; only the brown melanophoric pigments are left.

Variation: TL and BL of ten other tadpoles at stages 25–37 from the batches ZSM 808/2004 and ZSM 833/2004 are 22.2–39.3 mm and 8.2–14.1 mm respectively. The ratios vary in the following proportions: BW 119–135% of BH; ED 14–18% of BL; RN 64–106% of NP; NN 50–62% of PP; SS 49–57% of BL; TMH 48–63% of BH; TMH 46–60% of MTH; TMW 37–54% of BW; UF 36–41% of MTH; SU 79–108% of BL; LF 21–31% of MTH; MTH 97–117% of BH; ODW 25–35% of BL; ODW 38–51% of BW.

Eight other tadpoles at stage 25 from the batch ZSM 808/2004 have an average TL of 15.9 ± 2.5 mm (12.3–18.7, $n = 5$) and an average BL of 6.0 ± 1.3 mm (4.4–7.2, $n = 8$).

The naris can be at an equal distance from snout to pupil, or closer to the snout than to the pupil (RN/NP 64–116%). The spiracular opening can be at an equal distance from the snout to the body terminus. The caudal spots are less numerous on certain specimens, and the dots on the caudal muscle can extend to the tip of the tail.

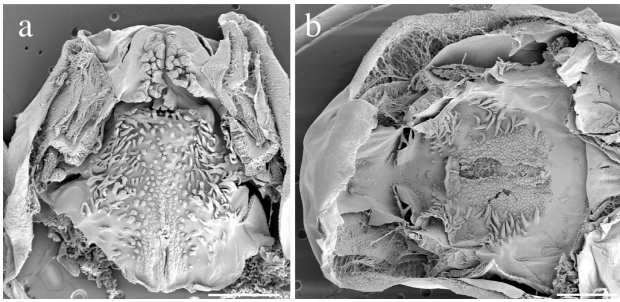


Figure 2. Buccal floor (a) and roof (b) of the tadpole of *Boophis picturatus* (ZSM 808/2004, stage 25, BL 5.4 mm, scale bar 1 mm).

Buccopharyngeal anatomy

The description of the buccal features is based on a specimen from the batch ZSM 808/2004 at stage 25 (BL 5.4 mm). Buccal floor (Fig. 2a). – Buccal floor trapezoid, as large as long. Prelingual arena as a longitudinal slit, surrounded by numerous densely arranged protuberances, pustulate on top (composed of tightly-spaced elements as protuberances of different sizes, flaps and even papillae that could be an agglomeration of papillae of the same kind as the pustulate buccal floor arena papillae); these protuberances form a truncated triangle whose tip is anterior, the largest of these protuberances anterior; at the posterior end of the arena lies a pair of flaps with jagged and pustular edges, the infralabial papillae, directed posterodorsally. Tongue anlage hidden in a cavity, seemingly very narrow and elongate, bearing a pair of small lingual papillae. Buccal floor arena trapezoid, delimited by less than one hundred tightly-spaced buccal floor arena papillae with the most anterior ones lying lateral to the tongue anlage and the most posterior ones near the free end of the ventral velum; the external buccal floor arena papillae are the largest (espe-

cially those in front of the buccal pockets), some are bifid, but always smooth, then their size decreases toward the centre of the arena with their tips becoming pustulate. The interior of the arena is occupied anteriorly by these smaller pustulate papillae, and the centre and the posterior parts are occupied by large and very densely arranged pustules that form a pustulate longitudinal ridge. Buccal pockets straight, fine, slightly oblique, not perforated, closer to tongue anlage than to medial end of the ventral velum; pre-pocket papillae mixed with the buccal floor arena papillae and not distinct from them. Ventral velum with spicular support, slightly waving, bearing no projection, a very deep median notch of about 20% of the buccal floor length, reaching the medial longitudinal pustulate ridge and bearing a pustulate flap on one side; secretory pits not visible. Glottis not observed (may have been damaged during dissection). Branchial baskets not observed.

Buccal roof (Fig. 2b). – Prenarial arena trapezoid; prenarial ridge missing or limited to a pair of small and smooth protuberances anterolateral against the anterior wall of the arena; interior smooth with only a few tiny pustules. Choanae fine, slightly anteromedially oriented; anterior wall bearing a fine prenarial papilla on its external half, orientated posteromedially above the opening, the edge of the anterior wall pustulate; narial valve smooth except for the pustulate tip, barely larger than the anterior wall. Post-narial arena with eight pustules of different forms and not uniformly arranged; a bunch of long and fine, sometimes bifid papillae (probably homologous to postnarial papillae) of the same type as the buccal floor and roof arena papillae. Median ridge triangular, low and pustulate on its free edge and on its posterior side. Lateral ridge papillae absent. Buccal roof arena round, delimited by 25–30 buccal roof arena papillae on each side of the same type as the buccal floor arena papillae, oriented roughly medially; interior of the arena densely covered with large pustules. Posterolateral ridges absent. Glandular zone not observed. Dorsal velum damaged during dissection.

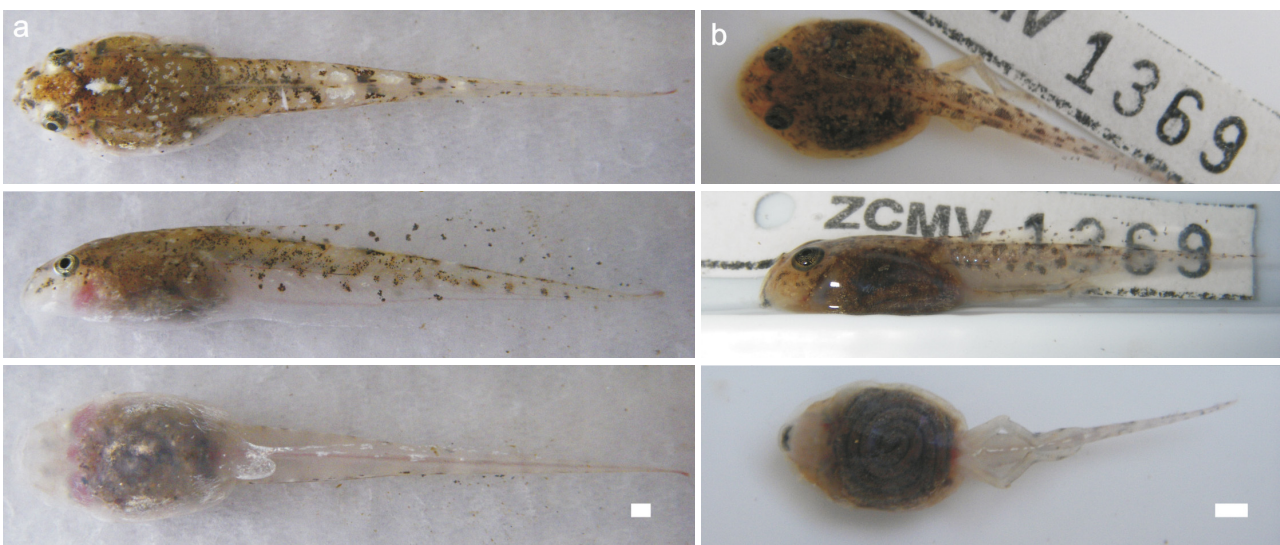


Figure 3. Photographs of live tadpoles of *Boophis picturatus* (T 08/0076 – uncatalogued) from Fompohonina IV (Ranomafana National Park) (a) and *Boophis majori* (ZSM 37/2007 – ZCMV 1369) from Ankidoanavo (Ranomafana National Park) (b). The scale bars represent 1 mm.

Genetic variation in *Boophis picturatus*

The available DNA sequences, all of the mitochondrial 16S rRNA gene, fell into two major groups, which were separated from each other by 3.0–3.9% uncorrected sequence divergences. The first of these clades contained samples from the Ranomafana area, including the Ambohitsara forest, and from Vevembe, in the southern central east of Madagascar. The second clade contained samples from An'Ala and Fierenana in the northern central east. Within the two clades, samples were uniform, with divergences of 0–0.5%, and with identical haplotypes shared by the populations of Ambohitsara and Ranomafana within the first clade and by An'Ala and Fierenana in the second clade. The single available sequence from Vevembe was also very similar to those from Ranomafana, differing by only 0.3–0.7%.

In the original description of *B. picturatus*, colour differences of adult specimens from the Ranomafana area (Vohiparara), as compared to those from An'Ala and Andasibe, were reported (GLAW et al. 2001), which would be in agreement with the assignment of these populations to two different genealogical lineages. In particular, specimens from Vohiparara had a middorsal stripe and showed less colour variability. However, recently collected adult material from various localities in the Ranomafana area (data not shown) indicates that these purported differences are not constant, and that there are no consistent differences in adult colouration between the two conspecific lineages of *B. picturatus*.

Variability of external morphology of *B. picturatus* tadpoles from different localities

Preserved *Boophis picturatus* tadpoles from the Ranomafana National Park, An'Ala, Ranomena, and Ambohitsara-Tsitolaka show the typical morphology of the species, making it easy to distinguish them from *B. majori* (ZSM 397/2008 – ZCMV 2641) tadpoles (Supplementary Table 4). No clear and consistent differences were found between tadpoles belonging to the two genealogical lineages identified by the molecular data. All tadpoles were characterised by the transparency of their integument, rendering most of their inner organs clearly visible. Their large nares are situated at about halfway between snout and eyes or closer to the snout, but they are always closer to the snout in *B. majori*. They also have a larger vent tube in comparison with *B. majori* (Supplementary Table 3). Only the tadpole from Ambohitsara appears to differ by having a smaller spiracle than the others. A variation in the configuration of the opening of the spiracle in numerous tadpoles (ZSM 808/2004, ZSM 0608/2007 – ZCMV 5189, ZSM 839/2004, ZSM 172/2008 – ZCMV 3807, ZSM 1711/2007 – ZCMV 3406, ZSM 196/2007 – ZCMV 5050, ZSM 77/2008 – ZCMV 4941) was observed. Tadpoles from Fierenana, Ranomena and some from the Ranomafana National Park were found having an inner wall that was free from the body and an aperture opening posteriorly; some tadpoles from the Ranomafana National Park and An'Ala, and those from Ambohitsara, also have an inner wall free from body, but with an aperture opening later-

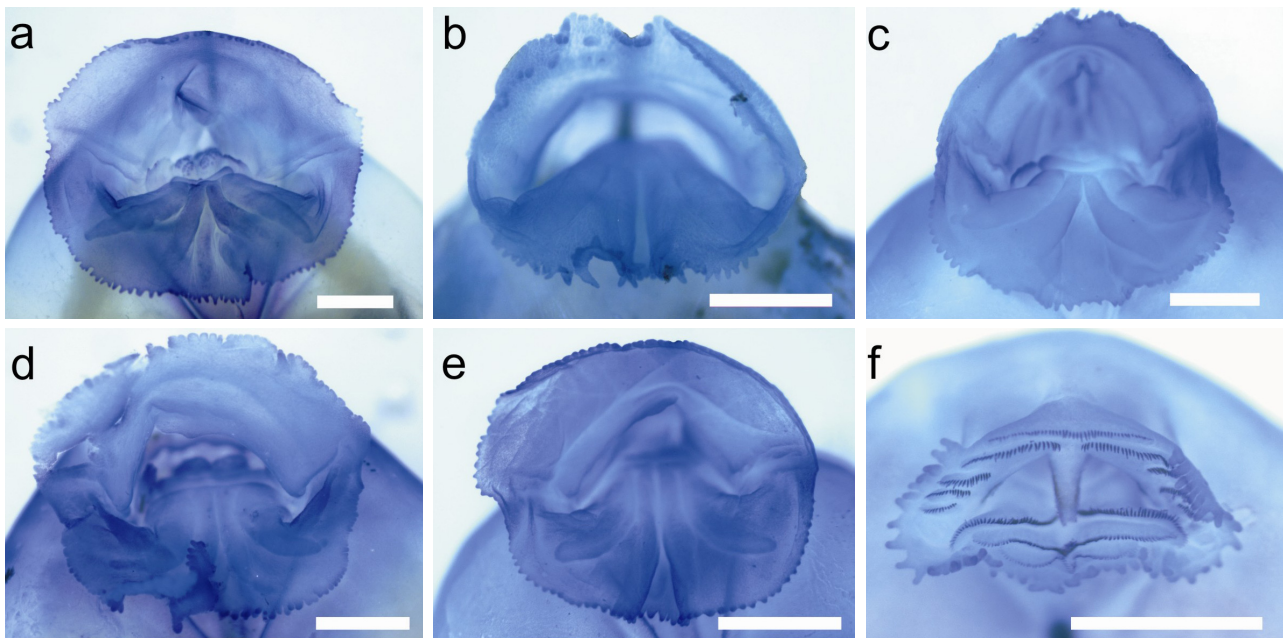


Figure 4. Photographs of the oral disc of five preserved tadpoles of *Boophis picturatus* discussed in this paper and one *Boophis majori* (stained with methylene blue). a) from Fierenana (ZSM 839/2004 – FGMV 2002.1664), b) from An'Ala (ZSM 1711/2007 – ZCMV 3406), c) from the Ranomafana National Park (ZSM 196/2007 – ZCMV 5050), d) from Ranomena (ZSM 172/2008 – ZCMV 3807), e) from Ambohitsara-Tsitola (ZSM 77/2008 – ZCMV 4941), f) *Boophis majori* from Vohiparara (ZSM 397/2008 – ZCMV 2641). The scale bars represent 1 mm.

Table 1. Characteristics of tadpoles in which gut contents were observed

ZSM	Species	Stage	BL (mm)	TL (mm)	Intestine length (mm)
680/2007	<i>B. picturatus</i>	27	12.9	22.0	110.0
680/2007	<i>B. picturatus</i>	26	13.6	23.2	106.5
172/2008	<i>B. picturatus</i>	27	14.5	23.3	89.6
953/2007	<i>B. majori</i>	27	10.5	16.2	144.3

ally instead of posteriorly, and an intermediate configuration was discovered in tadpoles from Fierenana, Ranomena and in some others from An'Ala.

All observed *B. picturatus* tadpoles (ZSM 839/2004–FGMV 2002.1664, ZSM 1711/2007 – ZCMV 3406, ZSM 196/2007 – ZCMV 5050, ZSM 172/2008 – ZCMV 3807, ZSM 77/2008 – ZCMV 4941) have a large, ventral, and non-emarginated oral disc with complex folds in the lower labium and lack all the typical keratinised components of a generalized oral disc (Supplementary Table 4; Fig. 4). Opening the oral disc maximally allows seeing a characteristic structure with the densely packed pustulate protuberances of the prelingual arena (Fig. 5).

Gut content

Three non-voucher tadpoles of *B. picturatus* (ZCMV 4017 – ZSM 680/2007) from the Ranomafana National Park and one *B. majori* (ZCMV 5398 – ZSM 953/2007) from Ranomena were dissected for gut content analysis (Fig. 6). *B. picturatus* tadpoles have a rather short intestine in com-

parison with *B. majori* (a mean of 100 mm vs. 144 mm; Table 1). As previously reported by ALTIG & MCDIARMID (2006), the whole intestine of *Boophis picturatus* is full of grains of sand, but the front part contains also organic matter (Fig. 6). External observation of all live *B. picturatus* tadpoles that we captured in the Ranomafana National Park in 2006 and 2007 reveals a similar situation. Analysis of the sand grains in the *B. picturatus* intestine shows a size range from 0.1 to 1.4 mm (Table 2). The general observation indicates a higher number of sand grains in the rear part except in the size range <0.2 mm. The majority of sand grains were between 0.2–0.4 mm, and those larger than 1 mm were rare. As is shown in Fig. 7a, in natural condition, the rear part of the intestine is exposed ventrally, with many sand grains being visible through the skin, which suggests a low quantity or even an absence of organic matter in the rear part after the digestion process. On the other hand, the whole intestine of *B. majori* tadpoles is full of organic matter, with many small sand grains <0.2 mm, but no prevalence of large sand grains (Table 2), and the rear part of the gut is not “cleaned out” after the digestion process (Fig 6e).

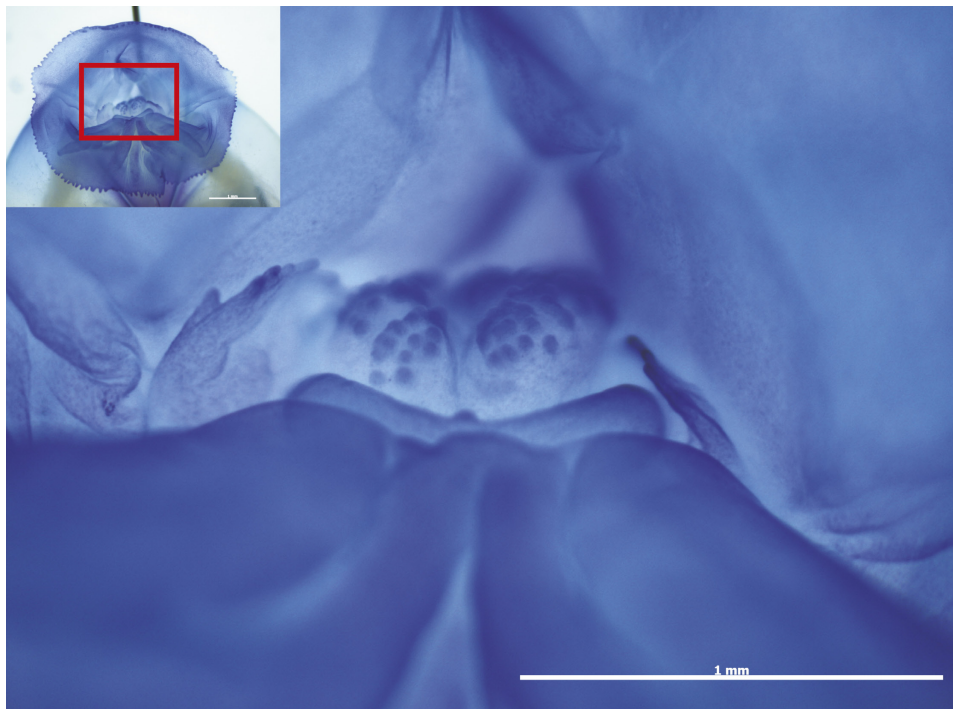


Figure 5. Photographs of the oral disc of the tadpole of *Boophis picturatus* (ZSM 808/2004 – FGMV 2002.1664) stained with methylene blue, showing the densely packed pustulate protuberances of the prelingual arena.

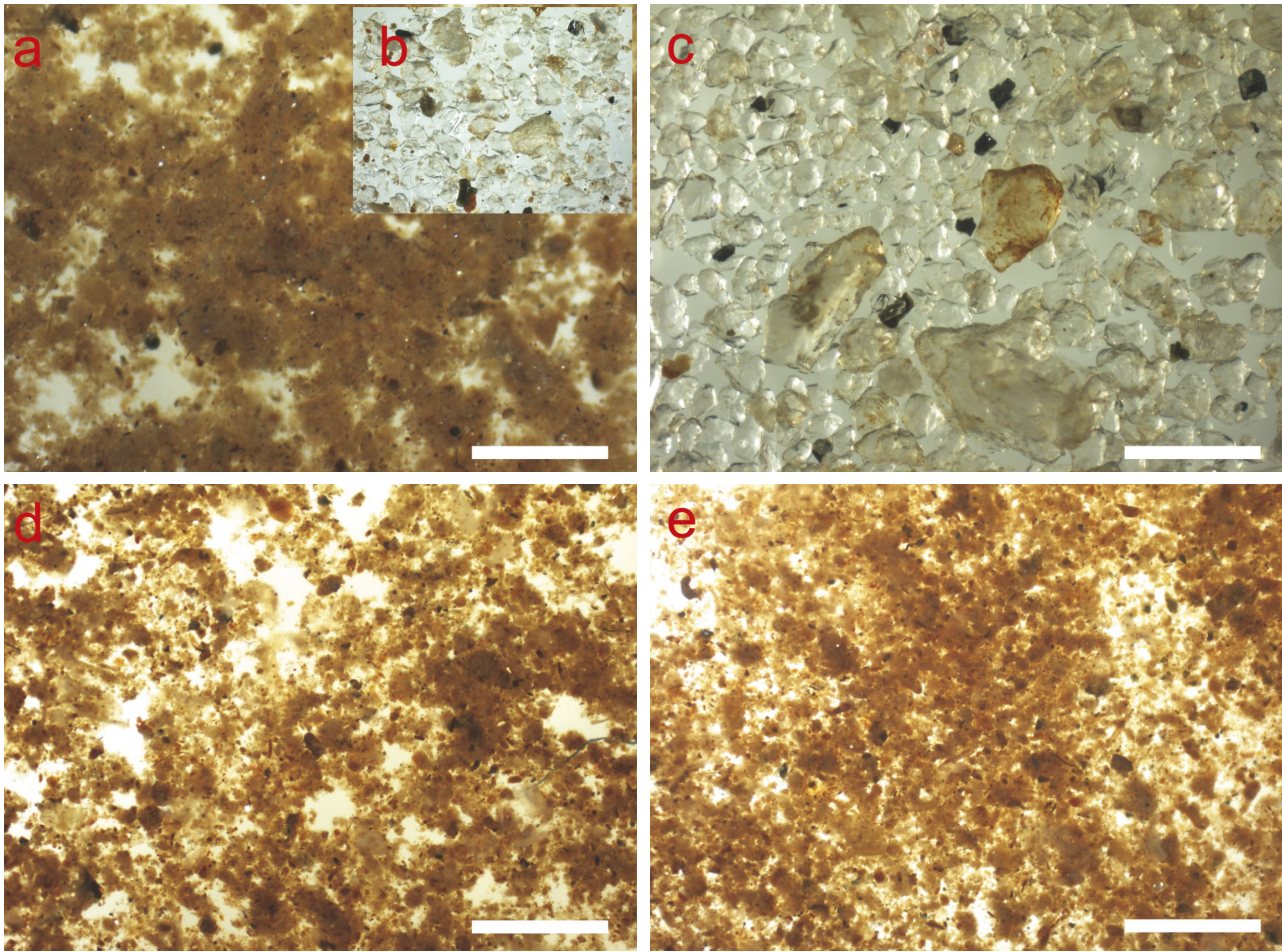


Figure 6. Photographs of the gut content of the tadpole of *Boophis picturatus* (ZSM 172/2008 – ZCMV 3807) and *Boophis majori* (ZSM 953/2008 – ZCMV 5398). a) organic matter in the front part of the *B. picturatus* intestine, b) sand grains in the front part of the *B. picturatus* intestine, c) sand grains in the rear part of the *B. picturatus* intestine, d) organic matter in the front part of the *B. majori* intestine, e) organic matter in the rear part of the *B. majori* intestine. The scale bars represent 1 mm.

Ecology

During the wet season of 2008, 33 streams in the RNP were sampled for tadpoles. In 15 of these streams, tadpoles of *Boophis picturatus* were found. Compared to other species in that area (GROSJEAN et al. 2011; RANDRIANIINA et al. 2011), tadpoles of this species were moderately abundant. There were 193 specimens in all 15 streams (range 1 to 35, mean of 13 specimens per stream). The same was true for the dry season, during which 6 out of 13 sampled streams contained tadpoles and the total number of 56 tadpoles was derived from 1 to 23 specimens per stream with a mean of 9. If *B. picturatus* tadpoles were observed in a stream in the dry season, tadpoles of this species were sampled in the wet season in the same stream, too. Also, streams without these tadpoles in the dry season had not harboured them in the wet season before either. Additionally, clutches of eggs that could be assigned to this species by DNA barcoding were found in and on two streams, respectively. One clutch, consisting of about 50 eggs (Fig. 8; ZCMV 9849), was attached to a twig under the water surface. Another clutch (ZCMV 9850) was attached to a broken-off stick lying on a rock just above the water surface.

To ordinate habitat characteristics of the streams available to *B. picturatus*, a PCA on the habitat variables of the stream and the surrounding forest was performed. It outlined three principal components that together explain 67.6% of the variation in the data. Based on the loadings of the PC and the results of the bootstrapped eigenvector method (PERES-NETO et al. 2003), we identified the following habitat variables being well represented: PC1 (33.9%) positive: slope and canopy cover of forest and stream, overhanging vegetation; negative: width and depth of the stream. The strongest contributors to PC2 (18%) were positive: incline of the stream and the number of shrubs in the forest; negative: number of trees and leaf litter depth, to PC3 (15.7%) were positive: number of small trees and shrubs in the forest and overhanging vegetation.

Using a multiple logistic regression with a stepwise deletion of non-significant terms, PC1 and PC3 were identified as not being of importance for breeding site choice (= presence of tadpoles) of *B. picturatus* frogs, as both terms were deleted from the full model (Fig. 9). PC2 remained as a factor of highly significant importance for breeding site choice (multiple logistic regression with binomial error structure; residual deviance: 31.3 on 31 d.f., $p < 0.01$; Fig. 9).

Table 2. Results of gut content examination, showing the number of sand grains by size classes recovered from the intestines of *Boophis picturatus* (ZSM 680/2007) and *Boophis majori* (ZSM 953/2007) tadpoles for each of the three square plots.

Sand grain diameter	< 0.2 mm	0.2–0.4 mm	0.4–0.6 mm	0.6–0.8 mm	0.8–1 mm	> 1 mm
ZSM 680/2007 (stage 27)	14	51	10	2	–	–
(front)	17	52	11	1	–	–
	13	73	8	2	–	–
ZSM 680/2007 (stage 26)	13	94	29	4	–	–
(front)	6	95	29	3	–	–
	5	69	21	3	3	1
ZSM 953/2007 (stage 27)	many	–	–	–	–	–
(front)						
ZSM 680/2007 (stage 27)	10	95	20	3	–	–
(rear)	6	85	26	3	1	–
	7	76	20	5	1	–
ZSM 680/2007 (stage 26)	3	40	24	3	3	–
(rear)	17	52	20	7	3	2
	6	73	23	6	3	1
ZSM 953/2007 (stage 27)	many	–	–	–	–	–
(rear)						

As PC2 was negatively correlated with *B. picturatus* tadpole incidence, this species prefers streams with a low incline in forest areas with a high number of trees but fewer shrubs and thick leaf litter.

Within the streams, *B. picturatus* tadpoles were unequally distributed across all microhabitats (Fig. 10). By far most of the specimens were found on sandy substrate, mainly in non-stagnant parts of the stream. Microhabitats with accumulations of dead leaves also harboured *B. picturatus* tadpoles, whereas almost none were found above stony substrates (rock, gravel). A true preference or avoidance can only be evaluated taking the availability of different microhabitats into account, and there was no significant preference for any kind of microhabitat. ANOVA

showed that the occurrence of *B. picturatus* tadpoles depended on the microhabitat, as the overall model was significant (ANOVA, $F_{7,56} = 4.2$, $p_{\text{model}} < 0.001$). In detail, it revealed that even the sand-fast microhabitat, where by far most of the *B. picturatus* tadpoles were found, was only used as often as a random tadpole distribution among all microhabitats would suggest. ($p_{\text{SandFast}} = 0.65$). However, for interpretation it needs to be noticed that sand-fast is by far the most commonly available habitat. The same is true for leaves-slow ($p_{\text{LeavesSlow}} = 0.42$) and sand-slow microhabitats ($p_{\text{SandSlow}} = 0.13$). All other microhabitats were used by this species significantly less often than expected if tadpoles would be randomly distributed among microhabitats (all $p < 0.01$).

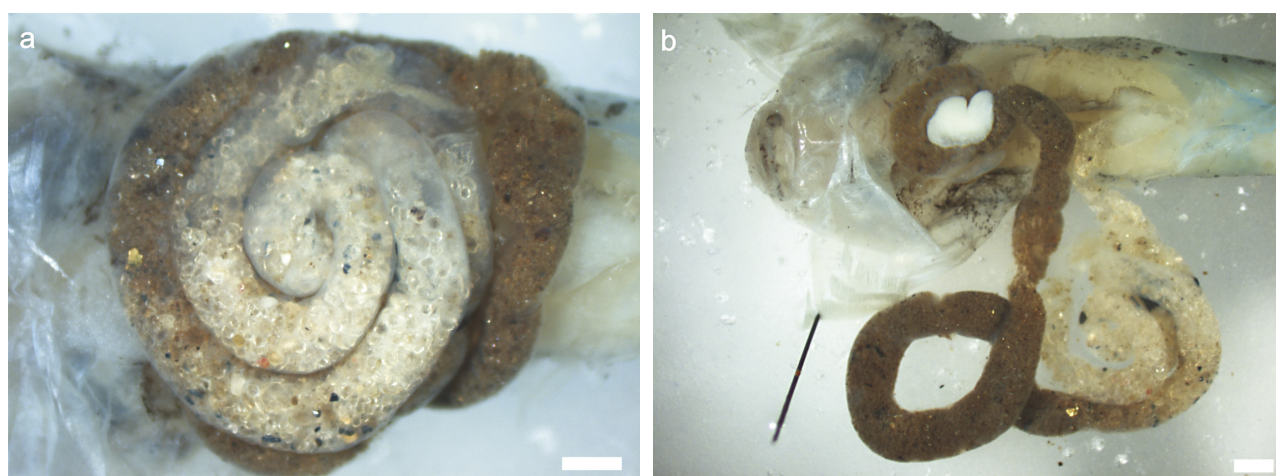


Figure 7. Photographs of the gut of the tadpole of *Boophis picturatus* (ZSM 172/2008 – ZCMV 3807) showing the different states in the front and the rear parts, a: coiled, b: uncoiled. The scale bars represent 1 mm.



Figure 8. Clutch of *Boophis picturatus* from a site named Fompohonina III. A few eggs were sampled from the clutch that was attached to a twig under water and identified by DNA barcoding (field number ZCMV 9849).

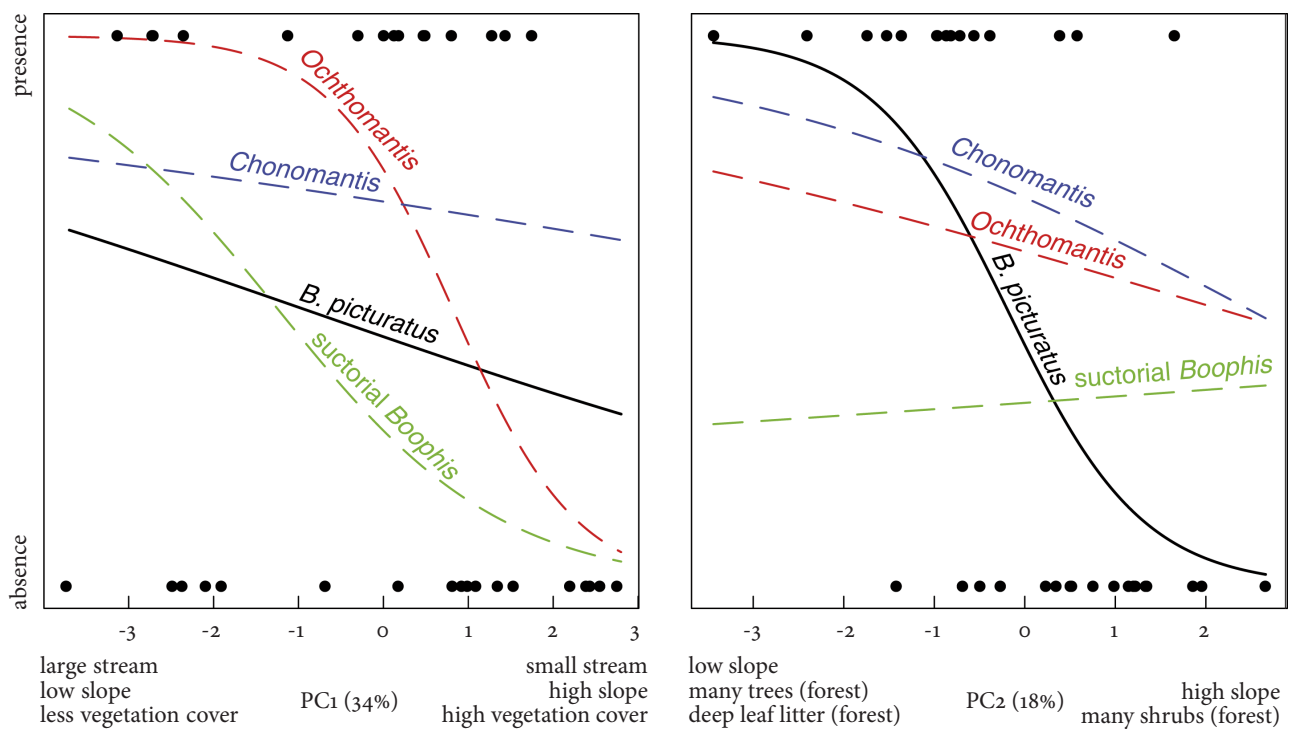


Figure 9. Breeding site choice of *Boophis picturatus* represented by incidence of tadpoles in streams of the RNP along the PC1 and PC2 gradients. Each symbol represents a stream; the regression line of the logistic regression is plotted as a black line. The logistic regressions of three other abundant tadpole groups are shown for comparison.

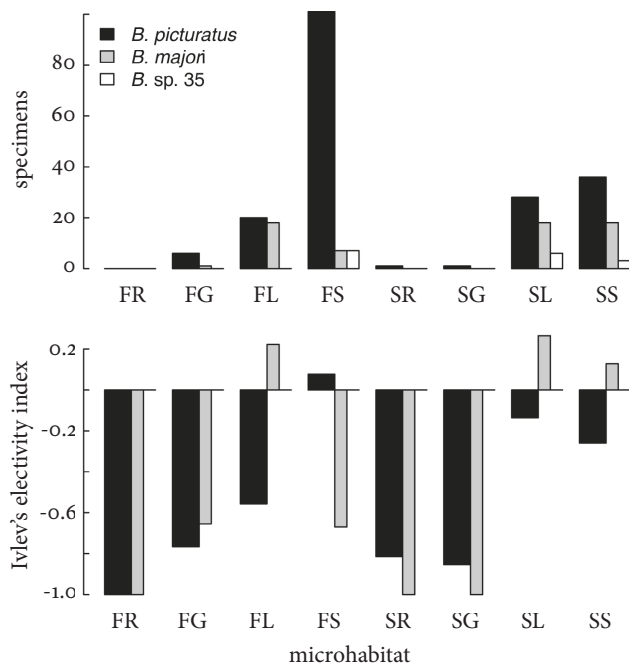


Figure 10. Distribution of *B. picturatus* tadpoles among eight microhabitats in the streams of the RNP, and distribution of *B. majori* and *B. sp. 35* tadpoles for comparison. Given are (upper graph) the absolute abundance values from all observations ($n=15$ streams) during the wet season of 2008 and (lower graph) calculated preference / avoidance for each microhabitat, taking microhabitat availability into account and using Ivlev's electivity index (E; IVLEV 1961). Only streams with at least eight *B. picturatus* specimens ($n=10$ streams) were used for the latter. No electivity index was calculated for *B. sp. 35*.

Discussion

Morphological comparisons

The tadpole of *Boophis picturatus* shows a highly derived and peculiar oral morphology among Mantellidae that is also appears to be unique amongst anuran larvae. It is devoid of keratinised structures (jaw sheaths and keratodonts) and includes unique labia of which the lower one is a flap of skin bearing radiating longitudinal low ridges and folds medially into a resting position. Most other *Boophis* have rather generalized oral morphologies with keratinised jaw sheaths and keratodonts being present and specializations limited mainly to the axis towards a suctional oral disc with increased numbers of keratodont rows and labial papillae (e.g., BLOMMERS-SCHLÖSSER 1979, RAHARIVOLOLOLONIAINA et al. 2006, RANDRIANIAINA et al. 2009a, b). Although a trend towards a reduction of keratinised structures is apparent in the closely related *B. majori* and *B. sp. 35* (as *B. sp. aff. majori*; SCHMIDT et al. 2008), the tadpole of *B. picturatus* is to date the only known tadpole in the mantellid subfamily Boophinae that is totally devoid of such structures.

ALTIG & MCDIARMID (2006) described the external morphology of the tadpole of *Boophis picturatus* and provided some information about its buccopharyngeal anatomy. Not unexpectedly, our description matches in all aspects the one published by ALTIG & MCDIARMID (2006), except that we found the lower jaw being very slightly

keratinised, a difference that can be attributed to a different appreciation of the same state by different observers. Even if the tadpole drawn by ALTIG & MCDIARMID seems more elongate, the proportions of their and our specimens agree, with our tadpole chosen for illustration having a particularly short tail. The colour pattern and the buccopharyngeal features described by ALTIG & MCDIARMID (2006) also agree very well with those of our specimens. Nevertheless, these authors described "Two large lingual papillae with large, clavate, papillate heads (...) arranged transversely on the oval tongue anlage and bordered posteriorly by four smaller but similarly structured papillae". These structures are indeed present in the buccal floor of our dissected specimen, but we rather interpret them as posterior infralabial papillae due to their position relative to the lower jaw. The tongue anlage, although not very clearly visible, seems to lie in a depression, being very narrow, and bearing a pair of small, simple and smooth lingual papillae.

Tadpoles of the genus *Boophis* are mostly pond to stream dwellers with classic adaptations to these habitats such as a strongly depressed body, low caudal fins, large oral disc with numerous keratodont rows and multiple rows of tightly spaced small marginal papillae of the torrent dwelling tadpoles (RAHARIVOLOLOLONIAINA et al. 2006, RANDRIANIAINA et al. 2009b). While the species of another large mantellid genus, *Mantidactylus*, show a very high ecomorphological diversity of tadpoles, very few cases of divergence from this pattern are known from among the members of the genus *Boophis*. Besides *B. picturatus*, only two other tadpoles show derived oral disc structures, i.e., those of *B. majori* and *B. sp. 35* (GLAW & VENCES 2007, SCHMIDT et al. 2008). Molecular analyses suggest that these three species belong to the same clade (VIEITES et al. 2009). These results indicate that the tadpoles of this lineage have a derived morphology and that their evolutionary history took a different course than that of the other members in the genus *Boophis*. The adult morphology of these species is very inconspicuous and has no obvious derived features compared to other *Boophis*. Although the tadpoles of *B. majori* and *Boophis sp. 35* possess derived oral structures (much more derived in the first species than in the second), these are very different from those of the larvae of *B. picturatus*. The most remarkable characters are the presence of a strong medial convexity on the upper jaw sheath (particularly long and narrow in *B. majori*), the presence of a few large marginal and submarginal papillae that are arranged in a radial pattern, and the folding of the lower labium at rest. Furthermore, the tadpole of *B. majori* has an interrupted upper keratodont row on the upper labium, which is extremely rare in anuran larvae. Other than that, the morphology of these two species is "classic" (note that certain ratios given for the tadpole of *B. majori* by SCHMIDT et al. [2008] seem to be faulty, such as BW 84% of BH or ED 29.3% of BL).

The unique tadpole of *B. picturatus* fits none of the ecomorphological guilds proposed by ALTIG & JOHNSTON (1989). It is exotrophic and lotic, but cannot be assigned to any of the lotic categories. The closest guilds are (1) the "psammonic" guild defined for the tadpole of *Otophryne robusta*, which lives buried in sand but feeds on micro-organisms living in the sand by passive filtering, has no kera-

todonts but possesses hypertrophied serrations on the jaw sheaths (WASSERSUG & PYBURN 1987), and (2) the “psammonektonic” guild recently defined for the lentic tadpoles of the genus *Scaphiophryne* (MERCURIO & ANDREONE 2006), which possess keratinised jaw sheaths but no keratodonts and which absorb sand particles and detritus during the day with half the body buried in sand and the body and tail axis at 35–40°, and swim through the water column filtering suspended particles at night. Hence, the tadpole of *B. picturatus* probably deserves the creation of a new guild within the exotrophic lotic group. In contrast, tadpoles of *B. majori* and *Boophis* sp. 35 likely belong to the “lotic clasping” guild of ALTIG & JOHNSTON (1989).

The clade containing *B. majori*, *Boophis* sp. 35 and *B. picturatus* also contains *B. miniatus*, *B. feonnyala* and further undescribed species whose tadpoles are not known at present. The knowledge of the morphology of the tadpoles of these species and especially of their oral specializations will certainly shed light on the evolutionary tendency of the ecology and morphology of the tadpoles of this lineage.

Ecological comparisons

Although the oral morphology is highly different between *B. majori* and *Boophis* sp. 35 on the one and *B. picturatus* on the other hand, these three species are closely related (VIEITES et al. 2009), live in the same kind of habitat (slow-flowing streams with a sandy bottom substrate), and from a preliminary observation of their gut contents they seem to ingest the sand particles for aiding digestion (ALTIG & McDIARMID 2006, SCHMIDT et al. 2008, this paper) even if at different proportions. Comparisons of the gut contents of *B. picturatus* and *B. majori* show a difference as to the presence of many large sand grains in *B. picturatus*. *B. majori* instead ingests many small sand grains of about 0.2 mm, with a maximal size of 0.5 mm.

In the rainforest of the Ranomafana National Park, the tadpoles of *Boophis picturatus* occur throughout the year with clearly higher abundances in the wet season. The same has also been noted for other species in this region (e.g., RANDRIANIINA et al. 2011, R.-D. RANDRIANIINA unpublished data). Compared to these and other species from the RNP region, *B. picturatus* tadpoles occur in moderately high abundance in a moderately large number of streams. Interestingly, streams typically harbouring *B. picturatus* tadpoles are characterised by different habitat variables of the stream and the surrounding forest than the ones often used by other prominent groups (Fig. 10). Habitat choice data are also available to us for the morphologically very different suctorial tadpoles of some species of *Boophis*, and for the tadpoles of the *Mantidactylus* subgenus *Ochthomantis*, which are characterised by a strong reduction of oral disc structures and therefore remotely comparable to *B. picturatus*. However, the streams chosen by these frogs for reproduction, and thus inhabited by their tadpoles, are characterised by different habitat characteristics than in *B. picturatus* (RANDRIANIINA et al. 2011). While all three groups choose streams with low inclines and therefore slow water currents, the main difference is that trees and leaf litter seem to be important for *B. picturatus* whereas it is more the size of the stream that

is important for the two other groups. A further group of specialized tadpoles with reduced keratinised oral structures is the funnel-mouthed tadpoles of the *Mantidactylus* subgenus *Chonomantis*. These species do not choose their breeding habitats according to parameters that would be comparable to those applicable to the other three groups, as no prediction of occurrence by habitat characteristics is possible for some species, and some (e.g., *Mantidactylus opiparis*) prefer combinations of habitat characteristics that are unfavourably represented in our PCs (GROSJEAN et al. 2011). Comparable analyses of breeding site choice of the closest relatives of *B. picturatus*, *B. majori* and *B. sp. 35* (VIEITES et al. 2009) in the RNP, are difficult due to their scarcity in the streams studied (found in four and two streams, respectively). They were always recorded from streams where *B. picturatus* tadpoles were also found, and as far as it is reasonable to state it for *B. majori*, their occurrence turned out to be not depending on PC1, but rather on PC2, as observed for *B. picturatus* (own unpublished data).

B. picturatus uses the same streams for reproduction throughout the year, and (based on a single observation) clutches are attached on structures like sticks in the water (Fig. 8). The second clutch observed attached to a stick lying on a rock was most likely washed there by the water current.

Within the streams, by far most of the *B. picturatus* tadpoles recorded occur in patches with sandy substrate, preferably where the water is non-stagnant (Fig. 10). Their concentration in this microhabitat differs from the pattern observed in other tadpoles in streams in the RNP (see also ALTIG & McDIARMID 2006), especially because most other species are very often found in accumulations of leaf litter (GROSJEAN et al. 2011; RANDRIANIINA et al. 2011), or also use stony parts of the streams (R.-D. RANDRIANIINA unpublished data). Tadpoles of *B. majori* and *B. sp. 35*, which show markedly lower abundances, do not share this clear distribution across microhabitats either (Fig. 10), although they also have derived oral disc structures and were observed to have ingested sand particles (SCHMIDT et al. 2008, this study). The dominant use of this kind of microhabitat is obviously related to the very particular feeding habits of *B. picturatus* tadpoles, given that also their colouration in life makes them cryptic on a sandy background (ALTIG & McDIARMID 2006). ALTIG & McDIARMID (2006) stated that sand grain size may be of importance for these tadpoles, a possible explanation for finding most tadpoles in the sand-fast microhabitat where sand grains are larger than in the slow-moving or almost stagnant parts of streams. This correlation is supported by the fact that both *B. majori* and *B. sp. 35*, which are most likely found in leaf accumulations or slow-moving sandy sections of the stream, have sand particles of smaller size in their guts. A number of *B. picturatus* tadpoles were also found where dead leaves had accumulated in the stream. They obviously do not use this microhabitat for feeding (ALTIG & McDIARMID 2006), and utilizing it as protection from predators seems unlikely, as there are no fish in these streams, and dragonfly larvae as well as larger crustaceans, which are the potential main tadpole predators in these streams (own unpublished data), can enter leaf accumulations.

However, relating pure abundance data with microhabitat availability does not reveal a true preference for sandy habitat. The microhabitats “sand-fast”, “sand-slow”, and also “leaves-slow”, were used as expected if tadpoles of *B. picturatus* would be randomly distributed among all microhabitats. The high abundance of this species in these microhabitats might possibly be viewed in part in a relative context owing to the microhabitat’s high availability. However, the calculation of preferences is based upon relative abundance values within a stream and these relative values could for their part mask extremely high abundances. While most specimens of *B. picturatus* were found in microhabitats of the type “sand-fast”, the percentage of specimens within each stream that used this microhabitat was similar to the percentage of availability of this microhabitat. A very obvious result is the strong avoidance of stony areas. This is also the case in many other species that have no clear adaptations that would enable them to attach to this kind of surface, especially in strong currents (RANDRIANIAINA et al. unpublished data). In general, it is striking that despite the amazing specializations of these and other tadpoles with respect to their oral structures and mode of feeding, their microhabitat choice is clearly not highly specific and restricted, and the ecological functions and evolutionary advantages of the numerous derived tadpole mouthparts observed among mantellids remain insufficiently understood.

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Supporting information

S. GROSJEAN, R. D. RANDRIANIAINA, A. STRAUSS & M. VENCES: Sand-eating tadpoles in Madagascar: morphology and ecology of the unique larvae of the treefrog *Boophis picturatus*. – Salamandra, 47: 63–76.

Supplementary Table 1. Collection data of tadpoles examined. RNP is used as abbreviation for Ranomafana National Park.

Abbreviations used for collectors: AFR (A.F. RANJANAHARISOA), AS (A. STRAUSS), ER (E. REEVE), JG (J. GLOS), CP (C. PATTON), DRV (D.R. VIEITES), JP (J. Patton), LR (L. RAHARIVOLONIAINA), MT (M. TESCHKE), MV (M. VENCES), PB (PARFAIT BORA), RDR (R.D. RANDRIANIAINA), TJR (T.J. RAZAFINDRABE), TRH (T. RASOLONJATOVO-HIOBIARILANTO), SN (S. NDRIANTSOA), YC (Y. CHIARI).

Species	Locality	Field number	ZSM	Coordinates	Date	Collectors
<i>B. picturatus</i>	Ambohitsara	ZCMV 4941	77/2008	21°21.431' S 47°48.941' E 294 m a.s.l.	03.03.2007	AS, JG, ER, RDR, TRH, SN
<i>B. picturatus</i>	An'Ala	ZCMV 3406	1711/2007	18°55.156' S 48°29.277' E 889 m a.s.l.	08.02.2006	CP, DRV, JP, LR, MV, RDR
<i>B. picturatus</i>	An'Ala	ZCMV 3487	1791/2007	18°55.156' S 48°29.277' E 889 m a.s.l.	08.02.2006	CP, DRV, JP, LR, MV, RDR
<i>B. picturatus</i>	Belle Vue (RNP)	ZCMV 5189	608/2007	21°15.582' S 47°25.320' E 963 m a.s.l.	12.03.2007	AS, JG, ER, RDR, TRH, SN
<i>B. picturatus</i>	Bibiango (RNP)	ZCMV 4329	172/2007	21°15.442' S 47°25.096' E 930 m a.s.l.	25.02.2007	AS, JG, ER, RDR, TRH, SN
<i>B. picturatus</i>	Fierenana	FG/MV 2002.1664	808/2004	18°32.600' S 48°26.933' E 948 m a.s.l.	2002	MT, MV
<i>B. picturatus</i>	Fierenana	FG/MV 2002.1664	839/2004	18°32.600' S 48°26.933' E 948 m a.s.l.	2002	MT, MV
<i>B. picturatus</i>	Fompohonina II (RNP)	ZCMV 4017	680/2007	21°16.088' S 47°25.423' E 996 m a.s.l.	15.03.2007	AS, JG, ER, RDR, TRH, SN
<i>B. picturatus</i>	Fompohonina IV (RNP)	T 08/0076	–	21°16.115' S 47°25.520' E 990 m a.s.l.	10.07.2008	RDR
<i>B. picturatus</i>	Vevembe	–	833/2004	22°47.686' S 47°11.228' E 581 m a.s.l.	10.02.2004	MV
<i>B. picturatus</i>	Ranomena (RNP)	ZCMV 3807	172/2008	21°12,1' S 47°27,4' E 970 m a.s.l.	28.02.2006	AFR, CP, DRV, JP, LR, MV, PB, TJR, RDR
<i>B. picturatus</i>	Sahamalaotra (RNP)	FG/MV 2002.1835	821/2004	21°14.112' S 47°23.767' E 1124 m a.s.l.	2002	MT, MV
<i>B. picturatus</i>	Sahamalaotra (RNP)	ZCMV 5050	196/2007	21°14.112' S 47°23.767' E 1124 m a.s.l.	06.03.2007	AS, JG, ER, RDR, TRH, SN
<i>B. majori</i>	Ankidoanavo (RNP)	ZCMV 1369	37/2007	21°13.537' S 43°22.217' E 1144 m a.s.l.	19.02.2007	AS, JG, ER, RDR, TRH, SN
<i>B. majori</i>	Ranomena (RNP)	ZCMV 5398	953/2007	21°12,1' S 47°27,4' E 970 m a.s.l.	19.03.2007	AS, JG, ER, RDR, TRH, SN
<i>B. majori</i>	Vohiparara	ZCMV 2641	397/2008	21°14.143' S 47°23.152' E 1118 m a.s.l.	24.02.2006	AFR, CP, DRV, JP, LR, MV, PB, TJR, RDR

Supplementary Table 2. Morphometric measurements (all in mm) of all DNA voucher specimens described and used in this paper. For abbreviations, see Materials and Methods.

Species	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. majori</i>
Field number	FG/MV 2002.1835	ZCMV 5050	ZCMV 3487	ZCMV 3807	ZCMV 4941	ZCMV 2641
ZSM	821/2004	196/2007	1791/2007	172/2008	77/2008	397/2008
Site	Ranomafana	Ranomafana	An'Ala	Ranomena	Ambohitsara	Vohiparara
Stage	26	25	26	27	25	28
BL	11.7	13.6	11.3	13.7	9.7	9.4
BW	6.8	8.5	7.6	7.4	5.7	5.2
SBW	6.4	9.8	8.0	9.3	5.7	3.6
BH	5.0	6.2	5.5	6.3	4.1	3.8
SBH	7.8	8.8	8.5	9.3	6.2	6.9
ED	1.6	1.6	1.3	1.6	1.1	1.2
SE	3.4	5.0	3.6	4.3	2.6	2.7
EH	3.7	5.0	3.7	4.9	3.3	2.8
PP	3.6	4.5	3.8	4.3	2.8	3.6
ND	0.6	0.7	0.5	0.7	0.4	0.3
NH	3.2	4.7	3.4	4.9	3.3	2.6
NN	2.3	2.8	2.3	2.6	2.0	1.6
RN	1.6	2.7	1.8	2.2	1.4	1.1
NP	1.8	2.3	1.9	2.1	1.2	1.6
SL	2.6	1.3	2.0	1.7	0.7	1.2
SS	7.8	8.6	6.7	7.8	4.9	5.2
SV	3.9	5.1	4.8	4.5	4.8	4.2
SH	1.8	2.5	2.2	2.7	1.9	1.6
VL	2.4	2.5	2.6	2.5	1.7	0.6
TAL	21.2	23.5	16.5	22.9	14.9	10.0
TMW	3.1	4.1	3.1	4.2	2.1	2.0
TMH	3.3	4.3	3.1	4.5	2.2	2.6
TH	4.4	5.7	4.7	5.8	3.7	3.7
TMHM	2.0	2.6	1.9	3.2	1.3	2.0
THM	4.5	6.4	4.1	6.2	3.1	3.5
MTH	5.0	6.8	4.3	6.3	3.6	3.8
DMTH	7.8	9.3	6.6	10.5	5.6	2.9
UF	1.6	2.3	1.2	1.8	0.8	0.8
LF	1.0	1.5	1.0	1.2	1.0	0.7
HAB	3.2	4.4	3.2	4.6	2.9	2.1
TL	32.9	37.2	27.8	36.6	24.6	19.4

Supplementary Table 3. Relative values (all in percent) of all morphometric parameters of all DNA voucher specimens described and used in this paper. For abbreviations, see Materials and Methods.

Species	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. majori</i>
Field number	FG/MV 2002.1835	ZCMV 5050	ZCMV 3487	ZCMV 3807	ZCMV 4941	ZCMV 2641
ZSM	821/2004	196/2007	1791/2007	172/2008	77/2008	397/2008
Site	Ranomafana	Ranomafana	An'Ala	Ranomena	Ambohitsara	Vohiparara
BW/BL	59	62	67	54	59	55
SBW/BL	55	72	71	68	59	39
BW/BH	138	138	139	118	138	136
SBH/BL	67	64	75	68	64	74
ED/BL	14	12	12	12	11	13
SE/BL	29	36	32	31	27	29
EH/BH	74	81	67	78	81	75
PP/BW	53	52	51	58	50	69
ND/BL	5.0	5.1	4.2	5.1	4.6	2.7
NH/BH	65	76	62	79	80	68
RN/NP	90	120	97	105	116	69
NH/EH	87	93	93	101	99	91
NN/PP	63	63	60	59	69	44
SL/BL	22	10	18	12	7	12
SS/BL	67	63	59	57	51	55
SH/BH	37	41	40	43	46	42
SH/HAB	57	57	68	59	66	77
VL/BL	21	18	23	18	18	6
TAL/BL	182	173	146	168	154	107
TMW/BW	46	48	41	56	36	39
TMH/BH	66	69	57	71	53	68
TMH/MTH	65	63	73	71	60	68
TH/BH	88	93	86	92	91	97
TMHM/THM	43	40	47	51	41	56
TMHM/MTH	39	37	45	50	36	52
THM/BH	91	103	74	98	76	92
THM/MTH	91	94	95	98	87	93
MTH/BH	101	111	78	100	88	99
DMTH/TAL	37	39	40	46	38	29
UF/TMHM	83	90	61	56	62	42
LF/TMHM	51	60	50	39	77	37
UF/LF	164	149	122	142	81	111
HAB/BH	65	72	59	73	70	55

Supplementary Table 4. Comparison of the oral disc characteristics of the voucher specimens described and used in this paper. JW, MC, DG, A₁, A₂, A_{2 gap}, A_{2 row + gap}, Kerat length, MP length and SMP length are in mm. ODW/BW, DG/ODW, JW/ODW, MCL/JW, A₁/ODW and A_{2 gap}/A_{2 row} are in percent. A₁ is density per millimetre. UR, LR, A₁ num, MP, SMP and Total papillae are numerical values. For abbreviations, see Materials and Methods. A: ventrally, B: anteroventrally, C: absent, D: reduced, F: very long, narrowly pointed, not app: not applicable, abs: absent.

Species	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. picturatus</i>	<i>B. majori</i>
Field number	FG/MV 2002.1835	ZCMV 5050	ZCMV 3487	ZCMV 3807	ZCMV 4941	ZCMV 2641
ZSM	821/2004	196/2007	1791/2007	172/2008	77/2008	397/2008
Site	Ranomafana	Ranomafana	An'Ala	Ranomena	Ambohitsara	Vohiparara
ODW	3.53	4.2	3.5	4.0	3.1	1.9
LTRF	not app	not app	not app	not app	not app	1:4+4/1+1:2
UR	abs	abs	abs	abs	abs	5
LR	abs	abs	abs	abs	abs	3
JL	abs	abs	abs	abs	abs	0.85
MC	abs	abs	abs	abs	abs	0.41
DG	abs	abs	abs	abs	abs	1.12
VG	abs	abs	abs	abs	abs	abs
A ₁ (R/L)	abs	abs	abs	abs	abs	0.86
A ₂	abs	abs	abs	abs	abs	0.52/0.50
A _{2 gap}	abs	abs	abs	abs	abs	0.11
A _{2 row + gap}	abs	abs	abs	abs	abs	1.13
A ₃ (R/L)	abs	abs	abs	abs	abs	0.28/0.26
A ₄ (R/L)	abs	abs	abs	abs	abs	0.24/0.24
A ₅ (R/L)	abs	abs	abs	abs	abs	0.20/0.21
P ₁ (R/L)	abs	abs	abs	abs	abs	0.62/0.69
P ₂	abs	abs	abs	abs	abs	1.05
P ₃	abs	abs	abs	abs	abs	0.91
Kerat length	abs	abs	abs	abs	abs	0.06
MP length	0.08	0.08	0.15	0.1	0.07	0.10
SMP length	not app	not app	not app	not app	not app	not app
ODW/BW	52	50	47	54	55	58
DG/ODW	not app	not app	not app	not app	not app	58
VG/ODW	not app	not app	not app	not app	not app	not app
JW/ODW	not app	not app	not app	not app	not app	44
MCL/JW	not app	not app	not app	not app	not app	48
A ₁ /ODW	not app	not app	not app	not app	not app	45
A _{2 gap} /A _{2 row}	not app	not app	not app	not app	not app	10
A ₁	abs	abs	abs	abs	abs	50
A ₂ (R/L)	abs	abs	abs	abs	abs	26/27
A ₃ (R/L)	abs	abs	abs	abs	abs	20/19
A ₄ (R/L)	abs	abs	abs	abs	abs	18/18
A ₅ (R/L)	abs	abs	abs	abs	abs	14/16
P ₁ (R/L)	abs	abs	abs	abs	abs	46/45
P ₂	abs	abs	abs	abs	abs	76
P ₃	abs	abs	abs	abs	abs	76
MP	163	113	97	144	118	50
SMP	abs	abs	abs	abs	abs	abs
Total papillae	163	113	97	144	118	50
A ₁ den	not app	not app	not app	not app	not app	58
A ₂ den	not app	not app	not app	not app	not app	98
A ₃ den	not app	not app	not app	not app	not app	81
A ₄ den	not app	not app	not app	not app	not app	75
A ₅ den	not app	not app	not app	not app	not app	73
P ₁ den	not app	not app	not app	not app	not app	69
P ₂ den	not app	not app	not app	not app	not app	72
P ₃ den	not app	not app	not app	not app	not app	70
OD orientation	A	A	A	A	A	A
Sheaths	C	C	C	C	C	D
MC	not app	not app	not app	not app	F	F