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Vogue or adaptive character? A tadpole's goatee helps to distinguish two cryptic treefrog species of the genus *Boophis*

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Abstract. We describe the tadpole of an undescribed candidate species of tree frog from Ranomafana National Park in the Southern Central East of Madagascar, *Boophis* sp. aff. *boehmei* "Ranomafana". This frog, in its adult stage, is almost indistinguishable from its allopatrically distributed close relative *Boophis boehmei* but differs from that species by a substantial divergence in mitochondrial DNA. The tadpole of *B.* sp. aff. *boehmei* "Ranomafana" is a stream-adapted, exotrophic larva of a relatively generalized morphology. However, its third posterior keratodont row is extremely short, with an average of only 7 keratodonts. This constitutes a distinct difference to *B. boehmei,* and as far as known to all other species of *Boophis*. This feature is constant across various localities, thus probably providing a relevant taxonomic character to recognize the distinctness of *B.* sp. aff. *boehmei* "Ranomafana" at the species level.

Key words. Amphibia, Mantellidae, *Boophis boehmei, Boophis* sp. aff. *boehmei* "Ranomafana", third keratodont row, tadpole morphology, Madagascar

Introduction

The remarkable diversity of endemic animals and plants, and in particular frogs, in Madagascar leads to a scientific responsibility to describe and classify them properly. Madagascar is considered a hotspot of biodiversity conservation (Myers et al., 2000, 2003) and the number of amphibian species known to science showed an impressive increase during the last years (Vences et al., 2007; Vieites et al., 2009). The family Mantellidae, endemic to Madagascar and the Comoro island of Mayotte, currently contains 12 genera (Glaw and Vences, 2006, 2007). So far, 174 mantellid species are known to science; however, there is a lack of knowledge on the larval stages of frogs. Because adults sometimes are hard to distinguish based on their morphology and call characteristics, tadpoles can provide key features for species identification.

Within the Mantellidae, the genus *Boophis* represents a group of treefrogs characterized by enlarged discs on fingers and toes as well as large eyes. As a difference to many other species in the Mantellidae, *Boophis* deposit eggs directly into the water and all known tadpoles of this genus are exotrophic. The subgenus *Boophis* contains those species laying eggs in running water whereas species in the subgenus *Sahona* breed in non-flowing water bodies (Vences et al., 2002). Fifty-eight nominal species and over 40 candidate species of *Boophis* are known, but tadpoles have only been described for 37 out of these (e.g., Andreone et al., 2002; Glos and Linsenmair, 2005; Thomas et al., 2005, 2006; Grosjean et al., 2006; Raharivololoniaina et al., 2006; Altig and McDiarmid, 2006; Glos et al., 2007; Schmidt et al., 2008; Randrianiaina et al., 2009).

Boophis tadpoles usually have a generalized oral disc, but some species show specializations such as very pronounced mouthparts in *Boophis marojezensis* (Raharivoloniaina et al., 2006) or completely reduced ones in *Boophis picturatus* (Altig and McDiarmid, 2006), passing through partial reductions of jaw sheath and keratodonts as in *Boophis majori* and *Boophis* sp. aff. *majori* "long calls" (Schmidt et al., 2008).

In many cases, species of *Boophis* are known to be very similar morphologically in their adult stage. Accordingly, larvae as basis for comparison have been useful as they provided more distinguishable morphological features than adults (Glos et al., 2007; Schmidt et al., 2008).

Boophis boehmei Glaw and Vences, 1992 is a small-sized (25-35 mm) member of the *Boophis goudoti* group that is uniformly light brown coloured and has an orange-red iris. A population in Ranomafana National Park was previously assigned to *Boophis boehmei* as it appears

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to be morphologically indistinguishable, except for a possible difference in iris color (Fig. 1). However, DNA sequences of a fragment of the mitochondrial 16S rRNA gene show a difference of 6.8% from *Boophis boehmei* specimens from the type locality Andasibe (Vieites et al., 2009), warranting its definition as candidate species under the name *Boophis* sp. aff. *boehmei* "Ranomafana" (Glaw and Vences, 2007) or *Boophis* sp. 16 (Vieites et al., 2009). In this species complex, larval stages have so far only been described from *B. boehmei* from Andasibe (Raharivololoniaina et al., 2006).

The goal of the present paper is to assess whether these two species show differences in their larval morphology. We present a description of the tadpole of the candidate species *Boophis* sp. aff. *boehmei* "Ranomafana" and compare it to published morphological measurements of *Boophis boehmei* tadpoles, complemented by additional own data on the latter species.

Materials and methods

Tadpoles were collected in the field, 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. This specimen is here named "DNA voucher". All detailed morphological tadpole characterizations and drawings are based on this DNA voucher, whereas variation is described based on further specimens of the series. After tissue collection, all specimens were preserved in 5% formalin or 70% ethanol. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM).

Tadpoles were identified using a DNA barcoding approach based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently variable among species of Malagasy frogs (Thomas et al., 2005). The ca. 550 bp fragment was amplified using primers 16Sar-L and 16Sbr-H from Palumbi et al. (1991) applying standard protocols, resolved on automated sequencers, and compared to a near-complete database of sequences of adult Malagasy frog species. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen, and clearly less similar to all sequences from other species. DNA sequences newly determined in this study were deposited in Genbank under accession numbers GQ904717- GQ904746; for accession numbers of comparative adult specimens, see Vieites et al. (2009).

The described tadpole voucher specimen of *B.* sp. aff. *boehmei* "Ranomafana" was collected on 15 March 2007 in the National Park of Ranomafana at Fompohonina II site (21°16.080'S, 47°25.433'E, 973 m a.s.l.). The additional tadpoles of which the mouth part is shown in Fig. 3 are as follows: *Boophis* sp. aff. *boehmei* "Ranomafana" (ZSM 443/2008) collected on 23 February 2006 in the National Park of Ranomafana at Imaloka site (21°14,529'S, 047°27,938'E, 957 m a.s.l.), *B. boehmei* (ZSM 1738/2007) collected on 8 February 2006 in An'Ala at Andohanisity site (18°55.156'S, 48°29.278'E, 889 m a.s.l.) in the first transect, *B. burgeri* (ZSM 1561/2007) and *B. rufioculis* (1807/2007) collected on 8 February 2006 in An'Ala at Andohanisity site (18°55.156'S, 48°29.278'E, 889 m a.s.l.) in the second transect, *B. reticulatus* (ZSM 962/2007) collected on 19 March 2007 in the National Park of Ranomafana at Ranomena site (21°12.736'S, 47°26.010'E, 1144 m a.s.l.) and *B.* sp. aff. *rufioculis* "Ranomafana" (ZSM 1151/2004) collected on 26 January 2004 in the National Park of Ranomafana at Maharira site (21°19.547'S, 47°24.147'E about 1200 m a.s.l.).

Developmental stages are described following Gosner (1960). Morphological measurements were taken by using a graduated ocular attached to a stereomicroscope, following landmarks, terminology and definitions of Altig and McDiarmid (1999). The formula of keratodonts (= labial tooth rows) is abbreviated LTRF and is given according to Altig and McDiarmid (1999). Color in life is presented in Figure 1, drawings and photographs of the preserved tadpoles are represented in Figures 2 and 3.

The following abbreviations are used: A_1 (first upper keratodont row), A_2 (second upper keratodont row), A_1W, (width of first upper keratodont row), A_2W (width of second upper keratodont row), A_{2gap} (medial gap in the second upper keratodont row), BL (body length), BH (body height), BW (maximum width of the body), DF (dorsal fin height), DG (dorsal gap of marginal papillae), ED (eye diameter), GOS (developmental stage as established by Gosner (1960)), IOD (inter-orbital distance), JL (jaw sheath length), LTD (keratodont density on A_1), LTRF (keratodont row formula), MTH (maximal tail height), NN (distance between nares), NP (narial-pupil distance), NS (nare size), ODW (maximal oral disc width), P_2 (second posterior keratodont row), P_2W (width of the second posterior keratodont row), P_3 (third posterior keratodont row), P_3W (width of the third posterior keratodont row), P_3KER (number of keratondonts in the third posterior keratodont row), PN (number of labial papillae), RN (rostro-narial distance), SP (spiracle size), SS (snout-spiracle distance), TAL (tail length), TH (tail height at the beginning of the tail), THM (tail height at mid-tail), TMH (tail muscle height at the beginning of the tail), TMHM (tail muscle height at mid-tail), TMW (tail muscle width), VF (ventral fin height), VG (ventral gap of marginal papillae), VL (vent tube length).

Results

Boophis sp. aff. *boehmei* "Ranomafana"

The following description refers to one tadpole in developmental stage 27, ZSM 0122/2007 (field number ZCMV 4491), TL 48.26 mm, BL 17.32 mm), from Fompohonina II in the National Park of Ranomafana. The 16S rDNA sequence of this specimen (GQ904717) was 99.8% identical to a reference sequence of an adult specimen of *Boophis* sp. aff. *boehmei* "Ranomafana" (accession AY848536) from Ranomafana.

In dorsal view, body ovoid, maximum body width attained between the proximal ½ and ¾ of the body (BW

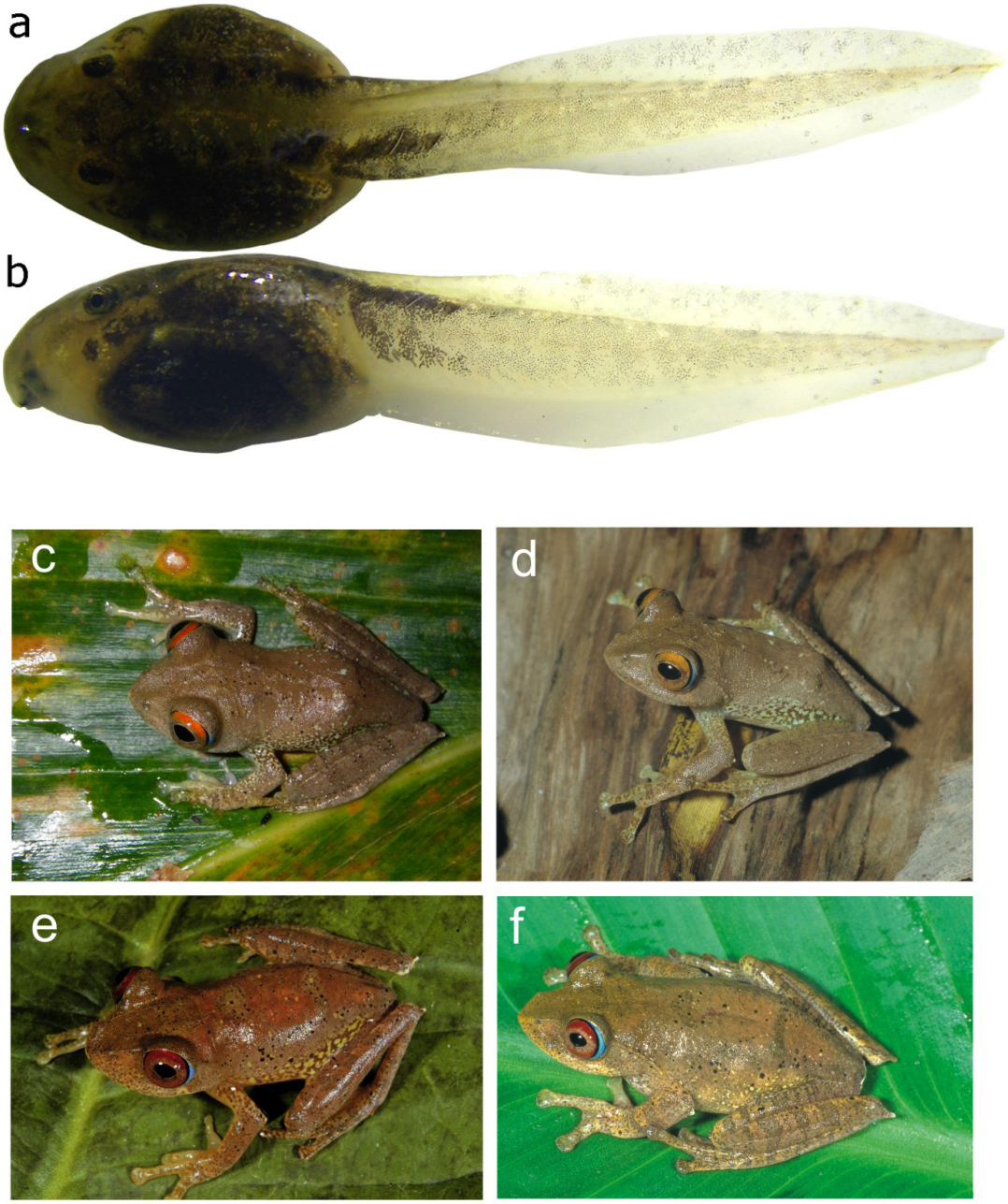

Figure 1. Color in life of the tadpole of *Boophis* sp. aff. *boehmei* "Ranomafana" in (a) dorsal and (b) lateral view (voucher tadpole, ZSM 1370/2007 from Ranomafana, Sakaroa site); of adult males of *B.* sp. aff. *boehmei* "Ranomafana" from Ambohitsara (c) and Ranomafana National Park (d); and of (e) an adult male and (f) a female of *B. boehmei* from Andasibe.

69% of BL), narrowly rounded snout. In lateral view, body depressed (BW 123% of BH), maximum body height attained in the ½ to ¾ of the body, rounded snout. Eyes of moderate size (ED 12% of BL), not visible from ventral view, positioned dorsally and directed laterally, situated between the proximal 3/10 and 4/10 proximal of the body, moderate distance between eyes (IOD 54% of BW). Ovoid and medium sized nares (NS 3.1% of BL), marked with a marginal rim, positioned dorsally and oriented anterolaterally, situated nearer to snout than to eye (RN 77% of NP), wide distance between nares (NN 63% of IOD), dark spot on the back of the nare present, small mediodorsal ornamentation present. Large sized sinistral spiracle (SP 22% of BL), visible from dorsal and ventral view, inner wall free from body and formed such that aperture opens laterally; rounded spiracular opening, directed posterodorsally, situated below the height of the point where the axis of the tail myotomes contacts the body and its aperture is between the proximal ½ to ¾ proximal of the body (SS 56% of BL). Dextral vent tube, inner wall present, short (VL 8% of BL), attached to ventral fin. No gland; Y mark in the tail basis absent. Short tail (TAL 179% of BL), maximal tail height lower than body height (MTH 97% of BH), tail height at midtail lower than body height and maximal tail height (THM 89% of BH and THM 92% of MTH), tail height at the beginning of the tail lower than body height (TH 92% of BH). Moderate sized caudal musculature (TMW 44% of BW, TMH 65% of BH, TMH 57% of MTH, TMHM 43% of THM, TMHM 40% of MTH). Tail muscle reaches tail extremity. Shallow fin type (DF 76% of TMHM, VF 57% of MTHM), dorsal fin higher than ventral fin (DF 134% of VF). Dorsal fin originates in dorsal body-tail junction, increases continuously to attain the maximum height before the midtail and then slopes slightly towards the tail tip. Ventral fin originates at the ventral terminus of the body, continues straight towards the maximum height before midtail, and then diminishes gradually towards the tail tip. Maximal tail height locates in the proximal ¼ and ½ of the tail, lateral line visible in the proximal ¾ of the tail. Tail tip narrowly rounded. Reduced and small sized oral disc (ODW 37% of BW), positioned ventrally and directed anteroventrally, emarginated, maximal width in the upper labium. Oral disc slightly visible from dorsal view, upper labium is a continuation of snout. Single row of marginal papillae interrupted by a wide gap on the upper labium (DG 74% of ODW) and a very narrow ventral gap (VG 11% of ODW); total number of marginal papillae 69 (34 left, 35 right). 34 submarginal papillae (16 left, 18 right) laterally and lateroventrally on the lower labium and laterally on upper labium. Medium sized conical papillae with rounded tips, marginal papillae measured 0.19 mm , submarginal papillae 0.24 mm, papillae visible in dorsal view. LTRF 3(2-3)/3(1). Very long A_1 (85% of ODW). The density of keratodonts varies from 18/mm to 67/mm. Very narrow gap in the first anterior interrupted row (A_{2gap} 9% of A_2). Rows alignment regular, posterior keratodont rows do not form a chevron; posterior keratodont rows not scattered. Very short P_3 (7% of P_2), containing only 3 keratodonts. Moderately sized keratodonts (0.14 mm), separated from each other. Distal keratodonts same length as those in the middle; considerable space between marginal papillae and keratodont rows. Fully keratinized jaw sheath. Pointed serrations; moderate sized jaw sheath (JL 47% of ODW), medial convexity absent. Lower jaw sheath V-shaped and partially hidden by the upper jaw sheath.

Coloration in life (Figure 1): In general yellowish. Dorsally: tanned with fine brown spots spread all over the body. Laterally: dorsolaterally with same color as dorsally, ventrolaterally transparent, intestinal coils visible, regular spiral-shaped. Ventrally: transparent, intestinal coil visible. Tail musculature: yellowish, with many dark brown spots. Fins transparent, dorsal fin less spotted than tail musculature, ventral fin poorly spotted. Yellowish color disappears when preserved.

Statistical comparison

In order to distinguish between *Boophis boehmei* and the genetically different candidate species *Boophis* sp. aff. *boehmei* "Ranomafana" (as initially identified by DNA barcoding; see Vieites et al., 2009) we identified the third posterior keratodont row as a potentially suitable attribute. *Boophis* tadpoles generally have three posterior keratodont rows of almost the same length. By contrast, *B.* sp. aff. *boehmei* tadpoles have a strongly shortened third keratodont row, only consisting of a small amount of keratodonts.

To further test the diagnostic value of this difference, we examined a larger number of tadpoles of the two species (*Boophis boehmei*, N=19; *Boophis* sp. aff. *boehmei* "Ranomafana", N=75). Besides the DNA-voucher tadpoles of several series (see below), this also included all other individuals assigned to the series. The average third posterior keratodont row length was 0.88 mm (0.4-1.7 mm) with an average number of keratodonts of 38.4 (23-63) in *B. boehmei*. In contrast,

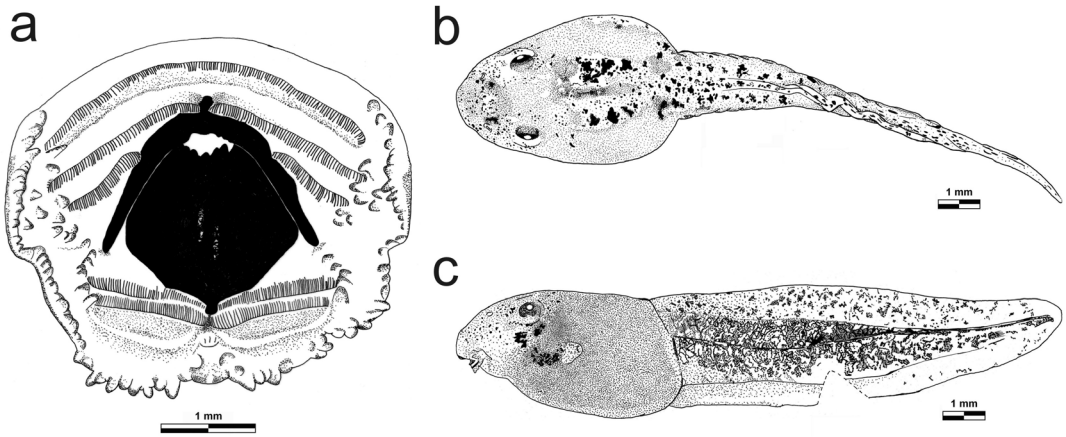

Figure 2. Drawings of *Boophis* sp. aff. *boehmei* "Ranomafana" (ZSM 443/2007) voucher tadpole. (a) oral disc with only few keratodonts at third lower labium, (b) dorsal view of the tadpole and (c) lateral view of the tadpole (note the cut at lower tail fin, missing piece used for DNA-analysis).

the third posterior keratodont row of *B.* sp. aff. *boehmei* "Ranomafana" measures on average 0.16 mm (0-0.47 mm) with an average number of keratodonts of 7 (0-15). Both of these difference are highly significant (Mann-Whitney-U-Test, $p<0.001$). In contrast, the first and second posterior keratodont rows showed no significant reduction in width or keratodont row number (see Fig. 3 for a comparison of relative width of P_2).

To exclude a possible effect of differences in the absolute size of specimens belonging to the two different species, we calculated size-corrected relative values for keratodont row length and number of keratodonts by dividing the absolute values by body length. Also after this correction, the observed differences remained highly significant (Mann-Whitney-U-Test, $p<0.001$).

The extremely reduced length of the third posterior keratodont row of *Boophis* sp. aff. *boehmei* "Ranomafana" is omnipresent among specimens from different localities. Indeed, besides specimens from different sites in Ranomafana National Park (ZSM 227/2008; ZSM 233/2008; ZSM 321/2008; ZSM 442/2008; ZSM 443/2008; ZSM 445/2008; ZSM 508/2008; ZSM 509/2008; ZSM 684/2007; ZSM 752/2007; ZSM 932/2007; ZSM 1010/2007; ZSM 1074/2007; ZSM 1106/2007; ZSM 1370/2007; ZSM 1153/2007; ZSM 1220/2007; ZSM 1682/2007; ZSM 1690/2007), our analysis included *Boophis* sp. aff. *boehmei* "Ranomafana" tadpole series from Tsitolaka forest (near Ambohitsara; ZSM 0073/2008, 0079/2008, 0083/2008 and 0084/2008). Of *B. boehmei*, the analysis included specimens from Andasibe (ZSM 534/2004), also studied by Raharivololoniaina et al. (2006), as well as newly studied specimens from An'Ala (ZSM 537/2004; ZSM 463/2008 ZSM 1750/2007; ZSM 1762/2007; ZSM 1765/2007; ZSM 1779/2007; ZSM 1812/2007; ZSM 1836/2007; ZSM 1847/2007).

We did not take comparative data on keratodont row lengths in other species of *Boophis* for the present study. However, a visual comparison of the length of the third posterior keratodont row among different species in the *Boophis madagascariensis* group (Figure 4) reveals the uniqueness of the reduced length observed in *B.* sp. aff. *boehmei* "Ranomafana".

Discussion

Based on our results, we can draw some limited hypotheses on the ecology of *Boophis* sp. aff. *boehmei* "Ranomafana" tadpoles. The relative short and low fin is an important characteristic for life in flowing water. This is also supported by the ventral position of the oral disc (Altig and McDiarmid, 1999) as well as the relatively high number of papillae (Hoff et al., 1999). The direction of the eyes, which are positioned dorsally, is typical for ground-living tadpoles (Altig and McDiarmid, 1999). Regarding the three defining characteristics of the three

guilds in the genus *Boophis* (A, B and C) defined by Raharivololoniaina et al. (2006), Guild A was defined by a ODW/BW ratio of 31-43%, a single row of 48-81 marginal papillae, and 58-144 keratodonts in row A_1. The respective values of *Boophis* sp. aff. *boehmei* "Ranomafana" fit neatly in this category: 87 keratodonts on A_1 (verified in ZSM 443/2008), a single row of 52-79 marginal papillae (Table 1) and an average ODW/BW value of 42.1% (Table 1). Also the tadpole of *B. boehmei* is assigned to this guild (Raharivololoniaina et al., 2006). It needs to be mentioned, however, that this guild system is not unambiguous. For example, the

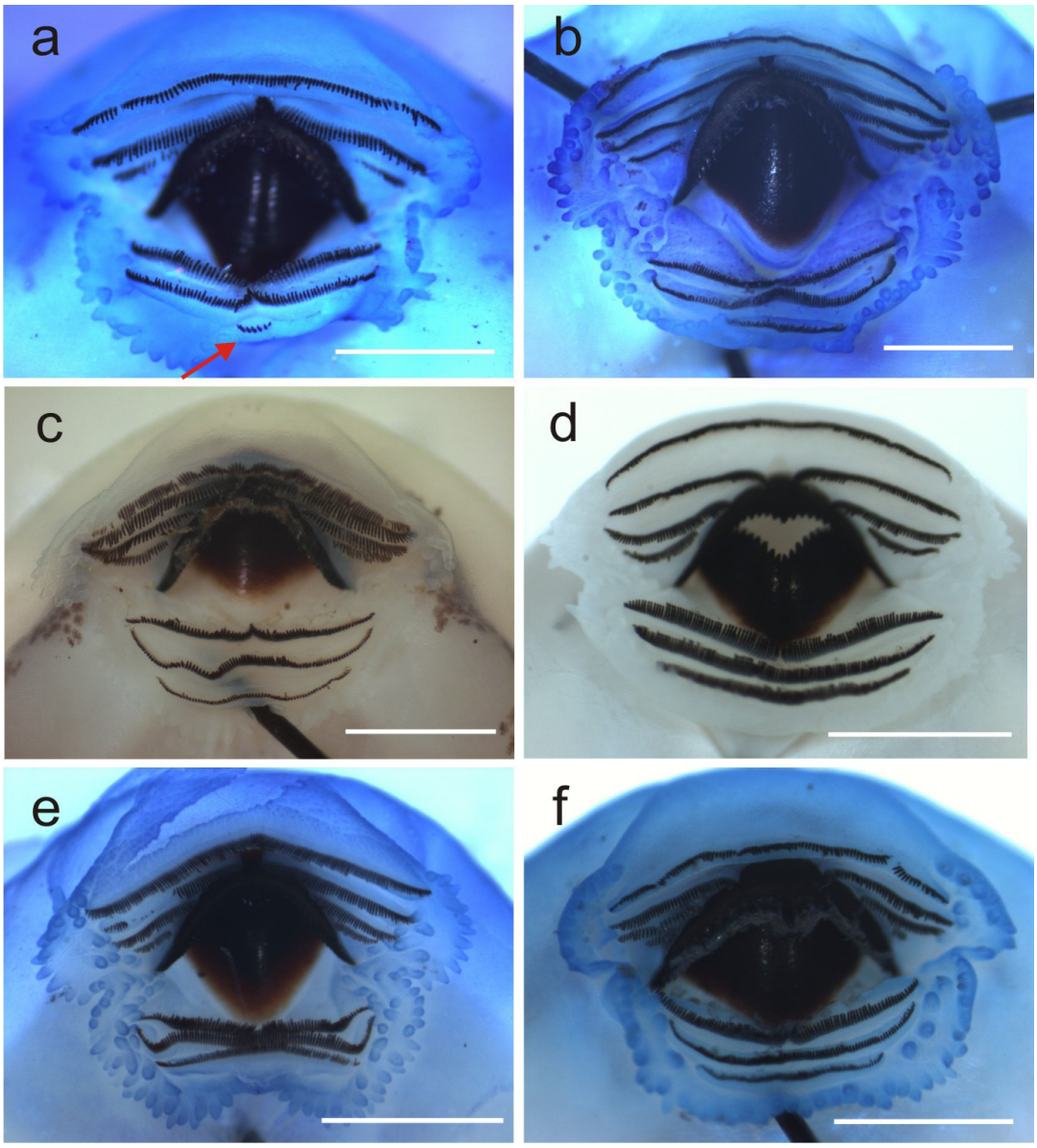

Figure 3. Photographs of oral disc of preserved DNA voucher tadpoles of (a) *Boophis* sp. aff. *boehmei* "Ranomafana" (ZSM 443/2008), (b) *B. boehmei* (ZSM 1738/2007), (c) *B. burgeri* (ZSM 1561/2007), (d) *B. reticulatus* (ZSM 962/2007), (e) *B.* sp. aff. *rufioculis* "Ranomafana" (ZSM 1151/2004), and (f) *B. rufioculis* (1807/2007). Note the unique, very short third lower (= posterior) keratodont row of *B.* sp. aff. *boehmei* "Ranomafana" (arrow) with only very few keratodonts, and the more weakly reduced length of this row in *B. boehmei.* Scale bar = 1 mm.

Boophis goudoti group contains some species which can be sorted in either guild A or B: *B. boehmei*, *B.* sp. aff. *boehmei* "Ranomafana" and *B. reticulatus* to guild A, and *B. madagascariensis* to guild B (Raharivololoniaina et al., 2006). On the other hand, *B. brachychir* can be sorted into guild A or guild B, depending on the character considered (Randrianiaina et al., 2009).

In general, *Boophis* sp. aff. *boehmei* "Ranomafana" as well as its relatives in the *B. goudoti* group show a rather generalized morphology and they lack the adaptations of some specialized tadpoles like the suctorial tadpoles of the *B. albipunctatus* group, *B. mandraka* group and some species of the *B. majori* group, or the species with strong reduction of keratinized oral structures, such as *B. majori* and *B. picturatus*. The generalized morphology is also in accordance with the fact that *B.* sp. aff. *boehmei* "Ranomafana" tadpoles occur in a large number of different brooks in Ranomafana National Park. They were found in 29 out of 30 streams, being present with a high density of 70 specimens per 30 m section on average (min: 2, max: 195; own unpublished data).

Boophis sp. aff. *boehmei* "Ranomafana" and its close relative *Boophis boehmei* appear to be allopatrically distributed. *Boophis* sp. aff. *boehmei* "Ranomafana" was commonly found in Ranomafana National Park (southern central east of Madagascar) whereas *B. boehmei* is reliably (i. e. identified by DNA barcoding) only known from the Andasibe/An'Ala area in the northern central east, without any data so far indicating that the ranges of the two species are in contact or overlapping in the intervening areas.

The obviously and constantly very short third posterior keratodont row of *B.* sp. aff. *boehmei* "Ranomafana" leads us to the question whether this character has an adaptive function. In fact, a slightly reduced third keratodont row can also be observed in *Boophis boehmei* as compared to other species in the *B. goudoti* group (as obvious from superficial examination of photographs in Figure 4). This tendency may be an adaptation to a particular habitat or type of food. This might also be related to the fact that *B. boehmei* has exceptionally large eggs compared to other *Boophis*, (Glaw and Vences, 1997) and thus possibly is particular in the ecology of its early larval stages, and this might also apply to *B.* sp. aff. *boehmei* although its eggs or embryonal stages are so far unknown. To verify this, more studies on the function of keratodont rows in tadpoles are necessary.

	ZSM 227/2008	**ZSM 233/2008**	**ZSM 321/2008**	**ZSM 442/2008**	**ZSM 443/2008**	**ZSM 445/2008**	**ZSM 508/2008**	**ZSM 509/2008**	**ZSM 1682/2007**	**ZSM 1690/2007**
GOS	27	25	25	27	26	27	26	28	27	25
BL	15.2	10.8	11.4	15.4	13.2	13	12.5	15	-	11.9
BH	9.3	6.8	6.8	8.3	6.4	5.6	5.7	7.4	-	5.4
BW	9.7	7	6.9	9.9	7.6	7.5	6.5	8	8	6.3
TL	39.8	25.5	-	38	35.4	34	32.5	41.6	-	-
TAL	24.6	14.7	-	22.6	22.2	21	20	26.6	23.2	-
TMH	5.9	3.7	-	5.3	4	3.7	3.6	4.5	4.5	-
TMW	5.2	3.3	2.9	4.5	3.3	3.7	3.1	4.1	4.1	2.8
MTH	7.4	4.8	-	7.5	5.3	5.5	4.9	6.7	7.3	-
TMHM	3.9	2.8	-	4.1	2.8	2.6	2.7	2.9	3.2	-
ODW	3.4	2.8	2.7	3.7	3.2	2.9	-	3.7	3.9	3
IOD	3.8	4	5.9	4.9	4.9	4.6	4.2	5.4	5.6	4.3
IND	3.2	2.8	2.5	3.3	2.8	2.9	2.7	3.2	3.4	2.7
ED	2	1.4	1.5	1.9	1.6	1.4	1.5	1.8	-	1.4
PN	61	59	55	52	57	55	66	79	67	75
LTRF	4(2-4)/3(1)	3(2-3)/3(1)	3(2-3)/2(1)	3(2-3)/3(1)	3(2-3)/3(1-2)	3(2-3)/3(1)	3(2-3)/3(1)	3(2-3)/2(1)	3(2-3)/3(1)	3(2-3)/3(1)
A_1KER	123	67	93	103	92	105	88	85	95	97
A_1W	2.5	1.9	1.8	2.8	2.4	2.1	2.2	2.9	2.9	2.1
A_2KER	82	57	76	105	79	93	83	81	88	73
P_2W	2	1.5	1.6	2.1	1.7	1.7	1.6	-	2.4	1.6
P_3W	0.3	0.3	-	0.4	0.2	0.2	0.2	aps	0.4	0.3
P_3KER	9	9	2	10	7	9	12	aps	10	10
LTD	50.2	34.9	51.9	37.5	38.2	49.8	40.7	29.7	32.5	45.8

Table 1. Original measurements and counts of DNA voucher specimens of tadpole series of *Boophis* sp. aff. *boehmei* "Ranomafana". For abbreviations, see Materials and Methods. Mensural variables (all except GOS, LTRF, PN, A1KER, A1W, A2KER, P2W, P3W, P3KER, LTD) in mm.

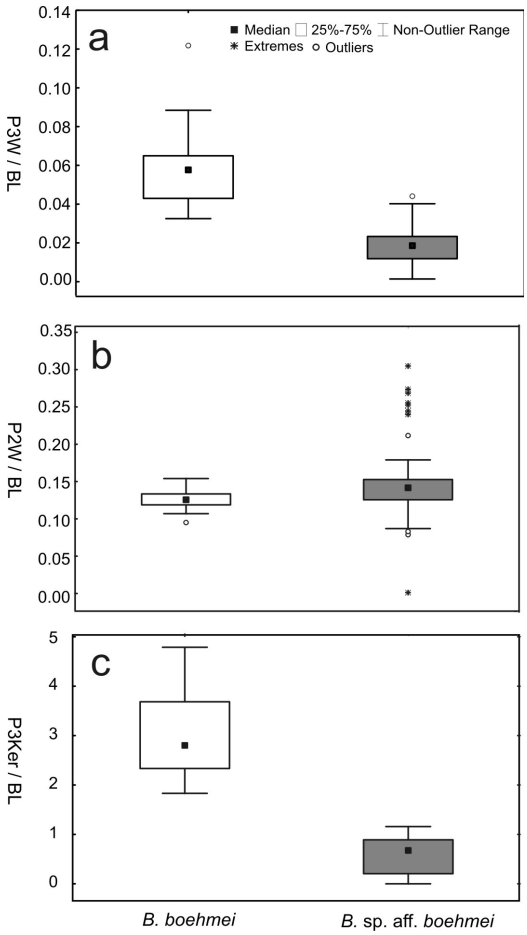

Figure 4. Boxplots of comparisons of oral discs between *Boophis boehmei* (N = 19) and *B.* sp. aff. *boehmei* "Ranomafana" (N = 75): (a) relative width of third lower (= posterior) keratodont row, (b) relative width of second posterior keratodont row and (c) number of keratodonts in third posterior row divided by body length. Observed differences in (a) and (c) are highly significant ($P < 0.001$; Mann-Whitney U-test); but are not significant in (b).

In conclusion, our data show that a difference in tadpole morphology can be used to distinguish the two cryptic species, *Boophis* sp. aff. *boehmei* "Ranomafana" and *B. boehmei*. As adults, there seem to be no relevant differences between these two species, neither in morphology nor in call characteristics. Adults only differ slightly in their eye coloration, *Boophis boehmei* showing a red outer and orange inner iris area while *Boophis* sp. aff. *boehmei* "Ranomafana" has a uniformly orange iris (Fig. 1). Although this differentiation is obvious in most specimens, in both species there can be individuals with slightly deviant colors; furthermore, this difference is only recognizable in living individuals. Instead, the detected difference in tadpole morphology is unambiguous. So far, two examples are known in which a pair of cryptic species of Malagasy frogs are virtually indistinguishable but their tadpoles show good taxonomic characters: *Boophis majori* and *B.* sp. aff. *majori*, both sympatric in Ranomafana, have distinct differences in tadpole oral morphology, with *B. majori* having a strongly derived oral disk with reductions of keratodont rows and of the jaw sheaths (Schmidt et al., 2008). On the other hand, *Boophis ankaratra* and *B. schuboeae* are very similar in their oral morphology but differ in life colouration, with a conspicuous striped pattern in *B. schuboeae* (Glos et al., 2007). *Boophis boehmei* and *B.* sp. aff. *boehmei* "Ranomafana" provide a third example of how tadpole characters can be helpful to distinguish species that are cryptic in their adult stage, and possibly, in combination with genetic data, can contribute to the crucial arguments to accept their distinctness at the species level.

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