

Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frogs

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(Accepted 11 July 2001)

Abstract

During a zoological survey in north-west Madagascar (Sahamalaza Peninsula) we documented aspects of the natural history of *Boophis occidentalis* (formerly a subspecies of *B. albilabris* and here raised to species level). Individual age assessed by skeletochronology ranged from 4 to 11 years. Breeding behaviour was observed at a seasonal stream after heavy rainfalls: five choruses of eight to 90 males aggregated in shallow brook sections. The males emitted low frequency calls and engaged in scramble battles. Only two females were found. A couple laid a large number of eggs, attached as a single layer on submerged stones. Tadpoles reared from these eggs had the typical morphology of brook-breeding *Boophis* species with a 1:5+5/3 keratodont formula and a relatively flattened body. Adult males had an anteroventrally oriented cloaca, a morphological trait shared with *B. albilabris*. Advertisement calls were similar in general structure to those of *B. albilabris* recorded at Anjanaharibe-Sud. They were unharmonious and consisted of pulsed notes emitted at irregular intervals. Much lower pulse rates in *B. albilabris* (30–38 pulses/s) than in *B. occidentalis* (90–109 pulses/s) support the specific distinctness of these taxa. The combination of characters related to reproductive biology in *B. occidentalis* and *B. albilabris* is unique in *Boophis*. The acontinuous breeding behaviour and large number of eggs per clutch are traits found in the pond-breeding species of the *B. tephraeomystax* group, while larval morphology and habitat are characteristic of brook-breeding species. Available morphological and molecular data place *B. albilabris* and *B. occidentalis* into the brook-breeding *Boophis* radiation. The acontinuous timing of reproduction and large egg number may be secondary adaptations that allowed the species to colonize arid regions of western Madagascar.

Key words: Madagascar, Mantellidae: Boophinae, *Boophis*, mating behaviour, tadpole, advertisement calls, skeletochronology, evolutionary relationships

INTRODUCTION

The anuran genus *Boophis* Tschudi, 1838 contains about 40 nominal species and is endemic to the Malagasy region. The genus is part of a speciose and diverse endemic radiation which also contains the genera *Aglyptodactylus*, *Laliostoma*, *Mantella*, and *Mantidactylus* (Richards & Moore, 1998; Vences, Glaw, Kosuch *et al.*, 2000) and is considered as family Mantellidae

(Vences & Glaw, 2001). *Boophis* are treefrogs inhabiting the eastern rainforest belt, although some species (mainly belonging to the *B. tephraeomystax* group) also colonized western deciduous forests and altered habitats.

Based on morphological and ecological traits seven infrageneric phenetic species groups were recognized by Glaw & Vences (1994): the *Boophis albilabris*, *B. luteus*, *B. rappiodes*, *B. difficilis*, *B. goudoti*, *B. microtympanum*, and *B. tephraeomystax* groups. The *B. albilabris* group is one of the least known of these groups according to Glaw & Vences (1994) and includes two taxa, *B. albilabris albilabris* and *B. a. occidentalis*. Although *B. albilabris* was described at the end of last century (Boulenger, 1888), its natural history remained unknown for > 100 years. Blommers-Schlösser & Blanc

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Fig. 1. Map of Madagascar with the Sahamalaza Peninsula, study area of *Boophis occidentalis* (1), and Anjanaharibe-Sud Massif (north-east), area from which the call of *B. albilabris* (2) has been recorded.

(1991) gave a general description of the species based on preserved specimens. Andreone (1993) provided preliminary life-history information, stressed differences between *B. albilabris* and species of the *B. luteus* group, and noted some differential characters of a specimen found by R. Jesu and L. Emanuelli at Tsingy de Bemaraha (west Madagascar). Blommers-Schlösser & Blanc (1993) presented a colour photograph, while Glaw & Vences (1994) placed *B. albilabris* in its own species group based on coloration and presence of a distinct prepollex, and described the western populations as a new subspecies *B. a. occidentalis*. Further observations and remarks by Cadle (1995) dealt mainly with the morphology and the peculiar sexual dimorphism of *B. albilabris*.

Recently, F. Andreone, J. E. Randrianirina and M. Vences had the opportunity to document the breeding behaviour, egg-laying and vocalizations of specimens belonging to a population of *B. a. occidentalis* during a field survey at Sahamalaza (north-west Madagascar). We provide here information about these traits

and raise the taxon to species rank (*B. occidentalis*) based on morphological and bioacoustic characters. Data on age structure based on skeletochronology are also reported. These new data on the reproductive biology of *B. occidentalis* and *B. albilabris* provided new insights into the phylogeny and adaptive radiation of the genus *Boophis*.

MATERIALS AND METHODS

Study sites and survey techniques

Field observations of *B. occidentalis* were made at Berara Forest (Sahamalaza Peninsula, north-west Madagascar), Mahajanga Province, Analalava Fivondronana, Amboloboza Firaisana and western part of the Befotaka Firaisana, 14°18.55'S and 47°54.92'E, 170 m a.s.l. (Fig. 1). The vegetation and climate of this area are transitional between those of the humid Sambirano region and those of the dry west

region, with an annual rainfall of *c.* 1600 mm (Projet ZICOMA, 1999; Andreone, Vences & Randrianirina 2001). Fieldwork took place during 13–23 February 2000, corresponding to the warm, rainy season, when most amphibians are at the peak of their activity. Other amphibians found at this site were *Aglyptodactylus securifer*, *Boophis jaegeri*, *Mantidactylus pseudoasper*, *M. ulcerosus*, *Mantella betsileo*, *Platypelis* sp., *Cophyla* sp., *Stumpffia* cf. *gimmeli* and *Plethodontohyla* sp. (Andreone *et al.*, 2001).

The observations of *B. albilabris* were made at Anjanaharibe-Sud, a massif situated south-west of Marojejy Massif and west of Andapa Basin. This typical mid-altitude rainforest receives a yearly rainfall of >2000 mm (Nicoll & Langrand, 1989). The specimens were observed and recorded next to campsite E1 (Raxworthy *et al.*, 1998), 14°45.3'S, 49°30.3'E.

Individuals were sexed based upon the examination of secondary sexual characters (e.g. keratinized spiculae covering several parts of the body, pointed prepollex and cloaca curved under the body in males and smoother skin and absence of nuptial pads in females). The snout–vent length (SVL, nearest 0.5 mm) and body mass (nearest 0.5 g) of the *B. occidentalis* specimens forming 2 choruses were measured in the field; voucher specimens were anaesthetized (immersion in chlorobutanol) and fixed (90% ethanol or 10% formalin), and later transferred to 70% ethanol. The morphological description is based on 9 preserved specimens from Berara, 1 specimen from Parc National d'Isalo, and observations of pictures taken in life.

Bioacoustic analysis

Vocalizations of *B. occidentalis* were recorded by F. Andreone and M. Vences with Sony TC-D3 and Sony WM-D6C professional recorders with external microphones. *Boophis albilabris* was recorded by F. Glaw with a Tensai recorder and a Vivanco EM-238 external microphone. Calls were analysed with the sound analysing device 'Medav 3' (*B. albilabris*) or on a PC with CoolEdit software (Syntrillium Corp.; *B. occidentalis*). Temporal measurements are given as the range (mean ± standard deviation, and number of measured temporal units given in parentheses).

Morphometry, eggs and larvae analysis, and used acronyms

A number of eggs from 1 *B. occidentalis* mating site at Berara were raised in a small aquarium. A developmental series was preserved in 10% formalin (specimens not individually tagged; see Appendix). Tadpoles were measured with a stereomicroscope with a micrometer to the nearest 0.1 mm. Tadpole developmental stages were given according to Gosner (1960). Terminology of tadpole morphology largely follows McDiarmid & Altig (1999) except for labial teeth

which are named keratodonts (Dubois, 1995). Keratodont formula follows Dubois (1995). Webbing formula of adults is given according to Myers & Duellman (1982). Morphological descriptions are based on Cadle (1995).

The following institutional abbreviations are used throughout the paper: MSNG, Museo civico di Storia naturale 'G. Doria', Genova; MRSN, Museo Regionale di Scienze Naturali, Torino; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; ZSM, Zoologische Staatssammlung, München.

Skeletochronological analysis

Skeletochronology was performed on 49 phalanges of the fourth toe of the specimens measured in the wild and on the femur of MRSN A2000. Phalanges were fixed with 70% ethanol, decalcified in 5% nitric acid for at least 2 h, cross-sectioned by a cryostat at 12–15 µm of thickness and stained with Ehrlich's haematoxylin for *c.* 30 min (Guarino, Angelini & Cammarota, 1995). Sections were observed with a Zeiss Axioscop light microscope, and images were captured with a Prog Res 3008 colour videocamera and KS 300 software interfaced with a personal computer.

RESULTS

Morphology of adults

Measurements of living males were recorded separately from two breeding aggregations at Berara. Specimens at the first chorus (*n* = 50) had a mean SVL ± SD of 56.6 ± 2.9 mm (range: 50.5–63.5 mm), and a mean weight ± SD of 10.7 ± 1.7 g (7.5–14.5 g). At the second chorus (*n* = 49) these values were respectively of 55.3 ± 3.3 mm (46.0–64.0 mm) and 10.9 ± 1.8 g (6.5–16.5 g). In nine preserved specimens from the second chorus, SVL was 54.9 ± 7.1 mm in the measured males and 51.0 mm in the single female. One male specimen (MRSN A2000) was peculiar in being much larger (84.6 mm) than the remaining males. Chromosome morphology and banding (G. Odierna, pers. comm.) and genetic analyses (mitochondrial DNA sequences; EMBL/GenBank accession numbers AJ314819–AJ314820) confirmed, however, that this specimen is conspecific with *B. occidentalis*.

The dorsal skin of males in breeding condition has cornified pointed spiculae on the head and body (Fig. 2). The keratinization on the back and upper head is apparently limited (at least in the examined specimens) to the area between the dorsolateral folds and from the snout tip to the cloaca. Two further distinct ventral regions are covered by spiculae: a sort of horse-shoe-shaped figure along the lower jaw and a transverse band between the forearms. These two keratinized patches are probably useful during amplexus, when males are embracing the female. The outer parts of the

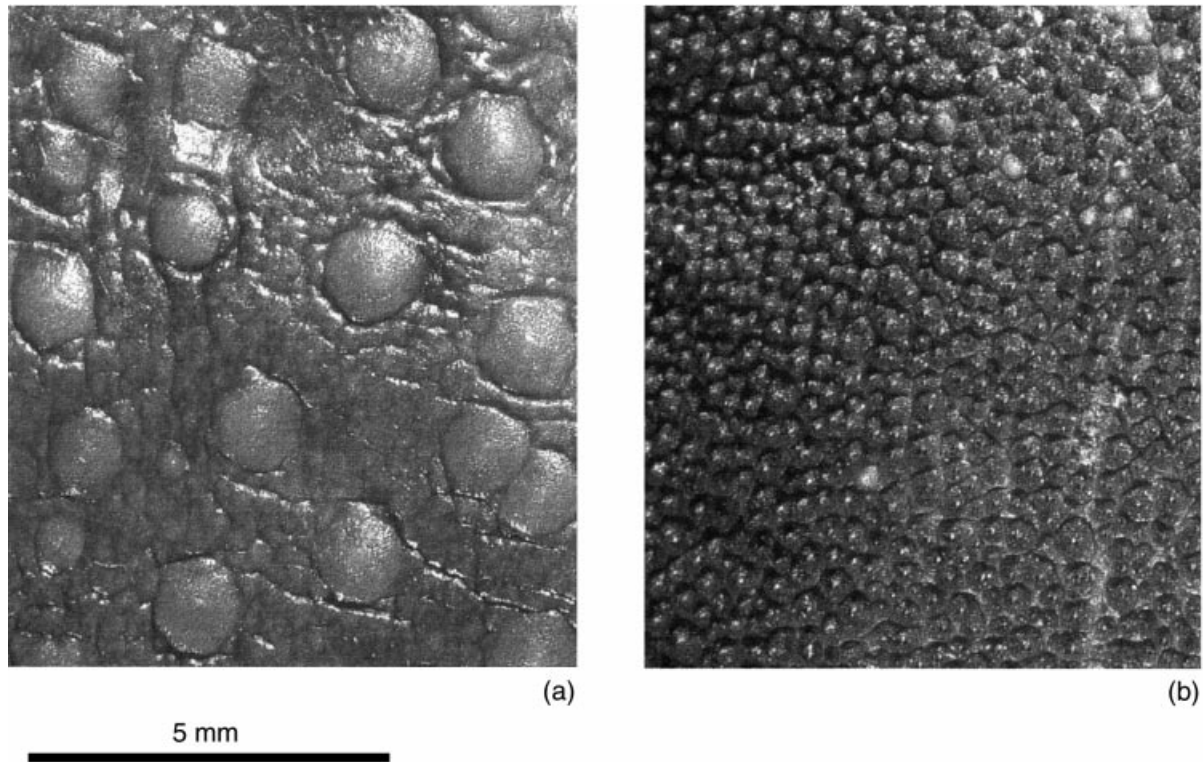


Fig. 2. Dorsal skin and keratinized spiculae in: (a) male *Boophis albilabris* (MRSN A1991); (b) *Boophis occidentalis* (MRSN A2002).

forearm, from the heel to the articulation of the hand, and the external parts of tibia, tarsus, foot, toes IV and V are also keratinized. The well-developed, blackish nuptial pads are extremely warty and occur on the first, second and part of the third finger. The enlarged prepollex extends to the proximal edge of the distal phalanx of finger I and protrudes away from thumb with a free distal end.

The single collected female (MRSN A1996) has a smoother skin totally without keratinized parts and a soft prepollex that is not distally oriented. In this specimen, and in a second female observed in the field, the cloacal opening is a simple curved slit positioned at about the mid-level of thighs. It has a short dorsal covering flap with ventrally directed opening. In males, the cloacal opening is clearly displaced ventrally and visible from the ventral surface of the thighs, and in most males it extends anteriorly below the thighs. As noted by Cadle (1995) in *B. albilabris*, the ventral displacement in males is caused by the ventral growth of the skin flap covering the cloacal opening. Cloacal sexual dimorphism does not seem to depend exclusively on ontogeny as assumed by Cadle (1995) for *B. albilabris*. MRSN A1997, A1999, A2000, and A2003 have a strongly anteroventrally directed opening, while MRSN A1998, A2002, and A2004 have a normal horizontal slit, although all have well-developed nuptial pads and keratinized spiculae on the body and therefore must be considered as sexually mature.

Coloration of adults

Two colour-morphs ('green' and 'brown') could be distinguished (Fig. 3a, b). Specimens were either more or less uniform light green dorsally, or had a brownish-beige coloration with some shadings of olive greenish. In both morphs the belly is yellowish, and the inner part of the legs is reddish.

In green males the area between the dorsolateral folds appears darker because of the presence of the blackish spiculae. The flanks, which lack spiculae, are lighter, shading to pinkish and whitish towards the ventral side. Small light greenish-white spots and reticulations are present on the posterior parts of the flanks anterior to the groin. The upper parts of the hands and feet are yellowish. In females, the supra-ocular yellowish stripes are visible, but are obscured by the spiculae in males. The upper lip is yellowish-white in both the sexes. The webbing between the toes and fingers is almost red, shading to purple. The iris is bronze-yellow to golden-yellow, with some brown spots, not bluish as in specimens from Isalo and Tsingy de Bemaraha (Glaw & Vences, 1994; R. Jesu, pers. comm.). The iris periphery is light blue.

In brown specimens the flanks are usually lighter than the back. The reddish webbing appears less contrasted and less bright. Within the two pooled chorus sample, brown specimens were more frequent than green specimens (67 vs 32 individuals; $\chi^2 = 11.68$, $P < 0.01$).

Similar to *B. albilabris* (Andreone, 1993; Glaw &

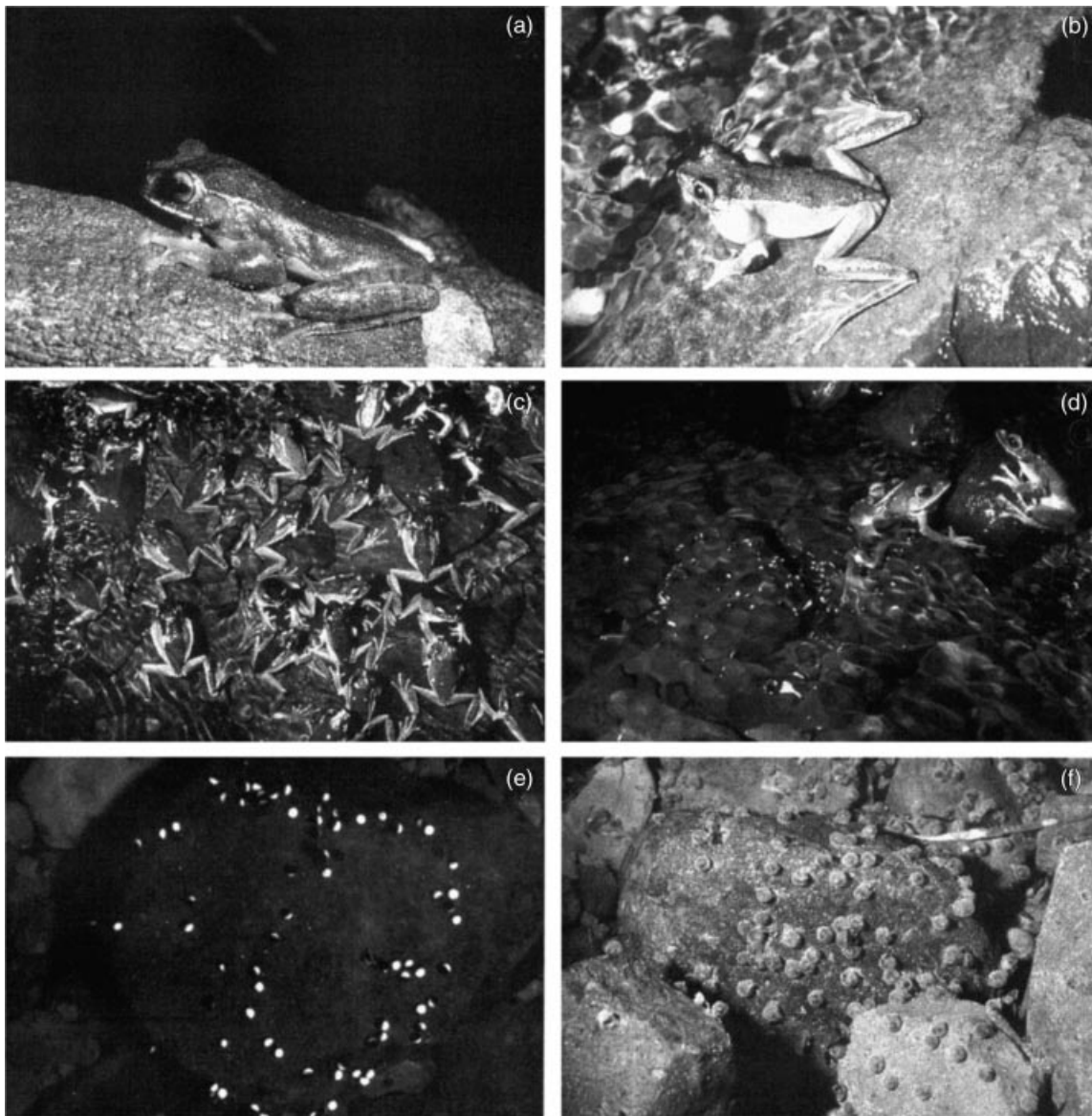


Fig. 3. General aspect and reproduction in *Boophis occidentalis* (all pictures from Berara, Sahamalaza Peninsula). (a) Male of the brown morph in lateral view; (b) calling male of the green morph; (c) *Boophis occidentalis* chorus; (d) amplexing couple of *Boophis occidentalis*; (e) eggs of *Boophis occidentalis* immediately after egg deposition; (f) eggs of *Boophis occidentalis* 12 h after deposition.

Vences, 1994; Cadle, 1995), specimens of *B. occidentalis* in liquid preservative have a rather different coloration than living individuals: green specimens changed into purple-violet, while brown specimens changed to purple-brownish.

Natural history

During the first day of our stay at Berara (13–23 February 2000), the study stream was a dry bed with a few isolated pools of stagnant water. After the heavy cyclonic rains on 14 February, it became almost filled before the water receded so that the deepest pools remained connected by small flowing sections. The

specimens were first observed on 14 February on trees along the stream at *c.* 2.5–3 m height. They did not exhibit any significant reproductive activity, and did not vocalize. The bulk of the activity was observed during 20 and 21 February when the males aggregated in choruses at 19:00–22:00. Five choruses were located at some rather small pools where the water ran slowly and was quite shallow (20 cm). The distance between consecutive choruses was *c.* 150, 150, 50, and 35 m. The first conspicuous aggregation was discovered on 20 February at the second chorus site and included *c.* 70 animals. Subsequently the animals were counted on 21 February: 20 males at the first chorus, 50 at the second (which became about 90 after a few h), eight at the third, 49 at the fourth, and 13 at the fifth.

At all the sites, the majority of observed males were sitting on the ground, half-submerged in the water, while a few others were found on trees. The frogs in the water (and less frequently also those in the trees) emitted a typical low-frequency 'rumbling' vocalization. Because of the high density of specimens in the water, it was difficult to distinguish and isolate the vocalizations of single individuals. The distance between the males was usually not > 20–50 cm and often < 5 cm (Fig. 3c). The pool of the second chorus had a surface of *c.* 2.5 m²; the area of the fourth chorus was 8 m² but the frogs were mostly concentrated on one bank. When alarmed by our presence or our lights, all males suddenly stopped calling. The vocalizations were emitted by expiration from the partly inflated body into the vocal sacs. These were paired, of an intermediate state between subgular and lateral (Fig. 3b). Several males showed a very loose skin all around the body. Males in the water engaged in a sort of scramble battle, which involved kicking and moving the forelegs with the pointed prepollax.

Mating was observed only once on the evening of 20 February. The female had just arrived within the chorus of *c.* 90 animals and was immediately clasped by a male. The pair was amplexed by several additional males that immediately rushed to the site. Egg deposition started just a few seconds after mating (Fig. 3d). Eggs left explosively from the female's cloaca in several groups. The female was totally submerged, and the male kept its curved cloaca closely adpressed to the female cloaca. The eggs immediately attached as a single layer on the surrounding submerged stones (Fig. 3e, f). Similar egg layers were also observed at the fourth site. The eggs were black with a large whitish pole immediately after oviposition (Fig. 3e), but gastrulated and became entirely black within a few h (Fig. 3f). Egg diameter (gametes without jelly layers) in the preserved series ZSM 192/2001 is 2.2–2.5 mm.

Boophis albilabris males were observed by F. Glaw at Anjanaharibe-Sud on 20 February 1995 at *c.* 21:00. About 10–15 individuals gathered in an area of *c.* 4 m² and were sitting at the edge of slowly moving water along an unpaved road. Males influenced each other in their calling behaviour. Neighbouring males often showed alternating calls. The vocal sac had a shape similar to that described and documented for *B. occidentalis*. Further observations corroborate that synchronous reproduction is the usual behaviour of *B. albilabris*. D. Rakotomalala (pers. comm.) observed large breeding aggregations of the species at Ranomafana National Park (Forêt de Vatoharanana, 21°17'S, 47°25'E, 1045 m elevation). F. Andreone, H. Randriamahazo and J. E. Randrianirina, during their survey at Anjanaharibe-Sud, western slope (Raxworthy *et al.*, 1998), observed an exceptionally large number of male *B. albilabris* during a single night (> 100 individuals counted within 3 h). These animals were seen on trees at low elevations from the ground. They were silent and did not show any mating behaviour. It is probable that these observations were made just one night (or a few h)

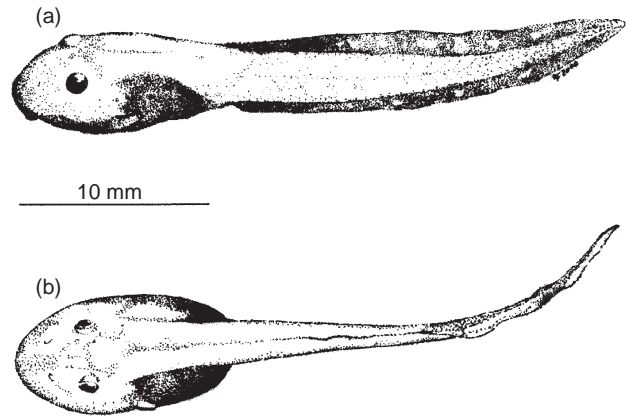


Fig. 4. Larva of *Boophis occidentalis*. Drawing taken from a photograph of MRSN A2009: (a) lateral view; (b) dorsal view.

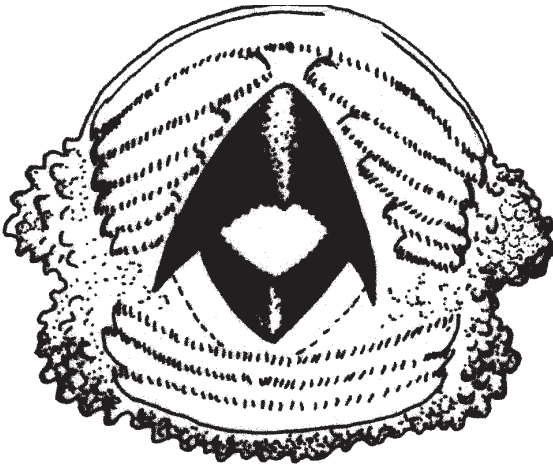
before the starting of the breeding aggregations. One female of *B. albilabris* from the Ambolokopatrika Corridor (see Andreone *et al.*, 2000; MRSN A2024; SVL 87 mm) contained 1342 oocytes, with a mean diameter \pm SD ($n = 50$) of 2.20 ± 0.23 mm.

Morphology and development of larvae

The tadpole description is from a specimen in batch MRSN A2009, and compared to four other specimens raised at the same time.

Ecological classification and external features

According to the categories of McDiarmid & Altig (1999) the tadpole of *B. occidentalis* can be defined of the lotic type, between clasping and adherent. The tadpole at stage 26 is 32.9 mm total length (Fig. 4). The tail length is 66.3% of total length. The maximum tail height is slightly greater than trunk height. The maximum height of the dorsal fin occurs at about two-thirds of the tail. The tail is euthoural with the extrapolated axis passing just below the eye. The height of the caudal muscles at the trunk is *c.* 70% of the maximum body height. The nostrils are large, oval, with a raised margin, and situated at a distance of about the diameter of the eye. The nostril passages are not visible dorsolaterally. The small pineal spot is visible slightly behind a line joining the front of the eyes. No orbitonasal line is present. The eyes are dorsolateral, with an extra-ocular proportion (i.e. a measure of the lateral position of the eyes, calculated as head width minus distance between the lateral limits of the eyes) of 0.60. The spiracle is sinistral with the opening visible dorsally and laterally and situated *c.* 50% along the body. The vent is medial, continuous with the ventral margin of the fin and with a small internal incision.



1 mm

Fig. 5. Mouthparts of *Boophis occidentalis* tadpole MRSN A2009.

Oral disc

The oral disc is not visible dorsally and is 70% of the width of the head at the plane of the disc. The mental gap in the oral papillae is *c.* 75% of the disc width. The oral papillae are present as a single row, with extra papillae in the angle of the mouth and on the inferior labium (Fig. 5). The rostradonts are finely serrated, with the outer half black, while the rest is quite reddish. The keratodont formula is 1: 5+5/3. The first superior keratodont line is continuous in all the specimens, while the other five lines are interrupted. The first of these seems continuous, but in reality it is interrupted. In one tadpole, the first continuous row of keratodonts appears only as a pigmented structure, in another one it is not possible to distinguish any keratodont.

Colour in life and in preservative

Several tadpoles photographed in life at later stages show a yellowish dorsal coloration. The tail is slightly darker with a variable amount of light brown to bronze markings. There are some irregular spots with a dark brownish ground colour with distinct small bronze dottings on the flanks. The belly is whitish. A few irregular scattered dark spots were visible on the back and part of the muscular tail. The dorsal fin was lightly mottled and the ventral fin was translucent. The eyes had a blackish iris and a greenish-iridescent pupil. In preservative the tadpoles became almost whitish, and it was possible to distinguish more clearly the internal organs.

Biometry

The measurements (mm) of the specimen are listed with the range of measurements from the batch of five individuals in parentheses. Total length 32.9 (30.2–32.9); head and trunk length 11.1 (11.1–11.4); trunk height 4.8 (4.4–4.8); trunk width 5.7 (5.2–6.2); head width at the disc 4.0 (3.0–4.0); disc width 2.5 (1.9–3.2); height of the tail 4.8 (4.8–5.1); height of tail muscles 3.2 (2.7–3.2); snout–nostril distance 1.3 (1.1–1.4); nostril–eye distance 1.0 (0.8–1.1); snout–spiracle distance 5.9 (5.6–5.9); inter-nostril distance 1.4 (1.4–1.7); inner inter-eye distance 1.7 (1.7–1.9); outer inter-eye distance 3.8 (3.3–4.1).

Acoustic repertoire

The advertisement calls of *B. occidentalis*, recorded at 24 °C air and water temperature, were rather unharmonious notes emitted at irregular intervals and largely in an interaction with other males of the chorus and not clearly arranged in regular series (Fig. 6). Notes were composed of 26–34 (30.1 ± 2.9 , $n = 8$) pulses. Note duration was 262–362 ms (309 ± 32 ms, $n = 8$). The last pulse of a note was generally more intense and more harmonious and can be described as a ‘click’ pulse. Duration of regular pulses was 3–5 ms (4 ± 1 ms, $n = 10$), duration of intervals between them was 4–8 ms (6 ± 1 ms, $n = 10$). Duration of click pulses was 5–18 ms ($n = 3$); sometimes they followed the regular pulse series after a longer interval of up to 33 ms. Pulse repetition rate was 90–109 (98 ± 7 , $n = 8$) pulses/s. Frequency was 900–2000 Hz and in some calls additional frequency bands were visible at 3800–4500 Hz and 6000–7000 Hz.

During handling, one male specimen emitted what were probably distress calls, which showed the typical pattern with several frequency bands of irregular modulation (compare Hödl & Gollmann, 1986). Note duration was 430–750 ms (584 ± 123 ms, $n = 5$), and notes contained 52–169 distinct pulses (85 ± 49 , $n = 5$). The differences in pulse number were caused by a highly different pulse repetition rate among the emitted notes that was probably related to an increasing state of stress in the specimen: 103–260 pulses/s (143 ± 66 , $n = 5$). Pulse duration was 2–5 ms (3.2 ± 1.4 ms, $n = 10$), duration of intervals between pulses was 3–6 ms (4.4 ± 0.8 ms, $n = 10$). Frequency ranged between 0 and 3000 Hz, with dominant bands between 500 and 2500 Hz.

The advertisement calls of *B. albilabris* also were recorded at 24 °C. The call had a general structure similar to that of *B. occidentalis*. The vocalizations consisted of irregular series of unharmonious notes with each note corresponding to one expiration. Notes were composed of 9–19 pulses (15 ± 3 pulses, $n = 15$) and were often followed by an isolated click (Fig. 7). Note duration was 339–596 ms (458 ± 94 ms, $n = 6$), but shorter notes with fewer pulses and not followed by a click note were also heard. Pulse duration was 8–22 ms, 17 ± 3 ms, $n = 14$, and duration of intervals between pulses was

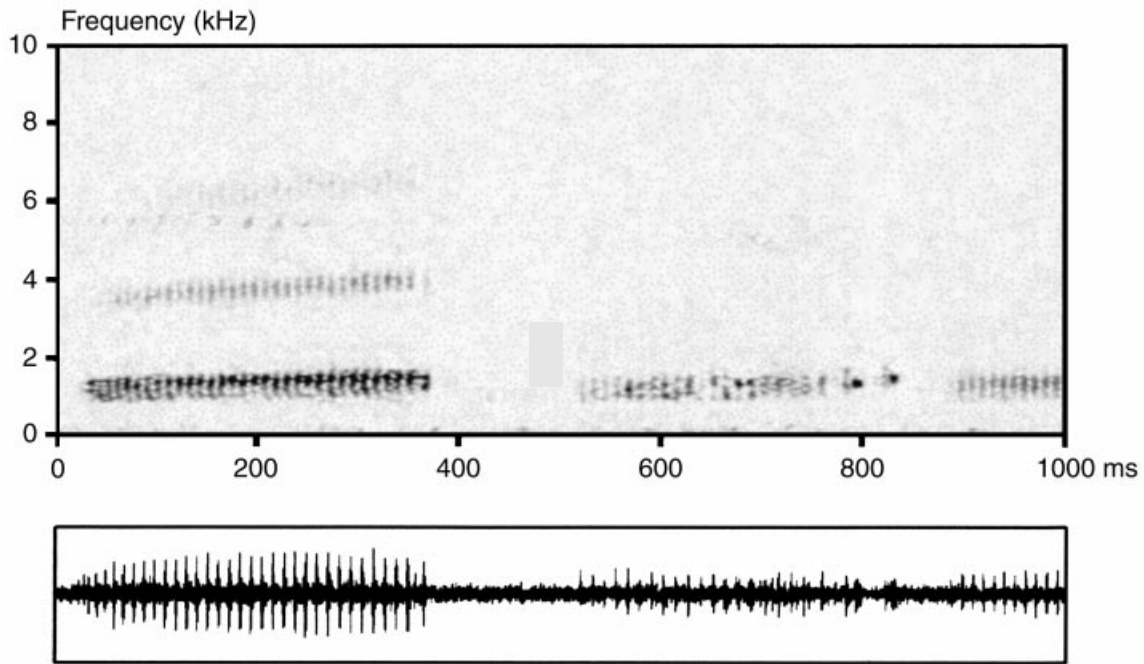


Fig. 6. Sonogram and time wave of the advertisement call of *Boophis occidentalis* (two complete and one uncomplete note) from Berara (Sahamalaza Peninsula). Recording air temperature = 24 °C.

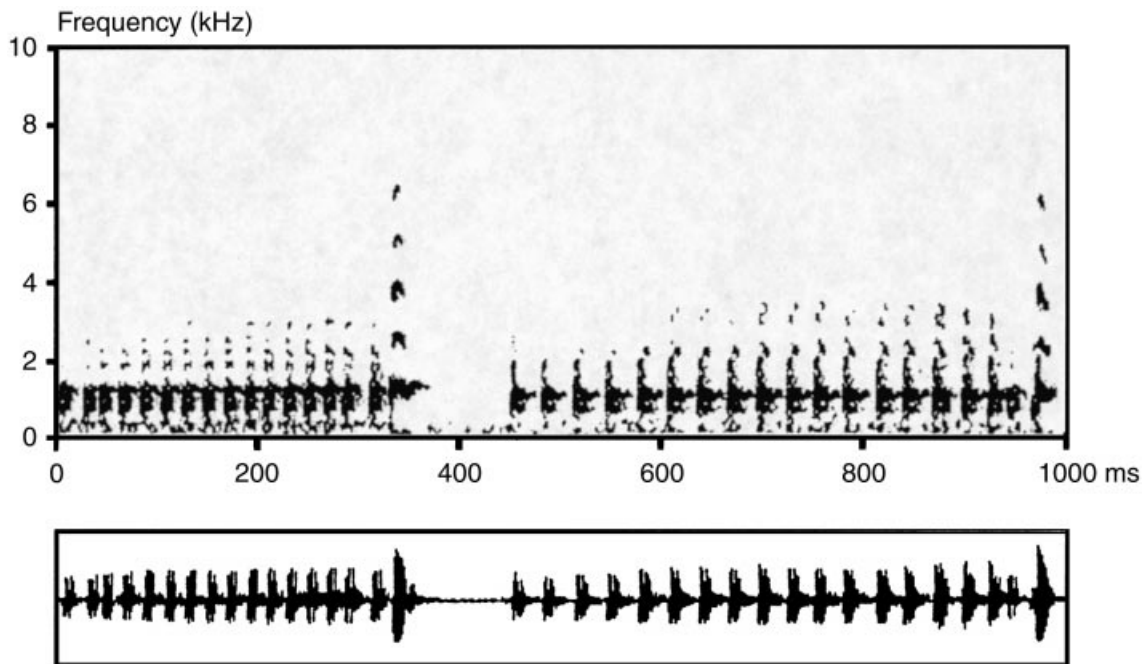


Fig. 7. Sonogram and time wave of the advertisement call (two notes) of *Boophis albilabris* from Anjanaharibe-Sud. Recording air temperature = 24 °C.

12–19 ms (15 ± 2 ms, $n = 12$). Duration of the isolated clicks was 18–27 ms ($n = 2$), duration of interval between end of pulsed note and click note was 34–41 ms ($n = 2$). The pulse repetition rate was much lower (30–38 pulses/s, 33 ± 3 , $n = 6$) than in *B. occidentalis* but single notes with higher repetition rates (to 49 pulses/s) also occurred, especially in alternating calls. Frequency of pulsed notes was 500–1200 Hz and the dominant fre-

quency 500–1200 Hz. Frequency of click notes included distinct frequency bands: one dominant band at 550–1500 Hz, further bands at 2400–2500, 3500–3800, 4800 and 6100 Hz.

Release calls of a male *B. albilabris* were recorded on 4 March 1995, 21:20, at 23 °C air temperature at Andapa. They were regular series of unharmonious notes that were structurally similar to the advertise-

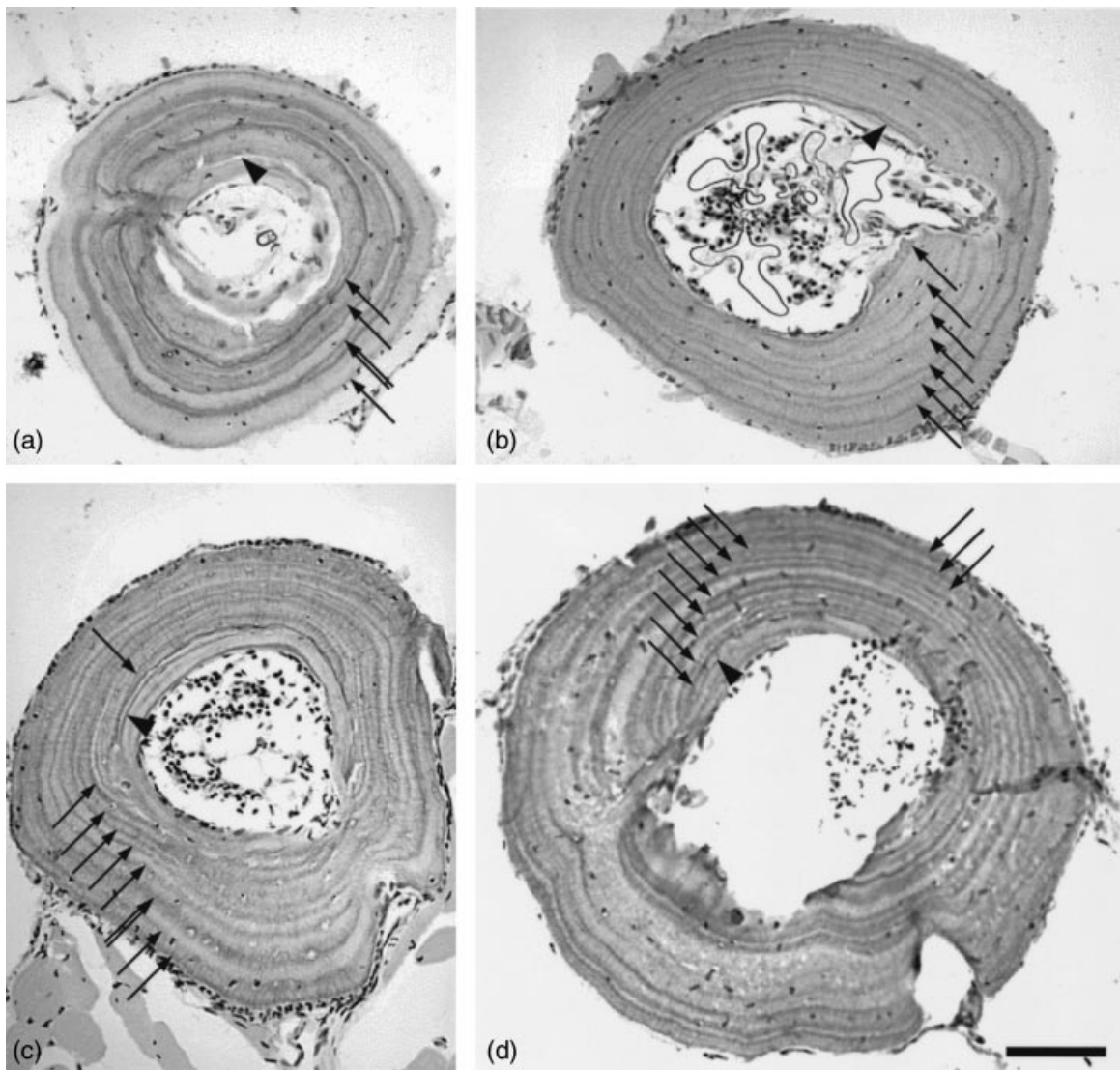


Fig. 8. Representative phalanx cross-sections from *Boophis occidentalis* stained with Erlich's hematoxylin: (a) male, SVL 57 mm, with four LAGs; (b) male, SVL 56 mm, with seven LAGs; (c) male, SVL 61 mm, with nine LAGs; (d) 'giant' male specimen, SVL 84.6 mm, with at least 11 LAGs. Arrow, LAGs; double arrow, double LAGs; arrowhead, reversal lines. Scale = 50 μ m, all parts.

ment calls but had a higher pulse repetition rate. Note duration was 215–278 ms (258 ± 18 ms, $n = 14$) and the duration of intervals between notes was 249–403 ms (330 ± 44 ms, $n = 13$). Notes consisted of 14–20 distinct pulses (17 ± 2 , $n = 11$). Dominant frequency was about 500–1200 Hz. Release calls of a female, triggered by claspings the specimen, were recorded on 10 March 1994 at Benavony (specimen ZFMK 57382, tentatively attributed to *B. albilabris*). In contrast to the male calls they showed no pulsed structure. Each note of a series consisted of 1–4 (2.5 ± 1.1 , $n = 12$) unharmonious subunits. Note duration was highly variable: 49–200 ms (131 ± 53 ms, $n = 12$). Duration of intervals between notes was 313–640 ms (419 ± 90 ms, $n = 11$). Frequency was 300–3250 Hz, dominant frequency 1000–1700 Hz.

Phalanx histology, age and size

The phalanx cross-sections in the mid-diaphyseal region consisted of two concentric bone layers separated by an irregular and strongly hematoxylinophilic resorption line. The innermost layer or endosteal bone surrounded the marrow cavity and was formed of lamellar bone tissue only. The outermost layer or periosteal bone consisted of pseudolamellar or lamellar bone tissue. The periosteal bone was always more developed than the endosteal bone and showed concentric hematoxylinophilic lines (Fig. 8) that were interpreted as lines of arrested growth (LAGs). In 62% of the samples, well-defined LAGs were observed (Fig. 8b), and in 24% the LAG number varied by about plus or minus one (Fig. 8d). In 14%, LAG number

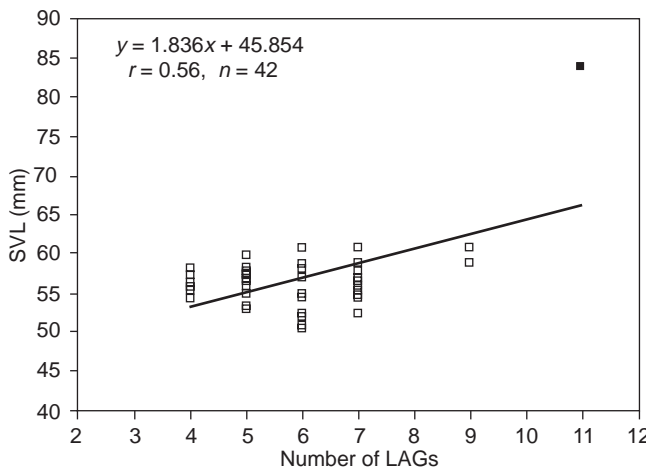


Fig. 9. Relationship between the LAG number and SVL in *Boophis occidentalis*. Closed square, 'giant' specimen. The seven males with a dubious LAG number are not considered.

could not be reliably determined and these were omitted from further analysis.

In most individuals the lamellar organization of the periosteal bone represented the main difficulty in the analysis because the LAGs were easily confused with bone lamellae (Fig. 8a, c). By observing 20–30 serial sections per phalanx, we were usually able to determine LAG number with certainty; false LAG were observed in 10% of the samples. Some LAGs were very close to one another (in *c.* 20% of the sample) and were considered as a single LAG (Fig. 8a, b). Finally, in some phalanges one LAG was locally split into several hematophilic lines, each of which was interpreted as a single LAG. The LAG number in our sample ranged from 4–11 (Fig. 8): 12 individuals with six LAGs; 11 with seven LAGs; 10 with five LAGs; six with four LAGs; two with nine LAGs; one with 11 LAGs (Fig. 9). The latter corresponded to the aberrant 'giant' specimen MRSN A2000. LAG number was significantly correlated to the SVL (Pearson's correlation, $r=0.51$, d.f. = 38, $P < 0.01$) (Fig. 9).

DISCUSSION

Age structure and longevity

The formation of LAGs occurs with an annual periodicity in several species of amphibians, mainly those inhabiting areas where climatic conditions force an arrest of activity (Castanet *et al.*, 1993). Climatic conditions prevailing at Berara are clearly marked by the seasonality of western Madagascar, with a prolonged dry season (Andreone *et al.*, 2001) during which amphibian activity is much reduced. An indication of this seasonal activity pattern is the fact that no specimen of *B. occidentalis* was recorded in a survey during the dry season (A. Raselimanana in 1996; see

Andreones *et al.*, 2001). For our sample it is therefore reasonable to assume that one LAG represents 1 year of growth and that age can be assessed by counting LAGs. However, bone remodelling near the marrow cavity might cause the complete destruction of the innermost periosteal LAG so that the visible LAGs do not correspond to the LAGs actually formed. In *Boophis* phalanges, very probably bone remodelling never totally destroyed the innermost LAG because it occurred in an asymmetrical fashion.

The majority of the males sampled (> 70%) are estimated to be 5–7 years of age; the maximum age ascertained is 11 years in one specimen. Considering the peculiar large size of this individual, its age may even be underestimated, as some of its LAGs may have been destroyed by bone remodelling. In conclusion, *B. occidentalis* is a more long-lived anuran in comparison to *Mantidactylus microtypanum*, the only other Malagasy amphibian that has been studied by skeletochronology (Guarino, Andreone & Angelini, 1998).

Identity of calls in the *B. albilabris* group

The calls of *B. albilabris* and *B. occidentalis* are based on direct and unequivocal observations of calling specimens. In contrast, the call descriptions of the two species given by Andreone (1993) and Glaw & Vences (1994) were based on indirect observations. Spectral and temporal call parameters differed greatly from the data provided herein and almost certainly refer to species of the *Boophis luteus* group which are known to occur at the respective recording localities (Andreone, 1993; Glaw & Vences, 1994; Hawkins, 1994).

Differential traits and relationships of *B. albilabris* and *B. occidentalis*

Boophis occidentalis, formerly regarded as subspecies of *B. albilabris*, was treated as a distinct species. This conclusion is based on the important bioacoustic differences, and on three morphological traits: keratinized dermal structures, body size, and webbing.

The keratinized areas in sexually active *B. albilabris* males do not show the sharply pointed spiculae of the *B. occidentalis* specimens from the Tsingy de Bemaraha (MSNG 48586; Andreone, 1993) and Berara. In contrast, the dorsal area of *B. albilabris* shows rather large rounded tubercles (Fig. 2). In *B. occidentalis* the spiculae have a diameter of 0.3–0.7 mm, while in *B. albilabris* the mean diameter of the tubercles is of 1.2–1.5 mm. This is clearly visible in the specimens MRSN A1991–1992 from Anjanaharibe-Sud Massif, MRSN A2025–2027 from Ambolokopatrika Forest, and in photographs in Cadle (1995) from Parc National de Ranomafana.

Boophis albilabris apparently reaches consistently a larger body size than *B. occidentalis*. Cadle (1995) quotes a mean SVL \pm SD of 85.3 ± 3.9 mm (males) and

85.0 ± 4.2 (females). In the measured *B. occidentalis*, the mean SVL was 57.9 ± 4.3 mm for the preserved males (except for MRSN A2000 with 84.6 mm SVL), 56.0 ± 3.17 mm for the live males, and 51.0 mm in the single preserved female.

The webbing in *B. occidentalis* is generally less developed than in *B. albilabris*, being rudimentary between the finger I and II and only partly developed between the other fingers (webbing formula I 2–2 II 1.5–3 III 1.5–1 IV). In *B. albilabris*, the webbing is I 2–2 II 1–1 III 1–1 IV.

The most relevant difference found between the two species concerns temporal call parameters. Although the calls were recorded at the same air temperature at Anjanaharibe-Sud and Berara, the pulse repetition rate of *B. occidentalis* was more than twice as fast. While several other minor differences between the calls from both localities (frequency, note duration) may be explained by the smaller size of the Berara males or by motivational influences, the highly different pulse repetition rates clearly indicate a separation of these populations at the species level.

Despite the important morphological and bioacoustic differences between *B. albilabris* and *B. occidentalis*, the affinities between these species are evident. Overall call structure and reproductive behaviour, highly developed keratinized areas, pointed prepollex and anteroventrally oriented cloaca in males emphasize their sister-group relationship. Also the presence of different colour morphs within a *Boophis* species, as observed in *B. occidentalis* at Berara and already known for *B. albilabris* (Glaw & Vences, 1994; Cadle, 1995; F. Andreone, pers. obs.) is exclusive of the two taxa. A further important trait is sexual size dimorphism. While females in *Boophis* are generally larger than males (Blommers-Schlösser, 1979; Glaw & Vences, 1994, 1997), in *B. albilabris* and *B. occidentalis* males seem to reach similar or larger sizes than females (Cadle, 1995; present study). Almost certainly this inversion of the usual size dimorphism evolved in the context of male combat behaviour, confirming a general trend in anurans (Shine, 1979).

Boophis albilabris has a distribution in the eastern rainforest belt from Andohahela in the South (Andreone & Randriamahazo, 1997) to the Anjanaharibe-Sud–Marojejy complex in the North (Raxworthy *et al.*, 1998, Andreone *et al.*, 2000; Raselimanana, Raxworthy & Nussbaum, 2000) and probably reaches into the Sambirano region in the north-west (female specimen ZFMK 57382 from Benavony; 71.7 mm SVL; lacking any light stripe on the head and anterior part of the back). It has not been found in the isolated northern Montagne d'Ambre (Raxworthy & Nussbaum, 1994). *Boophis occidentalis* is known from the western localities of Isalo, Tsingy de Bemaraha and Sahamalaza Peninsula. The apparent allopatric distribution of *Boophis albilabris* and *B. occidentalis* most probably reflects climatic changes with intermittent continuity and separation of the eastern and western faunas. A similar pattern is observed in the genus

Aglyptodactylus, with the species *A. madagascariensis* widespread along the eastern rainforests and in northern Madagascar and *A. securifer* and *A. laticeps* limited to western deciduous and transitional forests (Glaw, Vences & Böhme, 1998; Andreone *et al.*, 2001).

Relationships to other *Boophis* species

Boophis occidentalis (and probably also *B. albilabris*, although its tadpole is so far not reliably known) presents a unique combination of ecological and reproductive biological characters. Within *Boophis*, two main assemblages can be distinguished by reproductive mode: (1) the *B. tephraeomystax* group, with species reproducing in lentic water bodies and usually laying a large number of blackish eggs in single layers; (2) all other groups with species reproducing mainly in brooks and often laying coherent clutches of lower egg number (e.g. Glaw & Vences, 1997; Vences, Glaw & Zapp, 1999). *Boophis occidentalis* shows affinities to the brook-breeding species in rather large egg size and tadpole morphology; indeed the larva is more similar in body shape and oral morphology to that of other brook-breeding species (e.g. of the *B. goudoti* group) than to those of the pond breeders. Nevertheless, its breeding aggregations and large number of eggs are reminiscent of the pond-breeding *B. tephraeomystax* group.

Blommers-Schlösser & Blanc (1991) included *B. albilabris* in the *B. luteus* group, mainly because of the green colour in life and the presumably paired subgular vocal sac. This classification was based on preserved material only. As we have shown, in agreement with Cadle (1995), *B. albilabris* and *B. occidentalis* differ from the species of the *B. luteus* group by several characters: (a) relatively large size (*vs* small to medium-sized species); (b) inversed sexual size dimorphism (males being larger *vs* smaller than females); (c) almost lateral vocal sacs (*vs* subgular); (d) presence of dermal keratinized tubercles and keratinized patches in breeding males (*vs* lack of such structures); (e) presence of a keratinized and pointed prepollex in males, with an evident significance in male–male scramble competition; (f) pigmentation opaque green or brown in life (*vs* translucent green) and purple-violet in preservative (*vs* yellowish fading into whitish); (g) presence of green and brown colour morph (*vs* uniformly coloured green populations); (h) synchronous, explosive breeding behaviour (*vs* more or less continued breeding and calling during the hot–wet and even cold–dry seasons); (i) calling in choruses in shallow water (*vs* calling from isolated positions in the vegetation); (j) unharmonious, distinctly pulsed and rather irregularly repeated notes in advertisement calls (*vs* largely harmonious, not or indistinctly pulsed and regularly repeated notes); (k) eggs attached as single layer to underwater structures (*vs* coherent clutches).

As stressed by Cadle (1995), *B. albilabris* shows some similarities to the *B. goudoti* group. These include characters (a), (f), (j), and possibly also (k). Also

character (d) is found in at least one species tentatively included within the *B. goudoti* group (*B. periegetes*) and in *B. cf. brachychir* (F. Andreone, pers. obs.). The calls of *B. albilabris* and *B. occidentalis* are similar in some ways to those of *B. goudoti* which also shares a large number of eggs (Glaw & Vences, 1997). The derived absence of an anterolateral hyoid process observed in *B. albilabris* by Cadle (1995) is also shared by all *Boophis* groups except the *B. tephraeomystax* group (Vences et al., 2000), indicating that *B. albilabris* is probably not related to this assemblage. Obviously, *B. albilabris* and *B. occidentalis* share ecological traits with the large species belonging to the *B. goudoti* group and are possibly phylogenetically related to them.

Molecular data (Richards, Nussbaum & Raxworthy, 2000) support the phylogenetic position of pond-breeding *Boophis* (the *B. tephraeomystax* group) as sister group of all other representatives of the genus. The morphological plesiomorphies found in *B. tephraeomystax*, and the fact that its reproductive mode (breeding in lentic water) and habitat requirements (occurring in open areas, including dry regions) are similar to that of basal relict anurans of Madagascar (Vences et al., 2000), make it likely that pond breeding is the ancestral state for *Boophis*. In this scenario, the ecological key innovation of continuous breeding in lotic habitat initiated a rapid adaptive radiation of the brook breeding *Boophis* lineage into rainforest habitats. If *B. albilabris* and *B. occidentalis* are actually nested within the brook-breeding lineage, as indicated by their affinities to the *B. goudoti* group and by molecular data (Richards et al., 2000), it can be assumed that they secondarily returned to an acontinuous reproductive activity with breeding aggregations. During this differentiation and probably driven by sexual selection they acquired unique characters, such as the pointed prepollex structures of males, but maintained a specialization to lotic habitats. The acontinuous breeding may be advantageous in arid western Madagascar and thus explain the rather wide distribution of *B. occidentalis* in this region.

Acknowledgements

The work of F. Andreone and J. E. Randrianirina was carried out in collaboration with the PBZT, the WCS, and the AEECL. F. Glaw and M. Vences carried out their work throughout a co-operation between the Département de Biologie Animale (Antananarivo) and the Zoologische Staatssammlung (München), and were supported by the DAAD. The survey work was possible thanks to the agreement of the Malagasy Ministry of Eaux et Forêts, which delivered the requested authorizations. The authors thank L. Andriamampianina, S. M. Goodman, M. Hatchwell, M. Rakotondratsima, and Y. Rumpler, for the assistance, P. Bergò for the preparation of the specimens. G. Odierna and G. Aprea provided karyological information; R. Jesu shared with us unpublished information on the natural history of

B. occidentalis at Tsingy de Bemaraha; D. Rakotomalala contributed unpublished information on *B. albilabris* at Ranomafana. Thanks to G. Falcone for the graphic assistance and to C. Vollono for making the histological preparations. Ronald Altig and an anonymous reviewer improved an earlier draft of the manuscript by many suggestions.

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APPENDIX. SPECIMENS EXAMINED

***Boophis albilabris* (Boulenger, 1888)**

(A) *Metamorphosed specimens*: ZFMK 57382 (female, Benavony Forest, near Ambanja); MRSN A2022–2023 (males, Anjanaharibe-Sud Massif); ZFMK 59906 (male, Anjanaharibe-Sud Massif); MRSN A2024 (female, Ambolokopatrika Forest); MRSN A2025–2028 (males, Ambolokopatrika Forest); MRSN A2029–2030 (male and juvenile, Parc National de Masoala); MRSN A2031–2032 (juvenile, Parc National de Masoala); ZFMK 62291 (adult, between Vohiparara and Ambatolahy, Parc National de Ranomafana); MRSN A409 (male, Vohiparara, Parc National de Ranomafana); MRSN A411 and A758 (males, Ifanadiana); MRSN A567.1 (male, Parc National de Andohahela);

(B) *Eggs and larval stages*: ZFMK 59955 (eggs, Anjanaharibe-Sud Massif).

***Boophis occidentalis* Glaw & Vences, 1994**

(A) *Metamorphosed specimens*: MRSN A1996 (female, Berara Forest); MRSN A1997–2003 (males, Berara forest); MRSN A754 (sub-adult male?, Parc National d'Isalo); MSNG 48586 and 49085 (male and female, Réserve Naturelle Intégrale des Tsingy de Bemaraha); ZFMK 57383 (holotype, sub-adult male?, Parc National d'Isalo); ZSM 559/1999 (formerly ZFMK 57384) (paratype, sub-adult male?, Parc National d'Isalo).

(B) *Eggs and larval stages* (all from Berara): ZSM 192/2001 (eggs, laid on 20 February 2000, fixed after 22–24 h); ZSM 57/2001 (embryos, preserved on 22 February 2000, 10 h); ZSM 54/2001 (embryos, preserved on 23 February 2000, 9.15 h), ZSM 56/2001 (tadpoles, preserved on 24 February 2000, *c.* 22 h); MRSN A2005 (tadpoles, preserved on 26 February 2000, 11 h); MRSN A2006 (tadpoles, preserved on 4 March 2000, 15 h); MRSN A2007 (tadpoles, preserved on 7 March 2000, 14–16 h); MRSN A2008 (tadpoles, preserved on 9 March 2000, 18 h); MRSN A2009 (tadpoles, preserved on 26 March 2000, 20–22 h).