

Eye and webbing colouration as predictors of specific distinctness: a genetically isolated new treefrog species of the *Boophis albilabris* group from the Masoala peninsula, northeastern Madagascar

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Abstract. We describe a large and distinctive new treefrog species with blue webbing from the west coast of the Masoala peninsula in northeastern Madagascar. *Boophis masoala* sp. n. is morphologically similar to the other species of the *Boophis albilabris* group but can be distinguished from them easily by several chromatic characters of the eyes. Despite its similar morphology, it is genetically highly differentiated (10.5–13.3% pairwise p-distance in a segment of the 16S rRNA gene) from all other species in the *B. albilabris* group including the morphologically most similar *Boophis praedictus*. Both species share the blue webbing between toes and are distributed on the Masoala peninsula, but so far were not found in close sympatry. Although we recorded the new species only from the unprotected areas near the coast, we are confident that it also occurs within the adjacent Masoala National Park. We discuss the importance of eye colouration as a predictor of specific distinctness in the genus *Boophis* and that of webbing colouration as taxonomic characters of large treefrogs. Based on a micro-CT scan we provide a comprehensive description of the osteology of the new species, which is the first for any *Boophis* species, and furthermore describe its distress call which consists of three distinct sections corresponding to (1) the starting phase with closed mouth, (2) the opening of the mouth and (3) the final section with an open mouth.

Key words. Amphibia, Anura, Mantellidae, *Boophis masoala* sp. n., biogeography, distress call, osteology.

Introduction

Madagascar harbours an enormous diversity of amphibians. Currently 350 endemic species have been described from this island (AmphibiaWeb 2018) and numerous additional new species and candidate species are currently under study (e.g. LEHTINEN et al. 2018). Although arid western Madagascar also harbours several frog species (e.g. GLAW et al. 1998, GLOS 2003, RASELIMANANA 2008, BORA et al. 2010) the species diversity hotspots are clearly in the rainforests of humid eastern Madagascar, e.g. in the regions of Andasibe and Ranomafana, where more than 100 species can occur in an area of just a few square kilometres (VIEITES et al. 2009, BROWN et al. 2016, RAKOTOARISON et al. 2017).

Due to its relatively large expanses of remaining primary rainforest, the Masoala peninsula in northeastern Madagascar is assumed to represent another hotspot of herpetological biodiversity, yet the amphibians and reptiles of this region remain poorly studied. A number of new species described in the last decade (e.g. ANDREONE & GREER 2002, ANDREONE et al. 2006, FENOLIO et al. 2007) and recent dis-

coveries indicate that the percentage of still unrecognized species might be high in this region. A substantial portion of the peninsula's rainforest is now protected as Masoala National Park (KREMEN et al. 1999, KREMEN 2003), but there is also some ecotourist infrastructure in the unprotected areas along the coast, especially in the region around Tampolo (southeast of Maroantsetra) which we used for our study.

The treefrog genus *Boophis* Tschudi, 1838 forms the subfamily Boophinae in the family Mantellidae (VENCES & GLAW 2001). Most *Boophis* species have been described since the early 1990s (e.g. ANDREONE 1993, GLAW & VENCES 1994, GLAW et al. 2001, 2010, KÖHLER et al. 2007, 2008, 2011, VALLAN et al. 2010, VENCES et al. 2010, 2012, PENNY et al. 2014, HUTTER et al. 2015). BLOMMERS-SCHLÖSSER & BLANC (1991) distinguished only 28 species, whereas today 77 species are scientifically named and numerous additional candidate species have been identified (VIEITES et al. 2009, PERL et al. 2014, HUTTER et al. 2018). The genus is divided into the two subgenera *Boophis* and *Sahona* (GLAW & VENCES 2006), and the former is currently subdivided into nine species groups (HUTTER et al. 2018).

Currently, the *Boophis albilabris* group includes four nominal species: *Boophis albilabris* (BOULENGER, 1888), *B. occidentalis* GLAW & VENCES, 1994, *B. praedictus* GLAW et al., 2010, and *B. tsilomaro* VENCES et al., 2010. A candidate species from the Masoala peninsula, preliminarily listed as *Boophis* sp. aff. *albilabris* “reticulated lip” by GLAW & VENCES (2007), turned out to be genetically identical to *B. praedictus* (VENCES et al. 2010). However, photographs of ‘*Boophis praedictus*’ from low elevation of Masoala indicate that these populations have distinct characteristics delimiting them from other *B. praedictus* populations. The most distinctive characters are those of the eye: *B. praedictus* from the east (from Makira, Ambatovaky and Vevembe) have a red iris periphery whereas those from low elevation in Masoala are purple, and the areas around the pupil differ between both populations as well.

Boophis are notorious for lacking distinct morphological differences between closely related species and their intraspecific variability in body colour and pattern can be substantial (e.g. in *B. picturatus*, see GLAW et al. 2001). In contrast, the colouration of the eyes turned out to be species-specific for numerous *Boophis* species and therefore is a crucial character for their taxonomy (GLAW & VENCES 1997, AMAT et al. 2013). Similarly, the colour of the webbing between toes and fingers can be a reliable character to distinguish closely related species (e.g. GLAW et al. 2010). The relevance of eye colouration and webbing colouration for intraspecific species recognition and as prezygotic isolation mechanisms are still poorly understood given that *Boophis* are largely nocturnal. However, recent studies revealed that frogs have the unexpected ability to see colour at night (YOVANOVICH et al. 2017), thereby shedding new light on these unresolved questions. In this paper we describe a new species of the *Boophis albilabris* group with distinct eye colouration and blue webbing and discuss the possible roles of these remarkable colour traits. Furthermore, we study its genetic relationships to other *Boophis* species and provide a detailed description of its skeleton and its distress call.

Materials and methods

Frogs of the *Boophis albilabris* group were captured mostly at night and located by opportunistic searching, using torches and head lamps. Photographs were taken in life, either in the habitat or the next morning after capture. Specimens were euthanized and subsequently tissue samples for genetic analyses were preserved in 96% ethanol. Specimens were fixed in 96% ethanol, preserved in 70% ethanol, and deposited in the collections of the Université d’Antananarivo, Département de Biologie Animale, Antananarivo, Madagascar (UADBA), and the Zoologische Staatssammlung München, Germany (ZSM). Other institutional abbreviations used are: MRSN (Museo Regionale di Scienze Naturali, Torino, Italy), UMMZ (University of Michigan, Museum of Zoology, Ann Arbor, Michigan, USA), ZFMK (Zoologisches Forschungsmuseum Alex-

ander Koenig, Bonn, Germany). DRV, FGZC, RAX, and ZCMV refer to field numbers of D. R. VIEITES, F. GLAW, C. J. RAXWORTHY and M. VENCES, respectively. One specimen of the new species was collected, and tissue samples of two additional, differently coloured specimens were taken (ZSM-DNA 00289, green specimen, and ZSM-DNA 00290, brown specimen, both found on Masoala, around 15.7121°S, 49.9640°E, 21 m a.s.l.).

Morphological measurements (in millimetres) were taken by FG with a digital calliper to the nearest 0.1 mm. The definition of measurements, terminology and the description scheme follow GLAW et al. (2010) and VENCES et al. (2010), and GLAW & VENCES (1997) for eye colouration. Webbing formulae follow BLOMMERS-SCHLÖSSER (1979).

A micro-CT scan of the skeleton of the new species was produced following methods established in previous work (SCHERZ et al. 2017). Scanning was performed in a nanotom|µm cone-beam micro-CT scanner (GE Measurement & Control, Wunstorf, Germany), using a tungsten target and a 0.1 mm Cu filter. The specimen was mounted on polystyrene in a closed plastic vessel and anchored in place with small wooden struts to minimise movement. A small volume of 80% ethanol was added to the vessel to prevent desiccation. Scanning was performed at 140 kV and 80 µA with exposure times of 750 ms for a total of 30 minutes (2440 images). Scans were reconstructed in *datos|x* reconstruct (GE Measurement & Control), and visualised and refined in VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany). DICOM image stacks of the scans and rotational videos were deposited in MorphoSource at http://morphosource.org/Detail/ProjectDetail/Show/project_id/479. Osteological terminology follows TRUEB (1968, 1973).

Distress calls were recorded as a video with a Lumix DMC-FT5 digital camera with built-in microphone, saved in MTS format, converted to wav format and analysed using the software Cool Edit version 96 (Syntrillium Software Corporation). We used the Hanning windowing function at a resolution of 256 bands and a range of 55 dB to create the spectrogram. Call terminology follows KÖHLER et al. (2017).

For the genetic analysis, we used the 16S rRNA alignment of the *Boophis albilabris* group from VENCES et al. (2010) and added newly obtained sequences of three individuals of the new *Boophis* species and several individuals of *Boophis albilabris*, as well as further sequences downloaded from GenBank. We extracted DNA from tissue samples preserved in 100% ethanol and amplified and sequenced a fragment of the mitochondrial 16S rRNA gene using standard protocols (GLAW et al. 2010, VENCES et al. 2010), and with primers 16Sar-L and 16Sbr-H of PALUMBI et al. (1991). Sequences were resolved on an ABI 3130xl capillary sequencer and aligned using MEGA7 (KUMAR et al. 2016); the alignment required only a small number of indels. We carried out Maximum Likelihood phylogenetic inference under a GTR+I+G model as in VENCES et al. (2010), in MEGA7, testing robustness of nodes with 2000 bootstrap replicates. Sequence divergences were calculated as uncorrected pairwise distances (p-distances) in MEGA7.

All new sequences were submitted to GenBank (accession numbers MH628317–MH628325).

Molecular phylogeny and genetic divergences

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:058041C1-7D20-49B9-8329-6B1B33251DoF. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: www.salamandra-journal.com, Zenodo.org.

The three newly sequenced samples of the new species from Masoala, representing three different colour morphs, had identical 16S sequences. They differed by two substitutions (0.4% p-distance) from a previously published sequence from the Antalaha Province originally obtained by RICHARDS et al. (2000) and available from GenBank (specimen UMMZ 214050; accession number AF261264). All included sequences of *B. albilabris* are remarkably similar to each other (0.0–1.1% p-distance, Fig. 1) throughout the large distribution range of the species (Fig. 2).

The inferred phylogenetic tree (Fig. 1) based on a total of 505 aligned nucleotides reconstructed the following relationships among species of the *Boophis albilabris* group: *B. occidentalis* and *B. tsilomaro* were sister species, together forming the sister clade of *B. praedictus*, and the clade of these three species was sister to *B. albilabris*. This poorly supported branching pattern is congruent with a previous

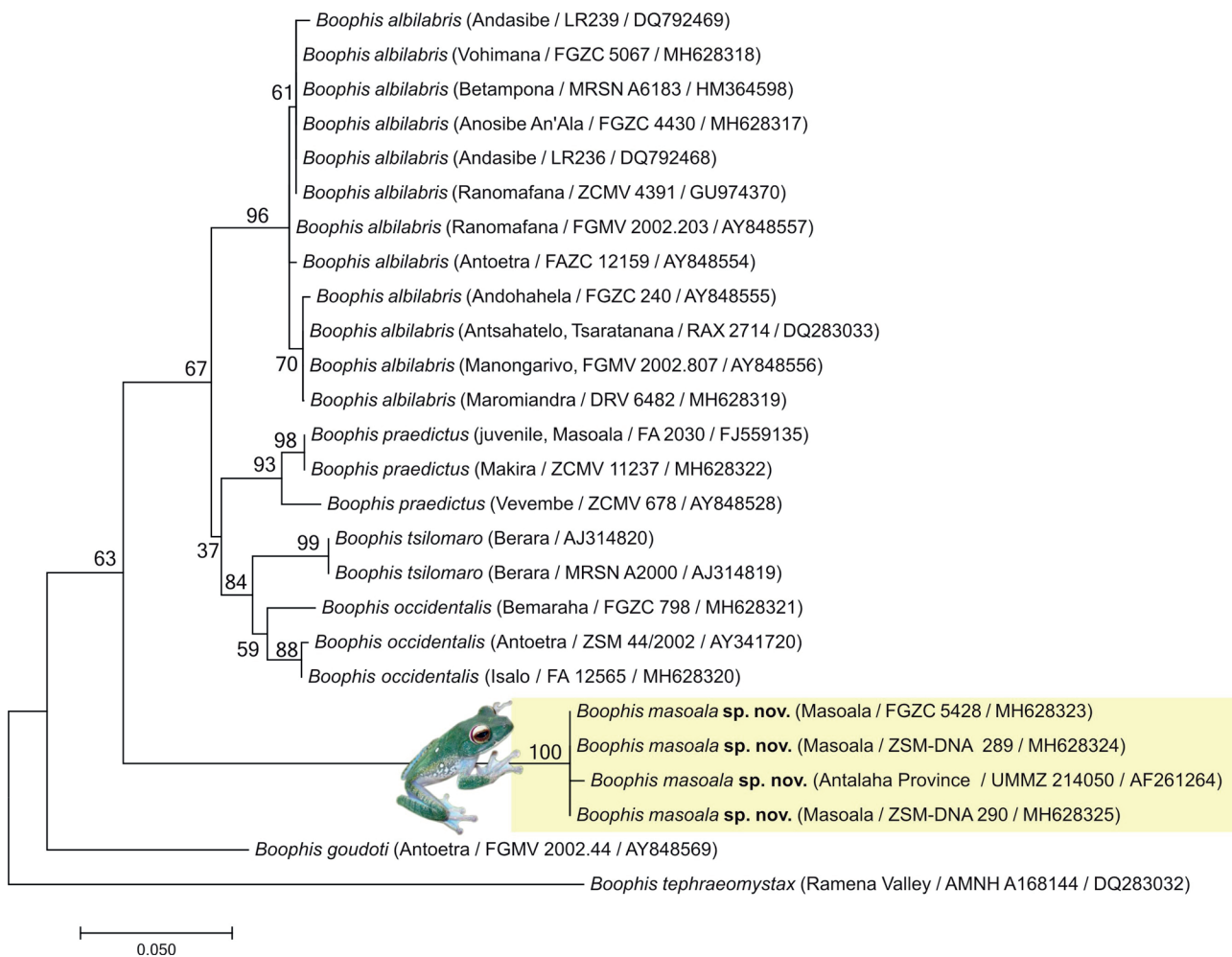


Figure 1. Maximum Likelihood tree of the *Boophis albilabris* group, based on an alignment of 505 bp of the mitochondrial 16S rRNA gene. Values at nodes are bootstrap support values in percent (2000 replicates). *Boophis tephraeomystax* (subgenus *Sahona*) and *B. goudoti* were used as the outgroup. For each individual, as far as available, the tree gives information on sampling locality, voucher specimen, and GenBank accession number.

study based on the 16S gene (VENCES et al. 2010), but differs from those of multigene phylogenies (WOLLENBERG et al. 2011, HUTTER et al. 2018) in which the branching pattern (((*B. occidentalis*, *B. tsilomaro*) *B. albilabris*) *B. praedictus*) was found.

The tree placed the new species from Masoala sister to all remaining species of the *B. albilabris* group, with a remarkably long branch indicating its extremely strong ge-

netic differentiation. The genetic divergences of the new species to all other species of the *B. albilabris* group were remarkably high: 11.1–13.3% pairwise uncorrected p-distance to *B. albilabris*, 10.5–12.4% to *B. occidentalis*, 12.8–13.3% to *B. tsilomaro* and 11.3–12.4% to *B. praedictus*. A comparison with homologous sequences of all other nominal species of *Boophis*, belonging to different species groups, revealed even higher genetic divergences to these. These results in-

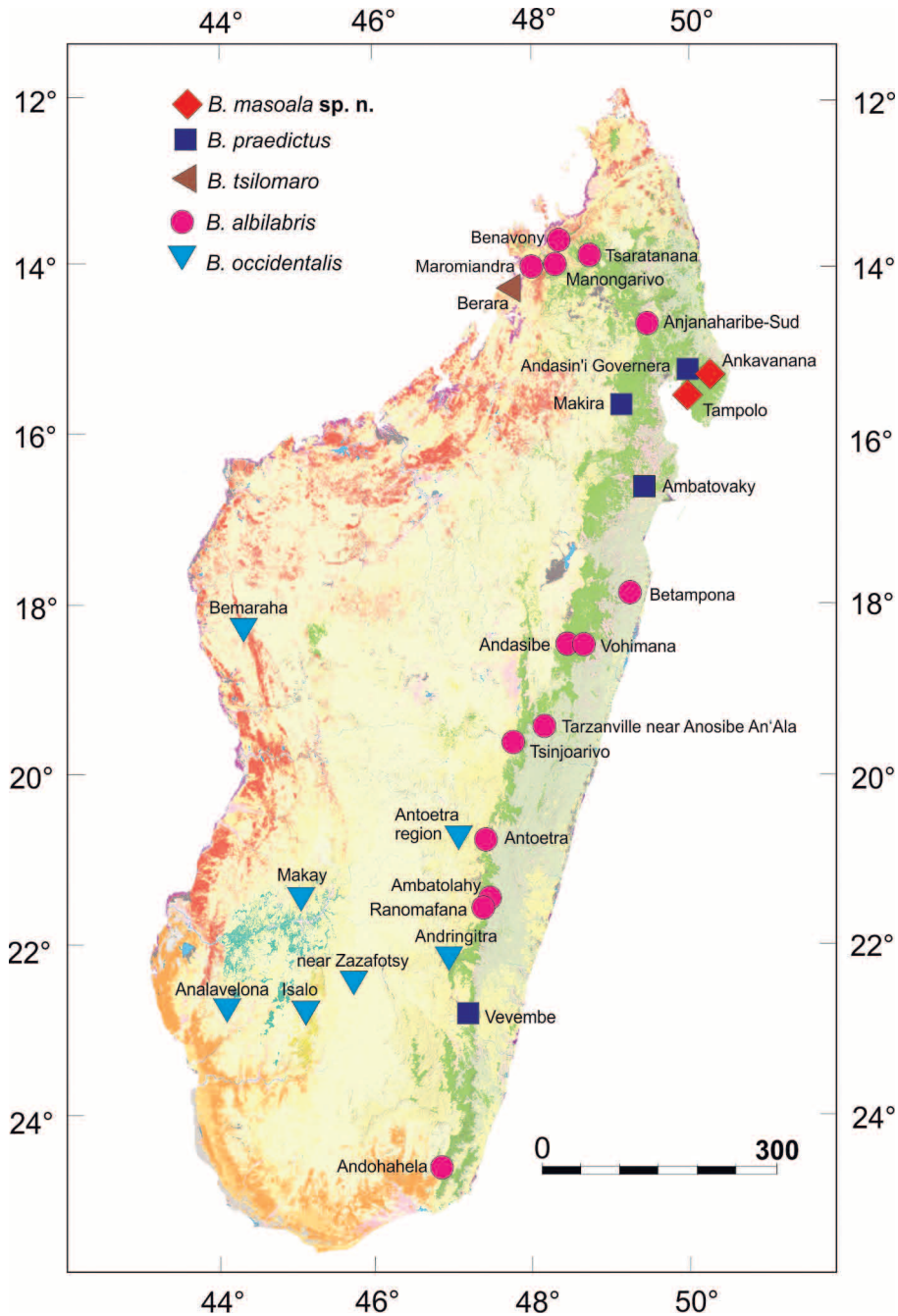


Figure 2. Map of Madagascar showing reliably identified locality records of the species in the *Boophis albilabris* group largely based on sequences used in Fig. 1 and on unambiguously identified voucher specimens or photographic records.

dicating a phylogenetically isolated position of these frogs from coastal Masoala, and strongly suggest that they represent a new species, which will be described below.

Updated distribution of species
in the *Boophis albilabris* group

Compared to the distribution map presented in VENCES et al. (2010) our updated map (Fig. 2) provides additional localities of *B. albilabris* (from near Ankaramy, Betampona, Tarzanville, Tsaratanana and Vohimana) which are confirmed by our own genetic sequences or others taken from GenBank (Fig. 1), and a few other records which were reliably identified by photographs or voucher specimens (from Benavony, Anjanaharibe-Sud and Tsinjoarivo). Records of *B. albilabris* from eastern and northern Madagascar include Vohimana (ZSM 119/2016 = FGZC 5067; 18.9257°S, 48.5069°E, ca. 830 m a.s.l.), Tarzanville near Anosibe An'Ala (ZSM 240/2010 = FGZC 4430; 19.3243°S, 48.2198°E, 881 m a.s.l.), Tsinjoarivo (ZSM 241/2010 = FGZC 4582; 19.7164°S, 47.8216°E, 1,300 m a.s.l., apparently the highest elevation recorded for this species), Betampona (Betakonana and Sa-haïndrana, 317 and 327 m a.s.l., see ROSA et al. 2012), Anjanaharibe-Sud (several records, including ZFMK 59906, see ANDREONE et al. 2002), Tsaratanana Reserve (RAX 2714, Antsahatelo Camp, 13.8597°S, 48.8664°E, ca. 720 m a.s.l., GenBank: DQ283033), and Andohahela (ZSM 130/2004 = FGZC 240, ca. 24.73°S, 46.83°E, ca. 500 m a.s.l.). Records from the Sambirano region in the northwest include Manongarivo Camp 1 (UADBA-FGMV 2002.807; 13.977°S, 48.422°E, 751 m a.s.l.), a forest near Benavony (ZFMK 57383, 13.7111°S, 48.4878°E, ca. 100 m a.s.l., the lowest reliable altitudinal record for the species), and the Maromiandra forest fragment near Ankaramy (ZSM 475/2014 = DRV 6482; 13.9965°S, 48.2177°E, 283 m a.s.l.). The straight-line distance between the Maromiandra forest fragment and the type locality of *Boophis tsilomaro* (given as 14°8'18.55"S, 47°8'54.92"E in the original description, but here corrected to 14°18'55"S, 47°54.92"E = 14.3092°S, 47.9153°E) is only 48 km, yet no indications of potential mitochondrial introgression were detected. We also slightly correct the position of the locality of a juvenile *Boophis praedictus* (MRSN A2030) which was reported by VENCES et al. (2010) from the west coast of Masoala close to the type locality of *Boophis masoala* and would have resulted in sympatric occurrence of *B. praedictus* and *B. masoala*. However, this juvenile *B. praedictus* used for the DNA sequencing was actually collected in the northwest of Masoala, at a campsite locally known as Andasin'i Governera Campsite (ca. 15°18'S, 50°01'E = 15.30°S, 50.02°E; coordinates according to ANDREONE & GREER 2002). An apparently adult *B. praedictus* with typically red iris periphery was photographed by F. ANDREONE in a nearby region, at Mont Beanjada (ca. 15°16'S, 49°59'E = 15.27°S, 49.98°E; coordinates according to ANDREONE & GREER 2002), confirming that 'typical' *B. praedictus* occur in the north of Masoala. On the other hand, the DNA sequence of '*Boophis albilabris*' published by RICHARDS et al.

(2000) is almost identical to the new species described below (Fig. 1), strongly suggesting that it actually belongs to this new species rather than to *B. albilabris* or *B. praedictus*. The corresponding specimen (UMMZ 214050) was collected at the Ankavanana river (15.3083°S, 50.2333°E, 80 m a.s.l., G. SCHNEIDER pers. comm.). These data demonstrate that both *B. praedictus*, and the new species described below, inhabit the Masoala peninsula, but so far are not known to occur in close sympatry. Locality records of *B. occidentalis* added to the map (Fig. 2) are Analavelona forest (based on a photograph of '*Boophis albilabris*' by H. SCHÜTZ in CADLE 2003: 918), Makay (based on ANDREONE et al. 2014: 124), and Tsaranoro Valley, near Andringitra massif (D. AUSTIN pers. comm.).

***Boophis masoala* sp. n.**

Figs 3 and 4

ZooBank LSID: urn:lsid:zoobank.org:act:7F77DDD6-84D6-4A8A-A6D1-75522D6D803B

Holotype: ZSM 251/2016 (FGZC 5428), adult female, collected between 'Eco-Lodge chez Arol' and 'Tampolo Lodge', coastal Masoala peninsula, Maroantsetra district, Analanjirofo Region, northeastern Madagascar, 15.7247°S, 49.9599°E, 14 m a.s.l., on 11 August 2016 by F. GLAW, D. PRÖTZEL, J. FORSTER, K. GLAW & T. GLAW. No paratypes.

Diagnosis: Assigned to the genus *Boophis* based on the presence of an intercalary element between ultimate and penultimate phalanges of fingers and toes (verified by external and osteological examination), enlarged terminal discs of fingers and toes, lateral metatarsalia separated by webbing, absence of outer metatarsal tubercle, molecular phylogenetic relationships (Fig. 1), and overall similarity to other *Boophis* species. Assigned to the *Boophis albilabris* group based on the following combination of characters: large size (snout-vent length of holotype 82.4 mm); well developed webbing between fingers; presence of vomerine teeth; presence of a white line along upper lip; molecular phylogenetic relationships; and overall morphological similarity to *B. praedictus* and *B. albilabris*.

Boophis masoala differs from all other *Boophis* species including all species of the *B. albilabris* group by its distinctive eye colouration (Fig. 3). It furthermore differs from all other *Boophis* species except *B. praedictus* by bluish (rarely yellowish) webbing between fingers and toes. It differs from most other *Boophis* species by the white line along the upper lip and distinctly larger size. It mostly resembles *B. praedictus* from which it differs by the colour of the iris periphery and by iris colour. An overview of diagnostic characters of the species in the *Boophis albilabris* group is provided in Table 1. In addition, *Boophis masoala* differs from the other species in the *Boophis albilabris* group by remarkable genetic differentiation, with pairwise 16S divergences of 10.5–13.3% to all other species of the group (and even higher divergences to all other species of *Boophis*).

Description of the holotype: Adult female (verified by dissection and presence of white oviducts, though without any oocytes), snout–vent length 82.4 mm. Body moderately slender; head length (28.8 mm) slightly shorter than width (31.7 mm), slightly wider than body; snout rounded in dorsal view, obtuse in lateral view, nostrils directed laterally, nearer to tip of snout than to eye (eye–nostril distance 7.7 mm, nostril–snout tip distance 6.9 mm), canthus rostralis moderately distinct, slightly concave in dorsal view, loreal region slightly concave; tympanum distinct (horizontal diameter 6.9 mm), rounded, tympanum diameter 74% of horizontal eye diameter (9.3 mm); supratympanic fold thin, distinct; vomerine odontophores prominent, well separated in two elongated patches, positioned posteromedial to choanae; choanae medium-sized, elongated. Tongue posteriorly bifid, free (left tip removed as tissue sample). Arms slender, with a poorly developed white dermal edge from elbow to the lateral base of the finger. Subarticular tubercles single, round; metacarpal tubercles not recognizable; fingers broadly webbed; webbing formula 1(1), 2i(1.5), 2e(0), 3i(1.5), 3e(0), 4(0); relative length of fin-

gers $1 < 2 < 4 < 3$ (finger 2 distinctly shorter than finger 4); finger discs strongly enlarged. No bony prepollex at the base of the first finger, nor any black keratinized nuptial pads on the base of the inner sides of fingers 1–3. Hindlimbs slender; tibiotarsal articulation reaching nostril when hindlimb is pressed along body; lateral metatarsalia separated by webbing; inner metatarsal tubercle small, distinct, elongated; no outer metatarsal tubercle; toes almost fully webbed; webbing formula 1(o), 2i(o), 2e(o), 3i(o), 3e(o), 4i(0.5), 4e(0.5), 5(o); relative length of toes $1 < 2 < 5 < 3 < 4$; toe discs enlarged. Skin smooth on dorsal surfaces. Cloaca distinct, slightly concealed by a skin fold. Skin partly folded on the flanks, almost smooth on throat and chest, slightly granular on belly and ventral surfaces of thighs.

After 1.5 years in preservative, ground colour of head and dorsum brown, marbled with few black spots on the posterior back and poorly delimited grey spots mostly on head and neck. Tympanic region grey. Iris grey with the dark pattern around the horizontal pupil still recognizable. Posterior iris periphery purple. A distinct and narrow white line along the entire upper lip and one (right) or

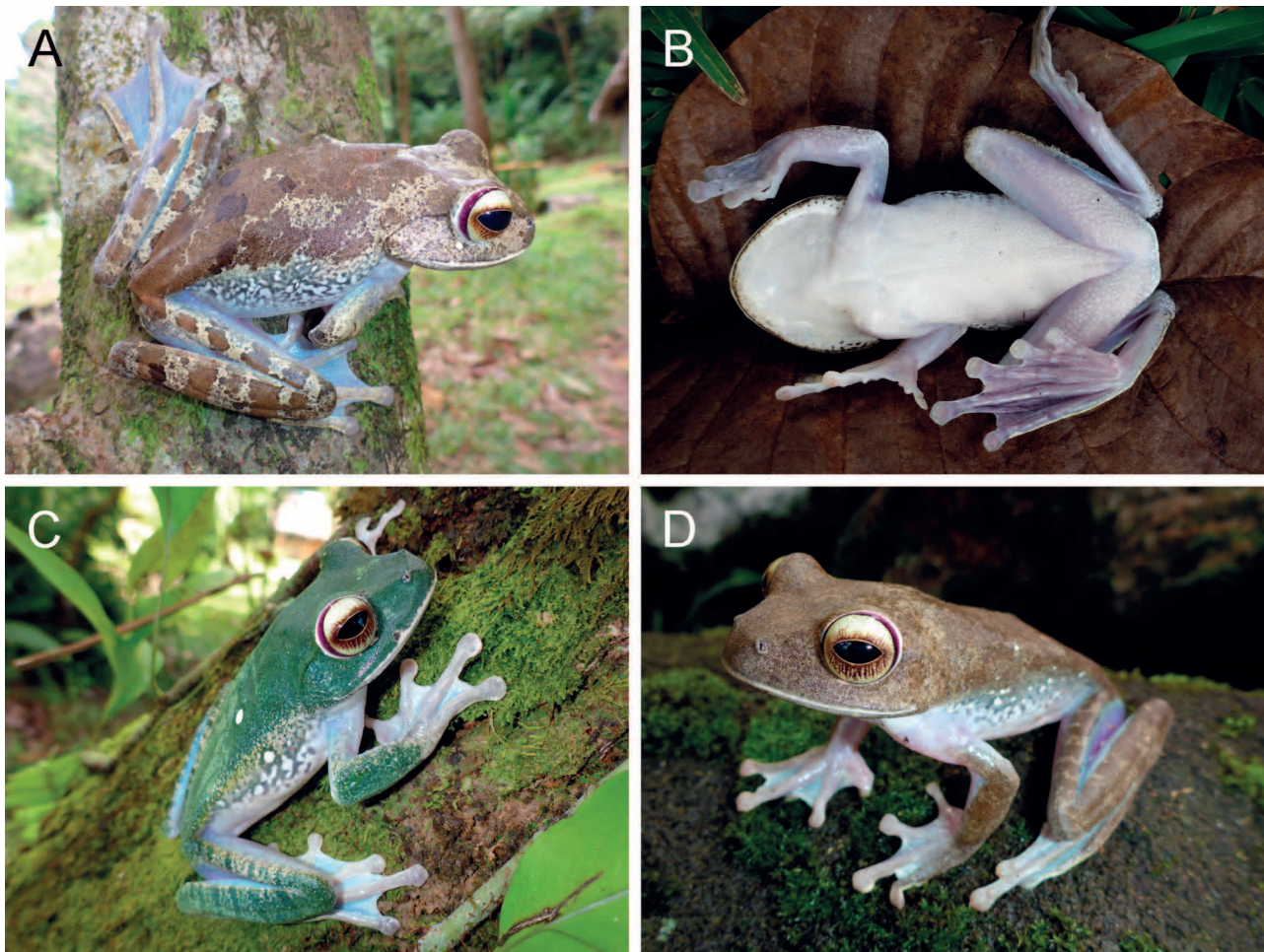


Figure 3. *Boophis masoala* sp. n.: (A) Holotype in dorsolateral and (B) ventral view. Two additional individuals were sampled and sequenced but not collected: (C) ZSM-DNA 00289 and (D) ZSM-DNA 00290, both representing different colour morphs.

Table 1. Diagnostic characters to distinguish the species of the *Boophis albilabris* group.

	<i>B. masoala</i> sp. n.	<i>B. praedictus</i>	<i>B. albilabris</i>	<i>B. occidentalis</i>	<i>B. tsilomaro</i>
outer iris colour	beige to whitish	golden-yellow	brown to yellow	blue	yellow to golden
inner iris colour	pattern of brown, almost vertical lines	reticulated brown	as outer iris or darker	brown	brown
iris periphery	purple	bright red	whitish to greyish	blue	blue?
white band behind iris periphery	present	absent	absent	absent	absent
green band above eye	absent	absent	mostly present	sometimes indistinct yellow band	usually absent
tympanum	moderately distinct	distinct	distinct	usually distinct	distinct
white ridge on lower arm and elbow	less distinct in observed specimens	very distinct	less distinct in males, distinct in females	distinct	less distinct in males
white ridge on lower tarsus	less distinct in observed specimens	very distinct	relatively indistinct	distinct	poorly developed
dorsal ground colour	green or brown with or without spots	green	very variable: green, brown or grey with or without dark or light spots or markings	bright green (rarely brown)	green to brown
flank colour	with distinct white spots or white marbling, no sharp border	rather sharp border between green dorsal and grey-white flanks with brown spots	variable	not very sharp border between green dorsal and grey/reddish flanks; flanks with or without white spots	not very sharp border
light dorsolateral stripe from eye to midbody	absent	absent	absent	usually distinct (yellow)	relatively short, sometimes absent
dorsal surface of upper arm	less pigmented	at least partly unpigmented	less pigmented	less pigmented	less pigmented?
supratympanic fold	mostly distinct	weak to moderately distinct	distinct	distinct mostly by colour	relatively distinct
webbing between toes (hindlimbs)	blue (rarely yellow)	blue	mostly yellowish	reddish to purple	reddish
fingertips (hand)	grey-brown	whitish to orange	variable: often grey or greenish	yellow to orange	yellow
white line along upper lip	distinct	distinct	distinct	thin, often indistinct or absent	narrow, but distinct
dorsal colour in ethanol	grey (n=1)	purple	purple or grey	purple	purple (with dense black spicules in breeding condition)

two (left) small white spots below the eye. Dorsal surfaces of hindlimbs with 3–4 alternating brown and grey crossbands, which largely extend from thighs to shanks and tarsus if limbs are placed in a ‘sitting position’. A white lateral line along tarsus and distal edge of outer toe. Dorsal surfaces of four inner toes light grey, outer toe brown-grey. Webbing between toes purple-grey. Hidden dorsal surfaces of thighs uniformly magenta without spots or dots. Forelimbs dorsally with three brown spots on the arm and grey colour in-between. A poorly developed whitish lateral line along lower arm and distal edge of outer finger. Dorsal surface of the three inner fingers dirty white, outer finger grey with indistinct brown spots. Webbing between fingers grey. Flanks are marbled with strongly contrasting grey and white. All ventral surfaces cream except the grey webbing.

Colouration in life (Fig. 3A, B) is generally similar to that in preservative, but the brown colour on the back was lighter, the grey spots were beige and the webbing and the hidden parts of the hindlimbs were blue. The iris was yellow with a brown area around the pupil and a purple posterior iris periphery. The ventral surfaces were largely white (Fig. 3B).

Osteology of the holotype (Fig. 4): Skull containing all of the typical anuran elements and no new elements; generally well ossified.

Cranium. Cranium widest near the posterior-most extension of the maxilla, at the level of the tip of the zygomatic ramus of the squamosal, highest at the level of the exoccipital-frontoparietal junction. Orbits relatively spacious, snout of moderate length, over one third of total

skull length. Braincase deepest at its posterior-most level, narrowing anteriorly to the sphenethmoid by the upward tendency of the parasphenoid (see below).

Neurocranium. The sphenethmoid is moderately ossified. It extends posteriorly under and the frontoparietals, maintaining contact with them, and is ventrally in contact with the parasphenoid. Medially it forms a septum anteriorly, and dorsally it is flattened and acts to extend the ossified roof of the skull beyond the anterior tip of the frontoparietals and the neopalatine, but not reaching the nasals. The otic capsule is rather well ossified. The prootic is fused medially with the frontoparietal and laterally with the otic ramus of the squamosal, ventrally in broad contact with the parasphenoid alae. It possesses a long lateral arm that extends along the ventral surface of the otic ramus but not to the body of the squamosal. The exoccipital is strongly

ossified and in contact with its contralateral. It is bound to the otic ramus of the squamosal through a poorly ossified, probably cartilaginous shelf dorsally. Three foramina are present in the lateral surface of the exoccipital, the lowest-most and largest of which is the otic foramen. Ventrally a further three foramina are present.

The septomaxilla is small and spiralled upwards counter-clockwise on the left and clockwise on the right. Its anterior ramus is thicker than the medial and lateral arms. It is oriented obliquely, situated near to the lingual shelf of the maxilla, roughly above the anterior-most extension of the vomer. As we have warned elsewhere (SCHERZ et al. 2017) we caution against the over-reliance on micro-CT data for the structure of this very fine bone, unless the snout itself is scanned specifically; physical investigation may be necessary to establish its detailed anatomy.

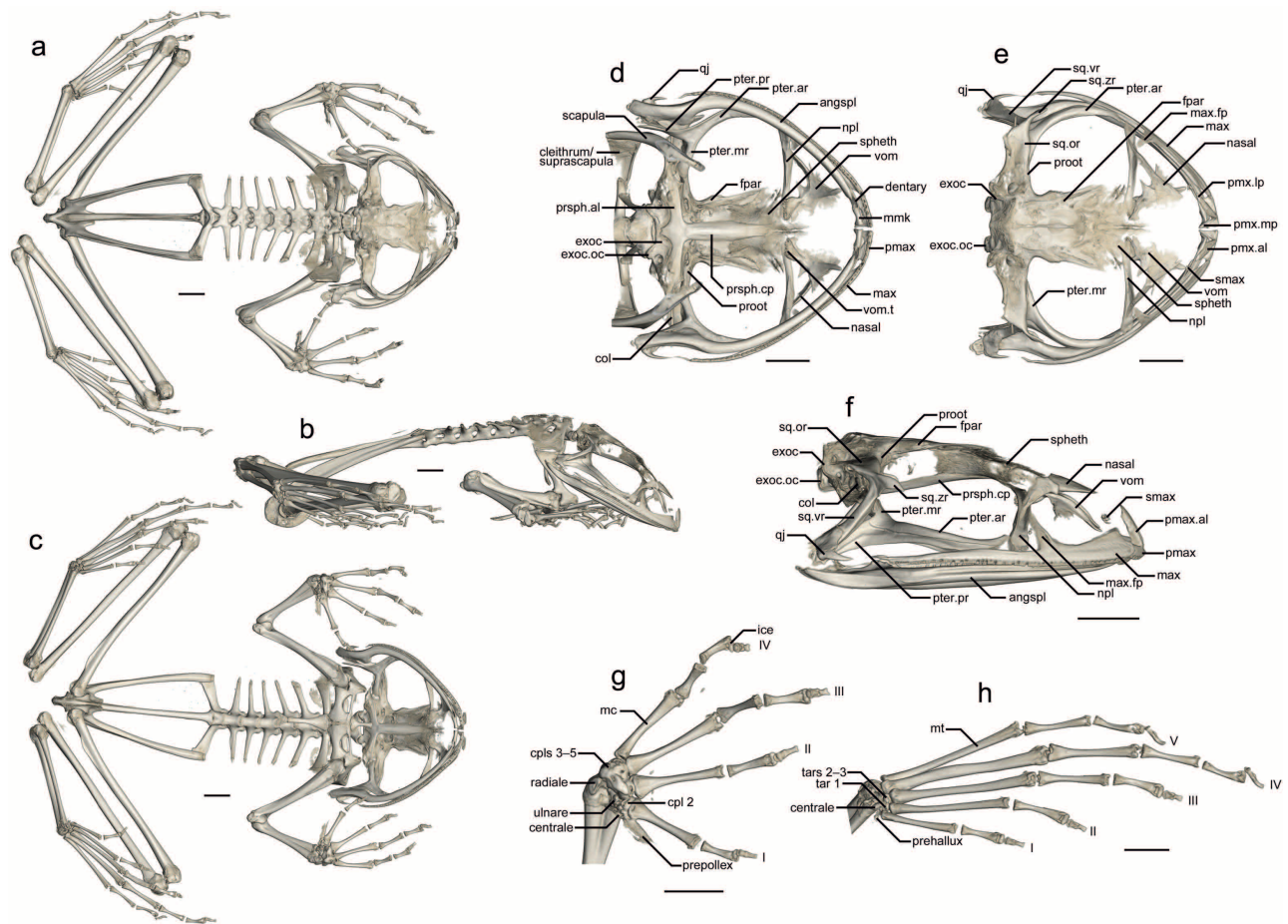


Figure 4. Osteology of the holotype of *Boophis masoala* sp. n. Full skeleton shown in (a) dorsal, (b) lateral, and (c) ventral view; skull shown in (d) ventral, (e) dorsal, and (f) lateral view; and (g) hand and (h) foot in ventral view. Scale bar indicates 5 mm. Abbreviations: angspl – angulosplenic; col – columella; cpl(s) – carpal(s); exoc – exoccipital; exoc.oc – occipital condyle of exoccipital; fpar – frontoparietal; ice – intercalary element; max – maxilla; max.fp – pars fascialis of maxilla; mc – metacarpal; mmk – mentomeckelian; mt – metatarsal; npl – neopalatine; pmax – premaxilla; pmx.al – alary process of premaxilla; pmx.lp – lateral process of premaxilla; pmx.mp – medial process of premaxilla; proot – prootic; prsph.al – parasphenoid alae; prsph.cp – cultriform process of parasphenoid; pter.ar – anterior ramus of pterygoid; pter.mr – medial ramus of pterygoid; pter.pr – posterior ramus of pterygoid; qj – quadratojugal; smax – septomaxilla; spheth – sphenethmoid; sq.or – otic ramus of squamosal; sq.vr – ventral ramus of squamosal; sq.zr – zygomatic ramus of squamosal; tar(s) – tarsal(s); vom – vomer; vom.t – dentigerous process of vomer.

The columella (stapes) is oriented perpendicular to the snout-vent axis, and is only slightly sloped upwards. The otic foramen is rather small, and the oval baseplate of the columella occupies most of its area.

Dorsal investing bones. The nasal is roughly triangular, and sits well isolated from its contralateral. Its medial area is not especially wide, and the posterolateral maxillary process is rather long and tapering, extending ventrally as it proceeds posterolaterally, at its posterior-most point reaching the anteroposterior level of the neopalatine, where it is approached from ahead and below by the maxillary pars facialis (described below), but the two are not in contact.

The frontoparietal is fairly elaborate in shape. Anteriorly it is broad, and it tapers posteriorly, at its narrowest (roughly half of its breadth anteriorly) at its posterior-most point, where it is in contact with the exoccipital. At the mid-level of the otic capsule, it possesses a small dorsal process. The posterior half of the lateral surface is in contact with the prootic's anterior ramus, so it lacks a ventral shelf itself. It is in medial contact with its contralateral.

Ventral investing and palatal bones. The parasphenoid is T-shaped. Its cultriform process is narrow, at its broadest point (near its middle) merely one third of the width of the frontoparietals. It broadens from the alae to the mid-point, beyond which it tapers more strongly and becomes increasingly thin and unmineralised. It does not reach the level of the neopalatine or the vomer. The anterior half of the cultriform process is in dorsal contact with the sphenethmoid. The parasphenoid alae are also narrow, narrowest medially and broadening laterally, becoming less mineralised toward their ends; they are slightly posterolaterally oriented. Anterodorsally they are contacted by the prootic, and posteriorly they are contacted by the exoccipital. The posteromedial process of the parasphenoid is pronounced, but does not reach the foramen magnum, being excluded by a mineralisation between the exoccipitals.

The vomer is a large and robust element, possessing four distinct rami: a thick anterior ramus extends towards the lingual shelf of the maxilla; a thinner, more acuminate lateral ramus is almost as long as the anterior ramus, but does not approach the maxilla; a posterolateral triangular ramus extends toward but does not achieve the level of the neopalatine; and a broadening posteromedial ramus bearing at least ten, clearly defined teeth along its posteroventral edge extends posteriorly to the level of the neopalatine, lying below but not in contact with this bone. It is broadly separated from its contralateral.

The neopalatine is an arched bone that lies perpendicular to the longitudinal body axis, running dorsomedially from the maxilla, with which it articulates but is not in direct contact (probably separated by cartilage), to a point just shy of the midline of the skull; it does not approach its contralateral. Around its midpoint, a strong ridge is present on the ventral surface of this bone, and dorsally its surface is sculpted.

Maxillary arcade. The maxillary arcade bears many small teeth on the premaxilla and maxilla. The premax-

illae are separated medially, but juxtaposing the maxillae laterally. The premaxilla bears a strong anterodorsal alary process that is long and dorsolaterally oriented, narrow at its proximal extent, broadening and then narrowing again dorsally. The pars palatina bears two well-defined processes, of which the medial or palatine process is thin and posterolaterally oriented, whereas the lateral process is thicker and curving posterolaterally, but both are of roughly equal length.

The maxilla is long, with a narrow pars palatina along its lingual margin, and a strongly developed, stark, long, triangular pars facialis that approaches but does not contact the posterolateral maxillary process of the nasal.

Suspensory apparatus. The tri-radiate pterygoid bears a short medial ramus with a posteriorly sculpted surface, a posterior ramus of moderate length that reaches the level of the quadratojugal, but does not exceed it (it is however exceeded by the articular surface of the mandible, see below), and a long and strongly curved anterior ramus with a strongly sculpted lateral surface, that articulates anteriorly with the mandible. On the lateral surface of the medial ramus, a large round flare is present that appears to articulate with the medial surface of the squamosal.

The quadratojugal appears reduced, and is not ossified along its full length; anteriorly it contacts the posterior-most tip of the maxilla weakly. It is robust at its posterior junction with the squamosal, but does not have any bulbous processes.

The squamosal is robust, with a distinct crest along its medial surface that articulates with the pterygoid, a short and curving zygomatic ramus, and a long, medially oriented otic ramus that comprises much of the dorsal surface of the otic capsule.

Mandible. The mandible is quite robust and edentate. The mentomeckelians are thin and arcuate, anteriorly in lateral contact with the dentary. The dentary is long and laminar, running along the lateral surface of the angulosplenic, and at least partly in contact with this bone. The angulosplenic is long and arcuate, strongly laterally sculpted where Meckel's cartilage runs along its length. The coronoid process is quite weak, at the level of the posterior-most extent of the maxilla. The articular surface is very long, dorsally flat, and smooth, extending beyond any other element of the skull.

Hyoid. The posteromedial processes of the hyoid are quite thin, spade-shaped bones, with their proximal edges broadened to be spatulate. They do not possess any strong crests. No ossified parahyoid is present.

Postcranium. Vertebral column. The vertebral column is diplasiocoelous with the first seven vertebrae being procoelous but the eighth presacral being apparently biconcave. No vertebrae are fused. The neural arch of the atlas (presacral I) is complete. The neural arches of presacrals II-IV bear weak neural spines. The transverse processes of presacrals II-IV are thick; those of presacral II are laterally but not ventrally oriented and rather short; those of presacral III are thick and oriented more ventrally; those of presacral IV are oriented more posteriorly and somewhat dor-

sally. The thinner transverse processes of presacrals V–VIII are also oriented somewhat dorsally, each becoming less posteriorly-oriented to the point of presacral VIII, which has laterally oriented transverse processes.

The sacrum bears weakly expanded, posteriorly oriented diapophyses. The leading and trailing edges are largely straight, and are not strongly sculpted. The urostyle is long, and possesses a moderately strong dorsal ridge along the anterior two thirds of its length. It lacks any major ornamentation at its head. Its articulation with the sacrum is bicondylar.

Pectoral girdle. The pectoral girdle possesses a well-ossified omosternum, clavicle, coracoid, and sternum, as well as an unossified xiphisternum. The omosternum is small and only very weakly bifurcated posteriorly without any real definition to the posterior arms. Its anterior neck broadens slightly anteriorly. It contacts but is not fused to the clavicles.

The clavicle is straight, medially strongly fused to the coracoid and in contact with its contralateral, laterally strongly fused with the scapula. The lateral scapula is broadened but its exact shape is difficult to distinguish in micro-CT scans due to the extent of mineralisation of this joint.

The coracoid is robust, strongly flared at either end with a rather narrow middle. The medial end is broader than the lateral end, anteriorly flattened at its fusion with the clavicle, medially broadly fused to its contralateral, and posteriorly in weak contact with the sternum. Together the coracoid and clavicle form a robust D-shaped foramen.

The sternum is anteriorly flared, tapering posteriorly to a square tip, itself as long as the prezoal and zonal elements of the girdle combined.

The scapula is long, with a short pars glenoidalis and longer and broader pars acromialis, forming a strongly angular notch in the glenoid socket. It borders and is contacted by the cleithrum dorsally.

The cleithrum is thin and laminar, thickest along its anterior edge and fading indistinctly posteriorly. The suprascapula is broad and poorly ossified, the greatest ossification being along the border with the suprascapula.

Forelimb and manus. The humerus bears moderate ventral and lateral cristae, and lacks a medial crista. The ventral crista is continuous with the caput humeri. The radius-ulna is fairly slender, with a distinct sulcus intermedius. The carpus is composed of a prepollex, a centrale, Element Y, carpal 2, and a large post-axial element formed by the fused carpals 3–5. The prepollex is half as long as the first metacarpal. The phalangeal formula is 2-2-3-3. There are very distinct intercalary elements between the ultimate and penultimate phalanges of each digit. The distal phalanges are long and have a small paired protuberance at the middle of their length, beyond which they are slightly arched and become weakly distally bilobed.

Pelvic girdle. The pelvic girdle is long. The iliac shafts pass ventral to the distal ends of the sacral diapophyses, but do not extend beyond them, making the iliosacral articulation type IIB sensu Emerson (1979). Their shafts pos-

sess strong dorsal crests arising at the strong dorsal prominence, and becoming lower anteriorly, disappearing just before the articulation with the sacrum. There is no oblique groove. The ilia are posteriorly fused with the ischium, and ventrally with the pubis. The pubis is ossified.

Hindlimb and pes. The femur is very weakly sigmoid. It is shorter than the tibiofibula, and bears a distinct posterior crest near the pelvic articulation. The tibiofibula has a weak sulcus intermedius. The tibiale and fibulare are proximally and distally fused, but much weaker distally than proximally. Two tarsals, T1 and T2+T3, a small centrale and a small prehallux are present, articulating with the first through third toes. The phalangeal formula is 2-2-3-4-3. Here too, intercalary elements are present. The distal phalanges of the toes are much the same as those of the fingers; see above.

Variation and distribution: Since only one specimen was collected, the variation described here is restricted to the colouration in life based on photographs of several specimens. The three different colour morphs observed by us are shown in Fig. 3. The dorsal ground colour can vary from green (Fig. 3C) to brown (Fig. 3D) with or without additional spots. Additional individuals of this species were figured on the Internet either as *Boophis albilabris*, e.g. photographs by N. GARBUTT (www.arkive.org, www.nickgarbutt.com) and by A. HYDE (www.alexhydephotography.com) or as *B. praedictus*, e.g. photographs by D. AUSTIN (www.iNaturalist.org) and by A. NÖLLERT (<https://calphotos.berkeley.edu>). All these photographs share the characteristic eye colouration of the species and all of them show bluish webbing, except one individual with distinctly reticulated flanks, which has yellow webbing (<https://nickgarbutt.photoshelter.com/image/I000068KPjyPvMpg>, accessed on 31 July 2018). As far as data are available, all these frogs were photographed at Masoala, suggesting that the species might be endemic to this peninsula. As already discussed above, the DNA sequence published by RICHARDS et al. (2000) suggests that *B. masoala* also occurs in the lowlands of northern Masoala (Fig. 2). The known elevational range of the species is within 0–100 m a.s.l.

Habitat and habits: The holotype was discovered at night perching on branches ca. 3 meters above the ground, in close proximity (ca. 30 m) to the sea shore, but not close to any other water body. Another individual was discovered at night in a tree, ca. 4–5 m above the ground at the edge of the trail that leads from the shore to the EcoLodge, just above a small stream and likewise not more than ca. 50 m distance from the shore. The third individual was found in the same area, but only ca. 2 m above the ground.

We did not notice any potential advertisement calls of this species during our survey in the dry season (from 9–15 August 2016). The eco-touristic region of the west coast of Masoala (between the EcoLodge and the Tampolo Lodge) appears to be well protected and we did not discover any significant traces of logging or other illegal activities in the unprotected coastal region. However, we have seen only a

small portion of the coast and did not enter the Masoala National Park, which is well-known to be heavily affected by illegal rosewood logging (BARRETT et al. 2010, INNES 2010).

Distress call: Upon capture by hand, the holotype emitted several loud distress calls (Fig. 5, Supplementary Material 1). Three recorded distress calls had durations of 1660 ms, 1717 ms and 1785 ms, respectively, and the intervals between two calls were 3322 ms and 7271 ms ($n=2$). Each of the three calls started with a section of increasing intensity with a duration of 227–327 ms ($n=3$) which was emitted when the mouth was still closed and had a frequency range between 0–7000 Hz. This short first section was followed by an intensive sound peak of 44–69 ms duration ($n=3$), which was obviously produced by the opening of the mouth. After this peak the long remaining section of the call was emitted with the mouth opened and its intensity remained relatively constant over most of the call until its decrease at the end. The frequency range of this section was mostly between 1000–7500 Hz. The three sections of the call were identified from a video (Supplementary Material 1, showing the second distress call of 1717 ms length) and are well recognizable in the audiospectrogram (Fig. 5).

Etymology: The specific epithet *masoala* is used as a noun in apposition and is composed of the Malagasy words ‘maso’ (meaning eye) and ‘ala’ (meaning forest), and is usually translated as ‘eye of the forest’ (e.g. RÜBEL 2003). In contrast to many other new *Boophis* species (e.g. *B. feonnyala*, meaning ‘voice of the forest’) which we noticed first by their distinctive advertisement calls, we discovered *B. masoala* by its large eyes shining many meters in the torchlight at night, and we did not hear its advertisement calls (in the dry season). In addition, its eye colour turned out to be species-specific, allowing us to distinguish *B. masoala* from all other species. The specific name furthermore refers to the known distribution of the new species, which might be endemic to the Masoala peninsula.

Available names: The problems with the identity of the *Boophis albilabris* holotype were extensively discussed in GLAW et al. (2010) and since then, no new data have become available that would challenge its attribution to the widespread species which occurs mostly at higher elevations of eastern Madagascar. Since the type locality of *B. albilabris* (‘eastern Imerina’) is in central eastern Madagascar, where *B. masoala* most likely does not occur, conspecificity of *B. masoala* and *B. albilabris* can be excluded with very high probability.

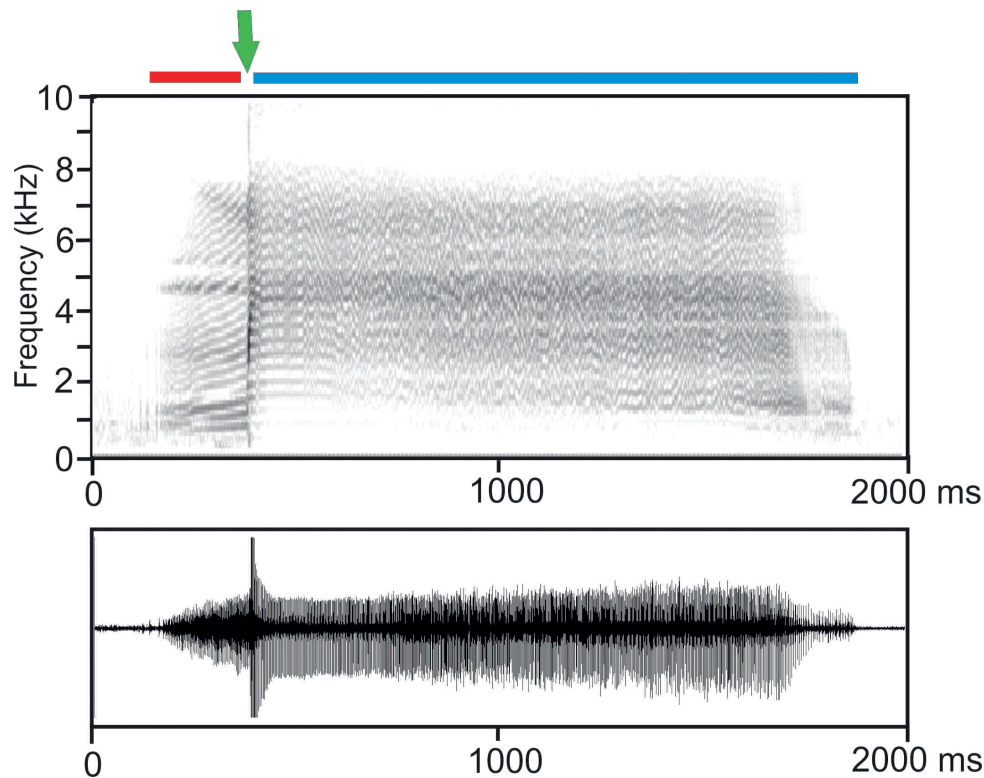


Figure 5. Distress call of the holotype of *Boophis masoala* sp. n., spectrogram (above) and corresponding oscillogram (below). Note that the call started with a short section (marked by red bar) that was emitted when the mouth was closed. This section was followed by a short intensive sound peak (marked by green arrow) obviously produced by the opening of the mouth and a long remaining section after this peak (marked by blue bar) emitted with an open mouth. The corresponding video of this distress call is shown in Supplementary Material 1.

Discussion

Boophis masoala is a distinctive new species that has an unexpectedly high genetic distance to any other *Boophis* species in the sequenced mitochondrial 16S gene fragment and also differs from almost all other species by distinct colourations of its eyes and webbing. In the following we discuss these traits in more detail.

Species-specific eye colouration: Adults of all five species of the *B. albilabris* group can be solely and reliably distinguished based on their eye colouration in life (Table 1) which appears to be rather constant within the species. In contrast, the body colouration of *B. albilabris*, *B. praedictus* and *B. tsilomaro* is known to vary substantially within and among populations. These observations confirm previous studies, which found species-specific eye colouration in frogs and especially in treefrogs (GLAW & VENCES 1997, AMAT et al. 2013). *Boophis* and other treefrog species appear to be largely nocturnal, especially when breeding, making it unlikely that the colourful eyes can be used as a prezygotic isolation mechanism. Although recent studies revealed the ability of nocturnal colour vision in frogs (YOVANOVICH et al. 2017), the colourful iris of the frog is only visible to the human eye during the day when light intensity is high and the pupil of the frog is small. If the frog eye is illuminated at night and the frog pupil is very large, only a small iris ring is visible. Thus it might be more reasonable to assume that the iris colour is used during the day when the frogs are usually sleeping high up in the trees. On days of heavy rainfalls, *Boophis* frogs sometimes start calling during the day and it is plausible that they could also use optical signals for mate recognition under such conditions. Using optical instead of acoustical signals for mate or competitor recognition during the day could help to avoid being captured by diurnal predators and also may explain why amplexant couples appear already at dusk when calling activity has only just started.

Species-specific webbing colouration: The recent discovery of nocturnal colour vision in frogs (YOVANOVICH et al. 2017) might also shed new light on a comparable phenomenon, i.e. the species specific colouration of webbing between toes (and fingers) which is also known to differ between several sister species pairs in *Boophis*.

All three individuals of *Boophis masoala* encountered by us and several photographs in the internet displayed blue webbing (Fig. 3), although a single photograph of this species on the internet with yellow webbing suggests possible sexual dichromatism or variation in this character. Blue webbing is rarely found in frogs, and in Madagascar it is restricted to *Boophis praedictus* and *B. masoala* in the *B. albilabris* group, both occurring on the Masoala peninsula. Outside of Madagascar, partial or complete black-bluish webbing can also occur in a few large Asian gliding treefrogs in the family Rhacophoridae (i.e. the *Rhacophorus nigropalmatus* clade, containing species such as *R. borneensis*, *R. helenae*, *R. kio*, *R. nigropalmatus*, *R. norhayatii*,

R. reinwardtii, and others; OHLER & DELORME 2006, MATSUI et al. 2013). Red webbing is known from the families Rhacophoridae (*Rhacophorus malabaricus*, *R. pardalis*, *R. dulitensis*), Hylidae (*Hypsiboas rufitelus*), Hyperoliidae (*Hyperolius*) and Mantellidae (e.g. *Boophis haematopus*, *B. picturatus*, *B. occidentalis*). The different colouration of the webbing is the most distinctive character to distinguish the closely related species *Rhacophorus kio* and *R. reinwardtii* (OHLER & DELORME 2006) as well as other species of this complex (MATSUI et al. 2013), and is generally an important feature to distinguish among *Rhacophorus* species from mainland Southeast Asia (ROWLEY et al. 2010). Webbing colouration is also a good character to distinguish *Boophis roseipalmatus* from the closely related *B. madagascariensis* (pink versus greyish webbing; GLAW et al. 2010), *B. pyrrhus* from *B. haematopus* (yellowish-brown versus red webbing; GLAW et al. 2001), and to differentiate several species in the *B. albilabris* group (Table 1). These observations confirm that webbing colour can be a species-specific character in treefrogs, similar to the colouration of and around the eye, and might also be an optical signals for mate recognition during the day (and possibly at night).

Distress calls: The genus *Boophis* contains more than 100 species and candidate species (HUTTER et al. 2018) and thousands of *Boophis* individuals were captured or handled by us over the last 30 years, yet only very few distress calls were heard or recorded. These are from *Boophis entingae* (VENCES et al. 2006 under the name *B. brachychir*, GLAW et al. 2010), *Boophis albilabris* (FORTI et al. 2018: suppl. Table S1, but calls not found in Fonozoo), *Boophis tsilomaro* (under the name *B. occidentalis*, interpretation as distress call uncertain, see ANDREONE et al. 2002) and *Boophis masoala* (reported herein). These species are among the largest *Boophis*, confirming the results of previous authors (e.g. HÖDL & GOLLMANN 1986, TOLEDO & HADDAD 2009, FORTI et al. 2018) who found that distress calls in anurans are more common in large species. The general spectral structure of the distress calls of *B. masoala* is similar to those reported previously (e.g. HÖDL & GOLLMANN 1986, TOLEDO & HADDAD 2009). However, in contrast to most other anuran distress calls which appear homogeneous in their structure and are entirely produced with an open mouth, the distress calls of *B. masoala* are unusual in consisting of three distinct sections, including (1) the starting phase with closed mouth, (2) the opening of the mouth and (3) the final section with an open mouth. This observation indicates that distress calls might be more variable than often assumed.

Biogeography of the *B. albilabris* group: Unlike most other *Boophis* species groups, the *B. albilabris* group inhabits a relatively wide variety of habitats, including both rainforest of the east as well as dry forests and even remains of gallery forests in western Madagascar. It also includes potential local or regional endemics (*B. tsilomaro*, *B. masoala*) as well as very widespread species (*B. albilabris*, *B. praedictus*, *B. occidentalis*). *Boophis albilabris* is one of only few Malagasy frogs which are widespread over most of the hu-

mid areas of Madagascar, ranging over more than 1200 km straight-line distance from Andohahela in the southeast to Tsaratanana and the Sambirano region in the north (but possibly excluding the Masoala peninsula) and with an elevational range of ca. 100–1300 m above sea level. Remarkably, the species is very homogenous in the mitochondrial 16S gene across its huge range (Fig. 1), suggesting that it might have evolved as a local endemic and then rapidly expanded its range over most of Madagascar only recently. However, the available data do not show any signal where its original distribution could have been and also the remarkable variability in its dorsal body colouration is not obviously correlated with biogeography.

Osteology: Expanding on the brief summaries for the whole genus given originally by GUIBÉ (1978) and updated by BLOMMERS-SCHLÖSSER & BLANC (1991), in this paper we have presented the first comprehensive osteological description of a particular *Boophis* species, and indeed the first such description of any member of the family Mantellidae. Previous work on the osteology of the mantellids has largely focussed on the roles of single bones – especially the elements of the pectoral girdle and characteristics of the manus and pes – in supraspecific systematic interpretations (e.g. LAURENT 1943, GUIBÉ 1978, BLOMMERS-SCHLÖSSER & BLANC, 1991, 1993, BLOMMERS-SCHLÖSSER 1993, GLAW et al. 1998, VENCES et al. 2002, MANZANO et al. 2007). To our knowledge, only one publication has ever published any images or illustrations of the articulated skeleton of a mantellid frog (GLAW & VENCES 1994, x-ray images of *Boophis goudoti* and *Mantidactylus guttulatus*). Detailed reference literature is important as it can provide a foundation for individuals for whom osteology is unfamiliar, serve as an anchor point for understanding osteological variation, be a source of taxonomic characters, and be crucial in interpreting large- and fine-scale evolutionary patterns within groups. Evolution of ecology within Mantellidae, as a single, highly-diverse, insular radiation, is an interesting topic of study (e.g. BOSSUYT & MILINKOVITCH 2000, WOLLENBERG et al. 2011, WOLLENBERG VALERO et al. 2017, HUTTER et al. 2018), and adding osteology to our investigation of this field will greatly augment our ability to understand the specific changes associated with ecological shifts and speciation among mantellid frogs, perhaps eventually shedding light on the functional underpinnings of the evolutionary patterns within these frogs.

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Supplementary Material 1

Video showing the distress call of the holotype of *Boophis masoala*. Available at http://www.salamandrajournal.com/images/stories/Glaw_et_al-1251-distress_call_B_masoala_short.mp4