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# Rediscovery of frogs belonging to the enigmatic microhylid genus *Madecassophryne* in the Anosy Massif, south-eastern Madagascar

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**Abstract**. Frogs assigned to the monotypic genus *Madecassophryne* (Anura, Microhylidae, Cophylinae), and possibly belonging to *Madecassophryne truebae,* were found in December 2016 in two low-altitude localities, Ambahavala and Kapilavato, in the Anosy Mountain in southeastern Madagascar. This poorly known genus was described in 1974 based on osteology, and neither verifiably identified photos of living specimens nor molecular information were available until now. We here update the available information on these enigmatic frogs and provide new data on morphology, osteology, bioacoustics and observations on their habitat, together with a preliminary molecular phylogenetic study, suggesting that *Madecassophryne* is highly divergent from other members of the cophyline clade.

Key words. Amphibia, Anura, Microhylidae, Cophylinae, *Madecassophryne truebae*, morphology, molecular genetics, 12S rRNA, 16S rRNA, Rag-1, evolutionary relationships.

## Introduction

The family Microhylidae is a group of frogs widely distributed in most tropical regions of the world (van der Meijden et al. 2007). The microhylids of Madagascar are subdivided into three subfamilies: Scaphiophryninae, Dyscophinae and Cophylinae (Andreone et al. 2005). Among these, the Cophylinae present the greatest species richness and display a high level of morphological and ecological diversity (Andreone et al. 2005, Glaw & Vences 2007, Van der Meijden et al. 2007), comprising arboreal, terrestrial, fossorial and rupicolous frogs (Blommers-Schlösser & Blanc 1991, Glaw & Vences 2007).

In recent years, considerable efforts have been made towards understanding the phylogenetic relationships of the Malagasy microhylids (Andreone et al. 2005, Wollenberg et al. 2008, Peloso et al. 2016, Scherz et al. 2016, Feng et al. 2017), but to date their systematics have not been satisfactorily resolved. Apparently scaphiophrynines, comprising the genera *Paradoxophyla* and *Scaphiophryne*, are the sister group of cophylines (e.g., van der Meijden et al. 2007, Feng et al. 2017), although this relationship requires further confirmation (e.g., Peloso et al. 2016). The cophylines remain one of the most enigmatic groups among Madagascar's amphibians, with disputed phylogenetic relationships (Peloso et al. 2016, 2017, Scherz et al. 2016, 2017a). One of the reasons why the phylogeny of cophylines is not yet resolved is the lack of tissue samples of *Madecassophryne* Guibé, 1974, a genus considered part of the Cophylinae subfamily (Blommers-Schlösser & Blanc 1991) but never studied for molecular characters.

*Madecassophryne* is a monotypic genus of microhylid frogs that was discovered by Charles P. Blanc (Guibé 1974) during the first 'Recherche Coopérative sur Programme n° 225' mission in 1971, in the Anosy mountain chain in the southeast of Madagascar (Paulian et al. 1973). The genus today still consists of a single species only, *Madecassophryne truebae* Guibé, 1974.

As reported in Paulian et al. (1973), H. Humbert first explored the Anosy mountain chain in 1928 and made botanical surveys in 1933 and 1934. Several additional botanical surveys were conducted afterwards, but the first zoological survey was not carried out until 1954, by R. Paulian and J. Arnoult. Eighteen years later (1971–1972), another multidisciplinary survey of the high elevation ecosystems was conducted in this area within the mission 225. The main goal of this expedition was to study the taxonomy and the ecology of the flora and fauna of the area (Paulian

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et al. 1973). The French team made an inventory of the mountain at different altitudes and established seven camp sites (called Ambana, Bekazaha, Sampanandrano [camp 3], Ranomandry, camps 5–7; Table 1). Paulian et al. (1973) provided a number of excellent maps of this area, with each of their campsites clearly indicated along the Mananjary and Ranomandry Rivers.

According to Blommers-Schlösser & Blanc (1993: 421) *Madecassophryne* is only known from the summit area of this mountain. However, Blommers-Schlösser & Blanc (1993: Table 14) and Stuart et al. (2008: 452) published a photo taken by C. J. Raxworthy in 1990 at Ambatovaky far north of the Anosy mountains, apparently referring to this species. This specimen is available at the Natural History Museum of London under catalogue number BMNH 1988.596 (Vertnet 2017) from -16.85°, 49.2667° (the edge of Ambatovaky Reserve). Several other specimens assigned to *Madecassophryne truebae* were collected at Andohahela RNI and reported in Nussbaum et al. (1999). These specimens are in the collection of the University of Michigan with collection numbers UMMZ 198827–198828, UMMZ 198830, UMMZ 198834, and UMMZ 221013 (Vertnet 2017). Specimens from Ambatovaky and Andohahela have apparently not yet been studied in detail (neither morphologically nor genetically) and their identification is in need of confirmation. In the IUCN Red List Assessment, the IUCN SSC Amphibian Specialist Group (2016) reported that the species is known only from extreme south-eastern Madagascar in the Anosyenne Mountains, Andohahela National Park and Tsitongambarika (north of Tolagnaro), between 700–1900 m asl and currently do not consider the Ambatovaky record as valid. BirdLife International (2011) listed *Madecassophryne truebae* for Tsitongambarika as an unrecorded potential species, and the Tsitongambarika record currently reported in Amphibian Specialist Group (2016) probably refer to this source. Ramanamanjato (2007) reported the presence of *Madecassophryne truebae* in Sainte Luce, without providing more detailed information.

The original description (Guibé 1974) and the MNHN catalogue list 25 specimens of *M. truebae* from 'mission 225' (holotype MNHN 1973.1149 (Fig. 1) and paratypes MNHN 1973.1150–1173). According to this catalogue these frogs were collected at different places around the summit (translated from French: 'summit H-B', 'basin/depression', 'waterfall', 'in moss', 'stream at the basin/depression'). According to Blommers-Schlösser & Blanc (1991) the following is known of the biology of this species: the male and female were observed close to a clutch of eggs; one clutch had 18 eggs, each about 4 mm in diameter without the membrane and 6 mm with the membrane; the yolk is pale yellow.

During 'mission 225', no photos or DNA samples were collected and since this expedition, no other attempts have been made to reach the type locality and consequently, lit-

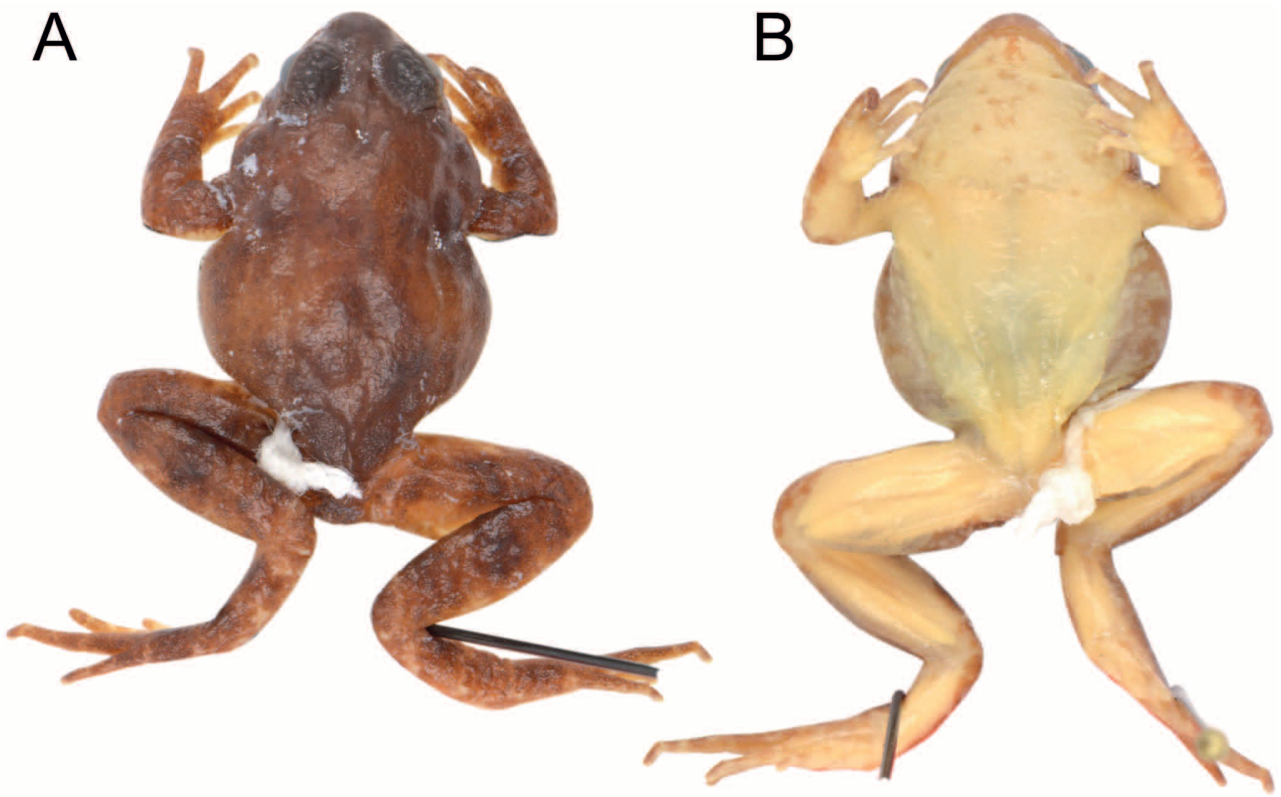


Figure 1. Preserved holotype of *Madecassophryne truebae* (MNHN 1973.1149) in (A) dorsal and (B) ventral view. Photographs courtesy of A. Ohler, 2016. Dark lines are pins used to fix the specimen in place.

Table 1. GPS coordinates from the fieldwork in 1971 and 2016. * localities around which *M. truebae* was found in 1971 (inferred from MNHN catalogue, not stated in Paulian et al. 1973 or original description).

| | Paulian et al. (1973), inferred | Field work 2016, GPS |
|---|---|---|
| Camp 1: Ambana | -24.17867°, 47.13960°, 90 m a.s.l. | -24.16934°, 47.13233°, 108 m a.s.l. |
| Camp 2: Bekazaha | -24.15325°, 47.11690°, 200 m a.s.l. | -24.15365°, 47.11639°, 214 m a.s.l. |
| Ambahavala | – | -24.14269°, 47.10573, 346 m a.s.l. |
| Kapilavato | – | ca.-24.1443°, 47.1051°, ca. 352 m a.s.l. |
| Camp 3: Sampanandrano | -24.14272°, 47.08888°, 700 m a.s.l. | -24.13994°, 47.07415°, 539 m a.s.l. |
| Camp 4: Ranomandry | -24.13529°, 47.07151°, 550 m a.s.l. | – |
| Camp 5* | -24.13053°, 47.05440°, 1050 m a.s.l. | – |
| Camp 6* | -24.13746°, 47.04472°, 1940 m a.s.l. | – |
| Camp 7* | -24.14069°, 47.03948°, 1900 m a.s.l. | – |

tle is known about this species. *Madecassophryne* is currently the only known cophyline genus that lacks samples for molecular analysis.

Based on the results of a recent expedition to the Anosy Mountains led by the first author, we report new information for this poorly known genus, including the first genetic data and new osteological data based on micro-computed tomography (micro-CT).

## Materials and methods

We reverse-engineered coordinates of each of the camp sites of Paulian et al. (1973) by superimposing their map onto the Mananjary and Ranomandry Rivers in GoogleEarth (Table 1). Alignment was based primarily on river bends and tributary entry points. Campsites proximal to the rivers were most reliably plotted; those away from the rivers have a larger margin of error. An expedition was then conducted by AR, accompanied by specialized guides, to the Anosy mountain chain in December 2016, based on the inferred coordinates of the camp sites of mission 225, to obtain new specimens and tissue samples from the historical collection sites of *Madecassophryne* (Table 1, Fig. 2). During this trip, it was possible to inventory just three of the seven camp sites visited by the French team (Ambana, Bekazaha and Sampanandrano [camp 3]) due to time limitations, bad weather, and extreme difficulty in accessing the other sites (Table 1, Fig. 2). Specimens were collected through opportunistic searches during the day and at night by searching in a variety of microhabitats, or guided by advertisement calls. Nocturnal searches were conducted using torches and headlamps. Specimens were euthanized in an overdose of MS 222 solution, fixed in 90% ethanol and preserved in 70% ethanol. Prior to fixation, tissue samples (thigh muscle) were taken and deposited in 99% ethanol.

The following morphological measurements on preserved specimens were taken with digital callipers to the nearest 0.1 mm by AR: snout–vent length (SVL), maximum head width (HW), head length (HL), horizontal tympanum diameter (TD), horizontal eye diameter (ED), eye–nostril distance (END), nostril–snout tip distance (NSD), nostril–nostril distance (NND), forelimb length (FORL), hand length (HAL), hindlimb length (HIL), foot length including tarsus (FOTL), foot length (FOL) and tibia length (TIBL). Terminology and description scheme follow Vences et al. (2010) and Glaw et al. (2012).

Calls were recorded in the field using a Tascam DR05 digital recorder at a sampling rate of 44.1 kHz and 24-bit resolution and saved as uncompressed files. Recordings were resampled at 22.05 kHz and 16-bit resolution and computer-analysed using the software Adobe Audition version 1.5. Frequency information was obtained through Fast Fourier Transformation (FFT, width 1024 points), and the audiospectrogram was obtained at Hanning window function with 256 bands resolution. Temporal measurements are given in milliseconds (ms) or seconds (s), as range, with mean ± standard deviation in parentheses. Terminology of the call description follows the note-centred description scheme of Köhler et al. (2017).

Total genomic DNA from tissue samples of *Madecassophryne* was extracted following a standard salt extraction protocol (Bruford et al. 1992). We sequenced three fragments of the mitochondrial 12S rRNA and 16S rRNA genes and one fragment of the nuclear Rag-1 gene, with primer combinations and cycling protocols as in Vences et al. (2003) and Rakotoarison et al. (2015). Standard polymerase chain reactions were performed in a final volume of 11 µl and using 0.3 µl each of 10 pmol primer, 0.25 µl of total dNTP 10 mM (Promega), 0.08 µl of 5 U/ml GoTaq, and 2.5 µl 5X Green GoTaq Reaction Buffer (Promega). PCR products were purified with ExoSAP-IT (Affymetrix) and directly used for cycle sequencing reactions using dye-labelled terminators (Applied Biosystems) with the amplification primers. Sequences were resolved on an ABI 3130 automated DNA sequencer (Applied Biosystems). The newly determined sequences were submitted to GenBank (accession numbers MF401953, MF401954, MF401955)

For a preliminary assessment of the phylogenetic relationships of *Madecassophryne* we selected DNA sequences of representatives (preferably type species) of all nominal genera of the cophylines, as well as *Breviceps* (Brevicipitidae) as the outgroup, and the microhylids *Kaloula* (Micro-

hylinae), *Dyscophus* (Dyscophinae) and *Scaphiophryne* (Scaphiophryninae) as hierarchical outgroups. Sequences of cophylines and *Scaphiophryne* were taken from the alignment of Scherz et al. (2016) and GenBank accession numbers can be found in that paper, whereas *Dyscophus antongilii* (GenBank accessions EU341120, EF396084), *Kaloula pulchra* (KC822624, EF396091) and *Breviceps mossambicus* (DQ283155, EF396076) were downloaded from GenBank. Sequences were aligned with MEGA7 (Kumar et al. 2016) and subsequently, all positions with gaps (insertions/deletions) in one or more species, and all positions with missing data in more than two species were excluded from analysis, corresponding to the most stringent settings in GBLOCKS (Castresana et al. 2000) but maintaining positions with missing data in one or two species. Our data set contains sequences of the almost complete mitochondrial gene fragments for 12S rRNA and 16S rRNA and the intervening tRNAVal genes (1227 bp after exclusion of variable sites), and of the nuclear Rag-1 gene (1380 bp). We determined a GTR+I+G model as most appropriate for the 12S/16S partition and a HKY+G model for the Rag-1 partition using jModeltest (Darriba et al. 2012) We ran a Bayesian Inference analysis, defining the two gene segments as separate partitions, with MrBayes 3.2.5 (Ronquist et al. 2012) for 10 million generations (starting with random trees) and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1000 generations. We verified stabilization and convergence of likelihood values in Tracer 1.4 (Rambaut & Drummond 2007), discarded 25% of the trees as burn-in, and computed a 50% majority-rule consensus tree with all compatible nodes retained.

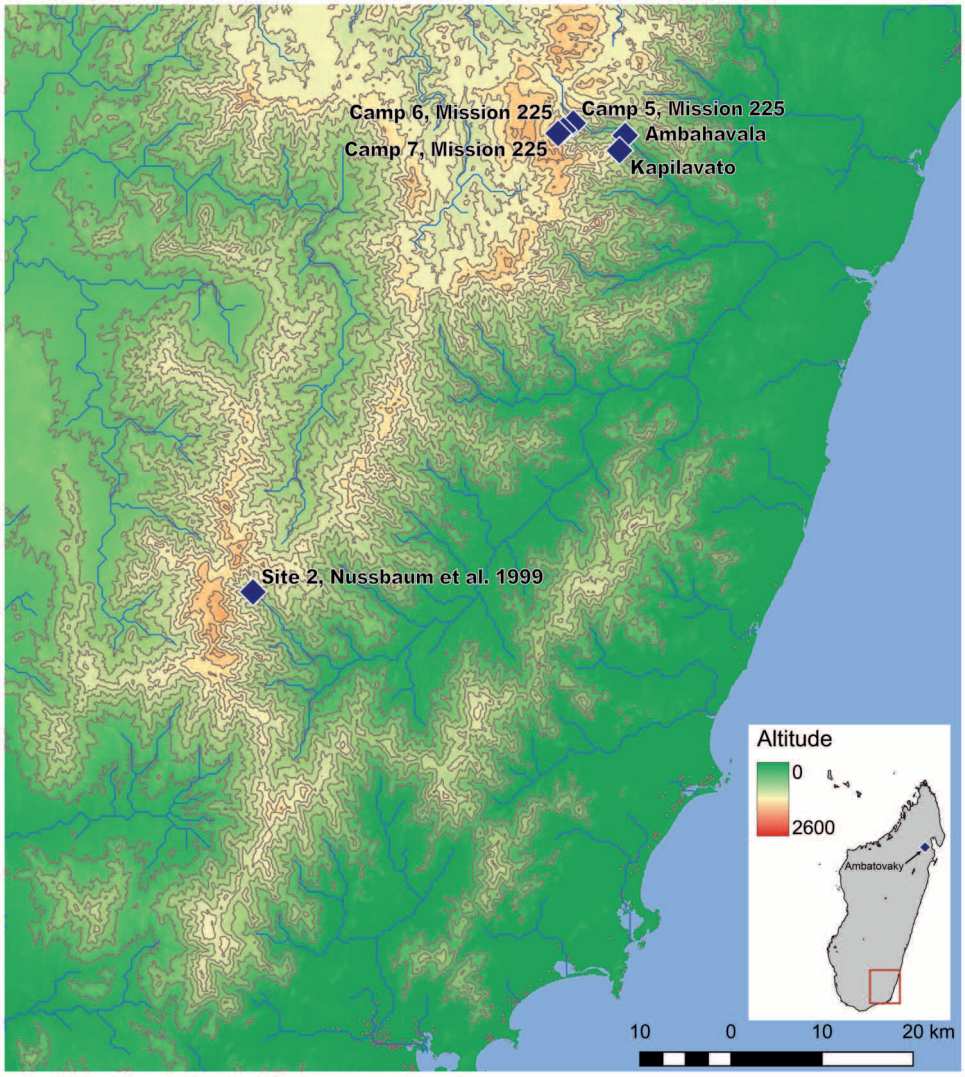


Figure 2. Map indicating known localities of *Madecassophryne truebae* including localities from Mission 225 (Camps 5–7; Paulian et al. 1973, Guibé 1974), from Nussbaum et al. (1999), and Ambahavala and Kapilavato visited by the expedition in December 2016. The type locality of *M. truebae* is presumably between Camps 5–7 but its precise coordinates are not known so is not indicated. The exact locality of the Tsitongambarika record is unclear and is therefore not considered here.

Table 2. Original morphometric measurements (all in mm) of representative specimens of *Madecassophryne* cf. *truebae* collected in 2016, and of two paratypes of *M. truebae* (originally from the MNHN collection; exchanged with ZSM). Relative hindlimb length (RHL) is given as the point reached by the tibiotarsal articulation when hindlimbs are adpressed along body: 0, eye; 1, nostril; 2, beyond tip of snout. ND means not determined, NR means not measured.

| Catalogue number (field number) | Sex | SVL | HW | HL | TD | ED | END | NSD | NND | FORL | HAL | HIL | FOTL | FOL | TIBL | RHL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZSM 301/2016 (ZCMV 14815) | ND | 15.2 | 6.5 | 5.2 | 0.5 | 2.6 | 1.2 | 0.9 | 1.2 | 9.1 | 5.1 | 27.3 | 10.6 | 7.3 | 9.1 | 2 |
| ZSM 302/2016 (ZCMV 14819) | ND | 15.4 | 6.6 | 5.4 | 0.6 | 2.3 | 1.1 | 1.0 | 1.1 | 7.8 | 3.1 | 22.1 | 8.2 | 6.3 | 9.0 | 2 |
| ZSM 303/2016 (ZCMV 14864) | ND | 12.3 | 4.3 | 4.1 | 0.5 | 1.8 | 1.1 | 1.1 | 1.0 | 6.7 | 3.3 | 19.8 | 8.8 | 4.9 | 6.6 | 1 |
| ZSM 304/2016 (ZCMV 14865) | ND | 12.6 | 4.9 | 4.7 | 0.5 | 1.8 | 1.2 | 1.0 | 1.0 | 7.5 | 3.7 | 20.1 | 8.9 | 6.0 | 6.6 | 1 |
| ZSM 305/2016 (ZCMV 14866) | ND | 15.7 | 7.1 | 5.4 | 0.5 | 2.3 | 1.0 | 1.0 | 1.2 | 9.8 | 4.3 | 26.2 | 12.0 | 7.2 | 9.4 | 1 |
| ZSM 745/2019 (ex MNHN 1973.1161) | M? | 20.7 | 8.1 | 6.7 | NR | 2.7 | 1.5 | 1.4 | 2.3 | 12.5 | 6.2 | 34.6 | 16.1 | 10.5 | 10.1 | 0 |
| ZSM 746/2019 (ex MNHN 1973.1166) | F | 24.3 | 8.5 | 7.9 | 1.1 | 3.2 | 1.6 | 1.5 | 2.2 | 15.5 | 7.7 | 40.6 | 19.5 | 13.1 | 12.6 | 0 |

Micro-CT data were obtained from a paratype of *M. truebae* (ZSM 746/2010, originally MNHN 1973.1166) and one newly collected specimen (ZSM 305/2016), following the methodology of previous work on cophyline osteology (Scherz et al. 2015). Scanning was performed with a tungsten target at 140 kV and 80 µA for 2440 projections of 750 ms each (30 min) in a phoenix|x nanotom m cone-beam micro-CT machine (GE Measurement & Control, Wunstorf, Germany). Files were reconstructed in datos|x reconstruct (GE Measurement & Control) and processed in VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany). Osteological terminology follows that used in the original description by Guibé (1974) translated into English, and generally follows the standards of Trueb (1968, 1973).

## Results

Seven specimens of *Madecassophryne* cf. *truebae* were collected in Ambahavala on 12 December 2016 (-24.14269°, 47.10573°, 346 m a.s.l.), by A. Rakotoarison, E. Rajeriarison and J. W. Ranaivosolo (Fig. 4A): UADBA-A 60290 (ZCMV 14814), ZSM 301/2016 (ZCMV 14815) [Figs 3D–E], UADBA-A 60291 (ZCMV 14816), UADBA-A 60292 (ZCMV 14817), ZSM 302/2016 (ZCMV 14819), UADBA-A 60293 (ZCMV 14820) and UADBA-A 60294 (ZCMV 14821). The site is located between Bekazaha and Sampanandrano. The specimens were found in a rocky wall covered by moss and moistened by a small waterfall (Fig. 4A). Three more specimens were found in a similar habitat at Kapilavato (Fig. 4B), a rocky area close to Ambahavala that was affected by evident deforestation activities, on 18 December 2016 (geographical coordinates not taken), by A. Rakotoarison, E. Rajeriarison and J. W. Ranaivosolo: ZSM 303/2016 (ZCMV 14864) [Fig. 3A], ZSM 304/2016 (ZCMV 14865) [Figs 3B–C] and ZSM 305/2016 (ZCMV 14866).

### Morphology

Description of the available specimens (Table 2): Small sized specimens (12.3–15.7 mm; mean 14.2 ± SD 1.6 mm; N = 5). Body round; head wider than long, narrower than body; snout short, rounded, slightly pointed in dorsal view, rounded in lateral view; nostrils directed laterally, not protuberant, equidistant to tip of snout and to eye; canthus rostralis slightly distinct, round; loreal region concave; tympanum slightly distinct, about 19.2–24.5% of eye diameter; supratympanic fold not visible; forelimbs slender; subarticular tubercles flat; outer metacarpal tubercle distinct, single, oval; prepollex either small or inner metacarpal tubercle present; hand without webbing; relative length of fingers 1<2=4<3, fourth finger subequal in length to second; finger tips not expanded into discs. Hind limbs slender; TIBL 52.3–56.8% of SVL; lateral metatarsalia strongly connected; inner metatarsal tubercle distinct, small, and oval; outer metatarsal tubercle absent; no webbing between toes; no toes reduced; relative length of toes 1<2<5<3<4; fifth toe shorter than third. Skin on dorsum without distinct dorsolateral folds. Ventral skin smooth. Tibiotarsal articulation reaching the nostril or beyond the tip of the snout. All specimens have very granular dorsal skin with distinct, regularly-arranged raised bumps. The dorsum of most specimens has a distinctive pattern formed of dark brown (sometimes with some greenish shading, Fig. 3B) markings and is ventrally whitish with brownish or white spots on the chin. Ventrally, a symmetrical colouration was

visible in life forming concave lines on either side of the midline at the level of the throat, becoming convex over the abdomen (Fig. 3E). This colouration appears to be due to internal organs (muscles) rather than pigmentation and its origin requires further study.

Osteologically, the new material closely resembles *M. truebae*, both as described by Guibé (1974), and based on a micro-CT scan of one of the paratypes: the vomerine and maxillary teeth are absent. Clavicles are absent. The vertebral column is procoelous. The vertebral centra are wider than they are long. The transverse processes of the sixth, seventh, and eighth presacrals are oriented anteriorly (only the last two are oriented anteriorly in the description of Guibé [1974] and in ZSM 746/2010). The urostyle articulation is bicondylar. The nasals are reduced and anterolaterally displaced. The frontoparietals are placed laterally on the braincase, leaving the frontoparietal fontanelle entirely exposed between them. The sphenethmoids are large and paired, exceeding the frontoparietals, although micro-CT does not reveal them to be as strongly anteriorly extended as originally described by Guibé (1974). The vomer is strongly reduced and lacks a post-choanal portion or posterior process. The neopalatines are well developed and separated at the midline, in dorsal contact with

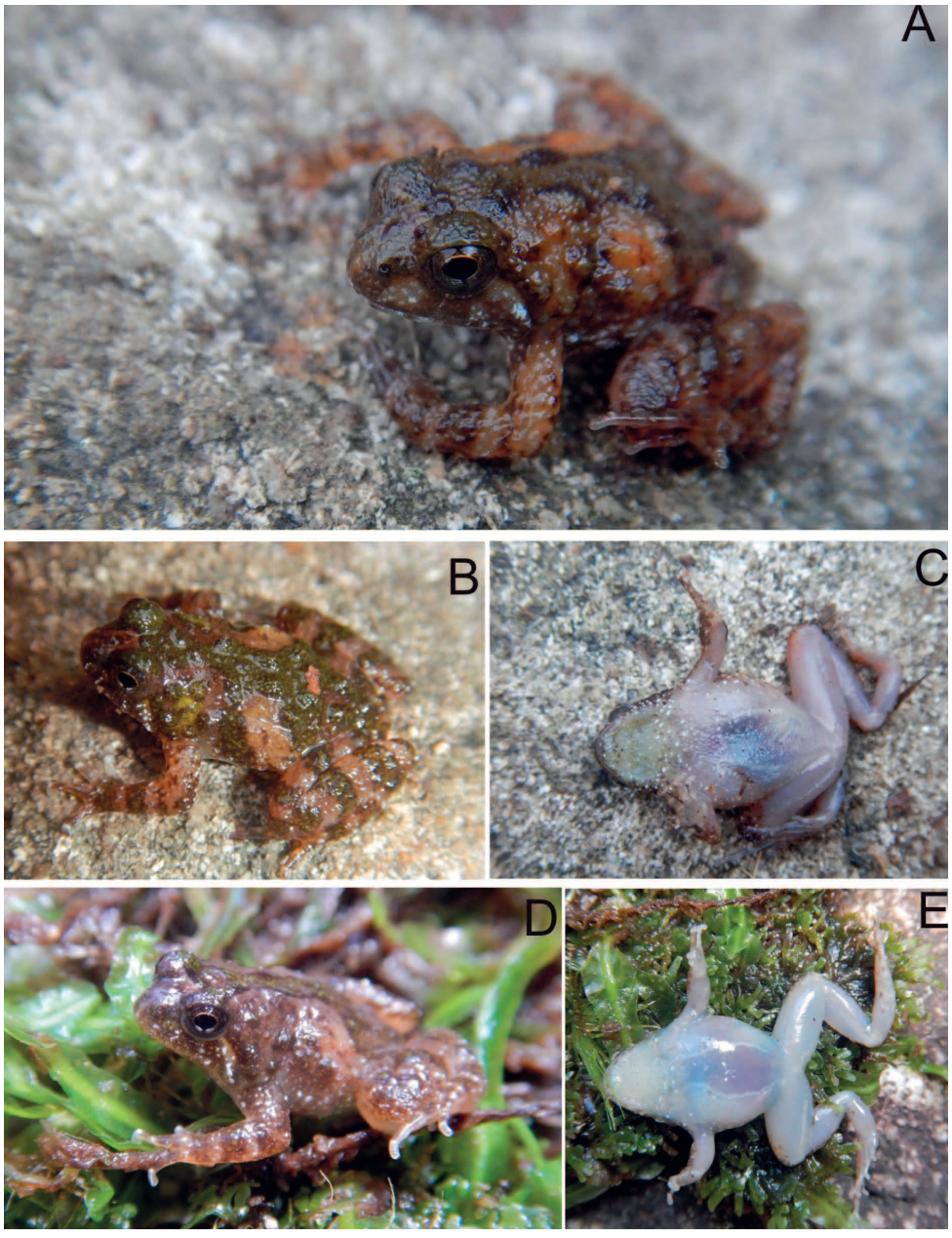


Figure 3. *Madecassophryne* cf. *truebae*: (A) ZSM 303/2016 from Kapilavato; (B–C) ZSM 304/2016 from Kapilavato; (D–E) ZSM 301/2016 from Ambahavala.


Figure 4. Microhabitat of *Madecassophryne* cf. *truebae*: (A) Ambahavala; (B) Kapilavato.

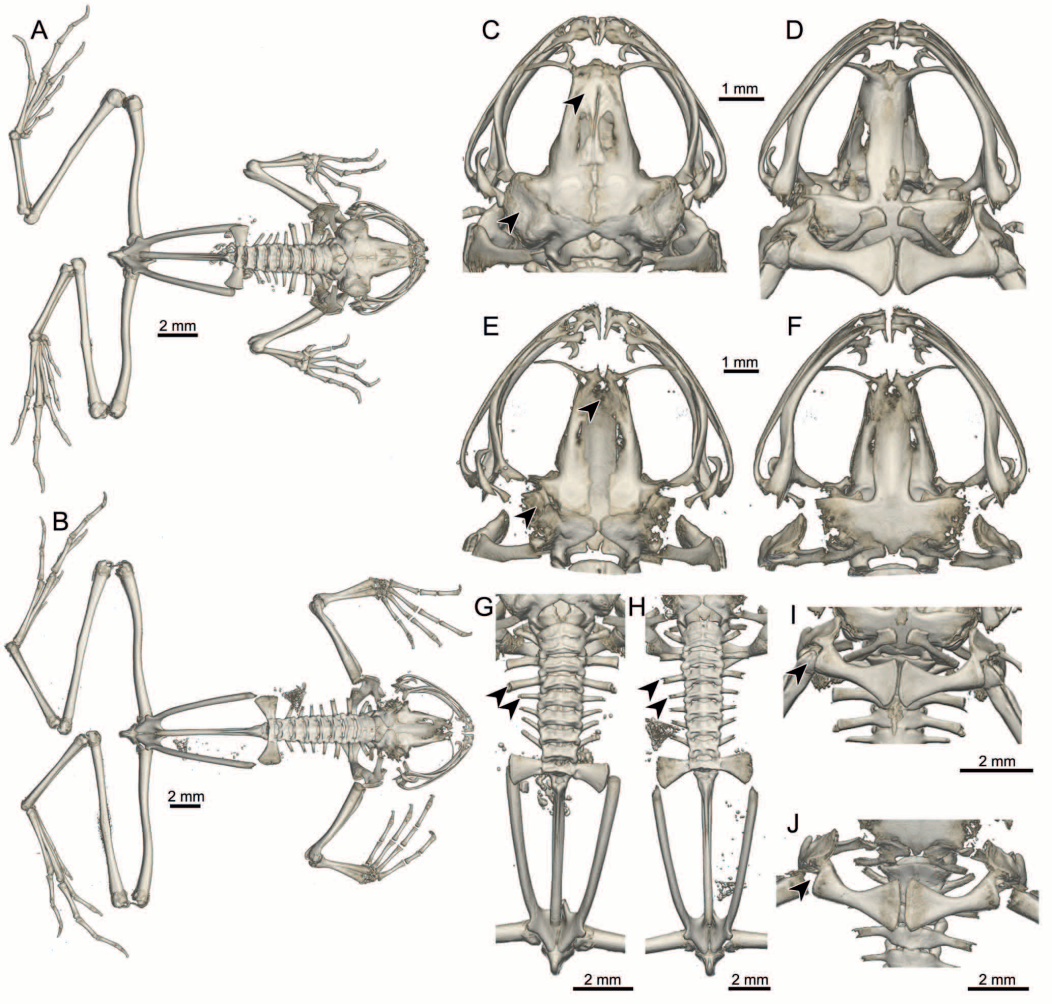


Figure 5. Osteological comparison of the newly collected specimen ZSM 305/2016 (A, C, D, G, I) with an adult female paratype of *Madecassophryne truebae* (ZSM 746/2010; B, E, F, H, J), showing (A, B) the full skeleton in dorsal view, (C–F) the skull in dorsal and ventral view, (G, H) the vertebral column in dorsal view, and (I, J) the pectoral girdle in ventral view. Arrows indicate features that differ between the two species possibly as a result of greater ossification of the recently collected ZSM 305/2016.

the sphenethmoid. In addition to those characters originally mentioned by Guibé (1974), the specimens examined share also the following characters that bear remark: The medial arm of the pterygoid is bilobate. The first and second presacral vertebrae are fused into a single element. The zygomatic ramus of the squamosal is dorsally curved. The terminal phalanges of the fingers are elongated and end in a small knob, with all fingers rather long and slender.

The only relevant osteological difference between the newly collected specimen ZSM 305/2016 and the paratype (ZSM 746/2010) is the extent of ossification. This is evident from several areas (indicated by arrows in Fig. 5), especially the frontoparietal fontanelle (Figs 5C, E), which is almost dorsally sealed by calcification of the brain case in ZSM 305/2016, the otic capsules, pubis, and head of humerus, which are ossified in ZSM 305/2016 but are unossified in ZSM 749/2010. There is also a slightly different ratio in the lengths of the transverse processes of presacrals IV and V, their lengths being more similar in ZSM 746/2010 than in ZSM 305/2016. This again could be attributed to the difference in ossification in the skeleton. ZSM 305/2016 possesses well-developed prepollex and prehallux, suggesting that it may be an adult male. ZSM 746/2010 is an adult female containing numerous oocytes. The minimal other differences in their osteology are within the scope of the variability observed in the few cophyline species for which information on intra-specific variability is available (e.g., see Scherz et al. 2017b).

## Vocalizations

Calls were recorded at Kapilavato by A. Rakotoarison and E. Rajeriarison on 18 December 2016 at around 13:00 local time (UTC+3), temperature not recorded (Fig. 6). The actual calling male was not seen. Therefore there is no certainty that the collected specimens were adults, and a minimal possibility remains that the calls were emitted by another, syntopic frog species (although no other frogs were encountered in this particular microhabitat). The sounds were heard from multiple individuals from the cracks on the rock, without any obvious social interaction among specimens, and are therefore here considered as the advertisement calls of *Madecassophryne* cf. *truebae*. They consisted of an irregularly emitted, short and distinctly but very rapidly pulsed note, with 28–40 pulses/note emitted. The call duration was 106–175 ms (153.5 ± 32 ms; N = 4), inter-call interval duration was 4427–11048 ms (8656 ± 3023 ms; N = 4), and the dominant frequency was 2627–3057 Hz (2831 ± 176 Hz, N = 4). Pulses were extremely short (between 1–2 ms), as is the interval between them (1–2 ms). The intensity and tightness of spacing of the pulses diminished gradually over the course of the call.

## Phylogenetic relationships

The phylogenetic trees based on 2607 bp of the concatenated mitochondrial and nuclear DNA sequences provides only a relatively poor resolution of phylogenetic relationships among Malagasy microhylids, similar to previous analyses based on the same genes such as that of Andreone et al. (2005). In particular, the sister group relationship of '*Stumpffia*' sp. Ca15 with *Plethodontohyla* (Scherz et al. 2016) is not retrieved, probably because only partial Rag-1 sequences of these taxa were available. Relationships of the main groups, however, were recovered in accordance with previous multigene analyses (e.g., Van der Meijden et al. 2007, Scherz et al. 2016). In agreement with all other analyses to date, the tree provides high support (1.0 posterior probability) for the monophyly of a group containing representatives of the cophyline genera *Anilany*, *Anodonthyla*, *Cophyla*, *Platypelis*, *Plethodontohyla*, *Rhombophryne*, and *Stumpffia*. However, in the tree, *Madecassophryne* (also considered to be in the Cophylinae; Blommers-Schlösser & Blanc 1991) is placed outside of

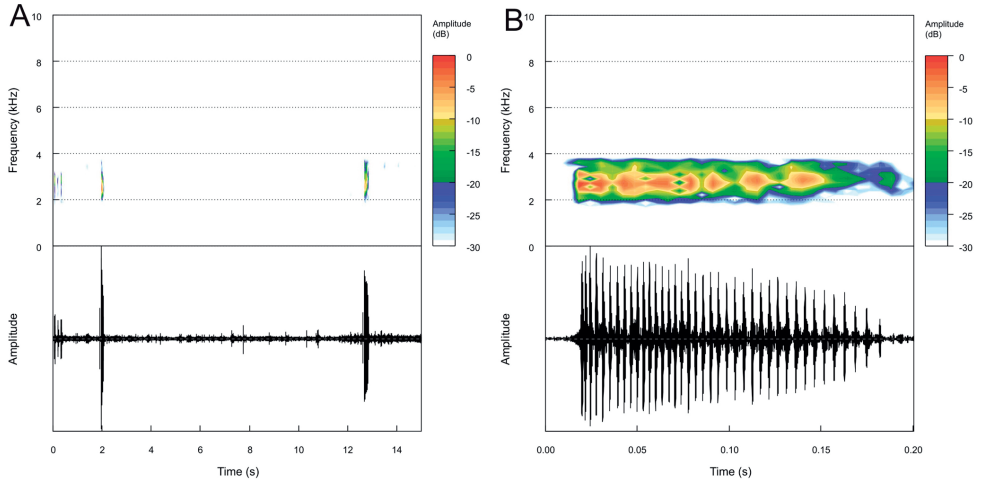


Figure 6. Spectrograms and oscillograms of advertisement calls probably emitted by *Madecassophryne* cf. *truebae* from Kapilavato, Anosy mountain chain: (A) 15 s duration section; (B) 200 ms duration section.

this highly supported group and is separated by a substantial branch length from it (Fig. 7). The clade containing *Madecassophryne* and the other cophylines is supported by a maximum posterior probability of 1.0. Thus, although the Cophylinae as currently understood (including *Madecassophryne*) is reconstructed as a monophyletic group in our preliminary analysis, *Madecassophryne* stands out as a genetically highly divergent member of this clade, not closely related to any other cophyline genus. Clearly, its relationships among Malagasy microhylids require further confirmation with wider taxon sampling and inclusion of multiple nuclear genes.

### Distribution, habitat and threats

At Ambahavala the specimens were found in a rocky wall covered by moss and moistened by a small waterfall (Fig. 4A). They were located either directly behind the moss or between cracks on the rock. The site at Kapilavato (Fig. 4B) is a rocky wall similarly moistened by a small waterfall, with several cracks, but not covered by moss. In this area, although affected by evident deforestation activities, several specimens were calling.

According to Paulian et al. (1973), the forest between 30–700 m.s.a.l was still a pristine forest during the 'mission 225'. This was not the case anymore in 2016. Intense deforestation was observed on our expedition. No forest remained from Analamary (-24.24110°, 47.22388°, 90 m a.s.l.) village until Bekazaha (-24.15365°, 47.11639°, 214 m a.s.l.). Some patches of forest were observed at Ambahavala and Sampanandrano, but several areas of logging were recorded (Fig. 8A). Slash and burn was implemented almost everywhere for agricultural purposes (Fig. 8B). In addition, several traps were observed in Sampanandrano for bushmeat hunting (lemurs, fish, and crayfish) (Figs 8C–E).

## Discussion

The comparison of the morphological descriptions of *Madecassophryne* in Guibé (1974) and Blommers-Schösser & Blanc (1991) with the newly collected specimens show that most of the studied characters are similar, except that the SVL of the specimens of *Madecassophryne* collected in December 2016 is much smaller (12–16 mm) than that of the MNHN specimens (20–23 mm), and that they differ in relative hindlimb length (see below) and degree of ossification. The specimens collected in 1971 were collected near the summit (1900 m a.s.l.) of the mountain (Blommers-Schlösser & Blanc 1993), whereas those collected in 2016 were collected at much lower elevation (350 m a.s.l.). The dissimilarity in size may mean that the new specimens are not conspecific with *M. truebae*, but belong instead to a separate species in the same genus, indicative of an elevational differentiation between species in the Anosy mountain chain similar to the case of *Cophyla* in Montagne d'Ambre, northern Madagascar (Rakotoarison et al. 2015). On the other hand, the observed size difference could also be due to age differences, as we cannot fully exclude that the specimens collected in 2016 were subadults. Osteological data suggests that this is unlikely however, as at least the largest specimen collected (ZSM 305/2016) shows signs of being an adult (strong ossification) male (large prepollex). Furthermore, hindlimb length of the specimens collected during the two expeditions also differs: According to Guibé (1974) the tibiotarsal articulation reaches the eye when hindlimbs are adpressed along the body, whereas those of the newly collected species are longer, reaching either the nostril or beyond the tip of the snout. Nevertheless, the granular skin and the darker coloration pattern in dorsum do agree with the specimens studied by Guibé, and for all our specimens, the toe 3 is longer than 5. Currently no morphological description is available from the Ambatovaky and

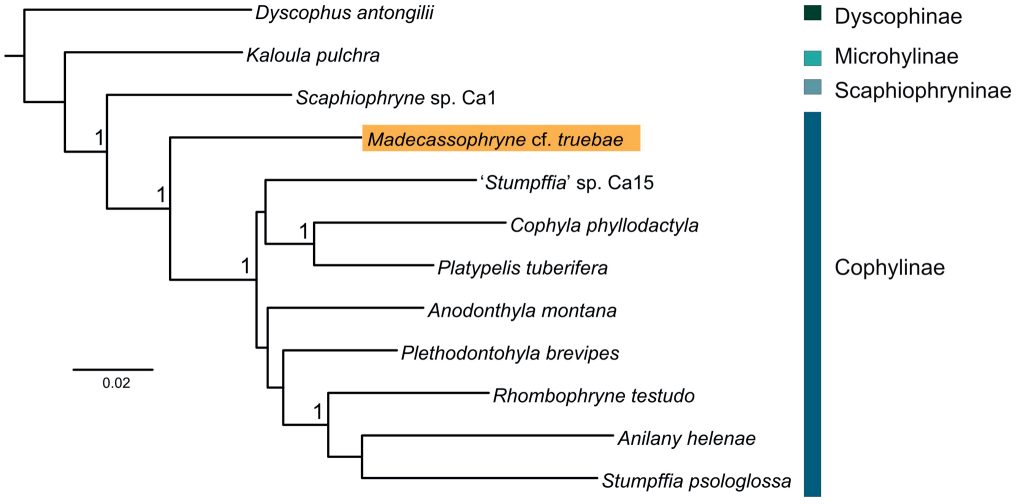


Figure 7. Bayesian Inference phylogenetic tree of representative Malagasy microhylids, reconstructed from DNA sequences of the mitochondrial 12S rRNA and 16S rRNA, and the nuclear Rag-1 gene fragments. Numbers at nodes are posterior probabilities (only shown for values > 0.95). The tree was rooted with *Breviceps mossambicus* (removed from figure for graphical reasons).

Andohahela RNI material, and the individuals from these sites were not available for the present study, so no comparison was possible. However, the Andohahela specimens were collected at 780 m a.s.l., which is at higher elevation than our collection sites but still considerably lower than the specimens collected on mission 225 by C. P. Blanc.

We found specimens of *Madecassophryne* inhabiting an exceedingly particular and special microhabitat. They were found in a rocky wall permanently moistened by a small waterfall. A specific description of the microhabitat in which the MNHN specimens were collected is not available, and the microhabitat of the specimens from Ambatovaky and Andohahela RNI has not been described. However, according to notes in the MNHN catalogue, most of the specimens from 'mission 225' were collected near a water source, and beneath moss, which more or less agrees with the microhabitat of our newly collected material. Until now, no cophylines have been known to inhabit such humid microhabitats. The arboreal cophylines *Anodonthyla*, *Cophyla*, *Platypelis* and a few *Plethodontohyla* occur and reproduce in water-filled tree-holes or leaf axils, while terrestrial cophylines such as *Anilany, Stumpffia*, *Rhombophryne*, and some *Plethodontohyla* species inhabit and as far as known reproduce in the leaf litter (Blommers-Schlösser 1975, Glaw & Vences 2007) and dyscophines and scaphiophrynines are fossorial with explosive reproduction in water. Unlike *Madecassophryne*, most microhylids in Madagascar come into regular contact with water only to reproduce.

Among all cophyline frogs, *Madecassophryne* is the second species with a distinctly pulsed call. Until now, *Stumpffia psologlossa* was the only cophyline emitting a clearly pulsed call, although its pulses are separated by much longer intervals. This would add still more variability to the bioacoustic repertoire of the Cophylinae (e.g., Glaw & Vences 1994, Lattenkamp et al. 2016, Lambert et al. 2017), if *Madecassophryne* is indeed confirmed as a member of this subfamily.

Guibé (1974) and Blommers-Schlösser & Blanc (1991) classified *Madecassophryne* in this subfamily based on osteological characters, especially the procoelous spine. While this character is confirmed by our study, several other features differ from other cophylines, such as the bilobate pterygoid, long phalanges and unusual shape of the squamosal (M. D. Scherz unpubl. data), while other features form a curious mosaic of characters seen in other cophyline genera, such as the laterally displaced frontals (otherwise found only in *Cophyla*: Rakotoarison et al. 2015, M. D. Scherz unpubl. data) and absence of posterior components to the vomers (a state similar to that seen

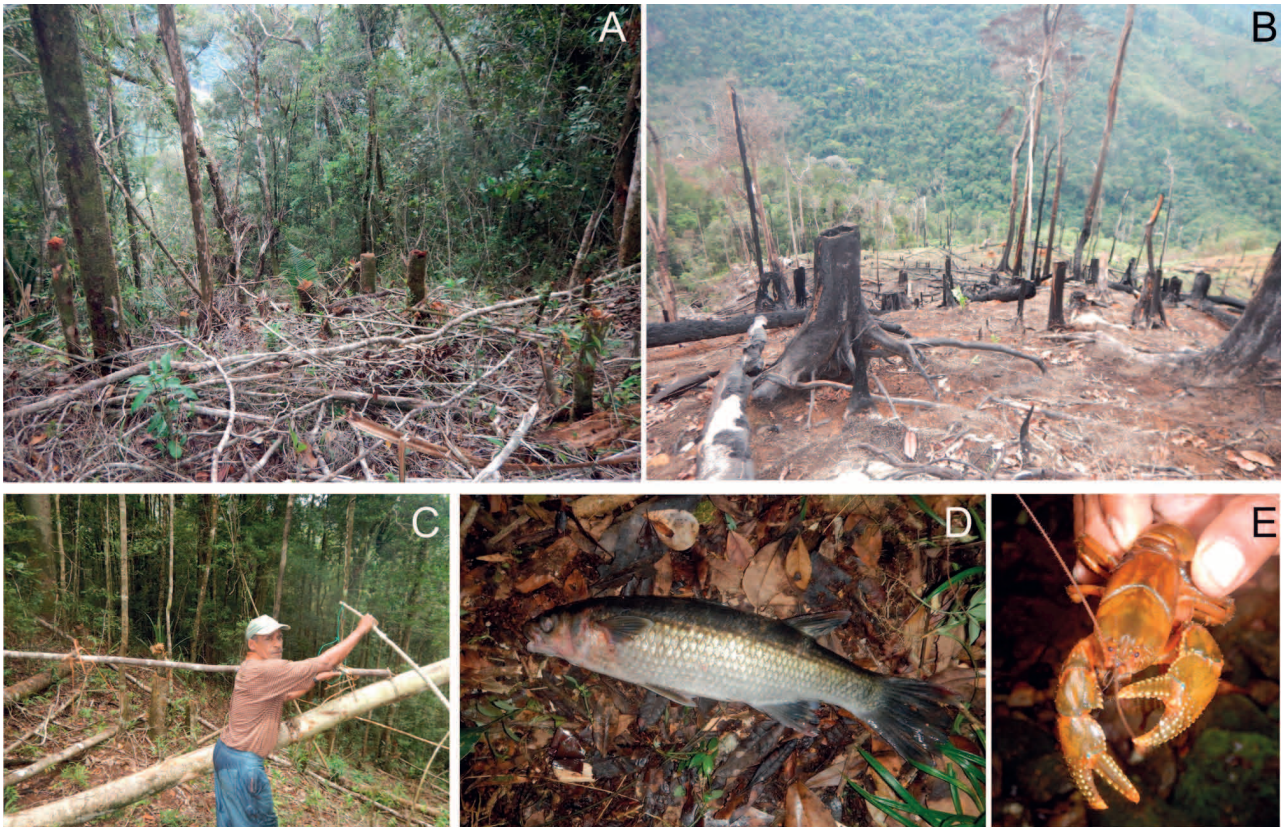


Figure 8. Photographs showing deforestation and nature exploitation in the Anosy mountain chain: (A) logging in forest; (B) slash and burn agriculture; (C) Lemur traps destroyed by E. Rajeriarison; (D) captured fish from Mananjary river; (E) crayfish, *Astacoides* cf. *petiti* in Sampanandrano.

in *Anodonthyla*: e.g., Noble & Parker 1926). From our preliminary phylogenetic tree, *Madecassophryne* appears to be the sister group of all the other cophylines, but with substantial divergence. Phylogenomic analyses with more extensive sampling of taxa and molecular markers will be necessary to reliably resolve the phylogenetic position of this distinct genus and the poorly understood relationships among the remaining cophyline genera.

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