## **PENSOFT**.



## Frogs of the genus *Platypelis* from the Sorata massif in northern Madagascar: description of a new species and reports of range extensions

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http://zoobank.org/7F7AC988-1EF8-4014-8DDC-0BE0ABA09B4F

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Academic editor: Johannes Penner • Received 5 October 2019 • Accepted 6 March 2020 • Published 5 June 2020

## Abstract

We describe a new species of arboreal microhylid frog, genus *Platypelis*, from northeastern Madagascar and report the expansion of distribution ranges of two other species. *Platypelis laetus* **sp. nov.** is small to medium-sized (24.3–25.6 mm snout-vent length) compared to other *Platypelis*, exhibits a greenish colored throat and was found in bamboo forest of the Sorata Massif. Its advertisement call consists of a single short tonal note repeated at regular intervals in long call series. Based on DNA sequences of a fragment of the mitochondrial 16S rRNA gene, the new species was placed in a clade with *Platypelis olgae* from the Tsaratanana Massif, and with two other, unconfirmed candidate species from the Sorata Massif and from Andravory, herein named *Platypelis* sp. Ca12 and Ca13. Molecular divergences among these lineages were substantial, amounting to 7.6–8.1% uncorrected 16S p-distance to the closest nominal species, *P. olgae*, from which the new species is also distinguished by a lack of allele sharing in the nuclear RAG-1 gene. We also provide new records of *Platypelis alticola* and *P. tsaratananaensis* from the Sorata Massif, supported by molecular analysis. This confirms a wider distribution of these two species that previously were considered to be endemic to the Tsaratanana Massif. However, their populations in Sorata were characterized by a certain degree of genetic differentiation from Tsaratanana populations suggesting they require more detailed taxonomic assessment.

## Key Words

Amphibia, Anura, Cophylinae, distribution, Microhylidae, molecular genetics, *Platypelis laetus* sp. nov., *Platypelis alticola*, *Platypelis tsaratananaensis*, Sorata, systematics

## Introduction

*Platypelis* Boulenger, 1882 is a moderately diverse genus of narrow-mouthed frogs (family Microhylidae Günther, 1858) in the subfamily Cophylinae Cope, 1889, endemic to Madagascar's humid forests. These arboreal frogs are generally characterized by small to medium body size (except *P. grandis* (Boulenger, 1889)), expanded terminal discs of their toes and especially their fingers, nidicolous endotrophic tadpoles (Blommers-Schlösser 1975), and a number of osteological characters (see Rakotoarison et al. 2012, 2015; Scherz et al. 2016 for details). There has been some debate considering the differentiation of *Platypelis* and *Cophyla* Boettger, 1880 (see Peloso et al.

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2016, 2017; Scherz et al. 2016, 2017b; Tu et al. 2018), but we here provisionally continue considering these as distinct genera on the basis of both probable reciprocal monophyly as recovered in most molecular studies, and presence of distinguishing characters in those species examined to date, particularly in their osteology (Scherz et al. 2016, 2017b; Tu et al. 2018), pending future revision.

At present, 15 species are recognized in the genus Platypelis: P. alticola (Guibé, 1974), P. ando Scherz, Köhler, Vences & Glaw, 2019, P. barbouri Noble, 1940, P. cowanii Boulenger, 1882, P. grandis, P. karenae Rosa, Crottini, Noel, Rabibisoa, Raxworthy & Andreone, 2014, P. mavomavo Andreone, Fenolio & Walvoord, 2003, P. milloti Guibé, 1950, P. olgae Rakotoarison, Glaw, Vieites, Raminosoa & Vences, 2012, P. pollicaris Boulenger, 1888, P. ranjomena Glaw, Scherz, Rakotoarison, Crottini, Raselimanana, Andreone, Köhler & Vences, 2020, P. ravus Glaw, Köhler & Vences, 2012, P. tetra Andreone, Fenolio & Walvoord, 2003, P. tsaratananaensis Guibé, 1974, and P. tuberifera (Methuen, 1920). Additionally, several candidate species have previously been identified (Andreone et al. 2005; Glaw and Vences 2007; Vieites et al. 2009; Perl et al. 2014; Scherz et al. 2016), and further fieldwork has yielded additional samples that likely constitute distinct species.

Generally, the tendencies of preferential elevation diverge between *Platypelis* and *Cophyla. Cophyla* are mostly found at lower elevations (except on Montagne d'Ambre, where three *Cophyla* species occur at relatively high altitude), whereas *Platypelis* species are spread across a broader elevational range. Some *Platypelis* indeed seem to be montane specialists (e.g. *P. olgae* and *P. alticola*), occurring exclusively above 2000 m a.s.l. (Rakotoarison et al. 2012). They belong to a cluster of *Platypelis* species that is restricted to the highlands and montane areas of northern Madagascar and that may form a clade, although their relationships so far are poorly resolved (Rakotoarison et al. 2012; Scherz et al. 2016; Tu et al. 2018). Due to their restricted distribution and the difficulty of performing fieldwork in these highland areas, little is known about these species.

In 2012, we conducted fieldwork on the rather remote massif of Sorata in the north of Madagascar. This massif rises from foothills at 400 m a.s.l. to nearly 1800 m a.s.l. and represents the northernmost end of the mountain escarpment that stretches southeast from Tsaratanana (comprising Madagascar's highest peak, Maromokotro, at 2876 m a.s.l.) toward the Antongil Bay, turning south-southwest in the area of Makira to form the mountain chain that runs the length of Madagascar's east coast. This fieldwork yielded a large number of new species from other taxa that have already been described elsewhere (see Scherz et al. 2015, 2017a, 2018a, b; Rakotoarison et al. 2017; Prötzel et al. 2018). Among the frogs, we recorded a diverse assemblage of Platypelis species, several of which belong to the highland cluster. Here, we describe that assemblage, reporting range extensions for two species, describing one new species, and providing cursory details on two further candidate species.

#### Materials and methods

Frogs were collected at night and by day by following the calling of males and through opportunistic searches in bamboo forest. Specimens were euthanized in MS-222 solution, fixed in 90% ethanol and preserved in 70% ethanol. Vouchers were deposited in either the Zoologische Staatssammlung München (ZSM) or in the amphibian collection of the Mention Zoologie et Biodiversité Animale of the University of Antananarivo (UADBA-A). FGZC, ACZC, AT, DRV, FGMV, and ZCMV, refer to field numbers of F. Glaw, A. Crottini, S. Megson, D.R. Vieites, F. Glaw and M. Vences, respectively. Measurements were taken to the nearest 0.1 mm by AR using digital calipers (except one specimen of P. alticola, ZSM 1615/2018, which was measured by H.J.-Lee). The measurement scheme follows that used by Rakotoarison et al. (2012): we measured snout-vent length (SVL), maximum head width (HW), tibia length (TIBL), hindlimb length (HIL), head length (HL), horizontal eye diameter (ED), eye-nostril distance (END), nostril-snout tip distance (NSD), nostril-nostril distance (NND), horizontal tympanum diameter (TD), hand length (HAL), foot length (FL), foot length including tarsus (FOTL), forelimb length (FORL). We also report the position of the tibiotarsal articulation when the hindlimb is adpressed along the body (RHL).

We took muscle tissue samples from the euthanized animals before fixation, and preserved them in 99% ethanol. We used a standard salt extraction protocol (Bruford et al. 1992) to extract genomic DNA and amplified a segment of the 5' end of the mitochondrial 16S rRNA gene (16S) with primers 16SL3 (AGCAAAGAHYWWACCTCG-TACCTTTTGCAT) and 16SAH (ATGTTTTTGATAAA-CAGGCG) from Vences et al. (2003), using the following PCR protocol: 90 s at 94 °C followed by 33 cycles of 45 s at 94 °C, 45 s at 52 °C, 90 s at 72 °C, and a final extension step of 300 s at 72 °C. For a selection of specimens we further amplified a fragment of the nuclear recombination-activating gene 1 (RAG-1) with primers Rag1 Coph F1 (CGTGATCGGGTAAAAGGTGT) and Rag1 Coph R1 (TCGATGATCTCTGGAACGTG) from Rakotoarison et al. (2015), with 120 s at 94 °C followed by 35 cycles of 20 s at 94 °C, 50 s at 53 °C, 180 s at 72 °C, and a final extension step of 600 s at 72 °C. PCR products were purified with 0.15 units of Shrimp Alkaline Phosphatase (SAP) together with 1 unit of Exonuclease I (New England Biolabs, Frankfurt am Main, Germany), incubating first for 15 min at 37 °C and subsequently for 15 min at 80 °C. PCR products were sequenced on automated DNA sequencers at LGC Genomics (Berlin), and chromatograms were checked with CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA). Newly obtained sequences were submitted to Gen-Bank (accession numbers MN864368-MN864395 and MN865083-MN865106).

Sequences were combined with those from previous studies, and alignments performed using the Clustal W algorithm in MEGA7 (Kumar et al. 2016). We separately

analyzed (1) an alignment of all available sequences of the 5' segment of the 16S gene for the focal group, combined with representative sequences of other species of *Platypelis* and *Cophyla*, and (2) an alignment of RAG-1 sequences for the focal group. This nuclear gene was analyzed separately from the mitochondrial DNA to obtain evidence from unlinked loci (mitochondrial versus nuclear) for genetic differentiation of lineages, which would add further support to their status as distinct species following a criterion of genealogical concordance (Avise and Ball 1990; Avise and Wollenberg 1997).

For the 16S dataset a maximum likelihood (ML) tree was calculated under a TN93+G substitution model determined by model testing in MEGA7 based on the Akaike Information Criterion, with node support assessed by 1000 full heuristic bootstrap replicates. Pairwise distances between sequences (uncorrected p-distances) were calculated in MEGA7.

For the RAG-1 data set, alleles (haplotypes) were inferred using the PHASE algorithm (Stephens et al. 2001) in DnaSP (version 5.10.3, Librado and Rozas 2009). A ML tree was then reconstructed from the phased and unpartitioned RAG-1 sequences in MEGA7 (Kumar et al. 2016) under a Jukes-Cantor substitution model, and this tree together with the phased alignment entered in the software Haploviewer, written by G. B. Ewing (http://www.cibiv. at/~greg/haploviewer), to build a network following the methodological approach of Salzburger et al. (2011).

Advertisement calls were recorded in the field using an apparently slightly damaged Tascam DR-07 digital recorder and although recordings appear and sound "normal", the possibility of artifacts in the recordings cannot be excluded. Recordings were saved as uncompressed files at a sampling rate of 44.1 kHz. Recordings were re-sampled at 22.05 kHz and 32-bit resolution and computer-analyzed using Adobe Audition 1.5. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points). Spectrograms were obtained at Hanning window function with 256 bands resolution. Temporal measurements are given as mean  $\pm$  standard deviation with range in parentheses. Terminology in call descriptions follows the note-centered approach of Köhler et al. (2017).

## Results

The Maximum Likelihood phylogenetic tree based on DNA sequences of the mitochondrial 16S rRNA gene (658 bp alignment length; Fig. 1) is, in general, to be seen as a visual representation of genetic divergences among lineages, and as a means to assign samples to main mitochondrial lineages. Most inter-species relationships in the phylogeny were poorly supported and will require confirmation by future phylogenomic studies.

Among the relationships rather strongly supported by the single-gene tree was the placement of the focal lineages from Sorata (previously referred to as "*P.* sp. CaNEW2" and "*P.* sp. CaNEW3" by Scherz et al. 2016) into one clade together with another lineage consisting of samples from Andravory, and with *P. olgae* (bootstrap value 99%) (see Fig. 2 for geographic location of sampling sites). For convenience, we will here refer to this assemblage of species as the *P. olgae* clade, and rename the lineages as follows: *P.* sp. CaNEW2 corresponds to the new species described herein as *P. laetus* sp. nov.; *P.* sp. CaNEW3 will be referred to as *P.* sp. Ca12; and the lineage from Andravory as *P.* sp. Ca13. The latter candidate numbers follow the consecutive numbering system of candidate species introduced by Vieites et al. (2009) and continued by Perl et al. (2014) and Glaw et al. (2020) to Ca11 for *Platypelis*.

Genetic divergences within the *P. olgae* clade were high. For the 5' fragment of the 16S gene, uncorrected pairwise genetic distances (p-distances) from the only nominal species in the clade, *P. olgae*, were 7.6–8.1% for *P. laetus* sp. nov., 9.2–9.3% for *P.* sp. Ca12, and 14.5% for *P.* sp. Ca13. Differences between *P. laetus* sp. nov. and *P.* sp. Ca12 were 8.0–8.6%, and *P.* sp. Ca13 differed from the other two candidate species by 13.6–14.8%.

Seven specimens from Sorata were placed with high confidence with topotypical specimens of P. tsaratananaensis (89%), and two were placed with P. alticola (79%). We also found the candidate species P. sp. Ca7 of Vieites et al. (2009) and Perl et al. (2014) from the Marojejy Massif to be part of the clade of P. tsaratananaensis from Tsaratanana and Sorata. Within this clade, distances are also high compared to overall trends in Madagascar's frogs: specimens from Sorata and Tsaratanana differed by 4.2-4.9%, Sorata from Marojejy by 5.2-5.6%, and Marojejy and Tsaratanana by 3.0-3.4%. Despite this strong differentiation, we think that these populations may represent deep conspecific lineages of P. tsaratananaensis, and therefore we assign Ca7 to that species, which was not included in Vieites et al. (2009); P. sp. Ca7 was also sister to P. tsaratananaensis in the trees presented by Perl et al. (2014) based on the COI gene, and by Scherz et al. (2016) in a multigene assessment.

Within the *P. alticola* clade, the specimens from Sorata differed from those from Tsaratanana by 6.2–6.6%. These specimens should be examined again in detail, and calls recorded and compared, but we here tentatively consider them conspecific.

In the RAG-1 allele network (based on 433 bp for 24 specimens), no haplotype sharing was detected among the specimens here assigned to *P. alticola*, *P. tsarata-nanaensis*, and *P. olgae*, respectively. Those specimens from Sorata and Marojejy that clustered with either *P. alticola* and *P. tsaratananaensis* in the mitochondrial tree were also placed close to topotypical samples of these species in the RAG-1 network, respectively (with allele sharing in the case of Sorata samples assigned to *P. tsaratananaensis*). In the focal group of our study, the *P. olgae* clade, *P. laetus* sp. nov. and *P. sp.* Ca12 did not share any allele with *P. olgae*, but one allele was shared between *P. laetus* sp. nov. and *P. sp.* Ca12 (no RAG-1 data were available for *P. sp.* Ca13).



**Figure 1.** Molecular differentiation of species of *Platypelis*, with an emphasis on samples related to *P. alticola*, *P. tsaratananaensis*, and *P. olgae* (the highland cluster, in the gray box). **a** Maximum Likelihood tree calculated from a 658 bp alignment of the mitochondrial 16S rRNA gene (5' segment of the gene). Numbers at nodes are support values from a bootstrap analysis (1000 replicates) in percent (not shown if below 50%). A sequence of *Stumpffia gimmeli* was included as the outgroup. For each sample, voucher number and if available, GenBank accession number is given. Samples outside the *P. olgae* clade (yellow box) that are highlighted in bold are newly sequenced specimens from the Sorata Massif. **b** Allele (haplotype) network based on 433 bp of the nuclear RAG-1 gene (phased sequences, hence each individual represented with two alleles in the network). In the *P. alticola* and *P. tsaratananaensis* clusters, an S marks alleles in samples from Sorata and an M marks samples from Marojejy.



**Figure 2.** Approximate geographical location of occurrence records of the focal *Platypelis* species in our study (modified from Rakotoarison et al. 2012; showing only records for which molecular data are available). The map indicates elevation (green, lowest; red-yellow, highest). Localities and species occurring at these sites are as follows: (1–4) campsites on the Mangindrano-Maromokotro trail, Tsaratanana Massif (Antevialambazaha, Matsabory Maiky, Bepia, Andranomadio); (5) Bemanevika forest; (6) Andrevorevo; (7) Marojejy; (8) Sorata; (9) Andravory. Occurrence records of *P. alticola* (at sites 1–3, and a deep genetic lineage at 8), *P. tsaratananaensis* (at sites 1–3, and deep genetic lineages at 7 and 8), *P. olgae* (between sites 3 and 4), *P. laetus* sp. nov. and *P.* Ca12 (site 8), and *P.* sp. Ca13 (site 9).

The concordant divergence in mitochondrial and nuclear DNA from the closest nominal species (*P. olgae*), the high genetic distance values to that species in the 16S gene, as well as morphological differences reported in the diagnosis below, provide conclusive evidence for a species-level differentiation of *P. laetus* sp. nov., for which we provide a formal description.

#### Platypelis laetus sp. nov.

http://zoobank.org/80E35235-9E39-48EC-ACB0-525952C098BC

**Remark.** This species has been listed as *Platypelis* sp. CaNEW2 Sorata by Scherz et al. (2016, 2017) and Peloso et al. (2017).

Holotype. Holotype ZSM 5652/2012 (FGZC 3761) (Figs 3a-c, 4), adult male, collected on 30 November 2012, in the Sorata Massif (near 13.6817S, 49.4411E, 1339 m a.s.l.), northern Madagascar, by F. Glaw, O.

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**Paratypes.** ZSM 5651/2012 (FGZC 3588) (Fig. 3d, e) collected on 26 November 2012 from the high elevation of the Sorata Massif (near 13.6745S, 49.4402E, 1541 m a.s.l.), northern Madagascar, by the same collectors as the holotype; ZSM 5653/2012 (FGZC 3762) (Fig. 3f, g), adult male, collected on 30 November 2012, from the same site and by the same collectors as the holotype; UADBA-A (FGZC 3767) and UADBA-A (FGZC 3768), both ovigerous adult females, collected on 30 November 2012 from the same site and by the same collectors as the holotype.

**Diagnosis.** Assigned to the genus *Platypelis* in the microhylid subfamily Cophylinae based on enlarged terminal discs on fingers and toes, absence of nuptial pads, and molecular phylogenetic relationships. The species can be identified among other cophylines by the combination of the following character states: (1) medium-sized species (adult male SVL 24.3–25.6 mm); (2) manus with second finger slightly shorter than fourth and pes with third toe much shorter to very slightly shorter than fifth; (3) males with prepollical tubercle but lacking a finger-like prepollex as typical for *Anodonthyla*; (4) throat greenish in life; (5) chest and anterior belly translucent gray, with distinct white spotting that is absent on the posterior belly; (5) absence of red color on limbs and ventral side.

Platypelis laetus sp. nov. can be distinguished from P. grandis and P. alticola by smaller body size (verified adult male SVL 24.3-25.6 mm vs 30-105 mm), and furthermore from P. grandis by a largely smooth dorsal skin (vs strongly granular and bumpy) and from P. tuberifera by dark pattern on the dorsum (vs uniformly beige-yellowish) and body not conspicuously flattened (vs flattened); from P. pollicaris by a slightly smaller body size (verified adult male SVL 24.3-25.6 mm vs 26–28 mm), third toe shorter than fifth (vs both toes of similar length) and less elongated body (vs conspicuously elongated); from P. tuberifera and P. cowanii by smaller body size (verified adult male SVL 24.3-25.6 mm vs 30-40 mm) and by third toe shorter than fifth (vs third toe longer than fifth); from P. tsaratananaensis by having a plump body (vs slender with a distinctively elongated "neck" region), absence of vomerine teeth (vs presence), and especially in call structure (see below); from P. tetra, P. barbouri, P. karenae, P. ravus, and P. ando by larger body size (SVL 24.3-25.6 mm vs 16-19 mm); from P. milloti by throat greenish colored (vs brownish) and by absence of red ventral color and of a very distinct dorsal pattern (vs presence); from P. barbouri and P. ranjomena by the absence of red color on ventral side or limbs (vs presence); from P. mavomavo by third toe shorter than fifth (vs third toe longer than fifth) and grayish with white spots on the chest (vs uniformly yellow on venter). Among the described species of Platypelis, the new species is phylogenetically closest to P. olgae. It differs from this species by larger body size (adult male SVL 24.3-25.6 mm vs 20.3-21.9



**Figure 3.** Photographs in life of *Platypelis laetus* sp. nov. from the Sorata massif: **a–c** ZSM 5652/2012 (FGZC 3761), holotype; **d**, **e** ZSM 5651/2012 (FGZC 3588), paratype; **f**, **g** ZSM 5653/2012 (FGZC 3762), paratype; **h** Eggs and embryos found in the same bamboo hole with the specimens ZSM 5652/2012 and ZSM 5653/2012.



Figure 4. Preserved holotype of Platypelis laetus sp. nov., ZSM 5652/2012 (FGZC 3761), in dorsal and ventral view.

mm), and by third toe often shorter than fifth (vs third toe equal to or longer than fifth).

Bioacoustically, *P. laetus* sp. nov. can be distinguished from most congeners by its rather short note duration of only

73–88 ms. The following *Platypelis* species all emit longer notes: *P. alticola* (411–466 ms), *P. ando* (424–441 ms), *P. barbouri* (142–160 ms), *P. karenae* (131–145 ms), *P. pollicaris* (160–180 ms), *P. ranjomena* (303–379 ms), *P. ravus* 

(384–443 ms), and *P. tuberifera* (280 ms) (Glaw and Vences 1994; Glaw et al. 2012, 2020; Rakotoarison et al. 2012; Rosa et al. 2014; Scherz et al. 2019). Calls of *P. grandis* are non-tonal, those of *P. milloti* are shorter in duration (55– 65 ms) and the call of *P. tsaratananaensis* consist of two notes, rather than a single note (Glaw and Vences 1994; Rakotoarison et al. 2012). Known calls of *Cophyla* species (*C. berara*, *C. fortuna*, *C. maharipeo*, *C. noromalalae*, *C. occultans*, *C. phyllodactyla*, *C. puellarum*) all exhibit a much longer note duration compared to the new species, ranging from 326 ms in the shortest to 1346 ms in the longest notes (see Rakotoarison et al. 2015, 2019b).

Description of the holotype. Adult male in good state of preservation, some muscle tissue removed from right thigh; snout-vent length 25.6 mm (for further measurements see Table 1); body plump; head slightly wider than long, not wider than body; snout slightly rounded in dorsal view and blunt in lateral view; nostrils not protuberant, nearer to tip of snout than to eye; canthus rostralis distinct, straight; loreal region straight, slightly oblique; tympanum distinct, 60% of eye diameter; supratympanic fold distinct, starting at the posterior border of the eye and ending anterior to the forelimb; tongue long, broadening posteriorly, attached anteriorly, not notched; maxillary teeth present and vomerine teeth absent; choanae rounded. Forelimbs robust; subarticular tubercles single, indistinct; outer metacarpal tubercle small, rounded; prepollex/inner metacarpal tubercle distinct, forming a distinct protuberance at base of first finger; hand without webbing; fingers distinctly broadly rounded to slightly bilobate, with lateral fringes, smaller than discs of fingers; relative length of fingers 1<2<4<3; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching between forelimb and tympanum when hindlimb adpressed along body; tibia length 47% of SVL; inner metatarsal tubercle small, oval; outer metatarsal tubercle absent; webbing between toes weakly developed, with traces of webbing between third and fourth toe; subarticular tubercles on toes single; toes flattened and their discs relatively broad, broadly rounded

to slightly bilobate; relative length of toes 1 < 2 < 3 < 5 < 4; third toe slightly shorter than fifth, dorsal skin smooth, without dorsolateral folds, ventral skin smooth on throat and chest and moderately granular on belly.

After nine years of preservation in 70% ethanol, the dorsum is light beige with a brown, teddy-bear-shaped patch flecked with cream from between the eyes to the inguinal region. The nostril is surrounded with brown. The lateral surface is homogenously light beige flecked with small brown dots. The flank coloration merges with the ventral coloration. The ventral trunk and the chin are light beige. The ventral thigh is beige spotted with brown in the cloacal region. The shank, tarsus and foot are ventrally beige. Dorsally, the thigh is beige with light brown crossbands. The posterodorsal surface of the thigh is beige spotted with light brown. The shank is beige with a brown crossband. The coloration of the distal portion of the shank merges with the coloration of the tarsus, extending to the toes. The arms are beige with a brown crossband. The coloration in life is shown in Fig. 3 (in dorsal, lateral and ventral view).

**Variation.** For variation in measurements, see Table 1. In general, the two examined adult paratypes agree well with the holotype, but with the following differences: The holotype and FGZC 3588 have plump bodies and FGZC 3762 is rather slim. Relative toe length is variable: the third toe is distinctly shorter than fifth on one foot, and slightly shorter on the other foot in FGZC 3588, slightly shorter in the holotype FGZC 3761, and very slightly shorter to equal in FGZC 3762. Overall, specimens in life have a dorsal surface with some irregular granules. In coloration, FGZC 3762 is the darkest specimen of the type series in preservative and FGZC 3588 is the lightest. The teddy-bear pattern of the dorsum of the holotype and FGZC 3588 is absent in FGZC 3762.

**Distribution.** The species is known only from the Sorata Massif, northern Madagascar, at elevations of 1339–1541 m above sea level (Fig. 2).

**Table 1.** Original morphometric measurements of *Platypelis* from the Sorata Massif, ND: Not determined. See Materials and Methods for other abbreviations. Specimens marked with UADBA-A are to be catalogued in the UADBA collection. The bolded row refers to the holotype of the new species.

Collection/	Field	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	RHL
Catalogue number	number																
P. laetus sp. nov.																	
ZSM 5651/2012	FGZC 3588	male	24.3	8.6	7.7	1.5	3.0	1.7	1.3	2.5	16.9	8.4	37.5	17.1	10.5	11.3	reaches tympanum
ZSM 5652/2012	FGZC 3761	male	25.6	8.6	8.0	2.0	3.3	1.4	1.2	2.5	16.2	7.7	36.8	17.4	10.0	12.0	reaches tympanum
ZSM 5653/2012	FGZC 3762	ND	23.0	8.3	7.4	1.7	3.4	1.8	1.2	2.1	16.2	7.0	34.3	16.7	9.5	10.8	reaches tympanum
P. sp. Ca12																	
UADBA-A	FGZC 3781	juvenile	16.7	5.6	5.4	1.4	2.7	1.2	1.0	1.7	12.6	5.0	20.4	9.0	5.2	6.5	reaches eye
UADBA-A	FGZC 3782	juvenile	12.3	4.1	3.9	0.7	2.1	0.8	0.8	1.4	9.4	3.4	18.6	6.7	2.7	3.8	reaches eye
P. tsaratananaensis																	
UADBA-A	FGZC 3646	ND	23.2	6.8	7.3	1.6	3.4	1.6	1.3	2.3	15.1	6.7	32.4	15.3	9.6	10.0	reaches tympanum
ZSM 5654/2012	FGCC 3647	ND	23.6	7.0	7.6	1.5	2.9	1.5	1.4	2.2	14.2	6.3	31.5	14.2	8.7	10.6	reaches tympanum
ZSM 5655/2012	FGZC 3648	ND	20.4	7.3	7.5	1.4	2.8	1.6	1.3	2.1	13.7	5.5	28.2	13.7	8.9	10.0	reaches tympanum
ZSM 5656/2012	FGZC 3649	NA	22.9	6.7	6.9	1.7	3.1	1.3	1.2	1.7	13.2	6.3	23.2	13.3	7.8	10.5	reaches tympanum
UADBA-A	FGZC 3765	ND	23.3	7.3	7.5	1.4	3.1	1.8	1.4	2.1	14.7	6.8	32.0	15.2	9.8	10.6	reaches tympanum
P. alticola																	
ZSM 1615/2018	FGZC 3669	ND	32.3	10.9	8.3	1.8	3.7	2.2	2.0	2.6	25.5	10.3	48.3	23.2	14.7	13.6	reaches tympanum

**Natural history.** *Platypelis laetus* sp. nov. occurs in rainforest on the Sorata Massif, but most of the specimens were collected in the bamboo forest of the massif. The holotype was calling from a bamboo hole at about 5 m above the ground. The bamboo node was occupied by another specimen, was water-filled, and contained 35 whitish eggs and embryos, probably of this species, in at least two different developmental stages (Fig. 3h). Another male was found on a palm tree at around 4 m above the ground. Calling occurred around dusk.

**Etymology.** The specific epithet is a masculine Latin adjective meaning "happy". The new species is so named in reflection of the joy and happiness of the first author to get to work on the cophyline microhylid frogs of Madagascar.

Vocalization. The advertisement call of *Platypelis laetus* sp. nov. consists of a single short tonal note (Fig. 5) repeated at regular intervals in long call series. Analysis of 43 calls belonging to three different males recorded on 30 November 2012 in Sorata revealed the following numerical parameters (ca. 15 °C air temperature): note duration (= call duration) 73–88 ms ( $83.4 \pm 4.3$  ms), inter-note interval 1465–2378 ms (1940  $\pm$  255 ms), and dominant frequency 4707–4793 Hz (4742  $\pm$  38 Hz). Each note exhibits some regular upward frequency modulation starting at around 4600-4700 Hz and terminating at 4770-4900 Hz. This narrow frequency band also constitutes the prevalent bandwidth of the call, although two additional rather weak frequency bands are present at around 2400 Hz and 7200 Hz. There is also a slight degree of amplitude modulation recognizable within notes, but the pattern of these modulations seems to be rather variable among notes (and possibly may constitute an artifact because of some recording device damage; see Materials and methods).

#### Range extensions of other Platypelis species

#### Platypelis alticola (Guibé, 1974)

**Identity.** Originally described by Guibé (1974) as *Platyhyla alticola* based on a single specimen (holotype MNHN 1973.693, SVL 37.7 mm). Rakotoarison et al. (2012:5) revised the taxonomy and stated that the species is characterized by "large body size (adult SVL 32–45 mm), uniform grayish color with absence of sharp border between dorsal and lateral color, third and fifth toes of similar length, vomerine teeth present, males with prepollical tubercle but lacking a finger-like prepollex as typical for *Anodonthyla*." Specimens collected on the Sorata massif (Fig. 6) agree with both the original description and this revised circumscription, as well as with the specimens depicted by Raxworthy et al. (2008) and Rakotoarison et al. (2012).

Given the substantial molecular divergence between the Tsaratanana and Sorata specimens (6.2–6.6% p-distance in the 16S gene), the taxonomy of the Sorata population will require thorough taxonomic revision in the future.



**Figure 5**. Spectrogram and oscillogram of one advertisement call of *Platypelis laetus* sp. nov. from a call series recorded on 30 November 2012 at the type locality in Sorata, northern Madagascar. Although not unequivocally verifiable, the depicted call most probably was emitted by the male holotype (ZSM 5652/2012).

**New material examined.** ZSM 1615/2012 (FGZC 3669) collected on 28 November 2012 from above the camp site in Sorata massif (close to a site with the coordinates 13.6778S, 49.4411E, 1423 m a.s.l.), northern Madagascar, by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M. Ratsoavina, and A. Razafimanantsoa.

**Distribution.** This species is known from Tsaratanana Strict Nature Reserve (Raxworthy et al. 2008; Rakotoarison et al. 2012) and the Sorata Massif (Fig. 2).

**Natural history.** In Tsaratanana, the species was found to breed in bamboo nodes (Raxworthy et al. 2008; Rakotoarison et al. 2012). In Sorata, we also found one individual in a water-filled bamboo node, at around 1 m from the ground, with 16 whitish young tadpoles (Fig. 6c). Calling activity in Tsaratanana was mostly observed at night (Rakotoarison et al. 2012); no data on advertisement calls are available from Sorata.

#### Platypelis tsaratananaensis Guibé, 1974

**Identity.** *Platypelis tsaratananaensis* was originally described by Guibé (1974) from the holotype specimen MNHN 1993.685 (originally A685). Rakotoarison et al. (2012) re-examined the holotype and studied newly observed and newly collected specimens from Tsaratanana. They stated that the species is characterised by "medium body size (adult SVL 22–33 mm), most specimens with colored patches above the eyes, absence of reddish or yellowish ventral color, no sharp border between dorsal and lateral color, third toe distinctly shorter than fifth, vomerine teeth present, males with prepollical tubercle but lacking a finger-like prepollex as typical for *Anodonthyla*."

Based on our molecular phylogeny we here extend this definition to also encompass specimens from the Sorata



Figure 6. Photographs in life of *Platypelis alticola* from the Sorata Massif. **a**, **b** ZSM 1615/2012 (FGZC 3669); **c** Young tadpoles found in the same bamboo hole as ZSM 1615/2012; **d**, **e** UADBA-A (FGZC 3652).



Figure 7. Photographs in life of *Platypelis tsaratananaensis* from the Sorata Massif. **a**, **b** ZSM 5655/2012 (FGZC 3648); **c**, **d** ZSM 5654/2012 (FGZC 3647); **e**, **f** ZSM 5656/2012 (FGZC 3649).

Massif (Fig. 7), as well as to those from the Marojejy Massif (the latter had previously been referred to as P. sp. 7 or P. sp. Ca7; Vieites et al. 2009; Perl et al. 2014), despite the substantial divergence among these populations of 3.0–5.6% p-distance in the 16S segment studied herein

(see molecular results above). The Marojejy population had also been tentatively assigned to the species by Rakotoarison et al. (2012). These populations will require thorough taxonomic revision in the future. Scherz et al. (2016) referred to the population from Sorata as *P*. sp. CaNEW1. They found these samples to be the sister clade to the sample AMNH 167233 PT-240, referred to as "*Cophyla occultans* Vohemar KM509119" by Peloso et al. (2016). The latter specimen is presumably from the Vohemar district in general, which includes part of Sorata.

**New material examined.** UADBA-A specimens (FGZC 3646, FGZC 3765), and ZSM 5654/2012–5656/2012 (FGZC 3647–3649), collected on 28 November 2012 above the camp site on Sorata massif (close to 13.6817S, 49.4411E, 1339 m a.s.l.), northern Madagascar, by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M. Ratsoavina, and A. Razafimanantsoa.

**Distribution.** This species is known from Tsaratanana Strict Nature Reserve (Raxworthy et al. 2008; Rakotoarison et al. 2012) as well as several nearby sites (Bemanevika, Andrevorevo), the Sorata Massif, and the Marojejy Massif (Fig. 2). Rakotoarison et al. (2012) reported additional sites in need of confirmation (Tsararano and Anjanaharibe-Sud) based on photographs only.

**Natural history.** Widely distributed in Tsaratanana Strict Nature Reserve, these frogs inhabit bamboo holes (Raxworthy et al. 2008; Rakotoarison et al. 2012). The specimens collected in the Sorata Massif were also discovered in bamboo holes, and some specimens were ovigerous or found with eggs in such holes (as in Tsaratanana; see also Rakotoarison et al. 2010). In Tsaratanana, calling activity was mostly observed at night and in the early morning (Rakotoarison et al. 2012), but no calls were heard in Sorata. As known from the Tsaratanana Massif, the dorsal pattern of the encountered specimens at Sorata was highly polymorphic (Fig. 7).

Undescribed candidate species

#### Platypelis sp. Ca12

**Remark.** This species has been listed as *Platypelis* sp. CaNEW3 Sorata in Scherz et al. (2016, 2017) and Peloso et al. (2017).

**Material.** UADBA-A (FGZC 3607) collected on 26 November 2012 at a site in the Sorata Massif (13.6817S, 49.4411E, 1339 m a.s.l.), northern Madagascar, by F. Glaw, O. Hawlitschek, T. Rajoafiarison, A. Rakotoarison, F.M. Ratsoavina, and A. Razafimanantsoa; UADBA-A (FGZC 3781) and UADBA-A (FGZC 3782) collected on 1 December 2012 from the camp site in the Sorata Massif (near 13.6851S, 49.4417E, 1279 m a.s.l.), northern Madagascar, by the same collectors.

**Identity.** Only three putatively juvenile specimens of this lineage were collected, and measurements of two of these are included in Table 1. As these were not recognized as

potentially taxonomically distinct in the field, no color photos and no field notes on color in life or habitat were taken. In preservative the dorsal color is brown, with a distinct light triangular spot at midback and a fine, light-coloured vertebral line (FGZC 3607), with an indistinct light dorsal marking (FGZC 3781) or a light-coloured vertebral stripe (FGZC 3782). The hindlimbs have dark crossbands dorsally and the ventral surfaces are brownish. An assessment of the status of this lineage will have to wait until additional material becomes available. However, due to its syntopic occurrence with P. laetus, strong mitochondrial divergence and limited RAG-1 haplotype sharing, it is very likely that this is yet another undescribed species of highland Platypelis, and we predict that it will likely differ in its advertisement call from P. laetus as do most frog species that occur in syntopy (Köhler et al. 2017).

#### Platypelis sp. Ca13

**Material.** UADBA-A (AEA 039) and UADBA-A (AEA 040) collected on 24 May 2016 in Andravory (13.9830S, 49.7500E, 1168 m a.s.l.), northern Madagascar, by S. Megson, J. Sawyer, R. Walker, W.-Y. Crawley, and T.H. Rafeliarisoa; UADBA-A (AEA 067) and UADBA-A (AEA 068) collected on 30 May 2016 in Andravory (13.005S, 49.7808E, 1168 m a.s.l.), northern Madagascar, by the same collectors.

**Identity.** Due to their phylogenetic placement within the *P. olgae* clade, we included sequences of these specimens from Andravory near Sorata (Fig. 2) in our molecular tree; however, the respective specimens were not available for morphological examination in this study as they are still uncatalogued in the UADBA collection. Based on the very high mitochondrial divergence of this lineage we hypothesize it represents a further distinct, undescribed species in the *P. olgae* clade.

### Other Platypelis species

During our surveys in Sorata, we also recorded specimens assigned to *P. grandis* (Fig. 1) and to *P. tetra* (Fig. 1). The taxonomic identity of these two lineages is uncertain and needs to be clarified by future studies.

## Discussion

This study adds a new species from northern Madagascar to the genus *Platypelis* and demonstrates that one third (5 of 16) of described *Platypelis* species and one undescribed candidate species (*P*. sp. Ca12) occur at higher elevations in the Sorata massif, plus another candidate (*P*. sp. Ca13) from the adjacent Andravory massif. This makes this massif a hotspot for *Platypelis* species diversity.

The new species is placed, along with two additional candidate species, in a clade with the recently described P. olgae, but it is genetically strongly differentiated from these. The collecting sites of all samples in the P. olgae clade confirm that these frogs represent a genetically distinctive group of Platypelis species from the highlands of northern Madagascar that remained unrecognized until 2012 when P. olgae was described. It is obvious that the species inventory of the northern massifs in Madagascar is still far from complete, and future expeditions will be necessary to better understand the faunal diversity of this region. The discovery of Platypelis sp. Ca12 also illustrates the need for comprehensive sampling on successive expeditions, as this candidate species would have passed unperceived if the respective juvenile specimens had not been collected despite not being recognized in the field as possibly taxonomically distinct.

Recently, several authors reported the affinity of species occuring in the Sorata Massif with those of the Marojejy National Park (Rakotoarison et al. 2017, 2019a; Prötzel et al. 2018; Scherz et al. 2015, 2017a, 2018a, b). This pattern is also seen between the Sorata Massif and Tsaratanana, as supported by the discovery of *P. alticola* and *P. tsaratananaensis* in Sorata, which were previously only known to occur in Tsaratanana Strict Nature Reserve. The new species described herein and *Platypelis* sp. Ca12, both from the Sorata Massif, belong to the *P. olgae* clade, which formerly was known only from Tsaratanana. Far greater sampling in this area of Madagascar will be needed to complete our picture of the biogeography of whole faunal communities over this geographically complex, hyperdiverse region.

The two lineages of the *Platypelis olgae* group occurring in the Sorata Massif (*P. laetus* and *P.* sp. Ca12) were found in the bamboo area of the Massif (around 1339–1541 m a.s.l). According to our observations, these bamboo forests of the Sorata Massif are under high pressure because local people use the area for cattle grazing. *Platypelis laetus* may therefore warrant consideration as Critically Endangered on the IUCN Red List.

## Acknowledgements

We are grateful to Angeluc Razafimanantsoa and Théo Rajaofiarison for their assistance in the field, to Meike Kondermann for help with laboratory work, to the Malagasy authorities for issuing permits for research, collection, and export of specimens. This work was carried out in the framework of collaboration accords of the Technische Universität Braunschweig and the Zoologische Staatssammlung München with the Université d'Antananarivo, Faculté des Sciences (Mention Zoologie et Biodiversité Animale), and funded by the Mohamed bin Zayed Species Conservation Fund (project 11253064) and the BIOPAT initiative (http://www. biopat.de).

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Zeitschrift/Journal: Zoosystematics and Evolution

Jahr/Year: 2020

Band/Volume: 96

Autor(en)/Author(s): Rakotoarison Andolalao, Scherz Mark D., Köhler Jörn, Ratsoavina Fanomezana Mihaja, Hawlitschek Oliver, Megson Steven, Vences Miguel, Glaw Frank

Artikel/Article: Frogs of the genus Platypelis from the Sorata massif in northern Madagascar: description of a new species and reports of range extensions 263-274