

# Two new plant-breeding frog species (Anura: Mantellidae, *Guibemantis*) from southeastern Madagascar

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Based on morphology, advertisement calls and DNA sequences, we describe two new species of plant-breeding frogs in the genus *Guibemantis* (subgenus *Pandanusicola*) from coastal rainforests in southeastern Madagascar. *Guibemantis annulatus* sp. nov. is most similar to *G. punctatus* but differs in having a lighter background colour on the dorsum, smaller and more regularly rounded spots on the dorsum, conspicuous light “rings” just proximal to each toe or finger disc, a longer femur, tibia and foot, a wider head, larger femoral glands (in males), a greater internarial distance and large genetic differences. *Guibemantis wattersoni* sp. nov. is most similar to *G. bicalcaratus* but differs in having a larger body size, a larger tympanum, two conspicuous parallel rostral lines and conspicuous light “rings” just proximal to each toe or finger disc, in the characteristics of the advertisement calls and in large genetic differences. Also, unlike the described character state of all other known *Pandanusicola*, these species have completely separated paired subgular vocal sacs. Both of these new species are only found in phytotelms (typically in *Pandanus* plants), where they breed. Both new species also appear to be regional endemics and are of immediate conservation concern. We also redescribe *G. punctatus* based on new topotypical material and discuss the identity of *G. bicalcaratus* and other available names.

*Key words:* Amphibia, *Guibemantis annulatus*, *Guibemantis wattersoni*, *Pandanusicola*, phylogeny, taxonomy

## INTRODUCTION

Madagascar is widely recognized as a biodiversity hotspot, with high levels of both species diversity and endemism. This is particularly well exemplified by frogs with 276 species currently described (all but one introduced species are thought to be endemic; Glaw & Vences, 2007). Amazingly, this is likely to be a substantial underestimate of the true diversity of frogs in Madagascar. On the basis of DNA sequences, acoustic analyses and morphological characters, Vieites et al. (2009) recently estimated that eventual species numbers of frogs in Madagascar may easily exceed 400.

In the last twenty years, there has been a dramatic increase in the number of new frog species descriptions from Madagascar (Glaw & Vences, 2007). However, one group of Malagasy frogs that remains poorly studied is that of the mostly plant-breeding frogs currently placed in the subgenus *Pandanusicola* of the genus *Guibemantis* in the family Mantellidae. At present there are six species in this subgenus, all of which were described between the late 19<sup>th</sup> and the late 20<sup>th</sup> century: *G. albolineatus* (Blommers-Schlösser & Blanc, 1991); *G. bicalcaratus* (Boettger, 1913); *G. flavobrunneus* (Blommers-Schlösser, 1979); *G. liber* (Peracca, 1893); *G. pulcher* (Boulenger, 1882); and *G. punctatus* (Blommers-Schlösser, 1979). Recent molecular phylogenetic analysis of this group (Lehtinen et al., 2007) confirmed that *G. liber* (as currently understood; see Vieites et al., 2009) is part of this clade (even though it breeds in ponds, not in the leaf axils of plants) and that

most described species comprise a number of genetically well differentiated forms. These data strongly suggested the existence of previously unrecognized species in this lineage.

Lehtinen et al. (2007) included in their analysis ten specimens initially attributed to *G. bicalcaratus*. These were resolved into three divergent clades located in northeastern Madagascar, in the vicinity of Ranomafana National Park and in coastal rainforests in the southeast. Sister to these *G. “bicalcaratus”* from southeastern Madagascar were sequences from two specimens attributed to *G. punctatus*, also from the southeastern part of the island. Using additional DNA sequence data, advertisement call recordings and morphological data (including data from the relevant type localities), we here describe these two forms from southeastern Madagascar as new. We also provide a detailed re-description of *Guibemantis punctatus* based on topotypic specimens and discuss the identity of *G. bicalcaratus* based on type specimens and new material from its type locality, Nosy Boraha.

## MATERIALS AND METHODS

The following morphological measurements were taken to the nearest 0.1 mm with digital calipers by the first author (RML): snout–vent length (SVL), femur length (FL), tibia length (TL), foot length (FOL), head length (HL), head width (HW), hand length (HAL), lower arm length (LAL), horizontal tympanum diameter (TD), horizontal eye diameter (ED), femoral gland size (length and width,

FGL and FGW (in males only)), maximum width of the third finger disc (W3FD), maximum width of the fourth toe disc (W4TD), interorbital distance (IOD), nostril–snout tip distance (NSD), eye–nostril distance (END), internarial distance (IND) and the mean diameter of dorsal spots (MDS, if any, based on measurements of ten spots per specimen). When mean values are presented, they are followed by the standard deviation (SD). Most abbreviations and definitions of morphological features follow Vences & Glaw (2005). We also examined all specimens for the presence, position and/or shape of the inner and outer metatarsal tubercles, subarticular tubercles, lateral metatarsalia, canthus rostralis, tongue, vomerine odontophores and the supratympanic fold. Finger and toe webbing was quantified following Blommers-Schlösser (1979). Overall dorsal and ventral coloration and skin texture were also recorded in detail for each specimen.

The following institutional abbreviations are used: Natural History Museum, London (BMNH); Field Museum of Natural History, Chicago (FMNH); Muséum National d'Histoire Naturelle, Paris (MNHN); Naturhistorisches Museum Wien (NMW); Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt (SMF); Université d'Antananarivo, Département de Biologie Animale, Antananarivo (UADBA); University of Michigan Museum of Zoology, Ann Arbor (UMMZ); Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK); Zoölogisch Museum Amsterdam (ZMA); and Zoologische Staatssammlung München (ZSM). FGMV, FGZC and ZCMV refer to F. Glaw and M. Vences field numbers. RAN refers to Ronald A. Nussbaum field numbers and UMFS refers to University of Michigan Field Series numbers.

DNA was extracted from muscle or liver tissues preserved in ethanol. We amplified a portion of the 16S mitochondrial gene (approximately 1000 bp) using the primers and protocols reported in Lehtinen et al. (2007). Using a subset of relevant 16S sequences from Lehtinen et al. (2007), we added sequences from three specimens assigned to *G. bicalcaratus* from Montagne d'Ambre (FG/MV 2002-905 [= ZSM 895/2003], GenBank accession number HQ456654) and Nosy Boraha (= Ile Sainte Marie – the type locality: ZCMV 3242 [= ZSM 427/2006] and ZCMV 3244 [= ZSM 429/2006], GenBank accession numbers HQ456652 and HQ456653, respectively). We added sequences from two specimens assigned to *G. punctatus* from Ambohitantely Jardin Botanique (the region of the type locality: ZCMV 5501 and 5502, [= ZSM 2337-2338/2007], GenBank accession numbers HQ456656 and HQ456657, respectively). We also added a sequence from an additional outgroup species, *G. timidus* from Manombo (UADBA-ZCMV 5479, GenBank accession number HQ456658). Sequences were aligned in Clustal X with the default gap insertion and gap extension penalties and the alignment further adjusted by eye. Approximately 40 bp in hypervariable regions that could not be confidently aligned were eliminated from the dataset.

Maximum parsimony analysis was conducted in PAUP\* version 4b10 (Swofford, 2002) using heuristic searches and 100 random addition sequence replicates. Characters were unordered and unweighted and sequences from *G.*

*cf. depressiceps* and *G. timidus* were used as outgroups. The tree-bisection-reconnection algorithm was used for branch swapping. Clade support was assessed with 1000 bootstrap replicates. The Bayesian analysis was conducted in MrBayes 3.1 using the GTR+I+G substitution model as determined by MrModeltest 2.3 (Nylander, 2004). Empirical base frequencies were: A=0.3711, C=0.2047, G=0.1619, T=0.2623. The proportion of invariable sites (I) was 0.3695 and the gamma distribution shape parameter was 0.6231. Priors were set using the default setting in MrBayes and the model parameters were estimated in the analysis. Two independent analyses with different random starting trees were run simultaneously with three heated chains and one cold chain. Each run was conducted for 1.4 million generations. We discarded the first 25% of the trees, corresponding to 350,000 generations, as burn-in. The analysis was terminated when the standard deviation of split frequencies was less than 0.01. We verified that tree length, amino acid model, log-likelihood score and alpha value of the gamma distribution had reached stationarity and that our burn-in was appropriate using the program Tracer version 1.5, which is part of the Beast package (Drummond & Rambaut, 2007), and further assessed convergence and mixing using AWTY (Nylander et al., 2008). Majority rule consensus trees were used to display the results of all analyses. For the Bayesian analysis, *G. cf. depressiceps* was arbitrarily chosen as the outgroup taxon as MrBayes allows only a single outgroup taxon to be designated.

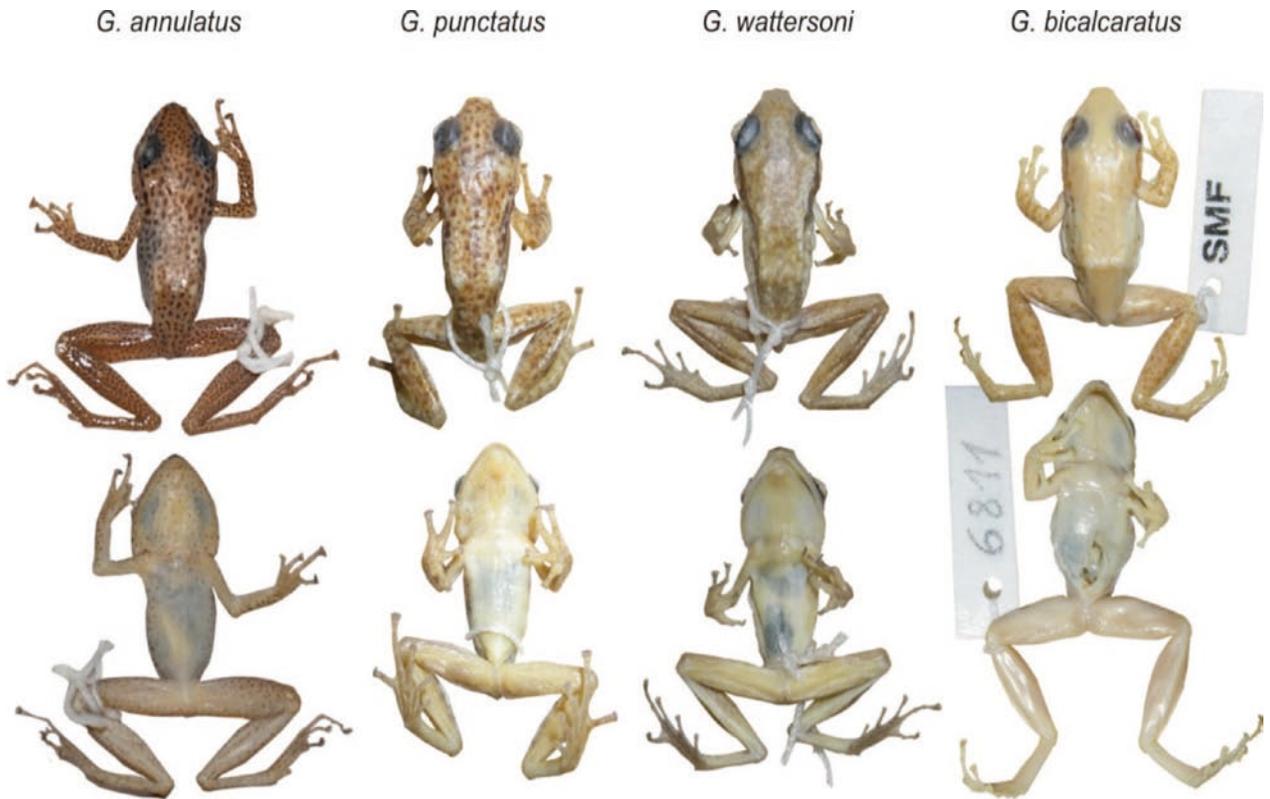
Calls were recorded using a Sony WM-D6C cassette recorder with a Sony ECM-MS957 external microphone with wind baffle. Vocalizations were digitized into Raven version 1.2.1 and Cooledit Pro version 2.0, where spectrograms and waveforms were produced for visualization.

## RESULTS

### Description of *Guibemantis annulatus* sp. nov. (Figs 1 & 2)

*Holotype*. UMMZ 227004 (UMFS 10877; Fig. 1), an adult female from Mandena littoral rainforest (24°57.095'S; 47°02.082'E, 10 m a.s.l.), southeast Madagascar, collected by Richard M. Lehtinen on 14 June 1999.

*Paratypes*. Twenty-one, all from southeast Madagascar: UMMZ 191561 (RAN 32720) and UMMZ 191564 (RAN 32762; both adult females) from Sainte Luce littoral rainforest, collected by Christopher J. Raxworthy on 19 and 24 October 1989 respectively; UMMZ 198081 (RAN 34303; adult female), UMMZ 198078 (RAN 34047; adult male) and UMMZ 198099 (RAN 34046; adult male) from Agnalaro Forest near Lake Ambavasohihy, collected by Ronald A. Nussbaum, Christopher J. Raxworthy, Achille Raselimanana and Jean-Baptiste Ramanamanjato on 3 October 1990; UMMZ 198098 (RAN 34551; adult female) from Mandena littoral rainforest collected by Christopher J. Raxworthy, Achille Raselimanana and Jean-Baptiste Ramanamanjato on 12 October 1990; ZSM 313/2005 (FGZC 2581; adult female), ZSM 314/2005 (FGZC 2582; adult female) and ZSM 315/2005 (FGZC 2586; subadult female) collected from Sainte Luce littoral rainforest



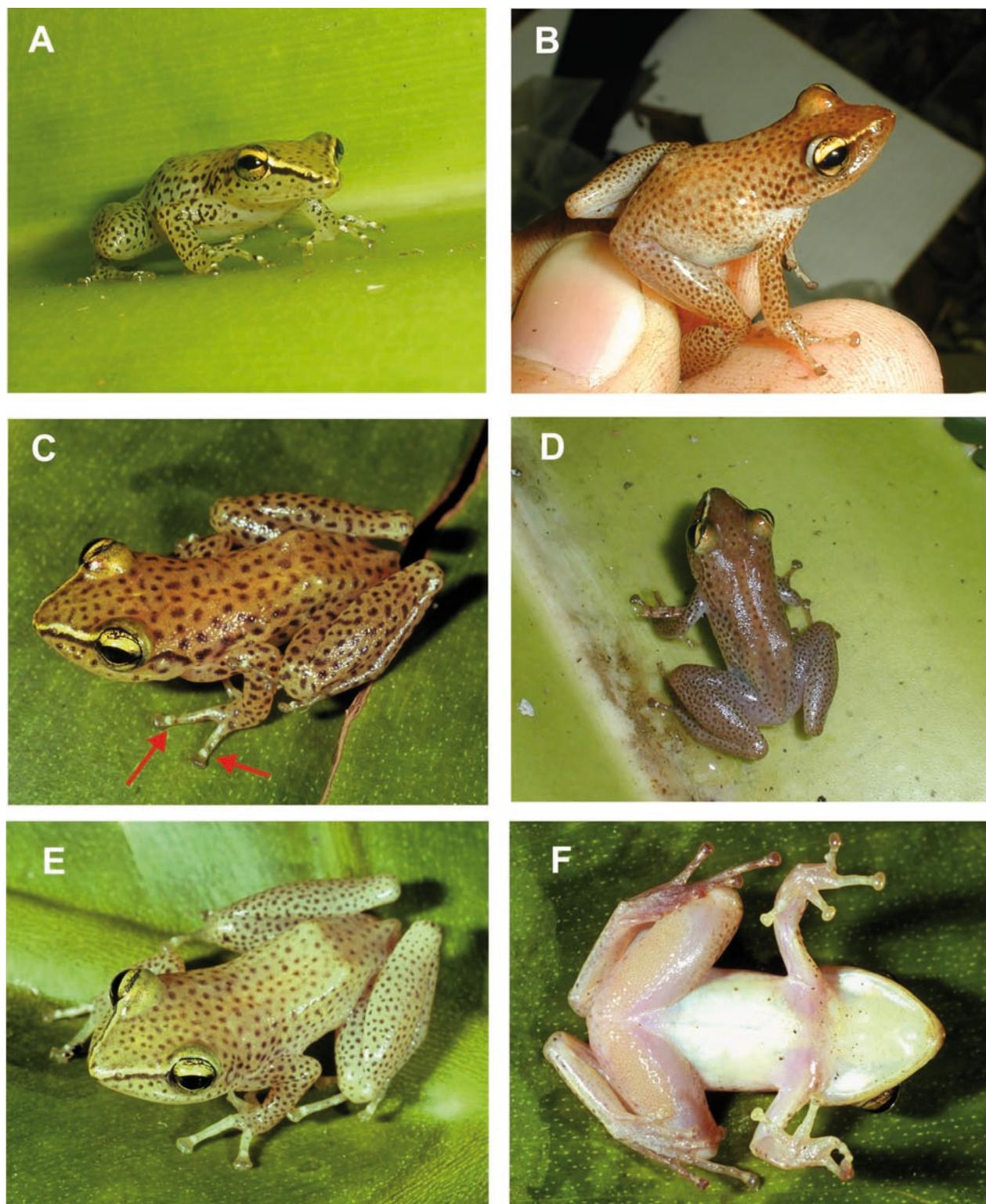
**Fig. 1.** Photographs of preserved type specimens in dorsal (upper row) and ventral view (lower row) of *G. annulatus* (holotype, UMMZ 227004, adult female), *G. punctatus* (paratype, ZSM 363/2004, originally ZMA 7170, adult male), *G. wattersoni* (holotype, ZSM 177/2005, adult male) and *G. bicalcaratus* (lectotype, SMF 6811, adult female). Not to scale (see Tables 1 and 2 for measurements).

(24°46.50'S; 47°09.05'E) by Frank Glaw and Parfait Bora on 4 February 2005; ZFMK 53704 (adult female) and 53705 (adult male) collected from a coastal locality several kilometres north of Tolagnaro by Joachim Müller and Frank Glaw on 5 January 1992; NMW 20360:3 and NMW 20360:8 (two adult males with clearly recognizable femoral glands), NMW 20360:1, NMW 20360:2, NMW 20360:5 and NMW 20360:7 (four adult females), NMW 20360:4, NMW 20360:6, NMW 20360:9 and NMW 20360:10 (four subadults) collected from “Fort Dauphin, S-Madagascar” by Franz Sikora in 1900.

**Etymology.** The specific name is used as a descriptive adjective and makes reference to the characteristic white rings found on both fingers and toes.

**Diagnosis.** Assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on small body size, diurnal, phytotelm-breeding habitats (in *Pandanus* plants), moderate webbing between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, type 2 femoral glands in males and DNA sequence similarities (Glaw & Vences, 2006). Within *Pandanusicola*, the new species is distinguished from *G. albolineatus*, *G. bicalcaratus*, *G. flavobrunneus*, *G. liber*, *G. pulcher*

and *G. wattersoni* sp. nov. (see below) by the abundance of conspicuous but small, round dark spots on a cream-coloured dorsum, a tibiotarsal articulation that extends well beyond the eye and by large genetic differentiation. By comparison, *G. pulcher* is a characteristic bright green in colour with very large irregular dark dorsal spots, *G. bicalcaratus* and *G. wattersoni* sp. nov. are yellowish dorsally with usually relatively few irregularly shaped spots (see below) and *G. albolineatus* has conspicuous white dorso-lateral lines. *Guibemantis flavobrunneus* is much larger (up to 38 mm SVL; Glaw & Vences, 2007) and *G. liber* has larger hands and feet (ratio HAL/SVL greater than 0.3 vs less than 0.3) and does not breed in *Pandanus* plants. The new species probably also differs from all known *Pandanusicola* (except its putative sister species *G. wattersoni*, see below) in having completely separated paired subgular vocal sacs, rather than single subgular as described by Blommers-Schlösser & Blanc (1991). The new species differs from *G. punctatus* by a lighter background colour on the dorsum (cream vs olive), by smaller and more regularly round-shaped spots on the dorsum (mean size 0.3 vs 0.6), the presence of conspicuous light “rings” just proximal to each toe or finger disc, the presence of a prominent and continuous gold (in life) rostral stripe medial to a black one, the pres-



**Fig. 2.** Photographs of *G. annulatus* in life taken at Sainte Luce coastal rainforest, southeast Madagascar by RML (A–D) and FG (E–F). Arrows in C point to typical light “rings” just proximal to each toe or finger disc.

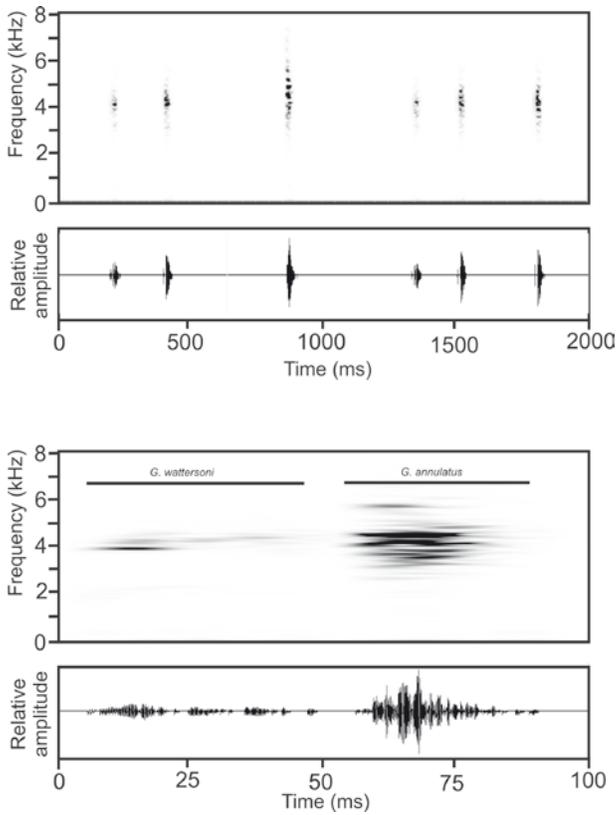
ence of small dark spots on the dorsal surface of arms and legs (vs thick bars), a relatively longer femur (FL/SVL mean =  $0.49 \pm 0.04$  vs  $0.46 \pm 0.02$ ), tibia (TL/SVL mean =  $0.55 \pm 0.03$  vs  $0.49 \pm 0.02$ ) and foot (FOL/SVL mean =  $0.48 \pm 0.03$  vs  $0.42 \pm 0.03$ ), longer femoral glands in males (FGL/SVL mean =  $0.27 \pm 0.02$  vs  $0.20 \pm 0.03$ ) and DNA se-

quence differences (mean pairwise sequence difference in the 16S rRNA gene >10%).

*Description of the holotype.* Adult female. Head wider than long and wider than body; snout somewhat pointed in dorsal, ventral and lateral views; canthus rostralis

**Table 1.** Morphometric data (in mm) of *Guibemantis punctatus* (n=13), *G. annulatus* sp. nov. (n=19) and *G. aff. annulatus* (n=4). HT = holotype, PT = paratype, SD = standard deviation; see Materials and Methods for other abbreviations. Not all paratypes are listed for *G. annulatus*.

Catalogue number	Sex	Status	SVL	FL	TL	FOL	HL	HW	HAL	LAL	TD	ED	FGLxFGW	W3FD	W4TD	IOD	NSD	END	IND	MDS
<i>Guibemantis punctatus</i>																				
ZSM 363/2004	M	PT	19.5	9.5	10.2	8.8	7.9	7.9	6.1	4.6	1.4	3.1	1.6 x 4.6	1.2	0.9	3.3	0.9	2.1	2.4	0.5
ZSM 2337/2007	F	-	21.4	10.0	10.8	8.9	7.6	7.2	5.6	4.5	1.4	2.8	-	1.1	1.0	3.5	0.9	2.2	2.1	0.6
ZSM 2338/2007	M	-	20.9	10.2	10.9	8.8	7.7	7.2	5.5	4.7	1.4	3.2	1.2 x 4.0	1.2	1.0	3.1	1.3	2.1	1.9	0.6
FMNH 260284	F	-	23.9	11.1	11.8	10.7	8.7	8.3	6.8	5.9	1.4	2.7	-	0.8	0.6	3.3	0.7	2.9	1.6	0.6
FMNH 260283	M	-	20.5	8.5	10.4	8.7	6.5	7.4	5.5	4.9	1.5	2.6	1.1 x 4.2	0.7	0.5	3.0	0.3	2.2	1.5	0.5
FMNH 260165	M	-	23.3	10.2	10.6	8.8	6.9	7.9	5.6	4.9	1.2	2.5	1.7 x 5.5	1.1	0.7	3.0	0.6	2.6	1.8	0.6
FMNH 260287	F	-	23.2	11.1	11.4	10.0	8.2	7.9	6.1	4.8	1.5	2.8	-	0.8	0.6	3.3	0.7	2.6	2.1	0.6
FMNH 260285	M	-	21.1	9.9	9.7	8.1	6.5	6.6	6.5	5.0	1.4	2.5	1.4 x 3.4	0.7	0.5	2.5	0.8	1.9	1.7	0.5
FMNH 260286	F	-	24.6	10.7	11.3	9.2	7.3	7.8	6.4	5.7	1.5	3.0	-	1.3	0.7	3.2	0.8	2.4	1.8	0.7
FMNH 260282	F	-	25.2	11.4	12.1	10.6	8.1	8.5	7.1	5.1	1.5	3.3	-	1.2	0.6	2.7	0.6	2.7	1.9	0.5
FMNH 260280	F	-	23.2	10.6	11.4	10.3	7.7	8.1	6.5	5.0	1.4	3.6	-	0.9	0.6	3.0	0.6	2.9	2.0	0.5
FMNH 260281	F	-	23.5	10.3	11.4	10.3	7.4	8.2	7.1	4.9	1.3	3.2	-	1.0	0.8	3.5	0.8	2.1	1.6	0.5
FMNH 259923	M	-	21.8	10.0	9.9	8.3	7.6	7.3	5.7	4.3	1.3	3.3	1.1 x 3.7	0.9	0.7	3.0	0.2	2.1	1.3	0.7
Mean (SD)			22.5 (1.7)	10.3 (0.8)	10.9 (0.7)	9.3 (0.9)	7.5 (0.6)	7.7 (0.5)	6.2 (0.6)	4.9 (0.4)	1.4 (0.1)	3.0 (0.3)	1.3 (0.3)/ 4.2 (0.7)	1.0 (0.2)	0.7 (0.2)	3.1 (0.3)	0.7 (0.3)	2.4 (0.3)	1.8 (0.3)	0.6 (0.1)
<i>Guibemantis annulatus</i>																				
UMMZ 227004	F	HT	24.2	12.4	13.3	11.4	8.0	9.2	7.0	5.8	1.5	3.2	-	1.0	0.9	4.0	1.2	2.9	2.8	0.3
UMMZ 191561	F	PT	24.2	12.6	13.8	11.6	7.9	8.9	5.7	5.2	1.1	3.2	-	1.3	1.0	3.4	1.2	2.6	2.8	0.4
UMMZ 191564	F	PT	27.6	13.0	14.1	11.1	8.9	9.9	7.6	6.0	1.5	3.6	-	1.2	1.2	3.6	1.0	3.1	2.7	0.4
UMMZ 198081	F	PT	25.9	12.9	14.0	13.3	8.2	9.7	7.0	6.2	1.4	3.6	-	1.1	1.1	4.1	1.2	2.5	2.9	0.3
UMMZ 198099	M	PT	23.3	11.7	12.9	11.8	7.4	8.7	7.2	6.1	1.4	3.2	1.7 x 6.3	1.2	0.9	3.5	1.4	2.7	2.8	0.4
UMMZ 198098	F	PT	23.1	11.1	12.7	10.6	8.1	9.0	6.3	5.2	1.1	3.3	-	1.0	0.9	3.4	1.0	2.5	2.7	0.4
UMMZ 191565	F	-	23.9	11.5	13.5	12.4	6.7	8.7	6.6	5.4	1.4	3.1	-	1.2	1.0	3.2	1.0	2.5	2.6	0.4
UMMZ 191562	F	-	19.7	9.4	10.7	8.8	6.3	7.4	4.9	4.5	1.1	2.7	-	0.8	0.8	2.9	0.9	2.1	2.3	0.2
UMMZ 198078	M	PT	22.3	11.6	12.8	11.4	7.2	8.3	5.9	4.7	1.1	2.9	1.6 x 6.4	0.7	0.7	3.6	1.2	2.4	2.5	0.4
UMMZ 191550	F	-	25.9	12.2	14.1	11.8	8.2	9.1	7.0	6.1	1.3	3.1	-	1.0	0.8	3.3	1.3	2.7	2.8	0.4
UMMZ 232191	F	-	25.4	12.5	12.7	11.7	7.5	8.3	6.0	5.0	1.4	3.3	-	1.0	0.8	3.3	0.9	2.9	2.5	0.3
UMMZ 198079	M	-	22.4	11.4	12.3	11.7	7.3	8.4	6.3	5.3	1.3	3.4	1.4 x 5.9	0.9	0.9	3.7	0.8	2.5	2.5	0.4
UMMZ 198090	F	-	25.2	12.6	14.0	11.9	7.8	8.9	6.8	5.2	1.1	2.8	-	0.8	0.7	3.2	1.1	2.8	2.7	0.4
UMMZ 232196	F	-	21.3	10.6	11.6	10.0	7.8	7.8	5.9	5.1	1.6	2.8	-	0.9	0.8	3.0	1.0	2.2	2.3	0.3
UMMZ 232193	F	-	25.3	11.3	13.7	11.7	8.9	8.9	5.6	5.2	1.4	3.2	-	1.0	0.7	3.5	1.0	2.9	2.7	0.3
UMMZ 232188	F	-	23.3	8.9	11.5	10.4	7.1	7.9	6.9	4.6	1.2	2.5	-	0.8	0.8	2.8	0.9	2.2	2.3	0.4
ZSM 315/2005	F	PT	17.0	9.9	10.7	9.0	6.4	6.6	5.5	4.4	1.1	2.8	-	0.5	0.5	3.1	0.8	2.2	2.3	0.4
ZSM 313/2005	F	PT	23.7	11.9	12.9	11.5	8.2	8.5	6.2	4.7	1.4	3.1	-	0.9	0.8	3.7	1.1	2.8	2.5	0.3
ZSM 314/2005	F	PT	21.7	10.9	12.9	11.5	7.1	8.2	6.1	5.0	1.4	3.3	-	1.0	1.0	3.7	1.1	2.6	2.7	0.3
Mean (SD)			23.4 (2.4)	11.5 (1.2)	12.9 (1.1)	11.2 (1.1)	7.6 (0.7)	8.5 (0.8)	6.3 (0.7)	5.2 (0.6)	1.3 (0.2)	3.1 (0.3)	1.6 (0.2)/ 6.2 (0.3)	1.0 (0.2)	0.9 (0.2)	3.4 (0.3)	1.1 (0.2)	2.6 (0.3)	2.6 (0.2)	0.4 (0.1)
<i>G. aff. annulatus</i>																				
UMMZ 214278	F	-	24.7	11.6	13.8	11.5	9.4	9.4	7.4	5.5	1.4	3.4	-	1.4	1.2	3.7	1.0	2.9	2.9	0.3
UMMZ 214279	F	-	24.0	11.6	12.6	10.7	8.0	8.4	7.1	4.8	1.3	3.3	-	1.3	1.2	3.4	1.0	2.9	2.6	0.3
UMMZ 214280	F	-	25.1	12.3	14.0	11.7	9.0	9.1	7.9	5.4	1.6	3.6	-	1.4	1.4	3.8	1.0	3.2	2.7	0.3
UMMZ 214281	M	-	23.9	10.6	12.0	10.1	8.7	7.7	6.5	4.8	1.2	2.8	1.9 x 5.8	1.4	1.1	3.0	1.0	2.9	2.7	0.3



**Fig. 3.** Spectrograms and oscillograms for *G. wattersoni* and *G. annulatus*. Upper graph shows an irregular series of six notes of *G. annulatus*. The lower graph shows a note of *G. wattersoni* directly followed by a note of *G. annulatus*. Recorded from Sainte Luce littoral rainforest by RML, February 2002.

rounded; nostrils much nearer to tip of snout than to eye and pointed forward, internarial distance 70% of interorbital distance; tympanum distinct, 47% of horizontal eye diameter; supratympanic fold present from posterior edge of eye around the tympanum nearly to the arm insertion, mostly dark-coloured with some lighter patches; one small round patch of vomerine odontophores medial between eye and choanae on either side; tongue stout, bifid at tip and free posteriorly. Arms thin, lower arm 83% of hand length; relative finger length  $1 < 2 < 4 < 3$ , finger discs moderately enlarged and squared off at tips in a somewhat rounded “T” shape, only traces of webbing between fingers, subarticular tubercles single, round and large. Hindlimbs slender but robust, tibiotarsal articulation reaches well beyond snout tip when hindlimbs are adpressed against the body; femur length 93% of tibia length, foot length 85% of tibia length; lateral metatarsalia connected; inner metatarsal tubercle oblong (1.1 in length, 0.4 in width); outer metatarsal tubercle round (0.4 in diameter); webbing formula between toes 1(1) 2i(1)

2e(1) 3i(2) 3e(1) 4i(2.75) 4e(2.75) 5(1); relative length of toes  $1 < 2 < 5 < 3 < 4$ ; toe discs moderately enlarged, width of fourth toe disc 90% of width of third finger disc. Cloaca not clearly recognizable; skin is uniformly smooth dorsally, ventral skin is finely granular. For full morphometric measurements, see Table 1.

After ten years in preservative, the dorsal background coloration appears as a medium cream color with many small, black, mostly round spots on the dorsal surface (mean diameter 0.3). A relatively thick conspicuous line between the snout tip and eye is black in colour and bordered medially by a conspicuously lighter parallel line. Dorsal colour of arms and legs is the same as on the dorsum, though the size of the spots is smaller. Hands and feet are of similar colour to the rest of the body (dorsally and ventrally) except for the small but conspicuous light “rings” found on all digits just proximal to the finger and toe discs. Venter is light cream-coloured with small to medium black spots along jawline, throat, chest and a few on the belly. The pupil appears black and the iris is white. The life coloration of the holotype is unknown.

*Coloration in life.* Based on field notes and numerous photographs of individuals from the Mandena and Sainte Luce localities (Fig. 2), much of the coloration in life is retained in preservative. Three exceptions to this include 1) a loss of gold coloration of the iris (appears white or grey in preservative), 2) the lighter line between the eye and snout-tip that appears as a dull white in preservative is iridescent gold in life and 3) the “rings” just proximal to the toe and finger discs are bright white in life (dull white in preservative). In males, femoral glands appear dull yellowish in life with small granules in the gland that are of somewhat lighter coloration (type 2, as defined by Glaw et al., 2000). Sexually mature males also possess white coloration on the ventral surface of the throat near the jaw angles (these are the paired subgular vocal sacs). A colour photograph of paratype ZFMK 53705 is shown in Glaw & Vences (1994, colour photo 79) and a photo of another specimen in Glaw & Vences (2007: 446).

*Variation.* On the basis of a much larger sample of specimens in a field study at Sainte Luce, Lehtinen (2009) reported no significant sexual size dimorphism in this species (female mean =  $21.0 \pm 2.2$ , range 17–25 mm SVL,  $n=146$ ; male mean =  $21.0 \pm 1.2$ ; range 18–23 mm SVL,  $n=64$ ). The largest known specimen is the female paratype NMW 20360:2 with 31 mm SVL. Older individuals have a faded appearance with the gold line between the eye and snout tip and the dorsal spots becoming less clearly demarcated from the background coloration (Fig. 2). The fine spots on the venter, while generally sparse, vary from nearly absent to fairly widely distributed (as in the holotype). The supratympanic fold can be conspicuously dark, intermittently dark/light or have little coloration associated with it. Some individuals differ markedly in the coloration on the supratympanic fold on the left and right sides. The tip of the tongue in all specimens examined was bifid. However in some specimens the tongue tip was very strongly bifid, in others this was less so, which might reflect variation or be due to different states of fixation

and dehydration. No individuals were observed to have any significant webbing on the hand. However, webbing on the foot was variable for some digits (range, where variable, given in parentheses): 1(1) 2i(1) 2e(0.5–1) 3i(2) 3e(1–1.75) 4i(2.5–3) 4e(2.5–3) 5(1–1.5). A few individuals had a relatively prominent raised area posterior to the tympanum, somewhat reminiscent of the parotoid gland of a toad. Most individuals, however, lacked this feature. One male specimen (UMMZ 198078) possessed a large rough area on the belly with raised white nodules of unknown function.

Four specimens from the Anjananaharibe-Sud Special Reserve in northeastern Madagascar (UMMZ 214278–214281; three females, one male – see Table 1) superficially resemble *G. annulatus*. However, we do not assign them to this species here, since they differ from the other *G. annulatus* specimens in several ways: 1) the ventral skin is much more coarsely granular, 2) the ventral surface is absolutely immaculate (rather than having various amounts of fine spots), 3) the dorsal background coloration is a much lighter shade of cream, 4) the darkly coloured supratympanic fold continues as a relatively conspicuous dorsal line to mid-body (ends at supratympanic fold in *G. annulatus*), 5) the dark line between the eye and snout tip is not bordered medially by a parallel gold line and 6) the size of the finger and toe discs is relatively larger (see Table 1). We hypothesize that these differences indicate yet another new species but cannot exclude the possibility that they may represent geographic variation within *G. annulatus*. More specimens and DNA sequences from these frogs are necessary to assess their taxonomic status.

**Distribution.** While it can be locally somewhat common, at present *G. annulatus* is known from only three well-defined low-elevation rainforest sites in extreme southeastern Madagascar: Mandena, Sainte Luce and the Agnalaro Forest near Lake Ambavasohihy (south of Sainte Luce; Fig. 7). The historical record from Fort Dauphin might refer to the region or this town of the same name. Note that for the purposes of suggesting a conservation status, we here exclude the specimens tentatively assigned to *G. annulatus* from the Anjananaharibe-Sud Special Reserve as these may represent another species.

**Advertisement call.** Male vocalizations were recorded at Sainte Luce littoral forest on 9 February 2002 at 26 °C (Fig. 3). To the human ear, the advertisement call of *G. annulatus* sounds like a simple, soft “click”. These are emitted as single notes or in a succession of twos or threes at frequencies between 3000 and 6060 Hz (mean frequency range 1907 Hz  $\pm$  669 SD,  $n=19$ ; Fig. 3). The peak intensity (dominant frequency) of recorded calls was approximately 4500 Hz. We assessed note duration and duration of inter-note intervals from recordings corresponding to a single individual male. Mean note duration of single notes in this recording was 28 ms ( $\pm$  7 SD,  $n=20$ ) and inter-note intervals averaged 700 ms  $\pm$  600 SD,  $n=19$ ). Calls of *G. punctatus sensu stricto* (from the Ambohitantely region in central Madagascar) have not yet been recorded and are unavailable for comparison.

**Natural history.** Breeding coincides with the rainy season in southeastern Madagascar (December to March). Males call from *Pandanus* plants only during the day. As reported in Lehtinen (2003, under the name *Mantidactylus punctatus*), eggs are laid above water-filled leaf axils in *Pandanus* plants. Freshly deposited eggs have a grey animal pole and a white vegetal pole. Individual eggs measured 1.9 mm in diameter in the field and 1.1 mm in diameter in one preserved gravid female specimen. Clutch size averaged 26 deposited eggs ( $n=14$ ). Males or females provide periodic egg attendance until hatchlings are washed into the leaf axil (Lehtinen, 2003). Early ontogeny is described in Lehtinen (2009). Lehtinen (2004) demonstrated that the elongated tadpoles are primarily detritivorous and estimated that the larval period lasts approximately 2–3 months. At metamorphosis, body size is approximately 9 mm SVL. Mark–recapture data reported in Lehtinen (2009) suggest a total life span of less than 14 months. The minimum size at sexual maturity for males was approximately 18 mm SVL (based on the appearance of external secondary sexual characteristics). Sex ratios at Sainte Luce were highly female-biased (0.307 females for each male; Lehtinen, 2009). Reported predators include an unidentified heteropodid spider and the snake *Ithyophis oursi* (Lehtinen, 2002).

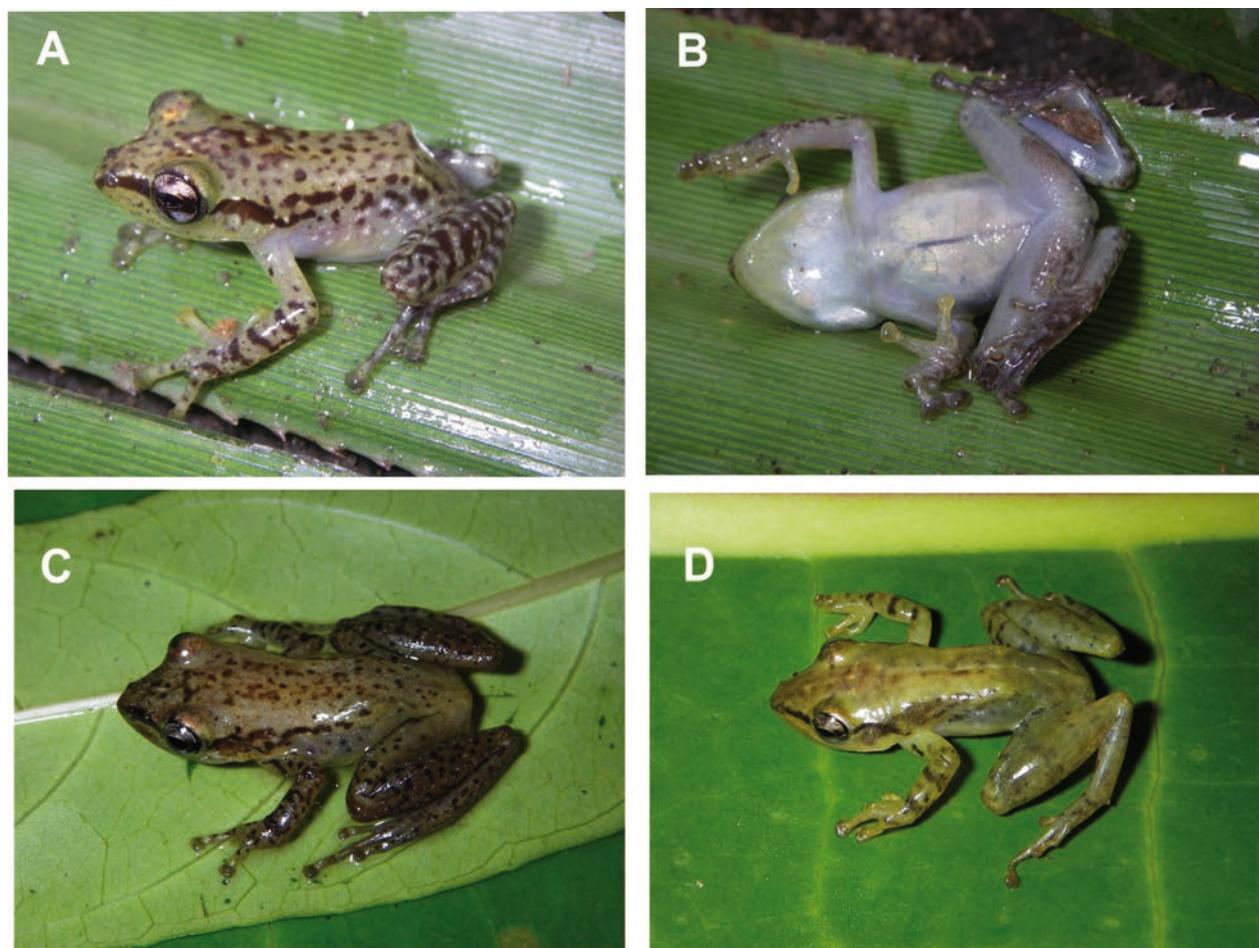
**Available names and comparisons.** We verified the identity of all nominal species of the subgenus *Pandanusicola* in the genus *Guibemantis* (*G. albolineatus*, *G. bicalcaratus*, *G. flavobrunneus*, *G. liber*, *G. pulcher*, *G. punctatus*) by morphological examination of their name-bearing types or topotypical paratypes and verified the current definition of all these species. All except *G. bicalcaratus* agree with current taxonomy as summarized in Blommers-Schlösser & Blanc (1991) and Glaw & Vences (2007). For a discussion of the status and identity of *G. bicalcaratus* and of three junior synonyms of *G. bicalcaratus* and *G. liber*, see “Available names” in the section on *G. wattersoni* below.

The dense and regular dorsal spots readily distinguish the new species from all *Guibemantis* except *G. punctatus*. To further clarify differences between *G. annulatus* and *G. punctatus sensu stricto*, below we redescribe *G. punctatus* based on recent material collected from its type locality.

### Redescription of *Guibemantis punctatus* (Blommers-Schlösser, 1979)

This species was originally described based on a male holotype (ZMA 7171A) and 15 paratypes (ZMA 7171B and ZMA 7170) collected from *Pandanus* leaf axils “in the relict forest in the gulleys of the Tampoketsa” at an elevation of 1600 m. Tadpoles and metamorphosed juveniles (ZMA 7169) were also available at the time of original description.

**Identity.** Twelve recently collected specimens from *Pandanus* at or very near to the type locality were available for study (five adult males, seven adult females; Table 1). All ZSM specimens were collected from the “Jardin Botanique” site in Ambohitantely Special Reserve by

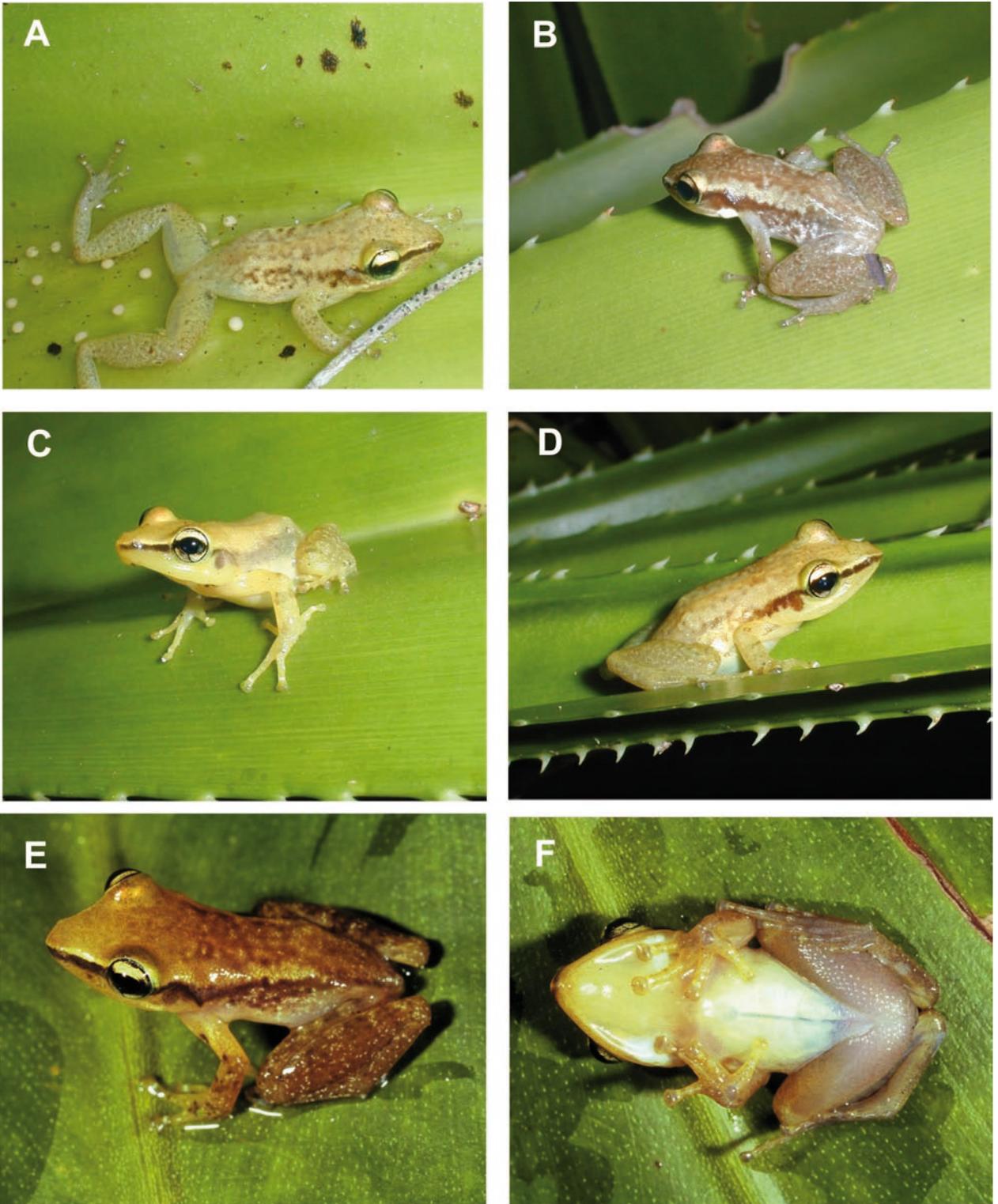


**Fig. 4.** Photographs of adult *Guibemantis punctatus* in life taken by MV at Ambohitantely Special Reserve (A–B) and of *Guibemantis* specimens from Nosy Boraha, the type locality of *G. bicalcaratus*, ZSM 425/2006 (ZCMV 3240) (C) and ZSM 395/2006 (ZCMV 3397) (D). Note that these specimens are probably not conspecific with each other and with *G. wattersoni* due to differences in the spotting of hindlimbs. ZSM 425/2006 is tentatively assigned to *G. bicalcaratus* but lacks the prominent and continuous gold (in life) rostral stripe medial to black one that probably occurs in at least some individuals of this species (as in *G. annulatus* and *G. wattersoni*).

M. Vences, P. Bora and collaborators in February 2007. All FMNH specimens were collected from “Forêt de Ambohitantely” by A. Graybeal, December 1997. These specimens agree well in nearly all features with the paratype we examined (ZSM 363/2004, originally ZMA 7170). For example, the horizontal eye diameter, the horizontal tympanum diameter, the size of dorsal spots, the size of femoral glands (in males) and the sizes of the limbs, hands, feet and toepads are all quite similar in the paratype and the 12 more recently collected specimens. The only character found to be notably dissimilar in this regard was the internarial distance (Table 1). Our phylogenetic data also provide strong evidence that *G. punctatus* should continue to be regarded as a distinct species. DNA sequences from FMNH 259923, ZSM 2337/2007 (ZCMV 5501) and ZSM 2338/2007 (ZCMV 5502) form a strongly supported monophyletic group, strongly divergent from all other nominal *Pandanusicola* species.

**Diagnosis.** Assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on small body size, phytotelm-breeding habitats (in *Pandanus* plants), moderate webbing

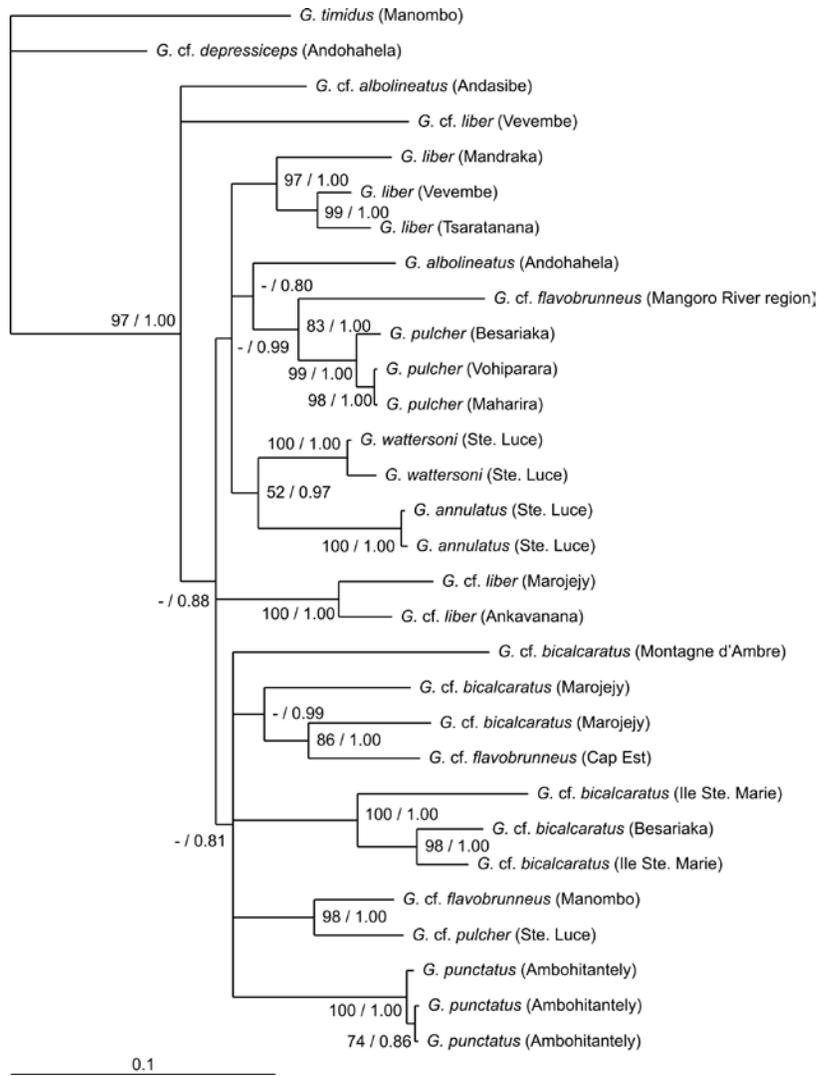
between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, type 2 femoral glands in males and DNA sequence similarities (Glaw & Vences, 2006). Within *Pandanusicola*, *G. punctatus* is distinguished from *G. albolineatus*, *G. flavobrunneus*, *G. liber*, *G. pulcher* and *G. wattersoni* sp. nov. (see below) by the abundant and conspicuous moderate-sized dark spots on an olive-coloured dorsum. By comparison, *G. pulcher* is a characteristic bright green in colour with very large irregular dark dorsal spots, *G. wattersoni* sp. nov. is yellow dorsally with usually relatively few spots (see below) and *G. albolineatus* has conspicuous white dorso-lateral lines. *G. flavobrunneus* is much larger (up to 38 mm SVL; Glaw & Vences, 2007) and *G. liber* has larger hands and feet and does not breed in *Pandanus* plants. As noted above, *G. punctatus* is distinguished from *G. annulatus* by shorter femur (FL/SVL mean =  $0.46 \pm 0.02$  vs  $0.49 \pm 0.04$ ), tibia (TL/SVL mean =  $0.49 \pm 0.02$  vs  $0.55 \pm 0.03$ ), foot (FOL/SVL mean =  $0.42 \pm 0.03$  vs  $0.48 \pm 0.03$ ) and femoral gland (FGL/SVL mean =  $0.20 \pm 0.03$  vs  $0.27 \pm 0.02$ ), a darker background dorsal colour (in preservative, olive vs cream), a lack of conspicuous light “rings” just proximal



**Fig. 5.** Photographs of *G. wattersoni* in life taken at Sainte Luce coastal rainforest, Madagascar by RML (A–D) and FG (E–F).

to each toe or finger disc, the lack of a continuous gold (in life) rostral stripe medial to black one, the presence of dark heavy bars on the dorsal surface of arms and legs (vs small spots only), probably a single subgular vocal sac in males (paired subgular in *G. annulatus*) and by DNA sequence differences (mean pairwise sequence difference in the 16S rRNA gene >10%).

*Redescription.* Based on ZSM 2338/2007 (ZCMV 5502), an adult male from Ambohitantely, Jardin Botanique. Specimen is in an excellent state of preservation (tissues taken from ventral surface of left thigh for molecular analysis). SVL is 20.9 (for other morphometric measurements, see Table 1). Head slightly longer than wide but wider than body; snout appears rounded in dorsal and



**Fig. 6.** Fifty percent majority rule consensus phylogram from Bayesian analysis of 983 bp of 16S mitochondrial DNA sequences. At each node, a bootstrap value from the parsimony analysis is given followed by the posterior probabilities to indicate the level of support for each node. Collection localities are given after each taxon name.

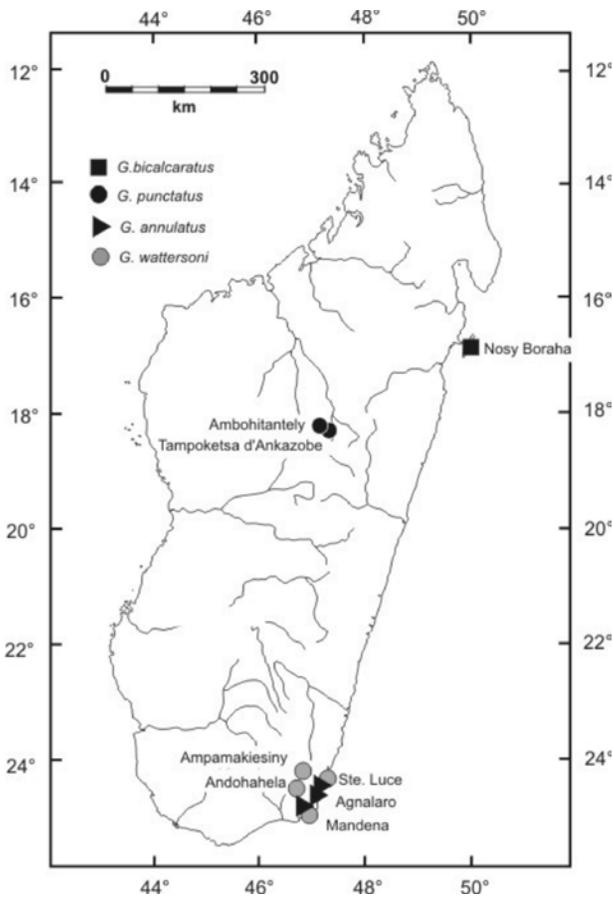
ventral views and somewhat pointed in lateral view; canthus rostralis rounded; nostrils closer to tip of snout than to eye and pointed anterior-laterally, internarial distance 61% of interorbital distance; tympanum distinct, 44% of horizontal eye diameter; a mostly dark and somewhat prominent supratympanic fold present from posterior edge of eye, ending posterior to the tympanum; one small round patch of vomerine odontophores on either side of head medial between eye and choanae; tongue short and stout, attached anteriorly and bifid at tip. Arms thin, lower arm 85% of hand length; relative finger length  $1 < 2 < 4 < 3$ , finger discs moderately enlarged and squared off at tips in a somewhat rounded “T” shape, a trace of webbing between fingers 3 and 4, subarticular tubercles single, round and large. Hindlimbs slender but robust, tibiotarsal articulation reaches to eye when hindlimbs are adpressed

against the body; femur length 94% of tibia length, foot length 81% of tibia length; lateral metatarsalia connected; inner metatarsal tubercle oblong (0.8 in length, 0.4 in width); outer metatarsal tubercle round (0.4 in diameter); webbing formula between toes 1(1) 2i(1) 2e(1) 3i(2) 3e(1.25) 4i(3) 4e(3) 5(1.5); relative length of toes  $1 < 2 < 5 = 3 < 4$ ; toe discs moderately enlarged and rounded, width of fourth toe disc 83% of width of third finger disc. Cloaca not clearly recognizable; skin is uniformly smooth dorsally, ventral skin is finely granular. Femoral glands distinct and oblong with indistinct small round granules; the entire structure is somewhat darker than adjacent areas on ventral surface of thigh.

After three years in preservative, the dorsal background coloration appears as a medium brownish-olive colour with many round or irregularly shaped spots. Spots are

**Table 2.** Morphometric data (in mm) of *G. wattersoni* sp. nov. ( $n=22$ ), of the type series of *G. bicalcaratus* and other specimens from the type locality of this latter species, Nosy Boraha. See text for further discussion of the identity of these specimens. HT = holotype, PT = paratype, PLT = lectotype, LT = lectotype, PLT = paratype, PT = paratype, PLT = lectotype, SD = standard deviation, ? = unknown sex; see Materials and Methods for other abbreviations.

Catalogue number	Status	Sex	SVL	FL	TL	FOL	HL	HW	HAL	LAL	TD	ED	FGL x FGW	W3FD	W4TD	IOD	NSD	END	IND
<i>Guibemantis wattersoni</i>																			
ZSM 0177/2005	HT	F	24.9	11.7	13.0	11.6	8.0	8.0	6.2	6.0	1.4	3.4	–	0.8	0.6	4.0	1.0	3.5	2.5
ZSM 0176/2005	PT	M	21.8	9.6	11.2	8.7	7.5	7.7	4.6	4.6	1.5	2.5	2.4 × 6.9	0.7	0.5	3.6	0.8	2.8	2.2
ZSM 0178/2005	PT	F	25.0	11.7	12.8	10.7	7.9	9.2	6.0	5.5	1.3	3.2	–	0.9	0.7	4.1	1.5	3.5	2.8
UMMZ 197485	PT	F	27.8	11.6	13.4	10.5	8.7	9.7	6.1	4.7	1.8	3.1	–	0.9	0.5	3.3	1.8	3.2	2.6
UMMZ 197513	PT	F	24.3	11.5	13.0	11.6	8.8	7.6	6.9	4.1	1.3	3.2	–	1.1	1.1	3.4	1.0	2.8	2.5
UMMZ 214504	PT	M	26.3	11.1	12.7	10.7	9.1	8.2	6.9	5.7	1.3	2.7	–	1.2	1.0	3.5	0.9	2.8	2.5
UMMZ 214505	PT	F	30.2	12.3	14.1	12.5	10.2	10.0	8.6	6.3	2.1	3.9	–	1.3	0.9	3.8	1.3	3.3	2.9
UMMZ 214506	PT	F	26.4	11.0	13.1	11.3	9.0	8.8	7.7	6.4	1.6	3.5	–	1.1	0.8	3.1	1.0	3.2	2.3
UMMZ 227001	PT	F	24.8	11.1	12.8	10.6	10.3	8.3	6.3	5.8	1.4	3.1	–	1.0	0.8	3.4	0.6	3.3	2.3
UMMZ 227002	PT	F	22.8	11.8	13.0	10.2	9.6	8.5	5.6	5.1	1.5	3.1	–	0.9	0.7	3.8	0.7	2.8	2.4
UMMZ 232152	–	F	26.5	11.8	13.3	11.1	10.0	9.1	7.0	5.5	1.5	3.3	–	0.9	0.7	3.6	0.7	3.1	1.9
UMMZ 232160	–	F	25.4	11.9	13.2	11.3	9.6	8.3	7.0	6.0	1.7	3.2	–	0.8	0.9	3.7	0.8	3.4	2.0
UMMZ 232149	–	F	26.2	11.0	13.1	10.4	10.5	8.0	6.4	5.6	1.6	3.0	–	1.2	0.8	3.3	0.5	3.0	2.4
UMMZ 232168	–	F	25.6	13.0	13.7	11.7	10.2	8.9	7.0	5.5	2.2	3.2	–	1.2	0.8	3.5	1.2	3.6	2.3
UMMZ 232166	–	F	22.9	9.4	12.0	10.8	9.1	7.4	6.3	4.6	1.4	3.0	–	1.2	0.7	3.2	0.9	3.3	2.2
UMMZ 232155	–	F	26.7	12.1	13.4	10.7	9.5	8.3	6.9	5.7	1.5	3.0	–	1.2	0.6	3.8	0.8	3.2	2.5
UMMZ 197482	–	F	22.5	10.5	11.6	10.0	8.4	7.2	6.3	4.0	1.4	2.9	–	0.9	0.8	2.7	1.1	2.7	2.4
UMMZ 191337	–	F	27.1	11.2	12.9	10.7	9.4	9.0	6.5	5.0	1.8	3.7	–	0.9	0.9	3.3	1.0	3.3	2.8
UMMZ 191335	–	F	25.8	10.6	13.0	10.2	8.7	8.1	5.9	4.9	1.7	3.4	–	1.0	0.7	3.1	1.0	2.9	2.2
UMMZ 191333	–	M	22.4	10.0	11.5	10.7	8.3	7.6	5.4	4.5	1.4	3.2	1.7 × 5.6	0.9	0.7	2.9	0.8	2.6	2.1
UMMZ 197503	–	F	21.6	10.4	11.4	7.9	7.5	7.5	6.1	4.1	1.2	3.5	–	0.9	0.9	3.1	1.0	2.6	2.2
UMMZ 197493	–	F	25.6	11.4	13.0	11.0	8.9	9.0	6.9	5.4	1.5	3.4	–	0.9	0.9	3.6	0.8	2.5	2.3
Mean±(SD)			25.1 (2.1)	11.2 (0.9)	12.8 (0.8)	10.7 (1.0)	9.1 (0.9)	8.3 (0.7)	6.5 (0.8)	5.2 (0.7)	1.6 (0.2)	3.2 (0.3)	1.9 (0.4)/ 6.1 (0.7)	1.0 (0.2)	0.8 (0.2)	3.4 (0.3)	0.9 (0.3)	3.0 (0.3)	2.4 (0.2)
<i>Guibemantis bicalcaratus</i>																			
SMF 6811	LT	F	24.3	11.2	12.9	11.2	8.8	9.4	7.7	5.5	1.4	3.4	–	1.4	0.9	3.0	1.5	3.1	2.3
SMF 6812	PLT	F	25.2	11	12.9	10.4	8.5	9.2	6.7	5.2	1.4	4.1	–	1.2	0.9	2.8	0.9	3.0	2.6
SMF 6813	PLT	F	23.1	10.5	12.7	10.1	8.7	7.6	5.2	4.7	1.2	3.3	–	1.1	0.8	3.5	1.4	3.0	2.3
BMNH 1953.1.2.93	–	?	23.6	–	12.7	10.6	6.3	8.3	6.4	4.1	1.3	3.7	–	1.1	0.8	3.7	–	2.9	–
ZSM 0429/2006 (ZCMV 3244)	–	M	21.2	9.2	10.9	8.8	8.3	7.3	4.7	4.2	1.2	2.6	1.9 × 5.2	0.9	0.6	2.8	0.8	2.8	2.7
ZSM 0427/2006 (ZCMV 3242)	–	F	23.8	11.0	11.9	10.6	9.7	8.1	6.3	4.6	1.4	2.7	–	1.1	0.8	4.1	1.4	2.9	2.6
ZSM 0425/2006 (ZCMV 3240)	–	F	19.0	9.0	10.3	8.9	7.7	6.5	4.8	4.1	1.2	2.8	–	1.0	0.7	3.5	1.0	3.2	2.2
NMW 3529	–	F	25.0	12.2	13.0	11.0	8.6	9.0	7.4	5.5	1.5	4.3	–	1.3	0.8	3.6	1.7	2.7	3.3
MNH 1929.209	–	?	21.7	10.3	10.8	8.5	8.5	7.6	5.5	4.4	1.5	3.2	–	0.8	0.6	3.3	1.2	3.1	2.2
Mean (SD)			23.0 (2.0)	10.6 (1.1)	12.0 (1.1)	10.0 (1.0)	8.3 (0.9)	8.1 (1.0)	6.1 (1.1)	4.7 (0.6)	1.3 (0.1)	3.3 (0.6)	–	1.1 (0.2)	0.8 (0.1)	3.4 (0.4)	1.2 (0.3)	3.0 (0.2)	2.5 (0.4)



**Fig. 7.** Map showing reliably known distribution records of *Guibemantis annulatus* and *G. wattersoni* and the two morphologically similar species *G. punctatus* and *G. bicalcaratus*. Many additional records assigned to *G. bicalcaratus* (e.g. Blommers-Schlösser & Blanc, 1991) belong to other undescribed species or are in need of confirmation. For graphical reasons, some distribution points in the southeast are slightly shifted and in reality are more fully overlapping.

moderately sized (most 0.5–0.7, mean diameter 0.6) and most appear black (but a few are lighter than the dorsal background colour and appear white). A relatively thick and conspicuous rostral stripe is black in colour. This rostral stripe is bordered medially by a conspicuously lighter parallel line but it is ragged and discontinuous. Dorsal colour of arms and legs is the same as on the dorsum, with dark heavy bars on both sets of limbs. Hands and feet are of similar colour to the rest of the body (dorsally and ventrally) and lack any conspicuous “rings” on digits just proximal to the finger and toe discs. Venter light cream-coloured with small to medium black spots along jawline, throat, chest and a few on the belly. The pupil appears black and the iris is white. Unlike some other species of

*Pandanusicola*, there does not appear to be any distinct coloration associated with vocal sacs in males, at least based on the material currently available.

**Coloration in life.** Based on photos from Ambohitantely (Fig. 4A,B), the dorsal background colour on the dorsum is a dull greenish-olive but on the limbs the background coloration appears light grey. On this background, the irregularly-shaped spots appear dark brown. Several small reddish areas are also evident on the hindlimbs and feet of this individual. In life, the pupil appears black and the iris is a metallic gold colour. The specimen shown in Fig. 4 is generally similar in colour and pattern to the specimen (ZMA 7170) shown in Blommers-Schlösser & Blanc (1993: Plate 17), except for its lighter background coloration.

**Variation.** The size and shape of dorsal spots was variable (see Table 1) but they were always larger and less regularly shaped than in *G. annulatus*. Some individuals we examined possessed a broken light rostral stripe, medial to the darker one (as in ZSM 2338/2007), but many specimens lacked this feature (it is always present and continuous in *G. annulatus*). The rostral stripe that begins at the snout tip extends just behind the tympanum in some individuals and in others it extends somewhat further, in some cases to mid-body. The fine spots on the venter, while generally sparse, vary from nearly absent to common on the lower jaw, throat, arm insertions, belly and/or chest. The supratympanic fold is consistently dark in most specimens and is generally prominently raised above the surface of the skin. However, in a few specimens the supratympanic fold was relatively weak. The webbing on the foot was variable for some toes (range, where variable, given in parentheses): 1(1) 2i(1) 2e(1) 3i(2) 3e(1–2) 4i(2.75–3) 4e(2.25–3) 5(1–1.5).

**Distribution.** So far only known from the Tampoketsa d’Ankazobe massif on which Ambohitantely Special Reserve is located. This site harbours at least two potentially endemic frog species (*Anodonthyla vallani* and *Stumpffia helenae*) and it is possible that *G. punctatus* is a local endemic as well.

**Natural history.** Only known from the leaf axils of *Pandanus* plants. The advertisement call is not known, but the tadpoles were described by Blommers-Schlösser (1979). The abdominal cavity of FMNH 260282 contained approximately ten eggs with a diameter of 1.8 mm.

#### Description of *Guibemantis wattersoni* sp. nov. (Figs 1 & 5)

**Holotype.** ZSM 177/2005 (FGZC 2596; Fig. 1) an adult female from Sainte Luce littoral rainforest (24°46.79’S; 47°10.28’E, 23 m a.s.l.), southeast Madagascar, collected by Frank Glaw and Parfait Bora on 4 February 2005.

**Paratypes.** Nine, all from southeast Madagascar. ZSM 178/2005 (FGZC 2616; adult female), same data as holotype; ZSM 176/2005 (FGZC 2588; adult male), from Sainte Luce rainforest (24°46.50’S; 47°09.05’E, 22 m a.s.l.), collected by Frank Glaw and Parfait Bora on

4 February 2005; UMMZ 197485 (RAN 34380; adult female) collected from Sainte Luce by Ronald A. Nussbaum, Christopher J. Raxworthy, Achille Raselimanana and Jean-Baptiste Ramanamanjato on 7 October 1989; UMMZ 197513 (RAN 36469; adult female) from Ampamakiesiny Pass collected by Christopher J. Raxworthy, Achille Raselimanana and Jean-Baptiste Ramanamanjato on 27 December 1990; UMMZ 214504 (RAN 52389; adult male) and UMMZ 214505-214506 (RAN 52390 and RAN 52442; adult females) from parcel 1 of the Reserve Naturelle Intégrale d'Andohahela, 12.5 km north-west of Eminiminy (24°35.6'S; 46°44.3'E, between 820 and 840 m elevation) collected by Achille Raselimanana and Jean-Baptiste Ramanamanjato on 30 October and 1 November 1995; UMMZ 227001-227002 (UMFS 10831 and UMFS 10878; adult females) from Mandena littoral rainforest fragment "M3" (24°57.095'S; 47°02.082'E) collected by Richard M. Lehtinen on 6 and 14 June 1999, respectively.

*Etymology.* The specific name is a patronym and is named in honour of William "Bill" Watterson II and his incomparable cartoon *Calvin and Hobbes*.

*Diagnosis.* Assigned to the subgenus *Pandanusicola* of the genus *Guibemantis* based on small body size, diurnal, phytotelm-breeding habitats (in *Pandanus* plants), moderate webbing between toes, connected lateral metatarsalia, the presence of both inner and outer metatarsal tubercles, type 2 femoral glands in males and DNA sequence similarities (Glaw & Vences, 2006). Within *Pandanusicola*, the new species is distinguished from *G. albolineatus*, *G. bicalcaratus*, *G. flavobrunneus*, *G. liber* and *G. pulcher* by the presence of conspicuous light "rings" just proximal to each toe or finger disc, probably by completely separated paired subgular vocal sacs (rather than single subgular as described by Blommers-Schlösser & Blanc, 1991), by laying unpigmented eggs (not known from all other species) and by large genetic differences. The new species differs from *G. annulatus* by the absence of regular, round dark spots on the dorsum (see above). It is further distinguished from *G. bicalcaratus* by the absence of regular spotting on the hindlimbs and probably by large genetic differences (>9% mean pairwise sequence difference in the 16S rRNA gene between *G. bicalcaratus* from its type locality and *G. wattersoni* from Sainte Luce; see Fig. 6 and the account on the identity of *G. bicalcaratus* below). A short 16S sequence from the holotype (GenBank accession number HQ456655) was identical to UMMZ 197485 and differed only at one position when compared to ZSM 176/2005 (both from Sainte Luce, see "Phylogenetic relationships" below).

*Description of the holotype.* Adult female. Head width equal to head length but wider than body; snout somewhat pointed in dorsal, ventral and lateral views; canthus rostralis rounded; nostrils much nearer to tip of snout than to eye and pointed forward, internarial distance 63% of interorbital distance; tympanum distinct, 41% of horizontal eye diameter; a relatively weak supratympanic fold present from posterior edge of eye around the tympanum,

uniformly dark in colour; one prominent circular patch of vomerine odontophores medial and just posterior to choanae on either side of head; tongue absent (removed for tissue sample). Arms thin, lower arm is 89% of hand length; relative finger length 1<2=4<3, finger discs moderately enlarged and rounded along distal edge, finger webbing is absent, subarticular tubercles single, round and large. Hindlimbs robust, tibiotarsal articulation reaches between eye and snout tip when hindlimbs are adpressed against the body; femur length 90% of tibia length, foot length 89% of tibia length; lateral metatarsalia connected; inner metatarsal tubercle oblong (1.0 in length, 0.5 in width); outer metatarsal tubercle round (0.4 in diameter); webbing formula between toes 1(1) 2i(1) 2e(1) 3i(2) 3e(1) 4i(2.5) 4e(2.5) 5(1); relative length of toes 1<2<5<3<4; toe discs moderately enlarged, width of fourth toe disc 75% of width of third finger disc. Cloaca not clearly recognizable; skin is finely granular dorsally, ventral skin is coarsely granular. For full morphometric measurements see Table 2.

After five years in preservative, the dorsal background coloration appears as a light yellowish brown with some scattered indistinct spots or blotches on the dorsal surface. A relatively thick and conspicuous black rostral line is present beginning at the snout-tip and extending just beyond the tympanum. This rostral line is bordered medially by another conspicuously lighter parallel line. Two indistinct whitish lines extend posteriorly from behind the eye orbits fading away about halfway down the dorsum. The dorsal colour of arms and legs is a uniform light yellowish brown with no conspicuous markings. Hands and feet are of similar colour to the rest of the body (dorsally and ventrally) except for the small but conspicuous light "rings" found on all digits just proximal to the finger and toe discs. Venter is light cream-coloured with a few small dark spots along throat and chest. Belly and adjacent lateral areas are semi-transparent. Femoral glands absent. The pupil appears black and the iris is white.

*Coloration in life.* Based on numerous photographs of individuals from Mandena and Sainte Luce (Fig. 5). There is substantial variation in dorsal coloration patterns but the dorsal background colour is always yellow to yellowish brown. Often there are few or no prominent dorsal markings but sometimes the dark line associated with the supratympanic fold continues down the dorsum as a ragged line to mid-body. In some individuals this dark ragged line on the dorsum is bordered medially with a lighter (nearly white) ragged parallel line. Very small scattered white spots are also found on the dorsum and dorsal surface of the legs of some individuals (this latter pattern is especially common in juveniles and recent metamorphs). In all individuals, the dark rostral line that begins at the snout tip is bordered by a conspicuously lighter metallic gold parallel line. Dorsal background colour of arms is the same as on the dorsum. Often, there are no markings on the arms but indistinct dark bands and/or spots are sometimes present. In some individuals the dark arm bands can be dark and conspicuous. The dorsal surface of the legs is similar to the dorsum in colour, typically with a scattering of dark or light spots but usually without

any prominent bands or spots. Hands and feet are of similar colour to the rest of the body (dorsally and ventrally) except for the conspicuous “rings” found just proximal to the finger and toe discs. In life, these are bright white and in some individuals the finger and/or toe discs appear as more darkly coloured than phalangeal surfaces proximal to the “rings”. The ventral coloration is typically a light cream and is immaculate with the exception of a small to moderate number of dark spots sometimes found along the jawline, throat and/or (rarely) chest. The belly and flanks of many individuals are semi-transparent. The femoral glands of males are usually no different in colour from the background ventral coloration, but on some specimens the femoral glands appear somewhat darker than surrounding areas on the thighs. Sexually mature males also possess white coloration on the ventral surface of the throat near the jaw angles (these are the paired sub-gular vocal sacs). The pupil appears black and the iris is a metallic gold that often has irregular dark jagged lines associated with it.

*Variation.* On the basis of a much larger sample of specimens in a field study at Sainte Luce, Lehtinen (2009) reported sexual size dimorphism in this species, with females being significantly larger, on average (female mean = 23.1±2.3 SD, range 17–28 mm SVL,  $n=324$ ; male mean = 20.7±1.5 SD, range 16–23 mm SVL,  $n=200$ ). As noted above, the dorsal coloration of *G. wattersoni* is much more variable than in *G. annulatus*. Many individuals have few markings dorsally but others have more abundant and conspicuous spots or lines. The length of the line associated with the supratympanic fold is particularly variable. In some specimens, this dorsal line only continues to the arm insertions, but in others it can continue to mid-body and is sometimes associated with a lighter area medial to it. The supratympanic fold is consistently dark in nearly all specimens, but how much the fold is raised from the lateral skin surface varies from being fairly low to very strongly elevated. The tip of the tongue is moderately to very strongly bifid. The degree of spotting and/or banding on the arms is also quite variable. The arms of some specimens lack any markings, while others have fairly prominent dark bands. Ventrally, some individuals are completely immaculate while others possess various amounts of fine dark pigment along the jawline, arm insertions, throat, chest and/or belly. Also ventral skin texture varies from coarsely granular to very coarsely granular. No individuals were observed to have webbing on the hand. However, webbing on the foot is variable for some toes (range, where variable, given in parentheses): 1(1) 2i(1) 2e(0.5–1) 3i(2) 3e(1–1.5) 4i(2.5–3) 4e(2.5–3) 5(1–1.25). The background dorsal coloration of the specimens from Réserve Naturelle Intégrale d’Andohahela (UMMZ 214504–214506) is notably darker than in most other specimens and these individuals were also relatively larger (Table 2). However, these specimens agree well with the other type specimens in all other features. These differences may represent geographic and/or elevational variation.

In males, femoral glands are typically oblong or oval with granules present inside the gland. However, several

males attributable to this species lack obvious granules in the femoral glands. Some individuals that are clearly male based on vocal sac coloration (e.g. UMMZ 214504) lack any clear macroscopic evidence of femoral glands from external view. As noted by Blommers-Schlösser (1979), it is probable that the appearance of the femoral glands differs depending on the time of year and physiological state of the individual. Also, femoral glands may become externally unrecognizable over time in preserved specimens (Glaw & Vences, 1994).

*Distribution.* *Guibemantis wattersoni* can be locally abundant and apparently has a wider geographic distribution than *G. annulatus*. Nonetheless, at the present time this species is only known from five localities in southeastern Madagascar (Fig. 7): Ampamakiesiny Pass, Mandena, Sainte Luce, the Agnalaro Forest near Lake Ambavahihy and lower elevations of parcel 1 of the Réserve Naturelle Intégrale d’Andohahela.

*Advertisement call.* Male vocalizations were recorded at Sainte Luce in February 2002 at temperatures between 21 and 27 °C (Fig. 3). The following call analyses are based on one series of vocalizations emitted by the same individual male, but calls from other specimens were heard and agreed in general structure. To the human ear, the advertisement call of *G. wattersoni* is quite similar to that of *G. annulatus*, resembling a simple, soft “click”. These are given as single notes or in succession of two or three at frequencies between 3240 and 4500 Hz (mean frequency range spanned 345 Hz ±116 SD,  $n=4$ ; Fig. 3). The notes are typically separated into 2–6 pulses (mean pulse length 6 ms ±9 SD,  $n=23$ ) with the total call length averaging 76 ms ±143 SD,  $n=7$ ). Peak intensity of recorded calls was at approximately 3800 Hz. Inter-note intervals averaged 0.9 s ±0.5 SD,  $n=4$ ). On several occasions, another call type from this species was heard at Sainte Luce that consisted of three soft “clicks” similar to those described above, but each of the three was given a notably higher pitch than the preceding “click”. Unfortunately, we were unable to record this call variant. While *G. annulatus* and *G. wattersoni* are sympatric and can even be found calling in the same plants together, their calls can be distinguished on the basis of: 1) a much larger range of frequencies spanned in *G. annulatus* (mean 1907 Hz) than in *G. wattersoni* (mean 345 Hz) and 2) the peak intensity of the vocalization is reached at a higher frequency in *G. annulatus* (approximately 4500 Hz) than in *G. wattersoni* (about 3800 Hz).

*Natural history.* Breeding coincides with the rainy season in southeastern Madagascar (December to March). Males call from *Pandanus* plants only during the day. One reproductive event was witnessed (described in Lehtinen, 2003, under the name *Mantidactylus bicalcaratus*) and, as in other mantelline frogs, no true amplexus occurred. Eggs are laid above water-filled leaf axils in *Pandanus* plants. Freshly deposited eggs were entirely white and measured 1.9 mm in diameter in the field and 1.2–1.4 mm in diameter in preserved gravid female specimens ( $n=5$ ). Clutch size averaged 45 deposited eggs ( $n=15$ ).

Males or females often provide periodic egg attendance until hatchlings are washed into the leaf axils (Lehtinen, 2003). Lehtinen (2004) demonstrated that the elongated tadpoles are primarily detritivorous and estimated that the larval period lasts approximately 2–3 months. At metamorphosis, body size is approximately 9 mm SVL and in males sexual maturity is attained at approximately 16 mm SVL based on the appearance of femoral glands and vocal sac coloration. Mark–recapture data reported in Lehtinen (2009) suggest a maximum total life span of less than one year. Sex ratios at Sainte Luce were highly female-biased (0.371 females for each male; Lehtinen, 2009). Reported predators include an unidentified heteropodid spider and the snake *Ithycyphus ousi* (Lehtinen, 2002).

*Identity and diagnosis of Guibemantis bicalcaratus.* As stated in the section of *G. annulatus*, we have ascertained the identity of all *Pandanusicola* species by examination of their name-bearing type specimens or of their paratypes. Thereby we confirmed that the species *G. albolineatus*, *G. flavobrunneus*, *G. liber*, *G. punctatus* and *G. pulcher* are currently (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 2007) correctly defined. However, the identity of *Guibemantis bicalcaratus* is less clear. The species (*Rhacophorus bicalcaratus* Boettger, 1913) was described on the basis of a series of syntypes (one male and two females) from Sainte Marie island (today Nosy Boraha), an island off Madagascar's east coast (Boettger, 1913). Measurements of the lectotype (SMF 6811) and the two paralectotypes (SMF 6812–6813) are included in Table 2 and the lectotype is shown in Fig. 1. Two additional specimens (BMNH 1953.1.2.93 and NMW 3529) from the London and Vienna museums were probably part of the same series of specimens but cannot be considered as original syntypes (and thus paralectotypes) because the original description gives no indication that these specimens were examined and only mentions three type specimens. All these specimens are comparatively large and characterized by a relatively uniform dorsum with distinct dark spotting which sometimes extends over the dorsum but is usually confined to the flanks and especially the hindlimbs. The lectotype in particular has a largely uniform dorsum, some spots along the flanks and a dense, regular fine spotting on the shanks. According to the original description, the dark rostral stripe can be bordered by a silver-white line, reminiscent of the state in *G. annulatus* and *G. wattersoni*. Very similar and almost certainly conspecific to the type series is a specimen collected and photographed by us in 1991 at the type locality Nosy Boraha and figured on p. 203, Fig. 4 in Glaw & Vences (2007). This specimen had no “rings” proximal to toes and fingers but instead a silver-white colour laterally on the finger disc itself, which we have not seen so far in any other *Pandanusicola* species.

During field work in 2006 we collected a number of *Pandanusicola* on Nosy Boraha that from molecular data (Fig. 6; see below) turned out to belong to at least two distinct species. None of the sequenced specimens (see Table 2 and Fig. 6) agreed fully with the *bicalcaratus* type series, but ZSM 425/2006 shared with those the fine spots on the shanks. Although this specimen lacks double

rostral stripes, we here assign this specimen tentatively to *G. bicalcaratus*. Independently from the identity of *G. bicalcaratus*, the lectotype differs in colour pattern (presence of distinct spots only on flanks and shanks) from that observed in both *G. annulatus* and *G. wattersoni* and we therefore exclude the possibility that any of these could be junior synonyms of *G. bicalcaratus*.

*Other available names.* Three nomina are currently considered to be junior synonyms of species in the subgenus *Pandanusicola* (Blommers-Schlösser & Blanc, 1991; Frost, 2010). Examination of their name-bearing types and original descriptions allowed us to exclude the possibility that they could constitute senior synonyms of *G. annulatus* and *G. wattersoni*, as reported in the following.

*Gephyromantis albogularis* was described by Guibé (1947) from “Madagascar” without precise locality information, based on one male holotype and one male paratype, collected by F. Sikora. The name has been considered as a junior synonym of *Guibemantis liber* (e.g. Blommers-Schlösser & Blanc, 1991). Upon examination in May 2010, the holotype and paratype were in extremely poor state of preservation, uniformly brown with no trace of their original colour or pattern and strongly emaciated. The holotype has SVL 23.8 mm and HAL 7.9 mm and therefore a HAL/SVL ratio of 0.33 (congruent with *G. liber* but not with the *Pandanusicola*-breeding species of *Guibemantis*). Two other arguments confirm the synonymy of *albogularis* with *liber*: 1) F. Sikora collected several specimens – e.g. the type of *Uroplatus sikorae* and *Rhacophorus sikorae* (synonym of *Spinomantis aglavei*) – from mid-elevations in the region around Moramanga and Andrangoloaka where *G. liber* is very common; 2) an immaculate white throat clearly distinct from a brownish belly was reported in the original description, which is diagnostic for *G. liber* among small-sized *Guibemantis*.

*Gephyromantis methueni* was described by Angel (1929) based on three syntypes, one from “Ambila (lagune), province de Tamatave”, the other two specimens from “Lac Alaotra”. The specimen MNHN 1929.209 from Ambila was later designated as lectotype (by implication; see Frost, 2010). This specimen (measurements in Table 2) has a HAL/SVL ratio of 0.25, thus agreeing with species of the subgenus *Pandanusicola* other than *G. liber*. Upon examination in May 2010, the specimen was uniformly brown without any trace of its original colour or pattern. The original description gives only a very general description of the pattern: brown, with light broad dorsolateral bands and irregular dark spots or markings; a dark line from nostril to eye continued above tympanum. However, because the description refers to the three syntypes, it is uncertain which of the mentioned characters actually refer to the name-bearing lectotype from Ambila. Nevertheless, the original description does not mention a dense spotting covering most of the body (as in *G. annulatus*) or a light stripe running above the nostril-eye stripe (as in both *G. annulatus* and *G. wattersoni*) and we therefore exclude the name *methueni* to be applicable to any of these species. For the time being, we suggest keeping this name in the synonymy of *G. bicalcaratus*.

*Gephyromantis variabilis* has been considered as a synonym of *G. liber* by Blommers-Schlösser & Blanc (1991) and Frost (2010). It was described by Millot & Guibé (1951) based on numerous specimens from different localities, including Perinet (=Andasibe) and Itremo. The original description does not list the type specimens and from the catalogue of the Paris museum it is also not fully clear which specimens should be considered as types. Blommers-Schlösser (1979) listed one specimen, MNHN 1953.116, as holotype of *G. variabilis* and this has been interpreted as a lectotype designation by implication (Frost, 2010). This lectotype is a juvenile frog of 15.1 mm SVL from Andasibe, in a good state of preservation but with completely faded colour and pattern. Its HAL/SVL ratio is 0.27 and thus in the range of *Pandanus*-breeding *Guibemantis* and outside the range of *G. liber*. Its collecting locality is at about 900 m above sea level, in the northern central-east of Madagascar, and is a well surveyed herpetological collection site.

The identity of *variabilis* remains uncertain, mainly because the juvenile lectotype with faded coloration does not allow for an unambiguous assignment. In Andasibe, other than *G. liber* (which can be ruled out as senior synonym of *variabilis* because of its longer hands), three species of *Pandanus* occur in Andasibe: *G. flavobrunneus*, *G. pulcher* and an undescribed candidate species, named *G. sp. aff. albolineatus* "Andasibe" by Glaw & Vences (2007) and *G. sp. 3* by Vieites et al. (2009). Of these, the candidate species is the most abundant at this locality and its typical colour pattern has been included in the description of *G. variabilis* as one of many colour variants supposedly occurring in this species. We do not propose here a nomenclatural change for *variabilis*, which will be the subject of a forthcoming paper. In fact, it will require more extensive study to understand whether the lectotype designation for this nomen (by implication) was valid and which specimens in fact belong to the original series of syntypes. In contrast to the lectotype as currently understood, all other specimens we have examined in the Paris museum which might have been part of the original syntype series, and some of which originate from localities other than Andasibe, clearly belong to *G. liber*. However, independent from this conundrum, we conclude that the name *variabilis* is not applicable as senior synonym to *G. annulatus* or *G. wattersoni* because 1) based on intensive surveys, we are confident in stating that no frogs morphologically resembling these two species occur in the wider region around Andasibe (Glaw & Vences, 2007; see Vieites et al., 2009 for a species list) and that the name *variabilis* is thus not applicable as senior synonym to these species, and 2) the colour variants described for *G. variabilis* in the original description do not at all match *G. annulatus* (no variant with regularly spotted dorsum) and also show some differences to *G. wattersoni* (no variant with largely uniform hindlimbs without crossbands or other dark pattern).

**Phylogenetic relationships.** Our 16S mtDNA tree is based on 983 base pairs of the 16S rRNA gene; 629 of these were constant and 229 were parsimony-informative. Some deeper nodes were not well resolved, but most relation-

ships among terminals were strongly supported in both Bayesian and parsimony analyses. Specimens attributed to *G. bicalcaratus* from the type locality (Ile Sainte Marie) were grouped with a mainland specimen from Besariaka with high support (100% bootstrap value, 1.00 posterior probability). As previously indicated, these specimens belong to at least two different species, which are apparently closely related. Nonetheless, *G. wattersoni* specimens from Sainte Luce (including the holotype) were not closely related to any *bicalcaratus*-like specimens from its type locality (Fig. 6). The mean uncorrected pairwise sequence difference (p-distance) between *G. bicalcaratus* from its type locality and *G. wattersoni* from Sainte Luce was 9.6%. Pairwise differences between the two Sainte Luce sequences from *G. wattersoni* was 0.09%.

Similarly, specimens attributed to *G. punctatus* from the type locality region (Ambohitantely) formed a well supported group (bootstrap support 100%, 1.00 posterior probability) but they were not closely related to *G. annulatus* from Sainte Luce (Fig. 6). The mean uncorrected pairwise sequence difference between *G. punctatus* from the type locality and *G. annulatus* from Sainte Luce was 10.7%. Pairwise differences between the two Sainte Luce sequences from *G. annulatus* was 0.1%. While bootstrap support for the node was low, *G. annulatus* and *G. wattersoni* appear to be each other's closest relative.

## DISCUSSION

Many species in Madagascar appear to be restricted to very small geographic ranges (Vences et al., 2009). Based on the information currently available, it appears that the two new frog species described here also have small geographic ranges and are endemic to the rainforests of the southeastern part of the island. *Guibemantis annulatus* is known from only three small, isolated and highly endangered coastal forest remnants. These forest fragments are subject to fires and uncontrolled timber harvesting for charcoal production and building materials. *Pandanus* leaves are also harvested for roofing of huts and mining operations are now active in this area. Surveys of other forests in southeastern Madagascar (Réserve Naturelle Intégrale d'Andohahela, Ampamakiesiny Pass, Malahelo Forest, Petriky; Andreone & Randriamahazo, 1997; Nussbaum et al., 1999; Ramanamanjato et al., 2002) have not found this species. Based on the extremely limited known distribution and the lack of any formal protection for these areas, (following the IUCN Red List criteria; IUCN, 2001) we suggest a conservation status of Endangered (G2) for this species.

*Guibemantis wattersoni* is currently known from five sites in southeastern Madagascar, including the Réserve Naturelle Intégrale d'Andohahela, which has formal protection. The other four sites are unprotected. This species is not known from the Malahelo transitional forest or from the littoral rainforest site at Petriky (Nussbaum et al., 1999; Ramanamanjato et al., 2002). Based on the limited known distribution and the lack of formal protection for most of these areas (following the IUCN Red List criteria; IUCN, 2001), we suggest a conservation status of Vulnerable (G3) for this species.

For more information on the effects of forest loss and fragmentation on these frogs see Ramanamanjato (2000), Lehtinen et al. (2003) and Lehtinen & Ramanamanjato (2006). Note also that *G. punctatus* and *G. bicalcaratus sensu lato* probably have a more restricted distribution than previously appreciated and their IUCN Red List status (currently Data Deficient and Least Concern, respectively) may need to be re-assessed.

Blommers-Schlösser (1979) and Glaw & Vences (1994) presented sonagrams from the locality of Fenoarivo attributed to *G.* (= *Mantidactylus*) *bicalcaratus* that show a much more distinctly pulsed vocalization and one given at a somewhat higher frequency than *G. wattersoni*. A further call from Vences et al. (2006) is listed as *G. sp. aff. bicalcaratus* and was recorded from Besariaka (south of Moramanga). This call more closely resembles that of *G. wattersoni* and *G. annulatus*. The fact that at least three species of *Guibemantis* have quite similar, poorly structured and rather irregular advertisement calls indicates that bioacoustic evidence (which so far is lacking for most *Pandanus*-breeding *Guibemantis*) might not be a very helpful taxonomic character for diagnosing species in the subgenus *Pandanusicola*. The call differences of *G. annulatus* and *G. wattersoni* are also very small compared to other syntopic sister species in Madagascar.

Based on the molecular data presented here and in Lehtinen et al. (2007) and based on other specimens already examined, it is clear that additional species from this group remain undiagnosed. Detailed morphological, acoustic and molecular studies are now needed to further elucidate the diversity in this group. We suspect that the true diversity of this group may be substantial and that plant-breeding may represent a key innovation facilitating the diversification of these frogs. It is relevant that, according to our analysis, the two newly described species are microendemic to a very small area of south-eastern Madagascar, occur in close sympatry across much of their range and are sister species in the molecular phylogeny. Similarly, some other sympatric sister taxa are also revealed by our phylogeny: the two lineages from Ile Ste Marie (of which one might correspond to *G. bicalcaratus*) are sister to each other and two deep lineages from the Marojejy massif also occur in one clade. The identity and possible species status of these lineages remains to be assessed. But these preliminary data might indicate that purely vicariant scenarios of speciation might not fully account for the island's amphibian species richness and that mechanisms of speciation in sympatry, or under parapatric conditions along ecotones (which are particularly sharp in the south-east; Vences et al., 2009), need to be evaluated.

#### Additional specimens

*Guibemantis annulatus* – from Sainte Luce littoral rainforest (24°46.50'S; 47°09.05'E); UADBA uncatalogued (FGZC 2583; adult male).

*Guibemantis wattersoni* – from Sainte Luce rainforest (24°46.50'S; 47°09.05'E, 22 m above sea level); UADBA uncatalogued (FGZC 2584, 2585, 2587 (males) and FGZC 2580 (female)).

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