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A NEW SPECIES OF *MANTIDACTYLUS* FROM THE EAST COAST OF MADAGASCAR AND ITS MOLECULAR PHYLOGENETIC RELATIONSHIPS WITHIN THE SUBGENUS *GUIBEMANTIS*

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We describe a new species of arboreal frog of the genus *Mantidactylus* from low altitude sites on the eastern coast of Madagascar. *Mantidactylus timidus* sp. n. has hitherto been considered as *Mantidactylus tornieri*, but some of the characters that distinguish it from typical populations of this species from mid-altitude localities had already been detected. The new species mainly differs from *M. tornieri* and all related species in the subgenus *Guibemantis* by a shorter relative hand length. Furthermore, it has shorter hind limbs, a smaller tympanum diameter and – at least in some populations – green egg pigmentation (as opposed to brown or white). Vocalizations of *M. timidus* were irregular and unstructured blasts and moans but the available recordings may not represent the real advertisement calls. A molecular phylogeny based on analysis of 539 nucleotides of the mitochondrial 16S rRNA gene placed the new species sister to a clade containing *M. tornieri, M. depressiceps* and *M. kathrinae*. Genetic differentiation from these related species was large, with uncorrected pairwise divergences of more than 8% in all cases. We discuss the recently increasing use of mitochondrial genetic markers to draw taxonomic conclusions and suggest that mitochondrial differentiation should not be used as an exclusive character to describe new amphibian taxa. Instead, phylogenetic placement of populations and morphological, ecological and behavioural arguments need to be carefully evaluated in each case to understand whether a population merits the status of a separate species.

Key words: Amphibia, Anura, Mantellidae, *Mantidactylus timidus* sp. n., *Mantidactylus tornieri*, cryptic species

INTRODUCTION

Frogs of the genus *Mantidactylus* belong to the family Mantellidae and are endemic to Madagascar and the Comoro island of Mayotte (Blommers-Schlösser & Blanc, 1991; Vences & Glaw, 2001). *Mantidactylus* is paraphyletic because the genus *Mantella* is nested within one *Mantidactylus* lineage (Richards *et al.*, 2000; Vences *et al.,* 2003). The large species diversity of currently about 85 scientifically named species is reflected by the current division of *Mantidactylus* into 12 subgenera (Dubois, 1992; Glaw & Vences, 1994). One of these, *Guibemantis,* had previously been considered as the *Mantidactylus depressiceps* group (Blommers-Schlösser, 1979; Blommers-Schlösser & Blanc, 1991). It has recently been reviewed by Glaw *et al.* (2000) who described a new species of these medium-sized, largely arboreal frogs. According to this account, the following species are currently assigned to *Guibemantis: Mantidactylus depressiceps, M. kathrinae, M. liber* and *M. tornieri.* Males of these species are characterized by a largely undifferentiated state of their femoral glands (Glaw *et al.,* 2000). These glands are typical for males of *Mantidactylus* and in some subgenera are also present in females (Blommers-Schlösser & Blanc, 1991). *Guibemantis* mainly reproduce in stagnant water. Males deposit their eggs on leaves or stones above ponds, the hatching tadpoles drop into the water and complete development in the ponds. Two species of *Guibemantis* (*Mantidactylus depressiceps* and *M. kathrinae*) are unique in having a white, non-transparent colour of the jelly of their clutches (Blommers-Schlösser, 1979; Glaw *et al.,* 2000). One species initially considered to belong to this group (Blommers-Schlösser & Blanc, 1991), *Mantidactylus elegans*, has been hypothesized to be possibly related to *Mantidactylus brunae* and *M. peraccae* instead, within the subgenus *Spinomantis* (Andreone *et al*., 1998), but a thorough test of this hypothesis is so far lacking.

In this paper we report on our discovery that populations assigned to *Mantidactylus* (*Guibemantis*) *tornieri* are in fact a complex of at least two species, one of which is described herein. We furthermore present a molecular phylogeny that includes most species belonging to *Guibemantis* or previously believed to be closely related to this subgenus, in order to assess monophyly and relationships among these frogs.

MATERIALS AND METHODS

In this study we focus on the medium- to large-sized species of *Guibemantis* and do not consider the small-sized and easily recognized *Mantidactylus liber.* Hence, morphological and bioacoustic comparisons refer to

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specimens and call data of *M. depressiceps, M. kathrinae* and *M. tornieri.* To assess the molecular phylogenetic relationships of these species, we further studied DNA sequences of other species that currently or in the past were considered to be related to them: *Mantidactylus liber*, three representatives of the subgenus *Blommersia* (*M. wittei, M. domerguei, M. blommersae*), *M. elegans*, and two species of the subgenus *Spinomantis* (*M.* aff. *peraccae* and *M. aglavei*) to which *M. elegans* has been hypothesized to belong (Andreone *et al.*, 1998). *Boophis tephraeomystax* was used as the outgroup.

Specimens were captured by locating calling males during the night. They were euthanised using chlorobutanol solution, fixed either in 5% formalin or 95% ethanol, preserved in 70% ethanol, and included in the herpetological collections of the Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK) and Zoölogisch Museum Amsterdam (ZMA). Further museum acronyms used herein are BMNH (The Natural History Museum, London), and Zoologisches Museum der Universität Berlin (ZMB).

The following morphometric measurements were taken by the senior author to the nearest tenth of a millimetre using a calliper: snout-vent length, SVL; maximum head width (HW); head length from tip of snout to posterior edge of snout opening (HL); horizontal tympanum diameter (TD); horizontal eye diameter (ED); distance between anterior edge of eye and nostril (END); distance between nostril and tip of snout (NSD); distance between both nostrils (NND); forelimb length, from limb insertion to tip of longest finger (FORL); hand length, to the tip of the longest finger (HAL); hind limb length, from the cloaca to the tip of the longest toe (HIL); foot length (FOL). The webbing formula is given according to Blommers-Schlösser (1979).

Muscle tissue samples were taken from freshly killed specimens in the field and preserved in 98% ethanol. DNA was extracted using different standard protocols and a fragment of the mitochondrial 16S rRNA gene amplified using the primers 16Sa-L and 16Sb-H of Palumbi *et al.* (1991). After purification with Qiaquick kits (Qiagen), the fragments were resolved on automated DNA sequencers (ABI 377 and ABI 3100). Sequences were validated and aligned with the software Sequence Navigator (Applied Biosystems), and deposited in Genbank (accession numbers of newly obtained sequences: AY684185-AY684191). The alignment required inclusion of gaps to account for indels in only three cases; one of these was a 1-3-gap interval in a hypervariable region, the others were single gaps and could unambiguously be aligned. We therefore included the complete dataset, after exclusion of gaps, in the phylogenetic analysis after assessing that exploratory analyses excluding ca. 70 b.p of hypervariable regions resulted in identical topologies.

Phylogenetic analysis was carried out using PAUP, version 4b10 (Swofford, 2002). We performed unweighted maximum parsimony heuristic searches, with tree-bisection reconnection branch swapping, and random sequence addition with 100 replicates. Furthermore, a maximum likelihood tree was constructed after determining the substitution model that best fits our data through hierarchical likelihood ratio tests as implemented in Modeltest (Posada & Crandall, 1998). Robustness of nodes was tested by full heuristic bootstrapping, with 2000 replicates (and 10 random addition sequence replicates) under maximum parsimony and 500 replicates under maximum likelihood.

RESULTS

VARIATION IN *MANTIDACTYLUS TORNIERI*

Blommers-Schlösser (1979) was the first to recognize, after thorough observations in the field, that the taxon previously considered as *Mantidactylus depressiceps* was heterogeneous. She revalidated *Mantidactylus tornieri* (Ahl, 1928) for a species with transparent jelly around the clutches, occurring sympatrically with *M. depressiceps*, with white clutches. The name *M. tornieri* was applied both to specimens from near sea level at the east coast of Madagascar (Foulpointe) and from the mid-altitude locality Andasibe, located at ca. 900 m above sea level. However, Blommers-Schlösser (1979) already noted distinct morphological differences between specimens from these two localities: the disk of the third finger was found to be larger than the tympanum in specimens from Andasibe and equal to the tympanum in specimens from Foulpointe; furthermore, the latter had comparatively shorter hind limbs and smaller eyes. Blommers-Schlösser (1979) concluded: "It is possible, that the specimens of Foulpointe represent a subspecies, but the material is not sufficient to decide this for the moment".

Glaw & Vences (1994) noted that clutches of *M. tornieri* from Andasibe contained brownish eggs, while eggs in clutches from an east coast locality (Nosy Boraha) were greenish. In addition, calls recorded from Nosy Boraha were unstructured single blasts whereas calls from Andasibe were always series of several short notes.

During recent fieldwork in 2003, at a locality north of Toamasina at the east coast, we again collected *M. tornieri*-like specimens and again only heard short unstructured calls. This prompted us to analyse the morphological differentiation between coastal and mid-altitude populations currently attributed to *M. tornieri.*

Original measurements of specimens assignable to *M. tornieri* sensu lato from the localities Andasibe, Ranomafana, Voloina, Foulpointe and Toamasina, as well as comparative data of all relevant type specimens and of the morphologically similar species *M. kathrinae* and *M. depressiceps*, are shown in Table 1. Comparing the east coast *M. tornieri*-like specimens from Foulpointe, Voloina and Toamasina to those of mid-altitude localities (Andasibe and Ranomafana) reveals a distinct morphological differentiation. East coast specimens have much smaller hands, which is reflected by the

TABLE 1. Morphometric measurements of specimens of *Mantidactylus timidus*, *M. tornieri*, *M. depressiceps* and *M. kathrinae* (all in mm). See Materials and Methods for abbreviations; additional abbreviations used: HT, holotype; PT, paratype; LT, lectotype; PLT, paralectotype. Asterisks mark types of two junior synonyms of *Mantidactylus depressiceps*: * holotype of *Mantidactylus acuticeps* Ahl, 1929; ** holotype of *Rhacophorus mocquardii* Boulenger, 1896.

Collection no.	Locality	Sex	Status	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOL
M. timidus sp. n.															
ZMA 19466	Toamasina	M	HT	35.8	12.3	13.5	2.2	4.0	3.4	1.9	3.3	21.1	10.4	54.0	16.7
ZMA 19492	Toamasina	M	PT	33.7	11.3	12.6	2.0	4.0	3.3	1.8	3.1	20.6	9.3	50.4	15.7
ZMA 7109 (814)	Foulpointe	M	PT	42.1	14.1	16.1	2.7	4.9	4.4	2.2	4.0	23.4	11.4	62.7	19.1
ZMA 7110 (706)	Foulpointe	M	PT	44.7	14.7	16.1	2.3	4.5	4.2	2.0	3.8	26.7	12.5	67.7	19.4
ZMA 7110 (707)	Foulpointe	M	PT	42.8	13.1	15.5	2.6	4.9	4.2	2.2	4.1	24.8	11.3	58.9	18.7
ZMA 7110 (708)	Foulpointe	F	PT	44.1	13.1	16.7	2.6	5.3	4.6	2.1	4.0	25.0	12.2	62.9	19.5
ZMA 7110 (558)	Foulpointe	M	PT	41.9	14.0	15.4	2.5	4.9	4.1	2.9	4.0	24.5	12.3	61.1	19.5
ZMA 7112 (686)	Foulpointe	M	PT	43.7	14.0	15.8	2.6	4.3	4.5	2.0	4.0	28.1	13.2	63.0	20.0
ZMA 7112 (475)	Foulpointe	M	PT	39.9	13.5	15.6	2.4	4.9	-	-	-	25.2	11.5	60.5	18.3
ZFMK 52698	Voloina	M	PT	55.4	19.8	21.0	3.2	6.4	5.7	3.1	4.9	36.2	16.7	86.4	26.9
M. tornieri															
ZMB 30533	Ankoraka	?	HT	47.9	16.7	18.6	2.5	5.3	5.2	3.3	4.9	30.2	16.0	75.9	23.4
ZFMK 52700	Andasibe	M	-	45.0	15.3	16.0	2.4	4.9	4.3	2.7	3.9	30.0	14.8	74.4	22.8
ZFMK 52699	Andasibe	M	-	42.0	15.0	16.0	2.4	4.5	4.2	2.2	4.0	27.6	14.7	64.8	21.6
ZMA 6986 (683)	Andasibe	M	-	43.1	14.8	16.4	2.0	5.5	4.4	2.4	4.5	30.5	14.6	68.8	22.2
ZMA 6987 (684)	Andasibe	M	-	47.9	16.8	17.3	2.0	5.4	4.7	2.9	5.1	30.4	15.9	75.3	25.5
ZMA 6988 (685)	Andasibe	M	-	47.5	16.0	17.7	2.5	5.0	4.6	3.0	5.0	30.6	15.8	74.7	23.6
ZMA 6989 (950)	Andasibe	M	-	45.7	16.0	17.4	2.2	5.4	4.7	2.4	4.2	31.1	15.2	75.7	22.7
ZMA 19402	Ranomafana	M	-	50.6	16.7	18.0	2.6	5.6	5.2	2.6	5.0	34.0	16.1	78.1	24.9
M. depressiceps															
BMNH 1947.2.27.50	East Betsileo	M	LT	39.6	13.2	13.7	2.4	4.2	3.7	2.4	3.9	27.4	13.2	69.6	20.9
BMNH 1947.2.27.51	East Betsileo	M?	PLT	36.7	11.8	12.2	2.2	4.0	3.2	2.2	4.1	22.0	12.16	1.0	19.0
BMNH 1947.2.27.52	Ankafana	M?	PLT	34.5	11.9	12.7	2.0	4.0	3.3	2.2	3.7	22.0	11.16	0.7	17.7
BMNH 1947.2.27.53	Ankafana	?	PLT	32.5	10.7	11.2	2.2	3.6	3.0	2.3	3.5	22.0	10.45	6.6	16.6
ZMB 30496*	Central Madagascar	M?	HT	35.1	11.8	12.6	2.0	4.0	3.2	2.4	3.8	23.9	11.76	3.7	19.3
BMNH 1947.2.8.62**	Sahembendrana	?	HT	33.0	12.3	13.6	1.8	3.7	3.4	2.1	4.0	21.9	10.45	4.9	15.6
ZMA 6973 (228)	Moramanga-Andasibe	M	-	32.2	10.8	12.7	2.0	4.1	3.0	1.7	3.6	19.9	9.6	50.8	15.0
ZMA 6974 (1154)	Mandraka	M	-	41.1	13.2	15.1	2.6	4.3	3.7	2.4	3.9	27.2	13.1	70.5	21.1
ZMA 6875 (120)	Ranomafana	F	-	35.0	11.8	11.6	2.3	4.4	3.5	2.2	4.0	21.4	11.3	57.4	17.3
ZMA 6976 (1072)	Mandraka	M	-	41.4	13.3	15.4	2.5	5.0	3.4	2.3	4.0	28.1	13.6	66.3	21.4
ZMA 6976 (1073)	Mandraka	M	-	42.4	13.6	15.7	2.4	4.8	3.9	2.3	4.0	29.3	14.4	73.0	23.4
ZMA 6976 (1076)	Mandraka	M	-	43.1	13.6	15.7	2.6	4.8	4.0	2.0	4.0	31.1	14.8	72.9	23.9
ZMA 6976 (1077)	Mandraka	M	-	37.4	12.3	14.8	2.3	4.1	3.5	2.3	3.7	25.4	12.6	64.6	19.7
ZMA 6976 (1078)	Mandraka	M	-	40.5	12.8	15.6	2.5	5.0	3.5	2.4	4.1	27.5	13.6	67.7	21.1
ZMA 6976 (1079)	Mandraka	M	-	44.9	14.7	16.5	2.6	4.8	3.9	2.7	4.7	27.5	13.5	69.9	21.5
ZMA 6982 (995)	Mandraka	M	-	39.7	13.0	14.7	2.3	4.5	3.3	2.0	4.0	28.0	13.2	68.0	20.8
ZMA 6979 (600)	Andasibe	M	-	41.1	13.7	15.1	2.6	5.0	4.0	1.9	4.2	28.0	12.7	67.7	21.2
ZMA 6983 (1050)	Mandraka	M	-	39.9	12.4	14.9	2.3	4.5	3.9	1.7	4.0	28.7	14.0	69.8	21.8
ZMA 19474	Ranomafana	M	-	39.1	12.6	15.0	2.3	3.9	3.7	2.2	3.9	23.8	12.9	59.1	18.2
M. kathrinae															
ZFMK 62264	An'Ala	M	HT	56.7	20.0	22.4	3.0	5.6	6.1	4.1	6.3	37.0	19.7	92.9	29.7
ZFMK 62263	An'Ala	M	PT	58.6	20.6	23.3	3.3	5.8	6.8	4.2	6.6	37.5	19.1	94.2	30.6
ZFMK 62266	An'Ala	M	PT	57.4	21.0	23.4	3.4	6.5	6.7	4.0	6.3	37.5	19.7	97.8	30.2

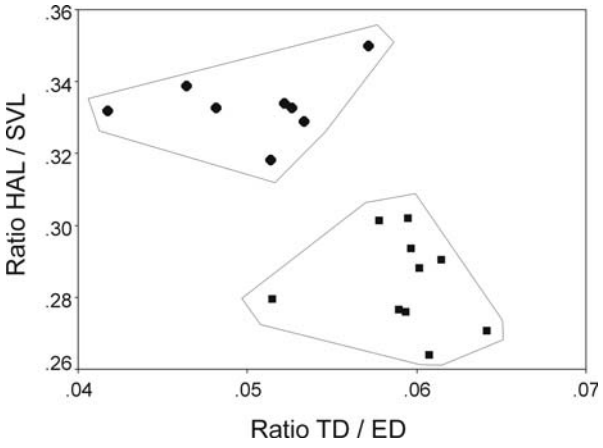

FIG. 1. Scatterplot of relative hand length (ratio HAL/SVL) vs. relative tympanum diameter (ratio TD/ED), showing the differentiation between *Mantidactylus timidus* (east-coast specimens previously attributed to *M. tornieri*; squares) to *M. tornieri* (circles).

absence of overlap of relative hand length values: ratio HAL/SVL, 0.28±0.01 (0.26-0.30) in east coast specimens vs. 0.33±0.01 (0.32-0.35) in mid-altitude specimens (Mann-Whitney *U*-test, $P<0.001$). Furthermore, the relative tympanum size is significantly larger in the east-coast specimens (*U*-test, $P<0.005$), although the values of the two forms do widely overlap (Fig. 1). The hind limb never reaches the anterior eye corner in the east coast specimens but it does sometimes in mid-altitude specimens, indicating a shorter relative hind limb length; the differences in the ratio HIL/SVL are highly significant (*U*-test; $P<0.001$). We did not detect a distinct difference in relative eye diameter as mentioned by Blommers-Schlösser (1979), but this might be due to the fact that eye diameter is difficult to measure reliably, especially among specimens in a different state of fixation. The holotype of *M. tornieri* agreed in relative hand length and other morphological characters with specimens from mid-altitudes.

Together with the observations provided by Blommers-Schlösser (1979), the available morphological evidence strongly suggests the east coast form to be differentiated from typical *M. tornieri* on the species level.

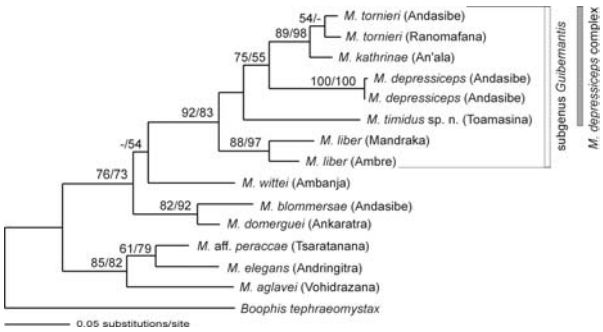

FIG. 2. Maximum likelihood phylogram based on 539 b.p of the mitochondrial 16S rRNA gene in species of the subgenus *Guibemantis,* and other *Mantidactylus* species previously thought to be related to these. The values show bootstrap support of nodes in percent (maximum likelihood, 500 replicates / maximum parsimony, 2000 replicates). Only values >50% are shown.

MOLECULAR PHYLOGENY

After exclusion of 10 gapped characters, the dataset comprised 539 characters, of which 386 were invariable and 104 were parsimony-informative. The molecular analyses produced well-resolved phylogenetic trees. The maximum likelihood topology (Fig. 2) agreed largely with the most parsimonious tree (not shown) that received a consistency index of 0.595 and a retention index of 0.604, and required 378 steps. Bootstrap support was high for most nodes (Fig. 2). Within *Guibemantis*, *M. liber* was most basal and the larger-sized *Guibemantis* species were arranged in one clade. The east-coast specimen previously attributed to *M. tornieri* was most basal in this clade, and *M. depressiceps* was sister to a group containing *M. tornieri* and *M. kathrinae*. Whereas the clade with *M. depressiceps, M. kathrinae* and mid-altitude *M. tornieri* was moderately well supported by bootstrap analysis, this was not the case for the placement of the east-coast *M. tornieri* as their sister group, which only received bootstrap supports <50%. *M. peraccae* was very clearly grouped with *M. aglavei,* corroborating its placement in the subgenus *Spinomantis* (Glaw & Vences, 1994; Andreone *et al.,* 1998), and *M. elegans* resulted to belong into the same clade and subgenus rather than into *Guibemantis.*

Uncorrected pairwise sequence divergence was 1.9% between the Ranomafana and Andasibe specimens of *M. tornieri,* 2.9-3.0% between these and *M. kathrinae,* 7.5-8.0% between *M. kathrinae* and *M. depressiceps,* and 8.0% comparing the *M. tornieri* individuals from Ranomafana and Andasibe to the specimen from Toamasina at the east coast. This high genetic differentiation of the east coast specimen, and its basal placement in the phylogeny, corroborate the status of these populations as separate species as indicated by the morphological analysis. We therefore describe the east coast populations in the following as:

MANTIDACTYLUS TIMIDUS SP. N. (FIG. 3-4)

Holotype. ZMA 19466, male, collected by M. Vences on 10 February 2003, less than 10 km north of Toamasina, eastern Madagascar (18°03'51"S, 49°22'39"E, 8 m above sea level).

Paratypes. ZMA 19492, one male, same locality and collecting data as holotype; ZMA 7109, one male (field number 814), collected by R. Blommers-Schlösser on 2 August 1972 at Foulpointe; ZMA 7110, three males (field numbers 556, 706, 707) and one female (field number 708), collected by R. Blommers-Schlösser on 13 February 1972 at Foulpointe; ZMA 7112, two males (field numbers 475 and 686), collected by R. Blommers-Schlösser on 13 October 1971 at Foulpointe; ZFMK 52698, collected by F. Glaw and M. Vences on 19 March 1991 at Voloina in the Antongil bay south of Maroantsetra.

Diagnosis and comparisons. The new species is considered as *Mantidactylus* based on the presence of an intercalary element between ultimate and penultimate phalanges between fingers and toes (as assessed by external examination), and molecular phylogenetic data (Fig. 2). Within *Mantidactylus,* the new species is allocated to the subgenus *Guibemantis* based on the structure of femoral glands in males (of type 1 sensu Glaw *et al.,* 2000), arboreal habits, molecular phylogeny (Fig. 2), white single subgular vocal sac, smooth dorsal skin without dorsolateral ridges, enlarged terminal disks of fingers and toes and complete separation of lateral metatarsalia. Within *Guibemantis*, it is distinguished from *M. depressiceps* and *M. kathrinae* by the transparent jelly of its clutches (versus white jelly) and by green eggs (versus white eggs), from *M. liber* by its distinctly larger size, and from *M. liber, M. depressiceps*, *M. kathrinae* and *M. tornieri* by its shorter relative hand length. From all these species it differs by its large genetic differentiation (Fig. 2).

Description of the holotype. Adult male in excellent state of preservation. A small amount of muscle tissue from left tibia removed for molecular analysis. For measurements see Table 1. Body relatively slender; head longer than wide, slightly wider than body; snout slightly pointed in dorsal and relatively truncate in lateral view; nostrils directed laterally, not protuberant; canthus rostralis distinct, straight; loreal region concave; tympanum distinct, relatively small, its diameter 55% of eye diameter; distinct and almost straight supratympanic fold; tongue ovoid, distinctly bifid posteriorly; vomerine teeth as one distinct oblong group posterolateral of each choana; choanae medium-sized, rounded. Forelimbs slender; subarticular tubercles single; inner and outer metacarpal tubercles present; a small prepollex (length ca. 1.4 mm) of about one fourth of the length of the first finger. Fingers with rudiments of webbing; relative finger length 1<2<4<3; finger disks strongly enlarged; nuptial pads absent. Legs slender, when legs are adpressed along body, the tibiotarsal articulation reaches the centre of the eye; lateral metatarsalia separated; inner metatarsal tubercle small, of same size of outer metatarsal tubercle which is distinctly recognizable; webbing formula of the foot 1(1), 2i(1.5-2); 2e(0.75); 3i(2); 3e(1); 4i/e(2.25), 5(0.75) (because only one subarticular tubercle is recognizable on the second toe, the relative extension of web on this toe can only be estimated); relative toe length 1<2<3<5<4. Skin on the dorsum smooth; ventral skin smooth on throat and chest, slightly granular on belly. Femoral glands indistinct, of type 1 as defined by Glaw *et al.* (2000).

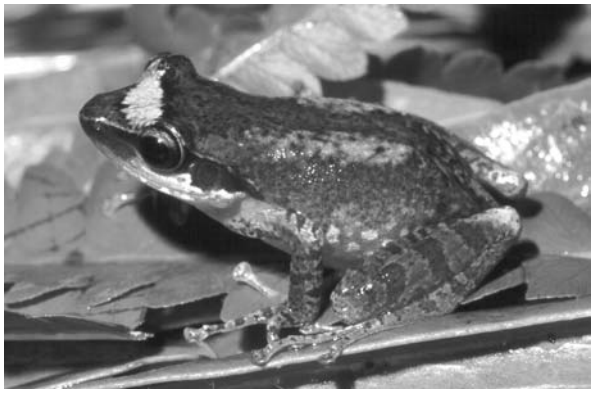

FIG. 3. Holotype of *Mantidactylus timidus* sp. n. in life. Photographed on 10 February 2003, ca. 10 km north of Toamasina, eastern Madagascar.

FIG. 4. Holotype of *Mantidactylus timidus* sp. n. in life, ventral view. Photographed on 10 February 2003, ca. 10 km north of Toamasina, eastern Madagascar.

Coloration of the holotype. General dorsal colour grey-brown, with a relatively diffuse grey-beige symmetrical longitudinal dorsolateral markings, and a distinct beige patch on the anterior head, between the eyes and the nostrils, bordered anteriorly and posteriorly by dark brown. A distinct dark brown tympanic patch, and a poorly contrasted trace of a light frenal stripe. Flanks marked with several small beige spots. Hind limbs light grey-brown with brown crossbands. Ventral side fading from bright white on the throat and dirty white on the chest to light brown on the limbs.

Variation. Morphometric measurements of all paratypes of *M. timidus* are found in Table 1. The size range is remarkable, from 33.7 mm SVL in a paratype from Toamasina to 55.4 mm in the single available specimen from Voloina. The mean SVL of males was 42.2 mm (± 6.1 mm standard deviation), thus being slightly smaller than that of the single female specimen (44.1 mm). Besides the morphometric characters highlighted in the diagnosis, it is conspicuous that from all localities of *M. timidus* there are specimens with a beige patch on the anterior head, running between the eyes, similar to that of the holotype (Fig. 3). This applies to ZFMK 52698 from Voloina, and very distinctly to ZMA 7109 from Foulpointe. We never observed such patches in other *Guibemantis*, except for one specimen of *M. tornieri* from Andasibe (ZMA 6987). When the hind limb is adpressed along the body, the tibiotarsal articulation reaches at most to the eye center, often only to the posterior eye corner or to the tympanum.

Etymology. The specific name is derived from the Latin adjective timidus = timid, and refers to the shy and inconspicuous calling behaviour of this species of which we only heard very few vocalizations on the two occa-

sions that we encountered it in the wild during its breeding season.

Advertisement call. We heard advertisement calls of this species on Nosy Boraha and near Toamasina. These were irregular short blasts and moaning sounds. They were often emitted from hidden position in dense vegetation, and after long silent intervals, which stands in contrast to the loud, regular and exposed calling behaviour of *Mantidactylus depressiceps, M. tornieri* and *M. kathrinae*. We therefore hypothesize that these vocalizations may not be the real advertisement calls as emitted by fully motivated males, and here refrain from a detailed description. Also Blommers-Schlösser (1979) did not refer to the calls of this species but only to those of *M. tornieri* at Andasibe.

Natural history. Specimens near Toamasina, on Nosy Boraha and Voloina were found in secondary vegetation and heavily degraded forest, sitting on leaves at heights of 1-3 m. The Toamasina site is far from any primary forest in an area of lowland swamps and rice fields. Egg masses are attached on leaves over water. According to Blommers-Schlösser (1979) and to our observations on Nosy Boraha (Glaw & Vences, 1994), the jelly is transparent and the eggs are greenish. Tadpoles of *M. timidus* have been described by Blommers-Schlösser (1979) under the name *Mantidactylus tornieri*.

Distribution. Voucher specimens of *M. timidus* are available from three localities, (1) the type locality N of Toamasina, (2) Foulpointe and (3) Voloina. We also assign specimens observed but not collected by us on the eastern offshore island (4) Nosy Boraha (Ile Ste. Marie) as belonging to this species.

Available older names. Rhacophorus mocquardii Boulenger, 1896, and *Mantidactylus acuticeps* Ahl, 1929, both considered to be junior synonyms of *Mantidactylus depressiceps*, could potentially be available as earlier names for *M. timidus.* Table 1 provides measurements of the holotypes of these taxa, and of the types of *M. depressiceps, M. tornieri* and *M. kathrinae.* All of these have large relative hand lengths, demonstrating their distinctness from *M. timidus*.

DISCUSSION

Recent studies have shown that many amphibian species that previously were thought to occur over wide geographic and altitudinal ranges of Madagascar actually are complexes of well differentiated species. Some of the newly recognized taxa appear to have an elevated degree of elevational endemism. Examples encompass the genus *Boophis* (B. *schuboeae* vs. *B. ankaratra;* Glaw & Vences, 2002*b*) as well as *Mantidactylus* (*M. sarotra* vs. *M. kely*; Glaw & Vences, 2002*a*). The data herein provide another example, showing that lowland populations previously considered as *M. tornieri* actually belong to another species, *M. timidus.*

Originally, the subgenus *Guibemantis* had been conceived as *Mantidactylus depressiceps* group to include only the two phenetically similar species *M. tornieri* and *M. depressiceps* (Blommers-Schlösser, 1979). Several other species were later added to the group (Blommers-Schlösser & Blanc, 1991), namely *M. peraccae, M. elegans* and *M. guibei*. While the relationships of *M. guibei* remain unstudied, the molecular results herein confirm that *M. peraccae* and also *M. elegans* belong into the subgenus *Spinomantis* (see Andreone *et al.,* 1998). *Mantidactylus liber*, which was added to *Guibemantis* by Glaw & Vences (1994) because of its femoral gland morphology and large relative hand length, is the sister taxon of other *Guibemantis* according to the results herein but may also be related to the leaf-axil breeding subgenus *Pandanusicola* (Vences *et al.,* 2003). Indeed, our study did not include any *Pandanusicola* species, but recent molecular studies by Lehtinen & Nussbaum (2003) suggested that *M. liber* was nested within that subgenus rather than in a clade with the larger-sized species of *Guibemantis*. Furthermore, not even the phylogenetic placement of *M. timidus* as sister group of the *M. depressiceps-kathrinae-tornieri* clade was sufficiently supported by the bootstrap analyses, emphasizing the need for more comprehensive molecular studies to fully clarify the phylogenetic relationships of these taxa.

An interesting aspect is the high intraspecific variability of body size in this lineage. According to the data presented herein, this regards both *M. timidus* (SVL of adult males 34-55 mm) and *M. tornieri* (42-51 mm). It also coincides with the rather large differences found between specimens of *M. kathrinae* from the type locality An'Ala (57-59 mm) and those from Andapa in north-eastern Madagascar (44-46 mm; Glaw *et al.,* 2000). In contrast, several other mantellids seem to have quite constant body sizes, and low differences in SVL can be used to distinguish among species (e.g., Glaw & Vences, 2002*a*). Our results suggest that body size differences in frogs must be studied carefully if they are to be used for taxonomic purposes (see also Andreone *et al.,* 2002).

Molecular data, more specifically the comparison of mitochondrial rRNA sequences, have shown to be a valuable tool to elucidate the taxonomic status and identity of certain frog populations, especially in taxa of inconspicuous advertisement calls (e.g., Glaw & Vences, 2004). Haplotype sharing between populations of mantellid frogs can exist (Vences *et al.,* 2004), but has so far only been observed among very closely related species or variants. The high divergence values found herein between *M. timidus* and other species of *Guibemantis* would be unprecedented if considered as within-species differentiation, and therefore alone already provide a strong indication of specific distinctness.

However, we discourage the uncritical use of mitochondrial divergences as only marker to draw taxonomic conclusions. Strongly divergent haplotypes are known to be shared among conspecific populations and individuals of some organisms (e.g. snails; Thomaz *et al.,* 1996). Threshold values of mitochondrial divergence have been proposed to distinguish species of

mammals in terms of a genetic species concept (Bradley & Baker, 2001). The identification of such thresholds in amphibians, above which two taxa can be regarded as not conspecific with a defined statistical probability, is a promising endeavour in terms of DNA taxonomy (Tautz *et al.,* 2003; Blaxter, 2004) or DNA barcoding (Hebert *et al.*, 2003). However, it needs to be stressed that this technique is based on genetic distances among individuals and therefore is prone to error whenever these are not representative of their populations, e.g. caused by phenomena of introgression, haplotype sharing, or non-monophyletic species. DNA barcoding holds a great potential for quick exploratory studies but should not be used as exclusive basis for the formal descriptions of new species. In our data set, also the rather strong divergences between individuals of *Mantidactylus liber* (from Mandraka and Montagne d'Ambre; uncorrected pairwise distance 4.6%) could be seen as indications for specific distinctness, but more detailed studies on their morphology and bioacoustics are necessary before formalizing such conclusions by the description of new taxa. Of course, in the case of *Mantidactylus timidus,* not only its strong molecular divergence but also its well corroborated isolated phylogenetic position supports a specific distinctness from *M. tornieri.* But in light of the surprisingly high number of non-monophyletic species that have been identified (Funk & Omland, 2003), also this evidence should not be used as exclusive taxonomic argument.

If we consider the subgeneric assignation of *Mantidactylus liber* and *M. guibei* as in need of confirmation and regard only the *M. depressiceps* complex (see Fig. 2), then the discovery of two new species, *M. kathrinae* and *M. timidus,* has led to a doubling of the species diversity of this clade. Considering the genetically divergent *M. tornieri* population from Ranomafana, it would not be surprising if future studies result in the discovery and identification of further new species in this lineage.

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