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Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution

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Abstract

Frogs of the subfamily Mantellinae (Amphibia: Anura: Mantellidae) are a species-rich and diverse lineage endemic to the Madagascan region. The major synapomorphy of this clade is a derived reproductive mode including an unusual mating behaviour (loss of strong mating amplexus, egg deposition outside of water) and associated morphological adaptations (evolution of femoral glands, loss of nuptial pads). However, the evolutionary steps towards this unique character complex remain obscure. We here describe a recently discovered new frog, *Tsingymantis antitra* **gen. nov., sp. nov.** from the moderately dry karstic massif Tsingy de Ankarana in northern Madagascar. The new species is not referable to any existing genus or species groups. A phylogenetic analysis, based on DNA sequences of four mitochondrial genes (12S and 16S rRNA, tRNAVal, cytochrome b) and one nuclear gene (rhodopsin) placed *Tsingymantis* without significant support as sister taxon of the Mantellinae which was found to be a well-defined monophyletic group (100% Bayesian and 99% bootstrap support). The position of *Tsingymantis* as the most basal clade of the Mantellinae is in agreement with several morphological and osteological characters, suggesting that this subfamily including *Tsingymantis* may be a monophyletic group whereas the Boophinae could represent the most basal clade of the Mantellidae. We therefore include *Tsingymantis* in the Mantellinae in a preliminary way, pending further study. In contrast to the large majority of recent mantellid species which are adapted to humid rainforests, the most basal clades of the three subfamilies show adaptations to relatively dry conditions, indicating that the climate during the early radiation of mantellids (probably in the Eocene) may have been drier than in recent times.

Key words: Anura, Mantellidae, *Tsingymantis antitra* **gen. nov.**, **sp. nov.**, molecular phylogeny, Madagascar

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Introduction

The amphibian fauna of Madagascar includes more described species (231) than any other African country (e. g. http://amphibiaweb.org) and has by far the highest rate of endemism as well (> 99% at species level). Most of these species belong to the Mantellidae which is endemic to Madagascar and Mayotte Island. This family is divided into the three subfamilies Mantellinae, Boophinae, and Laliostominae which can be diagnosed independently by both, non-molecular and molecular characters (Vences & Glaw 2001; Glaw & Vences 2006). With 106 described species and an enormous diversity in morphology, behaviour and habits, the subfamily Mantellinae represents the most successful lineage of Malagasy frogs. This clade is mainly characterized by a derived reproductive mode including behavioural and morphological apomorphies, but the evolutionary steps towards this unique reproductive biology remain obscure.

In the past 35 years a large number of new frog species has been discovered in Madagascar, most of them representatives of genera or species groups that were known since long. The most recent discoveries of new genera were *Madecassophryne* Guibé, 1974, based on a single species (*Madecassophryne truebae*), and *Paradoxophyla* Blommers-Schlösser & Blanc, 1991, based on a single species as well (*Microhyla palmata*). Both these species were discovered by Charles P. Blanc in 1971 in south-eastern Madagascar (Guibé 1974) and both are rather small (20–25 mm snout-vent length [SVL]). Very recently, the genus *Wakea* Glaw & Vences, 2006 was established. The single species of this genus was only discovered in 2001 and represents (with a SVL of 11–16 mm) the smallest mantellid known so far (Vences *et al.* 2002a). *W. madinika* is morphologically very similar to the species in the genus *Blommersia*, but turned out to be the sister group of the genus *Mantella* by genetic studies (Vences *et al.* 2003, see also Fig. 3). All other new genus group names that have been established for Madagascan frogs in the past decades (Dubois 1992; Glaw & Vences 1994, 2006; Glaw *et al.* 1998) were based on previously known species or species groups, indicating that all major lineages of Madagascan frogs might have been discovered. A similar situation is found on a global scale. Despite the enormous amount of amphibian species which have been described in recent years, the discovery of deeper new lineages, such as new genera, has been a rare event in the past decades and sometimes received remarkable attention (Biju & Bossuyt 2003; Min *et al.* 2005). Of the 35 extant (non-fossil) and valid amphibian genera named since 1990 (extracted from Dubois 2005) 22 include species that were discovered before 1990 (extracted from Frost 2004) and therefore do not represent discoveries of new lineages. They are the result of splitting of known genera, usually based on increased phylogenetic resolution (e.g., new genera erected by Frost *et al.* 2006). Only 13 genera were based on (and include only) species that were described since 1990 (*Minervarya*, *Nasikabatrachus*, *Protohynobius*, *Karsenia*, *Churamiti*, *Metaphryniscus*, *Truebella*, *Aromobates*, *Rupirana*, *Bryobatrachus*, *Spicospina*, *Altigius*, and *Ericabatrachus*).

During surveys in the Ankarana reserve in northern Madagascar in the years 2003 and

2004 we discovered four female specimens of a large new frog species (SVL 66–67 mm) that was not referable to any genus or subgenus of Madagascan frogs.

In the present paper we describe this remarkable new mantellid genus and species and investigate its phylogenetic relationships. We then discuss its potential to understand the evolution of the derived reproductive modes in mantellines and its relevance to resolve the relationships between the three mantellid subfamilies.

Materials and methods

Specimens were collected at night with the help of torches by opportunistic searching. Vouchers were fixed in 90% ethanol and subsequently stored in 70% ethanol. Museum acronyms used are UADBA (Université d'Antananarivo, Département de Biologie Animale), ZFMK (Zoologisches Forschungsmuseum A. Koenig, Bonn), and ZSM (Zoologische Staatssammlung, München).

The following morphological measurements were taken with dial calipers to the nearest 0.1 millimeter: SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus), TIBL (tibia length).

DNA was extracted from muscle tissue samples preserved in ethanol. We complemented the data set of Glaw & Vences (2006) which consists of partial fragments of four mitochondrial genes (12S rRNA, 16S rRNA, tRNAVal, cytochrome b) and one nuclear gene (rhodopsin) by amplifying and sequencing these fragments for the new species from Ankarana, from one crucial species missing in the previous dataset (*Gephyromantis pseudoasper*, a representative of the subgenus *Phylacomantis*), and by filling gaps of sequences that were previously missing. Detailed information on DNA extraction, primers, PCR and sequencing are given in Glaw & Vences (2006) and Vences *et al.* (2003). Newly obtained sequences were submitted to Genbank (accession numbers DQ901378- DQ901396; see appendix for a complete list of accession numbers).

Sequences were aligned using ClustalW (Thompson *et al.* 1994) as implemented in Bioedit (Hall 1999) and alignments were refined manually. Positions with gaps or positions that could not be aligned reliably were excluded from further analyses as were the third codon positions of cytochrome b. The complete dataset encompassed 58 sequences and 2303 positions.

Bayesian inference was performed using MrBayes 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on a partitioned dataset with partitions for each single gene and 1st and 2nd positions from cytochrome b. Models of sequence evolution and the analysis parameters (proportion of invariable sites and gamma-shape parameter alpha) were estimated using Modelgenerator (Keane *et al.* 2004). We ran 100,000

generations while sampling every 10th tree and a burn in value of 5000. The Maximum Likelihood analyses were performed with PhyML (Guindon & Gascuel 2003) with parameters estimated for the complete dataset. We ran 500 bootstrap replicates.

Results

Tsingymantis gen. nov.

Type species and only included species. *Tsingymantis antitra* **sp. nov.**

Etymology. Derived from "tsingy", the Malagasy word for eroded karstic limestone formations and the Greek word *mantis* = treefrog (see Vences *et al.* 1999 for the derivation of *mantis*). The genus name refers to the habitat, the tsingy formations of Ankarana. The gender of this genus is masculine.

Diagnosis. Large-sized species (female snout-vent length 66–67 mm) with a large tympanum (66–76% of eye diameter), toe 5>3, males unknown. Relatively little webbing between toes. No webbing between fingers. Lateral metatarsalia largely connected. Inner metatarsal tubercle very distinct, outer metatarsal tubercle absent. Finger tips strongly enlarged. Finger and toe pads with a complete circummarginal groove. First finger slightly shorter than second finger. Tibiotarsal articulation reaches the eye when the hind limb is adpressed along the body. Femoral glands not recognizable in females from external view. Tibial glands absent. Tongue bifid. For osteological characters see below. Habits terrestrial in tsingy formations. Activity nocturnal. Eggs pigmented (verified by dissection).

Tsingymantis **gen. nov.** does not show closer overall similarity to any other mantellid genus or species. Despite the absence of data on males, sexual dimorphism, and reproductive mode, there are still sufficient external characters to distinguish *Tsingymantis* from all other mantellid genera: *Tsingymantis* differs from the genus *Boophis* by a forked omosternum, largely connected metatarsalia, less webbing between toes and by general dissimilarity with any *Boophis* species; from *Aglyptodactylus* and *Laliostoma* by distinctly enlarged tips of fingers and toes, by the presence of a complete circummarginal groove on pads of fingers and toes, and by having the first finger shorter than the second; from *Mantella* (SVL 18–31 mm) and *Wakea* (SVL 11–16 mm) by much larger size, presence of maxillary teeth, and distinctly enlarged terminal finger disks; from *Boehmantis* and *Mantidactylus* sensu Glaw & Vences (2006), including the subgenera *Brygoomantis*, *Chonomantis*, *Hylobatrachus*, *Maitsomantis*, *Mantidactylus*, and *Ochthomantis*, by largely connected metatarsalia and in addition from most of these species by the absence of femoral glands in females and by less developed webbing between toes; from *Blommersia* (SVL 15–27 mm, tympanum/eye 35–55 %) by much larger size and larger relative tympanum size; from all *Guibemantis* (tympanum/eye up to 64 %) by larger relative tympanum size, furthermore from the subgenus *Guibemantis* by largely connected metatarsalia and less webbing between the toes, and from the subgenus *Pandanusicola*

(SVL 22–38 mm) by much larger size and very different habits; from *Spinomantis* (SVL 22–60 mm, tympanum/eye 33–60 %) by larger body size and larger relative tympanum size; and from all *Gephyromantis* (SVL 20–50 mm, tympanum/eye 30–67 %), including the subgenera *Duboimantis*, *Gephyromantis*, *Laurentomantis*, *Phylacomantis*, and *Vatomantis* by larger size and a relatively larger tympanum. In addition, *Tsingymantis* differs from most mantelline species by the following characters: bony part of the sternum longer than that of the omosternum, outer metatarsal tubercle absent, and toe 5 clearly longer than toe 3 (see Glaw & Vences 1994: 122–125). *Tsingymantis* is the only clade in the subfamily Mantellinae that is unknown from the rain forest areas (including the central high plateau) of Madagascar (where almost all mantelline species occur, except *Mantella expectata*, *M. viridis*, and *Gephyromantis corvus*) and is only known from a very seasonal and moderately dry habitat.

Justification. All hitherto known mantellid clades, including 164 described and many undescribed species, can all clearly be assigned to one of the three subfamilies Mantellinae, Laliostominae, or Boophinae based on their sequences of mitochondrial and nuclear genes, and strongly supported by high bootstrap values (see below and unpublished data). A clear attribution of all mantellid lineages to one of these three subfamilies is also possible based solely on morphological data (see Glaw & Vences 2006). In contrast, the phylogenetic position of *Tsingymantis antitra* **gen. nov. sp. nov.** is not significantly resolved by the molecular (Fig. 3) and morphological data although a basal sister group relationships to all other mantellines is indicated by the available overall evidence, and we propose preliminary inclusion of the new genus in the subfamily Mantellinae. Regarding this highly isolated position, the justification of the new genus is evident, but the possibility that future studies will reveal that *Tsingymantis* represents a fourth major lineage (subfamily) of the Mantellidae cannot be ruled out at present.

***Tsingymantis antitra* sp. nov.**

Holotype. ZSM 304/2004 (fieldnumber FGZC 589), collected on 27 February 2004 below the "Point de Vue Petit Tsingy", 12°57'25"S, 49°07'06"E, 117 m alt, Ankarana Special Reserve, northern Madagascar, by F. Glaw, M. Puente & R. D. Randrianiaina.

Paratypes. ZFMK 84436 (originally ZSM 305/2004), same data as holotype; ZSM 769/2003 (fieldnumber FG/MV 2002-0577), cleared and stained specimen, collected on 12 February 2003, below "Campement des Anglais" (now called Campement Anilotra), Ankarana Special Reserve, northern Madagascar, by F. Glaw, R. D. Randrianiaina & A. Razafimanantsoa; UADBA 24766 (fieldnumber FGZC 531), collected on 25 February 2004 close to the "Grotte des Chauve-souris", 12°57'S, 49°07'E, ca. 50 m alt, Ankarana Special Reserve, northern Madagascar, by F. Glaw, M. Puente & R. D. Randrianiaina.

Diagnosis. A large species of mantellid frogs representing an isolated and basal lineage within the family, based on a molecular analysis of mitochondrial and nuclear

genes (Fig. 3). Differs from all other mantellid genera and subgenera as outlined in the diagnosis of the genus *Tsingymantis*. It differs from all other large mantelline and laliostomine species that reach a SVL of more than 60 mm as follows: From *Mantidactylus mocquardi*, *M. grandidieri*, *M. guttulatus*, *M. ambohimitombi*, *Boehmantis microtympanum*, and *Aglyptodactylus madagascariensis* by largely connected lateral metatarsalia (versus separated), from *Aglyptodactylus laticeps* and *Laliostoma labrosum* by distinctly enlarged tips of fingers and toes, by the presence of a complete circummarginal groove on pads of fingers and toes, and by having the first finger shorter than the second.

Description of the holotype. Adult female (with oocytes in the body cavity), in good state of preservation but with a midventral slit. SVL 67.1 mm, for further measurements see table 1. Body slender; head wider than body; snout approximately rounded in dorsal and lateral views, nostrils directed laterally, protuberant, much nearer to tip of snout than to eye; canthus rostralis distinct, straight; loreal region concave; tympanum very distinct, rounded, 76 % of eye diameter; supratympanic fold distinct, curved; tongue was ovoid and bifid posteriorly (part of the tongue was removed as DNA sample); vomerine teeth present in two groups, maxillary teeth present; choanae relatively rounded. Arms slender, subarticular tubercles single; fingers without webbing; relative length of fingers 1<2<4<3; finger disks distinctly enlarged; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaches the eye when the hind limb is adpressed along the body; lateral metatarsalia largely connected; inner metatarsal tubercle distinct, outer metatarsal tubercle absent; webbing formula (according to Blommers-Schlösser 1979) between toes 1(1), 2i(1), 2e(0.5), 3i(1.5), 3e(1), 4i(2.5), 4e(2), 5(1); relative length of toes 1<2<3<5<4. Skin on the upper surface smooth, without folds or ridges. No distinct enlarged tubercles in the cloacal region; ventral skin smooth, finely granular on the shanks. No femoral glands.

Colouration of the holotype. After 1.5 years in preservative, back blackish with indistinct dark brown reticulations and a few small greyish spots above the insertion of the left arm. Upper surfaces of arms and legs dark brown with indistinct black markings. Flanks lighter brown than back, although there is no distinct colour border between flanks and back. Tympanum light brown in periphery, darker brown in center. Ventrally dirty cream-whitish on belly, with fine indistinct mottling. Throat brown, chest and ventral surfaces of arms light brown, ventral surface of hindlegs yellowish in the center becoming darker brown to periphery. Ventral side of lower leg and tarsus dark brown, foot and webbing brown.

The dorsal ground colour in life was brown with violet shade and with olive green spots (Fig. 1). The iris was silvery-grey with a brownish horizontal streak and a bluish iris periphery. The ventral surface was pinkish to brown (Fig. 2).

Variation (see table 1 for measurements). The ZFMK and ZSM paratypes are very similar to the holotype in size and morphology. The colouration of ZFMK 84436 is generally similar to that of the holotype, but sligthly lighter, that of ZSM 769/2003

(assessed before clearing and staining) distinctly lighter, especially on the ventral side. All three specimens have pigmented oocytes in the body cavity although they are relatively small in size and numbers. The UADBA paratype is similar to the other specimens in size and colour, but was not available for detailed studies.

Osteological features. A number of skeletal characters known to be relevant in mantellid systematics were assessed on the cleared and stained paratype ZSM 769/2003. Maxillary and vomerine teeth present. Omosternum forked, the greatest space between the arms being about two times the width of one arm, sternum unforked, bony part of sternum longer than that of omosternum. Hyoid with a distinct anterolateral and a small posterolateral process. Intercalary element present between ultimate and penultimate phalanges of all fingers and toes. Terminal phalanges distinctly Y-shaped, with rather broad and posteriorly serrated arms. Three free distal tarsals, the third tarsal being small.

Etymology. The specific name is derived from the Malagasy word "antitra" (meaning old) and refers to the presumed old age of the *Tsingymantis* lineage. The name is considered as an invariable noun standing in apposition to the genus name.

TABLE 1. Morphological measurements (all in mm) of type specimens of *Tsingymantis antitra*. For abbreviations of measurements see Materials and Methods. Additional abbreviations used: HT, holotype; PT, paratype; RHL, relative hindlimb length, given as point reached by the tibiotarsal articulation when hindlimb is adpressed along body.

Voucher number	ZSM 769/2003	ZFMK 84436	ZSM 304/2004
Status	PT	PT	HT
Sex	F	F	F
SVL	66.4	67.0	67.1
HW	24.4	23.9	23.9
HL	25.0	24.8	25.1
TD	5.1	5.0	5.2
ED	7.0	7.6	6.8
END	6.5	6.4	6.3
NSD	2.9	2.4	3.3
NND	4.0	3.6	5.1
FORL	45.0	45.4	43.0
HAL	21.0	19.4	20.0
HIL	102.0	100.8	96.4
FOTL	45.8	44.5	43.2
FOL	29.8	28.1	28.0
RHL	eye center	eye center	posterior eye corner
TIBL	32.1	31.6	29.4

FIGURE 1. *Tsingymantis antitra* **sp. nov.,** dorsolateral view of holotype.

FIGURE 2. *Tsingymantis antitra* **sp. nov.**, ventral view, probably of holotype.

Habitat and natural history. The Ankarana reserve consists mainly of bizarre, eroded tsingy limestone formations and moderately dry forest areas. It is crossed by four rivers and includes more than 100 km inventorized subterraneous passages and caves. The climate of Ankarana is "dry tropical" with a long dry season between May and December. The wettest months are January, February, and March. There are about 86–92 rainy days per year. The hottest month is March (36.2°C) and the coldest month is June (13.5°C) (http://www.parcs-madagascar.com/ankarana/index.htm, as of 20 August 2005). All four specimens of *Tsingymantis antitra* were exclusively found at night and associated with tsingy formations. ZSM 769/2003 was found along a small brook, whereas the other three specimens were not found associated with open waters (although not far from dry riverbeds). UADBA 24766 was sitting on the ground close to the entrance of a big cave. The two other specimens were found on the top of the tsingy limestone and in a cave of ca. 1 m depth in tsingy, respectively, both close to a dry riverbed. A further individual was photographed along a brook (Paul Freed, pers. comm.). However, it remains unclear if the species is mainly distributed along streams or widespread in the tsingy formations of Ankarana.

No unidentified frog calls were heard during the surveys and only small oocytes were found in the collected females, indicating that they were reproductively quiescent when collected. The period and mode of reproduction remain entirely unknown and at current no indications of cave breeding are known. ZSM 769/2003 had remains of a large orthopteran in the stomach.

Distribution and conservation status. *Tsingymantis antitra* is only known from the Ankarana Special Reserve in northern Madagascar which has a total surface of 182 km^2 (Hawkins *et al.* 1990). The types of *Tsingymantis antitra* are from two areas in the Ankarana reserve, around the "Petit Tsingy" and around the "Campment des Anglais". The specimen photographed by P. Freed was discovered in a third area close to the "Campement des Americains" (now called "Campement d'Andrafiabe") in the west of the reserve, indicating that the species has a wider distribution in the central part of Ankarana. Connecting these three localities to a triangle allows to estimate the known extent of occurrence (see http://www.redlist.org/info/categories_criteria2001 for definition) for this species as much smaller than 100 km^2 (this approach was also used to calculate the extent of occurrence of the other Malagasy amphibian species by the Global Amphibian Assessment, see Andreone *et al.* 2005). The actual range is certainly larger, but since the tsingy-dependent fauna and flora of Ankarana includes many presumed local endemics (e.g., the snake *Alluaudina mocquardi*) it appears likely that *T. antitra* is endemic to this reserve as well although it cannot be excluded that the species also occurs in other remote tsingy formations (e. g. Tsingy de Namoroka or Tsingy de Bemaraha) or other karstic areas (e. g. Analamera reserve). Due to its small assumed extent of occurrence (< 182 km^2), its very small known extent of occurrence (< 100 km^2), its very small known area of occupancy (< 10 km^2), its apparent rareness (only four specimens have been found), and the fact that it represents a very ancient relict lineage we consider *Tsingymantis antitra* as

"endangered" although its habitat currently seems to be relatively well protected.

Molecular phylogenetic relationships. The tree shown in Fig. 3 includes for the first time representatives of all known lineages (genera, subgenera, and species groups) of mantellid frogs. Bayesian analysis of the molecular data yielded a tree (Fig. 3) with high support for most nodes. Especially the deeper nodes received much higher support than in the tree shown in Glaw & Vences (2006) which was obtained from less a complete data matrix. Maximum Bayesian support (100%) was found for all genera as defined in Glaw & Vences (2006), and for many subgenera and species groups. The included species of the Mantellinae (to the exclusion of *Tsingymantis*) were highly supported as monophyletic group as well, with 100% Bayesian and 99% bootstrap support, and the same values were obtained for the species of the Boophinae. However, the relationships between the major mantellid lineages were not significantly resolved. The analysis placed *Tsingymantis* sister to the Mantellinae, and the Laliostominae (*Aglyptodactylus* and *Laliostoma*) sister to the *Tsingymantis*/Mantellinae clade. The Boophinae (genus *Boophis*) occupied the most basal position. However, none of these groupings received Bayesian support of 95% or higher, or bootstrap support of 70% or higher, and the molecular data can therefore merely seen as weak indication of possible relationships among the major mantellid lineages. In any case, *Tsingymantis* has a very isolated position.

Discussion

Relationships of mantellid subfamilies. Despite enormous efforts in DNA sequencing, the basal relationships of the three mantellid subfamilies Mantellinae, Laliostominae and Boophinae are still unresolved. Of the three possible relationships all were suggested by analyses of molecular data, though always with only moderate support. A ML analysis of 47 mantellid species of a dataset of 1875 bp of nuclear and mitochondrial genes (Vences *et al.* 2003: Fig. 1) and an earlier study (Bossuyt & Milinkovitch 2000) revealed the relationships (Boophinae (Laliostominae, Mantellinae)), as did the analyses presented in this paper (though without significant support), and Frost *et al.* (2006). On the other hand, a ML analysis based on 2625 bp of nuclear and mitochondrial genes (Vences *et al.* 2003: Fig. 2) with fewer mantellid species suggested the relationships (Mantellinae (Boophinae, Laliostominae)). This topology was also suggested by Richards *et al.* (2000) and Glaw & Vences (2006). The relationships (Laliostominae (Boophinae, Mantellinae)) was only recently suggested by an analysis including 2995 nucleotides and more nuclear genes, especially rag-1 and rag-2 (Van der Meijden *et al.* 2005). The discovery of *Tsingymantis* and its addition to the analysis did not significantly contribute to resolve the relationships between the three subfamilies, indicating that the major mantellid lineages separated from each other rather contemporary.

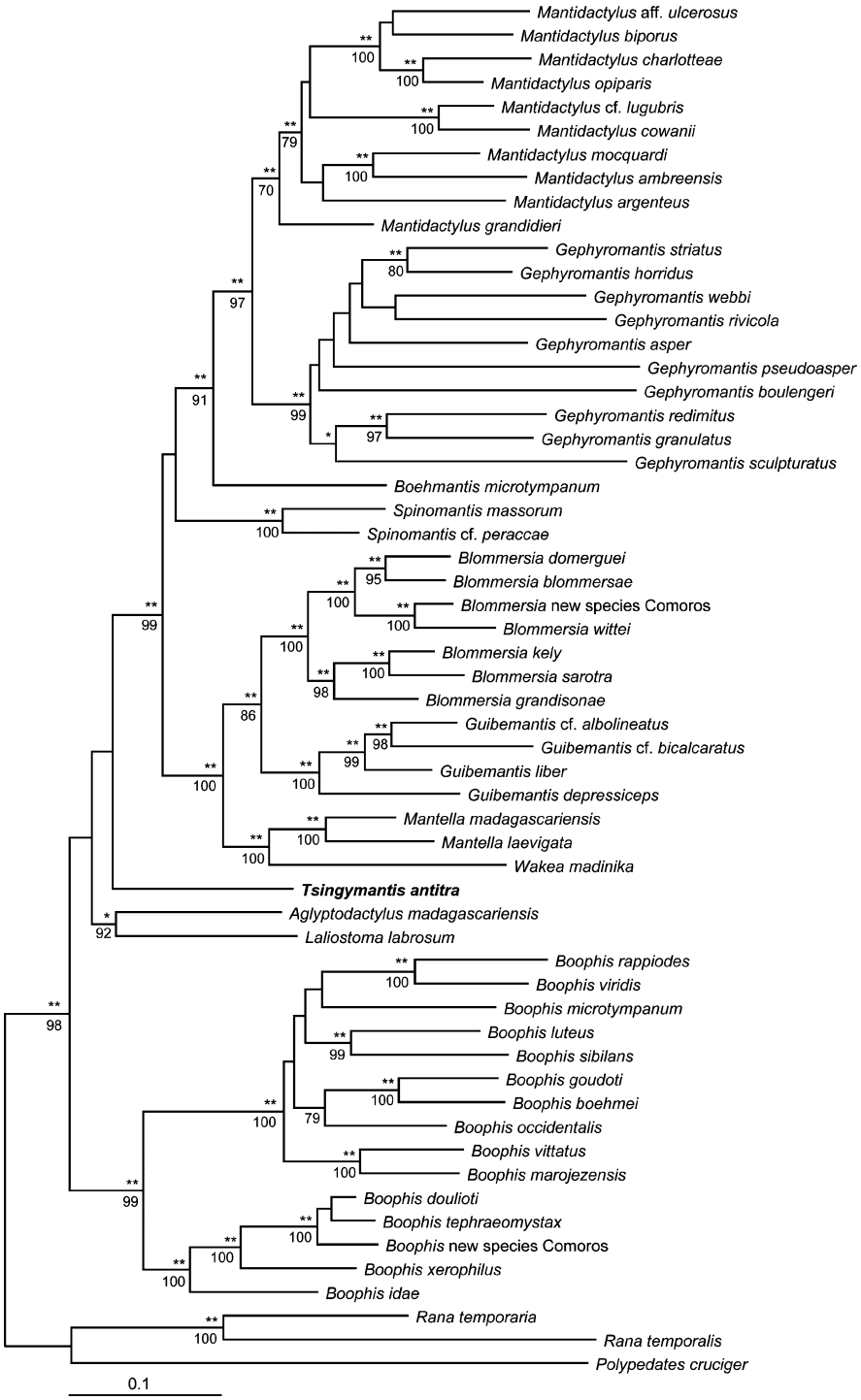

FIGURE 3. Phylogenetic tree of mantellids obtained by Bayesian Inference, based on 2303 nucleotide positions of four mitochondrial genes and one nuclear gene. Asterisks above branches indicate posterior probabilities (**=100, *=95–99). Values below branches are Maximum Likelihood bootstrap values.

Very recently Frost *et al.* (2006) considered the subfamily Laliostominae as a tribe within the subfamily Mantellinae, based on limited evidence from their molecular phylogenetic analysis (Bremer support = 21 in a very large data matrix). We here continue to recognize Laliostominae on the subfamilial level because (a) its placement sister to the Mantellinae is still controversial and, for instance, received only negligible support in the tree presented here; (b) the two lineages are separated both by very high genetic distances (see branch lengths in Fig. 3) and by distinct morphological and biological differences.

Climate change and early radiation of mantellids. Taking the topology of our molecular tree (Fig. 3) as a working hypothesis, the ancestor of the mantellid clade may have been adapted to relatively dry conditions with a reproductive mode including aquatic egg deposition in temporary stagnant water bodies with numerous small eggs per clutch as is still typical for the *Boophis tephraeomystax* group and the Laliostominae. Accepting the position of *Tsingymantis* as sister taxon of the other mantellines (which is also supported by sequences of the rag-2 gene, Hoegg unpublished) indicates that the ancestors of all three subfamilial radiations may have been adapted to dry conditions. Laliostomines as a whole show adaptations to arid environments (a large number of small eggs, explosive pond breeding), including *Aglyptodactylus madagascariensis* which lives in rainforest (Vences *et al.* 2000). The likewise pond-breeding *Boophis tephraeomystax* group is sister to a monophyletic stream-breeding group of all other *Boophis*, and within the genus is characterized by plesiomorphic features such as an anterolateral process of the hyoid and a third free tarsal in at least some species (Vences *et al.* 2002b). According to the molecular clock approach of Vences *et al.* (2003: Fig. 2) the splitting of the three major mantellid lineages might have occurred in the Eocene, some 40–50 million years ago. As a consequence of continental drift events, Madagascar experienced dry conditions from Cretaceous through Eocene times, starting in the north and moving to the south and these conditions probably filtered out nearly everything that was not drought-adapted (Wells 2003: 30). This scenario is in remarkable accordance with the early history of mantellids as suggested in Fig. 3 and with the occurrence of other putative relict amphibians (*Dyscophus*, *Scaphiophryne*) and several reptile groups (*Tracheloptychus*, *Erymnochelys*, oplurine iguanids) in dry western Madagascar (Lang 1990; Vences *et al.* 2000). The Malagasy rainforest probably originated in the Oligocene (Wells 2003). This climate change from predominantly dry to wet might have triggered the explosive radiation of those mantellines and boophines adapted to humid conditions which today include the large majority of mantellid species (more than 90%).

The continuous erosion of the limestone in tsingy areas permanently provides shelters like cracks and an extended network of caves. Such shelters with their humid microhabitats might be crucial to survive in cases of extreme dryness and might generally provide an efficient environmental buffer in periods of climate change. Finally, the subterraneous rivers that cross the Ankarana massif might warrant the persistence of waterbodies and humidity under extreme climatic conditions. Like islands, a number of

limestone massifs throughout the world are known to act as refugia for relict amphibian species. The new plethodontid salamander genus *Karsenia* from Korea (Min *et al.* 2005) was discovered in limestone habitats and the only surviving species of proteid amphibians in the palearctic region (*Proteus anguinus*) inhabits karstic limestone as well (Sket 1997). These conditions may have allowed for the survival of *Tsingymantis* as representative of one of the earliest mantellid lineages.

The shift from aquatic reproduction to the derived reproductive mode of mantellines, with terrestrial egg deposition, is unlikely to have evolved in a very dry or seasonal environment, but may have been advantageous in small subterraneous cave-like waterbodies with continuous high humidity of the air (as they occur in tsingy formations).

Due to the absence of data on the reproductive mode of *Tsingymantis* it remains unclear if this species already has evolved the derived characters which are typical for the other mantellines (evolution of femoral glands, loss of amplexus, loss of nuptial pads, and loss of release calls) or if it still retains the ancestral states found in laliostomines and boophines. Future studies of the natural history of this enigmatic taxon might therefore significantly contribute to understand the evolution of the derived reproductive biology of mantelline frogs and their morphological correlates.

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Appendix. Genbank accession numbers of DNA fragments used in molecular phylogenetic analysis.

Species	12S	16S-1	16S-2	cytb	rhod
Aglyptodactylus madagascariensis	AF249007	AY847959	AY341678	AF249068	AF249103
Blommersia blommersae	AY341584	AF317688	AY341638	DQ235418	AY341770
Blommersia domerguei	AY341582	AY848075	AY341636	DQ235416	AY341768
Blommersia grandisonae	AY454361	AF215315	AY341640	DQ901378	AY341771
Blommersia kely	AY341583	AF317690	AY341637	DQ235417	AY341769
Blommersia sarotra	AY341588	AY341704	AY341643	DQ235420	AY341773
Blommersia sp. (Comoros)	AY341585	AY330888	AY341639	AY341731	AY323742
Blommersia wittei	AY341586	AF317691	AY341641	AY341732	AY263291
Boehmantis microtympanum	DQ235455	AY848206	DQ235451	DQ901381	DQ235445
Boophis doulioti	AY341608	AY848465	AY341663	n.a.	AY341792
Boophis goudoti	AY341611	AY848572	AY341668	n.a.	AY341797
Boophis idae	AY341609	AY341715	AY341666	DQ901383	AY341795
Boophis luteus	AY341614	AJ315916	AY341671	DQ235434	AY341800
Boophis marojezensis	AY341617	AJ315923	AY341674	DQ235437	AY341803
Boophis microtympanum	AY341613	AJ315918	AY341670	DQ901384	AY341799
Boophis sp. (Comoros)	AY341610	AY341716	AY341667	AY341733	AY341796
Boophis occidentalis	AY341620	AY341720	AY341677	DQ235440	AY341806
Boophis rappiodes	AY341618	AY848664	AY341675	DQ235438	AY341804
Boophis sibilans	AY341615	AY848444	AY341672	DQ235435	AY341801
Boophis tephraeomystax	AF026344	AY848520	AY341664	AF249070	AY341793
Boophis viridis	AY341619	AJ314818	AY341676	DQ235439	AY341805
Boophis vittatus	AY341616	AY341719	AY341673	DQ235436	AY341802
Boophis xerophilus	AF249008	AF215335	AY341665	AF249069	AY341794
Guibemantis cf. *albolineatus*	AY341580	AY341701	AY341635	DQ235414	AY341766
Guibemantis cf. *bicalcaratus*	AY341581	AY848037	n.a.	DQ235415	DQ235443
Guibemantis depressiceps	AY341590	AF215326	AY341645	DQ235423	AY341775
Guibemantis liber	AY341589	AF317686	AY341644	DQ235422	AY341774
Gephryomantis boulengeri	DQ901389	DQ901391	DQ235450	DQ235442	DQ901396
Gephryomantis rivicola	DQ901388	DQ901390	DQ235449	DQ901386	DQ901395

.....continued on the next page

Appendix (continued)

Species	12S	16S-1	16S-2	cytb	rhod
Gephryomantis webbi	n.a.	DQ250677	DQ235448	DQ235441	n.a.
Gephryomantis redimitus	AY341593	AY341707	AY341648	DQ901379	AY341778
Gephryomantis granulatus	AY341594	AY848341	AY341649	n.a.	AY341779
Gephryomantis striatus	AY341595	AY848373	AY341650	n.a.	AY341780
Gephryomantis horridus	AY341596	AY341708	AF261253	n.a.	AY341781
Gephryomantis sculpturatus	AY341597	AY341709	AY341652	DQ901380	AY341782
Gephryomantis asper	AY341598	AJ314802	AY341653	DQ235426	AY341783
Laliostoma labrosum	AF249010	AF249037	AY341679	AF249096	AF249106
Mantella madagascariensis	AF249005	AF215301	AJ438892	AF249076	AY263284
Mantella laevigata	AY341607	AF215279	AJ438589	AY263292	AY263276|
Mantidactylus aff. *ulcerosus*	AF249006	AF215319	AY341654	AF249067	AF249102
Mantidactylus ambreensis	AY341603	AY848129	AY341659	DQ235431	AY341788
Mantidactylus argenteus	DQ235454	AY848130	DQ235447	DQ235421	DQ235444
Mantidactylus biporus	AY341599	AY848240	AY341655	DQ235427	AY341784
Mantidactylus charlotteae	AY341605	AY341713	AY341661	DQ901382	AY341790
Mantidactylus cowanii	AY341601	AY341711	AY341657	DQ235429	AY341786
Mantidactylus cf. *lugubris*	AY341600	AY341710	AY341656	DQ235428	AY341785
Mantidactylus grandidieri	AY341604	AY341712	AY341660	DQ235432	AY341789
Mantidactylus mocquardi	AY341602	AF261256	AY341658	DQ235430	AY341787 (*femoralis*)
Mantidactylus opiparis	AY341606	AY848289	AY341662	n.a.	AY341791
Spinomantis massorum	AY341591	AY341705	AY341646	n.a.	AY341776
Spinomantis cf. *peraccae*	AY341592	AY848415	AY341647	DQ235424	AY341777
Tsingymantis antitra	DQ901387	AY848213	DQ901392	DQ901385	DQ901393
Wakea madinika	AY341587	AY341703	AY341642	DQ235419	AY341772
Polypedates cruciger	AF249028	AF215357	AY341685	AF249089|	AF249124|
Rana temporaria	DQ283129	AF124135	AY341684	AF249078	AF249119
Rana temporalis	AF249022	AF215390	AY341683	AF249083	AF249118

