Advertisement call and tadpole morphology of the clutch-guarding frog *Mantidactylus argenteus* from eastern Madagascar

Silvia Vejarano[1], Meike Thomas[2], Frank Glaw[3] & Miguel Vences[1]*

[1]*Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Mauritskade 61, 1092 AD, Amsterdam, the Netherlands*

[2]*Institute for Genetics, Evolutionary Genetics, University of Cologne, Weyertal 121, 50931 Cologne, Germany*

[3]*Zoologische Staatssammlung, Münchhausenstr. 21, 81247 München, Germany*

Received 14 June 2005. Accepted 11 October 2005

We provide the first descriptions of the tadpole and advertisement call of *Mantidactylus argenteus*. Calls consist of 6–15 short and rather melodious notes, with a dominant frequency at 4100–4400 Hz and are emitted from arboreal positions during the day. The larva is a typical type IV tadpole, with oval body shape and dorsally positioned eyes. The oral disc is bordered with papillae, the labial tooth row formula is 2(2)/3(1–3), the upper jaw sheath is strongly curved and M-shaped, and the lower jaw is strongly curved in the middle and V-shaped. Specimens from different series present little variation of this general morphology, except some specimens with a labial tooth row formula of 2(2)/2(1–2) but the same general and oral disc morphology. Based on larval features, the position of *M. argenteus* within the subgenus *Blommersia* cannot be confirmed since only few characters are shared with other known tadpoles of this subgenus.

Key words: tadpoles, *Mantidactylus,* advertisement calls, morphology.

INTRODUCTION

Anuran larvae differ greatly from their adult form in morphology, ecology and habits (Altig & McDiarmid 1999) and this has been hypothesized to be one of the factors why frogs are such a successful group of organisms (Duellman & Trueb 1986). Therefore, studies on tadpole ecology and morphology have as much importance as those of adult frogs, but are usually hindered by the lack of reliable diagnoses and descriptions of the larvae.

Malagasy frogs show a wide variety of morphological adaptations which are especially apparent in the genus *Mantidactylus* (Blommers-Schlösser 1975, 1979; Glaw & Vences 1994; Lehtinen 2003, 2004). *Mantidactylus argenteus* is a species described more than 80 years ago (Methuen 1920), but very little is known about its biology and phylogenetic relationships. It has been tentatively included in the subgenus *Blommersia* (Dubois 1992), but shows little resemblance to other species in this subgenus. Natural history information on *M. argenteus* is scarce; Glaw & Vences (1994) reported parental care in this species, males guarding the egg clutches that are deposited on leaves hanging above rainforest streams. Such a behaviour is not found in other *Blommersia* species. Recently hatched tadpoles had a light greenish colouration and body lengths of 3–4 mm (Glaw & Vences 1994). We here complement these data by describing the advertisement call and tadpole morphology of *M. argenteus,* and discuss the relevance of these data to assess its phylogenetic relationships.

MATERIALS & METHODS

Specimens were collected in the field by opportunistic searching and by localizing calling males. Calls were recorded using a portable Tensai tape recorder with external microphone (Vivanco). They were analysed with a MEDAV sound analysing system using the software Spektro 3.2. Temporal and metric measurements are given as range, with mean ± standard deviation and number of measured units in parentheses.

Tadpoles were euthanased by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters. From each series one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. This specimen is here named 'DNA voucher'. After tissue collection, all specimens were preserved in 5% formalin. Specimens were deposited in the Zoologische Staatssammlung Munich (ZSM).

Tadpole identification followed a DNA barcoding approach based on a fragment of the mitochon-

*Author for correspondence. E-mail: vences@science.uva.nl

drial 16S rRNA gene, which is known to be sufficiently variable for species identification among Malagasy frogs (Thomas *et al.* 2005). The 550 bp fragment was amplified using standard protocols and primers 16Sa-L and 16Sb-H from Palumbi *et al.* (1991), resolved on an automated sequencer, and compared to a near-complete database of 16S sequences of adult Malagasy frog species. DNA sequences were deposited in Genbank (accession numbers DQ054489-DQ054491).

Drawings and descriptions of the tadpoles in this paper are based only on the DNA vouchers. All specimens of each series were examined to assess morphological variability, and the structures missing for the tissue sampling in the DNA vouchers were completed in the drawing based on the other specimens of the series. Developmental stages are based on Gosner (1960). The measurements taken using a stereomicroscope with measuring device, and subsequently converted into millimetres are: BL = body length (from the tip of the snout to the junction of the posterior body wall with the axis of the tail myotomes); BW = body width (the widest point of the 'head' right behind the eyes); ED = eye diameter; IOD = interorbital distance (from the centre of each pupil); TH = tail height (at the beginning of the tail); TMW = caudal muscle width (at the beginning of the tail); MTH = median tail height (including caudal fin); TMH = tail muscle height (at mid-tail); TAL = tail length; ODW = maximum oral disc width; DGMP = dorsal gap of the marginal papillae. The labial tooth row formula (LTRF) is presented in two ways: after Dubois (1995) and after Altig & McDiarmid (1999); the morphological description, in general, follows Altig & McDiarmid (1999). Mouth part features examined include: MP = number of marginal and SMP = sub marginal papillae; LMP = length of the marginal papillae; UTR = upper labial tooth row (1 is the most external); LTR = lower labial tooth row (1 is the most internal); NT = number of teeth; LLTR = length of the labial tooth row.

RESULTS

Calling males of *M. argenteus* were observed at An'Ala forest (Central Eastern Madagascar; 18°56'S, 48°28'E, altitude about 840 m), in December 1994 and February 1995, during the day in the vegetation along streams, usually at perch heights of 1.5–3 m (sometimes as low as 0.5 m). They were sitting on leaves or branches, at most a few metres from the water. The single subgular vocal sac was only slightly extended during the calls, and did not remain partly inflated between calls as sometimes occurs in other frog species. Advertisement calls were recorded at An'Ala on 20 December 1994 at an air temperature of 23°C, and on 11 February 1995 at 25°C. Calls from various males, sitting close to each other, could not unequivocally be assigned to each particular individual on the recording, largely because of the secretive and diurnal calling behaviour of these frogs which could only be seen while emitting their calls on few occasions. Calls therefore had to be pooled for analysis, but no major temporal or spectral differences were noted between those putatively from different individuals, nor between calls recorded at the two slightly different temperatures. Calls consist of 6–15 short and rather melodious notes ($n = 6$) and call duration can be up to 3210 ms (Fig. 1). Note duration was 15–42 ms (25 ± 9 ms, $n = 16$), duration of inter-note intervals was 155–249 ms (195 ± 33 ms, $n = 12$). Each note corresponded to one expiration. Two frequency bands were recognizable at 1900–2600 Hz and 3900–5500 Hz. The dominant frequency was at 4100–4400 Hz. The interval between the last and the second last note of a call can be much prolonged, and the last notes of a call can be of reduced intensity. When transporting several (3–4) male specimens in a collecting bag we heard a second note type of longer duration. The single recorded note of this type had the same frequencies as described above, but a clearly longer duration of 348 ms.

In two series of tadpoles, the DNA vouchers had 16S sequences identical to those of an adult of *Mantidactylus argenteus* from Mantadia National Park (ZSM 47/2002; Genbank accession number DQ054489), and strongly divergent from all other *c.* 700 sequences of mantellid species included in our database: ZSM 464/2004 (original field number FG/MV 2003.2009; DQ054491), a series of three specimens and ZSM 665–666/2004 (original field number FG/MV 2003.1993; DQ054490), a series of 10 specimens, all from An'Ala forest, collected on 1 March 2003 by M. Thomas, F. Glaw and M. Puente. This second series was subdivided and currently bears two separate ZSM numbers, ZSM 665/2004 and 666/2004. The DNA voucher specimen of this series is included in the sub-series ZSM 665/2004, and the following description is based on this DNA voucher specimen, a tadpole in stage 25. For morphometric data see Tables 1 and 2. Colour in preservative is brown with irregular spots on the body. The intestinal wall is also pigmented and the

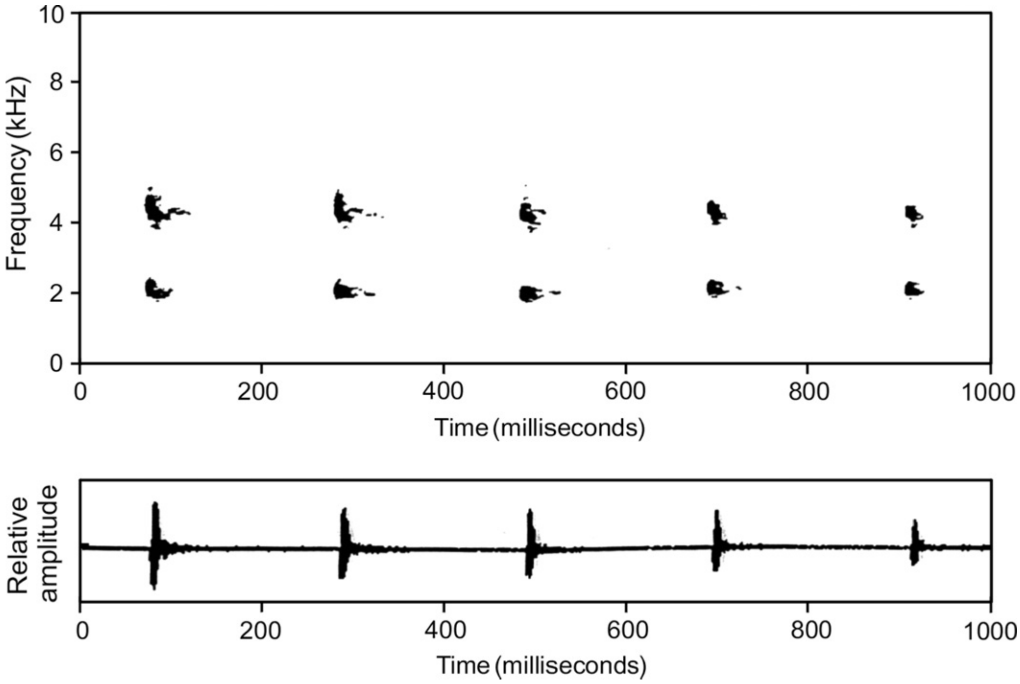

Fig. 1. Sonagram and oscillogram of a call of *Mantidactylus argenteus*, recorded at An'Ala, at an air temperature of 23°C.

intestinal spiral visible. Ventral part of the body without pigmentation. Proximal part of the tail with a continuous colourless dorsal stripe; sides of the tail strongly pigmented dorsally, and reducing in intensity towards the ventral part. Terminal portion of tail and caudal fin missing because of tissue sampling. Tadpole type IV (Orton 1953). Body shape oval. Snout in dorsal view gently pointed. Eyes medium-sized (about 0.08 of body length), dorsally positioned (Fig. 2a,b). Spiracle sinistral. Oral disc with two soft ventral emarginations (Fig. 2c, e); dorsal gap of the marginal papillae 0.88 mm; dorsal border of the oral disc almost at the front of the snout, and UTR_1 almost at this border (Fig. 2c). Oral disc bordered by 31 marginal papillae, the lateral ones being slightly shorter (0.075 mm) and more pointed than the more rounded ventral ones (0.12 mm); 1–2 submarginal papillae in each side of the upper lip, and 2–3 in the lower; two rows of submarginal papillae right below the LTR_2 (see Fig. 2d for close-up) and LTR_3 with 5–6 and 2–3 papillae, respectively. LTRF 1:1+1/3+3 (Dubois 1995) or 2(2)/3(1–3) (Altig & McDiarmid 1999); upper tooth rows with more teeth per millimetre (35–40) than the lower ones (30–34). UTR_2 is separated by the upper jaw sheath (Fig. 2c). Gap in LTR_1 and LTR_3 about three times larger than the one in LRT_2 (Fig. 2c). Jaw sheaths distinct, without any pigmentation and softly serrated edges (*c.* 80 in each jaw). Sheath of upper jaw strongly curved, M-shaped, and lower jaw strongly curved in the

Table 1. Morphometric data (in mm) of the two DNA voucher specimens of *Mantidactylus argenteus*. Some measurements on the tail could not be taken because of damage through tissue sampling.

	ZSM 665/2004	ZSM 464/2004
Stage:	25	28
BL	7.14	12.34
BW	4.00	5.78
ODW	1.38	2.05
DGMP	0.88	1.28
IOD	1.94	3.13
ED	0.63	1.09
TH	2.25	3.75
TMW	1.88	3.28
TMH	–	4.69
TAL	–	22.66
MP	0.1	0.08
UTR_1	0.95	1.08
UTR_2	0.10	0.30
LTR_1	0.25	0.33
LTR_2	0.38	0.50
LTR_3	0.13	0.08

Table 2. Number of marginal and submarginal papillae (MP, SMP) and number of labial teeth per row of two DNA voucher specimens of *Mantidactylus argenteus.*

	ZSM 665/2004	ZSM 464/2004
MP	31	29
SMP	23	21
UTR_1	34	42
UTR_2	4	8
LTR_1	8	10
LTR_2	13	23
LTR_3	4	3
LTRF	1:1+1/3+3* 2(2)/3(1–3)⁺	1:1+1/3+3* 2(2)/3(1–3)⁺

*Dubois (1995). ⁺Altig & McDiarmid (1999)

middle, V-shaped.

The series ZSM 666/2004 contains nine additional tadpoles in stage 25 except one in stage 26 and one in stage 24. Number of marginal papillae is variable (21–35). The submarginal papillae have the same arrangement as those of the DNA voucher and vary in number: 4–7 in the upper part and 6–13 below the lower tooth rows. LTRF is 1:1+1/3+3, except in two tadpoles with LTRF 1:1+1/2+2, and except the one in stage 24 where the oral disc is not completely developed yet and has only the upper tooth rows; this tadpole also differs by a much lighter colouration but has the same pattern. All other tadpoles agree in general and oral morphology with the DNA voucher. The series ZSM 464/2004 contains three tadpoles in stages 25, 26 and 28, the last-mentioned being the DNA voucher. LTRF is 1:1+1/3+3 except the one in stage 25 that has a single, central and short (four labial teeth) LTR_3. Marginal papillae 26–31; submarginal papillae have a similar arrangement in the oral disc, but the DNA voucher has only 15 such papillae below the lower tooth rows, while the other two specimens have 22 and 23. The three specimens of this series are very similar to each other in oral and general morphology.

DISCUSSION

Mantidactylus argenteus is a very unusual species among Malagasy frogs, and among frogs in general, in that it calls during the day while perched in bushes and trees. Among *Mantidactylus,* diurnal calling behaviour is regularly observed in stream-dwelling frogs of the subgenera *Brygoomantis* and *Chonomantis* (Blommers-Schlösser 1979; Glaw & Vences 1994), but these frogs call from hidden positions on the ground or at the water edge. The equally diurnal species of the *Mantidactylus boulengeri* group in the subgenus *Gephyromantis* call often from positions on the forest floor during the day, although at night they usually call from elevated perches as well. Species of the subgenus *Blommersia* often call during the day as well, but then usually from low and hidden positions. Nocturnal and arboreal calling behaviour is observed, among others, in *Mantidactylus* species of the subgenera *Guibemantis, Spinomantis* and *Phylacomantis,* which often have large and conspicuous vocal sacs that can remain partly inflated during the intervals between calls (Glaw & Vences 1994). Possibly, arboreal and diurnal calling behaviour confers a high risk of predation by optically oriented predators like birds and is therefore uncommon among frogs. The unconspicuous vocal sac of *Mantidactylus argenteus* and its cryptic green dorsal colour may have been advantageous to colonize the diurnal-arboreal bioacoustic niche in Malagasy rainforests.

With respect to tadpole morphology, the DNA voucher specimens from the two series are alike in all respects. The main variation is in the density of labial teeth; UTR_2 and LTR_1 of the specimen ZSM 665/2004 have 40 and 32 labial teeth per mm, respectively, while specimen ZSM 464/2004 has 26 and 30 labial teeth per millimetre; the number of labial teeth in the other tooth rows is similar in the two specimens. The colouration of the specimen ZSM 665/2004 is slightly lighter than the other, but has the same general pattern.

In conclusion, there is high homogeneity, not only between the two DNA voucher specimens, but also in the individuals within and among the series of *Mantidactylus argenteus,* indicating that the larval morphology described here is representative for the species. Tadpoles from other *Blommersia* species are not known except for *M. wittei* and *M. blommersae* (Blommers-Schlösser 1979); the described larvae measured 7–10 mm, respectively, 24–28 mm in total length, and had a variable labial tooth row formula of either 1:4+4/3 or 1:5+5/3. Therefore, especially their oral morphology is strikingly different from that of *M. sargenteus.* The reduced number of labial teeth of *M. argenteus* tadpoles, in combination with the unpigmented jaw sheaths, are unique among *Mantidactylus* as far as known (Blommers-Schlösser 1979; Glaw & Vences 1994), but reminiscent of larvae of the subgenus *Ochthomantis* where labial teeth are completely lacking in *Mantidactylus femoralis* (Blommers-Schlösser 1979). Vences & De la Riva (in press) have

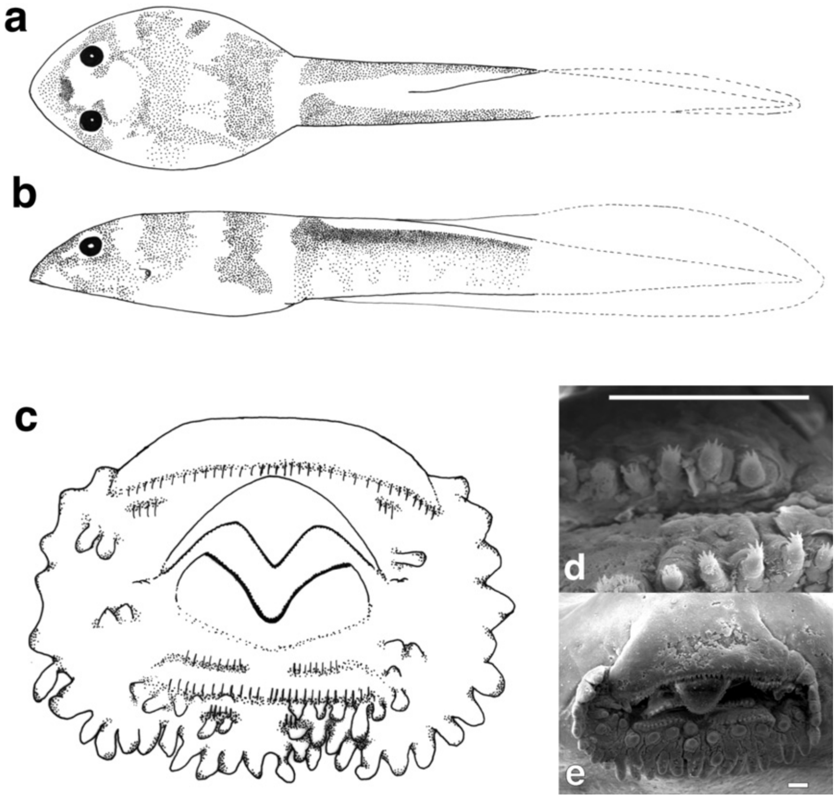

Fig. 2. Drawings of the preserved DNA voucher tadpole of *Mantidactylus argenteus* from series ZSM 665/2004, and SEM pictures of a specimen originating from the same series. **a**, Dorsal view; **b**, lateral view; **c**, oral disc. The SEM micrographs show (**d**) the labial teeth in LTR_1 and LTR_2, and (**e**) the complete oral disc. Scale bars = 100 µm.

recently observed deposition of egg clutches on leaves hanging above forest streams, and male guarding of egg clutches, in *Mantidactylus* (*Ochthomantis*) *majori*. This seems to indicate similar natural histories of *Mantidactylus argenteus* and species of the subgenus *Ochthomantis*, and indeed these relationships are confirmed by molecular markers (Glaw & Vences, in press). The tadpole morphology of *M. argenteus* as described here provides a further indication of its isolated phylogenetic position and possible relationships to *Ochthomantis*.

ACKNOWLEDGEMENTS

We acknowledge the help of Dirk Platvoet in the Zoological Museum of Amsterdam, who assisted with the SEM analyses, as well as Xu-Sen, who helped with the preparation of the material for the SEM. We are grateful to the Malagasy authorities for research and export permits. This work was supported by a grant of the Volkswagen Foundation.

REFERENCES

ALTIG, R. & McDIARMID, R.W. 1999. Body plan. Development and morphology. In: *Tadpoles: the Biology of Anuran Larvae,* (eds) R.W. McDiarmid & R. Altig, 1st edn, pp. 24–51. Chicago University Press, Chicago.

BLOMMERS-SCHLÖSSER, R.M.A. 1975. A unique case of mating behaviour in a Malagasy tree frog, *Gephyromantis liber* (Peracca, 1893), with observations on the larval development (Amphibia, Ranidae). *Beaufortia* **23**(296): 15–25.

BLOMMERS-SCHLÖSSER, R.M.A. 1979. Biosystematics of Malagasy frogs. I. Mantellinae (Ranidae). *Beaufortia* **29**(352): 1–77.

DUBOIS, A. 1992. Notes sur la Classification des Ranidae (Amphibiens, Anoures). *Bulletin de la Société Linnéenne de Lyon* **61**: 305–352.

DUBOIS, A. 1995. Keratodont formulae in anuran tadpoles: proposals for a standardization. *Journal of Zoological Systematics and Evolutionary Research* **33**: I–XV.

DUELLMAN, W.E. & TRUEB, L. 1986 *Biology of Amphibians.* McGraw-Hill, New York

GLAW, F. & VENCES, M. 1994. *A Fieldguide to the Amphibians and Reptiles of Madagascar,* 2nd edn. Vences and Glaw Verlag, Köln.

GLAW, F. & VENCES, M. In press. Phylogeny and genus-level classification of mantellid frogs. *Organic Diversity and Evolution*

GOSNER, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183–190.

LEHTINEN, R.M. 2003. Parental care and reproduction in two species of *Mantidactylus* (Anura: Mantellidae). *Journal of Herpetology* **37**: 766–768.

LEHTINEN, R.M. 2004. Test for competition, cannibalism, and priority effects in two phytotelm-dwelling tadpoles from Madagascar. *Herpetologica* **60**: 1–13.

METHUEN, P.A. 1920. Descriptions of a new snake from Transvaal, together with a new diagnosis and key of the genus *Xenocalamus,* and of some Batrachia from Madagascar. *Proceedings of the Zoological Society of London* **25**: 349–355.

ORTON, G.L. 1953. The systematics of vertebrate larvae. *Systematic Zoology* **2**: 63–75.

PALUMBI, S.R., MARTIN, A., ROMANO, S., McMILLAN, W.O., STICE, L. & GRABOWSKI, G. 1991. *The Simple Fool's Guide to PCR, Version 2.0.,* Privately published, University of Hawaii, Hawaii.

THOMAS, M., RAHARIVOLOLONIAINA, L., GLAW, F., VENCES, M. & VIEITES, D.R. 2005. Montane tadpoles in Madagascar: molecular identification and description of the larval stages of *Mantidactylus elegans, M. madecassus* and *Boophis laurenti* from the Andringitra Massif. *Copeia* **2005**: 174–183.

VENCES, M. & DE LA RIVA, I. In press. *Mantidactylus majori* (NCN). Male egg guarding. *Herpetological Review.*