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Nidicolous tadpoles rather than direct development in Malagasy frogs of the genus *Gephyromantis*

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Frogs in the genus *Gephyromantis* from Madagascar were assumed to have a direct developmental mode, i.e. the complete embryonic and larval development within the egg, but recently free-swimming, exotrophic tadpoles of a few species have been found. Herein we provide detailed morphological descriptions of the tadpoles of five more species of this genus, indicating a developmental mode other than direct development in species of *Gephyromantis*. Tadpoles of *Gephyromantis granulatus*, *G. sculpturatus*, *G. tschenki*, and *G. ventrimaculatus* were found free-swimming in streams, and tadpoles of *G.* sp. aff. *blanci* were raised after hatching from clutches found in the leaf litter. All tadpoles were identified by DNA barcoding. The oral discs of all five species are characterized by the lack of many typical morphological traits of exotrophic tadpoles (such as oral papillae and keratodonts). This indicates that these tadpoles are either non-feeding (endotrophic) or only facultatively feeding tadpoles. We classify these tadpoles as nidicolous based on the observation that the larvae of *G.* sp. aff. *blanci* stayed after hatching in the jelly nest until metamorphosis. It remains unclarified whether all species have strictly nidicolous tadpoles, and the larvae of the four species found in the streams were just accidentally washed into these streams; alternatively, some of these tadpoles might be nidicolous at first but in some species need to live in free water at later stages.

Keywords: Amphibia; *Gephyromantis*; oral disc; tadpole morphology; exotrophic; endotrophic; direct development; nidicolous development; carnivorous tadpoles; morphological clusters

Introduction

Tadpoles, the larval stages of anuran amphibians, are increasingly becoming the subject of biological research. There is a need for reliable identification of these larvae, particularly in tropical environments where amphibian diversity is highest. Tadpoles are present in aquatic habitats for longer periods than breeding adults and are often more easily collected. Understanding the diversity of tadpole morphology is a prerequisite for their successful identification. Appreciating how those morphologies are

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distributed across taxa and which tadpoles have been described in each group are important for further research (Altig and McDiarmid 1999a).

In the classification of developmental modes of amphibians, endotrophy is defined as the use of a maternal source of energy during larval development, and exotrophy is defined as the use of energy from food for development. Altig and Johnston (1989) defined a large number of developmental guilds for exotrophs, and six for endotrophs. Examples of the exotrophic developmental modes are typical lentic-benthic, filter-feeding nectonic, and carnivorous tadpoles that feed on macroinvertebrates, and conspecific and heterospecific tadpoles. The six endotrophic developmental modes proposed by Altig and Johnston (1989) are (1) viviparous (after exhaustion of vitellogenic yolk, the foetus in the oviduct feeds on oviducal materials to complete a modified development before birth as a froglet), (2) ovoviviparous (the embryo completes a modified development in the oviduct via only oogenic energy sources and is born as a froglet), (3) paraviviparous (embryo completes a modified development via oogenic energy source in a site other than the reproductive tract of the female and is "born" as a froglet), (4) exoviviparous (embryo develops via oogenic energy sources in a terrestrial egg before the hatchling moves to a site usually in or on the male parent's body, and a froglet eventually is born from that site), (5) direct development (embryo completes highly modified development via oogenic energy sources in a deposited egg that is not intimately associated with a parent's body and hatches as a froglet) and (6) nidicolous (terrestrial oviposition, and embryo develops from oogenic energy sources to produce various sorts of free-living, non-feeding larvae). Exotrophic development, in anurans, is supposed to be the ancestral reproductive mode, but endotrophic development is surprisingly common (Thibaudeau and Altig 1999).

Despite the existence of these quite precise definitions, in practice, hypotheses of endotrophic development in many species are based solely on the observation of clutches of only a few, large, usually non-pigmented ovarian eggs. Eggs of endotrophic species are usually larger than those of similarly sized frogs with exotrophic tadpoles, and are deposited in sites with sufficient moisture (Thibaudeau and Altig 1999). Based on the known relationship between egg size and pigmentation, also among species with exotrophic larvae breeding in different environments, this criterion should, however, be employed cautiously.

In the highly diverse frog fauna of Madagascar, with probably over 400 species including many as yet undescribed (Vieites et al. 2009), two clades of frogs are known to show endotrophic development: (1) the subfamily Cophylinae, a Madagascar-endemic clade of the family Microhylidae; its species have nidicolous development, with non-feeding tadpoles developing in water-filled tree holes, bamboo nodes or leaf axils, or in terrestrial jelly or foam nests (Blommers-Schlösser 1975); (2) the genus *Gephyromantis*, a species-rich genus in the Madagascar-endemic family Mantellidae, with currently over 50 species and candidate species (Blommers-Schlösser 1979; Glaw and Vences 1994; Vieites et al. 2009). Historically, and based mainly on observations of Blommers-Schlösser (1979) on eggs putatively belonging to *Gephyromantis asper*, and of Glaw and Vences (1994) on a clutch of *G. eiselti*, direct development has been stated to occur in this clade. A general prevalence of such a direct mode of development in the clade that is now considered to be the genus *Gephyromantis* (see Glaw and Vences 2006) has since generally been assumed.

However, the uniformity of developmental mode in *Gephyromantis* has been challenged already by the observation of Glaw and Vences (1994) of metamorphosing tadpoles likely to be assigned to *G. granulatus*. In addition, exotrophic tadpoles have become known from various species that, phylogenetically, are firmly embedded in the genus: *G. ambohitra* and *G. pseudoasper* have generalized and carnivorous tadpoles, respectively (Randrianiaina et al. 2007).

During a large-scale survey of tadpoles in Madagascar, based on reliable species identifications by DNA barcoding, we have been able to collect additional data on the tadpoles of *Gephyromantis*. We observed five additional species with exotrophic tadpoles: *G. asper*, *G.* sp. aff. *asper*, *G.* sp. aff. *ambohitra*, *G. corvus* and *G. azzurae*. The first three are generalized and the last two are carnivorous (see also Reeve et al. 2011). These larvae will be described in more detail in forthcoming papers. Here we focus on those tadpoles with strongly reduced oral discs and mouth openings that we hypothesize as endotrophic, and that we identified genetically as belonging to *G. granulatus*, *G. sculpturatus*, *G. tschenki*, *G. ventrimaculatus* and *G.* sp. aff. *blanci.* We describe the external morphology of these tadpoles and present data on the embryonic and larval development of *G.* sp. aff. *blanci* from a rearing experiment. The intention is to understand their developmental mode. We argue that none of these tadpoles qualifies as a direct developer and that probably only one kind of endotrophic developmental mode – nidicolous tadpoles – is present in Malagasy anurans.

Materials and methods

Tadpoles were collected using different kinds of nets having mesh sizes from 2 to 5 mm, depending on the size of stream, the strength of the current and the type of substrate. Specimens were euthanized 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 was taken and preserved in 99% ethanol. This specimen is here named "DNA voucher". All detailed morphological tadpole characterizations and drawings are based on this DNA voucher (Tables 1–3), whereas variation is described based on further specimens of the series, if such specimens exist. However, since the tadpoles described in this paper were mostly not common, in many cases, the series consist of single individuals only. After tissue collection, all specimens were preserved in 5% formalin or 70% ethanol. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM).

Tadpoles were identified using a DNA barcoding approach based on a fragment of the mitochondrial 16S ribosomal RNA (rRNA) gene, which is known to be sufficiently variable among species of Malagasy frogs (Thomas et al. 2005). The ca. 550 base pair (bp) fragment was amplified using primers 16Sar-L and 16Sbr-H from Palumbi et al. (1991) applying standard protocols (Vences et al. 2005), resolved on automated sequencers, and compared to a near-complete database of sequences of adult Malagasy frog species. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and clearly less similar to all sequences from other species. DNA sequences were deposited in Genbank (accession numbers GU975156, GU975158 and HQ188939–HQ188941).

Table 1. Morphometric measurements (all in mm) of all DNA voucher specimens described and included in this paper. For abbreviations, see *Materials and methods*.

Species	*G. granulatus*	*G. sculpturatus*	*G. tschenki*	*G. ventrimaculatus*	*G.* sp. aff. *blanci*	*G. ambohitra*	*G. pseudoasper*
Field number	Tad 2004-0075	ZCMV 4833	ZCMV 4335	ZCMV 4927	-	FG/MV 2002-1946	FG/MV 2002-1919
ZSM	298/2008	16/2008	142/2007	852/2007	649/2008	756/2004	707/2004
Site	Mt d'Ambre	Ranomafana	Ranomafana	Ranomafana	Ranomafana	Mt d'Ambre	Manongarivo
GOS	40	39	35	39	44	40	39
BL	5.8	6.0	4.6	6.4	5.3	9.7	14.0
BW	3.2	3.5	2.6	3.6	2.4	5.6	10.2
SBW	2.5	3.0	2.9	2.9	3.4	4.9	9.8
BH	2.4	2.5	2.3	3.1	2.4	4.5	7.4
SBH	3.6	3.8	2.8	4.2	3.8	6.8	9.6
ED	1.0	1.2	0.8	1.1	0.9	1.3	2.3
SE	1.5	1.4	0.9	1.5	1.2	2.5	5.1
EH	1.4	1.6	1.4	1.9	1.2	3.3	5.2
IOD	2.2	2.6	1.6	2.8	2.1	3.3	5.2
ND	0.08	0.10	0.09	0.11	0.1	0.29	0.33
NH	0.8	1.0	0.9	1.4	0.8	2.5	4.6
IND	1.2	1.2	1.1	1.3	0.2	1.6	3.2
RN	0.4	0.4	0.3	0.7	0.5	0.8	2.2
NP	1.0	1.1	0.6	0.8	0.7	1.7	2.9
SL	0.8	0.5	0.4	0.4	–	1.4	2.3
SS	3.4	3.8	2.3	3.5	–	5.5	10.3
SV	2.4	2.8	2.4	2.9	–	4.2	3.7
SH	0.9	0.9	0.9	1.0	–	1.9	3.0
VL	0.8	0.7	0.8	0.6	–	1.5	1.6
TAL	17.6	18.2	14.0	20.4	6.7	17.3	36.3
TMW	2.5	1.9	1.2	2.0	–	2.5	4.8
TMH	1.6	1.8	1.3	1.8	–	2.3	4.5
TH	1.6	2.0	1.7	1.9	–	3.9	5.7
TMHM	1.8	1.7	1.3	2.2	–	1.9	3.7
THM	2.6	2.6	2.0	3.0	–	4.0	7.3
MTH	2.7	2.6	2.2	3.0	–	4.5	7.4
DMTH	12.1	11.0	9.7	12.9	–	6.6	12.9
DF	0.3	0.3	0.3	0.4	–	1.1	2.0
VF	0.4	0.6	0.3	0.4	–	1.0	1.7
HAB	1.5	1.6	1.4	1.8	1.6	2.5	4.5
TL	20.7	21.6	16.7	24.0	9.0	22.9	46.4

Table 2. Morphometric ratios (in %) of all DNA voucher specimens described and included in this paper. For abbreviations see *Materials and methods*.

Species	*G. granulatus*	*G. sculpturatus*	*G. tschenki*	*G ventrimaculatus*	*G.* sp. aff. *blanci*	*G. ambohitra*	*G. pseudoasper*
Field number	Tad 2004-0075	ZCMV 4833	ZCMV 4335	ZCMV 4927	–	FG/MV 2002-1946	FG/MV 2002-1919
ZSM	298/2008	16/2008	142/2007	852/2007	649/2008	756/2004	707/2004
Site	Mt d'Ambre	Ranomafana	Ranomafana	Ranomafana	Ranomafana	Mt d'Ambre	Manongarivo
BW/BL	55	58	57	56	45	58	73
SBW/BL	44	50	64	45	64	51	70
BW/BH	132	138	117	117	102	123	137
SBH/BL	62	63	60	65	72	70	69
ED/BL	17	19	17	16	17	14	16
SE/BL	25	24	19	23	22	26	36
EH/BH	61	64	63	59	52	72	70
IOD/BW	68	74	62	76	86	59	51
ND/BL	1	2	2	2	2	3	2
NH/BH	33	41	40	45	34	55	62
RN/NP	42	34	55	86	70	50	76
NH/EH	55	63	65	75	66	77	89
IND/IOD	56	47	65	48	58	48	62
SL/BL	14	8	9	6	–	14	16
SS/BL	59	63	50	54	–	57	74
SH/BH	39	36	39	32	–	42	40
SH/HAB	61	56	64	54	–	75	66
VL/BL	14	11	18	10	–	15	12
TAL/BL	305	303	303	316	126	179	260
TMW/BW	80	53	47	54	–	45	47
TMH/BH	66	70	60	59	–	50	61
TMH/TH	100	88	81	94	–	60	61
TMH/MTH	58	66	60	60	–	51	61
TH/BH	66	80	73	62	–	86	77
TMHM/THM	72	66	68	72	–	48	50
TMHM/MTH	67	66	60	71	–	42	50
THM/BH	108	105	87	96	–	87	99
THM/MTH	94	100	88	98	–	89	99
MTH/BH	115	106	100	97	–	98	100
DMTH/TAL	69	61	69	64	–	38	35
DF/VF	81	61	114	86	–	110	117
DF/TMHM	18	20	25	18	–	57	54
VF/TMHM	23	32	22	20	–	52	46
HAB/BH	64	65	60	58	70	56	61

Table 3. Comparison of the oral disc characteristics of the voucher specimens described and included in this paper. JW, MCL, DG, $A_{1-5\,len}$, $P_{1-3\,len}$, A_{2gap}, A_{2len} + A_{2gap}, kerat length, MP length and SMP length are in mm. ODW/BW, UR, LR, $A_{1-5\,num}$, $P_{1-3\,num}$, MP, SMP and total number of papillae. DG/ODW, JW/ODW, MCL/JW, A_1/ODW and A_{2gap}/A_2 are in %. $A_{1-5\,den}$ and $P_{1-3\,den}$ are density (number/mm). A: ventrally, B: anteroventrally; C: absent, D: generalized, E: hypertrophied; F: very short and widely rounded, G: very short and widely pointed; H: upper labium has no connection with snout; I: upper labium is a continuation of the snout; J: small with rounded tip; K: moderately large with rounded tip; L: large with pointed tip; M: regular; not app: not applicable; abs: absent; $A_{2row+gap}$: length of A_2 including the length of the medial gap; Tot pap: total number of papillae. For abbreviations, see *Materials and methods*.

Species	*G. granulatus*	*G. sculpturatus*	*G. tschenki*	*G. ventrimaculatus*	*G.* sp. aff. *blanci*	*G. ambohitra*	*G. pseudoasper*
Field number	Tad 2004-0075	ZCMV 4833	ZCMV 4335	ZCMV 4927	–	FG/MV 2002-1946	FG/MV 2002-1919
ZSM	298/2008	16/2008	142/2007	852/2007	649/2008	756/2004	707/2004
Site	Mt d'Ambre	Ranomafana	Ranomafana	Ranomafana	Ranomafana	Mt d'Ambre	Manongarivo
ODW	0.5	0.3	0.3	0.7	1.0	2.1	3.5
LTRF	not app	not app	not app	not app	not app	5(2–5)/3(1)	1/2(1)
UR	not app	not app	not app	not app	not app	5	1
LR	not app	not app	not app	not app	not app	3	2
JW	not app	not app	not app	not app	not app	1.12	1.60
MCL	not app	not app	not app	not app	not app	0.02	0.06
DG	not app	not app	not app	not app	not app	1.58	1.03
VG	not app	not app	not app	not app	not app	abs	abs
$A_{1\,len}$	abs	abs	abs	abs	not app	1.78	0.96
$A_{2\,len}$	abs	abs	abs	abs	not app	0.87/0.84	abs
$A_{2\,gap}$	abs	abs	abs	abs	not app	0.10	abs
$A_{2\,len+A_2\,gap}$	abs	abs	abs	abs	not app	1.81	abs
$A_{3\,len}$	abs	abs	abs	abs	not app	0.69/0.65	abs
$A_{4\,len}$	abs	abs	abs	abs	not app	0.51/0.47	abs
$A_{5\,len}$	abs	abs	abs	abs	not app	0.25/0.18	abs
$P_{1\,len}$	abs	abs	abs	abs	not app	0.78/0.80	0.54/0.52
$P_{2\,len}$	abs	abs	abs	abs	not app	2.77	0.77
$P_{3\,len}$	abs	abs	abs	abs	not app	1.76	abs

Kerat length	not app	not app	not app	not app	not app	0.12	0.08
MP length	not app	not app	not app	not app	not app	0.12	0.57
SMP length	not app	not app	not app	not app	not app	0.11	0.27
ODW/BW	15	8	10	19	not app	38	34
DG/ODW	not app	not app	not app	not app	not app	75	30
VG/ODW	not app	not app	not app	not app	not app	abs	abs
JW/ODW	not app	not app	not app	not app	not app	53	46
MCL/JW	not app	not app	not app	not app	not app	2	4
A_1/ODW	not app	not app	not app	not app	not app	85	28
$A_{2\ gap}/A_{2\ len}$	not app	not app	not app	not app	not app	6	abs
$A_{1\ num}$	abs	abs	abs	abs	not app	150	40
$A_{2\ num}$	abs	abs	abs	abs	not app	62/62	abs
$A_{3\ num}$	abs	abs	abs	abs	not app	45/43	abs
$A_{4\ num}$	abs	abs	abs	abs	not app	36/33	abs
$A_{5\ num}$	abs	abs	abs	abs	not app	17/11	abs
$P_{1\ num}$	abs	abs	abs	abs	not app	53/52	24/21
$P_{2\ num}$	abs	abs	abs	abs	not app	132	40
$P_{3\ num}$	abs	abs	abs	abs	not app	143	abs
MP	abs	–	abs	abs	not app	63	39
SMP	abs	–	abs	abs	not app	5/5	72
Total papillae	abs	4	abs	abs	not app	73	111
$A_{1\ den}$	not app	not app	not app	not app	not app	84	42
$A_{2\ den}$	not app	not app	not app	not app	not app	93	abs
$A_{3\ den}$	not app	not app	not app	not app	not app	90	abs
$A_{4\ den}$	not app	not app	not app	not app	not app	70	abs

(*Continued*)

Table 3. (Continued).

Species	*G. granulatus*	*G. sculpturatus*	*G. tschenki*	*G. ventrimaculatus*	*G.* sp. aff. *blanci*	*G. ambohitra*	*G. pseudoasper*
Field number	Tad 2004-0075	ZCMV 4833	ZCMV 4335	ZCMV 4927	–	FG/MV 2002-1946	FG/MV 2002-1919
ZSM	298/2008	16/2008	142/2007	852/2007	649/2008	756/2004	707/2004
Site	Mt d'Ambre	Ranomafana	Ranomafana	Ranomafana	Ranomafana	Mt d'Ambre	Manongarivo
$A_{5\ den}$	not app	not app	not app	not app	not app	65	abs
$P_{1\ den}$	not app	not app	not app	not app	not app	66	42
$P_{2\ den}$	not app	not app	not app	not app	not app	48	52
$P_{3\ den}$	not app	not app	not app	not app	not app	81	abs
OD orientation	A	A	A	A	A	A	B
Sheaths	C	C	C	C	not app	D	E
MC	not app	not app	not app	not app	not app	F	G
Upper labium	H	H	H	H	not app	I	I
Papillae	abs	J	abs	abs	not app	K	L
Keratodont row	not app	not app	not app	not app	not app	M	M

Developmental stages were assigned following the scheme proposed by Del Pino and Escobar (1981) for endotrophic frogs. However, because of substantial differences in the development of different morphological structures in different endotrophic species, we also attempted to assign stages (GOS) according to the scheme of Gosner (1960) that is widely used for exotrophic tadpoles. Del Pino and Escobar (1981) and Gosner (1960) were used for *Gephyromantis* sp. aff. *blanci*, and only Gosner (1960) was used for *G. granulatus*, *G. sculpturatus*, *G. tschenki* and *G. ventrimaculatus*, because there is no equivalence of the developmental stage according to Gosner (1960) above 37 in the Del Pino and Escobar (1981) system. Description, morphological measurements and drawings were from pictures taken with a stereomicroscope Zeiss Discovery V12 connected to a computer, following landmarks, terminology and definitions of Altig and McDiarmid (1999b) and Randrianiaina et al. (2011), except that the term keratodonts is predominantly used instead of labial teeth. Measurements were taken digitally with Axiovision Rel. 4.8 software. The formula of keratodonts (= labial tooth row formula, LTRF) is given according to Altig and McDiarmid (1999b). Drawings and photographs of the preserved tadpoles are shown in Figures 1, 2 and 4–8. Comparing measurements, we consider them as almost equal if ratios of the measured values are 95–96% or 104–105%, as equal if they are in the range 97–103%, as almost "in the middle" if they are in the range 45–46% or 54–55%, and "in the middle" if they are in the range 47–53% (Randrianiaina et al. 2011).

The following abbreviations are used (for precise landmarks of measurements, see Figure 9 in Randrianiaina et al. 2011): A_1 (first upper keratodont row), A_2 (second upper keratodont row), A_{2gap} (length of medial gap in row A_2), A_3 (third upper keratodont row), A_4 (fourth upper keratodont row), A_5 (fifth upper keratodont row), $A_{1–5\ den}$ (density of the keratodonts in row $A_{1–5}$), $A_{1–5\ len}$ (length of the row $A_{1–5}$), $A_{1–5\ num}$ (number of keratodonts in row $A_{1–5}$), BH (maximal body height), BL (body length), BW (maximal body width), DF (dorsal fin height at mid-tail), DG (size of the dorsal gap of marginal papillae), DMTH (distance of maximal tail height from the tail–body junction), ED (eye diameter), EH (eye height – measured from the lower curve of the belly to the centre of the eye), GOS (developmental stages according to Gosner (1960)), HAB (height of the point where the axis of the tail myotomes contacts the body – measured from the lower curve of the belly), IND (inter-narial distance – measured from the centre), IOD (inter-orbital distance – measured from the centre), JW (maximal jaw sheath width), MC (medial convexity of the upper sheath), MCL (length of the medial convexity of the upper sheath), MP (marginal papillae), MTH (maximal tail height), ND (naris diameter), NH (naris height – measured from the lower curve of the belly to the centre of the naris), NP (naris-pupil distance), OD (oral disc), ODW (maximum oral disc width), P_1 (first lower keratodont row), P_2 (second lower keratodont row), P_3 (third lower keratodont row), $P_{1–3\ den}$ (density of the keratodonts in row $P_{1–3}$), $P_{1–3\ len}$ (length of the row $P_{1–3}$), $P_{1–3\ num}$ (number of keratodonts in row $P_{1–3}$), RN (rostro-narial distance), SBH (distance between snout and the point of maximal body height), SBW (distance between snout and the point of maximal body width), SE (snout–eye distance), SH (spiracle height – measured from the lower curve of the belly to the centre of the spiracle), SL (spiracle length – measured from the visible edges), SMP (submarginal papillae), SS (snout–spiracle distance), SV (spiracle–vent distance), TAL (tail length), TH (tail height at the beginning of the tail), THM (tail height at mid-tail), Thorn-pap (thorn-shaped papillae), TL (total length), TMH (tail muscle height at the beginning of the tail), TMHM (tail muscle height at mid-tail),

TMW (tail muscle width at the beginning of the tail), LR (number of the lower rows of keratodonts), UR (number of the upper rows of keratodonts), VF (ventral fin height at mid-tail), VG (size of the ventral gap of marginal papillae) and VL (vent tube length).

Results

Gephyromantis granulatus (Boettger, 1881)
(Figures 1A, 2)

Material examined

The following description refers to one tadpole in developmental stage Gosner 40 (field number Tad 2004-75 – ZSM 298/2008, BL 5.8 mm, TL 20.7 mm, accession number HQ188939) collected by R.D. Randrianiaina, M. Puente and F. Glaw on 19–23 February 2004 in Montagne d'Ambre National Park in a brook crossing the track "Voie des milles arbres" (coordinates at stream not taken, but not far from 12° 31.667' S, 49° 10.667' E, 1050 m a.s.l.). The 16S rRNA sequence of this specimen was 100% identical to the reference sequence of a *G. granulatus* adult specimen (accession AJ315926) in Genbank.

Description

In dorsal view, body ovoid, maximal body width attained between 2/5 and 3/5 of the body length (SBW 44% of BL), snout broadly rounded. In lateral view, body depressed (BW 132% of BH), maximal body height attained between 3/5 and 4/5 of body length (SBH 62% of BL), snout rounded. Eyes large (ED 17% of BL), visible from ventral view, positioned high (EH 61% of BH) laterally and directed laterally, situated between 2/10 and 3/10 of body length (SE 25% of BL). Distance between eyes wide (IOD 68% of BW). Nares small (ND 1.4% of BL), round, countersunk, positioned low (NH 33% of BH) laterally and oriented ventrally, situated nearer to snout than eye (RN 42% of NP) and below eye level (NH 55% of EH). Distance between nares moderately wide (IND 56% of IOD). Dark spot posterior to nares absent, other ornamentation absent. Spiracle sinistral, moderately large (SL 14% of BL), directed posteriorly, visible from ventral view and conspicuous from lateral view. Its inner wall absent and its aperture opens posteriorly. Opening elliptical, situated at 3/5 of body length (SS 60% of BL), located low on body (SH 39% of BH) and below height of contact of axis of tail myotomes with body (SH 61% of HAB). Vent tube medial, moderately long (VL 14% of BL), not attached to ventral fin. No dorsolateral glands visible. Tail very long (TAL 305% of BL). Maximal tail height higher than body height (MTH 115% of BH). Tail height at mid-tail higher than body height but lower than maximal tail height (THM 108% of BH and THM 94% of MTH). Tail height at beginning of tail lower than body height (TH 66% of BH). Caudal musculature well developed (TMW 49% of BW, TMH 66% of BH, TMH 100% of TH and 58% of MTH, TMHM 72% of THM, TMHM 67% of MTH). Tail muscle reaches tail tip. Fins very low (DF 18% of TMHM, VF 23% of TMHM), dorsal fin lower than ventral fin at mid-tail (DF 81% of VF). Dorsal fin originates on anterior 1/10 of tail musculature, rises progressively to attain its maximal height at maximal tail height, and then descends slightly towards tail tip. Ventral fin originates on caudal musculature just behind vent tube, increases

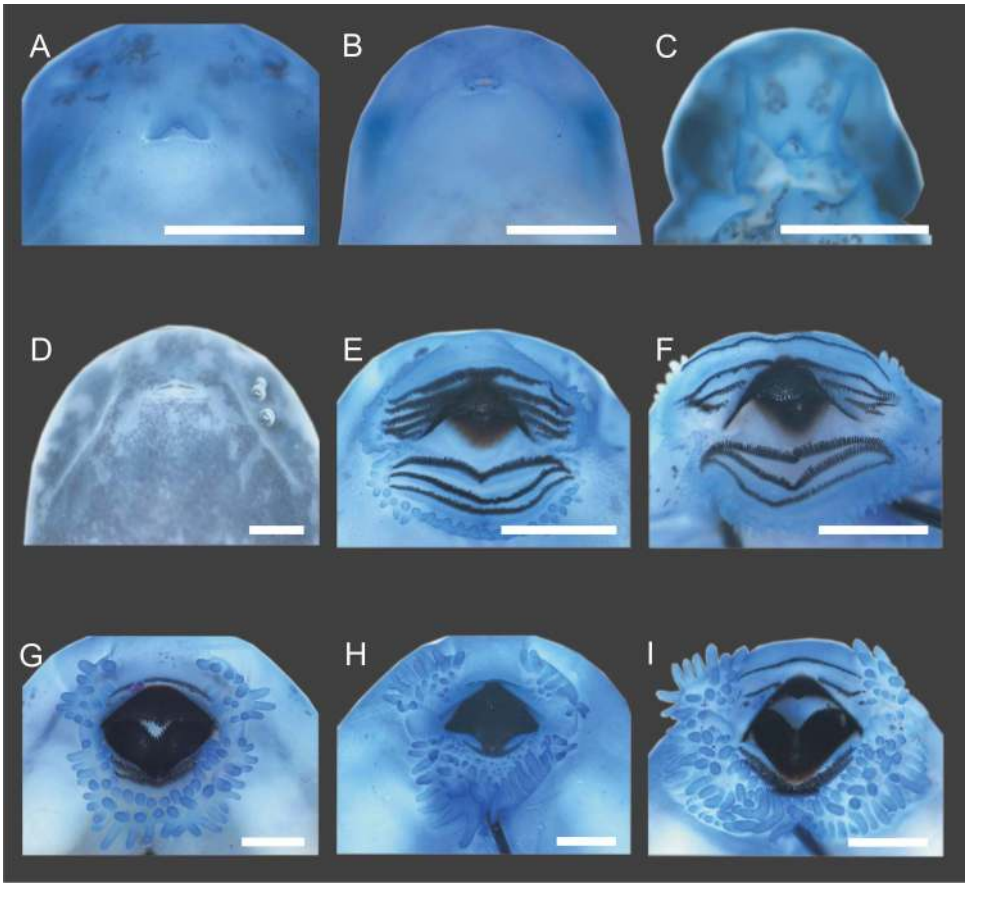

Figure 1. Photographs of the oral disc of the preserved voucher specimens of tadpoles described in this paper (stained with methylene blue). (A) *Gephyromantis granulatus* (ZSM 298/2008 – Tad 2004-75); (B) *G. sculpturatus* (ZSM 16/2008 – ZCMV 4833); (C) *G. tschenki* (ZSM 142/2007 – ZCMV 4335); (D) *G. ventrimaculatus* (ZSM 852/2007 – ZCMV 4927); (E) *G. ambohitra* (ZSM 756/2004 – FGMV 2003-1946); (F) *G. asper* (ZSM 1912/2007 – ZCMV 3401); (G) *G. azzurae* (ZSM 1922/2007 – T 2007-511); (H) *G. corvus* ZSM 0674/2008 – T 001); (I) *G. pseudoasper* (ZSM 707/2004 – FGMV 2003-1919). The scale bars represent 1 mm.

gradually to attain maximal height at maximal tail height, and then declines towards tail tip. Maximal tail height located between 3/5 and 4/5 of tail length (DMTH 69% of TAL). Caudal vein conspicuous all along tail, myosepta perceptible on anterior 1/2 of tail musculature. Point of contact of axis of tail myotomes with body located on upper half of body height (HAB 65% of BH), axis of tail myotomes parallel with axis of trunk. Tail tip narrowly rounded. Oral disc very small (ODW 15% of BW), positioned and directed ventrally, not visible from dorsal view, not connected to snout. Oral disc opening triangular. Lower labium absent, upper labium folded to form a triangular opening. Two protuberances on each base of upper labium. Papillae, jaw sheaths and keratodonts absent.

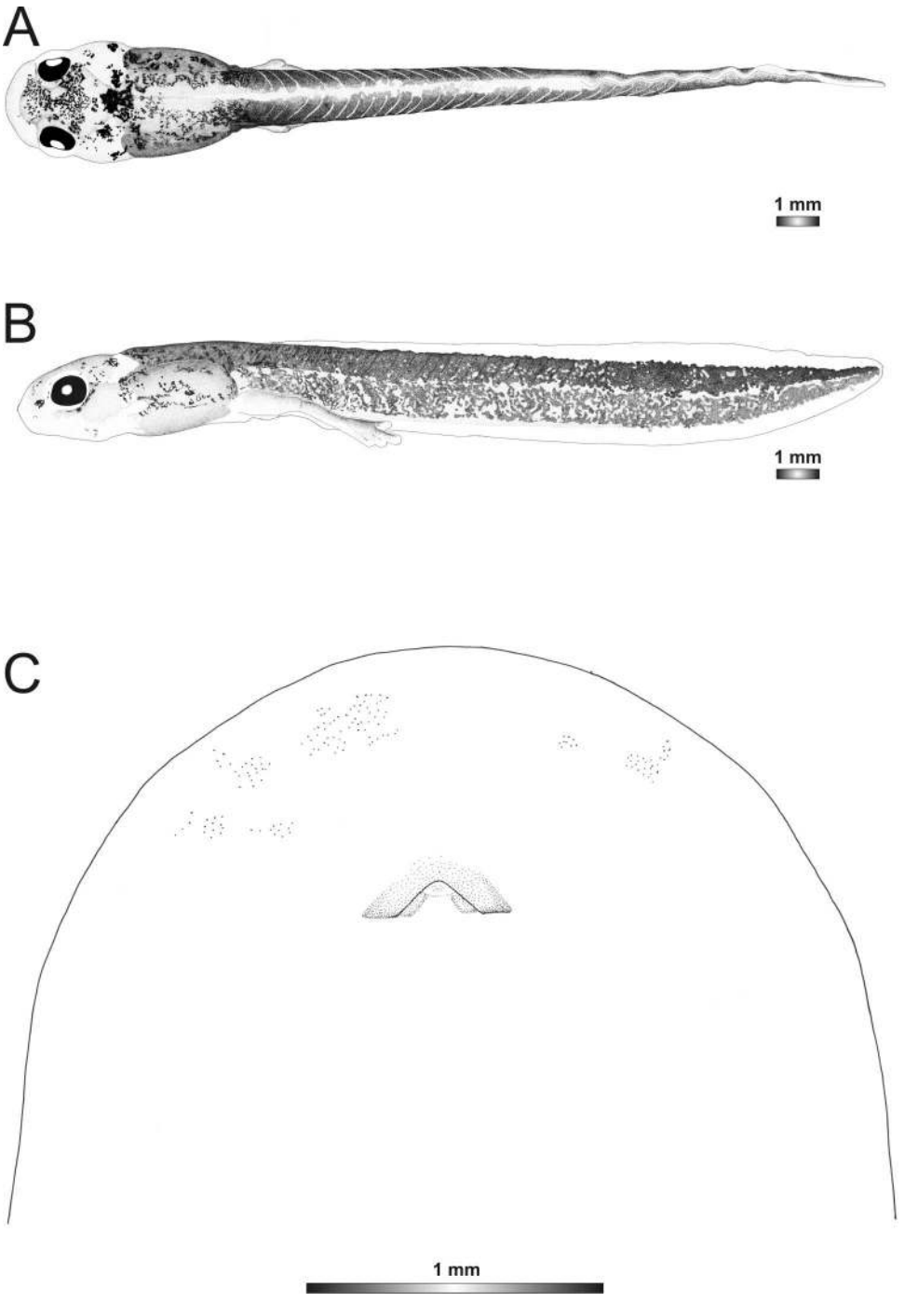

Figure 2. Drawings of the preserved DNA voucher tadpole of *Gephyromantis granulatus* (ZSM 298/2008 – Tad 2004/75). (A) Dorsal view; (B) lateral view; (C) oral disc.

Colouration in preservative

Predominantly pale brownish. Light, pale brown melanophoric pigment covers dorsum. Dark brown patches scattered irregularly on skin and condensed to form dark patches especially above neurocranium and whole dorsum. Laterally, jugal area and flank covered by light brown condensed reticulations, mainly between eye and spiracle, leaving obvious transparent spiracle on pale body wall. Tail musculature overlain by dense light brown reticulations leaving conspicuous lateral tail vein all along tail. Fins pale and unpigmented. Ventrally, oral disc, gular and branchial regions beige with a few blotches; venter covered by light brown condensed reticulations. Intestinal coils not visible.

Variation

All 19 non-DNA-voucher specimens of this series show the same external morphology as the voucher specimen.

Gephyromantis sculpturatus (Ahl, 1929)
(Figures 1B, 3A, 4)

Material examined

The following description refers to one tadpole in developmental stage Gosner 39 (field number ZCMV 4833 – ZSM 16/2008, BL 6 mm, TL 18 mm, accession number HQ188940) collected by R.D. Randrianiaina, T. Rasolonjatovo H., S.H. Ndriantsoa, E. Reeve, A. Strauß and J. Glos on 11 February 2007 in Ranomafana National Park at Piste X 175 site (21° 15.846' S, 47° 25.161' E, 966 m a.s.l.). The 16S rRNA sequence of this specimen was 100% identical to a reference sequence of a *G. sculpturatus* adult specimen (accession AY848432) from the same locality.

Description

In dorsal view, body elliptical. Maximal body width attained at mid-body (SBW 50% of BL), snout pointed. In lateral view, body depressed (BW 138% of BH), maximal body height attained between 3/5 and 4/5 of body length (SBH 63% of BL), snout broadly rounded. Eyes large (ED 19% of BL), visible from ventral view, positioned high (EH 64% of BH) laterally, directed laterally, situated between 2/10 and 3/10 of body length (SE 24% of BL). Distance between eyes wide (IOD 74% of BW). Nares small (ND 1.7% of BL), round, countersunk, positioned moderately high (NH 41% of BH) laterally, oriented ventrally, situated nearer to snout than eye (RN 34% of NP) and below eye level (NH 63% of EH). Distance between nares moderately wide (IND 47% of IOD). Dark spot posterior to nares absent, other ornamentation absent. Spiracle sinistral, small (SL 8% of BL), directed posterodorsally, visible neither from ventral nor from dorsal view, but perceptible laterally. Inner wall absent. Opening round, situated between 3/5 and 4/5 of body length (SS 63% of BL), located low on body (SH 36% of BH) and below height of contact of axis of tail myotomes with body (SH 56% of HAB). Vent tube medial, moderately long (VL 11% of BL), not attached to ventral fin. No dorsolateral glands visible. Tail very long (TAL 303% of

BL). Maximal tail height higher than body height (MTH 106% of BH). Tail height at mid-tail almost equal to body height and as high as maximal tail height (THM 105% of BH and THM 100% of MTH). Tail height at beginning of the tail lower than body height (TH 80% of BH). Caudal musculature well developed (TMW 53% of BW, TMH 70% of BH, TMH 88% of TH and 66% of MTH, TMHM 66% of THM, TMHM 66% of MTH). Tail muscle reaches tail tip. Fins very low (DF 20% of TMHM, VF 32% of TMHM), dorsal fin lower than ventral fin at mid-tail (DF 61% of VF). Dorsal fin originates on the anterior 1/4 of tail, increases progressively to attain maximal height at maximal tail height, and then descends slightly towards tail tip. Ventral fin originates on caudal musculature just behind vent tube, increases gradually to attain maximal height at maximal tail height, and then declines towards tail tip. Maximal tail height located at 3/5 of tail length (DMTH 60% of TAL). Caudal vein and myosepta not visible. Point of contact of axis of the tail myotomes with body located on upper half of body (HAB 65% of BH), axis of tail myotomes parallel with axis of trunk. Tail tip narrowly rounded. Oral disc very small (ODW 8% of BW), elliptical, positioned and directed ventrally. Four small papillae present, two each ventrolaterally of oral disc opening. Inner papillae larger (0.05 mm) than outer papillae (0.03 mm). Jaw sheaths and keratodonts absent.

Colouration in life (Figure 3A)

Typically yellowish. Dorsal skin covered by brown variegated melanophores and some silver iridophoric spots or patches. Dorsolateral and lateral regions with same pattern as dorsal. Non-pigmented spiracle perceptible. Tail musculature yellowish with irregular brown blotches grouped to form irregular patches; their density increases towards tail tip. Sporadic silver iridophoric blotches present. Fins transparent; dorsal fin with many dark patches, ventral unpigmented. Ventral side covered by silver iridophoric blotches, mainly on the venter. Oral disc and gular region transparent; branchial area reddish and beating heart visible. Venter yellow with some brown and iridophoric blotches on skin. Intestinal coils not visible.

Colouration in preservative

Largely brownish. Light, pale brown melanophoric pigment covers dorsum. Dark brown patches scattered irregularly on skin and condensed to form dark patches especially above neurocranium and vertebral region. Laterally, jugal area and flank with sporadic light brown blotches leaving a noticeable pale transparent spiracle on pale body wall. Tail musculature overlain with scattered light brown reticulations. Fins pale and with reticulations, mainly close to tail tip. Ventrally, oral disc, gular and branchial regions pale; venter covered by light brown reticulations, no intestinal coils visible.

Gephyromantis tschenki (Glaw and Vences, 2001)
(Figures 1C, 3B, 5)

Material examined

The following description refers to one tadpole in developmental stage Gosner 35, Del Pino and Escobar 23–25 (field number ZCMV 4335 – ZSM 142/2007, BL

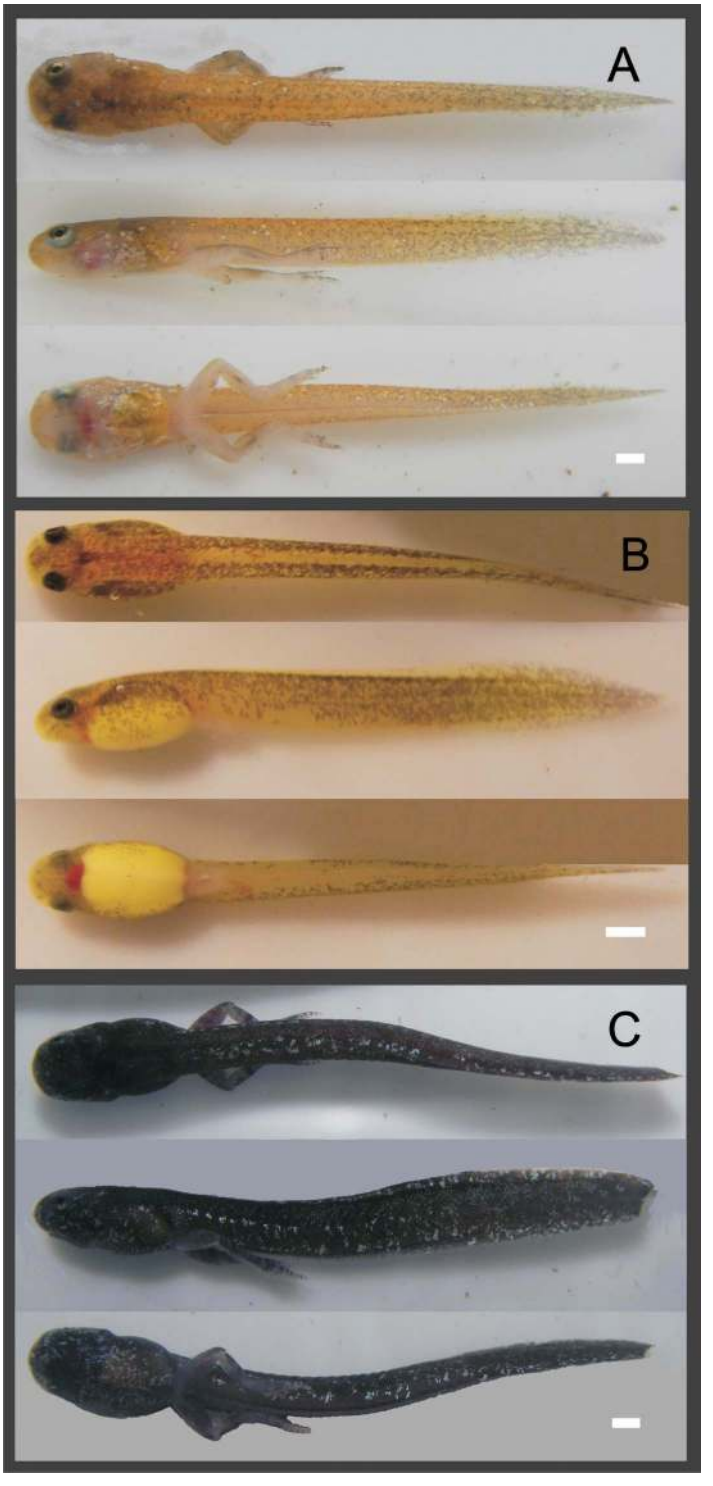

Figure 3. Colouration in life of tadpoles of three species of *Gephyromantis* in dorsal, lateral and ventral views. (A) *G. sculpturatus* (ZSM 16/2008 – ZCMV 4833); (B) *G. tschenki* (ZSM 142/2007 – ZCMV 4335); (C) *G. ventrimaculatus* (ZSM 852/2007 – ZCMV 4927). The scale bars represent 1 mm.

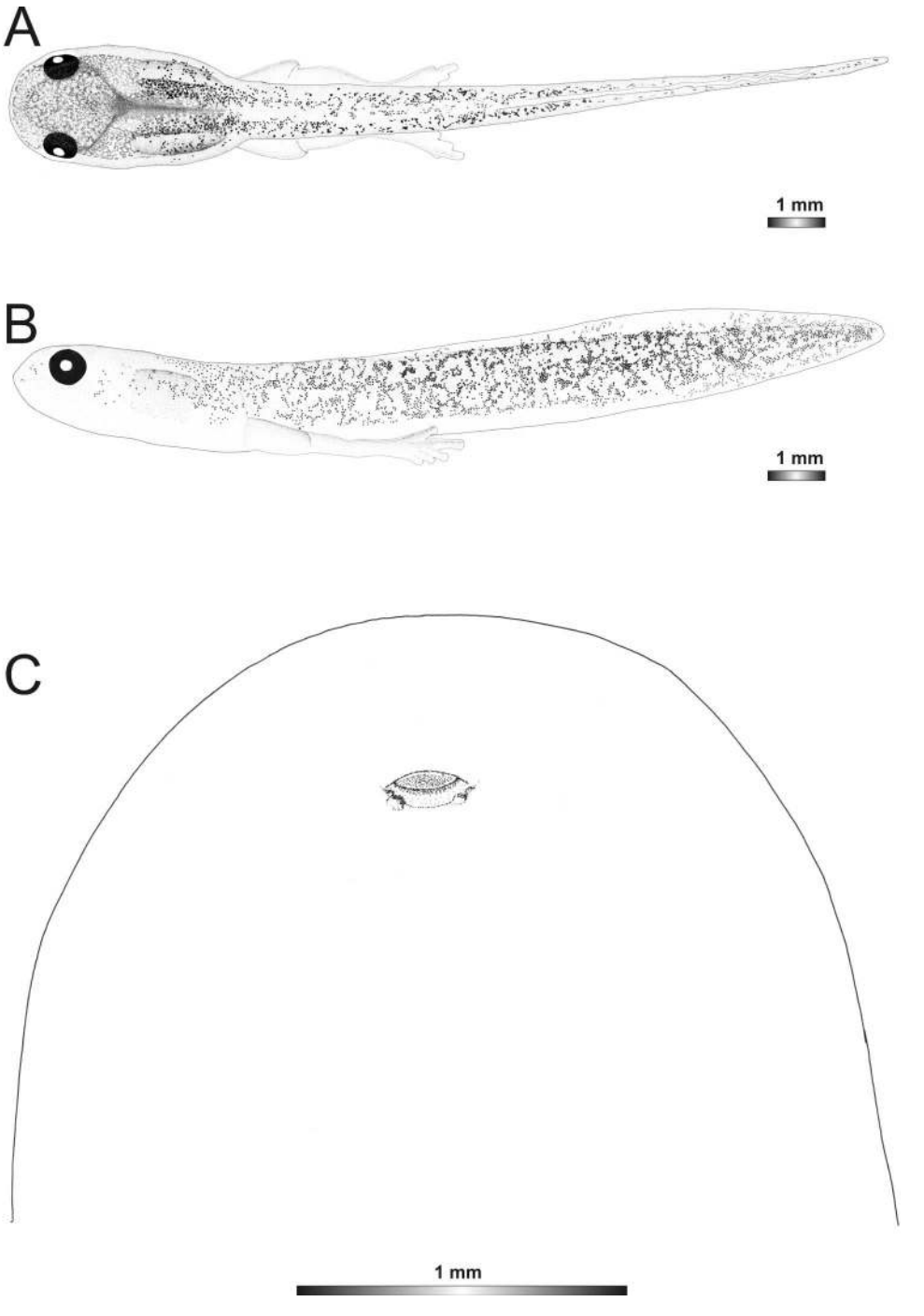

Figure 4. Drawings of the preserved DNA voucher tadpole of *Gephyromantis sculpturatus* (ZSM 16/2008 – ZCMV 4833). (A) Dorsal view; (B) lateral view; (C) oral disc.

4.6 mm, TL 16.7 mm, accession number GU975156) collected by R.D. Randrianiaina, T. Rasolonjatovo H., S.H. Ndriantsoa, E. Reeve, A. Strauß and J. Glos on 25 February 2007 in Ranomafana National Park at Bibiango site (21° 15.442' S, 47° 25.096' E, 962 m a.s.l.). The 16S rRNA sequence of this specimen was 98.5% identical to a reference sequence of a *G. tschenki* adult specimen (accession AY848374) from the same locality.

Description

In dorsal view, body elliptical, maximal body width attained between 3/5 and 4/5 of body length (SBW 64% of BL), snout broadly rounded. In lateral view, body depressed (BW 117% of BH), maximal body height attained at 3/5 of body length (SBH 60% of BL), snout pointed. Eyes large (ED 17% of BL), visible from ventral view, positioned high (EH 63% of BH) laterally, directed laterally, situated between 1/10 and 2/10 of body length (SE 19% of BL). Distance between eyes wide (IOD 62% of BW). Nares small (ND 2% of BL), round, countersunk, positioned moderately high (NH 41% of BH) laterally, oriented ventrally, situated nearer to snout than to eye (RN 55% of NP) and below eye level (NH 65% of EH). Distance between nares wide (IND 65% of IOD). Dark spot posterior to nares absent, other ornamentation absent. Spiracle sinistral, small (SL 9% of BL), directed posterodorsally, visible in ventral view and perceptible laterally. Its inner wall absent. Opening elliptical, situated at mid-body (SS 50% of BL), located low on body (SH 38% of BH) and below height of contact point of axis of tail myotomes with body (SH 64% of HAB). Vent tube medial, long (VL 18% of BL), not attached to ventral fin. No dorsolateral glands visible. Tail very long (TAL 303% of BL), maximal tail height as high as body height (MTH 100% of BH), tail height at mid-tail lower than body height and maximal tail height (THM 87% of BH and THM 88% of MTH), tail height at beginning of tail lower than body height (TH 73% of BH). Caudal musculature well developed (TMW 47% of BW, TMH 60% of BH, TMH 81% of TH and 60% of MTH, TMHM 68% of THM, TMHM 60% of MTH). Tail muscle reaches tail tip. Fins very low (DF 25% of TMHM, VF 22% of TMHM), dorsal fin higher than ventral fin at mid-tail (DF 114% of VF). Dorsal fin originates after dorsal body–tail junction, increases progressively to attain its maximal height at maximal tail height, and then descends slightly towards tail tip. Ventral fin originates on caudal musculature just behind vent tube, increases gradually to attain its maximal height at maximal tail height, and then declines towards tail tip. Maximal tail height located between 3/5 and 4/5 of tail length (DMTH 69% of TAL). Caudal vein visible on anterior 3/4 of tail, myosepta invisible. Point of contact of axis of tail myotomes with body located in upper half of body (HAB 60% of BH), axis of tail myotomes not parallel with axis of trunk. Tail tip narrowly rounded. Oral disc very small (ODW 15% of BW), positioned and directed ventrally, not visible from dorsal view and not connected to the snout. Oral disc opening triangular, lower labium absent and upper folded to form a triangular opening. Papillae, jaw sheaths and keratodonts absent.

Colouration in life (Figure 3B)

Typically yellowish. Dorsal skin covered by brown variegated melanophores. Dorsolateral and lateral regions with same pattern as dorsal part. Slightly

unpigmented spiracle perceptible. Tail musculature yellowish with irregular brown blotches grouping to form irregular networks. Their density increases towards tail tip. Fins yellow, reticulated. Ventrally, oral disc and gular region whitish with brown blotches, branchial area reddish and beating heart visible, venter yellow. Intestinal coils not visible.

Colouration in preservative

Largely brownish. Brown melanophoric pigment covers dorsum. Dark brown patches scattered irregularly across the skin and condense to form larger dark patches especially above neurocranium, vertebral and abdominal regions. Laterally, jugal area and flank with sporadic light brown blotches condensing to form reticulations. Spiracle difficult to discern. Tail musculature overlain by scattered light brown reticulations leaving lateral tail vein perceptible all along tail. Fins pale and reticulated, mainly close to tail tip. Ventrally, oral disc, gular and branchial regions pale with sporadic brown reticulations; venter pale, no intestinal coils visible.

Gephyromantis ventrimaculatus (Angel, 1935)
(Figures 1D, 3C, 6)

Material examined

The following description refers to one tadpole in developmental stage Gosner 39 (field number ZCMV 4927 – ZSM 852/2007, BL 6.4 mm, TL 20.4 mm, accession number GU975158) collected by R.D. Randrianiaina, T. Rasolonjatovo H., S.H. Ndriantsoa, E. Reeve, A. Strauß and J. Glos on 2 March 2007 in Ranomafana National Park at Sahateza site (21° 15.453' S, 47° 21.609' E, 1164 m a.s.l.). The 16S rRNA sequence of this specimen was 100% identical to a reference sequence of a *G. ventrimaculatus* adult specimen (accession FJ559200) from Ranomafana (Ranomafanakely).

Description

In dorsal view, body elliptical, maximal body width attained between proximal 2/5 and 3/5 of the body (SBW 45% of BL), snout broadly rounded. In lateral view, body depressed (BW 117% of BH), maximal body height attained between 3/5 and 4/5 of body length (SBH 65% of BL), snout rounded. Eyes large (ED 16% of BL), visible from ventral view, positioned moderately high (EH 59% of BH) laterally and directed laterally, situated between 2/10 and 3/10 of body length (SE 23% of BL). Distance between eyes wide (IOD 76% of BW). Nares small (ND 1.7% of BL), round, countersunk, positioned moderately high (NH 45% of BH) laterally and oriented ventrally, situated nearer to snout than to eye (RN 86% of NP) and below eye level (NH 75% of EH). Distance between nares moderately wide (IND 48% of IOD). Dark spot posterior to nares absent, other ornamentations absent. Spiracle sinistral, small (SL 6% of BL), directed dorsally, visible from ventral and lateral views, inner wall free from body,

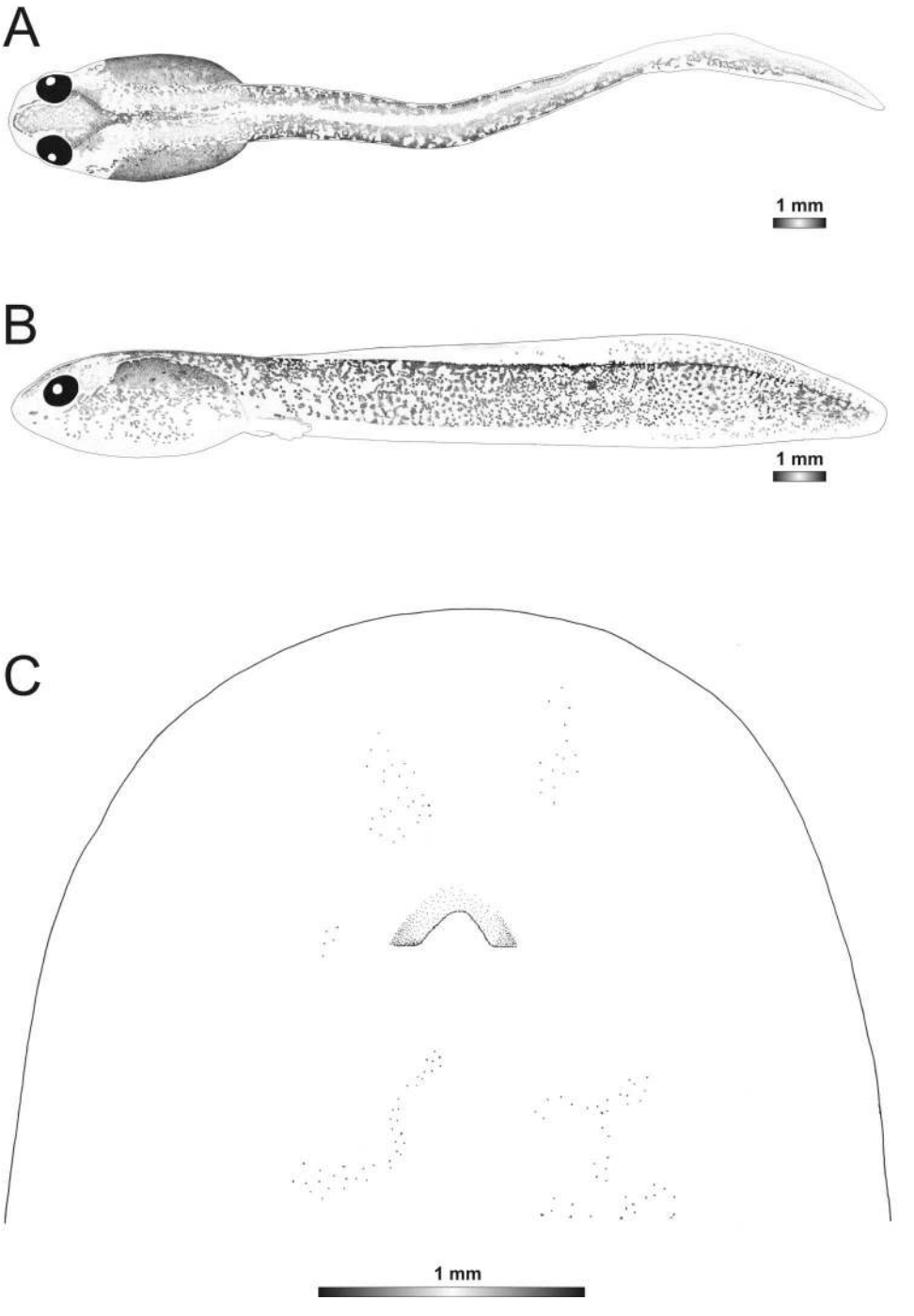

Figure 5. Drawings of the preserved DNA voucher tadpole of *Gephyromantis tschenki* (ZSM 142/2007 – ZCMV 4335). (A) Dorsal view; (B) lateral view; (C) oral disc.

aperture opens laterally. Opening ovoid, situated almost at mid-body length (SS 54% of BL), located low on body (SH 32% of BH) and below the height of the contact point of axis of tail myotomes with body (SH 54% of HAB). Vent tube medial, short (VL 10% of BL), not attached to ventral fin. No dorsolateral glands visible. Tail very long (TAL 316% of BL). Maximal tail height as high as body height (MTH 97% of BH). Tail height at mid-tail almost equal to body height and maximal tail height (THM 96% of BH and THM 98% of MTH). Tail height at beginning of tail lower than body height (TH 62% of BH). Caudal musculature well developed (TMW 54% of BW, TMH 59% of BH, TMH 94% of TH and 60% of MTH, TMHM 72% of THM, TMHM 71% of MTH). Tail muscle reaches tail tip. Fins very low (DF 18% of TMHM, VF 20% of TMHM), dorsal fin lower than ventral fin at mid-tail (DF 86% of VF). Dorsal fin originates on dorsal body–tail junction, rises progressively to attain its maximal height at maximal tail height, and then descends slightly towards tail tip. Ventral fin originates on caudal musculature just behind ventral terminus of body, increases gradually to attain maximal height at maximal tail height, and then declines towards tail tip. Maximal tail height located between 3/5 and 4/5 of tail length (DMTH 64% of TAL). Caudal vein and myosepta visible all along tail. Point of contact of axis of the tail myotomes with the body located on the upper half of the body (HAB 59% of BH), axis of tail myotomes not parallel with axis of trunk. Tail tip rather rounded. Oral disc very small (ODW 19% of BW), positioned and directed ventrally. Oral disc opening elliptical. Papillae, jaw sheaths and keratodonts absent.

Colouration in life (Figure 3C)

Typically black, covered by scattered silver iridophoric pigments.

Colouration in preservative

Largely black, area occupied by iridophoric pigment gives light pattern. Spiracle perceptible, intestine not visible.

Gephyromantis* sp. aff. *blanci
(Figures 7, 8)

Material examined

These data refer to a population of small terrestrial and diurnal frogs from Ranomafana National Park, considered as *G. blanci* by Vieites et al. (2009). However, our own unpublished molecular and bioacoustic data indicate that in fact this population represents an undescribed candidate species that we here refer to as *G.* sp. aff. *blanci*. A clutch of four eggs was collected by K.C. Wollenberg on 5 March 2007 in Ranomafana National Park, at a site locally known as Ranomafanakely (21° 14.921' S, 47° 22.307' E, 1134 m a.s.l.). Weather conditions were moist with constant rain at the time when the clutch was found. The site contained a forested slope, overgrown with lianae and moss. On the bottom of the slope, a ca. 10 cm thick layer of leaf litter covered the forest floor. Many males of *G. enki* were calling from there. At the more elevated positions of the slope, dead wood overgrown with moss was under the leaf

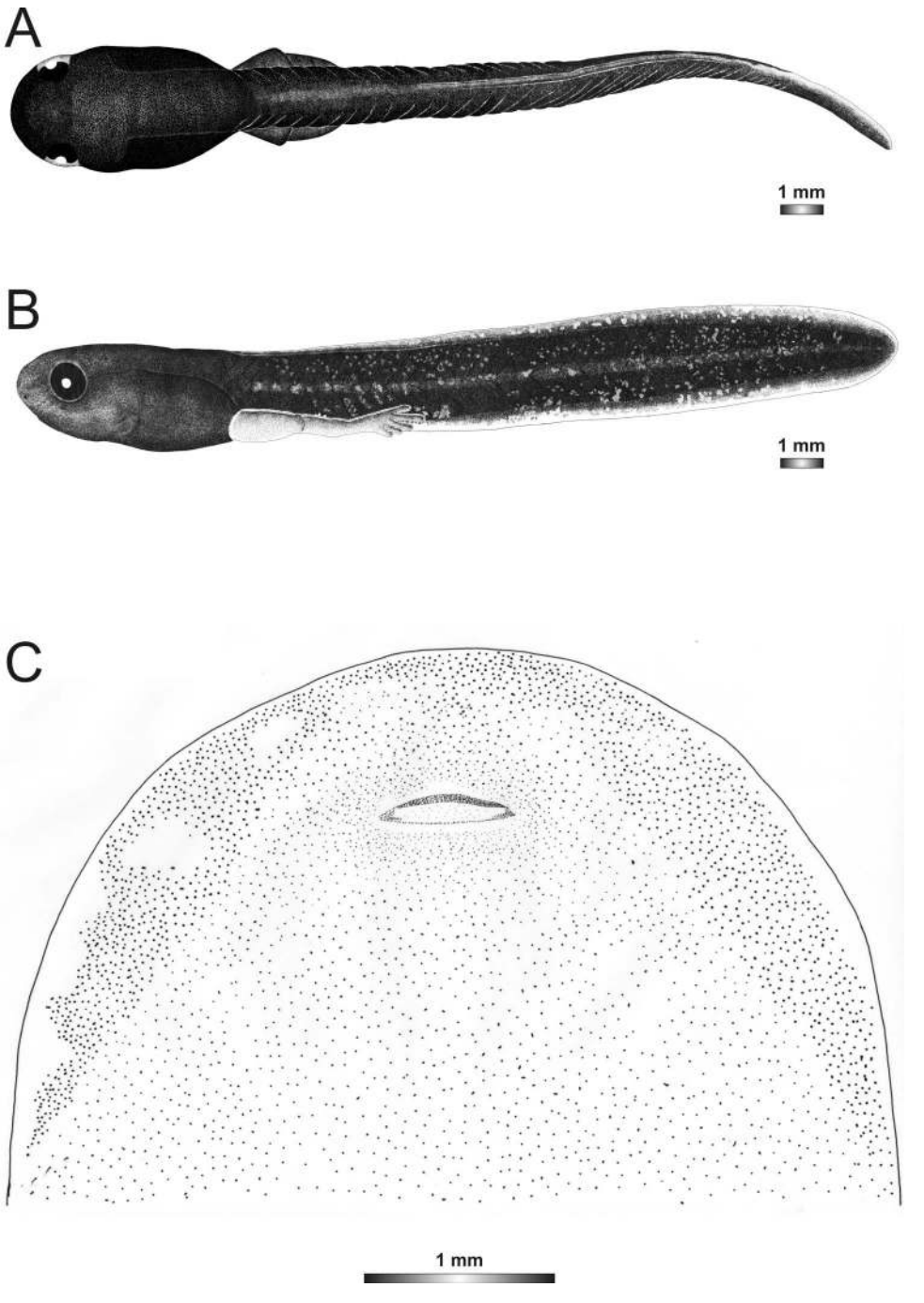

Figure 6. Drawings of the preserved DNA voucher tadpole of *Gephyromantis ventrimaculatus* (ZSM 852/2007 – ZCMV 4927). (A) Dorsal view; (B) lateral view; (C) oral disc.

litter. It was forming a thick, porous layer with many cavities of up to 1 m depth. The clutch was found on such a cavity, overgrown with moss but partially exposed to daylight and attached to the surface of a dead leaf. Rainwater was dripping on the clutch from the moss layer above it. The clutch was found while searching for a male specimen of *G.* sp. aff. *blanci* that was calling approximately 30 cm from the clutch from an elevated position. Other specimens of *G.* sp. aff. *blanci* were heard calling from other sites further up the slope. We suspect that the eggs might have been guarded by the male, as this behaviour has been observed in other species of the *G. boulengeri* group (K.C. Wollenberg personal observations). The clutch was taken to the lab, and one egg was immediately removed and preserved with field number ZCMV 5253 in 90% ethanol for species identification by DNA barcoding (accession number HQ188941). The remaining three eggs were left on the leaf, kept in a terrarium with leaf litter, and watered regularly. Their development was followed for 24 days until the last metamorph left the clutch. A single egg had a diameter of about 3 mm, the yolk being pale yellow in a transparent jelly.

Development of embryos and larvae (Figure 7)

The observation started on day 1 after collection (6 March). On this day, the embryos were visible and were a pale yellowish colour. The outline of the tail was starting to develop. They were developmental stage 17–18 according to Gosner (1960) or 13 following Del Pino and Escobar (1981).

On day 3, the embryos were weakly pigmented and the outline of the eye was visible.

On day 4, TAL reached about 120% of BL, ED about 8% of BL, and the tail had transparent fins. The beating heart was visible and the cornea was transparent. There was clear tail elongation allowing first movements (hatchling stage according to Gosner 1960; staging after DelPino and Escobar 1981 not applicable). Arteries across the yolk were formed.

On day 7, TAL was about 170% of BL, ED about 15% of BL. The toes began to differentiate and develop (indentation 4–5). The vitelline volume decreased. The cornea was visible and the dorsal body was pigmented.

On day 10, TAL was 193% of BL, ED 17% of BL. There were indentations in toes 2–3, the vitelline reduced by about 1/3 compared to day 7. The nares and the blood vessel in the posterior part of the caudal musculature were visible. The body was more pigmented and the jelly of the two tadpoles was united.

On day 11, TAL was about 180% of BL, ED 16% of BL. The transverse muscular structure was visible. The pigmentation increased laterally to ventrally and there were indentations in toes 1–2.

On days 13 and 14 (18 and 19 March), TAL was 177% of BL, ED 9% of BL, TAL 211% of BL and ED 15% of BL. Toes 3–5 were separated.

On day 16, TAL was 200% of BL, ED 12% of BL. All toes were separated and the outline of the mouth was visible.

On day 17, TAL 177% of BL, ED 15% of BL.

On days 19 and 20 TAL 210% of BL, ED 16% of BL, TAL 180% of BL and ED 15% of BL. The feet tubercles were visible.

On day 24, the last hatchling left the jelly with four well-developed legs; the tail length was about 126% of BL.

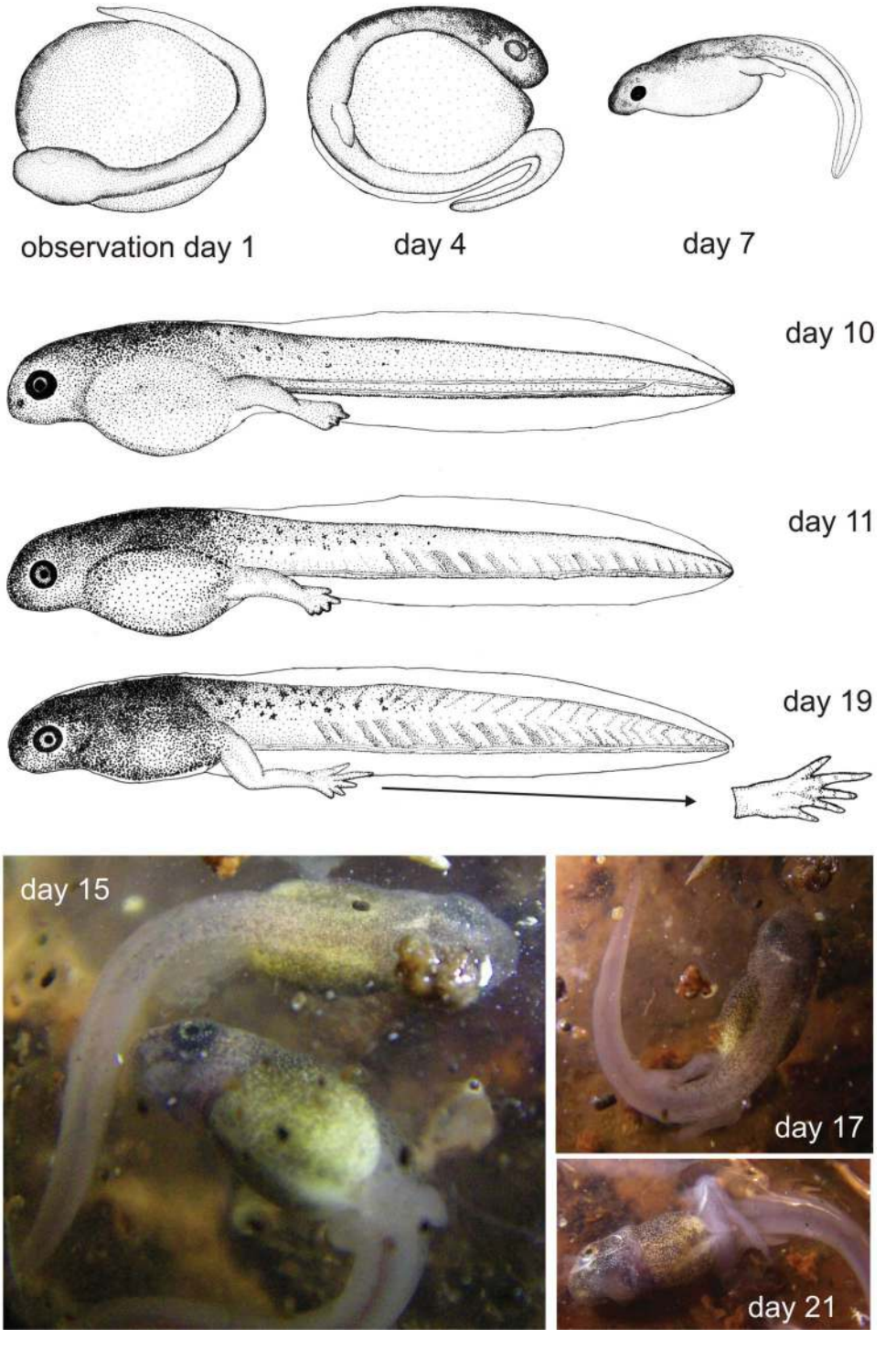

Figure 7. Larval development of *Gephyromantis* sp. aff. *blanci* from Ranomafana. Drawings were made on the basis of photographs of living specimens and are not to scale. Time is given as days after collection of the clutch; the actual time since egg deposition is unknown. Note in the photographs in ventral view that the larvae in comparatively early stages appear to have a developed mouth with jaws (no tadpole-like oral disc).

Description of metamorphosed froglet

The following description refers to one metamorphosed froglet in Gosner stage 44 (ZSM 649/2008, BL 5.3 mm TL 9 mm) from the batch described above. In dorsal view, body elliptical, maximal body width attained between 3/5 and 4/5 of body length (SBW 64% of BL), broadly rounded snout. In lateral view, body depressed (BW 112% of BH), maximal body height attained between 3/5 and 4/5 of the body length (SBH 72% of BL), snout round. Eyes large (ED 17% of BL), visible from ventral view, positioned moderately high (EH 52% of BH) laterally and directed laterally, situated between 2/10 and 3/10 of body length (SE 22% of BL). Distance between eyes wide (IOD 87% of BW). Nares small (ND 1.9% of BL), round, countersunk, positioned low (NH 34% of BH) laterally and oriented ventrally, situated nearer to snout than eye (RN 70% of NP). Distance between nares moderately wide (IND 58% of IOD). Dark spot posterior to nares absent, other ornamentation absent. Spiracle, vent tube not visible. Tail largely resorbed, therefore very short (TAL 126% of BL). Mouth opening moderately large (ODW 42% of BW), not connected to snout, positioned and directed ventrally, already being transformed into a frog mouth structure. Yellowish structure, probably the tongue, visible inside the mouth. All typical structures of tadpole oral disc absent.

Colouration in preservative

General colouration yellowish. Body dorsum, laterally, and abdominal surface covered by light brown reticulations. Gular and branchial regions brown, intestinal coils not visible, tail musculature covered by some blotches.

Discussion

Morphological diversity of Gephyromantis *larvae*

Blommers-Schlösser (e.g. 1975, 1979) first integrated life-history observations in the general systematic assessment of Malagasy anurans and, thereby, for the first time developed a classificatory system reflecting their biological (evolutionary) relationships. Species that nowadays are included in the genus *Gephyromantis* were assumed to have direct development. New observations (Glaw and Vences 1994; Vences and Glaw 2001) subsequently challenged this assumption and provided evidence for endotrophic as well as exotrophic development in this lineage. However, all these early observations were preliminary and not based on reliable identifications of the larvae or embryos examined. This changed with the work of Randrianiaina et al. (2007) who reliably reported (based on molecular species identification) exotrophy in tadpoles of *G. ambohitra* and *G. pseudoasper*, and provided detailed morphological descriptions of them.

Subsequently we have found further exotrophic tadpoles in *G. asper*, *G.* sp. aff. *asper*, *G.* sp. aff. *ambohitra* "Marojejy", *G. corvus*, and *G. azzurae* (authors' own observations, unpublished data). Some of these larvae are generalized tadpoles but the others appear to be carnivorous because of the presence of hypertrophied jaw sheaths. Carnivorous feeding as well as sound production has been demonstrated for the *G. azzurae* tadpole by Reeve et al. (2011).

Herein we provide the first detailed morphological descriptions of putatively endotrophic *Gephyromantis* tadpoles of five species that belong to three different

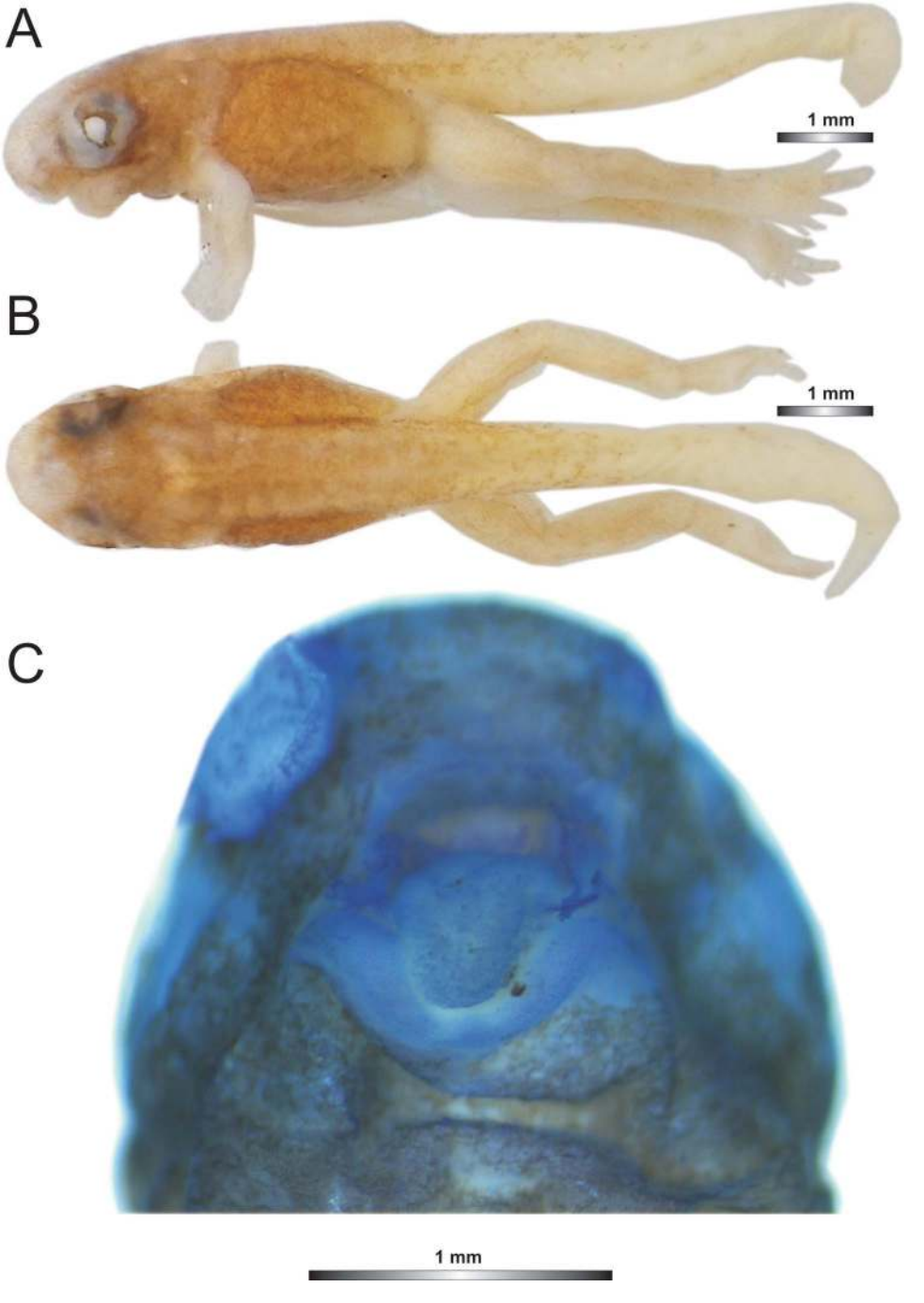

Figure 8. Pictures of the preserved DNA voucher tadpole of *Gephyromantis* sp. aff. *blanci* (ZSM 649/2008). (A) Dorsal view; (B) lateral view; (C) oral disc. Note that anterior limbs have been partly removed as tissue sample for DNA barcoding.

subgenera within the genus: *Duboimantis* (*G. granulatus*, *G. sculpturatus*, *G. tschenki*), *Laurentomantis* (*G. ventrimaculatus*) and the nominate subgenus *Gephyromantis* (*G.* sp. aff. *blanci*). The discovery and study of these larvae has yielded two main surprising insights: (1) none of these larvae had direct development, although this had been assumed at least for the subgenera *Gephyromantis* and *Laurentomantis* (e.g. Glaw and Vences 1994); and (2) the larvae of *Duboimantis* and *Laurentomantis* were found free-swimming in streams rather than in terrestrial nests.

The diversity of developmental modes within *Gephyromantis* is exceptional. Of the five existing subgenera (*Gephyromantis*, *Vatomantis*, *Laurentomantis*, *Phylacomantis* and *Duboimantis*) (Glaw and Vences 2006), the only subgenus for which no data on the larval development are available is *Vatomantis*. For one representative of this subgenus, *G. webbi,* it is known, however, that clutches of a few large eggs are deposited on rocks overhanging small streams (Andreone 1993; Glaw and Vences 1994). We therefore suspect that in this species too, endotrophic tadpoles will hatch from these eggs and complete their development free-swimming in the stream; similar to what we report here for *Laurentomantis* and *Duboimantis*. Three different ecomorphological clusters of tadpoles can thus be distinguished among the subgenera of *Gephyromantis*: the first comprises the generalized tadpoles of *G. ambohitra* (Randrianiaina et al. 2007), *G.* sp aff. *ambohitra* and *G. asper* (R.D. Randrianiaina pers. obs., unpublished data); the second comprises the carnivorous tadpoles of *G. pseudoasper* (Randrianiaina et al. 2007), *G. corvus* and *G. azzurae* (R.D. Randrianiaina pers. obs., unpublished data), and the third comprises the non-feeding tadpoles described in this study.

The first morphological cluster, "generalized", is characterized by a short tail, a moderately developed caudal musculature, a small to moderately large oral disc (ODW 38% of BW), moderately large and fully keratinized upper jaw sheaths (JW 51 to 53% of ODW), an upper jaw sheath having a very short, widely rounded medial convexity (MCL 2 to 5% of JW) and rounded serration, a half keratinized lower jaw sheath partially hidden by the upper ones, small (MP 0.12 mm, SMP 0.09 to 0.11 mm) and few papillae (MP 63 to 96, SMP 4 to 10) with rounded tips, a wide dorsal gap of papillae (DG 69 to 75% of ODW), an absence of a ventral gap of papillae, a lateral tooth row formula (LTRF) of 5(2-5)/3(1) (after Altig and McDiarmid 1999b), small keratodonts (0.12 mm), normal lower keratodont rows (not scattered as in a few other mantellids, e.g. *Mantidactylus femoralis*), and a very narrow A_{2gap} (6 to 8% of A_2). Tadpoles agreeing with this morphology have been described for *G. ambohitra* (Randrianiaina et al. 2007), and also for *G. asper*, *G.* sp. aff. *asper* and *G.* sp. aff. *ambohitra* (Figure 1; R.D. Randrianiaina unpublished data). These species have been provisionally classified in the subgenus *Duboimantis* (see Glaw and Vences 2006) but they together form a monophyletic group within the genus *Gephyromantis* whose affinities have not yet been solved (Vences and Glaw 2001; Vieites et al. 2009). Given that their developmental mode differs from other *Duboimantis*, these species should be placed in a separate subgenus.

The second morphological cluster of carnivorous tadpoles is characterized by a short tail, a moderately developed caudal musculature, a small to moderately large oral disc (ODW 34 to 42% of BW), moderately large and fully keratinized upper jaw sheaths (JW 46 to 57% of ODW), an upper jaw sheath having a very short, narrowly pointed medial convexity (MCL 3 to 4% of JW) and hypertrophied serration, a V-shaped fully keratinized lower jaw sheath partially hidden by the upper ones, large elongated (MP 0.42 to 0.57 mm, SMP 0.15 to 0.38 mm) and few papillae (MP 39 to

58, SMP 47 to 72) with rounded tips, a small to moderately wide dorsal gap of papillae (DG 30 to 48% of ODW), an absence of a ventral gap of papillae, a LTRF of 2(2)/1 and 3(2-3)/3(1) (after Altig and McDiarmid 1999b), small keratodonts (0.08 to 0.13 mm), normal lower keratodont rows, and a very narrow to very wide A_{2gap} (27 to 92% of A_2). All nominal species in the subgenus *Phylacomantis* have tadpoles agreeing with this morphology, and it is likely that similar tadpole morphology is also present in the so far undescribed candidate species assigned to this subgenus (see Vieites et al. 2009). These larval characters therefore seem to constitute synapomorphies for the clade including: *G. pseudoasper* (Randrianiaina et al. 2007), and *G. azzurae* and *G. corvus* (Figure 1; R.D. Randrianiaina unpublished data).

The third morphological cluster comprises the non-feeding tadpoles described in this study. These larvae are characterized by a small mouth opening (8 to 19% of BW) lacking the usual components of tadpole oral discs, except for *G. sculpturatus* which has four small papillae, a small body size (maximal BL 6.4, BW 3.6, BH 3.1 mm), a very long tail (TAL > 300% of BL), very low fins (DF 18–24% of TMHM, VF 20–23% of TMHM), laterally situated and directed eyes, laterally positioned and ventrally oriented nares. These putatively endotrophic *Gephyromantis* tadpoles are easily distinguished from other mantellid tadpoles by their small body size, small mouth, and their very long tail and very low fins. Their eyes are situated laterally and directed laterally. Having laterally positioned and ventrally oriented nares is unique to these tadpoles. The function of their small mouth opening is not clear, whether it is used only in gill irrigation, for air gulping, or to some degree or at some stage also for feeding. Tadpoles fitting this morphology occur in *G. granulatus*, *G. scuplturatus*, *G. tschenki* and *G. ventrimaculatus* (*Duboimantis* and *Laurentomantis*). Despite superficial morphological similarities, owing to the absence of appropriate preserved material it cannot be determined whether *G.* sp. aff. *blanci* tadpoles really can be considered to be in the same group as those of *G. sculpturatus*, *G. granulatus*, *G. tschenki* and *G. ventrimaculatus*.

Nidicolous tadpoles in Gephyromantis

Our assumption of endotrophic development in the species studied herein is based on the combination of comparatively small size, lack of visible intestinal coils and rudimentary mouthparts. However, in all of them, a mouth opening was recognizable and we thus cannot fully exclude that these larvae ingest some kind of food at some stage of larval development. However, in *G.* sp. aff. *blanci*, we did not observe food ingestion during the whole development.

Except for the larvae of *G.* sp. aff. *blanci*, which were reared from a clutch found close to an area where adult males of the species were calling, we captured all tadpoles in flowing streams and not in tree holes or terrestrial nests as is typical for the equally endotrophic cophyline microhylid tadpoles in Madagascar. We assume that these *Gephyromantis* larvae dwell some days in the stream and complete their development there, because we found series of tadpoles in stages ranging from newly hatched (without limbs) to close to metamorphosis (with four limbs but still with long tail). Since no unequivocally identified egg clutches of species in the subgenera *Duboimantis* or *Laurentomantis* have been found so far, it is at present not possible to ascertain whether it is (1) an integral part of their reproductive strategy that larvae complete their development in water, or (2) the tadpoles encountered by us

had just accidentally been washed into streams from their nests by heavy rainfall. *Gephyromantis* are semi-arboreal frogs that can be found on the forest floor but often climb onto low vegetation, and many species are regularly found in the vicinity of streams. It is obvious that the adults lay their eggs neither on leaves hanging above water bodies (Glaw and Vences 1994, 2007) like other, related, semi-arboreal or arboreal frogs in the genera *Blommersia*, *Guibemantis* and *Spinomantis* nor in any substrate close to the water, nor directly in the water. Clutches exposed on leaves would almost certainly not have passed unperceived during our intensive herpetological surveys in Ranomafana National Park and elsewhere in Madagascar.

Compared to most other tadpoles (Strauß et al. 2010), the endotrophic *Gephyromantis* tadpoles were very rare in the streams of Ranomafana National Park. Of the 7020 and 8399 tadpoles collected by us in the wet seasons of 2007 and 2008 respectively, and the 1201 tadpoles collected in the dry season of 2008, we found only six individuals of *G. ventrimaculatus*, two of *G. sculpturatus* and one of *G. tschenki* tadpoles, although at least two of these species (*G. sculpturatus* and *G. tschenki*) are common and easily observed frogs in the park. This corresponds to only 0.06% of all tadpoles sampled, with not a single one observed in the dry season. The rareness of these tadpoles supports the hypothesis that they arrive by accident in the stream, and that their normal development takes place in a nest in the leaf litter close to the stream bank, and the tadpoles are washed into the stream after heavy rains.

In contrast, at Montagne d'Ambre National Park we found 40 tadpoles of *G. granulatus* in a stream. Similar tadpoles had already been found before without intensive efforts (Glaw and Vences 1994). This supports the idea that, for this species, completing larval development in the free water of small streams is a very common event.

The observation of direct development in *Gephyromantis* (Blommers-Schlösser 1979; Glaw and Vences 1994) could not be confirmed by our data. This also applies to *G.* sp. aff. *blanci*, a representative of the subgenus *Gephyromantis*, in which males without exception call independent of streams or other types of free water. Within this subgenus, direct development has been reported by Glaw and Vences (1994) for *G. eiselti*. In *G.* sp. aff. *blanci*, the froglet did not hatch directly from the egg capsule of terrestrially deposited eggs, with the embryo developing immediately toward a frog morphotype, as is required by the definition of direct development (Altig and Johnston 1989). In this respect, the observation of Glaw and Vences (1994) should be interpreted with caution. It was based on the observations of a third person, who reared a clutch without photographically documenting the observations. We therefore hypothesize that the development in this species might have been similar to that here described for *G.* sp. aff. *blanci*, and that in fact no direct development occurs in mantellid frogs. The only remaining restriction to this hypothesis is the observation of Blommers-Schlösser (1979) of arboreal eggs from which a froglet directly hatched (purportedly of *G. asper* but almost certainly not belonging to this species; Randrianiaina et al. 2007). The existence of direct development in at least some *Gephyromantis* can therefore not be fully ruled out yet. However, we hypothesize that, in fact, nidicolous tadpoles (which sometimes become free-swimming in streams) are the only endotrophic developmental type found in *Gephyromantis* and in Malagasy frogs in general.

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