Comparative larval morphology of Madagascan toadlets of the genus *Scaphiophryne*: phylogenetic and taxonomic inferences

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The larval morphology of Madagascan frogs of the family Microhylidae, subfamilies Dyscophinae and Scaphiophryninae, is described based on material from the genera *Dyscophus* (*D. insularis*), *Paradoxophyla* (*P. palmata*) and five species of the enigmatic genus *Scaphiophryne*: *S. brevis*, *S. calcarata*, *S. madagascariensis*, *S. menabensis* and *S. spinosa*. The latter are known to have larvae that are intermediate between the filter-feeding larval type typical for most microhylids and the generalized tadpole of most ranoid and hyloid frogs. However, the two detailed descriptions available to date, referring to *Scaphiophryne calcarata* and *S. gottlebei*, pointed to important differences in size and oral morphology within *Scaphiophryne*. Our data confirm that all studied *Scaphiophryne* have horny beaks but lack keratodonts and are to be referred to the psammonektonic ecomorphological guild. *Scaphiophryne brevis* and *S. calcarata* have rather small tadpoles (up to 22 mm total length) whereas *S. madagascariensis*, *S. menabensis* and *S. spinosa*, as well as *S. gottlebei*, have larger tadpoles (up to 48 mm total length) with a striking distance between the skin and the internal organs, giving the head and body a balloon-like appearance. These two morphological tadpole groups agree with previously published molecular phylogenetic data and support the classification of these species in the two subgenera *Pseudohemisus* and *Scaphiophryne*. The larva of the genus *Paradoxophyla*, the sister group of *Scaphiophryne*, has a typical microhylid filter-feeding morphology and shares many synapomorphies with other microhylids. Since a convergent evolution of these features is unlikely, the ancestors of *Scaphiophryne* appear to have re-acquired their beak and other characters that at first view are plesiomorphic. © 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, **151**, 555–576.

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INTRODUCTION

Amphibians are an important component of the Madagascan fauna and are characterized by high species diversity and an extraordinary degree of endemicity. There are currently about 230 described and many more identified but still undescribed species (Glaw & Vences, 2003; AmphibiaWeb, 2006). As salamanders and caecilians are absent from Madagascar, the autochthonous Madagascan amphibian fauna is composed only of frogs, which belong to four families: Hyperoliidae, Mantellidae, Microhylidae and Ptychadenidae. All native species, and all native genera but one, are endemic to the region if defined as including the Comoro island of Mayotte, which harbours two endemic mantellids. This high endemism extends even to higher taxonomic levels, with one family (Mantellidae) and three subfamilies (Microhylidae:

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Cophylinae, Dyscophinae, and Scaphiophryninae) being restricted to this region. A wealth of recent studies have focused on the systematics of mantellids, hyperoliids and the Madagascan ptychadenid, *Ptychadena mascareniensis* (e.g. Richards & Moore, 1996; Bossuyt & Milinkovitch, 2000; Richards, Nussbaum & Raxworthy, 2000; Vences *et al.*, 2003a,b, 2004; Glaw & Vences, 2006; Glaw, Hoegg & Vences, 2006), whereas few novel data have become available regarding the relationships and taxonomy of the fourth anuran family represented in Madagascar, the Microhylidae. This family is represented, in the region, by three subfamilies: (1) the Dyscophinae with the single genus *Dyscophus* which has been considered to be a relative of the genus *Calluella* from Asia (Parker, 1934) until recently, but molecular data suggest that both genera are not closely related (Frost *et al.*, 2006; Van der Meijden *et al.*, 2007), (2) the probably monophyletic Cophylinae, a large and diverse radiation that colonized Madagascan rainforests and is characterized by non-feeding tadpoles developing in tree holes or terrestrial nests (Andreone *et al.*, 2004), and (3) the Scaphiophryninae defined by Blommers-Schlösser & Blanc (1991) as containing the genera *Scaphiophryne* and *Paradoxophyla*.

Although microhylid phylogeny is in general poorly resolved, the scaphiophrynines have long been especially enigmatic, mainly due to their unique larval morphology. Larvae of *Paradoxophyla* are characterized by the specialized filter-feeding morphology (Blommers-Schlösser & Blanc, 1991) defined as tadpole type II by Orton (1953). In contrast, as emphasized by Wassersug (1984), *Scaphiophryne* has an intermediate state between this and Orton's tadpole type IV (Orton, 1953) that is considered to be the generalized type of modern (neobatrachian) frogs. *Scaphiophryne* was placed within the family Ranidae until Guibé (1956) transferred them into the Microhylidae. Later, Savage (1973) proposed to include them into yet another family, the Hyperoliidae, while Dubois (1992) raised them to family rank as Scaphiophrynidae. However, as assessed by mitochondrial and nuclear DNA sequences (Van der Meijden, Vences & Meyer, 2004; Frost *et al.*, 2006), these toadlets belong with high support to a clade with other typical microhylids, although the basal relationships within this family are not yet resolved.

Historically, two separate genera were distinguished: *Scaphiophryne*, containing species with expanded terminal discs of fingers and, to a lesser degree, toes; and *Pseudohemisus*, encompassing species with undilated fingertips (Guibé, 1978). Blommers-Schlösser & Blanc (1991) recognized *Pseudohemisus* as a junior synonym of *Scaphiophryne*, a view accepted by Busse & Böhme (1992) who, however, proposed the continued use of *Pseudohemisus* as

a subgeneric name. The second scaphiophrynine genus, *Paradoxophyla*, was erected and assigned to the subfamily by Blommers-Schlösser & Blanc (1991) to accommodate a single species, *P. palmata*, that initially had been described as belonging to the Asian genus *Microhyla* by Guibé (1974). Considering the most recent revisions and descriptions (Busse & Böhme, 1992; Vences *et al.*, 2003b; Glos, Glaw & Vences, 2005; Andreone *et al.*, 2006), the following scaphiophrynine species are currently recognized: *Scaphiophryne boribory*, *S. brevis*, *S. calcarata*, *S. gottlebei*, *S. madagascariensis*, *S. marmorata*, *S. menabensis*, *S. spinosa*, *Paradoxophyla palmata*, and *P. tiarano*.

Recent studies have identified alarming trends of multicausal global amphibian declines, with over 30% of all amphibian species worldwide ranked as threatened according to IUCN criteria (Stuart *et al.*, 2004). In Madagascar, 55 of 223 evaluated species have been classified into one of the threatened categories, including also three species of *Scaphiophryne* (Andreone *et al.*, 2005). Rapid destruction and alteration of habitat are the most important factors threatening these species. Additionally, several of these toadlets are subjected to the international pet trade, which may constitute a further threat (Andreone & Luiselli, 2003; Andreone *et al.*, 2005). However, very few data are thus far available on the ecological requirements and population density of *Scaphiophryne* beyond mere distributional records (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 1994). It is remarkable that the tadpole morphology of *Scaphiophryne*, although extensively discussed in the literature, has long been known from a single species, *S. calcarata* (Blommers-Schlösser, 1975; Wassersug, 1984), with cursorial data on *S. madagascariensis* provided by Glaw & Vences (1994), and detailed information on a second species, *S. gottlebei*, published only recently (Mercurio & Andreone, 2006).

In an ongoing effort to improve the methodology for rapid assessment inventories of Madagascar's amphibian fauna, one goal is to provide basic morphological data for the identification of larval stages. By application of DNA barcoding techniques (Hebert *et al.*, 2003) based on a fragment of the mitochondrial 16S rRNA gene (Vences *et al.*, 2005a, b), we have been able to identify unambiguously the tadpoles of the majority of Madagascan amphibians, among them three species of *Scaphiophryne* and one species of *Paradoxophyla*. Tadpoles of a further two species of *Scaphiophryne*, and of the dyscophine *Dyscophus insularis*, became available through long-term studies on larval amphibian ecology in western Madagascar carried out by one of us (J.G.) over the past 7 years. Detailed examination of these specimens, and comparisons with other published data, confirmed the inter-

mediate type II–IV tadpole morphology as a common feature of all examined species of *Scaphiophryne*, but also revealed two distinct morphological subgroups within the genus. We here provide comparative morphological larval descriptions of five *Scaphiophryne*, one *Paradoxophyla* and one *Dyscophus* species, and discuss the phylogenetic and taxonomic implications of these data.

MATERIAL AND METHODS

COLLECTION OF SPECIMENS

We collected tadpoles in the field from a variety of lentic water bodies with dip nets adjusted to the specific conditions of each water body. Tadpoles were killed by immersion in chlorobutanol solution, and, by using a stereomicroscope immediately in the field, sorted into homogeneous series based on morphological characters. From each series, one specimen was selected, and from this individual, a tissue sample from its tail musculature or fin was taken and preserved in 99% ethanol. Because tadpoles of *Scaphiophryne* are immediately recognizable as such, at localities where only one species of this genus was known and no heterogeneity in shape or size was recognizable, we base our descriptions on the whole series and do not treat the DNA voucher specimen separately. For *Scaphiophryne calcarata* and *S. menabensis*, fertilized eggs from amplectant pairs were reared in plastic aquaria filled with rainwater and tadpoles were fed *ad libitum* with commercial fish food (TetraMinTabs). All specimens were preserved in 4% formalin and were eventually included in the herpetological collection of the Zoologische Staatssammlung München, Germany (ZSM). Comparative specimens were examined from the collection of the Zoological Museum Amsterdam (ZMA).

Altogether, the following materials were available (voucher specimen numbers in parentheses): *Scaphiophryne brevis*, 13 tadpoles (ZSM 617/2004-ZSM 629/2004) collected in the city of Tulear (23°24.811′S/43°45.285′E, 13 m above sea level) and 21 tadpoles (ZSM 631/2004-ZSM 644/2004), collected beside the road between Ambovombe and Fort Dauphin (24°59′32″S/46°30′23″E, *c.* 100 m above sea level); *S. calcarata*, 12 tadpoles (ZSM 410/2004), collected on 20 November 2001 at Kirindy; *S. madagascariensis*, 24 tadpoles (ZSM 595/2004-ZSM 601/2004) collected on the Andohariana plateau, Andringitra National Park (22°10′49″S/46°53′23″E; 2114 m above sea level); *S. spinosa*, 26 tadpoles (ZSM 602/2004-ZSM 616/2004) collected in Ranomafana National Park (near main Park entrance, 21°15′38″S/ 47°25′13″E, *c.* 800 m above sea level); *S. menabensis*, 13 tadpoles (ZSM 413/2004), collected on 2 February

2002 at Kirindy; *Dyscophus insularis*, eight tadpoles (ZSM 402/2004), collected on 29 January 2000 at Kirindy; *Paradoxophyla palmata*, 27 tadpoles (ZSM 645/2004-ZSM 656/2004), all collected in January and February 2003 in the region of Fierenana (18°34.900′S/48°28.128′E, 935 m above sea level), except for ZSM 565/2004 which was collected in January–February 2003 in Ranomafana National Park (at Kidonavo bridge, 21°13.497′S/47°22.213′E, 846 m above sea level). Additionally, we measured in the field body length and total length and assessed the developmental stage (Gosner, 1960) of 16 tadpoles of *Scaphiophryne calcarata* and 372 tadpoles of *Dyscophus insularis* at Kirindy. These tadpoles were subsequently released.

MOLECULAR IDENTIFICATION

Tadpoles were identified using a DNA barcoding approach based on a fragment of the mitochondrial 16S rRNA gene known to be sufficiently variable among species of Malagasy frogs (Thomas *et al.*, 2005). The fragment has a length of about 550 base pairs and was amplified with primers 16Sar-L and 16Sbr-H from Palumbi *et al*. (1991) and standard protocols. Sequences were resolved on automated sequencers and compared with a nearly complete database of sequences of adult Malagasy frog species which contains reference sequences of all scaphiophrynine species. Because the 16S rRNA gene sequence differences between *Scaphiophryne* species are relatively low due to their apparently slow mitochondrial substitution rate (Vences *et al.*, 2002; Glos *et al.*, 2005), we applied a strict criterion, with identification considered to be unequivocal only when the tadpole sequence was 100% identical to that of a reliably identified adult specimen. DNA sequences newly obtained in this study were deposited in GenBank (accession numbers DQ787110-DQ787114).

MORPHOLOGICAL EXAMINATION

Morphological terminology follows Altig & McDiarmid (1999) and developmental stages were determined according to Gosner (1960). Measurements were taken with a graduated ocular attached to a stereomicroscope except for the total length which was measured with a hand caliper to the nearest 0.1 mm. The landmarks are those shown in Altig & McDiarmid (1999: 26, fig. 3.1.); for others see Grosjean (2001). Drawings were made with the aid of a camera lucida.

The abbreviations used in the descriptions are as follows: BH, maximum height of body; BL, body length; BW, maximum width of body; DG, maximum size of dorsal papilla gap of the submarginal row; ED, maximum diameter of eye (and not the diameter of the eye-

ball formed by skin); LF, maximum height of lower tail fin; MTH, maximum tail height; NN, internarial distance; NP, naro-pupilar distance; ODW, oral disc width; PP, interpupilar distance; RN, rostro-narial distance; SS, distance from tip of snout to opening of spiracle; SU, distance from snout to beginning of upper tail fin; TL, total length; TMH, tail muscle height; UF, maximum height of upper tail fin. In *S. brevis* and *S. calcarata*, body height comprises the height of the upper tail fin which extends on the back as exact delimitation between body proper and fin was not possible.

Preparation for SEM examination (with a JEOL JSM-840A) comprised dehydration in a graded ethanol series, critical-point drying (liquid carbon dioxide) and gold sputter surface coating. Terminology of buccal structures follows Wassersug (1976).

The pictures of the oral discs were made from other individuals because the preparation for SEM examination requires destructive sampling.

RESULTS

Based on tadpoles obtained by rearing and molecular identification, we provide larval descriptions of seven species of microhylid frogs, five belonging to the genus *Scaphiophryne*, one to the genus *Dyscophus* and one to the genus *Paradoxophyla*. Within the genus *Scaphiophryne*, we identified a group of species possessing small tadpoles (*S. brevis* and *S. calcarata*) and a second group with large tadpoles (*S. madagascariensis*, *S. spinosa* and *S. menabensis*). This section gives detailed morphological description of one tadpole of the small tadpole group (*S. brevis*), an abbreviated description for *S. calcarata*, a detailed morphological description of one tadpole of the large tadpole group (*S. madagascariensis*) and abbreviated descriptions for the two other species of this group. A detailed description is provided for both *Dyscophus insularis* and *Paradoxophyla palmata*. The abbreviated descriptions summarize only the differences from the detailed morphological description of reference. TL and BL are provided for each available stage and for each species (Table 1).

SCAPHIOPHRYNE MADAGASCARIENSIS
(BOULENGER, 1882)

Specimens were collected in a shallow, ephemeral pond in the grasslands of the Andohariana plateau, Andringitra National Park. The bottom of the pond was covered with grass and the maximum depth was about 30–40 cm. The water was clear and cold. The description of external morphology was based on a specimen at a young stage 35, ZSM 595/2004 (TL and BL are 23.0 mm and 10.0 mm, respectively). Buccopharyngeal features are described based on a tadpole at stage 38, ZSM 599/2004.

External morphology: In dorsal view (Fig. 1A), body ovoid, widest at the level of gills, snout truncate. In profile (Fig. 1B), body depressed, almost flat below, BW 124% of BH, snout small and round. Eyes moderately small, ED 14% of BL, slightly bulging (caused by the presence of a space between the outer integument and the organs), not visible in ventral view, positioned more dorsally than dorsolaterally and directed laterally. Nares not open, positioned dorsally, closer to pupils than to snout, RN 127% of NP, very close to each other, NN 26% of PP. Spiracle sinistral but very low, very slightly conical, moderately large, entirely attached to body wall, inner wall absent, orientated almost posteriorly, closer to end of body than to tip of snout, SS 76% of BL; spiracular opening crescent-shaped, closer to the level of the opening of ventral tube than to the insertion of hindlimb. Tail musculature moderately weak, TMH 34% of BH and 33% of MTH, its maximum height reached before the proximal third of caudal muscle, then gradually tapering, not reaching tail tip. Tail fins of moderate height, UF 35% of MTH, LF 37% of MTH, convex, upper fin not extending onto body, SU 93% of BL; point of maximum height of tail located before the middle of tail length, MTH 102% of BH, tail tip round. Ventral tube moderately large, medial, conical but its posterior part folded against ventral fin, directed posteroventrally, its posterior part linked to ventral tail fin, opening medial. Neither lateral line organs nor glands visible.

Oral disc (Fig. 2A) in position and orientation subterminal, emargination very low, of moderate size, ODW 27% of BL and 39% of BW. An uninterrupted row of marginal papillae; a few submarginal papillae on a row laterally on the upper labium, a double row on the lower labium, the external one being interrupted shortly medially, a small group of smaller submarginal papillae at point of emargination on each side; marginal and submarginal papillae round, large, stocky, some of them blunt, those on the top of upper labium very small. No denticulate papillae. No keratodonts. Jaw sheaths of moderate breadth, very finely serrated; upper jaw sheath a large arch, flat on the most part with a weak median convexity, white; lower jaw sheath V-shaped, its distal third white, its proximal two-thirds light brown.

Coloration in preservative: Tadpole transparent, all underlying organs visible. External integument of upper side transparent except the snout, which is speckled with light brown; underlying tissues densely speckled with spots of the same colour. Flanks speckled in the same way as upper side, but with a dorsoventral gradation. Ventral side immaculate, except the part anterior to gills slightly speckled and the digestive tract brown coloured. Oral disc brown. Caudal

Table 1. Total length (TL) and body length (BL) of the tadpoles of *Scaphiophryne madagascariensis, S. spinosa, S. menabensis, S. brevis, S. calcarata, Dyscophus insularis* and *Paradoxophyla palmata* for the developmental stages 25–40

Stage	*S. madagascariensis* BL	*S. madagascariensis* TL	*S. spinosa* BL	*S. spinosa* TL	*S. menabensis* BL	*S. menabensis* TL	*S. brevis* BL	*S. brevis* TL	*S. calcarata* BL	*S. calcarata* TL	*Dyscophus insularis* BL	*Dyscophus insularis* TL	*Paradoxophyla palmata* BL	*Paradoxophyla palmata* TL
25					4.0 (1)	10.2 (1)			4.3 (1)	10.9 (1)	5.8 ± 1.4 (3.8–11.2) 214	14.2 ± 4.0 (9.0–27.0) 197	4.5 ± 0.7 (3.3–5.2) 9	
26					5.3 (1)	12.6 (1)					6.5 ± 0.5 (5.8–7.3) 9	18.8 ± 1.6 (16.8–19.0) 7		
27			10.0 ± 0.4 (9.5–10.4) 3	24.6 ± 1.4 (23.0–25.5) 3	6.1 (6.1–6.2) 2	15.2 (14.4–16.1) 2			4.6 (1)	12.4 (1)	7.2 ± 0.7 (6.0–8.5) 28	19.6 ± 3.1 (16.0–28.3) 26		
28	9.2 (2)	19.7 ± 0.9 (19.0–20.3) 2	10.0 ± 1.2 (8.7–10.9) 13	22.8 ± 1.2 (20.6–23.9) 6	7.0 ± 0.2 (6.8–7.2) 3	17.8 ± 0.5 (17.4–18.4) 3	6.1 (1)	16.8 (1)			7.6 ± 0.7 (5.3–8.5) 17	18.6 ± 4.8 (11.0–23.5) 15		
29	9.7 (1)	20.0 (1)	10.5 ± 0.5 (9.7–11.2) 7	24.8 ± 1.2 (22.6–26.7) 7			5.9 ± 0.3 (5.7–6.1) 2	16.2 ± 0.5 (15.8–16.5) 2	5.5 ± 0.2 (5.3–5.8) 3	14.0 ± 0.6 (13.3–14.5) 3	8.4 ± 0.7 (7.3–9.5) 10	23.5 ± 2.3 (19.5–26.5) 8		
30			10.3 ± 0.5 (9.7–10.7) 3	24.5 ± 0.6 (24.1–24.9) 2			6.9 ± 0.1 (6.8–7.0) 3	17.8 ± 0.5 (17.3–18.3) 3	6.0 (1)	14.5 (1)	8.6 ± 1.0 (6.8–10.0) 7	25.8 ± 1.9 (23.0–27.5) 4	5.8 (1)	
31	10.5 (1)	23.8 (1)					7.1 ± 0.5 (6.8–7.8) 4	18.7 ± 1.3 (17.3–20.4) 3	6.0 (1)	15.5 (1)	9.2 ± 0.9 (7.3–10.5) 14	25.2 ± 2.9 (20.5–29.0) 10	7.0 ± 0.3 (6.8–7.3) 5	
32		1					7.0 (4)	17.0 (4)	6.0 (5.9–6.2) 2	16.8 (16.7–17.0) 2	9.4 ± 0.9 (7.8–10.0) 5	25.6 ± 2.8 (21.5–28.0) 10	7.5 (5)	
33	10.8 ± 0.4 (10.2–11.2) 4	24.0 ± 1.5 (22.2–24.9) 3					7.5 (1)	18.4 (1)			9.6 ± 0.5 (9.0–10.5) 12	25.4 ± 2.3 (22.0–26.5) 11	7.3 (1)	
34	10.7 (1)	24.0 (1)							6.6 ± 1.1 (5.8–8.3) 4	16.5 ± 2.7 (14.5–20.5) 4	9.6 ± 1.2 (8.8–12.0) 10	26.6 ± 3.3 (22.3–32.8) 10	7.9 ± 0.3 (7.7–8.5) 7	
35	10.0 (1)	23.0 (1)			11.9 (1)	24.5 (1)			8.0 (8.0–8.0) 2	20.3 (20.0–20.5) 2	10.6 ± 0.9 (9.5–11.4) 7	29.0 ± 1.3 (26.9–30.3) 7	8.8 (1)	

Table 1. *Continued*

Stage	S. madagascariensis BL	TL	S. spinosa BL	TL	S. menabensis BL	TL	S. brevis BL	TL	S. calcarata BL	TL	Dyscophus insularis BL	TL	Paradoxophyla palmata BL	TL
36	10.8±0.1 (10.7–10.9) 2	24.7±1.3 (23.7–25.6) 2			11.0 1	22.9 1			6.8±0.4 (6.5–7.2) 3	18.3±0.8 (17.5–19.0) 3	10.6±1.4 (9.3–12.5) 7	27.5±3.9 (22.8–32.0) 6	10.0 1	
37										3	11.4±1.2 (9.8–14.0) 15	30.1±3.7 (24.5–35.0) 11	10.9 1	
38	11.8±0.4 (11.4–12.2) 3	28.3±1.1 (27.3–29.4) 3			12.5 1	27.3 1			7.0 1	20.0 1	11.0±1.6 (8.0–13.5) 9	29.5±3.2 (23.3–33.5) 7		
39	11.8±0.1 (11.7–11.9) 2	26.4±0.6 (25.9–26.8) 2			11.9±1.0 (11.0–12.0) 3	28.3±2.7 (25.7–31.0) 3			7.0 1	19.3 1	11.8±1.6 (10.5–13.5) 3	32.5±2.6 (30.5–35.5) 3		
40	11.6±0.4 (11.4–11.9) 2	27.9±0.6 (27.5–28.3) 2							7.5 (7.5–7.6) 2	22.1 (21.5–22.8) 2	14.1±0.4 (13.5–14.5) 8	38.0±1.6 (36.5–40.0) 6		
	SVL		SVL		SVL		SVL		SVL		SVL		SVL	
41											12.0±1.6 (10.0–13.3) 4			
42	12.9 1								7.9 1		12.0 1			
43									8.5 1					
44	11.4±0.4 (11.1–11.9) 4													

From stage 41 only snout–vent length (SVL) is measured. For each parameter the mean value in mm ± SD, range in parentheses (mm) and the number of specimens measured are given.
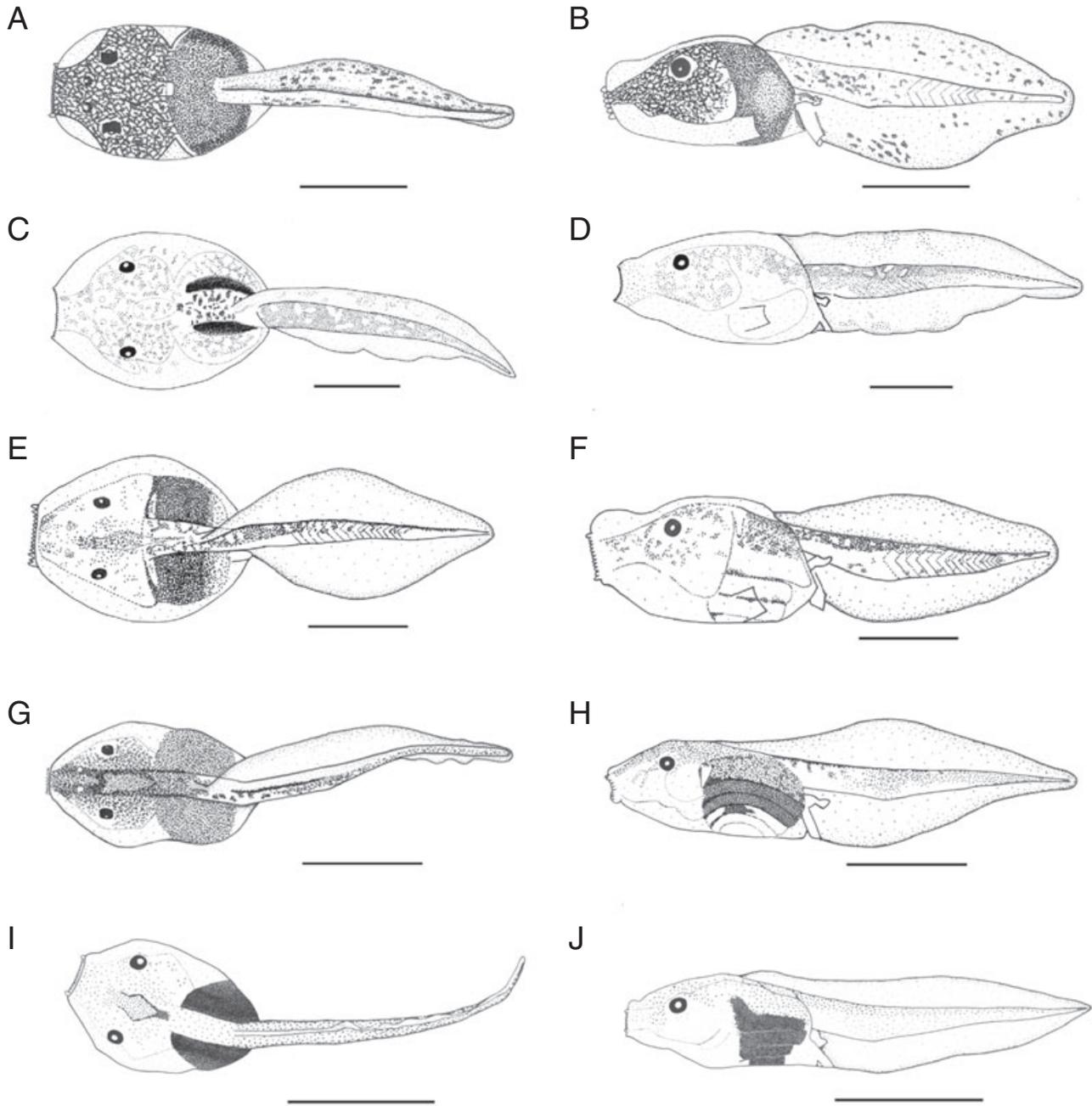
Figure 1. Dorsal and lateral views of tadpoles of *Scaphiophryne madagascariensis* (stage 35, ZSM 595/2004) (A, B), *S. menabensis* (stage 35, ZSM 413/2004) (C, D), *S. spinosa* (stage 29, ZSM 604/2004) (E, F), *S. brevis* (stage 31, ZSM 618/2004) (G, H) and *S. calcarata* (stage 31, ZSM 410/2004) (I, J). Scale bars represent 5 mm.

muscle coloured with the same tint. Fins bearing some scarce small spots, more numerous on the upper fin than on the lower. Upper side of hind limbs coloured with the same tint.

Variation: The ratios taken on three tadpoles at stages 34–36 (ZSM 696/2004-ZSM 698/2004) vary in the following proportions: BW 116–144% of BH; RN 129–133% of NP; NN 24–29% of PP; SS 78–79% of BL;

TMH 30–44% of BH; TMH 29–34% of MTH; UF 33–38% of MTH; SU 86–94% BL; MTH 104–128% of BH; ODW 24–27% of BL; ODW 35–39% of BW.

Buccal floor (Fig. 2B): Prelingual arena small; a pair of small prelingual papillae on the lateral wall of the arena, directed medially; a single medial curved gutter-shaped papilla originating from the base of the lower beak, bearing a vertical medial ridge on its
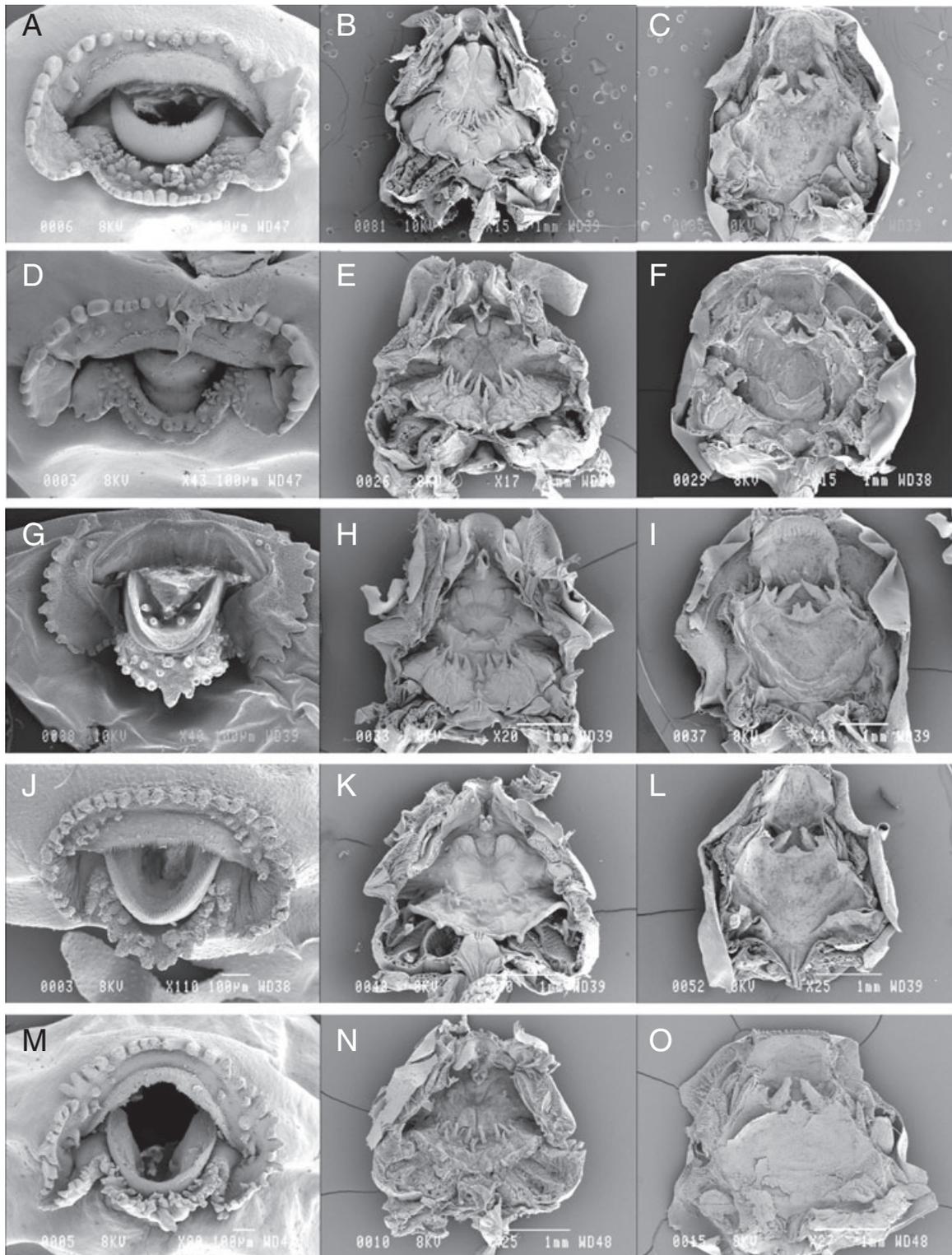
Figure 2. Oral disc (OD), buccal floor (BF) and buccal roof (BR) of tadpoles of *Scaphiophryne madagascariensis* (OD: ZSM 600/2004; BF, BR: stage 38, ZSM 599/2004) (A–C), *S. menabensis* (OD: stage 36 BF, BR: stage 36); all from specimens in batch ZSM 413/2004) (D–F), *S. spinosa* (OD: stage 28, ZSM 616/2004; BF, BR: stage 29, ZSM 612/2004) (G–I), *S. brevis* (OD: stage 34; BF, BR: stage 31, ZSM 619/2004) (J–L) and *S. calcarata* (OD: stage 34; BF, BR: stage 32; both from specimens in batch ZSM 410/2004) (M–O). Scale bars are indicated on the images.

distal part resulting in a short medial stub in posterior view. Tongue anlage prominent, without lingual papillae. Buccal floor arena round delimited anteriorly by few small papillae and posteriorly to the buccal pockets by a dense transversal row of large pustulate papillae, the largest medial; interior of arena smooth. Buccal pockets long, narrow, almost straight and obliquely orientated; four or five prepocket papillae of different size. Ventral velum with spicular support, bearing a pair of projections on each half above the 2nd and 3rd filter plate; medial notch present allowing the glottis to be fully exposed; glottis behind the velum, a papilla in front of the glottis and posterior to the row of the buccal floor arena papillae; secretory pits limited to the projections. Branchial baskets oblique, wider than long; with three filter cavities, filter plates obliquely arranged, filter mesh dense with tertiary folds.

Buccal roof (Fig. 2C): Prenarial arena rectangular, bearing two very small papillae in a transverse row in the centre of the arena. Choanae large, drop-shaped; anterior wall slightly elevated, smooth, without papilla; narial valve greatly enlarged posteromedially into a triangular structure curved dorsally and orientated anteromedially, extending above the choana, its lateral edge slightly jagged. Postnarial arena small bearing a pustule in central position. Median ridge triangular with an irregular median cleft. Two pairs of lateral ridge papillae, the larger one posterior to the narial valve, triangular with a jagged edge; the second pair (missing from Fig. 2C, visible on the buccal floor picture, Fig. 2B) lateral to the first, smaller, smooth and elongate. Buccal roof arena oval elongate; buccal roof arena papillae absent. A few small pustulations and papillae scattered across the buccal roof posteriorly to medial ridge; two small papillae and three pustules posteriorly in the arena. Posterolateral ridge present, lying relatively far anteriorly. Glandular zone present laterally, anterior to the dorsal velum. Dorsal velum interrupted medially, lateral edges curved anteriorly; secretory pits present on its posterior side. Two pressure cushions on each side.

SCAPHIOPHRYNE MENABENSIS
GLOS, GLAW AND VENCES, 2005

The tadpoles were collected from an ephemeral breeding pool in closed forest. This pool was medium sized (150 m²), shallow (< 10 cm) over 75% of its area, with clear to slightly muddy water and a sparse coverage of aquatic vegetation. The external morphological description is based on a specimen at stage 35, ZSM 413/2004 (TL and BL are 24.5 and 11.9 mm, respectively). Buccopharyngeal features are described on the basis of a tadpole at stage 36 included in the batch ZSM 413/2004.

External morphology: In dorsal view (Fig. 1C), body roughly ovoid. In profile (Fig. 1D), BW 131% of BH, snout small, vertical and directed slightly upward. Eyes small, ED 8% of BL, not bulging, directed almost laterally. RN 184% of NP, NN 23% of PP. Spiracle formed by a large square of skin, orientated posteriorly; spiracular opening clinging to the body wall, on a plane situated just above the opening of ventral tube. TMH 31% of BH and 32% of MTH, maximum height of tail musculature reached at the proximal quarter then slightly tapering to end, abruptly very finely. UF 35% of MTH, LF 35% of MTH, upper fin increasing slowly in height before decreasing abruptly in the distal third, extending slightly onto body, SU 79% of BL, lower fin roughly convex; point of maximum height of tail located at the proximal third of the tail length, MTH 97% of BH. Ventral tube short and relatively large, directed almost ventrally, its anterior part linked to body wall.

Oral disc (Fig. 2D) moderately small, in position and orientation terminal, ODW 20% of BL and 29% of BW. A row of four submarginal papillae separated from the row of marginal papillae on each side on the upper labium leaving a large medial gap anteriorly, a row of submarginal papillae separated from the row of marginal papillae on the lower labium; papillae small, marginal papillae round, submarginal papillae pointed. Jaw sheaths white; upper jaw sheath a large arch, flat on the most part; lower jaw sheath U-shaped.

Coloration in preservative: External tegument of the upper side and underlying tissues heavily pigmented by dark brown spots which form dots. Upper part of flanks less densely pigmented as the upper side of body except the snout which is well pigmented. Lower part of flanks and ventral side immaculate. Caudal muscle heavily pigmented with brown spots leaving some small parts immaculate which form white dots. The external half of fins slightly coloured with brown, the internal half immaculate. Upper part of hindlimbs pigmented with the same tint.

Variation: The ratios taken on 12 tadpoles at stages 25–39 vary in the following proportions: BW 115–164% of BH; SS 79–92% of BL; TMH 24–38% of BH; TMH 25–33% of MTH; UF 29–41% of MTH; LF 33–40% of MTH; MTH 84–136% of BH; ODW 24–35% of BL; ODW 28–40% of BW.

Buccal floor (Fig. 2E): Prelingual arena very narrow, gutter-shaped, two pustules anteriorly on the internal wall of the beak and two others laterally; a single medial curved gutter-shaped papilla originating just anterior to tongue anlage, directed posterodorsally, its distal part diamond-shaped with three projections corresponding to three angles, a small projection inside

the gutter medially, edges jagged. Buccal floor arena diamond-shaped, without ornamentation anteriorly, delimited posteriorly by a transversal row of about 15 large papillae, the largest medial. Buccal pockets wide, deep, transversely orientated, unperforated; two small prepocket papillae. Ventral velum bearing a pair of projections on each half medially (the most lateral above the second filter plate); medial notch present, a vertical papillae in front of the glottis; secretory pits not obvious. Branchial baskets wide, the fourth filter plate vertical, filter mesh very dense with tertiary folds.

Buccal roof (Fig. 2F): Prenarial arena small and round, bearing a small median papilla adjoining a small knob on each side laterally. Anterior wall of choanae pustulate; narial valve greatly enlarged posteromedially into a large and elongate structure, slightly jagged distally on its lateral side, dorsally and anteromedially directed, covering partially the choana. Postnarial arena small and concave, without ornamentation. Median ridge triangular, its extremity bifid. The larger pair of lateral ridge papillae posterolateral to the narial valves, triangular, stocky and smooth. Buccal roof arena non-existent, a few pustulations scattered within; one small papillae on each side anterolaterally, directed medially. Posterolateral ridges elevated, present through the buccal roof (if not a preservational artefact). Glandular zone present laterally between posterolateral ridges and dorsal velum, formed of only a few secretory pits wide. Dorsal velum straight; secretory pits not obvious on its posterior side.

SCAPHIOPHRYNE SPINOSA STEINDACHNER, 1882

Specimens were collected from an ephemeral pond in the forest of Ranomafana National Park. The muddy bottom of the pond was completely covered with dead leaves and it had a maximal water depth of 20 cm. The water was clear. The external morphological description is based on a specimen at stage 30, ZSM 602/2004 (BL is 9.7 mm, DNA voucher, with part of the tail missing for molecular determination), and a specimen at stage 30, ZSM 603/2004, which served for verification, calculation of the ratios and tail description (TL and BL are 22.6 and 10.4 mm, respectively). The drawings are based on a specimen at stage 29 (ZSM 604/2004). Buccopharyngeal features are based on a tadpole at stage 29, ZSM 612/2004. One specimen was used for the photographic documentation (Fig. 4).

External morphology: In dorsal view (Fig. 1E), body discoid. In profile (Fig. 1F), body almost flat above and below, BW 129% of BH, snout vertical and directed slightly upward. Eyes small, ED 7% of BL, not bulging. RN 167% of NP, NN 24% of PP. Spiracle formed by a square piece of skin, large, SS 80% of BL; spiracular opening clinging to the body wall, on a plane situated just above the opening of ventral tube. TMH 28% of BH and 30% of MTH, maximum height of tail musculature reached at the proximal quarter of the tail. UF 38% of MTH, LF 38% of MTH; point of maximum height of tail located between the proximal third and the middle of tail length, MTH 95% of BH. Ventral tube tubular but its posterior part folded against ventral fin.

Oral disc (Fig. 2G) in position and orientation terminal, ODW 32% of BL and 38% of BW. A row of submarginal papillae separated from the row of marginal papillae on the upper labium, a row of submarginal papillae separated from the row of marginal papillae on the lower labium with a cluster of small papillae between the extremities of this row and the lower jaw sheath; papillae round, stocky, some of them blunt, those on the top of upper labium very small, submarginal papillae positioned posterolaterally from the lower beak smaller. Jaw sheaths white; upper jaw sheath a large arch, flat on the most part; lower jaw sheath U-shaped, narrow.

Coloration in preservative: Tadpole transparent, all underlying organs visible. External tissue of upper side and upper flanks transparent, underlying tissues bearing numerous small light brown spots, more densely distributed around the digestive tract, a few small light brown spots on the external tissue at the level of gills laterally. Lower flanks and ventral side immaculate and perfectly transparent. Upper half of caudal muscle bearing a high density of the same small light brown spots, very few on the lower half. Fins immaculate except 2–3 blots on the upper fin near the caudal muscle.

Variation: The ratios taken on ten tadpoles at stages 27–30 (ZSM 604/2004-ZSM 613/2004) vary in the following proportions: BW 128–142% of BH; RN 127–167% of NP; NN 23–27% of PP; SS 75–86% of BL; TMH 27–37% of BH; TMH 24–33% of MTH; UF 34–40% of MTH; LF 35–40% of MTH; SU 93–100% of BL; MTH 106–116% of BH; ODW 29–34% of BL; ODW 35–43% of BW. The spiracle can be orientated in a more posterodorsal than almost posterior direction, but is never fully posterodorsal.

Buccal floor (Fig. 2H): Prelingual arena small, two pustules anteriorly on the internal wall of the beak and two small filiform papillae laterally; a pair of small prelingual papillae on the lateral wall of the arena, directed dorsally; a single medial curved gutter-shaped papilla originating from the floor of the prelingual arena, the laterodistal projections highly developed. Buccal floor arena round, delimited posteriorly by a transversal row of about 15 large papillae.

Buccal pockets wide, deep, transversely orientated, unperforated; two small prepocket papillae. A pair of projections on each half of the ventral velum medially, a small papillae in front of the glottis; secretory pits not obvious. Branchial baskets longer than wide.

Buccal roof (Fig. 2I): Prenarial arena wide and triangular. Anterior wall of choanae pustulate; narial valve greatly enlarged into an elongate structure not curved dorsally, covering the posterior end of the choana. Postnarial arena small and concave, without ornamentation. Median ridge irregularly shaped with a wide median cleft. The larger pair of lateral ridge papillae roughly triangular and smooth. Buccal roof arena non-existent, interior without ornamentation. Posterolateral ridge continuous through buccal roof, more prominent laterally. Glandular zone present anteriorly to dorsal velum, uninterrupted across buccal roof. Secretory pits not obvious on the posterior side of the dorsal velum.

SCAPHIOPHRYNE BREVIS (BOULENGER, 1886)

Specimens were collected from a large puddle (*c.* 5×2 m) beside the street in the city of Toliara. The water was very warm and muddy. The external morphological description is based on two specimens at stage 31, ZSM 617/2004 and ZSM 618/2004, the tail of the former served for DNA determination, the second for verification, calculation of the ratios, tail description and drawings (TL and BL are 18.5 and 6.8 mm, respectively). Buccopharyngeal features are described on the basis of one tadpole at stage 31 (ZSM 619/2004).

Additional specimens assigned to this species (ZSM 631/2004–644/2004) were collected from a pond beside the street within spiny forest, beside the road between Ambovombe and Tolagnaro. The bottom of this pond was completely covered with grass and the water was very warm. These tadpoles were not used for the following detailed description but one of them was photographed (Fig. 5).

External morphology: In dorsal view (Fig. 1G), body ovoid, widest at the level of gills, snout truncate. In profile (Fig. 1H), body depressed, almost flat above and below, BW 104% of BH, snout small, vertical and directed slightly upward. Eyes moderately small, ED 10% of BL, very slightly bulging (caused by the presence of a space between the outer integument and the organs), not visible in ventral view, positioned more dorsally than dorsolaterally and directed almost laterally. Nares not open, positioned dorsally, at the same distance to pupils than to snout, RN 100% of NP, very close to each other, NN 29% of PP. Spiracle sinistral but very low, tubular, moderately sized, entirely attached to body wall, inner wall absent, orientated posteriorly, slightly closer to end of body than to tip of snout, SS 59% of BL; spiracular opening a slit not clinging to the body wall, on a plane situated between the insertion of hindlimb and the opening of ventral tube. Tail musculature moderate, TMH 39% of BH and 36% of MTH, its maximum height reached before the proximal third then gradually tapering, not reaching tail tip. Upper fin moderately sized, UF 31% of MTH, convex, extending onto body, SU 70% of BL, lower fin moderately high, LF 38% of MTH, horizontal on the first third then straight toward tail tip before forming a rounded tip; point of maximum height of tail located between the proximal quarter and the midway of the tail length, MTH 108% of BH, tail tip round. Ventral tube moderately sized, medial, curved tubular, directed posteroventrally, entirely included in ventral tail fin, opening medial. Neither lateral line organs nor glands visible.

Oral disc (Fig. 2J) small, in position and orientation almost terminal, emargination very low, ODW 20% of BL and 29% of BW. An uninterrupted row of marginal papillae; three submarginal papillae separated from the row of marginal papillae on each side on the upper labium, DG 40% of ODW, a row of submarginal papillae separated from the row of marginal papillae on the lower labium; papillae of moderate size, conical with pointed tip, submarginal papillae claw-shaped. No denticulate papillae. No keratodonts. Jaw sheaths very finely serrated, white; upper jaw sheath a large arch, flat on the most part; lower jaw sheath V-shaped.

Coloration in preservative: Anterior part of upper side pigmented with brown, especially on the extension of caudal muscle on the back to an area between the eyes, digestive tract heavily pigmented with small brown spots. Flanks immaculate, digestive tract heavily pigmented with small brown spots. Ventral side immaculate. Caudal muscle pigmented with small brown spots. Fins immaculate.

Variation: The ratios taken on 11 tadpoles at stages 28–33 (ZSM 619/2004-ZSM 629/2004) vary in the following proportions: BW 108–125% of BH; RN 86–121% of NP; NN 23–33% of PP; SS 54–58% of BL; TMH 33–42% of BH; TMH 33–42% of MTH; UF 28–33% of MTH; LF 34–40% of MTH; SU 51–76% of BL; MTH 94–111% of BH; ODW 17–20% of BL; ODW 24–34% of BW; DG 34–57% of ODW.

Buccal floor (Fig. 2K): Prelingual arena very narrow, gutter-shaped; a pair of small prelingual papillae on the lateral wall of the arena, directed medially, another pair posterolaterally; a single medial curved gutter-shaped papilla originating just anterior to tongue anlage, directed dorsally, its distal part bearing three pustulate projections (one posterior and two lateral), a small projection on the posterior side. Tongue anlage prominent, without lingual papillae.

Buccal floor arena oval delimited anterolaterally by a small papilla on each side of the arena, by a papilla lateral to buccal pocket and posteriorly by a transversal row of more than 15 papillae, the largest lateral; interior of arena smooth. A half circle (the convexity anterior) consisting of five median pustules just behind the row of papillae, the centre of this hypothetical circle occupied by a papilla just in front of the glottis. Buccal pockets wide, deep, transversely orientated, unperforated; two small prepocket papillae. Ventral velum with spicular support, bearing four projections on each half, the most developed above the second filter plate, two above the third filter plate, the smaller of two median; velum interrupted medially by the glottis; secretory pits present on the second projection. Branchial baskets oblique, wide, with three filter cavities, filter plates obliquely arranged, filter mesh very dense with tertiary folds.

Buccal roof (Fig. 2L): Prenarial arena small and round, with a small median transversal ridge bearing two pustules. Choanae large, drop-shaped; anterior wall slightly elevated, pustulate, without papilla; narial valve greatly enlarged posteromedially into a triangular, elongate, large structure with pustulate lateral edge, dorsally and anteromedially directed, covering the posterior end of the choana. Postnarial arena small and flat, without ornamentation. Median ridge triangular. One pair of triangular lateral ridge papillae, pustulate on top, posterolateral to the narial valves. Buccal roof arena non-existent, pustulations scattered within, more densely posteriorly, buccal roof arena papillae absent. Posterolateral ridges slightly elevated, present laterally. Glandular zone well developed, continuous throughout the buccal roof, formed by about six secretory pits wide. Dorsal velum smooth, interrupted medially on about one-quarter of its length, secretory pits present on its ventral side. Two pressure cushions on each side.

SCAPHIOPHRYNE CALCARATA (MOCQUARD, 1895)

Amplectant pairs were collected at temporary breeding pools in open areas within Kirindy forest. The pools were small (< 10 m^2), shallow (< 10 cm) and had little coverage of aquatic vegetation. Subsequently, fertilized eggs from these amplectant pairs were reared in plastic aquaria. The description is based on one specimen at stage 32 (TL and BL are 16.7 and 5.9 mm, respectively) included in the batch ZSM 410/2004. Drawings are based on a specimen at stage 31 from the batch ZSM 410/2004. Buccopharyngeal features are described on the basis of one tadpole at stage 32 from the same batch.

External morphology: Dorsal view (Fig. 1I). In profile (Fig. 1J), body flat below, BW 135% of BH; snout small, vertical and directed slightly upward. Eyes moderately sized, ED 10% of BL. RN 103% of NP, NN 28% of PP. Spiracle square, large, closer to end of body than to tip of snout, SS 73% of BL; spiracular opening situated below the insertion of hindlimb. MC 35% of BH and of HT, proximal third parallel with a swelling at that point then gradually tapering, almost reaching tail tip. Fins moderately sized, UF 32% of HT, LF 36% of HT, straight on most part then decreasing in the distal third to form the end of the tail, SU 64% of BL; point of maximum height of tail located at the proximal third, HT 102% of BH, tail tip round but fine. Ventral tube tubular.

Oral disc (Fig. 2M) moderately sized, in position and orientation terminal, ODW 23% of BL and 31% of BW. The median marginal papillae of lower labium bigger than the other and directed forward; a row of 5–6 submarginal papillae on each side on the upper labium, DG 45% of ODW, a row of very small submarginal papillae on the lower labium; papillae moderately small to small, marginal papillae conical or round with rounded tip. Upper jaw sheath almost flat with a weak medial convexity, lower jaw sheath U-shaped very open.

Coloration in preservative: Upper side pigmented by numerous dark brown spots contained mainly in underlying tissues, a band from the snout through between the eyes and which enlarges to cover the upper part of intestine. Flanks immaculate except the orbitohyoideus muscle and the upper part of the intestine. Ventral side immaculate. Caudal muscle neatly pigmented with dark brown spots, the size of spots decreasing dorsoventrally (except a small immaculate anteroventral part). Fins immaculate except a few small spots on the upper part of the upper fin. Upper part of hindlimbs immaculate.

Variation: The ratios taken on eight tadpoles at stages 29–39, except for RN/NP, NN/PP, SU/BL and DG/ODW for which only two specimens were involved, vary in the following proportions: BW 121–138% of BH; RN 104–106% of NP; NN 28% of PP; SS 69–81% of BL; MC 33–42% of BH; MC 35–47% of HT; UF 30–39% of HT; LF 33–41% of HT; SU 56% of BL; HT 89–106% of BH; ODW 19–24% of BL; ODW 26–36% of BW; DG 50–63% of ODW.

Buccal floor (Fig. 2N): Prelingual arena non-existent, two pustules anterolaterally on the internal wall of the beak; a pair of small prelingual papillae posterolaterally to the beak, directed medially; a single medial gutter-shaped papillae originating just posterior to the beak, directed posteriorly and covering the tongue anlage, its distal part with three projections corresponding to the lateral and posterior end of the gutter. Buccal floor arena oval delimited anteriorly by

a papilla on each side anterolaterally, a papilla medially to buccal pocket and posteriorly by a transversal row of about 11 large papillae, the largest medial. Buccal pockets wide, deep, transversely orientated, unperforated; two small prepocket papillae. Ventral velum with spicular support, bearing a pair of projections on each half medially (the most lateral above the second filter plate); medial notch present, a vertical papillae in front of the glottis; secretory pits not obvious; glottis behind the velum.

Buccal roof (Fig. 2O): Prenarial arena wide and pentagonal, with a small median transversal ridge bearing two small papillae. Narial valve as a large and elongate structure. Postnarial arena small, covered by the medial ridge. Medial ridge triangular with its extremity bifid. One pair of triangular and smooth lateral ridge papillae, posterolateral to narial valve. Buccal roof arena non-existent, a very few pustulations scattered within. Posterolateral ridges few prominent, present through the buccal roof. Glandular zone present as a narrow granular band extending through the buccal roof; secretory pits not visible. Dorsal velum interrupted medially, lateral edges curved anteriorly.

DYSCOPHUS INSULARIS GRANDIDIER, 1872

Specimens were collected from an ephemeral breeding pool in the Kirindy forest. This pool was large (> 1000 m^2), with a depth of 80 cm and clear water. A large proportion of the pond area was covered with standing, floating and submerged aquatic vegetation. The external morphological description is based on a specimen at stage 34 (TL and BL are 26.9 and 9.5 mm, respectively) included in batch ZSM 402/2004. Buccopharyngeal features are described on the basis of a tadpole at stage 38 from the same batch.

External morphology: In dorsal view (Fig. 3A), body roughly ovoid, widest at the level of eyes, snout truncate. In profile (Fig. 3B), body depressed, BW 118% of BH, snout very small, round and almost acute. Eyes moderately sized, ED 13% of BL, bulging, visible in ventral view, positioned dorsolaterally and directed almost laterally. Nares not open, positioned dorsally, closer to snout than to pupils, RN 67% of NP, very close to each other, NN 21% of PP. Spiracle ventral, a large fold of skin free at the rear of the spiracular tube in the form of a half-circle, not attached to body wall, orientated posteriorly, very close to ventral tube, SS 89% of BL. Tail musculature moderately weak in the proximal third to weak in the distal two-thirds, TMH 32% of BH and 39% of MTH, the proximal quarter parallel then slightly tapering, almost reaching tail tip. Upper fin moderately high, UF 41% of MTH, convex, extending slightly onto body, SU 84% of BL, lower fin shallow, LF 34% of MTH, straight on the most part;

point of maximum height of tail located just after the ventral tube opening, MTH 83% of BH, tail tip a flagellum. Ventral tube small, tubular, medial, directed posteroventrally, its anterior part linked to body wall, its posterior part linked to ventral tail fin, opening medial. Neither lateral line organs nor glands visible.

Oral disc (Fig. 3E) moderately large, in position and orientation terminal, not emarginated, ODW 28% of BL and 36% of BW. Upper labium as a large flap of skin hanging down the lower labium, concave medially; lower labium flat with a large U-shaped extension medially covered in great part by upper labium, only the extremity of U going beyond it. No papillae, no denticulate papillae. No keratodonts. No jaw sheaths.

Coloration in preservative: Upper side uniformly punctuated with small dark brown spots. Upper labium coloured as the back. Upper part of flanks coloured as the back except an unpigmented area dorsolaterally at the back of body. Lower part of flanks and ventral side immaculate. Upper part of caudal muscle coloured as the back but less regularly, some rare spots on the lower part. Fins immaculate except the external half of the proximal part of the upper fin. A bright white area encompassing the caudal muscle and half of fins in the proximal third. Hindlimbs immaculate.

Variation: The ratios taken on seven tadpoles at stages 33–38 vary in the following proportions: BW 120–141% of BH; RN 36–89% of NP; NN 12–18% of PP; SS 87–94% of BL; TMH 29–43% of BH; TMH 24–46% of MTH; UF 28–42% of MTH; LF 29–40% of MTH; SU 84–96% of BL; MTH 86–141% of BH; ODW 23–37% of BL; ODW 29–41% of BW.

Buccal floor (Fig. 3F): Buccal floor wider than long, its maximum width at the level of the buccal pockets. Prelingual arena very short and narrow; two pairs of small pustules anterolaterally and two pairs of small prelingual papillae posterolaterally; part immediately posterior to prelingual arena vertical, forming a deep depression. Tongue anlage prominent, elongate, without lingual papillae, lying at the bottom of the depression. Buccal floor arena round, delimited by a papilla medially to buccal pocket and posteriorly by a transversal row of about 15–20 large papillae of equal size; interior of arena smooth except a few small papillae just anterior to the row of buccal floor arena papillae; a transversal row of two or three papillae between buccal pocket and tongue anlage. Buccal pockets wide, deep, almost transversely to obliquely orientated, unperforated; four prepocket papillae, one on the edge of the anterior wall of the buccal pocket, orientated posteriorly and above the buccal pocket; about ten small postpocket papillae. Glottis just posterior to the
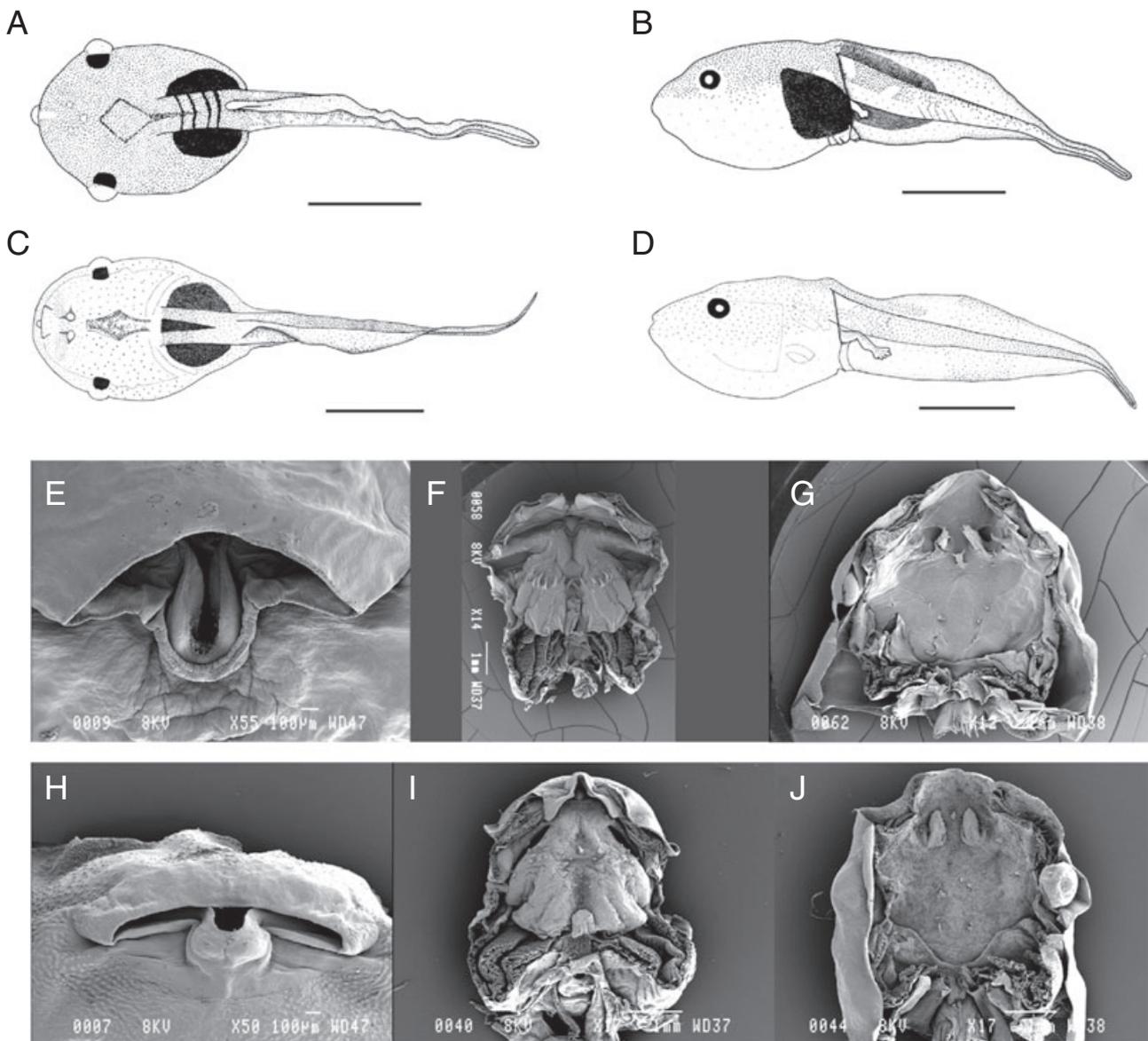
Figure 3. Dorsal and lateral views of tadpoles of *Dyscophus insularis* (A, B) (stage 34, ZSM 402/2004) and *Paradoxophyla palmata* (C, D) (stage 37, ZSM 647/2004). Oral disc of *D. insularis* (E) (stage 36, specimen from batch ZSM 402/2004) and *P. palmata* (H) (stage 35, ZSM 645/2004). Buccal floor (BF) and buccal roof (BR) of tadpoles of *D. insularis* (F, G) (stage 38, specimen from batch ZSM 402/2004) and *P. palmata* (I, J) (stage 37, ZSM 645/2004). Scale bars in A–D represent 5 mm, scale bars for E–J are indicated on the images.

row of buccal floor arena papillae, anterodorsal in orientation, far ahead of the end of the ventral velum. Ventral velum wide with spicular support, bearing an indistinct projection above each filter cavity (the most medial being the better defined); velum interrupted by the laryngeal anlage; secretory pits not visible. Branchial baskets almost straight, anteroposteriorly directed, longer than wide, with three filter cavities, filter plates almost vertically arranged, filter mesh very dense with tertiary folds.

Buccal roof (Fig. 3G): Prenarial arena wide and pentagonal, with 4–5 pustules. Choanae large, round; anterior wall slightly elevated, pustulate, without papilla; narial valve greatly enlarged into an anteromedially large and elongate structure with three projections distally, anteromedially and dorsally directed, covering the posterior end of the choana. Postnarial arena small, without ornamentation. Median ridge depressed anteroposteriorly, long and thin bearing three digitations on top, directed ventrally. One pair of

elongate and smooth lateral ridge papillae, posterolateral to narial valves. Buccal roof arena non-existent, a very few and small pustulations scattered within, three pairs of very small buccal roof arena papillae. Posterolateral ridges few prominent, present through the buccal roof, their lateral ends laying relatively far anteriorly. Glandular zone present at least laterally; secretory pits not visible. Dorsal velum continuous, medial part straight, directed ventrally. Two pressure cushions on each side.

PARADOXOPHYLA PALMATA (GUIBÉ, 1974)

Tadpoles used for the description were collected at a large pond in a dense, unprotected forest near Fierenana. The pond had a muddy bottom and many dead leaves as well as whole trees covered the ground. Most of the collected tadpoles already lacked the tip of their tails when they were collected. An additional series used for comparison (ZSM 656/2004) was collected in a small stream (about 50–100 cm wide) that included quiet pools as well as rapids. The external morphological description is based on a specimen at stage 37, ZSM 647/2004 (TL and BL are 27.7 and 10.0 mm, respectively). Buccopharyngeal features are described on the basis of a tadpole at stage 37 (ZSM 645/2004).

External morphology: In dorsal view (Fig. 3C), body oval, widest in the posterior part of gills; snout semicircular. In profile (Fig. 3D), body depressed with a rounded ventral side, BW 112% of BH, snout flat and oblique. Eyes moderately sized, ED 12% of BL, bulging, visible in ventral view, positioned dorsolaterally and directed laterally. Nares not open, positioned dorsally, closer to snout than to pupils, RN 77% of NP, very close to each other, NN 14% of PP. Spiracle ventral, a large fold of skin free at the rear of spiracle composed of two fringed flaps divided by a medial notch, each part bearing four lobes, not attached to body wall, orientated posteriorly, very close to the ventral tube, SS 97% of BL. Tail musculature weak, TMH 28% of BH and 47% of MTH, gradually tapering, reaching tail tip. Upper fin moderately sized, UF 35% of MTH, slightly convex, extending very slightly onto body, lower fin moderately sized, LF 38% of MTH, convex; point of maximum height of tail located at the proximal third of the tail length, MTH 81% of BH, tail tip being a flagellum. Ventral tube moderately small, fine and relatively long, tubular, medial but directed to the right, directed almost ventrally, its anterior part linked to body wall, its posterior part linked to ventral tail fin, opening lateral. Neither lateral line organs nor glands visible.

Oral disc (Fig. 3H) moderately sized, in position and orientation terminal, not emarginated, ODW 18% of BL and 22% of BW. Upper labium a large flap of skin

flattened circumflex accent-shaped hanging down the lower labium; lower labium flat with a large U-shaped extension medially covered in great part by the upper labium, only the extremity of the U going beyond it. No papillae, no denticulate papillae. No keratodonts. No jaw sheaths.

Coloration in preservative: Tadpole transparent. Upper side entirely punctuated by dark brown spots of different size; upper labium, nares, brain pan and upper side of intestine coil lighter brown. Flanks punctuated by small dark brown spots. Ventral side immaculate. Caudal muscle beige coloured by the same spots as the body, larger in the proximal quarter. Fins punctuated with these spots close to the caudal muscle in the proximal half, on the whole surface halfway. Upper side of hindlimbs densely punctuated by very small dark brown spots.

Variation: The ratios taken on nine tadpoles at stages 34–37 (ZSM 645/2004, ZSM 646/2004, ZSM 648/2004-ZSM 654/2004) vary in the following proportions: BW 109–118% of BH; RN 67–100% of NP; NN 13–21% of PP; SS 88–129% of BL; TMH 39–45% of BH; TMH 50–64% of MTH; UF 15–35% of MTH; LF 36–40% of MTH; MTH 70–81% of BH; ODW 18–26% of BL; ODW 24–33% of BW.

Buccal floor (Fig. 3I): Buccal floor as wide as long, its maximum width at the level of the buccal pockets. Prelingual arena very short and narrow; bearing a medial knob. Posteriorly to this knob, a large trapezoid area lacking ornamentation. Tongue anlage absent, a single medial papilla in the bottom of the depression present that may be homologous to papilla of tongue anlage. Buccal floor arena roughly triangular, delimited by about 15 stout and short papillae; interior of arena in a longitudinal depression, with no obvious pustulations; few secondary buccal floor arena papillae. Buccal pockets very long, extending from the prelingual arena to the middle of the buccal floor arena, deep, almost longitudinally orientated, seemingly perforated; some pustulations on the edge of the anterior wall of the buccal pocket; 2–5 small postpocket papillae. Glottis far ahead from the end of the ventral velum, orientated anteriorly. Ventral velum with spicular support, straight laterally, medial part damaged in dissection; secretory pits not visible. Branchial baskets almost round with a concavity posteromedially, three filter cavities, filter plates curved externally, long; filter mesh very dense with tertiary folds.

Buccal roof (Fig. 3J): Prenarial arena short and semicircular, with one short median pustule. Choanae large, round; anterior wall absent; narial valve greatly enlarged into a large and ventrally concave structure, anteriorly directed, covering entirely the choana. Postnarial arena absent. Median ridge and lateral ridge

Figure 4. Dorsal, lateral, and ventral views of a tadpole of *Scaphiophryne spinosa* (ZSM 606/2004) showing the typical morphology of the relatively large tadpoles of the *Scaphiophryne* (*Scaphiophryne*) type.

Figure 5. Lateral, dorsal, and ventral views of a tadpole of *Scaphiophryne brevis* (ZSM 632/2004) showing the typical morphology of the relatively small tadpoles of the *Scaphiophryne* (*Pseudohemisus*) type.

papillae absent. Buccal roof arena non-existent, interior of arena smooth, one pair of very small buccal roof arena papillae posteromedially to the buccal roof. Posterolateral ridges not visible. Glandular zone present at least medially; secretory pits visible on this band and on the ventral side of the dorsal velum. Dorsal velum continuous, its lateral parts extending anteriorly. Two small pressure cushions on each side.

DISCUSSION

RELATIONSHIPS WITHIN *SCAPHIOPHRYNE* INFERRED FROM LARVAL CHARACTERS

Tadpoles of *Scaphiophryne* are known to have a unique morphology (Blommers-Schlösser, 1975; Wassersug, 1984; Haas, 2003; Mercurio & Andreone, 2006), but comparative data on various species were not previously available. Our study provides clear evidence for the existence, within this genus, of two subgroups defined by larval characters of both external and buccopharyngeal morphology, here referred to as the small tadpole group and large tadpole group (Table 2). Polarizing these characters phylogenetically is difficult as the distinguishing states are not found in the immediate outgroup (*Paradoxophyla*) or are probably of poor phylogenetic value, such as body size and distance of epidermis from inner organs. As *Paradox-*

ophyla tadpoles are small and have no distinct separation of skin from inner organs, the latter two characters can tentatively be used phylogenetically and would indicate the larger size and large distance of skin from inner organs in the large tadpoles group of *Scaphiophryne* to be synapomorphic. This cavity may possibly represent an enlargement of lymphatic sacs or sinuses (see Viertel & Richter, 1999: 101), and is probably an extreme expression of the state also seen in other microhylids, and in several pipids (in particular in *Silurana*; Orton's tadpole type I). Only speculation is possible regarding the possible adaptive value of this character, but as these tadpoles are very fragile, a protective function is unlikely. Pipid larvae and typical microhylid larvae (Orton's types I and II) are often floating in midwater, and a function as balancer or float of these additional cavities may be possible. In this case, species of the large tadpoles group of *Scaphiophryne* may be more strongly adapted to such a floating behaviour, an hypothesis that could be tested by field observations or experimental data.

Other larval characters may also be useful to infer relationships among these species, such as the large dorsal bulges sometimes visible on the snout (e.g. *S. madagascariensis* and *S. spinosa* in Fig. 1). These bulges are not preservation artefacts and are sometimes also visible in living specimens, but we do not

Table 2. Morphological and anatomical differences between tadpoles of the large tadpoles group (*S. madagascariensis*, *S. spinosa* and *S. menabensis*) and those of the small tadpoles group (*S. brevis* and *S. calcarata*)

	Scaphiophryne Large tadpoles group (subgenus *Scaphiophryne*)	*Scaphiophryne* Small tadpoles group (subgenus *Pseudohemisus*)	*Paradoxophyla*
Size	Large (up to 29 mm TL)	Small (up to 22 mm TL)	Large
Skin	Very distant from internal organs	Less distant from internal organs	Less distant from internal organs
Position of nares	Closer to pupils than to snout (127–184%)	Equidistant between pupils and snout (100–104%)	Closer to snout than to pupils (67–100%)
Oral disc	Soft	Rigid	Not comparable
Spiracular position	Close to end of body (76–80% of BL)	Only slightly closer to end of body (56–59% of BL)	At the end of body
Spiracular opening	Clinging to body wall	Free from body wall	Clinging to body wall but not really comparable
Upper fin	Not extending onto body	Extending onto body	Extending very slightly onto body
Buccal floor arena papillae	Well developed	Less developed	Poorly developed
Lateral ridge papillae	Two pairs	One pair	Absent
Medial ridge	More or less regularly cut out medially	Without notch	Absent
Papillae anterolaterally to buccal roof arena	At least one pair of small papillae	No papillae	At least one pair of small papillae
Position of glottis in relation to the velum	In line with the velum but behind it due to a notch	Through the velum	Anterior to the velum
Pairs of projections on the margin of the ventral velum	One or two	Two or four	Absent

have sufficient data to ascertain their possible diagnostic value. Wassersug (1984) noted that the lower jaw sheath of *Scaphiophryne calcarata* (under the name *Pseudohemisus granulosus*) was serrated and the upper jaw sheath was not serrated. Our data (see Fig. 2) provide clear evidence of very fine serrations on both upper and lower jaw sheaths in all species, including *S. calcarata*.

The two subgroups can also be identified using adult morphological, bioacoustic and molecular characters. (1) *Scaphiophryne brevis* and *S. calcarata*, which are included in the small tadpole group, lack dilated terminal finger discs and have very loud, non-harmonious advertisement calls. (2) *Scaphiophryne madagascariensis*, *S. menabensis* and *S. spinosa* are included in the large tadpole group, and according to the tadpole description of Mercurio & Andreone (2006), the tetraploid species *S. gottlebei* belongs within this group as well. Indeed, this latter tadpole is of large size (up to 48.2 mm total length in Gosner stage 38), with nares positioned closer to eyes than to tip of the snout, a spiracle lacking an inner wall, and the upper fin not extending onto the body. However the tadpole of *S. gottlebei*, according to its description in Mercurio & Andreone (2006), may possess an oral disc that differs greatly from those of the other members of the genus. For instance, the emargination is lacking and the submarginal papillae are neatly arranged over the entire oral disc whereas the submarginal papillae are arranged in a similar way in all five tadpoles described herein (i.e. a short row along the lower jaw on the lower labium and a row largely interrupted medially on the upper labium and so composed of only a few submarginal papillae). It needs to be clarified whether these differences reflect biological reality or may just be due to a different perception of the same characters by different researchers.

All four species here included in the large tadpole group are characterized by a very similar advertisement call (Vences *et al.*, 2003b; Vences, Glaw & Marquez, 2006; Glos *et al.*, 2005), consisting of very rapidly repeated short melodious notes. They also form a very closely related monophyletic group based on molecular data (Glos *et al.*, 2005), together with the remaining two *Scaphiophryne* species (*S. boribory* and *S. marmorata*). Although the calls and larvae of the latter two species are undescribed, we can predict with some confidence that they will be similar in these characters to the other species of the large tadpole

group. Within this group, most species are characterized by dilated terminal discs of fingers and toes (*S. gottlebei*, *S. boribory*, *S. marmorata*, *S. menabensis*, *S. spinosa*) that are largely absent in *S. madagascariensis*. To some degree, the two subgroups correspond to the subgenera *Scaphiophryne* Boulenger, 1882 (large tadpole group), with type species *Scaphiophryne marmorata* Boulenger, 1882 and *Pseudohemisus* Mocquard, 1895, with type species *Hemisus obscurus* Grandidier, 1872 (small tadpole group). We therefore propose to continue to use these subgeneric names but emphasize that the expansion of terminal finger discs is not an unequivocal character to distinguish them. Instead, advertisement calls and especially larval morphology may become the major defining characters once these data are gathered for the two remaining species, *S. boribory* and *S. marmorata*.

ECOLOGY AND LARVAL NATURAL HISTORY

As assessed by Mercurio & Andreone (2006) for *S. gottlebei*, tadpoles of *Scaphiophryne* are not obligate suspension-feeders as most of the other members of the family Microhylidae but during the day display a singular feeding behaviour that consists of burrowing the anterior half of their body into the sand with their tail obliquely upwards. In contrast, at night, the tadpoles move throughout the water column, as typical obligate suspension-feeders. All *Scaphiophryne* tadpoles examined by us had sand in their guts, confirming the observations of Mercurio & Andreone (2006) and supporting the inclusion of *Scaphiophryne* tadpoles within a newly created psammonektonic ecomorphological guild.

Although we have no doubts that the differences in larval morphology between the two *Scaphiophryne* groups (Table 2) are of phylogenetic significance, they may also have adaptive components. Species of the small tadpole group are restricted to western and southern Madagascar and breed in temporary lentic waters in open, often semi-desertic areas. In such environments, many species have small larvae to allow for fast development and metamorphosis at small sizes before ponds dry up. In fact, our observations on *S. brevis* and *S. calcarata* at Kirindy in western Madagascar show that both species metamorphose at very small sizes (*S. calcarata*: mean BL in Gosner stage 45 and 46: 8.7 mm, range 6.1–11.0 mm, $n = 94$; *S. brevis*: mean BL: 6.0 mm, range 5.0–7.0 mm, $n = 7$).

In contrast, many species of the large tadpole group live in rainforests (*S. boribory*, *S. marmorata*, *S. spinosa*) or montane areas (*S. madagascariensis*). Although they share with all other *Scaphiophryne* a very explosive breeding behaviour in lentic waters, the duration of these ponds may often be longer and allow for a more extended larval development and larger metamorphic sizes. Also, the substrate in these ponds in forested montane areas may differ from that in semi-desert areas or deciduous western forests and may have had an influence on the evolution of the particular larval morphology of these species. However, two further species of the large tadpole group, *S. gottlebei* and *S. menabensis*, live in western Madagascar as well, and have large tadpoles. The breeding waters of *S. gottlebei* are mostly rather deep and comparatively permanent ponds remaining in the bed of temporary streams, often in canyons which sometimes also harbour rainforest remnants at Isalo. *Scaphiophryne menabensis* breeds in temporary ponds within deciduous forest at Kirindy. More research is necessary to ascertain whether the breeding habitats of these two species are more permanent than those of *S. calcarata* and *S. brevis*; regardless, *S. gottlebei* and *S. menabensis* seem to be more restricted to particular habitats in the west that are characterized by moist environments in canyons or deciduous forest.

DERIVED CHARACTERS IN TADPOLE MORPHOLOGY OF *SCAPHIOPHRYNE*

Microhylids, in the past, have sometimes been considered to be related to basal frog lineages, one argument for this placement being their larval morphology which is reminiscent of that of the archaic pipid and rhinophrynid frogs (e.g. Orton, 1953; Hecht, 1963; Inger, 1967). Current data are unambiguous with regard to placing the family, instead, firmly among modern (neobatrachian) frogs, and more precisely, as one of three major lineages in the superfamily Ranoidea (e.g. Van der Meijden *et al*., 2004), which is equivalent to the phylocode taxon Ranoides of Frost *et al*. (2006). Although most microhylids are characterized by filter-feeding tadpoles which are derived compared with the basic ranoid type, many microhylids have further derived larval morphologies, such as the surface-feeding tadpoles of some *Microhyla* (*M. heymonsi*, Wassersug, 1980; Chou & Lin, 1997; *M. achatina*, Smith, 1916), the non-feeding tadpoles of the subfamily Cophylinae (Blommers-Schlösser, 1975), the burrowing tadpoles of the South American *Otophryne* with keratinized 'teeth' in place of the beak and a sinistral spiracle (Wassersug & Pyburn, 1987), or a fully reduced larval stage such as in the directly developing Papuan taxa (e.g. Tyler, 1963; Zweifel, 1972; Menzies, 1976; McDiarmid & Altig, 1999).

The tadpoles of the genus *Scaphiophryne*, as emphasized by Blommers-Schlösser (1975) and Wassersug (1984), bear some characters typical of ranid tadpoles, others typical of microhylid tadpoles and others intermediate between the two families. Among

these intermediate characters are the structure and shape of branchial food traps, the position of the glottis, the shape of the ventral velum and, at the level of the oral disc, the presence of marginal papillae and of a beak but the absence of keratodonts, and an intermediate position of the spiracle. Further intermediate characters of *Scaphiophryne*, and their impact on morphological reconstruction of anuran phylogeny, are discussed by Haas (2003). It is tempting to interpret this morphology as an evolutionary transitory state, and scaphiophrynines as the most basal microhylid group. In fact, *Scaphiophryne* was placed basal to other microhylids in the molecular study of Van der Meijden *et al*. (2004), albeit with negligible statistical support.

This interpretation, however, is strongly questioned if the second scaphiophrynine genus, *Paradoxophyla*, is taken into account. This genus contained a single species, *P. palmata*, until recently, which was strongly divergent from *Scaphiophryne* based on its general habitus, its partially aquatic habits and fully webbed feet; the recent discovery of a second species, *P. tiarano*, with rudimentary foot webbing (Andreone *et al*., 2006) decreases the importance of these differences. Blommers-Schlösser & Blanc (1991) hypothesized relationships between *Scaphiophryne* and *Paradoxophyla* based on osteological similarities, especially on the presence of an undivided vomer and sphenethmoid. Molecular data by Van der Meijden *et al*. (2007) in fact support the monophyletic group constituted by these two genera. However, the tadpoles of *Paradoxophyla palmata* exhibit typical morphology and buccopharyngeal features of microhylids (Parker, 1934; Wassersug, 1980; Chou & Lin, 1997; Shimizu & Ota, 2003; S. Grosjean, pers. observ.), as obvious from their similarity to the tadpoles of *Dyscophus insularis* described herein (see Table 3). Tadpoles assigned to *Paradoxophyla tiarano*, decribed by Andreone *et al*. (2006), are of the same morphology, although buccopharyngeal features have not been examined. In the morphological phylogeny of Haas (2003), based on primarily larval characters, *Paradoxophyla* was grouped sister to the African microhylid *Phrynomantis* while *Scaphiophryne* was placed basal to all other microhylids, which emphasizes the strong morphological differences between these larvae and the morphological similarities of the *Paradoxophyla* tadpole to those of other microhylids.

Paradoxophyla appears to be the sister group of *Scaphiophryne* and shares with other microhylids their derived tadpole morphology, including detailed similarity in many specialized character states, indicating that the *Scaphiophryne* tadpoles have evolved their unique morphology from an ancestor of typical

Table 3. Summary of morphological character states distinguishing the tadpoles of *Scaphiophryne* from *Paradoxophyla*, *Dyscophus* and other microhylid tadpoles

	Scaphiophryne	*Paradoxophyla*	*Dyscophus*	Microhylinae and Gastrophryninae
Form of oral disc	Ovoid	T-shaped	T-shaped	T-shaped
Labial papillae	Present	Absent	Absent	Absent
Horny beak	Present	Absent	Absent	Absent
Form of body	Rounded transversally	Very depressed	Very depressed	Very depressed
Spiracle position	Lateroventral	Ventral	Ventral	Ventral
Eyes	Not visible in ventral view	Visible in ventral view	Visible in ventral view	Visible in ventral view
Eyes	Only slightly bulging	Bulging	Bulging	Bulging
Buccal floor	Larger posteriorly	Larger anteriorly	Larger anteriorly	Larger anteriorly
Medial gutter-shaped prelingual papilla	Present	Absent	Absent	Absent
Buccal pockets	Obliquely orientated	Almost longitudinal	Almost transversally orientated	Almost transversally orientated
Pustules on the anterior edges of the buccal pockets	Absent	Present	Present	Present
Glottis	In line with the medial portion of the ventral velum	Anterior to the medial portion of the ventral velum	Anterior to the medial portion of the ventral velum	Anterior to the medial portion of the ventral velum
Medial ridge	Triangular with a medial cleft	Absent	Straight, long with terminal projections	Straight, long with terminal projections or absent

microhylid larval morphology. Any other hypothesis would need the assumption that the filter-feeding microhylid tadpole has evolved twice independently, once in *Paradoxophyla* and once in the lineage leading to the remaining microhylid taxa. Given the close similarity between larvae of *Paradoxophyla* and other microhylids, which extends to many synapomorphic states not found in *Scaphiophryne*, and not found in pipid and rhinophrynoid filter-feeding tadpoles (such as the U-shaped lower labium, the unique medioventral spiracle lying near the ventral tube, the position of the glottis far anterior on the buccal floor and its anterodorsal orientation; Sokol, 1962, 1977; Gradwell, 1975), we consider this alternative as highly unlikely. Hence, we favour an evolutionary scenario in which the larvae of *Scaphiophryne* obtained their unique features by reversing some of the filter-feeding adaptations of their ancestors and adapting to their psammonektonic lifestyle which is not known from any other anuran species thus far, thereby constituting an astonishing exception from Dollo's rule. Together with probable examples of re-aquisition of larval stages from endotrophically developing ancestors (Duellman & Hillis, 1987; Vences & Glaw, 2001; Chippindale *et al.*, 2004), this provides remarkable evidence for the evolutionary plasticity of developmental modes and larval morphology in amphibians.

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