Resolving the confusion: *Amietia vertebralis* and *A. umbraculata* tadpole morphology

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Morphological similarities between the tadpoles of *Amietia umbraculata* and *A. vertebralis* have led to confusion and incorrect descriptions and identifications in the literature. Based on 33 body measurements and ratios we revised the morphological descriptions of the tadpoles of the two species. Tadpole identification was verified through DNA sequencing using mitochondrial (16S) gene fragments. A combination of four morphological characters proved to be informative and consistent in distinguishing between tadpoles of the two species. Tadpoles of *A. umbraculata* are characterized by having four labial tooth rows in the lower jaw, extensive tail mottling, a dorsal fin that originates well behind the body, reaching a maximum depth at 50% of the tail length, and an average tail length of 1.9 times body length. *Amietia vertebralis* tadpoles on the other hand are characterized by having five or more labial tooth rows in the lower jaw, tail mottling that is confined to the upper half of the tail musculature, a dorsal fin that originates at the body-tail junction but retains a low profile, rising abruptly to reach a maximum depth at about 40% of the tail length, and an average tail length of 1.5 times body length. These four characters identify the two species without ambiguity.

Key words: ***Amietia umbraculata, Amietia vertebralis,* identification key, Drakensberg, Maluti-Drakensberg, Maloti-Drakensberg, tadpole morphology.**

INTRODUCTION

The genus *Amietia* is a species-rich taxon with 15 species currently recognized (Frost 2011), of which eight are known from southern Africa. Although *Amietia angolensis* and *A. fuscigula* are widely distributed in southern Africa the other species known from the region are restricted to montane areas (Du Preez & Carruthers 2009). *Amietia* tadpoles have elongated bodies with muscular tails. They rely on camouflage as a first line of defence, but swiftly dart away when threatened. Montane species usually inhabit the calmer sections of streams and rivers, since they lack suctorial mouthparts.

The taxonomic status of two Maloti-Drakesberg endemic species *Amietia vertebralis* (Hewitt 1927) and *A. umbraculata* (Bush 1952) was clarified by Tarrant *et al.* (2008). Using both morphometric and genetic evidence Tarrant *et al.* (2008) showed that these are both valid species and that the name *A. vertebralis* had been confused in most recent literature with the taxon correctly known as *A. umbraculata* (Bush 1952). The holotype of *Strongylopus hymenopus* (Boulenger 1920), of unknown provenance, was not conspecific with *A. vertebralis* (Hewitt 1927) and was tentatively referred to *A. fuscigula* (Duméril & Bibron 1841).

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The adult of *A. umbraculata* is a large dark brown dorsoventrally flattened frog, restricted to cold mountain streams and rivers at altitudes of ≥1750 metres, in Afromontane grassland of the Drakensberg. It is predominantly aquatic and can survive beneath ice sheets that periodically cover rivers in winter (Du Preez & Carruthers 2009). *Amietia vertebralis* on the other hand is a small to medium-sized, light to dark brown frog with dark markings, and is found in seepage areas along rocky banks of gently flowing streams. While *A. umbraculata* is more widely distributed than *A. vertebralis,* they occur sympatrically in northeastern Lesotho (Minter *et al.* 2004; Tarrant *et al.* 2008).

Tadpoles of the Drakensberg species have been described by Hewitt (1927), Van Dijk (1966), Lambiris (1987, 1988, 1989) and Channing (2001). However, the identification of *A. umbraculata* and *A. vertebralis* tadpoles remains problematic, because they exhibit limited interspecies and considerable intraspecies morphological variation that was previously overlooked or unresolved. There is also a strong possibility that the existing descriptions were based on compound collections of tadpoles comprising more than one species (Tarrant *et al.* 2008). The aim of this study was to confirm the identity of *A. vertebralis* and *A. umbraculata* tadpoles, describe their morphology and quantify the

Table 1. List of specimens of *Amietia umbraculata* and *A. vertebralis* used for DNA sequencing.

Taxon	Specimen no.	Genbank accession no.	Location	Coordinates
Amietia umbraculata	AACRG1005	HQ203038	Sani Pass, Lesotho	29°34′53.0″S, 29°17′19.7″E
Amietia umbraculata	AACRG1182	HQ203039	Mont-Aux-Sources, South Africa	28°45′35.2″S, 28°53′55.8″E
Amietia umbraculata	AACRG1171A	HQ203040	Mont-Aux-Sources, South Africa	28°45′35.2″S, 28°53′55.8″E
Amietia umbraculata	AACRG1171B	HQ203041	Mont-Aux-Sources, South Africa	28°45′35.2″S, 28°53′55.8″E
Amietia umbraculata	AACRG1171C	HQ203042	Mont-Aux-Sources, South Africa	28°45′35.2″S, 28°53′55.8″E
Amietia vertebralis	AACRG1210	HQ203043	Mont-Aux-Sources, South Africa	28°45′35.2″S, 28°53′55.8″E

variation, and to develop a reliable identification key.

MATERIALS & METHODS

Collection of tadpoles

Tadpoles were collected from three localities in the Upper Sani and Mont-Aux-Sources areas (Table 1). Locality 1 was a clear, slow-flowing mountain stream at Upper Sani; Locality 2 a stagnant pool 1 km southwest of the Sentinel at Mont-Aux-Sources and Locality 3 the clear, slow-flowing head waters of the Bilanjil River at Mont-Aux-Sources.

Archived material examined was obtained from the Port Elizabeth Museum (PEM) and included *A. umbraculata* tadpoles collected by Robert Essex from a pool near the summit of Mont-Aux-Sources. A series of seven tadpoles from a tributary of upper Mokhotlong River, Lesotho, was obtained from the South African Institute for Aquatic Biodiversity (SAIAB).

Collected tadpoles were euthanazed in the field using MS222 (tricaine methane sulphonate) and the majority were fixed in 5% neutral buffered formalin. Tissue samples were taken in a manner that did not to jeopardize the taking of body measurements, and then preserved in 96% ethanol. Tadpoles and tissue samples were accessioned in the African Amphibian Conservation (AAC) herpetological collection at the North-West University, Potchefstroom.

Morphometrics

Morphological terminology follows McDiarmid & Altig (1999) and tadpole developmental stages are based on Gosner (1960). Descriptive terminology of oral apparatus arrangement, jaw sheath pigmentation and position of eyes, nostrils, spiracle and vent follows Anstis (2002). Labial tooth row formulae are given according to McDiarmid & Altig (1999). Anterior (upper) labial tooth rows are labelled A1–Ax, and posterior (lower) labial tooth rows P1–Px. Measurements of total length, body length and tail length were made using a teflon dial vernier calliper, accurate to 0.1 mm. The remainder of the morphometric measurements were made using a Nikon SMZ1500 stereo microscope fitted with a dedicated Nikon DXM 1200 digital camera connected to a personal computer with NIS Elements software (Nikon).

Abbreviations used in the descriptions (Fig. 1) include: TL, total length (distance from the tip of the snout to the tip of the tail, which is the sum of the body length and tail length); BL, total body length (distance from the tip of the snout to the body-tail junction, taken from where the hind limbs emerge); BW, maximum body width at the widest point; BH, maximum body height; TAL, tail length; ATS, anterior tail shaft height; DTS, deepest tail shaft height, measured at the point where the anterior fin is deepest; ADF, anterior dorsal fin height; DDF, deepest dorsal fin height; DVF, deepest ventral fin height; MTH, maximum tail height;TDP, tail deepest portion (expressed as percentage of tail where dorsal fin is deepest); SA,

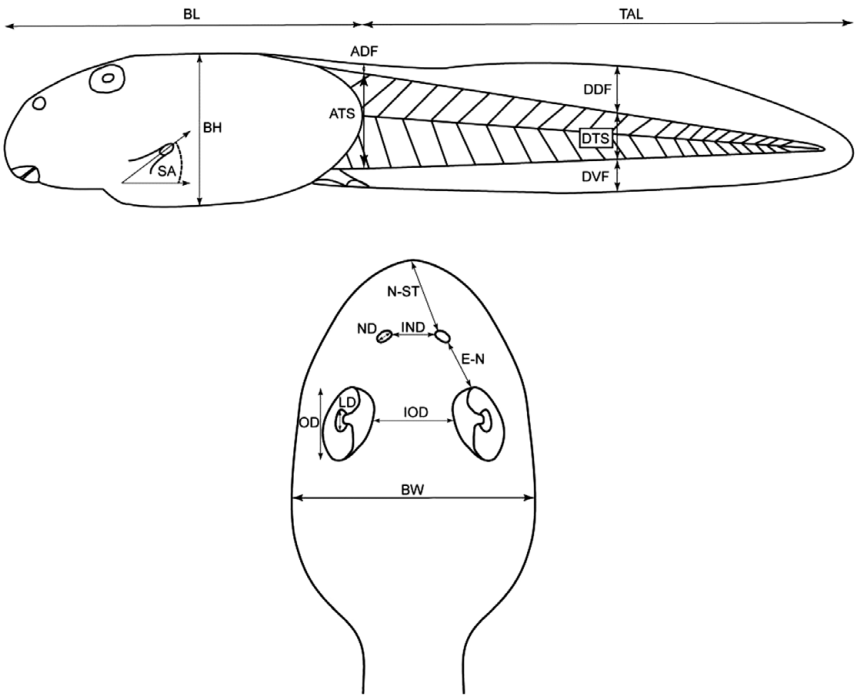

Fig. 1. Morphometric measurements obtained. Abbreviations: BL, body length; BW, maximum body width; BH, maximum body height; TAL, tail length; ATS, anterior tail shaft height; DTS, deepest tail shaft height; ADF, anterior dorsal fin height; DDF, deepest dorsal fin height; DVF, deepest ventral fin height; SA, spiracle angle; OD, ocular diameter; LD, lens diameter; IOD, inter-orbital distance; E–N, distance from the front of the eye to nostril; ND, nostril diameter; IND, inter-narial distance; N–ST, distance from nostril to snout tip.

spiracle angle; OD, ocular diameter; LD, lens diameter; IOD, inter-orbital distance; EL-ST, distance from front eye-line to snout tip; E-ST, distance from the front of the eye to the snout tip; E-N, distance from the front of the eye to nostril; ND, nostril diameter; IND, inter-narial distance; N-ST, distance from nostril to snout tip; ODW, oral disc width; LTRF, labial tooth row formula.

According to Hensley (1993) a tadpole's development is divided into a fast-growing phase when the tadpole increases in size and a second fast-developing phase (between Gosner stages 35–37) when the tadpole changes morphologically with the onset of metamorphosis. For this reason averages were taken for two groups of Gosner development stages (Gosner 26–34 and Gosner 35–40).

A geometrical construct was designed to quantify the rounding of the tail tip. Fixed points along the dorsal and ventral fin margin were assigned at positions 70% and 90% of the tail shaft (Fig. 2). Lines were drawn through the two dorsal and the two ventral points, respectively, and extended posteriorly to the point where the lines crossed. The distance from the tail tip to the line intersection (A in Fig. 2) was measured and expressed as a percentage of the tail length. This technique was performed only on specimens that had an intact tail fin with no signs of regrowth.

Data analyses

Statistical analyses of the data were performed using Statistica version 10 software (StatSoft,

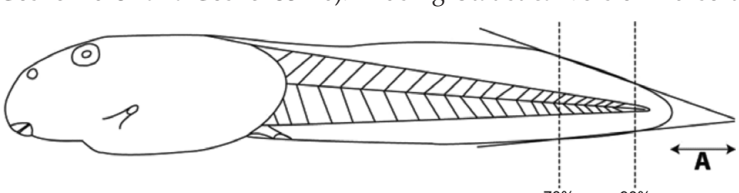

Fig. 2. Geometrical construct to quantify tailfin rounding.

Tulsa, OK). ANOVA was performed and statistical significant differences in the dataset were determined using the Tukey honest significant difference (HSD) test at a 95% confidence level. Various combinations of measurement ratios were tested for statistical significance. Only significant morphometric parameter differences were included in the results. *P*-values < 0.05 were taken to indicate significant differences.

Amietia umbraculata

Series examined. A total of 30 tadpoles was measured. These include 13 tadpoles from Sani Top, Dinakeng River tributary, Lesotho (AACRG1166–1170), four tadpoles from Mont-Aux-Sources, Bilanjil River, South Africa (AACRG1171a-d), seven tadpoles from rivers and their tributaries in Lesotho (SAIAB 87886; 87824; 87826; 87867; 87828) and six from Sani River, Lesotho and Sanqebethu above Mokhotlong, Lesotho (PEMT076; T070).

Taxonomic note. DNA sequencing was performed for four specimens (AACRG1171a-d and a specimen from batch AACRG1182). The DNA was matched with tissue from an adult specimen (AACRG1005). GenBank accession numbers are listed in Table 1.

Amietia vertebralis

Series examined. A total of 33 tadpoles was measured. These include 20 tadpoles (AACRG 1173) collected in a marsh at the base of Namahadi Pass, Mont-Aux-Sources, South Africa. Ten tadpoles (AACRG1170, 1172, 1174, 1210) were collected at the Bilanjil River, Mont-Aux-Sources, South Africa. Three tadpoles from the source of the Tugela River, Mont-Aux-Sources, South Africa were obtained from PEM (T294).

Taxonomic note. Ten tissue samples from AACRG1174 and 1210 were sequenced and matched the DNA sequences (unpubl.) from adult specimens belonging to *A. vertebralis.* One specimen was sequenced at the North-West University (see Table 1 for the Genbank Accession number).

Molecular analyses

To verify tadpole identification DNA sequencing was performed for 11 tadpoles of *A. vertebralis* and for four tadpoles of *A. umbraculata.* Tadpole tail tissue (preserved in 96% ethanol) was used for DNA extraction using the NucleoSpin Tissue Kit (Separations, Macherey-Nagel GmbH, Dueren, Germany) and following manufacturer's instructions. DNA quality and quantity were determined using a 1% (w/v) agarose gel and a Nano Drop Spectrophotometer ND-1000 v3.5.2 (NanoDrop Technologies, Delaware, USA). Mitochondrial 16S gene fragments for both *A. vertebralis* (10 samples) and *A. umbraculata* (five samples) were amplified using the primer pair 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi *et al.* 1991). PCR reaction mixtures contained1 X PCR master mix (4U/μl*Taq* DNA Polymerase in reaction buffer, 2 mM $MgCl_2$, 0.2 mMdNTP); 0.3 mM of each primer; 10-100ng DNA and PCR-grade water in a final reaction volume of 25μl. Cycling conditions were set at 95°C for 90 seconds followed by 34 cycles of 45 seconds of denaturation at 95°C, 45 seconds of annealing at 55°C, 90 seconds of extension at 72°C, and a 5 min final extension step at 72°C. PCR amplifications were confirmed on a 1.5% (w/v) agarose gel and subsequently purified using the NucleoSpin Extract II Kit (Separations, Macherey-Nagel GmbH, Dueren, Germany) prior to sequencing. All sequencing reactions were performed on a Genetic Analyzer 3130 (Applied Biosystems, California, U.S.A.). Sequences were matched with existing Genbank sequences using Mega 4 software and submitted to Genbank.

RESULTS

Amietia umbraculata (Fig. 3)

Description. Based on one tadpole (AACRG1165), Gosner stage 34. Specimen in an excellent state of preservation. BL 15.8 mm, TL 43.4 mm, for further measurements see Table 2. In dorsal view the body shape is ovoid. In lateral view (Fig. 3a) the body appears elongated and dorsoventrally flattened, BW 124% of BH, flattening towards blunt snout. Colouration pale brown with extensive dark brown mottling on the entire tail musculature. In dorsal view prominent broad transverse bands present on the tail musculature with bands becoming progressively narrower towards the tip of the tail. Dorsal tail fin margin with fine, brown mottling. Ventral fin unpigmented, except for a few inconspicuous chromatophores along the margin of the posterior 25%. Dorsal side of the body browner than the tail, with scattered dark brown spots. No pigmentation visible ventrally, becoming transparent when fixed. Nostrils narrowly spaced, with small ridge positioned midway between the snout tip and the eyes. IND 56% of IOD. Eyes positioned dorsolaterally, relatively

Table 2. Measurements for *Amietia umbraculata* and *A. vertebralis* tadpoles. See Materials and Methods for abbreviations.

Species	*A. umbraculata*	*A. vertebralis*
Accession no.:	AACRG1171A	AACRG1210
Gosner stage:	34	36
TL (mm)	43.4	40.6
BL (mm)	15.8	15.9
BW (mm)	7.8	7.5
BH (mm)	6.3	5.6
TAL (mm)	27.6	24.7
ATS (mm)	3.9	3.3
DTS (mm)	3.1	2.8
ADF (mm)	0.0	0.4
DDF (mm)	3.1	1.7
DVF (mm)	1.5	0.9
TDP (%)	45	46
SA (degrees)	30	43
OD (mm)	2.2	1.9
LD (mm)	0.8	0.5
IOD (mm)	3.4	3.2
EL-ST (mm)	3.5	3.4
E-ST (mm)	3.9	3.8
E-N (mm)	1.7	1.7
ND (mm)	0.4	0.3
IND (mm)	1.9	1.8
N-ST (mm)	1.6	1.7
ODW (mm)	3.9	4.1
LTRF	5(2-5)/4(1-2)	6(2-6)/5(1-2)

large, OD 14% of BL, not protuberant, elygium present. Snout rounded in lateral and dorsal view. Spiracle below body axis, directed at about 45°, visible in dorsal view. Spiracular opening constricted, inner wall attached to body. Intestinal spiral conspicuous in ventral view, not visible in dorsal view and partly visible in lateral view. Short, marginal vent tube, medial with right wall displaced dorsally. Tail musculature well developed, ATS 62% of BH, gradually tapering from base to the relatively blunt tip. Tail fin higher than body, MTH 122% of BH and DDF 40% of MTH. Dorsal fin very low at origin, gradually rising to reach the deepest point in the middle of the tail and gradually tapering to a blunt tip. Ventral fin relatively straight. Oral disc large, ODW 25% of BL and 50% of BW, transversally elliptical, directed ventrally, not visible in dorsal view, but margins visible in lateral view. Large rostral gap in marginal papillae, all papillae with a rounded tip, double row below and 2–3 rows laterally, above and below. LTRF 5(2–5)/4(1–2), with a small gap in A2, gradually widening to A5, tooth row length decreases from A2 to A5; large gap in adoral row (P1), resulting in a short tooth row, this tooth row also markedly further away from the other posterior tooth rows, situated closer to the angle of the mouth. Small gaps in P2; P3 and P4 continuous. Jaw sheaths moderately pigmented along the margin, upper jaw sheath M-shaped and lower jaw sheath V-shaped. Both jaws finely serrated, with three parts having different colourations: base not keratinized (unpigmented), medial part moderately keratinized (brown); edge black (Fig. 3c).

Variation. The series examined consisted of seven specimens in Gosner stages 25–34 and 23 specimens in stages 35–40. Table 3 shows the averages of the measurements of the series examined in each Gosner grouping with minimum and maximum values in brackets. Proportions vary as follows:

Gosner 25–34: BW 127–150% of BH, IND49–70% of IOD, OD 12–15% of BL, ATS59–72% of BH, ODW41–53% of BW, MTH 92–112% of BH, DDF 29–38% of MTH.

Gosner 35–40: BW 119–144% of BH, IND 42–79% of IOD, OD 11–15% of BL, ATS 59–76% of BH, ODW 29–51% of BW, MTH 94–125% of BH, DDF 31–42% of MTH.Tooth row formula variations are as follows: 5(2–5)/4(1–2); 6(2–6)/4(1–2).

Amietia vertebralis (Fig. 4)

Description. Description based on one tadpole (AACRG1210) in Gosner stage 36.

Specimen in a good state of preservation (a small ventral part of tail excised for DNA analysis). BL 15.9 mm, TL 40.6 mm, for further measurements see Table 2. Colouration dark brown with a few darker brown spots on the dorsal tail musculature, pigmentation extends to ventral aspect of musculature. Spots occur sporadically, varying in size with no discernible pattern. Dark brown bands on the dorsal view of the tail thinner than those of *A. umbraculata.* Mottling on tail fin more prominent on the upper half of the tail shaft. Body brown dorsally, with scattered dark brown mottling. Some pigmentation visible on ventral surface, but predominantly white (becomes translucent in fixative) with gold-coloured melanophores in the gular region. In dorsal view the body shape ovoid to pear-shaped in some specimens. In lateral view (Fig. 4a) body appears elongated and depressed, BW 134% of BH, flattening towards blunt snout. Nostrils narrowly spaced, not protuberant with small ridge, positioned approximately midway between the snout tip and the eyes. IND

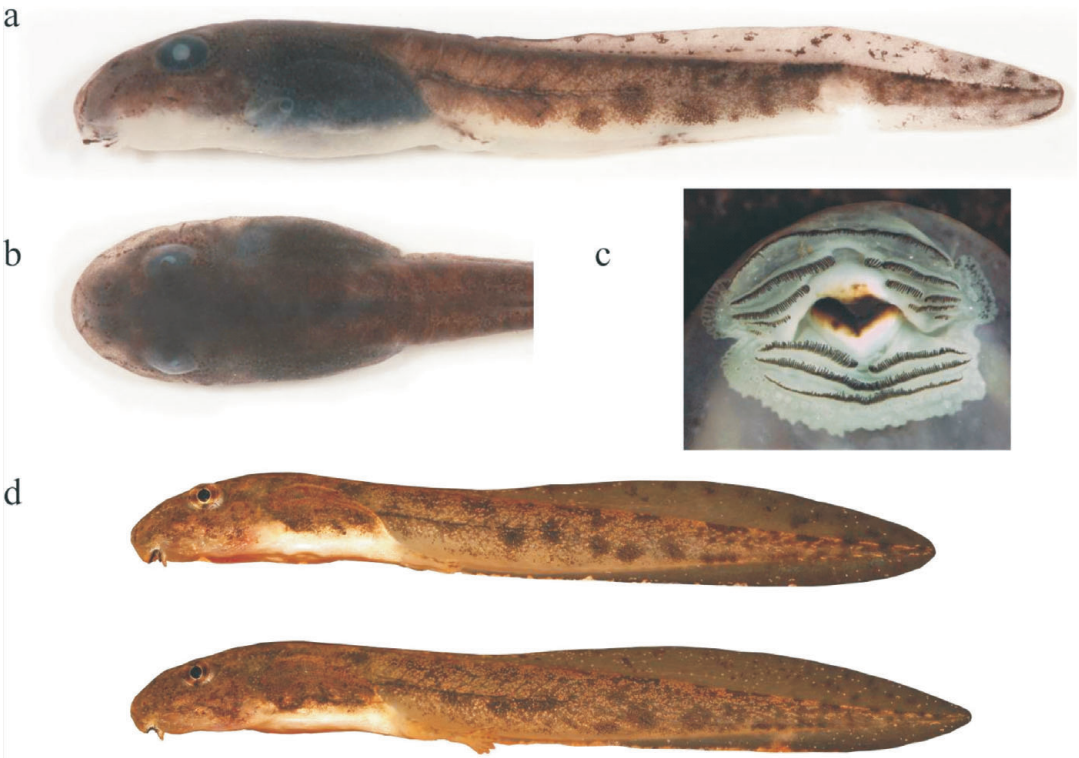

Fig. 3. *Amietia umbraculata* tadpole. **a**, Lateral view; **b**, dorsal view; **c**, oral disc of a 50.5 mm preserved specimen (AACRG1165), Sani Pass; **d**, two live specimens showing mottling of tail musculature.

56% of IOD. Eyes positioned dorsolaterally, relatively large, OD 12% of BL, not protuberant, elygium present. Snout rounded in lateral and dorsal view. Spiracle below body axis, directed at about 45° visible in dorsal view. Spiracular opening constricted, inner wall attached to body. Intestinal spiral conspicuous in ventral view, not visible in dorsal view and partly visible in lateral view. Short, marginal vent tube, medial with right wall displaced dorsally. Tail musculature well developed, ATS 59% of BH, gradually tapering from base to the bluntly rounded fin tip. Tail fin higher than body, MTH 104% of BH and DDF 31% of MTH. Dorsal fin initially very low at origin at the base of the tail, rising quite rapidly to about 46% of the tail length, tapering down to a blunt point. Oral disc large, ODW 0.26 of BL and 0.55 of BW, transversally elliptical, directed ventrally, not visible in dorsal view, but margins visible in lateral view. Rostral gap in marginal papillae, two to five rows on the sides above and below and a double row below, all papillae with a rounded tip. LTRF 6(2–6)/5(1–2), with a small gap in A2, gradually widening to A6, tooth row length decreases from A2 to A5; large gap in adoral row (P1), resulting in a short tooth row, the small gap in P2; P3, P4 and P5 is continuous. Beak moderately pigmented, upper jaw sheath almost M-shaped and lower jaw sheath V-shaped. Both jaws finely serrated, with three parts having different colourations: base not keratinized (unpigmented); medial part moderately keratinized (brown); edge well keratinized (black) (Fig. 4c).

Variation. The series examined consisted of 19 specimens in stages 25–34 and 14 specimens in stages 35–40. Table 3 shows the averages of the measurement of the series examined in each Gosner grouping with minimum and maximum values in brackets. Proportions vary as follows:

Gosner 25–34: BW 123–146% of BH, IND 52–70% of IOD, OD 9–13% of BL, ATS 49–65% of BH, ODW 41–77% of BW, MTH 100–117% of BH, DDF 23–33% of MTH.

Gosner 35–40: BW 123–146% of BH, IND 42–57% of IOD, OD 10–13% of BL, ATS 52–65% of BH, ODW 48–57% of BW, MTH 93–117% of BH, DDF 25–33% of MTH. Labial tooth row formula varia-

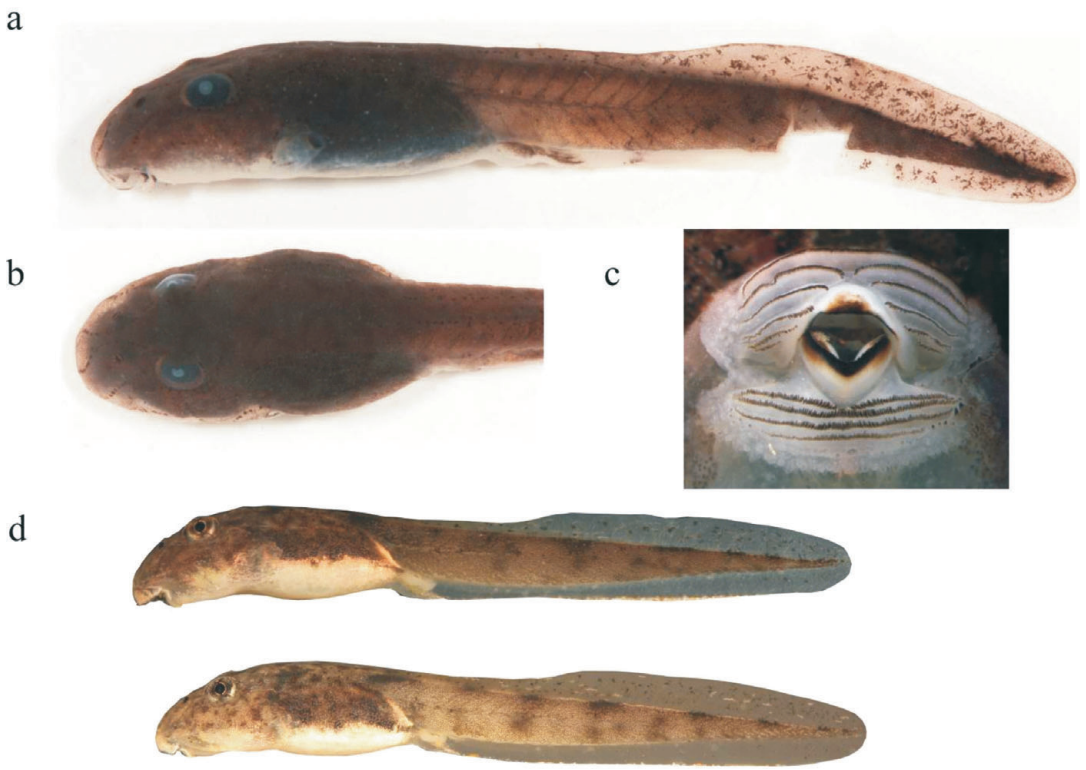

Fig. 4. *Amietia vertebralis* tadpole. **a**, Lateral view; **b**, dorsal view; **c**, oral disc of a 40.6 mm preserved specimen (AACRG1210), Mont-Aux-Sources; **d**, two live specimens showing mottling of tail musculature.

tions are as follows: 6(2–6)/5(1–2); 7(2–7)/6(1–3); 5(2–5)/5(1–2).

Interspecies morphometric differences

Identity of the tadpole specimens was confirmed using molecular analysis by comparison of sequence data of *A. umbraculata* and *A. vertebralis* to sequences from GenBank. Of the morphometric significance tests conducted for interspecies variation the following combinations were statistically significant: Gosner stage *vs* total length (Fig. 5a), body length *vs* tail length (Fig. 5b), tail curvature *vs* tail length (Fig. 5c), anterior tail shaft height/body length ratio *vs* tail deepest portion/tail length ratio (Fig. 5d).

A further significant difference was the size of the tadpole relative to its Gosner stage. We found that the tadpoles of *A. umbraculata* were significantly larger at any given developmental stage than those of *A. vertebralis*. *Amietia umbraculata* had a longer tail based on its body length to total length ratio, which was 15% (S.D. ± 7.8%) more than that of *A. vertebralis*.

The mottling of the tail musculature of *A. umbraculata* was more conspicuous than that of *A. vertebralis,* especially in the ventral half. This could be observed clearly in both live and fixed tadpoles (Figs 3d & 4d). The distribution of *A. vertebralis* is restricted to northern and northeastern Lesotho, not reaching Sani Pass on the northeastern border of Lesotho (Minter *et al.* 2004; Tarrant *et al.* 2008). *Amietia umbraculata* has a wider distribution and can be found throughout Lesotho at altitudes above 1750 m a.s.l. Geographic distribution can therefore be used as a distinguishing characteristic.

The labial tooth row formula of *A. vertebralis* was found to be highly variable. One distinct difference between the two species was that tadpoles of *A. vertebralis* never had fewer than five lower labial tooth rows (two divided, three undivided), but some had more. In comparison the tadpole of *A. umbraculata* showed no variation in lower labial tooth rows and all had four rows (two divided, two undivided). We found that for tadpoles of *A. umbraculata* the dorsal fin originated well behind the body whereas *A. vertebralis* had an

Table 3. Average body measurements for *Amietia umbraculata* and *A. vertebralis* tadpoles. For abbreviations, see Materials and Methods. All the measurements are averages of the indicated Gosner stages, with the minimum and maximum values in brackets.

	Amietia umbraculata		*Amietia vertebralis*	
	Gosner 25–34	Gosner 35–40	Gosner 25–34	Gosner 35–40
TL (mm)	50.6 (45.1; 55.5)	56.9 (47.6; 76.6)	32.5 (26.5; 43.4)	44.5 (40.2; 56.9)
BL (mm)	18.1 (16.0; 19.9)	20.8 (17.2; 27.7)	13.2 (10.7; 15.8)	17.1 (15.4; 22.0)
BW (mm)	9.5 (8.5; 10.5)	11.2 (9.1; 15.0)	6.5 (5.3; 7.8)	8.4 (7.4; 10.8)
BH (mm)	6.8 (5.9; 7.4)	8.3 (6.6; 10.4)	4.9 (4.1; 6.3)	5.8 (2.1; 8.9)
TAL (mm)	32.5 (29.1; 35.6)	36.1 (30.4; 48.9)	19.4 (15.8; 27.6)	27.5 (24.3; 34.9)
ATS (mm)	4.4 (3.9; 5.0)	5.7 (4.8; 6.7)	2.8 (2.1; 3.9)	3.6 (3.1; 5.1)
DTS (mm)	3.2 (3.0; 3.5)	4.0 (2.8; 4.7)	2.4 (1.7; 3.1)	3.2 (2.4; 4.6)
ADF (mm)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.4 (0.2; 0.5)	0.4 (0.0; 0.5)
DDF (mm)	2.4 (1.9; 2.8)	2.7 (2.1; 4.0)	1.3 (1.0; 1.6)	1.7 (1.4; 2.0)
DVF (mm)	1.1 (0.8; 1.3)	0.9 (0.1; 1.8)	0.9 (0.5; 3.1)	1.0 (0.5; 1.3)
TDP (%)	48 (42; 53)	56 (50; 60)	36 (28; 45)	42 (33; 48)
SA (°)	56 (42; 70)	43 (40; 45)	41 (30; 55)	41 (37; 55)
OD (mm)	2.3 (2.2; 2.5)	2.7 (2.2; 3.5)	1.5 (1.2; 2.2)	2.0 (1.8; 2.4)
LD (mm)	0.6 (0.6; 0.7)	0.8 (0.7; 0.9)	0.5 (0.4; 0.8)	0.6 (0.5; 0.8)
IOD (mm)	3.4 (3.0; 4.0)	3.9 (2.8; 5.7)	2.7 (2.1; 3.5)	3.5 (3.0; 4.4)
EL-ST (mm)	3.4 (3.0; 3.6)	4.1 (3.8; 4.2)	3.0 (2.4; 3.7)	3.9 (3.3; 5.6)
E-ST (mm)	4.0 (3.7; 4.2)	4.8 (4.3; 5.3)	3.4 (2.8; 4.1)	4.4 (3.8; 6.1)
E-N (mm)	1.9 (1.7; 2.1)	2.3 (1.9; 2.7)	1.4 (1.1; 1.8)	1.9 (1.6; 2.4)
ND (mm)	0.3 (0.3; 0.4)	0.4 (0.3; 0.6)	0.3 (0.2; 0.5)	0.4 (0.3; 0.5)
IND (mm)	2.1 (1.9; 2.2)	2.1 (1.9; 2.4)	1.6 (1.3; 2.4)	1.7 (1.6; 1.9)
N-ST (mm)	1.7 (1.4; 2.0)	2.0 (1.8; 2.5)	1.7 (1.2; 3.1)	2.0 (1.5; 3.1)
ODW (mm)	4.0 (3.7; 4.3)	4.9 (4.2; 6.2)	3.2 (2.4; 4.1)	4.4 (4.0; 5.3)
LTRF	5(2–5)/4(1–2); 6(2–6)/4(1–2)		6(2–6)/5(1–2); 7(2–7)/6(1–3); 5(2–5)/5(1–2); 8(2–8)/5(1–2)	

average ADF of 0.4 mm (Table 3). In a graph of Gosner stage against total length we found that *A. umbraculata* tadpoles were generally larger than those of *A. vertebralis* at any given Gosner stage (Fig. 5a). We also found a significant difference ($P < 0.05$) in the ratios of tail length to body length (Fig.5b). *Amietia umbraculata* had a longer tail than *A. vertebralis.* The body of *A. umbraculata* was on average 52% of the tail length and that of *A. vertebralis* 66%. In Fig. 5c the difference in tail fincurvature was plotted, showing that the tail fin of *A. umbraculata* was more rounded. The dorsal fin of *A. vertebralis* was less convex than that of *A. umbraculata* and reached its highest point sooner. The dorsal fin of *A. umbraculata* originated well behind the body and had a gradual slope tapering to a blunt point. In *A. vertebrali,* however, the anterior part of the dorsal fin averaged in the range of 0.4 mm at the tail base and rose abruptly one third into the tail before tapering gradually to a blunt tip.

For anterior tail shaft height/body length ratio against tail deepest portion/tail length ratio we found that large variation existed in the tail shaft height/body length ratio for *A. umbraculata,* while large variation in *A. vertebralis* was expressed by the tail deepest portion against tail length ratio (Fig. 5d). However, there was a significant difference ($P < 0.05$) in tail shaft height and tail deepest portion in these two species, respectively. The dorsal fin of *A. umbraculata* reached its maximum height well beyond the mid point of the tail, further back than in *A. vertebralis,* but some overlap did exist in smaller tadpoles. Furthermore, mottling on the tail musculature in *A. umbraculata* was much more extensive in both live and preserved specimens (Figs 3 & 4).

DISCUSSION

The tadpole of *A. umbraculata* was described by Hewitt (1927), who emphasized the importance of the width of the broad oral disc, the additional inconspicuous adoral tooth row, the tail fin and the labial tooth row formula in distinguishing it from

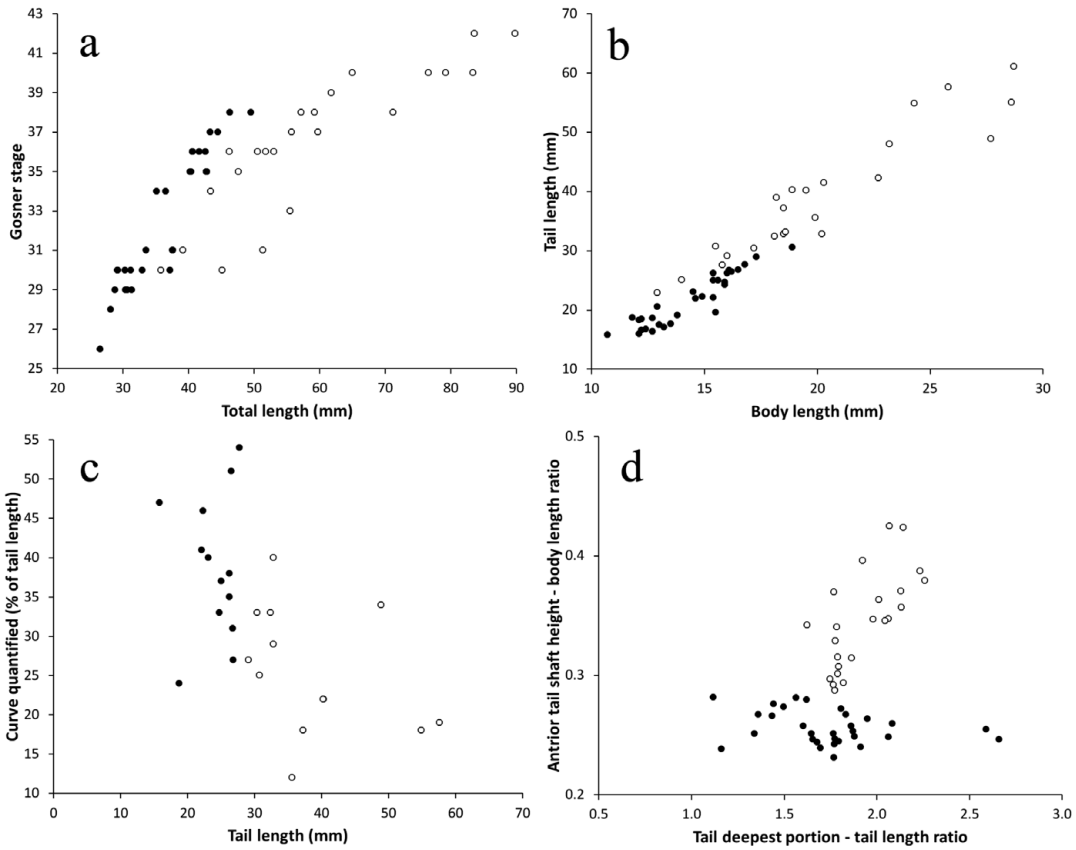

Fig. 5. Scatter plots of morphometric comparison between tadpoles of *Amietia umbraculata* (○) and *A. vertebralis* (●) to show (**a**) Gosner stage against total length, (**b**) tail length against body length, (**c**) tail curvature against tail length, and (**d**) anterior tail shaft height/body length ratio against tail deepest portion/tail length ratio.

other ranid relatives. There was, however, considerable variation in labial tooth row formula in the relatively small sample Hewitt studied. One of the five tadpoles had as many as eight upper tooth rows, whereas the rest of the sample had six and fewer. Inger (1959) agreed with Hewitt's description of the tadpole of *A. umbraculata* except that the inner row of papillae was not continuous in five specimens studied and that there were only six anterior tooth rows rather than eight.

Wager (1965) briefly described tadpoles that he referred to *R. vertebralis* and *R. hymenopus* (now *A. vertebralis*) on the basis of having been found in close proximity to the adults of these species. He did not succeed in rearing any of the tadpoles through metamorphosis and could therefore not confirm his identifications. He nevertheless assigned different labial tooth row formulae to each of the two species, with *R. hymenopus* having only three upper tooth rows (one continuous), and *R. vertebralis* up to five upper tooth rows, two being continuous. His description of *R. vertebralis* therefore differs considerably from that of Hewitt (1927) who found one continuous and as many as seven divided tooth rows above and four below, in this species.

According to Van Dijk (1966) *Strongylopus hymenopus* tadpoles are characterized by an elygium in the eye, the lower jaw sheath is deep and pigmented to the base, the vertical height of the tail is not greater than the height of the trunk and the tail is not mottled. He distinguished between *A. umbraculata* and *A. vertebralis* on the basis of differences in labial tooth row formulae, position of the lower adoral row, spiracular characters, neuromast organs and black pigmentation on the posterior part of the tail. Lambiris (1989) remarked that the variation in labial tooth rows noted by Van

Dijk might either be interspecific or intraspecific.

Lambiris (1987) described the tadpole of *A. vertebralis* from specimens collected at Bilanjil at 2960 m a.s.l. He identified a subset of these tadpoles as *A. umbraculata*, but could not identify the remainder because of inconsistencies between Wager's description and illustrations and Van Dijk's key. Lambiris preserved some of the tadpoles and kept the rest alive for rearing through to metamorphosis. He successfully raised the tadpoles to froglets and preserved some tadpoles at different developmental stages. Lambiris also re-examined the *A. vertebralis* tadpoles in the Natal Museum, which included Wager's specimens. These tadpoles could be divided into two groups: (1) tadpoles that were identical to those Wager described; and (2) the tadpoles that Lambiris reared and referred to as *A. vertebralis* based on post-metamorphic characteristics. He also noted the large variation that existed in the tooth row formulae. Lambiris (1989) described the tadpole of *S. hymenopus* as having a plump body; body and tail that is brownish grey with dark blotches; fins that are faintly stippled; anterior fifth of the tail that is narrow and rising to reach the deepest point just prior to the middle of the tail; a round non-constricted spiracle; an oral disk with a single row of papillae along the labial tooth rows and a double row in the corners of the mouth; jaw sheaths that are deep and pigmented to the base and a labial tooth row formula of 3(2-3)/3(1) or 3(2-3)/3.

Channing (2001) also described the tadpoles of *A. umbraculata* and *A. vertebralis. Strongylopus hymenopus* (now *Amietia vertebralis*) was described as heavy bodied dark tadpoles with the tip of the tail pale; elygium present; a large round spiracle; a double row of oral papillae and a labial tooth row formula of 3(2-3)/3 or 2(2-3)/3(1-2). *Amietia umbraculata* was described as having a slightly flattened body and a large mouth having a labial tooth row formula of 7(3–7)/4. Du Preez & Carruthers (2009) used the shape of the dorsal fin, as well as the position of the spiracle to separate the two species. They noted that the position of the spiracle in *A. umbraculata* was well below the lateral body axis and in the case of *A. vertebralis* only just below the lateral body axis. The labial tooth row formula for *A. vertebralis* given by Du Preez & Carruthers (2009) is incorrect. The three labial tooth rows indicated for the lower jaw should be five or more.

To add to the confusion a few incorrect illustrations were published. Based on the shape of the dorsal fin, the drawing of the *A. umbraculata* tadpole in Channing (2001) is probably that of *A. vertebralis.* Wager (1986) probably drew a tadpole of *Strongylopus grayii* for *S. hymenopus* (now *A. vertebralis*). In Passmore & Carruthers (1995) the photograph of *A. umbraculata* taken at Mont-Aux-Sources is that of an *A. vertebralis.*

Although the tadpoles of both *A. umbraculata* and *A. vertebralis* are morphologically very similar, it is possible to tell them apart. Identification in the field based on the pattern of the blotches on the tail will most likely be accurate for the trained eye but the identification has to be confirmed by studying the mouth parts and other body features at the microscopic level.

Key for identifying *Amietia umbraculata* and *A. vertebralis* tadpoles

1. Four labial tooth rows in lower jaw; extensive mottling on the entire tail musculature; dorsal fin originates well behind body and gradually rises to reach its maximum depth at about 50% of the tail length, then tapers gradually to a blunt tip; tail about 1.9 times body length . . . . . *Amietia umbraculata*

1′. Five or more labial tooth rows in lower jaw, mottling on tail musculature confined to upper half; dorsal fin originates on the body but remain low at first, rising abruptly to reach its maximum depth at about 40% of the tail length then slowly tapers to a blunt tip; tail about 1.5 times body length . . . . . *Amietia vertebralis*

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