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

Donnavan J.D. Kruger, Ché Weldon & Louis H. Du Preez*

School of Environmental Sciences and Development, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520 South Africa

Received 6 December 2010. Accepted 13 June 2011

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, 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-Drakensberg 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).

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
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 euthanized 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,

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Specimen no.</th>
<th>Genbank accession no.</th>
<th>Location</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amietia umbraculata</em></td>
<td>AACRG1005</td>
<td>HQ203038</td>
<td>Sani Pass, Lesotho</td>
<td>29°34'53.0&quot;S, 29°17'19.7&quot;E</td>
</tr>
<tr>
<td><em>Amietia umbraculata</em></td>
<td>AACRG1182</td>
<td>HQ203039</td>
<td>Mont-Aux-Sources, South Africa</td>
<td>28°45'35.2&quot;S, 28°53'55.8&quot;E</td>
</tr>
<tr>
<td><em>Amietia umbraculata</em></td>
<td>AACRG1171A</td>
<td>HQ203040</td>
<td>Mont-Aux-Sources, South Africa</td>
<td>28°45'35.2&quot;S, 28°53'55.8&quot;E</td>
</tr>
<tr>
<td><em>Amietia umbraculata</em></td>
<td>AACRG1171B</td>
<td>HQ203041</td>
<td>Mont-Aux-Sources, South Africa</td>
<td>28°45'35.2&quot;S, 28°53'55.8&quot;E</td>
</tr>
<tr>
<td><em>Amietia umbraculata</em></td>
<td>AACRG1171C</td>
<td>HQ203042</td>
<td>Mont-Aux-Sources, South Africa</td>
<td>28°45'35.2&quot;S, 28°53'55.8&quot;E</td>
</tr>
<tr>
<td><em>Amietia vertebralis</em></td>
<td>AACRG1210</td>
<td>HQ203043</td>
<td>Mont-Aux-Sources, South Africa</td>
<td>28°45'35.2&quot;S, 28°53'55.8&quot;E</td>
</tr>
</tbody>
</table>
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 fin. 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,
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’-CGCCTGTTTATCCAAACAT-3’) and 16Sbr (5’-CCGGTGCTGAACATCGATACGT-3’) (Palumbi *et al.* 1991). PCR reaction mixtures contained 1 X PCR master mix (4U/µlTag DNA Polymerase in reaction buffer, 2 mM MgCl2, 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...
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, IND 49–70% of IOD, OD 12–15% of BL, ATS 59–72% of BH, ODW 41–53% 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. 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
56% of IOD. Eyes positioned dorsolaterally, relatively large, OD 12% of BL, not protuberant, epygium 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-
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
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 fin curvature 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. vertebralis*, 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

### 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.

<table>
<thead>
<tr>
<th></th>
<th><em>Amietia umbraculata</em></th>
<th><em>Amietia vertebralis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gosner 25–34</td>
<td>Gosner 35–40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TL (mm)</td>
<td>50.6 (45.1; 55.5)</td>
<td>32.5 (26.5; 43.4)</td>
</tr>
<tr>
<td>BL (mm)</td>
<td>18.1 (16.0; 19.9)</td>
<td>13.2 (10.7; 15.8)</td>
</tr>
<tr>
<td>BW (mm)</td>
<td>9.5 (8.5; 10.5)</td>
<td>6.5 (5.3; 7.8)</td>
</tr>
<tr>
<td>BH (mm)</td>
<td>6.8 (5.9; 7.4)</td>
<td>4.9 (4.1; 6.3)</td>
</tr>
<tr>
<td>TAL (mm)</td>
<td>32.5 (29.1; 35.6)</td>
<td>19.4 (15.8; 27.6)</td>
</tr>
<tr>
<td>ATS (mm)</td>
<td>4.4 (3.9; 5.0)</td>
<td>2.8 (2.1; 3.9)</td>
</tr>
<tr>
<td>DTS (mm)</td>
<td>3.2 (3.0; 3.5)</td>
<td>2.4 (1.7; 3.1)</td>
</tr>
<tr>
<td>ADF (mm)</td>
<td>0.0 (0.0; 0.0)</td>
<td>0.4 (0.2; 0.5)</td>
</tr>
<tr>
<td>DDF (mm)</td>
<td>2.4 (1.9; 2.8)</td>
<td>1.3 (1.0; 1.6)</td>
</tr>
<tr>
<td>DVF (mm)</td>
<td>1.1 (0.8; 1.3)</td>
<td>0.9 (0.5; 3.1)</td>
</tr>
<tr>
<td>TDP (%)</td>
<td>48 (42; 53)</td>
<td>36 (28; 45)</td>
</tr>
<tr>
<td>SA (°)</td>
<td>56 (42; 70)</td>
<td>41 (30; 55)</td>
</tr>
<tr>
<td>OD (mm)</td>
<td>2.3 (2.2; 2.5)</td>
<td>1.5 (1.2; 2.2)</td>
</tr>
<tr>
<td>LD (mm)</td>
<td>0.6 (0.6; 0.7)</td>
<td>0.5 (0.4; 0.8)</td>
</tr>
<tr>
<td>IOD (mm)</td>
<td>3.4 (3.0; 4.0)</td>
<td>2.7 (2.1; 3.5)</td>
</tr>
<tr>
<td>EL-ST (mm)</td>
<td>3.4 (3.0; 3.6)</td>
<td>3.0 (2.4; 3.7)</td>
</tr>
<tr>
<td>E-ST (mm)</td>
<td>4.0 (3.7; 4.2)</td>
<td>3.4 (2.8; 4.1)</td>
</tr>
<tr>
<td>E-N (mm)</td>
<td>1.9 (1.7; 2.1)</td>
<td>1.4 (1.1; 1.8)</td>
</tr>
<tr>
<td>ND (mm)</td>
<td>0.3 (0.3; 0.4)</td>
<td>0.3 (0.2; 0.5)</td>
</tr>
<tr>
<td>IND (mm)</td>
<td>2.1 (1.9; 2.2)</td>
<td>1.6 (1.3; 2.4)</td>
</tr>
<tr>
<td>N-ST (mm)</td>
<td>1.7 (1.4; 2.0)</td>
<td>1.7 (1.2; 3.1)</td>
</tr>
<tr>
<td>ODW (mm)</td>
<td>4.0 (3.7; 4.3)</td>
<td>3.2 (2.4; 4.1)</td>
</tr>
<tr>
<td>LTRF</td>
<td>5(2–5)/4(1–2); 6(2–6)/4(1–2)</td>
<td>6(2–6)/5(1–2); 7(2–7)/6(1–3); 5(2–5)/5(1–2); 8(2–8)/5(1–2)</td>
</tr>
</tbody>
</table>
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

ACKNOWLEDGEMENTS

We are grateful to the South African Institute of Aquatic Biodiversity and the Port Elizabeth Museum for the specimens provided and the National Research Foundation of South Africa for financial support. Alan Channing, Karin Jordaan and Mathieu Badets assisted with molecular studies. We also thank Michael Cunningham, Les Minter and Jeanne Tarrant for valuable comments on the manuscript and Leon Meyer and Jeanne Tarrant for field assistance.

REFERENCES


Responsible Editor: P.le FN. Mouton