SALAMANDRA	42	1	1-12	Rheinbach, 20 February 2006	ISSN 0036-3375

The tadpole of *Tomopterna luganga* Channing, Moyer & Dawood, 2004 (Anura: Ranidae)

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Abstract. The study describes for the first time the larva of the Red Sand Frog, *Tomopterna luganga*. The description is based on colour photographs of living specimens, preserved specimens, and SEM images of the oral field and internal oral features. Larval stages were obtained from captive breeding experiments . The identity of the species was verified by DNA matching with the holotype and paratypes. *Tomopterna luganga* tadpoles were very similar to other described larvae within the genus. The Labial Tooth Row Formula 4(2-4)/3(1) of *T. luganga* distinguishes the species from some of its congeners. However, the currently available data does not allow the development of a reliable determination key for the tadpoles of *Tomopterna* species.

Keywords: Ranidae: *Tomopterna*; tadpole description; larva; morphology; oral disk.

Introduction

Nine species of Sand Frogs of the genus *Tomopterna* Duméril & Bibron, 1841 are currently recognized (Frost 2004). These stocky frogs of medium size inhabit sub-saharan Africa and are confined to areas where the soil is suitable for burrowing, but they are not restricted to sandy soils (Wager 1986, du Preez 1996, Channing 2001). Generally, very little is known about their biology. Sand frogs presumably can aestivate for considerable periods of dryness and emerge from their burrows after sufficient precipitation. Most of them breed in temporary pools (*T. natalensis* [Smith, 1849] in streams, Channing 2001), often remaining in riverbeds after the rainy season. Short embryonic development (2–3 days) and short larval periods (2–5 weeks) characterize the species of this genus (Passmore & Carruthers 1979, du Preez 1996). Beyond their robust body, a strongly developed metatarsal tubercle is indicative of their burrowing habits. Nine species most likely will not be the final number of species in the genus *Tomopterna*, because cryptic polyploid species have been discovered in the past (Bogart & Tandy 1976, Channing & Bogart 1996) and more cryptic species may yet be discovered in the future. Recently, a new species, *Tomopterna luganga* Channing, Moyer & Dawood, 2004, the Red Sand Frog, was described from central Tanzania (Channing et al. 2004). When compared to all the species in the genus *T. luganga* is most similar to *T. marmorata* (Peters, 1854). Descriptions of calls and genetic data for this new species were provided by Channing et al. (2004), but no tadpoles were collected along with the type adults.

In 2003, some of us (CM and AH) bought sand frogs from the pet trade. The locality given by the trading company was "Tanzania". The adults (Fig. 1) were sold as "*Tomopterna tuberculosa"* but did not match the descriptions in the standard key (Channing 2001). They were identified as belonging to the new species, *T. luganga*, then already in the process of description. We succeeded in breeding the individuals from the pet trade in captivity and collected the resulting larval stages. Thus, in this study we can supplement the earlier work by Channing et al. (2004) with data on the larva of *T. luganga*.

Materials and Methods

Terminology in describing tadpoles has not been standardized. We found it particularly helpful to consult and choose terms and descriptive characters eclectically from VAN DIJK (1966), ALTIG & MCDIARMID (1999a), and ANSTIS (2002). Terminology for internal oral features was adopted from WASSERSUG (1976). Tadpoles were staged according to the table of GOSNER (1960). Measurements were taken form microscopic digital images captured with a calibrated image acquisition system (Zeiss Axioplan microscope; analySIS software, Soft Imaging System). Voucher specimens were deposited at the Zoological Museum Hamburg, number ZMH A 07770.

The frog breeding colony was kept in semi-dry conditions in the terrarium. The temperature was adjusted to approximately 27 °C during the day and dropped only slightly during the night. Mating was induced by exposing the animals to artificial rain generated by a sprinkler system, after they had been kept dry and well fed for several weeks. Along with the rainfall, the photo period was set to approximately 14 hrs. per day. After these conditions had been applied for several days, we injected 200 µl human chorionic gondadotropin. Spawning occurred during the following night.

Because the breeding colony was obtained from the pet trade without an exact locality, several lines of evidence were followed to identify the species unequivocally: the colour pattern of the adults (Fig. 1) was exactly as described in CHANNING et al. (2004); second, the outer metatarsal tubercle was absent; the inner metatarsal tubercle was less than 140% of the second toe; the webbing notch between the fourth and fifth toes extended to distal subarticular tubercle of fifth toe; last and most importantly, comparison of 16S mtDNA with that from the type specimens showed only minimal differences.

We compared two larvae to the nine described *Tomopterna* species. We included *T. luganga* (TM 85107 and TM 85104), *T. cryptotis* (BOULENGER, 1907) (AC 1154 and AC 1100), *T. damarensis* DAWOOD & CHANNING, 2002 (TM 83916 and ZFMK 66403), *T. delalandii* (TSCHUDI, 1838) (AC 942 and AC 1144), *T. krugerensis* PASSMORE & CARRUTHERS, 1975 (AC 1506 and AC 1508), *T. marmorata* (AC 1387 and AC 1534), *T. natalensis* (AC 1391, AC 1451, ES 225, and ZFMK 68815), *T. tandyi* CHANNING & BOGART, 1996 (AC 2006, AC 1556 and AD 14), and *T. tuberculosa* (BOULENGER, 1882) (AC 1670 and AC 1672. See DAWOOD & UQUBAY (2004) and CHANNING et al. (2004) for specimen locality data except for ES 225 which was collected from Kyalami near Johannesburg, South Africa by LIZ SCOTT.

Genetic differentiation was based on the partial mitochondrial 16S rRNA gene. DNA was extracted from macerated muscle tissue according to standard methods and was stored at -20 °C (HILLIS et al. 1996). DNA amplification of partial 16S rRNA gene sequences was done on a Perkin-Elmer GeneAmp 2400 PCR system. The sense primer (16SA) 5'- CGCCTGTTTATCAAAAACAT - 3' and the anti-sense primer (16SB) 5'- CCGGTCTGAACTCAGATCACTG - 3' were chosen according to PALUMBI et al. (1991). They amplified a 582 base pair 16S rRNA fragment for further analysis. The cycling conditions for the amplification were: denaturation at 95 °C for 1:30 min; 33 cycles at 94 °C for 0:45 min, 55 °C for 0:45 min, and 72 °C for 1:30 min; then one extension cycle at 72 °C for 5:00 min. The PCR products were electrophoresed on an ethidium bromide stained agarose gel. The PCR products were purified using a Roche High Pure PCR purification kit and then cycle sequenced in the same PCR machine as used for amplification. The sequences were read from a 3100 ABI automated sequencer. Accession numbers for the sequences for the original *T. luganga* description (CHANNING et al. 2004) were AY547275-AY547276 and the number for *T. natalensis*, voucher ES 225 is AY547277. All the other specimens have their GenBank accession numbers given in DAWOOD and UQU-

BAY (2004) and CHANNING et al. (2004). The sequences for the two tadpoles sequenced in the present study were deposited in GenBank (National Center for Biotechnology Information [NCBI] of NIH) with numbers AY751302- and DQ017056.

The DNA sequences were aligned automatically using Clustal X (THOMPSON et al. 1997) and then checked visually. After alignment there were 449 unambiguously aligned sites for the 16S rRNA data. To determine percentage variation between the lab colony tadpole samples and the original type material, p-distances were calculated in PAUP*4.0b10 (SWOFFORD 2000).

Results from the sequencing study confirm that these larval specimens belong to *Tomopterna luganga*. The sequence divergence of tadpole samples from the types of *T. luganga* for 16S was 0% to 0.7%. Tadpole DNA was identical to one of the *T. luganga* paratypes, TM85107 (AC2060 in CHANNING et al. [2004]) and 0.7% different from the holotype TM85104 (AC2063 in CHANNING et al. [2004]). They differed by 4.3 to 4.5% from *T. marmorata*, a species that *T. luganga* can be confused with. In a parsimony analysis including all the species of *Tomopterna* from CHANNING et al. (2004) and using *Amietia vertebralis* (HEWITT, 1927) and *Afrana fuscigula* (DUMÉRIL & BIBRON, 1841) as outgroups, the tadpole samples formed a polytomy with the types of *T. luganga* and the *luganga* clade was the sister taxon to *T. marmorata* in the analyses.

For scanning electron microscopy tadpoles were anaesthetised using 0.1% chlorotone in tap water and fixed in 2.5% glutaraldehyde, 4% paraformaldehyde in 10% modified Steinberg's solution (MSS: 58 mM NaCl, 0.67 mM KCl, 0.34 mM $CaCl_2$, 0.83 mM $MgSO_4$, 4.6 mM HEPES; pH adjusted to 7.4). The heads were cut off, rinsed several times in 10% MSS and cleaned by 10 min ultrasonic treatment. The specimens were then postfixed in 1% OsO_4 in 10% MSS, dehydrated in acetone and critical point dried (Emitech K850). The dried specimens

Fig. 1. Adult female of *Tomopterna luganga* from the captive breeding group.

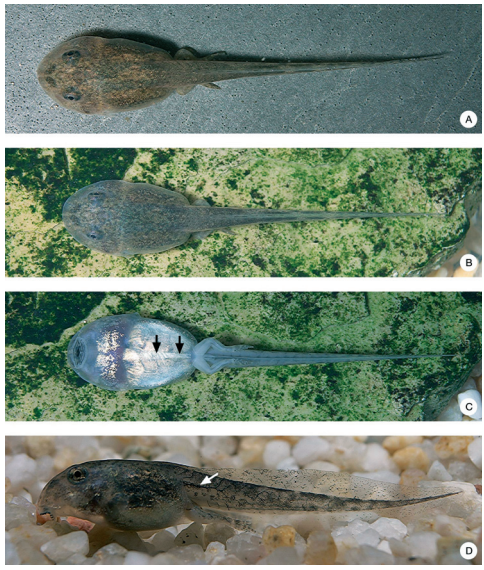

Fig. 2. Tadpole of *Tomopterna luganga*, coloration in life. **A** Dorsal view. Note the dark pigmentation of nasal sacs, brown mottled dorsal pattern, oval body shape, and dorsal eyes. **B** Second dorsal view to demonstrate the effect of backgrounds on the perceived colour pattern. **C** Ventral view showing medial belly streak (arrows) and subbranchial iridophore coloration. **D** Lateral view. Note in particular the dorsal fin height relative to ventral, invisibility of gut coils, lateral tail vein (arrow), pigmentation on muscular and fin parts of tail, and subterminal mouth.

were mounted and sputter-coated (Emitech K500) and were examined and digital images taken with a Philips XL30 ESEM scanning electron microscope.

Results

Advanced stages 36-40.

Colour in life (Fig. 2). The flanks and dorsum are mottled brown (Figs. 2a-b), with scattered golden iridophores and faint blueish iridescence. The nasal sacs are darkly pigmented and visible through the skin in dorsal view as crescentic brown blotches medial and posterior to the external naris. A pineal spot is absent. The venter is mostly silvery; gut coils are not visible in ventral view. There is a silver, longitudinal stripe in the midline of the venter (Fig. 2c). The iris is golden (occasionally greenish tints may occur). The skin ventral to the anterior parts of gill chambers has a silver-coppery tint (Fig. 2c). More posterior parts of the gills and heart are partly visible through the skin as red areas but mostly covered by a reflective iridophore cross band. The skin ventral to the buccal area is almost without pigmentation. The tail has blotches of dark pigmentation particularly along the dorsal edge of the tail musculature. Tail fins bear scattered melanophores. Some blood vessels of the tail fins are lined by melanophores making a broken network of vessels visible in most fully grown specimens (Fig. 2d). The *vena caudalis lateralis* and its side branches are clearly visible in first third of tail of most specimens (along 7–9 tail myomers, Fig. 2d). The *vena caudalis ventralis* is visible in ventral view, whereas a *vena caudalis dorsalis* is absent.

Colour in preservation (4% formalin) is similar to coloration in life, except for: There is no iridescence; silvery or golden iridophores have disappeared in preservation. The iris is dark brown and almost indistinguishable from the rest of the black eye. The median streak on venter disappears in preservation. The venter and buccal region are almost unpigmented and translucent, making gut coils, heart, gills, and superficial muscles visible in ventral view. The center of the gut coil is left of the mid-sagittal plane. There is deep, dark brown, subintegumental pigmentation that lines the peritoneum and obscures the lungs and upper gut coils in lateral view (Fig. 3). The skin of the flanks itself is rather poorly pigmented and mostly translucent.

External morphological features. The body shape is oval in dorsal view with a slight constriction of the body contour behind head (Fig. 2a). The body is wider than deep. The eyes are positioned dorsally. The external nares are slightly closer to the eyes than to the snout in lateral view (Fig. 3). The spiracle is sinistral and the spiracular orifice is fused to the body. The spiracle is directed dorsoposteriorly, opening just below the body axis (Fig. 3). The oral disk is subterminal and much narrower than the snout width. The marginal papillation of the oral disk is incomplete with a broad gap in the upper lip row (rostral gap) (Fig. 4). Oral papillae are arranged uniserially in the midventral part of the disk (lower lip), but biserially at the lateral part of the disk. Some submarginal papillae are located in lateral areas of the disk. Papillae terminate in blunt tips. The disk is divided into upper and lower lips by lateral indentations. The labial ridges bear uniserial keratodont rows. The Labial Tooth Row Formula (LTRF) is 4(2-4)/3(1). Keratodonts are spoon-shaped with 10-12 serrations along the edge (Fig. 4c). The jaws bear undivided beaks. The beaks are massive, darkly pigmented, and serrated along their edges.

The tail fin starts only at the end of the body. The tail tapers posteriorly into a narrow rounded tip. The dorsal tail fin is slightly convex in the posterior third of the tail (Fig. 3). The dorsal fin is higher than the ventral one (Fig. 3; Tab. 1). The maximum height of tail fins occurs at about 50-52% of the tail length. The anal siphon is located dextral on the ventral tail fin and opens at the margin of

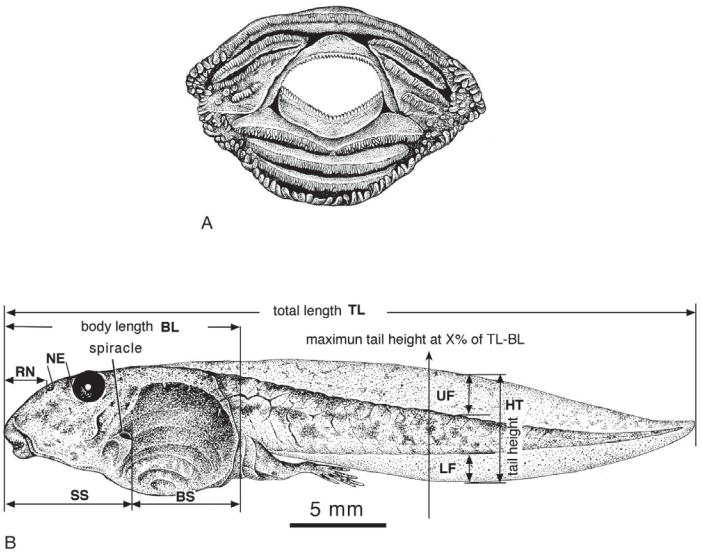

Fig. 3. *Tomopterna luganga* larva. **A** Larval oral disc. This drawing is a composite based on two SEM images, dorsal and ventral lip, respectively. For scale see bars in Fig. 4. **B** Stage 39 larva in lateral view, with indicators for the measurements taken in Tab. 1. – **A** and **B** not to scale.

the fin. The tail musculature is moderately strong: the height of the muscular tail at the base of the tail is approximately one-half of the body height (Fig. 3). The muscular part of the tail curves gently upwards (anaoural sensu VAN DIJK 1966). The epidermal melanocytes are dendritic in shape (Fig. 5).

Variation. The 3rd and/or 4th (proximal) upper labium tooth ridges can be fragmented into small, short ridges with reduced keratodont numbers. The first (proximal) lower labium tooth ridge is undivided in some specimens. Abnormalities also include fragmentation of third (distal) lower labial tooth ridge (Fig. 4a). Occasionally the lower labium papillation is biserial in the medial parts (uniserial in most specimens) (Fig. 4b) or reduced medially (Fig. 4d).

Internal oral features (Fig. 6). *Tomopterna luganga* possesses common soft tissue structures in the oral and buccal cavities. The prenarial area of the buccal roof bears an arched ridge. Two prenarial papillae are present along the anterior rim of each choana (not clearly visible in Fig. 6a). The single postnarial papilla is long and flat. It is flanked by two large, flat lateral ridge papillae. The median ridge separates the narial arena from the buccal roof arena. The buccal roof arena is oval; longer than wide. The lateral border of the buccal roof arena is marked by four papillae, whereas the center of the arena is covered with pustules.

In the oral floor region (Fig. 6b), papillae are present as two infralabial papillae on each side. The posterior one is particularly broad and bearing short side branches. The lingual complex is medially located on the anterior buccal floor and comprises a total of four lingual papillae (the region is damaged in Fig. 6b) and two pre-lingual papillae. The latter are anterior to the former, approximately at the transverse level of the infralabial papillae. The buccal floor between and posterior to the buccal pockets is rich in long papillae. The most anterior ones are located anteromedial to the buccal pocket, i.e. on the ceratohyal region. Buccal pocket ridge pa-

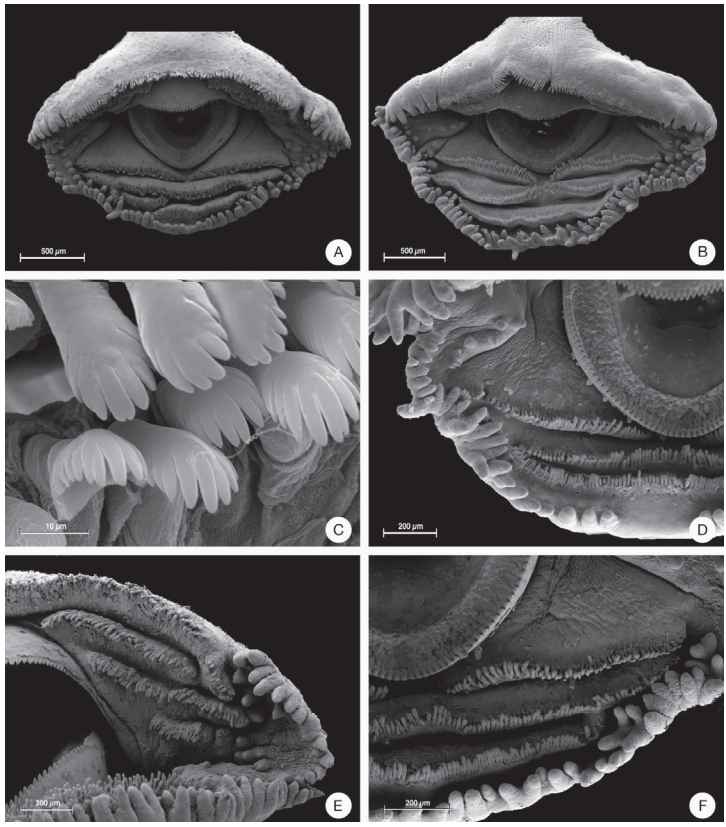

Fig. 4. Scanning electron microscopy images of *Tomopterna luganga* mouthparts. **A** Typical oral disc with broad papillation gap in the upper lip, uniserial papillation on the medial lower lip, and biserial papillation along the more lateral parts of the lower lip. **B** Individual with completely biserial papillation on lower lip. **C** Keratodonts. **D** Closer look at the lateral indention and the serration of beaks. **E** Details of the 4(2–3) keratodont rows of the upper lip and lateral papillae. **F** Details of the 3 (1) keratodont rows of the lower lip and transition from uni- to biserial oral disc papillation.

pillae are absent. Epithelial pustules are present between the buccal floor arena papillae, particularly dense in the posteromedial region of the buccal floor.

Discussion

Knowing the tadpoles in a biogeographic region can be very helpful in assessing anuran species richness in ecological surveys. They can be caught during daytime, often in small bodies of water that are easy to access. However, identification of tadpoles in the field, or even in the lab, can be a difficult task. For many groups, such as the genus *Tomopterna*, we do not have identification keys. If available at all, keys mostly rely on traditional characters from preserved specimens, but often neglect other features that might allow quick unequivocal identification in the field, such as coloration and colour patterns.

Recently tadpole descriptions have received increased attention, i.e., colour images of living specimens in combination

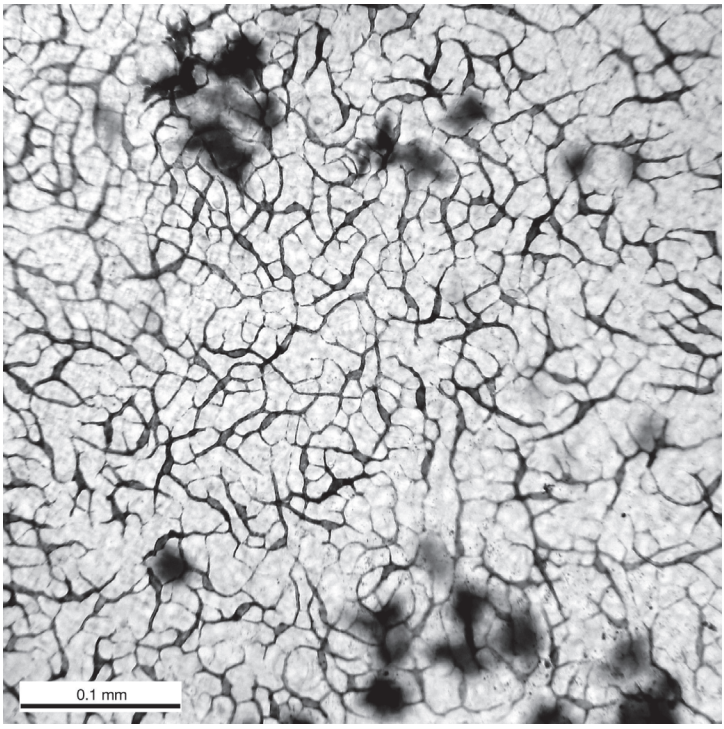

Fig. 5. Light microscopic image of dorsal epidermal melanocytes of *Tomopterna luganga*. Skin was removed from the interorbital region of a stage 38 preserved tadole. Epidermal melanocytes are long and branching (dendritic). Subepidermal melanocytes (dark blotches) are in deeper layers and out of focus in this image.

with detailed descriptions of those tadpole features traditionally used (for example, Chou & Lin 1999, Leong & Chou 1999, Anstis 2002). Anstis (2002) pointed out that the impression of coloration on images of living tadpoles is influenced by the setting (background, substrate, light, day/night time), particularly because reflecting iridophores are among the tadpole's pigment cells. We present a selection of backgrounds to give an impression of the animals in various settings (Fig. 2). Colour images of living tadpoles are necessary, because preserved specimens or even freshly killed ones will alter coloration (Anstis 2002). Population variability is beyond the control of the photographer and must be taken into consideration, just as in adults. Variation in pattern, hue and tint may occur in tadpoles related to population, daytime, water condition or presence of predators (Altig & Channing 1993, McCollum & Leimberger 1997). Variation of coloration as caused by water conditions has been reported for *T. tandyi* (Channing 2001). Power (1927) states that *T. delalandii* tadpoles "usually take the colour of the mud or sand on the bottom of the pool" and are hard to see in puddles unless they move; indicating camouflage as the major function of larval coloration in *T. tandyi*.

Beyond coloration, larval variation may occur in body size, size at metamorphosis, and tail length and height related to nutritional resources, predator presence, or population densities (Werner 1986, Smith & Van Buskirk 1995, Newman 1998, Relyea 2002,

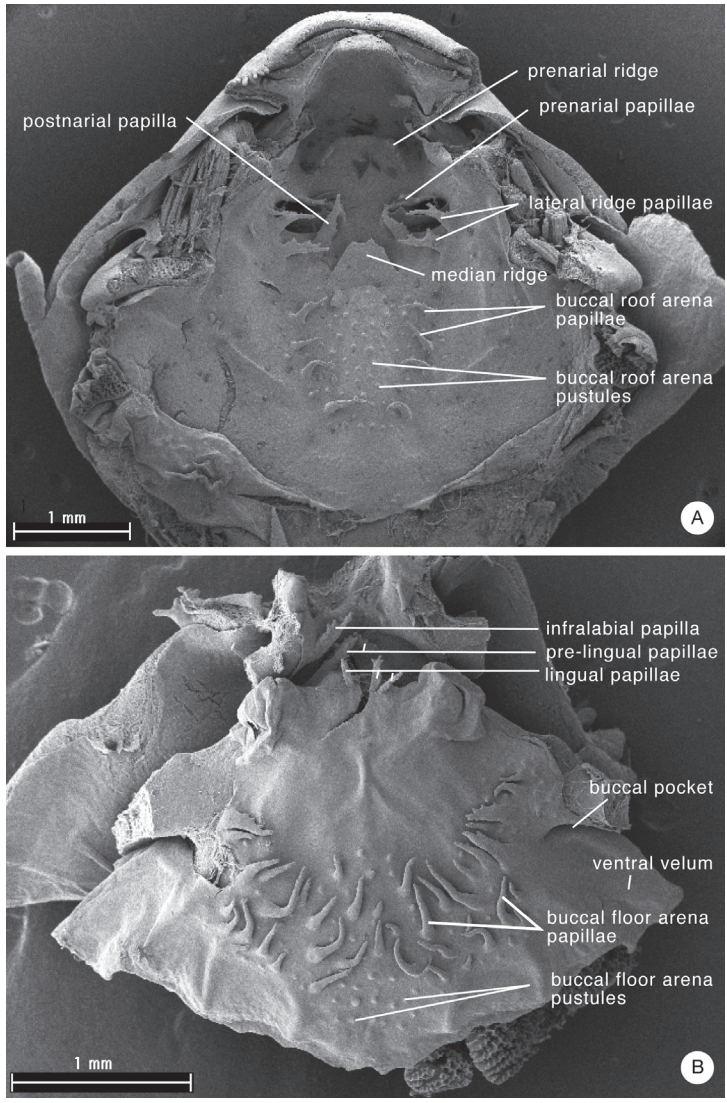

Fig. 6. Scanning electromicroscopic view of the buccal roof (**A**, stage 36) and buccal floor (**B**, stage 35) of *Tomopterna luganga* tadpole. See text for details.

2004). BALINSKY (1969) gives ranges of variation in tail to body lengths in *T. cryptotis* (as *T. delalandii)* and *T. natalensis* (1.25–2.11 and 1.57–2.33, respectively). Only extensive sampling from various habitats will show the norm and the range of variability, but is often, as in the present work, beyond feasibility. However, we have no grounds to assume that our tadpole sample from a captive breeding is less representative for the species than any single other sample of the same size from a wild population.

Tadpoles of some *Tomopterna* species still await description (CHANNING 2001). However, the tadpole of *T. luganga* fits the general description of *Tomopterna* tadpoles in

No.	Stage	TL	BL	SS	BS	RN	NE	HT	UF	LF	NN	PP
1	40	38.10	13.45	8.08	5.42	1.10	1.42	4.65	2.23	1.09	1.39	2.10
2	41	41.65	13.89	9.15	4.75	1.05	1.60	5.48	2.68	1.18	1.50	2.30
3	41	37.96	13.30	8.71	4.59	1.04	1.42	4.63	2.26	1.32	1.37	1.99
4	40	35.33	13.50	7.44	6.06	1.17	1.29	5.00	2.54	1.52	1.25	2.08
5	42	34.53	12.31	6.97	5.34	0.73	1.22	3.84	1.95	0.47	1.13	1.73
6	35	30.02	10.53	5.72	4.81	1.41	1.04	4.47	2.30	1.04	1.04	1.82
7	35	31.33	11.12	6.17	4.95	0.91	1.16	4.09	1.93	0.88	0.01	1.96
8	36	34.50	12.27	6.46	5.81	1.09	1.32	4.90	2.30	1.18	1.25	1.95
9	35	28.17	10.77	5.42	5.35	1.11	1.05	4.67	2.08	0.98	0.99	1.83
10	36	33.30	11.32	6.57	4.75	1.08	1.18	4.60	2.16	0.72	1.10	2.08
11	38	35.93	12.27	7.28	5.54	1.14	1.39	4.99	2.33	1.17	1.26	2.11
12	38	35.67	12.85	7.54	5.90	1.16	1.35	5.31	2.37	1.32	1.31	2.10
13	39	36.23	12.70	7.17	5.17	1.21	1.34	5.53	2.38	1.45	1.23	1.73
14	42	31.33	11.28	7.75	5.17	0.56	1.15	3.53	1.60	0.38	1.58	1.81
15	39	38.06	13.05	6.93	6.12	0.77	1.48	4.27	2.05	0.99	1.39	2.10

Tab. 1. Measurements of *Tomopterna luganga* larvae. BL, head-trunk length; BS, body end to spiracle distance; HT, maximum tail height; LF, ventral fin height at HT position; NE, naris to eye distance; NN, internarial distance; PP, interorbital distance; RN, rostro-narial distance (lateral projection); SS, snout-spiracle distance; TL, total length; UF, dorsal fin height (at HT position). All measurements in mm.

CHANNING (2001): it is heavy-bodied and the tail is less than twice as long as head and body. It is similar in body proportions to the larva of *T. delalandii* depicted in CHANNING (2001: Fig. 334) in having a dorsoposteriorly directed spiracle, a dorsal tail fin higher than the ventral fin, a *vena caudalis lateralis* visible over a number of tail myomers; but all these features might well apply to all larvae of *Tomopterna* species. For species of *Tomopterna*, the generic key of VAN DIJK (1966) lists a combination of characters: presence of rostral gap in papillation of oral disk, a marginal dextral vent, internarial distance 6x width of nostril or less, spur of hind foot present (in advanced larvae), and only one continuous row of keratodonts on upper lip. The combination of these characters distinguishes larvae of *Tomopterna* from other African genera. *Tompterna luganga* tadpoles described herein also fit the generic characterization in ALTIG & MCDIARMID (1999b), except for their description of the colour pattern as "uniformly dark", which is not an appropriate description for *T. luganga* (Fig. 2). POWER's (1927) description of *T. delalandii* (as *Pyxicephalus delalandii*) is not in acccord with the general generic description in ALTIG & MCDIARMID (1999) and VAN DIJK (1966) as it lists a median (rather than dextral) anus and one row of papillae "below and at the sides" of the lips.

There are differences in the LTRF between species. *Tomopterna cryptotis* possesses the LTRF 3(2-3)/3 (WAGER 1986, LAMBIRIS 1989, DU PREEZ 1996). GRILLITSCH et al. (1988) added for *T. cryptotis* that a fourth inner divided keratodont row on the upper lip occurred in 5 of the 20 specimens and half of their specimens had only two lower lip keratodont rows. If the third (distal) was present, then it was much shorter than the two proximal rows (GRILLITSCH et al. 1988: Fig. 3). *Tomopterna delalandii* has a LTRF of 2(1-2)/3(1) (VAN DIJK 1966, CHANNING 2001), *T. natalensis* 4(2-4)/3 or 5(2-5)/3 (VAN DIJK 1966, WAGER 1986, CHANNIG 2001), and *T. tandyi* 3(2-3)/3 (CHANNING 2001). From this list *T. luganga* and *T. natalensis* are the only species with four upper labium tooth ridges. *Tomopterna luganga* differs from *T. natalensis* in having a divided first (proximal) tooth ridge on the lower labium. The tooth row formulas given for *T. delalandii* in WAGER (1986; LTRF 3-4(2–4)/3) and POWER (1927; LTRF 3(2-3)/3(1)) differ from the other sour-

ces cited herein and needs verification. VAN DIJK (1966) reports presence of an elygium (dorsal eye pigmentation) in *Tomopterna natalensis*, but absence in *T. delalandii* and *T. marmoratus*. We found no trace of a elygium in preserved *T. luganga* tadpoles.

Tadpoles of all the *Tomopterna* species appear to be very similar in body proportions. GRILLITSCH et al. (1988) found no morphometric diagnostic feature to distinguish *T. cryptotis*, *T. delalandii*, and *T. natalensis*. The low ventral tail fin (Fig. 3) relates to the larval benthic life in all described species. *Tomopterna* tadpoles are known to lie on the bottom of pools and puddles and escape into the mud or under debris when disturbed (CHANNING 2001). Species identification by LTRF will not be reliable if only one or a few specimens are at hand, because variation may cause interspecific overlap in the character states. If larger samples allow to exclude aberrations, LTRF could be useful to distinguish between at least some of the *Tomopterna* species.

Whether or not there are diagnostic differences in the colour pattern between *T. luganga* and other species in the genus, cannot be answered, because most published accounts do not give much detail about colour pattern in life or preserved specimens. At least in *T. natalensis*, golden iridophores have been described as part of the dorsum and side coloration (CHANNING 2001). From the data presented herein, the ventral coloration in life seems particularly characteristic and easy to recognize (Fig. 2). However, we do not have any information if this pattern is shared with other species of the genus. For example, POWER (1927) describes the venter of *T. delalandii* as "very transparent, the spiral gut quite visible." But it is not clearly mentioned whether or not this referred to living or preserved specimens. Other pigmentation features remain unmentioned in some accounts. The drawings in GRILLITSCH et al. (1988) and CHANNING (2001) show that the *vena caudalis lateralis* is visible in *T. cryptotis* and *T. delalandii*, respectively, just as in *T. luganga*. *Tomopterna cryptotis* also shares pigmentation along blood vessels in the dorsal tail fin with *T. luganga*. But no details on that feature are available for the other species.

Recently, genetic barcoding, i.e. the identification of specimens by gene sequences, has been proposed as powerful tool in taxonomy (e.g., THOMAS et al. 2005; VENCES et al. 2005). As shown in the present case, matching tadpoles and adult *Tomopterna* by means of mitochondrial 16S gene sequences was highly reliable. However, genetic analyses are not always feasible and identification keys based on morphology remain to be important in general fieldwork. In the case presented herein, it was not possible to extract reliable diagnostic larval features for the species of *Tomopterna* from the available descriptions. There is preliminary evidence that some of the species could be distinguished by LTRF, but contradictory accounts from the literature make it necessary to verify published formulas. When using LTRF as diagnostic feature, it is necessary to consider that variation in larval mouthparts might be more widespread in tadpoles than generally thought (GRILLITSCH & GRILLITSCH 1989). In order to develop a reliable identification key for the larvae of the species in *Tomopterna*, it will be essential to collect new larval samples, identify them by DNA matching with adults, include colour pattern details in descriptions, and record some of the possible variation.

Acknowledgments

We would like to thank KATJA FELBEL for taking larval measurements from digital images. We are grateful to MONIKA HÄNEL who skilfully prepared the ink drawings.

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Manuscript received: 9 May 2005

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