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Tadpoles of *Mannophryne trinitatis* and *M. olmonae* (Anura: Aromobatidae): Further Description and Comparison

J.R. Downie[1*] and **M. Nokhbatolfoghahai**[2]

1. School of Life Sciences, Graham Kerr Building,
University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

2. Biology Department, Faculty of Science, University of Shiraz, Shiraz, Iran 71345.

* Corresponding author: *roger.downie@glasgow.ac.uk*

ABSTRACT

Mannophryne trinitatis and *M. olmonae* (Anura: Aromobatidae) are endemic to Trinidad and to Tobago, respectively. Previous descriptions have shown that tadpoles of these two species are very similar to one another. We examined a wide range of sizes and stages of tadpoles from one location in Tobago and three locations in Trinidad. Although some variability was found (in dentition, for example), no character alone could be used to distinguish any of the three Trinidad populations, and the Tobago specimens showed overlapping features. The only characters that appeared useful in distinguishing *M. trinitatis* and *M. olmonae* were pigmentation of the tail and origin of the dorsal fin. However, caution is recommended in identifying these species since tail characteristics vary.

Key words: Trinidad, Tobago.

INTRODUCTION

Lehtinen and Hailey (2008) first described the tadpole of the endemic Tobago stream frog, *Mannophryne olmonae*. They compared it with the tadpole of the closely related, endemic Trinidad stream frog, *M. trinitatis*. They were able to examine only two *M. trinitatis* specimens and found it impossible to distinguish them from *M. olmonae*. They reported a possible error in the description of the *M. trinitatis* tadpole provided by Kenny (1969) and suggested that examination of a bigger sample of the *M. trinitatis* tadpoles would be useful.

Mannophryne trinitatis inhabits two main localities, both in Trinidad: in the Northern Range, along the margins of small streams from just above sea level on the North Coast to hillside streams on the southern slopes; and in the Central Range, where known localities include Tamana Cave and several sites in the forest and hills to the east and west of Tamana Cave (Jowers and Downie 2004). Manzanilla *et al.* (2007) established that *M. trinitatis* is a separate species from the population found on the Paria Peninsula of Venezuela (called *M. venezuelensis*); these two are sister species and are more distantly related to Tobago's *M. olmonae* than they are to each other. Jowers *et al.* (2011) investigated the genetic and geographic structure of *M. trinitatis* throughout its range but found no evidence for genetic structuring related to geographical distribution.

In constructing his key to the tadpoles of Trinidad, Kenny (1969) relied on a number of morphological characters, principally body dimensions, tail shape and tail patterning, and features of the oral disc. A key feature is the dental formula, which describes the arrangement of the rows of labial teeth borne on the oral disc. Kenny gave the dental formula for *Mannophryne* (then *Phyllobates*) *trinitatis* as 1:2/1:1:1, meaning two anterior rows, the second of which is subdivided (designated 2), and three posterior rows (to the right of /), none of which is subdivided. Altig and McDiarmid (1999) attempted to standardise the presentation of dental formulae; in their system, *M. trinitatis* becomes 2(2)/3, meaning two anterior rows (to the left of the /), the second of which is subdivided (in brackets), and three undivided posterior rows. We follow Altig and McDiarmid's system, as did Lehtinen and Hailey (2008). We report here an analysis of *M. trinitatis* tadpoles from three localities, representing a wide ontogenetic range, and compare them with a new sample of *M. olmonae.*

MATERIALS AND METHODS

Mannophryne trinitatis tadpoles were collected from four sites: 1) a pool on El Tucuche (16/5/1982), 2) the stream through Tamana Cave (7/7/1982; 8/7/1994), 3) a stream by the side of the North Coast Road, a little west of Maracas Bay (8/7/1994), and 4) a stream beside the road a little south of Lopinot Village (26/7/2013). *Mannophryne olmonae* tadpoles were collected from an isolated freshwater pool close to a tributary of the Hermitage River near Charlotteville. Tadpoles were eutha-

nized by immersion in a lethal solution of benzocaine (0.01% aqueous) and then preserved in 10% formalin; two batches of *M. trinitatis* were preserved in Bouin's fluid. Specimens were examined by use of a Wild dissecting microscope and measured with callipers (for whole specimens) and by use of an eyepiece scale for fine features. Drawings were made with the aid of a microscope drawing tube. Tadpoles were staged by use of the method of Gosner (1960). The specimens used in this study have been deposited in the University of Glasgow's Hunterian (Zoology) Museum: *M. trinitatis* (GLAHM: 153253); *M. olmonae* (GLAHM: 153254).

To illustrate the mouthparts, specimens of both species were prepared for scanning electron microscopy by use of the methods described by Nokhbatolfoghahai and Downie (2005) and photographed by use of Image-slave for Windows (Meeco Holdings, Australia). Tadpole measurement comparisons were analysed by one-way ANOVA followed by post hoc Tukey tests, or by non-parametric Kruskal-Wallis tests when the data were not distributed normally.

RESULTS

Table 1 presents data on total length, snout to vent length (SVL), tail height, dorsal fin height, and oral disc width from the three different populations of *M. trinitatis* and from *M. olmonae*. No consistent differences were found that could be used to distinguish the *M. trinitatis* populations from each other or from *M. olmonae*, although a few of the features measured showed statistically significant differences in some cases.

Dental formulae from all populations of *M. trinitatis* and from *M. olmonae* were very similar, with little variability. All tadpoles had two anterior and three posterior tooth rows; no extra tooth rows were found in any specimen. Tooth rows A1, P2, and P3 were always undivided. In *M. trinitatis*, A2 was always divided (36 specimens examined). In some cases (25%), the gap between subrows could be detected only by moving the rows. In 11% of the cases, the gap was less than 40 μm; in 42% around 40 μm, and in 22% it was around 80 μm. P1 was undivided in 39% of specimens, but a division could be detected in the remainder. In nearly all specimens there was no gap, with a tiny gap (< 40μm) detectable in two specimens only. In *M. olmonae,* A2 was always divided and always had a detectable gap, around 80 μm in 88% of specimens and as narrow as 40 μm in one specimen only. P1 was undivided in one specimen and divided in the remainder, but with no gap. Representative oral discs are shown in Figs. 1 and 2.

Table 1. Comparison of quantitative features among three Trinidad *M. trinitatis* populations and one Tobago *M. olmonae* population. All features are presented as ratios: each feature length divided by total length (mm): mean ± SD; analysis conducted on arcsine-transformed ratios. * = data normally distributed; + = data not all normally distributed.

Species	Location	N	Stage range	SVL +	Maximum tail height +	Maximum dorsal fin height *	Oral disc width *
M. trinitatis	Lopinot (L)	10	26-39	0.65 ± 0.016	0.41 ± 0.019	0.22 ± 0.014	0.34 ± 0.016
	Tamana (T)	14	26-40	0.63 ± 0.013	0.42 ± 0.014	0.23 ± 0.013	0.31 ± 0.015
	North Coast (NC)	5	27-31	0.66 ± 0.006	0.40 ± 0.018	0.21 ± 0.02	0.34 ± 0.015
M. olmonae	Hermitage (H)	8	26-37	0.64 ± 0.027	0.40 ± 0.012	0.21 ± 0.016	0.32 ± 0.012
Significant differences at P < 0.05				T ≠ L	None	NC ≠ T	T ≠ NC
				T ≠ NC		H ≠ T	T ≠ L

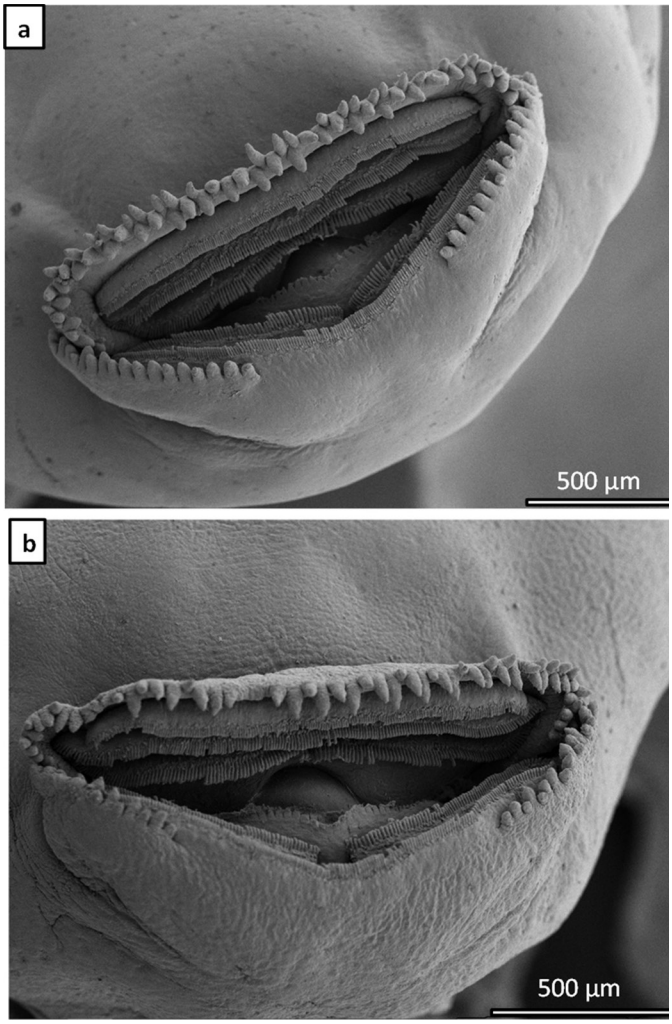

Fig. 1. Scanning electron micrographs of oral discs of a) *M. trinitatis* and b) *M. olmonae.*

Tails were tapered in both species and were highly muscular. Tail height is quite uniform over much of its length until it tapers at the end. The ventral fin is uniform over much of its length, but the dorsal fin is short anteriorly. One consistent difference found between the two species was that in *M. trinitatis* the dorsal fin begins level with the base of the hind limbs at the rear end of the body, whereas in *M. olmonae* the dorsal fin begins a little more posteriorly (Fig. 2). Another difference found was in tail pigmentation. In *M. trinitatis*, pigment blotches occur throughout the length of the tail, including the fins, whereas in *M. olmonae*, pigment blotches occur along the central axis of the tail and on the anterior part of the dorsal fin but were absent from the ventral fin and from the posterior part of the dorsal fin (Fig. 2).

DISCUSSION

Lehtinen and Hailey (2008) were concerned that Kenny's (1969) drawing of the *M. trinitatis* tadpole was in error because it erroneously depicted a large gap in tooth

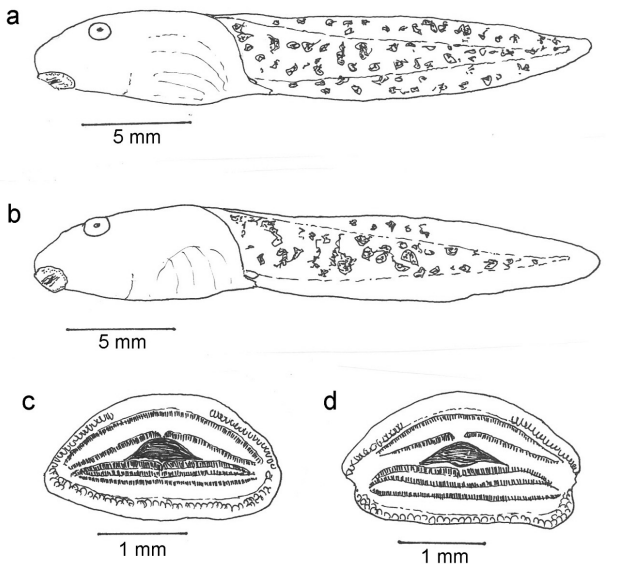

Fig. 2. Drawings of whole tadpoles in lateral view and of oral discs of *M. trinitatis* stage 27 (a, c) and *M. olmonae* stage 28 (b, d). Drawings made with the aid of a drawing tube using formalin-fixed specimens. Dorsal side above in all cases.

row A1. Kenny gave the dental formula as 1:2/1:1:1 [2(2)/3 in Altig and McDiarmid's terminology], indicating A1 as a complete undivided row, so the error was most likely simply in the illustration. Lehtinen and Hailey's (2008) account (page 261) of the *M. olmonae* dental formula noted that row A1 had a small gap (0.1 mm), as illustrated in their Fig. 2, but on page 263 they wrote that A1 is 'typically' undivided. Rick Lehtinen told us (pers. comm.) that there is indeed variation in their sample, with an undivided A1 being the more common condition. In our samples, we also found some variability but not in the same row as Lehtinen and Hailey (2008). We found A1, P2, and P3 always undivided in both species. A2 was always divided in both species, although the size of the gap was very variable. P1 was variable in both species, usually divided in both species but undivided in some individuals. We saw no evidence of an extra row, P4, as seen by Lehtinen and Hailey (2008) in one *M. olmonae* specimen.

It is not our aim here to provide complete descriptions of these two tadpole species, since these already have been written by Kenny (1969) and Lehtinen and Hailey (2008). Rather, we aimed to look for ontogenetic and regional variation in *M. trinitatis*, to clear up ambiguities in the literature, and to find characters that could be used to distinguish these two species of tadpole from each other.

Table 1 shows some evidence for inter-locality differences in *M. trinitatis*, particularly between the Tamana and Northern Range populations, but little consistency was found in differences between *M. trinitatis* and *M.*

olmonae. Cummins and Swan (1995) reported some reproductive differences, including tadpole size at deposition, between Northern Range and Tamana populations, and Downie *et al*. (2001) reported differences in tadpole deposition behaviour between North Coast and Northern Range southern slope populations. There may therefore be some fine-scale differences between different *M. trinitatis* populations, but as Jowers *et al.* (2011) and Lehtinen *et al*. (2011) found, these do not show up as consistent differences at the genetic level. Our size-corrected morphometric data showed very little variation over a considerable range of size and stage, indicating that shape characteristics alter little as tadpoles grow.

Regarding distinguishing features, we did detect consistent differences in tail origin between *M. trinitatis* and *M. olmonae* and differences in tail pigmentation. However, tail shape and tail pigmentation are characters known to vary with rearing conditions in some species (Viertel and Richter 1999; Relyea 2004), so such characters may not be reliable as distinguishing features. Overall, the best way to identify *M. trinitatis* and *M. olmonae* tadpoles is to know on which island they were collected.

As Lehtinen and Hailey (2008) noted, rather few tadpoles of the 19 species (Frost 2014) of *Mannophryne* have been described fully. It is surely time that larval descriptions are included alongside those of adult anurans.

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