

# The tadpole of the glass frog *Hyalinobatrachium orientale tobagoense* (Anura: Centrolenidae) from Tobago, West Indies

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**ABSTRACT** - We describe the tadpole of the Tobago glass frog *Hyalinobatrachium orientale tobagoense* for the first time. Like the few other *Hyalinobatrachium* species tadpoles described so far, it lives hidden in sand and gravel at the bottom of stream beds. The tadpoles have relatively long tails and slender lightly pigmented bodies with tiny eyes. They appear to grow very slowly and hind limb buds were not developed in the six week old Gosner stage 25 individuals we describe.

## INTRODUCTION

The glass frog *Hyalinobatrachium orientale* has been identified from two localities, the oriental sector of northeastern Venezuela and the north of the West Indian island of Tobago. Jowers et al (2014) felt that Hardy's (1984) original designation of the Tobago population as a sub-species, *H. o. tobagoense* was justified based on Braby et al.'s (2012) suggestion that the use of sub-species be 'restricted to evolving populations representing partially isolated lineages of a species that are allopatric, phenotypically distinct and have at least one fixed diagnosable character state.' Braby et al. felt that well characterised sub-species can be particularly useful in defining biodiversity units that need conservation.

As Hoffmann (2010) has noted, glass frog larvae are very difficult to locate in the field because, after hatching, the larvae burrow into the sand and gravel at the bottom of flowing streams and generally do not emerge until metamorphosis. Hoffmann's (2010) descriptions of 13 species of Costa Rican glass frog tadpoles are therefore mainly based on captive reared specimens. The tadpoles of *H. orientale* have not previously been described either from Venezuela or Tobago. Here, we provide a description based on captive reared specimens, using the same main characters as Hoffmann (2010).

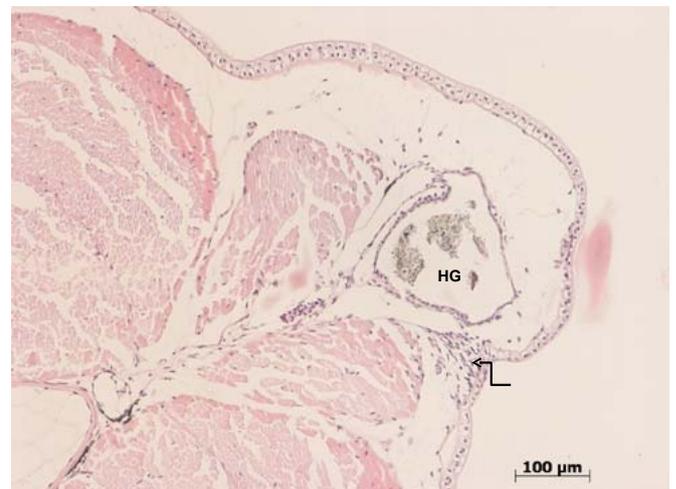
## METHODS

Two egg clutches of *H. orientale* were collected during June 2014 from the undersides of leaves overhanging a stream close to Spring Trail in Tobago's Main Ridge Forest Reserve (*H. orientale* is the only Tobago frog species showing this mode of reproduction: Hardy, 1984). The eggs were incubated until hatching and then the larvae were grown in tanks for six weeks as described by Nokhbatolfighahai et al. (submitted). Surviving larvae (9 from 15) were then recovered, lethally anaesthetised in benzocaine, then preserved in formol-saline. Specimens were examined and measured using a Wild M3Z dissecting microscope fitted with an eyepiece scale.

Photographs were taken using a Nikon D5100 DSLR camera with a Nikkor 40 mm lens. Two specimens were embedded in wax, sectioned and stained using H and E in order to examine limb development. For the labial tooth row formula, we have followed the recommendation of Altig and McDiarmid (1999a). The remaining specimens have been deposited in the University of Glasgow's Hunterian Zoology Museum, accession number 1437.

## RESULTS

During the six weeks we allowed the larvae to grow they had increased in length by 36.6% to a mean (+ SD) of 15.3+1.4 mm (from a hatching length of 11.2 +0.2, n=10) and in wet weight had tripled to a mean of 0.03+0.007 g (n=9). Their intestines were visibly full of particulate material. Even after six weeks growth, all specimens remained at Gosner (1960) stage 25, with no external sign of hindlimb development.



**Figure 1.** H & E stained transverse section of a stage 25 *H. orientale* tadpole in the hindgut region, showing a condensation of mesenchymal cells beneath the epidermis, but no protruding limb bud. HG = hindgut; arrow points to mesenchymal condensation.

Sections in the region where hind limb buds would be expected (junction of body and tail, close to vent tube) show condensations of mesenchymal cells near the hind gut, but no sign of any protruding buds, indicating that the limbs are in the pre-bud stage (Fig. 1). The detailed tadpole descriptions and measurements which follow are based on the five specimens with the most intact tails.

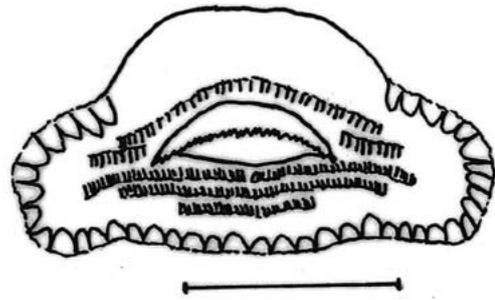
Figure 2 shows dorsal, left lateral and ventral views of one of the measured tadpoles. The body shape is flat in the dorso-ventral plane rather than rounded, with a mean width/height ratio of  $1.19 \pm 0.03$ . The tail is long and slender: tail length as a proportion of total length is 64%; tail width as a proportion of total tadpole length is 14.3%. The dorsal tail fin originates at the base of the tail and is initially low, increasing in height about half-way along the tail, as the musculature of the tail begins to narrow. The ventral tail fin originates at the vent tube and is a little higher initially than the dorsal fin, again widening posteriorly. At maximum height, the dorsal fin is 20.7% of tail width and the ventral fin is 22.0%. Maximal tail width is 22.4% of tail length. The tail tip is rounded after gradual narrowing.



**Figure 2.** Fixed, unstained complete *H. orientale* stage 25 tadpole showing a) left lateral with spiracle visible, b) ventral and c) dorsal views. See text for details.

The position of the sinistral spiracle on the body is slightly ventral to the midline and 58% along the body from the snout. The dorsal side of the body is fairly uniformly pigmented except for clear areas ventro-lateral to the eyes and lateral to the anterior of the intestine. The ventral side of the body is unpigmented and transparent. The lateral side of the tail has a discontinuous pigment line along the middle of the muscle half to two-thirds the length of the tail; at the tail tip, there is diffuse pigment covering the muscle. The tail fins are unpigmented. In dorsal view, the tail has a broad band of pigment along the midline, narrowing at the tip; in ventral view, the tail has a sparse discontinuous pigment line from base to tip.

The snout is rounded. The eyes are dorsally positioned and very small, 0.45 mm in diameter (in comparison,



**Figure 3.** Drawing of the oral disc of *H. orientale*, made with the aid of a drawing tube. Mouth opened out to make all tooth rows visible. Anterior above. Disc has a single row of papillae all round its margin except at anterior end. Scale line = 0.5 mm.

*Leptodactylus fuscus* stage 25 tadpoles of similar size have eyes of 1.3 mm in diameter; JRD, unpublished data). The ratio of interorbital width to maximum body width is 0.35, and the position of the interorbital axis is 20.4% along the body from the snout tip. The nares can just be seen with a dissecting microscope using intense lateral illumination and are about half-way between the interorbital axis and the snout tip.

Figure 3 shows the morphology of the oral disc. The disc is ventral in position, with a short length of snout sometimes protruding anterior to it in ventral view. The margin of the disc bears a single row of papillae laterally and posteriorly, but the anterior margin is papilla-free. The tooth row formula is 2(2)/3(1); i.e. the second anterior row has a gap, which is wide; the first posterior row has a narrow gap; the remaining rows are continuous; the lengths of the posterior rows reduce progressively from P1 to P3. The jaws have serrated edges. The mean ratio of oral disc width to maximum body width is  $0.46 \pm 0.008$ .

## DISCUSSION

In several respects: particularly body and tail shape, spiracle position, interorbital width and eye diameter, oral disc morphology and relative size - *H. orientale* tadpoles closely resemble other *Hyalinobatrachium* species described by Hoffmann (2010). Altig and McDiarmid (1999b) gave descriptions of the tadpoles of three glass frog genera, *Centrolene*, *Cochranella* and *Hyalinobatrachium*, noting that generic and specific differences are poorly known. Our findings fit well with the features they noted for *Hyalinobatrachium*.

As Hoffmann (2010) has noted, glass frog tadpoles remain relatively undescribed because of the problems of locating them in the field and the difficulties of rearing them under laboratory conditions. We present here the first description of the tadpole of the Tobago glass frog *H. orientale*. Our specimens were collected as eggs in the field, allowed to hatch then transferred to aquaria with river rocks and gravel covering the bottoms. The water was continually aerated and a pinch of tropical fish food flakes was provided every second day. The tadpoles were never visible in the water column (about 20 cm deep) and were only retrieved after removal of all the rocks and gravel: after 6 weeks, they were still at Gosner stage 25

but had grown significantly in length and weight, and their intestines were packed with particulate material, indicating food consumption. The growth rate achieved seems very slow. In comparison, several Trinidad frog species reach metamorphosis in 2-3 weeks (Downie, 2013). However, the rate is probably comparable with Hoffmann's (2010) findings, with two Costa Rican *Hyalinobatrachium* species taking 265 and 394 days respectively to reach Gosner (1960) stage 41, just before metamorphosis. The lack of progressive development of the hind limb buds in our specimens was striking and not noted in Hoffmann's (2010) descriptions. Relatively late development of the hindlimbs does occur in other species (for example, in *Mannophryne trinitatis*; Downie, unpublished observations), but it is not clear why this should happen. It will be of interest to attempt to grow *H. orientale* tadpoles as far as metamorphosis in order to determine how long it takes and when progressive limb development occurs.

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