Confirmation of nest building in a population of the gladiator frog *Hypsiboas crepitans* (Anura, Hylidae) from the island of Tobago (West Indies)

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Gladiator frogs (those in the *Hypsiboas faber* and *H. semilineatus* species groups; Faivovich et al., 2005) are named for the frequent fighting observed between males during the breeding season. Males are typically very territorial, have specialized agonistic vocalizations, and possess a well-developed pre-pollical spine capable of inflicting serious wounds. These highly aggressive behaviors are usually focused around nest sites, which are specifically constructed by males along the edges of ponds or streams. Males call from the constructed nest sites and eggs are laid therein. There are twelve described species in these two species groups and all but *H. albomarginata* have been reported to construct nests (although many species will also use natural depressions or re-use the nests made by other males; Höbel, 2008). On 31 July, 2012, we located an obviously constructed nest containing tadpoles (Fig. 1) in a swampy area adjacent to the Parlatuvier River on the island of Tobago (11.30°N, 60.65°W, 5 m elevation). The circular nest was constructed in fine silt amidst bamboo leaves, measured 19 x 21 cm at its widest points and had basin walls approximately 5 mm high. The nest contained approximately 500 tadpoles in developmental stage 25 (Gosner, 1960). However, no adult was present at the nest at the time of our observation.

The only gladiator frog previously reported from Tobago is *Hypsiboas crepitans* (Wied-Neuwied, 1824; see Murphy, 1997). However, there are only three literature records of nest construction in *H. crepitans*. Caldwell (1992) reported a single nest from a population in Venezuela and Lynch and Vargas Ramirez (2000) reported nests from a population in Columbia. Casal and Juncá (2008) briefly reported on an aquatic nest of somewhat different structure for a Brazilian population (however, this latter example may represent a different, but currently undescribed, species). Another possibility is that the nest we observed in Tobago could have been from either *H. geographicus* or *H. boans*, both of which are known to construct nests (Duellman, 2001; Höbel, 2008) and occur on the neighboring island of Trinidad. A third possibility was that the nest was from an undescribed species. Tobago is relatively well-studied herpetologically, however, new species have been recently described (e.g., Murphy et al., 2013). Therefore, it was a real possibility that a new or previously unreported species had constructed the nest.

To ascertain the identity of the nest-building frog, we generated DNA sequences from tadpoles collected from the nest. We used the 16S mitochondrial gene as this gene has been used extensively in anurans for barcoding (Vieites et al., 2009). We extracted genomic DNA from two ethanol-preserved tadpoles using a Qiagen DNeasy Blood and Tissue kit. Using universal vertebrate primers (16SL: 5’-GCCTGTTTATCAAAAAACAT-3’, 16SH 5’-CCGGTCTGAACTCAGATCGTGCT- 3’; Palumbi et al., 1996), we amplified a 550 bp fragment of 16S using a Qiagen Top Taq PCR kit. Successfully amplified PCR products were purified with a Qiagen Min-Elute kit and subsequently sequenced by Eurofins MWG Operon. Codon Code Aligner 4.2.4 was used to call bases and process the raw sequences. 16S sequences generated from the two tadpoles from the nest were then compared to the NCBI database using a standard nucleotide BLAST search (Altschul et al., 1990) to ascertain their identity.

Both sequences we generated (GenBank accession numbers KJ642639-KJ642640) were identical, and therefore the subsequent BLAST search results were also identical. The top three BLAST hits were *H. crepitans* (JN970653-JN970655) from Guyana. These were 99%
similar to the sequences we generated from Tobago. The fourth BLAST hit was also *H. crepitans* (AY843621), this time from Brazil (97% similar). All other BLAST hits were from other species and were 92% similar or less. An approximate cut-off for conspecific 16S sequences is 3% or less (Vieites et al., 2009).

We did not witness the construction of the nest and thus cannot provide further information on the sex of the individual involved or any details of the behavior. Nonetheless, the matches to our DNA sequences provide strong evidence that *H. crepitans* constructed the nest. Despite *H. crepitans* being a common and widely distributed species, this is only the fourth record of nest construction. As a number researchers have suggested that *H. crepitans* is actually a complex of cryptic species (e.g., Kluge, 1979; Duellman, 2001; Lynch and Suárez-Mayorga, 2001; Casal and Juncá 2008; Martins et al., 2009), discrepancies in the literature about whether this species constructs nests could result from more than one species being involved. Future work on assessing cryptic species in *H. crepitans* would help clarify this situation.

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**References**


**Figure 1.** Constructed nest containing tadpoles of *Hypsiboas crepitans* found adjacent to the Parlatuvier River on the island of Tobago. Photo by the author.


