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Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs

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ABSTRACT

Trypanosomes are a diverse group of protozoan parasites of vertebrates transmitted by a variety of hematophagous invertebrate vectors. Anuran trypanosomes and their vectors have received relatively little attention even though these parasites have been reported from frog and toad species worldwide. Blood samples collected from túngara frogs (*Engystomops pustulosus*), a Neotropical anuran species heavily preyed upon by eavesdropping frog-biting midges (*Corethrella* spp.), were examined for trypanosomes. Our results revealed sexual differences in trypanosome prevalence with female frogs being rarely infected (<1%). This finding suggests this protozoan parasite may be transmitted by frog-biting midges that find their host using the mating calls produced by male frogs. Following previous anuran trypanosome studies, we examined 18S ribosomal RNA gene to characterize and establish the phylogenetic relationship of the trypanosome species found in túngara frogs. A new species of giant trypanosome, *Trypanosoma tungarae* n. sp., is described in this study. Overall the morphometric data revealed that the trypomastigotes of *T. tungarae* n. sp. are similar to other giant trypanosomes such as *Trypanosoma rotatorium* and *Trypanosoma ranarum*. Despite its slender and long cell shape, however, 18S rRNA gene sequences revealed that *T. tungarae* n. sp. is sister to the rounded-bodied giant trypanosome, *Trypanosoma chattoni*. Therefore, morphological convergence explains similar morphology among members of two non-closely related groups of trypanosomes infecting frogs. The results from this study underscore the value of coupling morphological identification with molecular characterization of anuran trypanosomes.

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1. Introduction

Trypanosomes are protozoan parasites that are ubiquitous across invertebrate and vertebrate species. Indeed, trypanosomes infect species across all vertebrate classes. Anuran trypanosomes, however, have received considerably less attention than those in other vertebrates even though they infect frog and toad species worldwide (Bardsley and Harmsen, 1973; Desser and Yekutiel, 1986; Werner, 1993; Desser, 2001; Žičkus, 2002; Lemos et al., 2008). Since many anurans spend at least their early

developmental stages in aquatic environments and return to breed as adults, leeches have long been considered the main vectors of trypanosomes in this group (Reilly and Woo, 1982). As adults, however, many species of frogs are preyed upon by a variety of opportunistic and specialized hematophagous insects that may act as possible vectors of blood parasites. Phlebotomine sandflies (*Phlebotomus squamirostris*), for instance, transmit *Trypanosoma bocagei* França 1911 to European toads, *Bufo bufo* (Feng and Chung, 1940). Similarly, trypanosomes may be mosquito-borne parasites for anurans. Mosquitoes, such as *Culex territans*, that feed mainly on anuran hosts have been implicated in the transmission of *Trypanosoma ranarum* Lankester 1871 (Desser et al., 1973) but their role as trypanosome vectors is still controversial (Ferguson and Smith, 2012). Other mosquito species such as *Aedes aegypti* and *Culex pipiens* can transmit trypanosomes (*Trypanosoma rotatorium* Mayer

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1843 complex) to some frogs even though they do not usually feed on anurans (Ramos and Urdaneta-Morales, 1977). Closely related to mosquitoes, frog-biting midges (Corethrellidae) are small hematophagous flies specialized at feeding on anurans (Borkent, 2008). These midges are thus potentially important vectors of blood parasites of this vertebrate clade (McKeever and French, 2000). In fact, in the Southeastern United States, one species of frog-biting midge (*Corethrella wirthi*) transmits trypanosomes to green treefrogs, *Hyla cinerea* (Johnson et al., 1993). The family Corethrellidae contains 107 species of frog-biting midges, in which females are specialized in using the mating call of frogs to localize them and obtain a blood meal (Borkent, 2014). The frog's mating call is the main cue used by the midges for long-distance host detection (Bernal and de Silva, 2015). Further studies that examine the role of other species of frog-biting midges at transmitting trypanosomes are necessary to understand the evolutionary ecology of these interactions. In this study we investigate trypanosome infection in a Neotropical anuran species, the túngara frog (*Engystomops pustulosus*), which is heavily preyed upon by frog-biting midges.

Túngara frogs are small anurans that occur from southern Mexico to northern South America (Colombia, Venezuela, and Belize) and Trinidad and Tobago. Males aggregate during the rainy season at temporary puddles from where they produce mating calls (Ryan, 1985). While calling to attract a mate, túngara frog males also

attract frog-biting midges (*Corethrella* spp). These eavesdroppers prey upon túngara frogs in great numbers (Fig. 1a). A speaker broadcasting calls equivalent to those produced by a motivate túngara frog male, attracts up to 511 midges in 30 min (average = 142 midges/30 min; Bernal et al., 2006). Túngara frogs represent an ideal opportunity to investigate trypanosome infection potentially transmitted by frog-biting midges.

The goals of this study were twofold: firstly, to determine the presence of trypanosomes in túngara frogs along with characterizing these parasites, and secondly, to examine whether trypanosome prevalence differs between females and males. Since as in most anuran species, female túngara frogs do not produce mating calls (Ryan, 1985), eavesdropping frog-biting midges most likely only feed on male frogs. We thus expected differences in trypanosome prevalence between male and female túngara frogs reflecting the feeding habits of the frog-biting midges. As predicted, we found trypanosome infected male túngara frogs and thus implemented morphological and molecular methods to characterize and infer the phylogenetic relationship of this *Trypanosoma* species to other trypanosomes that parasitize other vertebrates that inhabit aquatic and marine environments. The characterization and phylogenetic relationships of this new *Trypanosoma* species provide new information on anuran trypanosomes, a group with poorly known taxonomic relationships (Martin et al., 2002). In

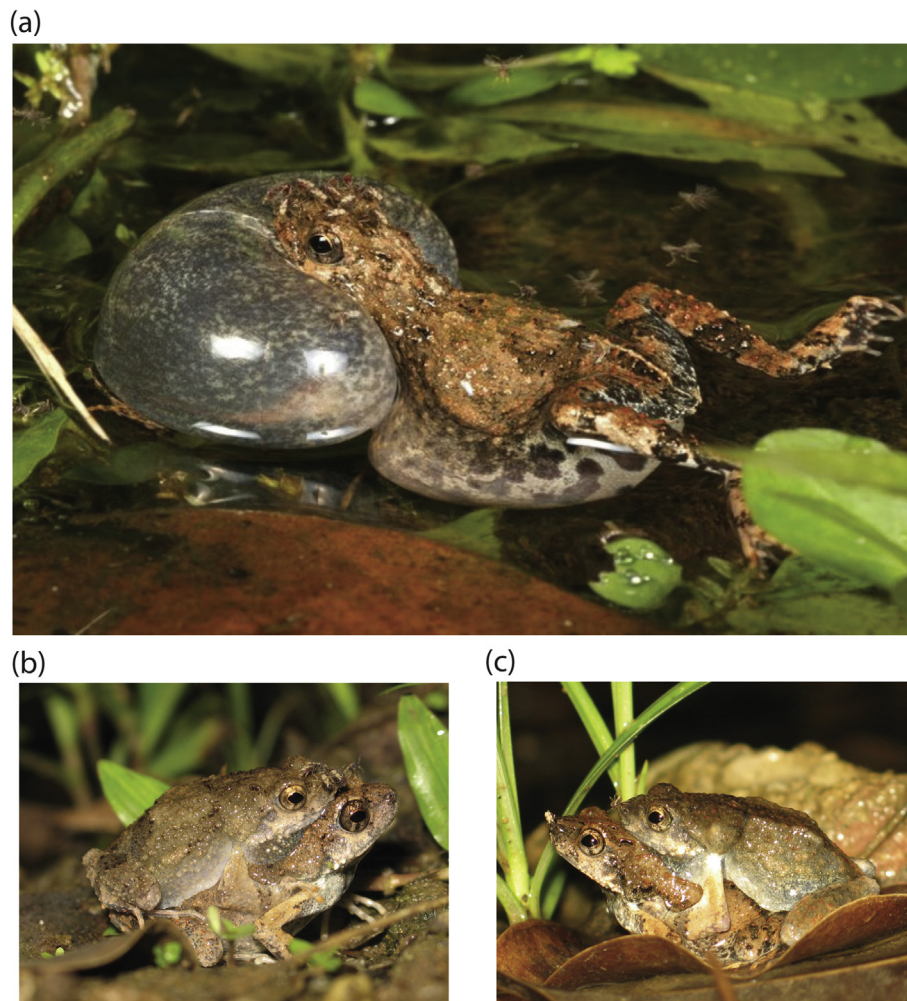


Fig. 1. Photographs of túngara frogs (*Engystomops pustulosus*) and frog-biting midges (*Corethrella* spp). (a) Calling male túngara frog preyed upon by frog-biting midges; (b) female (bottom) in amplexus with a male (top) covered with biting midges; (c) female (bottom) with a biting midge on her nostril that was passed from the male during amplexus. Túngara frogs are about 30 mm long while the frog-biting midges are only about 1.5 mm. Photos taken by Alexander Baugh (a) and Ximena E Bernal (b,c).

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