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Observations on the use of tarantula burrows by the anurans *Leptodactylus bufonius* (Leptodactylidae) and *Rhinella major* (Bufonidae) in the Dry Chaco ecoregion of Bolivia

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Abstract. Some species of anurans have been observed utilizing burrows of other animals, such as rodents and tarantulas. Here we report the observations of two anuran species, *Leptodactylus bufonius* and *Rhinella major*, utilizing the burrows of tarantulas (*Acanthoscurria* sp.; Family Theraphosidae) in the dry Chaco ecoregion of Bolivia. Both species of anurans never co-occurred with tarantulas in the burrows and used burrows that were wider in diameter and closer to breeding ponds as compared to the total available tarantula burrows in the area. These burrows may serve as refuges from predators, especially for conspicuous, calling males.

Keywords. *Acanthoscurria*, amphibian, Neotropics, predation, refuge, spider, Theraphosidae, tropical dry forest.

A number of animals are known to utilize cavities created by other animals, such as the many cavity-nesting birds that require tree cavities created by other animals for reproduction (Scott et al., 1977). Previous work has documented anurans using burrows of other animals including rodents, tortoises, and tarantulas (Gentry and Smith, 1968; Cocroft and Hambler, 1989; Witz et al., 1991; Dundee et al., 2012; Schalk, 2012). While spiders are known predators of anurans at both the larval (Schulze and Jansen, 2010) and post-metamorphic (Toledo, 2005) life stages, surveys of tarantula (Family Theraphosidae) burrows in both North America and South America have found that species of frogs in the family Microhylidae actually take refuge within an occupied tarantula burrow (Blair, 1936; Cocroft and Hambler, 1989; Dundee et al., 2012). A study of the skin chemistry of *Gastrophryne carolinensis* (Family Microhylidae) demonstrated that they produce toxic skin secretions making them unpalatable to spiders and thus allow them to coexist with the tarantulas within their burrows (Garton and Mushinsky, 1979). Although previous studies have focused on anuran use of burrows still occupied by the tarantula, anurans and other taxa can also utilize abandoned burrows (Witz et al., 1991).

During a 50-day survey in the Dry Chaco ecoregion of Bolivia, we observed two species of anurans, *Leptodactylus bufonius* (Family Leptodactylidae) and *Rhinella major* (Family Bufonidae) utilizing tarantula (*Acanthoscurria* sp., Family Theraphosidae) burrows. To our knowledge, this is the first instance of anuran species outside the family Microhylidae using tarantula burrows. Also, while anurans in the Gran Chaco have been observed utilizing the burrows of the Vizcacha (*Lagostomus maximus*; Family Chinchillidae) (Cei, 1980; Schalk, 2012), this is the first documentation of anurans in the Gran Chaco ecoregion utilizing tarantula burrows. The objectives of this study were to document if these anurans 1) overlapped temporally with tarantulas in their occupancy of a burrow, and 2) utilized a non-random subset of the available burrows based on microhabitat characteristics of the burrows.

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The study was conducted in an area of the Bolivian Gran Chaco within the vicinity of Yande Yari (18° 41' 30.516" S, 62° 18' 6.9474" W), a park guard camp in the Kaa-Iya of the Gran Chaco National Park, Cordillera Province, Santa Cruz Department, Bolivia. The climate of the Chaco is seasonal, having a wet, hot summer (November–March), and a dry, cool winter (April–October). Average rainfall and temperature for this area are 513 mm and 24.6 ºC, respectively (Navarro and Maldonado, 2002). The area is characterized by scrubby, short trees (e.g., *Schinopsis lorentzii* and *Aspidosperma quebracho-blanco*), while shrubs (*Capparis* sp., *Acacia* sp.), bromeliads, and cactii, (e.g., *Opuntia* sp., *Cleistocactus baumannii* and *Eriocereus guelichii*) are common understory plants (Navarro and Maldonado, 2002).

As part of an effort to document the reptiles and amphibians in the area, we conducted nightly time-constrained visual encounter surveys from 9 February 2012 to 29 March 2012 (Schalk et al., *in press*). Our visual encounter surveys were conducted between 20:00 h and 00:00 h along one of four trails at the camp. Each night we surveyed one of the four available trails, which was randomly chosen each night. While conducting these visual encounter surveys, we also conducted a two-step survey process of the occupants of the tarantula burrows found along the trail. The first step consisted of identifying the burrow to be that of a tarantula's by confirming it as an occupant. When we encountered a burrow, we shined our flashlight down a burrow to determine if it was occupied. When a burrow was occupied by a tarantula, we measured its diameter to the nearest mm using a ruler, and measured the distance to the nearest source of water to the nearest cm using a 50 m measuring tape. The burrow was then marked using bright flagging tied to a nearby plant or bush to assist in locating the burrow upon subsequent visits. The second step of the process consisted of revisiting the marked tarantula burrows at a later date during the nightly survey to determine if they contained an anuran as an occupant. If the burrow was found to be occupied by an anuran on a subsequent visit, we then measured the same variables previously mentioned. We measured burrow diameter to examine whether these anurans were excluded from utilizing burrows of smaller diameters due to their body size. We measured distance to a pond because we hypothesized that frogs might utilize burrows closer to breeding ponds as refuges from predators. Given that calling males are more conspicuous to predators (Ryan et al., 1981; Schalk and Morales, 2012), we hypothesized that those unoccupied tarantula burrows that were in close proximity to breeding sites may offer some form of protection to the frogs if they were approached by a predator. Inherent in this hypothesis is that the frogs exhibit a preference to utilize tarantula burrows closer to ponds; therefore we assumed that the likelihood of a burrow being abandoned by a tarantula and therefore, the availability of abandoned tarantula burrows was independent relative to the distance to a pond. Since the burrows occupied by frogs were previously documented to be occupied by tarantulas from the first step in the survey process, we did not include the frog-occupied burrows in our analysis of the tarantula burrows as it would be temporal pseudoreplication (Hurlbert, 1984). The data failed to meet the assumptions for parametric *t*-tests, thus we conducted a nonparametric Mann-Whitney U-test comparing the burrows occupied only by tarantulas to those occupied by anurans.

We found a total of 54 occupied tarantula burrows in the study area. Of the 54 burrows, 46 of the burrows only contained tarantulas as occupants, while eight of the 54 burrows were found to be utilized by anurans of two species when we re-surveyed the burrows. We did not observe frogs and tarantulas utilizing the same burrow simultaneously. Of the eight frog observations, six were male *L. bufonius*, while the other two were a male and female *R. major*. All of the male *L. bufonius* were calling from within the burrow or at the edge of the burrow and when approached, entered the burrow for refuge. The male *R. major* was not observed calling. During our surveys, we did witness a predation attempt in which a tarantula tried to capture a female *L. bufonius*. The burrows utilized by the frogs were significantly larger (median = 31.9 mm) in their diameter than those burrows in which we only found tarantulas (median = 23.7 mm; Fig. 1A; Mann-Whitney, $U = 86$, $P = 0.01757$). The burrows used by the frogs were also significantly closer (median = 3.5 m) to a source of water as compared to the burrows used only by tarantulas (median = 12.7 m; Fig. 1B; Mann-Whitney, $U = 75$, $P < 0.01$). All of the male *L. bufonius* used burrows less than four meters from the edge of a pond, whereas the male and female *R. major* were using burrows 5.4 m and 18.7 m from a pond, respectively.

Burrows often provide an amenable environment that offers shelter from harsh abiotic conditions or refuge from predators (Cocroft and Hambler, 1989; Witz et al., 1991). In the Chaco ecoregion, several anurans had been documented as being burrow associates of the Vizcacha (*L. maximus*); the known burrow associates included *L. bufonius* (Cei, 1980), as well as *Leptodactylus laticeps* (Cei, 1980), and *Physalaemus biligonigerus* (Schalk, 2012). The frogs utilized a subset of size classes of the total available burrows used by tarantulas in the area. Specifically, they used burrows which were larger in diameter and closer to a water source. Both *L. bufonius* and *R.*

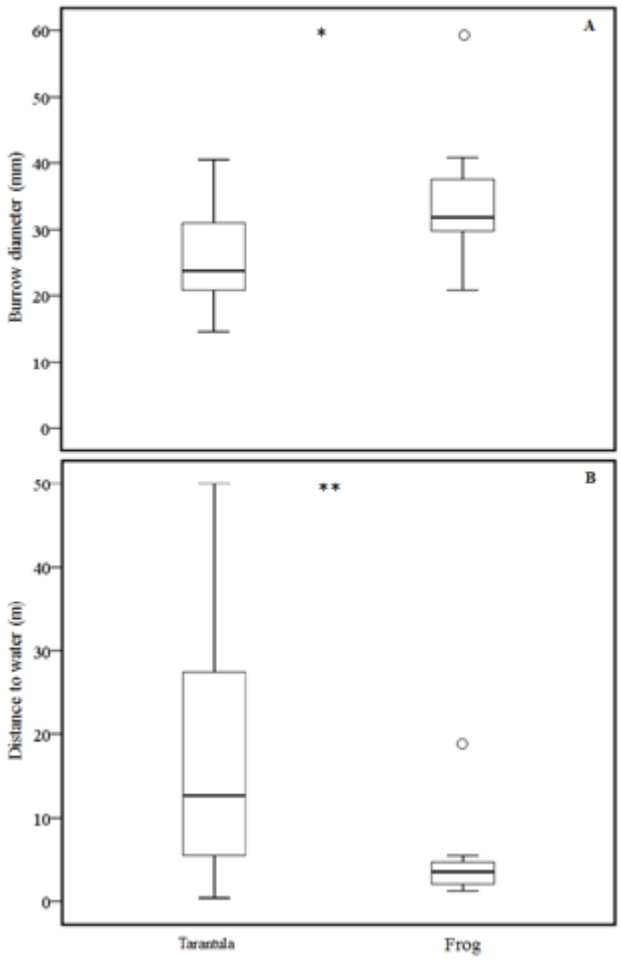

Fig. 1. Boxplots of the A) diameter of the burrows, and B) the distance to a pond used by tarantulas and frogs during the survey period. An asterisk (*) indicates P < .05, and a double asterisk (**) indicates P < .01. Outliers are depicted by an open circle symbol.

major are moderately sized anurans (mean SVL = 53 mm and 58.8 mm, respectively) and may be unable to utilize the smaller diameter burrows as a result.

All six male *L. bufonius* using the abandoned tarantula burrows were observed calling in or close to the tarantula burrow entrance, and all sought refuge inside the burrow when approached. Predation rates are often skewed towards male frogs at a breeding site as males are more conspicuous than females (Ryan et al., 1981; Lodé, 1996). These abandoned tarantula burrows may offer some form of protection to the more conspicuous males. In this region, documented predators of calling males include invertebrates (Schalk, 2010), foxes (Schalk and Morales, 2012), and other anurans (Schalk and Montaña, 2011). The breeding biology of *L. bufonius* may also permit it to utilize these burrows several meters from the pond's edge. The males of *L. bufonius* construct underground nest chambers over a meter from the pond's edge where the eggs are deposited and the nest is sealed with mud; the tadpoles can persist inside until the nest is flooded by heavy rains and the tadpoles enter the nearby pond (Cei, 1980; Reading and Jofré, 2003). *Rhinella major* call from the pond's edge (Schalk and Morales, 2012) and deposit eggs directly in the water (Cei, 1980), therefore they may be unable to utilize these burrows that are several meters away from the pond as refuges during breeding bouts.

The feeding experiments in Cocroft and Hambler's (1989) demonstrate that leptodactylid and bufonid frogs were palatable to the tarantula *Xenesthis immanis*. During our surveys, we observed an unsuccessful predation attempt on a female *L. bufonius* by a tarantula; the tarantula ambushed the *L. bufonius*, but it was able to escape quickly. We also never observed either species of frog and tarantulas co-occurring in the same burrow at the same time, suggesting that antagonistic interactions in the form of a predator-prey relationship may be occurring. However, without conducting feeding experiments (*sensu* Cocroft and Hambler, 1989; Dundee et al., 2012), we cannot comment on the palatability of *L. bufonius* or *R. major* to the tarantulas in the area.

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