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Microhabitat use and spatial distribution in Picado's Bromeliad Treefrog, *Isthmohyla picadoi* (Anura, Hylidae)

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Abstract

Microhabitat use and spatial distribution in Picado's Bromeliad Treefrog, *Isthmohyla picadoi* (Anura, Hylidae). *Isthmohyla picadoi* is a Neotropical hylid frog found in upper humid montane forests of Costa Rica and Panama. The species is of particular interest because it continues to persist in an area in which the amphibian community has otherwise been decimated by the pathogenic fungus, *Batrachochytrium dendrobatidis*. Ground search, ladder climbing, and tree climbing techniques were used to locate 32 individuals; including adult males and females, juveniles, and metamorphosing frogs. The majority of frogs were found in bromeliads, although some individuals were found on plants of the Euphorbiaceae, Musaceae, and Heliconiaceae families. Most frogs were found in larger bromeliads (45 cm or wider). There was a positive correlation between SUL and bromeliad width within the population but not within maturity classes (adult males, adult females, all adults, non-metamorphosing juveniles), suggesting that juvenile and adult frogs differ in bromeliad usage. Ranges of SUL and body weight in this particular population are much greater than those reported in previous species accounts.

Keywords: Anura, Hylidae, *Isthmohyla picadoi*, bromeliad, phytotelmata, upper humid montane forest, *Batrachochytrium dendrobatidis*.

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Resumen
Uso de microhábitats y distribución espacial de la rana arboricola de bromelia de Picado, *Isthmohyla picadoi* (Anura, Hylidae). *Isthmohyla picadoi* es una rana Neotropical de la familia Hylidae que habita en los bosques húmedos montanos de la parte alta de la región de Talamanca entre Costa Rica y Panamá. Esta especie es de interés en particular, porque continua sobreviviendo en una área afectada por el hongo patogénico, *Batrachochytrium dendrobatidis*. Buscamos ranas al nivel del suelo, y en árboles, subiendo tanto por escalera, como trepando por soga. Recolectamos datos correspondientes a 32 individuos, incluyendo machos y hembras adultos, juveniles, y ranas en metamorfosis. La gran mayoría de las ranas fueron encontradas en bromelias grandes (45 cm o más de anchura), y algunos individuos fueron localizados en plantas de las familias Euphorbiaceae, Musaceae, y Heliconiaceae. Se encontró una correlación positiva entre la longitud hocico-urostilo (LHU) y la anchura de la bromelia dentro de la población, pero ningúna correlación dentro de cada una de las clases de madurez (machos adultos, hembras adultas, todos los adultos, y juveniles que no están en metamorfosis), lo que sugiere que los adultos y juveniles usan diferentemente las bromelias. Estos datos también indican que los rangos de tamaño tanto en la longitud hocico-cloaca (LHC) como en el peso de esta población son mucho mas amplios que los descritos previamente para esta especie.

Palabras Claves: Anura, Hylidae, *Isthmohyla picadoi*, bromelia, phitotelmata, altos bosques húmedos, *Batrachochytrium dendrobatidis.*

Resumo
Uso de micro-habitats e distribuição espacial de *Isthmohyla picadoi* (Anura, Hylidae). *Isthmohyla picadoi* é um anuro neotropical da família Hylidae que habita os bosques úmidos da porção alta da região de Talamanca, entre a Costa Rica e o Panamá. Essa espécie é de particular interesse porque continua sobrevivendo em uma área em que outras espécies da comunidade de anfíbios foram dizimadas pelo fungo patogênico *Batrachochytrium dendrobatidis*. Os indivíduos foram localizados por procura no solo e por técnicas de escalada com escadas e escalada de árvores. Foram coletados dados de 32 indivíduos, incluindo machos e fêmeas adultos, juvenis e girinos em metamorfose. A grande maioria dos animais foi encontrada em bromeliáceas grandes (≥ 45 cm de largura), embora alguns indivíduos tenham sido localizados em plantas das famílias Euphorbiaceae, Musaceae e Heliconiaceae. Houve uma correlação positiva entre o comprimento rostro-cloacal dos anuros e a largura das bromeliáceas dentro da população mas não dentro das classes de maturidade (machos adultos, fêmeas adultas, todos os adultos, juvenis), sugerindo que os juvenis diferem dos adultos no uso das bromeliáceas. A amplitude de variação do comprimento rostro-cloacal e do peso corpóreo dessa população foi muito maior do que aquela registrada em estudos anteriores com essa espécie.

Palavras-chave: Anura, Hylidae, *Isthmohyla picadoi*, bromeliáceas, fitotelmata, floresta úmida alto-montana, *Batrachochytrium dendrobatidis.*

Introduction

Picado's Bromeliad Treefrog, *Isthmohyla picadoi* (Dunn 1937), formerly *Hyla picadoi*, is a medium-sized Neotropical hylid frog (SVL: ♂27-32 mm; ♀34-35 mm) which inhabits epiphytic bromeliads in humid montane forests of Costa Rica and Panama (Duellman 2001, Savage 2002, Faivovich *et al.* 2005). Tadpoles, metamorphosing juveniles, and adults have all been found together in bromeliads, suggesting the possibility of an entirely arboreal life cycle (Savage 2002). The rosette configuration of bromeliad axils form tank-like phytotelmata, a critical microhabitat for amphibians, plants, insects, arachnids, crabs, and annelids (Maguire 1971, Fish 1983, Catling and Lefkovitch 1989, Fragoso and Rojas-Fernandez 1996, Araújo *et al.* 1997). Physical aspects of these phytotelmata, specifically water temperature, are important to amphibian species (Piotrowski 2004). *Isthmohyla picadoi* inhabits primary, secondary, and transitional forests along the Central and Talamancan Cordilleras of Costa

Rica and western Panama from 2,000 to 2,200 m above sea level (asl) (Duellman 2001, Savage 2002, Solis *et al.* 2008). Scant information on the natural history of this species is limited to species accounts provided by (Duellman 2001, Savage 2002) and a description of their vocalization (Lindquist and Cossel 2007).

The population from the Cerro Punta region of the Chiriquí highlands of western Panama is of particular interest because it persists despite catastrophic declines and extinctions of amphibian species due to *Batrachochytrium dendrobatidis* (*Bd* hereafter). Species that disappeared rapidly after the 1994 *Bd* epidemic were primarily those whose life cycle involved direct contact with stream water (Lips 1999). Although *I. picadoi* has long been considered a canopy specialist, individuals have been found on rocks in streams on at least two occasions (Duellman 2001). These observations suggest a potential mechanism of vertical stream-to-canopy transfer as well as up-slope dispersion of *Bd*. As *I. picadoi* has been encountered within and near bromeliads containing high *Bd* zoospore concentrations (Cossel and Lindquist 2009), it is possible that individuals which travel between streams and bromeliads transmit the fungus to more terrestrial habitats.

This study presents data on the microhabitat use and spatial distribution of *I. picadoi* with respect to various physical and structural environmental parameters from a site in the Chiriquí Highlands of western Panama. This information may be crucial in understanding why this species has persisted after the decline or disappearance of the majority of amphibian species in this region, which is attributed to the *Bd* epidemic of 1994.

Materials and Methods

Study area

We studied a population of *I. picadoi* immediately north of Guadalupe Arriba, Chiriquí Province, in the Republic of Panama (8°52'10.0" N, 82°34'0.5" W) between 3 and 29 January 2008 (Figure 1). For the purpose of our study, we subdivided this area into four sites based on the location of vocalizing clusters of *I. picadoi*. These sites included primary, secondary, and transitional riparian humid forest, ranging in altitude from 1,950 to 2,300 m asl (Table 1). Site 1 contained a transition area between primary and secondary growth forest, sites 2 and 3 were situated within primary growth forest, and site 4 straddled the border of a secondary forest and the edge of a botanical garden and contained more bromeliads than any other site. Sites 1-3 were located in the forest preserve owned by Los Quetzales Lodge and Spa, and site 4 was located in a small forest tract in the Finca Dracula, owned by Andrés Maduro. Both properties border the Parque Internacional La Amistad and Parque Nacional Volcán Barú.

Locating individuals

We located frogs via two techniques: 1) thoroughly searching bromeliads (especially leaf axils, interaxil spaces, and phytotelmata) at random both at day and night; and 2) triangulating male position immediately after vocalizations were given (mostly from epiphytes) at night. Epiphytic vegetation (bromeliads and mosses) was searched at heights from 0 to 33.5 m above the ground. These epiphytes occupied tree limb and trunk surfaces primarily on *Sapium laurifolium* (Euphorbiaceae), *Alnus acuminata* (Betulaceae), and one *Schefflera* sp. (Araliaceae). Trees and epiphytes were searched using three methods: ground searches, ladder searches, and climbing using the Single Rope Technique (SRT) (Maher 2006). Ground searches entailed examining fallen bromeliads or those within standing reach. Ladder climbs employed a 7.3 m extension ladder to access higher bromeliads. Tree climbs using the SRT, which reached heights up to 33.5 m, were used to sample

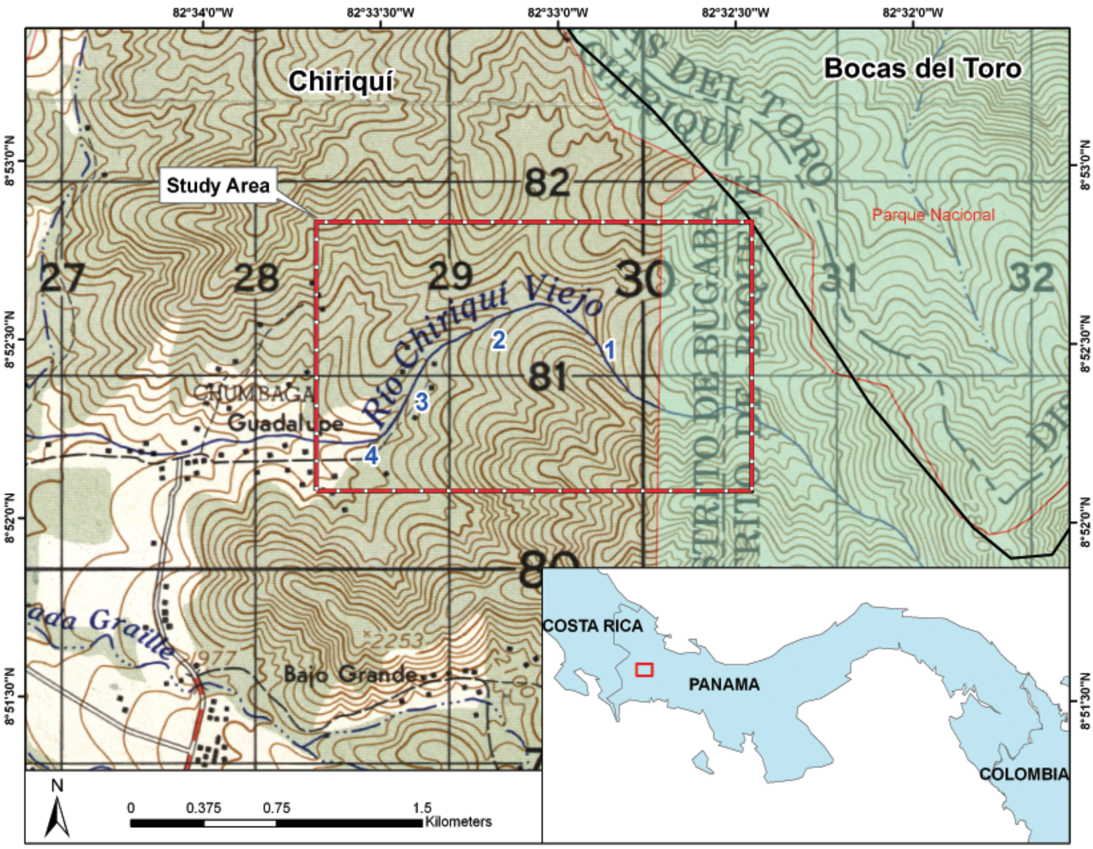

Figure 1 - Map of the sites 1-4 in the study area along the Río Chiriquí Viejo near Guadalupe Arriba, Chiriquí Province, Panama.

bromeliads otherwise inaccessible to ground and ladder searches. All bromeliads were measured at the widest point across to the nearest 5 cm increment using a pre-measured metal hook tool (29" Mark O'Shea Signature Series Hook, Midwest Tongs Inc.). Generally, the longest width was found tangential to the tree surface on which the bromeliad was anchored.

Data collection and animal care

To prevent further spread of *Bd*, latex gloves were worn while capturing and handling each frog and frogs were placed into individual plastic bags. Further, any objects that came into contact with a frog (i.e. hands, equipment, all surfaces) were wiped with 95% isopropyl alcohol. Water temperature was investigated in order to determine if physical aspects of the phytotelmata were thermally detrimental to *Bd* growth. For eleven of the bromeliads in which frogs were found (widths= 25, 45, 45, 50, 65, 70, 80, 80, 92, 95, and 105 cm), a thermometer was placed in the bromeliad's phytotelm for at least 24 hours to record the minimum and maximum water temperatures. As a control, a thermometer was also placed in the phytotelmata of eighteen randomly selected bromeliads for at least 24 hours. For each frog collected ambient air temperature, bromeliad

Table 1 - Size (SUL and SVL) and mass of males, females, and juveniles including mean ($\overline{x}$), standard deviation (SD), and range.

		SUL (mm)	SVL (mm)	Weight (g)
Males (n=15)	$\overline{x}$	31.50	33.95	2.07
	SD	2.37	2.80	0.40
	range	26.80-34.80	29.10-37.90	1.13-2.50
Females (n=3)	$\overline{x}$	35.05	38.23	2.83
	SD	2.18	2.09	0.07
	range	32.90-37.25	36.50-40.50	2.75-2.85
Juveniles (n=14)	$\overline{x}$	17.13	19.23	0.42
	SD	2.01	2.57	0.16
	range	14.20-21.10	16.85-24.95	0.25-0.88

water temperature, height above the ground, distance to canopy, calling activity, bromeliad (or other plant) width, collector's name, date and time of capture were recorded. Distance to canopy and ground measurements were taken using a Bushnell Yardage Pro Trophy 5x20 Rangefinder.

Frogs were taken back to our field base after collection. To promote ecdysis, a small amount of rainwater was added to each bag, where the frog remained for at least 24 hours. After 24 hours, shed skin was removed from the bag and placed in a labeled vial along with an epidermal swab of the individual for later detection of *Bd*. Using Savage's key (2002), individuals were positively identified as *I. picadoi* by the presence of two odontoids at the symphysis of the lower jaw. Frogs were also weighed, measured for snout-vent length (SVL) and snout-urostyle length (SUL), sexed noting the presence of nuptial pads in adult males or calling activity, and photographed. Although SUL was used in the analyses due to its lower variance, SVL is provided for comparisons to previous publications on *I. picadoi*.

After data and skin were collected from all individuals found in a study site, frogs were released back to the host tree on which they were found. Animal care protocols were reviewed and approved by the IACUC at the Smithsonian Tropical Research Institute prior to the commencement of our study, and research and collection permits were obtained through La Autoridad Nacional del Ambiente (ANAM). No frogs died in this study.

Results

During the study, 32 individuals were observed and captured from study areas 1 and 4 (n=2 and n=30, respectively): 15 males, 3 females and 14 juveniles; no tadpoles were found in this study (Table 1). Juveniles exhibited a body size (SUL) ranging from 14.20 to 21.10 mm. One juvenile appeared to possess nuptial pads (SUL = 18.7 mm) and four others were in the process of metamorphosis, exhibiting tail buds. A linear relationship between SUL and weight can be seen in Figure 2 (R^2=0.9595, p<0.0001).

Accessing frogs

A total of 1,376 bromeliads were inspected for frogs (site 1: 319, site 2: 248, site 3: 256, site 4: 553). Of these, 530 (38.5%) were

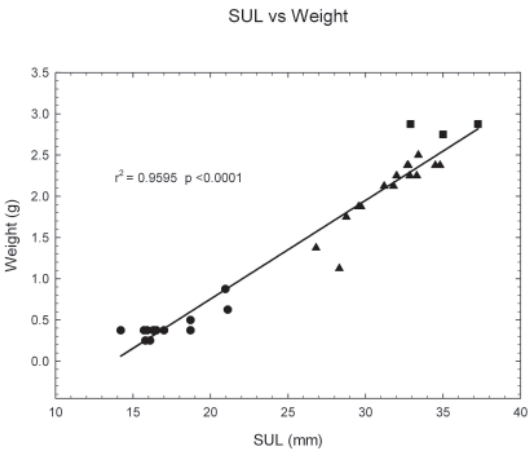

Figure 2 - XY scatter plot showing the linear relationship between SUL (mm) and weight (g) (R^2=0.9595, p<0.0001). Adult males, adult females, and juveniles are depicted as triangles, squares, and circles respectively.

examined from the ground, 260 (19%) were investigated using a ladder, and 586 (42.5%) were accessed via tree climbing (Table 2). Tree climbs resulted in 18 of the 32 frogs (56.25%), ladder climbs resulted in 7 frogs (21.875%), and ground searches found 7 frogs (21.875%) (Table 2). Frogs were encountered in only 1.7% of all bromeliads searched.

Figure 3 shows the number of *I. picadoi* found in bromeliads of different size classes relative to our search effort, which was representative of habitat availability in the sites. Bromeliads smaller than 10 cm in width are too small to create phytotelmata and as a result cannot be utilized by *I. picadoi*; therefore,

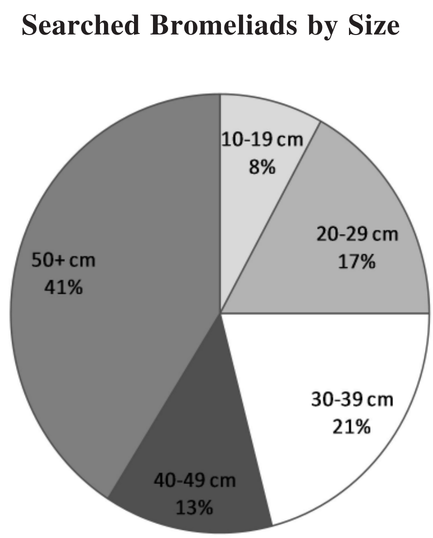

Figure 3 - Bromeliad width categories in which *I. picadoi* was encountered. Percentages lying within each width category represent the percentage of total bromeliads searched in this study.

Table 2 - Number of bromeliads searched by each method for each location. The number of bromeliads/plants containing frogs is indicated in parentheses.

Study Location	Elevation (m)	Ground Searches	Ladder Searches	Tree Searches	Non-bromeliad Plants with Frogs	Total Frogs/Site	Total Bromeliads Searched
1	2,180-2,270	118 (1)	56	145 (1)	0	2	319
2	2,120-2,150	76	37	135	0	0	248
3	2,070-2,120	126	45	85	0	0	256
4	1,990-2,060	210 (3)	122 (3)	221 (7)	3	30	553

bromeliads in this size range were not included in the data analyses. The remaining bromeliads were split into five categories for data analyses. No frogs were found in the smallest (10-19 cm) bromeliad size category, (n=109, 8% of total). In the 20-29 cm size category (n=238, 17% of total), a single frog (3.125%) was found. Six frogs (18.75%) were found in the 30-39 cm size category, (n=289, 21% of total). In the 40-49 cm size category, (n=181; 13% of total), 5 frogs (15.625%) were found. In the largest size category (50+ cm), (n=559, 41% of total), 16 frogs (50%) were found. Four frogs (12.5%) were found on plants other than bromeliads.

Phytotelmic associations

Frogs were primarily found in or on plants of the Bromeliaceae family (n=28; 87.5%); however, two frogs were found on *Heliconia lankisteria* (Heliconiaceae), one on *Ensete ventricosum* (Musaceae), and another on *Sapium laurifolium* (Euphorbiaceae). Widths of bromeliads containing frogs ranged from 25 to 105 cm.

There was a significant positive Spearman rank-order correlation between frog size (SUL) and bromeliad width (n=24, r_s=0.6697, p=0.0003, d.f.=22) (Figure 4). However, there was no statistical significance between SUL and bromeliad width within any of the maturity classes (adults, non-metamorphosing juveniles, adult males, or adult females). Metamorphic juveniles (n=4) with tail buds were not included in any statistical analyses relating to bromeliad choice as all were found emerging from the same natal bromeliad, indicating parental choice.

Phytotelmic water temperature

We found a significant difference in minimum phytotelmic water temperatures within bromeliads with and without frogs ($\bar{x}_{frog}$=10.04°C, $\bar{x}_{no\ frog}$=9.35°C, p=0.0455, z=1.69, n_{frog}=11, $n_{no\ frog}$=18; Mann-Whitney U test, one-tailed,). However, this was not the case for the thermal maxima of phytotelmic water ($\bar{x}_{frog}$=14.23°C, $\bar{x}_{no\ frog}$=13.86°C, p=0.1357, z=1.1, n_{frog}=11, $n_{no\ frog}$=18; Mann-Whitney U test, one-tailed). This suggests that in cool montane microhabitats, minimum phytotelmic water temperature may be an important environmental variable determining suitability of bromeliads to *I. picadoi*.

Spatial positioning

Vertical positioning for *I. picadoi* was particularly variable with respect to distance from the ground ($\bar{x}$=9.98 m; std. dev.=8.41 m; min=0 m; max=22.5 m; Spearman rank-order; n=28; r_s=0.3112; p=0.1069) (Figure 5) and distance to the canopy limit ($\bar{x}$=13.76 m; std. dev.=9.93 m; min=0 m; max=34 m; Spearman rank-order; n=28; r_s=-0.2908; p=0.1332) (Figure 6).

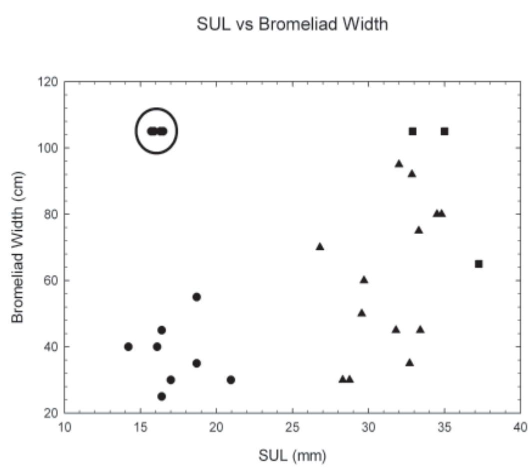

Figure 4 - The relationship between bromeliad width (cm) and SUL (mm) is shown below. The four metamorphic juveniles with tail buds excluded from the statistical trend analysis are circled. Adult males, adult females, and juveniles are depicted as triangles, squares, and circles respectively.

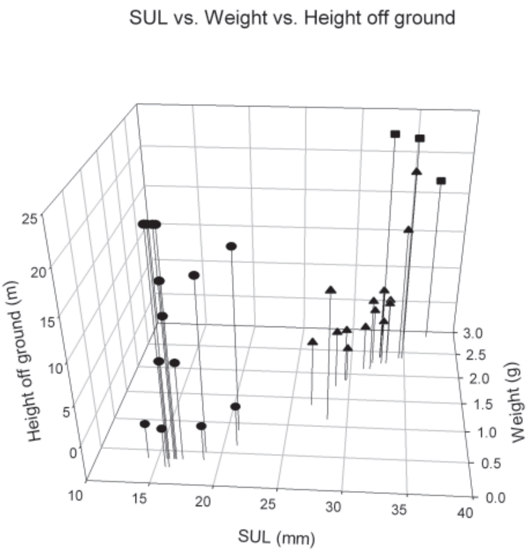

Figure 5 - SUL (mm) and weight (g) of each *I. picadoi* and their respective spatial distribution relative to the distance above the ground (m) at time and place of capture. Adult males, adult females, and juveniles are depicted as triangles, squares, and circles respectively.

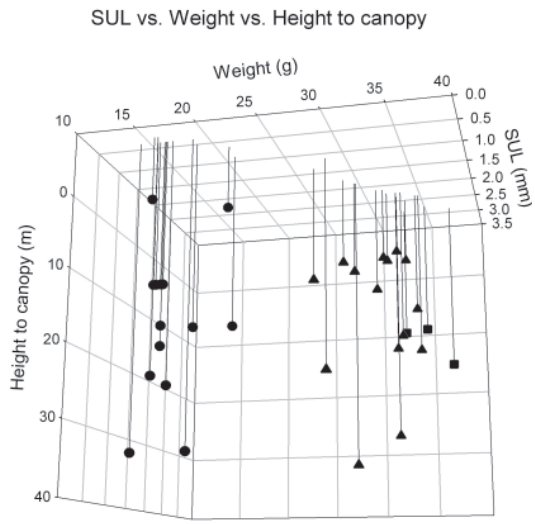

Figure 6 - SUL (mm) and weight (g) of each *I. picadoi* and their respective spatial distribution relative to the distance to the canopy limit (m) at time and place of capture. Adult males, adult females, and juveniles are depicted as triangles, squares, and circles respectively.

Discussion

This study provides new insights into the biology, microhabitat requirements, and spatial distribution of a species with scant natural history information available. This study reveals the species' overall size maximum (SVL) is larger than once thought, with individuals in our study population exceeding the sizes reported in Duellman (2001) and Savage (2002). The use of bromeliads as phytotelmic sources is critically important for this species, although individuals were found on plant species of three other families (Euphorbiaceae, Musaceae, and Heliconiaceae). No frogs were found in bromeliads smaller than 25 cm, perhaps indicating a minimum phytotelmic size requirement for the species. In addition, there was a direct correlation between SUL and bromeliad width in the overall population. However, this is not statistically significant within any of the maturity classes of these frogs (all adults, adult males, adult females, and non-metamorphosing juveniles) suggesting that the correlation is significant largely due to a juvenile preference for smaller phytotelmic hosts. This is potentially a result of differences in bromeliad usage between adults and juveniles. While adults likely use bromeliads for breeding – supported by the four metamorphosing juveniles found together in a relatively large bromeliad for their size – the focus of juveniles may be on feeding and finding refuge. A larger sample size of *I. picadoi* would further elucidate size choice in these amphibians across maturity classes and as a whole. Differences in bromeliad usage by age class may imply differences in lifestyles (i.e. a more mobile juvenile lifestyle and a more stationary, and perhaps territorial, adult lifestyle), which would have implications in both the species' survival in areas infected by *Bd* as well as transmission of the fungus.

Although collecting temperature data over a longer duration of time would be advantageous to clarify the interaction of temperature and phytotelmic water sources, temperature data gathered in this study indicate that these frogs may select for bromeliads containing a higher minimum phytotelmic water temperature. In effect, this could influence bromeliad size choice in this species as larger bromeliads, which contain more water, should moderate the effects of cool montane nights. Data from these phytotelmic water sources fall within the thermal optimal range for *Bd* in this study, and likely do not provide refugia for amphibians adversely affected by chytridiomycosis. Yet this *I. picadoi* population still thrives where many other species have disappeared, likely because of its primarily arboreal habits (pers. obs.).

The occurrence of metamorphic juveniles still possessing tail buds implied that our research in January coincided with the end of the breeding season in this region. We found no amplectant pairs, egg masses, or tadpoles, further supporting this hypothesis. Two adults were found on a large bromeliad (width=105 cm) containing four metamorphic juveniles; however, one escaped before a positive identification of sex could be determined. This may provide anecdotal evidence which supports K. Lips' report that parents remain close to their young through metamorphosis (Savage 2002). Many males were still actively calling despite the lack of observed breeding activity. Lastly, we recognize that our highly biased sex ratio was likely a result of our method of discovery—using vocalizations in some areas to increase sample sizes—and therefore is probably unrelated to actual operational sex ratios.

This study suggests that *I. picadoi* can utilize a much wider range of habitats than originally described. Individuals were encountered at variable heights above the ground (0 to 22.5 m) with maximum heights extending much higher than the shrub and stream heights previously reported (Duellman 2001, Savage 2002). Site 1, a transition area between primary and secondary growth forest, yielded 2 frogs. The vast majority of frogs (n=30) were found at site 4, which consisted predominantly of secondary forest and had a greater density of bromeliads than any other site. This site was the most disturbed of our four sites. Sites 2 and 3, regions of primary growth, yielded no frogs. This indicates that although *I. picadoi* was formerly thought to be a species of primary montane forest (Duellman 2001, Savage 2002), this species has a tolerance to disturbance and can survive in secondary and transitional forests as well as a botanical garden containing bromeliads or other species with phytotelmic water sources. This is significant for conservation efforts aimed at this species and may play a role in the species' persistence after the *Bd* epidemic in the region. Although much more is yet to be learned about the natural history of *Isthmohyla picadoi*, this study provides invaluable insights into microhabitat use and spatial distribution.

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