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MICROHABITAT USE AND OBSERVATIONS ON PARENTAL CARE IN *HYLOXALUS CEPEDAI* (ANURA: DENDROBATIDAE) FROM THE ANDEAN FOOTHILLS OF THE COLOMBIAN LLANOS

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Hyloxalus cepedai is an endemic frog from the Eastern Cordillera of the Colombian Andes, currently categorized as Vulnerable due to its restricted distribution and the ongoing transformation of its habitat. Despite its conservation status, ecological information about this species remains scarce. In this study, we characterized the microhabitat occupied by *H. cepedai* in four localities of the municipality of Aguazul, Casanare, in eastern Colombia, and provided observations on parental care. Fieldwork was conducted in April through daytime visual encounter surveys in gallery forests. For each individual, we recorded the substrate, vertical position, and horizontal position. A total of 72 individuals were observed. Most were recorded in leaf litter (n = 52), on the exposed surface of the substrate (n = 59), and in horizontal position I (n = 53). Chi-square analyses revealed that the use of these microhabitat variables was not random, but instead showed significant differences across all categories evaluated, indicating consistent patterns of space utilization. In addition, four males were observed carrying 8 to 10 larvae on their backs, moving across different substrates and at short distances (0–3 m) from the water. These records expand the knowledge of the ecology of *H. cepedai* by documenting both its non-random microhabitat use patterns and aspects of its parental care.

Keywords: Colombia, dendrobatid frogs, habitat, resources, reproductive strategies

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Hyloxalus cepedai je endemska vrsta žabe s Istočnih Kordiljera kolumbijskih Anda, trenutno svrstana u kategoriju ranjivih vrsta zbog svoje ograničene rasprostranjenosti i kontinuirane transformacije njenog staništa. Unatoč zaštićenom statusu ekološki podaci o ovoj vrsti i dalje su ograničeni. U ovoj studiji detaljno smo opisali mikrostanište koje *H. cepedai* zauzima na četiri lokaliteta u općini Aguazul, Casanare, u istočnoj Kolumbiji te donosimo opažanja o roditeljskoj skrbi. Terenski rad proveden je u travnju putem dnevnih vizualnih pregleda u šumama uz vodotoke. Za svaku promatranu jedinku bilježili smo podlogu, vertikalni položaj i horizontalni položaj. Ukupno su zabilježene 72 jedinke. Većina ih je pronađena u sloju lišća (n = 52), na izloženoj površini podloge (n = 59) i u horizontalnom položaju I (n = 53). Analize hi-kvadrat testom pokazale su da korištenje ovih mikrostaniš-

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nih varijabli nije nasumično, već su u svim ocjenjivanim kategorijama uočene značajne razlike, što ukazuje na konzistentne obrasce korištenja prostora. Dodatno su četiri mužjaka zabilježena noseći na leđima 8 do 10 ličinki, krećući se preko različitih podloga i na malim udaljenostima (0–3 m) od vode. Ovi rezultati doprinose razumijevanju ekologije *H. cepedai* dokumentiranjem nenasumičnih obrazaca korištenja mikrostaništa, kao i aspekata roditeljske skrbi kod te vrste.

Ključne riječi: Kolumbija, dendrobatidne žabe, stanište, resursi, reproduktivne strategije

INTRODUCTION

Habitat has been defined as the space that provides the resources and environmental conditions required for the presence, survival, and reproduction of a species (Hall *et al*., 1997; Krausman, 1999). In this sense, habitat establishes the relationship between organisms and the physical and biological features of the environment in which they occur (Hall *et al*., 1997). Within this broad scale, microhabitat refers to more specific units where individuals are located according to their ecological requirements (Inger, 1994). In amphibians, microhabitat selection is associated with requirements related to water balance, shelter, reproduction, and foraging, and it can be described through variables such as substrate type, vertical position and distance to water bodies (Wells, 2007; Urbina-Cardona & Reynoso, 2017; Acosta-Ortiz *et al*., 2024).

The availability and characteristics of microhabitats may influence the expression of reproductive strategies, including parental care (Wells, 2007). Factors such as structural heterogeneity of the substrate, the availability of shelters, and proximity to water sources determine the viability of embryos and the success of tadpole transport (McDiarmid, 1978; Haddad & Prado, 2005). These environmental conditions not only shape the occurrence of individuals but also influence the development of behaviors associated with offspring survival (Wells, 2007).

In dendrobatids, plasticity in microhabitat use has been associated with the diversification of reproductive strategies and the evolution of different forms of parental care (Weygoldt, 1987; Summers & Tumulty, 2014; Cossio *et al*., 2021). Within this group, species of the genus *Hyloxalus* use a wide variety of ground-level microhabitats, including herbaceous vegetation, mosses, leaf litter, exposed roots, substrate crevices, and small natural cavities, generally in close proximity to streams or shallow water bodies (Coloma, 1995; Pazmiño-Otamendi, 2012; Yánez-Muñoz *et al*., 2010; Rodríguez-Cardozo *et al*., 2016; Acosta-Galvis & Pinzón, 2018). In addition, space use in *Hyloxalus* may be associated with territorial behaviors in both males and females, as well as with movements related to different phases of the reproductive cycle, suggesting variation in microhabitat use linked to sex and reproductive function (Wells, 2007; Quiguango-Úbillus & Coloma, 2008; Pazmiño-Otamendi, 2012; Yánez-Muñoz *et al*., 2010; Acosta-Galvis & Pinzón, 2018).

Hyloxalus cepedai is a dendrobatid endemic to the humid forests on the eastern slopes of the Cordillera Oriental in Colombia, currently categorized as Vulnerable due to its restricted area of occupancy and the ongoing transformation of its habitat (Acosta-Galvis, 2025; IUCN, 2025). Given these conditions and its recognized sensitivity to environmental disturbances, it is considered a high-priority species for conservation (Acosta-Ortiz *et al*., 2025). However, ecological information available for this frog is limited, particularly regarding microhabitat use and reproductive behaviors, making it difficult to understand its environmental requirements. In this con-

text, the present study aimed to characterize the microhabitat occupied by *H. cepedai* in four localities of the municipality of Aguazul, Casanare, and to provide observations related to its parental care.

MATERIAL AND METHODS

Study area

The study was conducted in the municipality of Aguazul, located in the foothills of the Llanos in the department of Casanare, eastern Colombia (Fig. 1). The region has a warm climate with temperatures oscillating between 20 and 28.9 °C and a unimodal rainfall regime, with annual precipitation ranging from 1,000 to 3,200 mm (Díaz-Pérez *et al*., 2018). Four sampling localities were established in gallery forests along the Únete River (Fig. 1), located at the following coordinates: 5.176° N, –72.572° W; 5.176° N, –72.563° W; 5.180° N, –72.564° W; and 5.183° N, –72.575° W. The sampled gallery forests were characterized by a closed canopy that provided constant shade and high relative humidity; a heterogeneous understory composed of seedlings, juvenile palms, ferns, and lianas, which contributed to vertical complexity and spatial diversity; a forest floor covered by a continuous layer of leaf litter in different stages of decomposition, accompanied by rocks, fallen logs, moist branches, and trees with exposed roots; as well as the presence of canopy gaps generated by tree falls, which favored the establishment of herbaceous species.

Field work

In April, four field trips were conducted, one in each locality, during which a team of three people carried out diurnal searches (08:00–12:00 h and 14:00–15:00 h) through free-ranging walks in gallery forests. The detection of *H. cepedai* individuals was performed using the Visual Encounter Survey (VES) methodology (Crump & Scott, 1994). Each survey covered approximately one km of non-linear transects, ensuring the spatial representativeness of the sampling effort.

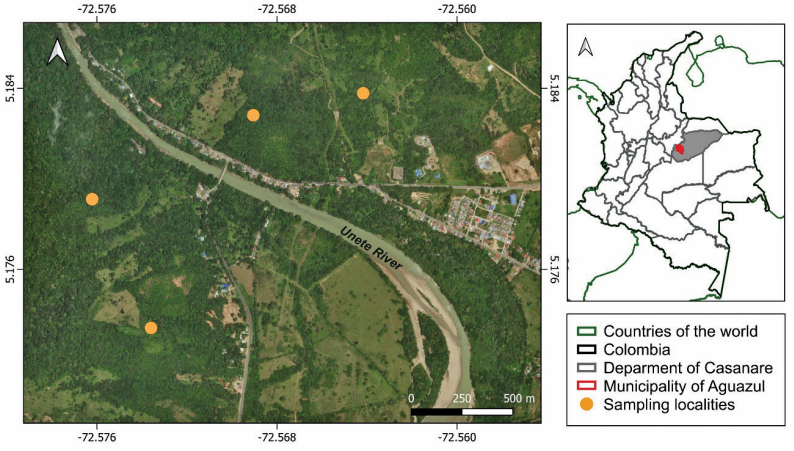


Fig. 1. Geographic location of the study area and sampling sites in the municipality of Aguazul, Casanare, Colombia.

Microhabitat description

For the characterization of microhabitat, three variables were considered: substrate, vertical position, and horizontal position (Inger, 1994; Acosta-Ortiz *et al*., 2024). For each individual, the use of these resources was recorded, including subdivisions defined as ecological categories (Colwell & Futuyma, 1971; Acosta-Ortiz *et al*., 2024). Vertical position was measured according to the individual's location relative to the substrate and classified into three categories: on the exposed surface of the substrate (Os), when the individual was visibly on the substrate; beneath the surface of the substrate (Bs), when the individual was buried or hidden under leaf litter, logs, rocks, or other surrounding material; and submerged (Su), when the individual was under water, either partially or completely (modified from Inger, 1994). Horizontal position was determined according to the distance of the individual from the nearest body of water and grouped into three categories: I (0–5 m), II (5–10 m), and III (10–15 m) (García *et al*., 2005). Finally, substrate corresponded to the type of surface on which each individual was located, concealed, or moving at the time of observation.

Microhabitat use analysis

To determine whether *H. cepedai* used microhabitat variables in a non-random manner, a chi-square (χ^2) goodness-of-fit test was applied to each resource category (vertical position, horizontal position, and substrate). Observed frequencies of individuals in each category were compared with expected frequencies under the null hypothesis of random use (Zar, 1999). Statistical significance was assessed at a 95% confidence level ($p < 0.05$), and all analyses were conducted in PAST v.4.17 (Hammer *et al*., 2001).

Parental care

Parental care observations were recorded incidentally during microhabitat characterization, based on direct encounters with individuals under natural activity conditions. Although these records were not part of a specific sampling design, they provide valuable insights into behaviors associated with parental care in the species.

RESULTS

A total of 72 individuals of *Hyloxalus cepedai* were recorded. Most observations corresponded to individuals located on the exposed surface of the substrate (Os), whereas records beneath the surface of the substrate (Bs) and under submerged conditions (Su) were comparatively less frequent (Fig. 2). In terms of horizontal position, category I concentrated the majority of records, while categories II and III showed lower representation (Fig. 2). Regarding substrate, leaf litter was the most frequently used, in contrast with the reduced number of records on roots, bare soil, water, and rocks (Fig. 2).

The chi-square goodness-of-fit test revealed significant differences in the frequency distribution of individuals among the categories of each resource evaluated. Non-random use was detected for vertical position ($\chi^2 = 70.25$; df = 2; $p < 0.05$), horizontal position ($\chi^2 = 56.08$; df = 2; $p < 0.05$), and substrate type ($\chi^2 = 121.4$; df = 4; $p < 0.05$).

Additionally, four individuals were recorded carrying larvae on their backs, with brood sizes ranging from 8 to 10 larvae per individual (Fig. 3). All four individuals were observed moving rapidly across the substrates, located between 0 and 3 m from the nearest water bodies. One male moved among tree roots (Fig. 4), another on a rock, and two others on leaf litter.

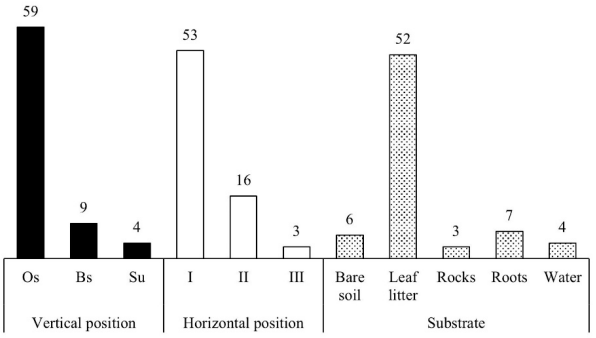


Fig. 2. Microhabitat resource use by *Hyloxalus cepedai*. Os: on the exposed surface of the substrate; Bs: beneath the surface of the substrate; Su: submerged.



Fig. 3. *Hyloxalus cepedai* individual exhibiting parental care on top of leaf litter. Photo: J. M. Acosta-Ortiz.

DISCUSSION

The vertical position pattern, with most individuals observed on the exposed surface of the substrate (Os), indicates that the species maintained its activity in the superficial soil layer during the hours corresponding to its natural activity period. Remaining in this vertical position may be facilitated by the humidity and shade provided by the forest canopy, conditions that reduce the risk of desiccation and allow for locomotion and foraging without the need to seek refuge beneath the substrate (Vásquez-Vélez *et al*., 2013; Posso-Peláez *et al*., 2017).



Fig. 4. *Hyloxalus cepedai* individual exhibiting parental care on a tree's superficial root. Photo: J. M. Acosta-Ortiz.

Regarding horizontal position, the highest concentration of records in categories closest to water bodies suggests a strong spatial affinity for these environments. This pattern agrees with expectations for small-bodied species such as *H. cepedai*, whose surface-to-volume ratio may increase water loss and restrict movement toward areas farther from water sources (Ashton, 2002; Blanco-Torres *et al*., 2018). Proximity to water not only ensures favorable conditions for maintaining daily activity but is also essential for reproductive development, particularly during larval transport to aquatic habitats (McDiarmid, 1978; Duellman & Trueb, 1994; Haddad & Prado, 2005). Thus, the distribution observed along the horizontal gradient may reflect an interaction between physiological constraints imposed by body size and the need to access reproductive resources (Duellman, 1967; Wells, 2007).

In terms of substrate, the predominance of individuals on leaf litter highlights the importance of this structural component of the microhabitat for terrestrial anurans. Leaf litter may provide a physically stable and environmentally constant substrate, offering support for locomotion, camouflage, and abundance of potential prey, while maintaining microenvironmental conditions that favor moisture retention (Duellman & Trueb, 1994; Vásquez-Vélez *et al*., 2013; Moskowitz *et al*., 2020). Furthermore, the use of this substrate may respond to both hydric and thermal requirements (Duellman & Trueb, 1994; Wells, 2007). In the first case, anurans are known to discriminate among substrates according to their water potential, selecting those that facilitate cutaneous water uptake and avoiding those that promote dehydration (Brekke *et al*., 1991; Nagai *et al*., 1999). In the second, the surface temperature of the leaf litter may favor thermoregulation given the ectothermic condition of the species (Duellman & Trueb, 1994). This pattern is consistent with the terrestrial habits of the species and with previous reports indicating that leaf litter constitutes the main substrate for daily activity (Angarita-Sierra *et al*., 2013; Aponte-Gutiérrez *et al*., 2021). Finally, although few individuals were recorded, the species also used other substrates such as

roots, bare soil, and rocks. These observations may reflect a plastic response to local environmental heterogeneity, evidencing some flexibility in substrate use depending on availability (Acosta-Ortiz *et al*., 2024).

Chi-square analyses confirmed that the use of microhabitat variables by *H. cepedai* was not random but rather showed significant differences across all evaluated categories. This indicates that the distribution of individuals in relation to vertical position, horizontal position, and substrate type follows consistent patterns of space utilization. These results indicate that certain microhabitat components are consistently associated with the occurrence of the species. The statistical evidence suggests that the observed records do not reflect a casual occupation, but rather reflect a non-random use of available microhabitats, potentially related to physiological constraints, reproductive requirements, and specific microenvironmental conditions.

The transport of larvae on the back observed in *H. cepedai* represents a form of parental care characterized by investment in a mobile developmental site, whereby the parent actively carries the offspring to suitable aquatic habitats for growth (McDiarmid, 1978; Blanco-Torres *et al*., 2018). In this species, this behavior is carried out by males, who assume responsibility for transporting larvae to favorable aquatic environments (Blanco-Torres *et al*., 2018; Aponte-Gutiérrez *et al*., 2021). Observations of four individuals moving across different substrates suggest that males may use multiple routes within the microhabitat to transport larvae, likely facilitating access to nearby water bodies and increasing the chances of successful transfer.

Larval transport is a well-documented behavior among dendrobatids, involving both males and females (Weygoldt, 1987; Crump, 1996). In some cases, females adopt more specialized strategies, such as provisioning unfertilized eggs to feed the larvae, thereby enhancing offspring survival (Weygoldt, 1987; Wells, 2007). Although transport on the back is the most common modality in these frogs, species from other families have evolved alternative forms of parental care. For instance, in *Rhinoderma darwinii*, tadpoles are housed within the adult's vocal sacs, while in *Assa darlingtoni*, larvae are transported in inguinal pouches (Weygoldt, 1987; Vági *et al*., 2019). This diversity of strategies indicates that parental care has evolved convergently across different anuran lineages, likely as a response to selective pressures favoring offspring survival in heterogeneous environments (McDiarmid, 1978; Crump, 1996; Wells, 2007).

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SUMMARY

Microhabitat use and observations on parental care in *Hyloxalus cepedai* (Anura: Dendrobatidae) from the Andean foothills of the Colombian Llanos

J.M. Acosta-Ortiz, A.S. Sierra-Rueda & E. Suárez-Rojas

Hyloxalus cepedai is an endemic frog species restricted to the Eastern Cordillera of the Colombian Andes, currently listed as Vulnerable (VU) due to its limited distribution range and the ongoing transformation of its habitat. Despite its conservation status, ecological information about this species remains scarce. Understanding its habitat use and behavioral patterns is essential to support conservation efforts, especially in areas where land-use changes are intensifying.

This study provides new data on the ecology of *H. cepedai* based on field surveys conducted in four localities of the municipality of Aguazul, Casanare, eastern Colombia. We characterized the microhabitat occupied by the species and documented observations of parental care. Fieldwork was carried out in April through daytime visual encounter surveys in gallery forests. For each individual, we recorded three ecological variables describing microhabitat use: substrate type, vertical position relative to the substrate, and horizontal position with respect to the nearest body of water.

A total of 72 individuals were recorded. Most were found on the exposed surface of the substrate and on leaf litter, at short distances from the water (0–5 m). Fewer individuals were observed beneath the substrate or submerged. Chi-square tests revealed that the use of each microhabitat variable was not random, showing significant differences among categories. These findings indicate consistent spatial patterns in habitat use by *H. cepedai*, suggesting specific use of the available microhabitat resources.

Additionally, four males were observed carrying larvae on their backs, with a load ranging from 8 to 10 larvae per individual. These males were seen moving across different substrates such as leaf litter, roots, and rocks at short distances (0–3 m) from nearby water sources. This behavior provides evidence of an active form of parental care, a characteristic observed in several dendrobatoid frogs and represents one of the few documented records of this behavior for the species.

The information presented here contributes to the understanding of *H. cepedai*'s ecology by documenting both its non-random use of microhabitat and aspects of its reproductive behavior.