

# Life on the Edge: A Comparative Study of Ecophysiological Adaptations of Frogs to Tropical Semiarid Environments

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Accepted 9/14/2017; Electronically Published 12/6/2017

*Dryad data:* <http://dx.doi.org/10.5061/dryad.jm838>.

## ABSTRACT

A key goal of ecology and evolution is to understand the relative contributions of environment and history in determining the geographic distribution of organisms. For the Neotropical lowlands, where temperatures are similar across landscapes, we hypothesize that water balance may be a critical but understudied factor in determining the distribution of species. Amphibians are especially sensitive to variation in precipitation due to their permeable skin. Here we focused on lowland frogs of northwestern South America and investigated variation among 17 species in potentially important ecologically relevant performance variables related to water balance, testing for possible adaptations to semiarid conditions within species. We studied frogs from coastal xeric, savannah, and wet forest biomes under common laboratory conditions and quantified rates of evaporative water loss, rates of water uptake, and variation in water-searching behavior and performance. We found significant differences in all three performance variables among species even after accounting for shared evolutionary history. A phylogenetic ANCOVA showed that categorizing species by ecological habit (terrestrial vs. arboreal) explained much of the ecoperformance trait variation among species. Secondly, environment explained additional variation; for example, coastal xeric species showed reduced rates of water loss, and terrestrial savannah amphibians showed lower rates of water uptake. Conspecific frog populations from different biomes exhibited similar performance. We compare our results with previous studies and conclude that ecological habit is the principle factor that

predicts ecophysiological trait variation and the possible geographic distribution of lowland Neotropical frogs.

*Keywords:* adaptation, amphibians, evaporative water loss, hydric biology, performance traits, water balance, water-finding ability, xeric habitat.

## Introduction

Many environmental factors can determine the distribution of species (Karr and Freemark 1983; Soberón and Nakamura 2009). Among these, temperature and humidity have direct effects on the ecological performance of individuals, so that combinations of thermal regimes and water availability potentially constrain the habitat available to a given species (Grace 1987; Lillywhite and Navas 2006). In this context, humidity includes all sources of available water, such as air or soil moisture, precipitation, and standing water. Specific adaptations to humidity depend on a species' natural history. Moisture gradients are known to drive the abundance and distribution of various lineages, and diversity generally declines as water becomes less available (Gaylard et al. 2003). Examples include plants (Grace 1987), birds (Smith et al. 2010), nematodes (Kennedy 1993), and, of course, amphibians (Scott 1976; Woinarski et al. 1999), a group in which geographical correlations between species richness and humidity could be hypothesized given their permeable skin. Relatively few studies, however, have focused on elucidating the functional traits that may underlie the limits to species' distributions (Cid et al. 2010; de Oliveira and Diniz-Filho 2010). Amphibians are particularly diverse in the wet tropical areas near the equator (Buckley and Jetz 2007), but assemblages of anuran species exist in different semiarid environments around the world (McClanahan et al. 1994). Furthermore, these amphibian assemblages may be divergent in their systematic composition and diverse in their natural history, and they may have adapted to semiarid conditions in multiple ways. Convergent adaptations—such as aestivation, for example—can involve fundamentally different physiological and ecological processes even when comparing syntopic species (Carvalho et al. 2010).

Anurans in semiarid environments must maintain water balance, yet a permeable skin is a fundamental aspect of their natural history (Bentley 1966; Toledo and Jared 1993). Therefore, water balance can be achieved through several ecological and physiological mechanisms that emphasize water uptake and water conservation (Lillywhite 2006; Titon et al. 2010), two approaches to maintaining water balance that likely have promoted evolutionary diversity. An enhanced ability to extract water from moist soil, as

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DOI: 10.1086/695705

opposed to standing water, may contribute to water balance in semiarid settings. Bentley (1966) reported the presence of pelvic patches of highly vascularized dermis around the ventral stomach and thigh areas that proved to be specialized areas enhancing water uptake (Roth 1973). Conversely, some anurans have evolved physiological and anatomical characteristics that allow them to minimize water loss (Withers et al. 1984), for example, via lipid secretion, a trait characterizing three genera of tree frog (Barbeau and Lillywhite 2005). In addition, some anurans avoid exposure to dry conditions by adopting fossorial habits (Toledo and Jared 1993), aestivating during the dry season (McClanahan et al. 1994), or remaining linked to humid refugia using specialized behaviors that may involve the ability to detect water (Navas et al. 2002). These diverse components of water balance are likely integrated and can be analyzed in the context of physiological attributes such as the water potential of plasma (Schmid 1965), overall blood flow (Adolph 1933, 1934), the presence of specialized skin patches (Braun 1951), and the density and variety of aquaporin proteins (Hasegawa et al. 2003). All of these variables may influence the total amount of liquid

water that may be absorbed per unit of time (Bentley et al. 1958) as well as the ability to extract water from the substrate in terms of water potential (Ruibal et al. 1969).

The purpose of this article is to enhance our understanding of the mechanisms allowing anurans to maintain water balance in semiarid environments and to integrate this information in the more general context of the geographic distribution of anurans. We contrast wet forest species with those from two Neotropical semiarid biomes of northwestern South America: the xeric coastal habitat (composed of seasonally dry tropical forests [Murphy and Lugo 1986], gallery forests, and open areas) and the savanna (plains, or *llanos*). These two biomes display low rainfall and marked seasonality in precipitation relative to the (Amazonian) wet forest (fig. 1; table A1). Neotropical wet forests are older than xeric habitats, which coalesced in the early Miocene (Graham 2012), suggesting that the ancestors of frogs from semiarid environments were denizens of wet forest. We quantify and analyze relevant water-related performance variables in the context of the following three hypotheses and predictions concerning water balance:

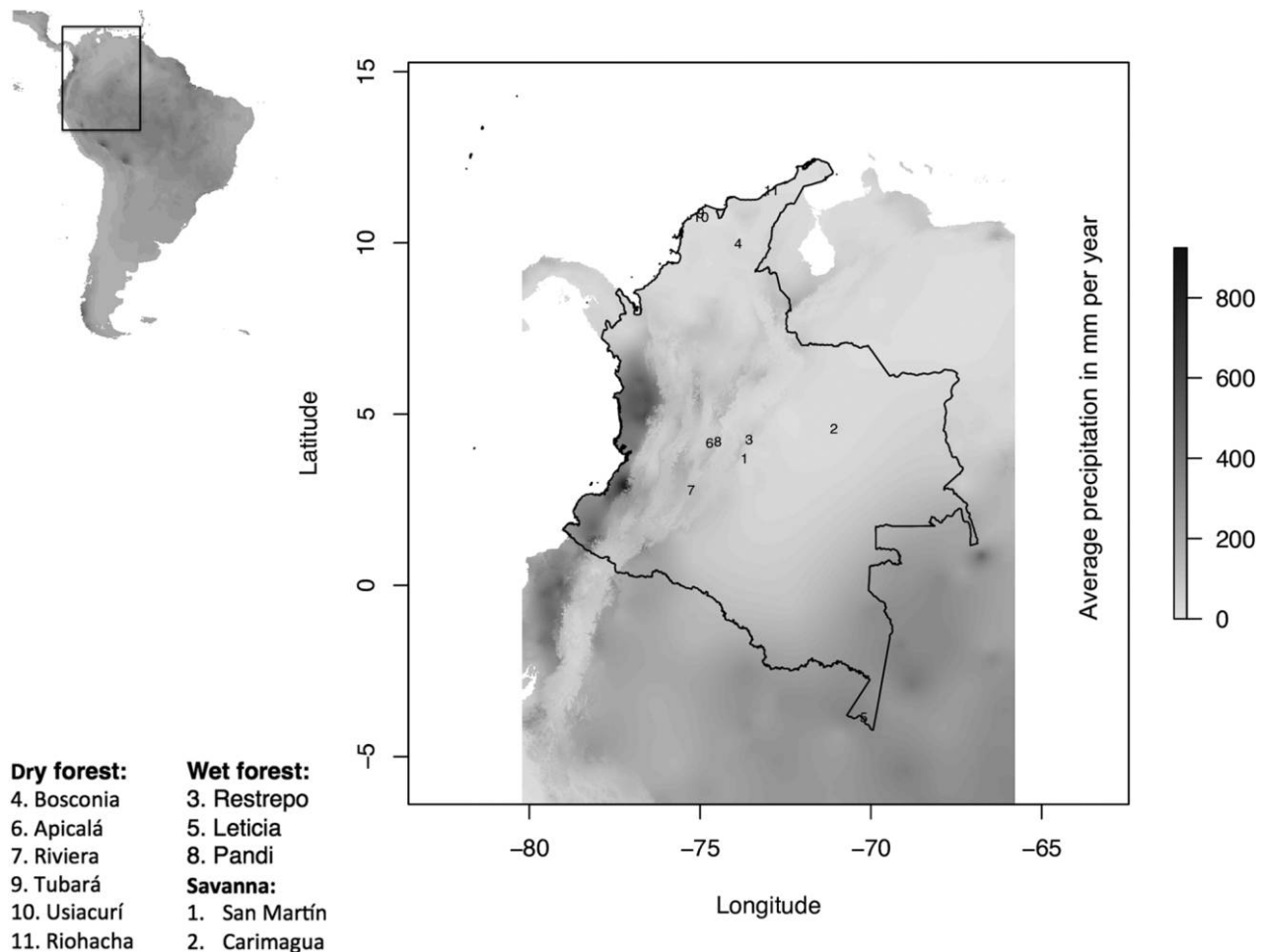


Figure 1. Map of annual precipitation (millimeters of rainfall per year) in northwestern South America, with the Colombia border outlined in black. Symbols indicate collecting localities. Names, geographic coordinates, and environmental variables for each locality are given in table A1.

1. *Water conservation hypothesis.* Frogs survive in semiarid (xeric coastal and savannah) habitats by minimizing water loss. Relative to wet forest species, semiarid species should have lower rates of evaporative water loss (EWL) through the dorsal skin.

2. *Water uptake efficiency hypothesis.* Frogs can survive in semiarid habitats through increased efficiency in taking up water through their ventral drink patch. Relative to wet forest species, semiarid frogs should have enhanced ability to take up water (WU), including from standing water and from moist substrates.

3. *Water-finding hypothesis.* Frogs can survive in semiarid environments through an increased ability to locate sources of water. Relative to wet forest species, frogs from semiarid biomes should show an enhanced ability to find water (AFW) in an experimental setup.

We test these hypotheses in arboreal and terrestrial species under the hypothesis that arboreal frogs may display enhanced water conservation (lower EWL or higher WU), since living in trees and other vegetation exposes arboreal frogs to dehydrating winds and other drying conditions relative to frogs with terrestrial habits (Young et al. 2005).

We collected performance data on 17 species of Colombian frogs (table 1) from 11 localities across our three biomes. We analyzed trait data under a phylogenetic framework and found that ecological habit (arboreal vs. terrestrial) is the principal factor that predicts variation in ecophysiological traits, with additional contribution from the type of habitat a species inhabits.

## Methods

### Ethical Considerations

All procedures were approved in September 2013 by the Institutional Committee on the Care and Use of Laboratory Ani-

mals (abbreviated CICUAL in Spanish) of the Universidad de los Andes, Bogotá. Study animals were handled in accordance with ethical guidelines for transportation, maintenance in captivity, and conducting experiments (Drummond 2009). Terraria were furnished with plants, water, and rocks for shelter. Frogs were fed crickets (*Gryllus* sp.), mealworms (*Tenebrio molitor*), and fruit flies ad lib. Captive study animals were not returned to the wild but were euthanized via an overdose of benzocaine anesthesia applied to the skin (Leary et al. 2013), and specimens were deposited in the Museo de Historia Natural ANDES (<http://museo.uniandes.edu.co/>), Universidad de los Andes, Bogotá.

### Field Sampling and Captive Conditions

Our study included two terrestrial toad species of the family Bufonidae (genus *Rhinella*), four terrestrial and semifossorial species of “ditch frog” of the family Leptodactylidae (genus *Leptodactylus*), three species of monkey frog in the family Phyllomedusidae (genus *Phyllomedusa*), and 11 arboreal species in the tree frog family Hylidae, comprised of three species of small yellow tree frog (*Dendropsophus* spp.), three species of gladiator frog (*Hypsiboas*), one species of slender-legged tree frog (*Osteocephalus planiceps*), and one snouted tree frog (*Scinax ruber*). These species were selected because of their distribution and high local abundance. We obtained live animals from 11 localities (table A1). Specimens were captured and transported to the Universidad de los Andes, Bogotá, in individual containers with water and vegetation.

Frogs were maintained in terraria in a heated room with a photoperiod of 0600 to 1730 hours. Temperature and humidity were recorded with data loggers. Temperature was set at 26°C, with a maximum recorded temperature of 28°C and a minimum of 24°C. Relative humidity was maintained around 75%, with a

Table 1: Summary of the 17 species collected for this study, with their taxonomic information, the type of environment they inhabit, and ecological habits (life history)

Species	Family	Type of habitat	Ecological habit
<i>Dendropsophus leali</i>	Hylidae	Wet forest	Arboreal
<i>Dendropsophus mathiassoni</i>	Hylidae	Savannah	Arboreal
<i>Dendropsophus microcephalus</i>	Hylidae	Coastal xeric	Arboreal
<i>Hypsiboas crepitans</i>	Hylidae	Coastal xeric and savannah	Arboreal
<i>Hypsiboas lanciformis</i>	Hylidae	Wet forest	Arboreal
<i>Hypsiboas pugnax</i>	Hylidae	Coastal xeric	Arboreal
<i>Leptodactylus colombiensis</i>	Leptodactylidae	Generalist (coastal xeric and savannah)	Terrestrial
<i>Leptodactylus fuscus</i>	Leptodactylidae	Generalist (coastal xeric and savannah)	Terrestrial
<i>Leptodactylus petersii</i>	Leptodactylidae	Wet forest	Terrestrial
<i>Leptodactylus poecilochilus</i>	Leptodactylidae	Coastal xeric	Terrestrial
<i>Osteocephalus planiceps</i>	Hylidae	Savannah	Arboreal
<i>Phyllomedusa bicolor</i> (juveniles)	Phyllomedusidae	Wet forest	Arboreal
<i>Phyllomedusa hypochondrialis</i>	Phyllomedusidae	Savannah (foothills)	Arboreal
<i>Phyllomedusa venusta</i>	Phyllomedusidae	Coastal xeric	Arboreal
<i>Rhinella humboldti</i>	Bufonidae	Generalist (coastal xeric and savannah)	Terrestrial
<i>Rhinella margaritifera</i>	Bufonidae	Wet forest	Terrestrial
<i>Scinax ruber</i>	Hylidae	Wet forest (but found more widely)	Arboreal

Note. For *P. bicolor*, we obtained reliable data only from juveniles. See the main text.

recorded maximum of 79% and a minimum of 71%. We fixed the maximum time in captivity at 2 mo, as captivity may alter the physiological performance of individual anurans (Navas and Gomes 2001). To achieve comparable acclimation among individuals to captive conditions, we tested all specimens after 1 wk in captivity. We observed all individuals carefully, and we used for experiments only those individuals that did not show any signs of atypical behavior. This was the case for all but two species. Adults of *Phyllomedusa bicolor* showed signs of distress under captive conditions, so data are reported here only for juveniles. A fourth species of gladiator frog, *Hypsiboas lanciformis*, appeared to be sensitive to captive conditions, so no data on this species are reported here. In total, we measured 137 individuals representing 17 species, seven genera, and three families.

#### Phylogenetic and Comparative Methods

Analysis of interspecific variation in performance traits (see below) should take into account the possible nonindependence of trait values due to common ancestry (Felsenstein 1985); thus, we required a time-calibrated phylogenetic hypothesis. For each study species plus the outgroup, *Elachistocleis ovalis* (Microhylidae), we obtained DNA sequence data from two mitochondrial gene fragments, ribosomal 16S and the 5' end of cytochrome oxidase subunit I (COI, the "barcode of life"; table A2). Primers and polymerase chain reaction conditions followed Crawford et al. (2013). Resulting sequences were assembled and trimmed using Sequencher (ver. 5.1; Gene Codes Corporation, Ann Arbor, MI). We used the software MUSCLE (ver. 3.7; Robert 2004) with default settings run on the web portal CIPRES (Miller et al. 2010) to align the 16S fragments. COI showed no length variation, and alignment was trivial after confirming amino acid translations in Mesquite (ver. 3.03; Maddison and Maddison 2015). We generated a relaxed-clock consensus time tree using the program BEAST (Drummond et al. 2012) with a birth-death incomplete sampling prior on trees (Stadler 2009). As our goal was not to reevaluate previous phylogenetic or temporal hypotheses using our two mitochondrial genes, we a priori assumed the topology and node ages consistent with Gomez-Mestre et al. (2012). The resulting time tree of our 17 species (removing the outgroup) was then assumed to be the true tree for performing phylogenetic size correction (Revell 2009) of dorsal surface area (for water loss analysis; see below) and ventral surface area (for water uptake analysis; see below) and for measuring phylogenetic signal using Blomberg's *K* (Blomberg et al. 2003) for traits related to finding water (see below), implemented in the R package phytools (Revell 2012) using R (ver. 3.2.0; R Core Team 2014).

#### Evaporative Water Loss

Measures of EWL were based on the weight loss of hydrated frogs relative to agar replicas. Each agar model mimicked the size and shape of each species and size class of frog, thus providing a null model for free-water flux via the surface of each frog. Frogs

showing an EWL rate lower than that of their agar counterpart suggests that the animal possesses some mechanism of reducing EWL relative to a free-water surface (Spotila and Berman 1976; Navas et al. 2002). For each species, we created six molds (except for *Phyllomedusa venusta* and *H. lanciformis*, for which only four were available per species), representing the smallest and largest size classes available and four individuals representing intermediate sizes, from formalin-fixed museum specimens preserved close to the water conservation posture. To create molds that better mimic the water conservation posture, the forelimbs and hind limbs of the specimens were tied with string to bring the limbs in closer to the body. This protocol worked well for all species of frog except hylids, whose forelimbs were relative large and hard to bring in close enough to mimic a live frog. We found that hylid models with no forelimbs were more similar to live hylids in the water conservation position, which tuck their limbs remarkably close to their bodies. Specimens were made available to us by the Museo de Historia Natural ANDES, Universidad de los Andes, Bogotá. Each specimen was placed on a flat base surrounded by a border of modeling clay to form a square with edges 4 cm high. We poured liquid alginate and waited until the hardening process completed. The alginate template was flipped and the specimen removed, leaving the shape of the frog specimen within. The alginate mold was subsequently filled with liquid agar (3 g of agar in 500 mL of water) and removed once solid, thus providing an agar cast of the original frog (Navas and Araujo 2000).

Because weight loss represents total EWL from the animal, we needed to calculate the surface area to obtain EWL per unit area. Some mass-to-area equations for anurans are available in the literature (e.g., McClanahan and Baldwin 1969). However, our 17 study species varied widely in shape, from robust toads to slender monkey frogs. Thus, we opted for measuring dorsal surface area directly. We defined dorsal surface as the area of exposed skin when the frog is in the water conservation posture, including the head, body, and the proximal part of each limb (brachia). To obtain the surface area, we removed the dorsal skin and placed it between a flat base and a piece of glass. We then photographed the skin and used ImageJ (Schneider et al. 2012) to calculate its total area. We measured each skin five times and used the mean value for all analyses.

We quantified EWL in an environmentally controlled room (see "Field Sampling and Captive Conditions" above) and followed the protocols of Titon et al. (2010) with some minor modifications. Before measuring EWL, we hydrated frogs for 10 min in plastic cups containing 2 cm of water. We then removed frogs, blotted them with a paper towel, induced bladder discharge by applying light pressure on the frog's ventral abdomen (Navas et al. 2002; Viborg et al. 2006), and measured initial body mass at 100% hydration. Next, we introduced the frog into its own wind tunnel and introduced the corresponding agar models based on the same species and of similar size (see above) into a separate wind tunnel. Most frogs were run simultaneously with their models, but in some cases we made models from the individual frog tested, in which case the EWL of the model was measured roughly 2 wk later. Airflow was maintained at a speed of 1.3 cm s<sup>-1</sup>. We reweighed the frog and model at 15, 30, 60, and 90 min after the

onset of testing or until the individual lost 30% of its fully hydrated weight (one exception was the genus *Leptodactylus*, which we dehydrated only to 20%, as beyond this point the animals showed signs of distress). Because activity during tests can enhance water evaporation rates, data were recorded only from frogs that remained inactive and in the water conservation posture during the experiment or that limited their movements to small adjustments in posture (Christian et al. 2017). If the frog changed location or orientation in the tunnel, the data were not included in our analyses. Including data from animals that made small changes in posture could increase the “noise” in our EWL measurements, and such error would make our task of detecting differences among species more difficult. Thus, if minor movements affected some EWL measurements, our statistical tests of among-species difference could be considered more conservative (lower type I error rate). For each individual frog or model, we calculated the rate of EWL from the slope of the curve of body mass per unit area against time ( $\text{g min}^{-1} \text{cm}^{-2}$ ). We tested for differences in the mean rate of EWL between frogs and agar models using a one-tailed randomization test with 1 million permutations.

We used phylogenetic comparative methods to explore whether variation in water balance variables was associated with habitat or ecological habits. We made a phylogenetic body size correction based on mass (Revell 2009) for EWL (and for water uptake rates; see below) using the package Phytools (Revell 2012) for the statistical analysis platform R (R Core Team 2014). As our EWL rates (and water uptake rates; see below) were already standardized by area, the phylogenetic size correction may reveal causes of interspecific trait variation apart from scaling factors (Revell 2009). The residuals were evaluated for normality and homogeneity of variance and used in a phylogenetic ANCOVA with type of habitat (wet forest, coastal xeric, or savannah) and ecological habit (arboreal vs. terrestrial) as categorical predictors. We refrained from analyzing habitat as a continuous variable (e.g., annual precipitation in millimeters) due to concerns about the lack of precise climatic data within poorly studied regions like the Colombian savannah, where weather stations are sparse to nonexistent. As our focus is on the challenge of water balance faced by adult frogs, we evaluated ecological habit, rather than reproductive mode or oviposition site, using the minimum number of unambiguous categories. We also compared rates of EWL (and water uptake rates, as defined below) among conspecific frog populations found in contrasting habitats (wet forest, coastal xeric, and savannah biomes) for three geographically widespread species, *Hypsiboas crepitans*, *Leptodactylus fuscus*, and *Rhinella humboldti*.

#### Water Uptake

Our estimate of water uptake was based on monitoring weight change in partially dehydrated frogs placed over a water source. To express this total value as a function of area, we required information on the ventral surface area of individual frogs, which we obtained by placing frogs on a glass surface, taking digital images of the ventral surface, calculating area using ImageJ, and

repeating this protocol five times. The forelimbs and feet were not included in this measurement. We then used the mean ventral area for all analyses. We measured water uptake in two types of trials. First, the maximum rate of standing water (1–2 cm depth) extracted per unit of time (WU) would correspond to, for example, an arboreal frog taking up rainwater from a bromeliad. Second, we measured the ability to uptake water from a moist paper towel (WUMT), which would correspond to, for example, a terrestrial frog extracting water from moist soil. Thus, we predicted these two performance measures would vary according to ecological habit (arboreal vs. terrestrial). Specifically, we expected terrestrial species to display similar values under both trials, whereas arboreal species would perform poorly in WUMT trials. The WU and EWL experiments (see above) occurred in sequence, so that the initial dehydration state for WU was the end point of the EWL test. WU and WUMT tests started with blotting the frog with a paper towel to remove any remaining excess water and then weighing it. To measure WU, we placed frogs in a pan with pure water 2 cm deep and then weighed frogs after 2, 5, 8, and 12 min, in sequence. WUMT was measured similarly, except a damp paper towel was the only source of water. All experiments took place in a room with controlled temperature and relative humidity (see “Field Sampling and Captive Conditions” above). We calculated WU and WUMT rates from the slope of the increasing body mass per unit area over time ( $\text{g min}^{-1} \text{cm}^{-2}$ ) for each individual. Area was calculated as ventral surface area (see above) assuming that absorption rates through nonventral surfaces are negligible relative to the ventral surface (McClanahan and Baldwin 1969). To test the null hypothesis that the difference between rates of WU and WUMT were zero within each species, we performed a randomization test with 1 million permutations. The species *Leptodactylus colombiensis* and *O. planiceps* lacked data on WUMT.

To explore whether the difference in WUMT and WU within species was related to habitat (wet forest, coastal xeric, or savannah) and ecological habit (terrestrial or arboreal), we created a new variable called the difference in water uptake (DWU) as the difference in phylogenetically size-corrected (see above) variables WU and WUMT. Subsequent statistical and comparative phylogenetic analyses were performed as with EWL rates.

Whatever anatomical or physiological adaptations prevent water loss dorsally could, in theory, have correlated effects than impede water uptake ventrally. This hypothesis predicts a trade-off between EWL and WU, that is, a positive correlation between rates of dorsal water loss and ventral water uptake. We evaluated this prediction via the correlation coefficient between phylogenetic residuals of EWL and DWU.

#### Ability to Find Water

To assess whether amphibians from semiarid biomes and the wet biome possess different abilities to find water, we followed the experimental design and protocols in Maia (2014). We conducted the following behavioral trials on 89 frogs representing 10 species. The experiments took place in an environmentally

controlled room (see “Field Sampling and Captive Conditions” above). We tested partially dehydrated frogs to insure they were motivated to find water. Dehydration was limited to a decrease of 5% in the frog’s fully hydrated body mass, as greater dehydration may negatively affect their locomotion (Titon et al. 2010). A labyrinth was made of Plexiglas and shaped like an eight-armed asterisk, with walls 10 cm high and a central arena 35 cm in diameter from which the eight arms radiated outward, with each arm being 13 cm long and 10 cm wide. Before each test, we placed 5 × 5-cm pieces of wadded paper towel at the end of each arm and randomly selected one to be previously soaked in water. On the basis of Maia (2014), we quantified two variables, “time to find water,” measured in seconds from starting the experiment to reaching the one moistened paper towel, and “number of arms visited before finding water.” For each test, a frog was introduced into the middle of the central arena with an arbitrary initial orientation. We started the experiment at 1800 hours and filmed for 5 h with an infrared Bell and Howell camcorder located 1.60 m above the arena. In the morning, we also noted in which arm the frog was resting.

We used Blomberg’s *K* (Blomberg et al. 2003) to test for phylogenetic signal in “time to find water” and “number of arms visited before finding water.” Because of their small size and the limited sensitivity of our infrared video, we could not digitally record species in three hylid genera: *Dendropsophus* (*D. microcephalus*, *D. mathiassoni*, *D. leali*), *Phyllomedusa* (*P. hypochondrialis*, *P. bicolor* [juveniles]), or *Scinax* (*S. ruber*).

To test the null hypothesis that frogs possessed no ability to detect water, we performed two statistical tests. First, we applied a simple binomial test assuming a probability of success of 1/8 (given that the arena had eight arms). Second, we tested for significant variation in AFW within the genera *Hypsiboas*, *Rhinella*, and *Leptodactylus* using ANCOVAs. We performed one ANCOVA for each genus, using species and number of arms as independent variables and “time to find water” as the dependent variable.

## Results

Summaries of raw ecophysiological performance data by species, along with data on body mass, surface area, and phylogenetically corrected performance and morphological data, are available in the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.jm838>; Cruz-Piedrahita et al. 2017).

### Evaporative Water Loss

EWL rates did not differ significantly between frogs and models in the arboreal species (hylids) *Dendropsophus leali*, *Hypsiboas lanciformis*, *Osteocephalus planiceps*, *Phyllomedusa bicolor*, and *Scinax ruber* or in the terrestrial species *Leptodactylus colombiensis*, *Leptodactylus petersii*, *Leptodactylus fuscus*, *Rhinella humboldti*, and *Rhinella margaritifera* (one-tailed randomization test:  $P > 0.05$  in all cases; fig. 2). Frogs displayed lower rates of EWL than their models in the arboreal species *Dendropsophus mathiassoni*, *Dendropsophus microcephalus*, *Hypsiboas crepitans*, *Hy-*

*psiboas pugnax*, *Phyllomedusa hypochondrialis*, and *Phyllomedusa venusta* and in the terrestrial species *Leptodactylus poecilochilus* (one-tailed randomization test:  $P < 0.05$  in all cases; fig. 2).

Analysis of phylogenetically size-corrected EWL rates showed that ecological habit (arboreal vs. terrestrial species) is the factor that best explained variation among species (ANCOVA:  $df = 1$ ,  $F = 12.11$ ,  $P < 0.0001$ ,  $n = 137$ , number of species = 17; fig. 3). The next most important term was the interaction between ecological habit and habitat (coastal xeric biome, savannah, and wet forest), but this was nonsignificant at the  $\alpha = 0.05$  level (ANCOVA:  $df = 2$ ,  $F = 2.58$ ,  $P = 0.079$ ,  $n = 137$ , number of species = 17). We found significant variation in the rate of EWL among species within the genera *Hypsiboas* (ANOVA:  $df = 2$ ,  $F = 12.11$ ,  $P < 0.0001$ ,  $n = 18$ , number of species = 3) and *Leptodactylus* (ANOVA:  $df = 3$ ,  $F = 14.57$ ,  $P < 0.0001$ ,  $n = 29$ , number of species = 4). We also found significant variation in the rate of EWL among species and among types of habitat within *Phyllomedusa* (ANOVA:  $df = 2$ ,  $F = 97.14$ ,  $P < 0.0001$ ,  $n = 11$ , number of species = 3). We did not find significant differences among conspecific populations of *H. crepitans*, *L. fuscus*, and *R. humboldti* located in distinct biomes (wet forest, coastal xeric habitat, and savannah).

### Water Uptake

We found that five of six terrestrial species absorbed water at similar rates from standing water (WU) compared with a moist paper towel (WUMT;  $P > 0.05$ ); the exception was *L. petersii*, which showed a higher WU rate (one-tailed randomization test:  $P = 0.004$ ,  $n = 6$ ; fig. 4). In contrast, 8 of 11 arboreal species showed faster WU than WUMT rates (one-tailed randomization test:  $P < 0.05$  for all), with the three exceptions being *D. microcephalus* (one-tailed randomization test:  $P = 0.308$ ,  $n = 6$ ) and *P. venusta* (one-tailed randomization test:  $P = 0.257$ ,  $n = 3$ ) from the coastal xeric habitat and *D. mathiassoni* from the savannah (one-tailed randomization test:  $P = 0.2974$ ,  $n = 3$ ). All subsequent analyses were performed on the phylogenetically size-corrected differences in water uptake values (DWU; see “Methods”). Ecological habit (terrestrial vs. arboreal) was the factor that best explained among-species variation in the DWU rate (ANCOVA:  $df = 1$ ,  $F = 7.34$ ,  $P = 0.008$ ,  $n = 133$ , number of species = 15; fig. 5). We found significant differences in DWU among species of the genus *Dendropsophus* (ANOVA:  $df = 2$ ,  $F = 4.71$ ,  $P = 0.025$ ,  $n = 19$ , number of species = 3) and of the genus *Phyllomedusa* (ANOVA:  $df = 2$ ,  $F = 4.67$ ,  $P = 0.045$ ,  $n = 9$ , number of species = 3); in both cases, the coastal xeric species showed a DWU closer to zero compared with savannah and wet forest species. We found a significant difference in the DWU rate among populations within the arboreal *H. crepitans* (ANOVA:  $df = 2$ ,  $F = 7.77$ ,  $P = 0.005$ ,  $n = 18$ , number of populations = 3); that is, *H. crepitans* from semiarid sites had lower residuals, implying they were equally efficient at WU and WUMT, similar to terrestrial frogs. Finally, we found that the phylogenetic residuals for EWL and DWU were positively related (Pearson’s correlation:  $df = 13$ ,  $P = 0.038$ ,  $R^2 = 0.255$ ), as predicted by the trade-off hypothesis.

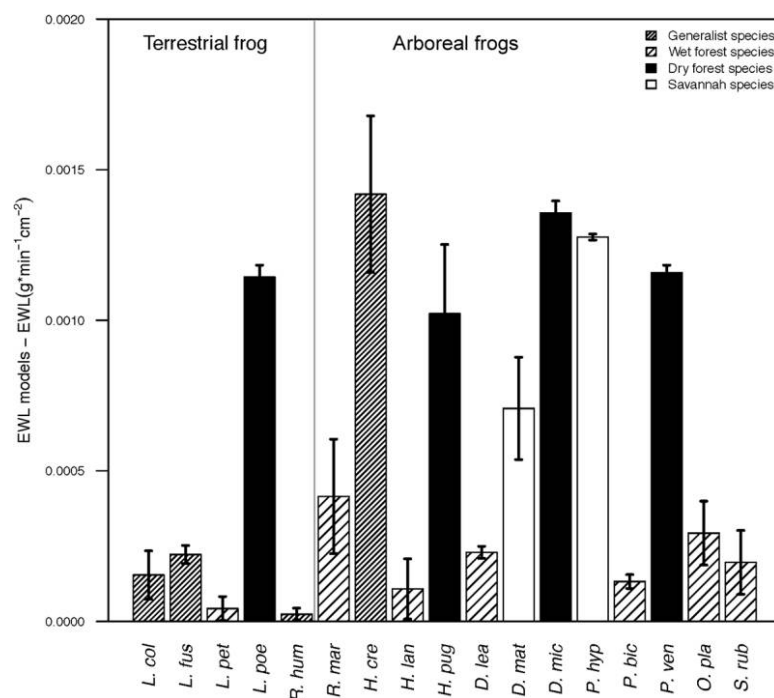


Figure 2. Difference in evaporative water loss (EWL) rates ( $\text{g min}^{-1} \text{cm}^{-2}$ ) for 17 anuran species versus their corresponding agar models: *Leptodactylus colombiensis* ( $n = 3$ ), *Leptodactylus petersii* ( $n = 6$ ), *Leptodactylus fuscus* ( $n = 15$ ), *Leptodactylus poecilochilus* ( $n = 6$ ), *Rhinella humboldti* ( $n = 26$ ), *R. margaritifera* ( $n = 6$ ), *Hypsiboas crepitans* ( $n = 20$ ), *Hypsiboas lanciformis* ( $n = 4$ ), *Hypsiboas pugnax* ( $n = 6$ ), *Dendropsophus leali* ( $n = 8$ ), *Dendropsophus mathiassoni* ( $n = 6$ ), *Dendropsophus microcephalus* ( $n = 5$ ), *Phyllomedusa bicolor* ( $n = 4$ ), *Phyllomedusa hypochondrialis* ( $n = 4$ ), *Phyllomedusa venusta* ( $n = 3$ ), *Osteocephalus planiceps* ( $n = 6$ ), and *Scinax ruber* ( $n = 6$ ). Each EWL measurement compares one frog against one agar model of approximately the same size and shape (see “Methods” for details). Species are organized by ecological habit with terrestrial frogs on the left and arboreal frogs on the right. The fill of each bar indicates the type of environment in which the species was collected, where “dry forest” refers to coastal xeric habitat and “generalist” means that the species was collected in both savannah and coastal xeric habitats.

### Ability to Find Water

In the AFW trials, frogs clearly did not end up in a random arm of the arena (binomial test:  $X = 87$ ,  $n = 92$ , estimated probability of success  $p = 0.884$ ,  $P < 0.0001$ ) but tended to find the arm with the water (wet paper towel) after exploring the labyrinth to diverse extents. We found relatively low phylogenetic signal for “time to find water” (Blomberg’s  $K = 0.3649$ ) and “number of arms visited before finding water” (Blomberg’s  $K = 0.4918$ ). The average elapsed time before frogs found the water was significantly correlated with habitat (phylogenetic ANOVA:  $df = 2$ ,  $F = 5.71$ ,  $P = 0.005$ ,  $n = 92$ ) and with life history (phylogenetic ANOVA:  $df = 1$ ,  $F = 4.44$ ,  $P = 0.038$ ,  $n = 92$ ), such that frogs from semiarid environments found water more quickly than their wet counterparts and terrestrial species found water more quickly than arboreal species (fig. 6). For the variable “number of arms visited before finding water,” life history was the only significant factor (ANOVA:  $df = 1$ ,  $F = 22.19$ ,  $P < 0.0001$ ,  $n = 92$ ): arboreal species tended to visit fewer arms than terrestrial ones (fig. 7).

### Discussion

We explored variation in potential adaptations to semiarid environments among 17 species of lowland South American

frogs. Our goals were first to establish whether there was variation among species in three ecophysiological traits relevant to water balance (EWL, WU, and AFW) and second to investigate whether variation among species in these traits was predictable from taxonomy, ecological habit (arboreal vs. terrestrial), or habitat (coastal xeric habitats, wet forest, and savannah) of each species or population. Our overarching question was whether any of these three characteristics reflect an adaptive signal to overcoming the hydric challenges of surviving in dry environments from the perspective of our three dimensions of the water balance process. To answer this question, we compared the ability of species from contrasting biomes to avoid water loss, to absorb water, and to find water. Certainly, other physiological or behavioral adaptations could also play an important role in surviving in semiarid habitats. Looking at only these three ecomorphological variables, however, we found wide variation among species along with clear patterns in terms of life history and environment, even after taking into account their shared evolutionary histories. Each ecophysiological trait reveals clues about potential adaptations to semiarid conditions, and together they begin to provide a richer understanding of how the hydric biology of tropical frogs may interact with environmental variables in determining species’ distributions.

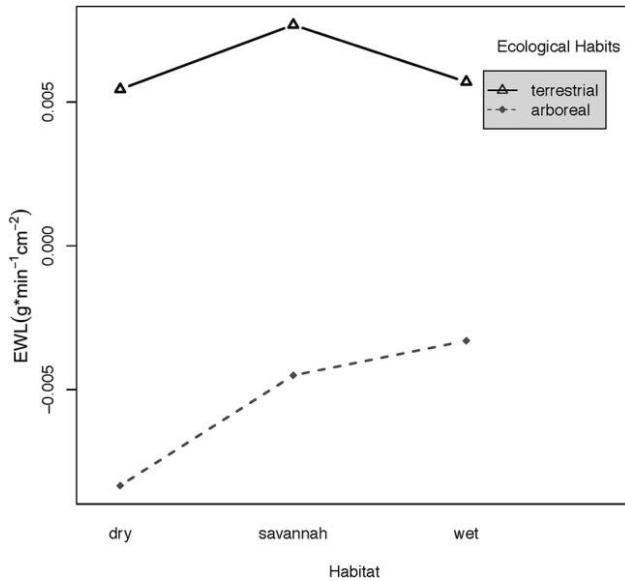


Figure 3. Variation in mean phylogenetic residuals of evaporative water loss (EWL) rates ( $\text{g min}^{-1} \text{cm}^{-2}$ ) among anuran species ( $n = 137$  individuals) partitioned by ecological habit (terrestrial vs. arboreal habits) and habitat (populations found in coastal xeric ["dry"], savannah, or wet forest). Ecological habit significantly affects the rate of EWL (ANCOVA:  $F = 12.11$ ,  $df = 1$ ,  $P < 0.0001$ ,  $n = 137$ , number of species = 17), with terrestrial species losing water faster than arboreal species. The interaction between ecological habit (arboreal vs. terrestrial) and habitat (coastal xeric biome, savannah, or wet forest) was nonsignificant at the  $\alpha = 0.05$  level ( $P = 0.0793$ ), as was habitat ( $P > 0.1$ ).

#### Water Conservation Hypothesis

Our findings reveal that life history predicts much of the variation in rates of EWL, even more so than habitat type. Previous work has suggested that species with arboreal habits generally have lower rates of EWL relative to terrestrial or semifossorial species (Wygoda 1984; Young et al. 2005; Titon and Gomes 2015). Our results support this trend, as five of the six terrestrial species showed EWL rates that were statistically indistinguishable from that of their paired agar models (fig. 2), while six of the 11 species with arboreal habits had significantly lower EWL than their agar models. This enhanced ability to retain water is likely associated with the windy and drying conditions of canopy environments frequented by arboreal species (Scheffers et al. 2013a, 2013b). Thus, even relatively small hylid frogs, such as species of *Dendropsophus*, possess comparatively lower rates of water loss despite their high area-to-volume ratio. The five remaining arboreal species that showed no apparent adaptations to resist water loss were all from wet habitats, such as the Amazonian forest (fig. 2).

After accounting for life-history variation, we find that habitat may still play a role in explaining rates of EWL, especially among arboreal species (fig. 3). Congeneric species tend to have similar rates of EWL; hence, the importance of a phylogenetic size correction before conducting among-species comparisons (Garland et al. 1993; Revell 2009). Our data show that all species that in-

habit coastal xeric and savannah habitats have a lower EWL rate than their congeners found in humid forest. These results support our first hypothesis that water conservation is an important factor in colonizing or surviving in semiarid Neotropical environments, although the effect of habitat is secondary to life-history considerations.

In this study, we used agar models as a null model of free-water evaporation to provide a common point of reference for comparison among frog species despite potential variation in physicochemical contexts among laboratory experiments. This approach requires two cautions: (1) agar models are not expected to display physicochemical properties identical to those of frogs and (2) shape identity with frogs is necessary, yet making agar models match perfectly real frogs in the water-conserving posture is challenging, especially for the forelimbs. Furthermore, frogs do have various physiological mechanisms to regulate water balance, and those influence water evaporation rates and perceived resistance, particularly in association with temperature regulation (Lillywhite 2006). Riddell et al. (2017) criticized the use of agar models for estimating EWL, preferring biophysical calculations of skin resistance to water loss. Christian et al. (2017) argued, however, that the results of Riddell et al. (2017) could potentially be explained by experimental error while also emphasizing the importance of measuring EWL from inactive amphibians. We assume that individual physiological adjustments are not accounted for by any such methods and use agar models under the reasonable assertion that slight variations in agar size or shape or minor modifications in posture are unlikely to severely affect measurements of EWL (Christian et al. 2017). However, significant changes in posture during the performance trial could compromise an experiment by overestimating water evaporation (Christian et al. 2017). In the present study, the size and shape of agar models were matched reasonably well to frogs, and movements were closely monitored during experiments. In this study, such minor sources of measurement error could not be eliminated completely, but our results should be interpreted not as an attempt to make precision estimates of resistance to water loss but as a valuable tool for revealing interpopulation and interspecific differences in EWL.

The development of strategies such as the water conservation posture (Prates and Navas 2009), coossified skin (Jørgensen 1997), or specialized glands in the skin (Barbeau and Lillywhite 2005) may result from environmental pressure related to the lack of water in semiarid environments (Bentley 1966). Despite the fact that all semiarid environment species have lower evaporative rates than wet forest species of the same genus, almost all terrestrial species lose water at higher rates; thus, life history is a key factor when comparing across all frogs. The only terrestrial species that showed enhanced resistance to EWL was *Leptodactylus poecilochilus*, which is endemic to the coastal xeric biome, suggesting that reduced EWL through the dorsal skin is likely an adaptive trait in this species (fig. 2). For the rest of the terrestrial species, other strategies—such as water uptake (Titon et al. 2010), microhabitat selection (Prates and Navas 2009), and aestivation (Amey and Grigg 1995; Vidal-García et al. 2013)—may be more



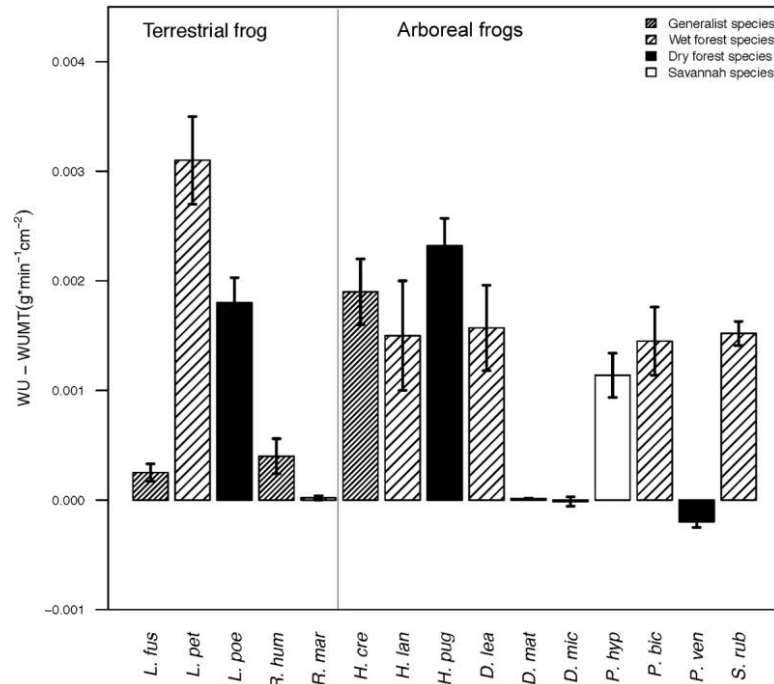


Figure 4. Difference in water uptake (DWU) rates between performance trials involving standing water (WU;  $\text{g min}^{-1} \text{cm}^{-2}$ ) versus a moist paper towel (WUMT;  $\text{g min}^{-1} \text{cm}^{-2}$ ) for 15 anuran species: *Leptodactylus petersii* ( $n = 6$ ), *Leptodactylus fuscus* ( $n = 15$ ), *Leptodactylus poecilochilus* ( $n = 6$ ), *Rhinella humboldti* ( $n = 26$ ), *Rhinella margaritifera* ( $n = 9$ ), *Hypsiboas crepitans* ( $n = 20$ ), *Hypsiboas lanciformis* ( $n = 4$ ), *Hypsiboas pugnax* ( $n = 6$ ), *Dendropsophus leali* ( $n = 8$ ), *Dendropsophus mathiassoni* ( $n = 6$ ), *Dendropsophus microcephalus* ( $n = 5$ ), *Phyllomedusa bicolor* ( $n = 4$ ), *Phyllomedusa hypochondrialis* ( $n = 4$ ), *Phyllomedusa venusta* ( $n = 3$ ), and *Scinax ruber* ( $n = 6$ ). DWU = WU - WUMT. Species are organized by ecological habit, with terrestrial frogs on the left and arboreal frogs on the right. The fill of each bar indicates the type of environment in which the species was collected, where “dry forest” refers to coastal xeric habitat and “generalist” means the species was collected in both savannah and coastal xeric habitats.

important to survival in xeric environments (Prates and Navas 2009).

Lower EWL rates in arboreal species relative to terrestrial species may have been an exaptation that originally evolved as a response to the extreme drying conditions of the forest canopy and subsequently allowed some hylid frogs to colonize xeric environments, such as seasonally dry tropical forests (Scheffers et al. 2013a, 2013b, 2014). Hylids are far more diverse in the Amazonian wet forest (Duellman 1999), however, which suggests that additional ecophysiological factors may exclude some arboreal frogs from semiarid habitats (e.g., hylids performed relatively poorly in water uptake; see below). Thus, reduced rates of EWL per unit area (figs. 2, 3) may be an adaptation to xeric environments. If so, dorsal water loss could be a limiting factor in determining species’ range limits that drive, in part, larger biogeographic patterns.

Two species showed an opposite pattern from our predictions and the general trends observed. First, we found that juveniles of *Phyllomedusa bicolor* lose water at the same rate as their model (fig. 2), which is surprising since members of this genus are remarkable for their wax-producing glands that minimize EWL dorsally. Perhaps *P. bicolor* is an interesting exception, or perhaps juvenile *Phyllomedusa* lack functional xeric glands. Ontogenetic changes in ecomorphological variables con-

stitute and important avenue of further research, as all life stages need to survive, of course, in any given environment. Second, we found that the arboreal habitat generalist *Scinax ruber* loses water at the same rate as its agar model. This species occurs in all three biomes studied here yet was the only generalist species to lose water at the same rate as the model. However, all individuals tested here were collected in the Amazonian wet forest locality of Leticia (table A1), and genetic evidence suggests that this ostensibly widespread taxon is actually a complex of species (Guarnizo et al. 2015). Therefore, a second challenge going forward would be obtaining ecomorphological data from more localities within more nominal species. An additional factor that could affect our results is seasonal variation in performance; for example, EWL rates are known to vary between wet and dry seasons in the African reed frog *Hyperolius viridiflavus* (Kobelt and Linsenmair 1986).

#### Water Uptake Efficiency Hypothesis

In general, terrestrial species show better water-absorbing performance than arboreal species (figs. 4, 5). Beuchat et al. (1984) reported that terrestrial species are generally better at absorbing water than species with aquatic habits, although a comparison between terrestrial and arboreal species has not been reported

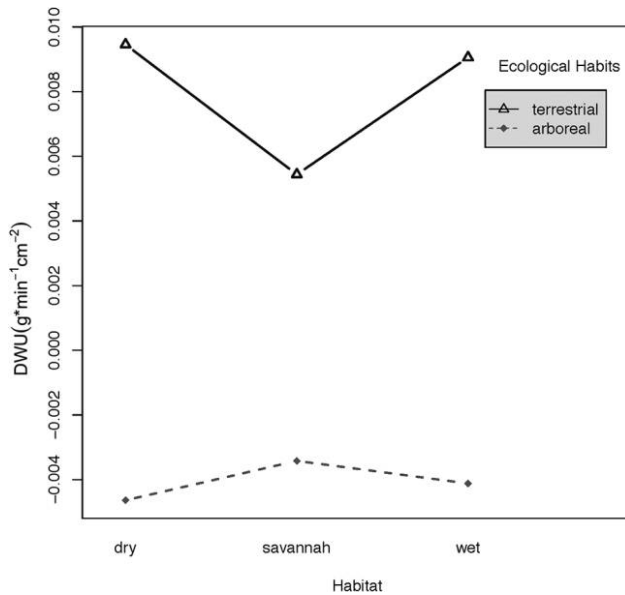


Figure 5. Phylogenetic residuals of differences in water uptake (DWU) rates ( $\text{g min}^{-1} \text{cm}^{-2}$ ) among 15 South American frog species, according to ecological habit (terrestrial or arboreal life history) and habitat (coastal xeric biome, savannah, or wet forest). Ecological habit significantly predicts the rate of water uptake minus water uptake from a paper towel (ANCOVA:  $df = 1$ ,  $F = 7.34$ ,  $P = 0.008$ ,  $n = 133$ , number of species = 15). Arboreal species tend to have positive DWU because the rate of absorption in standing water is higher than uptake rates from a moist paper towel.

previously in literature. However, two species of the arboreal genus *Hypsiboas*, *H. crepitans* and *H. pugnax*, that are endemic to coastal xeric and savannah habitats show rates of water uptake similar to those of terrestrial species. Five of the six terrestrial species can absorb water from a moist surface as quickly as from standing water, which we interpret as evidence of an adaptation to obtaining water via uptake from humid substrates. Toads in particular have long been noted for their ability to extract water from moist substrates, for example, toads of the southern part of Lake Michigan absorbing water from moist sand (Stille 1952; McClanahan and Baldwin 1969). Titon and Gomes (2015) report the presence of a more vascularized area in the ventral region of toads from semiarid habitats in Brazil, suggesting that this may be a morphological adaptation for increasing blood flow to the inguinal patch and thereby taking up more water. For most arboreal species, the rate of water absorption from a moist towel (WUMT) drops considerably relative to that from standing water (WU), with three notable exceptions to this trend: *Phyllomedusa venusta*, *Dendropsophus microcephalus*, and *Dendropsophus mathiassoni*. The first two are endemic to coastal xeric environments, while the latter is endemic to the savannah. While most arboreal species appear to rely on water conservation, we hypothesize that for these three arboreal species the enhanced ability to take water from a moist surface is an additional adaptation to the harsh, semiarid conditions in which they live.

Comparing across species, we found that the phylogenetic residuals for DWU and EWL were positively correlated, suggesting the existence of a trade-off between ventral uptake (DWU) and dorsal loss (EWL). While the dorsal and ventral skin are known to have distinctive gross morphologies, their basic ultrastructural components are the same (Goniakowska-Witalińska and Kubiczek 1998). We hypothesize that there may be developmental or physiological constraints preventing the simultaneous evolution of less permeable skin dorsally and more permeable skin ventrally, and this trade-off can be observed in a comparative context. Per our trade-off hypothesis, avoiding water loss—for example, as in hylid frogs (Wygoda 1984; Young et al. 2005)—would come at a cost of reduced WU efficiency, perhaps requiring standing water in order to rehydrate. In contrast, toads (*Rhinella*) showed the highest WU and WUMT rates, a likely adaptation to terrestrial, xeric environments (Viborg et al. 2006), but toads also showed EWL rates that were among the highest (fig. 2), supporting the trade-off hypothesis. We found that leptodactylids can also absorb water well from either type of surface and also have high EWL rates (comparable to their agar models, except for the coastal xeric endemic *L. poecilochilus*; fig. 2). The xeric arboreal species *H. crepitans* and *H. pugnax* showed high rates of WU but performed poorly in the WUMT trials. The difference between these two performance traits should indicate that while these species may inhabit dry environments, their distribution within these habitats may be restricted to sites with access to standing water.

Looking at within-species variation, conspecific populations of the terrestrial species *Rhinella humboldti* and *Leptodactylus fuscus* from wet environments absorbed water more slowly than

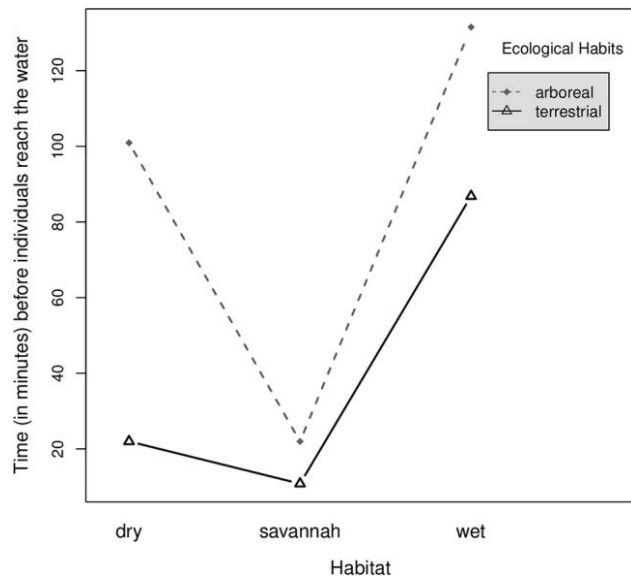


Figure 6. Average time elapsed for individuals ( $n = 92$ ) to find water (min) according to habitat (coastal xeric biome, savannah, or wet forest) and ecological habit (arboreal vs. terrestrial life history). The average elapsed time was significantly correlated with habitat (ANOVA:  $P = 0.005$ ) and ecological habit (ANCOVA:  $P = 0.038$ ).

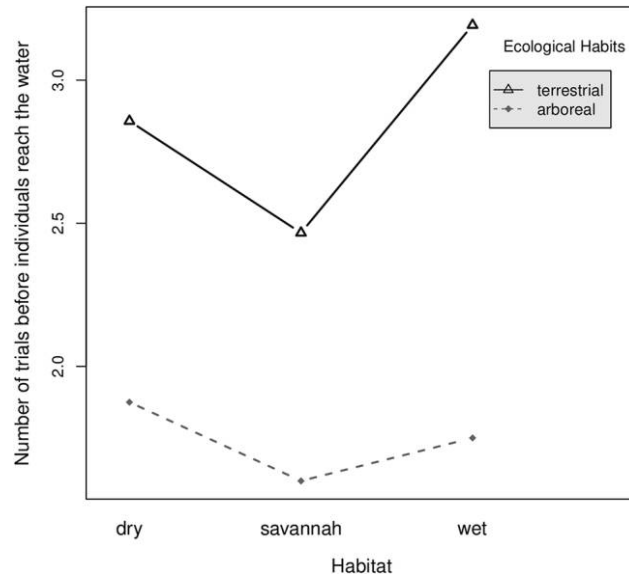


Figure 7. Number of arms visited on average before individuals reached water, according to habitat (coastal xeric biome, savannah, or wet forest) and ecological habit (arboreal vs. terrestrial frogs). The average number of arms visited was significantly predicted by ecological habit (ANCOVA:  $P < 0.0001$ ) but not by habitat ( $P > 0.05$ ).

populations from drier environments, although the differences were not statistically significant. Conspecific populations of the arboreal species *H. crepitans* from semiarid sites were equally efficient at WU and WUMT, similar to terrestrial frogs, implying that they may show local adaptation to absorbing water in a terrestrial environment. In WU trials, however, individuals from wet environments absorbed water more readily than populations from drier environments, that is, in the opposite direction from what we predicted. This unexpected result could perhaps be due to the hypothesized trade-off between the ability to retain versus absorb water, yet we found no evidence of reduced EWL in xeric versus wet-forest populations of *H. crepitans*. Thus, conspecific variation in eperformance traits requires further study.

#### Water-Finding Hypothesis (AFW)

The phylogenetic signal was weaker for traits related to detecting water, elapsed time, and number of arms visited relative to EWL and WU measures. As in the case of EWL and WU, AFW is explained primarily by the ecological habits of the species and secondarily by the type of habitat. For this trait, two contrasting strategies are observed. Terrestrial species (with the one exception of the wet-forest toad *Rhinella margaritifera*) visited several arms in a short period of time, while arboreal species (with the one exception of the Amazonian hylid *Osteocephalus planiceps*) waited a long time in the center of the radial design before moving, and they visited fewer arms (between one and three arms before reaching water). Arboreal species may be more

efficient at finding water, investing less energy despite requiring more time. We saw during our field trips that individuals with terrestrial habits were found during the dry season in patches of moist soil, suggesting that these species may sense water, although finding water may be the result of random search behavior. Frogs have at least two olfactory systems (Nowack 2011) and could potentially smell compounds associated with bodies of water. Toads in particular might sense humidity gradients using receptors located in their ventral skin, and further experiments could test this hypothesis. We note, however, that our experimental chamber contained only clean water and no humidity gradient.

#### Conclusions

We observed that maintaining, absorbing, and finding water are potentially important adaptations in the colonization and survival of frogs in xeric habitats. Perhaps the strongest signal for the importance of habitat came from our EWL experiments. Among our 11 arboreal species, for example, only those six species native to semiarid habitats showed significantly lower EWL than their agar models, whereas the five Amazonian species showed none. We found that water uptake rates may be an important factor in determining the presence of species in xeric habitats, but the relationship is less clear. Among five terrestrial species, only the Amazonian *Leptodactylus petersi* apparently needs liquid water to hydrate properly. Among 10 arboreal species, the only three species that absorbed water at similar rates in both trials (standing water and moist towel) were xeric habitat species. However, an additional three xeric species showed higher uptake rates in standing water (WU), highlighting a diversity of responses among species to a common environmental challenge. Arboreal frogs are able to maintain water (low EWL), are generally better at absorbing water from a standing source, and are perhaps better at detecting water. Terrestrial frogs do not resist water loss as well but seem adapted for faster water uptake, and they perhaps find water by scanning the substrate following a moisture gradient, although we were not able to further test this latter hypothesis. While some species showed intraspecific variation in performance traits in the direction predicted by habitat, more detailed studies are called for.

Considering recent concerns about the effect of global climate change on the distribution and survival of natural populations, we find that hydric biology has received little attention relative to thermal biology (but see, e.g., Tracy et al. 2014; Titon and Gomes 2015). Global average temperatures are increasing, although some uncertainty exists as to how fast it will increase in which environments. In contrast, global precipitation may change in drastic yet difficult-to-predict ways, due to spatially heterogeneous responses across the globe—that is, some regions may experience marked aridification or increased seasonality in precipitation, while other areas may experience increased rainfall or extreme weather events. Plans to mitigate the effects of changes in rainfall on animal populations will require a deeper understanding of hydric biology, especially concerning how animals may adapt—or fail to adapt—to semiarid conditions. This preliminary study of potential

adaptations in South American frogs to improve water balance under xeric conditions has revealed a variety of contrasting strategies among species, not fully predictable from their phylogenetic relationships. This study has also revealed some significant patterns, however, that will help inform predictions of how tropical frogs may respond to impending global changes in rainfall patterns.

### Acknowledgments

We thank Juan Manuel Renjifo and Juan Salvador Mendoza for help in developing some of the initial ideas that went into this project. Diego Gomez, Diana Stasiukynas, and Ángela Suárez-Mayorga provided substantial help during fieldwork, and numerous undergraduate volunteers helped maintain live frogs, create agar models, and measure frog area. Thanks to the CIMPAT (Centro de Investigaciones en Microbiología y Parasitología Tropical) laboratory at the Universidad de los Andes, Bogotá, for allowing us to use its environmental chamber. Gabrielle Genty provided valuable assistance with obtaining DNA sequence data. All animal experimentation procedures were approved in September 2013 by the Institutional Committee on the Care and

Use of Laboratory Animals (abbreviated CICUAL in Spanish) of the Universidad de los Andes. Research and collecting permission was provided by the Autoridad Nacional de Licencias Ambientales (ANLA) de Colombia (permiso de estudio con fines de investigación científica en diversidad biológica N° 27 de 22 de junio de 2012 and permiso de acceso a los recursos genéticos resolución N° 0377 de 11 marzo 2014 to A.J.C. plus permiso marco resolución N° 1177 to the Universidad de los Andes). Additional samples were provided by the Museo de Historia Natural ANDES, Universidad de los Andes. Financial support was provided by the Departamento Administrativo de Ciencia, Tecnología e Innovación (Colciencias), Programa Nacional en Ciencias Básicas, award 120456934310 to A.J.C. and by a Proyecto Semilla grant from the Facultad de Ciencias, Universidad de los Andes, to C.C.-P. and A.J.C. C.C.-P. was supported by a grant from the Fundación Alejandro Angel Escobar's Colombia Biodiversa program (to C.C.-P.) and by a scholarship jointly sponsored by Colciencias' Programa de Jóvenes Investigadores e Innovadores 2014 and the FAPA (Fondo de Apoyo para Profesores Asistentes) program of the Vicerrectoría de Investigaciones, Universidad de los Andes (to A.J.C.). During this research, C.A.N. was supported by FAPESP (São Paulo Science Foundation) grant 2008/57687-0.

APPENDIX

Table A1: Collecting localities with their corresponding department (equivalent to province), GPS coordinates (WGS84 datum), type of habitat, and environmental data

Locality	Lat., long.	Habitat type	Mean annual temperature (°C)	Mean annual precipitation (mm)	Seasonality in precipitation (CV)	Elevation (m asl)
San Martín, Meta	3.696946, -73.698613	Savannah	25.5	1,438	45	405
Carimagua, Meta	4.584983, -71.079697	Savannah	26.5	2,183	57	149
Restrepo, Meta	4.261667, -73.564167	Wet forest <sup>a</sup>	24.7	5,062	48	570
Bosconia, Cesar	9.976111, -73.8903	Coastal xeric habitat	27.9	1,030	52	117
Leticia, Amazonas	-3.86421, -70.20396	Wet forest	26.0	3,194	26	96
Carmen de Apicalá, Tolima	4.147778, -74.718611	Coastal xeric habitat (Upper Magdalena)	27.2	1,319	46	328
Rivera, Huila	2.777222, -75.255278	Coastal xeric habitat (Upper Magdalena)	24.8	1,250	49	705
Pandi, Cundinamarca	4.191111, -74.487778	Wet forest	23.6	1,300	39	103
Tubará, Atlántico	10.874167, -74.978611	Coastal xeric habitat	23.6	1,025	79	203
Usiacurí, Atlántico	10.75, -74.983333	Coastal xeric habitat	27.3	1,025	68	95
Riohacha, Guajira	11.544167, -72.906944	Coastal xeric habitat	28.0	800	99	5

Note: Environmental data (mean annual temperature, annual mean precipitation, precipitation seasonality, and elevation) were obtained from WorldClim (Hijmans et al. 2005). CV = coefficient of variation. <sup>a</sup>Restrepo, Meta, is a montane foothills site near the savannah that has high precipitation and whose fauna contains Amazonian influences; thus, we code this site as “wet forest” along with Leticia, Amazonas.

**Table A2: Voucher information for specimens providing mitochondrial DNA sequence data used to estimate the phylogenetic relationships of species in the present study**

Family	Species	Locality	Field no.	Museum		GenBank no.	
				voucher no.	COI (ProcessID)	16S	
Hylidae	<i>Dendropsophus leali</i>	Leticia, Amazonas	AJC 4803	ANDES-A 3830	MG030722 (DRYCO084-15)	MG030717	
Hylidae	<i>Dendropsophus mathiassoni</i>	Sabanalarga, Casanare	AJC 4070	ANDES-A 1066	KP149114 (BSECO021-11)	KP149310	
Hylidae	<i>Dendropsophus microcephalus</i>	Sabana de Torres, Santander	AJC 3887	ANDES-A 1296	KP149218 (BSECO315-11)	KP149423	
Hylidae	<i>Hypsiboas crepitans</i>	Sabanalarga, Casanare	AJC 4108	ANDES-A 1063	KP149168 (BSECO058-11)	KP149370	
Hylidae	<i>Hypsiboas lanciformis</i>	Sabanalarga, Casanare	AJC 3975	ANDES-A 1238	KP149169 (BSECO087-11)	KP149371	
Hylidae	<i>Hypsiboas pugnax</i>	San Vicente de Chucurí, Santander	AJC 3501	ANDES-A 1541	KP149202 (BSAMS110-12)	KP149407	
Leptodactylidae	<i>Leptodactylus colombiensis</i>	Corrales de San Luis, Tubará	AJC 5035	ANDES-A 3089	MG030719 (DRYCO090-15)	MG030714	
Leptodactylidae	<i>Leptodactylus fuscus</i>	Orocué, Casanare	AJC 2301	ANDES-A 1169	KP149194 (BSAMS081-12)	KP149398	
Leptodactylidae	<i>Leptodactylus petersii</i>	Leticia, Amazonas	AJC 4725	ANDES-A 2137	NA (DRYCO051-15)	MG030712	
Leptodactylidae	<i>Leptodactylus poecilochilus</i>	Corrales de San Luis, Tubará	AJC 4853	ANDES-A 3831	MG030721 (DRYCO382-17)	MG030716	
Hylidae	<i>Osteocephalus planiceps</i>	Montanita, Caquetá	AJC 5445	ANDES-A 2378	MG030720 (DRYCO196-15)	MG030715	
Hylidae	<i>Phyllomedusa bicolor</i> (juvenile)	Leticia, Amazonas	AJC 5130	ANDES-A 3832	NA (DRYCO083-15)	MG030713	
Hylidae	<i>Phyllomedusa hypochondrialis</i>	Miraflores, Meta	AJC 3453	ANDES-A 1212	KP149222 (BSECO230-11)	KP149427	
Hylidae	<i>Phyllomedusa venusta</i>	Bosconia, Cesar	JSM 176	ANDES-A 3829	MG030718 (BSAMS233-12)	MG030711	
Bufoidea	<i>Rhinella humboldti</i>	San Vicente del Chucurí, Santander	AJC 3533	ANDES-A 1213	KP149216 (BSAMS126-12)	KP149421	
Bufoidea	<i>Rhinella margaritifera</i>	Pajarito, Boyacá	LSB 380	ANDES-A 1261	KP149087 (BSECO270-11)	KP149282	
Hylidae	<i>Scinax ruber</i>	Sabanalarga, Casanare	AJC 3378	ANDES-A 1504	KP149243 (BSECO095-11)	KP149452	
Microhylidae	<i>Elachistocleis ovalis</i>	San Juan de Arama, Meta	AJC 1742	ANDES-A 1744	KP149259 (BSECO175-11)	KP149470	

Note. Field number acronyms are AJC (Andrew J. Crawford), JSM (Juan Salvador Mendoza), and LSB (Lucas S. Barrientos). ANDES-A indicates the amphibian collection of the Museo de Historia Natural ANDES, Universidad de los Andes, Bogotá, Colombia. GenBank accession numbers are provided for the cytochrome oxidase subunit I (COI) 5' DNA barcode and ribosomal 16S gene fragments, along with the ProcessID corresponding to the sample record in the International Barcode of Life database (<http://boldsystems.org>).

## Literature Cited

- Adolph E.F. 1933. Exchanges of water in the frog. *Biol Rev* 8: 224–240.
- . 1934. Influences of the nervous system on the intake and excretion of water by the frog. *J Cell Comp Physiol* 5: 123–139.
- Amey A.P. and G.C. Grigg. 1995. Lipid-reduced evaporative water loss in two arboreal hylid frogs. *Comp Biochem Physiol A* 111:283–291.
- Barbeau T.R. and H.B. Lillywhite. 2005. Body wiping behaviors associated with cutaneous lipids in hylid tree frogs of Florida. *J Exp Biol* 208:2147–2156.
- Bentley P.J. 1966. Adaptations of Amphibia to arid environments. *Science* 152:619–623.
- Bentley P.J., A.K. Lee, and A.R. Main. 1958. Comparison of dehydration and hydration of two genera of frogs (*Heleioporus* and *Neobatrachus*) that live in areas of varying aridity. *J Exp Biol* 35:677–684.
- Beuchat C.A., F.H. Pough, and M.M. Stewart. 1984. Response to simultaneous dehydration and thermal stress in three species of Puerto Rican frogs. *J Comp Physiol B* 154:579–585.
- Blomberg S.P., T. Garland, and A.R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.
- Braun R. 1951. Beiteäge zur Kenntnis vom Wasserhaushalt des Frosches. *Z Vergl Physiol* 33:266–300.
- Buckley L.B. and W. Jetz. 2007. Environmental and historical constraints on global patterns of amphibian richness. *Proc Biol Sci* 274:1167–1173.
- Carvalho J.E., C.A. Navas, and I.C. Pereira. 2010. Energy and water in aestivating amphibians. Pp. 141–169 in J.E. Carvalho and C.A. Navas, eds. *Aestivation*. Springer, Berlin.
- Christian K.A., C.R. Tracy, and C.R. Tracy. 2017. Physical calculations of resistance to water loss improve predictions of species range models: comment. *Ecology* 98:2962–2964.
- Cid F.D., M.B. Jofré, and E. Caviedes-Vidal. 2010. Spatial and temporal patterns of richness and abundance in the anuran assemblage of an artificial water reservoir from the semiarid central region of Argentina. *Amphib-Reptilia* 31:533–540.
- Crawford A.J., C. Cruz, E. Griffith, H. Ross, R. Ibáñez, K.R. Lips, A.C. Driskell, E. Bermingham, and P. Crump. 2013. DNA barcoding applied to ex situ tropical amphibian conservation programme reveals cryptic diversity in captive populations. *Mol Ecol Resour* 13:1005–1018. doi:10.1111/1755-0998.12054.
- Cruz-Piedrahita C., C.A. Navas, and A.J. Crawford. 2017. Data from: Life on the edge: a comparative study of ecophysiological adaptations of frogs to tropical semiarid environments. *Physiol Biochem Zool*, Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.jm838>.
- de Oliveira G. and J.A.F. Diniz-Filho. 2010. Spatial patterns of terrestrial vertebrates richness in Brazilian semiarid, northeastern Brazil: selecting hypotheses and revealing constraints. *J Arid Environ* 74:1418–1426.
- Drummond A.J., M.A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29:1969–1973.
- Drummond G.B. 2009. Reporting ethical matters in *The Journal of Physiology*: standards and advice. *J Physiol* 587:713–719.
- Duellman W.E. 1999. Distribution patterns of amphibians of South America. Pp. 255–328 in W.E. Duellman, ed. *Patterns of distribution of amphibians: a global perspective*. Johns Hopkins University Press, Baltimore, MD.
- Felsenstein J. 1985. Phylogenies and the comparative method. *Am Nat* 125:1–15.
- Garland T., Jr., A.W. Dickerman, M.J. Christine, and J.A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst Biol* 42:265–292.
- Gaylard A., N. Owen-Smith, and J. Redfern. 2003. Surface water availability: implications for heterogeneity and ecosystem processes. Pp. 171–188 in J.T. du Toit, K.H. Rogers, and H.C. Biggs, eds. *The Kruger experience: ecology and management of savanna heterogeneity*. Island, Washington, DC.
- Gomez-Mestre I., R.A. Pyron, and J.J. Wiens. 2012. Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* 66:3687–3700. doi:10.1111/j.1558-5646.2012.01715.x.
- Goniakowska-Witalińska L. and U. Kubiczek. 1998. The structure of the skin of the tree frog (*Hyla arborea arborea* L.). *Ann Anat* 180:237–246.
- Grace J. 1987. Climatic tolerance and the distribution of plants. *New Phytol* 106:113–130.
- Graham A. 2012. Sequencing New World ecosystems: comparison of the Cretaceous and Cenozoic appearance of habitats with biome-characterizing plant groups. *Ann Mo Bot Gard* 98:524–538. doi:10.3417/2011082.
- Guarnizo C.E., A. Paz, A. Muñoz-Ortiz, S.V. Flechas, J. Méndez-Narváez, and A.J. Crawford. 2015. DNA barcoding survey of anurans across the Eastern Cordillera of Colombia and the impact of the Andes on cryptic diversity. *PLoS ONE* 10: e0127312. doi:10.1371/journal.pone.0127312.
- Hasegawa T., H. Tanii, M. Suzuki, and S. Tanaka. 2003. Regulation of water absorption in the frog skins by two vasotocin-dependent water-channel aquaporins, AQP-h2 and AQP-h3. *Endocrinology* 144:4087–4096.
- Hijmans R.J., S.E. Cameron, J.L. Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978.
- Jørgensen C. 1997. 200 years of amphibian water economy: from Robert Townson to the present. *Biol Rev* 72:153–237.
- Karr J.R. and K.E. Freemark. 1983. Habitat selection and environmental gradients: dynamics in the “stable” tropics. *Ecology* 64:1481–1494. doi:10.2307/1937503.
- Kennedy A.D. 1993. Water as a limiting factor in the Antarctic terrestrial environment: a biogeographical synthesis. *Arct Alp Res* 25:308–315.
- Kobelt F. and K.E. Linsenmair. 1986. Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. I. The skin of *Hyperolius viridiflavus nitudulus* in wet and dry season conditions. *Oecologia* 68:533–541.

- Leary S., W. Underwood, R. Anthony, S. Cartner, D. Corey, T. Grandin, C.B. Greenacre, S. Gwaltney-Bran, M.A. McCrackin, and R. Meyer. 2013. AVMA guidelines for the euthanasia of animals: 2013 edition. American Veterinary Medical Association, Schaumburg, IL.
- Lillywhite H.B. 2006. Water relations of tetrapod integument. *J Exp Biol* 209:202–226. doi:10.1242/jeb.02007.
- Lillywhite H.B. and C.A. Navas. 2006. Animals, energy, and water in extreme environments: perspectives from Ithala 2004. *Physiol Biochem Zool* 79:265–273.
- Maddison W.P. and D.R. Maddison. 2015. Mesquite: a modular system for evolutionary analysis. Ver. 3.04.
- Maia M.R. 2014. Intra and interspecific variation in microhabitats selection and its relevance to the maintenance of water balance in anurans. Master's thesis, University of São Paulo.
- McClanahan L. and R. Baldwin. 1969. Rate of water uptake through the integument of the desert toad, *Bufo punctatus*. *Comp Biochem Physiol* 28:381–389.
- McClanahan L.L., R. Ruibal, and V.H. Shoemaker. 1994. Frogs and toads in deserts. *Sci Am* 270:82–88.
- Miller M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in Proclamations of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA. doi:10.1109/GCE.2010.5676129.
- Murphy P.G. and A.E. Lugo. 1986. Ecology of tropical dry forest. *Annu Rev Ecol Syst* 17:67–88.
- Navas C.A. and C. Araujo. 2000. The use of agar models to study amphibian thermal ecology. *J Herpetol* 34:330–334.
- Navas C.A. and F.R. Gomes. 2001. Time in captivity as a confounding variable in herpetological research: an example from the metabolic physiology of treefrogs (*Scinax*). *Herpetol Rev* 32:228–230.
- Navas C.A., C. Jared, and M.M. Antoniazzi. 2002. Water economy in the casque-headed tree-frog *Corythomantis greeningi* (Hylidae): role of behaviour, skin, and skull skin co-ossification. *J Zool (Lond)* 257:525–532.
- Nowack C. 2011. Functional anatomy of the lateral nasal gland in anuran amphibians and its relation to the vomeronasal organ. *J Herpetol* 45:511–515. doi:10.1670/10-188.1.
- Prates I. and C.A. Navas. 2009. Cutaneous resistance to evaporative water loss in Brazilian *Rhinella* (Anura: Bufonidae) from contrasting environments. *Copeia* 2009:618–622. doi:10.1643/CP-08-128.
- R Core Team. 2014. R: a language and environment for statistical computing. Vienna.
- Revell L.J. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* 63:3258–3268.
- . 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217–223.
- Riddell E.A., E.K. Apanovitch, J.P. Odom, and M.W. Sears. 2017. Physical calculations of resistance to water loss improve predictions of species range models. *Ecol Monogr* 87:21–33.
- Robert C.E. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.
- Roth J.J. 1973. Vascular supply to the ventral pelvic region of anurans as related to water balance. *J Morphol* 140:443–460.
- Ruibal R., L.J. Tevis, and V. Roig. 1969. The terrestrial ecology of the spadefoot toad *Scaphiopus hammondi*. *Copeia* 1969:571–584.
- Scheffers B.R., R.M. Brunner, S.D. Ramirez, L.P. Shoo, A. Diesmos, and S.E. Williams. 2013a. Thermal buffering of microhabitats is a critical factor mediating warming vulnerability of frogs in the Philippine biodiversity hotspot. *Biotropica* 45:628–635.
- Scheffers B.R., D.P. Edwards, A. Diesmos, S.E. Williams, and T.A. Evans. 2014. Microhabitats reduce animal's exposure to climate extremes. *Glob Change Biol* 20:495–503.
- Scheffers B.R., B.L. Phillips, W.F. Laurance, N.S. Sodhi, A. Diesmos, and S.E. Williams. 2013b. Increasing arboreality with altitude: a novel biogeographic dimension. *Proc Biol Sci* 280:20131581. doi:10.1098/rspb.2013.1581.
- Schmid W.D. 1965. Some aspects of the water economies of nine species of amphibians. *Ecology* 46:261–269.
- Schneider C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675.
- Scott N.J., Jr. 1976. The abundance and diversity of the herpetofaunas of tropical forest litter. *Biotropica* 8:41–58. doi:10.2307/2387818.
- Smith J.A.M., L.R. Reitsma, and P.P. Marra. 2010. Moisture as a determinant of habitat quality for a nonbreeding Neotropical migratory songbird. *Ecology* 91:2874–2882.
- Soberón J. and M. Nakamura. 2009. Niches and distributional areas: concepts, methods, and assumptions. *Proc Natl Acad Sci USA* 106:19644–19650.
- Spotila J.R. and E.N. Berman. 1976. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. *Comp Biochem Physiol A* 55:407–411.
- Stadler T. 2009. On incomplete sampling under birth-death models and connections to the sampling-based coalescent. *J Theor Biol* 261:58–66.
- Stille W.T. 1952. The nocturnal amphibian fauna of the southern Lake Michigan beach. *Ecology* 33:149–162. doi:10.2307/1930635.
- Titon B. and F.R. Gomes. 2015. Relation between water balance and climatic variables associated with the geographical distribution of anurans. *PLoS ONE* 10:e0140761.
- Titon B., C.A. Navas, J. Jim, and F.R. Gomes. 2010. Water balance and locomotor performance in three species of Neotropical toads that differ in geographical distribution. *Comp Biochem Physiol A* 156:129–135.
- Toledo R. and C. Jared. 1993. Cutaneous adaptations to water balance in amphibians. *Comp Biochem Physiol A* 105:593–608.
- Tracy C.R., T. Tixier, C. Le Nöene, and K.A. Christian. 2014. Field hydration state varies among tropical frog species with different habitat use. *Physiol Biochem Zool* 87:197–202.



- Viborg A.L., T. Wang, and S.D. Hillyard. 2006. Cardiovascular and behavioural changes during water absorption in toads, *Bufo alvarius* and *Bufo marinus*. *J Exp Biol* 209:834–844.
- Vidal-García M., P.G. Byrne, J.D. Roberts, and J.S. Keogh. 2013. The role of phylogeny and ecology in shaping morphology in 21 genera and 127 species of Australo-Papuan myobatrachid frogs. *J Evol Biol* 61:181–192.
- Withers P.C., S.S. Hillman, and R.C. Drewes. 1984. Evaporative water loss and skin lipids of anuran amphibians. *J Exp Zool* 232:11–17.
- Woinarski J.C.Z., A. Fisher, and D. Milne. 1999. Distribution patterns of vertebrates in relation to an extensive rainfall gradient and variation in soil texture in the tropical savannas of the Northern Territory, Australia. *J Trop Ecol* 15:381–398.
- Wygoda M. 1984. Low cutaneous evaporative water loss in arboreal frogs. *Physiol Zool* 57:329–337.
- Young J.E., K.A. Christian, S. Donnellan, C.R. Tracy, and D. Parry. 2005. Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. *Physiol Biochem Zool* 78:847–856.