Title

Amphibian Conservation in Working and Natural Landscapes: Investigating the Impacts of Disease, Bioacoustics, and Natural History

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By

Rebecca Marie Brunner

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Committee in charge:

Professor Claire Kremen, Co-Chair
Professor Damian Elias, Co-Chair
Professor Iryna Dronova

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Abstract

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Amphibians are among the world's most endangered vertebrates, especially in the tropics. Land-use change remains the most significant driver of amphibian declines worldwide; natural history information on most tropical species is severely lacking, rendering conservation strategies difficult. Human actions have become one of the dominant forces driving the outcomes of a classic question in ecology: Why are species found where they are? Some frog species are tolerant to habitat disturbance, inhabiting urban areas or various types of agriculture, while others are only found in relatively untouched habitats like primary forests. Still other species are so understudied that we are unable to assess their distributions. This dissertation investigates the role of natural history traits in disturbance tolerance, as well as in the discovery of undescribed frog species and behaviors in Ecuador. Chapter 1 introduces these concepts in more detail. Chapter 2 investigates the effect of land management and species traits on pathogen prevalence, specifically the aquatic fungus *Batrachochytrium dendrobatidis* (*Bd*)—another major driver of global amphibian loss. At the community level, body size and lifetime aquatic index (level of water dependence for breeding) were the strongest predictors of *Bd* infection. The results introduce nuance to the theory that amphibian populations experience higher disease risk in natural habitats. In Chapter 3, I investigate how land-use change influences sound propagation, which has direct implications for amphibian communication. If the change in vegetation causes a habitat to become acoustically inhospitable, frogs with certain call properties would be rendered unable to attract mates. I found that species with high-pitched calls in some families (Hylidae and Strabomantidae) were more likely to be found in disturbed habitats—as hypothesized—but, unexpectedly, species with high-pitched calls in Centrolenidae were more likely to be found in undisturbed habitats. This work highlights the need to consider the acoustic environment when assessing a species' vulnerability to habitat disturbance. In Chapter 4, I describe the call and visual signaling behavior of *Sachatamia orejuela*, an elusive glassfrog species that occupies a noisy acoustic niche. Documenting these new natural history traits provides a fascinating example of behavioral convergent evolution and further evidence that signals are shaped by their environment. Finally, Chapter 5 describes two new glassfrog species that are phenotypically very similar to other glassfrog species but are genetically and acoustically distinct. We recommend that both be listed as Endangered, given the rate of deforestation in their only known habitat. Chapter 6 discusses conservation challenges in the Ecuadorian Andes, especially for amphibians.

Dedication

This dissertation is dedicated to my mother, best friend, and hero, Linda Marie Brunner. Thank you for sacrificing so much to support me—especially my crazy, inexplicably innate desire to explore rainforests despite growing up in a cornfield. I love you more than words can say.

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It would take another dissertation-length piece to properly thank all the people who have helped me get to this point. I have dedicated this work to my mother, Linda Marie Brunner, who has encouraged my curiosity and passion every step of the way—even in moments I was too young to remember. I cannot think of a more selfless and courageous woman than my mom. Thank you for sacrificing so much so that I could pursue my dreams, even when the path wasn't clear. Thank you for listening, for making me laugh, for teaching me how to be a good person, for instilling a sense of justice and for a million other things I haven't realized you've done for me. You are my best friend, sister, and role model. I aspire to be even half the person you are.

My father died of brain cancer when I was three. While it was just mom and I for my formative years, she eventually married another wonderful man, Patrick Sweeney. Thank you, Pat, for being the father I never had, for supporting me through my college and grad school years. I am beyond lucky that you came into our lives. Thank you for learning how to sew so that you could help me make mesh cages for sound experiments, for putting on a hazmat suit to spray my clothes with permethrin before my jungle excursions, and for every other crazy thing you did to support my adventures in science.

About halfway through my PhD, my advisor left UC Berkeley to pursue an opportunity at University of British Columbia in Vancouver, Canada. I did not follow, mainly for funding reasons. Around the same time, an old colleague and friend of mine from Cornell, David Rodriguez, offered for me to be a visiting scholar in his lab at Texas State University to complete my genetic work. I accepted and moved to Texas—probably the last place in the world I thought I'd ever live. Thank you, David, for taking me under your wing and giving me a place to call home while finishing my PhD. David is also the reason I started working in Ecuador in the first place. I actually began my PhD fieldwork in Madagascar, which I ceased due to complications with an extremely toxic collaborator. The prospect of starting over was one of the lowest points of my PhD journey. I called David to ask for his advice. He not only convinced me not to quit, but also gave me the opportunity to help him teach a study abroad course in Ecuador, which would double as a way for me to scout potential field sites for my future research. I am so thankful, because my work in Ecuador has been the most rewarding of my life thus far. Thank you, David, for believing in me when I didn't, and for being the catalyst for what I hope is a lifelong connection to Ecuador.

This unexpected move to Texas also enabled me to meet the love of my life, Colin Cox. Dating during the Covid-19 pandemic, while strange and terrifying, actually allowed us to get to know each other much faster than normal. Colin, I would never have believed that someone like you actually exists: you have such a beautiful soul and an empathetic heart; you are kind, funny, and nerdy in all the right kinds of ways; you value nature, wildlife, and social justice in such a deep and inspiring manner. I truly believe we are kindred spirits, and I am so thankful that we found each other. Thank you for supporting me—in every way—through the hardest phase of my PhD. I love you so much, and I can't wait to start this new chapter with you by my side.

Unfortunately, I need to break this narrative form for the sake of space, but there are so many more people to thank:

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Chapter 1. Introduction

REEXAMINING A CLASSIC ECOLOGICAL QUESTION IN THE ANTHROPOCENE

Human actions have become one of the dominant forces driving the outcomes of a classic question in ecology: Why are species found where they are? The distribution of any given species over space and time is influenced by the interplay between its traits and environment. Anthropogenic change continues to drastically alter the latter, especially in the context of habitat loss and degradation. Currently, the most pervasive threat to biodiversity is the conversion of natural ecosystems to agricultural crops (Global Forest Resources Assessment Report 2021; Tilman et al. 2001). The negative effects of habitat loss on species diversity and abundance have been relatively well-studied (e.g., Chase et al. 2020; Pimm 2008; Brooks et al. 2002).

However, agriculture is not always an inhibitable 'ocean' amidst forest 'islands' (Diamond 1975). Work by my PhD advisor, Claire Kremen, and many others have published on the conservation potential of various agricultural practices and types (e.g., hedgerows for native bees, shade-grown coffee for birds), situating agriculture as a 'matrix' with varying levels of suitability for wildlife (e.g., Driscoll et al. 2013; Franklin et al. 2009). More recently, the concept of working landscapes (Kremen & Merenlender 2018) has surfaced as an exciting paradigm in which the matrix can work for both people and nature if planned creatively and informed by ecological and traditional knowledge. For example, the quality of the matrix has been shown to have a higher impact on species richness than patch size in many systems (Kremen 2015). Some matrices function as habitats, benefiting select species by providing increased resources. Due to their structural similarity to natural forests, agroforests (ideally a diversity of crops such as coffee, interspersed with native shade trees) especially hold potential to aid in connectivity and serve as habitat for many species.

Yet species/populations react to disturbance in different ways: some seem to persist (and sometimes even thrive) in human-dominated landscapes, while many others remain exclusively in fragments of their once contiguous habitats (Edwards et al. 2010; Brashares et al. 2001). The suitability of a modified landscape for a given species depends in part on the breadth of its niche with respect to microhabitat, climate, resource dependence (food, breeding and/or nesting sites); its phenotype (body size, mobility, coloration); and biotic interactions with its community (competition, mutualisms, predation, parasitism). However, it is fairly common for species in the same family or genus to share many of these characteristics (breeding strategy, diet, coloration, etc.) and still exhibit different responses to habitat conversion. For example, in the family Centrolenidae (glassfrogs), some species are only found in primary forests (e.g., *Centrolene lynchi*) while others (e.g., *Espadarana prosoblepon*) are found in many habitat types along a disturbance gradient, from primary forest to pastures (Kime et al. 2000). These two species are arboreal, have extremely similar coloration and body size, lay a similar number of eggs on leaves above streams, and are found at similar elevations and climate profiles. So what, then, drives their differences in disturbance tolerance?

Chapters 2 and 3 of my dissertation attempt to investigate the shift in advantages or disadvantages conferred by certain traits and the environment in tropical forests and agricultural matrices, with a focus on disease prevalence and bioacoustics. The majority of studies related to conservation in working landscapes have focused on birds (e.g., Ortega-Álvarez et al. 2021; Karp et al. 2013), mammals (e.g., Silva et al. 2020; Cassano et al. 2014), and bees (e.g., Guzman et al. 2019; Klein et al. 2007); I have therefore chosen to focus on frogs, which are indicator species (Weygoldt 1989) for water health, likely providers of pest control services (Khatiwada et al. 2016), and essential components of food chains in their ecosystems (Kupfer et al. 2006).

UNEXPECTED DISCOVERIES AND THE IMPORTANCE OF NATURAL HISTORY

When I began my fieldwork, I planned to survey frogs in primary forests as a baseline for the species distributions and environmental properties I was comparing to those in working landscapes. To accomplish that goal, I needed to learn the natural history of the species in my study regions throughout Ecuador. Gaining this understanding turned out to be much more difficult than I had anticipated: we know next to nothing about most tropical frog species–even basic biology such as advertisement calls (which are essential for species identification–both for female frogs and biologists). In response, I became determined to fill in as many natural history gaps as possible. While I expected that any new data I collected would be supplementary to my thesis, to my surprise, my discoveries were important enough to stand on their own. The last two chapters of my dissertation represent these discoveries. Chapter 4 consists of a description of the acoustic and visual signals of an elusive glassfrog species, which together provide compelling evidence of convergent trait evolution in loud acoustic environments–proof of concept for the acoustic adaptation hypothesis that I explore in the preceding Chapter 3. Chapter 5 fulfills a childhood dream: a description of two new glassfrog species that I helped discover while familiarizing myself with the species of Mashpi Reserve. This research contributes to the conservation of Ecuadorian forests and their species. We cannot conserve species that we do not know exist, and charismatic taxa like glassfrogs actually impact the public's opinion towards preserving forests. Increasing our understanding of an animal's behavior also aids in conservation efforts: especially calls, which are essential for identifying species in real-time and in passive acoustic surveys.

STUDY SITES

I collected data for this dissertation in many locations throughout Western Ecuador (Manabí and Pichincha provinces). My two main field sites were forested reserves surrounded by heterogeneous working landscapes, dominated mostly by cacao, coffee, banana, and cattle pastures. The first, Jama-Coaque Reserve (JCR; 0°06'29.5"S 80°07'06.5"W; ~200-700m elevation range in this study), conserves a crucial portion of the last remaining coastal moist evergreen forests and premontane cloud forests of the Tumbes-Chocó-Magdalena biodiversity hotspot among agricultural communities. The second, Mashpi Reserve (0°09'57.9"N 78°52'45.5"W; ~500-1000m elevation range in this study), conserves a large stand of mature rainforest within the Tropical Andes biodiversity hotspot. Its neighboring community of the same name hosts a locally operated sustainable agroforestry and reforestation network, including Reserva/Bosque Escuela Pambiliño, Reserva Mashpi Shungo, and Reserva Chontaloma. I also sampled in two other working landscapes without adjacent forests: private lands in Mompiche (0°30'23.1"N 80°00'55.1"W;~0-50m elevation range in this study) and Calceta (0°50'10.0"S 80°08'54.4"W; ~50m-500m elevation range in this study), both small villages that have experienced high rates of deforestation in the past 10 years.

OVERVIEW OF DISSERTATION

My dissertation research consists of four chapters. In Chapter 2, "Natural history traits are the strongest predictors of pathogen prevalence across working and natural landscapes: implications for amphibian conservation," I examine how land-use change and functional traits influence disease prevalence in frogs, specifically focusing on the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). This study is the first to focus on *Bd* dynamics in working landscapes like agroforests. I also highlight the importance of natural history traits in examining the drivers of species distribution shifts with land-use change.

In Chapter 3, "Soundscapes of conservation: call characteristics influence disturbance tolerance in Ecuadorian frog species," I investigate the role that acoustic space plays in influencing whether species are tolerant to land-use change. When agriculture replaces forest, the structural aspects of the landscape inherently change (e.g., the density and position of trees), which in turn modifies the acoustic environment. Since the acoustic environment affects the propagation of frog calls, I hypothesize that land-use change can cause a habitat to become acoustically inhospitable for some species (i.e., rendering them unable to effectively communicate). The lack of certain soundscape structures in a habitat may help explain why only a subset of vertebrates are found in agricultural areas, even when other resources are abundant.

Chapter 4, "Nocturnal visual displays and call description of the cascade specialist glassfrog *Sachatamia orejuela*," supplements Chapter 3 by providing strong evidence for evolutionary adaptations to acoustic environments. *Sachatamia orejuela* is an elusive glassfrog species with a strict waterfall niche. Already published in the journal *Behaviour*, this chapter catalogs: 1) the first advertisement call record for the species, which is extremely high-pitched, contrary to what we would predict based on its large body size, and 2) the first record of visual signaling in a glassfrog species. Both findings provide compelling support for the acoustic adaptation hypothesis, which predicts that species' calls have evolved optimal characteristics for transmission in their native environments.

Chapter 5, "Two new glassfrogs (Centrolenidae: Hyalinobatrachium) from Ecuador, with comments on the endangered biodiversity of the Andes," exemplifies the importance of biodiversity surveys for conservation. During my fieldwork, I helped discover two new frog species (*Hyalinobatrachium mashpi* and *Hyalinobatrachium nouns*), the former by recognizing a new call among familiar frog calls along my transects. These new species are examples of cryptic diversity: while they are phenotypically very similar to other glassfrog species, their genetic and acoustic traits clearly differentiate them from their sister species. In our publication of this work in the journal *PeerJ*, we recommend that both *H. mashpi* and *H. nouns* be listed as Endangered, following IUCN criteria. These new species provide further evidence that the Andes fosters much more biodiversity than we have the resources to catalog. Threatened by mining and other exploitative industries, these glassfrogs and many other yet-to-be-discovered Andean species highlight the dire need for effective conservation measures.

Chapter 6, "Conservation challenges & opportunities in the Ecuadorian Andes," concludes this dissertation by reiterating the importance of the Andes for biodiversity conservation, with Ecuador and its constitutional rights for nature as a case study.

Chapter 2. Natural history traits are the strongest predictors of pathogen prevalence across working and natural landscapes: implications for amphibian conservation

ABSTRACT

Land-use change and pathogens–especially the aquatic fungus *Batrachochytrium dendrobatidis* (*Bd*)—are currently considered the main drivers of amphibian declines worldwide. Yet many working landscapes support relatively high levels of amphibian diversity. Since declining species tend to share traits that increase vulnerability, we sought to understand how natural history traits mediate pathogenicity across different land management types. We tested for *Bd* prevalence in 658 individuals from 32 species and 6 families sampled from pastures, agroforests, polycultures, secondary forests, and mature forests throughout Western Ecuador. At the community level, we found that body size and lifetime aquatic index (level of water dependence for breeding) were the strongest predictors of *Bd* infection. While we found that frogs were more likely to be infected in pastures, we encourage future studies to characterize land-use with measures such as management level rather than canopy cover, which was not an accurate proxy for differentiating multiple working landscape types (including pastures) or even natural habitats in our models. Our study introduces nuance to the theory that amphibian populations experience high disease risk in natural habitats and provides compelling evidence that natural history traits and land-use are powerful predictors of *Bd* prevalence.

INTRODUCTION

Although land-use change remains the largest driver of amphibian declines and extinctions (Nori et al. 2015; Becker et al. 2007), disease is not far behind. *Batrachochytrium dendrobatidis* (*Bd*), an aquatic fungal pathogen, has given rise to the single greatest documented loss of diversity attributed to a non-human species (Fisher et al. 2009). As the causal agent of chytridiomycosis, *Bd* has contributed to the worldwide decline of more than 500 amphibian species, leading to at least 90 extinctions and >90% abundance loss in another 124 species since 2019 (Scheele et al. 2019). However, the relationship between these two main drivers of amphibian decline—land-use change and pathogen prevalence—is poorly understood (Hof et al. 2011). This is especially true in tropical landscapes, which are difficult to categorize remotely due to their structural complexity and varied management practices. Natural history traits may also be an overlooked mediating factor in species loss or extinction: declining species tend to share traits that increase vulnerability to disturbance (Davies et al. 2000) or disease (Lips et al. 2003). Considered together, natural history traits and land-use characteristics may provide powerful insights for predicting disease risk in amphibian communities.

Human-dominated habitats can support relatively high levels of amphibian diversity — especially those that blur the distinction between disturbed and natural habitats, such as agroforests and polycultures (Brüning et al. 2018). The concept of fragmentation has developed well beyond the binary definition it once held in the context of island biogeography (Diamond 1975), where a forest patch was considered an island in a sea of uniform, uninhabitable human-modified landscapes. The 'conservation of working lands' (Kremen & Merenlender 2018) and 'countryside biogeography' (Daily et al. 2001) paradigms explore the habitat potential of the ‘matrix,’ or land-use types that occur adjacent to forest patches (e.g., comparing shade-grown cacao vs. a pineapple monoculture). Some matrix types, especially those associated with deforestation like monocultures or cattle pastures, eliminate vital resources and greatly alter abiotic conditions, leading to drastically increased local extinction rates (Curado et al. 2011). However, other matrix types function as habitats, benefiting select species by providing increased resources (Franklin & Lindenmayer 2009; Ricketts 2001). The quality of the surrounding matrix has since been shown to have a higher impact on species richness than forest patch size in many systems (Kremen 2015; Mendenhall et al. 2014).

Yet working landscape matrices are rarely considered in studies of amphibian pathogen dynamics. Frog species in Costa Rica, Australia, and the US exhibited lower disease risk in disturbed habitats, likely due to suboptimal temperatures for *Bd* and/or differences in species richness (Becker et al. 2012; Becker & Zamudio 2011). However, rather than management practices, these and other studies defined ‘disturbance’ with canopy cover or satellite imagery, which can be insufficient when characterizing matrix types. For example, human-modified land-use types in the tropics like agroforests (e.g., shade-grown cacao) can generate canopy cover percentages indistinguishable from forests (Fujiki et al. 2016). Similarly, palm oil monocultures were found to be mistakenly categorized as forest (Tropek et al. 2014) in a global forest cover study (Hansen et al. 2013) that relied on high-resolution satellite imagery to define disturbance. To better predict *Bd* prevalence, land-use should be defined more specifically, according to vegetation structure, management practice, and microclimate.

Trait diversity also influences the interplay between land-use and host-pathogen dynamics (McKenzie 2007). For example, since *Bd* zoospores are motile and aquatic, a frog’s contact with water during its life cycle is likely to influence prevalence (Lips et al. 2003). While numerous tropical species call from and breed in water bodies, many others only need water for one life stage (e.g., Centrolenidae; egg clutches placed on vegetation but with aquatic tadpoles) or not at all (e.g., Strabomantidae; eggs develop completely on land or vegetation). Both water presence (e.g., rivers, irrigation ditches) and temperature—which promotes *Bd* growth when between 17-25°C (Piotrowski et al. 2004)—are variable across working landscapes. Furthermore, traits mediate shifts in amphibian community composition in response to habitat change (Riemann et al. 2017); body size can predict species presence in working landscapes (Meurling et al. 2021).

Our study is one of the first to investigate the effect of both natural history traits and multiple land management practices on *Bd* prevalence. Most *Bd* studies to date have focused on: 1) a few species, limiting our ability to predict prevalence at the community level, and 2) comparing prevalence in deforested versus ‘natural’ landscapes, without investigating *Bd* presence in transitionary management types such as agroforests and polycultures. Addressing these gaps is crucial for informing conservation initiatives; for instance, increasing our understanding of host-pathogen dynamics across management practices can help inform forest restoration projects and their effects on native wildlife. In this study, we sought to determine the factors that best predict *Bd* prevalence across amphibian communities within typical land management types found throughout the tropics. We hypothesized that natural history traits and land management would be the most significant predictors of community-level *Bd* prevalence.

METHODS

Sampling localities and land-use types

We sampled frog communities in four localities (Jama-Coaque, Mashpi, Mompiche, and Calceta) across the lowlands and Andean foothills of Western Ecuador (Figure 1) during the rainy season (January-May) and dry season (June-July) in 2018, 2019, and 2021. These localities collectively represent common management types typical of the tropics: a) livestock pastures; b) agroforests (i.e., shade-grown coffee and/or cacao trees); c) rustic polycultures (i.e., agroforests with a mostly natural forest canopy); d) secondary forests (i.e., selectively logged over the past two decades); e) mature forests (i.e., contiguous forest with minimal signs of human intervention and large hardwoods). Land management classifications (Table 1) were adapted from Philpott et al. 2008. At each locality, we surveyed frogs along multiple 500m transects per available land-use type, each separated by at least 1km. To account for the possible effect of water presence, we ensured that approximately half of the transects per locality (distributed equally among land-use types) bordered streams and/or ponds where amphibians are known to breed.

Two large localities consist of forests surrounded by working landscapes. The first, Jama-Coaque Reserve (JCR; 0°06'29.5"S 80°07'06.5"W; ~200-700m elevation range in this study), conserves a crucial portion of the last remaining coastal moist evergreen forests and premontane cloud forests of the Tumbes-Chocó-Magdalena biodiversity hotspot among agricultural communities. Within JCR and its surrounding matrix, we sampled frogs across 20 transects (5 Pasture, 5 Agroforest, 5 Polyculture, 5 Secondary Forest). Streams bordered half of the 20 transects, separated by land-use (2 Pasture, 2 Agroforestry, 3 Polyculture, 3 Secondary Forest).

The second locality, Mashpi (0°09'57.9"N 78°52'45.5"W; ~500-1000m elevation range in this study), includes a forest reserve that conserves a large stand of mature rainforest within the Tropical Andes biodiversity hotspot. Its neighboring community of the same name hosts a locally operated sustainable agroforestry and reforestation network, including Reserva/Bosque Escuela Pambiliño, Reserva Mashpi Shungo, and Reserva Chontaloma. Within Mashpi Reserve and community, we sampled frogs across 20 transects (5 Pasture, 5 Agroforest, 5 Polyculture, 5 Mature Forest). Streams bordered approximately half of the 20 transects (2 Pasture, 2 Agroforest, 2 Polyculture, 3 Mature Forest).

The other two localities consisted of working landscapes without adjacent forests: private lands in Mompiche (0°30'23.1"N 80°00'55.1"W;~0-50m elevation range in this study) and Calceta (0°50'10.0"S 80°08'54.4"W; ~50m-500m elevation range in this study), both small villages that have experienced high rates of deforestation in the past 10 years. We sampled along two pasture and two agroforest transects (neither with streams) in Mompiche, and two pasture and two agroforest transects (with streams present) in Calceta, for a total of eight transects.

All transects were sampled by the author and at least one other person for 5+ hours each night. We captured every adult frog encountered visually or acoustically within a maximum distance of 4m from the transect line. We also noted the height we found each frog (e.g., 0.5m on a leaf), as well as elevation (Garmin GPS units). To avoid cross-contamination, we used a new set of gloves to capture each individual. Frogs were kept in individual bags and were released within 5m of their original capture point after processing.

Pathogen sampling and host measurements

To sample *Bd* zoospore presence within the skin, we swabbed the ventral area of each frog using a sterile cotton tip dry swab (Medical Wire & Equipment, model MW113) following established procedures (Rodriguez et al. 2012; Hyatt et al. 2007). Swabs were stored dry in screw-cap tubes with o-rings until we had access to a freezer (~3 months later). After swabbing, we weighed and measured the snout-to-vent length (SVL) of each frog. We later checked natural history databases (e.g., AmphibiaWeb, BioWeb) and field guides (e.g., Arteaga et al. 2013; Lynch & Duellman 1997) to ensure that the SVL of each individual was within the range of an adult for its species. All frogs with an SVL in the juvenile range were not included in further analyses. We cross-referenced the same guides with our field notes to determine the 'strata' of each species: terrestrial, arboreal, or mixed.

qPCR and pathogen prevalence

We extracted DNA from swabs by adding 50 mL of PrepMan Ultra reagent (Applied Biosystems) to each swab tube, following procedures in Becker et al. 2016. To determine the presence of *Bd*, we used quantitative PCR (protocol by Boyle et al. 2004, modified by Kriger et al. 2006) on 1:10 dilutions of swab extracts. We performed reactions using Taq-Man1 Fast Advanced Master Mix (Applied Biosystems) on a QuantStudio 3 system (Applied Biosystems). Our standard curve ranged from 0.5 to 5,000 genomic equivalents (*Bd*-GPL isolate JEL423). We calculated *Bd* prevalence by dividing the number of positive samples by the total number of individuals tested.

Lifetime aquatic index (LAI)

Since *Bd* is an aquatic pathogen, life history stages that increase contact with water are likely to be relevant to infection probability. We therefore assigned a lifetime aquatic index value (modified from Lips et al. 2003; Table 2) to each species based on the aquatic dependence of its egg, larval, and adult stages. In this study, 0 represents species that do not contact water bodies to breed at any life stage (direct developers), 1 represents species in which the adults do not call/mate from nor lay eggs in water but have aquatic tadpoles (in this study, only glassfrogs), and 2 represents species that call from/mate in water, lay eggs in water, and have aquatic tadpoles (water breeders).

Environmental measurements

We measured canopy cover every 20m along each 500m transect. Using a 15mm, 170° wide-angle attachment lens (Moment, Inc.) for an iPhone X, we took standardized photographs 1m above the ground with the camera positioned level at 180° towards the sky. We then transformed each photograph into a binary image (black for vegetation and white for open sky) with the processing program ImageJ (v.1.53). To calculate canopy cover percentage for each image, we divided the number of black pixels by the total number of pixels and multiplied by 100.

We also obtained daily temperature data for each locality using two data loggers (HOBO and iButtons) placed at the center mark of each transect (250m), on the ground and at 2m. Loggers recorded temperature at each hour mark over a 24-hour period. We then calculated the mean of temperatures from both loggers to obtain the average for each day. When data loggers failed, we used data from weather stations—from privately-owned stations within Jama-Coaque and Mashpi Reserves and from stations managed by the Ecuadorian government in Calceta and Mompiche. We obtained daily humidity and average monthly rainfall from the same weather stations. Humidity was so uniform (~90%) across transects and localities that we did not include it as a variable in our models.

Statistics

We ran path analyses implemented within a Bayesian framework to investigate the effects of natural history traits and land management on *Bd* prevalence. Because larger frogs tend to be found in pastures and depend on water for breeding, the first model layer included the direct effects of land management type (pasture, agroforest, polyculture, secondary forest, primary forest) and lifetime aquatic index (LAI; 0, 1, or 2— described above) on body size (snout-to-vent length, SVL). The second model layer included the following explanatory variables to test their effect on *Bd* prevalence (0 or 1), assuming Binomial error: a) land management type; b) adjacent forest (0 or 1); c) percent canopy cover; d) elevation; e) average daily temperature (per transect); f) average monthly precipitation (per transect); g) SVL; h) strata of adults (terrestrial, arboreal, or mixed); and i) LAI. All numerical variables were scaled. We included both management type and canopy cover as explanatory variables because canopy cover was significantly different across (Kruskal-Wallis test; p-value < 2.2e-16) and between (pairwise Wilcoxon test; p-values < 0.00) land-use types. Elevation was not correlated with average daily temperature (Kendall's correlation; tau = -0.078) or average monthly precipitation (tau = 0.167), so we included all three variables. We included sampling month and species as random effects.

Using the statistical modeling platform Stan via the R 4.1.0 (R Core Team 2022) package brms (Buerkner 2017), we ran three Markov chain Monte Carlo (MCMC) chains for 10^4 iterations after an initial burn-in of 10^2 iterations. We used standard practices for assessing convergence, including chains, effective sample size, and Rhat values.

RESULTS

We measured and swabbed 658 individuals from 32 species and 6 families. Overall, 33% were infected with *Bd*. Infection prevalence by family is as follows: Bufonidae (25%), Centrolenidae (62%), Dendrobatidae (82%), Hylidae (18%), Leptodactylidae (2%), and Strabomantidae (35%). Table 2 provides infection prevalence, natural history traits, and land management type(s) that served as habitat for each species.

Table 3 provides results from our Baysian path analyses. The first model layer provided strong evidence that frogs are likely to be larger in pastures compared to other working landscapes (agroforests and polycultures) and that frogs that rely on water for all breeding stages (LAI 2) are also likely to be larger. The second model layer provided strong evidence that frogs are less likely to be infected in pastures compared to all other land management types (other working landscapes as well as natural landscapes). In terms of environmental factors, the model found that frogs are less likely to be infected in areas with higher temperatures, lower precipitation, and lower canopy cover. In terms of natural history traits, arboreal frogs are less likely to be infected than terrestrial species and frogs that occupy both (mixed strata). Frogs with a larger body size (SVL) and with LAI 0 or LAI 2 were less likely to be infected than smaller frogs and/or species with LAI 1.

DISCUSSION

Our study provides compelling evidence that natural history traits are powerful predictors of *Bd* prevalence throughout tropical landscapes, regardless of management type. Across 32 species, smaller body size (snout-to-vent length; Figure 4) and minimal water dependence (LAI 1; Figure 3) were the strongest positive predictors of *Bd* infection. This finding has important implications for conservation, as it suggests that *Bd* prevalence can be estimated at the community-level based on trait information that is generally readily available in the literature.

Studies that concentrate on taxonomically similar species have also found that smaller body size increases susceptibility to *Bd* (Meurling et al. 2021; Burrow et al. 2017). Our results expand this pattern to the level of community (Figure 4). *Bd* essentially hardens the keratin within the skin, rendering respiration and water uptake more difficult (Fisher et al. 2009). Given a constant rate of spread, species with a smaller surface area may be more rapidly overtaken by zoospores. The smallest species in this study are represented in Centrolenidae (glassfrogs) and Dendrobatidae (dart frogs), which are also the families that showed the highest infection prevalence (Figure 2; Table 2). It may also be the case that species within these families do not have enough bacterial skin symbionts, which help fend off *Bd* infection (Piovia-Scott et al. 2017). Recent studies of other taxa (e.g., bees) in working landscapes (Cohen et al. 2021) also found that smaller body size was associated with higher parasite prevalence at the community level.

We found that *Bd* prevalence was lower in pastures, which agrees with other findings (Becker et al. 2012; Becker & Zamudio 2011); since the ideal temperature range for Bd growth is between 17-25° C, increased exposure to higher temperatures in extremely open areas like pastures likely suppresses *Bd* growth. Higher temperatures and lower precipitation levels were also associated with lower *Bd* prevalence in our study, similar to patterns reported in many others (e.g., Brem & Lips 2008).

However, our results introduce nuance to the theory that amphibian populations experience higher disease risk in natural habitats. Our study shows that frogs in agroforests and polycultures experience very similar *Bd* prevalence rates to those in natural forests—and even more shaded pastures. This is further indication that working landscapes can function as habitats for biodiversity, including pathogens. We also show that vegetation cover is not necessarily an accurate proxy for management type. This finding is important, because many studies (e.g., Beyer et al. 2015) use vegetation cover—measured via canopy cover and/or satellite imagery—as the main or only measure of habitat disturbance. In our study, canopy cover was statistically independent both across management types and within the same management type (see examples in Table 1). Additionally, out of all the significant environmental variables in our Baysian model, canopy cover had the weakest support. We therefore strongly suggest that future studies characterize land-use by additional measures beyond canopy cover, such as management and structural complexity (Table 1).

Instead, we recommend relying more on natural history traits when predicting *Bd* prevalence patterns. Our findings highlight the importance of sampling representative species within the same community rather than considering just a few species–especially when those species do not represent the full spectrum of aquatic indices (level of water dependence for breeding). One of the only other community-level *Bd* studies in Ecuador (Guayasamin et al. 2014), which focused exclusively on frogs in natural habitats, found that frogs with aquatic reproductive modes (the equivalent of LAI 1) showed lower *Bd* prevalence than directdeveloping frogs (LAI 0). Our results mirror this finding in forests: glassfrogs (LAI 1) were least likely to be infected in forests, whereas direct developers were most likely to be infected in forests (Figure 3). However, our study highlights the importance of contextualizing *Bd* prevalence patterns by land management type, since we observed the opposite pattern in working landscapes: glassfrogs were more likely to be infected in polycultures (the only working landscape type they occupied in this study), whereas direct developers were less likely to be infected in working landscapes, especially pastures.

We recommend that future studies work to further contextualize the relationship between land management and natural history traits—especially body size and water contact. Our layered Baysian model showed that frogs are likely to be larger in pastures (fig 4), which could conflate their low likelihood of infection. Similarly, direct developers (LAI 0) were less likely to be infected than glassfrog species (LAI 1), which tend to be smaller—although body size and LAI were not directly correlated in this study. We therefore cannot yet determine whether body size is driving the association between *Bd* infection and other traits.

However, we suspect that *Bd* presence in water and an individual's contact with it—whether from bodies like streams or the soil on the ground or in epiphytes in the canopy—may mediate these patterns. Although not as significant as body size or LAI, strata (arboreal, terrestrial, or mixed) was a predictor of *Bd* prevalence: arboreal species were less likely to be infected than those occupying terrestrial habitats or species that occupy both arboreal and terrestrial habitats during the non-breeding season (e.g., *Pristimantis achatinus*). Glassfrogs (Centrolenidae) and treefrogs (Hylidae) are arboreal, yet Centrolenidae was one of the most vulnerable families in terms of *Bd* infection. Treefrogs in this study were all categorized as LAI 2, since they call, breed, and lay eggs in the water; however, outside of their breeding season, treefrogs tend to remain in the canopy. Species in Hylidae also tend to be larger overall. In contrast, glassfrogs are categorized as LAI 1 because they only contact water as tadpoles—they do not call from, breed, or lay eggs in water (except for *Sachatamia orejuela*, whose niche is the spray zone of waterfalls; Brunner & Guayasamin 2020); they also do not sit in water bodies as adults, and they tend to be smaller. Future studies should investigate if glassfrogs are more vulnerable to *Bd* because they rarely come in contact with water, which has limited their ability to develop resilience.

Overall, our results have important conservation implications. We have shown the value of defining land management beyond 'disturbance' when predicting the factors that influence disease prevalence in amphibian communities. Integrating natural history traits into that framework strengthens our ability to understand disease dynamics, especially for frogs—the world's most threatened vertebrates. As humans continue to alter landscapes in increasingly nuanced ways through different forms of land management, it is imperative that we consider these complexities when researching anthropogenic impacts on wildlife.

FIGURE 1. SAMPLING LOCALITIES THROUGHOUT WESTERN ECUADOR

Colored circles by each locality represent the combination of land management types sampled there.

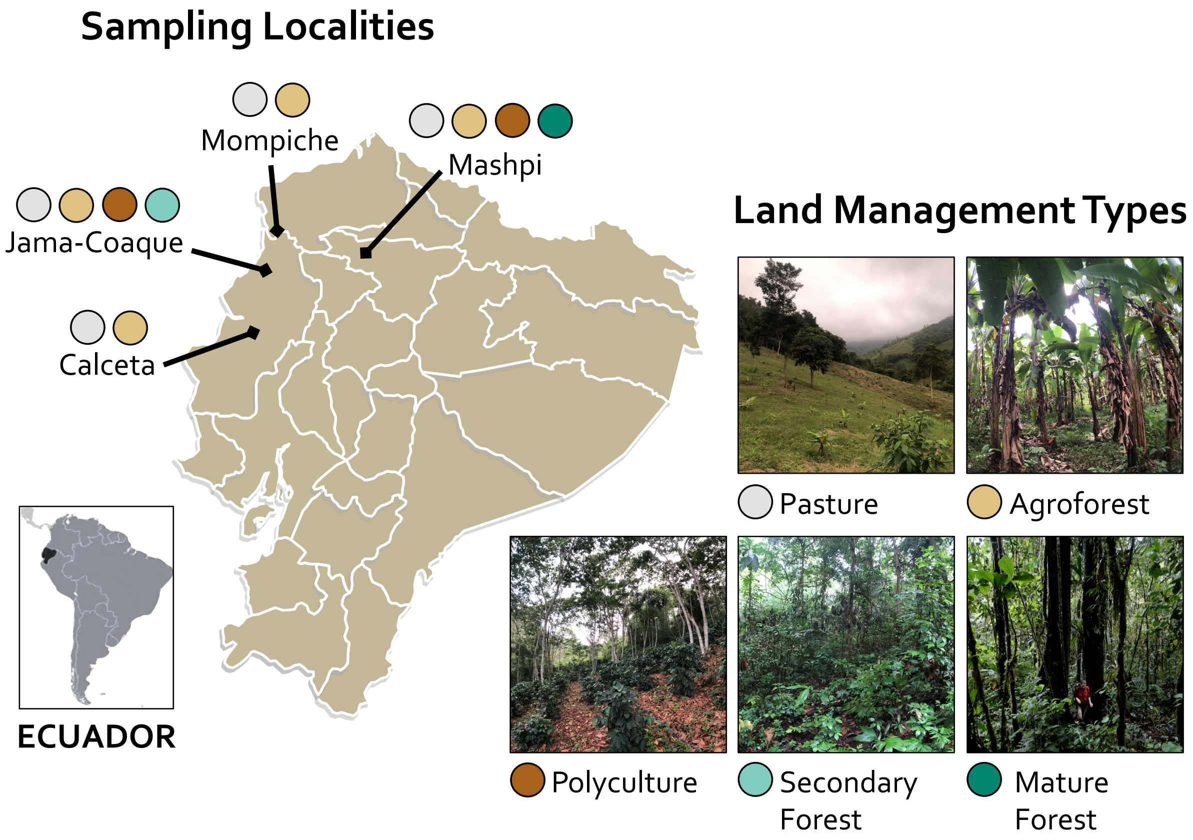


FIGURE 2. *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*) PREVALENCE BY LAND MANAGEMENT TYPE AND ANURAN FAMILY

Left y-axis shows *Bd* prevalence (number of infected individuals divided by the total number of captured individuals) per land management type. Mean prevalence and 95% confidence intervals per land-use type are shown in red within each bar. Mean prevalence across all five management types is depicted with the red dotted line. Right y-axis shows the number of frogs captured in each management type, color-coded by family.

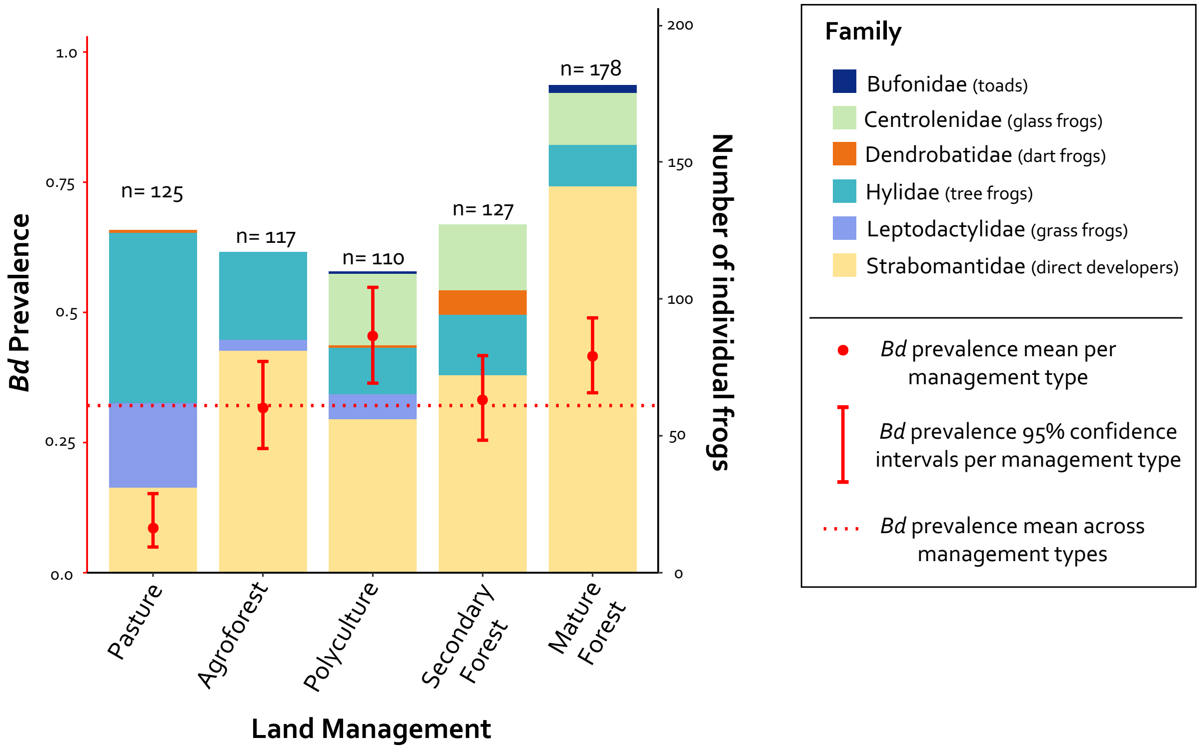


FIGURE 3. *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*) PREVALENCE, LIFETIME AQUATIC INDEX (LAI), AND LAND MANAGEMENT

Bd prevalence across species within each lifetime aquatic index (LAI) **(A)** and across land management types for **(B)** direct developers (aquatic index 0), which do not need to interact with water bodies at any point in their life cycle, **(C)** glassfrogs (aquatic index 1), which do not contact water bodies as eggs or adults, but that have aquatic tadpoles, and **(D)** frogs with aquatic tadpoles that also require water for breeding and laying eggs (aquatic index 2). Mean *Bd* prevalence is depicted by a red dotted line and 95% confidence intervals are shown in red within each bar.

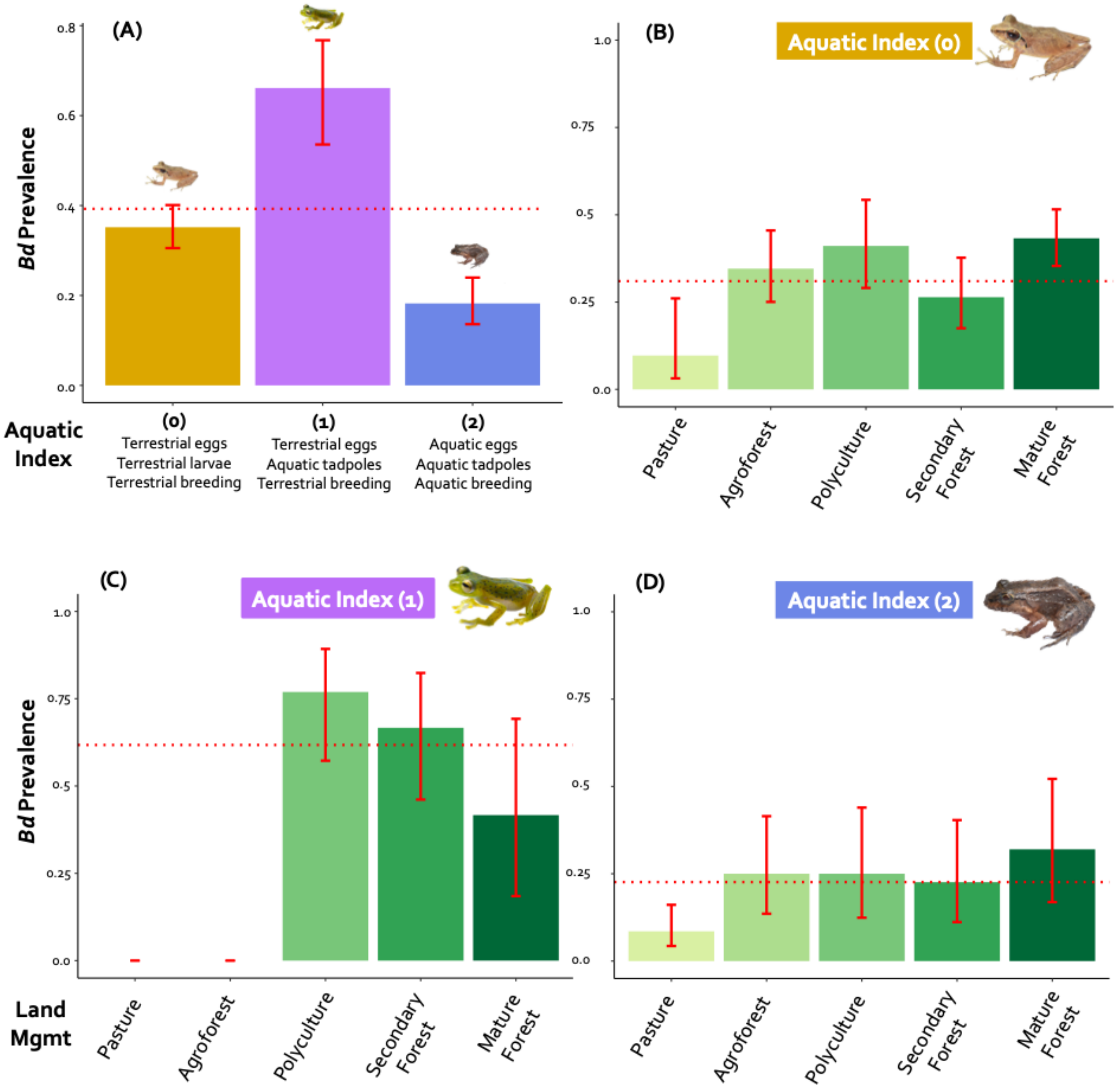


FIGURE 4. *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*) PREVALENCE, BODY SIZE, AND LAND MANAGEMENT

Body size (snout-to-vent length; SVL) relative to *Bd* prevalence (left) and land management (right). Larger frogs are more likely to remain uninfected and to be found in pastures. Yellow dots represent individual means; black dotted line represents population mean. Within each boxplot, horizontal lines represent the median and vertical lines represent the maximum and minimum values (excluding outliers). Results from non-parametric statistical tests are provided above each plot; p-values indicate that the means are significantly different in both tests. In the left plot, black stars denote level of significance.

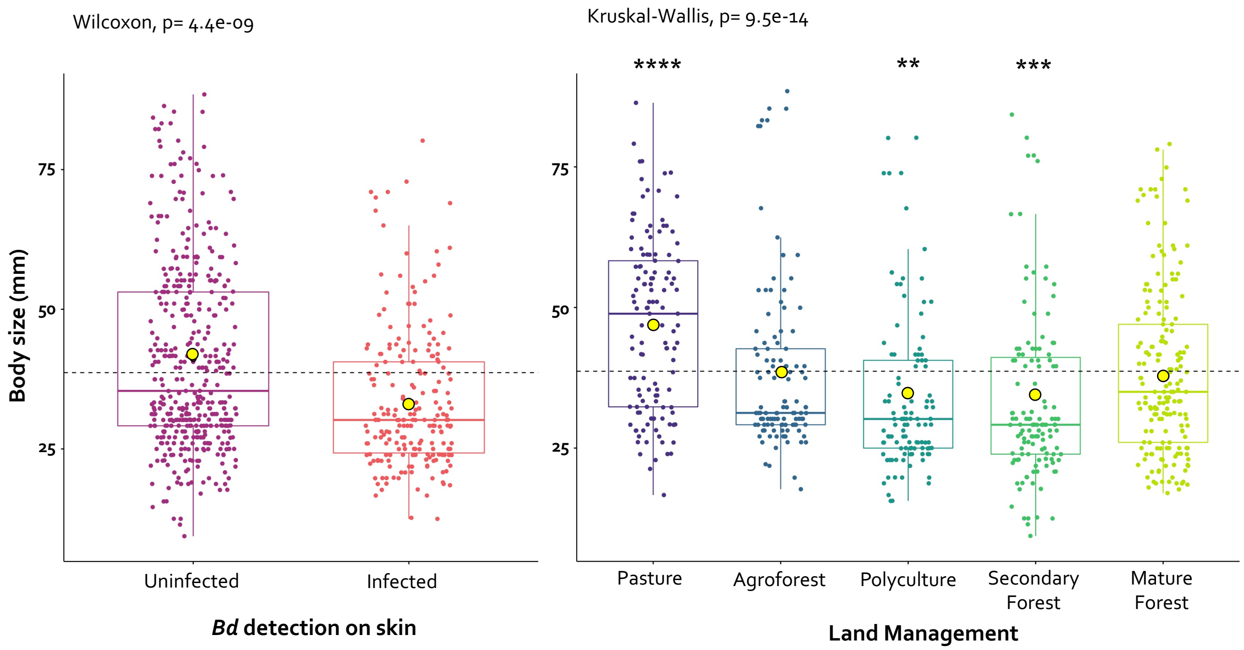


TABLE 1. LAND MANAGEMENT CLASSIFICATION CRITERIA

Criteria used to classify land management type in this study (adapted from Philpott et al. 2008), with photographic examples of canopy cover variability. Rows in the last column contain the low, medium, and high ranges of canopy cover for that management type.

Land-use Type	Tree Composition	Main Crop(s)	Shade Strata	Management Level	Canopy Cover Variability within the Same Land-use Type
Pasture	Isolated patches, sparse canopy	Grass (cows) ± sparse banana ± pineapple	0-1	Regular removal of plants (grass competitors); cow damage; daily to weekly activity	 5%  25%  55%
Shade-grown Agroforest	Mostly planted canopy trees	Coffee ± cacao ± banana ± fruit trees ± timber trees	1-2	Canopy pruning; occasional epiphyte removal; weekly human activity	 30%  65%  75%
Rustic Polyculture	Mostly native forest canopy	Coffee ± cacao ± banana	3	Minimal canopy intervention; weekly human activity	 60%  75%  90%
Secondary Forest	Native forest canopy, some pioneers and second-growth species	N/A	2-3	Minimal human activity; some reforestation activities	 55%  60%  80%
Mature Forest	Native forest canopy	N/A	3	Isolated from most human intervention	 60%  75%  85%

TABLE 2. *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*) PREVALENCE AND NATURAL HISTORY TRAITS FOR EACH SPECIES IN THIS STUDY

Details of Ecuadorian frog species sampled in this study, including *Batrachochytrium dendrobatidis* (*Bd*) prevalence and relevant natural history traits. **SVL**= snout-to-vent length, including size range of individuals captured in this study and the mean in parentheses; **Lifetime Aquatic Index** = 0 represents species that do not contact water bodies to breed (direct developers), 1 represents species that do not contact water bodies as eggs or adults, but that have aquatic tadpoles (glassfrogs), and 2 represents species that breed and lay eggs directly in the water with aquatic tadpoles; ***Bd* Positive/Total** = number of individuals detected positive for *Bd* over the total number of individuals captured; **Prevalence** = percentage of individuals that tested positive for *Bd*, with 95% confidence intervals (binomial distribution) in parentheses. **Management Type(s)** = land-use(s) where each species was found in this study (P = Pasture; A = Agroforest; R = Rustic Polyculture; S = Secondary Forest; M = Mature Forest). Percentages in parentheses indicate the proportion of individuals found in each management type.

Family	Species	Snout-to-vent length (mm)	Lifetime Aquatic Index	*Bd* Positive/ Total	*Bd* Prevalence (95% CI)	Mgmt Type(s)
Bufonidae	*Rhaebo haematiticus*	72.85-79.07 ($\bar{x}$ = 76)	2	1/4	25% (3-76%)	R (25%) M (75%)
Centrolenidae	*Espadarana prosoblepon*	22.36-27.54 ($\bar{x}$ = 24.7)	1	27/39	69% (53-82%)	R (49%) S (36%) M (15%)
	Hyalinobatrachium aureoguttatum	18.72-20.79 ($\bar{x}$ = 20)	1	2/5	40% (10-80%)	M (100%)
	Hyalinobatrachium mashpi	21.8	1	0/1	0% (0-98%)	M (100%)
	Hyalinobatrachium [fleischmanni] tatayoi	20.81-24.94 ($\bar{x}$ = 22.4)	1	8/13	62% (34-83%)	R (31%) S (69%)
	Sachatamia albomaculata	22.9-26.04 ($\bar{x}$ = 24.5)	1	4/4	100% (40-100%)	R (100%)
	Sachatamia orejuela	29.0-33.32 ($\bar{x}$ = 32.1)	2	1/5	20% (3-69%)	M (100%)
Dendrobatidae	*Epipedobates boulengeri*	16.7	2	1/1	100% (3-100%)	P (100%)
	Hyloxalus awa	12.48-20.78 ($\bar{x}$ = 17.8)	2	8/10	80% (46-95%)	R (10%) S (90%)
Hylidae	*Boana pellucens*	37.45-62.45 ($\bar{x}$ = 46.4)	2	10/35	29% (16-45%)	P (49%) A (37%) R (14%)
	Boana picturata	48.89-69.0 ($\bar{x}$ =58.9)	2	0/2	0% (0-84%)	M (100%)
	Boana rosenbergi	75.96-88.46 ($\bar{x}$ =81.3)	2	1/10	10% (1-47%)	P (10%) A (40%) R (10%) S (40%)
	Hyloscirtus mashpi	21.0-39.0 ($\bar{x}$ = 31.84)	2	4/11	36% (8-64%)	M (100%)

Family	Species	Snout-to-vent length (mm)	Lifetime Aquatic Index	*Bd* Positive/ Total	*Bd* Prevalence (95% CI)	Mgmt Type(s)
	Scinax quinquefasciatus	32.25-38.49 ($\bar{x}$ = 35.2)	2	2/13	15% (4-45%)	P (100%)
	Scinax tsachila	25.99-38.5 ($\bar{x}$ = 30.9)	2	3/6	50% (17-83%)	P (33%) A (67%)
	Smilisca phaeota	31.43-80.14 ($\bar{x}$ = 57.3)	2	5/49	10% (4-22%)	P (31%) A (16%) R (20%) S (31%) M (2%)
	Trachycephalus jordani	45.77-99.91 ($\bar{x}$ = 76.1)	2	1/19	5% (1-29%)	P (58%) A (16%) R (5%) S (16%) M (5%)
	Trachycephalus [typhonius] quadrangulum	64.53-72.82 ($\bar{x}$ = 67.3)	2	0/3	0% (0-71%)	P (100%)
Leptodactylidae	*Leptodactylus labrosus/ ventrimaculatus*	34.34-73.98 ($\bar{x}$ = 48.5)	2	1/44	2% (0-14%)	P (70%) A(10%) R (20%)
Strabomantidae	*Barycholos pulcher*	22.88-30.2 ($\bar{x}$ = 27.4)	0	3/10	30% (10-62%)	P (40%) S (60%)
	Pristimantis achatinus	12.49-52.82 ($\bar{x}$ = 32.6)	0	79/237	33% (25-37%)	P (11%) A (34%) R (22%) S (25%) M (8%)
	Pristimantis colomai	19.0	0	0/1	0% (0-98%)	M (100%)
	Pristimantis sp. nov. [jamacoaquensis]	24.99-33.33 ($\bar{x}$ = 29.2)	0	0/2	0% (0-84%)	S (100%)
	Pristimantis latidiscus	18.73-43.0 ($\bar{x}$ = 30.5)	0	8/17	47% (23-71%)	S (6%) M (94%)
	Pristimantis [labiosus] lipsus	18.0-71.0 ($\bar{x}$ = 40.3)	0	35/92	38% (28-48%)	M (100%)
	Pristimantis luteolateralis	15.6-19.0 ($\bar{x}$ = 16.1)	0	2/4	50% (0-98%)	R (50%) M (50%)
	Pristimantis mindo	18.71-36.43 ($\bar{x}$ = 27.6)	0	1/2	50% (6-94%)	M (100%)
	Pristimantis muricatus	17.0-43.74 ($\bar{x}$ = 30.37)	0	0/2	0% (0-84%)	M (100%)
	Pristimantis ornatissimus	40.0	0	0/1	0% (0-98%)	M (100%)
	Pristimantis subsigillatus	23.92-69.73 ($\bar{x}$ = 24.3)	0	1/3	33% (4-85%)	M (100%)
	Pristimantis walkeri	9.38-26.02 ($\bar{x}$ = 17.3)	0	3/8	38% (13-72%)	R (38%) S (62%)

TABLE 3. BAYESIAN PATH ANALYSIS RESULTS

Predictors of body size (snout-to-vent length, SVL) (Model 1.1) and *Batrachochytrium dendrobatidis* (*Bd*) prevalence (Model 1.2) listed with parameter estimates, upper and lower 95% confidence intervals, bulk effective sample size, and proportion of posterior samples >/< 0. Species and month were included as random effects in the models. ** = strong support, * = support. Interpretations are provided in the last column.

	Variable	Estimate	Standard Error	Lower 95%	Upper 95%	Bulk_ESS	p > 0	p < 0	Interpretation
Model 1.1 **Body Size (SVL)**	Pastures vs. Agroforests	-0.1	0.08	-0.26	0.07	4921.59	0.12	0.88*	Frogs are likely to be smaller in agroforests (compared to pastures)
	Pastures vs. Polycultures	-0.09	0.09	-0.27	0.08	4444.98	0.15	0.85*	Frogs are likely to be smaller in polycultures (compared to pastures)
	Pastures vs. Secondary Forests	-0.05	0.09	-0.23	0.13	4881.35	0.29	0.71	
	Pastures vs. Mature Forests	0.15	0.12	-0.09	0.39	4924.49	0.78	0.22	
	Direct Developers (LAI 0) vs. Glassfrogs (LAI 1)	0.01	0.5	-0.95	1	1529.97	0.51	0.49	
	Direct Developers (LAI 0) vs. Water Breeders (LAI 2)	1.2	0.39	0.42	1.98	929.31	1**	0	Water breeders (LAI 2) are likely to be larger (compared to direct developers, LAI 0)
Model 1.2 ***Bd* Prevalence**	Pastures vs. Agroforests	1.79	0.75	0.35	3.31	2225.38	0.99**	0.01	Frogs are more likely to be infected in agroforests (compared to pastures)
	Pastures vs. Polycultures	2.29	0.88	0.63	4.07	2145.63	1**	0	Frogs are more likely to be infected in polycultures (compared to pastures)
	Pastures vs. Secondary Forests	2.02	0.87	0.38	3.76	2211.72	0.99**	0.01	Frogs are more likely to be infected in secondary forests (compared to pastures)
	Pastures vs. Mature Forests	1.66	0.99	-0.24	3.62	2706.19	0.95**	0.05	Frogs are more likely to be infected in mature forests (compared to pastures)
	Forest Adjacent to Management Type (y/n)	0.25	0.45	-0.61	1.18	7823.76	0.71	0.29	
	Elevation	-0.09	0.32	-0.72	0.53	8283.93	0.38	0.62	
	Canopy Cover	-0.43	0.32	-1.05	0.18	2383.78	0.09	0.91*	Frogs are less likely to be infected in areas with lower canopy cover
	Average Monthly Temperature	-1.33	0.47	-2.28	-0.41	4974.7	0	1**	Frogs are less likely to be infected in areas with higher temperatures
	Average Monthly Precipitation	-0.3	0.24	-0.78	0.19	5378.42	0.11	0.89*	Frogs are less likely to be infected in areas with lower precipitation
	Arboreal vs. Mixed	1.04	0.7	-0.28	2.52	3381.85	0.94*	0.06	Frogs that occupy mixed strata are more likely to be infected than arboreal frogs
	Arboreal vs. Terrestrial	0.97	0.75	-0.42	2.55	3524.31	0.92*	0.08	Terrestrial frogs are more likely to be infected than arboreal frogs
	Direct Developers (LAI 0) vs. Glassfrogs (LAI 1)	1.92	0.85	0.35	3.72	3485.79	0.99**	0.01	Glassfrogs (LAI 1) are more likely to be infected than direct developers (LAI 0)
	Direct Developers (LAI 0) vs. Water Breeders (LAI 2)	0.11	0.62	-1.09	1.37	3886.44	0.56	0.44	
	Body Size (SVL)	-0.16	0.15	-0.45	0.13	5799.37	0.04	0.96**	Larger frogs are less likely to be infected

Chapter 3. Soundscapes of conservation: call characteristics influence disturbance tolerance in Ecuadorian frog species

ABSTRACT

What if an animal's call influences whether it can tolerate land-use change? A large body of research has demonstrated that human-generated noise such as traffic and oil drilling have negative effects on animals that rely on acoustic communication. These studies provide evidence that sound plays an important role in habitat suitability; however, noise is only one component of an acoustic space (i.e., soundscape). Soundscapes are created through the interactions of structures (e.g., vegetation) and abiotic factors (e.g., wind) that can either impede or enhance the propagation of sound waves. We hypothesize that the physical changes attending deforestation can cause a habitat to become acoustically inhospitable for the calls of some species, rendering them unable to effectively communicate—and by extension, unable to breed. In this study, we experimentally test how different sound frequencies attenuate in pastures, agroforests (e.g., coffee, cacao) and forests throughout Ecuador. We also test the hypothesis that frog species with higher-pitched calls are more likely to be found in disturbed habitats. This pattern held true across Ecuadorian species in Hylidae (treefrogs) and Strabomantidae (rainfrogs; we found the opposite pattern for species in Centrolenidae (glassfrogs). Overall, this work highlights the need to consider the acoustic environment when assessing a species' vulnerability to habitat disturbance.

INTRODUCTION

Species across many different taxa (e.g., frogs, birds, arthropods, mammals) use vocalizations or substrate-borne vibrations to communicate. Acoustic signals can affect crucial aspects of a species' fitness: obtaining a mate (Welch et al. 1998), intercepting resources (Kalan et al. 2015), maintaining social cohesion (Ryan et al. 1981), etc. An immense diversity of acoustic signals exists, even distinguishing among congeners and sub-species; much of this variation has evolved to promote species recognition and/or cater to mate preferences (Gerhardt 1991). However, another selective force on acoustic signals has been vastly overlooked until recently: the environment.

Soundscape ecology (Pijanowski et al. 2010) and ecoacoustics (Farina & Gage 2017) are relatively new fields that investigate how structural and abiotic factors of a habitat influence how sounds are transmitted, and in turn, how transmission effectiveness influences the evolution of call characteristics. For example, higher frequency airborne calls tend to transmit more efficiently in open spaces like prairies compared to dense forests. Higher frequencies attenuate much faster than low frequency calls because shorter wavelengths are more vulnerable to reverberation (bouncing off objects, usually resulting in a loss of energy), which is minimized in open areas with less vegetation (Farina 2013). One landmark study of 177 bird species (Morton 1975) found that forest birds had an average dominant frequency (frequency with the highest amplitude) of 2.5 kHz, whereas grassland birds had a much higher average of 4.5 kHz. There is even evidence that the environment promoted call divergence between two frog subspecies that occur in habitats with different vegetation densities (Ryan & Wilczynski 1990).

The acoustic adaptation hypothesis (Ey & Fischer 2009; Mullet et al. 2017) postulates that the elements of a habitat differentially affect the transmission of acoustic signals depending on their characteristics (e.g., frequency, rate), which in turn can promote call divergence among species—perhaps even as a form of niche differentiation. Dominant frequencies and other call characteristics have evolved as a result of the interaction between an animal and its environment to maximize the efficiency of an emitted sound (Farina 2013). This is particularly relevant for anuran calls: because frog calls are not learned, call characteristics such as dominant frequency and pulse rate are largely species-specific attributes and are not as plastic over an individual's lifetime compared to birds and mammals (Cocroft & Ryan 1995). Birds, for instance, have been found to increase the frequency of their calls to communicate over lower-frequency traffic noise (Nemeth et al. 2013). In contrast, most frog species and individuals must rely on a change in amplitude, timing, and/or calling position to be heard over acoustic disturbance (Bee & Swanson 2007). Given this lack of plasticity, anuran advertisement calls (e.g., calls emitted to attract a mate) are more likely to require an acoustic space resembling that in which their call evolved.

Investigating how soundscapes drive evolution likely has crucial conservation implications for animals that communicate with sound, especially frogs. There is growing evidence that fragmentation alters the soundscape of a habitat (e.g., Laiolo 2010). Compatibility of vocalizations within a new vegetation structure may be a missing factor in assessments of species tolerance for disturbance, especially on finer spatial scales—the relevant scale for critical social interactions. Land transformation inherently changes the spatial aspects of the landscape by altering the density and position of trees (from forest to pasture, for instance), which in turn modifies the acoustic environment. Consequently, habitat change could cause new habitats to become unsuitable for some species if the new soundscape causes certain species' calls to propagate with significantly less efficiency—even if other resources such as nesting sites or food remain. For example, if female frogs are unable to hear or successfully locate calling males, they will not mate, which would have negative individual fitness and population level effects. Habitat loss is also likely to drive the evolution of new or altered calls.

For its size, Ecuador has the highest annual deforestation rate in the Western Hemisphere; most Ecuadorian forests are converted for agricultural uses (Global Forest Watch 2022). Yet Ecuador also has the highest diversity of herpetofauna in the world per acre (CI 2019). While few Ecuadorian frog species can survive complete habitat destruction, many species can tolerate shaded working landscapes such as agroforests (e.g., cacao, banana, coffee) (Brunner et al., Chapter 2). The traits and habitat elements that differentiate disturbance-tolerant species from those only found in pristine habitats remains mostly unknown. Better understanding the factors that drive this distinction could help inform future conservation management decisions.

In this paper, we sought to answer two questions: 1) How efficiently do various frequencies propagate along a gradient of human disturbance? We hypothesized that higher frequencies would propagate more effectively in pastures compared to forests and agroforests and that lower frequencies would propagate best in forested environments, consistent with previous research (e.g., Laiolo 2010). 2) Are frog species with certain call characteristics more likely to be found in human-dominated landscapes (e.g., pastures, roadsides)? We hypothesized that species with higher frequency calls would be more likely to be found in disturbed (e.g., more open) habitats.

METHODS

Acoustic experiment

We conducted acoustic experiments in two localities in Western Ecuador where forest is surrounded by agroforests and pastures. The first locality, Jama-Coaque Reserve (JCR; 0°06'29.5"S 80°07'06.5"W; ~200-700m elevation range in this study), conserves a crucial portion of the last remaining coastal moist evergreen forests and premontane cloud forests of the Tumbes-Chocó-Magdalena biodiversity hotspot and is situated among agricultural communities. The second locality, Mashpi (0°09'57.9"N 78°52'45.5"W; ~500-1000m elevation range in this study), includes a forest reserve that conserves a large stand of mature rainforest within the Tropical Andes biodiversity hotspot. Its neighboring community of the same name hosts a locally operated sustainable agroforestry and reforestation network, including Reserva/Bosque Escuela Pambiliño, Reserva Mashpi Shungo, and Reserva Chontaloma. At each locality, we conducted acoustic experiments in 10 forest sites, 8 agroforest sites, and 8 pasture sites (20 forests, 16 agroforests, and 16 pastures in total). Before conducting each experiment, we measured canopy cover. Each experiment was conducted at night, between the months of January-May (the rainy season). Sites at each locality were at least 500m apart.

At each site, we identified the flattest area closest to the center of the habitat. The setup consisted of one speaker (Sony SRS-XB31)—which represented a calling male frog—and eight microphones (Audiomoths, Open Acoustic Devices 2019)—which represented listening females (Figure 1A). We measured a 35m line with a meter tape, placing the speaker at 0m. We then placed a pair of microphones at 5m, 15m, 25m, and 35m from the speaker, one on the ground and the other 2m above ground (Figure 1C). To begin the experiment, we played a broadcast with the speaker on the ground. The broadcast consisted of three elements: a pure tone, a frequency sweep from 20 Hz to 20 kHz, and another pure tone (Figure 1B). We then played the same broadcast again, but with the speaker 2m above the ground. To account for structural variability within the same site, we repeated this methodology perpendicular to the first line (within the same site). We therefore completed four broadcasts at each site. Microphones recorded at a sampling rate of 44.1 kHz and were randomized (in terms of height and placement along the transect) for each experiment.

We extracted frequency data from each microphone recording via Fast Fourier Transform (FFT) analyses using the Python package Librosa (McFee et al. 2015). We matched FFT size to our sample rate (44100) and averaged the amplitudes of each frequency bin to obtain a single spectrogram for each recording. We compared and averaged spectrogram data using the classes AudioAnalyzer and SpetrumCompare (Dizon 2020). Soundscape characterization was limited to a description and no statistical tests due to equipment limitations (see Discussion).

Frog data & call analyses

We obtained as many Ecuadorian frog species call recordings as were publicly available for Centrolenidae, Hylidae, and Strabomantidae; we downloaded calls from the museum collections of Universidad San Francisco de Quito (USFQ) and Pontificia Universidad Católica del Ecuador (PUCE), as well as Fonoteca Zoológia from the Museo Nacional de Ciencias Naturales of Madrid. We also received some recordings from private collections, and a few were recorded by the author (RMB). Unfortunately, calls remain undescribed or unrecorded for many species, so we were unable to analyze calls for every Ecuadorian species in each family. We were only able to obtain one recording per species (with very few exceptions).

We analyzed calls using the Raven sound analysis software (Cornell Lab of Ornithology). Measurements and definitions of acoustic variables follow Köhler et al. (2017). Calls were divided into two classes—pulsatile or tonal—based upon distinct waveforms in the oscillogram. Pulsed notes have one or more clear amplitude peak(s) and amplitude modulation (i.e., increases and decreases in amplitude throughout the call). Tonal notes have no clear amplitude peak (Dautel et al. 2011). Harmonics (multiples of the fundamental frequency) were noted when observed, although poor recording quality can mask their presence.

To obtain natural history data pertaining to each species' tolerance to disturbance, we used species-habitat data collected for Chapter 2 and natural history resources for species that were not familiar to the author, including biodiversity databases (e.g., AmphibiaWeb, BioWeb) and field guides (e.g., Arteaga et al. 2013; Lynch & Duellman 1997). When a species was listed as being found in human-dominated areas (e.g., near houses or roads) or heavily converted landscapes (e.g., pastures or other non-shared agricultural areas)—even if it is also found in pristine habitats—we categorized that species as 'disturbance tolerant'. Species that are only found in primary or secondary forest (or if habitat adaptability was not specified) were categorized as 'not disturbance tolerant'.

Statistics

We ran Bayesian linear mixed models to investigate whether species with certain call characteristics were more likely to be found in natural vs. altered habitats, with family as a random effect. We also ran Bayesian linear models for each frog family separately. Each model tested the direct effects of disturbance tolerance (0 or 1) on dominant frequency, maximum frequency, minimum frequency, frequency range, and call duration. Using the statistical modeling platform Stan via the R 4.1.0 (R Core Team 2022) package brms (Buerkner 2017), we ran three Markov chain Monte Carlo (MCMC) chains for 10^4 iterations after an initial burn-in of 10^2 iterations. We used standard practices for assessing convergence, including chains, effective sample size, and Rhat values.

RESULTS

Averaged broadcast experiment results for each habitat (Figure 2) showed different attenuation patterns for low (0-2 kHz), mid-range (3-4 kHz), and high (5-8 kHz) frequencies. Mid-range frequencies propagated effectively in all habitat types, low frequencies propagated effectively in agroforests and forests, and high frequencies propagated most effectively in pastures (and least effectively in agroforests).

We analyzed 164 frog recordings (Table 1). Among calls for species in Centrolenidae (Figure 3A), the mean dominant frequency was 5.4 kHz (1.1 SD), the mean maximum frequency was 5.9 kHz (1.2 SD), and the mean frequency range was 1.2 kHz (0.9 SD). For Hylidae calls (Figure 3B), the mean dominant frequency was 2.0 kHz (1.0 SD), the mean maximum frequency was 3.0 kHz (1.4 SD), and the mean frequency range was 1.8 kHz (1.2 SD). Calls in Strabomantidae (Figure 3C) had a mean dominant frequency of 3.0 kHz (0.96 SD), a mean maximum frequency of 3.8 kHz (1.3 SD), and a mean frequency range of 1.4 kHz (0.93 SD). The mean call durations (Figure 4) were 0.3 s (0.37 SD) for Centrolenidae, 0.6 s (1.5 SD) for Hylidae, and 0.5 (0.3 SD) for Strabomantidae.

Tables 2-5 provide the results from our Bayesian GLMMs. All Rhat values were 1. When all species were considered together, the models provided strong evidence that calls with a higher dominant frequency, a higher maximum frequency, and a wider frequency range are more likely to tolerate disturbance. The Hylidae (Model 3, Table 4) models showed the same pattern for all three characteristics: dominant frequency, maximum frequency, and frequency range. Strabomantidae (Model 4, Table 5) showed the same pattern for dominant frequency; duration was the only other significant variable, suggesting that species with longer calls are more likely to be tolerant to disturbance. However, the Centrolenidae models (Model 2, Table 3) showed contradictory results compared to the others: glassfrogs that have calls with a higher dominant frequency are *less* likely to tolerate disturbance.

DISCUSSION

This study provides compelling evidence that: 1) land-use change affects the propagation of biologically relevant sound frequencies, and 2) advertisement call characteristics affect whether certain frog species are found in human-altered habitats. Our hypothesis that species with higher frequency calls would be more likely to be found in disturbed habitats was supported both experimentally and statistically for models in which all species were considered together, as well as for species separated by family in Hylidae (treefrogs) and Strabomantidae (rainfrogs). These findings make sense, given that high-pitched sounds are more susceptible to reverberation and undisturbed habitats have more vegetation structure. Even in natural environments, species with higher-pitched calls are more likely to call from open areas such as clearings caused by tree falls or wide riverbeds (e.g., Bosch & De la Riva 2004). It follows, then, that males from those same species could successfully communicate in open areas caused by land-use change.

However, we found the opposite pattern for Centrolenidae: glassfrog species that call with a higher dominant frequency were less likely to be found in disturbed habitats. We have identified two main explanations for this difference in pattern: a potential reliance on reverberation for propagation, and/or an adaptation to the noise levels along streams, over which almost every glassfrog species calls.

Glassfrog calls are high-pitched compared to most other frog species. In this study, the mean dominant frequency for glassfrog calls was 5.4 kHz, which was much higher than the mean dominant frequency for treefrogs and rainfrogs (2.0 and 3.0 kHz, respectively). The upper range of frequencies for Hylidae and Strabomantidae calls are in the lower range for Centrolenidae calls (Figure 3). Previous studies examining the effects of soundscape on bird calls cite 'high' frequencies for open-habitat species as <4 kHz and 'low' frequencies for forest species as <2 kHz (e.g., Morton 1995). Thus, by multiple measures, glassfrog calls have comparatively high frequencies. While relatively high frequencies do not propagate well in forested environments, there may be a threshold over which very high frequencies cease to propagate efficiently in any habitat *without* reverberation. Constructive interference (Figure 5) occurs when two sound waves occupy the same space (in this case, sounds emitted from the same individual reverberating off a surface) and the peaks are aligned in such a way that enhances the amplitude (Bradbury & Vehrencamp 2011). Many glassfrog species, especially in the genus *Hyalinobatrachium,* call upside down with their vocal sacs facing their leaf perch (e.g., Guayasamin & Brunner et al. 2022; Chapter 5), which could be a behavioral adaptation to enhance amplitude via constructive interference (via reverberation off the leaf). Precedence for behavioral amplitude enhancement in other species exists. For example, Bornean tree-hole frogs (*Metaphrynella sundana*) actively exploit the acoustic properties of their tree cavities, tuning their calls to the resonant frequency of the hole (Lardner & bin Lakim 2002). Glassfrogs could therefore rely on the vegetation structures, both on a microhabitat and larger habitat scale, to effectively propagate their calls. These structures may be more reliably common in undisturbed habitats; this theory requires further investigation, through a combination of experimental and in situ tests.

A second explanation for the association between high-pitched glassfrog calls being associated with undisturbed habitat may be related to another acoustic element of a habitat: noise. Species have adapted to natural sources of noise for millennia, whether biological (e.g., interference from other species like cicadas) or geophysical (e.g., rushing water). The acoustic adaptation hypothesis (Ey & Fischer 2009; Mullet et al. 2017) postulates that signals have evolved optimal characteristics for transmission in the sender's native environment. My coauthors and I have found evidence of this in my own field research with *Sachatamia orejuela*, an elusive glass frog species with an extreme habitat: roaring waterfalls. We discovered that *S. orejuela* has the *highest* frequency call of any species in Centrolenidae by two orders of magnitude (~100 Hz) (Brunner & Guayasamin 2020; Chapter 4), despite being double the size of the average glassfrog (larger body size is usually correlated with *lower* frequency calls; Gringras et al. 2013). The acoustic niche of *S. orejuela* likely holds the answer to this exception: only a high-pitched call can be detected by a female over the noise of their specialized waterfall habitats. Although most glassfrog species do not call from waterfalls, many call near rushing water (females lay eggs on leaves directly over streams) (Guayasamin et al. 2020), which produces sound in a mixture of frequencies. To be heard over that noise, those species may have evolved higher-pitched calls. Since streams with more waterflow are more likely to be found in undisturbed habitats (Davies et al. 2008; Bunn et al. 1999), the glassfrog species associated with them are likely to be as well.

Another unexpected result from this study was that rainfrog species with longer call durations were more likely to be found in disturbed areas (Table 5). The information in longer notes is more likely to be lost to wind and noise in open areas (Naguib 2003). There is also evidence that reverberations improve the sound transmission of longer notes (Nemeth et al. 2006). Many examples of these trends exist for birds; for example, grassland thrush species (e.g., *Turdus fumigatus*) have shorter calls compared to their forest counterparts (e.g., *Turdus albicollis*) (Farina 2013). However, frog calls are generally much simpler and shorter in comparison to birds, especially passerines (Wells 2001). In some cases, males may prioritize simply being heard by a female over details of the call being conveyed. Strabomantidae calls also tend to be repetitive (Lynch & Duellman 1997), which also increases the likelihood of being heard.

Unfortunately, we are unable to expound on our experimental results. After we completed fieldwork, we discovered anomalies in the data that we later attributed to the speaker emitting the broadcasts with inconsistent energy/power. Equipment malfunctions are a risk in fieldwork, especially in extremely wet places like cloud forests. We currently do not know if differences in magnitude were a result of habitat variability or our equipment. However, we decided to share the general attenuation patterns (Figure 2) for each habitat in case they are useful for future tests of this nature. If at least some of the variability in the data can be attributed to the environment rather than the equipment, then there are interesting differences in sound propagation in agroforests that warrant further investigation.

Overall, this work highlights the need to consider the acoustic environment when assessing a species' vulnerability to habitat disturbance. If the ability to effectively broadcast calls influences which species are tolerant to land-use change, we may eventually be able to recreate the acoustic space of sensitive species in working landscapes.

FIGURE 1. BROADCAST EXPERIMENT

(A) In this experiment, the speaker broadcast represented a calling male frog, while the microphones represented listening females at different distances. **(B)** Example of a broadcast recording used for every run consisted of three elements: a pure tone, a frequency sweep from 20 Hz to 20 kHz, and another pure tone. Other elements present in this recording represent natural sounds commonly encountered in each site (e.g., calling cicadas). **(C)** The experimental design consisted of two runs: Run 1, where the speaker broadcast (B) from the ground (0 m) and Run 2, where the speaker broadcast (B) from 2m above ground. Microphones were placed at four distances away from the speaker (5m, 15m, 25m, and 35m), one on the ground (0m) and one 2m above the ground at each distance, for a total of eight. This experiment was repeated at 10 sites per habitat type (pasture, agroforest, and forest), for a total of 30 sites and 60 runs. Illustrations by Natalia Golovanova.

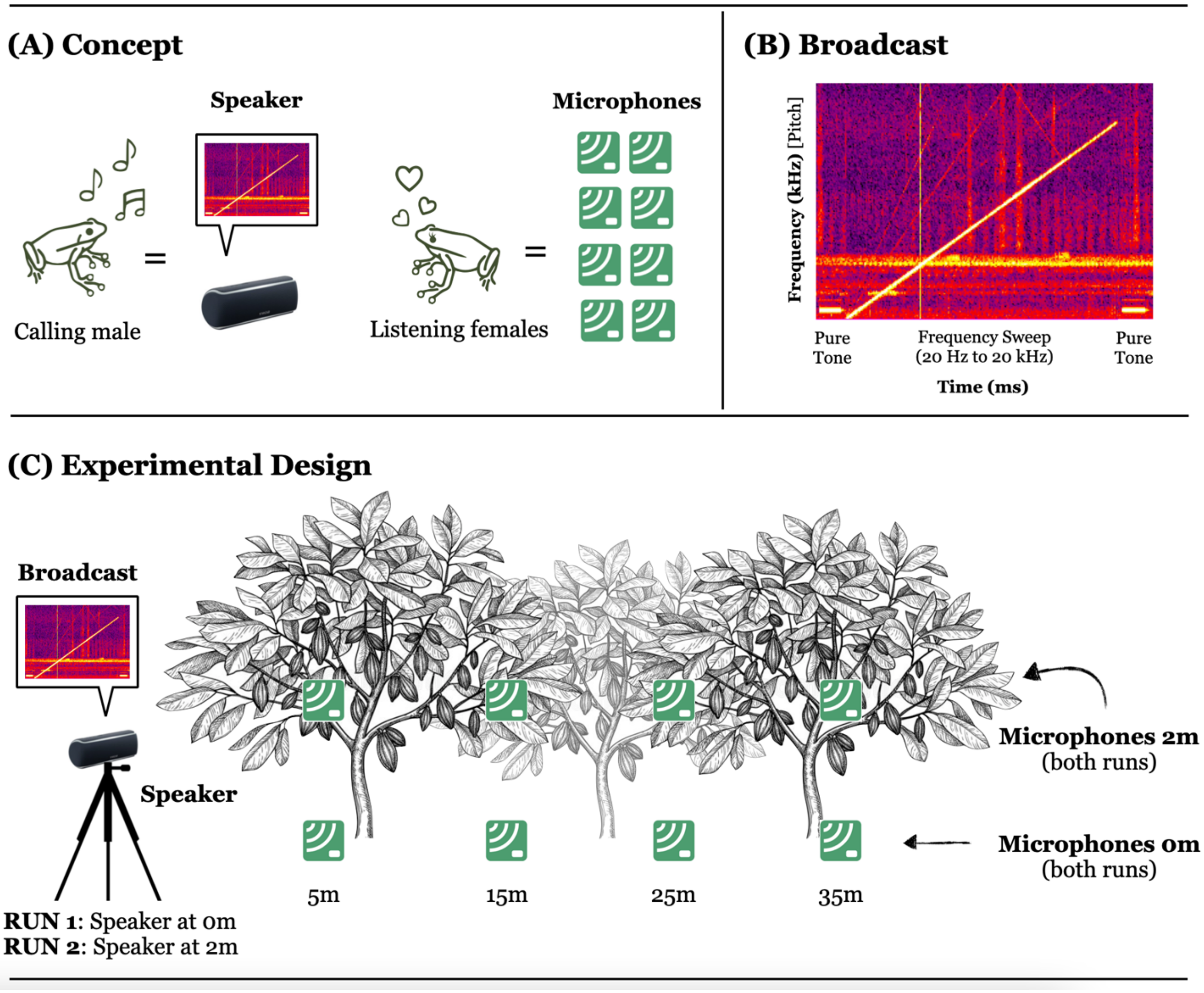


FIGURE 2. RESULTS FROM BROADCAST EXPERIMENT

Frequency attenuation averaged across all sites per habitat type: **(A)** pastures, **(B)** agroforests, and **(C)** forests. Blue lines represent average attenuation from microphones 15m away from the speaker per habitat, pink lines represent average attenuation from microphones 25m away from the speaker per habitat, and red lines represent attenuation from microphones 35m away from the speaker per habitat. Grayscale rectangles represent biologically relevant frequency bins for this study: low frequencies (black), mid-range frequencies (dark gray), and high frequencies (light gray). These results represent a preliminary description of the soundscape in each habitat, limited by broadcast inconsistencies with our equipment.

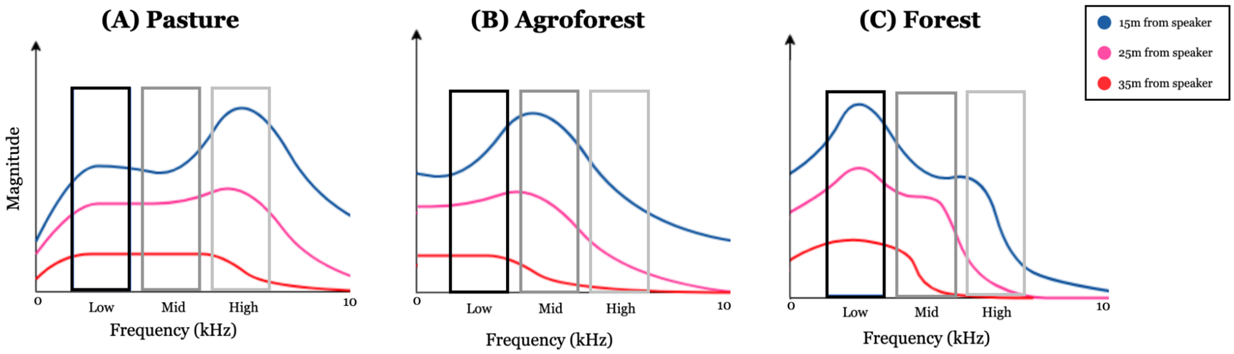


FIGURE 3. COMPARISON OF CALL FREQUENCY METRICS BY DISTURBANCE TOLERANCE

Comparison of dominant frequency (frequency with the highest amplitude), maximum frequency, and minimum frequency per call in **(A)** glassfrogs (Centrolenidae), **(B)** treefrogs (Hylidae), and **(C)** rainfrogs (Strabomantidae). Visual examples of each metric are shown in the spectrogram (time vs. frequency, with higher amplitudes in warmer colors) on the left. Blue boxplots represent species only found in undisturbed habitats (forests); yellow boxplots represent species found in disturbed habitats (pastures, non-shaded agriculture).

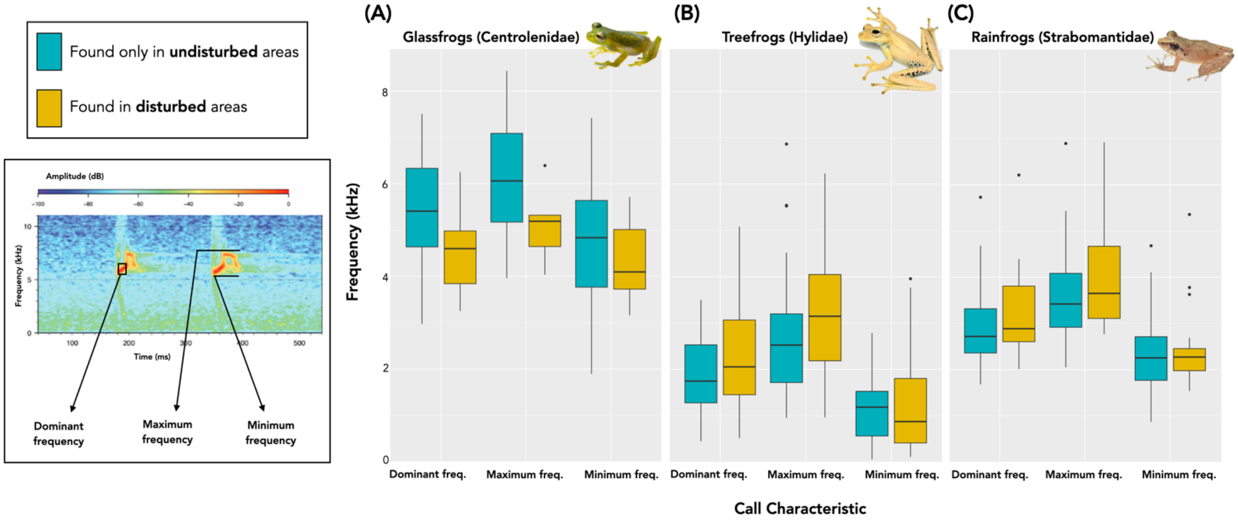


FIGURE 4. COMPARISON OF CALL FREQUENCY RANGE AND DURATION BY DISTURBANCE TOLERANCE

Comparison of call **(A)** frequency range and **(B)** duration among all species in this study and between families (glassfrogs, Centrolenidae; treefrogs, Hylidae; and rainfrogs, Strabomantidae). Visual examples of each metric are shown in the spectrogram (time vs. frequency, with higher amplitudes in warmer colors) on the left. Blue boxplots represent species only found in undisturbed habitats (forests); yellow boxplots represent species found in disturbed habitats (pastures, non-shaded agriculture).

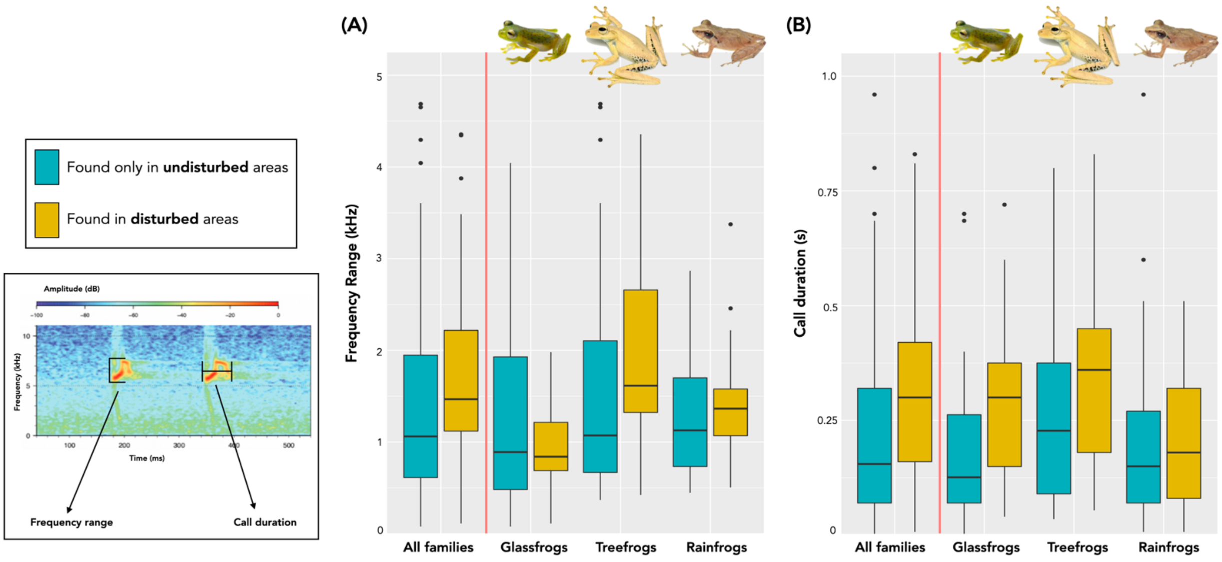
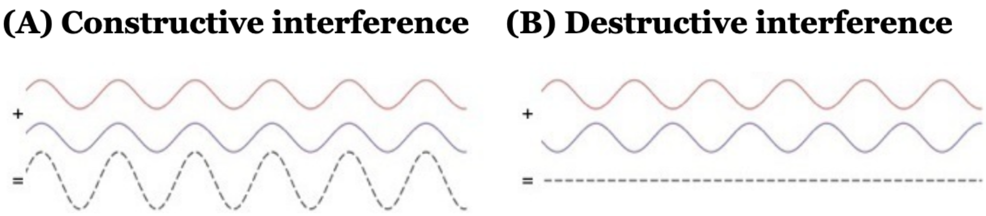


FIGURE 5. CONSTRUCTIVE VS. DESTRUCTIVE SOUND INTERFERENCE

When two sound waves occupy the same space, they can affect the resulting wave in different ways: **(A)** constructive interference, which increases the overall amplitude of a sound vs. **(B)** destructive interference, which cancels out the amplitude altogether. Adapted from the Science Learning Hub, University of Waikato.



Same frequency and amplitude aligned peak to peak = higher amplitude

Same frequency and amplitude aligned peak to trough = amplitude canceled out

TABLE 1. CALL CHARACTERISTICS PER SPECIES

Call characteristics and disturbance tolerance of each species analyzed in this study. Numeric values represent the mean of all calls analyzed for each species.

Frog Species	**Family**	**Dom Freq (Hz)**	**Min Freq (Hz)**	**Max Freq (Hz)**	**Duration (s)**	**Call Structure**	**Harmonics present?**	**Tolerates disturbance?**
Chimerella mariaelenae	Centrolenidae	7510	6460	8441	0.6	pulsatile	N	Y
Sachatamia orejuela	Centrolenidae	7433	7219	7688	0.167	tonal	N	N
Teratohyla midas	Centrolenidae	7285	7210	7390	0.082	pulsatile	N	N
Rulyrana flavopunctata	Centrolenidae	6931	5685	7924	0.4	pulsatile	Y	N
Teratohyla spinosa	Centrolenidae	6664	6469	6937	0.13	pulsatile	Y	N
Sachatamia albomaculata	Centrolenidae	6600	6100	7100	0.001	tonal	N	N
Sachatamia ilex	Centrolenidae	6522	7422	7500	0.08	pulsatile	N	N
Centrolene peristicta	Centrolenidae	6471	3235	7278	0.087	pulsatile	Y	N
Espadarana prosoblepon	Centrolenidae	6252	5712	6394	0.04	pulsatile	Y	Y
Hyalinobatrachium valerioi	Centrolenidae	6197	5599	6718	0.72	tonal	Y	Y
Espadarana audax	Centrolenidae	6146	5254	7063	0.38	pulsatile	Y	N
Teratohyla amelie	Centrolenidae	6034	4984	7085	0.013	pulsatile	Y	N
Centrolene sanchezi	Centrolenidae	5996	4688	6656	0.11	pulsatile	Y	N
Hyalinobatrachium munozorum	Centrolenidae	5812	5625	6000	1.7	tonal	Y	N
Hyalinobatrachium pellucidum	Centrolenidae	5690	5163	5943	1.28	tonal	N	N
Espadarana callistomma	Centrolenidae	5577	5343	5812	0.03	pulsatile	N	N
Centrolene lynchi	Centrolenidae	5296	4996	5599	0.2	tonal	Y	N
Hyalinobatrachium yaku	Centrolenidae	5284	5219	5330	0.3	tonal	N	Y
Nymphargus balionotus	Centrolenidae	5081	3818	6097	0.04	tonal	N	N
Vitreorana ritae	Centrolenidae	5044	4312	6029	0.685	pulsatile	N	N
Nymphargus siren	Centrolenidae	4977	4651	6115	0.24	pulsatile	Y	N
Hyalinobatrachium adespinosai	Centrolenidae	4855	4645	5203	0.38	pulsatile	Y	N
Hyalinobatrachium iaspidiense	Centrolenidae	4710	3700	5850	0.7	pulsatile	N	N
Centrolene heloderma	Centrolenidae	4682	4393	5082	0.161	pulsatile	Y	Y
Hyalinobatrachium tatayoi	Centrolenidae	4600	3800	5300	0.3	tonal	N	Y

Hyalinobatrachium chirripoi	Centrolenidae	4565	1895	5082	0.25	pulsatile	N	N
Nymphargus manduriacu	Centrolenidae	4268	4052	4447	0.1	pulsatile	N	N
Centrolene geckoidea	Centrolenidae	4187	3468	4187	0.155	pulsatile	N	N
Nymphargus griffithsi	Centrolenidae	4107	3790	4307	0.122	tonal	N	N
Cochranella granulosa	Centrolenidae	4100	3700	4500	0.3	pulsatile	N	Y
Nymphargus mariae	Centrolenidae	3766	3234	4299	0.018	pulsatile	Y	N
Nymphargus lasgralarias	Centrolenidae	3691	3445	3962	0.0257	pulsatile	N	N
Nymphargus cariticommatus	Centrolenidae	3656	3070	4159	0.3	tonal	N	N
Nymphargus grandisonae	Centrolenidae	3588	3159	4039	0.115	pulsatile	Y	Y
Dendropsophus riveroi	Hylidae	5082	3956	6226	1.8	tonal	Y	Y
Dendropsophus brevifrons	Hylidae	4552	3765	5099	0.68	pulsatile	Y	Y
Dendropsophus shiwiarum	Hylidae	4393	3188	5512	0.18	tonal	Y	Y
Dendropsophus bokermanni	Hylidae	4240	3439	4828	0.38	pulsatile	Y	Y
Dendropsophus parviceps	Hylidae	4057	2929	4465	0.14	pulsatile	Y	Y
Dendropsophus gryllatus	Hylidae	3639	2937	3912	0.46	pulsatile	Y	Y
Scinax quinquefasciatus	Hylidae	3531	688	5033	0.7	pulsatile	N	Y
Scinax garbei	Hylidae	3503	1243	5539	1.24	pulsatile	N	N
Scinax cruentomma	Hylidae	3348	2271	4930	0.42	pulsatile	N	Y
Dendropsophus bifurcus	Hylidae	3273	1810	4026	1.3	pulsatile	Y	Y
Scinax funereus	Hylidae	3178	1422	3731	0.14	pulsatile	N	N
Dendropsophus marmoratus	Hylidae	3135	1256	3899	2.8	pulsatile	Y	N
Boana punctata	Hylidae	3101	570	4055	0.2	tonal	Y	Y
Dendropsophus rhodopeplus	Hylidae	3027	2211	6862	0.46	pulsatile	N	N
Dendropsophus ebraccatus	Hylidae	2959	2165	3688	0.21	pulsatile	Y	Y
Hyloscirtus mashpi	Hylidae	2944	2784	3152	0.34	tonal	N	N
Hyloscirtus alytolylax	Hylidae	2903	2616	3277	0.49	pulsatile	N	N
Hyloscirtus phyllognathus	Hylidae	2756	2533	2999	1.5	tonal	Y	N
Dendropsophus reticulatus	Hylidae	2713	1662	3161	0.41	pulsatile	N	Y
Boana nympha	Hylidae	2699	2285	2942	0.37	pulsatile	N	N
Dendropsophus carnifex	Hylidae	2695	1809	3132	0.17	pulsatile	Y	Y

Dendropsophus triangulum	Hylidae	2625	1640	2944	0.57	pulsatile	Y	Y
Hyloscirtus palmeri	Hylidae	2614	1049	2990	0.8	pulsatile	N	N
Dendropsophus sarayacuensis	Hylidae	2584	1783	3324	0.4	pulsatile	Y	N
Boana rufitela	Hylidae	2433	1790	3066	0.077	pulsatile	N	Y
Hyloscirtus psarolaimus	Hylidae	2406	2114	2601	0.18	pulsatile	Y	Y
Osteocephalus fuscifacies	Hylidae	2369	717	3214	0.32	pulsatile	N	N
Osteocephalus deridens	Hylidae	2196	916	4521	1.28	pulsatile	Y	N
Hyloscirtus albopunctulatus	Hylidae	2186	1834	2526	1.48	pulsatile	Y	N
Hyloscirtus torrenticola	Hylidae	2186	1777	2525	0.05	pulsatile	Y	N
Boana alfaroi	Hylidae	2153	1543	2880	0.15	pulsatile	Y	N
Boana maculateralis	Hylidae	2153	986	3584	0.312	pulsatile	Y	Y
Dendropsophus minutus	Hylidae	2110	1548	5904	0.83	pulsatile	Y	Y
Sphaenorhynchus lacteus	Hylidae	2110	1291	2758	0.38	pulsatile	N	Y
Boana appendiculata	Hylidae	2084	343	3377	0.15	pulsatile	N	Y
Trachycephalus jordani	Hylidae	2025	201	3584	0.38	pulsatile	N	Y
Agalychnis hulli	Hylidae	1959	1400	2599	0.225	tonal	Y	N
Scinax ruber	Hylidae	1956	762	6129	0.19	pulsatile	N	Y
Boana fasciata	Hylidae	1955	1324	3257	0.15	pulsatile	Y	Y
Boana almendarizae	Hylidae	1895	831	2551	0.39	pulsatile	Y	Y
Boana calcarata	Hylidae	1895	464	3011	0.07	tonal	Y	N
Boana tetete	Hylidae	1895	1689	2195	0.09	tonal	Y	N
Trachycephalus macrotis	Hylidae	1827	112	3023	0.45	pulsatile	N	Y
Boana picturata	Hylidae	1823	792	4668	0.28	pulsatile	Y	Y
Agalychnis buckleyi	Hylidae	1766	1175	2229	0.27	pulsatile	Y	N
Hyloscirtus pacha	Hylidae	1729	1480	1933	0.17	tonal	Y	N
Scinax tsachila	Hylidae	1701	1064	3801	0.16	pulsatile	N	Y
Phyllomedusa tomopterna	Hylidae	1649	958	2757	0.05	pulsatile	N	N
Phyllomedusa vaillantii	Hylidae	1636	917	2713	0.08	pulsatile	N	Y
Hyloscirtus criptico	Hylidae	1614	1381	1773	0.18	tonal	Y	N
Hyloscirtus staufferorum	Hylidae	1594	1375	1810	0.14	tonal	Y	N
Boana	Hylidae	1593	403	2119.7	0.054	pulsatile	Y	Y

pellucens								
Boana cinerascens	Hylidae	1568	1197	3669	0.46	tonal	Y	N
Hyloscirtus pantostictus	Hylidae	1550	1326	1741	0.39	pulsatile	Y	N
Phyllomedusa ecuatoriana	Hylidae	1547	1123	2090	0.04	pulsatile	Y	N
Phyllomedusa palliata	Hylidae	1522	838	5526	0.035	pulsatile	Y	N
Hyloscirtus tapichalaca	Hylidae	1494	1163	1708	0.08	tonal	Y	N
Hyloscirtus lindae	Hylidae	1464	1236	1658	0.38	pulsatile	N	Y
Osteocephalus taurinus	Hylidae	1450	555	1981	0.81	pulsatile	N	Y
Nyctimantis rugiceps	Hylidae	1206	441	1667	0.26	pulsatile	N	N
Boana lanciformis	Hylidae	1167	468	2384	0.36	pulsatile	Y	Y
Trachycephalus coriaceus	Hylidae	1120	183	1337	0.69	pulsatile	N	Y
Osteocephalus mutabor	Hylidae	1094	540	3320	1.64	pulsatile	N	Y
Boana geographica	Hylidae	1033	560	1616	0.46	pulsatile	N	N
Osteocephalus yasuni	Hylidae	1031	665	1373	0.3	pulsatile	Y	Y
Hyloscirtus condor	Hylidae	982	573	1502	0.54	tonal	Y	N
Trachycephalus cunauaru	Hylidae	962	60	2220	0.25	pulsatile	N	N
Agalychnis spurrelli	Hylidae	947.5	443.5	1309	0.178	pulsatile	N	Y
Osteocephalus cannatellai	Hylidae	828	538	1698	0.2	pulsatile	N	N
Boana boans	Hylidae	801	181.4	1795	0.28	pulsatile	N	Y
Osteocephalus buckleyi	Hylidae	785	512	1510	0.08	pulsatile	N	N
Osteocephalus planiceps	Hylidae	775	385	1847	0.39	pulsatile	N	Y
Tepuihyla tuberculosa	Hylidae	750	242	1329	13.3	pulsatile	N	N
Cruziohyla craspedopus	Hylidae	689	258	1705	0.09	pulsatile	N	N
Boana rosenbergi	Hylidae	613.7	280.8	1497	0.55	pulsatile	Y	Y
Phyllomedusa tarsius	Hylidae	603	287	1748	1.5	pulsatile	N	Y
Osteocephalus vilmae	Hylidae	562	213	955	0.35	pulsatile	N	N
Osteocephalus verruciger	Hylidae	517	353	965	0.5	pulsatile	N	Y
Boana ventrimaculata	Hylidae	448	201	1003	0.23	pulsatile	N	N
Smilisca phaeota	Hylidae	430	166	3034	1.8	pulsatile	N	Y
Pristimantis achatinus	Strabomantidae	4101	1676	5051	0.33	tonal	Y	Y
Pristimantis andinodiabolus	Strabomantidae	1766	1327	2239	0.17	tonal	N	N

Pristimantis andinogigas	Strabomantidae	1743	1551	2052	0.15	tonal	Y	N
Pristimantis andinognomus	Strabomantidae	4680	4107	5431	0.07	pulsatile	Y	N
Pristimantis apiculatus	Strabomantidae	2906	2681	3132	0.51	tonal	N	N
Pristimantis appendiculatus	Strabomantidae	2012	1540	2775	0.007	pulsatile	Y	Y
Pristimantis balionotus	Strabomantidae	2485	2084	3266	0.07	tonal	Y	N
Pristimantis bicantus	Strabomantidae	3047	2689	3651	0.16	pulsatile	Y	Y
Pristimantis buenaventura	Strabomantidae	3687	3059	4132	0.2	pulsatile	Y	N
Pristimantis calcarulatus	Strabomantidae	3125	2844	3459	0.08	pulsatile	Y	N
Pristimantis conspicillatus	Strabomantidae	2702	2372	3075	0.37	tonal	Y	N
Pristimantis crenunguis	Strabomantidae	2719	873	3307	0.96	pulsatile	Y	N
Pristimantis diadematus	Strabomantidae	4393	3303	8694	0.27	pulsatile	Y	N
Pristimantis eremitus	Strabomantidae	2633	1770	3012	0.07	tonal	Y	N
Pristimantis eugeniae	Strabomantidae	3782	3387	4269	0.12	pulsatile	N	N
Pristimantis festae	Strabomantidae	2167	1944	2403	0.15	tonal	Y	N
Pristimantis ganonotus	Strabomantidae	6208	5356	6918	0.29	pulsatile	Y	Y
Pristimantis gloria	Strabomantidae	2412	2251	2769	0.12	tonal	Y	Y
Pristimantis jimenezi	Strabomantidae	3003	2378	3648	0.2	tonal	Y	Y
Pristimantis lacrimosus	Strabomantidae	2928	2712	3158	0.19	pulsatile	N	N
Pristimantis latericius	Strabomantidae	4393	3777	5322	0.11	tonal	Y	Y
Pristimantis leoni	Strabomantidae	2625	2294	3011	0.07	tonal	Y	Y
Pristimantis lutzae	Strabomantidae	2361	2254	2700	0.18	tonal	Y	N
Pristimantis lymani	Strabomantidae	3229	2763	3508	0.32	pulsatile	Y	N
Pristimantis mindo	Strabomantidae	2605	1937	2944	0.27	tonal	Y	N
Pristimantis nyctophylax	Strabomantidae	2156	1830	3283	0.42	pulsatile	Y	Y
Pristimantis omeviridis	Strabomantidae	2664	2248	3940	0.007	pulsatile	N	N
Pristimantis orestes	Strabomantidae	2865	2028	4246	0.02	tonal	Y	Y
Pristimantis paquishae	Strabomantidae	2282	2019	2778	0.01	pulsatile	Y	N
Pristimantis parvillus	Strabomantidae	5728	4679	6890	0.08	pulsatile	N	N
Pristimantis pecki	Strabomantidae	2638	2072	3474	0.36	pulsatile	Y	N
Pristimantis peruvianus	Strabomantidae	3281	1317	3785	0.15	pulsatile	Y	N
Pristimantis petersi	Strabomantidae	4288	3626	5024	0.2	pulsatile	N	Y

Pristimantis phoxocephalus	Strabomantidae	2906	2088	3716	0.51	pulsatile	Y	Y
Pristimantis pyrrhomerus	Strabomantidae	2427	2159	2835	0.03	tonal	Y	N
Pristimantis roni	Strabomantidae	3359	2584	4307	0.07	pulsatile	N	N
Pristimantis samaniegoi	Strabomantidae	3317	2523	5390	0.02	pulsatile	Y	N
Pristimantis saturninoi	Strabomantidae	2993	2394	3802	0.6	pulsatile	Y	N
Pristimantis simonbolivari	Strabomantidae	2713	2314	3419	0.02	tonal	Y	Y
Pristimantis tiktik	Strabomantidae	3187	2683	4066	0.01	tonal	Y	N
Pristimantis tinguichaca	Strabomantidae	1981	1745	2606	0.03	tonal	Y	N
Pristimantis totoroi	Strabomantidae	2541	2307	2813	1.7	tonal	Y	Y
Pristimantis unistrigatus	Strabomantidae	2756	1811	3141	15.2	tonal	Y	Y
Pristimantis verecundus	Strabomantidae	3919	3429	5023	0.008	tonal	Y	N
Pristimantis verrucolatus	Strabomantidae	1902	1516	2259	0.45	tonal	Y	N
Pristimantis versicolor	Strabomantidae	1723	1229	3563	0.08	pulsatile	N	N
Pristimantis vidua	Strabomantidae	3445	2628	5326	0.03	tonal	Y	N
Pristimantis w-nigrum	Strabomantidae	1679	960	3382	0.3	pulsatile	Y	N
Barycholos pulcher	Strabomantidae	3710	2092	4550	0.46	pulsatile	N	Y

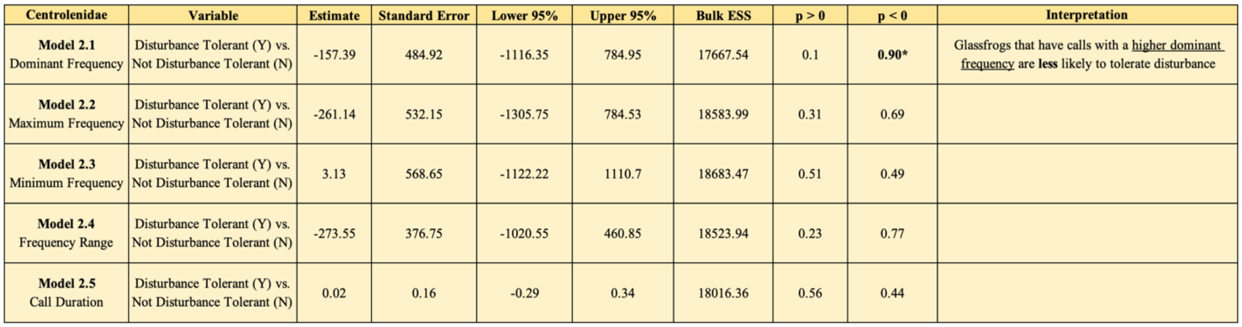
TABLE 2. BAYESIAN LINEAR MIXED MODEL RESULTS: ALL SPECIES (MODEL 1)

Model 1 represents analyses conducted for each call characteristic across all species, with family as a random effect. Habitat (disturbance) predictors of each call characteristic are listed with parameter estimates, upper and lower 95% confidence intervals, bulk effective sample size, and proportion of posterior samples >/< 0. ** = strong support, * = support. Interpretations are provided in the last column.

All Families	Variable	Estimate	Standard Error	Lower 95%	Upper 95%	Bulk ESS	p > 0	p < 0	Interpretation
Model 1.1 Dominant Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	240.77	171.92	-88.24	582.78	15103.56	**0.92***	0.08	Species that have calls with a higher dominant frequency are **more** likely to tolerate disturbance
Model 1.2 Maximum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	324.16	220.43	-108.24	752.81	14204.18	**0.93***	0.07	Species that have calls with a higher maximum frequency are **more** likely to tolerate disturbance
Model 1.3 Minimum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	83.87	168.28	-246.67	413.98	15478.8	0.69	0.31	
Model 1.4 Frequency Range	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	273.54	171.73	-59.78	614.13	10483.03	**0.95****	0.05	Species with calls that have more frequency range are **more** likely to tolerate disturbance
Model 1.5 Call Duration	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	0.18	0.26	-0.32	0.7	9558.81	0.76	0.24	

TABLE 3. BAYESIAN LINEAR MODEL RESULTS: CENTROLENIDAE (MODEL 2)

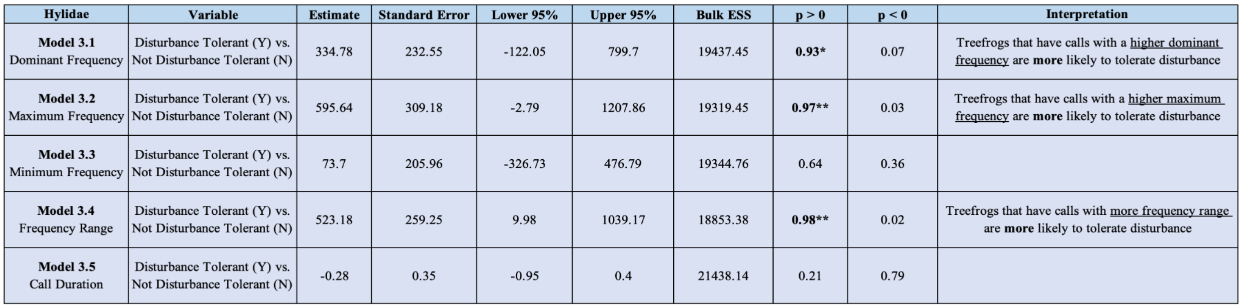
Model 2 represents analyses conducted for each call characteristic across glassfrog species (family Centrolenidae). Habitat (disturbance) predictors of each call characteristic are listed with parameter estimates, upper and lower 95% confidence intervals, bulk effective sample size, and proportion of posterior samples >/< 0. ** = strong support, * = support. Interpretations are provided in the last column.



Centrolenidae	Variable	Estimate	Standard Error	Lower 95%	Upper 95%	Bulk ESS	p > 0	p < 0	Interpretation
Model 2.1 Dominant Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	-157.39	484.92	-1116.35	784.95	17667.54	0.1	**0.90***	Glassfrogs that have calls with a higher dominant frequency are **less** likely to tolerate disturbance
Model 2.2 Maximum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	-261.14	532.15	-1305.75	784.53	18583.99	0.31	0.69	
Model 2.3 Minimum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	3.13	568.65	-1122.22	1110.7	18683.47	0.51	0.49	
Model 2.4 Frequency Range	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	-273.55	376.75	-1020.55	460.85	18523.94	0.23	0.77	
Model 2.5 Call Duration	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	0.02	0.16	-0.29	0.34	18016.36	0.56	0.44	

TABLE 4. BAYESIAN LINEAR MODEL RESULTS: HYLIDAE (MODEL 3)

Model 3 represents analyses conducted for each call characteristic across treefrog species (family Hylidae). Habitat (disturbance) predictors of each call characteristic are listed with parameter estimates, upper and lower 95% confidence intervals, bulk effective sample size, and proportion of posterior samples >/< 0. ** = strong support, * = support. Interpretations are provided in the last column.



Hylidae	Variable	Estimate	Standard Error	Lower 95%	Upper 95%	Bulk ESS	p > 0	p < 0	Interpretation
Model 3.1 Dominant Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	334.78	232.55	-122.05	799.7	19437.45	**0.93***	0.07	Treefrogs that have calls with a higher dominant frequency are **more** likely to tolerate disturbance
Model 3.2 Maximum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	595.64	309.18	-2.79	1207.86	19319.45	**0.97****	0.03	Treefrogs that have calls with a higher maximum frequency are **more** likely to tolerate disturbance
Model 3.3 Minimum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	73.7	205.96	-326.73	476.79	19344.76	0.64	0.36	
Model 3.4 Frequency Range	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	523.18	259.25	9.98	1039.17	18853.38	**0.98****	0.02	Treefrogs that have calls with more frequency range are **more** likely to tolerate disturbance
Model 3.5 Call Duration	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	-0.28	0.35	-0.95	0.4	21438.14	0.21	0.79	

TABLE 5. BAYESIAN LINEAR MODEL RESULTS: HYLIDAE (MODEL 4)

Model 4 represents analyses conducted for each call characteristic across rainfrog species (family Strabomantidae). Habitat (disturbance) predictors of each call characteristic are listed with parameter estimates, upper and lower 95% confidence intervals, bulk effective sample size, and proportion of posterior samples >/< 0. ** = strong support, * = support. Interpretations are provided in the last column.

Strabomantidae	**Variable**	**Estimate**	**Standard Error**	**Lower 95%**	**Upper 95%**	**Bulk ESS**	**p > 0**	**p < 0**	**Interpretation**
Model 4.1 Dominant Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	305.98	297.5	-282.04	889.89	17322.15	**0.9***	0.1	Rainfrogs that have calls with a higher dominant frequency are **more** likely to tolerate disturbance
Model 4.2. Maximum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	194.33	405.82	-594.93	992.72	19532.36	0.68	0.32	
Model 4.3 Minimum Frequency	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	165.63	276.28	-365.1	708.53	17512.8	0.73	0.27	
Model 4.4 Frequency Range	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	31.67	292.49	-543.24	602.77	17279.48	0.55	0.45	
Model 4.5 Call Duration	Disturbance Tolerant (Y) vs. Not Disturbance Tolerant (N)	1.05	0.66	-0.25	2.36	18470.29	**0.94***	0.06	Rainfrogs that have calls with longer duration are **more** likely to be tolerant to disturbance

Chapter 4. Nocturnal visual displays and call description of the cascade specialist glassfrog *Sachatamia orejuela*

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ABSTRACT

Although most male frogs call to attract females, vocalizations alone can be ineffective long-range signals in certain environments. To increase conspicuousness and counter the background noise generated by rushing water, a few frog species around the world have evolved visual communication modalities in addition to advertisement calls. These species belong to different families on different continents: a clear example of behavioral convergent evolution. Until now, long-distance visual signaling has not been recorded for any species in the glassfrog family (Centrolenidae). *Sachatamia orejuela*, an exceptionally camouflaged glassfrog species found exclusively within the spray zone of waterfalls, has remained poorly studied. Here, we document its advertisement call for the first time—the frequency of which is higher than perhaps any other glassfrog species, likely an evolutionary response to its disruptive acoustic space—as well as a sequence of non-antagonistic visual signals (foot-flagging, hand-waving, and head-bobbing) that we observed at night.

INTRODUCTION

Communication is crucial for mating success in frogs. To attract females, most males rely on advertisement calls, which can convey information related to a male's location and/or characteristics (e.g. body size). On a basic level, acoustic signals facilitate positive phonotaxis, enabling females to locate males across long distances (Gerhardt & Shwartz, 2001). Calls often function as form of territory delimitation as well, especially when suitable egg deposition sites are limited. Some well-studied systems have shown that a male's advertisement call can also convey information about his 'quality' in the context of female mate choice (Welch et al., 1998). For example, lower frequency variations of a frog call can be an honest signal for larger body size, which is preferred by females. Similarly, call length and pulse rates (syllable period) can convey information about performance and energetic constraints; females in playback experiments have been shown to prefer longer calls and higher pulse rates (Klump & Gerhardt, 1987).

However, vocalizations alone can be ineffective signals in certain environments. For example, loud streams and waterfalls create acoustic interference that can render a male's call difficult for listening females (or competing males) to receive. To avoid such interference, a few species produce calls in ultrasonic frequencies (Boonman & Kurniati, 2011; Narins et al., 2007). Others have evolved visual communication modalities to counter background noise, such as foot-flagging or hand-waving, where a male raises its hand and/or foot above its body in a repetitive motion (Hödl & Amezquita, 2001). Some well-known examples include *Hylodes japi* from Brazil (de Sá et al., 2016), *Micrixalus saxicola* from India (Preininger et al., 2013), and *Staurois latopalmatus* from Borneo (Grafe & Wanger, 2007). Each of these species call and visually display near rushing water, but belong to different families on different continents: a clear example of behavioral convergent evolution.

Sachatamia orejuela is a large glassfrog from Ecuador and Colombia that is found exclusively on rock walls or boulders within the spray zone of cascades. Due to its dark green-grey coloration and highly reflective skin (Figure 1A), this species blends exceptionally well into the mossy, wet rock crevices of its hard-to-reach habitat (Figure 1B). *S. orejuela* has thus remained an elusive and poorly studied species; none of the literature to date includes information on its breeding biology (Duellman & Burrowes, 1989; Rada et al., 2017). Therefore, the aim of this study was to document its advertisement call and any other associated behaviors.

METHODS & OBSERVATIONS

Fieldwork was conducted within Mashpi Reserve (0.167082° N, -78.871437° W; 950m a.s.l.), part of the Tropical Andes hotspot of Ecuador. At least four times per week over the course of three months (February-April 2019), our three-person team extensively surveyed known cascade sites within the reserve (~250 sample hours). During this time, we observed a total of 18 *S. orejuela* individuals on moss and/or wet rocks at four waterfalls >500m apart, along different streams. Frogs were consistently in the spray zone, within a two- to ten-meter radius of the cascade. Most individuals (n = 14) were observed >3.5 meters above the water level. Those found lower (n = 4) were in or near hard-to-reach rock crevices over deep pools of water.

Despite extensive surveying efforts, we only heard *S. orejuela* calling at one of the four cascade sites. On 6 April 2019, between 19:15 and 22:15, we observed an *S. orejuela* individual calling from a concave rock face next to a rushing waterfall (~3 m above the pool below). To record its call (Roland R-26 digital audio recorder, Sennheiser ME67 directional microphone; 44.1 kHz sampling rate), RMB climbed to the only accessible viewing spot for this display: a small, slippery rock ledge ~4m across from the rock face above the same pool. In the process of recording its call, she observed cyclical visual displays that included foot-flagging, head-bobbing, and hand-waving (Figure 2)—all well after sundown. RMB took videos of these behaviors from the same ledge across from the displaying frog using the camera that was on-site at the time (Nikon 7100 with 105mm macro lens), as these observations were not expected.

We also observed another male exhibiting the same behaviors >6.5 m away from the individual described above, on the other side of the waterfall. We visually searched for more conspecifics in the area (with and without binoculars) during the same time period, but did not hear or see any other individuals.

Call parameters were analyzed in Raven Pro 1.5 (Center for Conservation Bioacoustics, Cornell Lab of Ornithology 2014) without filters or transformations.

RESULTS

The *Sachatamia orejuela* advertisement call (Figure 3A; Audio 1) is high-pitched, even for a glassfrog (mean peak frequency = 7.433 ± 0.1203 SD; range = 7.219–7.688 kHz; n = 13 notes from 2 individuals; minimal amplitude modulation). Generally emitted as a single ‘peep’, each note in its call had a duration of 0.137–0.189 sec (0.167 ± 0.018, n = 13). Time between individually emitted notes ranged from 12.1–57.0 sec (32.4 ± 12.9, n = 21). The male would occasionally emit two notes in quick succession (Figure 3A; between-note interval 0.455–0.548 sec, n = 2); although we only recorded it twice, we heard the double note call multiple times each hour. We also observed that *S. orejuela* has paired vocal sacs (Figure 3B), the first record of such in glassfrogs.

In addition to calling, the same individual would occasionally wave its leg(s), wave its arm(s), and bob its head. The male would rotate through one of four signals every 5 to 60 seconds (20 seconds on average, based on observations of two individuals over the course of > 2 hours). Although the signal sequence was not consistent, the most common pattern was as follows: calling bout/vocal sac inflation (2–4 times), head bob, hand wave, leg wave (Figure 2). It repeated these behaviors over the course of two hours, interspersed with ‘rest’ periods where it would vocalize without foot or hand movements. Video 1 clearly shows each of these behaviors in succession, with shakiness edited out (RMB had to balance on one foot while on the adjacent ledge). Video 2 shows the same succession at half speed for better visualization. The full-length, unedited recording is provided in Video 3.

DISCUSSION

Since *S. orejuela* vocalizes near loud cascades, it is unsurprising that its call is high-pitched. Waterfalls are a source of wide-spectrum noise, but the lower frequencies contain the highest amplitudes (see power spectrum in Figure 3A). Therefore, higher frequency sounds, like the *S. orejuela* call, experience less interference. A recent review found the mean peak frequency across 72 glass frog species to be 4.88 kHz (range: 2.71-7.41 kHz) (Sulbarán et al., 2019); the peak frequency of the *S. orejuela* call is above this mean by at least 2 kHz, and in many cases higher than the upper limit of the peak frequency range in other species (e.g. 7.688 kHz).

As a comparison, the call of a sympatric glassfrog species, *Espadarana prosoblepon*, has a peak frequency (i.e. the frequency with the highest amplitude) of 5.758 ± 0.232 kHz (Jacobson, 1985), while *S. orejuela* has a peak frequency well above 7 kHz (both vocalize in Audio 2). This difference is especially interesting given that *S. orejuela* is larger (mean SVL= 31.35 ± 1.68 mm; mean mass = 2.52 ± 0.49 g; n = 9) than *E. prosoblepon* (mean SVL= 24.77 ± 1.84 mm; mean mass = 0.844 ± 0.22 g; n = 60), based on individuals we caught during the same field season. In bioacoustics, an individual’s size is typically inversely correlated with the peak frequency of its call (Wilkins et al., 2013), meaning that larger species tend to make sounds with lower frequencies—yet *S. orejuela* calls with a much higher frequency than the smaller *E. prosoblepon*. This suggests that *S. orejuela* may have evolved higher-pitched calls in order to communicate near loud waterfalls, thus being able to occupy an otherwise inaccessible acoustic niche. *E. prosoblepon* calls much farther away above stream sections where lower frequencies can propagate with less attenuation.

Information is more likely to reach its intended receiver(s) when paired with multiple modes of signaling. We observed two *S. orejuela* individuals calling, bobbing, and limb-flagging at night, >6.5 meters away from one another (and on different sides of the waterfall); no other conspecifics were visible or audible. Descriptions of nocturnal visual signaling have been limited to situations in which conspecifics are in close proximity. For example, species in the genus Hyla wave their limbs reciprocally during courtship (Hartmann et al., 2004). In Centrolenidae (glassfrogs), the only records of nocturnal visual signals—rocking back and forth (*Hylainobatrachium fleishchmanni;* McDiarmid & Adler, 1974) or limb-lifting (*Vitreorana uranoscopa*; Hartmann et al., 2005)—involve direct territorial challenges. However, our observations of visual signals in *S. orejuela* bear greater resemblance to diurnal foot-flagging displays, which generally serve as a territorial spacing mechanism (Preininger et al., 2009) in the presence of acoustic interference—not as a directly antagonistic or aggressive response to an encroaching male. If further research confirms this function, our observations add Centrolenidae to the short list of anuran families that have evolutionarily converged to counter background noise with visual signals.

Non-antagonistic visual displays are rarely observed at night. Since they communicate by waterfalls, which generally have less canopy cover, it is possible that *S. orejuela* relies on moonlit evenings for visual displays (e.g. Grant et al., 2009). However, recent experiments have demonstrated that frogs are able to distinguish blue from green light in almost complete darkness (Yovanovich et al., 2017), confirming that anuran vision functions remarkably well in the dark. Our observations emphasize the need for further research on long-range visual display systems, particularly at night. Similar behaviors may have been overlooked in other nocturnal species inhabiting disruptive acoustic spaces.

In addition to bobbing and limb-waving, vocal sac inflation is considered a visual signal in anuran communication (Wells, 2010). For example, females of *Engystomops pustulosus* prefer the coupling of call and vocal sac inflation to the same sound stimulus without the visual vocal sac component (Rosenthal et al., 2004). *Sachatamia orejuela* has paired vocal sacs (Figure 3B)—the first record of such in a glassfrog. This modification may have evolved to maintain its visual role in intraspecific communication, as the lateral extension of each vocal sac likely enhances visibility from multiple angles (e.g. directly above the calling male). If this is the case, it stands to reason that other behaviors evolved to enhance signal effectiveness as well.

Although it is not clear why the observed males did not maintain the flagging signals over the entire duration of our observations, it is likely related to energy expenditure and/or predation avoidance. Calling is already an energetically expensive endeavor for male frogs. Presumably the addition of visual signals presents even more of an energy trade-off, and is thus not sustainable to maintain for long periods of time. It is also possible that the observed male began to incorporate visual signals in response to the communication efforts of the other calling male nearby. Presumably visual cues also increase predation risk, although their habitats are incredibly slippery and hard to access; waterfall spray may even disrupt bat echolocation (e.g. Halfwerk et al., 2014).

Future studies should attempt to address whether these behaviors constitute true multimodal signaling by testing their communicative relevance in intra-specific interactions (Hödl & Amezquita, 2001; Feng et al., 2006). It is possible that the behaviors we observed do not elicit a behavioral or physiological response in females or competing males; although some studies have found this to be the case in certain displays of presumed aggression (Furtado et al., 2019), the flagging behaviors of *S. orejuela* did not appear to occur in close proximity to another male. Further work on this species should also investigate whether its calls contain harmonics in ultrasonic frequencies by recording with a sample rate higher than 44.1 kHz.

These observations inspire further study into how the acoustic environment effects the evolution of multimodal signaling. Increasing our understanding of how species overcome signal interference through other modes of communication may inform future studies of adaptation and conservation.

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FIGURE 1. SPECIES AND HABITAT

(A) *Sachatamia orejuela*. (B) This species is found exclusively in the spray zone of waterfalls.

FIGURE 2. VISUAL SIGNALING SEQUENCE OF *SACHATAMIA OREJUELA*

Positions: (A) not calling or displaying, (B) vocal sacs inflated while calling, (C) arm-waving, (D) double foot-flagging. Illustrations by Robert Tyler.

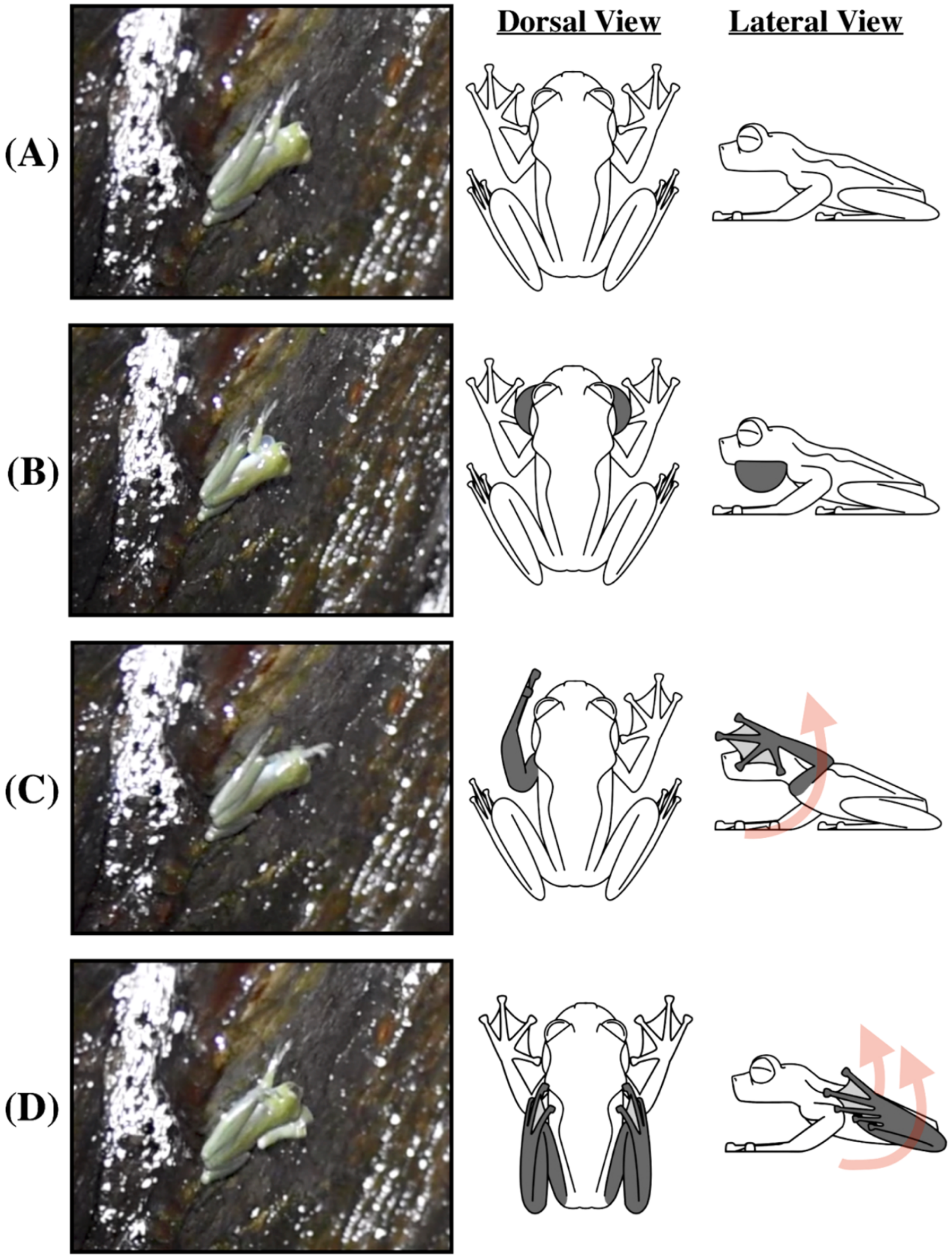
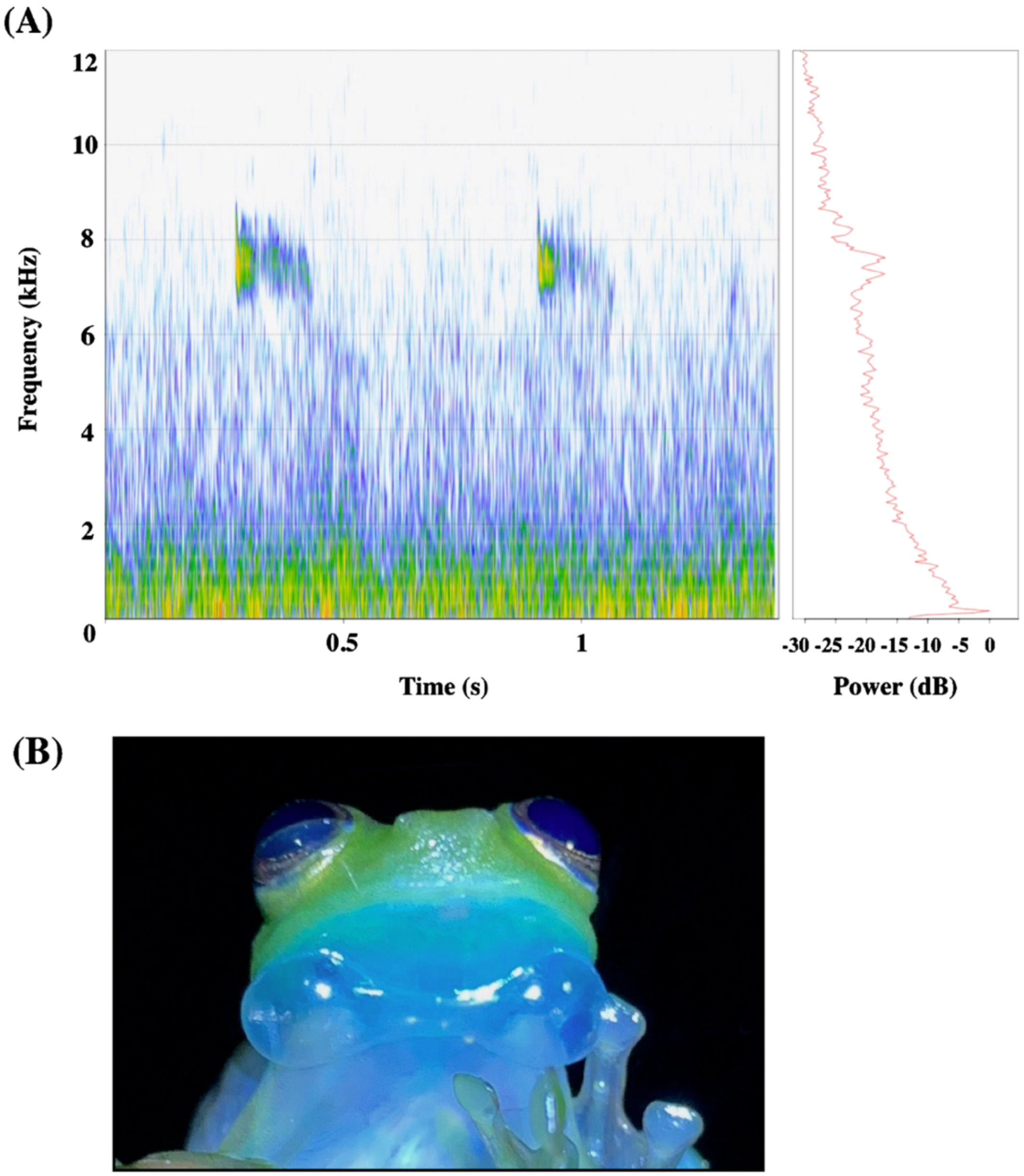


FIGURE 3. ***SACHATAMIA OREJUELA*** **ADVERTISEMENT CALL**

(A) Spectrogram (frequency vs. time; warmer colors indicate higher amplitude) and power spectrum (frequency vs. power) of the *Sachatamia orejuela* advertisement call within its loud cascade habitat. The majority of the power (dB) is located <2 kHz (noise generated by the waterfall) and between 7-8 kHz (the double-note *S. orejuela* call, which registers above much of the acoustic interference). Spectrogram was generated with a window length of 128 samples. Power spectrum was generated with a window length of 1024 samples; all powers (dB) are relative to 0, which was set as the maximum power of the recording. (B) A close-up of the *S. orejuela* paired vocal sac, the first record of such for glassfrogs.



Chapter 5. Two new glassfrogs (Centrolenidae: *Hyalinobatrachium*) from Ecuador, with comments on the endangered biodiversity of the Andes

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ABSTRACT

Background: The Tropical Andes is the world's most biodiverse hotspot. This region contains >1,000 amphibian species, more than half of which are endemic. Herein we describe two new glassfrog species (Centrolenidae: *Hyalinobatrachium*) that we discovered within relatively unexplored and isolated localities of the Ecuadorian Andes.
Methods: We employed morphological, acoustic, and molecular methods to test the hypothesis that *Hyalinobatrachium mashpi* sp. nov and *H. nouns* sp. nov. are species new to science. Following standard methods, we generated mitochondrial sequences (16S) of 37 individuals in the genus *Hyalinobatrachium*. We inferred the phylogenetic relationships of the two new species in comparison to all other glassfrogs using Maximum Likelihood. In addition to describing the call of *H. mashpi* sp. nov., we performed a discriminant analysis of principal components (DAPC) with the advertisement call characteristics of several congeners.
Results: Based on an integrative taxonomy approach, we describe two new species. Morphological traits and the inferred phylogeny unambiguously place the new taxa in the genus *Hyalinobatrachium*. Both species are distinguished from other glassfrogs mainly by their dorsal coloration (i.e., dorsum lime green with small light yellow spots, head usually with interorbital bar) and transparent pericardium (i.e., the heart is visible through the ventral skin). The new species exhibit a high morphological similarity (i.e., cryptic) and occur within relatively close geographical proximity (closest aerial distance = 18.9 km); however, their uncorrected p distance for the mitochondrial gene 16S is 4.6–4.7%, a value that greatly exceeds the genetic distance between closely related species of centrolenid frogs. The DAPC revealed that the advertisement call of *H. mashpi* sp. nov. is acoustically distinct.
Discussion: Our findings are congruent with several previous studies that report a high degree of endemism in the Toisán mountain range, which appears to be isolated from the main Andean cordillera for some amphibian groups. We recommend that both *H. mashpi* sp. nov. and *H. nouns* sp. nov. be listed as Endangered, following IUCN criteria. These new species provide another example of cryptic diversity in the Andes—further evidence that the region fosters much more biodiversity than we have the resources to catalog. Threatened by mining and other exploitative industries, these glassfrogs and many other yet-to-be-discovered Andean species highlight the dire need for effective conservation measures—especially in northwestern Ecuador.

INTRODUCTION

The diversity of glassfrogs (Family Centrolenidae) is concentrated in the northern Andes, which hosts more than half (83 taxa) of the species in the family (Guayasamin et al. 2020). The linearity of the Andes, combined with its topographical and climatic complexity, has facilitated numerous diversification events—dominated by allopatric speciation, niche conservatism, and few ecological shifts (Hutter et al. 2013; Castroviejo-Fisher et al. 2014; Guayasamin et al. 2020). As a consequence, glassfrogs tend to occupy narrow distribution ranges in this biogeographic region, often restricted by elevation and river valleys (Guayasamin et al. 2020).

Within Centrolenidae, *Hyalinobatrachium* is particularly charismatic due to its peculiar morphological and behavioral traits. All species in the genus have ventral transparency (Ruiz-Carranza & Lynch 1991; Cisneros-Heredia & Mcdiarmid 2007; Guayasamin et al. 2009) and extended paternal care—a derived trait that has evolved at least twice in the family (Delia et al. 2017). Although *Hyalinobatrachium* species have been the focus of numerous behavioral and ecological studies (Vockenhuber et al. 2008; Delia et al. 2010; Mangold et al. 2015; Delia et al. 2017; Valencia-Aguilar et al. 2021), their taxonomy is complex because they exhibit remarkable morphological conservatism (Castroviejo-Fisher et al. 2009, 2011; Guayasamin et al. 2009). Additionally, locating *Hyalinobatrachium* spp. in the Andean cloud forests is challenging, as they typically occupy high vegetation along steep streams and rivers. Our recent work in Andean localities of northwestern Ecuador has provided enough data to describe two new (and beautiful) glassfrog species. Because the habitat is severely fragmented and experiences constant deforestation and mining pressures, both species are of conservation concern.

MATERIALS AND METHODS

Ethical statement

Research was conducted under permits MAE-DNB-CM-2015-0017, 019-2018-IC-FAU-DNB/MAE, and MAE-DNB-CM-2018-0105, issued by the Ministerio del Ambiente del Ecuador. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field research (Beaupre et al. 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). We confirm that out study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). Access to field sites was granted by Mashpi Reserve and Fundación Ecominga.

Taxonomy and species concept

Glassfrog taxonomy follows the proposal by Guayasamin et al. (2009). Species are considered separately evolving lineages, following the conceptual framework developed by Simpson (1951, 1961), Wiley (1978), and De Queiroz (2007). Determining if a given population is an independent lineage is a non-trivial task and requires an integrative approach to assess species hypotheses (Dayrat 2005; Padial et al. 2010).

Morphological data

For the diagnosis and description of the new species, we follow Lynch & Duellman (1973), Cisneros-Heredia & Mcdiarmid (2007), and Guayasamin et al. (2009). Webbing formula follows Savage & Heyer (1967), as modified by Guayasamin et al. (2006). We compared *Hyalinobatrachium* specimens housed at the following collections (Material S1): Centro Jambatu de Investigación y Conservación de Anfibios, Quito, Valle de San Rafael, Ecuador (CJ); Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN); University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA (KU); Museo de Zoología, Universidad Tecnológica Indoamérica, Quito, Ecuador (MZUTI); National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (USNM); and Museo de Zoología, Universidad San Francisco de Quito, Quito, Ecuador (ZSFQ). We obtained morphological data with a Mitutoyo® digital caliper to the nearest 0.1 mm, as described below (Fig. 1): (1) snout–vent length (SVL) = distance from tip of snout to posterior margin of vent; (2) femur = distance from cloaca to knee; (3) tibia = length of flexed leg from knee to heel; (4) foot = distance from proximal edge of Toe I to tip of Toe IV; (5) head length = distance from tip of snout to posterior angle of jaw articulation; (6) head width (HW) = width of head measured at level of jaw articulation; (7) interorbital distance (IOD) = shortest distance between upper eyelids, a measurement that equals to the subjacent frontoparietal bones; (8) eye = distance between anterior and posterior borders of the eye; (9) tympanum = distance between anterior and posterior borders of tympanic annulus; (10) arm = length of flexed forearm from elbow to proximal edge of Finger I at the level of articulation with arm; (11) hand = distance from proximal edge of Finger I to tip of Finger III; (12) Finger I = distance from outer margin of hand to tip of Finger I; (13) Finger II = distance from outer margin of hand to tip of Finger II; and (14) width of Finger III = maximum width of Finger III measured at distal end. We determined sexual maturity of examined frogs by the presence of vocal slits in museum specimens and calling activity in males during fieldwork.

Evolutionary relationships

We sequenced mitochondrial 16S in 37 individuals, including the two new taxa described below, as well as the morphologically similar *H. valerioi* (Dunn, 1931) and *H. aureoguttatum* (Barrera-Rodriguez & Ruiz-Carranza 1989). Extraction, amplification, and sequencing protocols are described in Guayasamin et al. (2008) and Peñafiel et al. (2019). The obtained data were compared with homologous sequences from all available species in the genus *Hyalinobatrachium* and its sister taxon *Celsiella* (Guayasamin et al. 2008), downloaded from GenBank (https://www. ncbi.nlm.nih.gov/genbank/) and generated mostly by Guayasamin et al. (2008), Castroviejo-Fisher et al. (2014), and Twomey et al. (2014). We also included data from the following newly described species: *H. yaku* (Guayasamin et al. 2017a), *H. muiraquitan* (De Oliveira & Hernández-Ruz 2017), and *H. adespinosai* (Guayasamin et al. 2019a). Sequences were aligned using MAFFT v.7 (Multiple Alignment Program for Amino Acid or Nucleotide Sequences: http://mafft.cbrc.jp/alignment/ software/), with the Q-INS-i strategy. The software Mesquite (Maddison & Maddison 2019) was used to visualize the alignment (no modifications were necessary). Maximum likelihood trees were estimated using GARLI 0.951 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). GARLI uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously (Zwickl 2006). Default values were used for other GARLI settings, as per recommendations of the developer (Zwickl 2006). Bootstrap support was assessed via 1,000 pseudoreplicates under the same settings used in tree search. Genetic distances were calculated using PAUP (Swofford 2002).

Bioacoustics

We describe the call of the new *Hyalinobatrachium* species found in Mashpi and Tayra Reserves, as well as the vocalizations from morphologically and/or phylogenetically similar species: *Hyalinobatrachium adespinosai*, *H. aureoguttatum*, *H. chirripoi*, *H. pellucidum*, *H. tatayoi*, and *H. valerioi*. Calls of the new species were recorded with a Tascam DR-05; calls of *H. adespinosai*, *H. aureoguttatum*, *H. chirripoi*, *H. pellucidum*, and *H. tatayoi* were obtained with an Olympus LS-10 Linear PCM Field Recorder and/or a Roland R-26 digital recorder with a Sennheiser ME 67 directional microphone. All vocalizations were recorded in WAV format with a sampling rate of 44.1 kHz/s with 16 bits/sample. Recordings of *Hyalinobatrachium valerioi* by Roy McDiarmid in Costa Rica were obtained from the Macaulay Library (ML) of the Cornell Lab of Ornithology. We unfortunately were unable to record the new *Hyalinobatrachium* species from the Toisán Mountain Range, despite several attempts (i.e., males were not calling when located in the field). Measurements and definition of acoustic variables follow Köhler et al. (2017). Notes were divided into two classes—pulsed or tonal—based upon distinct waveforms in the oscillogram. Pulsed (or peaked) notes are defined as having one or more clear amplitude peak(s) and amplitude modulation (i.e., visible increases and decreases in amplitude on the oscillogram throughout the call); tonal notes are defined as having no clear amplitude peak (Dautel et al. 2011). To determine if major call characteristics (peak frequency, maximum frequency, minimum frequency, call duration, and inter-call duration) cluster by species, we performed a discriminant analysis of principal components (DAPC; Jombart et al. 2010), using the R package adegenet.

DAPC maximizes differentiation between pre-defined groups (in this case, the new and related *Hyalinobatrachium* species listed above, except for *H. chirripoi*, due to lack of sufficient data), by transforming data via principal components analysis (PCA) and subsequently identifying clusters via discriminant analysis (DA).

New zoological taxonomic names

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:0C4888D5-2DB9-4421-A96E-7E41C17EC82F. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

RESULTS

Evolutionary relationships

The phylogeny (Fig. 1) confirms the placement of the two new species within the genus *Hyalinobatrachium* with significant support (bootstrap support = 96). The two new species show considerable genetic divergence (uncorrected p distance = 4.6–4.7% for the mitochondrial gene 16S), especially considering that they are found only 18.9 km apart (but with the Intag-Guayllabamba river valley between them). *Hyalinobatrachium mashpi* sp. nov. is sister to unidentified populations from Colombia (MAR 2147, 2222); further analyses of the Colombian populations (identified as *H. cf. talamacae* by Díaz-Ricaurte & Guevara-Molina 2020) is necessary to determine if they are conspecific with *H. mashpi* sp. nov. *Hyalinobatrachium nouns* sp. nov. is sister to the clade formed by *H. mashpi* sp. nov. and the Colombian populations; genetic distances to Colombian populations are also considerable (4.7–5.1%). More distantly related taxa include two species from Central America, *H. vireovittatum* (Starrett & Savage 1973) and *H. talamancae* (Taylor 1952).

(1) ***Hyalinobatrachium mashpi*** new species
LSID: 0815B7E6-33FB-42D9-A367-4FB50885C256

Suggested English name: Mashpi Glassfrog
Suggested Spanish name: Rana de Cristal de Mashpi

Holotype: CJ11642, adult male from San Vicente River (0.16334 N, 78.86736 W; 1,040 m a.s.l.), Mashpi Reserve, Pichincha Province, Ecuador, collected by Jaime Culebras and Carlos Morochz on 28 September 2019.

Paratopotypes: CJ11643–44, adult males with same data as holotype.

Paratypes: MZUTI-3921, adult male from Amagusa River (0.15469 N, 78.85322 W; 1,137 m a.s.l.), Amagusa Reserve, Pichincha Province, Ecuador, collected by Carlos Morochz and Lucas Bustamante on 14 December 2014. CJ11645, adult male from tributary of the Mashpi River (0.11463 N, 78.88307 W; 1,126 m a.s.l.), Tayra Reserve, Pichincha Province, Ecuador, collected by Jaime Culebras on 28 October 2019.

Generic placement

The new species is placed in the genus *Hyalinobatrachium* (Ruiz-Carranza & Lynch 1991), as modified by Guayasamin et al. (2009), on the basis of morphological and molecular data. The molecular phylogeny (Fig. 2) places the new species within the genus *Hyalinobatrachium* with high confidence. Phenotypically, the main diagnostic traits of *Hyalinobatrachium* are: (1) completely transparent ventral parietal peritoneum; (2) digestive tract and bulbous liver are covered by iridophores; (3) absent humeral spines; (4) white bones in life; (5) males call from the undersides of leaves; (6) females place the eggs on the undersides of leaves; (7) males provide extended parental care; and (8) tympanum with an orientation that places it almost on a horizontal plane (instead of a more lateral plane as observed in other glassfrog genera). All the aforementioned characteristics are present in *Hyalinobatrachium mashpi* sp. nov. We note that we have observed males on the same leaves as egg clutches for continuous days, but additional studies are necessary to confirm that these observations actually represent extended paternal care.

Diagnosis

Hyalinobatrachium mashpi sp. nov. is distinguished from other species in the genus mainly by its dorsal coloration (i.e., head with light yellow spots that may form an interorbital bar; dorsum lime green with small light yellow spots) and by its transparent pericardium (i.e., red heart visible in ventral view). *Hyalinobatrachium mashpi* sp. nov. is most similar to *H. aureoguttatum*, *H. talamancae*, *H. valerioi*, *H. vireovittatum*, and the new species described below. Differences among these species are indicated in Table 1 and Figs. 3–5. The new species is morphologically cryptic with *Hyalinobatrachium nouns* sp. nov. (described below); however, the two new species exhibit a considerable genetic distance (16S; 4.6–4.7%), which is particularly remarkable given that they are found at relatively close geographic proximity (straight distance = 18.9 km), but separated by the Intag-Guayllabamba river valley.

Characterization

The following combination of characters are found in *Hyalinobatrachium mashpi* sp. nov.: (1) dentigerous process of the vomer lacking teeth; (2) snout truncate in dorsal view and slightly protruding in lateral view; (3) tympanum oriented almost horizontally; tympanic annulus barely visible, hidden under skin; tympanic membrane differentiated, with coloration similar to that of surrounding skin; (4) dorsal skin shagreen; (5) ventral skin areolate; cloacal ornamentation absent, paired round tubercles below vent absent; (6) parietal peritoneum and pericardium translucent (in life, red heart visible in ventral view); liver, viscera and testes covered by iridophores; (7) liver bulbous; (8) humeral spines absent; (9) hand webbing formula: I (2_{+}–$2_{1/2}$)—(3_{-}–3) II (2–2_{-})—(3_{+}–$3_{1/4}$) III (2–2_{+})—($1_{3/4}$–2) IV; (10) foot webbing moderate; webbing formula: I 1_{+}—(2–2_{+}) II (1–$1_{1/3}$)—$2_{1/4}$ III ($1_{1/3}$–$1_{1/2}$) —(2_{+}–$2_{1/4}$) IV ($2_{1/2}$–$2_{1/3}$)—1 V; (11) fingers and toes with thin lateral fringes; ulnar and tarsal folds absent; (12) nuptial excrescence present as a small pad on Finger I (Type V), prepollex not enlarged; prepollical spine not projecting (spine not exposed); (13) when appressed, Finger I longer than II; (14) diameter of eye about 2 times wider than disc on Finger III; (15) coloration in life: dorsal surfaces lime green with small light yellow spots; (16) coloration in preservative: dorsal surfaces creamish white, with minute lavender melanophores; (17) eye coloration in life: iris yellow to golden-yellow; pupil surrounded by lavender ring; (18) melanophores absent from fingers and toes, except Toes IV and V; (19) males call from underside of leaves; advertisement call consisting of single pulsed note, with duration of 0.37–0.46 s, peak frequency at 5.25–5.60 kHz, maximum frequency at 5.46–5.81 kHz, and minimum frequency at 4.62–4.92 kHz; (20) males attend egg clutches located on the underside of leaves overhanging streams; clutch size of 31 or 32 embryos (n = 2); (21) SVL in adult males 19.7–20.9 mm (mean = 20.5; n = 5); females unknown; and (22) enameled tubercles absent from sides of head.

Description of holotype

CJ11642, adult male with SVL 20.6 mm. Head wider than long (head width 39% of SVL; head length 78% of head width). Snout truncate in dorsal view and slightly protruding in lateral view.

Loreal region concave, nostrils slightly protuberant, elliptical; internarial region concave; canthus rostralis not well defined. Eyes small, directed anterolaterally, eyes about 50° relative to midline (where anterior-facing eyes would be 90° relative to midline). Tympanum barely visible, oriented almost horizontally; tympanic membrane differentiated and pigmented as surrounding skin. Dentigerous processes on vomers absent; choanae large, oval, separated widely (distance about the same as between nostrils); tongue round, white in preservative, anterior 4/5 attached to mouth; posterior border of tongue widely notched; vocal slits present, extending along floor of mouth lateral to tongue; enameled glands absent from lower part of upper jaw. Ulnar fold absent; humeral spine absent. Relative length of fingers: II < I < IV < III; finger discs rounded, about the same size as discs on toes, disc on Finger III 42% of eye width; hand webbing reduced between Fingers I–III, moderate between Fingers III and IV, with formula I 2^{+}—3^{-} II 2^{-}—$3^{1/5}$ III 2^{+}—$1^{3/4}$ IV. Prepollex concealed; subarticular tubercles round, faint; few small supernumerary tubercles present, palmar tubercle round, of moderate size and difficult to see, thenar tubercle ovoid; nuptial excrescences present as a small pad on external edge of Finger I (Type V). Hind limbs slender, tibia length 55% of SVL; tarsal fold absent; discs of toes round; inner metatarsal tubercle small, outer metatarsal tubercle round, both very difficult to distinguish. Webbing formula of feet: I 1_{+}—2 II 1—$2_{1/4}$ III $1_{1/2}$—2_{+} IV $2_{1/2}$—1 V. In preservative, dorsal skin creamish white, with minute dark lavender melanophores (only visible under the stereomicroscope); dorsal skin shagreen; skin on venter areolate; cloacal opening at level of upper thighs, small and non-enameled cloacal warts present. Parietal peritoneum and pericardium translucent (in life, the red heart is visible ventrally); urinary bladder lacking iridophores; liver, viscera, and tested fully covered by iridophores. Kidneys rounded, approximately bean-shaped; liver bulbous.

Coloration in life

Dorsal surfaces apple green to yellowish green with diffuse yellow spots; head with light yellow spots that may form an interorbital bar. Melanophores absent from fingers and toes, except Toes IV and V. Ventrally, parietal peritoneum and pericardium transparent, with a red heart always visible. Gall bladder and urinary bladder covered by translucent peritonea; hepatic and visceral peritonea covered by white iridophores; ventral vein red. Iris yellow, with numerous minute lavender spots. Bones white.

Coloration in preservative

Dorsal surfaces creamish white dotted with minute dark lavender melanophores; venter uniform cream, with partial translucence; pericardium translucent; visceral peritoneum covered by iridophores. Iris white with minute lavender melanophores. Melanophores absent from hands and feet, except from some present on dorsal surfaces of Toes IV and V.

Measurements of holotype (in mm)

CJ11642, adult male. SVL = 20.6, femur length = 11.4, tibia length = 11.3, foot length = 9.6, head length = 6.2, head width = 8.0, interorbital distance = 2.4, eye diameter = 2.6, tympanum diameter = 0.6, arm length = 4.2, hand length = 6.3, Finger I length = 4.6, Finger II length = 4.1, width of Finger III = 1.1.

Vocalizations

We measured call variables from two individuals, each from a different locality, Mashpi Reserve (CJ11642; call code LBE-C-051) and Tayra Reserve (CJ11645; call code LBE-C-052). The call of *Hyalinobatrachium mashpi* sp. nov. (Fig. 6) consists of a single pulsed (amplitude-modulated) note, which starts with one lower-frequency pulse followed by ~9 more consistent pulses at a slightly higher dominant frequency. We analyzed variables from both individuals: four calls from CJ11645 and eight calls from CJ11642. Calls in our field recordings had a duration of 0.373–0.461 s (mean = 0.425 ± 0.027 SD, n = 12). Time between calls ranged from 10.07–17.48 s (mean = 12.80 ± 2.166 SD, n = 10); intervals between *H. mashpi* calls were longer when a sympatric glassfrog (*Espadarana prosoblepon*) called in the interim period. Peak frequency was 5.25–5.6 kHz (mean = 5.38 kHz ± 0.12 SD; n = 12), with a maximum frequency of 5.46–5.81 kHz (mean = 5.38 kHz ± 0.11 SD; n = 12) and a minimum frequency of 4.62–4.92 kHz (mean = 4.79 kHz ± 0.10 SD; n = 12).

We compared the calls of *H. mashpi* sp. nov. to those of phenotypically and/or genotypically similar species within the same genus: *H. adespinosai*, *H. aureoguttatum*, *H. pellucidum*, *H. tatayoi*, and *H. valerioi* (Table 2). The call of *H. adespinosai* is a single pulsed (amplitude-modulated) note, consisting of ~12 pulses (mean call duration = 0.54 s ± 0.007 SD, n = 10). Time between calls ranged from 10.87–30.04 s (mean inter-call interval = 20.12 s ± 8.77 SD). Mean peak/fundamental frequency was 4.94 kHz (±0.07 SD; range = 4.87–5.04 kHz), with a mean maximum frequency of 5.11 (±0.08 SD; range = 5.0–5.25 kHz) and a mean minimum frequency of 4.57 (±0.15 SD; range = 4.32–4.75 kHz).

The call of *H. aureoguttatum* (Fig. 6; Table 2) consists of a very short, single tonal note (mean call duration = 0.082 s ± 0.002 SD, n = 24). Time between calls ranged from 1.99–5.20 s (mean inter-call interval = 3.24 s ± 0.79 SD, n = 23). Mean peak/fundamental frequency was 6.86 kHz (±0.25 SD; range = 6.55–7.41 kHz; n = 24 calls). Two harmonics are present. We measured call variables from individuals recorded in Canandé (0.5112 N, 79.1343 W; 457 m), Esmeraldas Province, Ecuador, in December 2018 by AVA (LBE- 053–55), and in Mashpi Lodge Reserve (0.17057 N, 78.888 W; 721–723 m) in March 2019 by RMB (LBE-056, 057).

The call of *H. chirripoi* is a single pulsed (amplitude-modulated) note, consisting of ~12 pulses (mean call duration = 0.255 s ± 0.03 SD, n = 2). Since our recording only included two bouts of calling, we were unable to include H. chirripoi in the DAPC analysis. The interval between the two calls was 84.3 s. Peak/fundamental frequency was 4.48 kHz, with a maximum frequency of 4.99–5.77 kHz and a minimum frequency of 4.16–4.21 kHz. We measured call variables from one individual recorded in Reserva Itapoa (0.51307 N, 79.134 W; 321 m), Esmeraldas Province, Ecuador, in July 2016 by JC (LBE-019).

The call of *H. pellucidum* consists of a short, single tonal note (mean call duration = 0.129 s ± 0.009 SD, n = 41). Time between calls ranged from 1.67–5.35 s (mean inter-call interval = 2.94 s ± 0.79 SD). Mean peak/fundamental frequency was 5.70 kHz (±0.06 SD; range = 5.60–5.86 kHz), with a mean maximum frequency of 6.0 (± 0.06 SD, range 5.86–6.14 kHz) and a mean minimum frequency of 5.16 (±0.07 SD, range 5.05–5.32 kHz). We measured call variables from one individual (USNM 286708) recorded at Río Azuela, Napo Province, Ecuador, by Roy McDiarmid on 23 February 1979.

The call of *H. tatayoi* consists of a short, single tonal note (mean call duration = 0.143 s ± 0.04 SD, n = 26). Time between calls ranged from 2.05–21.68 s (mean inter-call interval = 7.64 s ± 4.92 SD). Mean peak/fundamental frequency was 4.82 kHz (±1.77 SD; range = 4.45–5.11

kHz), with a mean maximum frequency of 5.14 (±0.17 SD, range 4.83–5.40 kHz) and a mean minimum frequency of 4.24 (±0.34 SD, range 3.30–4.61 kHz). We measured call variables from four individuals recorded in Jama Coaque Reserve (0.108264 S, 80.117701 W; 700 m), Manabí Province, Ecuador, in March 2019 by RMB.

The call of *H. valerioi* (Fig. 6) consists of a single tonal note (mean call duration = 0.079 s ± 0.01 SD, n = 70). Time between calls ranged from 1.76–8.00 s (mean inter-call interval = 4.27 s ± 1.2 SD). Mean peak frequency was 6.77 kHz (±0.19 SD; range = 6.46–7.24 kHz). Harmonics are likely present but are difficult to discern in the available recordings. We measured call variables from three individuals recorded in Costa Rica (Limón and Rincón de Oso) by Roy McDiarmid. We used the following recordings from the Macaulay Library at the Cornell Lab of Ornithology: ML212787, ML212788, and ML213430.

Results from the discriminant analysis of principal components (DAPC) revealed that the calls of *H. mashpi* sp. nov. cluster separately, and are thus acoustically distinct from *H. adespinosai*, *H. aureoguttatum*, *H. pellucidum*, *H. tatayoi*, and *H. valerioi* (Fig. 7). Overlap occurred between *H. aureoguttatum* and *H. valerioi* clusters, as well as between *H. tatayoi* and *H. adespinosai* clusters. This suggests that the calls of these pairs may not be adequate for species identification alone; more field recordings with genetic verification of the calling species are thus recommended for future studies. Nearly all (99.9%) of the variance was retained by three principal components. Table 3 lists the eigenvalues and variable loadings of each principal component.

Natural history

Most individuals of *Hyalinobatrachium mashpi* sp. nov. were found on the underside of leaves among riverine vegetation (Figs. 8, 9). These frogs are difficult to observe because they are found 3–14 m above ground along steep creeks. Males have been observed calling in the months of April, May, June, August, September, October and November. Males that were guarding egg clutches while calling were observed during the rainy season (18 February 2019; 7 May 2021) and dry season (October 2014, June 2015, and August 2021). Examined egg clutches contain 31–34 eggs (n = 3). A female with mature eggs visible through the skin was observed on 27 May 2015.

Distribution

Hyalinobatrachium mashpi sp. nov. is only known from the following localities (Fig. 10) in the Mashpi river basin, Pichincha Province, Ecuador: (i) Mashpi Lodge Reserve (San Vicente River, 1,040–1,101 m; Laguna River, 1,069 m); (ii) Amagusa Reserve (Amagusa River, 1,137 m; Mashpi Chico River, 1,130 m); and (iii) Tayra Reserve, 976–1,126 m. Unidentified and closely related frogs from Colombia (Departamento de Risaralda, MAR 2147; Departamento de Valle del Cauca; MAR 2222; Fig. 2) may prove to be conspecifics of *H. mashpi*.

Evolutionary relationships

Our phylogenetic analyses (Fig. 2) reveal *Hyalinobatrachium mashpi* sp. nov. as sister to undetermined haplotypes from the Colombian Andes (MAR 2147, 2222) and a new species from the Toisán Mountain Range, described below. Other closely related taxa are endemic to Central America: *H. vireovittatum* and *H. talamancae* (Fig. 2).

Etymology

The specific epithet mashpi is used as a noun in apposition and refers to the Mashpi area in northwestern Ecuador. There are several conservation efforts to preserve the last patches of forest remaining in Mashpi (e.g., Mashpi Lodge Reserve, Tayra Reserve, Amagusa Reserve, Mancomunidad del Chocó Andino, Chocó Andino Biosphere Reserve). Mashpi is a Yumbo word that means 'friend of water', an apt description of this glassfrog, which depends on healthy streams for its reproduction.

Conservation status

We recommend that *Hyalinobatrachium mashpi* be listed as Endangered, following IUCN Red List criteria B1ab(iii): extent of occurrence estimated to be less than 5,000 km^2; known to exist at no more than 5 localities; and continuing decline, observed, inferred or projected, in area, extent, and/or quality of habitat. The main threats for this species are habitat loss and contamination due to cattle ranching, agriculture, and mining activities (see Discussion).

(2) *Hyalinobatrachium nouns* new species
LSID: 1A908651-9A82-4DCA-9960-E8DC525F5ADF

Suggested English name: Nouns' Glassfrog
Suggested Spanish name: Rana de Cristal de Nouns

Holotype: MZUTI 3299, adult male from stream in Bosque Protector Los Cedros (0.310 N, 78.781 W; 1,420 m a.s.l.), Cordillera de Toisán, Imbabura Province, Ecuador, collected by Mariela Palacios, Jaime Culebras and Juan M. Guayasamin, on 12 March 2012.

Paratypes: CJ7703, adult male from stream in Bosque Protector Los Cedros (0.30241 N, 78.78558 W; 1,229 m a.s.l.), Cordillera de Toisán, Imbabura Province, Ecuador, collected by Morley Read and Arturo Guasti on 8 November 2017. CJ7722, adult male from stream in Bosque Protector Los Cedros (0.30191 N, 78.78513 W; 1,241 m a.s.l.), Cordillera de Toisán, Imbabura province, Ecuador, collected by Morley Read and Arturo Guasti on 11 November 2017. CJ7723, adult male from stream in Bosque Protector Los Cedros (0.30302 N, 78.78674 W; 1,313 m a.s.l.), Cordillera de Toisán, Imbabura province, Ecuador, collected by Morley Read and Arturo Guasti on 11 November 2017. ZSFQ-0537, adult male from stream in Río Manduriacu Reserve (0.31126 N, 78.8588 W; 1,254 m a.s.l.), Cordillera de Toisán, Imbabura province, Ecuador, collected by José Vieira, Scott Trageser, and Ross J. Maynard on 10 February 2018. ZSFQ-3906, metamorph from stream in Río Manduriacu Reserve (0.3099 N, 78.8567 W; 1,202 m a.s.l.), Cordillera de Toisán, Imbabura province, Ecuador, collected by Ross J. Maynard and Jaime Culebras on 23 November 2019.

Generic placement

Based of morphological and molecular data, the new species is placed in the genus *Hyalinobatrachium* sensu Ruiz-Carranza & Lynch, as modified by Guayasamin et al. (2009). The molecular phylogeny (Fig. 2) places the new species within the genus *Hyalinobatrachium* with high confidence. Phenotypically, *Hyalinobatrachium nouns* sp. nov. shares the following diagnostic traits of the genus *Hyalinobatrachium*: (1) completely transparent ventral parietal peritoneum; (2) digestive tract and bulbous liver are covered by iridophores; (3) absent humeral spines; (4) white bones in life; (5) males call from the undersides of leaves, (6) females place the eggs on the undersides of leaves; (7) males provide extended parental care; and (8) tympanum with an orientation that places it almost on a horizontal plane (instead of a more lateral plane as observed in other glassfrog genera). All the aforementioned characteristics are present in *Hyalinobatrachium nouns* sp. nov. We note that we have observed males on the same leaves as egg clutches for consecutive days, suggesting the possibility of parental care, but additional studies are necessary to confirm that these observations actually represent extended paternal care as observed in other *Hyalinobatrachium* species (see Delia et al. 2017).

Diagnosis

Hyalinobatrachium nouns sp. nov. is distinguished from other species in the genus mainly by its dorsal coloration (i.e., head with light yellow spots that may form an interorbital bar; dorsum lime green with small light yellow spots) and by its transparent pericardium. *Hyalinobatrachium nouns* sp. nov. is most similar to *H. aureoguttatum*, *H. mashpi* sp. nov., *H. talamancae*, *H. valerioi*, and *H. vireovittatum*. Differences among these species are indicated in Table 1 and Figs. 2–4. The new species is morphologically cryptic with *Hyalinobatrachium mashpi* sp. nov. (described above), but they exhibit a considerable genetic distance (16S; 4.6–4.7%), which is remarkable given that they are found at relatively close geographic proximity (straight distance = 18.9 km), but separated by the Intag-Guayllabamba river valley.

Characterization

The following combination of characters are found in *Hyalinobatrachium nouns* sp. nov.: (1) dentigerous process of the vomer lacking teeth; (2) snout truncate in dorsal view and slightly protruding in lateral view; (3) tympanum oriented almost horizontally; tympanic annulus barely visible, hidden under skin; tympanic membrane differentiated, with coloration similar to that of surrounding skin; (4) dorsal skin shagreen; (5) ventral skin areolate; cloacal ornamentation absent, paired round tubercles below vent absent; (6) parietal peritoneum and pericardium translucent (in life, red heart visible in ventral view); liver, viscera and testes covered by iridophores; (7) liver bulbous; (8) humeral spines absent; (9) hand webbing formula: I (2^{+}–2)—(2–2$^{1/2}$) II (1^{+}–1$^{1/2}$)—(3–3^{+}) III (2–2^{+})—(1$^{1/2}$–1$^{3/4}$) IV; (10) foot webbing moderate; webbing formula: I (1–1^{+})—(2^{-}–2) II (1–1^{+})—(2^{+}–2$^{1/2}$) III 1—(2^{+}–2$^{1/3}$) IV (2$^{1/4}$–2$^{1/3}$)—(1^{+}–1$^{1/3}$) V; (11) fingers and toes with thin lateral fringes; ulnar and tarsal folds absent; (12) nuptial excrescence present as a small pad on Finger I (Type V), prepollex not enlarged; prepollical spine not projecting (spine not exposed); (13) when appressed, Finger I longer than II; (14) diameter of eye about 2 times wider than disc on Finger III; (15) coloration in life: dorsal surfaces lime green with small light yellow spots; (16) coloration in preservative: dorsal surfaces creamish white, with minute lavender melanophores; (17) eye coloration in life: iris yellow to golden-yellow; pupil surrounded by lavender ring; (18) melanophores absent from fingers and toes, except Toes IV and V; (19) males call from underside of leaves; advertisement call unknown; (20) parental care unknown; clutch size unknown; (21) SVL in adult males 19.1–21.3 mm (mean = 20.3; n = 4), females unknown; and (22) enameled tubercles absent from sides of head.

Description of holotype

MZUTI 3299, adult male with SVL 19.1 mm. Head wider than long (head width 39% of SVL; head length 80% of head width). Snout truncate in dorsal view and slightly protruding in lateral view. Loreal region concave, nostrils slightly protuberant, elliptical; internarial region concave; canthus rostralis not well defined. Eyes small, directed anterolaterally, eyes about 50 relative to midline (where anterior-facing eyes would be 90 relative to midline). Tympanum visible, oriented almost horizontally; tympanic membrane differentiated and pigmented as surrounding skin. Dentigerous processes on vomers absent; choanae large, oval, separated widely (distance about the same as between nostrils); tongue round, white in preservative, anterior 4/5 attached to mouth; posterior border of tongue slightly notched; vocal slits present, extending along floor of mouth lateral to tongue; enameled glands absent from lower part of upper jaw. Ulnar fold absent; humeral spine absent. Relative length of fingers: II < I < IV < III; finger discs rounded, about the same size as discs on toes, disc on Finger III 41% of eye width; hand webbing reduced between Fingers I–III, moderate between Fingers III and IV, with formula $\mathrm{I}\ 2_{+}\text{—}2_{1/2}\ \mathrm{II}\ 1_{1/2}\text{—}3_{+}\ \mathrm{III}\ 2_{+}\text{—}1_{3/4}\ \mathrm{IV}$. Prepollex concealed; subarticular tubercles round, faint; few small supernumerary tubercles present, palmar tubercle round, of moderate size and difficult to see, thenar tubercle ovoid; nuptial excrescences present as a small pad on external edge of Finger I (Type V). Hind limbs slender, tibia length 59% of SVL; tarsal fold absent; discs of toes round; inner metatarsal tubercle small, outer metatarsal tubercle round, both very difficult to distinguish. Webbing formula of feet: $\mathrm{I}\ 1\text{—}2^{-}\ \mathrm{II}\ 1\text{—}2_{1/2}\ \mathrm{III}\ 1\text{—}2_{1/3}\ \mathrm{IV}\ 2_{1/4}\text{—}1_{1/3}\ \mathrm{V}$. In preservative, dorsal skin creamish white, with minute dark lavender melanophores (only visible under the stereomicroscope); dorsal skin shagreen; skin on venter areolate; cloacal opening at level of upper thighs, small and non-enameled cloacal warts present. Parietal peritoneum and pericardium translucent (in life, the red heart is visible ventrally); urinary bladder lacking iridophores; liver, viscera, and tested fully covered by iridophores; kidneys rounded, approximately bean-shaped; liver bulbous.

Coloration in life

Dorsal surfaces apple green to yellowish green with diffuse yellow spots; head with light yellow spots that may form an interorbital bar. Melanophores absent from fingers and toes, except Toes IV and V. Ventrally, parietal peritoneum and pericardium transparent, with a red heart always visible. Gall bladder and urinary bladder covered by translucent peritonea; hepatic and visceral peritonea covered by white iridophores; ventral vein red. Iris yellow, with numerous minute lavender spots. Bones white.

Coloration in preservative

Dorsal surfaces creamish white dotted with minute dark lavender melanophores; venter uniform cream, with partial translucence; pericardium translucent; visceral peritoneum covered by iridophores. Iris white with minute lavender melanophores. Melanophores absent from hands and feet, except from some few present on dorsal surfaces of Toes IV and V.

Measurements of holotype

MZUTI-3299, adult male. SVL = 19.1, femur length = 11.2, tibia length = 11.3, foot length = 8.8, head length = 5.9, head width = 7.4, interorbital distance = 2.2, upper eyelid = 1.5, internarial distance = 1.5, eye diameter = 2.2, tympanum diameter = 0.6, radioulna length = 4.0, hand length = 6.0, Finger I length = 4.4, Finger II length = 3.9, width of disc of Finger III = 0.9. Natural History. At Bosque Protector Los Cedros, individuals were found on the underside of riparian leaves 1–5 m above stream level during the months of November and March. At Río Manduriacu Reserve, during the rainy season (February), a male was found on the underside of a leaf 6 m above a stream; the male was calling next to an egg clutch. At the same reserve, metamorphs have been found perched on leaves 50–150 cm above streams in October and November.

Distribution

Hyalinobatrachium nouns sp. nov. is only known from Río Manduriacu Reserve and Bosque Protector Los Cedros at elevations of 1,177–1,420 m a.s.l. The reserves are located adjacent to one another and are situated within the Toisán Mountain Range, Imbabura Province, Ecuador (Fig. 10), and protect premontane wet tropical forest and cloud forest (Fig. 11) in an area where illegal deforestation and mining are constant threats (see Discussion).

Evolutionary relationships

Our phylogenetic analyses (Fig. 2) place *Hyalinobatrachium nouns* sp. nov. as sister to a clade formed by *H. mashpi* sp. nov. and unidentified haplotypes from the Colombian Andes (MAR 2147, 2222). However, this relationship has low support (bootstrap support = 60). Other closely related taxa are endemic to Central America: *H. vireovittatum* and *H. talamancae* (Fig. 2). Etymology. The specific epithet honors Nouns DAO, a global decentralized autonomous organization ("DAO") composed of owners of Nouns characters, which are digital art creations that live on the blockchain. The mission of Nouns DAO is to promote and build the Nouns brand throughout the physical and digital world. One of the ways Nouns DAO accomplishes this is by building public works and funding philanthropic projects that support the wonder of nature. Conservation status. We recommend that *Hyalinobatrachium nouns* sp. nov. be listed as Endangered, following IUCN (2012) criteria B1ab(iii): extent of occurrence estimated to be less than 5,000 km^2; known to exist at no more than five localities; and continuing decline, observed, inferred or projected, in area, extent, and/or quality of habitat. The main threats for this species are habitat loss and contamination due to cattle ranching, agriculture, and mining activities (see below).

DISCUSSION

Hidden diversity in the Andes

The striking homogeneity exhibited by glassfrog in the genus *Hyalinobatrachium* (sensu Guayasamin et al. 2009) probably is related to evolutive success of traits such as color pattern (related to camouflage) and reproductive strategies (e.g., breeding associated with streams, eggs placed on underside of leaves, extended parental care). Morphological similarity is also expected among closely related glassfrogs because they mainly speciate by allopatry (Castroviejo-Fisher et al. 2014; Guayasamin et al. 2020), retaining the ancestral ecological niche (Wiens 2004; Hutter et al. 2013). Therefore, considering morphological traits alone is likely to provide an underestimation of the true species richness within the genus. Congruently, vocalizations and molecular data have been shown to be robust tools to reveal morphologically cryptic taxa in Centrolenidae, as shown herein and previous studies (Castroviejo-Fisher et al. 2011; Hutter & Guayasamin 2012; Twomey et al. 2014; Guayasamin et al. 2020; Escalona-Sulbarán et al. 2019).

The topographical complexity of the Andes, with numerous pronounced river valleys, has favored population structure within species and, ultimately, speciation (Gentry 1982; Lynch & Duellman 1997; Madriñán et al. 2013; Pérez-Escobar et al. 2017; Polato et al. 2018; Guayasamin et al. 2017b, 2020).

Our study provides additional evidence of the biological uniqueness within the Toisán mountain range, which is separated from the western Andes by the Intag-Guayllabamba river valley in the south and the Mira river valley in the north. These valleys seem to be an important dispersal barrier; as a consequence, several anuran sister species are found in the Toisán mountain range and the nearby western Andes: (i) *Hyalinobatrachium nouns* sp. nov. + *H. mashpi* sp. nov., (ii) *Noblella worleyae* + *N. mindo* (Reyes-Puig et al. 2021), (iii) *Pristimantis cedros* + *P. pahuma* (Hutter & Guayasamin 2015), (iv) *Hyloscirtus princecharlesi* + *H. ptychodactylus* (Coloma et al. 2012), and (v) genetically differentiated populations of *P. mutabilis* (Guayasamin et al. 2015). The high levels of endemism exhibited by amphibians in the Toisán mountain range likely also apply to other taxa with limited dispersal abilities (e.g., flightless invertebrates and small mammals). The two new glassfrog species described herein, although inhabiting forests that are only 18.9 km apart (Fig. 10), have a considerable genetic distance (4.6–4.7%), which is much higher that the intraspecific variation observed in the family, even in species with broad distributional ranges (< 3%; Castroviejo-Fisher et al. 2011; Guayasamin et al. 2020).

An unexpected result from our study is that the calls of the sister species *Hyalinobatrachium aureoguttaum* and *H. valerioi* are very similar (Figs. 6, 7). Given the importance of calls in species recognition (Wells, 2010), two scenarios explain the observed data: (i) the two species are fully allopatric and the ancestral call traits have been retained, or (ii) the two species actually represent one evolutionary lineage. Based on our current dataset, we tend to favor the first hypothesis, because there are color (Figs. 3–5) and genetic differences (Fig. 1) between *H. aureoguttaum* and *H. valerioi*. Nevertheless, full clarification would require more sampling (especially in Colombia) and studies in potential contact areas. Finally, within lowland populations of *H. aureguttatum* in Ecuador, we found two clades (Fig. 2); further analyses should determine if these genetic differences are the result of different evolutionary trajectories or retained ancestral polymorphisms (Nichols 2001).

Amphibians are the most threatened Andean vertebrates. Amphibian diversity and endemicity are particularly accentuated in the Andes—roughly 70% of the 1,120 reported species are endemic (CEPF 2021). The Andes also boasts the highest rate of new amphibian species discoveries of any biogeographic region in South America (Vasconcelos et al. 2019; Womack et al. 2021). Yet amphibians are particularly susceptible to anthropogenic impacts (Duellman & Trueb 1994; Lips et al. 2006; Pounds et al. 2006; Scheele et al. 2019), which are immense in the Andes. Currently, only 8% of Andean amphibian species are well-protected (Bax

& Francesconi 2019). An array of human pressures continues to diminish the integrity of Andean terrestrial and freshwater ecosystems (Myers et al. 2000; Knee & Encalada 2014; Roy et al. 2018; Bax & Francesconi 2019; CEPF 2021; Torremorell et al. 2021). As a result, taxonomic groups such as glassfrogs—where a majority of members are endemic to the Tropical Andes, and individual species often have highly restricted distributions—are especially at risk of population declines and extinction (Aguilar et al. 2012; Guayasamin et al. 2019b, 2020; Ortega-Andrade et al. 2021).

Baseline data for amphibians and many Andean taxa—if not most—do not exist. It is therefore difficult to fully appreciate the potential extent of regional biodiversity loss if human landscape modification continues without the implementation of effective mitigation measures (Moura & Jetz, 2021 Pérez-Escobar et al. 2022). Although many tropical areas lack the resources necessary to establish and manage protected areas (Lessmann et al. 2016), the presence of community or non-governmental nature reserves in the Andes can play a crucial role in the protection of amphibians and other threatened species. Notably, our records of *Hyalinobatrachium nouns* sp. nov. were all collected within the boundaries of mining concessions (i.e., Reserva Los Cedros and Río Manduriacu Reserve), and records for *H. mashpi* sp. nov. are either within or adjacent to mining concessions (Roy et al. 2018).

Given the plethora of evidence that supports the importance of biodiversity of the Andean region, the decision by the last governments (2007 to present) to encourage large-scale mining operations throughout Andean Ecuador is alarming. Nonetheless, communities in the Intag-Toisán Region and Chocó Andino of northwest Ecuador have demonstrated how unified action, voting for local politicians who support and legislate environmental policies, and partnering with a diverse network of NGOs can result in the ability to meaningfully contest the progression of mining in and around their territories (Avci & Fernández-Salvador 2016; Roy et al. 2018; Guayasamin et al. 2019b, 2021; Freile et al. 2020). Los Cedros Reserve has become a landmark legal case premised on the rights of nature; the recent ruling by Constitutional Court of Ecuador in favor of Los Cedros opens up the possibility of a domino effect favoring biodiverse areas in the Ecuadorian Andes (Guayasamin et al. 2021).

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FIGURE 1. **Morphological measurements as obtained in this study**

Measurements are described in the text. SVL = snout-vent length; HW = head width; IOD = interorbital distance. Illustrations by Valentina Nieto Fernández.

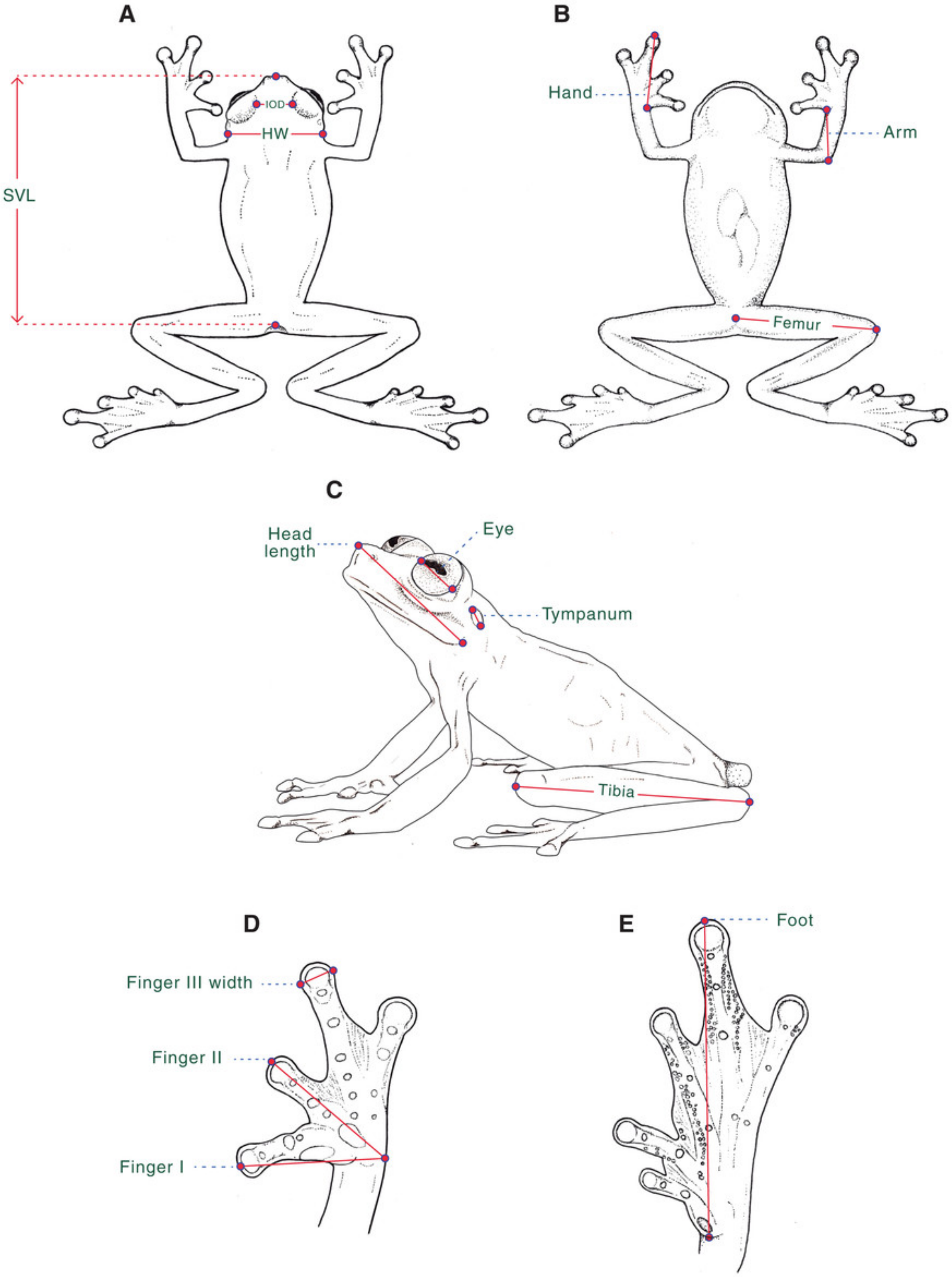


FIGURE 2. **Phylogenetic position of *Hyalinobatrachium mashpi* sp. nov. and *H. nouns* sp. nov.**

Phylogenetic relationships of *Hyalinobatrachium* inferred from the 16S mitochondrial gene under ML criterion. Genbank codes are listed next to each terminal, if applicable. Associated locality data is available at Genbank, as well as in Guayasamin *et al*. (2008, 2020), Castroviejo-Fisher *et al*. (2014), and Twomey *et al*. (2014).

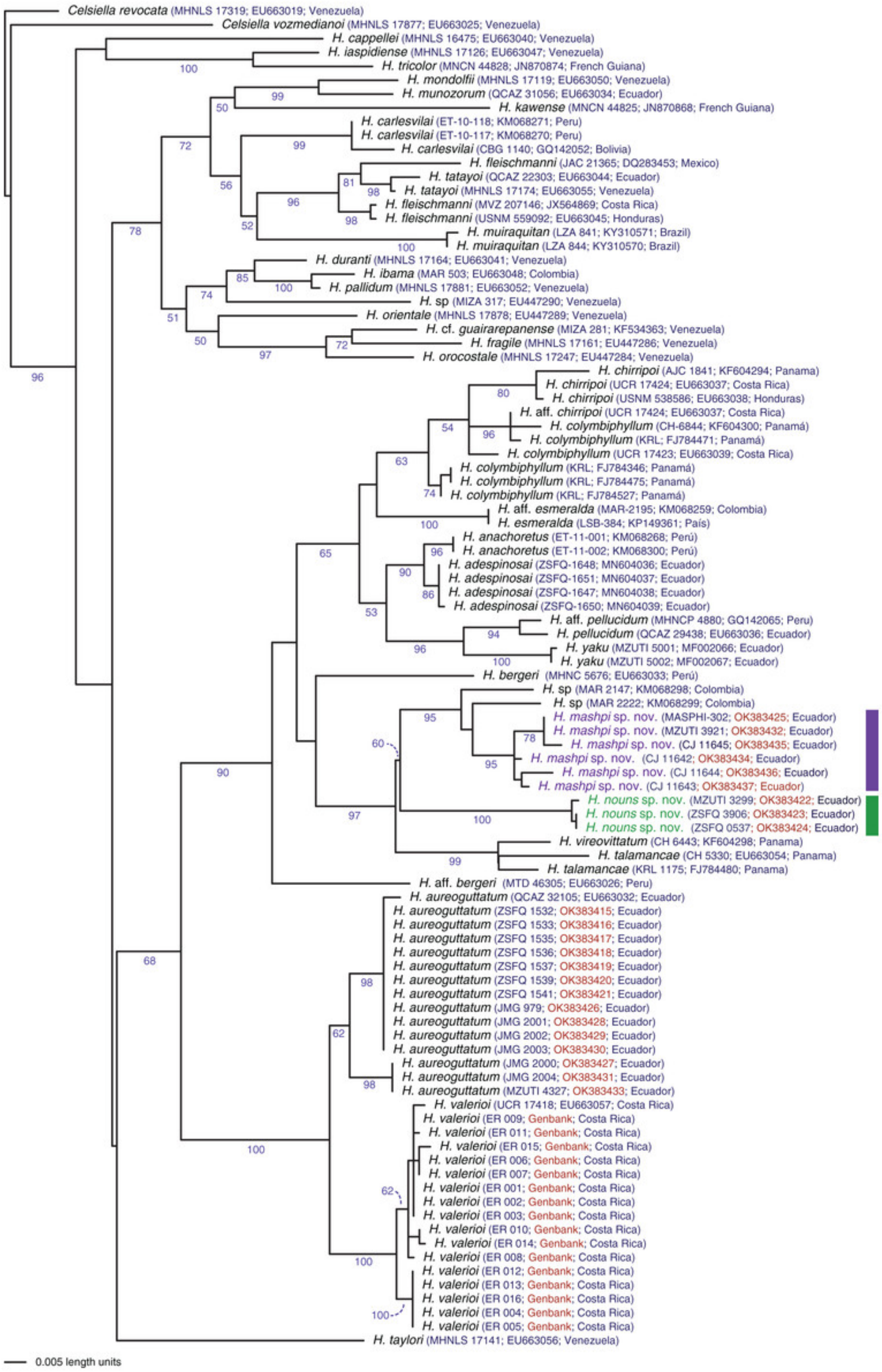


FIGURE 3. **Dorsal and ventral photos of *Hyalinobatrachium mashpi* sp. nov., *H. nouns* sp. nov., and *H. aureoguttatum* in life.**

(A) Male of *Hyalinobatrachium mashpi* sp. nov., CJ11642 (holotype). **(B)** Gravid female of *H. mashpi* sp. nov., Mashpi Reserve, Ecuador. **(C)** Male of *H. nouns* sp. nov., ZSFQ0537. **(D)** Male of *H. nouns* sp. nov., MZUTI3299 (holotype). **(E)** Male of *H. aureoguttatum*, Ecuador. **(F)** Gravid female of *H. aureoguttatum*, Ecuador. Photos by Jaime Culebras (A, B, D, E, F) and Ross Maynard (C).

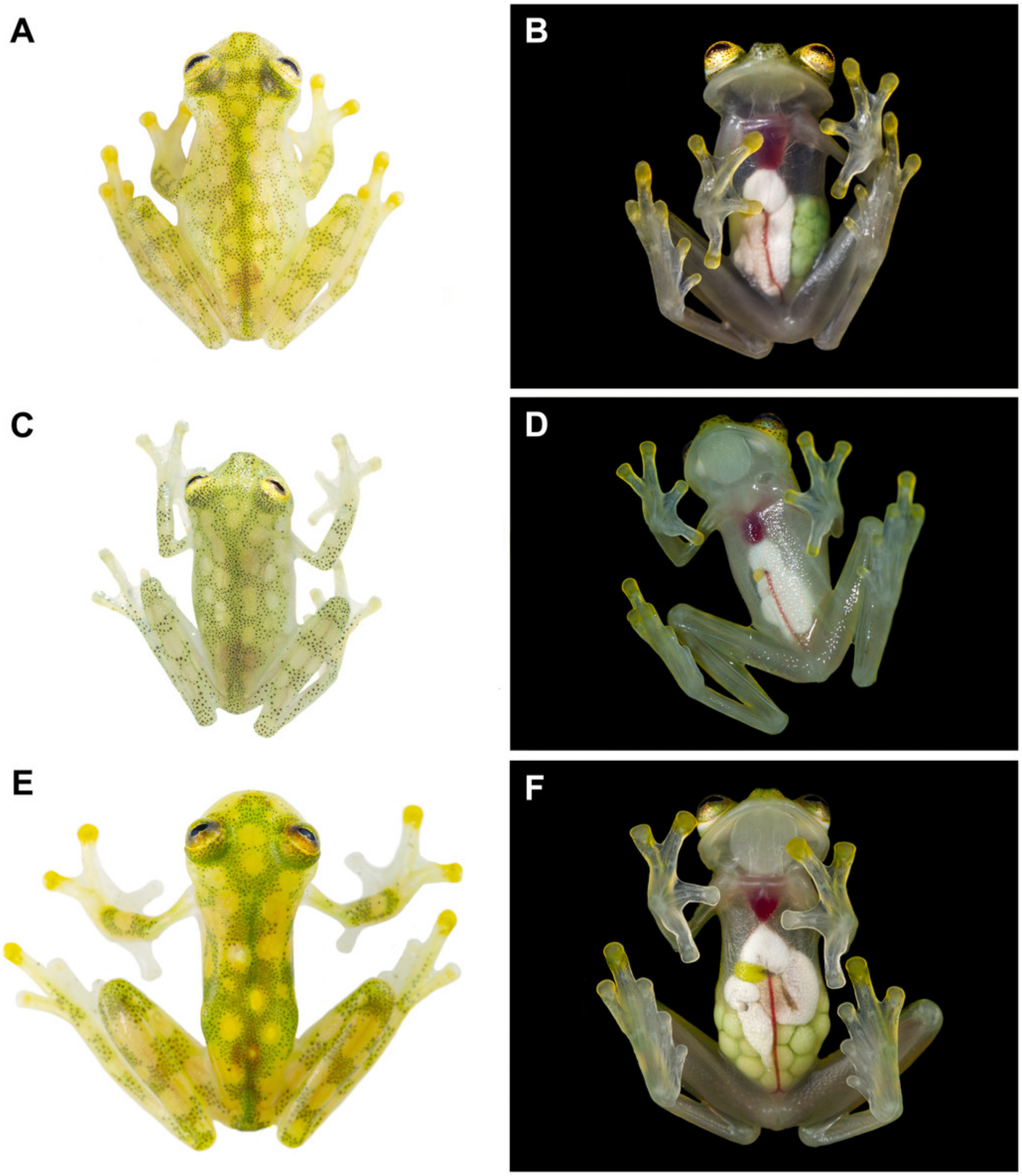


FIGURE 4. Dorsal and ventral photos of *Hyalinobatrachium vireovittatum, H. talamancae,* and *H. valerioi* in life.

(A, B) Male of *Hyalinobatrachium vireovittatum*, Costa Rica. **(C, D)** Male of *H. talamancae*, Costa Rica. **(E, F)** Male of *H. valerioi*, Costa Rica. Photos by Jaime Culebras (A, C, D, E, F) and Josué Alberto Vargas (B).

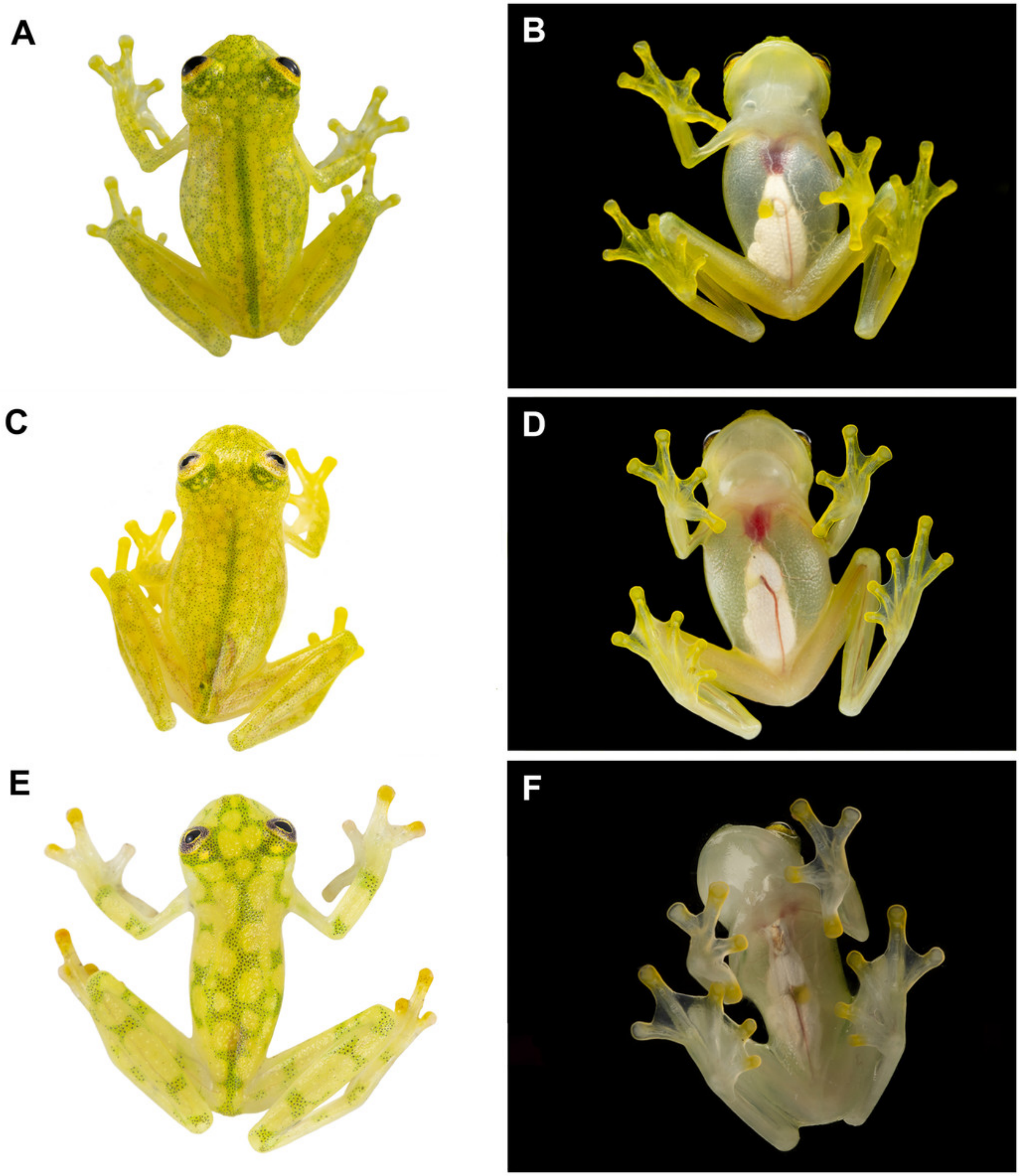


FIGURE 5. Dorsal comparisons of closely related glassfrog species.

(A) *Hyalinobatrachium mashpi* sp. nov., CJ11642 (holotype). **(B)** *H. nouns* sp. nov., ZSFQ0537. **(C)** *H. aureoguttatum*, SC 435. **(D)** *H. talamancae*, Costa Rica. **(E)** *H. vireovittatum*, Costa Rica. **(F)** *H. valerioi*, Costa Rica. Photos by Jaime Culebras (A, D, E, F), Jose Vieira (B), and Luis Coloma (C).

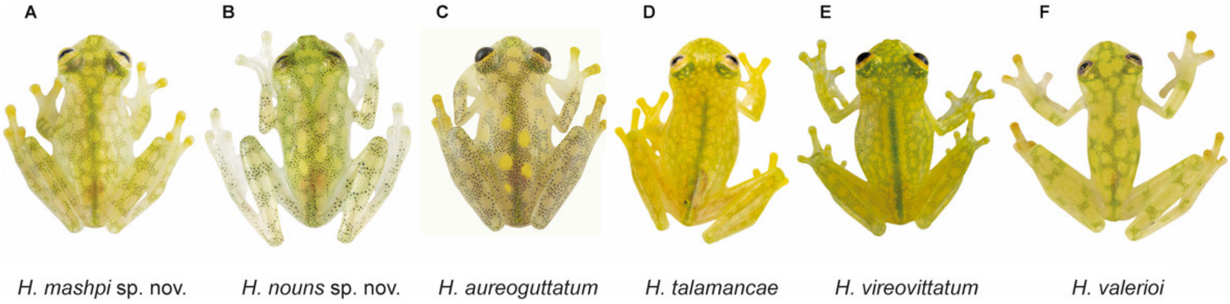
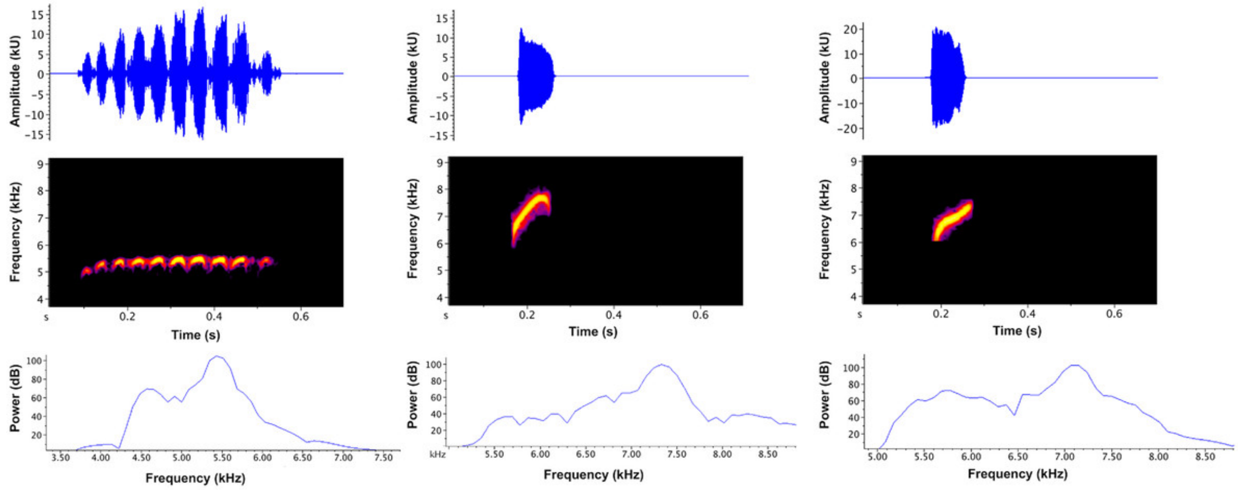


FIGURE 6. Visual representation of *Hyalinobatrachium mashpi* sp. nov. advertisement call, with comparisons of two similar species.

The call of each species is depicted in three forms: **(Top)** oscillograms, waveforms representing amplitude changes over time; (**Middle)** spectrograms, plots of frequency over time, with higher amplitudes represented by brighter colors; and (**Bottom)** power spectra, representing the relative amplitude of each frequency.



H. mashpi sp. nov.

H. aureoguttatum

H. valerioi

FIGURE 7. Discriminant analysis of principal components (DAPC) of glassfrog species' calls.

Variables analyzed include: peak frequency, maximum frequency, minimum frequency, call duration, and inter-call duration. Sample size as follows: *H. adespinosai* (1 individual, 10 calls); *H. aureoguttatum* (6 individuals, 24 calls); *H. mashpi* sp. nov. (2 individuals, 12 calls); *H. pellucidum* (1 individual, 41 calls); *H. tatayoi* (4 individuals, 26 calls); *H. valerioi* (3 individuals, 70 calls).

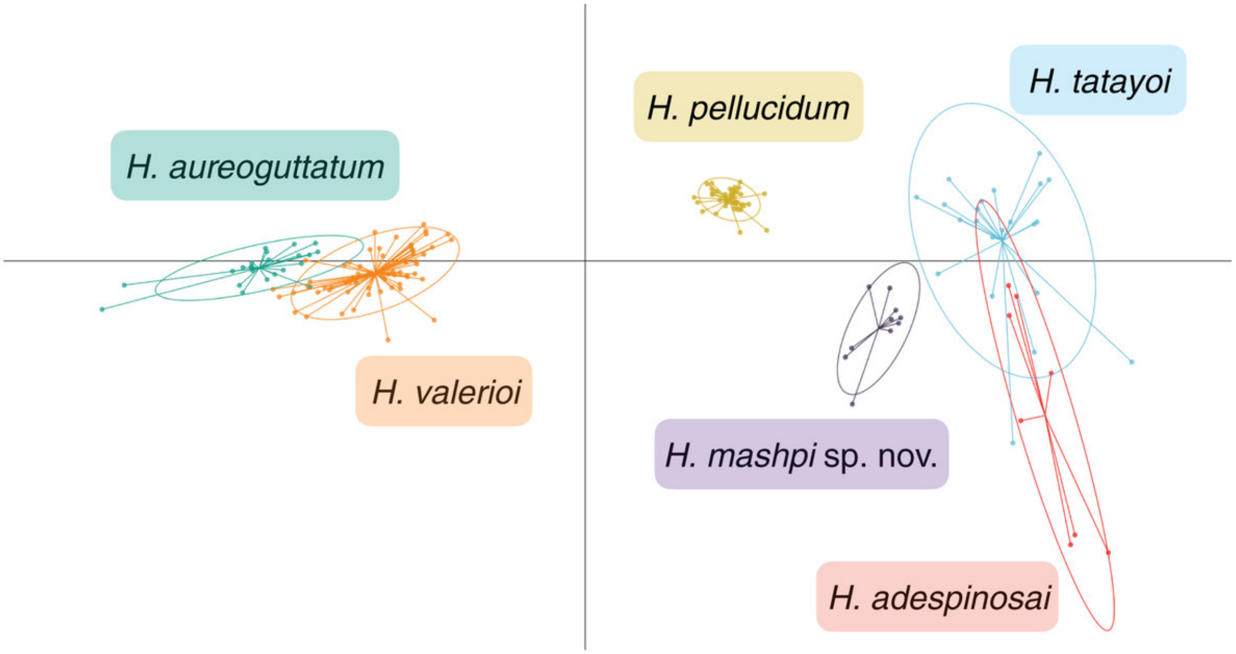


FIGURE 8. Males of *Hyalinobatrachium mashpi* sp. nov. guarding eggs.

(A) Male calling at San Vicente River, Mashpi Reserve, Pichincha Province, Ecuador. **(B)** Male at tributary of the Mashpi River, Tayra Reserve, Pichincha Province, Ecuador. Photos by Carlos Morochz (A) and Jaime Culebras (B).

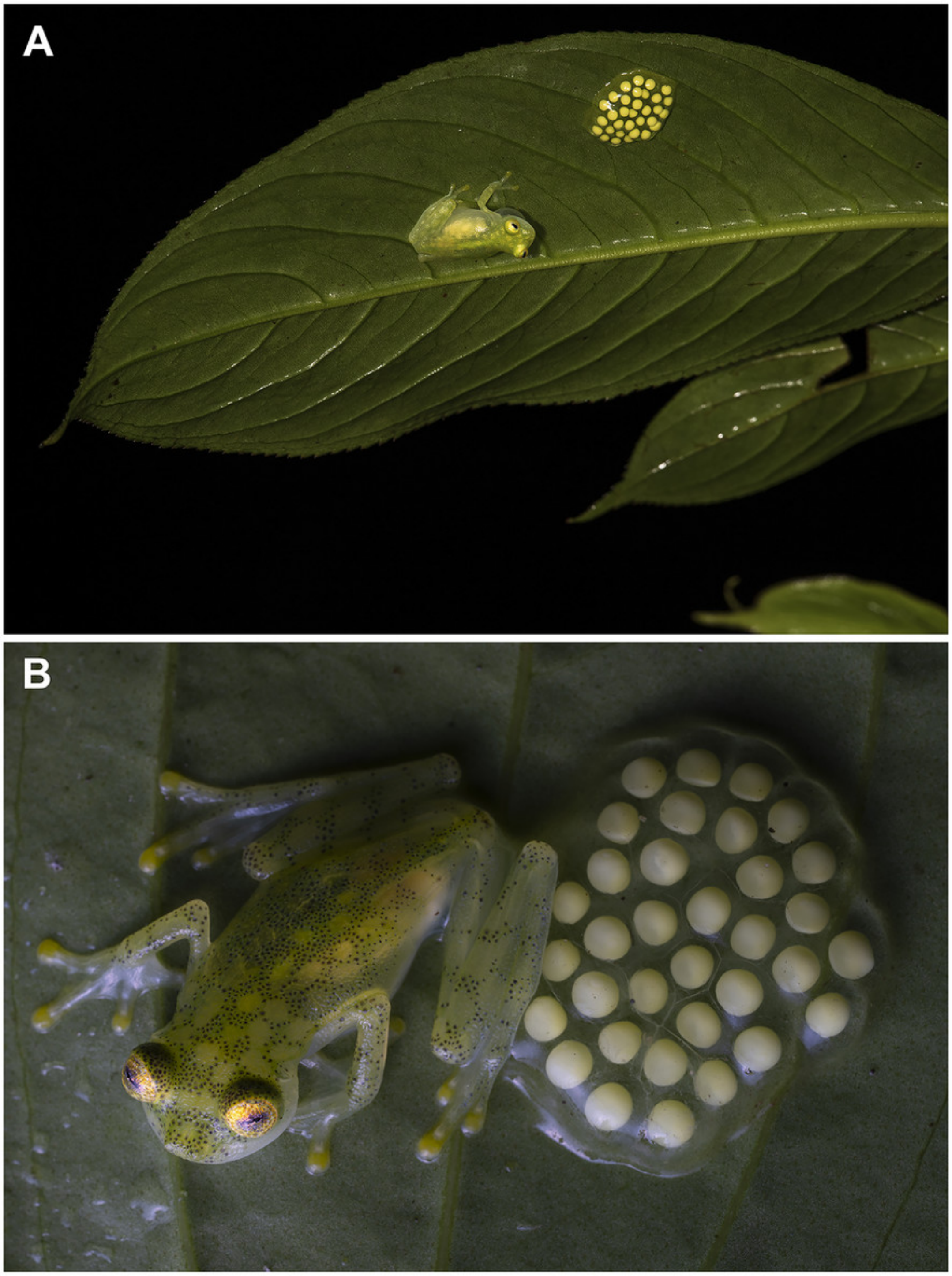


FIGURE 9. Habitat of *Hyalinobatrachium mashpi* sp. nov.

(A) Tributary of the Mashpi River, Tayra Reserve, Pichincha Province, Ecuador. **(B)** Mashpi Reserve, Pichincha Province, Ecuador. **(C)** Tayra Reserve, Pichincha Province, Ecuador. **(D)** Habitat loss in the vicinity of Tayra Reserve, Pichincha Province, Ecuador. Photos by Jaime Culebras.

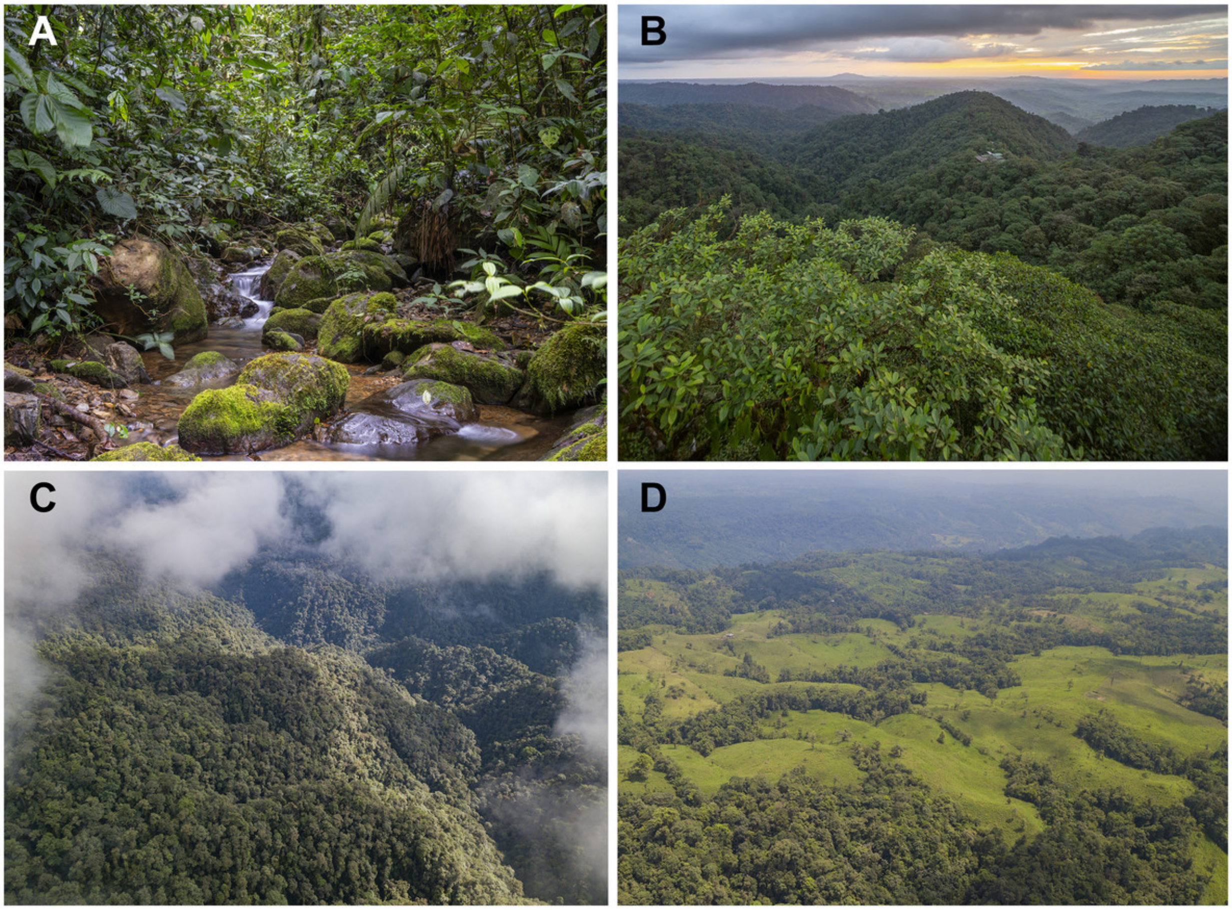


FIGURE 10. Distribution of *Hyalinobatrachium mashpi* sp. nov. and *H. nouns* sp. nov. in Ecuador.

Note that localities of the two new taxa are separated by the Intag-Guayllabamba valley.

FIGURE 11. Habitat of *Hyalinobatrachium nouns* sp. nov.

(A) Tributary of the Manduriacu River, Río Manduriacu Reserve, Imbabura Province, Ecuador. **(B)** Tributary of the Manduriacu River, Río Manduriacu Reserve, Imbabura Province, Ecuador. **(C)** Río Manduriacu Reserve, Imbabura Province, Ecuador. **(D)** Habitat loss in the vicinity of Los Cedros Reserve, Imbabura Province, Ecuador. Photos by Jaime Culebras.

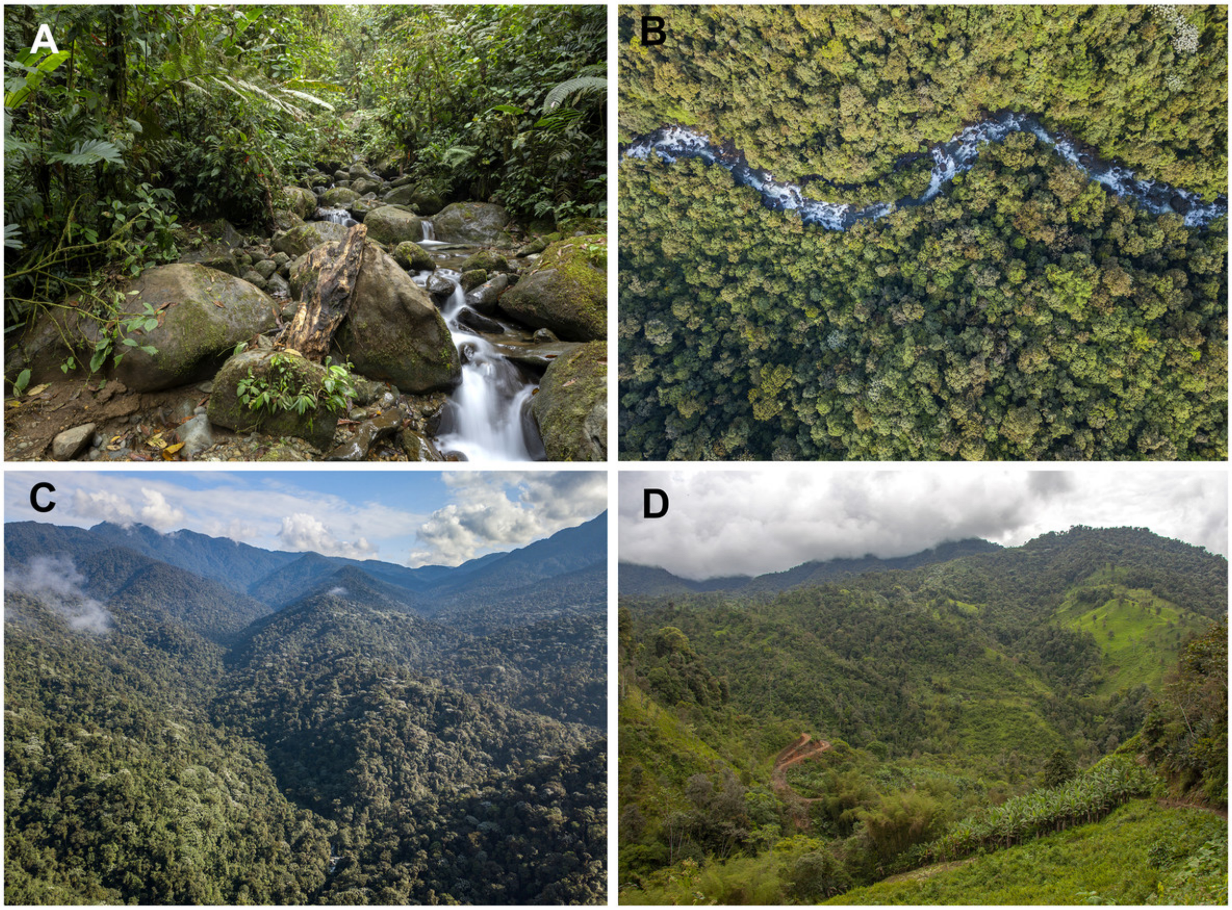
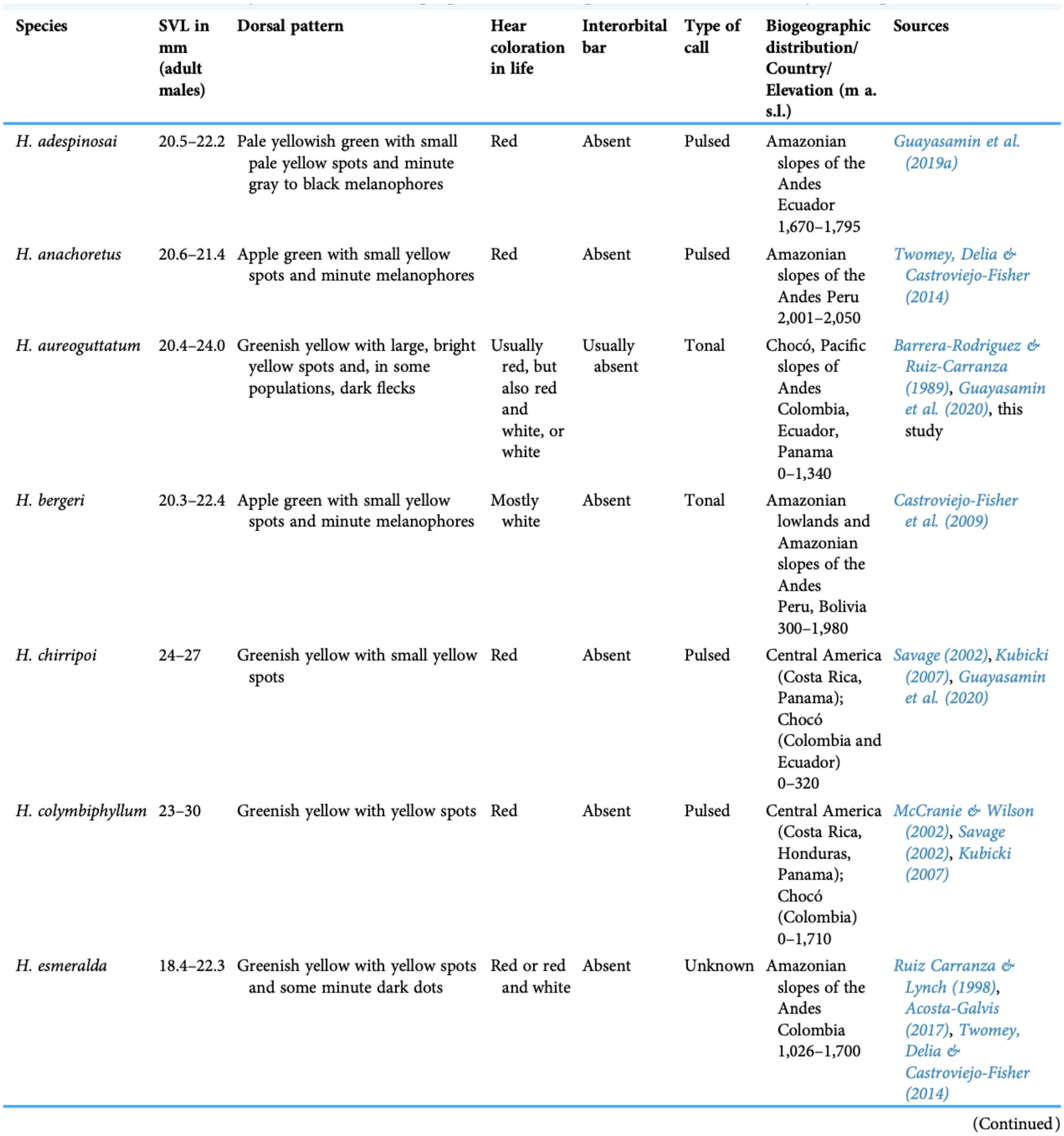


TABLE 1. Trait differences between *Hyalinobatrachium mashpi* sp. nov., *H. nouns* sp. nov., and similar and closely related species.



Species	SVL in mm (adult males)	Dorsal pattern	Hear coloration in life	Interorbital bar	Type of call	Biogeographic distribution/ Country/ Elevation (m a. s.l.)	Sources
H. adespinosai	20.5–22.2	Pale yellowish green with small pale yellow spots and minute gray to black melanophores	Red	Absent	Pulsed	Amazonian slopes of the Andes Ecuador 1,670–1,795	*Guayasamin et al. (2019a)*
H. anachoretus	20.6–21.4	Apple green with small yellow spots and minute melanophores	Red	Absent	Pulsed	Amazonian slopes of the Andes Peru 2,001–2,050	*Twomey, Delia & Castroviejo-Fisher (2014)*
H. aureoguttatum	20.4–24.0	Greenish yellow with large, bright yellow spots and, in some populations, dark flecks	Usually red, but also red and white, or white	Usually absent	Tonal	Chocó, Pacific slopes of Andes Colombia, Ecuador, Panama 0–1,340	*Barrera-Rodriguez & Ruiz-Carranza (1989)*, *Guayasamin et al. (2020)*, this study
H. bergeri	20.3–22.4	Apple green with small yellow spots and minute melanophores	Mostly white	Absent	Tonal	Amazonian lowlands and Amazonian slopes of the Andes Peru, Bolivia 300–1,980	*Castroviejo-Fisher et al. (2009)*
H. chirripoi	24–27	Greenish yellow with small yellow spots	Red	Absent	Pulsed	Central America (Costa Rica, Panama); Chocó (Colombia and Ecuador) 0–320	*Savage (2002)*, *Kubicki (2007)*, *Guayasamin et al. (2020)*
H. colymbiphyllum	23–30	Greenish yellow with yellow spots	Red	Absent	Pulsed	Central America (Costa Rica, Honduras, Panama); Chocó (Colombia) 0–1,710	*McCranie & Wilson (2002)*, *Savage (2002)*, *Kubicki (2007)*
H. esmeralda	18.4–22.3	Greenish yellow with yellow spots and some minute dark dots	Red or red and white	Absent	Unknown	Amazonian slopes of the Andes Colombia 1,026–1,700	*Ruiz Carranza & Lynch (1998)*, *Acosta-Galvis (2017)*, *Twomey, Delia & Castroviejo-Fisher (2014)*

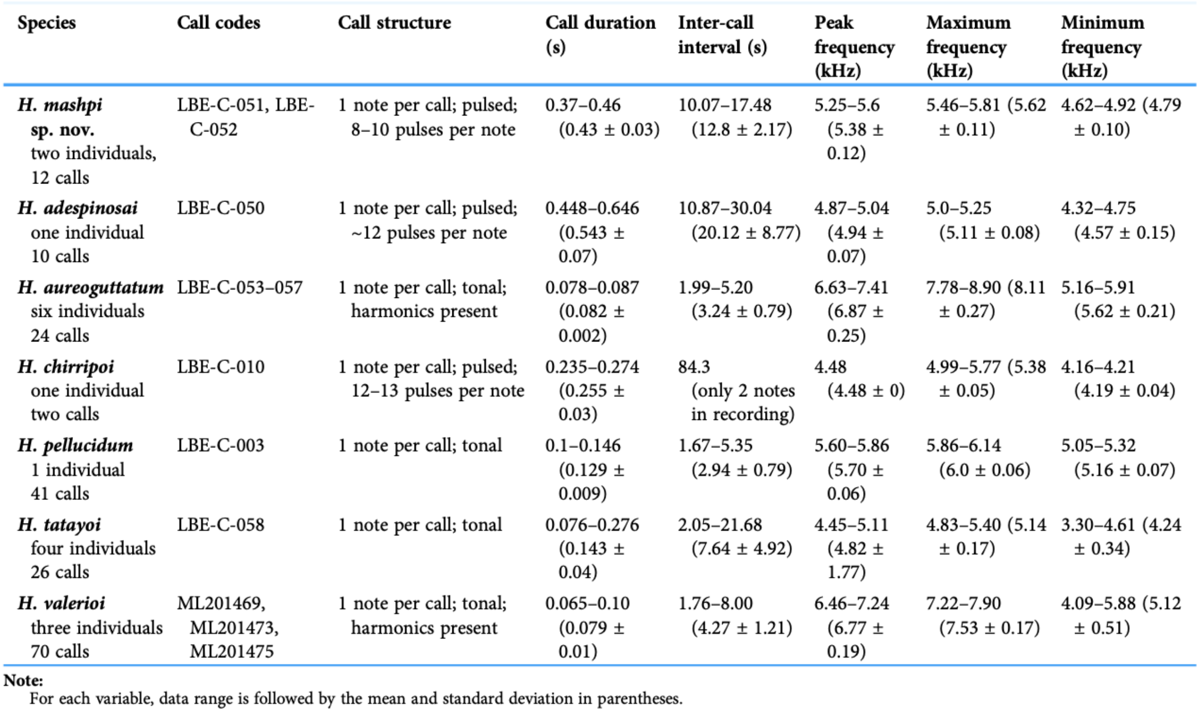
(Continued)

Table 1 (continued)

Species	SVL in mm (adult males)	Dorsal pattern	Hear coloration in life	Interorbital bar	Type of call	Biogeographic distribution/ Country/ Elevation (m a. s.l.)	Sources
***H. mashpi* sp. nov.**	19.7–20.9	Greenish yellow with small and diffuse yellow spots	Red	Usually present	Pulsed	Pacific slopes of the Andes Ecuador 976–1,137	This study
***H. nouns* sp. nov.**	19.1–21.3	Greenish yellow with small and diffuse yellow spots	Red	Usually present	Unknown	Pacific slopes of the Andes Ecuador 1,177–1,420	This study
H. pellucidum	20.4–21.4	Greenish yellow with small yellow spots	White	Absent	Tonal	Amazonian slopes of the Andes Ecuador, Peru 523–1,740	*Lynch & Duellman (1973)*, *Guayasamin et al. (2020)*
H. valerioi	19.5–24.0	Greenish yellow with large and diffuse yellow spots	Red or red and white	Usually absent	Tonal	Central América, Chocó, Pacific slopes of the Andes Costa Rica, Colombia, Ecuador 0–1,500	*Savage (2002)*, *Kubicki (2007)*, *Guayasamin et al. (2020)*
H. vireovittatum	21.5–23.0	Greenish yellow with small yellow spots. Dark green middorsal stripe outlined by yellow paravertebral stripes	Red	Present or absent	Tonal	Central America Costa Rica 250–1,957	*Savage (2002)*, *Kubicki (2007)*, *Campos-Villalobos et al. (2020)*
H. talamancae	24–26	Greenish yellow with small yellow spots. Dark green middorsal stripe present.	Red	Present or absent	Tonal	Central America Costa Rica 475–1,600	*Kubicki (2006*, *2007)*; *Zamora-Roda, Herrera-Martínez & Salazar (2021)*
H. yaku	20.8–22.3	Green with small yellow spots and minute melanophores; posterior head and anterior half of the body with few small dark green spots placed middorsally	Red	Absent	Tonal	Amazonian lowlands Ecuador 300–360	*Guayasamin et al. (2017a)*

Note:
Sources of traits are indicated in the last column.

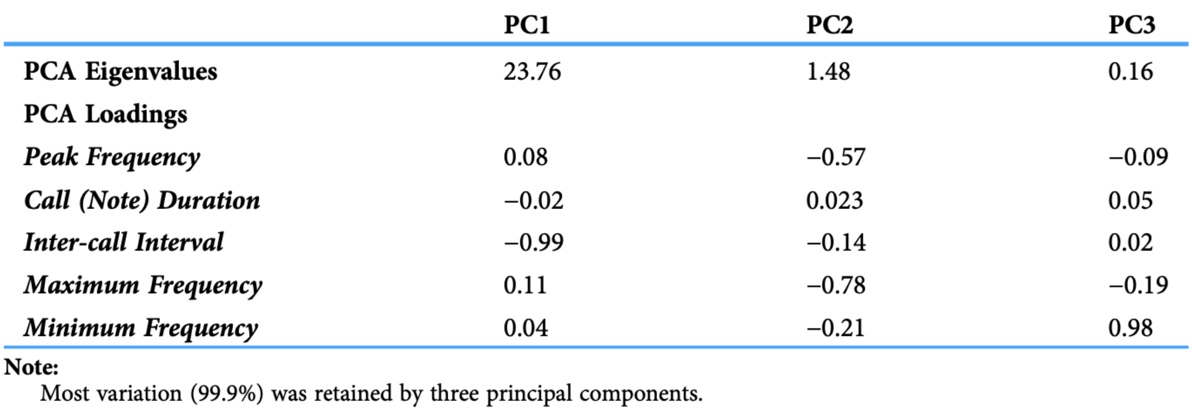
TABLE 2. Acoustic differences between *Hyalinobatrachium mashpi* sp. nov. and related species.



Species	Call codes	Call structure	Call duration (s)	Inter-call interval (s)	Peak frequency (kHz)	Maximum frequency (kHz)	Minimum frequency (kHz)
***H. mashpi* sp. nov.** two individuals, 12 calls	LBE-C-051, LBE-C-052	1 note per call; pulsed; 8–10 pulses per note	0.37–0.46 (0.43 ± 0.03)	10.07–17.48 (12.8 ± 2.17)	5.25–5.6 (5.38 ± 0.12)	5.46–5.81 (5.62 ± 0.11)	4.62–4.92 (4.79 ± 0.10)
H. adespinosai one individual 10 calls	LBE-C-050	1 note per call; pulsed; ~12 pulses per note	0.448–0.646 (0.543 ± 0.07)	10.87–30.04 (20.12 ± 8.77)	4.87–5.04 (4.94 ± 0.07)	5.0–5.25 (5.11 ± 0.08)	4.32–4.75 (4.57 ± 0.15)
H. aureoguttatum six individuals 24 calls	LBE-C-053–057	1 note per call; tonal; harmonics present	0.078–0.087 (0.082 ± 0.002)	1.99–5.20 (3.24 ± 0.79)	6.63–7.41 (6.87 ± 0.25)	7.78–8.90 (8.11 ± 0.27)	5.16–5.91 (5.62 ± 0.21)
H. chirripoi one individual two calls	LBE-C-010	1 note per call; pulsed; 12–13 pulses per note	0.235–0.274 (0.255 ± 0.03)	84.3 (only 2 notes in recording)	4.48 (4.48 ± 0)	4.99–5.77 (5.38 ± 0.05)	4.16–4.21 (4.19 ± 0.04)
H. pellucidum 1 individual 41 calls	LBE-C-003	1 note per call; tonal	0.1–0.146 (0.129 ± 0.009)	1.67–5.35 (2.94 ± 0.79)	5.60–5.86 (5.70 ± 0.06)	5.86–6.14 (6.0 ± 0.06)	5.05–5.32 (5.16 ± 0.07)
H. tatayoi four individuals 26 calls	LBE-C-058	1 note per call; tonal	0.076–0.276 (0.143 ± 0.04)	2.05–21.68 (7.64 ± 4.92)	4.45–5.11 (4.82 ± 1.77)	4.83–5.40 (5.14 ± 0.17)	3.30–4.61 (4.24 ± 0.34)
H. valerioi three individuals 70 calls	ML201469, ML201473, ML201475	1 note per call; tonal; harmonics present	0.065–0.10 (0.079 ± 0.01)	1.76–8.00 (4.27 ± 1.21)	6.46–7.24 (6.77 ± 0.19)	7.22–7.90 (7.53 ± 0.17)	4.09–5.88 (5.12 ± 0.51)

Note:
For each variable, data range is followed by the mean and standard deviation in parentheses.

TABLE 3. Results from the discriminant analysis of principle components (DAPC), comparing the advertisement calls of *Hyalinobatrachium mashpi* sp. nov. with those of closely related species (See Fig. 7).



	PC1	PC2	PC3
PCA Eigenvalues	23.76	1.48	0.16
PCA Loadings			
Peak Frequency	0.08	−0.57	−0.09
Call (Note) Duration	−0.02	0.023	0.05
Inter-call Interval	−0.99	−0.14	0.02
Maximum Frequency	0.11	−0.78	−0.19
Minimum Frequency	0.04	−0.21	0.98

Note:

Most variation (99.9%) was retained by three principal components.

Chapter 6. Conservation challenges & opportunities in the Ecuadorian Andes

With more than 34,000 species of plants and vertebrates—and a high level of endemism—the Tropical Andes Hotspot is the most biodiverse region in the world (e.g., Hutter et al. 2017). Despite this overwhelming diversity, Andean ecosystems have more species listed as threatened than any other global hotspot (CEPF 2015), due in part to anthropogenic pressures in a landscape also defined by high rates of endemism, especially on a micro-scale (e.g., Pomar-Gómez et al. 2021).

While the region's diversity patterns apply to most major Andean groups of flora and fauna, they are particularly accentuated in amphibians, with roughly 70% of more than 980 species endemic to the region and greater than 500 threatened species (CEPF 2020). The region also boasts the highest rate of new amphibian species discoveries compared to all other biogeographic regions in South America (Vasconcelos et al. 2019). The limited distributions of many amphibians render them especially sensitive to anthropogenic impacts (Aguilar et al. 2012; Guayasamin et al. 2019, 2020). As a result, amphibians are the most threatened vertebrates in the Andes (CEPF 2020).

Without the help of Andean countries and their governments to support research and conservation, population declines and extinction events are inevitable. Currently, only 8% of Andean amphibian species are well protected (Bax & Francesconi 2019). Nonetheless, human pressures continue to diminish the integrity of Andean terrestrial and freshwater ecosystems (Roy et al. 2018; Bax et al. 2019; Torremorell et al. 2021). As a result, taxonomic groups such as glassfrogs—most of which are endemic to the Tropical Andes—are especially at risk of population declines and extinction (Aguilar et al., 2012; Guayasamin et al., 2019, 2021). The lack of baseline data for amphibians and many (if not most) Andean taxa renders it difficult to fully appreciate the potential extent of regional biodiversity loss if human landscape modification continues without the implementation of effective mitigation measures.

Ecuador represents an important case study within this broader dynamic, not only in terms of imminent threats to Andean biodiversity, but also as an example of how to implement innovative management that empowers local communities while mitigating species decline and loss. Once again, amphibians serve as a proxy for what is more broadly at stake for Ecuadorian biodiversity in the Andes. Amphibian diversity, rates of endemism, and density of threatened species are all highest in the Andes of Ecuador (Ortega-Andrade et al. 2021). Mapping the distribution of taxa by individual categories demonstrates that threatened amphibians are most concentrated in Andean Ecuador regardless of status, but especially so for Critically Endangered and Endangered amphibians (Ortega-Andrade et al. 2021). Moreover, the region is a prominent source of new species discovery, the two glassfrogs described in Chapter 5 being the latest examples. As a result of this dynamic in Ecuador, a recent analysis identified the central and northern Andes as a conservation priority for anurans based on species composition, phylogenetic diversity, and the human footprint index (Vasconcelos et al. 2019).

Even though the Constitution of Ecuador grants rights to nature (Artículo 71 de la Constitución de la República del Ecuador), paradoxically, the short-term benefits of extractive industries supersede the protection of critical habitats for threatened species (Roy et al. 2018). The government of Ecuador has sold off scores of mining concessions to international corporations in recent years, most of which are situated along Andean slopes (Roy et al. 2018; Capparelli et al. 2021). Many of these concessions were not modified to exclude protected areas, and local communities were not consulted in advance of their auctioning (Roy et al. 2018).

Northwestern Ecuador is home to many other threatened animals, including two of the most endangered primates in the world (*Ateles fusciceps fusciceps* and *Cebus aequatorialis*; Cortes-Ortíz et al. 2020, Moscoso et al 2021), *Panthera onca centralis* (Critically Endangered; Cervera et al. 2016), and *Espizaetus isidori* (Endangered; BirdLife International 2016), and several plant species (Pérez-Escobar et al. 2022). However, many tropical areas lack the resources necessary to establish and manage protected areas (Lessmann et al. 2016).

The presence of non-governmental nature reserves in the Andes can play a key role in the protection of amphibians and other taxa. In northwestern Ecuador, several local and indigenous territories, government-recognized protected forests (i.e., bosques protectores), and private reserves represent the only known strongholds for a growing list of threatened species. For example, the protected cloud forests of the Junín community in the Intag Valley lies within the Llurimagua mining concession; two species of frog listed as Critically Endangered are currently known to exist within this community reserve and nowhere else (i.e., *Atelopus longirostris* and *Ectopoglossus confusus*; Freile et al. 2020). The Río Manduriacu and Los Cedros Reserves, which are adjacent to one another at the west end of the Intag Region in Imbabura Province, harbor the only known extant populations of *Rhaebo olallai* (CR), *Noblella worleyae* (Not Evaluated), *Nymphargus manduriacu* (CR*), Pristimantis cedros* (EN), as well as the only extant Ecuadorian population of *N. balionotus* (EN); both reserves are within, and surrounded by, mining concessions (e.g., Reyes-Puig et al. 2020; Maynard et al. 2020). Dracula Reserve in the province of Carchi is also encompassed by mining concessions and home to the only extant population of *Atelopus coynei*, among other threatened amphibians (Reyes-Puig et al. 2019; Yanez-Muñoz et al. 2020). Notably, our records of *Hyalinobatrachium nouns* were all collected within the boundaries of mining concessions (i.e., Los Cedros and Río Manduriacu Reserves); *H. mashpi* only occurs within or adjacent to concessions. The distributions of both new species are also within Key Biodiversity Areas: Bosque Protector Los Cedros and Mashpi-Pachijal (Key Biodiversity Areas Partnership 2020).

Given the plethora of evidence supporting the importance of Andean biodiversity, the decision by the last governments (2007-2018) to encourage large-scale mining operations throughout Andean Ecuador have been alarming. Nonetheless, communities in the Intag-Toisan Region and Choco Andino of northwest Ecuador have demonstrated how unified action, voting for local politicians that support and legislate environmental policies, and partnering with a diverse network of NGOs can result in the ability to meaningfully contest the progression of mining in and around their territories (e.g., Avci & Fernández-Salvador 2016; Roy et al. 2018). Garnering strong public support, financial resources, and scientific backing has resulted in successful legal challenges to mining in the community reserve of Junín (Freile et al. 2020), based on projected violations to the Rights of Nature. Los Cedros Reserve has litigated a similar legal case premised on the rights of nature.

While the long-term outcomes of these legal challenges are yet to be seen, the example of Ecuador and the actions of Intag-Toisan and other local communities exemplify measures that can be adopted, and adapted, by the constituents and governing bodies of neighboring tropical Andean countries, and elsewhere. Promoting local empowerment, extending Rights to Nature, and appealing to governments to legislate progressive environmental policies are necessary to shift the trajectory of the region towards more sustainable practices in all sectors of society (Bax & Francesconi 2019; Torremorell et al. 2021). Without such measures, the ability to navigate socio-ecological challenges, including the conservation management of Tropical Andean biodiversity, will become increasingly difficult into the future.

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CHAPTER 6

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