Tadpoles in lotic waters, habitat specialization, and human population density lead tree frogs (Hylinae) to higher extinction risk

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A B S T R A C T

Analyzing patterns of vulnerability to extinction across clades and traits that make species more prone to extinction are crucial steps towards prioritization of conservation efforts. Here we identified genera more vulnerable to extinction than expected by chance within the anuran subfamily Hylinae. We also investigated a broad range of intrinsic and extrinsic factors and their interactions as predictors of a proxy for extinction risk (IUCN Red List status). By conducting simulation analyses, we found that binary threat status is not randomly distributed across Hylinae genera, with *Charadrahyla*, *Hyloscirtus*, *Isthmohyla*, *Plectrohyla*, and *Sarcohyla* containing more threatened species than expected by chance. By using model selection and average approaches, we found strong support for increased likelihood of threatened status as habitat diversity decreases and human population density increases, and for species with larvae inhabiting lotic waters to be more threatened than those with larvae inhabiting lentic waters. Overall, our results were robust to different treatments of Data Deficient species (threatened or non-threatened). However, results were affected by the inclusion of geographic range size as another predictor, with habitat diversity becoming a non-significant predictor of threatened status. Our results highlight the importance of simultaneously analyzing both intrinsic and extrinsic predictors in correlative studies of extinction risk. Additionally, they underscore the need for conservation research and programs focused on both larvae and adults of organisms with biphasic life cycles and the establishment and evaluation of effectiveness of natural refuges far from areas under high human pressures, such as privately and community protected areas.

1. Introduction

Conservationists and ecologists widely recognize that knowledge of patterns of vulnerability to extinction of species across clades and regions, as well as their intrinsic and extrinsic correlates, are crucial steps towards prioritization of conservation efforts (Caughley, 1994; Fisher and Owens, 2004). In this sense, the International Union for the Conservation of Nature (IUCN) created and continually updates the Red List of Threatened Species (IUCN, 2022). This list classifies species according to their extinction risk based on risk factors, such as small population size, small geographic range size, high spatial fragmentation of populations, and population decline (IUCN, 2012). This classification provides a scale of extinction risk that has been used in numerous studies at broad phylogenetic scales (e.g., Cooper et al., 2008; Böhm et al., 2016; Wang et al., 2018). Some studies have found a pattern of non random distribution of threatened conservation status among clades, which has been mostly analyzed among families of different groups of organisms such as amphibians and birds (Bielby et al., 2006; Wang et al., 2018). This pattern suggests that species within certain clades share biological traits that make them more prone to extinction than species in other clades (Jones et al., 2003; Böhm et al., 2013).

Among these traits, large body size is frequently correlated with an increase in extinction risk at different phylogenetic scales (Terzopoulou et al., 2015; Wang et al., 2018; but see Chichorro et al., 2019). This relationship has been mainly explained by indirect effects of large body size on extinction risk through low population densities and slow life

cycles and recovery rates, although the importance of body size may depend on the threat, e.g. larger bodied species may be more vulnerable to exploitation (Bielby et al., 2006; Wang et al., 2018; Chichorro et al., 2019). Moreover, many ecological traits also have been found to predispose species to extinction. For instance, Sodhi et al. (2008) found that aquatic and arboreal habit, ovoviviparous reproductive mode, and unseasonal breeding are correlated with extinction risk across amphibians. On the other hand, behavioral traits in general have been less considered in correlative studies of extinction risk (Chichorro et al., 2019), despite some of them potentially playing a role as predictors of extinction risk. For example, territorial defense of resources may lead to lower abundance (as a consequence of higher spacing among individuals) and thus be indirectly related with proneness to extinction (Wiegand et al., 2002). Nonetheless, territoriality has been rarely considered in comparative studies addressing correlates of extinction risk (e.g., Suárez-Tovar et al., 2019).

In addition to biological intrinsic traits, external factors such as climatic conditions are also correlated with extinction risk. For example, amphibian species inhabiting regions with lower temperatures and pronounced climatic seasonality tend to have higher extinction risk (Cooper et al., 2008; Sodhi et al., 2008). Non-exclusive hypotheses invoked to explain these patterns are that lower temperatures promote slower life histories and that global climate change exerts a pressure over species adapted to specific environmental conditions (Cooper et al., 2008; Sodhi et al., 2008). Moreover, other external factors commonly interact with intrinsic traits to affect vulnerability to extinction of species. For instance, habitat disturbance is known to increase the extinction risk of habitat-specialist bird species (Owens and Bennett, 2000; Norris and Harper, 2004). These patterns overall indicate that external factors—both climatic and anthropogenic—can act solely but also synergistically with biological intrinsic traits to drive species to increased extinction risk. The inclusion of these factors in correlative studies of extinction risk is therefore important to increase the predicting power of the models generated, and better inform proactive conservation actions (Cardillo et al., 2008).

Since amphibians are ectothermic organisms and many of them have a biphasic life cycle, they are an ideal model to evaluate the effect of intrinsic and extrinsic factors on extinction risk. Within this vertebrate group, the subfamily Hylinae is further suitable to analyze how a wide range of traits are correlated with vulnerability to extinction. Firstly, this frog clade exhibits a remarkable variation in intrinsic traits that are theoretically or empirically correlated with vulnerability to extinction, such as body size (Duellman et al., 2016), different aspects of reproductive mode (Wells, 2007), and territoriality (Kluge, 1981; Wells and Schwartz, 1984). Secondly, this subfamily is widely distributed, being found from North to South America, temperate Eurasia, Japan, and extreme northern Africa (Frost, 2023), which exposes its species to a wide range of climatic conditions. Thirdly, Hylinae has a high number of species that are threatened and/or experienced recent population declines (Stuart et al., 2004; Frías-Alvarez et al., 2010; IUCN, 2022): 23 % of the 632 Hylinae species that had their conservation status assessed by the IUCN (2022) are threatened with extinction.

In this study, we conducted the first extensive analysis of patterns of extinction risk in the anuran subfamily Hylinae. Particularly, we first examine whether extinction risk is randomly distributed among the genera of Hylinae. We then identify which genera are more and less threatened with extinction than expected by chance. Finally we investigate which intrinsic and extrinsic factors—as well as which of their interactions—better predict extinction risk in this anuran subfamily.

2. Materials and methods

2.1. Data collection and processing

We downloaded IUCN Red List status from the IUCN Red List v. 2022.2 (IUCN, 2022) as our response variable of extinction risk. We treated this variable as both ordinal and binary variables. For the ordinal classification, we translated Red List category into 0 for Least Concern (LC), 1 for Near Threatened (NT), 2 for Vulnerable (VU), 3 for Endangered (EN), and 4 for Critically Endangered (CR); Extinct or Extinct in the Wild species were not present in our dataset. For the binary classification, species in LC and NT categories were considered as non-threatened (0), and species in VU, EN, and CR were considered as threatened (1). Data Deficient (DD) species were treated in three ways (modified from Böhm et al., 2013): 1) we excluded them from all ordinal and binary analyses (Cooper et al., 2008); 2) we included all of them in our binary analyses as threatened; and 3) we included all of them in our binary analyses as non-threatened (results for approaches 2 and 3 are presented in the supplemental materials). Moreover, we downloaded population trend information from the IUCN Red List (IUCN, 2022) as another proxy for vulnerability to extinction (see supplemental materials). Population trend refers to trends over a period of ca. three years around the present (IUCN, 2013). This proxy was treated as a binary variable, with species coded as having experienced recent population declines (1) or not (0), considering in the latter category species that have stable or increasing populations.

We obtained eight intrinsic and 12 extrinsic variables for each species, based on hypotheses derived from the literature (Table S1). Most intrinsic traits were compiled from the literature (peer-reviewed articles and books): 1) mean male snout–vent length (SVL) in mm as a measure of body size (or mean of range when mean was not available), 2) oviposition site (aquatic, on vegetation, or in phytotelmata), 3) larval habitat (lentic, lotic, or phytotelmata), 4) reproductive pattern (explosive or prolonged according to Wells, 1977), 5) territorial call present/absent, 6) physical combat present/absent, 7) habitat diversity (as a proxy for habitat specialization), and 8) geographic range size (in km^2). The absence of records of behavioral traits (such as territorial call and physical combat) in the literature can be the result of lack or limited research effort and not genuine absence of the trait. Therefore, we considered lack of territorial call and physical combat (i.e., absent) in a species only if these two behaviors are not mentioned in studies that describe in detail other mating behaviors. Such mating behaviors include nest building, mate searching, advertisement call, satellite behavior, and breeding aggregation (Han and Fu, 2013). We generated Minimum Convex Polygons from IUCN Red List species range maps (IUCN, 2022) and projected them in QGIS 3.4 (QGIS, 2019). As some species had only point occurrences rather than shapefiles, we used a buffer of 0.5° (about 55 km at the equator) for species with less than two records and a Minimum Convex Polygon for species with three or more records, both clipping out potential occurrence in the ocean. This allowed us to generate discrete ranges for analyses. For habitat diversity, we overlaid geographic range shapefiles with the most recent layer of terrestrial habitat types (Jung et al., 2020), calculated the number of cells within each habitat type, and estimated habitat diversity with the Shannon true diversity index (Jost, 2006). Geographic range size was calculated with the shapefile generated for each species.

The extrinsic (climatic and anthropogenic) variables considered were: 1) mean annual temperature (in °C; BIO1 in WorldClim 2; Fick and Hijmans, 2017), 2) temperature seasonality (in °C; BIO4), 3) mean temperature of warmest quarter (in °C; BIO10), 4) mean temperature of coldest quarter (in °C; BIO11), 5) annual precipitation (in mm; BIO12), 6) precipitation seasonality (BIO15), 7) precipitation of wettest quarter (in mm; BIO16), 8) precipitation of driest quarter (in mm; BIO17), 9) net primary productivity (in g m^{-2} year^{-1}; Imhoff et al., 2004), 10) mean human population density (as people km^{-2}; CIESIN, 2005), 11) human footprint (Venter et al., 2018), and 12) percent of anthropogenic habitat loss. Extrinsic variables, except for percent of anthropogenic habitat loss, were extracted by overlaying shape files of species' geographical ranges with a 0.5° × 0.5° grid, and recording the median grid cell value within each species' geographical range using "Zonal Statistics" in QGIS 3.4 (Hijmans et al., 2005; Chen et al., 2019; QGIS, 2019). For percent of anthropogenic habitat loss, we overlaid species geographic ranges with

a recent layer of terrestrial habitat types (Jung et al., 2020) and calculated the percentage of cells occupied by anthropogenically modified habitat types. We considered "anthropogenically modified habitats" as those classified as "artificial" in Jung et al. (2020), which includes arable land, pastureland, plantations, and urban areas. Raw data on conservation status, population trend, and the eight intrinsic and 12 extrinsic variables for each species are shown in Table S2.

2.2. Statistical analyses

We conducted simulation analyses to evaluate whether binary threatened conservation status and recent population declines are randomly distributed across Hylinae genera, following the general procedure described by Bennett and Owens (1997). Excluding DD species, 148 Hylinae species have a threatened conservation status, out of 545 non-DD Hylinae species in total. Therefore, we randomly selected 148 from the 545 non-DD Hylinae species. We recorded to which genera these species belonged to and calculated the proportion of randomly selected species per genus. We repeated this procedure 10,000 times and drew a frequency histogram of the mean number of genera in each of ten proportion classes from 0 to 1 (with a range of 0.1 each) across all 10,000 simulations (Wang et al., 2018). We considered this histogram as the predicted distribution of extinction risk. If extinction risk is randomly distributed among genera, then one would expect to find that the observed distribution does not differ from the predicted distribution (Wang et al., 2018). We tested this null hypothesis with a chi-square (χ^2) test (Sokal and Rohlf, 1995).

We used the binomial distribution to find which genera contained more and less threatened or declining species than expected by chance (over- and underthreatened genera, respectively; Bennett and Owens, 1997; Bielby et al., 2006). Under the null hypothesis that species in each genus are randomly threatened, the probability of a species being threatened is 0.272 (148 threatened species out of a total of 545 non-DD species). Since we independently tested it for each of 43 Hylinae genera, adjusted critical values were calculated with the Dunn–Sidak method (Sokal and Rohlf, 1995).

We tested the relationship of species' extinction risk (separately as ordinal and binary variable) and population trend with intrinsic and extrinsic variables with generalized linear mixed-effects models (GLMMs), with a Poisson and binomial error structure for ordinal and binary variables, respectively. We included genus nested within tribe as random intercept effects in our models to control for phylogeny. Additionally, numeric predictors were log-transformed, except for percent of anthropogenic habitat loss that was logit transformed (Warton and Hui, 2011) and habitat diversity that was treated as a raw variable. We then scaled all the numeric predictors to have a mean of 0 and a standard deviation of 1 (Kittelberger et al., 2021). The categorization of 123 out of the 148 threatened species within Hylinae (83.1 %) is based on criteria in which geographic range size is an important variable (criteria B and D2; IUCN, 2012). Thus, correlating conservation status with range size has the risk of circularity. To deal with this risk, we carried out the subsequent analyses without considering geographic range size and then included this variable as another predictor to determine whether its inclusion overwhelmed other variables (Chen et al., 2019). Results including geographic range size are presented in the supplemental materials.

Firstly, we conducted univariate GLMMs between each of our predictors and extinction risk separately. Afterwards, we tested for collinearity among numerical predictors that were significant in univariate models, and only retained one predictor for those pairs that were highly correlated (Spearman's $\rho \geq 0.60$; Mertler and Vannatta, 2001). We then built all possible combinations of models using the retained predictors as well as plausible first-order interactions among intrinsic and extrinsic variables (Table S1). We ranked these models according to their Akaike Information Criterion value for small sample size (AICc), considering substantially supported models as those with AICc values within two

units in relation to the best fitted model (Burnham and Anderson, 2002). Multicollinearity among numerical and categorical predictors in substantially supported models was tested by generalized variance inflation factor analyses (GVIF), which yielded values <5 in all cases (GVIFmax = 2.44), indicating sufficient independence among predictors. Since we always obtained more than one supported model, we used the model-average method to account for model selection uncertainty (Burnham and Anderson, 2002). All statistical analyses were conducted in R 4.0.3 (R Core Team, 2020), using the packages car (Fox and Weisberg, 2019), lme4 (Bates et al., 2015), and MuMIn (Barton, 2020).

3. Results

According to the IUCN Red List v. 2022.2, 45 (7.1 %) species of the subfamily Hylinae are Critically Endangered, 60 (9.5 %) are Endangered, 43 (6.8 %) are Vulnerable, 29 (4.6 %) are Near Threatened, 368 (58.2 %) are Least Concern, and 87 (13.8 %) are Data Deficient. Considering conservation status and population trend as binary variables, 148 species (27.2 %) are threatened and 198 (42.1 %) have experienced recent population decline. Only 3.6 % of the threatened species were non-declining but 42.8 % of the declining species were in a non-threatened conservation status, excluding DD species. Among threatened species, 4.8 and 3.6 % were non-declining but 40.4 and 46 % of the declining species were in a non-threatened category, considering DD species as threatened and as non-threatened, respectively (Fig. 1). Excluding DD species, highest species richness of Hylinae treefrogs was located in the Amazon Basin and the Atlantic Rainforest in South America (Fig. 2A), whereas highest proportions of threatened species were located in southern Mexico, Central America, northern South America, and some Greater Antilles islands (Fig. 2B). Similar patterns were observed when including DD species in species richness (Fig. S1A), and for the proportion of threatened species considering DD species as threatened (Fig. S1B) and as non-threatened (Fig. S1C). Highest proportions of declining species were located in central United States, southern Mexico, Central America, northern South America, some Greater Antilles islands, southern Europe, and southeastern China (Fig. S1D), but the high proportion of declining species in the regions of North America, Europe, and Asia was mainly due to the low species richness in these regions.

Excluding DD species, the observed and predicted frequency distributions of the proportion of threatened species per genus differed significantly ($\chi^2 = 24.25$, $p = 0.002$, Fig. 3), which indicates that extinction risk is not randomly distributed among Hylinae genera. The observed and predicted frequency distributions did not differ when all DD species were considered as threatened ($\chi^2 = 15.38$, $p = 0.13$; Fig. S2A), but they differed when considering them as non-threatened ($\chi^2 = 25.69$, $p = 0.007$; Fig. S2B). The observed and predicted frequency distributions of the proportion of declining species per genus significantly differed ($\chi^2 = 19.1$, $p = 0.02$, Fig. S2C). Excluding DD species, 16 genera had twice or more of the percentage of threatened species than expected by chance. Specifically, *Charadrahyla*, *Hyloscirtus*, *Isthmohyla*, *Plectrohyla*, and *Sarcohyla* contained more threatened species than expected by chance (Table 1). The genera showing lower proportions of threatened species than expected by chance were *Boana*, *Dendropsophus*, and *Scinax* (adjusted $p < 0.01$ in all cases). Similar results were obtained considering DD species as threatened and non-threatened, as well as considering population trend as a proxy for vulnerability to extinction (Table S3).

Excluding DD species and geographic range size as predictor, the best model explained 57 % and 64 % of total variation in ordinal and binary conservation status, respectively (Table S4). We found from four to eight substantially supported models depending on how conservation status was treated, which suggests model selection uncertainty in all cases (Table S4). The model-average method indicated that threatened conservation status increases as habitat diversity decreases ($p < 0.001$ in all cases) and as human population density increases ($p < 0.001$ in all

cases), and that species with larvae inhabiting lotic waters are more threatened than those with larvae inhabiting lentic waters ($p < 0.001$ in all cases), regardless of how conservation status was treated (Table S5, Fig. 4). Qualitatively similar results were obtained for the binary conservation status considering DD species as threatened and as non-threatened, as well as for population trend. The best model explained from 39 to 56 % of total variation, depending on the case (Table S4). Model selection yielded from two to seven substantially supported models, depending on the case (Table S4), and model-average identified the same significant predictors ($p < 0.01$ in all cases; Table S5, Fig. S3).

When we included geographic range size as an additional predictor, the best model explained a higher percentage of total variation (from 76 to 84 %; Table S6) in almost all cases. Thus, range size overwhelmed other variables in these cases. The exceptions to this pattern were for binary conservation status considering DD species as non-threatened and for population trend, for which percentage explained was similar between the best model including and excluding range size as a predictor (Tables S4 and S6). Model selection including range size yielded from three to ten substantially supported models depending on the variable related to vulnerability to extinction (Table S6). Overall, the model-average method indicated that threatened status and population decline were not significantly correlated with habitat diversity ($p > 0.15$ in all cases), but were negatively correlated with geographic range size ($p < 0.01$ in all cases) and positively correlated with human population density ($p < 0.05$ in almost all cases). Additionally, species with larvae

inhabiting lotic waters were more likely to be threatened and declining than those with larvae inhabiting lentic waters ($p < 0.01$ in almost all cases). The only exception to this pattern was for the binary conservation status considering DD species as non-threatened, for which human population density ($p = 0.6$) and larval habitat ($p = 0.36$) were not significant predictors (Table S7).

4. Discussion

Here, we conducted the first comprehensive analysis of extinction risk in the anuran subfamily Hylinae. As this subfamily is one of the most speciose anuran clades (Frost, 2023), we were able to analyze patterns of extinction risk and decline across genera and test a broad range of intrinsic and extrinsic (climatic and anthropogenic) correlates of extinction risk. Overall, we found that extinction risk was not randomly distributed across genera, with a few of them containing more threatened species than expected by chance. We also found that vulnerability to extinction is best explained by larval habitat, habitat specialization (both intrinsic factors), and human population density (extrinsic anthropogenic factor). Therefore, a combination of both intrinsic and extrinsic anthropogenic factors best predicted vulnerability to extinction in the subfamily Hylinae.

Many studies have found that extinction risk is not randomly distributed across families in many animal taxa (Bennett and Owens, 1997; Bielby et al., 2006; Bland et al., 2012). Such analyses have rarely
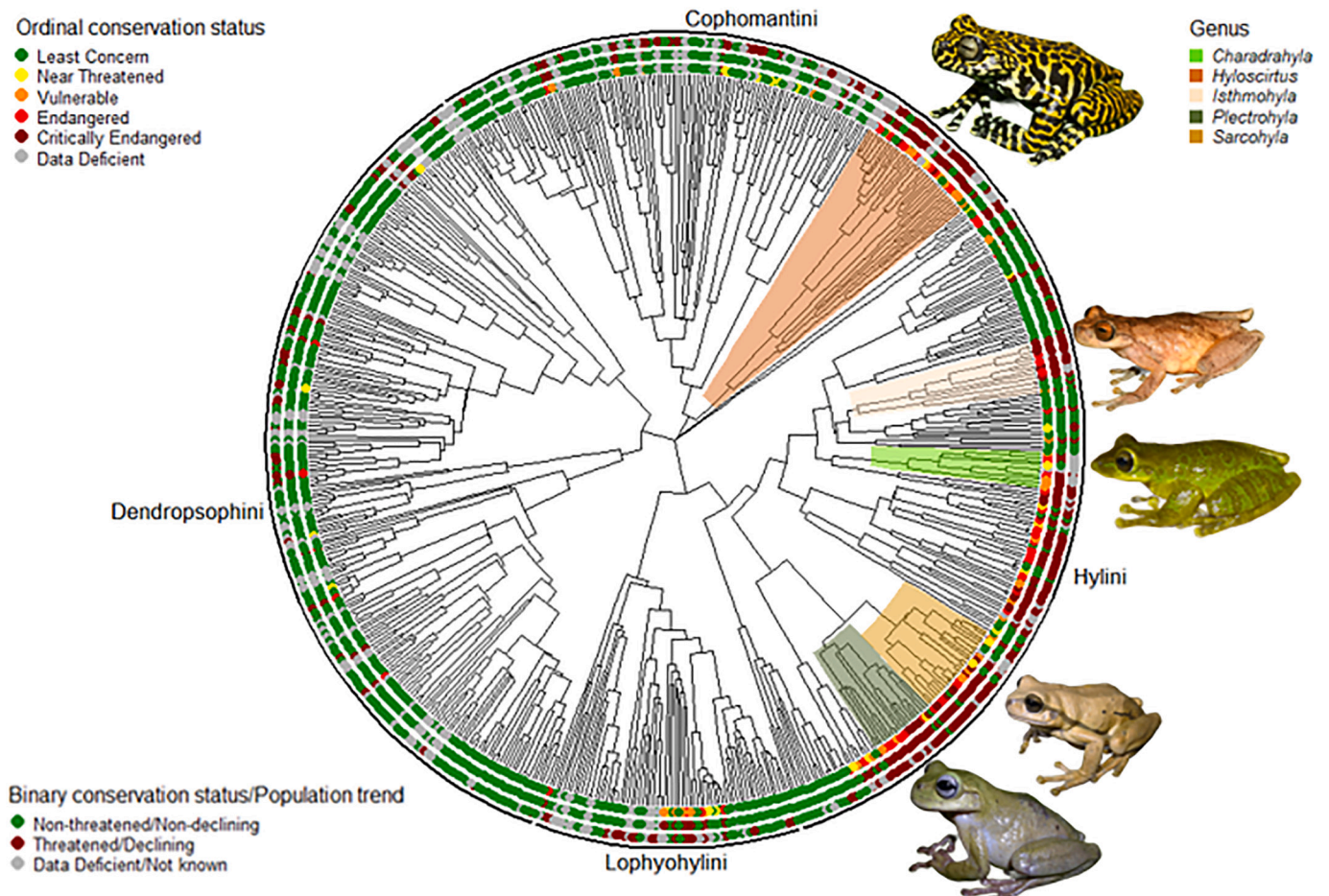
Fig. 1. Phylogeny of Jetz and Pyron (2018) for 630 Hylinae frog species, with ordinal conservation status (inner ring), binary conservation status (middle ring), and population trend (outer ring), according to the IUCN (2022). Genera with more threatened species than expected by chance (see Methods) are highlighted, along with an image of a threatened species for each of them: *Charadrahyla taeniopus* (Vulnerable, photo credit Ricardo Luría-Manzano), *Hyloscirtus tigrinus* (Endangered, photo credit Mario Fernando Montezuma and Francisco López-López), *Isthmohyla rivularis* (Endangered, photo credit Andrew R. Gray), *Plectrohyla exquisita* (Critically Endangered, photo credit Franklin Castañeda), and *Sarcohyla labeculata* (Endangered, photo credit Luis Canseco-Márquez).
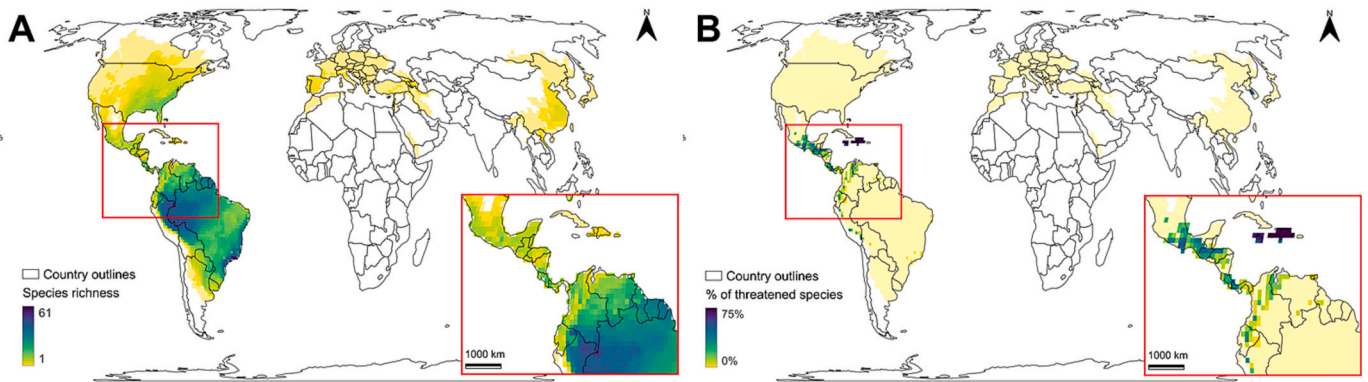
Fig. 2. Distribution of global species richness (A) and the proportion of threatened species within the subfamily Hylinae per grid cell (B), excluding DD species.
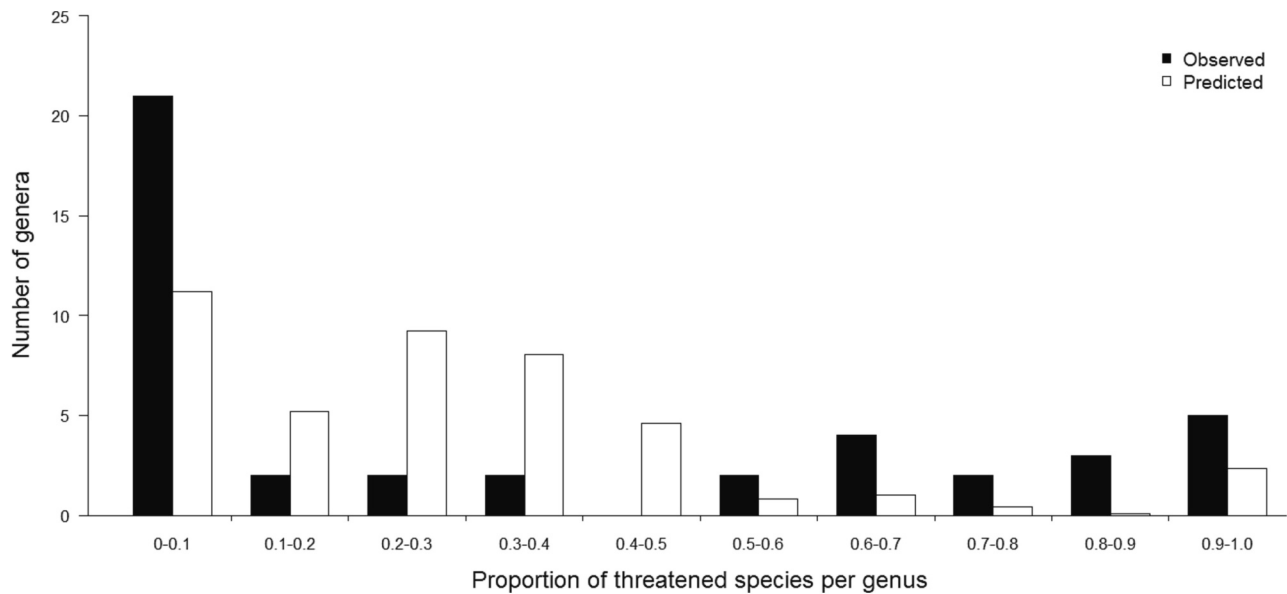

Fig. 3. Observed and predicted frequency distributions of the proportion of threatened species per genus in the anuran subfamily Hylinae, excluding DD species.

been conducted at lower taxonomic levels (e.g., genera), but the limited evidence suggests similar patterns (Purvis et al., 2000). Here we found the same pattern for genera within the subfamily Hylinae, which reinforces the idea that species within certain clades likely share biological traits predisposing them to extinction risk (Bennett and Owens, 1997), not only in deeper evolutionary times but also in more recent ones. This result is particularly interesting since Bielby et al. (2006) found that Hylidae (the family to which Hylinae belongs) is an underthreatened family within amphibians. Therefore, our study highlights the importance of analyzing patterns of vulnerability to extinction across genera in underthreatened lineages, which can uncover vulnerable genera even in these lineages.

Overall, we found that the genera *Charadrahyla*, *Hyloscirtus*, *Isthmohyla*, *Plectrohyla*, and *Sarcohyla* were more threatened than expected by chance, regardless of the exclusion of DD species and their consideration as threatened or non-threatened. These five genera exhibit a clumped distribution across the phylogeny, because almost all of them belong to the tribe Hylini, with the exception of *Hyloscirtus* which belongs to the tribe Cophomantini (Fig. 1). Since many amphibian functional traits are often phylogenetically conserved (Campos et al., 2019), our results suggest that the extinction of these species could likely impact group-specific ecosystem services and functional diversity (Oliveira et al., 2020; Toussaint et al., 2021). Additionally, the three overthreatened genera of Hylinae distributed in Mexico (*Charadrahyla*,

Plectrohyla, and *Sarcohyla*) were also found to be highly threatened within amphibians in that country (Frías-Alvarez et al., 2010). These results combined indicate that these genera are of conservation concern at broad geographical and phylogenetic scales. It is important to note that *Plectrohyla* reaches northern Central America, and that *Hyloscirtus* and *Isthmohyla* are distributed from Central America to northern South America and in Central America, respectively (Frost, 2023). Thus, overthreatened genera in Hylinae are concentrated across Central America, including Mexico, which roughly coincides with regions harboring the highest proportion of threatened species within this subfamily. This geographical concentration could allow for the development of conservation strategies in countries with overthreatened genera, since these strategies are usually established at the national scale (Moilanen et al., 2013).

Ninety five percent species within overthreatened genera in Hylinae have larvae inhabiting lotic waters, which contributed to our consistent finding that species with larvae inhabiting these environments are more prone to extinction than those with larvae inhabiting lentic waters. This result agrees with studies at broad phylogenetic and geographical scales from other freshwater animal taxa (Clausnitzer et al., 2009 for dragonflies and damselflies; Böhm et al., 2021 for molluscs), as well as studies with amphibians at smaller geographical scales (Lips et al., 2003; Hero et al., 2005). Three non-exclusive explanations that can account for this pattern are easier spread of aquatic infectious diseases in flowing
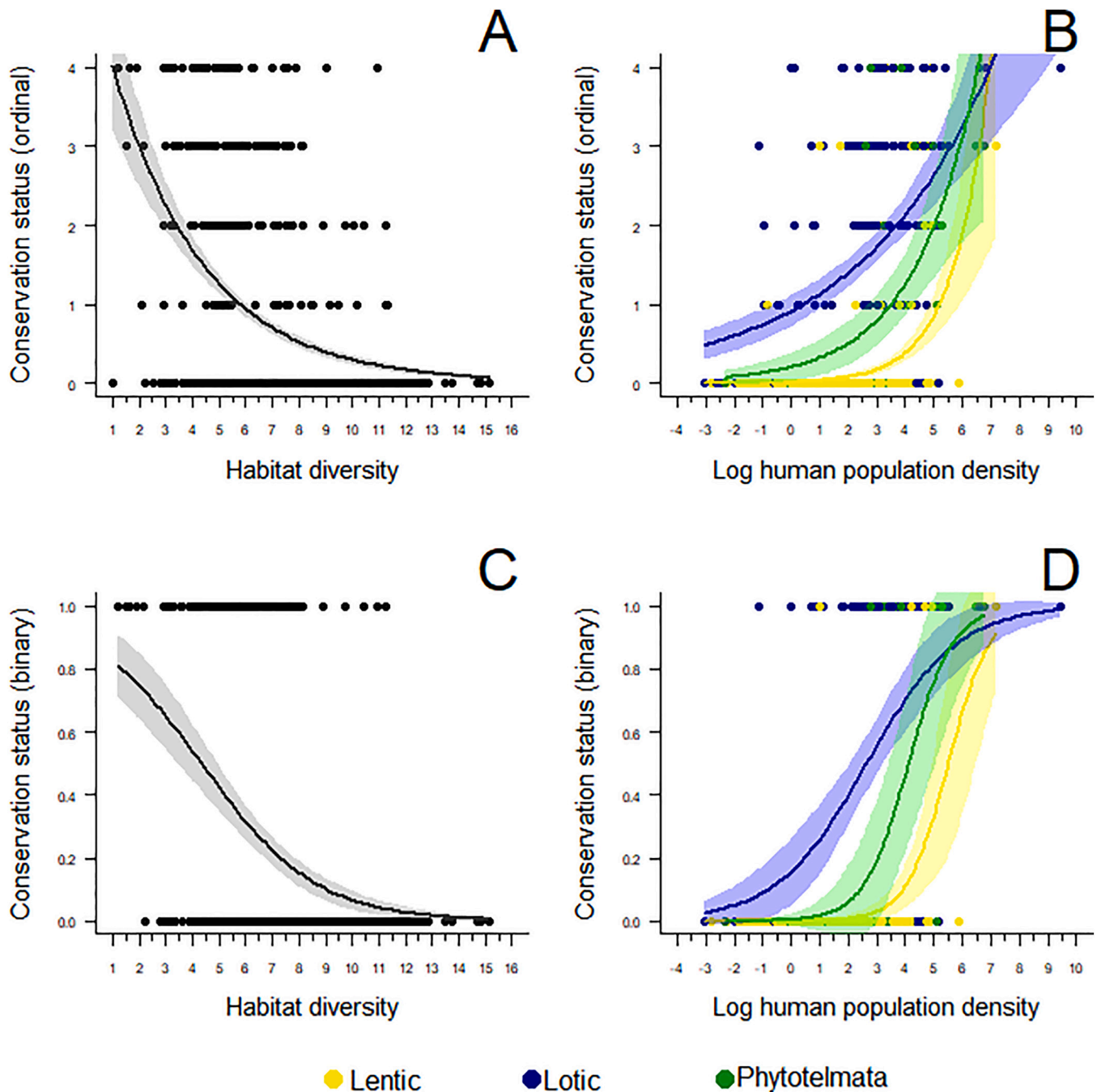
Fig. 4. Relationship of ordinal (A and B) and binary conservation status (C and D) with habitat diversity, larval habitat (lentic, lontic, or phytotelmata), and human population density in the anuran subfamily Hylinae, excluding DD species. Shadow areas represent the 95 % confidence intervals.

waters (particularly chytridiomycosis in amphibians; Lips et al., 2003; Li et al., 2021), lower dispersal capacity of adults of species breeding in this type of water bodies (Marten et al., 2006; Clausnitzer et al., 2009), and lower thermal tolerance (Carilo Filho et al., 2021). Regardless of the mechanism behind the pattern, our results provide further evidence for lotic systems to deserve particular conservation concern, because they harbor high numbers of vulnerable-to-extinction species from multiple animal taxa. In this sense, since larval habitat is a spatially-defined trait, consistent conservation actions could be applied to lotic systems in which larvae of many threatened species co-occur.

In addition to larval habitat, we found that habitat specialization (another intrinsic factor) and human population density (an anthropogenic factor) were also important predictors of extinction risk, with species occupying few habitats and distributed in areas with higher human population density being more vulnerable to extinction. These results agree with studies at a global scale (Owens and Bennett, 2000; Cardillo et al., 2004) and particularly with analyses in tropical regions (Williams and Hero, 1998; Tingley et al., 2013; Böhm et al., 2016; but see Davies et al., 2006 for human population density). Habitat-specialist species are more likely to be adversely affected by anthropogenic habitat loss (e.g., for agriculture, cattle raising, urbanization; Owens and Bennett, 2000; González-Suárez et al., 2013), a factor that is worryingly high in tropical regions across the globe (Laurance, 2007; Bradshaw et al., 2009), where human population density is also high (Davies et al., 2006). Since overthreatened genera within Hylinae are restricted to the Neotropics (Frost, 2023) and this region is inhabited by a high

Table 1

Hylinae genera with twice or more of the percentage of threatened species than expected by chance (see Methods) and/or with an unexpectedly large number of threatened species, excluding DD species. Significant *p* values are boldfaced.

Genus	No. species[a]	No. threatened	Proportion threatened	Binomial probability
Atlantihyla	2	2	1.00	0.07374
Bromeliohyla	3	2	0.67	0.16116
Charadrahyla	9	8	0.89	**0.00019****
Duellmanohyla	10	6	0.60	0.02371
Ecnomiohyla	10	7	0.70	0.00505
Exerodonta	9	5	0.56	0.05239
Hyloscirtus	32	23	0.72	**1.54e-07****
Isthmohyla	13	10	0.77	**0.00024***
Megastomatohyla	4	4	1.00	0.00544
Nesorohyla	1	1	1.00	0.27156
Osteopilus	8	5	0.63	0.03197
Phytotriades	1	1	1.00	0.27156
Plectrohyla	19	17	0.89	**2.16e-08****
Ptychohyla	6	4	0.67	0.04329
Quilticohyla	4	4	1.00	0.00544
Sarcohyla	25	21	0.84	**4.60e-09****

[a] Number of species with their conservation status evaluated according to the IUCN (2022).

* Significant at 5 % level adjusted p value (1.19e-03) for multiple comparisons (see Methods).

** Significant at 1 % level adjusted p value (2.34e-04) for multiple comparisons (see Methods).

proportion of threatened species, our results support these explanations.

Contrary to previous studies (Sodhi et al., 2008; Jeppsson and Forslund, 2014), we found that the same predictors best explained both conservation status and population trend. This result is even more unexpected given the asymmetric concordance between threatened conservation status and population decline in the subfamily Hylinae, with a high number of declining species being in a non-threatened conservation status but a low number of threatened species being non-declining. This asymmetric concordance is similar to the results of Becker and Loyola (2008) and the idea that many declining species would qualify as threatened in the future if species-threatening processes continue at similar rates (Jeppsson and Forslund, 2014). Therefore, if it applies to threats that we found to directly affect Hylinae frogs (e.g., human population density), they will continue to be prevalent factors imperiling the existence of this group of anurans in the near future, as they currently do.

Overall, our results were robust to the treatment of DD species as threatened or non-threatened, which is similar to the findings of Purvis et al. (2000) analyzing patterns of vulnerability to extinction across avian and mammalian genera. Our finding of a similar pattern in Hylinae frogs is particularly relevant given the relatively high percentage of species listed as DD in this subfamily at the moment (13.8 %), compared with that of birds (0.8 %) and mammals (5.3 %; Bielby et al., 2006) around the time of Purvis et al. (2000). The only exception to the robustness of our results to different treatments of DD species was when we evaluated if vulnerability to extinction is randomly distributed among Hylinae genera considering all DD species as threatened, finding a random distribution among genera. Eighty five percent of DD amphibians are likely to be threatened (Borgelt et al., 2022), so considering all DD species as threatened seems to be a more realistic scenario than considering all of them as non-threatened. However, even in the first case we identified nearly the same overthreatened genera as when we excluded DD species or considered all of them as non-threatened.

When geographic range size was used as a predictor (see Table S5), our results changed, with habitat diversity becoming a non-significant predictor of vulnerability to extinction across analyses, and also with human population density and larval habitat becoming non-significant for binary conservation status considering DD species as non-threatened. Since small geographic range size (criteria B and D2;

IUCN, 2012) was used to categorize 123 (83.1 %) of the threatened species in the subfamily Hylinae, circularity when correlating conservation status with range size is high, as well as the probability of range size to overwhelm other predictors (Böhm et al., 2016; Chen et al., 2019). On the other hand, since geographic range size is not a criterion to categorize a species as declining or not, our results based on population trend do not have such risk of circularity. This may explain why the inclusion of range size as an additional predictor did not increase the explained percentage of total variation in population trend. Since significant predictors were the same regardless of the proxy for extinction risk we used, we recommend to account for range size as predictor in correlative studies of extinction risk.

Larval habitat, habitat specialization, and human population density explain a high percentage of variation in extinction risk among a broad range of intrinsic and extrinsic factors, which has implications for conservation research and action. For instance, larval habitat is a trait that is shared to some degree with adults in most species during the breeding season, since the latter usually inhabit overhanging vegetation surrounding the aquatic habitat in which larvae occur (Wells, 2007). Thus, dependence on lotic waters may lead species to higher extinction risk through negative effects on both larvae and adults of groups with biphasic life cycles, highlighting the need for conservation research and programs focused on both life stages. However, it is notable that such conservation programs are scarce for amphibians (Nolan et al., 2023). Additionally, given the importance of human population density as a predictor of extinction risk, there is also a need to establish new (or expand) and evaluate the effectiveness of protected areas far from areas under high human pressures, to aid in survival of threatened species. In this sense, social initiatives for conservation such as privately and community protected areas could be a reasonable solution, given their growing but still limited number in countries with high proportions of threatened species (e.g., Colombia, Mexico; Bingham et al., 2017), and their effectiveness to protect them in case studies (Ortiz-Lozada et al., 2017; Simón-Salvador et al., 2021). Effective conservation actions are urgently needed considering that habitat specialists are more prone to extinction because they are less adaptable to rapid changes in the environment, changes that are commonly associated with ongoing threats imperiling long-term conservation of amphibians.

5. Conclusions

In summary, our results highlight the importance of analyzing patterns of vulnerability to extinction in relatively underthreatened clades, which can uncover geographically concentrated and phylogenetically clumped overthreatened taxa. Additionally, our finding of both intrinsic and extrinsic (anthropogenic) drivers to be relevant predictors of vulnerability to extinction in Hylinae frogs highlights the importance of simultaneously analyzing both types of variables in similar studies, particularly in those focused on taxa exhibiting high species richness and proportions of threatened species in regions highly impacted by anthropogenic processes. Correlative studies of vulnerability to extinction at broad geographical and phylogenetic scales are important to identify general current and possible future drivers of extinction risk, mainly in taxa lacking detailed information on population trends over many years. Additionally, the results of these studies can also inform conservation prioritization in order to design and implement conservation actions for high-risk species.

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CRediT authorship contribution statement

Ricardo Luría-Manzano: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. **Filipe C. Serrano:** Formal analysis, Methodology, Software, Visualization, Writing –

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data are available in supplemental materials

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