Contributed Paper

Conservation threats and the phylogenetic utility of IUCN Red List rankings in *Incilius* toads

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Abstract: *Phylogenetic analysis of extinction threat is an emerging tool in the field of conservation. However, there are problems with the methods and data as commonly used. Phylogenetic sampling usually extends to the level of family or genus, but International Union for Conservation of Nature (IUCN) rankings are available only for individual species, and, although different species within a taxonomic group may have the same IUCN rank, the species may have been ranked as such for different reasons. Therefore, IUCN rank may not reflect evolutionary history and thus may not be appropriate for use in a phylogenetic context. To be used appropriately, threat-risk data should reflect the cause of extinction threat rather than the IUCN threat ranking. In a case study of the toad genus* Incilius, *with phylogenetic sampling at the species level (so that the resolution of the phylogeny matches character data from the IUCN Red List), we analyzed causes of decline and IUCN threat rankings by calculating metrics of phylogenetic signal (such as Fritz and Purvis' D). We also analyzed the extent to which cause of decline and threat ranking overlap by calculating phylogenetic correlation between these 2 types of character data.* Incilius *species varied greatly in both threat ranking and cause of decline; this variability would be lost at a coarser taxonomic resolution. We found far more phylogenetic signal, likely correlated with evolutionary history, for causes of decline than for IUCN threat ranking. Individual causes of decline and IUCN threat rankings were largely uncorrelated on the phylogeny. Our results demonstrate the importance of character selection and taxonomic resolution when extinction threat is analyzed in a phylogenetic context.*

Keywords: amphibian, Bufonidae, extinction threat, Mesoamerica, phylogeny

Las Amenazas para la Conservación y la Utilidad Filogenética de las Clasificaciones de la Lista Roja de la UICN de los Sapos *Incilius*

Resumen: *El análisis filogenético de la amenaza de extinción es una herramienta emergente en el campo de la conservación. Sin embargo, existen problemas con los métodos y los datos que se usan comúnmente. El muestreo filogenético generalmente se extiende hasta el nivel de familia o género, pero las clasificaciones de la Unión Internacional para la Conservación de la Naturaleza (UICN) están disponibles solamente para especies individuales, y aunque especies diferentes puedan tener la misma clasificación de la UICN, la especie puede estar clasificada así por razones distintas. Por eso, la clasificación de la UICN puede no reflejar la historia evolutiva y por lo tanto no ser la adecuada en un contexto filogenético. Para que se use adecuadamente, la información de amenaza de riesgo debe reflejar la causa de la amenaza de extinción en lugar de la clasificación de amenaza de la UICN. En un estudio de caso del género de sapos* Incilius, *con muestreo filogenético a nivel de especies (para que la resolución de la filogenia sea igual a datos de características de la Lista Roja de la UICN), analizamos las causas de la declinación y las clasificaciones de amenazas de la*

UICN al calcular las medidas filogenéticas (Fritz y Purvis D*). También analizamos el alcance al cual la causa de la declinación y la clasificación de la amenaza se traslapan mediante el cálculo la correlación filogenética entre dos tipos de datos de características. Las especies del género* Incilius *variaron ampliamente tanto en la clasificación de la amenaza como en la causa de la declinación; variación que se perdería con una resolución taxonómica más burda. Encontramos muchas más señales filogenéticas, probablemente en correlación con la historia evolutiva, para la causa de la declinación que las clasificaciones de riesgo de la UICN. Las causas individuales de la declinación y las clasificaciones de amenaza de la UICN no estuvieron correlacionadas en su mayoría con la filogenia. Nuestros resultados demostraron la importancia de la selección de carácter y la resolución taxonómica cuando la amenaza de extinción es analizada en un contexto filogenético.*

Palabras Clave: amenaza de extinción, anfibio, Bufonidae, filogenia, Mesoamérica

Introduction

As extinction rates continue to increase (Pimm et al. 2014) and anthropogenic threats to biodiversity become better understood, a new consensus has emerged: the patterns and causes of decline tend not to be phylogenetically random (Purvis 2008). In particular, elevated levels of amphibian decline and extinction are now a well-corroborated phenomenon (Stuart et al. 2004; Wake & Vredenburg 2008). The causes of amphibian declines are multiple, and surely are synergistic in many cases (Collins & Storfer 2003; Sodhi et al. 2008; Collins & Crump 2009). An assessment of the conservation status of all known amphibian species (Stuart et al. 2004) is available on the International Union for Conservation of Nature (IUCN) Red List (www.redlist.org), though the list is somewhat outdated in terms of threat assessment and taxonomic completeness. An important development since the publication of this assessment is that a relatively complete and robust phylogeny of the major clades of amphibians now exists (Frost et al. 2006; Pyron & Wiens 2011).

These two classes of information—data regarding the level and types of threats faced by each species and a fully resolved, robust phylogeny with complete species-level sampling—are required for identification of phylogenetic patterns in extinction risk. This has led to the use of amphibians in studies of phylogenetic patterns of extinction risk (e.g., Corey & Waite 2008; Bielby et al. 2008, 2010). These studies are based, at least in part, on the phylogeny of Frost et al. (2006) and information on species' conservation status and threats from IUCN Red List. However, no complete phylogeny for the over 7000 species of amphibians exists. Phylogenetic studies of amphibian extinction risk are therefore limited to higher taxonomic categories, as is the case with other taxa (Brooks et al. 2005). Phylogenetic studies of threat ranking are therefore limited to broader patterns among major clades, which are often referred to as taxonomic families or genera in the literature (e.g., Bennett et al. 2005; Brooks et al. 2005). In such cases, the concept of taxonomic distinctiveness may be conflated with phylogenetic distinctiveness (Avise 2005) inasmuch as supraspecific taxonomic categories are arbitrary and may be inconsistently applied. Furthermore, clades of the same age can differ greatly in species richness (Ricklefs et al. 2007); the amount of species-level data that is lost for each major clade (e.g., variation in IUCN threat rankings) may therefore vary significantly between higher-level clades such as Linnaean families.

Another problem facing phylogenetic studies of threat ranking is the incompleteness or non-comparability of threat information available in the rank listings (e.g., IUCN Red List). The criteria and categorical definitions used by IUCN are intended to be as objective as possible (IUCN 2012), such that 2 species relegated to the same category (e.g., critically endangered) may be assumed to be in similar situations in terms of population trajectories and extent of geographic range occupied (either reduced by conservation threats or naturally very small). Between clades or geographic regions, comparisons may be made regarding the number of species in a category; however, it is not possible to reconcile the actual causes of decline with threat rankings in any standardized manner. This means that if two species are ranked equivalently as critically endangered, one may be under threat from water pollution whereas the other may be threatened by over-harvesting for human consumption. In other words, although the equivalent ranks indicate that both species are similarly endangered, the causes can be wholly different (Davidson et al. 2009). In our opinion, this violates the concept of homology in phylogenetic systematics, in that one should not map critically endangered as a character onto a phylogeny because fundamentally different processes may underlie an equivalent threat ranking. Homologous characters reflect similarity based on common ancestry and are appropriate for use in the study of character evolution, whereas homoplastic characters are those that superficially appear to be similar but may have arisen through different mechanisms. For example, one would not code the character green coloration as equivalent in a frog and a bird because the equivalent condition is not produced by homologous mechanisms.

We approached the concept of phylogeny and conservation on a finer scale than most previous studies, which allowed us to use a taxonomically complete and fully resolved phylogeny of a clade, the Mesoamerican

toad genus *Incilius* (Mendelson et al. 2011). Our analysis of the genus begins with a replication of the methods used in previous studies: we calculated a number of autocorrelation metrics to measure phylogenetic signal in threat rankings. In addition to these conventional analyses, we also reconstructed the acknowledged cause or causes of decline to each species. Finally, we analyzed the correlation between different causes of decline, and between cause of decline and threat ranking. We present this as a case study demonstrating the advantages of working on a finer scale than most phylogeny-conservation studies and the advantages of considering cause of decline in the context of homology rather than using the threat rankings as a potentially homoplastic proxy.

Methods

We used three methods to determine whether phylogeny can be used to predict extinction risk. First, we calculated two traditional metrics of phylogenetic autocorrelation (Pagel's λ and Abouheif's test), following the methods of previous studies of extinction risk in amphibians (Corey & Waite 2008). Second, we calculated Fritz and Purvis' (2010) *D*, a new metric that measures phylogenetic signal of binary traits and may be better suited to discrete data. This metric has been used in more recent studies of phylogeny and extinction risk (Fritz & Purvis 2010; Turvey & Fritz 2011; Yessoufou et al. 2012). Lastly, we measured the phylogenetic correlation of the different causes that underlie threat rankings and calculated the correlation between cause of decline and threat ranking.

Our analyses incorporated 39 *Incilius* species: all currently recognized species except *I. intermedius*, whose taxonomic status is questionable; no natural population, extinct or extant, has been assigned to this taxon (Frost 2015). We used the phylogeny from Mendelson et al. (2011) (their Fig. 6) as a starting point for our phylogenies. Because several species of *Incilius* evidently are extinct and several are extremely rare, some species were missing in the analyses presented by Mendelson et al. (2011), so the authors inferred the phylogenetic positions of these species and illustrated them as minimally resolved (as polytomies) in their final tree. We used that tree and further resolved those polytomies with the following justifications. *Incilius mccoyi* was placed as sister to *I. occidentalis* based on their similarity and evident relationship (Santos-Barrera & Flores-Villela 2011). The clade ((*guanacaste*)(*chompipe, epioticus*)) was resolved to follow a hypothesized north-to-south vicariance model of speciation. Resolving it as a south-to-north model would have no effect on our analyses. *Incilius gemmifer* was placed as sister to *I. mazatlanensis* based on morphological similarity and a vicariant model of speciation. *Incilius holdridgei* was placed as sister to *I. fastidiosus* based on

morphological and ecological similarity and proximate, but allopatric, distributions. The pairing of *I. majordomus* and *I. peripatetes* was based on their close affiliation presented in the recent description of *I. majordomus* (Savage et al. 2013).

For branch lengths, we used the Bayesian analysis of the combined molecular and morphological data from Mendelson et al. (2011) (their Fig. 5). For the added taxa, we used branch lengths (brlen) comparable to their nearest neighbors. (*I. mccoyi* brlen = 0.05 because *I. occidentalis* brlen = 0.0558; *I. gemmifer* brlen = 0.02 because *I. mazatlanensis* = 0.0212; *I guanacaste* 0.04 because "*Crepdiophryne*" was 0.0475. For *I. chompipe* and *I. epioticus*, we used brlen = 0.0475 because the "*Crepdiophryne*" of Mendelson et al. [2011] was a chimera of *epioticus* and *chompipe*. *I. periglenes, I. majordomus, I. peripatetes,* and *I. holdridgei* were all set to brlen = 0.04 because *I. fastidiosus* = 0.0467.) We used the value 0.001 to connect branch lengths of all added taxa. To make the tree ultrametric, we used TreeEdit (Rambaut & Charleston 2001) and chose the nonparametric rate smoothing method (Sanderson 1997). Because of uncertainty in the immediate outgroup to *Incilius* (Mendelson et al. 2011), we conducted all analyses with 2 trees: the first, henceforth RR, rooted on the branch leading to the *Rhaebo–Rhinella* clade, and the second, henceforth Ana, rooted on the branch leading to *Anaxyrus* (Fig. 1). All tests of phylogenetic signal were performed on both phylogenies.

We used two sets of threat rankings: IUCN (2014) Red List and a new data set (hereafter updated rankings) containing our re-assessment of the conservation status of the included species (but using IUCN categories) and our evaluation of the general consensus of the principal cause or causes of decline for each species (Supporting Information). The new data set was compiled based on our own field experience with these toads; nevertheless, our conservation-threat evaluations were largely consistent with the IUCN Red List. For each data set, we compiled binary data matrices for each threat ranking: not evaluated/data deficient, least concern, near threatened, vulnerable, endangered, critically endangered, and extinct. The threat rankings endangered, critically endangered, and extinct were also analyzed jointly in a severe threat category, as has been done in previous studies (Corey & Waite 2008). In addition, we compiled binary data matrices for the three categories of principal cause of decline in *Incilius* species: small range, habitat destruction, and a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). For principal cause of decline, unlike the IUCN and updated threat rankings, a single species can belong to more than one category. We included small range as a cause of decline because the correlation between small range and extinction probability is a well-supported and generally accepted phenomenon (Purvis et al. 2000). If a species has very small range then its entire habitat can
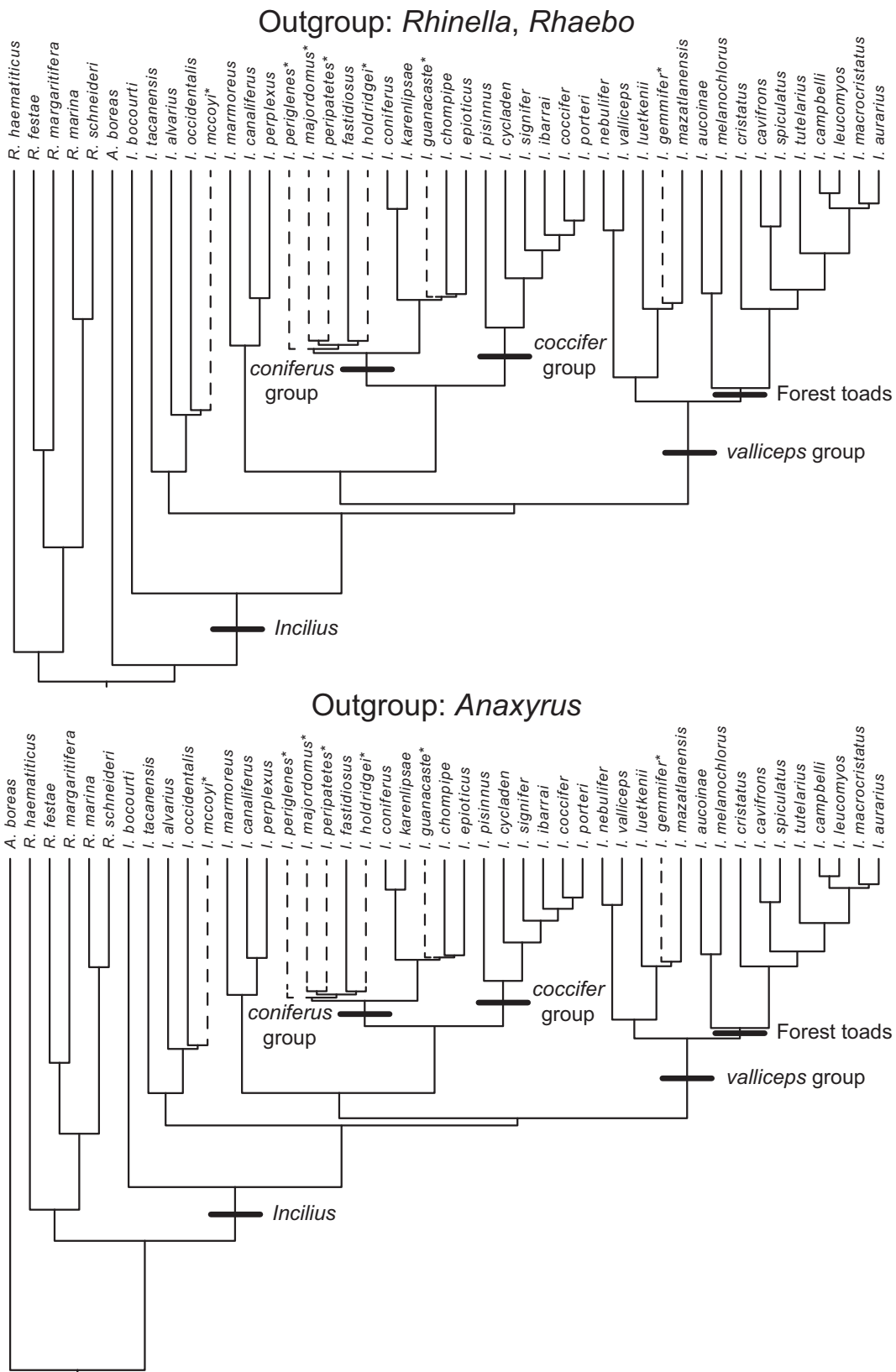
Figure 1. The 2 Incilius *phylogenies, with different outgroups, analyzed (adapted from Mendelson et al.' [2011] Fig. 6) with resolved polytomies. The dashed lines and asterisks indicate post-hoc placement of species for which no samples of DNA are known to exist and that were therefore not included in the original analyses by Mendelson et al. (2011) (see Methods for justification).*

very easily be destroyed, and we considered this a threat to the species. In the case of *Incilius*, several species are known only from their type locality.

Phylogenetic Signal

We used 2 traditional tests of phylogenetic autocorrelation: Pagel's λ (Pagel 1999) and Abouheif's test (Abouheif 1999). Pagel's λ detects random and Brownian phylogenetic distributions of continuous traits well (Münkemüller et al. 2012) and is one of the few measures of phylogenetic signal that can be used to measure discrete traits. Abouheif's test is a qualitative implementation of Moran's *I* (Pavoine et al. 2008), which has been used in previous studies of extinction risk in amphibians (Corey & Waite 2008) and has been recommended for such studies (Hardy et al. 2012). We calculated both metrics in R (R Development Core Team 2013) with the `geiger` package for Pagel's λ (Harmon et al. 2008) and the `adephylo` package for Abouheif's test (Jombart et al. 2010). We then used a likelihood ratio test to compare the negative log likelihoods of two values of λ: the maximum likelihood estimate of λ and a λ of zero (which removes branch lengths, transforming the tree into a polytomy and thus erasing all phylogenetic signal).

Another, more recent measure of phylogenetic signal in extinction threat is Fritz and Purvis' *D* (Fritz & Purvis 2010), which we also calculated in R with the `caper` package (Orme 2012; R Development Core Team 2013). The resulting *p* value measures whether *D* is significantly < 1, indicating strong phylogenetic signal.

Because a single species can fall into multiple categories of causes of decline, we calculated the phylogenetic correlation of different causes of decline in the `Discrete` module of `BayesTraits` (Pagel & Meade 2013). Using the same methods, we also performed correlation analysis between each threat ranking and each cause of decline. We compared two models: a 4-parameter model of independent evolution and an 8-parameter model of dependent evolution. Analyses were performed with a maximum likelihood algorithm. We used a likelihood ratio test approximated by a chi-squared distribution with four degrees of freedom and compared the negative log likelihoods of the independent and dependent models of evolution.

For all the metrics calculated here, we used binary data matrices to examine 1 or 2 characters in isolation; therefore, the total number of characters considered did not affect our results. For example, the inclusion of small range as a cause of decline had no effect on the *p* values obtained for habitat destruction and *B. dendrobatidis*. Similarly, the inclusion of the severe threat category (combined endangered, critically endangered, and extinct) had no effect on the *p* values obtained for the other threat rankings.

Table 1. Phylogenetic autocorrelation, based on Pagel's λ and Abouheif's tests, of extinction risk and cause of decline in *Incilius* toads and 2 selected outgroups. Calculated two traditional metrics of phylogenetic autocorrelation.

	Pagel's λ[a]		Abouheif's test[a]	
	RR	*Ana*	*RR*	*Ana*
Cause of decline				
habitat destruction	0.000***	0.000***	0.001**	0.001**
small range	0.033	0.034	0.001**	0.001**
Chytridiomycosis	0.895	0.505	0.019	0.006*
IUCN rank[b]				
NE, DD	1.000	1.000	0.793	0.781
LC	0.039	0.038	0.108	0.102
NT	0.532	0.321	0.146	0.069
VU	1.000	1.000	0.912	0.894
EN	1.000	1.000	0.168	0.169
CR	1.000	1.000	0.011	0.015
EX	1.000	1.000	0.532	0.553
EN-CR-EX	1.000	1.000	0.047	0.053
Updated rank[b]				
NE, DD	1.000	1.000	0.834	0.815
LC	0.024	0.023	0.011	0.010
NT	0.116	0.073	0.012	0.005*
VU	1.000	1.000	0.039	0.040
EN	1.000	1.000	0.892	0.896
CR	1.000	1.000	0.055	0.049
EX	1.000	1.000	0.175	0.186
EN-CR-EX	1.000	1.000	0.018	0.016

[a]*Phylogenies: RR*, Incilius *phylogeny rooted with* Rhaebo *and* Rhinella *as outgroups; Ana,* Incilius *phylogeny rooted with* Anaxyrus *as the outgroup. Significance:* $p < 0.05$; **$p < 0.005$; ***$p < 0.001$.
[b]*The IUCN Red List categories: NE, not evaluated; DD, data deficient; LC, least concern; NT, near threatened; VU, vulnerable; EN, endangered; CR, critically endangered; EX, extinct. None of the taxa analyzed here are extinct in the wild (EW).*

Results

We obtained fairly similar results from the 2 differently rooted phylogenies and from both sets of threat rankings. With Pagel's λ (Table 1), we found four significantly autocorrelated traits for each tree: habitat destruction as a cause of decline, small range as a cause of decline, least concern threat ranking according to the IUCN, and least concern threat ranking according to our new data set. However, the threat-ranking traits were no longer significantly autocorrelated when the Bonferroni correction was applied. Habitat destruction as a cause of decline was a very highly autocorrelated trait on both phylogenies ($p < 0.0001$). This was the only trait that remained significantly autocorrelated when the Bonferroni correction was applied to the data.

Abouheif's test was the only measure for which we found significant differences between the two phylogenies (Table 1). This test was also far less conservative than Pagel's λ because it showed the highest number of significantly autocorrelated traits. However, when the Bonferroni correction was applied,

Table 2. Fritz and Purvis' *D* as a measure of phylogenetic signal in extinction risk and cause of decline of *Incilius* toads.

	RR^a		Ana^a	
	D	p	D	p
Cause of decline				
habitat destruction	−0.986	<0.001***	−1.068	<0.001***
small range	−2.548	<0.001***	−2.613	<0.001***
Chytridiomycosis	−0.062	0.018	−0.154	0.018
IUCN rank^b				
NE, DD	1.507	0.895	1.495	0.869
LC	0.628	0.130	0.618	0.129
NT	0.397	0.181	0.379	0.205
VU	1.701	0.857	1.669	0.836
EN	0.743	0.265	0.766	0.264
CR	−0.313	0.033	−0.348	0.031
EX	−2.401	0.085	−1.295	0.077
EN-CR-EX	0.464	0.070	0.438	0.072
Updated rank^b				
NE, DD	4.815	0.869	3.106	0.872
LC	0.209	0.014	0.196	0.014
NT	−0.587	0.009	−0.776	0.008
VU	0.195	0.057	0.214	0.054
EN	1.017	0.475	1.002	0.467
CR	0.240	0.045	0.224	0.046
EX	0.575	0.201	0.559	0.221
EN-CR-EX	0.221	0.017	0.202	0.019

^{a}*Phylogenies: RR,* Incilius *phylogeny rooted with* Rhaebo *and* Rhinella *as outgroups; Ana,* Incilius *phylogeny rooted with* Anaxyrus *as the outgroup. The p values indicate the probability that D < 1 (departure from random phylogenetic distribution of the trait).*
^{b}*Threat categories are identified in Table 1.*

Table 3. Phylogenetic correlation between cause of decline and threat level in *Incilius* toads.

	RR^a			Ana^a		
	Bd	HD	SR	Bd	HD	SR
IUCN rank^b						
NE, DD	0.568	0.582	0.809	0.528	0.561	0.848
LC	0.061	0.193	0.791	0.058	0.199	0.837
NT	0.688	0.291	0.787	0.287	0.343	0.949
VU	0.904	0.798	0.623	0.907	0.805	0.663
EN	0.575	0.837	0.794	0.575	0.837	0.834
CR	0.032	0.875	0.364	0.033	0.861	0.340
EX	0.377	0.769	0.949	0.380	0.802	0.979
EN-CR-EX	0.028	0.861	0.620	0.027	0.855	0.648
Updated rank						
NE, DD	0.481	0.769	0.956	0.411	0.802	0.979
LC	0.070	0.001	0.442	0.081	0.002	0.472
NT	0.620	0.333	0.894	0.487	0.333	0.949
VU	0.565	0.009	0.300	0.681	0.009	0.324
EN	0.954	0.289	0.475	0.956	0.275	0.506
CR	0.264	0.512	0.812	0.269	0.451	0.855
EX	0.009	0.258	0.264	0.010	0.275	0.254
EN-CR-EX	0.002	0.409	0.666	0.002	0.377	0.670

^{a}*Phylogenies: RR, the* Incilius *phylogeny rooted with* Rhaebo *and* Rhinella *as outgroups; Ana, the* Incilius *phylogeny rooted with* Anaxyrus *as the outgroup. Other abbreviations: Bd: B. dendrobatidis, commonly known as the amphibian chytrid fungus; HD, habitat destruction; SR, small range.*
^{b}*Threat categories are identified in Table 1.*

only one threat ranking (near threatened under the updated rankings) was significantly autocorrelated.

Habitat destruction and small range yielded significant *p* values with Pagel's λ and Abouheif's test, and negative values of *D* (Table 2). These are the only two traits for which the probability of a Brownian phylogenetic distribution was >0.95 under Fritz and Purvis' *D* and are the only two traits that remained significantly autocorrelated when the Bonferroni correction was applied to the results of *D*. Chytrid fungus as a cause of decline yielded a *p* of 0.018 with both trees; this value was marginally non-significant with the Bonferroni correction. Threat rankings that yield significant *p* values resulting from Fritz and Purvis' *D*—critically endangered according to the IUCN and a number of threat rankings under our new data set (Table 1)—were no longer significant when the Bonferroni correction was applied.

With or without the Bonferroni correction, habitat destruction and small range were the only cause-of-decline categories that were significantly correlated with each other (Supporting Information). Among cause of decline and threat ranking correlations, 12 of the 96 pairings yielded significant *p* values without the Bonferroni correction; however, with the Bonferroni correction, none of these results were significant (Table 3). When the

Bonferroni correction was not applied, the significant results were the same from both phylogenies. Notably, chytrid fungus was associated with severe threat (endangered, critically endangered, or extinct), and small range, a rare cause of decline (affecting only 3 *Incilius* species), was not significantly associated with any single threat ranking.

Discussion

We found far more phylogenetic signal in cause of decline than in threat ranking. Given that 96 tests of phylogenetic autocorrelation were conducted on threat ranking (Pagel's λ, Abouheif's test, and Fritz and Purvis' *D*; two phylogenetic hypotheses; two data sets of rankings; eight rankings), one would expect between 4 and 5 false positives at α = 0.05. Yet, with the Bonferroni correction, only one of these 96 tests yielded a significant *p* value. Our results therefore strongly suggest that there is no species-level phylogenetic signal in threat ranking within *Incilius*; the different threat rankings were widely dispersed across the phylogeny (Supporting Information).

In contrast, a very different picture emerges from the results pertaining to cause of decline (Fig. 2). All metrics very strongly supported the designation of an *Incilius* clade sensitive to habitat destruction. Two of our three metrics we used strongly supported the designation of
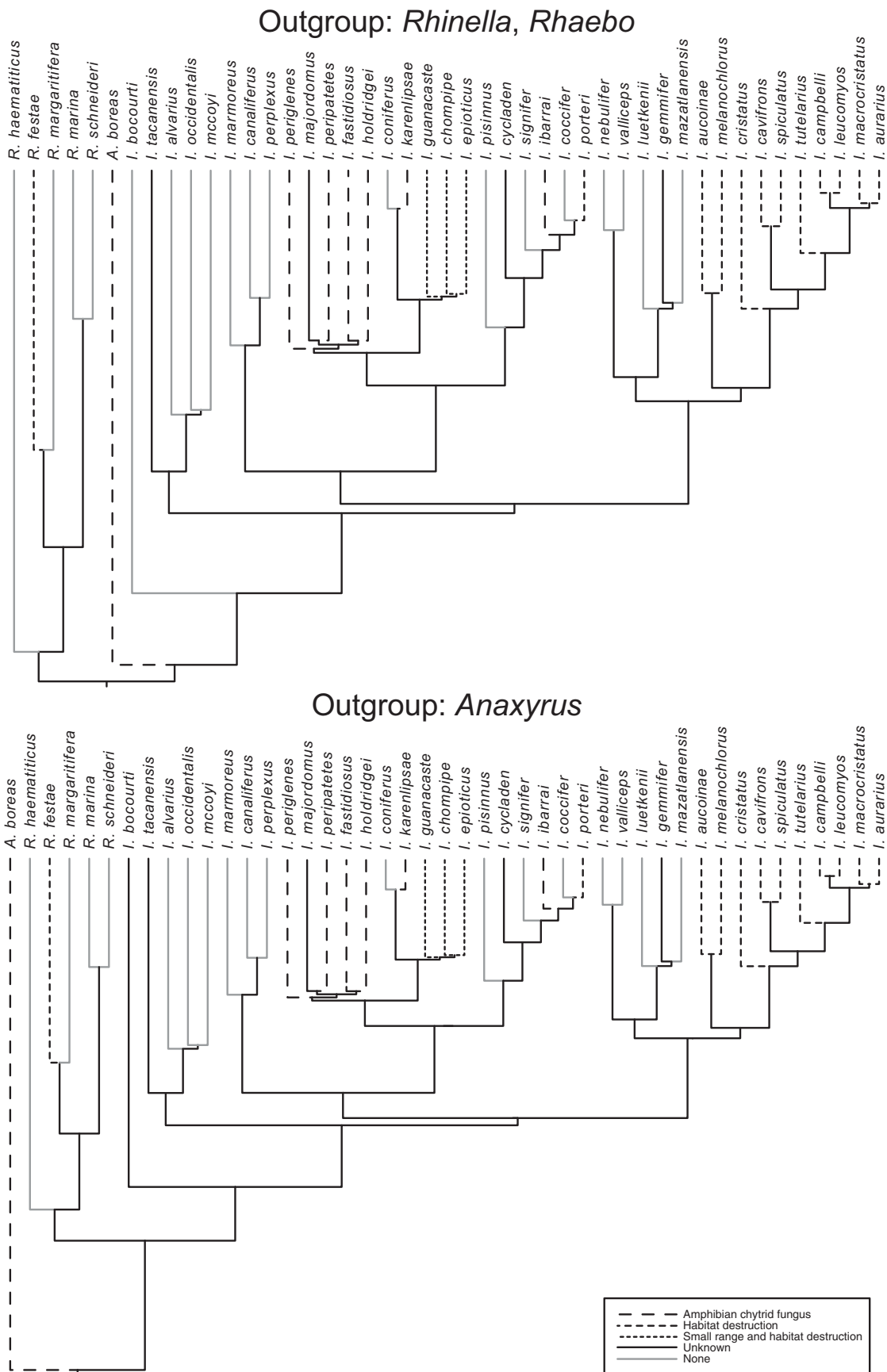
Figure 2. Causes of decline in Incilius *in a phylogenetic context.*

a small-range-endangered *Incilius* clade. The 3 species that are threatened due to small range — *I. guanacaste*, *I. chompipe*, and *I. epioticus*—formed a monophyletic clade in both phylogenies; we therefore suspect that the lack of strong support from Pagel's λ was due to the small size of this clade. Our correlation analysis showed a high degree of overlap between these 2 causes of decline (habitat destruction and small range), which is unsurprising because the 3 species threatened due to small range are also threatened due to habitat destruction. The clade that is sensitive to small range and habitat destruction was not nested within the clade that is sensitive to habitat destruction only. Our results support the designation of an *Incilius* clade that is endangered due to habitat destruction—a group often referred to as the forest toads—with a separate clade nested within the *coniferus* group that is endangered due to both habitat destruction and small range. It is highly unlikely that this phylogenetic signal is an artifact of spatial autocorrelation because most *Incilius* species are parapatric or allopatric and closely related species are particularly unlikely to co-occur in a given area (Supporting Information). We are aware of only 2 instances of syntopy (*I. valliceps* and *I. marmoreus*, in Veracruz, Mexico, and *I. perplexus* and *I. marmoreus* in west-central Mexico). However, researchers using our method would want to take spatial autocorrelation into account if they are studying species that show significant amounts of sympatry.

The data regarding the chytrid fungus were inconclusive because different tests yielded non-significant, marginally significant, and significant *p* values for autocorrelation of this trait. We cannot confidently say whether or not the data supported the designation of a chytrid-endangered clade within *Incilius.* Smith et al. (2009) did find such a pattern working at the community level in Costa Rica and Panama. In *Incilius*, the chytrid fungus threatens *I. ibarrai*, which is in the *coccifer* group, as well as a number of species within the *coniferus* group. Together, the *coccifer* and *coniferus* groups form a monophyletic clade. The notable lack of support for a chytrid-endangered clade according to the Pagel's λ may be due to the fact that one outgroup lineage, *A. boreas*, is also endangered due to the chytrid fungus.

In addition to our autocorrelation results, which show that cause of decline and threat ranking contain different amounts of phylogenetic signal, the results of our correlation analysis strongly suggest that cause of decline is decoupled from threat ranking. This is because cause of decline and threat ranking tend not to be correlated, such that threat ranking (which is a homoplastic character) appears not to be an appropriate proxy for cause of decline (a homologous character).

A recurring problem for estimates of phylogenetic signal in conservation-threat assessment is the lack of a complete and fully resolved phylogeny. Some researchers had access to nearly complete phylogenies (e.g., Isaac et al.

2007, Mammalia) or created a complete phylogeny of focal taxa and documented well or experimentally controlled causes of threat (e.g., Smith et al. 2009; Hammond et al. 2012). Those studying other groups, such as the amphibians, had to seek patterns at a broad scale because of incomplete sampling or the coarse resolution of the phylogeny available to them. Because we found that significant differences in threat ranking existed at the species level, our results demonstrate the importance of complete sampling and fine resolution when searching for phylogenetic signal in threat rankings. Results at broader scales tend to be relatively uninformative. For example, a key result from Corey and Waite (2008) is that certain families of hyloid frogs are statistically correlated in terms of the IUCN threat ranking critically endangered and cause of decline is "enigmatic decline" (latter data from Stuart et al. 2004). Thus, taxonomic families such as Bufonidae (approximately 575 species, worldwide distribution) are flagged wholesale as critically endangered by enigmatic declines as a result of their shared evolutionary history. This conclusion clearly is inapplicable to many species within Bufonidae. At a smaller phylogenetic scale, with better-defined causes of declines, we identified phylogenetic patterns that should inform protection and recovery programs far better. For example, our results clearly indicate a clade in need of progressive habitat conservation programs and suggest that another clade may bear the synapomorphy, or shared trait inherited from a common ancestor, of special sensitivity to chytrid fungal infections. Our data also highlight interesting exceptions that may be important in terms of conservation. For example, the species *I. gemmifer*, from Mexico, has not been found in over 40 years, despite repeated searches, yet its threat ranking as either critically endangered or extinct would not be predicted by its phylogenetic position.

We also found that homologous characters pertaining to cause of extinction contained strong phylogenetic signal, whereas homoplastic data pertaining to severity of extinction threat (e.g., IUCN threat rankings) did not reflect phylogeny. This difference between homologous and homoplastic characters has implications for the application of evolutionary history to conservation. Although our study is limited to a single genus, our findings are highly relevant to a large number of studies that report potential effects of shared evolutionary history in analyses of susceptibility or that identify clade-level influences of specific conservation threats (e.g., Cooper et al. 2007; Bielby et al. 2008; Davies et al. 2008; Isaac et al. 2012). Our approach could be applied to any taxon, and additional studies should examine the characters considered here among species in larger clades. We suggest that future studies incorporate the important distinction between the homoplastic trait "proneness to equivalent IUCN rankings" as opposed to the homologous trait "proneness to extinction due to shared susceptibility to specific causes of decline."

The IUCN threat rankings are essential for purposes of immediate, short-term conservation activities. However, phylogenies reflect evolutionary history over very long periods and are based on the concept of homology. Our results strongly suggest that phylogenetic studies meant to guide long-term conservation strategy should use homologous characters relating to cause of decline, rather than homoplastic designations derived from IUCN threat rankings.

Acknowledgments

Supporting Information

The current IUCN Red List categories for *Incilius* spp., our recommended updated categories for those species, and principal cause of decline (Appendix S1); phylogenetic correlation of cause of decline of *Inclius* toads (Appendix S2); rankings of extinction risk for *Incilius* (Appendix S3); and general distributions of *Incilius* species (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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