Received: 31 January 2019 | Revised: 1 April 2019 | Accepted: 7 April 2019

DOI: 10.1111/zsc.12354

ORIGINAL ARTICLE

Zoologica Scripta | WILEY

Evaluation of species boundaries in sympatric and parapatric populations of Mesoamerican toads

Thomas J. Firneno Jr. | **Josiah H. Townsend**

Department of Biology, Indiana University of Pennsylvania, Indiana, Pennsylvania

Correspondence
Thomas J. Firneno Jr., Department of Biology, University of Texas at Arlington, Box 19498, Arlington, TX 76019-0498.
Email: thomas.firneno@uta.edu

Abstract

Approaches that integrate multiple independent, yet complimentary, lines of evidence have been effectively utilized to identify and evaluate species diversity. Integrative approaches are especially useful in taxa that exhibit cryptic diversity and are highly morphologically conserved, as well as organisms whose distributions may be sympatric or parapatric. The *Incilius coccifer* complex in Honduras is comprised of three putative taxa: *I. coccifer*, *I. ibarrai* and *I. porteri*. The taxonomy of the *I. coccifer* complex has been a source of debate among specialists, with some recognizing three species, while others choose to recognize one widespread taxon. To assess species boundaries and evaluate the taxonomic structure for the *I. coccifer* complex, we utilized a combination of comprehensive field sampling, molecular phylogenetics and macroecological modelling. Using 58 samples representing all three putative taxa, we generated sequence data from the mitochondrial loci 16S and COI in order to assess genetic diversity and phylogenetic relationships, and tested putative species boundaries using General Mixed Yule-Coalescent models. To evaluate macroecological differences in the distribution of putative taxa, we utilized maximum entropy modelling and identified areas of suitable and non-suitable habitat, as well as identifying potential areas of overlap between species habitats. We recovered three clades that broadly correspond to the three named taxa that, while being monophyletic, are separated by relatively small genetic distances. Species distribution models revealed that *I. coccifer* is macroecologically different than the other two taxa, but that *I. ibarrai* and *I. porteri* are highly similar. We uncovered cases of sympatry between pairs of species in at least three localities in Honduras, suggesting the potential for hybridization in these closely related lineages.

KEYWORDS

Incilius, *Incilius coccifer* complex, integrative taxonomy, species boundaries

1 | INTRODUCTION

Species boundaries are often unclear and have caused the taxonomic status of many organisms to continuously be disputed. One of the main issues stems from conflicting concepts of what a species "is," based on different organismal criteria such as reproductive isolation (Mayr, 1942), ecological distinctiveness (Van Valen, 1976), evolutionary fate (Wiley, 1978) and morphological diagnosability (Bisby & Coddington, 1995; Cronquist, 1978). Many taxonomists have tried to remedy this situation by adopting and using the General Lineage Concept (GLC; de Queiroz, 2005a, 2005b) under which species are separately evolving lineages and that multiple criteria may be used to identify them. Ultimately,

the greater number of species criteria that are satisfied by a group, the more likely it is that the group is a distinct lineage (de Queiroz, 2007). Though the GLC has alleviated many of the issues facing species delimitation, there are still issues that taxonomists face in distinguishing and evaluating species boundaries (Barley, White, Diesmos, & Brown, 2013; Brown et al., 2007).

To overcome some of these obstacles, methodological approaches to delimiting species boundaries, including more sophisticated genomic tools, more powerful statistical approaches to defining morphological variation and ecological uniqueness, and increased computational power, have been developed (Luo, Ling, Ho, & Zhu, 2018; Zapata & Jiménez, 2012). These methodological advances have allowed for the integration of multiple lines of evidence (e.g., morphology, genetics, ecology) to be utilized in the inference of species boundaries under what has been deemed integrative taxonomy (Dayrat, 2005; de Queiroz, 2007; Padial, Miralles, Riva, & Vences, 2010; Padial & de la Riva, 2006; Schlick-Steiner et al., 2010). These integrative methods are also attempting to decrease the degree of subjectivity that exists in many traditional taxonomic practices and are moving towards increasing the objectivity of such methods and practices (Camargo, Morando, Avila, & Site, 2012; Fujita, Leaché, Burbrink, McGuire, & Moritz, 2012). Ultimately, integrative taxonomy has helped accelerate the discovery and documentation of biodiversity, as well as the evaluation of species boundaries of confounding species complexes. This has been especially true with regard to closely related and/or cryptic species, which tend to be highly morphologically conserved organisms with independent evolutionary histories (Agapow et al., 2004; Bickford et al., 2007; Grismer et al., 2013; Meier, Tan, Ang, Lim, & Ismail, 2010; Padial & de la Riva, 2009).

Mesoamerican toads (Anura: Bufonidae: *Incilius*) are a diverse evolutionary radiation of amphibians that inhabit virtually the full range of terrestrial habitats in Central America, from coastal dry forests to montane cloud forests (McCranie & Wilson, 2002; Mendelson, Mulcahy, Williams, & Sites, 2011). Many putative species exhibit highly conserved morphology, thus making them difficult to analyse using traditional taxonomic methods forests (McCranie & Wilson, 2002; Mendelson, Williams, Sheil, & Mulcahy, 2005; Mendelson et al., 2011). Six putative species are recognized in the *Incilius coccifer* group, with three of those species occurring Honduras (*I. coccifer, I. ibarrai* and *I. porteri*), also referred to as the *I. coccifer* complex (Mendelson et al., 2005, 2011). Because these three taxa represent distinct but closely related species whose distributions are apparently in broad contact, Mendelson et al. (2005) suggested parapatric relationships between *I. coccifer* (lowland dry forest inhabitant) and *I. ibarrai* and *I. porteri* (upland pine-oak and cloud forest inhabitants). The *I. coccifer* complex provides an excellent system for studying closely related species with possible parapatric and/or sympatric lineages due to their inferred zones of contact in southern and southwestern Honduras.

The evolutionary and taxonomic relationships within the *I. coccifer* complex have been the source of debate among specialists, with some recognizing only a single species in Honduras (*I. coccifer*) and considering the other two taxa as junior synonyms (McCranie & Castañeda, 2007; McCranie & Wilson, 2002). Much of the criticism of the current taxonomy involves perceived discrepancies between morphology and molecular phylogenetics (McCranie, 2015), represented by a maximum of three individuals from each nominal taxon, as well as a lack of comprehensive sampling throughout Honduras (McCranie, 2009, 2015; McCranie & Castañeda, 2007). However, those who criticize the current taxonomy never present evidence (morphological, molecular or otherwise) against it.

Extensive sampling by the authors and collaborators throughout Honduras over the past decade has led to the collection of additional samples representing the nominal taxa recognized by Mendelson et al. (2005). Here we utilize a more robust sample size of individuals from all three nominal taxa to carry out phylogenetic, demographic and species delimitation analyses of a mitochondrial DNA genetic data set from across the potential contact zones, and species distribution modelling based on verified localities of all three taxa, in order to: (a) evaluate species boundaries within this complex; (b) reinforce the current taxonomic hypothesis for this species complex; and (c) identify the proposed/potential zones of sympatry and/or parapatry among populations within the complex. The results of our study demonstrate the utility and success of integrative techniques when applied to complicated biological systems such as the *I. coccifer* complex.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and sequencing

Fifty-eight genetic samples representing the three nominal taxa (*I. coccifer*, *I. ibarrai* and *I. porteri*) were collected throughout Honduras and Nicaragua from 2006 to 2015 (Figure 1). *Incilius pisinnus, I. cycladen* and *I. signifier*, the other species three of the *I. coccifer* group (Mendelson et al., 2011), were used as outgroup taxa. Taxa and samples used in this study, along with their associated voucher numbers, locality data, GenBank accession numbers and Barcode of Life Database (BOLD) accession numbers, are presented as Table S1.

A segment of 492 base pairs (bp) from the 16S large subunit RNA (16S) gene was amplified using primers 16Sar-L (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr-H (5′-GGTTTGAACTCAGATCATGT-3′) (Palumbi et al., 1991). 16S amplifications were carried out in 20 μl containing 1× PCR buffer (200 mM Tris HCl [pH 8.4], 500 mM KCL),

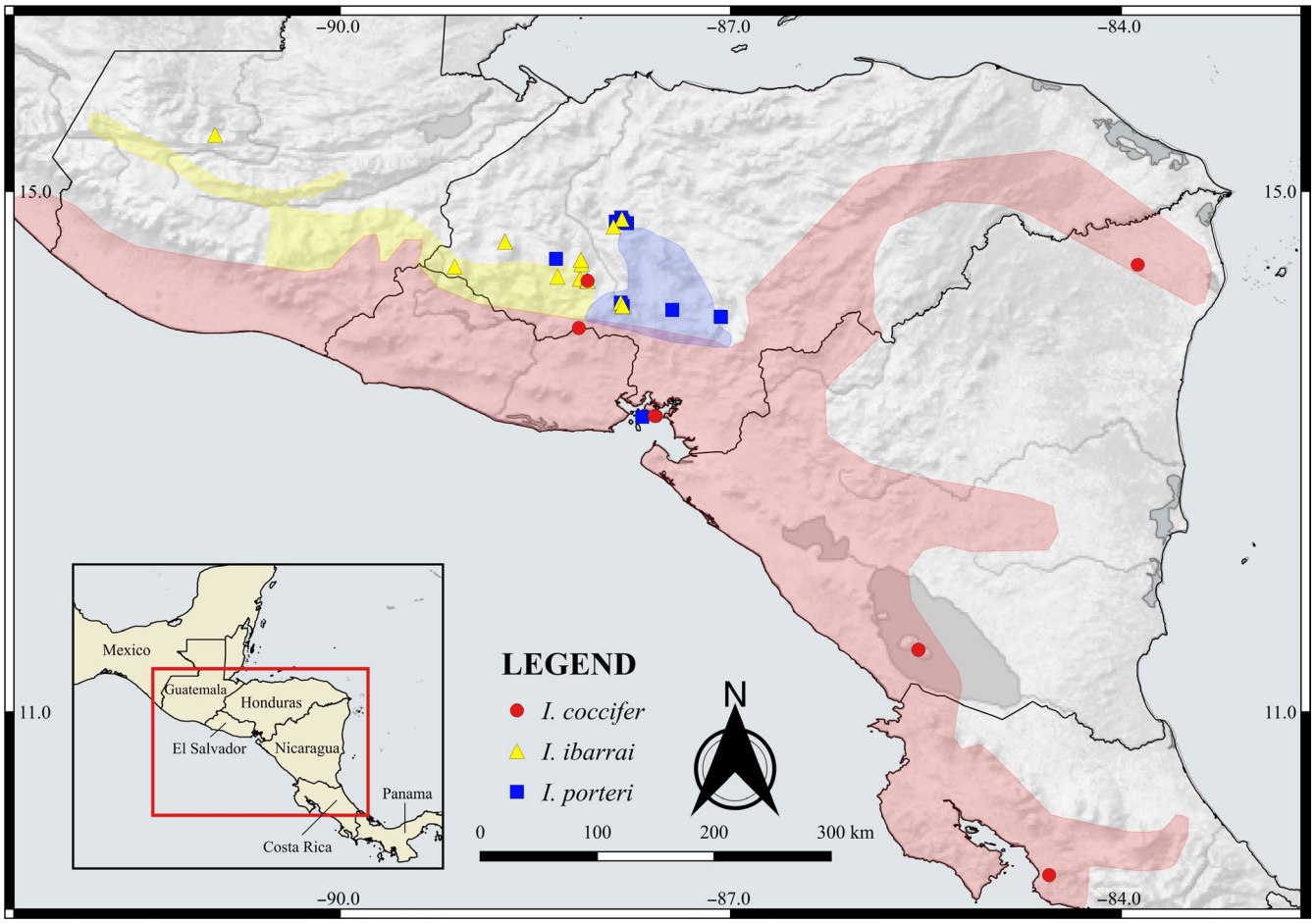

FIGURE 1 Map showing genetic sampling localities for the three focal taxa (*Incilius coccifer*, *Incilius ibarrai*, and *Incilius porteri*). Historical distributions for each taxon are also denoted (*I. coccifer* = red; *I. ibarrai* = yellow; *I. porteri* = blue)

1.5 mM $MgCl_2$, 0.3 mM dNTPS, 0.4 μM of each primer, 0.05 U of AmpliTaq (Thermo-Fisher) and 500 ng of genomic DNA. The following cycling parameters were used: 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 45 s, with a final extension of 72°C for 5 min.

A segment of 658 bp from the cytochrome oxidase subunit I (COI) gene was amplified using primers LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). Cytochrome oxidase subunit I amplifications were carried out in 25 μl containing 1× PCR buffer, 2 mM $MgCl_2$, 0.3 mM dNTPS, 0.4 μM of each primer, 0.05 U of AmpliTaq (Thermo-Fisher) and 500 ng of genomic DNA. The following cycling parameters were used: 94°C for 1.5 min, followed by 37 cycles of 94°C for 40 s, 50°C for 40 s, 72°C for 40 s, with a final extension of 72°C for 6 min.

PCR products were cleaned using 2 μl of ExoSAP-IT per sample. PCR product was sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit (ABI) and electrophoresed on an ABI 3730xl DNA Analyzer at the Smithsonian Institution Laboratory of Analytical Biology (SI-LAB).

2.2 | Sequence alignment and model selection

A data set containing all available sequences of *I. coccifer, I. ibarrai* and *I. porteri* (newly sampled and previously published) was generated, and sequences were aligned using CLUSTALW (Thompson, Higgins, & Gibson, 1994) as implemented within the program package MEGA7.0 (Kumar, Stecher, & Tamura, 2016) using the default parameters. We partitioned the data set by gene (16S, which codes for RNA) and by codon position (1st, 2nd, 3rd) for COI (protein-coding gene) to account for potential substitution saturation at the third codon position. Best fit models of nucleotide substitution were estimated for each gene and each partition using jModeltest 2.0 (Darriba, Taboada, Doallo, & Posada, 2015), which uses PHYML 3.0 (Guindon & Gascuel, 2003) to estimate models under a likelihood framework. The number of

substitution schemes was set to eight to limit the number of models to 88.

2.3 | Mitochondrial DNA analyses

Uncorrected (*p*-distance) pairwise sequence divergence was calculated for all samples and for each gene to provide an estimate of intraspecific versus interspecific variation. Sequence divergence estimation was performed in MEGA7.0 (Kumar et al., 2016). Maximum likelihood (ML) analysis was carried out in RAxML v8.0 (Stamatakis, 2014), with 1,000 bootstrap pseudoreplicates under the GTR + GAMMA substitution model. Bayesian Inference (BI) was performed using MRBAYES3.2.2 (Huelsenbeck & Ronquist, 2001) and consisted of two parallel runs of four Markov chains (three heated, one cold) run for 20×10^6 generations and sampled every 10,000 generations, with a random starting tree and the first 2×10^6 generations discarded as burnin.

2.4 | Species delimitation analyses

An ultrametric mtDNA tree was generated using BEAST v2.3.1 (Bouckaert et al., 2014) for our combined mitochondrial data set (16S and COI), using a strict clock model, Coalescent constant population and a random starting tree, with a Markov Chain Monte Carlo (MCMC) run for 20 million generations, sampling trees every 1,000 generations. Substitution models were unlinked and appropriate models (as selected by jModeltest 2.0) were applied to each partition. To estimate species boundaries within the complex, a single threshold General Mixed Yule-Coalescent (GMYC) model was implemented on the phylogeny obtained from BEAST in R using the package "splits."

2.5 | Haplotype networks and demographic analyses

A median-joining haplotype network was constructed using POPART (Leigh & Bryant, 2015). The network was constructed from the combined COI and 16S data set for all samples within the *I. coccifer* complex (excluding outgroups). Genetic diversity (nucleotide and haplotype diversity and mean number of pairwise differences) was calculated within the three species groups with the program ARLEQUIN 3.5 (Excoffier & Lischer, 2010). Fu's *Fs*, Ramos and Razos R_2, and Tajima's *D* neutrality tests were also implemented to assess for population expansion (Fu, 1997; Ramos-Onsins & Rozas, 2006; Zhang, Rao, Yang, Yu, & Wilkinson, 2010). Assuming an infinite sites model, a stationary population will exhibit a mismatch distribution that is ragged and often multimodal, whereas an expanding population will exhibit a distribution that is smooth and often unimodal (Harpending et al., 1998; Harpending, 1994; Rogers & Harpending, 1992).

2.6 | Species distribution modelling

Occurrence data for 414 morphologically verified individuals were compiled from published localities (from the appendix of Mendelson et al., 2005) and genetically verified unpublished localities from 2006 to 2015 fieldwork conducted by the authors and colleagues (Figure S2; data file provided as Table S2). Bioclimatic grid data layers at a 30 arc-second resolution were acquired from the WorldClim database (http://www.worldclim.org) and imported into ARCGIS 10.2 (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005).

Pairwise correlation coefficients were calculated and compared for each bioclimatic layer using SDMToolbox within ARCGIS 10.2 (Brown, 2014). A Pearson correlation coefficient of ±0.75 was used to identify and remove highly correlated variables. The following seven variables remained in the data set: BIO1 = annual mean temperature; BIO2 = mean diurnal range (mean of monthly [max temp − min temp]); BIO3 = isothermality (mean diurnal range/annual temperature range); BIO12 = annual precipitation; BIO15 = precipitation seasonality (coefficient of variation); BIO18 = precipitation of warmest quarter of the year; BIO19 = precipitation of the coldest quarter of the year.

Spatially correlated occurrence records were eliminated using the "Spatially Rarify Occurrence Data" tool in SDMToolbox. A threshold of 500 m was used as a threshold for rarefication. This reduced the original occurrence data set from 414 to 124 spatially independent samples, with 36 of these corresponding to genetic localities.

Species distribution models for *I. coccifer*, *I. ibarrai* and *I. porteri* were constructed using the maximum entropy method executed in MAXENT 3.3 (Phillips, Dudîk, & Schapire, 2004). Separate models were created for each of the nominal taxa with 1,000 bootstrap replicates, a random test percentage of 33% and a maximum of 5,000 iterations (all other

TABLE 1 Within and between-species sequence divergence (uncorrected *p*-distance) for *Incilius coccifer* complex

	Intraspecific		Interspecific	
	16S	**COI**	**16S**	**COI**
Incilius coccifer	0.000–0.010	0.000–0.021	0.006–0.016	0.026–0.045
Incilius ibarrai	0.000–0.008	0.000–0.015	0.008–0.021	0.019–0.045
Incilius porteri	0.000–0.010	0.000–0.017	0.006–0.021	0.019–0.038

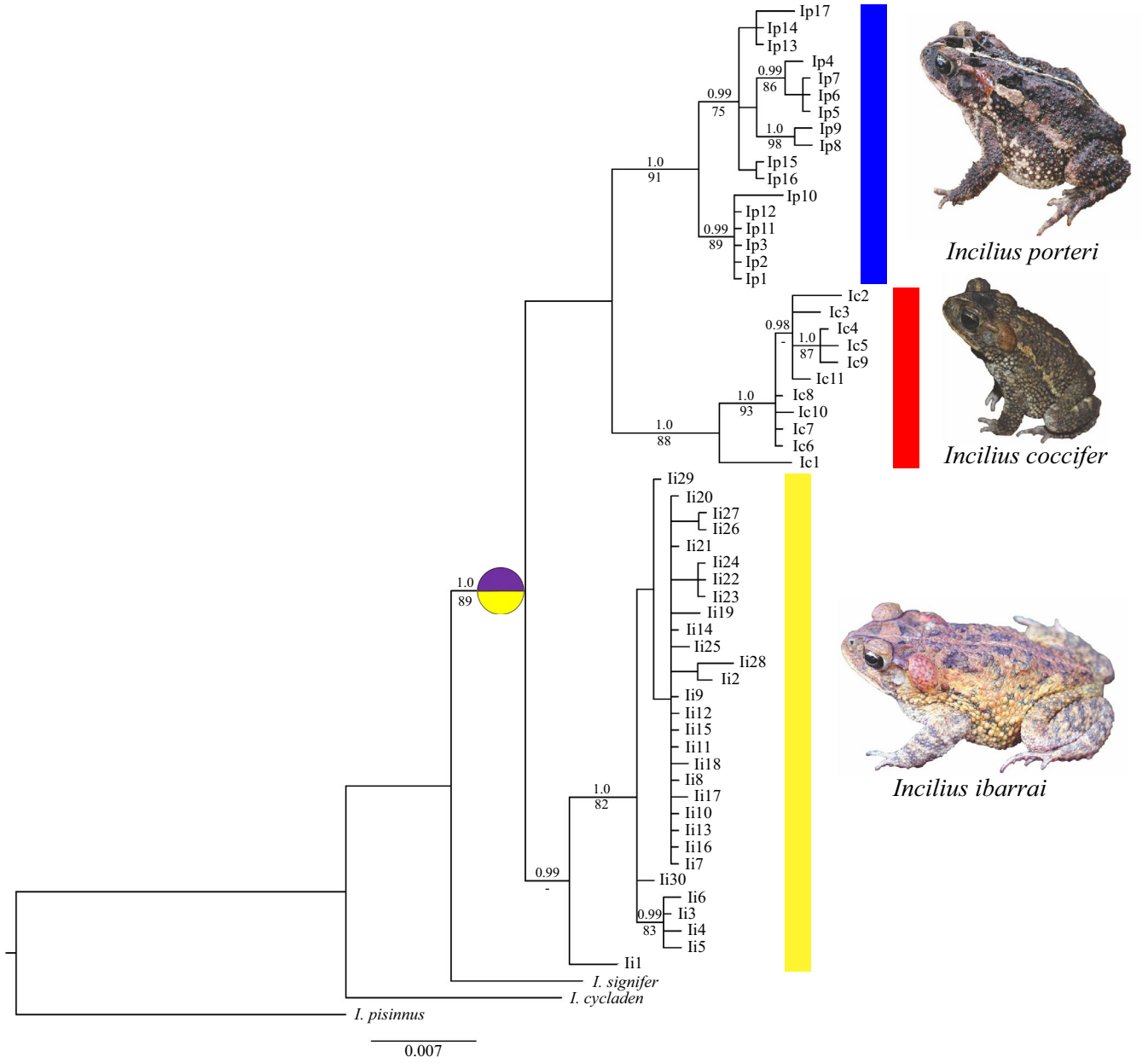

FIGURE 2 Bayesian phylogram showing inferred relationships among Honduras samples from the *Incilius coccifer* complex based on combined and partitioned 16S and COI data set. Posterior probabilities are shown above the branch and bootstrap values from ML analysis are shown below. The split circle indicates the species split estimated by the single model GMYC analysis into a *coccifer-porteri* clade (purple) and an *ibarrai* clade (yellow). Scale bar represents the genetic divergence distance. Photos by Thomas J. Firneno, Jr. and Josiah H. Townsend

parameters were left as default). Following the approach used by Luque-Montes et al. (2018), models were reclassified into binary files of suitable and non-suitable habitat based on the maximum training sensitivity plus specificity logistic thresholds due to its more conservative estimates over the minimum training presence logistic threshold (Table S4; Liu, Berry, Dawson, & Pearson, 2005; Phillips et al., 2004).

Model performance was evaluated using an analysis of the value of the "area under the curve" (AUC) and the unregularized training gain. The goodness-of-fit for each of the models' predictions was evaluated using the partial area under the curve (pAUC) procedure since the use of the whole AUC of the receiver operating curve has been criticized (Barve, 2008; Lobo, Jiménez-Valverde, & Real, 2008; Peterson, Papeş, & Soberón, 2008) using the following parameters: 1,000 repetitions, 95 per cent confidence interval, and two independent data sets.

To evaluate niche overlap, the niche overlap function in ENMTools was used to calculate Schoener's *D* by pairwise comparison (Rödder & Engler, 2011; Warren, Glor, & Turelli, 2008), which gives an output value from 0 to 1, where a value of 0 indicates no overlap between niches and a value of 1 indicates that the niches are identical.

3 | RESULTS

3.1 | Phylogenetic analyses, species delimitation models and haplotype networks

Best fit nucleotide substitution models varied by gene and codon position, supporting the use of a gene- and codon-based partitioning strategy (Table S3). Distance-based analyses of each gene yielded ambiguous results concerning delimited species-level lineages and clusters. Nucleotide variability was 4.3% for the 492 bp of 16S and 10.2% for the 658 bp of COI. Interspecific and intraspecific divergence distances slightly overlapped, with interspecific divergence ranging from 0.6% to 2.1% for 16S and 1.9% to 4.5% for COI, and intraspecific divergence ranging from 0.0% to 1.0% for 16S and 0.0% to 2.1% for COI (Table 1).

Both the ML and BI methods recovered three well-supported, genetically distinct clades assignable to *I. coccifer*, *I. ibarrai* and *I. porteri*, albeit with relatively short genetic distances between clades (Figure 2). *Incilius coccifer* and *I. porteri* were recovered as sister lineages, with *I. ibarrai* sister to the *I. coccifer-porteri* clade. Two samples collected from different elevations on Isla del Tigre (Valle), represented *I. coccifer* and *I. porteri* (Firneno, Luque-Montes, & Townsend, 2017). Eight samples collected in near sympatry from northern Comayagua form divergent haplogroups within two species-level clades (*I. porteri* and *I. ibarrai*). Within the *I. ibarrai* clade, there is a high degree of divergence (1.3%–1.9% for COI and 0.4%–1.0% for 16S) between a single sample from central Guatemala and all remaining samples from Honduras. The eleven samples of *I. coccifer* vary across a wide geographic range (southwestern El Salvador to central Costa Rica). There is a high degree of divergence (1.3%–1.9% for COI and 0.8%–1.2% for 16S) between the samples from Honduras, Nicaragua and El Salvador, compared to the sample from Costa Rica. Ten samples collected in sympatry from San Pedro la Loma (Intibucá) at 2,015 m elevation formed two divergent haplogroups, both associated with the taxa *I. ibarrai* and *I. coccifer*. A single sample representing the *I. porteri* clade was collected from the Cordillera de Opalaca (Intibucá) at 1,985 m elevation.

The corresponding haplotype network also revealed three groups, consistent with the results of the phylogenetic analysis (Figure 3). *Incilius coccifer* and *I. porteri* clades differed by 13 mutations, *I. coccifer* and *I. ibarrai* clades differed by 21 mutations and *I. ibarrai* and *I. porteri* clades differed by 17 mutations. Nine unique haplotypes were revealed for both *I. coccifer* and *I. porteri*, with 16 unique haplotypes being revealed for *I. ibarrai* (Figure 3).

The phylogeny output from BEAST had an identical topology to the ML/BI methods. The single GMYC model estimated two species units, with the split being between the *I. ibarrai* clade and the *I. coccifer* + *I.porteri* clade (Figure 2).

3.2 | Genetic diversity and demographic history

Genetic diversity estimates and neutrality tests within populations are shown in Table S5. Within the separate clades and among all individuals, nucleotide diversity (*n*) is relatively low and haplotype diversity (*h*) is high, suggesting that modern populations have very low levels of gene flow among them, and that populations have evolved in relative isolation from each other.

Fu's *Fs* tests (Table S5) were non-significant for the clades, supporting sequence evolution consistent with the expectation of selective neutrality and stable demographic history. Negative Tajima's *D* values may indicate that the populations have recently begun to expand or there is evidence

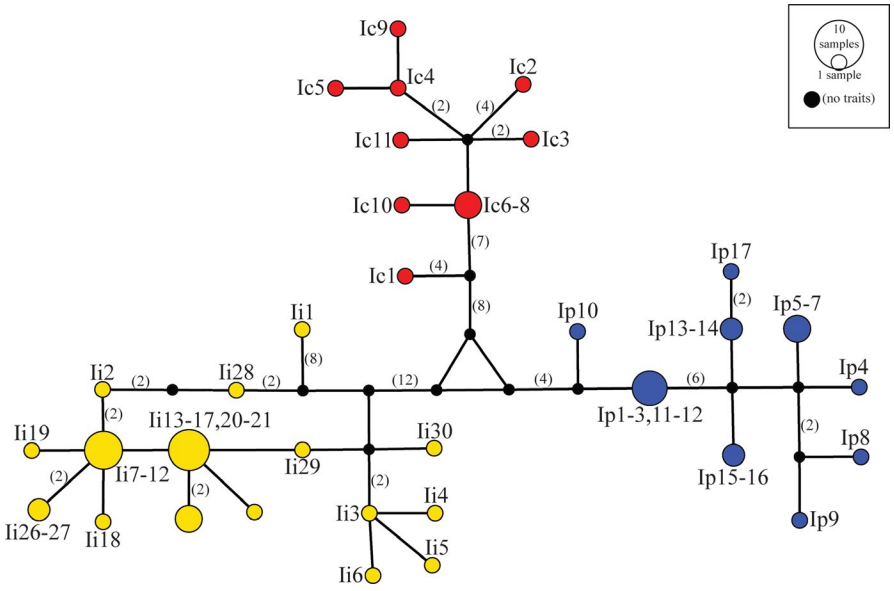

FIGURE 3 Median-joining phylogenetic network showing inferred relationships among Honduran samples of the *Incilius coccifer* complex based on a combined and partitioned 16S and COI data set. Connecting lines represent single mutations unless indicated otherwise (in parentheses). *I. coccifer* clade is denoted in red, *Incilius ibarrai* in yellow and *Incilius porteri* in blue

for purifying selection at a locus. Ramos and Rozas R_2 statistics were all small and positive, and non-significant, also supporting these hypotheses of population expansion and/or demographic stability.

Mismatch distributions were generated for all three species (Figure S1). The three clades showed multimodal distribution patterns (*I. coccifer* raggedness index $r = 0.0413$, $p = 0.627$; *I. ibarrai* $r = 0.0454$, $p = 0.006$; *I. porteri* $r = 0.176$, $p = 0.001$), which indicate stable or slowly declining populations and long-term demographic stability (Rogers & Harpending, 1992).

3.3 | Species distribution modelling

Model performance was high based on their AUC values, partial ROC values and other performance statistics (Table S4). All three mean AUC ratios were well above 0.5, indicating that the models ran better than random (Figure S3).

The relative contribution of the variables to the models differed between the taxa, though some similarity was seen (Figure S4). The variables that contributed most (80.8%) to the *I. coccifer* model were precipitation seasonality (61.5%), mean diurnal range (11.6%) and precipitation of the warmest quarter of the year (7.7%); most (90.7%) to the *I. ibarrai* model were annual mean temperature (72.9%), precipitation of the coldest quarter (13.6%) and isothermality (4.2%); and most (92.5%) to the *I. porteri* model were annual mean temperature (63.5%), isothermality (22.3%) and precipitation seasonality (6.7%). Based on the known geographic distributions of the species, very little geographic overestimation occurred in the *I. coccifer* and *I. ibarrai* models; whereas, what seems like a significant amount of geographic overestimation occurred in the *I. porteri* model (Figure 4, Figure S3). It should be noted that breaks occur in the predicted distribution for *I. coccifer* (Figure 4) at the head of the Grijalva Valley at/along the border of Mexico and Guatemala, and around Lake Xolotlán in Nicaragua. These breaks are most likely an artefact of the low number of samples from these regions used to create the model (Figure S2).

Binary maps indicating presence/absence of suitable habitat (Figure 4) revealed little to no distributional overlap between *I. coccifer* and *I. ibarrai* or *I. porteri*, but did reveal broad zones of overlap between *I. ibarrai* and *I. porteri*. Niche overlap tests showed significant similarity between the *I. ibarrai* and *I. porteri* niches ($D = 0.6688$), and very little similarity between *I. coccifer* and *I. porteri* or *I. ibarrai* niches ($D = 0.2850$ and 0.2214, respectively).

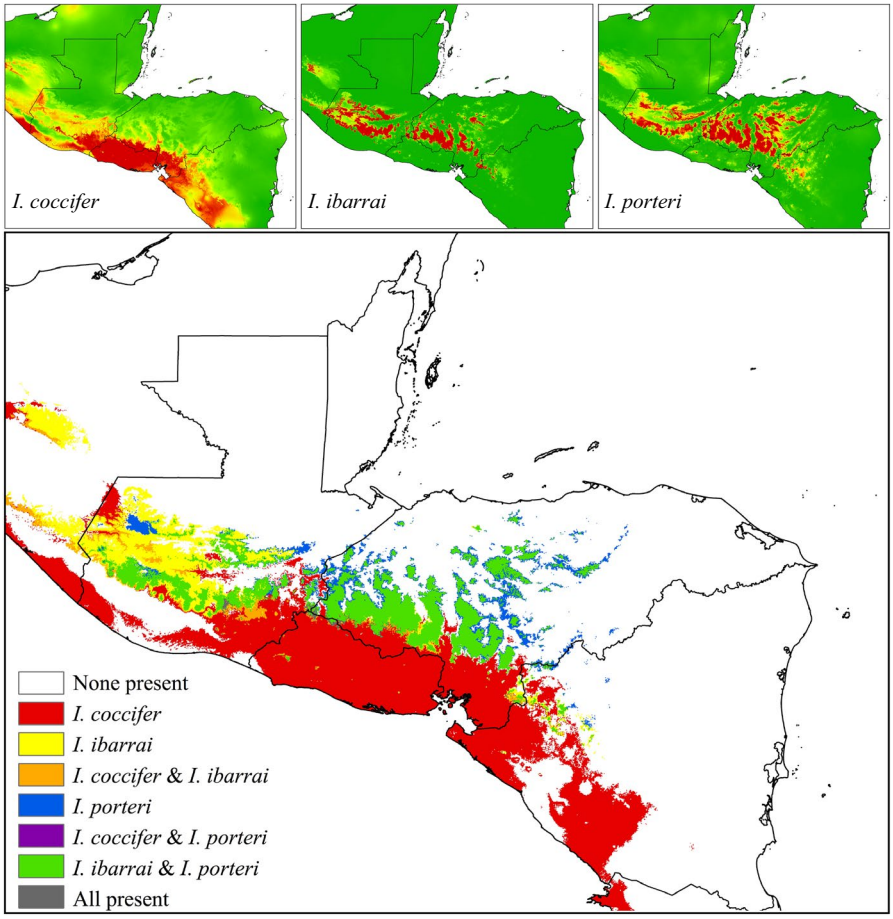

FIGURE 4 Species distribution models (top) for the three focal taxa showing the predicted fundamental niche for each. Warmer colours indicate areas of high probability of occurrence, whereas cooler colours indicate a lower probability of occurrence. Combined presence/absence map (bottom) showing potential zones of overlap between the three focal taxa generated from the species distribution models

4 | DISCUSSION

4.1 | Taxonomic implications

Sequence divergence data from 16S and COI revealed relatively low genetic differentiation for both intra- and interspecific ranges (Table 1). However, the phylogenetic analyses recovered three well-supported, reciprocally monophyletic groups within the *I. coccifer* complex, supporting the hypothesis of Mendelson et al. (2005), Mendelson et al. (2011) of three distinct, albeit closely related, species (*I. coccifer*, *I. ibarrai* and *I. porteri*) with sympatric and parapatric distributions in central and southern Honduras.

Based on our sampling, the extent to which populations of all three species are sympatrically and/or parapatrically distributed seems to be much more extensive than first predicted by Mendelson et al. (2005), Mendelson et al. (2011). While bufonids are often viewed as rampant hybridizers both in the laboratory and in nature where their distributions overlap (Blair, 1941, 1972; Masta, Sullivan, Lamb, & Routman, 2002), this has been recognized as a skewed generality that seems to primarily occur in *Anaxyrus spp.* and may also not be as extensive in nature as it has been believed to be (Malone & Fontenot, 2008; Vogel & Johnsons, 2008; Mendelson et al., 2011). The only known hybridization to occur with any species of *Incilius* is with *Anaxyrus spp*. within the southeastern United States (specifically *I. nebulifer* and *A. fowleri*); however, it has never been shown to occur, nor is there an indication that it occurs between any species of *Incilius* (Blair, 1972; Vogel & Johnson, 2008; Mendelson et al., 2011). There is no indication of hybridization occurring in the *I. coccifer* complex based on our data, as none of the specimens that were morphologically identified as their respective species have the mitochondrial DNA of a different species within the complex. Since our results are based on uniparentally inherited mitochondrial genes, identifying any instances of hybridization or introgression where species within the *I. coccifer* complex are found in sympatry/parapatry would benefit from the addition of other types of data (e.g., nuclear loci, fitness data) in order to be more adequately explored.

Our data also supports Mendelson et al.'s (2011) suggestion that *I. ibarrai* from Guatemala and Honduras may represent two species, with all Honduran samples forming a monophyletic "eastern" clade sister to a single sample (UTA A-52528) from Quiche, Guatemala. More extensive sampling from Guatemalan populations is required to further evaluate phylogeographic structure within the nominal taxon *I. ibarrai*. It is possible that a single sample of *I. coccifer* (TCWC 83998) from the Valle Central region of Costa Rica may represent a distinct species from the rest of *I. coccifer* from Honduras, Nicaragua and El Salvador. More extensive sampling from Costa Rican populations is required to further evaluate phylogeographic structure within the nominal taxon *I. coccifer*.

4.2 | Species delimitation

The GMYC analysis supports the recognition of two species within the *I. coccifer* complex (*I. ibarrai* and *I. coccifer* + *I. porteri*). General Mixed Yule-Coalescent identifies the transition between within-species coalescence and between-species coalescence, and uses that threshold to delimit species. It has been noted that GMYC has the potential to underestimate species number due to low interspecific differences (Talavera, Dinca, & Vila, 2013), which offers a potential explanation for the lack of delimitation between *I. coccifer* and *I. porteri*, two ecologically distinct entities under this analytical model. A concern with GMYC is that it utilizes only a single locus, in this case we used a combined mitochondrial data set (here considered a single locus because it is inherited as a single marker), which can be sorted faster in GMYC and has a higher mutation rate than nuclear loci. Additional data, including genome-wide SNPs that are analysed using coalescent-based species delimitation techniques (e.g., BFD*) (Leaché, Fujita, Minin, & Bouckaert, 2014) and demographic methods (Portik et al., 2017; Streicher et al., 2014) can provide deeper insight into the species boundaries and demographic histories of these species.

Though it was not one of our formal species delimitation methods, it is worth noting that our BOLD sequences for the focal taxa were clustered into three Barcoding Index Numbers (BINs; see Table S1). The BIN system in BOLD clusters barcode sequences that show high concordance with certain species, which can then in turn be used to specify species identifications (Ratnasingham & Hebert, 2013). Since our sequences are separated into three BINs in BOLD, this suggests that three species exist within this complex.

4.3 | Geographic distribution and demography

As mentioned before, our sampling suggests that the distribution and interaction among these three taxa are more complex than previously recognized by Mendelson et al. (2005), Mendelson et al. (2011), and apparently includes sympatric populations, in at least two pairs of species and possibly among all three. Samples representing haplotypes of both *I. ibarrai* and *I. porteri* were collected <5 km apart in northern Comayagua, suggesting sympatry or near sympatry. Haplotypes of *I. ibarrai* and *I. porteri* were found in direct sympatry in Guajiquiro, Depto. La Paz, where 13 *I. ibarrai* and two *I. porteri* were collected while active at night along an unpaved road through disturbed Mixed Cloud Forest from about 1,730 to 2,160 m elevation. Two samples from Isla del Tigre (Depto. Valle), a volcanic island off the southern Pacific coast, represented haplotypes of *I. coccifer* and *I. porteri* (Firneno et al., 2017). However, additional sampling from

Isla del Tigre was carried out in June 2014, including the collection of four specimens from the summit of the volcanic cone and a sea level lagoon (Table S1), yielded no additional haplotypes of *I. porteri*. The single sample of *I. porteri* collected from the Cordillera de Opalaca (Depto. Intibucá) represents a haplotype that is found extremely far west, outside of their range, but within the range of *I. ibarrai*. However, this was the only sample collected from this region; therefore, it is not known if any other haplogroups (e.g., *I. ibarrai*) exist there as well.

Very broad zones of potential range overlap were identified between *I. ibarrai* and *I. porteri* (Figure 4). Phylogenetic evidence has revealed areas of possible sympatry between these two taxa. Smaller zones of contact between *I. coccifer* and the other two taxa could indicate potential parapatric populations, as suggested by Mendelson et al. (2005). It is also not surprising that the niches for *I. ibarrai* and *I. porteri* are highly similar; both are upland and/or montane inhabitants, and that both are highly dissimilar in ecological association to *I. coccifer*, a lowland dry forest inhabitant. Though *I. ibarrai* and *I. porteri* share similar niches according to the models, these models do not take into consideration the historical contingency of these species—they originated in separate locations (indicated by the phylogenetic analyses and not being sister taxa), they are coming back together via secondary contact (indicated by the demographic analyses), and they are potentially preventing each other from invading one another's niche space in geographic position. Therefore, this provides further evidence that these two species are distinct.

Our demographic analyses indicate that these species/their populations are relatively stable or may be undergoing population expansion. Caution is warranted, however, for the interpretation of these analyses. These demographic analyses are based on two mitochondrial genes that may be subject to random variation and/or lineage selection. Several nuclear loci that were screened across the *I. coccifer* complex toads were invariant and were deemed uninformative for this study. As genomic resources have become more available, multilocus approaches have the potential to produce more accurate estimates of these population parameters, which will provide a more in-depth understanding of the existence and maintenance of species boundaries in the *I. coccifer* complex.

5 | CONCLUSION

Our results reinforce Mendelson et al.'s (2005) three species taxonomic composition for the *I. coccifer* complex, as well as revealing quite a bit about these three species that was previously unknown. Phylogenetically, these taxa exhibit three distinct lineages that have relatively shallow divergence, and whose distributions and interactions are much more complex than previously expected due to the extent to which they are found in sympatry. While our demographic analyses point towards stable or expanding populations, it would be useful in the future construct a robust genomic data set that includes nuclear markers to test demographic and biogeographic hypotheses for this species complex. Ultimately, the *I. coccifer* complex seems to be composed of three relatively young species that have recently diverged, whose populations have come into secondary contact where no hybridization is evident. Since hybridization is not evident even in the face of sympatric distributions, this supports that these three closely related species are distinct entities that have developed some sort of reproductive isolation barriers. Due to the complexity of the geographic/geologic history of this region of Central America, along with the biological complexity of the *I. coccifer* complex, it may be interesting to further investigate the possibility of gene flow within/between these three species, how the species boundaries within the complex are maintained, and the mechanisms of divergence that have played a role in the diversification of this species complex. It is our hope that this study provides an example of the utility of integrative taxonomy to delimit species boundaries in cryptic or highly morphologically conserved species, such as bufonids, as well as highlighting the importance of using integrative techniques for organisms that may be overlooked in conservation related efforts due to their apparent prevalence in many habitats.

ACKNOWLEDGEMENTS

We thank S. Lainez (Section of Protected Areas and Wildlife, Instituto Nacional de Conservación y Desarrollo Forestal, Áreas Protegidas y Vida Silvestre [ICF]) for issuing research and exportation permits in 2014; fieldwork was carried out under research permits issued by ICF (Resolución DE-MP-095-2014 and Dictamen Tecnico ICF DVS-112-2014). Fieldwork was also aided by Mancomunidad de Municipios del Parque Nacional Montaña de Celaque (MAPANCE), and particularly H. Vega; as well as Centro Zamorano de Biodiversidad, and particularly K. Lara, C. Funes, O. Komar and X. Moya. Fieldwork and all work with animal subjects was carried out under IACUC #04-1314. We would like to thank all those who aided in the fieldwork for this project including J. Sunyer, I. Luque-Montes, C. Cerrato, A. Hess, M. Itgen, M. Kenney, C. Krygeris, G. McCormick, K. Weinfurther, F. Pereira, L. Munguía and J. Vásquez. Sequencing of the 16S and COI was provided by the Smithsonian Institution Laboratory of Analytical Biology (SI-LAB) as part of the project "Barcoding the Herpetofauna of eastern Nuclear Central America"; we thank A. Driskell, D. Mulcahy and A. Ormos for support in the laboratory. We would finally like to thank the two anonymous reviewers for their insightful and detailed reviews of our manuscript.

ORCID

Thomas J. Firneno https://orcid.org/0000-0002-4975-2794
Josiah H. Townsend https://orcid.org/0000-0002-6232-4065

REFERENCES

Agapow, P., Bininda-Emonds, O., Crandall, K., Gittleman, J., Mace, G., Marshall, J., & Purvis, A. (2004). The impact of species concept on biodiversity studies. *The Quarterly Review of Biology*, *79*(2), 161–179. https://doi.org/10.1086/383542

Barley, A. J., White, J., Diesmos, A. C., & Brown, R. M. (2013). The challenge of species delimitation at the extremes: Diversification without the morphological change in Phillipine sun skins. *Evolution*, *67*(12), 3556–3572. https://doi.org/10.1111/evo.12219

Barve, N. (2008). *Tool for partial-ROC*. Lawrence, KS: Biodiversity Institute.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., …Das, I. (2007). Cryptic species as a window into diversity and conservation. *Trends in Ecology and Evolution*, *22*, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Bisby, F. A., & Coddington, J. (1995). Biodiversity from a taxonomic and evolutionary perspective. In V. H. Heywood, & R. T. Watson (Eds.), *Global biodiversity assessment* (pp. 27–56). Cambridge, UK: Cambridge University Press.

Blair, A. P. (1941). Variation, isolating mechanisms, and hybridization in certain toads. *Genetics*, *26*(4), 398–417.

Blair, W. F. (1972). *Evolution in the genus* Bufo. Austin, TX: University of Texas Press.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., … Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*(4), 1–6. https://doi.org/10.1371/journal.pcbi.1003537

Brown, D. M., Brenneman, R., Koepfli, K., Pollinger, J., Milá, B., Georgiadis, N., … Wayne, R. (2007). Extensive population genetic structure in the giraffe. *BMC Biology*, *5*, 1–13. https://doi.org/10.1186/1741-7007-5-57

Brown, J. (2014). SDMtoolbox: A python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution*, *5*(7), 694–700. https://doi.org/10.1111/2041-210X.12200

Camargo, A., Morando, M., Avila, L., & Site, J. W. (2012). Species delimitation with abc and other coalescent-based methods: A test of accuracy with simulations and an empirical example. *Evolution*, *66*(9), 2834–2849. https://doi.org/10.5061/dryad.4409k652

Cronquist, A. (1978). Once again, what is a species? In L. V. Knutson (Ed.), *Biosystematics in agriculture* (pp. 3–20). Montclair, NJ: Allenheld Osmin.

Darriba, D., Taboada, G., Doallo, R., & Posada, D. (2015). jModelTest2: More models, new heuristics and high-performance computing. *Nature Methods*, *9*(8), 6–9. https://doi.org/10.1038/nmeth.2109. jModelTest

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, *85*, 407–415.

de Queiroz, K. (2005a). Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences*, *102*, 6600–6607. https://doi.org/10.1073/pnas.0502030102

de Queiroz, K. (2005b). Different species problems and their resolution. *BioEssays*, *27*(12), 1262–1269. https://doi.org/10.1002/bies.20325

de Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, *56*(6), 879–886. https://doi.org/10.1080/10635150701701083

Excoffier, L., & Lischer, H. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Firneno, T. J. Jr, Luque-Montes, I., & Townsend, J. H. (2017). An enigmatic record of *Incilius porteri* (Anura: Bufonidae) from Isla del Tigre, Honduras. *Salamandra*, *53*, 160–162.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299. https://doi.org/10.1371/journal.pone.0013102

Fu, Y. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*(2), 915–925. https://doi.org/genetics.org//147/2/915

Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A., & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution*, *27*(9), 480–488. https://doi.org/10.1016/j.tree.2012.04.012

Grismer, L., Wood, P., Anuar, S., Muin, M. A., Quah, E., McGuire, J. A., … Hong Tai, P. (2013). Integrative taxonomy uncovers high level of cryptic species diversity in *Hemiphyllodactylus* Bleeker, 1860 (Squamata: Gekkonidae) and the description of a new species from Peninsular Malaysia. *Zoological Journal of the Linnean Society*, *169*(4), 849–880. https://doi.org/10.1111/zoj.12064

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*(5), 696–704. https://doi.org/10.1080/10635150390235520

Harpending, H. C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, *66*(4), 591–600.

Harpending, H. C., Batzer, M. A., Gurven, M., Jorde, L. B., Rogers, A. R., & Sherry, S. T. (1998). Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences*, *95*(4), 1961–1967. https://doi.org/10.1073/pnas.95.4.1961

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land area. *International Journal of Climatology*, *25*(15), 1965–1978. https://doi.org/10.1002/joc.1276

Huelsenbeck, J., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Leaché, A. D., Fujita, M. K., Minin, V. N., & Bouckaert, R. R. (2014). Species delimitation using genome-wide SNP data. *Systematic Biology*, *63*(4), 534–542. https://doi.org/10.1093/sysbio/syu018

Leigh, J., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Liu, C., Berry, P. M., Dawson, T. P., & Pearson, R. G. (2005). Selection thresholds of occurrence in the prediction of species distributions. *Ecography*, *28*, 385–393. https://doi.org/10.1111/j.0906-7590.2005.03957.x

Lobo, J. M., Jiménez-Valverde, A., & Real, R. (2008). AUC: A misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, *17*(2), 145–151. https://doi.org/10.1111/j.1466-8238.2007.00358.x

Luo, A., Ling, C., Ho, S., & Zhu, C. (2018). Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Systematic Biology*, *67*(5), 830–846. https://doi.org/10.1093/sysbio/syy011

Luque-Montes, I., Austin, J. D., Weinfurther, K. D., Wilson, L. D., Hofmann, E. P., & Townsend, J. H. (2018). An integrative assessment of the taxonomic status of putative hybrid leopard frogs (Anura: Ranidae) from the Chortís Highlands of Central America, with description of a new species. *Systematics and Biodiversity*, *16*(4), 340–356. https://doi.org/10.1080/14772000.2017.1415232

Malone, J. H., & Fontenot, B. E. (2008). Patterns of reproductive isolation in toads. *PLoS ONE*, *3*(12), e3900. https://doi.org/10.1371/journal.pone.0003900

Masta, S. E., Sullivan, B. K., Lamb, T., & Routman, E. J. (2002). Molecular systematics, hybridization, and phylogeography of the *Bufo americanus* complex in Eastern North America. *Molecular Phylogenetics and Evolution*, *24*, 302–314. https://doi.org/10.1016/S1055-7903(02)00216-6

Mayr, E. (1942). *Systematics and the origin of species*. New York, NY: Columbia University Press.

McCranie, J. R. (2009). *Amphibians and reptiles of Honduras. Listas Zoológicas Actualizadas UCR*. San Pedro, Costa Rica: Museo de Zoología UCR. Retrieved from http://museo.biologia.ucr.ac.cr/Listas/LZAPublicaciones.htm

McCranie, J. (2015). A checklist of the amphibians and reptiles of Honduras, with additions, comments, on taxonomy, some recent taxonomic decisions, and areas of further study needed. *Zootaxa*, *3931*(3), 352–386.

McCranie, J. R., & Castañeda, F. E. (2007). *Guia de Campo de los Anfibios de Honduras*. Salt Lake City, UT: Bibliomania!

McCranie, J. R., & Wilson, L. D. (2002). *The amphibians of Honduras*. Ithaca, NY: Society for the Study of Amphibians and Reptiles.

Meier, R., Tan, D., Ang, Y., Lim, G. S., & Ismail, M. (2010). From "cryptic species" to integrative taxonomy: An integrative process involving DNA sequences, morphology, and behavior leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera). *Zooloiga Scripta*, *39*(1), 51–61. https://doi.org/10.1111/j.1463-6409.2009.00408.x

Mendelson, J. R. III, Williams, B. L., Sheil, C. A., & Mulcahy, D. G. (2005). Systematics of the *Bufo coccifer* complex (Anura: Bufonidae) of Mesoamerica. *Scientific Papers: Natural History Museum the University of Kansas*, *38*, 1–27. https://doi.org/10.1007/s11084-011-9248-z

Mendelson, J. R. III, Mulcahy, D. G., Williams, T. S., & Sites, J. W. (2011). A phylogeny and evolutionary natural history of Mesoamerican toads (Anura: Bufonidae: Incilius) based on morphology, life history, and molecular data. *Zootaxa*, *34*(3138), 1–34.

Padial, J. M., & de la Riva, I. (2006). Taxonomic inflation and the stability of species lists: The perils of ostrich's behavior. *Systematic Biology*, *55*(5), 859–867.

Padial, J. M., & de la Riva, I. (2009). Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society*, *155*, 97–122.

Padial, J. M., Miralles, A., de la Riva, I., & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, *7*, 1–14. https://doi.org/10.1186/1742-9994-7-16

Palumbi, S. R., Martin, A. P., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR*. Honolulu, HI: Special Publication of the Department of Zoology, University of Hawaii.

Peterson, A. T., Papeş, M., & Soberón, J. (2008). Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological Modelling*, *213*(1), 63–72. https://doi.org/10.1016/j.ecolmodel.2007.11.008

Phillips, S., Dudîk, M., & Schapire, R. (2004). A maximum entropy approach to species distribution modeling. In *Proceedings of the twenty-first international conference on machine learning – ICML -04, 83*. https://doi.org/10.1145/1015330.1015412

Portik, D. M., Leaché, A. D., Rivera, D., Barej, M. F., Burger, M., Hirschfeld, M., … Fujita, M. K. (2017). Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Molecular Ecology*, *26*(19), 5245–5263. https://doi.org/10.1111/mec.14266

Ramos-Onsins, S. E., & Rozas, J. (2006). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, *23*(8), 1642. http://www.ncbi.nlm.nih.gov/pubmed/12446801

Ratnasingham, S., & Hebert, P. D. N. (2013). A DNA-based registry for all animal species: The barcode index number (BIN) system. *PLoS ONE*, *8*(8), e66213. https://doi.org/10.1371/journal/pone.0066213

Rödder, D., & Engler, J. (2011). Quantitative metrics of overlaps in Grinnellian niches: Advances and possible drawbacks. *Global Ecology and Biogeography*, *20*(6), 915–927.

Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, *9*(3), 552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H., (2010). Integrative taxonomy: A multisource approach to exploring biodiversity. *Annual Review of Entomology*, *55*(1), 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogeneties. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Streicher, J. W., Devitt, T. J., Golberg, C. S., Malone, J. H., Blackmon, H., & Fujita, M. K. (2014). Diversification and asymmetrical gene flow across time and space: Lineage sorting and hybridization in polytypic barking frogs. *Molecular Ecology*, *23*(13), 3273–3291. https://doi.org/10.1111/mec.12814

Talavera, G., Dinca, V., & Vila, R. (2013). Factors affecting species delimitation with the GMYC model: Insights from a butterfly survey. *Methods in Ecology and Evolution*, *4*(12), 1101–1110. https://doi.org/10.1111/2041-210X.12107

Thompson, J., Higgins, D., & Gibson, T. (1994). ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Van Valen, L. (1976). Ecological species, multispecies, and oaks. *Taxon*, *25*(2), 233–239.

Vogel, L. S., & Johnson, S. G. (2008). Estimation of hybridization and introgression frequency in toads (genus: *Bufo*) using DNA sequence variation at mitochondrial and nuclear loci. *Journal of Herpetology*, *42*(1), 16–75. https://www.jstor.org/stable/40060483

Warren, D., Glor, R., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution*, *62*(11), 2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Wiley, E. O. (1978). The evolutionary species concept reconsidered. *Systematic Zoology*, *27*(1), 17–26. https://doi.org/10.2307/2412809

Zapata, F., & Jiménez, I. (2012). Species delimitation: Inferring gaps in morphology across geography. *Systematic Biology*, *61*(2), 179–194. https://doi.org/10.1093/sysbio/syr084

Zhang, M., Rao, D., Yang, J., Yu, G., & Wilkinson, J. A. (2010). Molecular phylogeography and population structure of a mid-elevation montane frog *Leptobrachium ailaonicum* in a fragmented habitat of southwest China. *Molecular Phylogenetics and Evolution*, *54*(1), 47–58. https://doi.org/10.1016/j.ympev.2009.10.019

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Firneno TJ Jr., Townsend JH. Evaluation of species boundaries in sympatric and parapatric populations of Mesoamerican toads. *Zool Scr*. 2019;00:1–12. https://doi.org/10.1111/zsc.12354