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An enigmatic record of *Incilius porteri* (Anura: Bufonidae) from Isla del Tigre, Honduras

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Determining what a species is and defining species boundaries are core questions in the field of taxonomy and evolutionary biology. They are also the focus of major controversies in those fields. Practitioners in the growing field of "integrative taxonomy" have been striving to utilize more than one single line of evidence (e.g., morphology, molecular, ecological, or behavioural) to describe a species (Hey et al. 2003, Sites & Marshall 2004, Wiens 2007, Padial & De la Riva 2009, Padial et al. 2009). Padial et al. (2009) suggested rejection of fixed thresholds of genetic divergence as a means of species delimitation (such as the 3% threshold suggested by Fouquet et al. [2007]), favouring use of divergence distance as heuristic criteria (e.g., Padial et al. 2009, Lim et al. 2012, Paez-Moscoso & Guayasamin 2012, Köhler et al. 2014). One challenge facing many studies of phylogenetic systematics is the necessity of relying on small sample sizes in many understudied groups. Along with singular lines of evidence, small sample sizes can often impair or bias taxonomic resolution, particularly when it comes to identification of parapatric or sympatric populations as a component of understanding of the speciation process.

Incilius porteri is a medium-sized toad (max. snout–vent length [SVL] 60 mm in males and 76 mm in females) known from 1,584–2,100 m a.s.l. in the departments of Comayagua, Francisco Morazán, and La Paz, in south-central Honduras (Mendelson et al. 2005). This species is closely related to, and often confused with, *I. coccifer* (max. SVL 62 mm in males and 82 mm in females, and found from sea level to 1,435 m; Mendelson et al. 2005, Mendelson et al. 2011), with some authorities recognizing only *I. coccifer* as a valid taxon (McCranie & Wilson 2002, McCranie & Castañeda 2007, McCranie 2009). The taxonomic revision by Mendelson et al. (2005), later supported by the multilocus phylogenetic analysis by Mendelson et al. (2011), delimited *I. porteri* to accommodate highland populations in south-central Honduras and restricted *I. coccifer* to populations inhabiting xeric lowlands from Mexico to Costa Rica. Within this distribution, *I. coccifer* has been documented previously to occur on Isla del Tigre, a volcanic island in the Golfo de Fonseca off the south coast of Honduras (Lovich et al. 2010). The topography of Isla del Tigre consists of the cone of a single extinct volcano, rising to 783 m in altitude, and the associated alluvial plain.

On 16 August 2010, two immature female *I. coccifer*-like toads were collected by IRL and JHT during a brief sampling trip to Isla del Tigre. Both were found during the day after a rain shower while they were active along the trail leading from the community of Caracol to the communication towers at the summit, one (USNM 578693) at about 150 m a.s.l. and the second (USNM 578694) at about 450 m. Tissue samples from USNM 578693 and 578694 were amplified for 16S and COI using primers 16Sar-L and 16Sbr-H (Palumbi et al. 1991), and LCO-1490 and HCO-2198 (Folmer et al. 1994), respectively. The samples were sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit (ABI) on an ABI 3730xl DNA analyzer at the Smithsonian Institute Laboratory of Analytical Biology (SI-LAB). Our samples were combined with the comparative sequences used by Mendelson et al. (2011) and available from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), representing all described species of the *I. coccifer* complex (Table 1). Sequences were aligned using ClustalW (Thompson et al. 1994) within the program package MEGA 6.06 (Tamura et al. 2013), using default parameters. We partitioned the dataset by gene (16S, RNA coding) and codon position (1st, 2nd, 3rd) for COI (protein-coding gene) to account for the potential for substitution saturation at the third codon position. Best-fit models of nucleotide substitution were

Table 1. Samples used in phylogenetic analyses; CR – Costa Rica; ES – El Salvador; GT – Guatemala; HN – Honduras; PA – Panama.

			Genbank Accession #	
Taxon	Locality	Voucher	16S	COI
I. coccifer	CR: San José: Montañas Jamaic	TCWC83993	AY929302	JN867964
I. coccifer	ES: Morazán	KU 290030	AY927856	JN867963
I. coccifer	HN: Valle: Isla del Tigre	USNM 578693	KR736044	KR736042
I. ibarrai	HN: Ocotepeque	UTA-A-53662	AY927854	JN867970
I. ibarrai	GT: Quiché	UTA-A-52528	AY927855	JN867971
I. porteri	HN: Francisco Morazán: Cerro Uyuca	UF-JHT2249	HM563882	JN867987
I. porteri	HN: Valle: Isla del Tigre	USNM 578694	KR736045	KR736043
I. signifer	PA: Coclé: El Copé	UTA-A-JRM4968	HM563883	JN867988

estimated with jModeltest 2.0 (Darriba et al. 2012); the number of substitution schemes was set to eight to limit the number of possible models to 88. Uncorrected (p-distance) pairwise sequence divergence was calculated for all samples and each gene to provide an estimate of intra-versus interspecific variation. Sequence divergence estimation was performed in MEGA 6.06 (Tamura et al. 2013). Bayesian Inference (BI) was performed using MrBayes 3.2.2 (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 burn-in.

Best-fit nucleotide substitution models varied by gene and codon positions, supporting the use of a gene- and codon-based partitioning strategy (Table 2). Nucleotide variability was 3.45% for the 493 bp of 16S and 7.62% for the 669 bp of COI. Interspecific and intraspecific divergence distances slightly overlapped, with interspecific divergence ranging from 0.8–1.9% for 16S and 0.7–4.2% for COI, and intraspecific divergence ranging from 0.0–1.0% for 16S and 0.0–1.8% for COI. Our phylogenetic analysis indicates that

Table 2. Models of nucleotide substitution chosen for phylogenetic analysis of taxa using Akaike Information Criterion (AIC) and Bayesian Information Criterion (lnL) values.

Partition	Model	AIC	lnL
16S	HKY+I	1633.5023	-797.751
COI (1st)	K80	669.8427	-319.921
COI (2nd)	F81	594.5685	-280.284
COI (3rd)	TIM2+G	1121.5536	-539.776

the sample from lower altitude (150 m; USNM 578693) represents the widespread lowland form *I. coccifer*, whereas the higher-altitude sample (450 m; USNM 578694) clusters with the single available sequence of *I. porteri* from the vicinity of the type locality (Fig. 1).

The presence of haplotypes representing both *I. porteri* and *I. coccifer* on Isla del Tigre is the first evidence of possible sympatry, or near sympatry, between these two putative taxa in Honduras. This is a problematic case of sympatry, considering that *I. porteri* is a highland species previously found only on the mainland at higher altitudes, and would
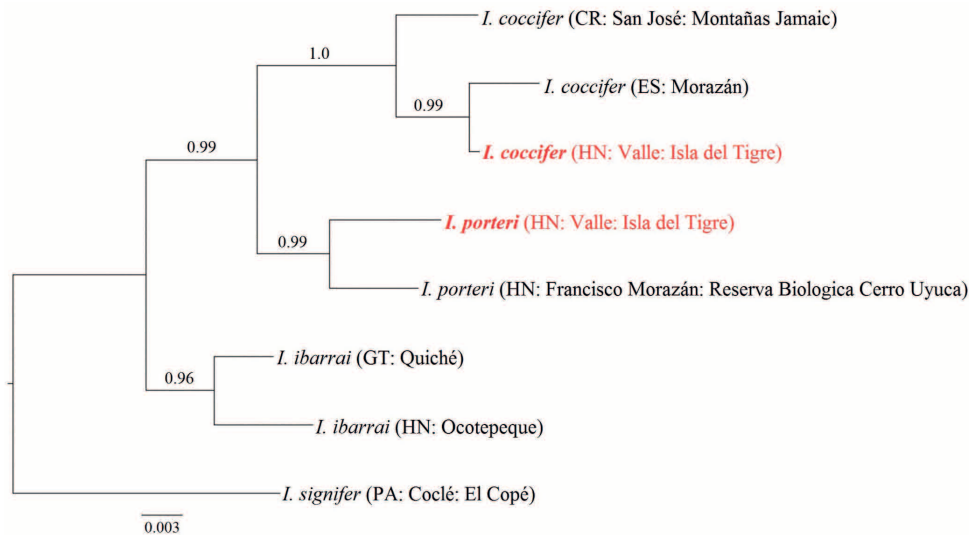

Figure 1. Bayesian phylogram showing the phylogenetic relationships of the *I. coccifer* and *I. porteri* samples from Isla del Tigre (highlighted in red). Locality information in parenthesis. Bayesian posterior probabilities are shown when > 0.75.

appear unlikely to inhabit an offshore volcanic island isolated from other populations by a relatively wide lowland plain. The phylogeny that we have generated from our data has recovered very well supported clades for all three species (*I. coccifer*, *I. ibarrai*, and *I. porteri*), albeit with all three species exhibiting relatively low divergence distances. While these phylogenetic results continue to support the taxonomy proposed by Mendelson et al. (2005), the addition of this single sample of *I. porteri* from Isla del Tigre suggests that the distribution and evolution of lineages in the *I. coccifer* complex is more complicated than initially suggested. Investigation based on comprehensive geographic sampling, larger sample sizes per locality, multiple mitochondrial and nuclear loci, and additional lines of evidence are needed to elucidate the evolutionary history of *I. coccifer*, *I. ibarrai*, and *I. porteri* in their region of parapatry in Honduras.

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