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PENSOFT

Range expansion of the Gulf Coast Toad, *Incilius valliceps* (Wiegmann, 1833) (Anura, Bufonidae), from Costa Rica

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Abstract
We report the range expansion of the Gulf Coast Toad, *Incilius valliceps* (Wiegmann, 1833), from tropical moist forests to tropical wet forests and towards southeastern Costa Rica. We evaluated erroneous reports of this species in databases on the web. Also, we discussed that movements to areas with wet conditions for a species known from humid and xeric habitats can be explained by the trends in deforestation and increments of the dry conditions during recent decades in the country.

Keywords
Central America, DNA barcoding, herpetofauna, Neotropics, species distribution.

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Introduction

The genus *Incilius* (Cope, 1863), often referred to as the Mesoamerican Toads, currently contains 35 species which range from the southern edge of the United States down to the northern edge of Colombia (Frost et al. 2009). *Incilius valliceps* (Wiegmann, 1833) belongs to the *Incilius valliceps* group, which is characterized by having cranial crests, medium to small parotoid glands, presence of an omosternum, and a fully developed auditory apparatus (Savage 2002; Mendelson et al. 2011). This species presents one of the largest distributional ranges among toads of this genus, extending from central Veracruz in Mexico to the northern part of Costa Rica (Mendelson et al. 2011). Even though *I. valliceps* mainly inhabits humid lowland forests, this species also seems to adapt very well to open and human-altered areas (Leenders 2016).

In Costa Rica, *I. valliceps* was previously known only from the most north-central part of the country, close to the San Juan River and the frontier with Nicaragua (Leenders 2016). Related species that inhabit Costa Rica are *I. melanochlorus* (Cope, 1877), *I. leutkenii* (Boulenger, 1891), and *I. aucoinae* (O'Neill & Mendelson, 2004). Among these species, the ones that can occur sympatrically with *I. valliceps* are *I. melanochlorus,* in the Atlantic versant, and *I. luetkenii*, across northern Costa Rica (Savage 2002; Leenders 2016). Herein, we present new records of *I. valliceps* from Costa Rica, expanding the current southern limit of distribution of this species.

Methods

We carried out the fieldwork in La Tirimbina Biological Reserve and nearby sites located in Sarapiquí, Heredia, Costa Rica. We collected the specimens during standard visual encounter surveys (Crump and Scott 1994). We euthanized the toads with lidocaine and further collected and preserved the liver tissue in 96% ethanol. The specimens were fixed with 10% buffered formaldehyde and stored in 70% ethanol in the Museo de Zoología de la Universidad de Costa Rica (MZUCR). The collection was conducted under the permit R-SINAC-PNI-ACAHN-27-2019.

We produced a distribution map (Fig. 1) for *Incilius valliceps* based in the herpetological records of the MZUCR (Table 1). We followed Holdridge (1967) and considered three life zones: tropical dry forests (5–6 dry months per year), tropical moist forests (1–4 dry months per year) and tropical wet forests (0 dry months per year). This classification holds true for Costa Rican lowlands (i.e. 0–500 m a.s.l.) (Savage 2002). We obtained climatic and Holdridge life zones shapefiles from the Atlas Digital de Costa Rica project (Ortíz-Malavassi 2015).

Molecular analysis. We determined partial sequences of the large subunit ribosomal RNA (16S) mitochondrial gene for one specimen of *I. melanochlorus* and one specimen of *I. valliceps*, both from La Tirimbina Biological Reserve and nearby sites located in Sarapiquí, Heredia, Costa Rica (Fig. 1). We compared the sequences obtained herein with those available in GenBank for *I. valliceps, I. aucoinae* and *I. melanochlorus.* The protocols for DNA extraction, amplification, sequencing, and editing follow those of Arias et al. (2018). We deposited the obtained sequences in GenBank under the accession numbers MT180485 (UCR 23196) and MT180486 (UCR 23197). Sequence alignments used the MAFFT software (Katoh et al. 2017) under the "auto" strategy and default parameters and were trimmed to the point where a majority of the taxa had sequence data. We used the maximum likelihood analysis to infer the phylogenetic relationships using RAxML–HPC v8 (Stamatakis 2014) with the GTR + G model of nucleotide substitution and the *-f a* option, which searches for the best-scoring tree and performs a rapid bootstraps analysis (i.e. 1000 bootstraps) to estimate node support by resampling the

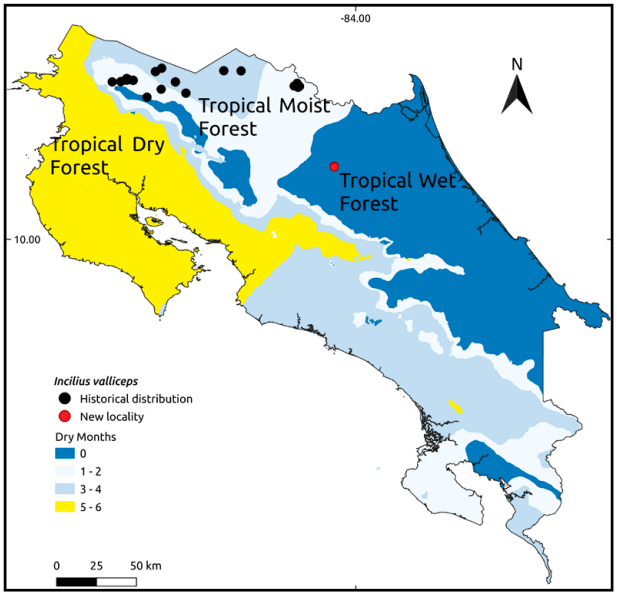

Figure 1. Distribution map of the locality records of *Incilius valliceps* in Costa Rica. Dry months layer from the life zones shapefile shows that the new locality (red dot) is in a wetter area than the previous records (black dots).

Table 1. Records of *Incilius valliceps* from the Museo de Zoología de la Universidad de Costa Rica.

Voucher	Latitude	Longitude	Altitude (m a.s.l.)	Distribution status
UCR9774	10.9583	−084.7500	100	Historical distribution
UCR9775	10.9583	−084.7500	100	Historical distribution
UCR9924	10.8944	−085.0250	54	Historical distribution
UCR9925	10.8944	−085.0250	54	Historical distribution
UCR9926	10.8944	−085.0250	54	Historical distribution
UCR9927	10.8944	−085.0250	54	Historical distribution
UCR9933	10.9533	−085.1383	46	Historical distribution
UCR9934	10.9533	−085.1383	46	Historical distribution
UCR9935	10.9533	−085.1383	46	Historical distribution
UCR9936	10.9533	−085.1383	46	Historical distribution
UCR9937	10.9533	−085.1383	46	Historical distribution
UCR9938	10.9533	−085.1383	46	Historical distribution
UCR10257	10.8306	−084.9653	120	Historical distribution
UCR10258	10.8306	−084.9653	120	Historical distribution
UCR10445	10.9533	−085.1383	46	Historical distribution
UCR10446	10.9533	−085.1383	46	Historical distribution
UCR10447	10.9533	−085.1383	46	Historical distribution
UCR10539	10.9035	−085.2649	450	Historical distribution
UCR10540	10.9035	−085.2649	450	Historical distribution
UCR10541	10.9035	−085.2649	450	Historical distribution
UCR10542	10.9035	−085.2649	450	Historical distribution
UCR10685	10.9722	−085.1028	30	Historical distribution
UCR12776	10.8694	−084.3194	192	Historical distribution
UCR12777	10.8861	−084.3333	70	Historical distribution
UCR12778	10.8861	−084.3333	70	Historical distribution
UCR12779	10.8639	−084.3278	182	Historical distribution
UCR12780	10.8639	−084.3278	182	Historical distribution
UCR12781	10.8639	−084.3278	182	Historical distribution
UCR15611	10.9139	−085.3017	344	Historical distribution
UCR15624	10.9139	−085.3017	344	Historical distribution
UCR15660	10.8961	−085.3353	491	Historical distribution
UCR15661	10.9039	−085.3031	344	Historical distribution
UCR15722	10.9139	−085.3017	491	Historical distribution
UCR17729	10.9078	−085.2922	343	Historical distribution
UCR17733	10.9078	−085.2922	343	Historical distribution
UCR17740	10.9078	−085.2922	343	Historical distribution
UCR17753	10.8946	−085.3842	500	Historical distribution
UCR17754	10.9078	−085.2922	343	Historical distribution
UCR19620	10.8964	−085.3356	400	Historical distribution
UCR19805	10.8711	−084.3501	90	Historical distribution
UCR20588	10.9572	−084.6518	51	Historical distribution
UCR20756	10.8770	−084.3288	100	Historical distribution
UCR20789	10.8078	−085.1865	400	Historical distribution
UCR22634	10.8528	−085.1059	179	Historical distribution
UCR23197	10.4155	−084.1226	142	New locality
UCR23198	10.4155	−084.1226	146	New locality
UCR23259	10.4113	−084.1219	150	New locality

data. All phylogenetic analyses were performed on the CIPRES portal (Miller et al. 2010).

Results

***Incilius valliceps* (Wiegmann, 1833)**
Figures 2, 3A, C

New records. COSTA RICA • 1 ♂; Heredia Province, Cantón de Sarapiquí, La Virgen, private property next to La Tirimbina Biological Reserve; 10.4155°N, 084.1225°W; 145 m a.s.l.; 01 Feb. 2019; Jeremy Klank, Gerardo Chaves leg.; GenBank: MT180486; UCR 23197 • 1 ♂; Heredia Province, Cantón de Sarapiquí, La Virgen, private property next to La Tirimbina Biological Reserve; 10.4155°N, 084.1225°W; 145 m a.s.l.; 02 Feb. 2019; Jeremy Klank, Gerardo Chaves leg.; UCR 23198 • 1 juvenile; Heredia Province, Cantón de Sarapiquí, La Virgen, La Tirimbina Biological Reserve; 10.4113°N, 084.1219°W; 150 m a.s.l.; 05 Oct. 2019; Jeremy Klank, Gerardo Chaves leg.; UCR 23259.

Figure 2. *Incilius valliceps* (UCR23198). Adult male calling at the breeding site.

Remarks. Both specimens collected in February 2019 (UCR 23197, UCR 23198) were part of an aggregation consisting of four more males of *I. valliceps* that were calling at night on low moist grass surrounding a small pond destined for growing Tilapia Fish (*Tilapia* sp.) and

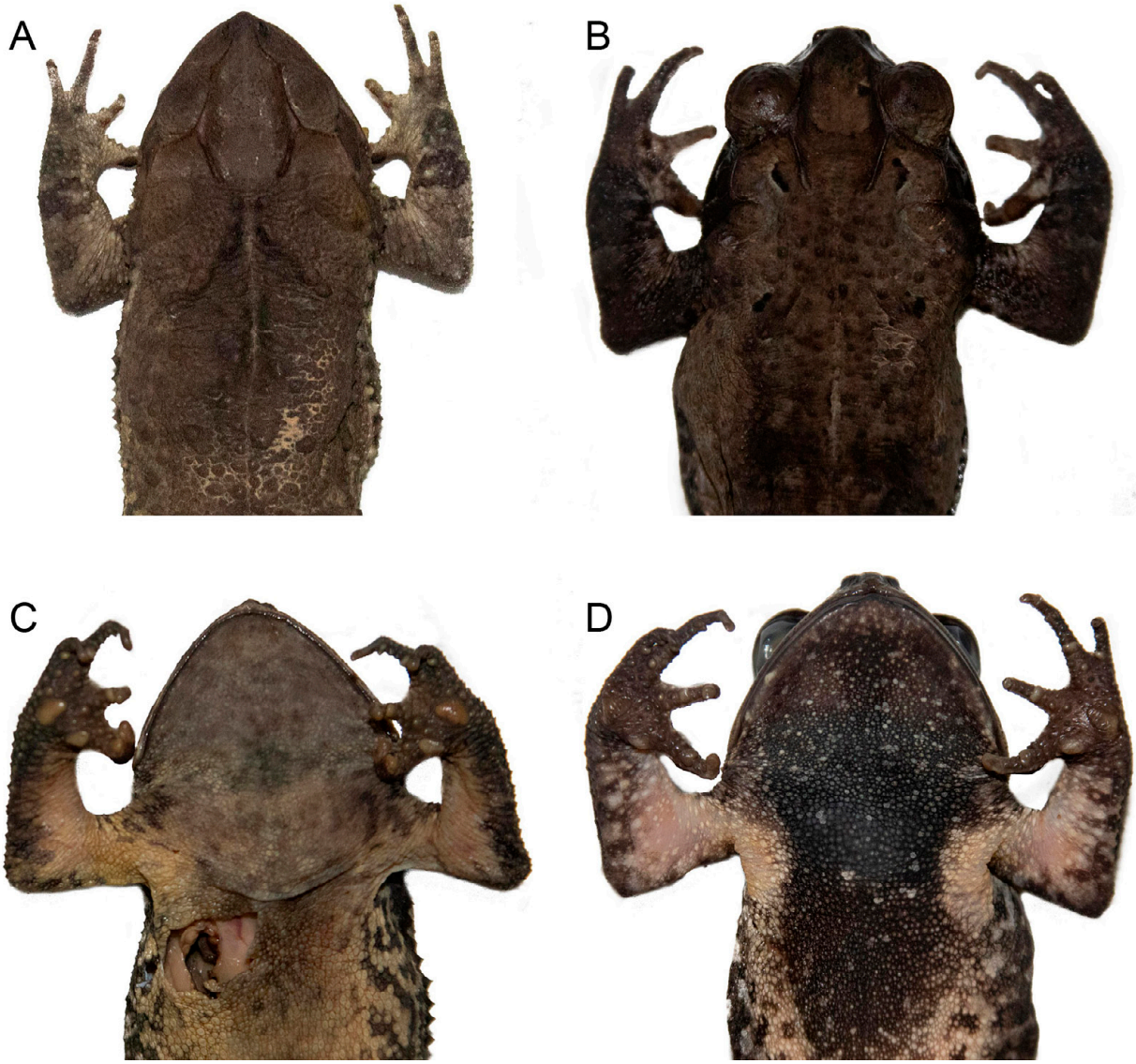

Figure 3. **A, C.** *Incilius valliceps* (UCR 23198). **A.** Dorsal view **C**. Ventral view. **B, D.** *Incilius melanochlorus* (UCR 23196). **B.** Dorsal view **D.** Ventral view.

a house located in the border of La Tirimbina river. The other specimen collected in October 2019 (UCR 23259) was found in a human-kept, open, grassy area, which is bordered by secondary forest.

Identification. We followed Savage (2002) for morphological identification. The presence of well-defined cranial crests, a dark lateral stripe with pointy light warts, and oblong to semi-triangular shaped parotoid glands that are similar in area to its upper eyelid differentiate *I. valliceps* (Fig. 3A) from the rest of toad species in Costa Rica. It is most easily distinguished from *I. melanochlorus* by its creamish gular area (Fig. 3C), which is usually black or dark brown in the latter (Fig. 3D). The parotoid glands of *I. melanochlorus* (Fig. 3B) tend to be smaller than their upper eyelid area and more oval-shaped than those of *I. valliceps.* Also, *I. valliceps* presents a subacuminate snout (Fig. 3A, C) while *I. melanochlorus* presents a truncated snout (Fig. 3B, C).

The phylogenetic analysis data matrix includes 38 sequences, with a total sequence length of 568 bp, including gaps. The phylogeny obtained (Fig. 4) resulted in three well-supported clades, corresponding to *I. aucoinae*, *I. melanochlorus*, and *I. valliceps.* The morphological identification of the two specimens of *Incilius* from La Tirimbina Biological Reserve was supported by the molecular analysis, corroborating the presence of *I. melanochlorus* and *I. valliceps* in the site.

Discussion

The new records of *Incilius valliceps* expand the distribution of the species to 54 km to the south from the last known record of the MZUCR. In the Global Biodiversity Information Facility (GBIF 2020), we found 14 specimens of *I. valliceps* reported from Colombia and two from Panama, collected between the decades of 1940 and 1960. The specimens from Colombia are deposited in the Museum of Comparative Zoology of Harvard (MCZ) and the ones from Panama are in the Royal Belgian Institute of Natural Sciences and in the Natural History Museum of Los Angeles County. In the revision of the Costa Rican herpetofauna, Savage (2002) did not include those specimens in the distribution of *I. valliceps*, which possibly implies that those specimens have an identification error. Specimens from Colombia likely correspond to *I. coniferus* (Cope, 1862), given that is the only species of this genus reported from there (Acosta-Galvis 2019). Nevertheless, inspection of the specimens housed in the MCZ is still needed to confirm their identification.

VertNet database (VertNet 2020) reported a specimen of *I. valliceps* deposited in the Cleveland Museum of Natural History (CMNH 115293) that was collected in 1986 in the road between Guápiles and Limón in the Caribbean versant of Costa Rica. This specimen was collected at least 100 km to the south from our observations. We compared photos of the specimen (CMNH 115293) with other Costa Rican *Incilius* species and concluded that it belongs to *I. coniferus* because it presents a similar length between the first and second fingers, and has an extensive membrane between the toes, morphological features relative to its arboricole habits (Savage 2002).

The new locality reported in this study for *I. valliceps*, a tropical wet forest without a dry season, is a new life zone for this species, which usually occurred in

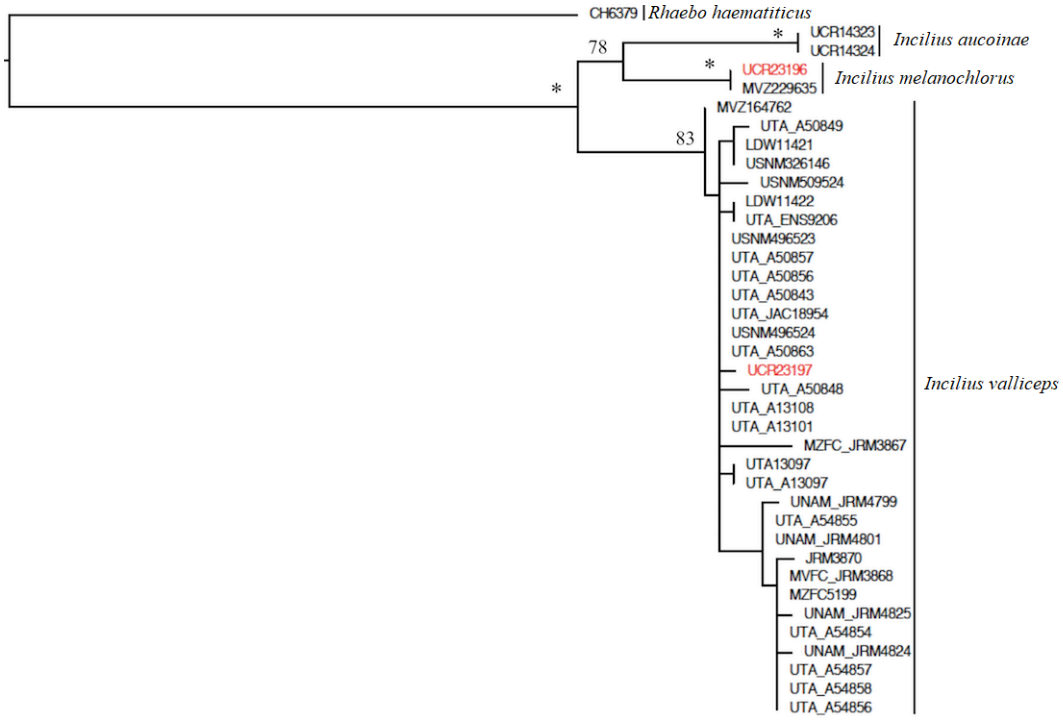

Figure 4. Maximum likelihood phylogram showing the the phylogenetic placement of the two specimens from La Tirimbina Biological Reserve within the three *Incilius* species analyzed: *I. aucoinae*, *I. melanochlorus*, and *I. valliceps*. Values above branch represent bootstraps proportions. The scale bar refers to the estimated substitution per site. Asterisks represent support >95.

comparative driest life zones in the rest of Mesoamerica (Khatun et al. 2013). Such cases have been reported for two other Costa Rican species previously found in arid zones of the Pacific versant, that were recently recorded in the wet forests of the Caribbean versant: *Leptodactylus poecilochilus* (Cope, 1862) (Crawford et al. 2003) and *Engystomops pustulosus* (Cope, 1864) (Weigt et al. 2005). Also in several other groups of amphibians that have expanded their distribution from lower to higher altitude zones (Pounds et al. 1997; Pounds et al.1999; Acosta-Chaves et al. 2015; Acosta-Chaves et al. 2019). We believe that the deforestation and global drier conditions during the last decades (Sanchez-Azofeifa et al. 2001; Pounds et al. 2006) forced the movement of xeric species to current altered wet forests.

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Authors' Contributions

GC and JK collected the specimens and did the fieldwork. JK took the photographs, GC made the maps. EA did the molecular analysis. All three authors wrote the manuscript. This work is a contribution from Museo de Zoología de la Universidad de Costa Rica.

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