

A new species of *Craugastor* (Anura: Craugastoridae) from the montane rainforest of the Cordillera de Talamanca, Costa Rica

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Abstract

A new species of *Craugastor* (Anura: Craugastoridae) from the montane rainforest of the Cordillera de Talamanca, Costa Rica. A new dirt frog of the *Craugastor podiciferus* Species Group is described from Costa Rica; it is restricted to elevations between 2330 and 2700 m a.s.l. in the montane rainforest of the Cordillera de Talamanca. Analysis of DNA sequences of the mitochondrial 16S rRNA (16S) and cytochrome oxidase I (COI) genes reveals a distinct lineage within the *C. podiciferus* Species Group. Additional morphological and morphometric analyses support the distinctiveness of this lineage that is described as a new species herein. The species is distinguished from other members of the *C. podiciferus* Species Group by its unique coloration: a violet-brown to blackish brown venter with white pigment forming blotches, and dark brown palmar surfaces with prominent white folds between subarticular tubercles in the adults. The genetic divergence of the species from other members of the *C. podiciferus* Species Group is significant (higher than 9.2% in 16S and 13.3% in COI). Although not closely related, it resembles *C. podiciferus* morphologically, a species that also inhabits montane rainforest. The discovery of this new species highlights the importance of montane rainforest as a center of species richness and endemism.

Keywords: Brachycephaloidea, Central America, *Craugastor podiciferus* Species Group, Panama, Terrarana.

Resumen

Una especie nueva de *Craugastor* (Anura: Craugastoridae) del bosque montano lluvioso en la Cordillera de Talamanca, Costa Rica. Se describe una especie nueva para Costa Rica de rana de hojarasca perteneciente al grupo de especies *Craugastor podiciferus*, restringida a elevaciones entre 2330–2700 m s.n.m. en el bosque montano lluvioso de la Cordillera de Talamanca. Análisis de las secuencias del ADN de los genes mitocondriales 16S ARNr (16S) y citocromo oxidasa I (COI) reveló un linaje distinto dentro del grupo de especies *C. podiciferus*. Los análisis complementarios

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de morfología y morfometría apoyaron la diferenciación de este linaje, el cual describimos aquí como una especie nueva. Esta especie se distingue de los miembros del grupo de especies *C. podiciferus* por su coloración única: vientre violeta-marrón a marrón negruzco con pigmento blanco formando manchas, la superficie palmar en adultos es marrón oscuro con pliegues blancos prominentes entre los tubérculos subarticulares. Genéticamente esta nueva especie es significativamente divergente de los demás miembros del grupo de especies *C. podiciferus* (mayores a 9.3% en el 16S y 13.3% en COI). Aunque no están estrechamente relacionadas, la nueva especie es morfológicamente similar a *C. podiciferus*, especie que también habita en el bosque montano lluvioso. El descubrimiento de esta nueva especie resalta la importancia del bosque montano lluvioso como un centro de riqueza de especies y endemismos.

Palabras clave: América Central, Brachycephaloidea, grupo de especies *Craugastor podiciferus*, Panamá, Terrarana.

Resumo

Uma nova espécie de *Craugastor* (Anura: Craugastoridae) do bosque montano chuvoso da Cordilheira de Talamanca, Costa Rica. Descrevemos aqui uma nova espécie do grupo de *Craugastor podiciferus* para a Costa Rica, restrita a altitudes entre 2330–2700 m acima do nível do mar no bosque montano chuvoso da Cordilheira de Talamanca. Análises das sequências de DNA dos genes mitocondriais 16S ARNr (16S) e da citocromo oxidase I (COI) revelaram uma linhagem distinta dentro do grupo de espécies de *C. podiciferus*. Análises morfológicas e morfométricas complementares apoiaram a diferenciação desta linhagem, que descrevemos aqui como uma espécie nova. Essa espécie distingue-se dos membros do grupo de espécies de *C. podiciferus* por sua coloração única: ventre marrom-violeta a marrom enegrecido com pigmento branco formando manchas e superfície palmar nos adultos marrom escura com pregas brancas proeminentes entre os tubérculos sub-articulares. Geneticamente esta nova espécie é significativamente divergente dos demais membros do grupo de espécies de *C. podiciferus* (maiores em 9.3% no 16S e 13.3% no COI). Ainda que não estejam estreitamente relacionadas, a nova espécie é morfológicamente similar a *C. podiciferus*, que também habita o bosque montano chuvoso. A descoberta dessa nova espécie ressalta a importância do bosque montano chuvoso como um centro de riqueza de espécies e endemismos.

Palavras-chave: América Central, Brachycephaloidea, grupo de espécies de *Craugastor podiciferus*, Panamá, Terrarana.

Introduction

The highlands of isthmian Central America are characterized by a high level of species richness and endemism. The isthmian highlands (1000–3820 m a.s.l.) are an isolated topographic unit in Central America (Campbell 1999, Gutiérrez-García and Vázquez-Domínguez 2013) formed by the Guanacaste, Tilarán, Central, and Talamanca mountain ranges. The Cordillera de Talamanca extends from the central valley in Costa Rica to western Panama and contains both the highest mountain peaks of the isthmus (Campbell 1999, Savage 2002) and the most endemic amphibians of Costa Rica (Campbell

1999, Olson *et al.* 2001, Savage 2002, Boza-Oviedo *et al.* 2012). The summits of the Cordillera de Talamanca (ranging from 2500–3500 m a.s.l.) are dominated by Montane Rainforest Life Zone (Holdridge 1967, Bolaños *et al.* 2005), which is extremely fragmented and isolated. The Talamanca montane rainforest (TMR) is relatively poorly studied and is thought to have a lower species diversity than the premontane forest of Talamanca Range (Kubicki 2008, Santos-Barrera *et al.* 2008, Arias and Bolaños 2014). Nevertheless, the TMR is home to several micro endemic amphibians—*viz.* *Atelopus chirripoensis* Savage and Bolaños, 2009, *Bolitoglossa kamuk* Boza-Oviedo, Rovito,

Chaves, García-Rodríguez, Artavia, Bolaños, and Wake, 2012, *B. pesrubra* Taylor, 1952, *B. pygmaea* Bolaños and Wake, 2009, *B. robinsoni* Bolaños and Wake, 2009, *B. splendida* Boza-Oviedo, Rovito, Chaves, García-Rodríguez, Artavia, Bolaños, and Wake, 2012, and *Diasporus ventrimaculatus* Chaves, García-Rodríguez, Mora, and Leal, 2009.

During recent fieldwork in the TMR we found frogs of the *Craugastor podiciferus* Species Group Hedges *et al.* (2008). The anurans were collected at the summits of Cerro Arbolado, Cerro Hakú, Cerro Utyum, and Caribbean slopes of Cerro Pando. The population of Cerro Utyum is near (~ 10 km airline distance) to the type locality of *C. podiciferus* (Cope, 1875) (Cope 1875, Arias and Chaves 2014) and both species of anurans are morphologically similar. *Craugastor podiciferus* is abundant, with a broad distribution across the highlands of all the mountain ranges of Costa Rica and western Panama (1000–2650 m a.s.l.); it has been thought to represent a species complex with several unnamed species (Savage 2002, Streicher *et al.* 2009). Molecular analyses of two mitochondrial genes showed that despite the morphological resemblance to *C. podiciferus*, the recently discovered populations from the TMR are not *C. podiciferus*, but instead represent a new member of the species group, which is described here based on molecular and morphological data.

Materials and Methods

Taxon Sampling

Frogs of the new species were collected at five localities along the Cordillera de Talamanca, at the summits of Cerro Arbolado, Cerro Hakú, Cerro Utyum, and the Caribbean slopes of Cerro Pando in southwestern Costa Rica (Figure 1). In addition, we collected several specimens from highlands of Costa Rica and western Panama, including several populations referred to *C. podiciferus*, *Craugastor* sp.B of Crawford & Smith (2005), and two populations that we

record herein as unnamed species (*Craugastor* sp.1 and *Craugastor* sp.2). The frogs were euthanized in the field and extracted liver or muscle tissue was preserved in 95% ethanol or RNAlater. Voucher specimens were fixed in 10% formalin, stored in 70% ethanol, and deposited at the Museo de Zoología, Universidad de Costa Rica (UCR) and Senckenberg Research Institute and Nature Museum, Frankfurt, Germany (SMF). Museum codes follow those of Frost (2018), with the addition of CRARC in reference to the Costa Rica Amphibian Research Center private collection; EAP denotes field numbers of Erick Arias.

Amplification and Sequencing

We extracted total genomic DNA from the preserved tissue samples using the Animal Genomic DNA Kit (BioBasic Canada Inc.), DNeasy Blood & Tissue Kit (Qiagen), or the phenol-chloroform standard protocol (Sambrook & Russell 2006). We amplified the large subunit ribosomal RNA (16S) and cytochrome oxidase subunit I (COI) mitochondrial genes. The primers 16Sar and 16Sbr (Palumbi *et al.* 1991) were used for 16S and dgLCO and dgHCO (Meyer 2003) for COI. The PCR amplifications were performed using a total volume of 15 µL, which contained 1 µL DNA template (*c.* 50 ng/µL), 0.75 U Taq polymerase (Amplificasa®, Biotecnologías Moleculares), 1X PCR buffer with 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3–0.5 µM forward and reverse primers. The PCR conditions are as follow: 16S, an initial cycle of 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 30 s at 50 or 55°C, 45 or 120 s at 72°C, plus a final cycle of 3 min at 72°C; COI, an initial cycle of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, 45 s at 72°C, plus a final cycle of 3 min at 72°C. PCR products were cleaned with ExoSap-IT (USB Corporation) and sequenced in both directions using the original amplification primers and BigDye termination reaction chemistry (Applied Biosystems). The cycle-

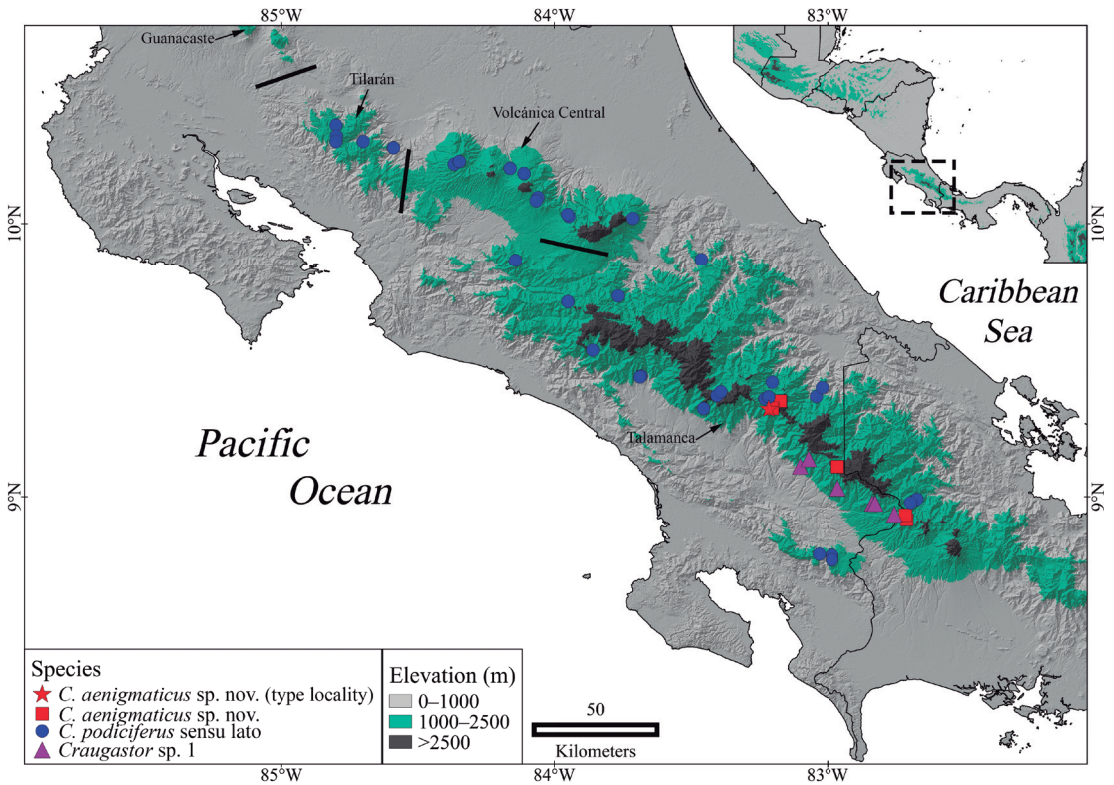


Figure 1. Map showing the known populations of *Craugastor aenigmaticus* sp. nov. (red star = type locality) in the Cordillera de Talamanca, and the populations of *C. podiciferus* used in our molecular analysis. Solid (black) lines depict the geographical limits between each mountain range.

sequencing products were column-purified with Sephadex G-50 (GE Healthcare) and run on an ABI 3500xL Genetic Analyzer (Applied Biosystems). Consensus sequences for each individual were constructed using SEQUENCHER 5.3 (Genes Codes Corp.).

Phylogenetic Analyses

The sequences obtained were compared to those available in GenBank for the *Craugastor podiciferus* Species Group and sequences of *C. gollmeri* (Peters, 1863) were used as the outgroup. See Appendix I for the list of DNA voucher and GenBank Accession numbers used in this study. Sequence alignments were

performed using the MUSCLE 3.7 software (Edgar 2004) with default parameters and trimmed to the point at which a majority of taxa had sequence data. We partitioned the sequence data by gene, and further partitioned COI by codon position. We used PartitionFinder v1.1.1 (Lanfear *et al.* 2012) and the Bayesian Information Criterion (BIC) to select the best partition scheme and the best model of sequence evolution for each partition. We used a single set of branch-lengths across all partitions (branchlengths = linked); the search of the best partition scheme was using a heuristic search (scheme = greedy). We defined, *a priori*, four partitions: one for 16S and three for COI (one for each codon).

We performed analyses using both the maximum likelihood (ML) and Bayesian analyses (BA). For ML we used Garli 2.01 (Zwickl 2006), with 10 search replicates with the following default setting values changed: streefname = random, attachmentspertaxon = 24, genthreshfortopoterm = 100000, significanttopochange = 0.00001. For bootstrapping, we ran 1000 replicates with the previous settings with the following changes: genthreshfortopoterm = 10000, significanttopochange = 0.01, treerejectionthreshold = 20, as suggested in the Garli manual to speed up bootstrapping. The bootstrap consensus tree was performed using Sumtrees (Sukumaran and Holder 2010a) from DendroPy packages Version 4.4.0 (Sukumaran and Holder 2010b). Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 (Ronquist *et al.* 2012) with the partition scheme and the model of sequence evolution for each partition as selected previously. Two separate analyses were run; each consisted of 20 million generations, sampled every 1000 generations, and four chains with default heating parameters. We examined a time-series plot of the likelihood scores of the cold chain to check stationarity using Tracer 1.6 software (Rambaut *et al.* 2014). We discarded the first 25% of trees as burn-in and used the remaining trees to estimate the consensus tree along with the posterior probabilities for each node and each parameter. The ML and Bayesian analyses were run on the CIPRES portal (Miller *et al.* 2010). Estimates of pairwise evolutionary genetic divergence between species were computed using MEGA7 (Kumar *et al.* 2016), assuming uncorrected distances based on the Tamura 3-parameter model (Tamura 1992), with rate variation among the sites modeled as a gamma distribution with the shape parameter = 4 as the default of the software.

Morphometric Analyses

We performed a morphometric analysis to compare the new species with *Craugastor*

podiciferus because the taxa closely resemble one another and both inhabit the TMR (allopatrically). In addition we compared the new species with a third undescribed species (*Craugastor* sp.1) because populations of this frog are located nearby (~ 5 km airline distance) (Figure 1). We examined 20 specimens of the new species from four localities, 86 *C. podiciferus* from several localities in Costa Rica, and 19 *Craugastor* sp.1 (Appendix II). Specimens are deposited at UCR. The following morphological measurements were recorded, as described by Savage (2002), Duellman and Lehr (2009), and Arias *et al.* (2016): snout–vent length (SVL), head length (HL), head width (HW), interorbital distance (IOD), width of upper eyelid (EW), intercanthal distance (IC), internarial distance (IN), upper lip–nostril distance (TN), eye–nostril distance (E–N), eye diameter (ED), tympanum diameter (TY), ulna length (UL), hand length (HaL), lengths of the Fingers I (F1) and III (F3), femur length (FL), tibia length (TL), tarsus length (TaL), foot length (FoL), and lengths of the Toes III (T3) and V (T5). Measurements were taken with dial calipers and were rounded to the nearest 0.1 mm.

We transformed the morphometric data using the method of Leonart *et al.* (2000) to avoid allometric effects relative to the differences in the size and shape between species and between individuals. In this method, a logarithmic transformation of the continuous variables is performed to reduce the extreme values. All transformed variables are used in the allometric transformation by means of equation $Y_i^* = Y_i (X_0/X_i)^b$, where Y_i^* is the value of each of the dependent variable corrected for size and shape; Y_i is the value of each of the dependent morphometric variable; X_0 is the average of the SVL variable for all populations; X_i is the SVL value for each individual; and b is the regression line intercept with the Y-axis resulting from the regression of each dependent variable with X_0 . The intercept is used as an allometric transformation factor and is unique for each

variable. The additional proportions reported here include: EW/IOD, IOD/HW, TY/ED, EN/ED, ED/HL, IC/HL, IN/EN, IN/TN, FL/TL, TL/TaL, TaL/FoL, T3/FoL, T5/FoL, UL/HaL, F1/HaL, and F3/HaL. The sex of individuals was determined by gonadal morphology. Specimens with opaque seminal vesicles were assumed to be adult males, and those with developed oviducts were assumed to be adult females. The general terminology for the morphological characteristics follows that of Duellman and Lehr (2009). We adopted Savage's (2002) usage of the term "supernumerary tubercles" to refer to the tubercles on the phalanges (between subarticular tubercles); this differs from the tubercles denoted as accessory palmar or plantar tubercles.

We calculated the mean, standard deviation, and range for each morphometric variable without correction. We performed a discriminant analysis to determine whether the morphometric variables were effective to predict the species, using the following variables: EW/IOD, IOD/HW, TY/ED, EN/ED, ED/HL, IC/HL, IN/EN, IN/TN, FL/TL, TL/TaL, TaL/FoL, T3/FoL, UL/HaL, F1/HaL, and F3/HaL. We also conducted a Principal Component Analysis (PCA) to explore the degree of structure within the sample and which variables have more loads in the segregation of groups. All analyses were performed using R v3.3.3 (R Core Team 2017).

Results

Molecular Data

The data matrix includes 56 sequences, with a total sequence length of 1222 bp, including gaps: 565 bp for 16S and 657 bp for COI. Three partition schemes were identified with the following substitution models: GTR + I + G for 16S and COI codon position 3, K80 + I + G for COI codon position 1, and HKY+I for COI codon position 2. Genetic distances between the new species and other members of the *Craugastor podiciferus* Species Group are of 9.2–18.5% for

16S and 18.9–24.8% for COI. Specifically, the new species is separated by a mean-uncorrected genetic distance to *C. podiciferus* of 11.57–16.15% for 16S and 19.16–24.35% for COI and to *Craugastor* sp.1 of 12.25–12.9% in 16S and 23.57–24.72% in COI.

The phylogenies inferred by Garli and MrBayes were mostly congruent (Figure 2), with six well-supported clades. The three most basal clades represent three unnamed species (*Craugastor* sp.B, *Craugastor* sp.1, and *Craugastor* sp.2) that occur in the highlands of the southwestern end of Cordillera de Talamanca. The fourth clade contains the samples from Cerro Hakú, Cerro Utyum, and Cerro Pando at Cordillera de Talamanca (Figure 1). A fifth major clade includes seven species of the *C. podiciferus* Species Group that mainly occur from the lowlands to intermediate elevations from eastern Honduras to central Panama. The sixth major clade is formed by all the samples of *C. podiciferus* sensu lato that are broadly distributed in the highlands of Costa Rica and western Panama (Figure 1).

The main differences between the ML and Bayesian topologies are in the relationships of *Craugastor* sp.B, *Craugastor* sp.1, and *Craugastor* sp.2 within the phylogeny. Their placement is unresolved in the ML tree, whereas in the Bayesian tree (not shown), *Craugastor* sp.1 is the most basal taxon for the entire group. *Craugastor* sp.1 + *Craugastor* sp.B form a clade that is sister to the rest of the species group.

Morphometric Analysis

Morphometric variation among the species is shown in Table 1. The PCA did not differentiate specimens of the new species from those of *Craugastor podiciferus* and *Craugastor* sp.1. The discriminant analysis correctly classified 87.9% of the specimens to the species, showing a clear separation between the specimens of the new taxon and the specimens referred to *C. podiciferus* and *Craugastor* sp.1 (Figure 3).

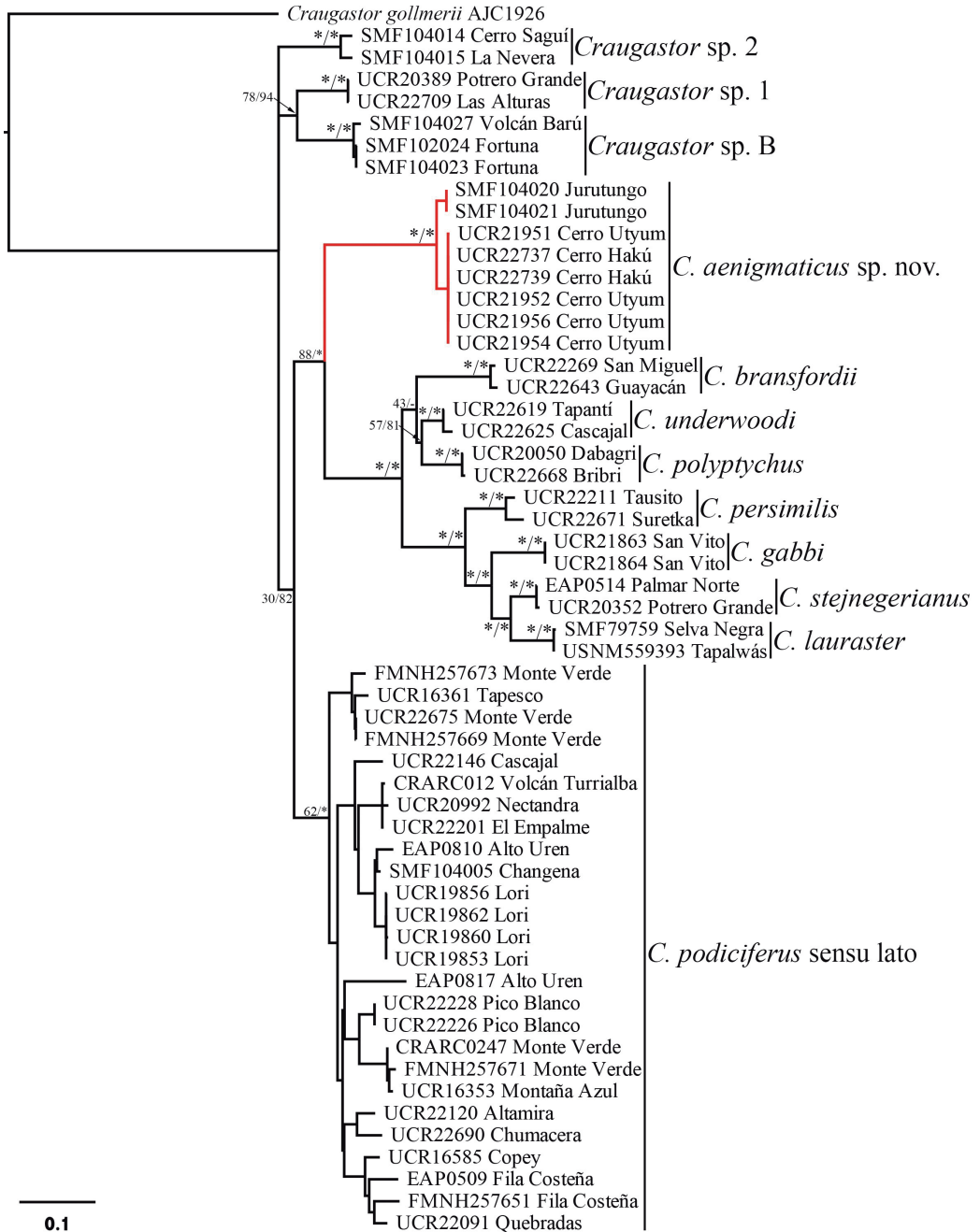


Figure 2. Maximum likelihood phylogeny of *Craugastor podiciferus* Species Group based on 16S and COI mitochondrial DNA genes. Bootstraps proportions are before the slash, and posterior probabilities (multiplied by 100) from MrBayes analysis are following the slash. The scale bar refers to the estimated substitutions per site. The support values of any node within species are not shown. The asterisks represent support of > 95.

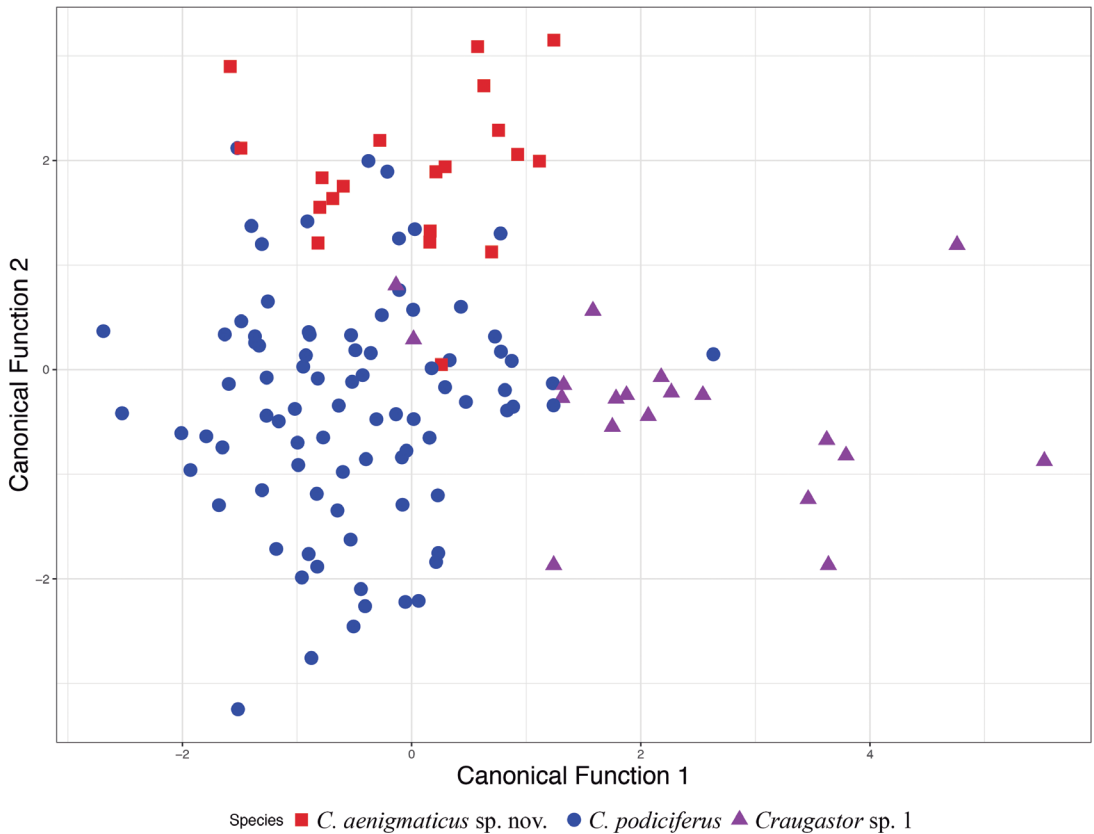


Figure 3. Linear discriminant analysis showing the morphological separation among the 20 individuals of *Craugastor aenigmaticus* sp. nov. and the individuals of *C. podiciferus* and *Craugastor* sp.1.

***Craugastor aenigmaticus* sp. nov.**

Montane Dirt Frog
(Figures 4–6)

Holotype.—UCR 22961 (EAP 0762), an adult female from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: summit of Cerro Arbolado, Parque Internacional La Amistad, (09°19'12.0" N, 83°12'57.6" W; 2600 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 19 October 2016.

Paratopotypes.—UCR 22957 (EAP 0758), subadult male; UCR 22958 (EAP 0759) and

UCR 22960 (EAP 0761), adult females; UCR 22962 (EAP 0763), subadult female; UCR 22959 (EAP 0760), juvenile; same date as the holotype.

Paratypes.—UCR 21951 (EAP 0303), adult female from Costa Rica: Provincia de Limón: Cantón de Talamanca: Distrito de Telire: Caribbean slopes of Cerro Utyum, Parque Internacional La Amistad, (09°20'56.4" N, 83°10'30.0" W; 2700 m a.s.l.), collected by Erick Arias, Gerardo Chaves, Olmer Cordero, and Omar Zúñiga on 12 July 2013. UCR 22414 (EAP 0490) and UCR 22415 (EAP 0491), adult females, same data as UCR 21951 but collected on 29 March 2013. UCR 22737 (EAP 0674) and

Table 1. Mean \pm SD and range (in mm) for morphometric variables by species. Abbreviations: SVL, snout-vent length; HL, head length; HW, head width; IOD, interorbital distance; EW, width of the upper eyelid; IC, intercanthal distance; IN, internarial distance; TN, upper lip-nostril distance; E-N, eye-nostril distance; ED, eye diameter; TY, tympanum diameter; UL, ulna length; HaL, hand length; F1, length of Finger I; F3, length of Finger III; FL, femur length; TL, tibia length; TaL, tarsus length; FoL, foot length; T3, length of Toe III; T5, length of Toe V.

Variable	<i>Craugastor aenigmaticus</i> sp. nov.		<i>Craugastor podiciferus</i>		<i>Craugastor</i> sp.1	
	Mean \pm SD	Min-Max	Mean \pm SD	Min-Max	Mean \pm SD	Min-Max
SVL	26.38 \pm 9.33	16.10–41.10	24.27 \pm 4.76	15.10–35.10	20.79 \pm 3.94	13.80–26.50
HL	10.65 \pm 3.52	6.20–15.95	9.91 \pm 1.76	6.45–13.60	8.44 \pm 1.47	5.70–10.30
HW	10.61 \pm 3.68	6.25–16.50	9.66 \pm 1.88	6.20–14.15	8.22 \pm 1.64	5.60–10.70
IOD	3.265 \pm 1.10	1.80–5.55	3.23 \pm 0.53	2.10–4.55	2.74 \pm 0.51	1.80–3.70
EW	2.08 \pm 0.70	1.20–3.20	1.80 \pm 0.38	1.15–2.90	1.62 \pm 0.34	1.15–2.10
IC	5.20 \pm 1.64	3.25–8.00	5.02 \pm 0.77	3.55–6.95	4.22 \pm 0.73	3.15–5.30
IN	3.29 \pm 0.99	2.15–4.85	3.04 \pm 0.51	2.20–4.30	2.57 \pm 0.39	1.95–3.25
TN	1.54 \pm 0.46	1.00–2.30	1.25 \pm 0.24	0.90–2.10	1.04 \pm 0.18	0.70–1.25
EN	2.59 \pm 0.77	1.55–3.70	2.43 \pm 0.46	1.50–3.60	1.99 \pm 0.42	1.30–2.60
ED	3.05 \pm 0.95	2.05–4.90	2.88 \pm 0.49	1.90–3.90	2.46 \pm 0.32	1.90–2.90
TY	2.01 \pm 0.73	0.95–3.15	1.75 \pm 0.41	1.00–2.70	1.96 \pm 0.32	1.55–2.95
UL	6.08 \pm 2.34	3.10–9.65	5.56 \pm 1.09	3.50–8.20	4.80 \pm 0.99	3.35–6.30
HaL	6.93 \pm 2.57	3.70–11.00	5.83 \pm 1.24	3.55–9.50	5.03 \pm 0.99	3.10–6.55
F1	2.62 \pm 1.31	1.10–4.85	2.10 \pm 0.55	1.10–3.95	1.93 \pm 0.56	0.90–2.95
F3	4.16 \pm 1.62	2.20–6.50	3.46 \pm 0.79	1.75–6.10	2.97 \pm 0.62	1.80–3.90
FL	14.73 \pm 5.85	7.90–23.00	12.40 \pm 2.80	7.60–20.30	10.67 \pm 1.96	7.05–14.00
TL	16.75 \pm 6.54	8.50–25.85	14.07 \pm 3.04	8.25–22.50	12.25 \pm 2.26	8.25–15.25
TaL	9.44 \pm 3.38	5.45–14.25	8.28 \pm 1.66	5.10–12.85	7.36 \pm 1.35	5.10–9.30
FoL	14.87 \pm 5.76	7.95–23.50	12.79 \pm 2.75	7.65–20.15	10.94 \pm 2.24	7.05–14.15
T3	5.40 \pm 2.32	2.05–9.70	4.46 \pm 0.99	2.60–7.25	3.79 \pm 0.77	2.45–5.05
T5	4.27 \pm 1.82	1.70–7.00	3.67 \pm 0.95	1.90–6.70	2.99 \pm 0.58	1.90–3.80
EW/IOD	0.64 \pm 0.07	0.46–0.75	0.56 \pm 0.09	0.38–0.89	0.59 \pm 0.08	0.44–0.76
EN/ED	0.86 \pm 0.11	0.67–1.02	0.84 \pm 0.07	0.64–1.04	0.81 \pm 0.10	0.63–0.98
ED/HL	0.29 \pm 0.03	0.25–0.35	0.29 \pm 0.02	0.25–0.33	0.29 \pm 0.02	0.26–0.33
IOD/HW	0.31 \pm 0.03	0.27–0.38	0.34 \pm 0.03	0.27–0.43	0.34 \pm 0.02	0.30–0.38
TY/ED	0.66 \pm 0.15	0.45–1.03	0.61 \pm 0.10	0.41–1.00	0.81 \pm 0.17	0.62–1.34
IC/HL	0.49 \pm 0.02	0.43–0.53	0.51 \pm 0.03	0.45–0.61	0.50 \pm 0.05	0.36–0.58
IN/EN	1.27 \pm 0.12	1.03–1.55	1.26 \pm 0.11	0.95–1.53	1.31 \pm 0.16	1.10–1.62
IN/TN	2.14 \pm 0.13	1.94–2.44	2.45 \pm 0.20	1.90–2.94	2.50 \pm 0.22	2.21–3.00
FL/TL	0.88 \pm 0.03	0.80–0.94	0.88 \pm 0.05	0.54–0.96	0.87 \pm 0.03	0.79–0.92
TL/TaL	1.76 \pm 0.10	1.51–1.94	1.70 \pm 0.07	1.48–1.83	1.67 \pm 0.07	1.58–1.91
TaL/FoL	0.64 \pm 0.04	0.58–0.77	0.65 \pm 0.03	0.58–0.73	0.68 \pm 0.03	0.59–0.72
T3/FoL	0.36 \pm 0.04	0.24–0.46	0.35 \pm 0.02	0.30–0.39	0.35 \pm 0.01	0.32–0.36
T5/FoL	0.28 \pm 0.04	0.18–0.34	0.28 \pm 0.02	0.22–0.38	0.27 \pm 0.02	0.22–0.31
UL/HaL	0.88 \pm 0.05	0.77–0.95	0.96 \pm 0.06	0.81–1.16	0.96 \pm 0.08	0.82–1.11
F1/HaL	0.36 \pm 0.06	0.27–0.47	0.36 \pm 0.04	0.24–0.43	0.38 \pm 0.05	0.29–0.46
F3/HaL	0.60 \pm 0.02	0.56–0.64	0.59 \pm 0.04	0.49–0.67	0.59 \pm 0.04	0.51–0.68

UCR 22747 (EAP 0684), adult males from Costa Rica: Provincia de Puntarenas: Cantón de Buenos Aires: Distrito de Buenos Aires: summit of Cerro Hakú, Parque Internacional La Amistad, (09°19'19.2" N, 83°12'10.8" W; 2660 m a.s.l.), collected by Erick Arias and Omar Zúñiga on 28 December 2015.

Group assignment.—Assigned to the genus *Craugastor* based on possessing the following characters: differentiated tympanum; absence of cranial crest; and Toe III longer than Toe V. Assigned to the *C. podiciferus* Species Group based on having a narrow head (HW/SVL = 36.1–43.64%) and a rugose dorsum, but lacking inner tarsal folds, webbing between the toes, nuptial pads, and vocal slits.

Diagnosis.—The combination of the following characteristics distinguish *Craugastor aenigmaticus* from its congeners (Figure 4 and 5): (1) skin on venter smooth, but with large granules laterally; (2) vocal slits absent; (3) nuptial pads absent; (4) toes lacking webbing; (5) heels lacking enlarged calcar tubercle, but can have one to three small tubercles or granules on heels; (6) supernumerary, accessory palmar, and plantar tubercles absent; and (7) unique coloration consisting of a dark brown, olive, olive-brown or dark violet-brown dorsal ground color, violet-brown to blackish brown venter with white to white-bluish pigment forming blotches, dark brown palmar surface in adults with prominent white folds between subarticular tubercles, and in some specimens, white bones apparent through the skin (Figure 6C).

Comparisons with other species.—*Craugastor aenigmaticus* differs from all the other craugastorids of isthmian Central America except for those in the *C. podiciferus* Species Group by having a narrow head (HW 36.1–43.64% SVL) and toes that lack webbing. *Craugastor aenigmaticus* differs from other members of the *C. podiciferus* Species Group by having the following characteristics (condition



Figure 4. *Craugastor aenigmaticus* sp. nov. Photograph taken by EA.

for *C. aenigmaticus* in parentheses). In *C. bransfordii* (Cope, 1886), *C. gabbi* Arias, Chaves, Crawford, and Parra-Olea, 2016, *C. lauraster* (Savage, McCranie, and Espinal, 1996), *C. persimilis* (Barbour, 1926), *C. polyptychus* (Cope, 1886), *C. stejnegerianus* (Cope, 1893), and *C. underwoodi* (Boulenger, 1896): (1) the venter is cream (olive-brown in life, and dark brown in ethanol); (2) the venter, as well as the midline, is completely areolate to tuberculate (smooth, at least in the midline in *C. aenigmaticus*); and (3) they range in altitude from 0–1600 m a.s.l. (range 2330–2700 m a.s.l.). *Craugastor jota* (Lynch, 1980) differs from *C. aenigmaticus* by having a prominent calcar tubercle on the heel (evident calcar tubercle absent, although some individuals have one to three small tubercles). *Craugastor podiciferus* differs from *C. aenigmaticus* by: (1) having a prominent calcar tubercle on the heel (Figure 7) (evident calcar tubercle absent although some individuals could have one to three small tubercles); (2) venter yellow, orange, grayish or olive in adults (adults with venter violet-brown with white blotches); and (3) by lack of white prominent folds between subarticular tubercles on hands of adults (prominent white folds between the subarticular tubercles on the hands of adults) (Figure 6D).

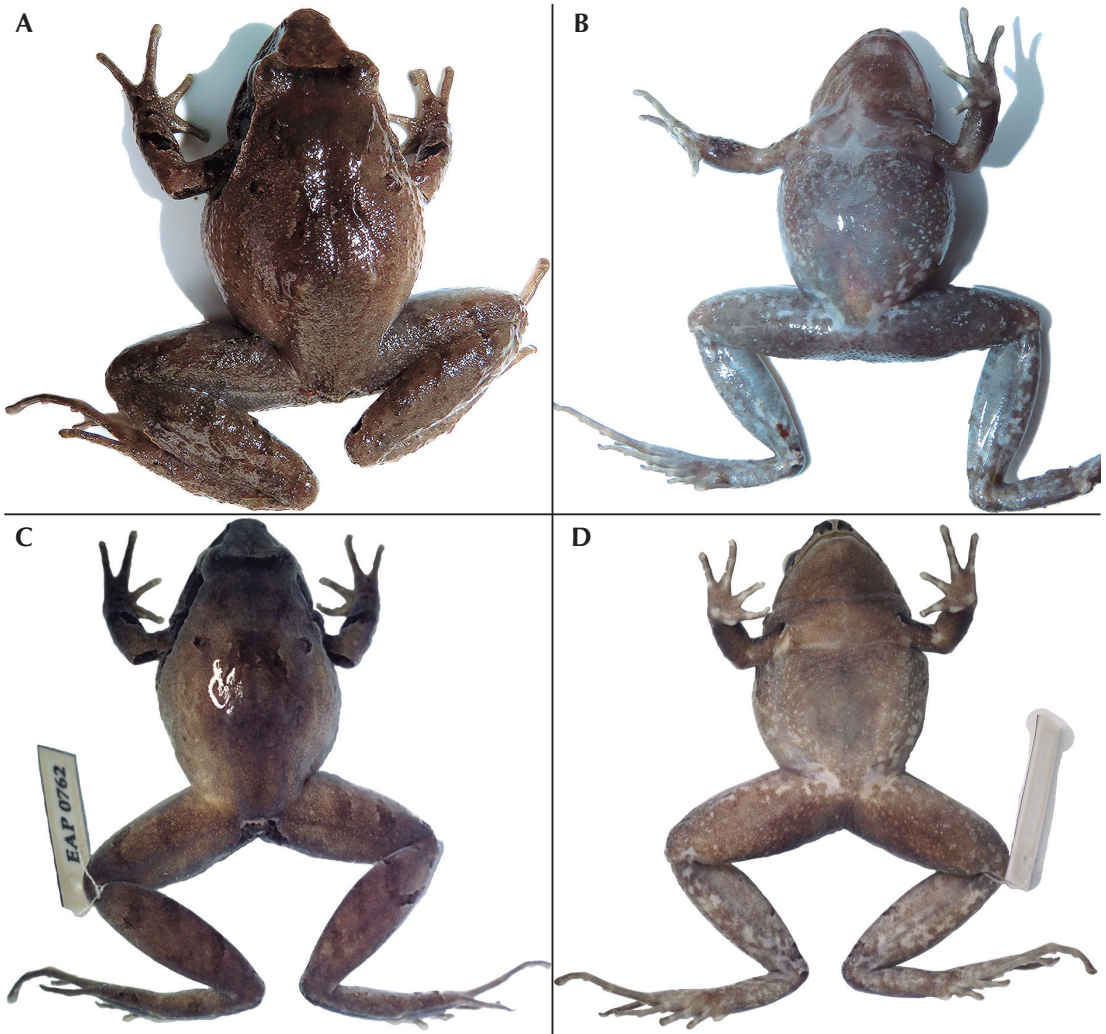


Figure 5. Dorsal (right) and ventral (left) color in life (above) and in ethanol (below) of the holotype (UCR 22961) of *Craugastor aenigmaticus* sp. nov. Photographs taken by EA.

Description of the holotype.—Adult female, SVL = 40.1 mm (Figures 4, 5). Head relatively narrow, width = 41.15% SVL; snout subovoid in dorsal view, rounded in profile; snout relatively long (HL = 15.6 mm, 38.9% SL), with nostrils directed laterally; in ventral view, tip of snout protruding markedly beyond edge of lower lip. Internarial area convex (IN 4.85 mm); canthus

rostralis rounded; intercanthal area flat (IC = 8.0 mm); loreal region slightly concave; vomerine teeth transverse, in two fascicles well behind the choanae. Tongue round, lacking a distinct posterior notch; teeth absent; choanae moderately large, rounded on posterior half, but flat on anterior half, hemispherical; vocal slits absent. Eye moderate (EW = 92.75% E-N), not

protruding beyond dorsal and ventral outline of head, directed laterally. Tympanum distinct, round, and covered by skin; tympanic annulus prominent, round, small (54.12% of ED). Skin on all dorsal and lateral surfaces of head

moderately granular. Upper eyelid granular, without superciliar or supraocular tubercles. Postrictal tubercles fused forming short ridge posteroventral to tympanum. Skin on dorsum and limbs weakly granular. Skin of chest smooth,

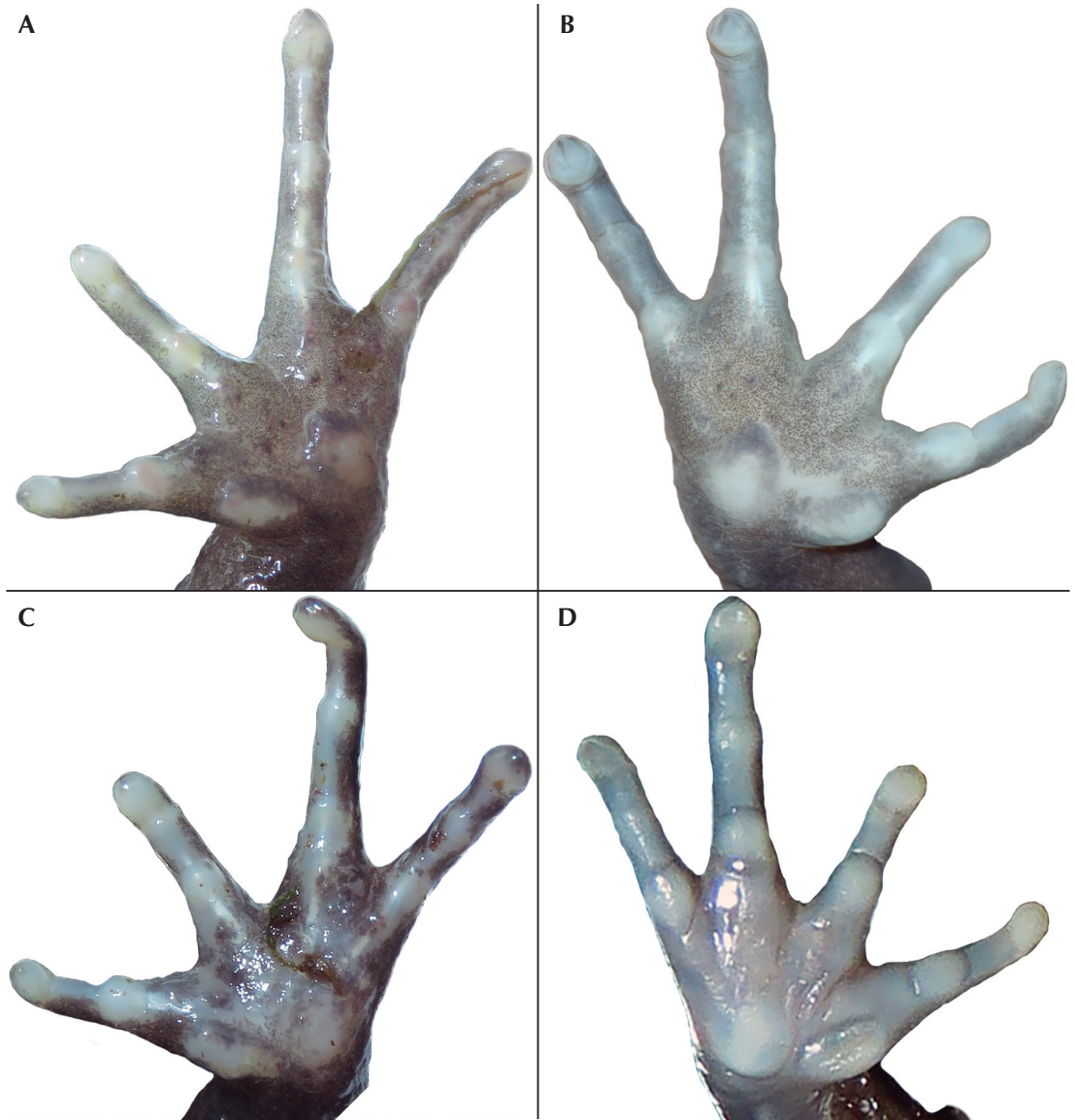


Figure 6. Variation of the ventral views of the hands for comparison. (A) *Craugastor aenigmaticus* sp. nov. holotype in life (UCR 22961); (B) holotype (UCR 22961) in ethanol; (C) paratopotype (UCR 22958); (D) *C. podiciferus*. Photographs taken by EA.

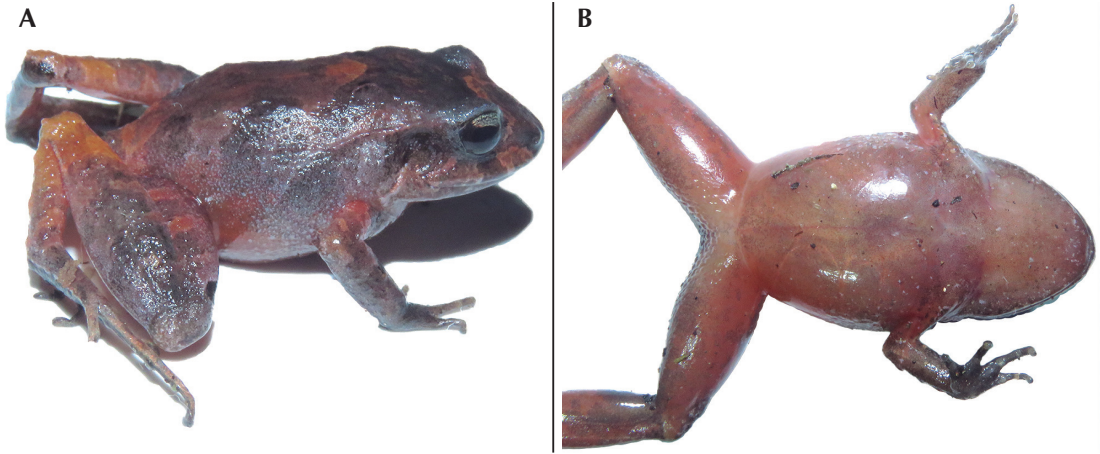


Figure 7. Dorsal (A) and ventral (B) color in life views of *Craugastor podiciferus* (EAP 0803), for comparison. Note the enlarged calcar tubercle and coloration on dorsum and venter. Photographs taken by EA.

venter smooth and encroached on laterally by low granules; ventral surfaces of thighs smooth to weakly granular; skin of groin and ventral surfaces of arms and lower legs nearly smooth. Flanks areolate, especially along the anteroventral flank region; skin on chin smooth. A pair of complete hourglass-shaped dorsal ridges present between posterior margin of eye and sacrum. Pair of supratympanic folds extending from posterior margin of eye, above tympanum, bifurcating at the axillary level, one fold continues laterally and other continues to the venter. Discoidal fold complete.

Forelimb relatively short and robust; fingers moderately long and slim without lateral fringes. Discs absent; fingers with grooves; tips of fingers unexpanded, rounded in dorsal view; pads ovoid. Proximal subarticular tubercles indistinct. Supernumerary tubercles absent; accessory palmar tubercles absent; distal subarticular tubercles rounded in basal outline, flattened, and globular in profile; thenar tubercle ovoid, flattened, palmar tubercle rounded, flattened; thenar tubercle much smaller than palmar. Ulnar tubercles and fold absent. Fingers are not webbed.

Legs relatively long and robust; heel granular but lacking enlarged tubercles. Discs absent; toes with grooves; tips of toes unexpanded, lanceolate in dorsal view; pads triangular. Supernumerary tubercles absent; plantar tubercles absent; subarticular tubercles ovoid in basal outline, flattened, and globular in profile; inner metatarsal tubercle elongate, globular; outer metatarsal tubercle rounded, barely discernible, flattened; outer metatarsal tubercle much smaller than inner; inner tarsal fold absent; toes are not webbed. Cloacal opening directed posteriorly at midlevel of thighs.

Coloration of the holotype in life.—Dorsal ground color is uniform dark brown, but with two dark spots on the scapular region (Figure 4). A dark interorbital mark is present, lying just posterior to an adjacent paler area. Dorsal surfaces of the legs and arms with dark bars. The surfaces below the canthus rostralis and the supratympanic fold, from the snout to the axilla, are darker (forming a type of mask); the dark pigment of the mask continues posteriorly bordering the supratympanic fold. The upper lip has dark bars with white pigment in form of

faded bars. The flanks are the same color as dorsum, but with white pigment in form of small spots. Ventral surfaces of the body and legs are violet-brown with bluish-white pigment in the form of blotches; ventral surface of tibia-tarsus grayish; ventral surface of the throat violet-brown is relatively uniform, slightly paler than the venter (Figure 5). The palmar surface of the hands with violet coloration, especially on thenar, palmar, and proximal subarticular tubercles; the fingers are whitish, with a white prominent fold between subarticular tubercles (Figure 6A).

Coloration of the holotype in ethanol.—After 2 years in ethanol (70%), the overall dark brown dorsum has changed little from its color in life. The violet-brown on ventral surfaces of the body and legs has faded to dark brown and the bluish-white blotches have changed to light brown. The overall patterns of the blotches and other markings on the holotype have remained identical to those that were observed while alive.

Measurements of holotype (mm).—SL 40.1; HL 15.6; HW 16.5; IOD 4.65; EW 3.2; IC 8.0; IN 4.85; TN 2.3; EN 3.45; ED 4.25; TY 2.3; UL 9.65; HaL 10.25; F1 4.85; F3 6.5; FL 22.65; TL 25.85; TaL 14.25; FoL 23.5; T3 8.1; T5 7.0. Measurements in related percentages: EW/IOD 68.82%; IOD/HW 28.18%; TY/ED 54.12%; EN/ED 81.12%; ED/HL 27.24%; IC/HL 51.28%; IN/EN 140.58%; IN/TN 210.87%; FL/TL 87.62%; TL/TaL 181.40%; TaL/FoL 60.64%; T3/FoL 34.47%; T5/FoL 29.79%; UL/HaL 94.15%; F1/HaL 47.32%; F3/HaL 63.42%.

Variation.—Morphometric variation is summarized in Table 1. *Craugastor aenigmaticus* has a relatively high level of intraspecific polymorphisms (Figure 8). The skin on venter ranges from almost completely smooth to heavily areolate, but always with a smooth midline. Some frogs have supraocular tubercles; additionally, some individuals have two postrictal tubercles that are not fused. The palmar tubercle

is heart shaped in some specimens, but ovoid in others; the proximal subarticular tubercles are not visible in some specimens. In the adult male, UCR 22747, an accessory palmar tubercle is visible. In some specimens, the heel is areolate; in others one to three small tubercles are visible. Some specimens have flattened subarticular tubercles, whereas others have projecting subarticular tubercles. Some individuals have globular subarticular tubercles in profile, whereas others have obtuse subarticular tubercles. UCR 21951 and UCR 22734 have a pattern of dark brown lateral stripes on a pale brown background (Figure 8E); UCR 21956 has an olive-green dorsum (Figure 8F). The mask is absent in some specimens. The throat is a uniform cream coloration in some frogs, whereas it is uniform violet-brown, heavily mottled, or uniformly dark brown in others. The venter usually is dark-brown or grayish in coloration, but in some individuals, especially in juveniles, it is cream (Figure 8D). The juveniles have light brown arms in contrast to the dark brown dorsum (Figure 8C). The palmar surfaces of the hands in adults usually are dark brown with prominent white folds between subarticular tubercles, but in some frogs, the white bones are visible beneath the skin; in juveniles, the palmar surfaces are dark brown or cream.

Habitat and natural history notes.—*Craugastor aenigmaticus* inhabits the montane rainforest of the Cordillera de Talamanca (Holdridge 1967, Bolaños *et al.* 2005), which has a short dry season (1 or 2 mo), annual precipitation ranging between 2200 and 4500 mm, and annual temperatures ranging from 6–12°C. The type locality (Cerro Arbolado) and the other known localities consist of primary forest dominated by oak trees (genus *Quercus* L.) that are abundantly covered with bryophytes and epiphytes (Figure 9). The forest floor is covered by a thick layer of leaf litter and other types of decomposing organic material. Little is known about the natural history of *C. aenigmaticus*, but it is important to note that the species was abundant

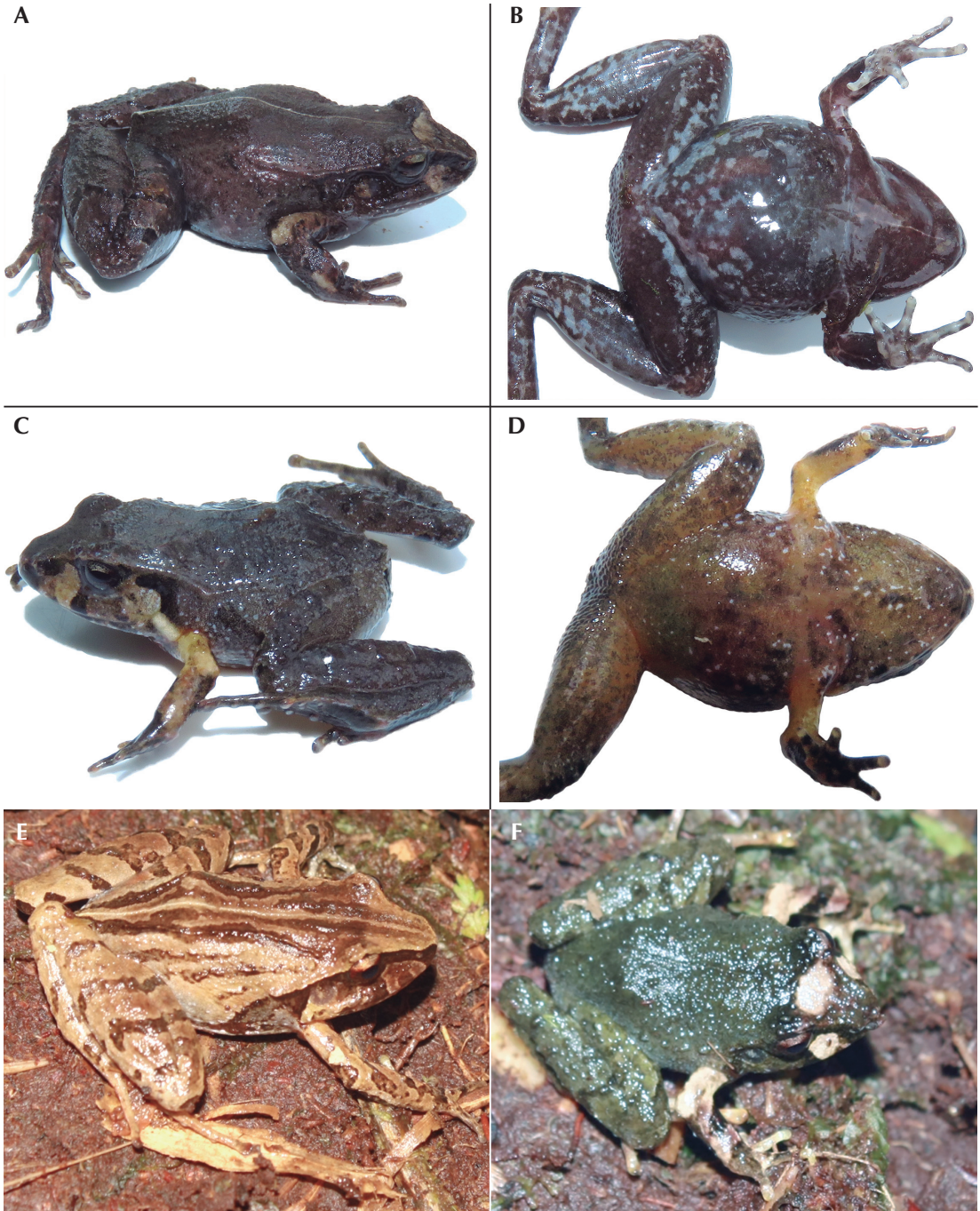


Figure 8. Variation on dorsum and venter of *Craugastor aenigmaticus* sp. nov. (A, B) Paratopotype UCR 22958, adult female; (C, D) paratopotype UCR 22959, juvenile; (E) paratype UCR 21951, adult female; (F) UCR 21956, juvenile. Photographs taken by EA.



Figure 9. Cloud forest at type locality of *Craugastor aenigmaticus* sp. nov., summit of Cerro Arbolado at 2600 m a.s.l. Photograph taken by Omar Becerra Soria.

during our fieldwork in the months of April and July 2013, December 2015, and October 2016. During December 2015 and October 2016, gravid females were observed. Juveniles were observed during all the periods of fieldwork; the smallest size recorded was with a SVL of 16.1 mm. All specimens of *C. aenigmaticus* collected were discovered as they were jumping on the forest floor. We did not record any vocalization that we could attribute to *C. aenigmaticus*, though we think that the frog may vocalize. *Craugastor aenigmaticus* is sympatric with *Diasporus ventrimaculatus* in Cerro Arbolado, Cerro Hakú, on the Caribbean slopes of Cerro Utyum, and Valle del Silencio, which is the type locality of *D. ventrimaculatus*.

Distribution.—*Craugastor aenigmaticus* is restricted to the summit of Cerro Arbolado, Cerro Hakú, Caribbean slopes of Cerro Utyum, Valle del Silencio, and Caribbean slopes of Cerro Pando (Figure 1). The altitudinal range of this taxon is 2330–2700 m a.s.l. The species occurs in primary forest; and the populations on Cerro Arbolado, Cerro Hakú, Cerro Utyum, and Valle del Silencio are within the La Amistad International Park and that on Cerro Pando is within the Zona Protectora Las Tablas. The distribution of *C. aenigmaticus* is fragmented along a line of ~ 80 km. We did not find this species during fieldwork carried out on Cerro Kamuk and Cerro Echandi. More fieldwork is required to assess the range of this species more

accurately. Given that this species is found at Cerro Pando, which is on the Costa Rica–Panama border, *C. aenigmaticus* is recognized as having a distribution within southeastern Costa Rica and at least for now, marginally into Panama.

Etymology.—The specific name is derived from the Latin word for enigmatic. We propose this name in light of the taxonomic confusion surrounding this species. *Craugastor aenigmaticus* was first collected in 2009 but was erroneously identified as *C. podiciferus* because of its great morphological resemblance to the latter, and its close proximity to the type locality of *C. podiciferus*. However, based on molecular data, we confirmed that this taxon is highly divergent genetically from other species in the *C. podiciferus* Species Group and represents a new species.

Discussion

With the recognition of *Craugastor aenigmaticus*, the *C. podiciferus* Species Group now comprises 10 species, all of which are endemic to Costa Rica and western Panama (Savage 2002, AmphibiaWeb 2018). However, the diversity within the *C. podiciferus* Species Group is underestimated and several species remain unnamed (Streicher *et al.* 2009). The high genetic divergence between *C. aenigmaticus* and all other members of the *C. podiciferus* Species Group strongly supports the distinctiveness of *C. aenigmaticus*. The above-mentioned genetic distances are greater than those suggested by Fouquet *et al.* (2007) for recognizing new taxa of Neotropical frogs, which is 3% 16S divergence as threshold to define candidate species. However, it is important to point out that although not closely related, *C. aenigmaticus* and *C. podiciferus* are morphologically similar. Furthermore, unlike other members of the group, both of these species inhabit the montane rainforest, although *C. podiciferus* is not restricted to it.


The montane rainforest of the Cordillera de Talamanca is naturally fragmented in two relatively large patches (Bolaños *et al.* 2005). One extends from Cerro Vueltas to Cerro Dúrika and the other from Cerro Arbolado to Cerro Ehandi, and both are separated by a depression (2300 m a.s.l.). *Craugastor aenigmaticus* inhabits the patch that extends from Cerro Arbolado to Cerro Ehandi. *Bolitoglossa kamuk*, *B. pygmaea*, *B. robinsoni*, and *B. splendida* are endemic to the same patch of montane rainforest. *Craugastor aenigmaticus* occurs in sympatry with *D. ventrimaculatus* in four localities, with the former only being known from one additional locality (Cerro Pando).

The restricted distribution of *Craugastor aenigmaticus* to montane rainforest highlights the importance of this habitat as center of diversification and endemism, and suggests that the highlands of the Cordillera de Talamanca have had an important role in the process of speciation, possibly in association with climatic fluctuations (Savage 2002, Streicher *et al.* 2009). At least eight amphibians are restricted to montane rainforest in Cordillera de Talamanca (Savage 2002, AmphibiaWeb 2018), a habitat that was naturally reduced along the last 2 million years and that is fragmented (Foster 2001).

Further studies are needed to help us resolve the phylogenetic relationships of *Craugastor aenigmaticus* within the *C. podiciferus* Species Group and to evaluate the biological significance of the high genetic distances that exist within the group. Additionally, it is important to continue inventory work in the montane rainforest to justify the need to protect the flora and fauna of this life zone, much of which may remain unknown.

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Appendix I. Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Museum codes follow those of Frost (2018), with the addition of CRARC in reference to the Costa Rica Amphibian Research Center private collection and EAP denotes field numbers of Erick Arias.

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number	
					16S	COI
<i>C. aenigmaticus</i> sp. nov.	SMF: 104020	Jurutungo, Changuinola, Bocas del Toro, PA	8°54' N, 82°42' W	2388	MK211615	MK211577
<i>C. aenigmaticus</i> sp. nov.	SMF: 104021	Jurutungo, Changuinola, Bocas del Toro, PA	8°55' N, 82°42' W	2330	MK211621	-
<i>C. aenigmaticus</i> sp. nov.	UCR: 21951	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2700	MK211616	MK211578
<i>C. aenigmaticus</i> sp. nov.	UCR: 21952	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2690	MK211618	-
<i>C. aenigmaticus</i> sp. nov.	UCR: 21954	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2627	MK211620	-
<i>C. aenigmaticus</i> sp. nov.	UCR: 21956	Cerro Utyum, Talamanca, Limón, CR	9°20' N, 83°10' W	2690	MK211619	-
<i>C. aenigmaticus</i> sp. nov.	UCR: 22737	Cerro Hakú, Buenos Aires, Puntarenas, CR	9°19' N, 83°12' W	2660	MK211617	MK211579
<i>C. aenigmaticus</i> sp. nov.	UCR: 22739	Cerro Hakú, Buenos Aires, Puntarenas, CR	9°19' N, 83°12' W	2660	MK211622	-
<i>C. bransfordii</i>	UCR: 22269	San Miguel, Alajuela, Alajuela, CR	10°18' N, 84°10' W	466	KT950295	MK211571
<i>C. bransfordii</i>	UCR: 22643	Guayacán, Siquirres, Limón, CR	10°03' N, 83°32' W	537	MK211610	MK211572
<i>C. gabbi</i>	UCR: 21863	Fila Costeña, Coto Brus, Puntarenas, CR	8°47' N, 82°57' W	1200	KT950271	MK211567
<i>C. gabbi</i>	UCR: 21864	Fila Costeña, Coto Brus, Puntarenas, CR	8°47' N, 82°57' W	1200	KT950272	MK211568
<i>C. lauraster</i>	SMF: 79759	Selva Negra, Matagalpa, Matagalpa, NI	12°59' N, 85°54' W	1300	MK211608	MK211565
<i>C. lauraster</i>	USNM: 559393	Bodega del Río, Tapalwás, Gracias a Dios, HN	14°55' N, 84°32' W	150	KU323364	MK211566
<i>C. persimilis</i>	UCR: 22211	Tausito, Paraiso, Cartago, CR	9°47' N, 83°45' W	1050	KT950293	MK211570
<i>C. persimilis</i>	UCR: 22671	Suretka, Talamanca, Limón, CR	9°34' N, 82°56' W	121	MK211609	MK211569
<i>C. podiciferus</i> s.l.	CRARC: 012	Volcán Turrialba, Turrialba, Cartago, CR	10°01' N, 83°42' W	2250	MK211633	MK211589
<i>C. podiciferus</i> s.l.	CRARC: 247	Monte Verde, Tilarán, Guanacaste, CR	10°21' N, 84°48' W	1470	MK211645	-
<i>C. podiciferus</i> s.l.	EAP: 509	Fila Costeña, Golfito, Puntarenas, CR	8°47' N, 83°01' W	1546	-	MK211605
<i>C. podiciferus</i> s.l.	EAP: 810	Alto Uren, Talamanca, Limón, CR	9°21' N, 83°02' W	1860	MK211640	MK211596
<i>C. podiciferus</i> s.l.	EAP: 817	Alto Uren, Talamanca, Limón, CR	9°23' N, 83°01' W	1500	MK211630	MK211586

Appendix I. Continued.

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number	
					16S	COI
<i>C. podiciferus s.l.</i>	FMNH: 257651	Fila Costeña, Coto Brus, Puntarenas, CR	8°47' N, 82°59' W	1350	EF562367	-
<i>C. podiciferus s.l.</i>	FMNH: 257669	Monte Verde, San Ramón, Alajuela, CR	10°18' N, 84°47' W	1500	EF562372	MK211598
<i>C. podiciferus s.l.</i>	FMNH: 257671	Monte Verde, San Ramón, Alajuela, CR	10°18' N, 84°47' W	1500	EF562374	MK211599
<i>C. podiciferus s.l.</i>	FMNH: 257673	Monte Verde, San Ramón, Alajuela, CR	10°18' N, 84°47' W	1500	EF562343	MK211603
<i>C. podiciferus s.l.</i>	SMF: 104005	Changena, Changuinola, Bocas del Toro, PA	8°59' N, 82°40' W	1766	MK211641	MK211597
<i>C. podiciferus s.l.</i>	UCR: 16353	Montaña Azul, Sarapiquí, Heredia, CR	10°12' N, 84°09' W	1500	EF562349	MK211602
<i>C. podiciferus s.l.</i>	UCR: 16361	Tapesco, Alfaro Ruiz, Alajuela, CR	10°13' N, 84°22' W	1930	EF562371	-
<i>C. podiciferus s.l.</i>	UCR: 16585	Copey, Dota, San José, CR	9°32' N, 83°51' W	1400	MK211647	-
<i>C. podiciferus s.l.</i>	UCR: 19853	Lori, Talamanca, Limón, CR	9°21' N, 83°13' W	1817	MK211639	MK211595
<i>C. podiciferus s.l.</i>	UCR: 19856	Lori, Talamanca, Limón, CR	9°21' N, 83°13' W	1817	MK211637	MK211593
<i>C. podiciferus s.l.</i>	UCR: 19860	Lori, Talamanca, Limón, CR	9°21' N, 83°12' W	2108	MK211636	MK211592
<i>C. podiciferus s.l.</i>	UCR: 19862	Lori, Talamanca, Limón, CR	9°21' N, 83°12' W	2108	MK211638	MK211594
<i>C. podiciferus s.l.</i>	UCR: 20992	Nectandra, Alfaro Ruiz, Alajuela, CR	10°13' N, 84°20' W	2143	MK211632	MK211588
<i>C. podiciferus s.l.</i>	UCR: 22091	Quebradas, Pérez Zeledón, San José, CR	9°26' N, 83°40' W	1488	MK211646	MK211604
<i>C. podiciferus s.l.</i>	UCR: 22120	Altamira, Buenos Aires, Puntarenas, CR	9°19' N, 83°27' W	1821	MK211642	-
<i>C. podiciferus s.l.</i>	UCR: 22146	Cascajal, Coronado, San José, CR	10°01' N, 83°56' W	1700	MK211635	MK211591
<i>C. podiciferus s.l.</i>	UCR: 22201	El Empalme, El Guarco, Cartago, CR	9°42' N, 83°56' W	2395	MK211634	MK211590
<i>C. podiciferus s.l.</i>	UCR: 22226	Pico Blanco, Escazú, San José, CR	9°51' N, 84°08' W	2242	MK211644	MK211601
<i>C. podiciferus s.l.</i>	UCR: 22228	Pico Blanco, Escazú, San José, CR	9°51' N, 84°08' W	2242	MK211643	MK211600
<i>C. podiciferus s.l.</i>	UCR: 22675	Monte Verde, Puntarenas, Puntarenas, CR	10°19' N, 84°47' W	1726	-	MK211606
<i>C. podiciferus s.l.</i>	UCR: 22690	Chumacera, Pérez Zeledón, San José, CR	9°19' N, 83°28' W	1793	MK211631	MK211587
<i>C. polyptychus</i>	UCR: 20050	Dabagri, Talamanca, Limón, CR	9°37' N, 83°16' W	900	MK211614	MK211576
<i>C. polyptychus</i>	UCR: 22668	Bribri, Talamanca, Limón, CR	9°36' N, 82°54' W	198	MK211613	MK211575

Appendix I. Continued.

Species	Institutional vouchers	Collection locality	Geographic coordinates	Elevation (m a.s.l.)	GenBank Number	
					16S	COI
<i>C. stejnegerianus</i>	EAP: 0514	Palmar Norte, Osa, Puntarenas, CR	8°57' N, 83°26' W	45	MK211607	MK211563
<i>C. stejnegerianus</i>	UCR: 20352	Potrero Grande, Buenos Aires, Puntarenas, CR	9°05' N, 83°06' W	900	KT950284	MK211564
<i>C. underwoodi</i>	UCR: 22619	Tapantí, Paraíso, Cartago, CR	9°45' N, 83°46' W	1412	MK211611	MK211573
<i>C. underwoodi</i>	UCR: 22625	Cascajal, Coronado, San José, CR	10°01' N, 83°56' W	1708	MK211612	MK211574
<i>Craugastor</i> sp.1	UCR: 20389	Potrero Grande, Buenos Aires, Puntarenas, CR	9°06' N, 83°06' W	1500	MK211625	MK211581
<i>Craugastor</i> sp.1	UCR: 22709	Las Alturas, Coto Brus, Puntarenas, CR	8°58' N, 82°49' W	1980	MK211626	MK211582
<i>Craugastor</i> sp.2	SMF: 104014	Cerro Saguí, Nole Duima, Ngöbe Buglé, PA	8°33' N, 81°49' W	1762	MK211623	-
<i>Craugastor</i> sp.2	SMF: 104015	La Nevera, Nole Duima, Ngöbe Buglé, PA	8°30' N, 81°46' W	1700	MK211624	MK211580
<i>Craugastor</i> sp.B	SMF: 102024	Fortuna, Gualaca, Chiriquí, PA	8°40' N, 82°11' W	1730	MK211627	MK211583
<i>Craugastor</i> sp.B	SMF: 104023	Fortuna, Gualaca, Chiriquí, PA	8°40' N, 82°12' W	1280	MK211628	MK211584
<i>Craugastor</i> sp.B	SMF: 104027	Volcán Barú, Bugaba, Chiriquí, PA	8°50' N, 82°30' W	2134	MK211629	MK211585

Appendix II. *Specimens used in the morphometric analysis. Museum collection acronyms follow Frost (2018), with the addition of EAP refers to Erick Arias field numbers.*

Craugastor aenigmaticus sp. nov. COSTA RICA: LIMÓN: Cerro Utyum, Telire, Talamanca (UCR: 21951–21952, 22413–22415); Valle del Silencio, Telire, Talamanca (UCR: 21194, 21931–21932). PUNTARENAS: Cerro Arbolado and Cerro Hakú, Buenos Aires, Buenos Aires (EAP: 758–763; UCR: 22731–22734, 22737, 22747).

Craugastor podiciferus. COSTA RICA: ALAJUELA: Poasito, Sabanilla, Alajuela (UCR: 21317–21319); Zarcero, Palmira, Alfaro Ruiz (UCR: 20992–20994); Los Alpes, Piedades Sur, San Ramón (UCR: 13961–13962). Santa María, Aguas Claras, Upala (UCR: 10528). CARTAGO: Coris, Quebradilla, Cartago (UCR: 3490–3491); Empalme, San Isidro, Guarco (UCR: 22200, 22202, 22204–22205); Tapantí, Orosí, Paraíso (UCR: 11542, 12010, 21708); Vereh, Chirripó, Turrialba (EAP: 748–750). HEREDIA: San José de la Montaña, Barva (UCR: 21299, 21352); Cerro Chompipe, Varablanca, Heredia (UCR: 18403–18404); La Legua, Varablanca, Heredia (UCR: 17464, 17478); Montaña Azul, Cureña, Sarapiquí (UCR: 16354). LIMÓN: Fila Matama, Matama, Limón (UCR: 20177, 20213); Cerro Pat, Telire, Talamanca (EAP: 0792, 801–803, 807, 810, 814, 817–818); Sabanas Dúrika, Telire, Talamanca (UCR: 21662, 21682, 21825, 21836); Transtalamanca, Telire, Talamanca (UCR: 19849, 19853, 19860, 19870, 19874, 19895, 19933). PUNTARENAS: Cerro Quemado, Biolley, Buenos Aires (UCR: 21191, 21700); Las Cruces, San Vito, Coto Brus (UCR: 12934, 12936, 13239); Las Tablas, Sabalito, Coto Brus (UCR: 8379, 8381–8382, 10738, 10747–10748, 12560); Monte Verde, Puntarenas (UCR: 13646–13647, 17243, 21718, 22674–22676). SAN JOSÉ: Pico Blanco, San Antonio, Escazú (UCR: 22228); Tinamaste, Barú, Pérez Zeledón (UCR: 14510, 14513, 14518); Quebradas, San Isidro del General, Pérez Zeledón (EAP: 738–740; UCR: 16359–16360, 22606); Chumacera, San Pedro, Pérez Zeledón (EAP: 721–723; UCR: 22119); Cascajal, Vazquez de Coronado (UCR: 16090, 16092, 21918).

Craugastor sp.1. COSTA RICA: PUNTARENAS: Las Alturas, Pittier, Coto Brus (UCR: 22703–22704, 22709–22710); Tres Colinas, Potrero Grande, Buenos Aires (EAP: 725, 727–729; UCR: 20257–20258, 20389, 20395, 20401, 20411, 20419, 20421, 20423, 20428); road to Las Tablas, Sabalito, Coto Brus (EAP: 0823).