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Deep cryptic diversity in the *Craugastor podiciferus* species group (Anura: Craugastoridae) of Isthmian Central America revealed by mitochondrial and nuclear data

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The *Craugastor podiciferus* Species Group contains eleven species of terraranan frogs distributed from eastern Honduras to eastern Panama. All species have remarkable color pattern polymorphisms, which may contribute to potential taxonomic problems. We performed exhaustive sampling throughout the distribution of the group to evaluate the phylogenetic relationships and biogeographic history of all named species based on two mitochondrial markers and nuclear ddRAD loci. We also implemented various species delimitation methods to test for the presence of unconfirmed candidate species within the group. Molecular phylogenetic analyses showed that the group contains four major clades. All currently named species are supported by molecular data, yet species richness within the group is clearly underestimated. Species delimitation was discordant between the mitochondrial and nuclear datasets and among analytical methods. Adopting a conservative approach, we propose that the *C. podiciferus* species group contains at least 12 unconfirmed candidate species. Ancestral area reconstruction showed that the group originated and diversified in the highlands of the Talamancan montane forest ecoregion of Costa Rica and western Panama.

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Abstract

The *Craugastor podiciferus* Species Group contains eleven species of terraranan frogs distributed from eastern Honduras to eastern Panama. All species have remarkable color pattern polymorphisms, which may contribute to potential taxonomic problems. We performed exhaustive sampling throughout the distribution of the group to evaluate the phylogenetic relationships and biogeographic history of all named species based on two mitochondrial markers and nuclear ddRAD loci. We also implemented various species delimitation methods to test for the presence of unconfirmed candidate species within the group. Molecular phylogenetic analyses showed that the group contains four major clades. All currently named species are supported by molecular data, yet species richness within the group is clearly underestimated. Species delimitation was discordant between the mitochondrial and nuclear datasets and among analytical methods. Adopting a conservative approach, we propose that the *C. podiciferus* species group contains at least 12 unconfirmed candidate species. Ancestral area reconstruction showed that the group originated and diversified in the highlands of the Talamancan montane forest ecoregion of Costa Rica and western Panama.

Keywords: Ancestral area reconstruction, Costa Rica, historical biogeography, candidate species, species delimitation, systematics, taxonomy

Introduction

Species diversity is not homogeneously distributed over the globe. The American tropics have among the highest biodiversity in the world, including seven of 25 biodiversity hotspots (*Myers et al., 2000*). The Mesoamerica biodiversity hotspot includes the Isthmian Central America

(ICA) region centered in Costa Rica and Panama and is renowned for its exceptional biodiversity and endemism (*Bagley & Johnson, 2014*). This region hosts more species of amphibians (*Savage 2002; AmphibiaWeb 2023*), reptiles (*Savage 2002; Solórzano 2022*), birds (*Anger & Dean 2010; Garrigues & Dean 2014*), insects (*Doré et al. 2021*), orchids (*Bogarin et al. 2013; Crain & Fernández 2020*) and vascular plants (*Davis et al. 1997*) per area unit than almost any other place in the world. The high biodiversity of the ICA has been attributed to two major factors. First, the closure of the Isthmus of Panama allowed the Great American Biotic Interchange (GABI) between North America and South America, resulting in the coexistence of long-independent lineages of mammals and other organisms (*Savage, 1966; Vanzolini & Heyer, 1985; Marshall, 1988; Webb, 2006; Pinto-Sánchez et al., 2012*). Second, the long history of volcanic and orogenic activity in the ICA resulted in high geographic and climatic heterogeneity (*Weyl, 1980; Herrera, 1985; Bagley & Johnson, 2014; Montes et al., 2015; García-Rodríguez et al., 2021*) that has promoted *in situ* diversification and high levels of endemism associated with diverse habitats (*Savage, 2002; Boza-Oviedo et al., 2012; Bogarin et al., 2013; Doré et al., 2021; Solórzano, 2022*).

Given its small area and the relatively large and active multinational taxonomic communities working in the ICA, new species are continually discovered and described. However, extensive areas remain unexplored, and some particular groups, such as amphibians, still lack taxonomic resolution. Previous molecular systematic studies on direct-developing frogs (i.e., terraranans such as *Craugastor*, *Diasporus*, and *Pristimantis*) and direct-developing plethodontid salamanders (especially *Bolitoglossa*) have revealed extremely high levels of genetic diversity in the highlands and lowlands of the ICA (*García-París et al., 2000; Crawford, 2003; Crawford, Bermigham & Polanía-S, 2007; Wiens et al., 2007; Wang, Crawford &*

Bermigham, 2008; Streicher, Crawford & Edwards, 2009; Batista et al. 2016; García-Rodríguez, Arias & Chaves, 2016).

The *Craugastor podiciferus* Species Group (Anura: Craugastoridae; *Hedges, Duellman & Heinicke, 2008*) is currently composed of eleven described species (*Arias, Hertz & Parra-Olea, 2019*). This group occurs from eastern Honduras to eastern Panama, covering a wide variety of habitats and ranging in elevation from sea level to 2700 m (*Savage & Emerson, 1970; Savage, 2002*). Previous molecular studies on the systematics and taxonomy of the *C. podiciferus* species group support the presence of several undescribed species (*Crawford & Smith, 2005; Arias, Hertz & Parra-Olea, 2019*). *Crawford (2003),* as inferred from the high genetic divergences found between populations of *C. stejnegerianus* in the ICA lowlands on the Pacific coast. *Streicher, Crawford & Edwards (2009)* mentioned that the name *C. podiciferus* could mask a species complex formed by up to six distinct taxa and supported the existence of an undescribed species (*Craugastor* sp. B) related to *C. podiciferus*. According to mitochondrial sequences and morphological data, *Arias et al. (2016)* showed that populations formerly considered part of *C. stejnegerianus* from southwestern Costa Rica and western Panama belong to a different species, *C. gabbi*.

The *Craugastor podiciferus* species group represents an ideal model for studies of amphibian cryptic diversity, given its high local abundance, collectively wide geographic distribution, high genetic diversity, and high levels of polymorphism. To our knowledge, no molecular studies have extensively evaluated the phylogenetic relationships and the potential cryptic diversity of an amphibian species group restricted to ICA, including populations both from highlands and lowlands and using mitochondrial markers and an extensive nuclear dataset. Here, we use mitochondrial gene sequences and a genome-scale dataset to 1) infer the

phylogenetic relationships of the *C. podiciferus* species group, 2) determine the existence of overlooked species within the currently recognized species, and 3) identify the center of origin for the group and suggest a possible historical framework for its diversification.

Materials & Methods

Taxon sampling

Tissue samples were collected from all eleven named species of the *C. podiciferus* species group in all countries where the group occurs: Honduras, Nicaragua, Costa Rica, and Panama (Fig. 1, Appendix I). All of the specimens collected for this study were humanly euthanized through the use of a 20% lidocaine hydrochloride (Xylocaine) injection, and all efforts were made to minimize suffering. The specimens were then fixed in a 10% formalin solution, and transferred to 70% ethanol for long-term storage. Tissue samples used for genetic analyses were preserved in 96% ethanol or in RNAlater™. Vouchers were deposited at the Museo de Zoología, Universidad de Costa Rica (UCR), the Division of Amphibians and Reptiles at the Field Museum of Natural History, Chicago, USA (FMNH), Círculo Herpetológico de Panamá (CH), and Senckenberg Research Institute and Nature Museum, Frankfurt, Germany (SMF). Museum collection acronyms follow *Frost (2023)*, with the addition of the following three collectors' field numbers: AJC referring to Andrew J. Crawford, AH referring to Andreas Hertz, and EAP referring to Erick Arias

Collecting permits

The Costa Rican Ministry of Environment and Energy (MINAE) provided the corresponding scientific collection permits for this research (SINAC-SE-GAS-PI-R 007-2013 and 59-2015). Collecting permits for Panama SE/A-30-08, SC/A-8-09, SC/A-28-09, and SC/A-21-10, as

well as the corresponding exportation permits, were issued by the Ministerio de Ambiente (MiAmbiente), Panama City, Panama. Collecting permits for Nicaragua No. 006–062009 was issued by Ministerio del Ambiente y los Recursos Naturales, Managua, Nicaragua.

Mitochondrial data

Amplification and sequencing

We extracted total genomic DNA from the preserved tissue samples using the Animal Genomic DNA Kit (BioBasic Canada Inc.), the DNeasy Blood & Tissue Kit (Qiagen), or the phenol–chloroform standard extraction protocol (*Sambrook & Russell, 2006*). We amplified fragments of two mitochondrial genes: the large subunit ribosomal RNA (16S) and the 5'-end of cytochrome oxidase subunit I (COI), also known as the DNA barcode of life (*Hebert et al., 2003*). The primers 16Sar and 16Sbr were used to amplify 16S (*Palumbi et al., 1991*) and dgLCO plus dgHCO for COI (*Meyer, 2003*). Amplifications were performed using a total volume of 15 µL, which contained 1 µL DNA template (*approx.* 50 ng μL^{-1}), 0.75 U Taq polymerase (Amplificasa®, Biotecnologias Moleculares), 1X PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3 – 0.5 µM forward and reverse primers. The PCR conditions were as follows. For 16S, we employed an initial cycle of 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 30 s at 50°C or 55°C, and 45 s or 120 s at 72°C, plus a final extension cycle of 3 min at 72°C. For the COI fragment, we used 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 amplification primers and BigDye termination reaction chemistry (Applied Biosystems). The cycle-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

We generated 16S and COI sequences for the *C. podiciferus* species group members. We used sequences of *C. loki* to root mtDNA trees, based on *Crawford & Smith (2005)*. See Appendix 1 for a list of the examined material, their localities, museum voucher, and GenBank accession numbers. Sequences of each gene were trimmed at the 3' and 5' ends until a majority of operational taxonomic units (OTUs) had sequence data for a given character. The two genes were aligned independently using MUSCLE 3.7 (*Edgar, 2004*) with default parameters and then concatenated. We used PartitionFinder v2.1.1 software (*Lanfear et al., 2017*) 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*), and the search for the best partition scheme used a heuristic search (*scheme=greedy Lanfear et al., 2012*). We defined, *a priori,* four subsets: one for 16S and three for COI (partitioned by codon position). The selected partition scheme and substitution selected by PartitionFinder were used in Bayesian MCMC phylogenetic inference.

We used maximum likelihood (ML) and Bayesian MCMC methods to infer phylogenetic trees from the concatenated loci. All phylogenetic analyses were run on the CIPRES portal (*Miller, Pfeiffer & Schwartz, 2010*). We performed ML analyses using RAxML-HPC v8 (*Stamatakis, 2014*) with an unpartitioned GTR + GAMMA model of nucleotide substitution (the default model of RAxML) and the –f an option, which searches for the best-scoring tree and performs a rapid bootstrap analysis (1000 bootstrap replicates) to estimate node support by

resampling characters with replacement. A partitioned Bayesian MCMC phylogenetic analysis was performed using MrBayes 3.2.6 (*Ronquist et al., 2012*) with the previously selected partition scheme and substitution model (see above). Two separate analyses were run, each consisting of 50 million generations, sampling trees every 1000 generations and using 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 *allcompat* tree along with the posterior probabilities for each node and each parameter.

We used the program BEAST v1.8.3 (*Drummond et al., 2012*) to estimate a concatenated ultrametric phylogenetic tree (timetree) using an uncorrelated lognormal relaxed clock, a birth-death process tree prior, and with the partition scheme and nucleotide substitution model selected previously. We ran the analysis for 50 million generations, sampling trees every 1000 generations, and discarded the first 5000 samples as burn-in when estimating a consensus tree.

Nuclear data

ddRADseq data collection

We generated ddRADseq data for 48 samples of the *C. podiciferus* species group plus one sample of *C. rhodopis* as an outgroup. We followed the protocol described by *Peterson et al. (2012)* and modified by *Leaché et al. (2015)*. High-molecular-weight genomic DNA was further purified with RNase A, examined for quality on agarose gels, and quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific). We used 1000 ng of genomic DNA for each sample, except for three samples that had just 241–630 ng. We double-digested the genomic DNA with 20 units each of a rare 8-cutter, SbfI (restriction site 5′-CCTGCAGG-3′), and a common 4-

cutter, MspI (restriction site 5′-CCGG-3′), in a single reaction with the manufacturer recommended buffer (New England Biolabs) for 2 h at 37 °C. Postdigestion fragments were purified with Serapure 1.5X and quantified with a Qubit 2.0 fluorometer before ligating barcoded Illumina adaptors onto the fragments.

The oligonucleotide sequences used for barcoding and adding Illumina indexes during library preparation were those employed in *Leaché et al. (2015)*. The barcodes differed by at least two base pairs to reduce the chance of errors caused by inaccurate base calls subsequent to barcode assignment. Equimolar amounts of each sample were pooled in a 96-well plate format, with each pool containing up to eight unique barcoded samples. Each pool was purified with Serapure 1.5X, rehydrated in 50 μL, and quantified with a Qubit 2.0 fluorometer before size selection. The pooled libraries were size-selected (~500 bp) on an e-gel (Invitrogen) according to the manufacturer's instructions. The two external and internal lanes (next to the leader lane) in the e-gel were not used, while in the four available lines, only two different libraries were run, with a maximum of 500 ng per line. The size-selected libraries were purified again with a Qubit 2.0 fluorometer and amplified using PCR with the primers designed by *Leaché et al. (2015)* and Phire Hot Start II polymerase (Thermo Fisher Scientific). The amplified libraries were purified with Serapure 1.5X and quantified with a Qubit 2.0 fluorometer.

The fragment size distribution and concentration of each pool were determined on an Agilent BioAnalyzer, and qPCR was performed to determine sequenceable library concentrations before multiplexing equimolar amounts of all 6 pools for sequencing on a single Illumina HiSeq 2000 lane (100 bp, single-end run) at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley.

ddRADseq bioinformatics

We processed raw Illumina reads with the software pipeline ipyrad v0.5.15 (Eaton, 2014), which consists of seven steps. We first demultiplexed the samples using their unique barcode and adapter sequences. Before the second step, six of the 48 samples had <50,000 reads passing the quality filter and were excluded from further analyses. In the second step, the remaining 42 samples were edited and filtered. The 6-bp restriction site overhang and the 5-bp barcode were removed. Bases with an accuracy of < 99% (Phred quality score = 20) were converted to 'N' characters, and reads with >9 Ns (~10% Ns) were discarded.

During steps 3–6, the reads from each sample were clustered using the program VSEARCH version 1.11.1 (https://github.com/torognes/vsearch). The first clustering step establishes homology among reads within samples. We determined the optimal value for the clustering parameter using the clustering threshold series approach described by *Ilut, Nydam & Here (2014)*. This method, which maximizes the number of clusters inferred to contain two distinct haplotypes, seeks to assemble reads into loci such that false homozygosity (splitting reads from a single locus into two) and false heterozygosity (due to clustering of paralogs) are minimized (i.e., the optimum clustering threshold). We generated a clustering threshold series (sensu *Ilut, Nydam & Here, 2014*) using similarity thresholds ranging from 0.85 to 0.98 for 19 randomly chosen samples. The optimal clustering threshold was 0.9 (Fig. S1a), which was used within and between sample clustering. After clustering reads within samples, we estimated the error rate and heterozygosity from the base counts at each site across all clusters, and these values were used to generate consensus sequences for each cluster. Consensus sequences were then clustered across samples and aligned with MUSCLE version 3.8.31 (*Edgar, 2004*). Within steps 3–6, we also discarded loci that had >4 ambiguous or heterozygous sites (default ipyrad

settings) or >2 haplotypes (to filter out paralogs) and used a minimum depth of coverage of 6 for genotype calls.

Following *Nieto-Montes de Oca et al. (2017)*, we performed multiple replicates of the seventh step to determine the optimal value for the parameters: maximum numbers of SNPs allowed in a locus, maximum number of insertions/deletions allowed in among-sample clusters, and the maximum proportion of samples allowed to share a heterozygous site. The number of retained loci increased linearly with higher numbers of SNPs allowed until it began to plateau at a maximum of 30 SNPs per locus (Fig. S1b). We selected a maximum of 20 SNPs per locus (default ipyrad settings) for the assembly of the final dataset under the rationale that above this value, the small number of additional loci that were retained potentially represented paralogs. The number of loci retained increased with higher numbers of indels allowed until a maximum of 8 indels (default ipyrad settings; Fig. S1c). Thus, we permitted a maximum of 8 insertions/deletions per locus for the assembly of the final dataset. We also examined the sensitivity of the final dataset to changing the maximum proportion of samples allowed to share a heterozygous site (between 0–0.5). The number of loci retained increased roughly linearly (Fig. S1d) until it first plateaued at a value of 0.1 (corresponding to 4 samples), which we again chose for the final value under the rationale that loci exhibiting higher shared heterozygosity potentially represented paralogs. We set the minimum proportion of ingroup samples with data for a given locus to be retained in the final dataset to 26% (11 samples).

Phylogeny reconstruction

We used RAxML and Bayesian MCMC methods to estimate phylogenetic trees from the concatenated ddRAD loci, which contained 697,771 characters for 42 samples. We ran MrBayes

253 and BEAST analyses using the same priors as in the mitochondrial data analyses (see above),
254 except only one GTR+I+G model was used for analyzing the unpartitioned RADseq data. To
255 evaluate the effect of the number of terminals in the topology, we also performed RAxML,
256 MrBayes, and BEAST analyses for each of the three major clades within the *C. podiciferus*
257 species group (*C. bransfordii* clade, *C. podiciferus* clade, and *C. stejnegerianus* clade; see
258 Results). To perform the analyses by clade, we replicated the pipeline in ipyrad for each clade,
259 maximizing the number of retained loci according to the same filters applied to the complete
260 dataset (see above).
261
262 ***Relaxed molecular clock analysis***
263 We used the program BEAST v1.8.3 (*Drummond et al., 2012*) to estimate a concatenated
264 ultrametric phylogenetic tree (timetree) using the nuDNA dataset with the same prior as in
265 *Mitochondrial data: Phylogenetic analyses*. We use this analysis to perform a time calibration
266 for the *C. podiciferus* S.G. The lack of *C. podiciferus* in the fossil record makes dating
267 divergences based on molecular sequence data difficult. We used the dating for the group
268 estimated by *Streicher, Crawford & Edwards (2009)* to estimate divergence times within the
269 species group. Following *Streicher, Crawford & Edwards (2009)* and citations therein, we
270 assumed that ICA emerged ~25 Mya and used a secondary calibration of 20 Mya for the crown
271 age of the *C. podiciferus* species group using a normal prior distribution with a mean of 20 Mya
272 and SD of 2 Mya to place 95% of the prior distribution on 16.7–23.3 Mya.
273
274 **Species delimitation**

We used six methods to address species boundaries in the *C. podiciferus* species group and evaluated the effect of the phylogeny assumed. We performed analyses separately on the mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) datasets; however, these analyses were performed similarly. Species delimitation based on the nuclear dataset was run using both the complete phylogeny and the partial phylogenies (independent analyses of each of the three main ingroup clades [see *Results*]: the *C. bransfordii*, *C. podiciferus,* and *C. stejnegerianus* clades), except for the BPP analysis, where only the partial phylogenies were used. Using mtDNA, 24 combinations were performed to combine three tree inputs and six methods; 37 combinations were performed using nuDNA.

We used three species discovery methods, GMYC, PTP, and mPTP, that infer putative species limits on a given phylogenetic tree. The GMYC method (*Pons et al., 2006; Fujisawa & Barraclough, 2013*) infers the transition point between interspecific (Yule process) and intraspecific (coalescent process) branching rates on a time-calibrated ultrametric tree. We ran the GMYC analyses separately on each of the two datasets, concatenated mitochondrial and concatenated ddRADseq data, using the web server (http://species.h-its.org/gmyc/) under the single threshold and multiple threshold GMYC models assuming the respective timetree from the BEAST analyses.

The PTP method (*Zhang et al., 2013*) uses the number of substitutions to identify significant changes in the rate of branching in a phylogenetic tree (which may or may not be ultrametric). We ran PTP in the web server (http://species.h-its.org/ptp/) for 500,000 generations, with thinning = 100 and burn-in = 10%. Following a conservative approach, we considered candidate species those clades with a posterior delimitation probability less than 0.01 (mitochondrial) or 0.05 (nuclear), where the posterior probability indicates that the clade in

298 question forms a single species. Given that PTP uses any completely bifurcating tree, we used
299 the RAxML, MrBayes, and BEAST trees to evaluate the effect of the input tree. As with the
300 GMYC method, we performed PTP separately on the mitochondrial DNA (mtDNA) and nuclear
301 DNA (nuDNA) datasets. All analyses were replicated excluding the out-group; thus, we
302 conducted a total of twelve PTP analyses.

303 The third method, mPTP (*Kapli et al., 2016*), is similar to PTP but incorporates different
304 rates of coalescence within clades, allowing different levels of intraspecific genetic diversity.
305 Similar to PTP, mPTP runs both ML and MCMC analyses. MCMC analyses were run for 100
306 million generations, sampling once every 10,000 generations, and the first 2 million generations
307 were discarded as burn-in. All ML and Bayesian analyses were run as both *single* and *multiple*
308 rates of coalescence among species and resolved any polytomies in the input tree randomly by
309 adding a branch of length 0.0001. For the MCMC analyses, we conservatively considered clades
310 with a delimitation support value greater than 0.99 (mitochondrial) or 0.95 (nuclear) as species.
311 As with the PTP analysis, we used our RAxML, MrBayes, and BEAST (consensus) trees to
312 evaluate the effect of the assumed tree; all analyses were performed including the out-group.

313 We used a fourth method, automatic barcode gap discovery (ABGD; *Puillandre et al.,*
314 *2012*). Unlike the three tree-based methods, ABGD uses genetic distances estimated from an
315 alignment of DNA sequences. This method was used only for the mitochondrial dataset, and
316 analyses were performed separately for 16S and COI. The method seeks to quantify the possible
317 'barcode gap' location that separates intraspecific from interspecific distances. We used Pmin
318 (0.01), Pmax (0.1), JC69 corrected distances, and a relative gap width of 1.5 (default).

319 For our fifth method of species delimitation, we used BPP version 3.1 (*Yang, 2015; Yang*
320 *& Rannala, 2010, 2014*) to jointly perform species delimitation and species tree inference under

the multispecies coalescent model. We used method A10, which evaluates species delimitation from a guide tree, using a rjMCMC algorithm (*Rannala & Yang, 2013*). Each individual is assigned to a putative species, and the rjMCMC algorithm evaluates subtrees generated by collapsing or splitting nodes on the guide tree without performing any type of branch swapping. We used the ultrametric topology of the BEAST analyses as a user-specified guide tree and analyzed mtDNA and nuDNA datasets independently. The analysis was run for 500,000 generations (sampling interval of 5) with a burn-in period of 1000 generations. We evaluated the influence of the ancestral population size (θ) and root age (τ_0) considering three different combinations of parameter values, as in *Leaché & Fujita (2010)*. The first combination of prior distributions is $\theta \sim$ gamma prior G (1, 10) and $\tau_0 \sim$ G (1, 10). The second combination of priors is $\theta \sim$ G (2, 2000) and $\tau_0 \sim$ G (2, 2000). The third combination is a mixture of priors: $\theta \sim$ G (1, 10) and $\tau_0 \sim$ G (2, 2000). When using the mtDNA tree as the guide tree, we used algorithm 0 with the fine-tuning parameter ε=15 each with 500,000 generations (saving each fifth sample) and a burn-in of 10,000 generations. With the nuclear dataset, the number of generations was 100,000, and the other parameters were the same as above. Each analysis was run at least twice to confirm consistency between runs. To be conservative, only speciation events simultaneously supported by posterior probabilities ≥ 0.99 for all three combinations of priors were considered for species delimitation.

As our sixth and final approach to species delimitation, we used simple genetic distances based on each mtDNA dataset separately. Although not considered a formal species delimitation algorithm, genetic distance has been used as an indicator of candidate species. For amphibians, the 16S gene fragment has been suggested as a DNA barcode marker for diversity inventories in amphibians (*Vences et al., 2005*) to complement the newer standardized marker COI-5' used for

344 animals (*Smith et al., 2008*). *Fouquet et al. (2007)* suggested a threshold of 3% in the 16S marker
345 to identify candidate species. *Vences et al. (2005)* suggested a threshold of 10% in the COI
346 barcode for identifying candidate species. Genetic distances (uncorrected p-distances, Table 1)
347 were computed using MEGA7 (*Tamura et al., 2013*) for each gene separately.
348 Given that it is almost impossible that all the different species delimitation methods agree
349 on the same results, we should interpret these results using our expertise on the group and take
350 into account other data such as their morphology and ecology. To propose a more accurate
351 number of species within the *C. podiciferus* S.G., we iteratively compared the results from the
352 mtDNA and nuDNA datasets and prioritized the following: 1) We recognized all 11 currently
353 named species within the *C. podiciferus* S. G; 2) additional unnamed species can be suggested if
354 they are supported by all the combinations of methods within a dataset (mtDNA or nuDNA); 3)
355 additional unnamed species can be suggested to reconcile discordant results among mtDNA and
356 nuDNA to avoid synonymizing named species that were supported by a dataset (mtDNA or
357 nuDNA); and 4) additional unnamed species can be suggested if morphological evidence
358 distinctively supports monophyletic clades found in both mtDNA and nuDNA.
359
360 **Ancestral area reconstruction**
361 We used the nuDNA BEAST time-calibrated tree to infer the ancestral areas. The distribution
362 range of the *Craugastor podiciferus* species group was divided into five areas based on the
363 terrestrial ecoregions of the world proposed by *Olson et al. (2001)*: (A) Costa Rican seasonal
364 moist forest, (B) Isthmian-Atlantic moist forest, (C) Isthmian-Pacific moist forest, (D)
365 Talamancan montane forest and (E) Choco-Darien moist forest. We used the R package
366 BioGeoBEARS (*Matzke, 2014*) and implemented the DEC model (*Ree & Smith, 2008*) within a

maximum likelihood framework. Furthermore, a founder-event speciation parameter, J, was added to each of these models. Because no species is distributed over more than four defined areas, we set the maximum number of areas to four.

Results

Phylogeny of the *Craugastor podiciferus* Species Group

Mitochondrial phylogeny

The resulting mtDNA data matrix included 96 sequences with a total alignment length of 1216 bp, including gaps (559 bp of 16S and 657 bp of COI). PartitionFinder recommended a GTR+I+G substitution model for 16S and recommended codon positions 1 through 3 of COI receive a K80+I+G, HKY+I, and GTR+G, respectively. The pairwise mitochondrial genetic distances are shown in Table 1.

The mtDNA phylogenies inferred with maximum likelihood and Bayesian analysis from MrBayes (Fig. 2) were almost identical in topology. The clade *C. aenigmaticus* (red) was consistently inferred as the sister clade to all other species within the species group. The *C. podiciferus* clade (blue) contained two well-supported subclades, one including only species restricted to the highlands of southwestern Costa Rica and western Panama (*C. blairi, C. sagui,* and *C. zunigai*) and a second highly structured subclade containing *C. podiciferus* and six additional populations from the highlands of Costa Rica. The *C. bransfordii* clade (purple) was not supported as monophyletic, but six other clades were well-supported. *C. bransfordii* was the sister clade to an unnamed species from the Caribbean slope of the Talamanca Mountain range. An unnamed species from Panamá was consistently supported; *C. underwoodi* was supported as the sister clade to an unnamed species from Caribbean Costa Rica. A sixth unnamed clade from

the Pacific slopes of Costa Rica was supported as the sister clade to all *C. stejnegerianus* clades. The *C. stejnegerianus* clade (green) contained four well-supported clades, including *C. persimilis,* which was consistently supported as the sister clade to all other species within the *C. stejnegerianus* clade. *Craugastor gabbi* was supported as the sister clade to the clade formed by *C. stejnegerianus* + *C. rearki.*

The Bayesian MCMC timetree obtained using BEAST (Fig. 3) was similar to phylogenies inferred by RAxML and MrBayes (Fig. 2); the main difference was that the Bayesian analysis moderately supported the monophyly of the *C. bransfordii* clade (pp = 0.88). Within the *C. bransfordii* clade, the unnamed clade from the Pacific slopes of Costa Rica was supported as the sister clade to all species within the clade. The next branching clade was the clade formed by *C. bransfordii* + an unnamed species from the Caribbean highlands of Costa Rica. Finally, an unnamed species from Panamá was consistently supported as the sister clade to the clade formed by *C. underwoodi* + an unnamed species from Caribbean Costa Rica.

In summary, the three phylogenies inferred using mtDNA recovered strong support for a monophyletic *C. podiciferus* species group relative to our outgroup, *C. loki*, and strong support for three major clades: a monotypic *C. aenigmaticus* clade, plus *C. podiciferus* and *C. stejnegerianus* clades. Formal species delimitation results are presented below, while here, we count 'unnamed lineages' as the smallest possible number of monophyletic groups that will neither lump nor split named species.

Nuclear phylogeny

The Illumina HiSeq2500 lane generated 66,364,543 reads demultiplexed to 42 samples. The nuclear data matrix contained 7,770 loci and 697,771 characters, with 64.4% missing data (total

count of Ns in the matrix). RAxML, MrBayes, and BEAST recovered the same topology (Fig. 4). As in the BEAST analysis of the mtDNA data (see above), the ddRADseq data supported the *C. podiciferus* species group being composed of four major clades, with only minor differences among inference methods for relationships within each of the four clades. *Craugastor aenigmaticus* (red) was again sister to all other samples within the species group. As with the mtDNA analyses, the *C. podiciferus* clade (blue) was composed of 2 subclades, one containing the three highland species, *C. blairi, C. sagui*, and *C. zunigai*, and a second highly structured subclade containing *C. podiciferus* and six additional populations from the highlands of Costa Rica (1000–2700 m a.s.l.). The nuclear analysis did not include several populations phylogenetically close to *C. podiciferus* in the mitochondrial phylogeny.

The *C. bransfordii* clade (purple) contains five of the six clades recorded in the mtDNA analyses, *C. bransfordii, C. underwoodi*, and three additional unnamed lineages, but the relationships within this clade differed in the nuclear DNA topology. In the ddRADseq tree, *Craugastor* sp. Panama is sister to the other lineages (essentially from Costa Rica; compare with Fig. 2-3). The *C. stejnegerianus* clade (green) supported the monophyly of *C. gabbi, C. persimilis,* and *C. rearki*. The monophyletic clade *Craugastor stejnegerianus* inferred by mtDNA analysis (see *Craugastor stejnegerianus, Craugastor* sp. Neilly, and *Craugastor* sp. Quepos in Fig. 2-3) was split into three clades in the nuDNA phylogenies; one was the sister clade to *C. rearki,* a second clade (*C. stejnegerianus* [sensu stricto]) was the sister clade to the clade formed by *C. gabbi* + a third clade unnamed.

Species delimitation

We found considerable differences between the mitochondrial and nuclear datasets and among the various delimitation methods (Figs. 3 and 4). Based on the mitochondrial data, using some methods, such as GMYC and PTP, we identified > 60 candidate species, while using others, such as mPTP Bayesian delimitation, resulted in only 13 candidate species. The nuclear data based on 42 specimens showed several differences among the species delimitation methods; some (*e.g.*, mPTP Bayesian on MrBayes and RAxML trees) only recognized a single species for the entire complex, whereas others (*e.g.*, mPTP maximum likelihood) identified up to 36 species. We also found incongruences when comparing results for the same clade based on the complete phylogeny versus those based on clade-specific subsets of the data. For example, within the *C. stejnegerianus* clade, with the GMYCm and PTP methods, we identified six species using the clade-specific phylogeny versus the three identified species using the complete phylogeny. In contrast, within the *C. podiciferus* clade, we identified 3–5 species using the GMYCm and PTP methods over the complete phylogeny versus 1-2 species identified using a clade-specific phylogeny (Fig. 4). These results highlight the impact of the selection of the initial phylogeny on the results obtained by GMYC, PTP, and mPTP, which are tree-based methods.

According to our priorities for species delimitation, the 12 named species within the *C. podiciferus* S.G. were supported as monophyletic in mtDNA and nuDNA. In addition, in the mtDNA analyses, almost all the combinations of methods supported the 12 species as different species; therefore, we validated the 11 named species as different.

Following our second criterion, four additional clades, all within the *C. bransfordii* clade, were supported by all the combinations of methods in the mtDNA analyses, and therefore, we recognized it as an unconfirmed candidate species. These four clades are also supported by our third criterion, which reconciles the mtDNA trees and nuDNA tree. According to our third

458 criterion, two additional clades were recognized as unconfirmed candidate species within the *C.*
459 *stejnegerianus* clade to reconcile the mtDNA trees and nuDNA tree. In the mtDNA analyses,
460 these two clades were supported as monophyletic but delimited as *C. stejnegerianus*; however, in
461 the nuDNA analyses, they were not monophyletic (see above).
462 Finally, in our fourth criterion, we evaluated the populations contained within the 17
463 lineages identified above. We found that the nominal species *C. podiciferus,* as identified by the
464 species delimitation methods, is highly variable, but this variation is fixed in monophyletic
465 clades; therefore, we suggested that this clade contains at least 6 additional unconfirmed
466 candidate species. However, these were not delimitated in the mtDNA or nuDNA analyses. We
467 found morphological and acoustic evidence (*E Arias, 2023, unpublished data*) that supported the
468 distinction of these monophyletic clades. In addition, some of these clades have been previously
469 suggested as different species (*Streicher, Crawford & Edwards,* 2009; *Arias, Hertz & Parra-*
470 *Olea,* 2019). In summary, by integrating multiple species delimitation methods and our
471 taxonomic expertise in this species group, we suggest that it is formed by at least 23 lineages of
472 named species and unconfirmed candidate species.
473
474 **Biogeography**
475 Ancestral area reconstruction assuming the BEAST tree for ddRADseq data inferred a
476 Talamancan origin for the *C. podiciferus* species group during the middle Miocene (Fig. 5). A
477 Talamancan origin for the *C. podiciferus* clade was also supported, with a dispersal event to
478 Isthmian-Pacific moist forest (lowland) and another dispersal event to Costa Rican seasonal
479 moist forest. Our data support the origin of the *C. stejnegerianus* + *C. bransfordii* clade in
480 Isthmian-Atlantic moist forest, with five independent dispersal events from the Isthmian-Atlantic

moist forest to Talamancan montane forest during the Pliocene-Pleistocene and three dispersal events to Isthmian-Pacific moist forest, explaining the current patterns of distribution. The two clades that are sister to all other clades, *Craugastor aenigmaticus* clade (in red) and *C. podiciferus* clade (in blue), are restricted to highlands (1000–2700 m), yet all 23 lineages have populations above 750 m in the Talamancan montane forest (Figs. 1–2). Lineages found in the highlands have narrower elevational ranges, except *C. podiciferus* and *C. blairi* (Fig. 1–2); all lineages distributed under 1000 m have altitudinal ranges greater than 750 m in extent, but four out of 15 lineages restricted to highlands have altitudinal ranges smaller than 250 m. In addition, the highlands of the Pacific versant are more structured; in the Pacific versant, eight lineages are distributed exclusively over 1000 m a.s.l. However, in the Atlantic versant, only five lineages are restricted to highlands.

Discussion

Systematics and biogeography of the *C. podiciferus* species group

The highlands of the ICA played an important role in the diversification of several groups of vertebrates (*García-París et al., 2000; Savage, 2002; Castoe et al., 2009; Boza-Oviedo et al., 2012; Duellman, Marion & Hedges, 2016; Arias, Chaves & Parra-Olea, 2018; Arias, Hertz & Parra-Olea, 2019*), and given that the basal clades within the nuDNA and mtDNA trees are restricted to the highlands of the ICA (Figs. 2 and 5), the *C. podiciferus* species group appears to follow this pattern. We used the results of *Streicher, Crawford & Edwards (2009)* as our prior for the time of origin for the most recent common ancestor (MRCA) of the *C. podiciferus* species group between 16.8 and 27.8 Mya when the ancestor dispersed from Nuclear Central America to the ICA when the latter emerged as a peninsula at its current position (*Montes et al., 2015*). Our posterior estimates matched our priors, indicating that our data are at least consistent with the

four major clades within the *C. podiciferus* species group having diverged during the Miocene. Diversification within the ICA during the Miocene and Pliocene has been found in other northern lineages of anurans (*Duellman, Marion & Hedges, 2016*), pitvipers (*Castoe et al., 2009*), and freshwater fishes (*Říčan et al., 2013*).

All identified lineages of the *C. podiciferus* species group are distributed from eastern Honduras to eastern Panama in an area smaller than 40,000 km^2. *Craugastor aenigmaticus* is restricted to Talamanca montane forest, ranging from 2330–2700 m, the highest elevational distribution for any species in this group (*Arias, Chaves & Parra-Olea, 2018*). *Craugastor aenigmaticus* is sister to the rest of the species group, and according to our ancestral area reconstruction, the ancestor was distributed in the Talamanca montane forest and diversified to lower elevations (Fig. 5). The *C. podiciferus* clade, as suggested by *Streicher, Crawford & Edwards (2009)*, possibly diversified due to climatic fluctuations that isolated the suitable habitat on peaks in the mountain ranges. The *C. bransfordii* + *C. stejnegerianus* clade contains species ranging from sea level to 1600 m elevation. The *C. bransfordii* clade diversified mainly on the Caribbean slopes of Nicaragua, Costa Rica, and Panama. Only one lineage was found on the Pacific slopes of Costa Rica. No obvious barriers separate the species of the *C. bransfordii* clade, except for paleoclimatic differences. The *C. stejnegerianus* clade contains more species on the Pacific slope of Costa Rica, with only two species distributed on the Caribbean slopes of Nicaragua, Costa Rica, and Panama. Possibly, the drier conditions found on the Pacific slope of Costa Rica during the Pleistocene (*Crawford, Bermigham & Polanía-S, 2007*) allowed for the diversification of this clade in this region, isolating the ancestors in areas with more humidity.

In the lowlands, the climatic oscillations and the fluctuations in sea level during the Pliocene may have fragmented distribution ranges, isolating populations and restricting gene

528 flow. These climatic fluctuations mainly affected the Pacific slopes (*Savage, 1966; Crawford,*
529 *Bermigham & Polanía-S, 2007*). If these fluctuations were long enough, they could explain the
530 high genetic structure found here, with species having narrow elevational and latitudinal ranges
531 (Figs. 2 and 5). The Pacific slopes have more highland lineages than the Caribbean slope. This
532 could be due to the climatic heterogeneity found there; for example, the mean annual
533 precipitation varies from ~1 800 mm in the Northern Pacific to nearly 5 000 mm in the Southern
534 Pacific in less than 400 lineal km (*Savage, 1966; Coen, 1991*). Recently, this heterogeneity in the
535 Pacific slopes has been suggested to promote speciation in three species of *Anolis* lizards
536 distributed along the Central and South Pacific (*Chaves et al. In press*).
537
538 **Species delimitation and taxonomic comments**
539 Seven named species and candidate species (*C. aenigmaticus, C. blairi, C. persimilis, C.*
540 *podiciferus, C. sagui, C. zunigai,* and *Craugastor* sp. Panama) were supported as distinct
541 evolutionary lineages in most analyses. Nevertheless, we found substantial differences in
542 delimitation results between mitochondrial and nuclear analyses and among methods. We
543 identified 23 lineages (Figs. 6–7), including named species and unconfirmed candidate species
544 (Appendix I). Our approach aims to minimize taxonomic instability while recognizing the full
545 biodiversity contained within this group. Below are details of the major clades and their species.
546
547 ***Craugastor aenigmaticus***
548 This species (Fig. 7A) was consistently supported as a distinct evolutionary lineage in all
549 mtDNA and nuDNA analyses. This species is notably separated from other *C. podiciferus*
550 species group members by mean uncorrected genetic distances greater than 13.3% in 16S and

19.3% in COI (Table 1).

The Craugastor podiciferus clade

The species *C. blairi* (Fig. 7B)*, C. sagui* (Fig. 7C), and *C. zunigai* (Fig. 7D) are each confirmed as distinct species from the topotypic *C. podiciferus* by several delimitation methods. The mitochondrial and nuclear phylogenies placed them as a monophyletic group sister to *C. podiciferus* + candidate species (Figs. 2–4). They are separated from each other by mean uncorrected genetic distances of at least 3.4% in 16S and 17.3% in COI (Table 1). *Craugastor blairi, C. sagui*, and *C. zunigai* are allopatric and distributed latitudinally in southwestern Costa Rica and western Panama (*Arias, Hertz & Parra-Olea, 2019*). *Craugastor blairi* corresponds to *Craugastor* sp. B of *Crawford & Smith (2005)* and clade G of *Streicher, Crawford & Edwards (2009)*.

The number of species masked under the name *C. podiciferus* remains unclear. We consistently identified several candidate species with mitochondrial sequence data, but only some were included in the species delimitation analyses based on nuDNA data. *Streicher, Crawford & Edwards (2009)* performed a phylogenetic analysis for this clade, suggesting that it was composed of six species. Of the seven candidate species in our mitochondrial phylogeny, only three were included in the nuclear dataset, which were supported as separate species in BPP analyses.

We included samples from the type locality of *C. podiciferus* (Fig. 7E-F). The type locality was discussed by *Savage (1970)* and *Arias & Chaves (2014)*, who later corrected the type locality to the Caribbean slope of Cerro Kamuk. Here, we restrict *C. podiciferus* to the populations of Cordillera Volcánica Central from Costa Rica and the Cordillera de Talamanca of

Costa Rica and western Panama. In the Cordillera de Talamanca, *C. podiciferus* is restricted to the Caribbean slopes.

Based on mitochondrial data, we identified seven lineages within the name *C. podiciferus*, some of which also differ morphologically (*E Arias, 2023, unpublished data*). These lineages are separated from each other by mean uncorrected genetic distances of 2.0–10.5% in 16S and 7.3–23.4% in COI (Table 1). Thus, we suggest that these seven lineages represent separate species (*C. podiciferus sensu stricto* and 6 unconfirmed candidate species). *Streicher, Crawford & Edwards (2009)* included samples for four of these taxa: *C. podiciferus sensu stricto* corresponds to clades C and D of *Streicher, Crawford & Edwards* (2009), *Craugastor* sp. Monte Verde (Fig. 7G) corresponds to clade A of *Streicher, Crawford & Edwards* (2009), a species restricted to the Cordillera de Tilarán and Cordillera Volcánica Central. *Craugastor* sp. San Gerardo (Fig. 7H) corresponds to clade B of *Streicher, Crawford & Edwards (2009)*, a species distributed on the Cordillera de Tilarán and Volcánica Central. In Monte Verde *Craugastor* sp. Monte Verde and *Craugastor* sp. San Gerardo are possibly in sympatry, but this has not yet been confirmed. Sympatry between *C. podiciferus* and *Craugastor* sp. Monte Verde near Zarcero might also be possible. *Craugastor* sp. Fila Costeña (Fig. 7I) corresponds to clades E and F of *Streicher, Crawford & Edwards (2009)*, a species restricted to southern Pacific Costa Rica. The remaining unconfirmed candidate species *Craugastor* sp. Siola (Fig. 7L), *Craugastor* sp. Pico Blanco (Fig. 7J), and *Craugastor* sp. Chumacera (Fig. 7K) have not been included in any previous work. *Craugastor* sp. Pico Blanco is known only from one site in Valle Central. *Craugastor* sp. Chumacera is known only from one site on the Pacific slope of the Cordillera de Talamanca, and *Craugastor* sp. Siola is known from a single population on the Caribbean slope of the Cordillera de Talamanca.

Streicher, Crawford & Edwards (2009) estimated a time to MRCA of the *C. podiciferus* clade of between 4.70 and 8.18 Ma. We hypothesize that the lack of support for the distinctness of these taxa in some species delimitation methods (*e.g.,* mPTP, BPP) may reflect the fact that they are recently derived, as shown by their lower genetic divergence. However, morphological and acoustic evidence (*E Arias, unpublished data*) suggests that they are nonetheless on evolutionarily independent trajectories and therefore should be recognized as separate species.

The Craugastor bransfordii clade

The nuclear phylogeny supports the monophyly of the *C. bransfordii* clade but not the RAxML and MrBayes of the mitochondrial analyses. We suggest that the *C. bransfordii* clade is composed of six separate species, only five of which were included in the nuclear analysis. These six lineages are separated from each other by mean uncorrected genetic distances between 4.1–11.3% in 16S and 9.5–17.3% in COI (Table 1). *Craugastor bransfordii* (Fig. 7M) samples included a specimen (UCR 20559, ID 78) collected near the type locality, San Juan River, on the border between Costa Rica and Nicaragua. *Craugastor bransfordii* is distributed from northern Nicaragua to central Caribbean Costa Rica. The nominal species *C. polyptychus* was described with specimens from the same type locality as *C. bransfordii* and in the same publication (*Cope, 1886*). It was later recognized as a synonym of *C. bransfordii* (*Savage & Emerson, 1970; Miyamoto, 1983*) until *Savage (2002)* resurrected this name and assigned it to specimens from Caribbean Costa Rica but noted that further taxonomic work is needed to clarify the status of the species. Since only one lineage within the *C. bransfordii* clade was found in northern Costa Rica and southern Nicaragua, we suggest that *C. polyptychus* should be referred to as a junior synonym of *C. bransfordii. Craugastor* sp. Fila Carbón (Fig. 7N) corresponds –in part– to *C.*

620 *polyptychus* of *Savage (2002)*, but since the type locality of *C. polyptychus* is outside of the
621 range of our *C.* sp. Fila Carbón, this unconfirmed candidate species, will require a new formal
622 description and name. Based on all but one (mPTPbs) of the 24 mtDNA species delimitation
623 analyses, including genetic distance, this lineage represents an unconfirmed candidate species
624 distributed in southeastern Caribbean Costa Rica and western-most Panama.
625 *Craugastor underwoodi* (Fig. 7O) includes specimens from Vázquez de Coronado (ID 82),
626 near the type locality. This species is distributed in the premontane forest of the Cordillera de
627 Guanacaste, Cordillera de Tilarán, Cordillera Volcánica Central, and the northern edge of the
628 Cordillera de Talamanca. *Craugastor* sp. Quebradas (Fig. 7P) includes specimens from the only
629 locality known on the Pacific slope for a member of the *C. bransfordii* clade. We consider that
630 *Craugastor* sp. Quebradas represents a separate species due to its allopatric distribution and the
631 large genetic distances from all other samples, with 15.3% or higher in COI (Table 1).
632 *Craugastor* sp. Vereh (Fig. 7Q) includes specimens from two localities in the premontane forest
633 in the central Caribbean of Costa Rica. Finally, *Craugastor* sp. Panama (Fig. 7R) is composed of
634 specimens from Panama that were assigned to *C. bransfordii* (*Leenders, 2016*), but based on the
635 mitochondrial and nuclear phylogenies, this clade from Panama is not closely related to *C.*
636 *bransfordii.* We suggest that these populations from Panama represent an unconfirmed candidate
637 species.
638
639 ***The Craugastor stejnegerianus clade***
640 Within the *C. stejnegerianus* clade, large differences were found between the mitochondrial and
641 nuclear phylogenies, mainly in the relationships between the recently described *C. gabbi* and *C.*
642 *stejnegerianus.* We suggest that the *C. stejnegerianus* clade is composed of six independent

643 lineages. *Craugastor persimilis* (Fig. 7U) is represented here by specimens from the type
644 locality, Suretka, Cantón de Talamanca (ID 43). *Craugastor persimilis* is distributed on the
645 central and southern Caribbean slopes of Costa Rica (Fig. 6). The specimens from Honduras (ID
646 58) and Nicaragua (ID 51–52) that were tentatively referred to as *C. lauraster* (*McCranie, 2006*;
647 type locality in Honduras) are closely related to specimens from the central Caribbean coast of
648 Costa Rica (ID 50, 55–57) that correspond to *C. rearki* (*Taylor, 1952*), synonymized earlier
649 under *C. bransfordii* by *Savage & Emerson (1970)*. We included one specimen from Siquírres
650 (ID 50), near the type locality of *C. rearki*, and one specimen from Pococí (ID 55), a locality of
651 paratypes of *C. rearki.* These specimens agree morphologically with *C. rearki.* We suggest that
652 the name *C. rearki* (Fig. 7V) should be resurrected to include populations from the Caribbean of
653 Costa Rica, Nicaragua, and Honduras, and the newer name, *C. lauraster*, should be referred to as
654 a junior synonym of *C. rearki.*

655 There are considerable differences between the mitochondrial and nuclear phylogenies on
656 the relationship of *C. stejnegerianus* from the Pacific slopes and the other members of the clade.
657 *Arias et al. (2016)* supported the distinctiveness of *C. stejnegerianus* (Fig. 7S) and *C. gabbi* (Fig.
658 7T) based on mitochondrial phylogeny, morphology, and ecological preferences. Cossel *et al.*
659 (2018) recorded differences in advertisement calls among *C. gabbi, C. stejnegerianus,* and
660 *Craugastor* sp. Quepos (referred to as *C. stejnegerianus* Northern). The mitochondrial phylogeny
661 clusters *C. stejnegerianus* with *Craugastor* sp. Neilly and *Craugastor* sp. Quepos, whereas in the
662 nuclear phylogeny, *C. gabbi* is sister to *C. stejnegerianus sensu stricto.* Based on both molecular
663 analyses, we recognize *Craugastor* sp. Neilly (Fig. 7 W), restricted to the southeast Pacific of
664 Costa Rica, and *Craugastor* sp. Quepos (Fig. 7X) (the sister to *C. rearki*) are distributed in the
665 central Pacific and Central Valley of Costa Rica. These three species are separated from *C.*

persimilis, C. gabbi, and *C. rearki* by mean uncorrected genetic distances between 4.2–9.0% in 16S and 11.6–17.2% in COI (Table 1).

Conclusions

The diversity within the *Craugastor podiciferus* species group is vastly underestimated, as revealed by the presence of several undescribed species recovered from the phylogenetic and species delimitation analyses. An exhaustive morphological review of the genetic lineages may show morphological characteristics that would allow for the differentiation of the molecular lineages. Comprehensive studies are needed on habitat use, acoustics, behavior, and other data to better understand the taxonomy of all lineages revealed here.

Based on our mitochondrial and nuclear analyses, we recovered 23 lineages, 11 with names and 12 unconfirmed candidate species. Based on our results, we propose the following taxonomic changes:

- We restrict *C. podiciferus* to populations of Cordillera Volcánica Central from Costa Rica and the Cordillera de Talamanca of Costa Rica and western Panama. In the Cordillera de Talamanca, *C. podiciferus* is restricted to the Caribbean slopes.
- *Craugastor polyptychus* is referred to as a junior synonym of *C. bransfordii.*
- *Craugastor rearki* is resurrected to include wide-ranging populations from the Caribbean versant of Costa Rica, Nicaragua, and Honduras.
- *Craugastor lauraster* is referred to as a junior synonym of the older name, *C. rearki.*
- Finally, we want to highlight the need to continue exploring remote areas in the ICA, especially in the Talamanca Mountain range. The fieldwork performed in this area has resulted in the discovery of several new species or new records for the region. Therefore, more fieldwork and laboratory work are necessary to improve the knowledge of

biodiversity in this region to perform informed strategies of conservation.

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Gabriela Parra Olea is an Academic Editor for PeerJ.

Author Contributions

- Erick Arias conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Andrew J. Crawford contributed reagents/materials/analysis tools, authored or reviewed drafts of the article, and approved the final draft.
- Andreas Hertz contributed reagents/materials/analysis tools.
- Gabriela Parra-Olea conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the article, and approved the final draft.

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Figure 1

FIGURE 1. Geographic distribution of the *Craugastor podiciferus* Species Group

Geographic distribution of the *Craugastor podiciferus* Species Group from Honduras (top) through Nicaragua, Costa Rica, to central Panama (right side). The mountains of southeastern Costa Rica are referred to as the Talamanca in the text. The purple shapes correspond to the *Craugastor bransfordii* clade; green shapes = *C. stejnegerianus* clade; blue shapes = *C. podiciferus* clade; red shape = *C. aenigmaticus* clade**.**

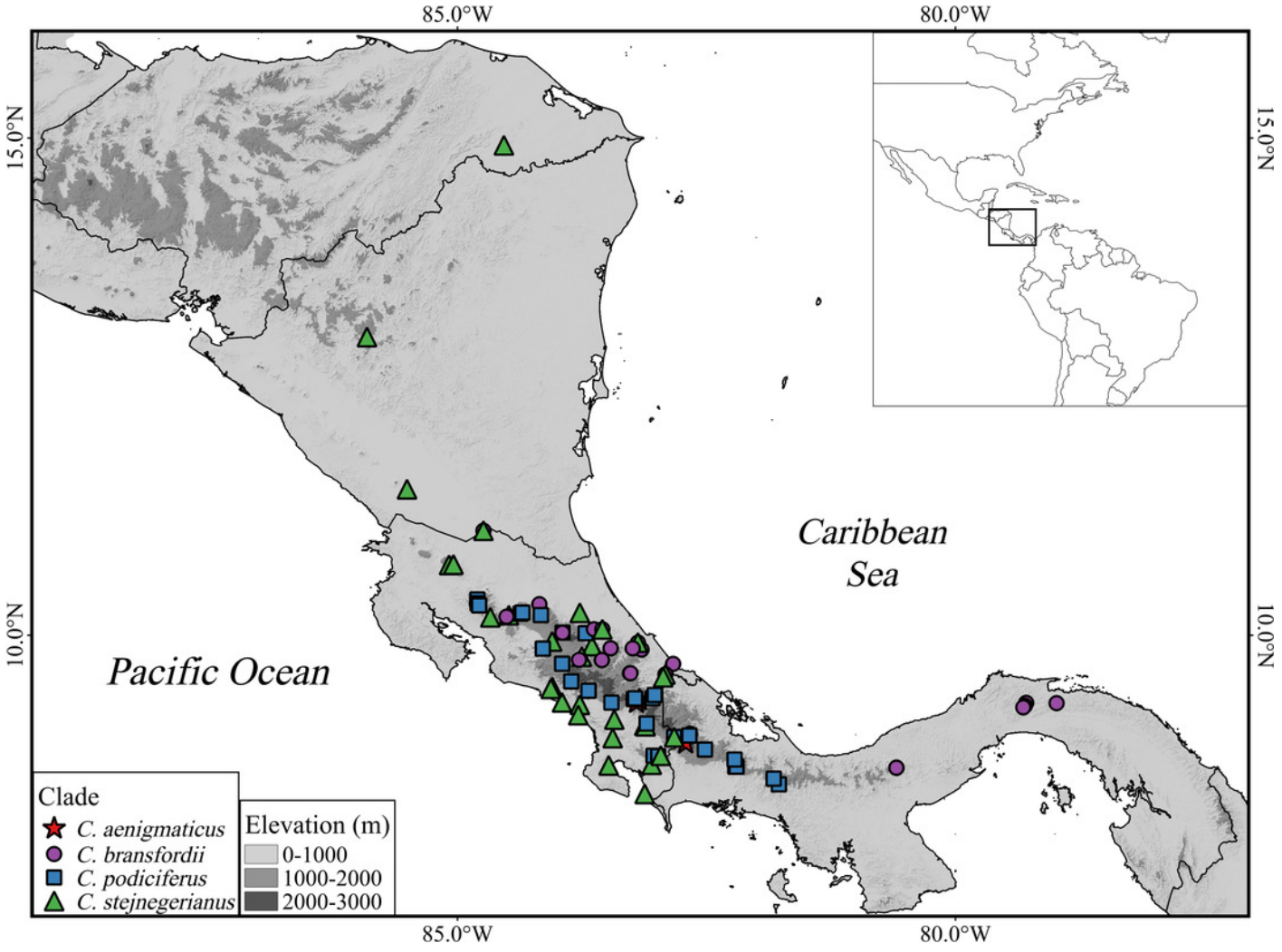

Figure 2

FIGURE 2. Bayesian phylogram derived from MrBayes of the *Craugastor podiciferus* Species Group based on the 16S and COI mitochondrial DNA gene markers

Bayesian phylogram derived from MrBayes of the *Craugastor podiciferus* Species Group based on the 16S and COI mitochondrial DNA gene markers. Bootstraps proportions are shown above branches. Below the branches are shown posterior probabilities (multiplied by 100) from MrBayes analysis (left) and posterior probabilities (multiplied by 100) from BEAST analysis (right). The scale bar refers to the estimated substitutions per site. The asterisks represent posterior probability values > 0.95. Colors represent the following groups identified as clades (Fig. 1): Purple (*Craugastor bransfordii* 'clade'), green (*C. stejnegerianus* clade), blue (*C. podiciferus* 'clade'), and red (*C. aenigmaticus* clade). Numbers in parentheses correspond individual ID number provided in Appendix I. In the insert rectangle (left) is show the elevational distribution of lineages within of the *Craugastor podiciferus* Species Group; black bars correspond with those lineages from Pacific slopes, brown bars correspond with those lineages from Caribbean slopes, and yellow bars correspond with those lineages from both slopes.

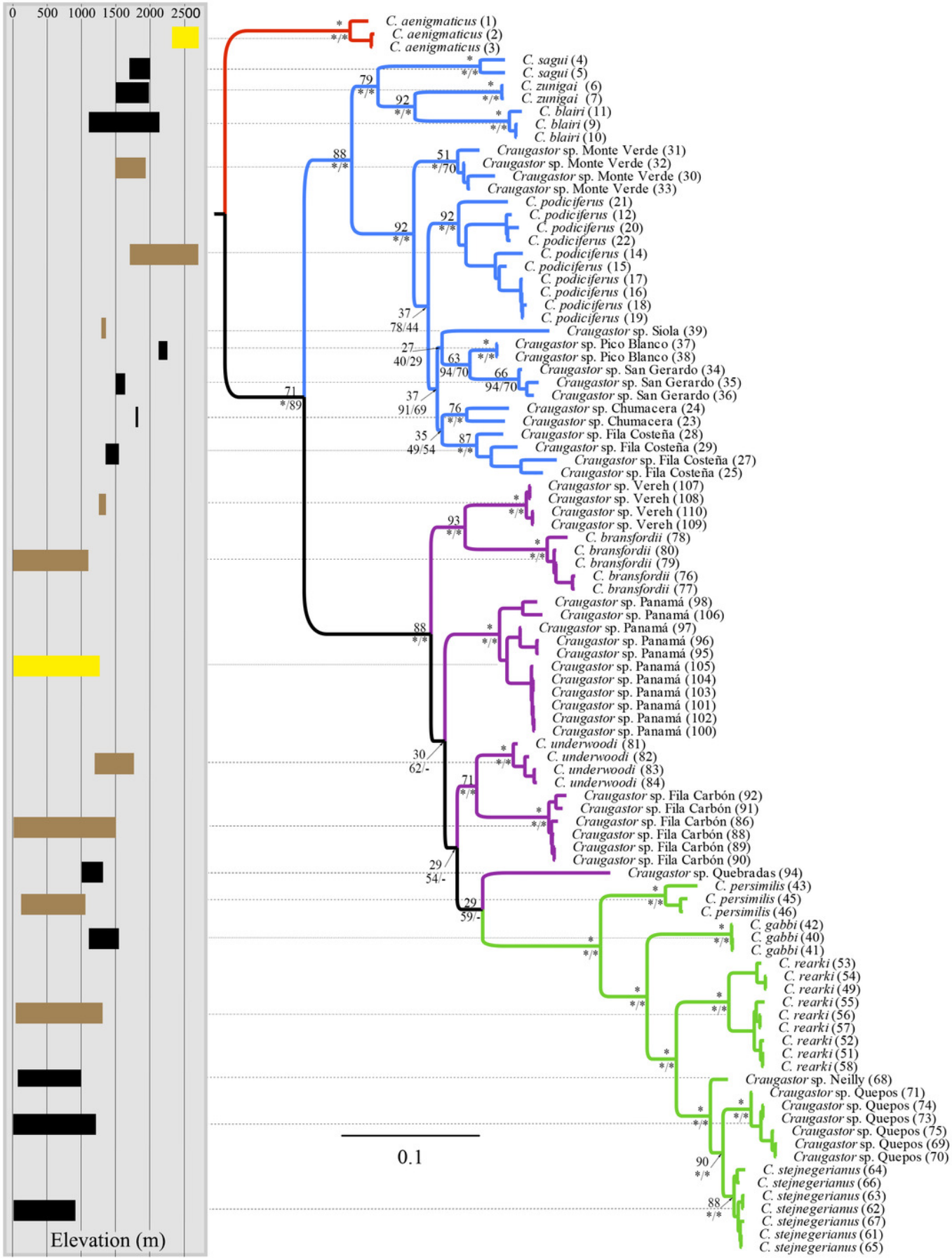
0
500
1000
1500
2000
2500
Elevation (m)
C. aenigmaticus (1)
C. aenigmaticus (2)
C. aenigmaticus (3)
C. sagui (4)
C. sagui (5)
C. zunigai (6)
C. zunigai (7)
C. blairi (11)
C. blairi (9)
C. blairi (10)
Craugastor sp. Monte Verde (31)
Craugastor sp. Monte Verde (32)
Craugastor sp. Monte Verde (30)
Craugastor sp. Monte Verde (33)
C. podiciferus (21)
C. podiciferus (12)
C. podiciferus (20)
C. podiciferus (22)
C. podiciferus (14)
C. podiciferus (15)
C. podiciferus (17)
C. podiciferus (16)
C. podiciferus (18)
C. podiciferus (19)
Craugastor sp. Siola (39)
Craugastor sp. Pico Blanco (37)
Craugastor sp. Pico Blanco (38)
Craugastor sp. San Gerardo (34)
Craugastor sp. San Gerardo (35)
Craugastor sp. San Gerardo (36)
Craugastor sp. Chumacera (24)
Craugastor sp. Chumacera (23)
Craugastor sp. Fila Costeña (28)
Craugastor sp. Fila Costeña (29)
Craugastor sp. Fila Costeña (27)
Craugastor sp. Fila Costeña (25)
Craugastor sp. Vereh (107)
Craugastor sp. Vereh (108)
Craugastor sp. Vereh (110)
Craugastor sp. Vereh (109)
C. bransfordii (78)
C. bransfordii (80)
C. bransfordii (79)
C. bransfordii (76)
C. bransfordii (77)
Craugastor sp. Panamá (98)
Craugastor sp. Panamá (106)
Craugastor sp. Panamá (97)
Craugastor sp. Panamá (96)
Craugastor sp. Panamá (95)
Craugastor sp. Panamá (105)
Craugastor sp. Panamá (104)
Craugastor sp. Panamá (103)
Craugastor sp. Panamá (101)
Craugastor sp. Panamá (102)
Craugastor sp. Panamá (100)
C. underwoodi (81)
C. underwoodi (82)
C. underwoodi (83)
C. underwoodi (84)
Craugastor sp. Fila Carbón (92)
Craugastor sp. Fila Carbón (91)
Craugastor sp. Fila Carbón (86)
Craugastor sp. Fila Carbón (88)
Craugastor sp. Fila Carbón (89)
Craugastor sp. Fila Carbón (90)
Craugastor sp. Quebradas (94)
C. persimilis (43)
C. persimilis (45)
C. persimilis (46)
C. gabbi (42)
C. gabbi (40)
C. gabbi (41)
C. rearki (53)
C. rearki (54)
C. rearki (49)
C. rearki (55)
C. rearki (56)
C. rearki (57)
C. rearki (52)
C. rearki (51)
C. rearki (58)
Craugastor sp. Neilly (68)
Craugastor sp. Quepos (71)
Craugastor sp. Quepos (74)
Craugastor sp. Quepos (73)
Craugastor sp. Quepos (75)
Craugastor sp. Quepos (69)
Craugastor sp. Quepos (70)
C. stejnegerianus (64)
C. stejnegerianus (66)
C. stejnegerianus (63)
C. stejnegerianus (62)
C. stejnegerianus (67)
C. stejnegerianus (61)
C. stejnegerianus (65)
0.1

Figure 3

FIGURE 3. Maximum clade credibility tree (left) from the BEAST analysis of *Craugastor podiciferus* Species Group

Maximum clade credibility tree (left) from the BEAST analysis of *Craugastor podiciferus* Species Group based on concatenated 16S and COI mitochondrial DNA gene fragments. Clade colors represent the following: purple (*Craugastor bransfordii* clade), green (*C. stejnegerianus* clade); blue (*C. podiciferus* clade) and red (monotypic *C. aenigmaticus* clade). Above the branches are shown posterior probabilities (multiplied by 100) from BEAST analysis; the asterisks represent support of >0.95 posterior probability. Numbers in parentheses correspond to individual ID numbers provided in Appendix I. Comparison of species delimitation results (right) based on the concatenated 16S and COI mitochondrial DNA markers, and for each gene separately (right-most four columns). The six different bar colors correspond to six species delimitation methods used (see section 2.4); the bars with same color represents different parameter settings for a given delimitation algorithm. The missing (white) patches in ABGD represent combination of clustering that cannot evaluate in this tree.

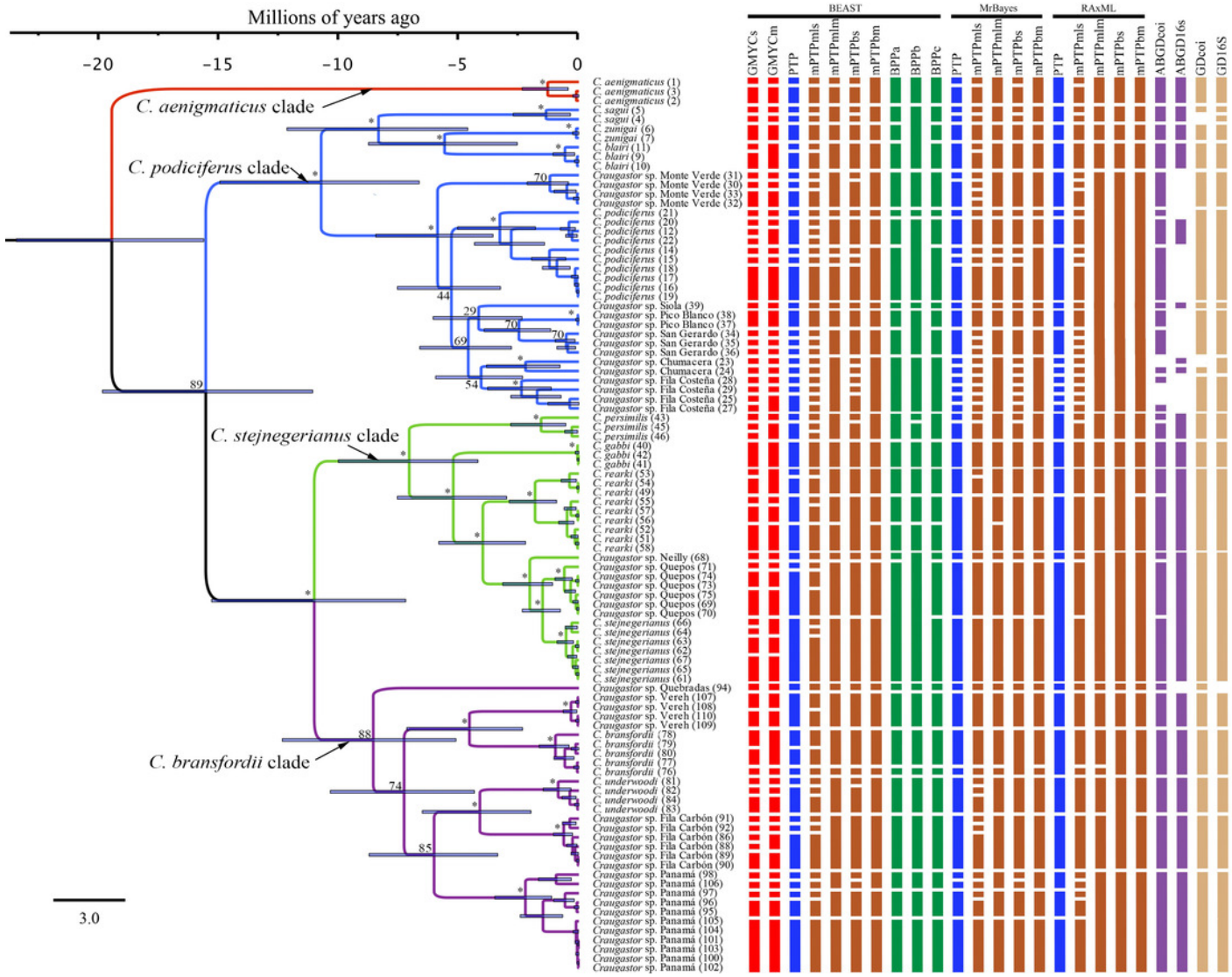
Millions of years ago
-20
-15
-10
-5
0
C. aenigmaticus clade
C. podiciferus clade
C. stejnegerianus clade
C. bransfordii clade
3.0
BEAST
MrBayes
RAxML
GMYCs
GMYCm
PTP
mPTPmls
mPTPmlm
mPTPbs
mPTPbm
BPPa
BPPb
BPPc
PTP
mPTPmls
mPTPmlm
mPTPbs
mPTPbm
PTP
mPTPmls
mPTPmlm
mPTPbs
mPTPbm
ABGDcoi
ABGD16s
GDcoi
GD16S
C. aenigmaticus (1)
C. aenigmaticus (3)
C. aenigmaticus (2)
C. sagui (5)
C. sagui (4)
C. zunigai (6)
C. zunigai (7)
C. blairi (11)
C. blairi (9)
C. blairi (10)
Craugastor sp. Monte Verde (31)
Craugastor sp. Monte Verde (30)
Craugastor sp. Monte Verde (33)
Craugastor sp. Monte Verde (32)
C. podiciferus (21)
C. podiciferus (20)
C. podiciferus (12)
C. podiciferus (22)
C. podiciferus (14)
C. podiciferus (15)
C. podiciferus (18)
C. podiciferus (17)
C. podiciferus (16)
C. podiciferus (19)
Craugastor sp. Siola (39)
Craugastor sp. Pico Blanco (38)
Craugastor sp. Pico Blanco (37)
Craugastor sp. San Gerardo (34)
Craugastor sp. San Gerardo (35)
Craugastor sp. San Gerardo (36)
Craugastor sp. Chumacera (23)
Craugastor sp. Chumacera (24)
Craugastor sp. Fila Costeña (28)
Craugastor sp. Fila Costeña (29)
Craugastor sp. Fila Costeña (25)
Craugastor sp. Fila Costeña (27)
C. persimilis (43)
C. persimilis (45)
C. persimilis (46)
C. gabbi (40)
C. gabbi (42)
C. gabbi (41)
C. rearki (53)
C. rearki (54)
C. rearki (49)
C. rearki (55)
C. rearki (57)
C. rearki (56)
C. rearki (52)
C. rearki (51)
C. rearki (58)
Craugastor sp. Neilly (68)
Craugastor sp. Quepos (71)
Craugastor sp. Quepos (74)
Craugastor sp. Quepos (73)
Craugastor sp. Quepos (75)
Craugastor sp. Quepos (69)
Craugastor sp. Quepos (70)
C. stejnegerianus (66)
C. stejnegerianus (64)
C. stejnegerianus (63)
C. stejnegerianus (62)
C. stejnegerianus (67)
C. stejnegerianus (65)
C. stejnegerianus (61)
Craugastor sp. Quebradas (94)
Craugastor sp. Vereh (107)
Craugastor sp. Vereh (108)
Craugastor sp. Vereh (110)
Craugastor sp. Vereh (109)
C. bransfordii (78)
C. bransfordii (79)
C. bransfordii (80)
C. bransfordii (77)
C. bransfordii (76)
C. underwoodi (81)
C. underwoodi (82)
C. underwoodi (84)
C. underwoodi (83)
Craugastor sp. Fila Carbón (91)
Craugastor sp. Fila Carbón (92)
Craugastor sp. Fila Carbón (86)
Craugastor sp. Fila Carbón (88)
Craugastor sp. Fila Carbón (89)
Craugastor sp. Fila Carbón (90)
Craugastor sp. Panamá (98)
Craugastor sp. Panamá (106)
Craugastor sp. Panamá (97)
Craugastor sp. Panamá (96)
Craugastor sp. Panamá (95)
Craugastor sp. Panamá (105)
Craugastor sp. Panamá (104)
Craugastor sp. Panamá (101)
Craugastor sp. Panamá (103)
Craugastor sp. Panamá (100)
Craugastor sp. Panamá (102)

Figure 4

FIGURE 4. Maximum clade credibility tree (left) from the BEAST analysis

Maximum clade credibility tree (left) from the BEAST analysis of *Craugastor podiciferus* Species Group based on 697,771 bp (7,770 loci) from ddRAD dataset. Clade colors represent the following: purple (*Craugastor bransfordii* clade), green (*C. stejnegerianus* clade); blue (*C. podiciferus* clade) and red (monotypic *C. aenigmaticus* clade). Bootstraps proportions are shown above branches. Below the branches are shown posterior probabilities (multiplied by 100) from MrBayes analysis (left) and posterior probabilities (multiplied by 100) from BEAST analysis (right). The asterisks represent support of >0.95 posterior probability. Numbers in parentheses correspond to individual ID numbers provided in Appendix I. Comparison of species delimitation results (right) based on the concatenated nuDNA dataset. The four different bar colors correspond to four species delimitation methods used (see section 2.4); the bars with same color represents different parameter settings for a given delimitation algorithm.

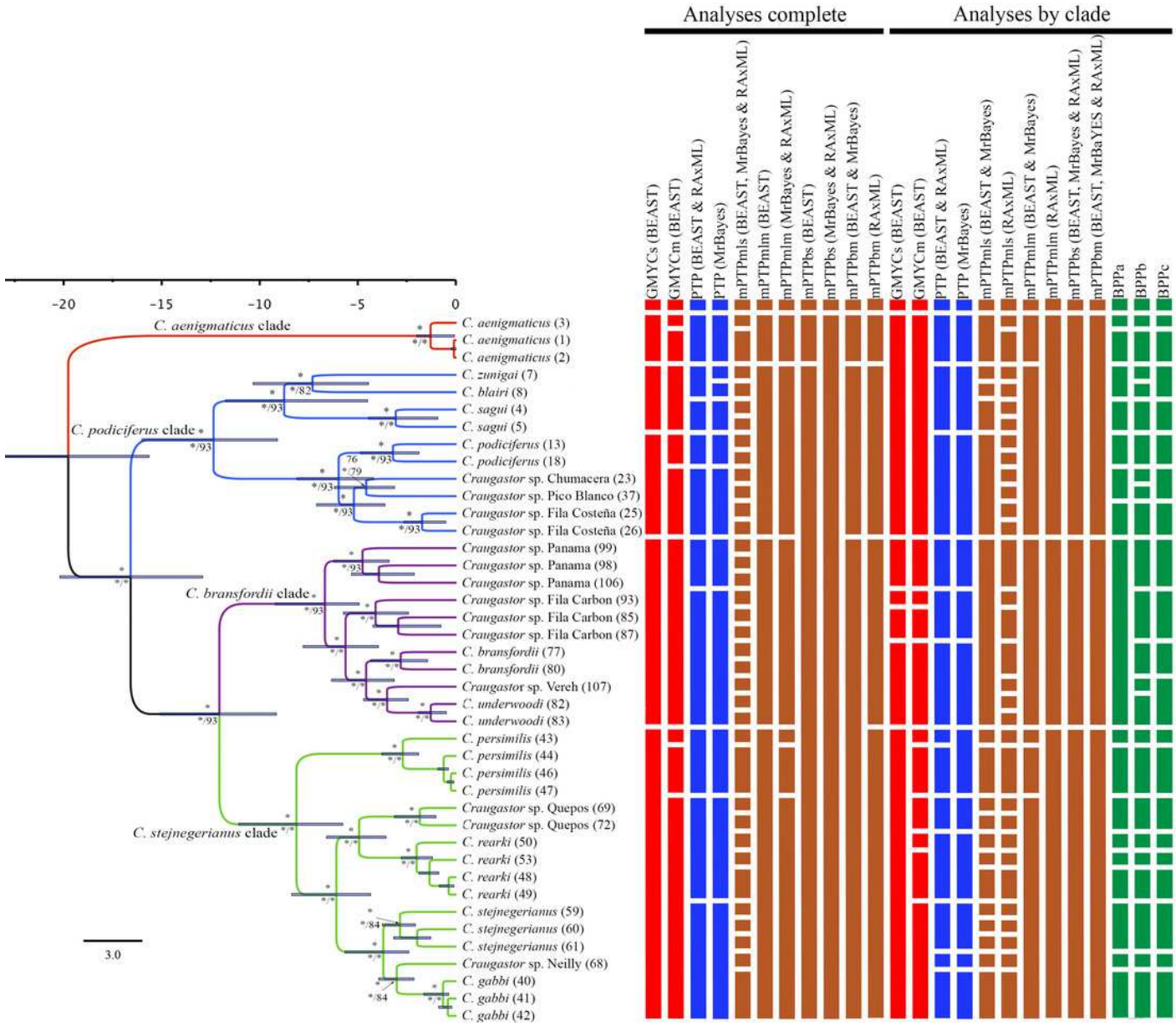
Analyses complete
Analyses by clade
GMYCs (BEAST)
GMYCm (BEAST)
PTP (BEAST & RAxML)
PTP (MrBayes)
mPTPmls (BEAST, MrBayes & RAxML)
mPTPmlm (BEAST)
mPTPmlm (MrBayes & RAxML)
mPTPbs (BEAST)
mPTPbs (MrBayes & RAxML)
mPTPbm (BEAST & MrBayes)
mPTPbm (RAxML)
GMYCs (BEAST)
GMYCm (BEAST)
PTP (BEAST & RAxML)
PTP (MrBayes)
mPTPmls (BEAST & MrBayes)
mPTPmls (RAxML)
mPTPmlm (BEAST & MrBayes)
mPTPmlm (RAxML)
mPTPbs (BEAST, MrBayes & RAxML)
mPTPbm (BEAST, MrBaYES & RAxML)
BPPa
BPPb
BPPc
-20
-15
-10
-5
0
C. aenigmaticus clade
C. podiciferus clade
C. bransfordii clade
C. stejnegerianus clade
C. aenigmaticus (3)
C. aenigmaticus (1)
C. aenigmaticus (2)
C. zumigai (7)
C. blairi (8)
C. sagui (4)
C. sagui (5)
C. podiciferus (13)
C. podiciferus (18)
Craugastor sp. Chumacera (23)
Craugastor sp. Pico Blanco (37)
Craugastor sp. Fila Costeña (25)
Craugastor sp. Fila Costeña (26)
Craugastor sp. Panama (99)
Craugastor sp. Panama (98)
Craugastor sp. Panama (106)
Craugastor sp. Fila Carbon (93)
Craugastor sp. Fila Carbon (85)
Craugastor sp. Fila Carbon (87)
C. bransfordii (77)
C. bransfordii (80)
Craugastor sp. Vereh (107)
C. underwoodi (82)
C. underwoodi (83)
C. persimilis (43)
C. persimilis (44)
C. persimilis (46)
C. persimilis (47)
Craugastor sp. Quepos (69)
Craugastor sp. Quepos (72)
C. rearki (50)
C. rearki (53)
C. rearki (48)
C. rearki (49)
C. stejnegerianus (59)
C. stejnegerianus (60)
C. stejnegerianus (61)
Craugastor sp. Neilly (68)
C. gabbi (40)
C. gabbi (41)
C. gabbi (42)
3.0

Figure 5

FIGURE 5. Ancestral area reconstruction from BioGeoBEARS

Ancestral area reconstruction from BioGeoBEARS, using the DEC+J model, and the tree derived from the Bayesian analyses of ddRAD dataset. Most likely biogeographic areas are shown in the pies, and the colors in the squares indicate the current species distribution. Numbers in parentheses by sample names correspond to individual ID numbers provided in Appendix I. The map shows the location of the areas used.

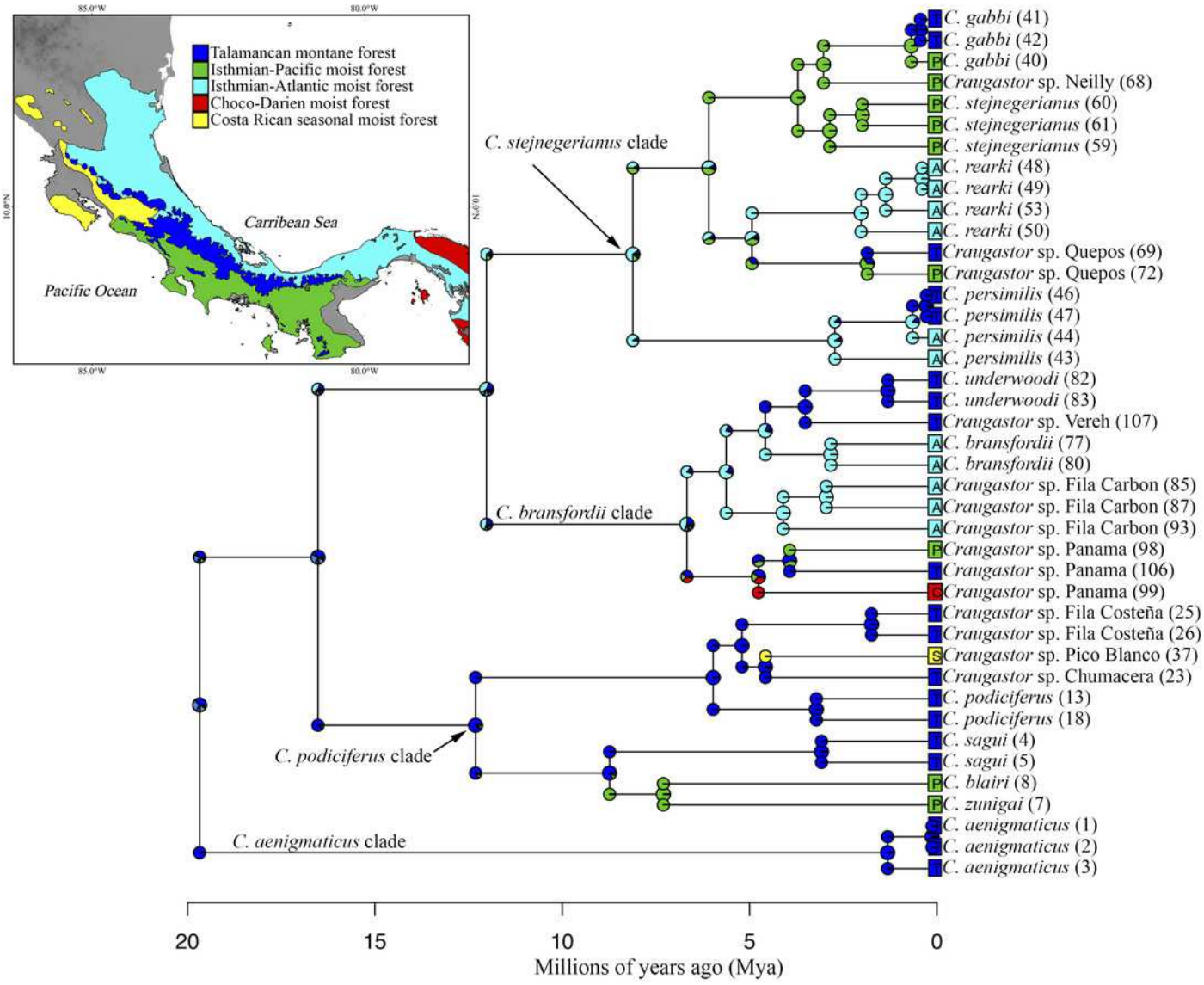

Figure 6

FIGURE 6. Geographic distribution of Craugastor *podiciferus* Species Group

Geographic distribution of the all named species and candidate species within of Craugastor *podiciferus* Species Group in Costa Rica and eastern Panama. The mountains of southeastern Costa Rica are referred to as the Talamanca in the text. The purple shapes correspond to the *Craugastor bransfordii* clade; green shapes = *C. stejnegerianus* clade; blue shapes = *C. podiciferus* clade; red shape = *C. aenigmaticus* clade.

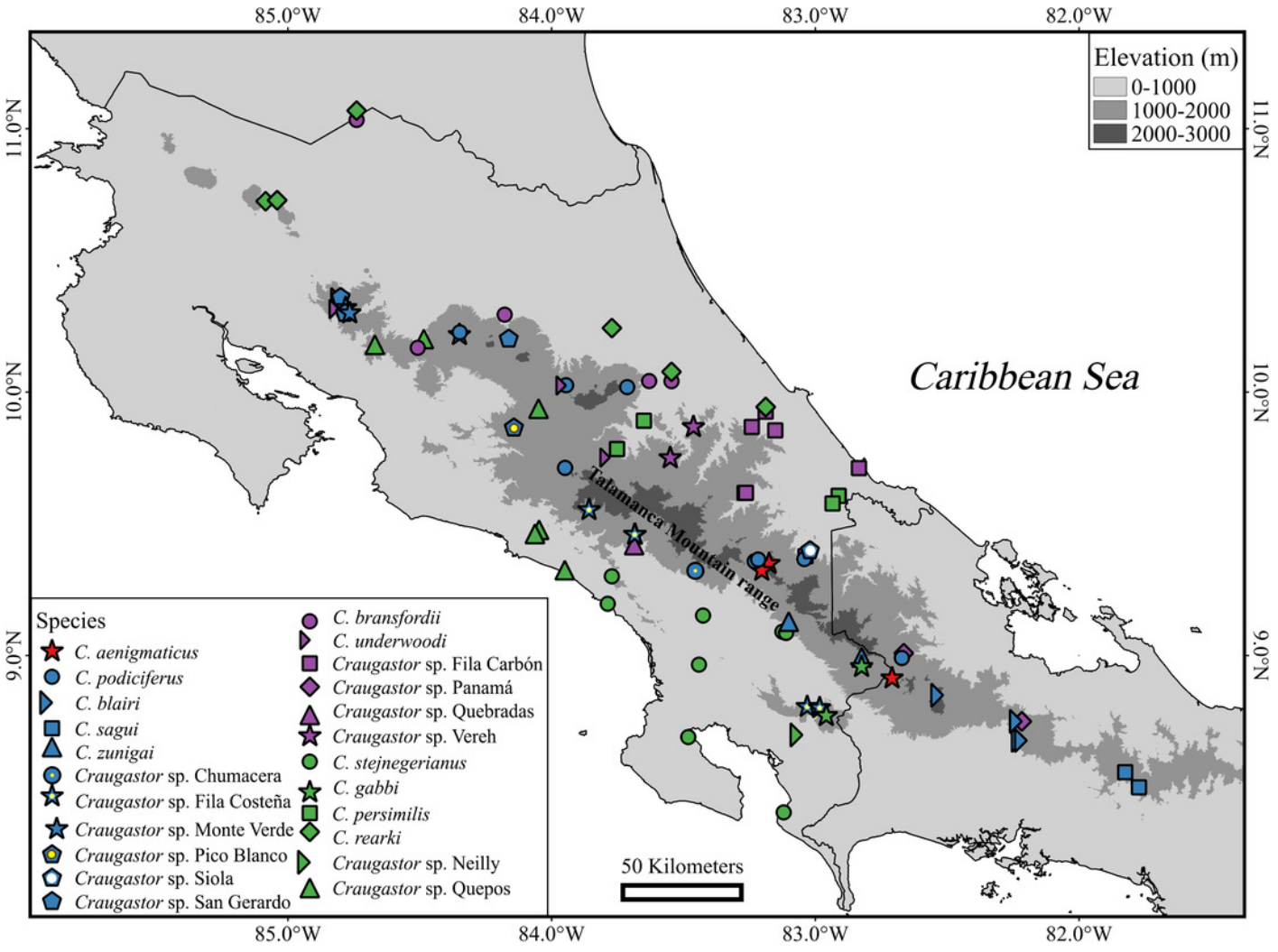

Figure 7

FIGURE 7. Photographs in life of Craugastor podiciferus species complex

. Photographs in life of (A) *Craugastor aenigmaticus* (UCR 22961) from Cerro Arbolado, Puntarenas, CR, (B) *C. blairi* (SMF 104032) from Fortuna, PA, (C) *C. sagui* (SMF 104018) from La Nevera, PA, (D) *C. zunigai* (UCR 20389) from Potrero Grande, Puntarenas, CR, (E-F) *C. podiciferus* (UCR 23155, 23159) from Caribbean slopes of Cerro Kamuk, Limón, CR, (G) *Craugastor* sp. Monte Verde from Monte Verde, Puntarenas, CR, (H) *Craugastor* sp. San Gerardo (CRARC 0247) from San Gerardo, Guanacaste, CR, (I) *Craugastor* sp. Fila Costeña (UCR 23028) from Quebradas, San José, CR, (J) *Craugastor* sp. Pico Blanco from Escazú, San José, CR, (K) *Craugastor* sp. Chumacera (UCR 23011) from Chumacera, San José, CR, (L) *Craugastor* sp. Siola (UCR 23169) from Siola, Limón, CR, (M) *C. bransfordii* from Siquirres, Limón, CR, (N) *Craugastor* sp. Fila Carbon (UCR 23127) from Amubri, Limón, CR, (O) *C. underwoodi* from Cascajal, San José, CR, (P) *Craugastor* sp. Quebradas from Fila Costeña, Puntarenas, CR, (Q) *Craugastor* sp. Vereh (UCR 23040) from Vereh, Cartago, CR, (R) *Craugastor* sp. Panama from Rambala. PA, (S) *C. stejnegerianus* (UCR 22976) from Palmar Norte, Puntarenas, CR, (T) *C. gabbi* (UCR 22998) from San Vito, Puntarenas, CR, (U) *C. persimilis* from Siquirres, Limón, CR, (V) *C. rearki* from Nicaragua, (W) *Craugastor* sp. Neilly (UCR 22985) from Río Claro, Puntarenas, CR, and (X) *Craugastor* sp. Quepos from Balsa, Alajuela, CR. Photos by E. Arias (A,E,F,I,K,L,N,Q,S,T, and W), Andreas Hertz (B-C), Eduardo Boza-Oviedo (D and O), Avocat (G), Brian Kubicki (H, M, and U), Tico Haroutiounian (J), Raby Nuñez (P), Marcos Guerra (R), Todd Pierson (V), and Emmanuel Rodríguez-Rojas (X).


A
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Table 1(on next page)

Table 1. Mean uncorrected genetic distances among lineages within the *Craugastor podiciferus* Species Group

Mean uncorrected genetic distances among lineages within the *Craugastor podiciferus* Species Group based on mitochondrial genes 16S (above the diagonal) and COI (below the diagonal). Genetic distances within the three major clades are highlighted in blue, yellow, and gray along the diagonal. Values less that the thresholds (3% in 16S and 10% in COI) are shown in red.

1

ID	16S/COI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*Craugastor aenigmaticus*	—	17.5	13.3	15.6	15.0	14.2	14.4	15.1	14.8	14.8	17.3	—	14.2	16.7	16.3	16.2	16.8	18.7	20.3	19.6	18.9	19.6	21.7
2	*Craugastor sagui*	21.8	—	7.9	7.4	7.3	7.1	7.4	9.5	8.2	8.4	9.1	—	11.1	17.5	12.0	11.1	12.1	12.8	16.1	16.2	16.2	16.3	18.0
3	*Craugastor zunigai*	25.0	17.3	—	3.4	7.2	6.2	7.3	9.3	9.4	9.6	8.9	—	12.3	17.0	13.2	13.7	13.1	13.8	16.3	16.4	15.7	16.4	19.4
4	*Craugastor blairi*	20.2	20.3	18.3	—	7.6	7.4	7.6	10.5	8.3	9.9	9.4	—	13.3	17.7	12.0	12.5	13.0	14.1	17.0	17.1	17.0	17.2	21.0
5	*Craugastor* sp. Monte Verde	19.5	16.6	18.7	19.9	—	2.5	2.0	3.7	6.6	4.4	4.5	—	9.1	14.9	9.9	11.1	10.4	11.5	14.6	14.7	14.6	14.8	16.6
6	*C. podiciferus*	22.2	17.8	18.5	19.8	12.0	—	2.7	4.1	6.6	4.6	5.1	—	7.6	13.6	10.1	11.2	9.6	11.5	13.2	13.2	13.1	13.2	15.9
7	*Craugastor* sp. Pico Blanco	20.8	18.4	20.7	17.2	11.3	10.0	—	2.6	6.9	4.7	3.1	—	9.8	14.5	10.4	11.7	9.9	11.8	15.2	15.3	15.4	15.3	14.9
8	*Craugastor* sp. San Gerardo	21.9	18.4	21.4	20.5	12.8	12.4	7.3	—	7.8	5.8	4.5	—	9.8	14.2	10.9	11.7	9.2	13.4	15.3	15.4	15.5	15.4	13.7
9	*Craugastor* sp. Siola	24.0	19.8	22.8	19.6	12.8	12.9	11.4	13.7	—	8.0	9.0	—	11.0	15.8	9.2	10.3	10.5	11.8	13.0	11.5	11.5	11.6	16.1
10	*Craugastor* sp. Chumacera	19.3	19.3	21.3	18.3	12.4	11.8	9.9	12.8	11.6	—	7.4	—	11.2	17.6	11.8	12.1	12.7	14.2	17.8	16.8	16.8	16.9	19.9
11	*Craugastor* sp. Fila Costeña	21.2	17.8	23.4	19.9	12.5	12.3	12.0	14.4	13.8	9.6	—	—	12.0	16.5	11.4	12.7	10.9	12.7	14.2	15.9	15.8	16.0	16.0
12	*Craugastor* sp. Quebradas	25.4	19.2	22.0	24.5	24.2	22.6	24.2	25.3	26.3	22.8	25.2	—	—	—	—	—	—	—	—	—	—	—	—
13	*Craugastor* sp. Vereh	26.9	20.2	23.4	23.9	19.7	22.6	23.8	23.7	26.0	20.8	22.6	16.2	—	5.4	6.0	6.8	5.0	9.5	11.6	10.2	10.2	10.3	10.2
14	*C. bransfordii*	25.3	18.8	21.1	23.0	21.1	19.9	20.6	22.3	24.0	20.8	21.2	16.7	11.7	—	10.4	11.3	9.4	14.4	14.1	14.1	14.1	14.2	13.4
15	*C. underwoodi*	23.5	19.1	21.8	19.9	19.4	19.7	19.7	22.4	22.9	20.9	21.2	15.3	14.5	13.2	—	4.1	5.4	8.5	11.5	10.9	10.9	11.0	12.1
16	*Craugastor* sp. Fila Carbón	22.9	20.7	23.4	22.9	22.4	23.3	23.9	26.3	24.9	22.5	23.0	17.3	14.0	15.6	9.5	—	4.3	7.7	12.2	11.6	11.5	11.6	11.6
17	*Craugastor* sp. Panama	21.3	18.2	22.5	22.2	21.1	21.1	19.8	23.4	24.1	21.5	22.1	17.3	14.3	13.2	11.9	14.0	—	7.6	11.8	10.5	10.4	10.5	8.8
18	*C. persimilis*	24.2	22.4	26.5	24.0	23.0	23.8	24.9	27.4	24.1	26.2	26.4	23.8	20.8	21.8	19.1	19.6	20.9	—	7.6	6.3	7.0	6.5	9.0
19	*C. gabbi*	25.4	26.7	27.6	24.3	24.1	23.5	24.7	26.3	26.7	26.7	26.9	22.0	20.1	23.3	20.6	19.6	21.5	16.7	—	4.2	4.3	4.3	8.0
20	*Craugastor* sp. Neilly	24.9	27.5	27.4	22.6	24.6	27.2	26.9	28.9	29.3	29.8	29.2	24.2	22.4	22.9	20.5	20.4	22.6	16.3	14.1	—	1.1	0.2	6.0
21	*Craugastor* sp. Quepos	23.0	27.3	27.4	22.6	24.6	25.3	26.0	26.2	27.3	28.4	26.2	23.6	23.4	22.7	19.1	20.1	22.2	15.3	15.1	6.9	—	1.1	6.1
22	*C. stejnegerianus*	21.8	27.5	25.2	22.3	25.1	25.5	25.8	27.0	28.7	27.8	27.2	22.6	21.6	21.6	20.9	20.3	21.9	16.2	14.7	5.6	5.5	—	6.0
23	*C. rearki*	22.6	25.4	24.9	23.8	23.6	28.0	27.6	28.6	28.3	27.9	27.7	23.4	20.8	22.0	22.2	20.0	21.1	17.2	15.7	11.9	11.6	13.8	—

2