



## A new species of dink frog, genus *Diasporus* (Anura: Eleutherodactylidae), from the Caribbean foothills of the Cordillera de Talamanca, Costa Rica

ERICK ARIAS<sup>1,2,6</sup>, GERARDO CHAVES<sup>2</sup>, STANLEY SALAZAR<sup>3</sup>,  
JOSÉ ANDRÉS SALAZAR-ZÚÑIGA<sup>2,4</sup> & ADRIÁN GARCÍA-RODRÍGUEZ<sup>1,2,5</sup>

<sup>1</sup>Departamento de Zoología, Instituto de Biología, UNAM, AP 70-153 Ciudad Universitaria, CP 04510, Ciudad de México, México.

<sup>2</sup>Escuela de Biología, Universidad de Costa Rica, San Pedro, 11501-2060 San José, Costa Rica.

<sup>3</sup>La Esperanza, Horquetas, Sarapiquí, Limón, Costa Rica.

<sup>4</sup>Veragua Foundation for the Rainforest Research, Limón, Costa Rica.

<sup>5</sup>Departamento de Ecología, Universidade Federal do Rio Grande do Norte, Natal - RN, Brasil, 59078-900.

<sup>6</sup>Corresponding author: E-mail: [eapiedra@gmail.com](mailto:eapiedra@gmail.com)

### Abstract

A new dink frog (Eleutherodactylidae: *Diasporus*) is described from the Tropical Wet Forest, in the northeastern foothills of Cordillera de Talamanca in Costa Rica at an elevation of ca. 1000 m. Analysis of DNA sequences of the 16S rRNA (16S) and cytochrome oxidase 1 (COI) mitochondrial genes revealed a distinct lineage within the genus *Diasporus*. Additional morphological, morphometric, and acoustic analyses support the differences of this lineage, which we recognize as a new species. This new taxon is distinguished from other members of the genus *Diasporus* inhabiting Isthmian Central America by its unique coloration: dorsum brown to brown-grayish and venter gray-bluish with pale blotches. The new species is distinguished from other members of the genus *Diasporus* by very significant genetic distances (higher than 5.6 % in 16S and 16.7 % in COI). The new taxon is most closely related to *D. vocator* from the south pacific of Costa Rica and to an unnamed taxon from western Panama.

**Key words:** Brachycephaloidea, Isthmian Central America, Panama, Taxonomy, Terrarana

### Resumen

Se describe una nueva especie de rana campanita (Eleutherodactylidae: *Diasporus*), del bosque muy húmedo tropical del pie de montaña de la vertiente noreste de la Cordillera de Talamanca en Costa Rica, a una elevación aproximada de 1000 m. Análisis de secuencias de los genes mitocondriales 16S rARN (16S) y citocromo oxidasa 1 (COI) revelaron un linaje distinto dentro del género *Diasporus*. Análisis adicionales de morfología, morfometría y acústica apoyaron la diferencia de este linaje, que aquí reconocemos como una nueva especie. Este nuevo taxón se diferencia de sus congéneres en América Central Istmica por sus características cromáticas únicas: dorso marrón a marrón-grisáceo y vientre gris-azulado con manchas claras. La nueva especie está separada de otros miembros del género *Diasporus* por distancias genéticas significativas (mayores a 5.6 % en 16S y 16.7 % en COI). Este nuevo taxón está relacionado filogenéticamente a *D. vocator* del Pacífico Sur de Costa Rica y a un innombrado taxón del oeste de Panamá.

**Palabras clave:** América Central Istmica, Brachycephaloidea, Panamá, Taxonomía, Terrarana

### Introduction

Isthmian Central America (ICA), defined herein as Costa Rica and Panama, is a highly biodiverse region: it hosts more species of amphibians, reptiles, birds, insects and vascular plants per area unit than almost any other place in the world (Davis *et al.* 1997; Anger & Dean 2010; Garrigues & Dean 2014; AmphibiaWeb 2018). Approximately 218 species of anurans have been documented in the region thus far, and near a third of that diversity is represented by direct-developing frogs (AmphibiaWeb 2018). Direct-developing frogs of the genus *Diasporus* Hedges, Duellman, & Heinicke, 2008 represent the unique non-introduced clade of the family Eleutherodactylidae in ICA, and

forms the sister group to the genus *Eleutherodactylus*, a large clade of mostly Caribbean species (Hedges *et al.* 2008; Padial *et al.* 2014; AmphibiaWeb 2018). *Diasporus* species occur from eastern Honduras to western Ecuador, and 12 of the 15 currently named species are distributed on ICA (Savage 2002; Padial *et al.* 2014; Batista *et al.* 2016; AmphibiaWeb 2018). Nine of these 12 species have been named during the last 20 years (Savage 1997; Lynch 2001; Chaves *et al.* 2009; Batista *et al.* 2012; Hertz *et al.* 2012; Batista *et al.* 2016); this progress in our understanding of *Diasporus* diversity reveals significant levels of microendemism and suggests the potential existence of additional unnamed species in the region (Batista *et al.* 2016; García-Rodríguez *et al.* 2016).

This genus also contains some of the smallest frogs from ICA, and its species are often quite difficult to identify based on morphology alone (Batista *et al.* 2016). On the other hand, high levels of chromatic, ecological, behavioral, and acoustic variation have been observed within nominal species in the genus (Arias and García-Rodríguez unpublished data). Some nominal species are suspected to mask additional unnamed species (Savage 2002), some of them rather cryptic, at least in the functional definition (Pérez-Ponce de León & Nadler 2010), that is, those species treated as cryptic by the systematists although it is possible that morphological differences will be found when a more detailed morphological investigation serve as reciprocal illumination (Pérez-Ponce de León & Nadler 2010). This inadequate understanding of the species limits and variation in their traits requires an integrative approach to delimitate species boundaries (Padial *et al.* 2010; Batista *et al.* 2016). Indeed, Batista *et al.* (2016) recently discovered four new species from eastern Panama using an integrative perspective, while García-Rodríguez *et al.* (2016) also used multiple lines of evidence to support the delimitation of two closely related species from southeastern Costa Rica and western Panama.

In this same region, stands the Cordillera de Talamanca, the highest elevation mountain system and one of the least explored areas of ICA (Kappelle *et al.* 1992; Renjifo *et al.* 1997; Boza-Oviedo *et al.* 2012). The few expeditions conducted in Talamanca in recent decades have resulted in the discovery of several new species of amphibians, many of them only known from their respective type localities (Savage 1997; Hanken *et al.* 2005; Wake *et al.* 2007; Bolaños & Wake 2009; Chaves *et al.* 2009; Savage & Bolaños 2009; Boza-Oviedo *et al.* 2012; Kubicki & Arias 2016; Arias & Kubicki 2018), suggesting a high degree of endemism in this geomorphological unit. The slopes of Talamanca's northeastern portion lie within the central Caribbean region of Costa Rica, which contains one of the highest concentrations of amphibian species in the world (Kubicki 2016). These foothills harbor such levels of species diversity, in part because of the confluence of lowland and montane biotas (Savage 2002), but also because of the high levels of isolation and endemism promoted by the complex orography and isolated slopes of this mountain range. Just in the last years, three new species of plethodontid salamanders were discovered from the Caribbean foothills of Cordillera de Talamanca in Costa Rica (Kubicki 2016; Kubicki & Arias 2016; Arias & Kubicki 2018).

During recent expeditions to the Caribbean foothills in the northeastern portion of Cordillera de Talamanca, we found a population that we tentatively associated with species in the genus *Diasporus*, but that differed significantly from any known species in terms of their morphology, ecology, and behavior. In this study, we assess the taxonomic status and affinities of this population by performing acoustic, morphological, and molecular phylogenetic analyses. Based on the evidence presented herein we propose the recognition of this premontane population as a distinct species. The results of our detailed analyses show that this species is most closely related to *D. vocator* (Taylor, 1955), which is restricted to the Pacific slope of Southwestern Costa Rica and Northwestern Panama.

## Materials and methods

**Species criterion.** Our view of species follows the general metapopulation lineage species concept (Simpson 1951; Wiley 1978; de Queiroz 2007). Since we adhere to this concept, we recognize a species when there is evidence of the separation of metapopulation lineages, preferably based on multiple lines of evidence following a consensus protocol for integrative taxonomy (Dayrat *et al.* 2005; Padial *et al.* 2010).

**Field-work.** During the beginning of 2013, Stanley Salazar (SS) discovered two adult individuals of frogs on the northern edge of the Matama Ridge (on an unnamed peak; 9.817, -83.188, 1030 m a.s.l.) and on Plátano peak (9.864, -83.236; 1000 m a.s.l.) that were tentatively identified as belonging to the genus *Diasporus*. These two individuals were considered to represent an unnamed species due to the fact that they were quite different from the other known members of the genus *Diasporus* in Costa Rica and Panama. During the months of March and November 2013 and June 2015, three subsequent surveys were carried out along a 600 m elevation gradient on the 11 km

trail between Veragua Rainforest Eco Adventure (9.925, -83.191; 419 m a.s.l.) and Plátano peak. We recorded the presence of the putative new species by its distinctive advertisement call. Specimens collected were euthanized and subsequently fixed in 10% formalin, and later stored in 70% alcohol. Tissue samples were stored in 95% ethanol. Vouchers are now housed at the herpetological collection of Museo de Zoología at the Universidad de Costa Rica (UCR). Museum collection acronyms follow Frost (2018). We recorded advertisement calls from four males using a shotgun microphone (Sennheiser ME66) coupled to a solid-state digital recorder (Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 24 bit; file format: WAV) from an approximate distance of 1 m. We also deposited photo and audio voucher at the Fonoteca Zoológica Animal Sound Library at the Museo Nacional de Ciencias Naturales of Madrid, Spain ([www.fonozoo.com](http://www.fonozoo.com) –Fz SOUND CODE 11240).

**Amplification and sequencing.** We extracted total genomic DNA from the preserved tissue samples using the Animal Genomic DNA Kit (BioBasic Canada Inc.). We obtained partial sequences of 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  $\mu$ L, which contained 1  $\mu$ L DNA template (*c.* 50 ng  $\mu$ L<sup>-1</sup>), 0.75 U Taq polymerase (Amplificasa®, Biotecnologías Moleculares), 1X PCR buffer with 1.5 mM MgCl<sub>2</sub>, 0.2 mM deoxynucleotide triphosphates (dNTPs), and 0.3–0.5  $\mu$ M forward and reverse primers. The PCR conditions were 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 55°C, 45 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-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 compared the sequences here obtained with sequences of the 16S rRNA (16S) and cytochrome oxidase 1 (COI) mitochondrial genes, for 84 specimens of the genus *Diasporus* that were available in GenBank. We used sequences of *Eleutherodactylus johnstonei* and *E. planirostris* as outgroups, and *Adelophryne maranguapensis* to root all trees based on the results of Padial *et al.* (2014). The list of vouchers and GenBank Accession numbers used in this study are provided in Appendix I. Sequence alignments were performed using the MAFFT software (Kato *et al.* 2017) under the “auto” strategy, default parameters and trimmed to the point where 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 conducted both maximum likelihood (ML) and Bayesian analyses (BA). We performed the maximum likelihood analysis using Garli 2.01 (Zwickl 2006). To find the best tree, 10 search replicates were ran with the following default setting values: *streefname=random*, *attachmentspertaxon=24*, *gentreshfortopoterm=100000*, *significanttopochange=0.00001*. For bootstrapping, we ran 1000 pseudoreplicates with the previous settings and with the following changes: *gentreshfortopoterm=10000*, *significanttopochange=0.01*, *treerejectionthreshold=20*, as suggested in the Garli manual to speed up bootstrapping. From these bootstraps, we obtained a majority rule consensus tree using Sumtrees (Sukumaran & Holder 2010b) from DendroPy packages version 4.4.0 (Sukumaran & Holder 2010a). We performed a Bayesian phylogenetic analysis 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 allcompat 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). Genetic distances (uncorrected *p*-distances) were computed using MEGA6 (Tamura *et al.* 2013). Based on the results of our phylogenetic analyses we used *Diasporus vocator* to perform morphometric and bioacoustic comparisons with the putative species (see below).

**Morphometric analyses.** We examined 6 individuals from Plátano peak and 16 specimens representing several populations referred to *D. vocator*. All material was deposited at the Museo de Zoología (UCR), Universidad de Costa Rica, San José, Costa Rica. We recorded the following morphological measurements, as described by Arias *et al.* (2016), Savage (2002), and Duellman & Lehr (2009): snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), inter orbital distance (IOD), tympanum diameter (TY), width of upper eyelid (EW), eye-nostril distance (E-N), tibia length (TL), lengths of toes (T1, T2, T3, T4, T5), and lengths of fingers (F1, F2, F3, F4). To avoid allometric effects relative to the difference in the size and shape between species and between individuals we transformed the data using the method of Leonart *et al.* (2000). In this method a logarithmic transformation to the continuous variables is performed to reduce the extreme values. All transformed variables are used in the allometric transformation by means of equation:  $Y_i^* = Y_i \left[ \frac{X_0}{X_i} \right]^b$ , whereas  $Y^*$  corresponds to the value of each of the dependent variable corrected for size and shape,  $Y_i$  corresponds to the value of each of the dependent morphometric variables,  $X_0$  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. Additional proportions reported here include: EW/IOD, E-N/ED, ED/HL, IOD/HW, and T4/TL. We followed Savage (2002) in our usage of the term “supernumerary tubercles,” which we used to refer to the tubercles on the phalanges (between subarticular tubercles); this is different from the tubercles referred to here as accessory palmar or plantar tubercles.

**Acoustic analysis.** We conducted a spectro-temporal analysis for a total of 16 calls, corresponding to four males of the new species from the Plátano peak, including one collected voucher (UCR 21842, SVL = 18.0 mm). We identified these calls in the recordings through an automatic detection procedure based on amplitude and duration thresholds, applied within the species frequency range. From the detected calls, we selected the ones with the highest signal-to-noise ratio (mean = 4 calls per individual). We visually inspected the spectrograms of the detected calls and removed undesired sounds or calls that overlapped in time with other sounds. Next, we measured the following 13 acoustic parameters: duration, mean frequency (average frequency of the spectrum), skewness (asymmetry of the spectrum), kurtosis (peakedness of the spectrum), spectral entropy (energy distribution; pure tone ~ 0; noisy ~ 1), mean dominant frequency (average dominant frequency throughout the call), minimum dominant frequency, maximum dominant frequency, frequency range (max. dominant - min. dominant), modulation index (accumulated absolute difference between adjacent measurements of dominant frequencies divided by the frequency range), start dominant frequency (dominant frequency at the start of the call), end dominant frequency (dominant frequency at the end of the call), frequency slope (slope of the change in dominant through time ([end dominant - start dominant]/duration)). All these procedures were conducted in R using the packages tuneR (Ligges *et al.* 2016), Seewave (Sueur *et al.* 2008), and warbleR (Araya-Salas & Smith-Vidaurre 2016). Finally, we produced spectrograms and oscillograms using the R package Seewave (Sueur *et al.* 2008).

In order to set acoustic differences between the new species described herein and *D. vocator* we repeated all the previous analyses for 82 calls of *D. vocator*. These calls correspond to 10 males recorded at Rincón de Osa, Altamira de Biolley, and San Vito de Coto Brus [type locality (the two latter localities are included in our molecular analysis, Fig. 1)]. Additionally, we analyzed a single call of *Diasporus* sp., from Santa Fe, Panama; this call was of very limited quality, therefore spectro-temporal analysis not was performed; only a spectrogram and oscillogram were produced for it.

**Statistical analysis.** We calculated the mean, standard deviation, and range for each morphometric and acoustic variable. We conducted two Principal Component Analysis (PCA), one for morphometric and another for acoustic analysis, using all the variables to explore the grade of structure within the sample and which variables better define this. We used multivariate analysis of variance (MANOVA) to test for differences between the putative species and *Diasporus vocator*. In the morphometric analysis, prior to MANOVA we used the Variance Inflation Factor (VIF) to eliminate multicollinearity (>70 %); normality was evaluated with Royston test ( $\alpha = 0.05$ ), and homoscedasticity was tested with BoxM test ( $\alpha = 0.001$ ). We applied the square root transformation when the assumption of normality failed and there was heteroscedasticity. All the analyses were performed using R v3.3.3 (R Core Team 2017).

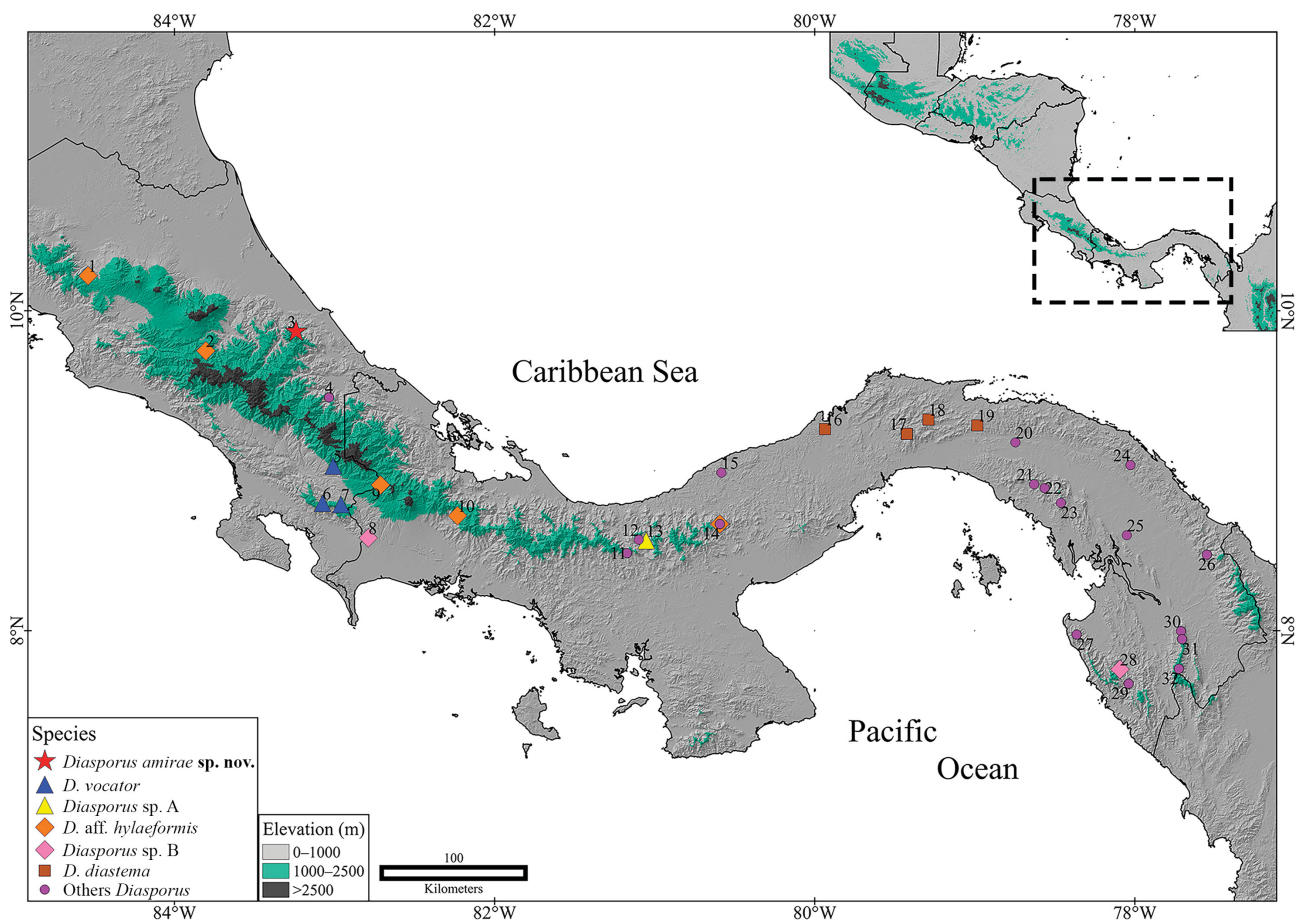
## Results

**Molecular phylogenetics.** The resulting data matrix had a total sequence length of 1218 bp, including gaps; 560 bp for 16S and 658 bp for COI. The better strategy partition contains four partitions, 16S and one for each codon



in COI. The following substitution models were selected: GTR+I+G for 16S, GTR+G for COI codon position 1, SYM+I+G for COI codon position 2, and GTR+I for COI codon position 3. Genetic distances between the specimens from Plátano peak and other members of the genus *Diasporus* are of 5.6–11.2 % for 16S and 16.7–22.4 % for COI. Interestingly, the genetic distances between the specimens from Plátano peak and the sister species are larger than those between the first and other species of the genus *Diasporus*. Specifically, the specimens from Plátano peak are separated from *D. vocator* by a genetic distance of 10.1–11.2 % for 16S and 17.45 % for COI and from *Diasporus* sp. A by 10.1 % in 16S (sequences of COI were not available for *Diasporus* sp. A so we are unable to report herein the amount of divergence for that gene).

The phylogenies inferred by Garli and MrBayes were very similar (Fig. 2) and show four well-supported clades. The basal clade contains three species, *D. majeensis*, *D. sapo*, and *D. darienensis*, which occur in eastern Panama (see Figure 1). A second clade contains three groups, an unnamed species from El Pantano, Santa Fe, Panama, the three specimens from Plátano peak forming a well-supported clade, and *D. vocator*. The third clade contains an unnamed species from Panama and the specimens referred to herein as *Diasporus* aff. *hylaeformis*. Finally, a fourth major clade is formed by several named species (*D. pequeno*, *D. tinker*, *D. tigrillo*, *D. diastema*, and *D. citrinobapeus*) and several clades referred to as *Diasporus* sp. C, *Diasporus* aff. *quidditus*, and *Diasporus* aff. *diastema*. The main differences between ML and Bayesian (not shown) topology is that in the Bayesian tree the second and third clade were not grouped, the second clade was the sister clade to the group formed by third clade + fourth clade. In addition, in the Bayesian tree the sister clade to *D. tigrillo* was *D. aff. diastema* EPL.



**FIGURE 1.** Map of Costa Rica and Panama, showing the type locality for *Diasporus amirae* sp. nov. and the populations of *Diasporus* sampled in our molecular phylogenetic analysis. Numbers correspond to locality IDs in Appendix I.

**Morphometric analyses.** Morphometric variation and comparisons among the species are shown in Table 1. The PCA efficiently differentiated the specimens from Plátano peak and the populations referred to *Diasporus vocator* (Fig. 3a). The first principal component (PC1) explained 82.58 % of the total variance and the second explained 5.25 %. The PCA showed that all but three variables were related to the putative species, IOD/HW was related to

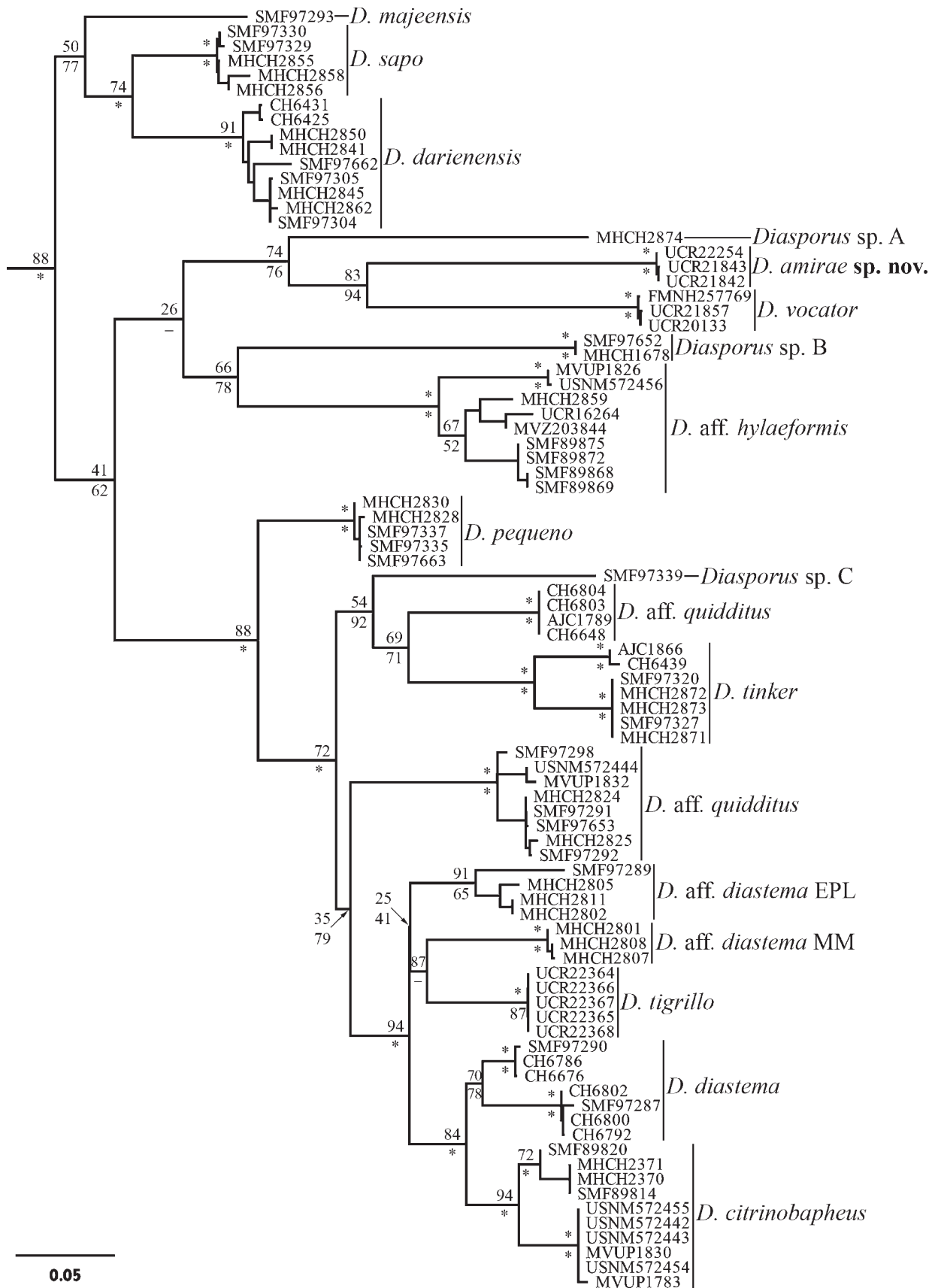
*D. vocator*, while ED/HL and E-N/ED were not relative to any species. Only IOD and EW/IOD were subject to MANOVA, both of them were normalized by square root transformation. The residuals of the MANOVA were normal ( $H = 1.55$ ,  $p = 0.44$ ) and homoscedastic ( $X^2 = 12.62$ ,  $p = 0.006$ ). The MANOVA supported the differences between the specimens from the putative species and *D. vocator* (Pillai's trace = 0.930,  $F = 58.47$ ,  $p = <0.01$ ).

**TABLE 1.** Morphometric comparison between *Diasporus vocator* and *D. amirae* **sp. nov.** See the text for the statistical results.

Variable	<i>Diasporus amirae</i> <b>sp. nov.</b> (n = 6)		<i>Diasporus vocator</i> (n = 16)	
	Mean ± S.D.	Range	Mean ± S.D.	Range
SVL	21.0±2.0	18.0–23.0	14.6±0.9	13.2–16.4
HL	6.1±0.7	5.5–8.0	5.1±0.3	4.5–5.8
HW	8.2±0.7	7.0–8.5	5.0±0.4	4.2–5.8
ED	2.4±0.3	3.0–4.0	2.0±0.2	1.7–2.5
IOD	2.5±0.3	2.1–2.8	2.0±0.2	1.6–2.4
TY	1.2±0.2	1.0–1.2	0.7±0.2	0.5–1.0
EW	1.6±0.3	1.2–2.0	1.1±0.2	0.8–1.6
E-N	1.2±0.1	1.2–2.0	1.1±0.2	0.9–1.4
TL	9.1±0.4	9.2–10.0	6.5±0.5	5.5–7.6
T1	2.4±0.4	2.0–2.9	1.4±0.2	1.2–1.9
T2	3.3±0.3	3.0–3.8	2.1±0.3	1.6–2.6
T3	4.8±0.3	4.2–5.5	3.2±0.4	2.5–3.7
T4	6.9±0.4	7.0–7.1	4.8±0.6	3.5–5.6
T5	5.3±0.4	5.0–5.1	3.8±0.4	3.1–4.6
F1	2.1±0.2	1.9–2.1	1.2±0.2	1.0–1.5
F2	2.8±0.2	2.2–3.0	1.7±0.2	1.3–2.0
F3	3.7±0.3	3.1–4.0	2.3±0.2	1.9–2.7
F4	3.1±0.3	2.5–3.5	1.8±0.2	1.4–2.1
EW/IOD	0.66±0.14	0.46–0.90	0.57±0.09	0.46–0.81
E-N/ED	0.53±0.10	0.43–0.71	0.56±0.10	0.40–0.82
ED/HL	0.39±0.02	0.35–0.42	0.39±0.03	0.33–0.45
IOD/HW	0.31±0.05	0.25–0.39	0.39±0.04	0.31–0.47
T4/TL	0.34±0.03	0.73–0.80	0.27±0.02	0.52–0.84

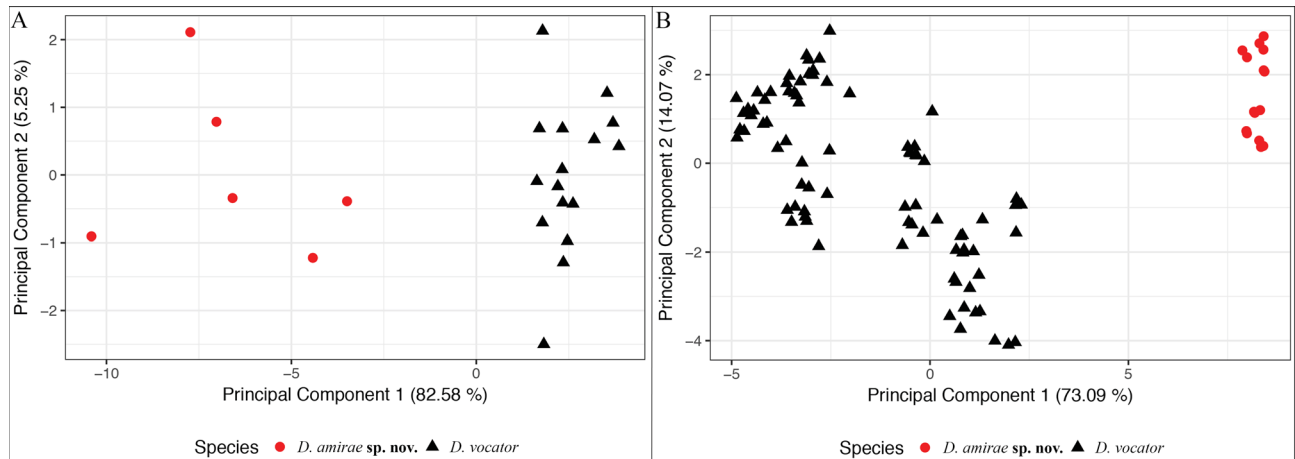
**TABLE 2.** Mean values, standard deviation, and range of the spectro-temporal characteristics in the advertisement calls of *Diasporus amirae* **sp. nov.** (n = 16 calls) and *D. vocator* (n = 82 calls).

Call Feature	<i>Diasporus amirae</i> <b>sp. nov.</b>		<i>Diasporus vocator</i>		p-value
	Mean±S.D.	Range	Mean±S.D.	Range	
Duration (s)	0.26±0.02	0.23–0.27	0.04±0.01	0.02–0.08	< 0.001
Mean Frequency (KHz)	3.24±0.06	3.15–3.34	4.86±0.41	4.32–5.50	< 0.001
Skewness	3.23±0.13	3.05–3.47	2.39±0.40	1.66–3.36	< 0.001
Kurtosis	13.11±1.30	11.17–15.60	7.83±2.16	4.27–13.58	< 0.001
Spectral Entropy	0.76±0.02	0.72–0.79	0.71±0.05	0.57–0.80	< 0.001
Mean Dominant Frequency (KHz)	3.08±0.04	3.01–3.16	4.83±0.43	4.19–5.46	< 0.001
Minimum Dominant Frequency (KHz)	2.87±0.04	2.81–2.96	4.53±0.37	3.96–4.80	< 0.001
Maximum Dominant Frequency (KHz)	3.27±0.05	3.16–3.35	5.08±0.47	4.22–5.86	< 0.001
Frequency Range (KHz)	0.40±0.04	0.32–0.43	0.55±0.18	0.01–0.95	0.001
Modulation Index	0.26±0.03	0.25–0.34	0.62±0.26	0–1.00	< 0.001
Dominant frequency at the start (KHz)	2.87±0.04	2.81–2.96	4.53±0.37	3.96–4.50	< 0.001
Dominant frequency at the end (KHz)	3.27±0.05	3.16–3.35	5.06±0.46	4.22–5.86	< 0.001
Slope in dominant frequency	1.57±0.10	1.41–1.72	13.97±5.20	7.02–20.80	< 0.001

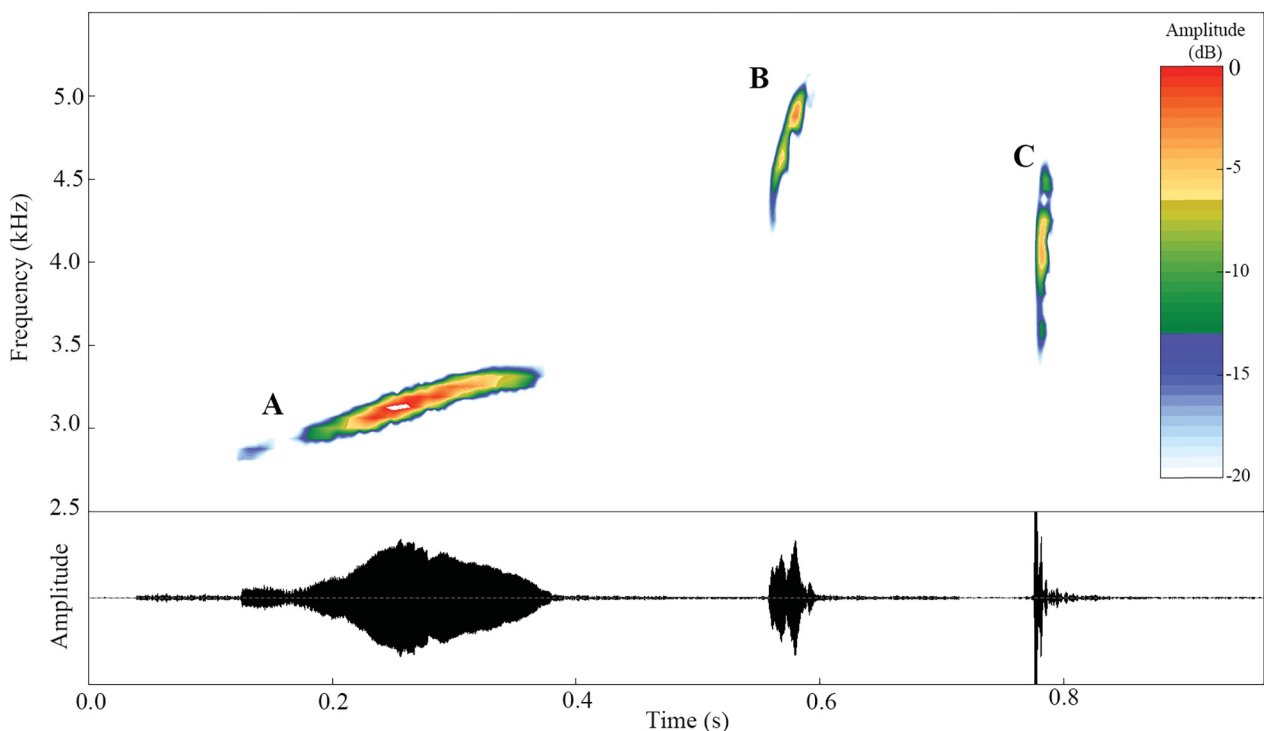


**FIGURE 2.** Maximum likelihood phylogeny (log likelihood = -8942.363171) of the relationships of *Diasporus amirae* sp. nov. within the *Diasporus* genus based on 16S and COI mitochondrial DNA genes. Bootstraps proportions are shown above branches and posterior probabilities (multiplied by 100) from MrBayes analysis are shown below branches. The scale bar refers to the estimated substitutions per site. The asterisks represent support of >95.

**Acoustic divergence.** In the genus *Diasporus* some species produce exclusively pure tones while others vocalize with modulated calls. The latter is the case of the new species described herein and *D. vocator*, which are most closely related according to the topology of our phylogeny (Fig. 2), however we found a deep divergence between their vocalizations (Fig. 4). The PCA efficiently differentiated the specimens from Plátano peak and the populations referred to *D. vocator* (Fig. 3b). The first principal component (PC1) explained 73.09 % of the total variance and the second explained 14.07 %. We found significant differences in the 13 spectro-temporal features we measured for each advertisement call after conducting the MANOVA (Wilk's lambda=0.001597, F=5439.1, p<0.01) (see Table 2).



**FIGURE 3.** Principal component analysis showing morphometric (A) and acoustic (B) differences among individuals of *Diasporus amirae* sp. nov. (red circles) from the Plátano peak and individuals of *D. vocator* (black triangles) from the Pacific of Costa Rica.



**FIGURE 4.** Spectrogram and oscillogram of the advertisement call of (A) *Diasporus amirae* sp. nov., (B) *D. vocator*, and (C) *Diasporus* sp. A.

The combination of the above mentioned genetic, morphometric, and acoustic differentiation and the distinct phenotypic characteristics (see below) of the specimens from Plátano peak provide us sufficient evidence to recognize it as an independent evolutionary lineage and propose the following as a new species.



***Diasporus amirae* sp. nov.**

Amira's dink frog

(Figures 5–6)

urn:lsid:zoobank.org:act:4C5AE14B-3B6C-4846-946C-CC6BB15FF42

**Holotype.** UCR 21843, an adult male from Costa Rica: Provincia de Limón: Cantón de Limón: Distrito de Matama: Cerro Plátano (9.870°, -83.240°; 1000 m a.s.l.) in the northeastern of the Cordillera de Talamanca; collected by Gerardo Chaves, Adrián García-Rodríguez, Stanley Salazar, and Gilbert Alvarado on 8 March 2013.

**Paratypes.** UCR 21842, adult male, same data as the holotype. UCR 22010–2, adult males, from of Plátano peak, near of type locality (9.869°, -83.241°; 1121 m a.s.l.), collected by Gerardo Chaves, José Andrés Salazar-Zúñiga, Julissa Gutiérrez, Diego Salas, and Ruperto Madrigal on 6 September 2013. UCR 22554, adult male from Plátano peak, near of type locality (9.863°, -83.236°; 1000 m a.s.l.), collected by Gerardo Chaves, José Andrés Salazar-Zúñiga, and Juan Ignacio Abarca on 18 June 2015.

**Generic placement.** This small species is assigned to the genus *Diasporus* based on its phylogenetic position, its characteristic “dink” call and the presence of palmate disc covers on some fingers and toes.

**Diagnosis.** A small species of the genus *Diasporus* with the following characteristics: (1) skin on dorsum smooth to having scattered tubercles; head having scattered tubercles; venter smooth in the midline but weakly areolate in the transition to flanks; flanks weakly areolate to granular; posterior surface of hind limbs surrounding cloaca strongly areolate; without dorsolateral, lateral, discoidal or supratympanic folds (Fig. 5); (2) tympanic membrane round, small, and indistinct; annulus partially evident through the skin; (TY/ED = 18.5–41.9 %); (3) snout subovoid in dorsal view, rounded in profile; loreal region concave; canthus rostralis usually rounded (Fig. 6); (4) eyelid areolate (EW/IOD = 57.7–65.2 %); cranial crests absent (5) vomerine teeth on oblique odontophores, barely distinct; (6) vocal slits large in adult males; single external subgular vocal sac present; nuptial pads absent; (7) Finger II larger than Finger I; discs and terminal transverse grooves present on all the fingers; tips symmetric, disc covers palmate on Finger III, sometimes also on fingers II and IV, others fingers with disc covers rounded; pads usually broadened, sometimes triangular on Finger III (Fig. 6c); (8) fingers lack lateral fringes; webbing absent; thenar tubercle elongate, palmar rounded, thenar and palmar tubercles flattened and similar in size; supernumerary and accessory palmar tubercles absent; subarticular tubercles round in basal outline, flatted in form and globular in profile; (9) ulnar tubercle and fold absent; (10) heel smooth; inner tarsal folds absent; (11) toes lack lateral fringes; inner metatarsal tubercle elongate, outer rounded, much smaller than inner, inner and outer metatarsal tubercles projecting; supernumerary tubercles and plantar absent; subarticular tubercles ovoid in basal outline, flatted in form and globular in profile; (12) Toe V larger than Toe III; discs and terminal transverse grooves present on all the toes; tips symmetrical, disc covers palmate on Toe IV, sometimes also on toes III and V, others toes with disc covers rounded; pads broadened; webbing absent; (13) dorsum brown with a black spots or brown-grayish with several black spots, almost uniform; venter pale gray-bluish with pale blotches forming a pattern of pale gray blotches; throat uniform dark gray-bluish; forelimbs and hind limbs with dark bars; some specimens with a dark supratympanic stripe; mask, occipital, labial, or lateral marks absent; (14) SVL in males 18.0–23.0 mm.

**Comparisons with other species.** *Diasporus amirae* differs from other species of *Diasporus* in the following characters (condition for *D. amirae* in parentheses). *Diasporus anthrax* (Lynch, 2001) differs from *D. amirae* by having red blotches on the surfaces of limbs (limbs brown-grayish to gray-blackish with black stripes). *Diasporus citrinobapheus* Hertz *et al.*, 2012 has a yellow to bright orange dorsum and translucent venter with white mottling (dorsum brown to brown-grayish and venter gray-bluish with pale blotches). *Diasporus darienensis* Batista *et al.*, 2016 usually has a slightly enlarged conical supraocular tubercle (eyelid areolate but never with a conical supraocular tubercle), a brown to reddish dorsum, usually with a pair of red or pale dorsolateral lines (dorsum brown to brown-grayish uniform never with dorsolateral lines). *Diasporus diastema* (Cope, 1875) has a pale gray to tan dorsum and yellow venter and groin, usually with a pair of light dorsolateral stripes extends from the shoulders to the pelvis (dorsum brown to brown-grayish and venter and groin gray-bluish with pale blotches, without a pair of dorsolateral stripes). *Diasporus gularis* (Boulenger, 1898) has a pale brown dorsum, cream venter, and a yellow groin, and usually shows a significant amount of red in its color pattern (dorsum brown to brown-grayish and venter and groin gray-bluish with pale blotches, without red in its color pattern), usually with a dark bar between the eyes (without a dark bar between the eyes). *Diasporus hylaeformis* (Cope, 1875) has a pale yellow to orange venter and groin (venter and groin gray-bluish with pale blotches). *Diasporus igneus* Batista *et al.*, 2012 shows rounded

scattered tubercles in the dorsum (dorsum smooth to scattered tubercles), an enlarged and conical supraocular tubercle (eyelid areolate but never with a conical supraocular tubercle), yellow to orange reticulations in the dorsum and a yellow venter (dorsal coloration brown to brown-grayish without light reticulations, venter nearly uniform, gray with dark brown pigment). *Diasporus majeensis* Batista *et al.*, 2016 has smooth dorsal skin with small-dispersed tubercles (dorsum smooth to areolate), dorsum brown to reddish and venter translucent (dorsum brown to brown-grayish and venter gray-bluish with pale blotches). *Diasporus pequeno* Batista *et al.*, 2016 has a rounded supraocular tubercle (eyelid areolate but never with a conical supraocular tubercle), unguual flap lanceolate to papillate (ungual flap palmate to rounded), dorsum brown to cream, with dark reticulations, venter translucent (dorsum brown to brown-grayish and venter and groin gray-bluish with pale blotches). *Diasporus quidditus* (Lynch, 2001) and *D. tinker* (Lynch, 2001) have the unguual flap lanceolate to papillate on fingers II-III (ungual flap palmate to rounded), dorsum gray to brown and venter brown to orange (dorsum brown to brown-grayish and venter and groin gray-bluish with pale blotches). *Diasporus sapo* Batista *et al.*, 2016 has snout rounded in dorsal view (snout subovoid in dorsal view), dorsal skin slightly tuberculate (dorsum smooth to scattered tubercles), dorsum reddish and venter translucent (dorsum brown to brown-grayish and venter gray-bluish with pale blotches). *Diasporus tigrillo* (Savage, 1997) has dorsum with low pustules and yellow to bright orange in coloration (dorsum smooth to scattered tubercles and brown to brown-grayish in coloration), venter white (venter gray-bluish with pale blotches). *Diasporus ventrimaculatus* Chaves *et al.*, 2009 has venter typically blotched and contrasting or with light to red coloration (venter gray-bluish with pale blotches, without contrasting blotches). *Diasporus vocator* (Taylor, 1955) differs from *D. amirae* by: a) having a dark interorbital bar with a paler area anterior to it (Fig. 7a, [without interorbital bar]), b) its smooth head (head scattered tubercles), c) its snout pointed (snout rounded); d) its disc covers in fingers and toes oblong ending with a distinct point (disc covers in fingers and toes palmate or rounded), and e) smaller, SVL in adults 14–18 mm (larger, SVL in adults 18.1–21.8 mm). *Diasporus* sp. A has a pale interorbital bar (Fig. 7d, [without interorbital bar]), and its smooth head (head scattered tubercles).

**Description of holotype.** Adult male having a SVL of 18.5 mm (Fig. 6). Head narrow, HW 37.8 % of SVL; HL 33.5 % SVL; snout subovoid in dorsal view, rounded in profile; canthus-rostralis indistinct; loreal region slightly concave; nostrils small, directed laterally; vocal slits large, single external subgular vocal sac present; vomerine teeth barely discernable, positioned transversely in two fascicles well posterior of choanae. Eye large, diameter equal to two and half of E-N. Tympanic membrane indistinct, covered in skin; tympanic annulus weak to partially discernable below skin, small (33.3 % of ED), round. Skin on dorsum smooth, venter smooth in the midline but becomes weakly areolate towards flanks, throat and head smooth, flanks weakly areolate; without dorsolateral, lateral, discoidal, supratympanic or interocular folds; eyelid smooth; inguinal gland and postriatal tubercles absent.



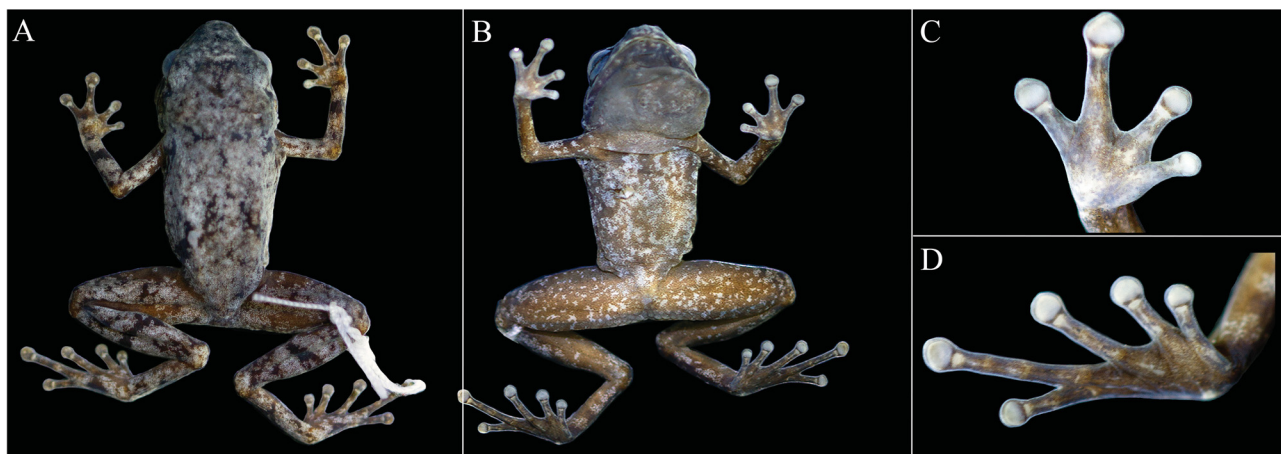
**FIGURE 5.** Color in life view of *Diasporus amirae* sp. nov. **A)** Dorsum and **B)** venter in nighttime coloration, and **C)** dorsum in daytime coloration. Photos A–B by S. Salazar and C by J. A. Salazar-Zúñiga.

Forelimbs relatively short and slim. Fingers moderately long and robust, proximal section slightly wider than distal section; without lateral fringes; Finger II larger than Finger I. Discs and terminal transverse grooves on all the fingers; tips symmetric, disc covers on Finger I and IV round, palmate on fingers II and III; disc pads broadened. Thenar tubercle elongate, palmar rounded, thenar and palmar flattened and similar in size. Supernumerary and accessory palmar tubercles absent; subarticular tubercles on fingers barely discernable, rounded basally, flattened in form and globular in profile. Ulnar tubercles and fold absent. Fingers lack interdigital webbing.

Hind limbs relatively short and slim. Toes without lateral fringes; Toe III shorter than Toe V. Discs and terminal transverse grooves on all the toes; tips symmetrical; disc covers rounded on toes I, II, and V, palmate on toes III and IV; disc pads broadened. Inner metatarsal tubercle elongated; outer metatarsal tubercle rounded, much smaller than inner, both inner and outer metatarsal tubercles projecting. Supernumerary and plantar tubercles absent; subarticular tubercles on toes ovoid basally, flattened in form and globular in profile. Heel smooth, inner tarsal fold absent. Toes lack interdigital webbing.

**Coloration in life.** *Diasporus amirae* is metachromatic and shows a high-level of color variation between day and night. While active at night the dorsum was observed to be dark brown with black marks (Fig. 5a-b), but while inactive, in the daytime, the dorsum was observed to change to a bronze coloration with bluish black marks that form thin lines across the girdles and in the interorbital area (Fig. 5c). We consider that the dark brown coloration of the dorsum is more characteristic of this species because the dorsal background of the preserved specimen in ethanol is gray (Fig. 6). The venter is usually gray-bluish with pale blotches. The dorsal surfaces of the forelimbs and hind limbs are similar to the dorsum, and present black-bluish marks. The flanks and groins are similar in coloration to that of the venter. The iris is brown.

**Coloration of the holotype in ethanol.** After five years in ethanol (70 %), the dorsum and head is gray-blackish with several black blotches; a narrow pale interorbital band present; a dark supratympanic stripe extending from the orbit to the suprascapular shoulder; venter gray with dark brown pigment, forming a pattern of pale blotches on dark background due to the lack of dark brown pigment; throat black, vocal sac uniformly gray-blackish; flanks and groin similar to dorsal background; dorsal surface of forelimbs and hind limbs similar to dorsal background with darker marks; ventral surfaces of forelimbs and hind limbs dark brown with pale blotches; ventral surfaces of hands and feet dark brown, with pale blotches on fingers and toes; posterior and anterior surfaces of hind limbs uniform dark brown (Fig. 6).



**FIGURE 6.** A) Dorsum, B) venter, C) right hand, and D) right foot of the holotype (UCR 21843) of *Diasporus amirae* sp. nov. Photos by E. Arias.

**Measurements of holotype (mm).** SVL 18.5; HL 6.2; HW 7.0; ED 3.0; IOD 2.8; TY 1.0; EW 1.5; E–N 1.2; F1 2.0; F2 2.2; F3 3.6; F4 3.0; T1 2.9; T2 3.5; T3 5.0; T4 7.1; T5 5.1; TL 9.5.

**Morphometric and morphological variation of paratypes.** We did not find evident morphological variation among the five paratypes, and little variation was observed in their coloration. Dorsal background in UCR 22010, 22012, and 22554 is nearly uniform black (in ethanol). The dorsal background coloration of UCR 22011 is paler than the holotype. The ventral surfaces of UCR 22010 are paler than the holotype, with little dark brown pigment.

**Natural history notes.** We have found only two populations of *Diasporus amirae*, both above 1000 m a.s.l., on the Plátano peak and a nearby unnamed peak. Both peaks are covered by primary forest, and are categorized as Tropical Wet Forest (Holdridge 1967), lacking a dry season, having an annual precipitation range between 3000 to 4000 mm, and annual temperatures oscillating between 18 to 28 °C. On Plátano peak *D. amirae* was detected only along the northern slope, in the last 200 m of the ascent to Plátano’s summit. The new species has been observed calling during the night from low vegetation (less than 1 meter above the ground) or within the leaf litter near the walls of small stream canyons. However, we also detected some males calling from trees, up to 6 meters high. This species appears to be very shy; when disturbed, males stop calling for several minutes, such behavior is rare in other



species of *Diasporus* in which males keep quiet when disturbed, but start over their calling activity almost immediately. In relation to the calling activity, *D. amirae* behaves similar to many other anuran species that call during the night but also can vocalize during the afternoon on rainy days (Savage 2002). According to the call activity during the sampled months, *D. amirae* seems to be less common than the sympatric *D. aff. diastema* in the area. We did not find females, juveniles or eggs in spite of 158 man-hours of searching effort during a total of eight nights of fieldwork in three different months.

**Vocalizations.** The advertisement call of *Diasporus amirae* consists of a single modulated note (Fig. 4) with a mean duration of  $0.256 \pm 0.015$  s. Dominant frequency in this species ranges in mean from  $2.871 \pm 0.039$  kHz at the beginning of the call to  $3.273 \pm 0.051$  kHz at the end of the call. Compared to other modulating species in the genus, the duration of the call of *D. amirae* is more than six times the duration of the call of *D. vocator* (Table 2) and twice the duration of the calls of *D. tigrillo* and *D. citrinobapheus* (García-Rodríguez *et al.* 2016). This is the most evident difference to the human ear, due to the fact that the narrow frequency range and the long duration results in a lower slope in dominant frequency making modulation less perceptible (Table 2). In contrast, other species such as *D. vocator* or *D. tigrillo* use wider frequency ranges in shorter calls, giving their vocalization a whistle-like tone.

**Geographic distribution.** *Diasporus amirae* is currently known to be restricted to the headwaters of the Río Banano on the northeastern foothills of Cordillera de Talamanca, Caribbean slope, Costa Rica (Fig. 1). The known altitudinal range of the new species is 1000–1121 m a.s.l. The known populations of *D. amirae* are within La Amistad International Park. We believe that more fieldwork is needed to better understand the potential distributional range of this species. *Diasporus amirae* is sympatric with *D. aff. diastema*.

**Etymology.** The name *amirae* is a matronym dedicated to Stanley Salazar's daughter, Amira Salazar who was born in May 2017.

## Discussion

**Phylogenetic relationships of *Diasporus amirae* and its sister species.** Our phylogenies showed that *Diasporus amirae* is most closely related to *D. vocator* and to an unnamed species from western Panama (Fig. 2), previously referred as *D. aff. vocator* by Batista *et al.* (2016) but that we prefer to refer as *Diasporus* sp. A to avoid confusion. However, the phylogenetic position of *D. vocator* within the genus remains uncertain given that in our phylogeny, although with a weak support, it is closely related to *D. aff. hylaeformis*. This relationship contrasts with the findings of Batista *et al.* (2016) and García-Rodríguez *et al.* (2016). The phylogenetic position only is not enough to support the evolutionary distinctiveness of *D. amirae*, because the hierarchical structure of character variation could be explained by geographical structure due to processes such as isolation by distance or drift/selection of small metapopulations (Padial *et al.* 2010; Castroviejo-Fisher *et al.* 2017). However, we have shown robust morphometric, acoustic, and phenotypic evidence supporting that *D. amirae* clearly differs from *D. vocator*, its sister taxon. Thus, we conclude that the evidence provided here supports the recognition of *D. amirae* as a different evolutionary lineage.

In addition, although genetic distances are not adequate for species delimitation (Hamilton *et al.* 2014; Yu *et al.* 2017), we consider that deep genetic distances, between phylogenetically related species constitute evidence of their different evolutionary trajectory as species. The genetic distances found herein are above the thresholds of 3% in 16S and 10% in COI mitochondrial genes suggested by Fouquet *et al.* (2007) and Vences *et al.* (2005), respectively. These genetic distances also are greater than some of those reported by Batista *et al.* (2016) between four new species of *Diasporus* from eastern Panama (see in Batista *et al.* 2016, page 270 Table 1). For amphibians, the 16S gene fragment has been suggested as a DNA barcode marker for amphibian diversity inventories (Vences *et al.* 2005) to complement the standard COI-5' marker used in general for animals (Smith *et al.* 2008).

With the recognition of *Diasporus amirae*, the population from El Pantano, Santa Fé on western Panama (MHCH 2874) cannot be referred to any currently named species. This specimen was referred as *D. aff. vocator* by Batista *et al.* (2016), however in their phylogeny this clade is not closely related to *D. vocator* from the type locality (FMNH 257769). This population is in need of more detailed morphological and acoustical analyses in order to assign a valid taxonomic status. We consider that the phylogenetic position of *Diasporus* sp. A provides significant evidence of its independent evolutionary trajectory as a species beyond the observation that it resembles *D. vocator* in morphology.



The distribution range of *D. vocator* is uncertain. To date only a limited number of populations near the type locality were phylogenetically grouped to *D. vocator sensu stricto* (see Fig. 1–2). Although it has been suggested that this species occurs in Costa Rica, Panama, and Colombia (Savage 2002; AmphibiaWeb 2018; Frost 2018) the distribution range shown by the IUCN (2015) is restricted to Costa Rica and western Panama. Batista *et al.* (2016) included, in their Appendix 2, a specimen (SMF 97339) from central Panama referred as *D. vocator*, however, they did not show it in their phylogeny; we found that this specimen (herein shown as *Diasporus* sp. C) is not phylogenetically related to *D. vocator*. We consider it necessary to obtain genetic data from other populations referred to as *D. vocator* in order to precisely delineate the distributional range of this species. Specimens from Bocas del Toro on the Caribbean slope of Panama, for example, are very similar to *D. vocator sensu stricto* in coloration (see available photos on AmphibiaWeb). The same situation occurs in Cerro Azul, central Panama, where this species is abundant (Sosa & Guerrel 2013, Fig. 7b,c); however, this locality is not included in the distribution range delineate by the IUCN (2015).



**FIGURE 7.** Dorsal color in life views of *Diasporus vocator* from **A**) type locality in San Vito, Costa Rica (EAP0730), **B-C**) Cerro Azul, Panama (not voucher), and *Diasporus* sp. A from **D**) Santa Fe, Panama. Photo A by E. Arias, B-C by Angel Sosa, and D by Abel Batista.

**Diversity of the genus *Diasporus*.** With the description of *Diasporus amirae*, the genus reaches a total of 16 species (Frost 2018). Our results support previous findings (Savage 2002; Batista *et al.* 2016), suggesting that the diversity within the genus is underestimated and that several species remain unnamed. For example, in our phylogeny we recovered two clades referred as *D. aff. quidditus*, three samples (*Diasporus* sp. A, *Diasporus* sp. B, and

*Diasporus* sp. C) are not robustly assigned to any named species, and two clades are referred as *D. aff. diastema*. It is necessary to obtain sequences of *D. quidditus* from the type locality (Buenaventura, Valle del Cauca, Colombia), which is separated by ~500 linear km from the specimens here shown (eastern Panama), to clarify this situation. Sequences of *D. quidditus sensu stricto* could help to disentangle the taxonomic status of the clades currently assigned to *D. aff. quidditus* and determine whether any of them correspond to *D. quidditus* or both represent unnamed species.

Batista *et al.* (2016) included two samples (SMF97287 and SMF97290) of *D. diastema* from central Panama (type locality region), however those individuals were not monophyletic. We included other sequences from central Panama used by Paz *et al.* (2015) and found that all sequences from central Panama are clustered. These samples from central Panama were collected near the type locality of *D. diastema*, thus we consider this clade may represent *D. diastema sensu stricto*. Although both clades within of *D. diastema* (see Fig. 2) are strongly structured, we recognize it as one species due to its monophyly and the lack of other evidence suggesting the presence of separated lineages. In this case the phylogenetic position is not enough to support its evolutionary distinctiveness, in turn drift/selection of small metapopulations can explain its hierarchical structure (Padial *et al.* 2010; Castroviejo-Fisher *et al.* 2017). In this case, other types of evidence of lineage divergence, such as non-overlapping morphological character, sympatry between clades, mating calls, or combined analyses of multiple loci are necessary (Padial *et al.* 2010). Our suggestion, recognized these clades as *D. diastema sensu stricto*, will be tested with additional evidence, especially comparing mating calls of both populations herein assigned to *D. diastema*.

As with *Diasporus amirae*, other *Diasporus* species have small ranges in montane regions (Chaves *et al.* 2009; Batista *et al.* 2012, 2016; García-Rodríguez *et al.* 2016). It is very likely that several species of *Diasporus* remain undiscovered within Isthmian Central America, in montane regions that have been poorly explored so far (Savage 1997; Hertz *et al.* 2012; Batista *et al.* 2016; García-Rodríguez *et al.* 2016). Further efforts must be oriented to visit other remote areas and integrate multiple lines of evidence to facilitate species discovery and description in this and other taxa with restricted distributions.

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**APPENDIX I.** Institutional voucher numbers, locality information, and GenBank accession numbers for the specimens used in the molecular phylogenetic analyses. Numbers in the ID column correspond to the collection sites indicated in Figure 1.

ID	Species	Institutional vouchers	Collection locality	Geographic coordinates	GenBank Number	
					16S	COI
13	<i>Diasporus</i> sp. A	MHCH2874	El Pantano, Santa Fe, Veraguas, PA	8.563°, -81.052°	KT186622	—
8	<i>Diasporus</i> sp. B	MHCH1678	Breñón, Renacimiento, Chiriquí, PA	8.580°, -82.789°	—	KT186574
28	<i>Diasporus</i> sp. B	SMF97652	Jingurudó, Sambú, Emberá-Wounaan, PA	7.759°, -78.092°	—	KT186562
15	<i>Diasporus</i> sp. C	SMF97339	Coclé el Norte, Donoso, Colón, PA	8.988°, -80.582°	—	KT186573
24	<i>D. aff. diastema</i> EPL	MHCH2802	Wargandí, Pinogana, Darién, PA	9.035°, -78.026°	KT186617	KT186563
20	<i>D. aff. diastema</i> EPL	MHCH2805	Mudugandí, Chepo, Panamá, PA	9.177°, -78.746°	—	KT186555
25	<i>D. aff. diastema</i> EPL	MHCH2811	Metetí, Pinogana, Darién, PA	8.597°, -78.049°	—	KT186571
26	<i>D. aff. diastema</i> EPL	SMF97289	Manuel Ortega, Cémaco, Emberá-Wounaan, PA	8.476°, -77.549°	—	KT186586
21	<i>D. aff. diastema</i> MM	MHCH2801	Unión Santeña, Chimán, Panamá, PA	8.916°, -78.629°	KT186624	KT186572
22	<i>D. aff. diastema</i> MM	MHCH2807	Tortí, Chepo, Panamá, PA	8.892°, -78.563°	—	KT186580
22	<i>D. aff. diastema</i> MM	MHCH2808	Tortí, Chepo, Panamá, PA	8.892°, -78.563°	KT186627	KT186578
10	<i>D. aff. hylaeiformis</i>	MHCH2859	Hornito, Gualaca, Chiriquí, PA	8.719°, -82.232°	KT186614	KT186558
14	<i>D. aff. hylaeiformis</i>	MVUP1826	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784390	FJ766809
2	<i>D. aff. hylaeiformis</i>	MVZ203844	Orosi, Paraíso, Cartago, CR	9.750°, -83.804°	EU186682	—
9	<i>D. aff. hylaeiformis</i>	SMF89868	Valle del Risco, Almirante, Bocas del Toro, PA	8.911°, -82.714°	JQ927337	—
9	<i>D. aff. hylaeiformis</i>	SMF89869	Valle del Risco, Almirante, Bocas del Toro, PA	8.911°, -82.714°	JQ927338	—
9	<i>D. aff. hylaeiformis</i>	SMF89872	Valle del Risco, Almirante, Bocas del Toro, PA	8.911°, -82.714°	JQ927339	—
9	<i>D. aff. hylaeiformis</i>	SMF89875	Valle del Risco, Almirante, Bocas del Toro, PA	8.911°, -82.714°	JQ927340	—
1	<i>D. aff. hylaeiformis</i>	UCR16264	Ángeles, San Ramón, Alajuela, CR	10.220°, -84.540°	JN991418	JN991347
14	<i>D. aff. hylaeiformis</i>	USNM572456	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784369	FJ766810
17	<i>D. aff. quidditus</i>	AJC1789	Chilibre, Panamá, Panamá, PA	9.228°, -79.419°	KR863218	KR862963
17	<i>D. aff. quidditus</i>	CH6803	Chilibre, Panamá, Panamá, PA	9.231°, -79.419°	KR863219	KR862964
17	<i>D. aff. quidditus</i>	CH6804	Chilibre, Panamá, Panamá, PA	9.231°, -79.419°	KR863216	KR862961
17	<i>D. aff. quidditus</i>	CH6648	Chilibre, Panamá, Panamá, PA	9.228°, -79.419°	KR863212	KR862957
30	<i>D. aff. quidditus</i>	MHCH2824	Camoganti, Chepigana, Darién, PA	7.997°, -77.711°	KT186621	KT186569
30	<i>D. aff. quidditus</i>	MHCH2825	Camoganti, Chepigana, Darién, PA	7.992°, -77.709°	KT186560	—
14	<i>D. aff. quidditus</i>	MVUP1832	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784405	—
30	<i>D. aff. quidditus</i>	SMF97291	Camoganti, Chepigana, Darién, PA	7.992°, -77.709°	KT186613	—

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APPENDIX 1. (Continued)

ID	Species	Institutional vouchers	Collection locality	Geographic coordinates	GenBank Number	
					16S	COI
30	<i>D. aff. quidditus</i>	SMF97292	Camoganti, Chepigana, Darién, PA	7.987°, -77.707°	KT186634	KT186589
24	<i>D. aff. quidditus</i>	SMF97298	Wargandí, Pinogana, Darién, PA	9.035°, -78.026°	KT186625	—
28	<i>D. aff. quidditus</i>	SMF97653	Jingurudó, Sambú, Emberá-Wounaan, PA	8.799°, -78.462°	—	KT186567
14	<i>D. aff. quidditus</i>	USNM572444	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784326	—
3	<i>D. amirae</i> sp. nov.	UCR21842	Matama, Limón, Limón, CR	9.870°, -83.240°	MK457180	—
3	<i>D. amirae</i> sp. nov.	UCR21843	Matama, Limón, Limón, CR	9.870°, -83.240°	MK457181	MK471387
3	<i>D. amirae</i> sp. nov.	UCR22254	Matama, Limón, Limón, CR	9.870°, -83.240°	MK457182	MK471388
11	<i>D. citrinobapheus</i>	MHCH2370	El Paredón, Ñürüm, Ngäbe-Buglé, PA	8.485°, -81.173°	JQ927335	—
11	<i>D. citrinobapheus</i>	MHCH2371	El Paredón, Ñürüm, Ngäbe-Buglé, PA	8.485°, -81.173°	JQ927336	—
14	<i>D. citrinobapheus</i>	MVUP1783	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784338	—
14	<i>D. citrinobapheus</i>	MVUP1830	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784395	—
11	<i>D. citrinobapheus</i>	SMF89814	El Paredón, Ñürüm, Ngäbe-Buglé, PA	8.485°, -81.173°	JQ927333	—
12	<i>D. citrinobapheus</i>	SMF89820	Santa Fe, Santa Fe, Veraguas, PA	8.569°, -81.099°	JQ927334	—
14	<i>D. citrinobapheus</i>	USNM572442	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784425	—
14	<i>D. citrinobapheus</i>	USNM572443	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784484	—
14	<i>D. citrinobapheus</i>	USNM572454	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784423	—
14	<i>D. citrinobapheus</i>	USNM572455	El Harino, La Pintada, Coclé, PA	8.667°, -80.592°	FJ784424	—
32	<i>D. darienensis</i>	CH6425	Boca de Cupe, Pinogana, Darién, PA	7.762°, -77.724°	KR863223	KR862968
32	<i>D. darienensis</i>	CH6431	Boca de Cupe, Pinogana, Darién, PA	7.764°, -77.722°	KR863222	KR862967
31	<i>D. darienensis</i>	MHCH2841	Camoganti, Chepigana, Darién, PA	7.988°, -77.708°	KT186618	—
31	<i>D. darienensis</i>	MHCH2845	Camoganti, Chepigana, Darién, PA	7.988°, -77.708°	—	KT186561
29	<i>D. darienensis</i>	MHCH2850	Jingurudó, Sambú, Emberá-Wounaan, PA	7.683°, -78.038°	KT186626	KT186576
31	<i>D. darienensis</i>	MHCH2862	Camoganti, Chepigana, Darién, PA	7.987°, -77.708°	—	KT186579
31	<i>D. darienensis</i>	SMF97304	Camoganti, Chepigana, Darién, PA	7.963°, -77.704°	—	KT186581
31	<i>D. darienensis</i>	SMF97305	Camoganti, Chepigana, Darién, PA	7.973°, -77.708°	—	KT186582
31	<i>D. darienensis</i>	SMF97662	Camoganti, Chepigana, Darién, PA	7.947°, -77.704°	—	KT186564
18	<i>D. diastema</i>	CH6676	Chilibre, Panamá, Panamá, PA	9.320°, -79.289°	KR863214	KR862959
17	<i>D. diastema</i>	CH6786	Chilibre, Panamá, Panamá, PA	9.231°, -79.419°	KR863213	KR862958
17	<i>D. diastema</i>	CH6792	Chilibre, Panamá, Panamá, PA	9.231°, -79.424°	KR863217	KR862962

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APPENDIX 1. (Continued)

ID	Species	Institutional vouchers	Collection locality	Geographic coordinates	GenBank Number	
					16S	COI
17	<i>D. diastema</i>	CH6800	Chilibre, Panamá, Panamá, PA	9.231°, -79.419°	KR863211	KR862956
17	<i>D. diastema</i>	CH6802	Chilibre, Panamá, Panamá, PA	9.231°, -79.419°	KR863215	KR862960
16	<i>D. diastema</i>	SMF97287	Cristóbal, Colón, Colón, PA	9.260°, -79.935°	—	KT186566
19	<i>D. diastema</i>	SMF97290	El Llano, Chepo, Panamá, PA	9.284°, -78.984°	—	KT186577
23	<i>D. majeensis</i>	SMF97293	Río Congo Arriba, Chepigana, Darién, PA	8.799°, -78.462°	—	KT186565
26	<i>D. pequeno</i>	MHCH2828	Manuel Ortega, Cémaco, Embera-Wounaan, PA	8.476°, -77.549°	—	KT186556
26	<i>D. pequeno</i>	MHCH2830	Manuel Ortega, Cémaco, Embera-Wounaan, PA	8.479°, -77.528°	—	KT186559
26	<i>D. pequeno</i>	SMF97335	Manuel Ortega, Cémaco, Embera-Wounaan, PA	8.479°, -77.528°	—	KT186583
26	<i>D. pequeno</i>	SMF97337	Manuel Ortega, Cémaco, Embera-Wounaan, PA	8.479°, -77.528°	—	KT186570
26	<i>D. pequeno</i>	SMF97663	Manuel Ortega, Cémaco, Embera-Wounaan, PA	8.479°, -77.528°	—	KT186575
27	<i>D. sapo</i>	MHCH2855	Garachiné, Chepigana, Darién, PA	7.976°, -78.363°	KT186619	KT186568
27	<i>D. sapo</i>	MHCH2856	Garachiné, Chepigana, Darién, PA	7.976°, -78.363°	KT186616	—
27	<i>D. sapo</i>	MHCH2858	Garachiné, Chepigana, Darién, PA	7.976°, -78.363°	KT186628	—
27	<i>D. sapo</i>	SMF97329	Garachiné, Chepigana, Darién, PA	7.976°, -78.363°	—	KT186557
27	<i>D. sapo</i>	SMF97330	Garachiné, Chepigana, Darién, PA	7.976°, -78.363°	KT186630	KT186584
4	<i>D. tigrillo</i>	UCR22364	Telire, Talamanca, Limon, Costa Rica	9.458°, -83.034°	KT438502	—
4	<i>D. tigrillo</i>	UCR22365	Telire, Talamanca, Limon, Costa Rica	9.458°, -83.034°	KT438503	—
4	<i>D. tigrillo</i>	UCR22366	Telire, Talamanca, Limon, Costa Rica	9.458°, -83.034°	KT438504	—
4	<i>D. tigrillo</i>	UCR22367	Telire, Talamanca, Limon, Costa Rica	9.458°, -83.034°	KT438505	—
4	<i>D. tigrillo</i>	UCR22368	Telire, Talamanca, Limon, Costa Rica	9.458°, -83.034°	KT438506	—
32	<i>D. tinker</i>	AJC1866	Boca de Cupe, Pinogana, Darién, PA	7.774°, -77.733°	KR863221	KR862966
32	<i>D. tinker</i>	CH6439	Boca de Cupe, Pinogana, Darién, PA	7.764°, -77.722°	KR863220	KR862965
29	<i>D. tinker</i>	MHCH2871	Jingurudó, Sambú, Emberá-Wounaan, PA	7.669°, -78.038°	KT186620	—
29	<i>D. tinker</i>	MHCH2872	Jingurudó, Sambú, Emberá-Wounaan, PA	7.669°, -78.038°	KT186623	—
29	<i>D. tinker</i>	MHCH2873	Jingurudó, Sambú, Emberá-Wounaan, PA	7.669°, -78.038°	KT186615	—
29	<i>D. tinker</i>	SMF97320	Jingurudó, Sambú, Emberá-Wounaan, PA	7.693°, -78.042°	KT186632	KT186587
29	<i>D. tinker</i>	SMF97327	Jingurudó, Sambú, Emberá-Wounaan, PA	7.669°, -78.038°	KT186635	—
7	<i>D. vocator</i>	FMNH257769	San Vito, Coto Brus, Puntarenas, CR	8.790°, -82.960°	JN991419	JN991348
5	<i>D. vocator</i>	UCR20133	Biolley, Buenos Aires, Puntarenas, CR	9.026°, -83.030°	KT438508	—
6	<i>D. vocator</i>	UCR21857	Limoncito, Coto Brus, Puntarenas, CR	8.797°, -83.075°	KT438507	—