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An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama

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We have applied an integrative taxonomic approach, including bioacoustics, ecology, morphology, and molecular genetics (barcoding and phylogeography), to explore species richness in the genus *Diasporus* in eastern Panama, from where only *Diasporus quidditus* (Lynch, 2001) was previously known. During fieldwork in eastern Panama in 2011 and 2012 we found six additional species, four of which we are describing here as new to science, plus two species that are new for this region. We have evaluated the presence of *Diasporus diastema* (Cope, 1875) in eastern Panama by comparing morphological, genetic, and bioacoustic characters of specimens from near the type locality in central Panama with specimens from eastern Panama. We further describe and compare male advertisement calls of most *Diasporus* species. The phylogeographic analysis suggests the allopatric speciation of *Diasporus* species in eastern Panama following the completion of the Panamanian isthmus in the middle Miocene. Subsequent geological events concur with the vicariant evolution of different lineages *in situ*, suggesting eastern Panama to be a centre of endemism for this group of frogs. We present an integrative analysis of the species from eastern Panama and include an identification key for all species of the genus.

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ADDITIONAL KEYWORDS: barcoding – bioacoustics – *Diasporus* – *Diasporus darienensis* sp. nov. – *Diasporus majeensis* sp. nov. – *Diasporus pequeno* sp. nov. – *Diasporus sapo* sp. nov. – ecology – integrative taxonomy – Panama – phylogeography.

INTRODUCTION

Cryptic species are defined as ‘two or more distinct species that are erroneously classified under one species name’ (Bickford *et al.*, 2007). Frogs of the genus *Diasporus* are a good example of cryptic diversity, as most species resemble each other externally, and are difficult to distinguish by morphological methods alone; however, integrative analysis (the combination of several methods and lines of evidence) reveals the true diversity in this species complex (Hertz *et al.*, 2012). In recent years, three new species of the genus *Diasporus* (formerly *Eleutherodactylus diastema* group; *sensu* Hedges, Duellman & Heinicke, 2008) have been described from Costa Rica and western Panama (Chaves *et al.*, 2009; Batista, Ponce &

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Hertz, 2012; Hertz *et al.*, 2012), and several more species presumably await discovery and description (Lynch & Duellman, 1997; Ibáñez, Rand & Jaramillo, 1999; Savage, 2002; Hertz *et al.*, 2012). There is evidence of divergence between populations in traits other than morphology, for example on geographic isolation, ecology, bioacoustics, and molecular genetics, but it is difficult to distinguish them based on morphology alone. The application of an integrative approach offers a more balanced solution (Dayrat, 2005; Vieites *et al.*, 2009; Padial *et al.*, 2010; Hertz *et al.*, 2012) that can solve the taxonomic problems within this genus.

The genus *Diasporus* comprises small frogs with short limbs and expanded disc pads, with or without lanceolate or papillate tips, that inhabit rainforests from eastern Honduras to north-western South America as far as north-western Ecuador (Hedges *et al.*, 2008). Adult males possess well-developed vocal slits and a single external subgular vocal sac. Their characteristic 'dink' call (subjective general acoustic description), which can be more like a whistle in some species, is commonly heard in wet forests throughout the distribution range of the genus. Males usually call from vegetation growing at ground level up to several metres above the ground (Savage, 2002).

To date, the genus *Diasporus* contains 11 described species (Frost, 2014). Seven species are currently known to be endemic to Central America: *Diasporus citrinobapheus* Hertz *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus diastema* (Cope, 1875), widespread between central Panama and Honduras; *Diasporus hylaeformis* (Cope, 1875), cordilleras (mountain ranges) of Costa Rica and western Panama; *Diasporus igneus* Batista *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus tigrillo* (Savage, 1997), Atlantic slopes of the Cordillera de Talamanca, Costa Rica; *Diasporus ventrimaculatus* Chaves *et al.*, 2009; Cordillera de Talamanca, Costa Rica; and *Diasporus vocator* (Taylor, 1955), western Panama and southern Costa Rica. *Diasporus quidditus* (Lynch, 2001) occurs in eastern Panama and north-western Colombia (Lynch, 2001; Köhler, 2011). The remaining three species are distributed along the Pacific side of northern South America, from Colombia to north-western Ecuador: *Diasporus anthrax* (Lynch, 2001), along the eastern foothills of the Cordillera central and the western slope of the Cordillera Oriental, Colombia; *Diasporus gularis* (Boulenger, 1898), lowlands of western Colombia and north-western Ecuador; and *Diasporus tinker* (Lynch, 2001), Pacific slopes of Colombia (IUCN, 2013; Frost, 2014).

Eastern Panama (EP) is known as an important biodiversity hot spot with high endemism, as a result of the great variety of habitats. Still largely unknown forests, that are suffering from rapid habitat loss (Parker, Carrión & Samudio, 2004). The high endemism in EP is likely to be the result of the complex geohistory of the Isthmus of Panama. EP represents the northernmost part of the Chocó biogeographical region (Duque-Caro, 1990), and can be subdivided into three main geographical units: the massifs of the Dabeiba Arc in the north-east (San Blas and Darién mountain ranges) and the Baudó Arc in the south-west (Jingurudó, Majé, Pirre, and Sapo mountain ranges). Between these mountains lies the Chucunaque Basin, a sedimentary basin that forms the central part of the Choco Block, with the drainage of Chucunaque River and Tuira Basin in the lowlands (Duque-Caro, 1990; Coates & Obando, 1996). The uplift of the Choco Block is the result of the collision of the Panama Arc with South America since the middle Miocene (as early as 11 Mya; Farris *et al.*, 2011). The continuous uplift of the Choco Block shallowed the water depth in the Atrato and Chucunaque basins, as they were steadily filled with sediments (Duque-Caro, 1990; Coates *et al.*, 2004). These geohistorical dynamics periodically separated land masses (e.g. the isolation of the mountains in the Baudo and Dabeiba arcs in EP during the Middle Miocene) that promoted speciation events and an increased species diversity in this region (Batista *et al.*, 2014b).

During the last 4 years we have conducted several expeditions and collected numerous specimens and associated materials of amphibians and reptiles across large parts of EP. Besides two recent discoveries and publications on regional anuran fauna (Batista *et al.*, 2014a,b), preliminary barcoding analysis of *Diasporus* frogs from different localities in EP revealed several distinct lineages. Herein, we apply the first integrative approach on *Diasporus* taxa to evaluate the status of these lineages. We use information from bioacoustics, ecology, morphology, and biogeography to evaluate the divergence among genetic lineages and draw taxonomic conclusions. Furthermore, we discuss the distribution pattern of *Diasporus* taxa from EP in a biogeographical context by comparing analyses of regional geological events and molecular clock calibrations.

MATERIAL AND METHODS

Fieldwork was carried out in the Chucunaque and Tuira basins of the eastern Panamanian lowlands (EPLL), and in all major eastern Panamanian mountain ranges. Eastern Panama (EP) defines the eastern half of the country, corresponding to the area east of the Panama Canal. It comprises two important ecoregions of the western hemisphere, the eastern Panamanian montane forests (EPMF) in the highlands and the Chocó-Darién moist forests (CDMF) in the lowlands (Fund, 2014). The EPMF is further split into several mountain ranges: San Blas mountain range (SBM), Darién mountain range (DM), Jingurudó-Sapo mountain range (JSM), Majé mountain range (MM), and Pirre mountain range (PM) (Figs 1 and 2). The lowlands (EPLL) are dissected by a few large rivers, the Balsas, Chucunaque, Sambú, and Tuira rivers, within the CDMF. There are a few additional, smaller (<500 m a.s.l.) mountain ranges, such as the Filo del Tallo-Canglón in middle of Darién, and the Bagre in the south-east of Darién along the Sambú River. All geographical coordinates were recorded in the WGS 1984 datum given in decimal degrees. The maps were created with ArcGIS 10 (ESRI, 2009). The voucher specimens collected were killed with agent T61 and subsequently fixed with a preservative mixture of 5 mL of formalin (40%) in 1 L of ethanol (94%), and then stored in ethanol (70%). All figures have been digitally modified for improved visibility and combined using Adobe CS3. For candidate species and their delimitation we follow the integrative concept for amphibians of Vieites *et al.* (2009).

MORPHOMETRICS

Morphological nomenclature, measurements, and diagnoses follow Duellman & Lehr (2009). All measurements were made using digital calipers and were rounded to the nearest 0.01 mm. Measurements are given as mean ± SD and range in parentheses (Table 1). Specimens were deposited in the Museo Herpetológico de Chiriquí at the Universidad Autónoma de Chiriquí, David, Panama, and at the Senckenberg Research Institute and Nature Museum, Frankfurt, Germany. The abbreviations for museum collections follow Sabaj Pérez (2013), with field numbers AB from the abbreviated name Abel Batista. Morphological data of similar *Diasporus*

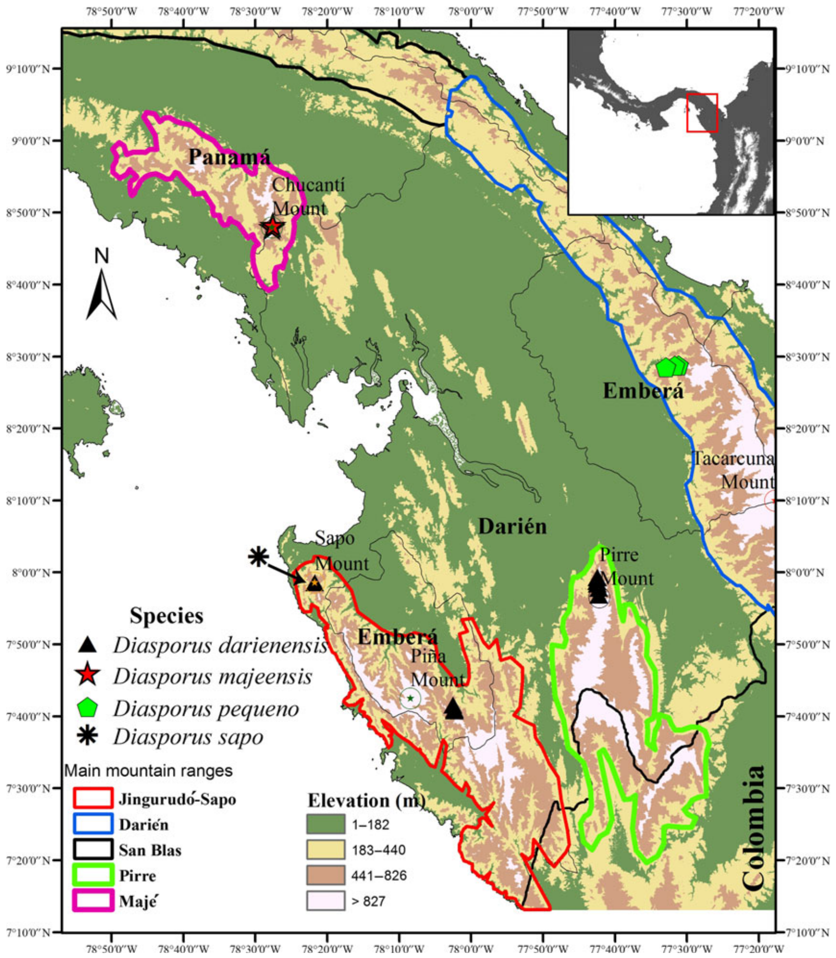

Figure 1. Map of the Darién region, eastern Panama, showing the distribution of the species of *Diasporus* described herein.

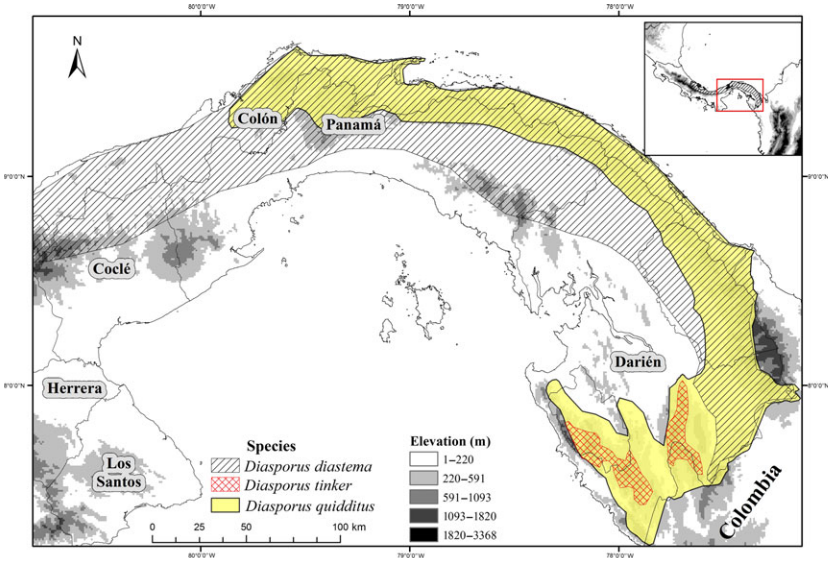

Figure 2. Map of eastern Panama (EP), showing the distribution of EP species that inhabit variable elevations, including lowlands: *Diasporus diastema* complex, *Diasporus* aff. *quidditus*, and *Diasporus tinker*.

Table 1. Genetic p–distances (in percentages) in the *16S* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)											
	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*D. majeensis* sp. nov.												
2	*D. darienensis* sp. nov.	5.0											
3	*D. pequeno* sp. nov.	10.3	8.5										
4	*D. sapo* sp. nov.	6.5	2.6	9.5									
5	*D. diastema* CWP	10.0	9.1	9.6	8.3								
6	*D.* aff. *diastema* EPL	11.0	9.2	9.7	9.2	4.6							
7	*D.* aff. *diastema* MM	10.8	8.0	7.1	8.0	5.0	4.2						
8	*D. citrinobapheus*	11.0	9.0	9.7	9.2	3.8	5.1	4.6					
9	*D. hylaeformis*	8.4	5.5	10.0	6.5	10.7	10.6	10.5	10.1				
10	*D.* aff. *quidditus*	10.2	6.9	9.2	7.0	7.0	6.3	5.2	7.0	8.9			
11	*D. tinker*	9.9	7.2	8.5	8.0	7.8	7.4	7.1	7.5	9.6	7.4		
12	*D. vocator*	10.5	8.9	11.1	8.3	10.7	10.8	9.8	10.1	8.2	9.2	10.6	

species for comparison were taken from holotypes (*D. anthrax*, *D. citrinobapheus*, *D. igneus*, *D. quidditus*, and *D. tinker*), as well as from original descriptions and published literature (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013). A list of specimens examined is presented in Appendix 1.

The following morphometric measurements were taken (with abbreviations indicated, see Duellman & Lehr, 2009): snout-vent length (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW), measured between angles of jaws; interorbital distance (IOD); eye length (EL), measured from anterior to posterior edge of externally accessible eye; hand length (HAL), measured from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), the distance from knee to distal end of the tibia; foot length (FL), measured from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW), at penultimate phalanx just anterior to disc; width of disc of third finger (3FD), at greatest width; width of third toe (3TW), at penultimate phalanx just anterior to disc; width of disc of third toe (3TD), at greatest width; width of fourth toe (4TW), at penultimate phalanx just anterior to the disc; width of disc of fourth toe (4TD), at greatest width; tympanum diameter (TD), measured horizontally, based on an estimated circular tympanum; and body width (BW), at greatest width of body. We determined the sex of adults by the presence of vocal slits in males and the presence of eggs in females. Specimens without vocal slits or eggs, and with the correspondingly smaller SVL (relative to the standard size of confirmed adult females or males for each species), were classified as juveniles. For the description of the ungual flap we followed Lynch (2001) and Savage (2002). The ungual flap is known as the 'more or less free anterior portion of the disk cover' (see Savage, 1987), it represents the anteriormost margin of the finger/toe tip, which can be hanging in a loose flap (sheet of skin). Generalized coloration summaries were derived directly from live specimens or indirectly from photos of live specimens. For the standardized color descriptions of selected individuals, the capitalized color and color codes (with the latter in parentheses) are taken from Köhler (2012).

Bioacoustics

Male advertisement calls were recorded using a Marantz Professional (PMD 620) and/or a Panasonic RR-XS410 digital recorder, with a Sennheiser ME 66 shotgun microphone capsule and a Sennheiser K6 powering module. The microphone was positioned between 0.5 and 1.5 m from the calling frog. Recordings were made at a sampling rate of 44 kHz with 16–bit resolution in uncompressed pulse-code modulation (PCM) format and saved as .wav files. The spectral and temporal parameters were analysed and the power spectra were calculated in RAVEN PRO 1.4 (Blackman DFT window; 2048 samples; 3–dB filter bandwidth of 158 Hz; grid spacing of 21.5 Hz; overlap 70.1%; Charif, Clark & Fristrup, 2004). The lowest and highest frequencies were measured at 20 dB below the peak frequency, thereby avoiding overlapping with background noise (Simões & Lima, 2011). Because our original recordings of two male *Diasporus* sp. nov. from Bajo Pequeno were lost, we extracted the calls from the background of another recording that was targeted at a *Pristimantis* sp. nov. (A. Batista, unpubl. data), using Adobe Audition 5.0. We amplified the sound (using the amplitude function) to extract details that were difficult to see in the original file. The terminology used in the description of advertisement calls follows Duellman & Trueb (1994), and follows Ursprung, Ringler & Hödl (2009) for the description of calling in bouts. The call rate was calculated as (total number of calls − 1)/(time from the beginning of the first call to the beginning of the last call) (Cocroft & Ryan, 1995). Because the dominant frequency (DF) is correlated with SVL in many frog species (Gerhardt & Huber, 2002; Bradbury & Vehrencamp, 2011), we used the non-standardized residuals between these variables for the statistical analyses. The environmental temperature and humidity were measured using an Oakton digital thermo-hygrometer. Because temporal parameters are temperature-dependent in many frog species, statistical adjustments are required (Gerhardt & Huber, 2002). In cases where we found no correlation between temperature and temporal parameters among species, we used raw data (e.g. call rate, note duration, note interval). We ran a discriminant function analysis to classify the advertisement calls of different species. The species for which SVL and/or temperature were not available were excluded from this analysis; however, all specimens were included in scatter plots of raw data that correlate DF against call rate and DF against note duration (Gerhardt & Huber, 2002; Padial *et al.*, 2008). The statistical analyses were performed using SPSS 21.0. Acoustic data for *D. anthrax* and *D. ventrimaculatus* were taken from Chaves *et al.* (2009) and Jiménez *et al.* (2013), respectively. As the terms DF, peak frequency, and high frequency were obviously confused by Jiménez *et al.* (2013), we re-estimated the real DF value from the spectrogram shown in that publication. The spectrogram figure was produced with the SEEWAVE package in R.

Molecular laboratory work and phylogenetic inference

DNA was extracted from fresh muscle or liver tissue in the Grunelius-Möllgaard Laboratory for Molecular Evolution, Senckenberg, Germany, using the protocol of Ivanova, Dewaard & Hebert (2006). The samples were amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany), performing an initial denaturation for 1 min at 94 °C, followed by 35 steps with denaturation for 9 s at 94 °C, annealing for 27 s at 45 °C, and with elongation for 1.5 min at 72 °C. Final elongation proceeded for 7 min at 94 °C. For the nuclear *recombination activating gene 1* (*RAG1*), we used: one cycle of 2 min at 96 °C; 45 cycles of 20 s at 95 °C, 25 s at 52 °C, and 2 min at 72 °C; and one cycle of 7 min at 72 °C. The reaction mixture contained 1 μL of mitochondrial DNA (mtDNA) template, 2.5 μL of reaction buffer ×10 (PeqGold), 4 μL of 2.5 mM dNTPs, 0.4 μL (containing 2.5 units) of Taq Polymerase (PeqLab), 14.1 μL of H_2O, 1 μL of 25 mM $MgCl_2$, and 1 μL per primer for *16S* (10 pmol; forward primer, L2510, 5′-CGCCTGTTTATCAAAAACAT-3′; reverse primer, H3056, 5′-CCGGTCTGAACTCAGATCACGT-3′; eurofins MWG Operon), and 3 μL per primer for *RAG1* (forward, R182, 5′-GCCATAACTGCTGGAGCATYAT-3′; reverse, R270, 5′-AGYAGATGTTGCCTGGGTCTTC-3′; eurofins MWG Operon (Heinicke, Duellman & Hedges, 2007). Sequencing of the *16S* rRNA and *RAG1* was performed in the molecular laboratory of the Senckenberg Biodiversität und Klima Forschungszentrum (BIK–F), Germany. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene was sequenced in the Southern China DNA Barcoding Center. In total we extracted DNA from 48 *Diasporus* frogs from EP and received 41 sequences for *16S*, 30 sequences for *COI*, and 45 sequences for *RAG1* (Appendix 2). We compared the mtDNA data of our specimens with *Diasporus* sequences published in GenBank. The sequences were aligned with CLUSTAL W (Larkin *et al.*, 2007) and were edited by eye using GENEIOUS 4.8.5 (Drummond *et al.*, 2010). A list of specimens included in the genetic analysis with corresponding GenBank accession numbers is presented in Appendix 2. GenBank sequences of *Colostethus panamansis* (Dunn, 1933), *Craugastor opimus* (Savage and Myers, 2002), *Craugastor fitzingeri* (Schmidt, 1857), *Eleutherodactylus planirostris* (Cope, 1862), *Eleutherodactylus thorectes* Hedges, 1988, and *Pristimantis caryophyllaceus* (Barbour, 1928) were used as out-groups. The final alignment (including GenBank sequences) of the *16S* mtDNA comprised 66 sequences (excluding out-groups) and consisted of 449 positions, of which 251 sites were variable and 158 sites were parsimony-informative. The final alignment of *COI* comprised 38 sequences consisting of 552 positions, of which 250 sites were variable and 224 sites were parsimony-informative. The final alignment of *RAG1* comprised 48 sequences consisting of 571 positions, of which 172 sites were variable and 76 sites were parsimony-informative. A total of 73 samples, combining the mitochondrial genes and the nuclear *RAG1*, were obtained (excluding out-groups), consisting of 1537 positions, of which 630 sites were variable and 447 were parsimony-informative. Using MEGA 6 (Tamura *et al.*, 2011) we calculated uncorrected pairwise genetic p–distances for *COI* and *16S* both separately and combined. For each gene and for the combined gene data set (*COI*, *16S*, and *RAG1*). We selected the substitution model for the Bayesian analysis using JModeltest 0.1.1 (Posada, 2008) under the corrected Akaike information criterion (AICc; Akaike, 1974). The three-parameter model with rate heterogeneity, TPM1uf+I+G (Kimura, 1981), was implemented for the combined gene data set. We ran a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) for 10 000 000 generations with four default chains, sampling every 1000 generations. In the final consensus tree 25% were discarded as burn-in. To test species delimitation among the *Diasporus* species, we applied the automatic barcode gap discovery (ABGD) algorithm (Puillandre *et al.*, 2011) with the following settings: 20 steps; distance calculated using the Kimura two-parameter model with a transversion/transition ratio of 2.0; and the setting for the minimum relative gap width (*X*) was set to different values between 0 and 1.5.

PHYLOGEOGRAPHY AND DIVERGENCE TIME ANALYSIS

We have used defined biogeographic areas of EP (see first paragraph in the Material and methods section) in the phylogeographic analysis. The phylogenetic relationships and divergence times were estimated for the mtDNAs *16S* and *COI* and nuclear DNA *RAG1* combined (1537 bp, 20 *Diasporus* samples included) in BEAST 1.5.4 (Drummond & Rambaut, 2007), with a relaxed clock, allowing substitution rates to vary according to an uncorrelated log-normal distribution, assuming a Yule tree prior (Drummond *et al.*, 2006). The prior distribution on substitution parameters was set to the default. To calibrate the root and one node age respectively, we used the age of approximately 57 Mya with a standard deviation of 15 Myr for the most common recent ancestor between *Pristimantis* and *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009; Pinto-Sánchez *et al.*, 2012); and with the estimated crown ages of 32 Mya for the *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009), and 17 Mya for *D. vocator* and *D. hylaeformis* (Pinto-Sánchez *et al.*, 2012). Parameters were estimated using 100 million generations with a burn-in of 50 million generations and trees were sampled every 10 000 generations. Results were visualized and compared using TRACER 1.5 (Rambaut & Drummond, 2009), and summary trees were generated using TreeAnnotator 1.5.4.

RESULTS

The phylogenetic analysis of the two mitochondrial genes revealed substantial molecular differences between groups with p–distances above 4.0% in *16S* mtDNA, 11% in *COI* mtDNA (Tables 1–3), and 7.0% when both genes were combined (Table 3). The divergence of these groups are further supported by substantial differences in male advertisement call and morphometric characters. The ABGD analysis generated 12 distinct phylogenetic groups for *16S* with a divergence threshold of 0.033 and a relative barcoding gap of 0.05 (*X*–value). For *COI*, it produced 12 groups assuming an a priori intraspecific divergence threshold of 0.021 and a relative gap width of 0.05 (*X*–value). The two analyses (ABGD for *16S* and *COI*) lumped all samples in one group, with a prior intraspecific divergence of 0.050 and 0.010, respectively. For both mitochondrial genes, the groups resulting from the ABGD analysis were consistent with the species units named in this study, except for the lumping of *Diasporus* sp. nov. from Cerro Sapo and *Diasporus* sp. nov. from DM and JSM, which is also present at Cerro Sapo.

The Bayesian consensus tree of all genetic markers combined (*16S*, *COI*, and *RAG1*) as well the divergence time analyses showed two well-differentiated clades of *Diasporus* in EP, with one containing the highland species (MM, PM, and JSM) that are monophyletic and well supported (Bayesian probability >0.95). The second clade includes species primarily distributed in the lowlands, including also *D. citrinobapheus* (from western Panama, WP), various lineages of *D.* aff. *diastema* (from central Panama, CP, the eastern Panamanian lowlands, EPL, and Majé MM, below 1000 m a.s.l.) and *D.* aff. *vocator* (from WP and south-eastern Costa Rica, CR). The divergence time analyses indicated that the EP lineages split from the WP lineages around 15 Mya (95% confidence interval, 95% CI, 6–29 Myr).

Most *Diasporus* species can be differentiated morphologically by a combination of SVL, disc shape, and color pattern (Table 4). In bioacoustics, a discriminant function analysis correctly classified all species included ($P < 0.01$; $N = 26$; Table 5). These results proved that seven species of the genus *Diasporus* occur in EP and can be diagnosed by some selected traits (e.g. SVL, ungual flap, color pattern, dominant frequency, and distribution; for more details, see Figs 3–7 and Table 6), with a new record for the species *D. tinker* in Panama. A detailed comparison of the type series of *D. tinker* from Colombia left no doubt that the specimens collected in EP are conspecific. We took advantage of these new records and include molecular, bioacoustic, and morphological data of *D. tinker* to increase the knowledge of morphological variation in this species in Panama. Furthermore, we used this data to distinguish *D. tinker* from other *Diasporus* species in EP. Addi-

Table 2. Genetic p–distances (in percentages) in the *COI* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)										
	Species	1	2	3	4	5	6	7	8	9	10	11
1	*D. majeensis* sp. nov.											
2	*D. darienensis* sp. nov.	12.3										
3	*D. pequeno* sp. nov.	15.2	16.9									
4	*D. sapo* sp. nov.	13.0	10.0	14.7								
5	*D. diastema* CP	16.1	17.6	13.9	16.5							
6	*D.* aff. *diastema* EPL	16.7	16.5	14.4	17.0	10.4						
7	*D.* aff. *diastema* MM	15.2	16.0	12.3	16.2	9.6	10.0					
8	*D. hylaeformis*	18.3	19.7	18.3	18.7	18.9	19.1	17.0				
9	*D.* aff. *quidditus*	16.7	16.9	14.5	17.5	12.6	14.3	13.1	19.0			
10	*D. tinker*	16.4	16.9	14.6	17.1	14.7	14.1	14.0	20.6	16.2		
11	*D. vocator*	17.8	18.7	17.7	17.6	18.5	20.1	16.5	19.1	21.1	18.6	

Table 3. Genetic p–distances (in percentages) in the *16S* and *COI* mtDNA combined between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)						
	Species	1	2	3	4	5	6	7
1	*D.* aff. *diastema* EPL							
2	*D. majeensis* sp. nov.	14						
3	*D.* aff. *quidditus*	10.4	14.3					
4	*D. darienensis* sp. nov.	13.5	9.9	12.6				
5	*D. tinker*	11.5	14.1	12.2	13.2			
6	*D. sapo* sp. nov.	13.4	10.3	12.7	7	13.6		
7	*D. pequeno* sp. nov.	12.1	14.1	12.4	14.3	12.5	12.9	

tionally, four species that clearly belong to the genus *Diasporus* on the basis of molecular (Figs 8, 9), bioacoustic, and morphological evidence cannot be assigned to any described species. Thus, we describe them as new species hereafter.

SPECIES ACCOUNTS

DIASPORUS DARIENENSIS SP. NOV.

FIGS 10, 14A, B
ELEUTHERODACTYLUS DIASTEMA – MYERS 1969: FIG. 19B

Holotype
SMF 97304 (original field number AB 1144), an adult female (Fig. 10), collected by Abel Batista & Konrad Mebert on the ridge of Pirre mountain range, ~3 km north from the peak of Cerro Pirre, Parque Nacional Darién (PND), Distrito de Pinogana, Darién, Panama, on 8 December 2012 at 20:35 h (7.97312 N, 77.70785 W; 1143 m a.s.l.).

Paratypes
MHCH 2840–41, 2844–47, 2862, SMF 97303, 97314, 97306–08, 97661–62, with same collecting data as holotype; MHCH 2850–52, SMF 97309–10, 97312, collected by Abel Batista & Milan Veselý in the Jingurudo mountain range, on a ridge between Aldo Creek and Sambú River, between the Comarca Emberá-Wounaan and the PND, Distrito de Sambú, Darién, Panama, 26–30 September 2011 (7.69271 N, 78.04200 W; 869 m a.s.l.); SMF 97313, collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 22:06 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Diagnosis
Diasporus darienensis sp. nov. is characterized by the following combination of traits (see Table 1): (1) dorsal skin texture smooth and/or with rounded or pointed scattered tubercles; (2) tympanic annulus concealed by skin, tympanic membrane absent; (3) snout acuminated in dorsal view and rounded in profile; (4) usually with a slightly enlarged and conical supraocular tubercle, cranial crests absent; (5) dentigerous processes of vomers triangular, diagonal to the eyes, near to the middle of the mouth and posteriorly separated about three-quarters of their total length from each other; (6) vocal sac moderately developed, with longitudinal gular folds evident, vocal slits present on the posterior part of the jaw, halfway under the tongue and ending at the midlevel of the eyes, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, and more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles, palmar accessory tubercles small, rounded, and almost indistinguishable; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, between one and three non-protuberant subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown to reddish, some specimens with pale reticulations on a dark background color; usually with a pair of red or pale dorsolateral lines, venter translucent or suffused with dark color, vocal sac yellow; (14) SVL 18.1 ± 21.3 (14.9–22.9, $N = 21$), males 17.1 ± 1.11 (14.9–18.5, $N = 15$), females 20.7 ± 1.86 (18.5–22.9, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note (49.1–51.7 ms) with harmonic structure. The dominant frequency is also the fundamental frequency, with most energy emitted at 3.34–3.81 kHz.

Description of the holotype
An adult female (SVL 17.40, Fig. 10) with slender body; dorsal skin smooth with small scattered tubercles, ventral skin smooth, discoidal fold not evident, low anal warts present; one small conical supraocular tubercle; eye twice as long as snout; tympanum of moderate size, ratio TD/EL 34%; tympanum indistinguishable, annulus tympanicus concealed by skin, tympanic membrane absent, positioned above the junction of jaws and behind the orbit; head as long as wide (HL/HW 1.03), greatest head width between angles of jaw 40% of SVL; snout subacuminate from above and rounded in profile; nares situated near tip of snout and slightly dorsolaterally directed, clearly visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes of vomer clearly visible, orbit in an oblique outline in frontal of eyes, each with five teeth; vocal slits absent; tongue long (25% of SVL) and knobbed at the end, first third attached to floor of mouth; hands moderate in size, 20% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II subequal in size to finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles;

Table 4. Morphological proportions for *Diasporus* species

Species (*N*)	SVL	HW	HL
D. anthrax (2)*	16.5–18.7	–	–
D. tigrillo (2)*	16.8–17.5		
D. citrinobapheus (7)	19 ± 1.41 (17.3–21.8)	7 ± 0.47 (6.5–7.8)	7.6 ± 0.54 (7–8.7)
D. darienensis sp. nov. (21)	18.1 ± 2.13 (14.9–22.9)	6.5 ± 0.82 (5.2–8.4)	6.5 ± 0.64 (5.6–7.9)
D. aff. *diastema*, CP (49)	19.2 ± 2.78 (14.6–27.7)	7 ± 1.2 (5.4–10.9)	7.6 ± 0.99 (6–10.6)
D. aff. *diastema*, MM (5)	19.8 ± 3.11 (16–24.5)	7.3 ± 0.93 (6.1–8.7)	7.3 ± 1.04 (6–8.9)
D. aff. *diastema*, EPL (20)	21.3 ± 1.82 (18.3–25.2)	7.9 ± 0.83 (6.1–9.6)	7.4 ± 0.63 (6.7–8.4)
D. gularis (3)	22.1 ± 1.75 (20.4–23.9)	–	–
D. hylaeformis (28)	20.3 ± 1.51 (16.9–23.1)	7.4 ± 0.74 (5.9–8.8)	8 ± 0.71 (6.8–9.5)
D. igneus (4)	26.1 ± 0.5 (25.5–26.6)	9.9 ± 0.17 (9.7–10.1)	8.5 ± 0.31 (8.2–8.8)
D. majeensis sp. nov. (15)	21.5 ± 2.64 (15.3–25.5)	8.1 ± 0.92 (6.2–9.7)	7.8 ± 0.84 (6–9.4)
D. pequeno sp. nov. (13)	19.3 ± 2.38 (16.9–24.8)	7.2 ± 0.96 (6.2–8.9)	7.2 ± 1.04 (5.9–9.1)
D. aff. *quidditus* (51)	14.5 ± 1.44 (11.5–17.9)	5.4 ± 0.48 (4–6.5)	5.6 ± 0.48 (4.5–6.3)
D. sapo sp. nov. (11)	22.6 ± 2.86 (18.8–29.1)	8 ± 0.82 (7.1–9.7)	8.2 ± 1.05 (6.6–10.2)
D. tinker (39)	17.3 ± 1.55 (14.6–20.4)	6.4 ± 0.42 (5.6–7.3)	6.2 ± 0.58 (5.3–7.2)
D. ventrimaculatus (4)	22.8 ± 1.89 (20.2–24.7)	–	–
D. vocator (12)	14.4 ± 1.46 (12–17.2)	4.9 ± 0.41 (4–5.4)	5.4 ± 0.66 (4.4–6.3)
Species	**TL**	**HAL**	**HW/SVL**
D. anthrax	–	–	–
D. tigrillo		–	0.36 (0.34–0.37)
D. citrinobapheus	7.9 ± 0.68 (7–9.2)	–	–
D. darienensis sp. nov.	7.7 ± 0.73 (6.5–9.5)	6.8 ± 0.89 (5.4–9)	4.4 ± 0.39 (3.7–5.1)
D. aff. *diastema*, CP	8 ± 1.32 (5.5–12)	4.5 ± 0.93 (3.2–6)	0.4 ± 0.02 (0.3–0.4)
D. aff. *diastema*, MM	8.4 ± 0.96 (7.3–9.9)	4 ± 0.58 (3.5–5)	0.4 ± 0.01 (0.4–0.4)
D. aff. *diastema*, EPL	8.3 ± 0.77 (7.3–9.4)	3.9 ± 0.5 (3.4–4.8)	0.4 ± 0.02 (0.3–0.4)
D. gularis	–	–	–
D. hylaeformis	8 ± 0.75 (6.6–10)	–	–
D. igneus	11.7 ± 0.5 (11.3–12.4)	11.5 ± 0.27 (11.1–11.7)	11.9 ± 0.36 (11.5–12.2)
D. majeensis sp. nov.	9.1 ± 1.14 (6.8–10.9)	8.3 ± 1.01 (5.9–9.6)	5.1 ± 0.63 (3.5–6.1)
D. pequeño	8.4 ± 0.71 (7.5–10)	7.1 ± 0.69 (6.1–8.5)	4.4 ± 0.51 (3.7–5.5)
D. aff. *quidditus*	6.9 ± 0.56 (5.9–8)	5.5 ± 0.54 (4.3–6.3)	3.3 ± 0.32 (2.7–4)
D. sapo sp. nov.	10.4 ± 1.02 (8.3–12.1)	9.5 ± 1.07 (7.3–11.5)	5.5 ± 0.6 (4.2–6.4)
D. tinker	7.3 ± 0.66 (5.3–8.8)	6.2 ± 0.42 (5.3–7.4)	3.8 ± 0.31 (3.2–4.6)
D. ventrimaculatus	–	–	–
D. vocator	5.8 ± 0.43 (5.2–6.6)	4.9 ± 0.88 (3.9–5.5)	2.9 ± 0.38 (2.5–3.2)
Species	**HW/HL**	**HL/SVL**	**TL/SVL**
D. anthrax	–	–	–
D. aff. *tigrillo*	0.92 (0.85–0.99)	0.39 (0.38–0.40)	0.48 (0.46–0.50)
D. citrinobapheus	0.9 ± 0.04 (0.9–1)	0.4 ± 0.02 (0.4–0.4)	0.4 ± 0.01 (0.4–0.4)
D. darienensis sp. nov.	1 ± 0.06 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *diastema*, CP	0.9 ± 0.07 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.04 (0.4–0.6)
D. aff. *diastema*, MM	1 ± 0.02 (1–1)	0.4 ± 0.01 (0.4–0.4)	0.4 ± 0.03 (0.4–0.5)
D. aff. *diastema*, EPL	1 ± 0.05 (0.9–1.1)	0.4 ± 0.01 (0.3–0.4)	0.4 ± 0.02 (0.4–0.4)
D. gularis	–	–	–
D. hylaeformis	0.9 ± 0.05 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. igneus	1.2 ± 0.03 (1.1–1.2)	0.3 ± 0.01 (0.3–0.3)	–
D. majeensis sp. nov.	1 ± 0.03 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. pequeño	1 ± 0.05 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *quidditus*	0.9 ± 0.07 (0.8–1.2)	0.2 ± 0.19 (0–0.4)	0.5 ± 0.03 (0.4–0.5)
D. sapo sp. nov.	1 ± 0.05 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.5 ± 0.02 (0.4–0.5)
D. tinker	1 ± 0.06 (0.9–1.2)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. ventrimaculatus	–	–	–
D. vocator	0.9 ± 0.07 (0.8–1.1)	0.4 ± 0.03 (0.3–0.4)	0.4 ± 0.04 (0.4–0.5)

Mean ± SD (range); see Material and methods for abbreviations. Numbers in parenthesis next to the species names represents the number of specimens analysed.

*Measurements taken from original descriptions and literature.

Table 5. Variations in advertisement call parameters in 11 species of *Diasporus*

	Traits				
Species	DF (kHz)	Low freq. (Hz)	High freq. (Hz)	Delta freq. (Hz)	Note duration (s)
*D. anthrax**	3.81	3.19 ± 0.35 (2.94–3.44)	4.45 ± 0.29 (4.25–4.65)	1.31–1.22	0.06
D. citrinobapheus (2)	2.86–3.04	2.77–2.95	2.95–3.42	0.2–0.5	0.11–0.17
D. darienensis sp. nov. (2)	3.57 ± 0.33 (3.34–3.81)	3 ± 0.29 (2.79–3.21)	4.07 ± 0.33 (3.83–4.3)	1.07 ± 0.04 (1.04–1.09)	0.05 ± 0 (0.05–0.05)
D. aff. *diastema*, EPL (7)	3.3 ± 0.12 (3.2–3.5)	2.9 ± 0.07 (2.8–3)	3.8 ± 0.11 (3.6–3.9)	0.9 ± 0.05 (0.8–0.9)	0.1 ± 0.01 (0.1–0.1)
D. aff. *diastema*, MM (4)	3.1 ± 0.2 (3–3.4)	2.7 ± 0.1 (2.7–2.9)	3.5 ± 0.15 (3.4–3.7)	0.8 ± 0.06 (0.7–0.9)	0.1 ± 0 (0.1–0.1)
D. aff. *diastema*, CP (7)	3.3 ± 0.16 (3.2–3.5)	2.97	3.82	0.86	0.09
*D. igneus** (1)	2.4	2	2.7	0.7	0.05–0.10
D. majeensis sp. nov. (1)	2.47–2.71	2.38–3.03	2.85–3.14	0.50–0.93	0.01–0.02
D. pequeño (1)	3.44–3.48	3.20–3.23	3.67–3.63	0.39–0.46	0.09–0.15
D. aff. *quidditus* (22)	4.81 ± 0.14 (4.55–5.08)	4.56 ± 0.18 (4.35–4.84)	4.97 ± 0.19 (4.77–5.29)	0.41 ± 0.02 (0.39–0.45)	0.34 ± 0.04 (0.25–0.38)
D. tinker (9)	3.5 ± 0.19 (3.14–3.71)	3.16 ± 0.16 (2.84–3.32)	3.8 ± 0.2 (3.42–4.07)	0.64 ± 0.06 (0.56–0.75)	0.17 ± 0.02 (0.14–0.19)
*D. ventrimaculatus**	2.50–2.61	2.14	2.9	0.76	0.07
D. vocator (5)	4.6 ± 0.3 (4.35–5.1)	3.83 ± 0.17 (3.71–3.94)	4.94 ± 0.25 (4.77–5.12)	1.12 ± 0.08 (1.06–1.18)	0.02 ± 0 (0.01–0.02)
Species	**Note interval (s)**	**Call rate (calls/min)**	**Notes/bouts**	**Bout duration**	**Interbout duration**
D. anthrax	0.55–5.77	23.4–44.2			
D. citrinobapheus	16.58 ± 0.47 (16.25–16.91)	3.61 ± 0.1 (3.54–3.68)	19–30	19.30–55.50	38.47–156.43
D. darienensis sp. nov.	3.08 ± 0.9 (1.65–4.84)	20.29 ± 6.17 (12.04–34.41)	–	–	–
D. aff. *diastema*, EPL	3.1 ± 0.68 (2.2–3.9)	19.7 ± 4.43 (14.8–25.8)	8.5	28.4	40.26
D. aff. *diastema*, MM	3.4 ± 1.08 (2.5–4.8)	17.9 ± 5.02 (12–22.8)			
D. aff. *diastema*, CP	1.65	34.41	10.2	17.33	30.25
D. igneus	6.40–9.67	8.18	–	–	–
D. majeensis sp. nov.	2.67–6.02	12.32	–	–	–
D. pequeño	3.51–6.85	11.61	–	–	–
D. aff. *quidditus*	4.71 ± 0.78 (3.27–5.44)	12.19 ± 2.24 (10.35–16.71)	–	–	–
D. tinker	2.96 ± 2.05 (1.35–7.55)	25.38 ± 11.84 (7.76–39.16)	11–13	15.97–27.85	19.71–30.58
*D. ventrimaculatus**	5.15	11.45			
D. vocator	1.91 ± 0.57 (1.4–2.52)	32.98 ± 9.35 (23.7–42.39)	13.23 ± 10.51 (5.8–20.67)	19.25 ± 13.31 (9.84–28.66)	311.02 ± 304.7 (95.56–526.47)

Mean ± SD (range). Number in parentheses next to the species names represents the number of individuals analysed. DF, dominant frequency.
*Information obtained from literature.

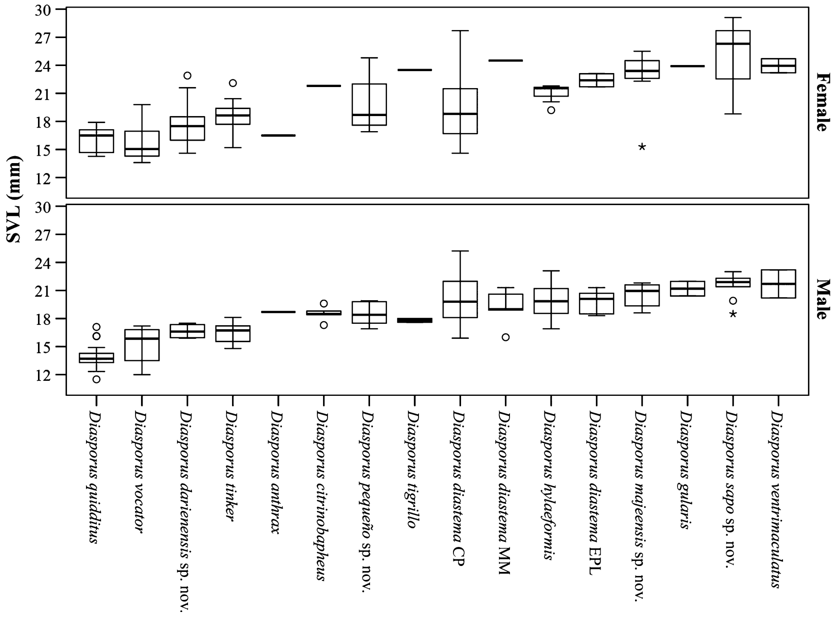

Figure 3. Differences in snout-vent length (SVL) of *Diasporus* species, separated by sex. The bottom and top of the box are the first and third percentile, and the band inside the box is the median, whiskers are the extreme values; open circles above or below the boxes represent outliers.

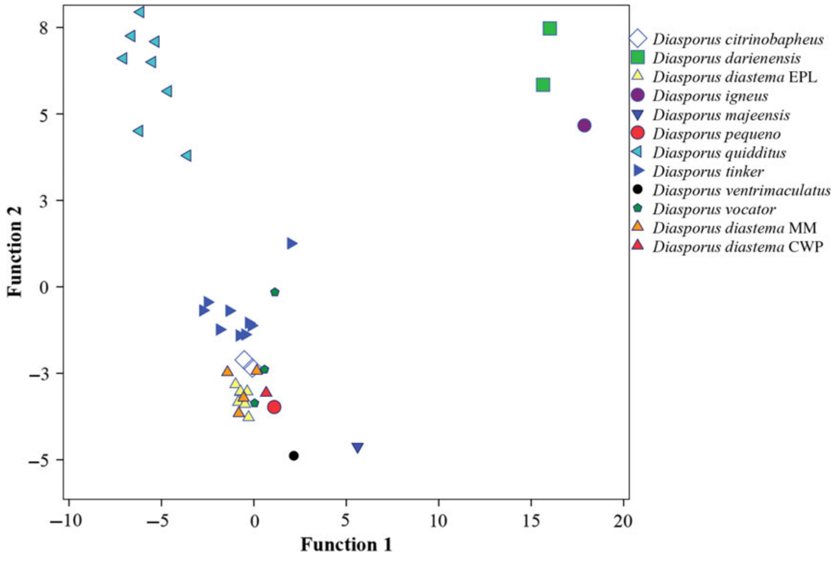

Figure 4. Discriminant function analyses of the acoustic characters of *Diasporus* species. Variables included in the analysis: note duration, note interval, dominant frequency (DF, corrected by snout-vent length), low frequency, high frequency, and call rate (temporal characters are corrected for temperature).

palmar and plantar accessory tubercles indistinguishable; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches to tubercle of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; between one and three nonprotuberant subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle ovoid; outer metatarsal tubercles slightly pointed and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even, broadened; ungual flap expanded, almost

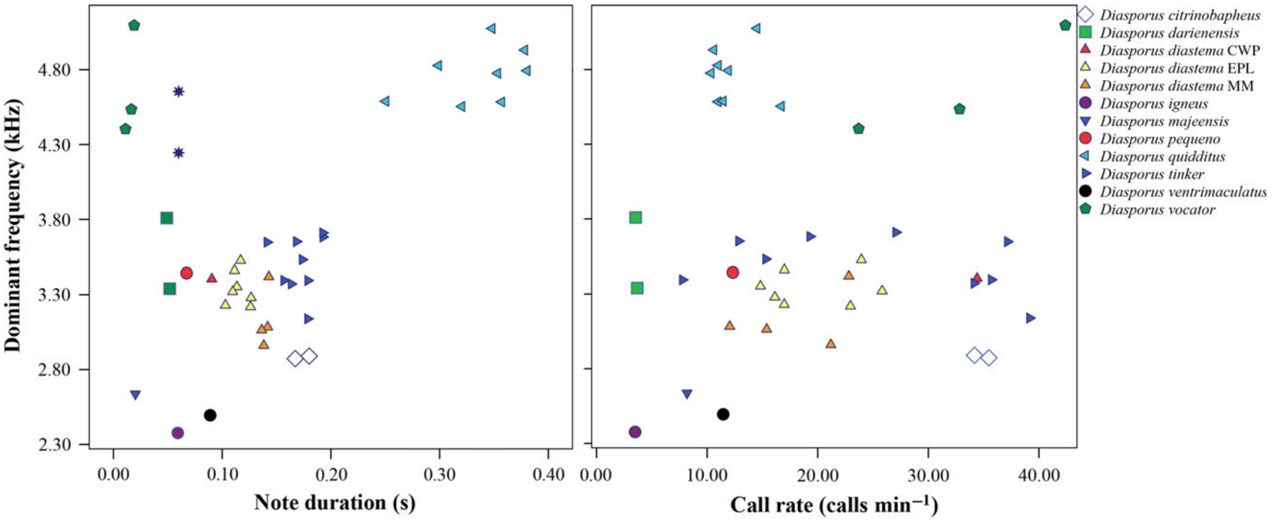

Figure 5. Scatter plot for dominant frequency/note duration (left) and dominant frequency/call rate (right) in 11 species of *Diasporus*.

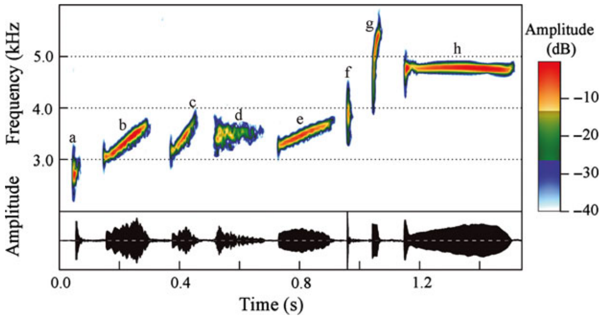

Figure 6. Spectrograms (only the harmonic containing the dominant frequency is shown) and oscillograms (below) of the advertisement calls of *Diasporus* species from eastern Panama (EP): a, *Diasporus majeensis* sp. nov. (SMF 97658); b, *Diasporus* aff. *diastema* MM (MHCH 2809); c, *Diasporus diastema* from Colón, Panama (SMF 97287, 9.26020 °N, 79.93540 °W, 36 m a.s.l.; ~9 km south-west from type locality); d, *Diasporus pequeno* sp. nov. (Bajo Pequeño, Cerro Pechito Parado, not collected); e, *Diasporus tinker* (SMF 97315); f, *Diasporus darienensis* sp. nov. (SMF 97313); g, *Diasporus vocator* (not collected; from Celmira, Bugaba, Panama, 8.55348 °N, 82.81525 °W, 242 m a.s.l.; ~60 km east from type locality); h, *Diasporus* aff. *quidditus* (SMF 97292).

rounded; pads broadened and globular in profile (Figs 7A, 10).

Coloration of holotype in life

Holotype (SMF 97304; Figs 10, 14A, B) recorded as follows: iris light Pratt's rufous (71) with a couple of lateral and irregular lines tawny (60), lumbar region tawny (60), bordered by two lines light buff (2); flanks brick red (36); groin, axilla, and ventral areas mottled with brick red (36).

Coloration in preservative

Dorsal ground color raw amber (23), with a couple of dorsolateral lines light buff (2); groin and ventral areas buff (5), with small points sepia (279); ungual flaps dark drab (45).

Measurements of holotype (mm)

SVL 17.40; HL 6.70; HW 6.30; IOD 3.24; EL 2.72; TD 0.92; FL 6.42; TL 7.50; HAL 3.47; 3FW 0.40; 3FD 0.64; 3TW 0.36; 3TD 0.49; 4TW 0.31; 4TD 0.67; BW 5.22 (for variation of the species, see Table 1).

Vocalization

The calls produced by two specimens from Cerro Sapo (Fig. 6; Table 2), one paratype (SMF 97313, environmental temperature 21.5 °C; humidity 84%; 22:06 h) and an uncollected specimen (environmental temperature 21.7 °C; humidity 80%; 21:00 h) were analysed. The calls consist of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.04–0.05 s, with an interval between calls of 16.91–16.25 s, and with a call rate of four calls per minute. The peak frequency band ranges from 2.79 to 4.30 kHz; the first harmonic contains the dominant frequency at 3.34–3.81 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) along the PM and JSM (Fig. 1). The vegetation consists predominantly of trees covered with moss, bromeliads (*Werauhia* spp.

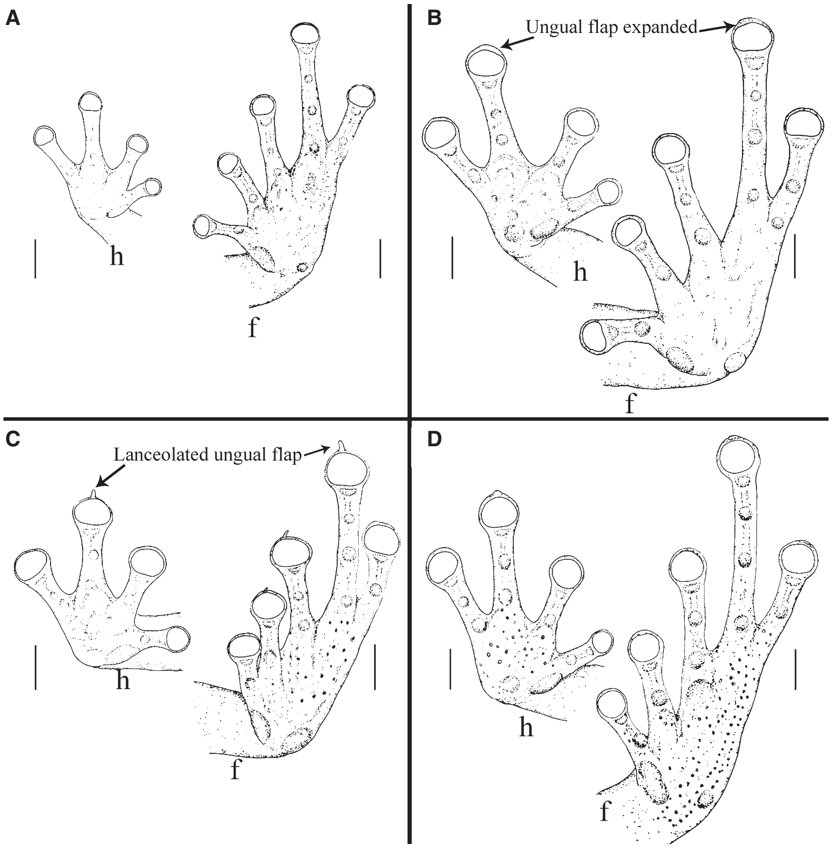

Figure 7. Drawings of ventral view of right hand and left foot of the new *Diasporus* species described here; h, hand; f, foot. Arrows indicate two examples of ungual flap shape. A, *Diasporus darienensis* sp. nov. (MHCH 2852). B, *Diasporus majeensis* sp. nov. (MHCH 2835). C, *Diasporus pequeno* sp. nov. (MHCH 2826). D, *Diasporus sapo* sp. nov. (SMF 97331). Scale bars: 1 mm.

and *Guzmania* spp.), giant ferns (*Cyathea* spp.), and orchids. *Diasporus darienensis* sp. nov. is an inhabitant of the cloud forest (869–1169 m a.s.l.), usually found 1–5 m above ground. During the day, specimens seek retreats between bromeliad leaves. At night they actively move across tree bark and bromeliads. Only two males were encountered calling, both during the end of the rainy season (December) at the top of Cerro Sapo. One male (SMF 97313) was observed calling from the underside of a leaf in a tree about 5 m above ground, the other was calling from a branch on a ridge 3 m above the ground on the same day. Diet is not known, but as with other *Diasporus* it may eat small arthropods (Batista, 2009).

Etymology

The species name is derived from the province name Darién where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species is known to occur only in this province, and it is present in the main mountain ranges of the region.

DIASPORUS MAJEENSIS SP. NOV.

FIGS 11, 14C, D

Holotype

SMF 97293 (original field number AB 1030), an adult male (Fig. 11) collected by Abel Batista & Konrad Mebert on the top of Cerro Chucantí, at Majé mountain range, Río Congo Arriba, Distrito de Chepigana, Darién, Panama, on 2 December 2012 at 20:35 h (8.79936 N, 78.46156 W; 1380 m a.s.l.).

Paratypes

MHCH 2832–39, SMF 97655–60, with same collection data as the holotype.

Diagnosis: Diasporus majeensis sp. nov. is characterized by the following combination of characters (Figs 11, 14C, D; Table 1): (1) dorsal skin smooth with small dispersed warts, ventral skin smooth; (2) only lower part of the tympanic annulus barely visible, tympanic membrane absent; (3) snout

Table 6. Main diagnostic characters and character states to differentiate members of the *Diasporus* genus in Central and South America

Species	SVL	Ungual flap	Dorsal color pattern	Ventral color pattern	DF (kHz)	Distribution
*D. anthrax**	16.5–18.7	Palmate to rounded, or expanded	Dark or blackish	Black with white flecks	3.81	North-western Ecuador and Colombia
*D. tigrillo**	16.8–17.5	Spadate	Yellow to orange	White, granules on venter with base greyish	No data	Alto Lari, SE Costa Rica, 300–400 m a.s.l.
D. citrinobapheus	19 ± 1.41 (17.3–21.8)	Palmate to rounded, or spadate	Yellowish	Almost transparent	2.86–3.04	Western Panama, 680–790 m a.s.l.
D. darienensis sp. nov.	18.1 ± 2.13 (14.9–22.9)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Reddish or small black speckles	3.57 ± 0.33 (3.34–3.81)	Serranía de Pirre and Jingurudó-Sapo, Darién, Panama, 869–1169 m a.s.l.
D. aff. *diastema*, CWP	19.2 ± 2.78 (14.6–27.7)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.16 (3.2–3.5)	Lowlands of central and western Panama
D. aff. *diastema*, MM	19.8 ± 3.11 (16–24.5)	Palmate to rounded	Yellowish	White with dark spots	3.1 ± 0.2 (3–3.4)	Serranía de Maje, Panama
D. aff. *diastema*, EPL	21.3 ± 1.82 (18.3–25.2)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.12 (3.2–3.5)	Lowlands of eastern Panama
D. gularis	22.1 ± 1.75 (20.4–23.9)	Palmate to rounded, or spadate	Pale brown with vague markings	Cream with brown stippling on throat	No data	Lowlands of western Colombia and north-western Ecuador
D. hylaeformis	20.3 ± 1.51 (16.9–23.1)	Palmate to rounded	Suffused with pink or red	Translucent, suffused with yellow or red	No data	Serranía de Talamanca Costa Rica and Panama, 1500–2500 m a.s.l.
D. igneus	26.1 ± 0.5 (25.5–26.6)	Palmate to rounded	Brownish with yellow to orange reticulations	Yellow	2.4	Western and eastern slopes of Cerro Santiago, Panama, above 1500 m a.s.l.
D. majeensis sp. nov.	21.5 ± 2.64 (15.3–25.5)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Unpigmented venter	2.47–2.71	Top of Cerro Chucantí, Panama, 1400 m a.s.l.
D. pequeno sp. nov.	19.3 ± 2.38 (16.9–24.8)	Lanceolate to papillate	Brown, cream, with dark reticulations	Venter translucent, with a dark speckle and sky blue spots	3.44–3.48	Serranía de Darién, Panama, above 472 m a.s.l.
D. aff. *quidditus*	14.5 ± 1.44 (11.5–17.9)	Lanceolate to papillate	Brown	Brown	4.81 ± 0.14 (4.55–5.08)	Eastern Panama and north-western Colombia, above 100 m a.s.l.

Table 6. *Continued*

Species	SVL	Ungual flap	Dorsal color pattern	Ventral color pattern	DF (kHz)	Distribution
D. sapo sp. nov.	22.6 ± 2.86 (18.8–29.1)	Palmate to rounded, or spadate	Uniform red	Translucent, suffused with red	No data	Cerro Sapo, Darién, Panama, 1169 m a.s.l.
D. tinker	17.3 ± 1.55 (14.6–20.4)	Lanceolate to papillate	Grey	Brown to orange	3.5 ± 0.19 (3.14–3.71)	Eastern Panama to north-western Colombia, in Panama 800–1350 m a.s.l., in Colombia, up to 1880 m a.s.l.
D. ventrimaculatus	22.8 ± 1.89 (20.2–24.7)	Spadate	Red to pink	White with red spots in males and white with dark spots in females	2.50–2.61	Valle del Silencio, Costa Rica, 2550 m a.s.l.
D. vocator	14.4 ± 1.46 (12–17.2)	Lanceolate to papillate	Pigmented with dark mottling and light areas	Brown	4.6 ± 0.3 (4.35–5.1)	South-western Costa Rica to central Panama, 2–1220 m a.s.l.

*Information obtained from literature. DF, dominant frequency.

rounded in dorsal and profile view; (4) conical supraocular tubercle or cranial crests absent; (5) dentigerous processes of vomers with between one and four teeth each, straight in outline, in frontal to the orbit; (6) vocal sac small, but with visible longitudinal gular folds, vocal slits present, situated beside the tongue, from the middle side of the tongue to near the junctions of jaws, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; one or two supernumerary tubercles, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown to reddish, some specimens with dark reticulations on a reddish background color, venter translucent, vocal sac same color as venter (Fig. 14C–D); (14) SVL 21.5 ± 2.64 (15.3–25.5, $N = 15$), males 19.9 ± 2.1 (15.3–21.8, $N = 9$), females 23.9 ± 1.22 (22.3–25.5, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note with duration of 0.01–0.02 s, and with the DF ranging between 2.47 and 2.71 kHz (Fig. 6; Table 2).

Description of the holotype

An adult female (SVL 20.90), with slender body; dorsal skin smooth with small dispersed warts, ventral skin smooth, discoidal fold not evident; eye 1.30 times longer than snout; tympanum small, ratio TD/EL 21%; only lower part of the tympanic annulus barely visible, tympanic membrane absent, positioned 2 mm behind orbit; head slightly wider than long (HL/HW 0.85), greatest head width between angles of jaw 38% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, and also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes barely visible, in frontal of the orbit of eyes in a straight outline, each with four teeth; vocal slits absent; tongue long (20% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 23% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller

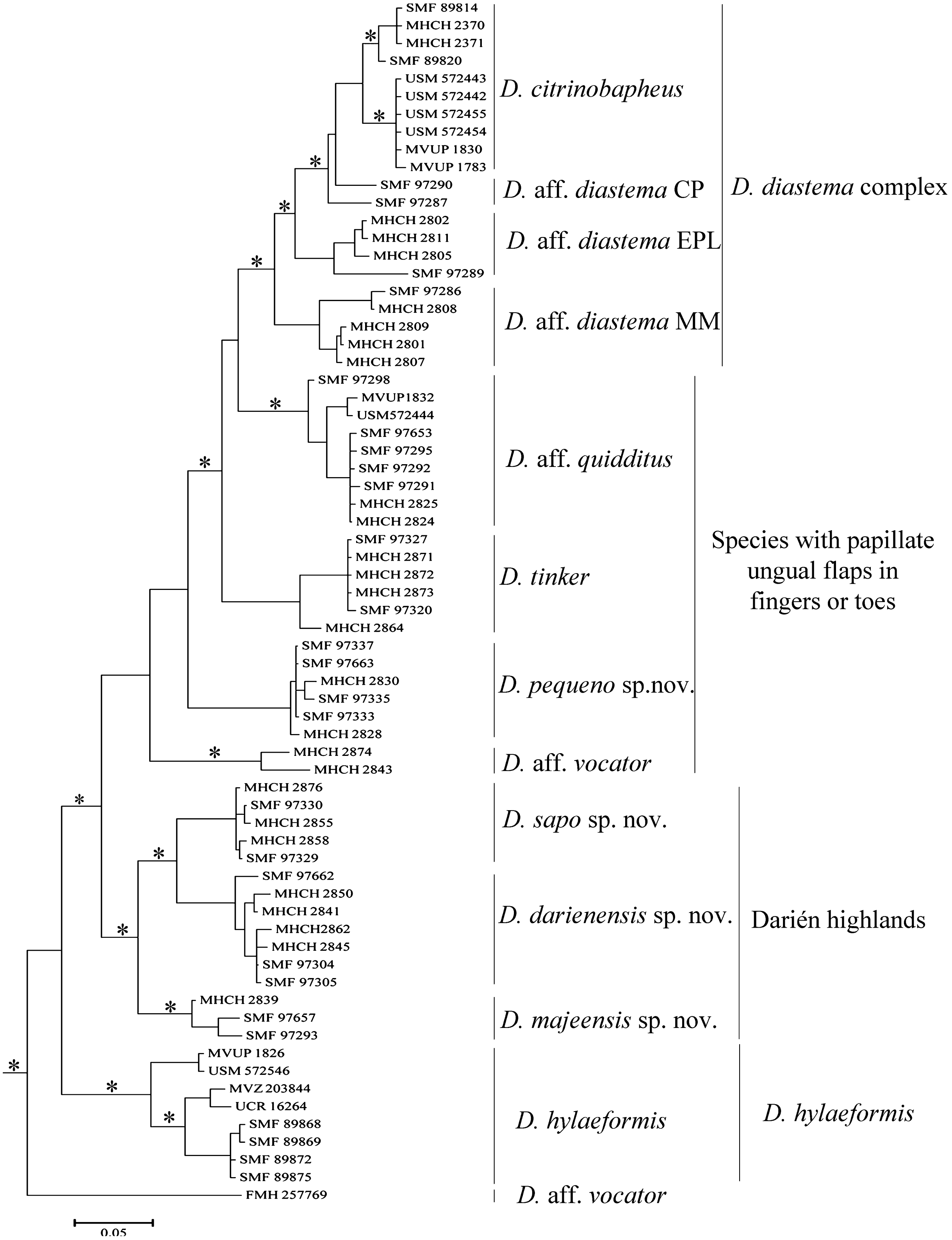

Figure 8. Bayesian consensus tree of the genus *Diasporus* based on *16S*, *COI*, and *RAG1* genes. Out-groups are not shown (*Pristimantis caryophyllaceus*, *Craugastor gollmeri*, *Craugastor fitzingeri*, *Colostethus pratti*, *Eleutherodactylus planirostris*, and *Eleutherodactylus thorectes*). Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.90$.

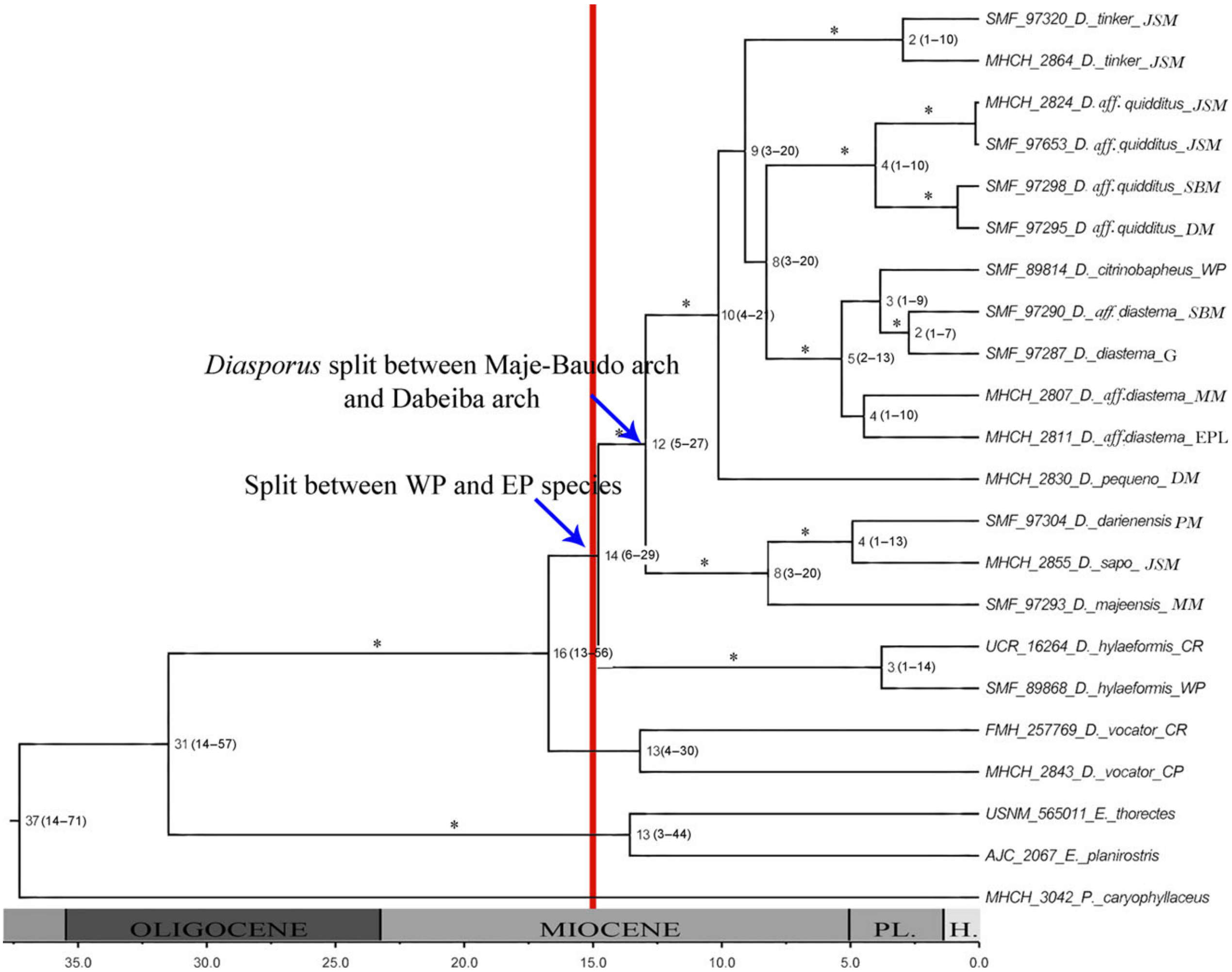

Figure 9. A chronogram of *Diasporus* species based on *16S*, *COI*, and *RAG1*, derived from a relaxed-clock Bayesian analysis, using BEAST software. The scale indicates time in Mya. The red line indicates the hypothesized completion, 15 Mya, of the Isthmus of Panama. Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.95$. Numbers at nodes represent estimated ages of diversification (SD in parenthesis). Letters at the end of species names represent biogeographic areas (for an explanation, see Material and methods); CR, Costa Rica; CP, central Panama; DM, Darién mountain range; G, Gatún lake at CP; JSM, Jingurudó-Sapo mountain range; MM, Majé mountain range; PM, Pirre mountain range; SBM, San Blas mountain range; WP, western Panama.

than finger VI, finger II reaching the base of disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 46% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more visible than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs slightly triangular; ungual flap expanded, even, rounded; pads globular in profile (Fig. 7B).

Coloration of holotype in life

Holotype (SMF 97293, Fig. 11) recorded as follows: iris light orange yellow (7) with middle area light Pratt's rufous (71); dorsal ground color chestnut (30) with peach red (70) areas in the occipital, flanks, and

Figure 10. Holotype of *Diasporus darienensis>* sp. nov.: A, frontal view; B, ventral view; C, left foot ventrally; D, right hand ventrally; E, flanks; F, posterior side of thighs and rear.

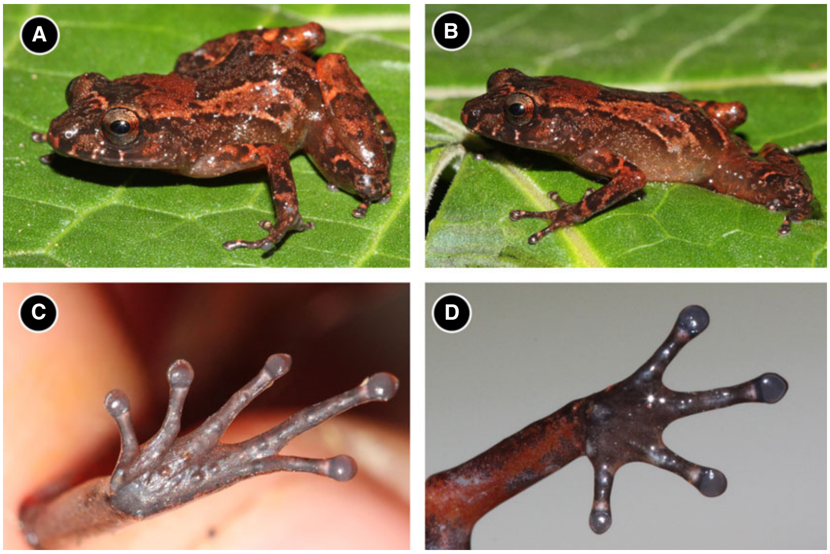

Figure 11. Holotype of *Diasporus majeensis* sp. nov.: A, B, frontal and lateral view, respectively; C, left foot ventrally; D, right hand ventrally.

lumbar region; a spectrum red (67) interorbital band, bordered posteriorly by a sepia (286) band; axilla and groin slightly pigmented with chestnut (30); limbs same as dorsum; ventral areas translucent slightly pigmented with sepia (286); ventral part of fingers and toes dark carmine (61).

Coloration in preservative

Dorsal ground color burnt sienna (38) with flesh ocher (57) areas in the occipital, flanks, and lumbar region; interorbital band flesh ocher (57), groin and venter light buff (2), ventral surfaces of limbs light orange yellow (7).

Measurements of holotype (mm)

SVL 20.90; HL 6.53; HW 7.68; IOD 2.21; EL 2.97; TD 0.62; FL 8.41; TL 9.51; HAL 4.79; 3FW 0.47; 3FD 0.75; 3TW 0.47; 3TD 0.65; 4TW 0.42; 4TD 0.53; BW 6.77 (for variation of the species, see Table 1).

Vocalization

The calls produced by one specimen (SMF 97658, environmental temperature 18.5 °C; 3 December 2012, 18:19 h) were analysed. The call consisted of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.01–0.02 s, with an interval between calls of 2.67–6.02 s and a call rate of 12.32 calls/min; the low frequency was 2.38–3.03 kHz, the high frequency was 2.85–3.14 kHz, and the first harmonic contains the dominant frequency at 2.47–2.71 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Majé mountain ranges (Fig. 1). Cloud forest in this area has vegetation consisting predominantly of trees covered with moss and a large variety of understory bromeliads (*Werauhia* spp. and *Guzmania* spp.). At night, *D. majeensis* sp. nov. was found 0.5–2.0 m above ground on tree bark in bromeliad foliage. During the daytime, individuals were found hiding between bromeliad leaves. At the top of Cerro Chucantí, males were calling during the end of the rainy season (December). The recorded male was observed calling between dry bromeliad leaves 1.5 m above ground. The diet is not known, but as with other *Diasporus* it is likely to eat small crickets, cockroaches, ants, and isopods (Batista, 2009).

Etymology

The species name is derived from the name of the mountain range, Majé, where the holotype was found, with the Latin suffix *-ensis* donating a place or locality.

DIASPORUS PEQUENO SP. NOV.

FIGS 12, 14 E, F

Holotype

SMF 97663 (original field number AB 857), an adult female (Fig. 12) collected by Abel Batista, Marcial Sabugara, and Amadiel Chaquí at Cerro Pechito Parado, at the Darién mountain range, Río Tuquesa, Bajo Pequeño, Cémaco, Comarca Embera Wounaan, Darién, Panama, on 5 November 2012 at 22:35 h (8.47553 N, 77.54883 W; 472 m a.s.l.).

Paratypes

SMF 97333–34, same locality as holotype; MHCH 2828–31, SMF 97635–38, collected at Cerro Pechito Parado on 7 November 2012 at 19:00–

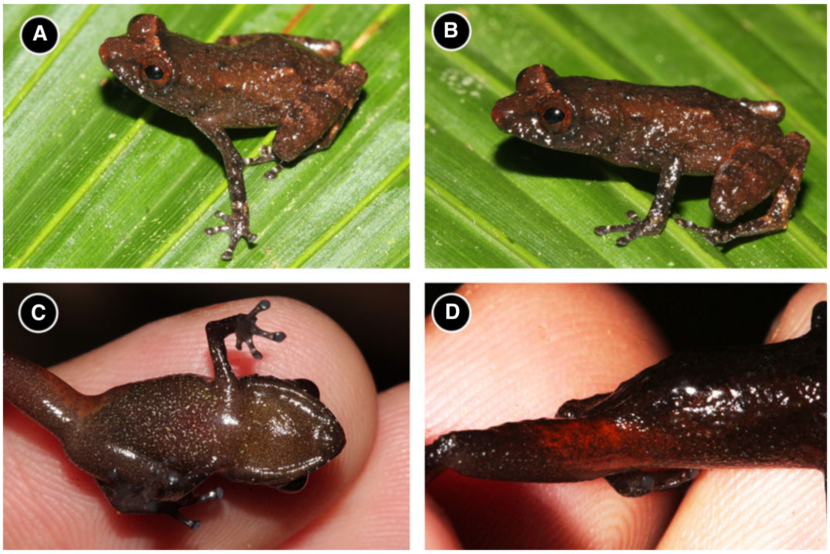

Figure 12. Holotype of *Diasporus pequeno* sp. nov.: A, B, frontal and lateral view, respectively; C, ventral view; D, flanks.

00:30 h (8.47911 N, 77.52799 W; 718 m a.s.l.), with same collectors as for holotype; MHCH 2826–27 collected at Cerro Pechito Parado, on 6 November 2012 at 19:00–01:30 h (8.47996 N, 77.51941 W; 858 m a.s.l.), with same collectors as for holotype.

Diagnosis

Diasporus pequeno sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal and ventral skin texture smooth, with small scattered tubercles, anal warts present; (2) tympanic annulus present, but with only the lower part clearly visible, tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) rounded supraocular tubercle present, cranial crests absent; (5) dentigerous processes of vomers with between four and six teeth each, straight in outline, near to the frontal border of the orbit; (6) vocal sac well developed (Fig. 14E, F), vocal slits present, situated under the centre of the orbit level, no nuptial pads; (7) finger II longer than finger I; disc pads even broadened; ungual flap expanded, lanceolate, more evident on finger III (Fig. 7C); (8) no fringes or webbing on fingers; (9) palmar tubercle rounded, flattened, and larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles barely evident, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap expanded, lanceolate, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown, cream, with dark reticulations, venter translucent (Wolffian duct visible), with a dark speckle and sky-blue spots, vocal sac bright yellow (Fig. 14E, F); (14) SVL 19.3 ± 2.38 (16.9–24.8, $N = 13$), males 18.2 ± 1.09 (16.9–19.9, $N = 10$), females 22.9 ± 1.69 (21.5–24.8, $N = 3$); (15) advertisement call composed of a single, amplitude-modulated short note (0.09–0.15 s) with harmonic structure, and with most energy emitted with the first harmonic call (3.44–3.48 kHz).

Description of the holotype

An adult female (SVL 22.68) with a slender body; dorsal skin smooth with scattered tubercles, with a small supraciliary tubercle, ventral skin smooth, discoidal fold not evident; eye 1.70 times longer than snout; tympanum of moderate size, ratio TD/EL 36%, tympanic annulus present, but only the lower part clearly visible, tympanic membrane absent; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 39% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes in front of the orbit of eyes, perpendicular in direction to the centre of roof of mouth, in a straight outline, each with seven teeth; vocal slits absent; tongue long (18% of SVL) and broadening to the tip, first third attached to floor of mouth; hands moderate in size, 18% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller than finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 2.16 times wider than distal end of adjacent phalanx; palmar tubercle rounded to ovoid, larger than thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles rounded and small; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV expanded, 1.73 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more evident than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened; ungual flap expanded, fingers and toes III and IV lanceolated; pads globular in profile (Fig. 7C).

Coloration of holotype in life

Holotype (SMF 97663; Fig. 12) recorded as follows: iris geranium (66) with fine sepia (286) reticulations; dorsal ground color walnut brown (27), with sepia (286) blotches, and small sky-blue (192) dots; a flesh ocher (57) interorbital band, bordered posteriorly by a sepia (286) band; groin Pratt's ruby (68); axilla and venter walnut brown (27) mottled with pale pinkish buff (3), throat suffused with buff (5); fingers and toes with a pale buff (1) band just before the disc cover.

Coloration in preservative

Dorsal ground color drab (19), with a pair of dorsolateral lines light orange yellow (7); groin and ventral areas light buff (2), with small points sepia (279); ungual flaps cinnamon–drab (50).

Measurements of holotype (mm)

SVL 22.68; HL 8.43; HW 8.89; IOD 2.52; EL 3.33; TD 1.20; FL 8.12; TL 9.75; HAL 4.49; 3FW 0.50; 3FD 1.08; 3TW 0.51; 3TD 0.91; 4TW 0.52; 4TD 0.90; BW 7.81 (see Table 4).

Vocalization

Through call amplification of a recording containing several species (4–dB amplified; for an explanation, see Material and methods), we were able to extract the calls of three species: *Pristimantis* sp. (DF 2.76 kHz), *D. diastema* (DF 2.99 kHz), and *D. pequeno* sp. nov. (environmental temperature 24 °C; 8 October 2012, 18:17 h). Eleven calls were analysed (because the similarity of the call intervals indicates a single individual), consisting of single, short, monophasic notes that are reminiscent of a 'tink' (Fig. 6). Note duration is 0.09–0.15 s, with an interval between calls of 3.51–6.85 s and a call rate of 11.61 call/min; the low frequency was 3.20–3.23 kHz, the high frequency was 3.63–3.67 kHz, and the fundamental frequency is also the dominant frequency at 3.44–3.48 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Darién mountain range (Fig. 1). Most specimens were found at 0.2–1.0 m above ground, over green leaves, between branches with dry leaves or in bromeliads. At the first location (472 m a.s.l.) the understory was open. The predominant vegetation were palms, vines, and small trees; at the second location above 700 m a.s.l. bromeliads were predominant; *D.* aff. *pequeno* sp. nov. was found to be sympatric with *D. diastema* and *D. quidditus,* and all species were actively calling.

Etymology

The species name *pequeno* is derived from the name Bajo Pequeño (or Bajo Chiquito), the last village at Río Tuquesa, where this species was found.

DIASPORUS SAPO SP. NOV.

FIGS 13, 14G, H

ELEUTHERODACTYLUS SP. – MYERS 1969: FIG. 19C.

Holotype

SMF 97329 (original field number AB 429), an adult female (Fig. 13) collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 20:00 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Paratypes

MHCH 2853–58, SMF 97328, SMF 97330–32; same collecting data as for holotype.

Diagnosis

Diasporus sapo sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal skin texture slightly tuberculate, venter smooth; (2) tympanum indistinguishable, annulus tympanicus and tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) conical supraocular tubercle and cranial crests absent; (5) dentigerous processes of vomers with between seven and 11 teeth each, straight in outline, from the centre of the orbit to the centre of the roof of mouth; (6) vocal sac and vocal slits not differentiated, only a slightly differentiated fold beside the tongue, no nuptial pads; (7) finger II longer than finger I, ungual flap expanded, spadate, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid or rounded, flattened and almost the same size as thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular; two or three supernumerary tubercles; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap on toes expanded, spadate, more evident on toes IV and V; (12) plantar tubercle indistinguishable, subarticular tubercles rounded and globular (one on toes I and II, two on toes III and V, and three on toe IV); foot without supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and

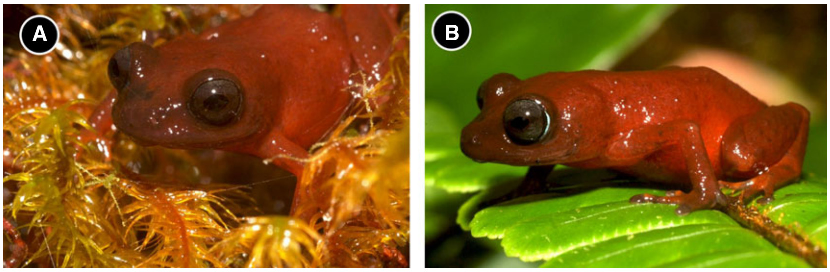

Figure 13. Holotype of *Diasporus sapo* sp. nov.: A, B, frontal and lateral views, respectively.

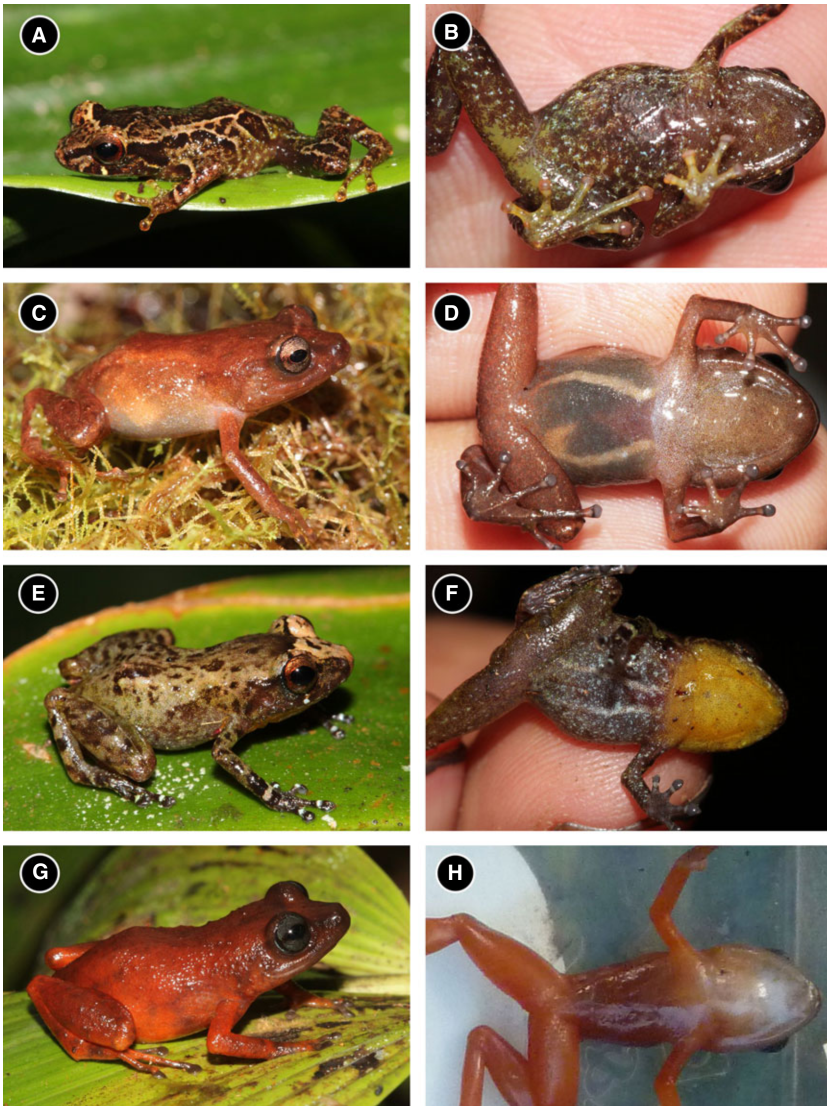

Figure 14. Color variation of the new *Diasporus* species: A, B, *Diasporus darienensis* sp. nov. (SMF 97305); C, D, *Diasporus majeensis* sp. nov. (SMF 97658); E, F, *Diasporus pequeno* sp. nov. (MHCH 2830); G, H, *Diasporus sapo* sp. nov. (G, not collected; H, MHCH 2854).

globular, smaller than inner; tarsal ridge absent; (13) dorsal ground color in life reddish and patternless, venter translucent, vocal sac not visible (Fig. 14H); (14) SVL 22.6 ± 2.86 (18.8–29.1, $N = 11$), males 22.6 ± 2.59 (19.9–29.1, $N = 9$), females 22.6 ± 5.3 (18.8–26.3, $N = 2$); (15) advertisement call unknown.

Description of the holotype

An adult female (SVL 28.91), with slender body; dorsal skin texture slightly tuberculate, venter skin smooth, discoidal fold not evident; protuberant eyes 1.78 times longer than snout; tympanum small, ratio TD/EL 23%; tympanum indistinguishable, annulus tympanicus and tympanic membrane absent, positioned 2.6 mm behind the orbit; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 35% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region concave; dentigerous processes of vomers with ten (right) and eight (left) teeth each side, straight in outline, from the centre of the orbit to the centre of the roof of mouth, and separated by a space of half of its total length; vocal slits absent; tongue long (26% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 22% of SVL; relative lengths of adpressed fingers

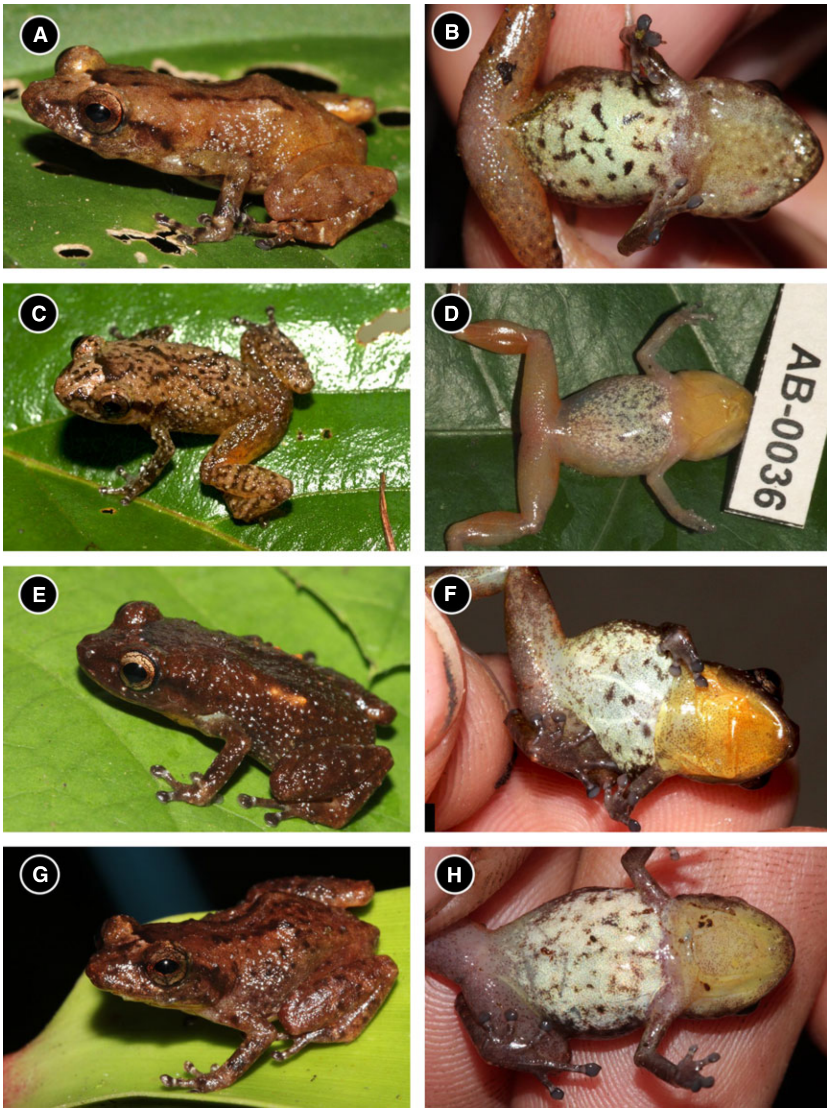

Figure 15. *Diasporus* aff. *diastema*, dorsal and ventral views: A, B, Majé mountain range (MM), near Ambroya (MHCH 2801); C, D, Eastern Panamanian lowlands (EPL), Río Mono, near Bayano (MHCH 2806); E, F, Gatún, Colón, near type locality (SMF 97287); G, H, Darién mountain range (DM), Bajo Pequeño, Río Tuquesa (SMF 97289).

I < II < IV < III; finger II smaller than finger VI, finger II reaching the middle of disc on finger IV when adpressed; finger III disc 2.07 times wider than distal end of adjacent phalanx; palmar tubercle ovoid to rounded, flattened, and almost the same size as thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; two palmar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the disc base of toe II; disc of toe IV expanded, 2.11 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles rounded and globular (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles rounded, globular, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened and slightly globular in profile (Fig. 7D); ungual flap on toes expanded, spadate, more evident on toes IV and V.

Coloration of holotype in life

Coloration recorded as follows (Fig. 13): iris medium neutral gray (298) with reticulations sepia (286), iris periphery jet black (300), eye periphery sky blue (192); dorsal ground color uniform Pratt's ruby (68), becoming darker to the front as dark carmine (61); venter and limbs chrome orange (74), throat pale buff (1).

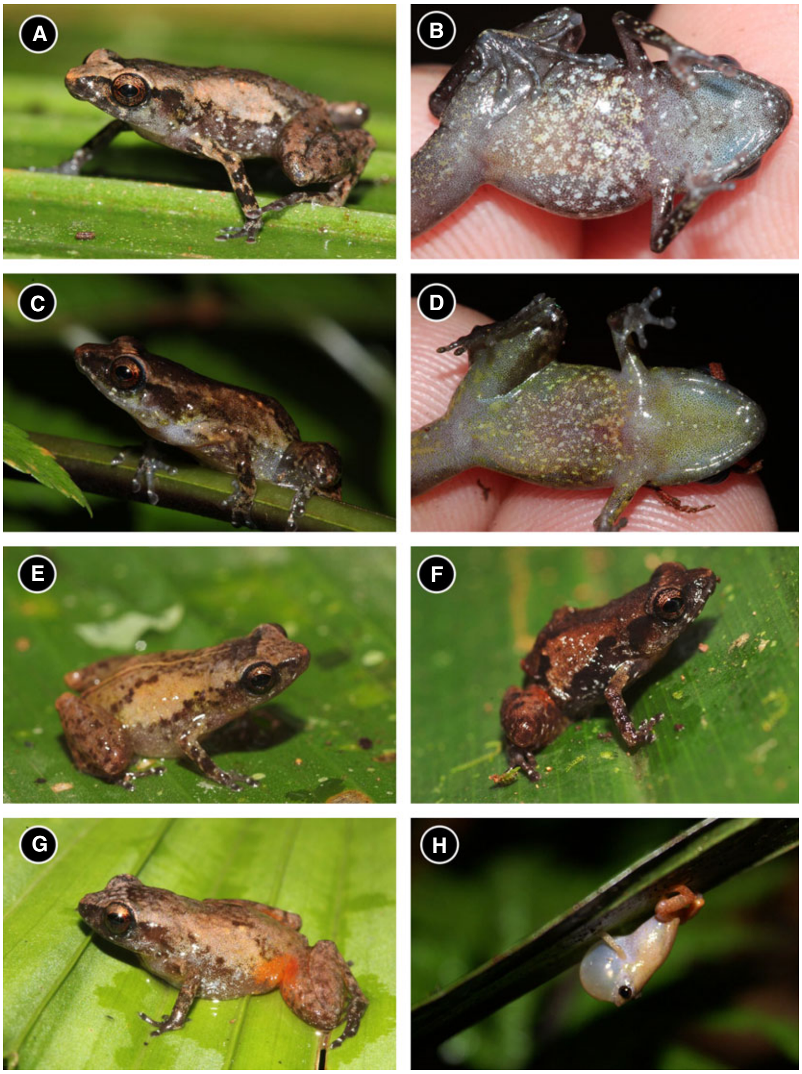

Figure 16. *Diasporus* aff. *quidditus*: A, B, Pirre mountain range (PM), Perresenico stream (MHCH 2824); C, D, Jingurudó-Sapo mountain range (JSM), near Pavarandó (SMF 97653); E, PM, Cana Field Station (MHCH 2813); F, PM, Pirre ridge (SMF 97292); G, San Blas mountain range (SBM), Taintidu river (SMF 97298); H, calling male under leaf, 20 cm from ground, SBM, Burbayar Private Reserve.

Coloration in preservative

Dorsal ground color cinnamon–drab (50), becoming darker to warm sepia (40) to the tip of snout; limbs and venter cream color (12), throat buff (5), hand and foot drab (19).

Measurements of holotype (mm)

SVL 28.91; HL 9.63; HW 10.19; IOD 2.93; EL 4.29; TD 0.98; FL 11.58; TL 12.33; HAL 6.37; 3FW 0.52; 3FD 1.8; 3TW 0.61; 3TD 1.12; 4TW 0.57; 4TD 1.20; BW 9.99 (for variation in the species, see Tables 4–6).

Natural history

This species is known only from the top of Cerro Sapo, which is covered by elfin forest. The vegetation predominantly consists of small trees (roughly 10 m in height) fully covered with moss and bromeliads. *Diasporus sapo* sp. nov. was most often found at 1–2 m above ground during the night; individuals were seen walking over tree branches and tree bark.

Etymology

The species name is derived from the name of Cerro Sapo, where the species was found.

DISCUSSION

The application of an integrative approach has resulted in the description of four new species within the genus. Additionally, the historical reconstruction

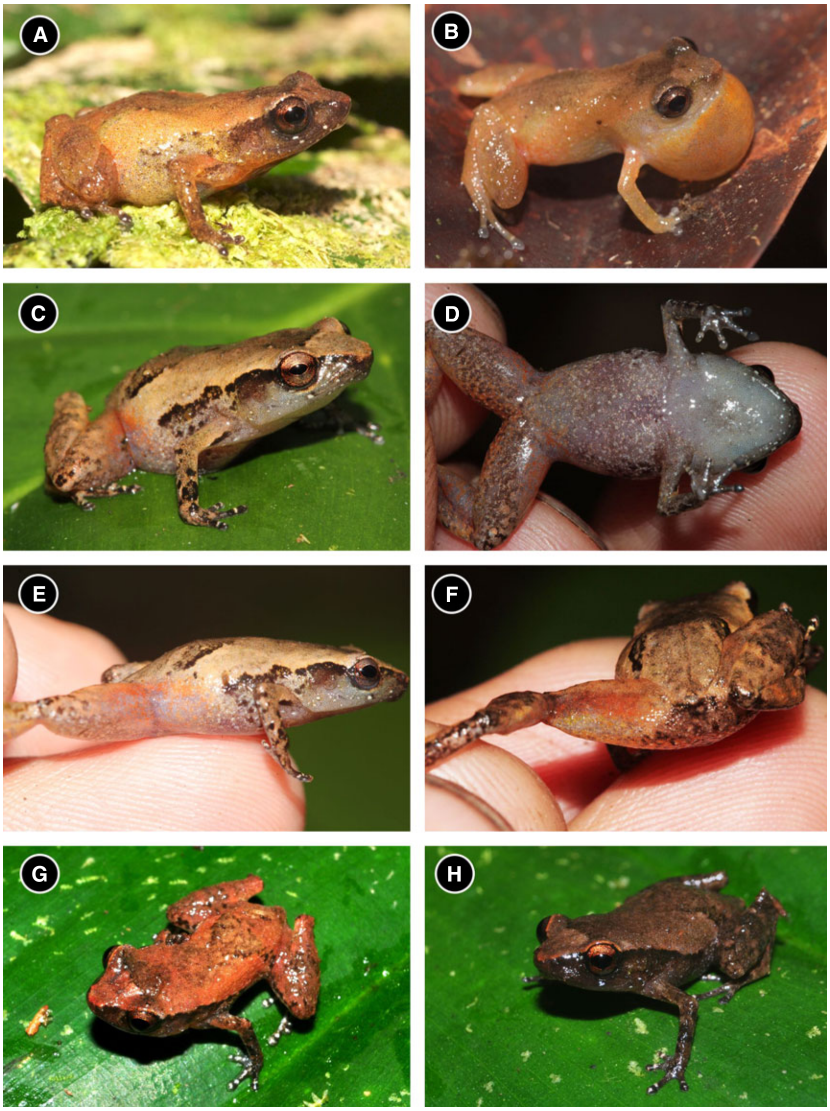

Figure 17. *Diasporus tinker*: A, B, Pirre mountain range (PM), Pirre ridge (MHCH 2864); C, F, PM, Pirre ridge (SMF 97324); G, H, Jingurudó-Sapo mountain range (JSM), showing metachrosis (same specimen); G, night coloration; H, day coloration.

of the biogeography for the *Diasporus* species confirms a Middle American origin of the genus, as revealed by previous studies (e.g. Pinto-Sánchez, Crawford & Wiens, 2014), as the older clade is represented by species distributed in western Panama (Fig. 2). Moreover, the 11 amphibian species endemic to EP (Ibáñez & Crawford, 2004; Crawford, Ryan & Jaramillo, 2010a; Crawford, Lips & Bermingham, 2010b; Batista *et al.*, 2014a; this paper) support the hypothesis of EP being a centre of endemism rather than just a pathway between two continents during the Great American Biotic Interchange (GABI; Crawford *et al.*, 2010a,b).

MORPHOLOGY AND ECOLOGY

Most diagnostic characters used to differentiate species within the genus *Diasporus* are morphological traits: usually SVL (body size), ungual flap (disc cover), and color pattern (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012). Although SVL appears to be helpful in identifying species because of remarkable interspecific variation (Fig. 3), this character is valid only to differentiate between species with no overlap in SVL, or supported along with other morphological characters. In EP there are two groups of *Diasporus*, separated by the ungual flap shape (see results, Fig. 3). The lanceolate or papillate ungual flap present in *D. pequeno* sp. nov., *D. quidditus*, *D.* aff. *vocator*, and *D. tinker* seems to have evolved from one common ancestor. Although those species build a monophyletic clade with members of the *D. diastema* complex, the latter do not possess lanceolate or papillate ungual flaps. This possibly means that a lanceolate/papillate ungual flap was subsequently lost in *D. diastema* and *D. citrinobapheus*. The function of the ungual flap in these frogs is not known, but interestingly all species with lanceolate or papillate ungual flaps are inhabitants of the lower understory up to 1 m above ground (including the western Panamanian *D. vocator*), and only populations of *D. quidditus* from Colombia are usually found above this height. There are other rain frogs or Terrarana (Hedges *et al.*, 2008; former genus *Eleutherodactylus*) with lanceolate or papillate ungual flaps [*Craugastor gollmeri* group, *Pristimantis chalceus* (Peters, 1873), *Pristimantis scolodiscus* (Lynch & Burrowes, 1990), *Noblella* spp.] that are also inhabitants of the forest floor or the low understory (Savage, 1987; Lynch & Duellman, 1997; Duellman & Lehr, 2009). In contrast, lanceolate or papillate ungual flaps are usually not present in other Terrarana members with more arboreal habits (e.g. *Pristimantis* spp.; Savage, 2002; Duellman & Lehr, 2009). So we suggest that the expression of this character is related to the understory habit of these frogs.

Diasporus diastema has more palmate than spadate disc covers, as stated by Savage (1997, 2002), Lynch (2001), and Köhler (2011); however, we found specimens at the mouth of Chagres River, Colón, Panama (near the type locality) and in EP with spadate rather than palmate finger disc covers. We presume that the authors mentioned above did not examine *D. diastema* specimens from central Panama, but only examined specimens from Costa Rica that are likely to represent at least one different species (Savage, 1997, 2002: plate 127; Lynch, 2001: fig. 2D KU 35149; Köhler, 2011: figs 437, 39). Moreover, the disc cover shape can be highly variable as a result of different preservation techniques (Lynch, 2001). Such high variation in disc shape has been described within and between populations of *Pristimantis caryophyllaceus* (Barbour, 1928) (Batista *et al.*, 2014b), among other species of Terrarana, leaving this trait as a controversial tool to identify species in preserved specimens.

Most species of *Diasporus* are inhabitants of the understory of tropical forests. Nevertheless, there are differences in the spatial distribution of species: whereas *D. pequeno* sp. nov., *D. quidditus* (Fig. 16), and *D. tinker* (Fig. 17) occupy the zone from near ground level up to 1 m above the forest floor, *D. diastema*, *D. majeensis* sp. nov., and *D. sapo* sp. nov. are usually found higher, 1.0–2.5 m above the ground, and *D. darienensis* sp. nov. has been found even higher, from 1 m up to 5 m above ground. There is also controversy about the smallest species, *D. quidditus*, which was originally described from Colombia, with specimens from Panama referred to this species (Lynch, 2001). Although the populations from Panama and Colombia are almost similar in appearance (size and color), they vary substantially in ecology. Specimens from Colombia are reported to inhabit the mid-level of the forest, with males calling from the undersides of leaves at 1.5–4.0 m above ground (Lynch, 2001). After more than 3 years of field experience with this species in EP and observing more than a hundred calling males, we never encountered *D. quidditus* (Fig. 16) calling from a position higher than 0.5 m above the ground. Unfortunately, we have neither acoustic nor molecular data of topotypic specimens of *D. quidditus* to compare with our specimens. Therefore, the taxonomic relevance of these geographical behavioural variations remains unclear. Thus, we consider the populations from EP as *D.* aff. *quidditus* until more detailed molecular and bioacoustics comparison between the populations from EP and Colombia become available.

BIOACOUSTIC

To the human ear, the calls of *Diasporus* species are very similar to each other. A typical advertisement call was described as a simple 'tink tink' or 'dink dink' (Savage, 1997, 2002; Lynch, 2001). With such a simple subjective call description it was difficult to distinguish among the calls of different species; however, in recent years detailed descriptions of advertisement calls for 12 of the 15 described species have been published (Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013; this paper). It has been shown that comparison of acoustic parameters is a powerful tool for species identification in the genus *Diasporus*. To date, only call descriptions of *D. gularis*, *D. sapo* sp. nov., and *D. tigrillo* are pending.

Spectral and temporal parameters of the calls have been used successfully to evaluate the taxonomic status in Terrarana frogs. Even with a limited sample size these parameters show differences between *Diasporus* spp. (Padial *et al.*, 2008; Figs 4 and 5). The discriminant function analysis properly sorted species when we compared dominant frequency (DF) against note duration, but not if we used DF against call rate (Figs 4 and 5). Some species, such as *D. citrinobapheus*, *D. diastema* (at least for Panamanian populations of *D.* aff. *diastema*), *D. tinker*, and

D. vocator have calls that are organized in bouts. In species with calls organized in bouts, the note interval decreases from the beginning towards the end of the call group. Thus, the high variation of DF versus call rate in those species is a result of this modulation of the note interval. We recommend that more call recordings are made in future studies (with at least ten individuals and ten calls/individual per population) to evaluate the effect of the note interval modulation on the call rate. Four species of *Diasporus* are known to call in bouts, but we did not evaluate whether this is a phylogenetic character of related species. Usually, temporal parameters can be related to evolutionary constraints or to environmental selection (Ryan, Cocroft & Wilczynski, 1990; Bosch & De la Riva, 2004); however, calling in bouts is often used to facilitate note alternation between conspecific neighbours (Schwartz, 1991), as well as to restore energetic deficits during a calling session (Leary *et al.*, 2004). It is known that orthopterans that call in bouts can affect female preferences (Hendrick, 1986), but in *Diasporus* spp. the reason for this calling pattern remains unknown.

The call of *D. diastema* from central Panama was described by Fouquette (1960) and Wilczynski & Brenowitz (1988), but their temporal and spectral measurements are highly variable, which might be an artifact of involving several species in the recording (Hertz *et al.*, 2012). We have analysed a sample of *D. diastema* from a site near the type locality (Figs 6C and 16E, F), and its DF ranged between 3.34 and 3.47 kHz, which agrees well with data from Wilczynski & Brenowitz (1988). Nevertheless, some incongruence has been detected in the note duration (Hertz *et al.*, 2012). According to our experiences, measuring temporal parameters can sometimes lead to erroneous results. This is especially true when trying to determine the end point of the call on the oscillogram, or if the recording includes considerable background noise. The *D. diastema* specimen (SMF 97287; Fig. 15E, F) that we found at the mouth of the Chagres River was the only *Diasporus* specimen found within a radius of 2 km from this site, which is approximately 9 km from the type locality near Margarita, Colón, Panama (Dunn, 1942; Taylor, 1955; Savage, 1973; Hertz *et al.*, 2012; Fig. 16E, F). Therefore, we assume that this specimen belongs to *D. diastema* as originally described by Cope (1876). Nevertheless, it is difficult to test for conspecificity by comparing the morphology of our specimen with that of the holotype of *D. diastema*, as it is in poor condition (Cochran & Goin, 1970; A. Hertz, pers. observ.); however, it should be noted that the specimen from the mouth of Chagres River and the holotype of *D. diastema* are at least of almost similar size (*D. diastema* holotype, SVL 20.0 mm; *Diasporus* sp. from Chagres River, SVL 19.0 mm). In this area the only other congeneric frog is *D.* aff. *vocator* (see also Ibáñez *et al.*, 1999), which is significantly smaller than *D. diastema*, (Fig. 3; Table 4), and calls at a higher DF.

BARCODING AND PHYLOGENETIC INFERENCE

DNA barcoding is a useful tool for species identification (Hebert *et al.*, 2004; Crawford *et al.*, 2010b; Jörger *et al.*, 2012; Paz & Crawford, 2012); however, the straightforward application of this approach could yield misleading interpretations of biodiversity (see Trewick, 2008; Huang *et al.*, 2013; Shen, Chen & Murphy, 2013). DNA sequence information in the absence of other lines of evidence should never be used for species delimitations (DeSalle, 2006). Here we are using molecular barcoding along with other methods to reveal unnamed species within the genus *Diasporus* from EP. We found high genetic divergence between lineages above the suggested threshold to identify candidate species in Neotropical amphibians (>3.0% in *16S* and >10% in *COI*; Vences *et al.*, 2005; Fouquet *et al.*, 2007; Crawford *et al.*, 2013), and most of them were supported by the barcoding analysis (ABGD; Puillandre *et al.*, 2011). Differences in the barcoding genes were additionally supported by bioacoustics, ecology, morphology, and phylogeography. According to the integrative analysis, most lineages identified as species showed considerable genetic distances and are monophyletic in the reconstructed tree; however, the polyphyly in members of the *D. diastema* complex is problematic (Fig. 8). Whereas *D. citrinobapheus* is monophyletic, although consisting of two subclades, and is well differentiated from *D. diastema*, *D. diastema* itself is paraphyletic. We included two specimens from central Panama, of which SMF 97287 is most probably a 'true' *D. distema*, whereas SMF 97290 could represents another genetic lineage. We were not able to clearly distinguish between separate lineages of *D.* aff. *diastema* from MM or EPL, nor raise any of them to species level (Tables 4–6). Despite the fact that they showed a genetic distance above the threshold used to recognize candidate species within this group (e.g. >4.0% genetic p–distance in mtDNA *16S*; Table 1), we prefer treating these populations as unconfirmed candidate species (Vieites *et al.*, 2009), and label all specimens other than SMF 97287 as *Diasporus* aff. *diastema* until more comparative data from more widespread populations become available.

PHYLOGEOGRAPHY

The results of our chronological tree indicate that species from EP are younger than those from WP

(Fig. 9). Thus, the ancestors of the genus *Diasporus* have originated somewhere in Lower Central America (see also Pinto-Sánchez *et al.*, 2014). Recent hypotheses on the formation of the land bridge between South America and North America suggest that the Isthmus of Panama was connected with the north-western landmass of South America via an island arc during the mid-Miocene around 15 Mya (Montes *et al.*, 2012a,b). *Diasporus* species from WP and EP split around this time (15 Mya), promoting the subsequent evolution into numerous *Diasporus* spp. within EP. Later on, another vicariant event took place *in situ*, probably induced by eustatic fluctuations during the middle and late Miocene (as early as 11 Mya), such as the flooding of the Atrato and Chucunaque basins (Duque-Caro, 1990; Coates *et al.*, 2004). This consequently separated populations, including the predecessors of the three closely related species *D. darienensis* sp. nov., *D. majeensis* sp. nov., and *D. sapo* sp. nov. that became isolated on separate land masses of EP during this period (5–8 Mya), and evolved allopatrically into distinct species. Around the same time (5−6 Mya), *D.* aff. *diastema* from MM split from the rest of the *D.* aff. *diastema* complex and remained isolated within the foothills of MM, evolving only minor morphological changes.

A hypothetical route of colonization and speciation for *Diasporus* frogs in EP is as follows: *Diasporus* ingresses into EP through the San Blas peninsula when it started to uplift (around 20 Ma, Montes *et al.*, 2012a). The Chucunaque and Atrato basins isolated the islands of Maje-Baudo in the south and Dabeiba (e.g. the San Blas mountain range) in the north. *Diasporus* populations colonized those islands either by over sea dispersal (debris rafting) and/or via a temporary land connection. The clade of *D. dariensis*, *D. sapo* sp. nov., and *D. majeensis* sp. nov. evolved on the southern island, whereas the remainder species (*D.* aff. *diastema*, *D. pequeno* sp. nov., *D.* aff. *quidditus*, and *D. tinker*) evolved on the northern island. *Diasporus pequeno* sp. nov. is the oldest lineage of the latter clade, whereas the other species from the southern island dispersed more recently when continuing tectonic events and sedimentation allowed occasional migrations over more shallow and narrow water bodies. Ancestors of *D. tinker* expanded east and crossed the Atrato corridor. The ancestors of *D.* aff. *quidditus* evolved during an extended period on the northern island, and began dispersing into South America when the Isthmus of Panama was nearly completed. The *D.* aff. *diastema* clade including *D. citronobapheus* expanded in both western and eastern directions.

CONCLUSION

Many species within the genus *Diasporus* in EP have been difficult to differentiate: as they were based solely on external appearance, a new approach became necessary. Based on a comprehensive analysis of *Diasporus* samples from EP, we bring new insights into bioacoustics, ecology, molecular diversification, and morphology, and reconstruct the phylogeography of the genus in this region. All new species described herein were well supported by the integration of these approaches. Although this study substantially raises the number of known species of the genus, the diversity of *Diasporus* spp. still promises to grow in the future. During the last 4 years seven species have been described (including those described here); therefore, we can expect that integrative taxonomical approaches on the genus in western Panama, Costa Rica, and Colombia may further raise the number of species.

KEY TO THE SPECIES OF THE GENUS *DIASPORUS*

1a. Ungual flap lanceolate or papillate at least on the third finger or third and fourth toe. 2

1b. Fingers and toes with rounded or spadate ungual flap. 6

2a. Very small frogs; SVL usually <17 mm. 3

2b. Small frogs; SVL usually >17 mm 4

3a. Dorsum shagreen; fingers without thick lateral fringes; toe V not partially fused with toe IV; SVL of adult males 14.0–16.0 mm, adult females 16.5–18.0 mm; calls with DF of 4.35–5.10 kHz . . . . *Diasporus vocator*

3b. Dorsum with scattered low warts; fingers with thick lateral fringes; toe V partially fused with toe IV; SVL of adult males 10.9–14.8 mm, adult females 13.2–16.9 mm; calls with DF of 4.55–5.08 kHz . . . *Diasporus* aff. *quidditus* (populations from EP)

4a. Disk expanded with cuspidate pads, skin smooth aside from low flattened warts, no perianal warts; vocal sac pale brown or orange in males; calls with DF of 3.14–3.71 kHz . . . . *Diasporus tinker*

4b. Disk expanded with rounded pads, skin texture smooth, with small scattered tubercles, perianal warts may or may not be present, vocal sac bright yellow in males 5

5a. Finger III with a small papillate ungual flap; dorsal pattern brownish with dark blotches; ventral areas translucent with dark speckles and small sky-blue blotches; males with bright yellow vocal sac; calls with DF of 3.44–3.48 kHz . . . . *Diasporus pequeno* sp. nov.

5b. Fingers without papillate ungual flap; dorsal color yellowish tan, with brown markings; ventral surfaces white . . . . *Diasporus gularis*

6a. Reddish color pattern on dorsum, venter translucent or with distinct black and white blotches 7

6b. Dorsal color pattern variable, pale, dark brown, or yellowish, venter white or cream in color, with dark blotches or suffused (or speckled) with dark color 10

7a. Venter translucent. 8

7b. Venter usually with distinct black and white blotches, males have white venters with red spots and females have white venters with black spots; calls with DF of 2.50–2.61 kHz. . . . . *Diasporus ventrimaculatus*

8a. Outer edge of the tibia and forearm smooth, without a series of tubercles. 9

8b. Outer edge of the tibia and forearm covered with a series of tubercles; calls with DF of 2.4 kHz . . . .*Diasporus igneus*

9a. Dorsal color uniform red; eye periphery sky blue; species restricted to the Sapo-Jingurudó mountain range . . . .*Diasporus sapo* sp. nov.

9b. Dorsal color reddish with brown or pale reticulations; eye periphery black; species restricted to the Majé mountain range; calls with DF of 2.47–2.71 kHz . . . .*Diasporus majeensis* sp. nov.

10a. Dorsal pattern yellowish, usually suffused with pink or red; venter translucent without blotches, or with speckled pattern 11

10b. Dorsal pattern dark or pale brown, venter cream with dark spots or dark with white flecks 13

11a. Dorsum uniformly bright yellow to orange, color of posterior surface of thigh same color as dorsum; adults with vomerine teeth 12

11b. Posterior surface of thigh often suffused with pink or red in life; adults without vomerine teeth; calls with DF of 2.35–3.05 kHz. . . . *Diasporus hylaeformis*

12a. Dorsum smooth, uniformly bright yellow to orange, sometimes with irregularly distributed dark blotches; distal subarticular tubercle on finger I and toe I flat and rounded; SVL of adult males 17.3–19.7 mm; calls with DF of 2.86–3.04 kHz . . . . . *Diasporus citrinobapheus*

12b. Dorsum with scattered low pustules, dorsum yellow to orange with dark-brown spots confined to pustules; distal subarticular tubercle on finger I and toe I weakly bifid; SVL of adult males 16.0–17.5 mm. . . . . . . *Diasporus tigrillo*

13a. Dorsal pattern pale brown or reddish; venter cream with dark spots or suffused with reddish color; axilla and groin cream in color or same color as dorsum 14

13b. Dorsal pattern black with short red lines; axilla and groin scarlet; ventral surfaces black with white flecks; calls with DF of 3.81 kHz . . . .*Diasporus anthrax*

14a. Dorsal pattern pale brown with dark spots; venter cream with dark spots; axilla and groin cream in color; calls with DF of 2.96–3.55 kHz.. . . . .*Diasporus* aff. *diastema* (populations from CP and EP)

14b. Dorsal pattern reddish with pale lines or blotches; venter suffused with reddish color; axilla and groin unpigmented or same color as dorsum; calls with DF of 3.34–3.81 kHz . . . . . *Diasporus darienensis* sp. nov.

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APPENDICES

Appendix 1. Details of the museum voucher numbers (when available) and collecting locality for all *Diasporus* samples used in this study.

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 89819	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
MHCH 2373	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
LSt 018	*D.* aff. *citrinobapheus*	Cerro Mariposa, Santa fé National Park	Panama	8.51545	81.119	930
LSt 085	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.50128	81.11868	1215
LSt 120	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.52556	81.13168	652
ICN 41696 (holotype)	*D. anthrax*	Campamento la Miel II, near junction of quebrada Tasajos with Río la Miel, km 23 carretera la Victoria-Samaná; Caldas, Colombia	Colombia			700
ICN 41697 (paratype)	*D. anthrax*	Bosque de San Rafaél, Municipio San Rafael, Antioquia, Colombia	Colombia			1200
MHCH 2840	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
MHCH 2841	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2844	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2845	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98771	77.70783	1149
MHCH 2846	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97976	77.70843	1133
MHCH 2847	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98	77.70839	1139
MHCH 2850	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68289	78.03846	959
MHCH 2851	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67942	78.03868	946
MHCH 2852	*D. darienensis* sp. nov.	Bailarín mount, Jingurudó mountain range	Panama	7.69312	78.04226	865
MHCH 2862	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98724	77.70785	1140
SMF 97303	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
SMF 97304	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97305	*D. darienensis* sp. nov.	From mirador 2 to Perresenico Stream to Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field Station	Panama	7.97312	77.70785	1143
SMF 97306	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98887	77.70739	1100
SMF 97307	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97864	77.70851	1112

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97308	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97935	77.70872	1085
SMF 97309	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97310	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97312	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68357	78.03848	948
SMF 97313	*D. darienensis* sp. nov.	Sapo Mountain	Panama	7.97632	78.36269	1152
SMF 97314	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98741	77.70787	1137
SMF 97661	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97662	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field Station, Pirre mountain range	Panama	7.94719	77.7042	1326
MHCH 2801	*D. diastema*	Amborlla, La Javillosa ridge	Panama	8.91587	78.62897	906
MHCH 2802	*D. diastema*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2803	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2804	*D. diastema*	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2805	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.1772	78.74551	107
MHCH 2806	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.17816	78.74582	100
MHCH 2807	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89224	78.56029	943
MHCH 2808	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89182	78.56268	788
MHCH 2809	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89427	78.56509	622
MHCH 2810	*D. diastema*	Maje mountain range, Amborlla	Panama	8.8972	78.56762	610
MHCH 2811	*D. diastema*	La Moneda, Meteti, Darién	Panama	8.5974	78.04934	69
SMF 97286	*D. diastema*	Maje mountain range, Amborlla	Panama	8.91681	78.61779	485
SMF 97287	*D. diastema*	400 m W from the Gatún Locks, Colón	Panama	9.26021	79.9354	36
SMF 97288	*D. diastema*	Maje mountain range, Amborlla	Panama	8.92267	78.6253	852
SMF 97289	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97290	*D. diastema*	Río Terable, Chepo	Panama	9.28399	78.98383	322
MHCH 1440	*D. diastema*	Donoso, Colón	Panama			
MHCH 1469	*D. diastema*	Donoso, Colón	Panama			
SMF 80781	*D. diastema*		Panama			
SMF 81961	*D. diastema*		Panama			
SMF 79796	*D. diastema*		Panama			
SMF 79797	*D. diastema*		Panama			
SMF 83391	*D. diastema*		Panama			
SMF 85135	*D. diastema*		Panama			
SMF 78965	*D. diastema*		Panama			
SMF 82033	*D. diastema*		Panama			

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 82032	*D. diastema*		Panama			
SMF 82035	*D. diastema*		Panama			
SMF 81812	*D. diastema*		Panama			
SMF 78187	*D. diastema*		Panama			
SMF 78188	*D. diastema*		Panama			
SMF 78189	*D. diastema*		Panama			
SMF 78190	*D. diastema*		Panama			
SMF 78186	*D. diastema*		Panama			
SMF 78191	*D. diastema*		Panama			
LSt 018	*D. diastema*		Panama			
LSt 085	*D. diastema*		Panama			
MHCH 1360	*D. diastema*	Bocas del Toro Island, Bocas del Toro	Panama			
MHCH 1379	*D. diastema*	Donoso, Colón	Panama			
MHCH 1427	*D. diastema*	Donoso, Colón	Panama			
SMF 85938	*D. diastema*		Panama			
SMF 79794	*D. diastema*		Panama			
SMF 79800	*D. diastema*		Panama			
SMF 79799	*D. diastema*		Panama			
SMF 83390	*D. diastema*		Panama			
SMF 83389	*D. diastema*		Panama			
SMF 85068	*D. diastema*		Panama			
SMF 84997	*D. diastema*		Panama			
SMF 80977	*D. diastema*		Panama			
SMF 80978	*D. diastema*		Panama			
SMF 80979	*D. diastema*		Panama			
SMF 82034	*D. diastema*		Panama			
SMF 82031	*D. diastema*		Panama			
SMF 29859	*D. diastema*		Panama			
SMF 29874	*D. diastema*		Panama			
SMF 81811	*D. diastema*		Panama			
SMF 78561	*D. diastema*		Panama			
SMF 78185	*D. diastema*		Panama			
SMF 78184	*D. diastema*		Panama			
SMF 77231	*D. diastema*		Panama			
LSt 123	*D. diastema*		Panama			
SMF 85939	*D. diastema*		Panama			
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
ICN 19306	*D. gularis*	Quebrada La Miquera, Vereda Venados, Parque Natural Nacional Las Orquideas, Antioquia	Colombia	6.53802	76.30345	1060
ICN 53771	*D. gularis*		Colombia			
ICN 45169	*D. gularis*	Centro Forestal Bajo Calima, Valle del Cauca	Colombia	3.98333	76.95	50
AH 242	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1820
AH 244	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1872
AH 245	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1780
HAU 012	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82511	82.49813	1778
HAU 013	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82595	82.49904	1800
HAU 018	*D. hylaeformis*	Cerro Guayaba, Comarca Ngöbe buglé	Panama	8.75797	82.2572	1358
AH 486	*D. hylaeformis*	Cerro Sagui, Comarca Ngöbe buglé	Panama	8.5639	81.8221	2033
AH 175	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 178	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 176	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 177	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 115	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 116	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 117	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
AH 118	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 380	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
HAU 007	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1810
HAU 011	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1750
AH 381	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
SMF 89872	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91138	82.71288	2068
SMF 89867	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89874	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89873	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
MHCH	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89868	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89869	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89875	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
SMF 89876	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
AH 042	*D. hylaeformis*	La Nevera, Comarca Ngöbe buglé	Panama	8.49966	81.77238	1700
AH 343	*D. hylaeformis*	La Nevera/Cerro Santiago Westhang	Panama	8.49546	81.76718	1815
SMF 89871	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
SMF 89870	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
NH 0034	*D. hylaeformis*	Lost and Found, Reserva Forestal Fortuna	Panama	8.67445	82.2193	1283
AH 236	*D. hylaeformis*	Volcán Barú/Sendero Quezales	Panama	8.84944	82.51538	2134
MHCH 1327 (holotype)	*D. igneus*		Panama			
MHCH 1388 (paratype)	*D. igneus*		Panama			
MHCH 2072 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*	La Nevera, Comarca Ngöbe buglé	Panama	8.49546	81.76718	1815
MHCH 2832	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2833	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2834	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2835	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2836	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2837	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
MHCH 2838	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2839	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97293	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97655	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97656	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97657	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97658	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97659	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97660	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2826	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859
MHCH 2827	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859
MHCH 2828	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2829	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2830	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2831	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97333	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97334	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97335	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97336	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97337	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97338	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97663	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2813	*D. quidditus*	Rio Cana, Cana field station, Chimenea trail	Panama	7.75602	77.68565	525
MHCH 2814	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68338	78.03844	943
MHCH 2815	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67978	78.03856	947
MHCH 2816	*D. quidditus*	Sapo Mountain	Panama	7.98013	78.35546	796
MHCH 2817	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2818	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2819	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2820	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03434	78.022	228
MHCH 2821	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2822	*D. quidditus*	From Taintidu River to the ridge, San Blas mountain range	Panama	9.04897	77.99753	433
MHCH 2823	*D. quidditus*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2824	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99706	77.71084	558
SMF 97291	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99241	77.70941	871

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97292	*D. quidditus*	Pirre mountain range	Panama	7.98728	77.70785	1135
SMF 97294	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67962	78.03859	956
SMF 97295	*D. quidditus*	Camp 2 Pícuro River	Panama	8.04887	77.37004	787
SMF 97296	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97297	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97298	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
SMF 97299	*D. quidditus*	San Blas mountain range	Panama	9.05929	77.98421	553
SMF 97300	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47553	77.54884	472
SMF 97301	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47997	77.51941	859
SMF 97302	*D. quidditus*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station	Panama	7.9632	77.70432	1267
SMF 97653	*D. quidditus*	Pavarandó, from camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
SMF 97654	*D. quidditus*	Pavarandó, from Camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
MHCH 2825	*D. quidditus*	Pirre mountain range	Panama	7.99207	77.70947	871
ICN 38150 (paratype)	*D. quidditus*	3 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38151 (paratype)	*D. quidditus*	4 km. NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38152 (paratype)	*D. quidditus*	5 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 45173 (holotype)	*D. quidditus*	Centro Forestal Bajo Calima, Buenaventura, Valle del Cauca	Colombia	3.98333	76.94999	50
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
MHCH 2853	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97616	78.36097	1063
MHCH 2854	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97614	78.36285	1148
MHCH 2855	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2856	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2857	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2858	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97328	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97329	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97606	78.36289	1158
SMF 97330	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97589	78.36254	1160

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97331	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97594	78.36265	1158
SMF 97332	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2812	*D. tinker*	Pirre mountain range	Panama	7.98923	77.7074	1149
MHCH 2848	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2849	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2863	*D. tinker*	Pirre mountain range	Panama	7.96258	77.70401	1253
MHCH 2864	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
MHCH 2865	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
MHCH 2866	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
MHCH 2867	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68423	78.03867	971
MHCH 2868	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969
MHCH 2869	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2870	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2871	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2872	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2873	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97311	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
SMF 97315	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97316	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97317	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
SMF 97318	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97319	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97320	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97321	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97322	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67947	78.03861	955
SMF 97323	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97324	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9615	77.70426	1303
SMF 97325	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.) to stream; Rancho Frío Field station, Pirre mountain range	Panama	7.95947	77.70365	1230
SMF 97326	*D. tinker*	Pirre top (1400) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94719	77.7042	1326
SMF 97327	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
ICN 45174 (holotype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45175 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45176 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45177 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45178 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45179 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45181 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
UCR 20491	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20492	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20493	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20504	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
MHCH 2874	*D. vocator*	Narices Mountain, Santa fé National Park	Panama	8.56315	81.05242	841
SMF 97339	*D. vocator*	San Lucas, Donoso, colón	Panama	8.98843	80.58243	149
SMF 89949	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 89950	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
AH 364	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50555	82.52417	5
SMF 89865	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50814	82.52843	5
SMF 89820	*D. citrinobapheus*	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
SMF 89814 (holotype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2370 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
SMF 89816 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2371 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2372	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681
SMF 89817	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681

n.a. = voucher number not available.

Appendix 2. Voucher numbers and GenBank accession numbers for all *Diasporus* samples included in the phylogenetic analyses.

Species	Museum no.	Field number	GenBank accession no.			Country	Coordinates	
			16S	*COI*	*RAG1*		Latitude	Longitude
D. aff. *diastema*	MHCH 2801	AB 637	KT186624	KT186572	KT119471	Panama	8.91587	78.62896
D. aff. *diastema*	MHCH 2802	AB 675	KT186617	KT186563	KT119472	Panama	9.03547	78.02637
D. aff. *diastema*	MHCH 2805	AB 035		KT186555	KT119461	Panama	9.17720	78.74551
D. aff. *diastema*	MHCH 2807	AB 073		KT186580	KT119438	Panama	8.89224	78.56029
D. aff. *diastema*	MHCH 2808	AB 084	KT186627	KT186578	KT119439	Panama	8.89182	78.56268
D. aff. *diastema*	MHCH 2809	AB 086	KT186633	KT186588		Panama	8.89427	78.56509
D. aff. *diastema*	MHCH 2811	AB 218		KT186571	KT119459	Panama	8.59740	78.04934
D. aff. *quidditus*	MHCH 2824	AB 1130	KT186621	KT186569	KT119443	Panama	7.99706	77.71084
D. aff. *quidditus*	MHCH 2825	B 131		KT186560	KT119454	Panama	7.99207	77.70947
D. pequeno sp. nov.	MHCH 2828	AB 822		KT186556	KT119475	Panama	8.47553	77.54884
D. pequeno sp. nov.	MHCH 2830	AB 860		KT186559	KT119478	Panama	8.47911	77.52799
D. majeensis sp. nov.	MHCH 2839	AB 1065	KT186629		KT119442	Panama	8.80462	78.45951
D. darienensis sp. nov.	MHCH 2841	AB 1268	KT186618		KT119449	Panama	7.98771	77.70783
D. vocator	MHCH 2843	AB 1240			KT119448	Panama	8.86528	80.64383
D. darienensis sp. nov.	MHCH 2845	AB 151		KT186561	KT119456	Panama	7.98771	77.70783
D. darienensis sp. nov.	MHCH 2850	AB 329	KT186626	KT186576	KT119460	Panama	7.68289	78.03846
D. sapo sp. nov.	MHCH 2855	AB 430	KT186619	KT186568	KT119464	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2856	AB 431	KT186616		KT119465	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2858	AB 439	KT186628		KT119467	Panama	7.97618	78.36263
D. sp.	MHCH 2859	AB 032	KT186614	KT186558		Panama	8.71893	82.23161
D. darienensis sp. nov.	MHCH 2862	AB 159		KT186579	KT119458	Panama	7.98724	77.70785
D. tinker	MHCH 2871	AB 1270	KT186620		KT119451	Panama	7.66911	78.03802
D. tinker	MHCH 2872	AB 1271	KT186623		KT119452	Panama	7.66911	78.03802
D. tinker	MHCH 2873	AB 1272	KT186615		KT119453	Panama	7.66911	78.03802
D. vocator	MHCH 2874	AB 564	KT186622		KT119469	Panama	8.56315	81.05242
D. diastema	SMF 97287	AB 602		KT186566	KT119470	Panama	9.26021	79.93540
D. diastema	SMF 97289	AB 818		KT186586	KT119474	Panama	8.47553	77.54884
D. diastema	SMF 97290	AB 979		KT186577	KT119481	Panama	9.28399	78.98383
D. aff. *quidditus*	SMF 97291	AB 138	KT186613		KT119455	Panama	7.99241	77.70941
D. aff. *quidditus*	SMF 97292	AB 158	KT186634	KT186589	KT119457	Panama	7.98728	77.70785
D. majeensis sp. nov.	SMF 97293	AB 1030		KT186565	KT119440	Panama	8.79936	78.46156
D. aff. *quidditus*	SMF 97295	AB 499			KT119468	Panama	8.04887	77.37004
D. aff. *quidditus*	SMF 97298	AB 689	KT186625		KT119473	Panama	9.03547	78.02637
D. darienensis sp. nov.	SMF 97304	AB 1144		KT186581	KT119445	Panama	7.96320	77.70432
D. darienensis sp. nov.	SMF 97305	AB 1134		KT186582	KT119444	Panama	7.97312	77.70785
D. darienensis sp. nov.	SMF 97312	AB 323	KT186631	KT186585		Panama	7.68357	78.03848
D. darienensis sp. nov.	SMF 97313	AB 425			KT119462	Panama	7.97632	78.36269
D. tinker	SMF 97320	AB 308	KT186632	KT186587		Panama	7.69271	78.04200

Appendix 2. *Continued*

Species	Museum no.	Field number	GenBank accession no.			Country	Coordinates	
			16S	*COI*	*RAG1*		Latitude	Longitude
D. tinker	SMF 97326	AB 1184			KT119446	Panama	7.94718	77.70420
D. tinker	SMF 97327	AB 1269	KT186635		KT119450	Panama	7.66911	78.03802
D. sapo sp. nov.	SMF 97329	AB 429		KT186557	KT119463	Panama	7.97606	78.36289
D. sapo sp. nov.	SMF 97330	AB 435	KT186630	KT186584	KT119466	Panama	7.97589	78.36254
D. pequeno sp. nov.	SMF 97335	AB 856		KT186583	KT119476	Panama	8.47911	77.52799
D. pequeno sp. nov.	SMF 97337	AB 861		KT186570	KT119479	Panama	8.47911	77.52799
D. vocator	SMF 97339	AB 028		KT186573		Panama	8.98843	80.58243
D. aff. *quidditus*	SMF 97652	AB 931		KT186562		Panama	7.75898	78.09228
D. majeensis sp. nov.	SMF 97653	AB 1031		KT186567	KT119480	Panama	8.79936	78.46156
D. majeensis sp. nov.	SMF 97657	AB 1033			KT119441	Panama	8.79936	78.46156
D. darienensis sp. nov.	SMF 97662	AB 1185		KT186564	KT119447	Panama	7.94719	77.70420
D. pequeno sp. nov.	SMF 97663	AB 857		KT186575	KT119477	Panama	8.47911	77.52799
D. citrinobapheus	SMF 89814	AH 449	JQ927333			Panama	8.48500	81.17300
D. citrinobapheus	SMF 89820	AH 211	JQ927334			Panama	8.56900	81.09900
D. citrinobapheus	MHCH 2370	AH 450	JQ927335			Panama	8.48500	81.17300
D. citrinobapheus	MHCH 2371	AH 452	JQ927336			Panama	8.48500	81.17300
D. hylaeformis	SMF 89868	AH 267	JQ927337			Panama	8.93100	82.71400
D. hylaeformis	SMF 89869	AH 268	JQ927338			Panama	8.93100	82.71400
D. hylaeformis	SMF 89872	AH 124	JQ927339			Panama	8.91100	82.71300
D. hylaeformis	SMF 89875	AH 282	JQ927340			Panama	8.91200	82.71300
D. citrinobapheus	USNM 572442	KRL 0902	FJ784425			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572443	KRL 1181	FJ784484			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572454	KRL 0900	FJ784423			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572455	KRL 0901	FJ784424			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1783	KRL 0694	FJ784338			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1830	KRL 0840	FJ784395			Panama	8.66700	80.59200
D. quidditus	USNM 572444	KRL 0647	FJ784326			Panama	8.66700	80.59200
D. quidditus	MVUP 1832	KRL 0856	FJ784405			Panama	8.66700	80.59200
D. vocator	FMNH 257769	AJC 0127	JN991419	JN991348		Costa Rica	8.79000	82.96000
D. hylaeformis	USNM 572546	KRL 0782	FJ784369	FJ766810		Panama	8.66700	80.59200
D. hylaeformis	MVUP 1826	KRL 0831	FJ784390	FJ766809		Panama	8.66700	80.59200
D. hylaeformis	MVZ 203844	1999	EU186682			Costa Rica	9.75000	83.80400
D. hylaeformis	UCR 16264	AJC 0468	JN991418	JN991347		Costa Rica	10.22000	84.54000