Research Article

Calling activity of the common tink frog (*Diasporus diastema*) (Eleutherodactylidae) in secondary forests of the Caribbean of Costa Rica

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Abstract

Calling activity in terrestrial frogs can be used as a measure of habitat suitability for reproduction. We evaluated the calling activity of the common tink frog *Diasporus diastema* (Eleutherodactylidae) in 12 secondary forest sites that vary in age of recovery, and three old growth sites in the Caribbean of Costa Rica. We used visual and acoustic surveys along transects, and we identified only 26 individuals in a total of 12 hours of censuses for each site. We also used automated species identification from recordings, and we detected *D. diastema* in 10,605 of 49,273 recordings. The peak of the daily calling activity occurred from 2100h to 0100h, and the peak of monthly activity occurred in May. Calling activity did not vary among the different forest sites, suggesting that secondary forests can provide suitable habitat for *D. diastema* reproduction.

Keywords: automated species identification, calling activity, Costa Rica, *Diasporus diastema*, secondary forests

Resumen

La actividad de canto en ranas terrestres puede ser utilizada como una medida de la calidad del hábitat para su reproducción. Nosotros evaluamos la actividad de canto de la rana martillito *Diasporus diastema* (Eleutherodactylidae) en 12 bosques secundarios de diferentes edades y tres bosques maduros en el Caribe de Costa Rica. Nosotros realizamos muestreos visuales y acústicos en transectos, y solamente detectamos la presencia de 26 individuos para un total de 12 horas de censos visuales en cada uno de los sitios. Sin embargo, detectamos la presencia de cantos *D. diastema* en 10,605 de 49,273 grabaciones, utilizando la identificación automática de especies en las grabaciones. El pico de actividad de canto ocurrió entre las 2100h y la 0100h, y el pico de actividad mas alto fue durante el mes de mayo. La actividad de canto no vario entre los diferentes bosques, lo cual sugiere que los bosques secundarios puede proveer hábitats adecuados para la reproducción de *D. diastema*.

Palabras clave: actividad de canto, bosques secundarios, Costa Rica, *Diasporus diastema*, identificación automática de especies

Introduction

Male frog calling activity usually occurs in areas suitable for reproduction [1]. For example, species with aquatic larvae usually call from vegetation near creeks, rivers and ponds, while species with terrestrial development (e.g. leaf-litter frogs) usually call from the leaf-litter or shrubs [2, 3]. In general, terrestrial species reproduce in the same habitat where they live, while species with aquatic larvae tend to move from forests to water bodies for reproduction [4]. For terrestrial frogs, calling activity can be an indicator of habitat quality [5], and thus, calling activity can be used as a measure of species recovery in a habitat (*e.g.,* secondary forests).

Secondary forests are often suitable and valuable habitats for amphibian populations. For example, secondary forests in India had similar amphibian communities in comparison with old-growth forests [6]. Amazonian primary, secondary, and plantation forests had different species richness among them, but similar amphibian abundances [7]. Puerto Rican secondary forests achieved similar species richness with old-growth forests, but had different species abundances [8, 9]. In general, these studies show the ability of many species to recover in these secondary forests. In contrast, a study of the common tink frog (*Diasporus diastema*) showed poor recovery in secondary forests (10 years old) in the Pacific region of Costa Rica [10], confirming this species' preference for older forest habitats [2, 3].

This study evaluates *D. diastema* calling activity in a chronosequence of secondary forests in the Caribbean region of Costa Rica. We used visual and acoustic surveys in 12 secondary forests that varied on their age of recovery, and in three old-growth forests. We hypothesized that calling activity would increase as forest age increases in the chronosequence, as was found in the Pacific region of Costa Rica [10]. Therefore, old-growth forests would have higher calling activity than secondary forests, and older secondary forests would have higher calling activity than younger secondary forests, highlighting the importance of older forests as a suitable habitat for *D. diastema*.

Methods

Study sites

We selected 15 forests sites in lowlands in the Caribbean region of Costa Rica (10°26' N, 86° 59' W), Sarapiquí, Heredia. Specifically, we selected 12 secondary forests that represent different ages of forest recovery: three 10-16 years old, three 17-23 years old, three 24-28 years old, three 36-48 years old, and three old-growth forests (Fig. 1). Ten of the secondary forest sites were located between 700-2500 m away from old-growth forests, while two sites were adjacent to old forests. Six of our forest sites were located in La Selva Biological Station (LSBS). None of the sites included water sources (e.g. rivers, creeks or permanent ponds), habitats that can have very high densities of *D. diastema* [10]. The main land uses in this region are agricultural (e.g. banana and pineapple plantations), cattle pastures, and secondary forests [11-15]. All secondary forest sites were used as cattle pastures before being abandoned [15].

Study species

Diasporus diastema (Eleutherodactylidae) is a common and abundant arboreal, nocturnal species that inhabits humid forests from Nicaragua to western Ecuador, and occurs from sea level to 1600 m [2, 3] (Fig. 2). This species is normally found in old-growth forests, but it also has been reported in forest fragments and in disturbed habitats (Fig. 2) [14]. Despite its common metallic "tink" call (Fig. 2), this species is difficult to locate because it calls from sites hidden by vegetation (*e.g.,* base of *Heliconia* spp. leaves), and because of its small size (26 mm

snout-vent length) [3]. Little is known about its reproduction, which has been reported to occur from May to November [2].

Fig. 1. The location of the 15 study sites and the La Selva Biological Station (LSBS) in northeastern Costa Rica. The sites in the five age classes are: 10-16 years old: Bejuco, JEnriquez, and LG6; 17-23 years old: Arrozal, LG16, and STR2450; 24-28 years old: LOC600, LG5, and Tirimbina; 36-48 years old: Aceituno, Manu, and Botarrama; old-growth forests: Selvaverde, SHA50, and SJ1700. Forest and landscape characteristics of each site are in Appendix 1.

Sampling design

Acoustic surveys were conducted in the 15 forest sites using automated recording devices in 2010 during the dry season (Fig. 2) (February and March), and the wet season (April, May, June, August, and October). We placed one recording device in the center of each forest site, at a minimum of 50 m from the forest edge. The automated recorders were developed by our research group Automated Remote Biodiversity Monitoring Network (ARBIMON) [16]. We recorded one minute of audio every 10 minutes, 24 hr per day. Recordings were collected for two weeks during each month. Although detection will vary depending on the power of each species, in general, amphibians will be detected at a radius of 50 m (*i.e.,* 7850 m^2 sampling area). In addition, we spent a total of 12 hours conducting visual encounter surveys (VES) [17] along transects (2 x 50 m) in each site during the day (0800h to 1200h) and night (1900h to 2400h), to compare with the acoustic surveys.

Data analysis

We visually and acoustically evaluated the presence or absence of *D. diastema* calls in 2,383 1-minute recordings, using spectrograms. This information was used to validate the results of an automated identification model. Within each recording an algorithm identifies "regions of interest" (*i.e.,* area of high energy). Then, using the visualizer web application [17], we developed a species-specific identification model. We adjusted the frequency and call duration filters to optimize the model. We validated the final model, and we obtained its accuracy and precision with a confusion matrix. This model was then used to identify the species' presence in 49,273 1-minute recordings from the forest chronosequence surveys.
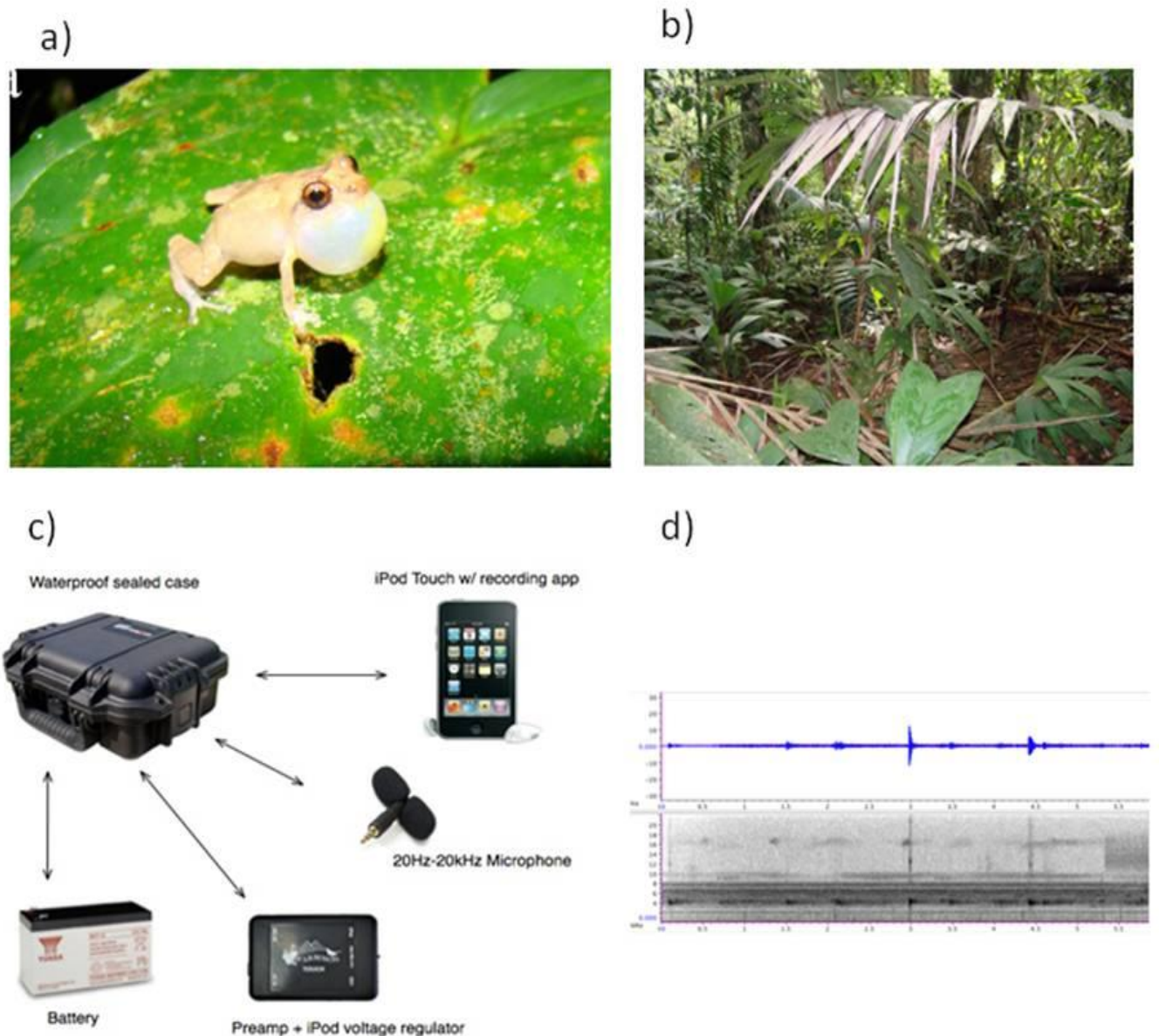
Fig. 2. a) the common tink frog (*Diasporus diastema*) b) the habitat for the common tink frog in the Caribbean c) the recording device d) the oscillogram and spectrogram of *D. diastema* call.

We used a linear regression model to determine if there was a relationship between the density of the species in the transect surveys and the detection frequency (number of recordings with a positive detection/total number of recordings) in the acoustic surveys for the same days. To determine if there was an effect of site age (five age classes) or season (dry, wet) on the detection frequency (*i.e.,* calling intensity), we used a Friedman nonparametric two way ANOVA. Given that there was no effect of age, we combined the call detections from all sites for each month to explore the effect of season. For this analysis, we related the detection frequency with monthly values of precipitation, relative humidity, and air temperature provided by LSBS meteorological station [18] using a multiple regression model.

To determine if other site factors could explain the variation in the detection frequency among sites, we conducted another multiple regression that included six forest structure variables (aboveground biomass, stem density, basal area, tree species richness [15], leaf-litter depth, and understory shrub cover) and six landscape variables (percentage of forest cover within 250, 500, 750 and 1000 m of each site, distance of site to the nearest old-growth forest, and the area of each forest site) (Appendix 1). All statistical analyses were performed in R (version 2.12.2) [19].

Results

Transects versus automated recording

Detection of *D. diastema* was very low in the transect surveys; we detected only 26 individuals during a total of 12 person-hours of censuses. On the other hand, the automated recorders along with the species identification model detected *D. diastema* calls in 10,605 of the 49,273 1-minute recordings. Although the detection model had high levels of accuracy (*i.e.,* the total number of predictions that were correct, 82%) and precision (*i.e.,* the proportion of the predicted positive cases that were correct, 86%), the true positive rate (the proportion of positive cases that were correctly identified) was only 56% (Appendix 2). The major cause of the false negatives was calls with low energy (*i.e.*, calling far from the microphone), which were not detected by the species detection algorithm, but could be observed in the spectrogram by the author. There was a positive relationship between densities obtained from the transect surveys and the detection frequency in the audio recordings (F = 4.48, df = 1, 42, P = 0.04), but this relationship explained only 7% of the variation. All further analyses are based on the presence/absence data from the recordings.

Effects of site age and season on calling activity

The peak in daily calling activity occurred between 2200h and 0100h (Fig. 3a). During this period, *D. diastema* was detected in approximately 30% of the recordings. Between 1900h and 2100h and 0200h and 0500h, this species was detected in more than 25% of the recordings (Fig. 3a). As expected for most amphibians, calling activity was low during the day (0600h-1600h).

There was no significant difference in calling activity among the five forest age classes (Friedman two way Anova – F = 7.6, df = 4, P = 0.10), but there was a significant effect of season (F = 5.0, df = 2, P = 0.02) (Fig. 4). Regardless of the site age, calling activity was low during the "dry season" (February-March) with detections in only ~5% of the recordings. During the wet season (April-October), there was again no difference in call detection among the forest age class, but calling activity was much greater (5-30% of the recordings).

Given that there was no effect of site age, we combined the observation of sites for each month to better understand the effects of season. In a multiple regression the detection frequency was not related with mean monthly rainfall (P = 0.25) or mean monthly relative humidity (P = 0.18), but it was positively related with temperature (P = 0.008) (R^2_{adj} = 0.861) (Appendix 3 Fig. 5). April through October were the months with highest temperature (Fig. 5c) and highest calling activity (Fig. 3b), and the peak of calling activity occurred in May when *D. diastema* was detected in approximately 45% of the recordings (Fig. 3b).

Effects of site characteristics on calling activity

In the previous analysis, we showed that site age was not an important factor in explaining the variation in calling activity of *D. diastema*, but it is possible that other site characteristics could

influence calling behavior. However, forests characteristics and landscape variables did not explain the variation in *D. diastema* calling activity (Forest - *F* = 0.68, df = 7, 7, *P* = 0.67; Landscape - *F* = 0.48, df = 5, 5, *P* = 0.77, respectively).
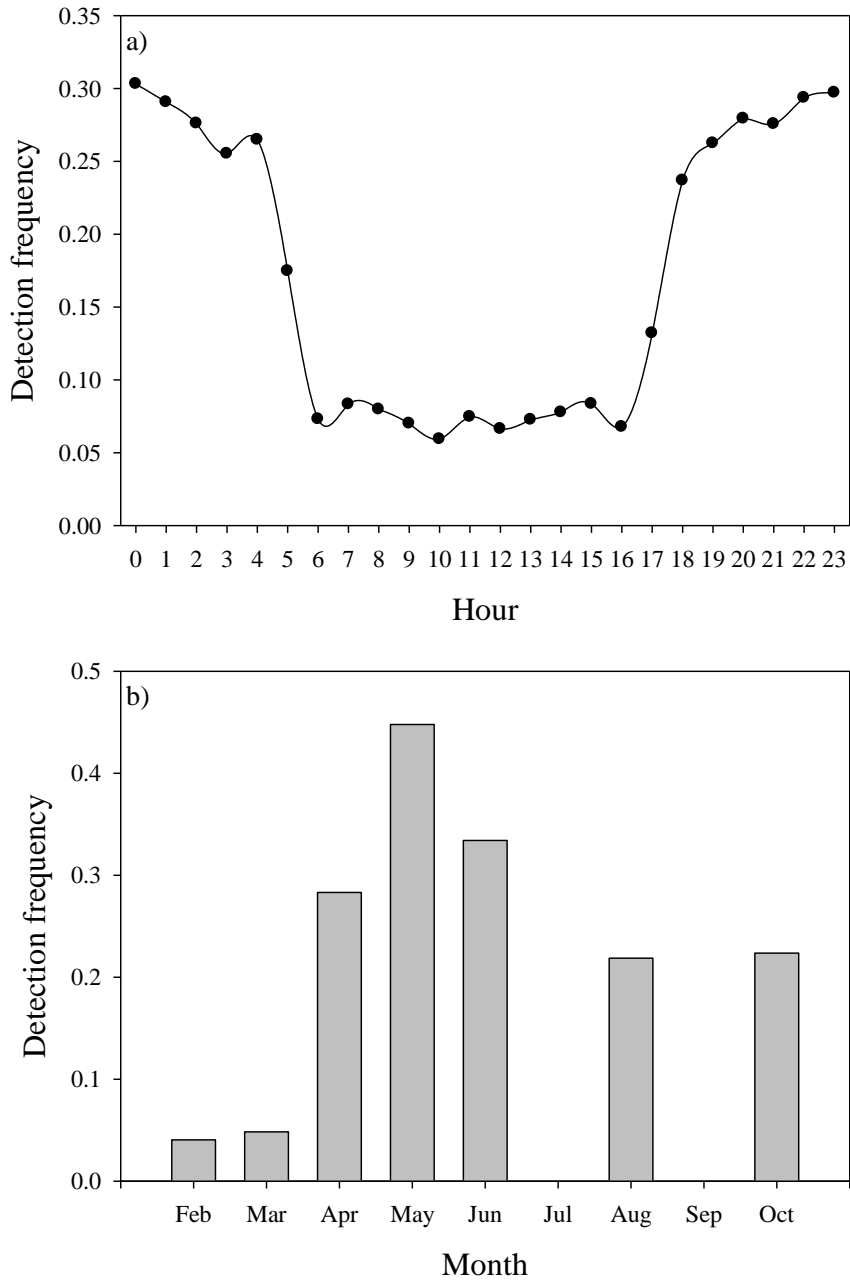

Fig. 3. a) daily and b) monthly calling activity of *D. diastema* based on 10,605 detections from audio recordings. Data from all sites were combined.

Discussion

Acoustic surveys were much better for detecting *D. diastema* in comparison with visual surveys. The species' small size and its preference for calling from hidden sites make it difficult to observe in the field [3]. In contrast, its "tink" call is loud and easily differentiated from other species. By using the automated recorders, we were able to sample many sites at the same time, and thus avoid the effect of sampling on different days, which may vary in rainfall, temperature, or humidity. In addition, the automated species identification model allowed us to analyze a large data set (approximately 50,000 recordings), providing detailed information to establish clear calling activity patterns. For example, the majority of calling activity of *D. diastema* occurred during the night [20], but calls were also detected during the day, possibly after heavy rainfall, as has been reported before (Fig. 3a) [3].
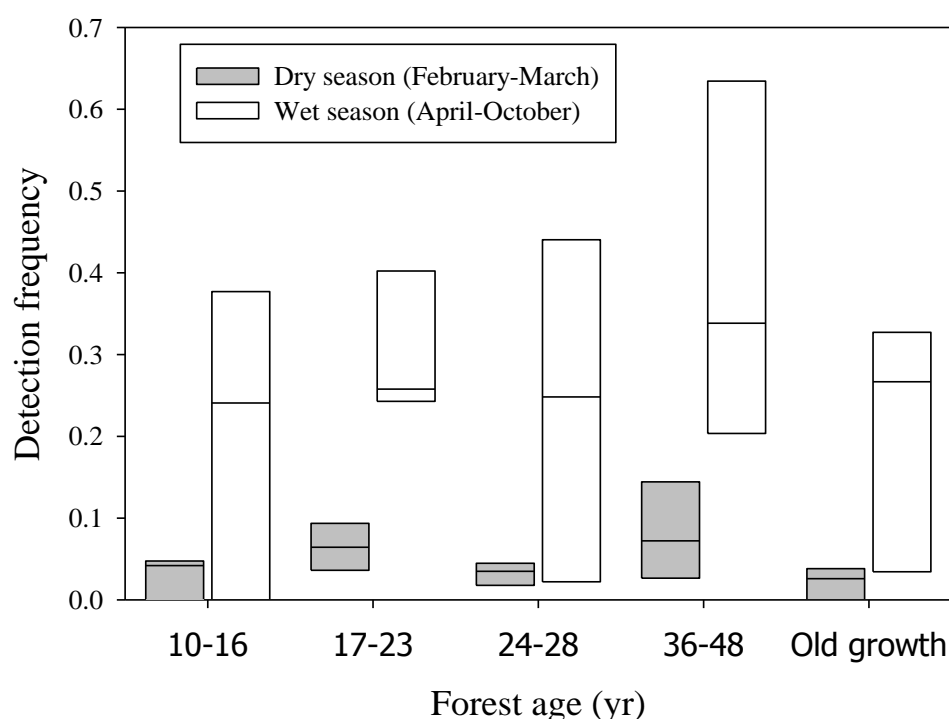

Fig. 4. A comparison of the detection frequency of calling behavior of *D. diastema* between the five forest ages classes during the dry season and wet season. Each age class included three replicates. The line within the box represents the median value and the box represents the 25 and 75 percentiles.

In the Caribbean region of Costa Rica, amphibian reproductive behavior (*i.e.,* male vocalization) can occur during the dry or the wet season depending on their food and substrate needs [21]. Calling activity of *D. diastema* was low during February and March (normally the driest months in this region) [22], and high in April through October (wet season) (Fig. 3). Nevertheless, calling activity was not related to rainfall or relative humidity, but instead it was related to temperature. Higher temperatures may promote reproduction in *D. diastema*. Previous studies of closely related species indicated that higher temperatures can accelerate embryo development in eggs, but it can also retard hatching when temperatures are extreme (>24°C) [23]. An alternative explanation may be related to plant phenology and the impact on leaf-litter arthropods. In the study region, higher temperatures occur in April and May when solar radiation incidence is at its maximum for this latitude [24]. Drought-tolerant tree species tend to drop old leaves and flush new leaves during this peak of solar

radiation [24]. The accumulation of leaf-litter increases the abundance of leaf-litter arthropods (*e.g.,* orthopterans and ants) [25], which are very important components in the diet of *D. diastema*. This peak of food abundance could be responsible for the higher calling activity in April and May.

Calling activity was similar among the different forest sites, suggesting that all sites provide suitable habitats for reproduction. Although these sites vary in age of recovery, at a minimum they share a closed canopy. These results contrast with a recent study of a population of *D. diastema* in the Pacific lowlands of Costa Rica, which reported much lower densities in secondary forests compared with old-growth forests [10]. In contrast, in our study, *D. diastema* had similar levels of calling activity in both secondary forests of different ages and old growth forest. Given that the secondary forests were abandoned pastures, and *D. diastema* has been reported as a forest species [2, 3], it appears that the species has recovered rapidly in these sites. A possible difference between these studies is that in the Pacific study a large river separated the mature and secondary forest, which may have limited colonization [10], while in our study, riparian vegetation, live fences, or recently abandoned pastures may have facilitated the recolonization of these secondary forest [26].

Implications for conservation

To better understand the impacts of global change it is essential that we have reliable data from many sites. The present study showed the usefulness of automated recording surveys and call identification techniques for a common but hard to observe species. These techniques allowed us to determine the daily and monthly calling activity of *D. diastema* in 15 sites simultaneously. In contrast to our initial hypothesis, forest age did not explain the variation in calling activity of *D. diastema*, suggesting that secondary forests can provide suitable characteristics for reproduction for this species. Given the extent of amphibian decline [27], there is a small glimpse of hope that these secondary forests can help maintain populations of this and other amphibians [26].

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Appendix 1. Forest sites and landscape characteristics.

Forest sites	Age	# of trees	# of stems	# of tree species	Tree basal area (m2/ha)	Aboveground biomass (Mg/ha)	Understory shrub cover	Mean leaf-Litter depth (cm)	Forest cover 0-250 m (%)	Forest cover 0-500 m (%)	Forest cover 0-750 m (%)	Forest cover 0-1000 m (%)	Distance to OG forest (m)	Area (ha)
					Forest characteristics						**Landscape characteristics**			
Bejuco	14	288	340	70	22.2	117.4	3	2.5	65.7	42.8	26.1	34.2	1203.7	16.8
JEnriquez	14	261	357	72	10.8	50.8	2	2.4	74.0	44.7	32.4	34.2	1203.7	34.3
LG6	15	330	570	61	18.3	99.7	4	2.3	100.0	100.0	100.0	100.0	983.5	2.4
Arrozal	20	431	431	92	19.9	108.5	3	3.7	93.6	92.4	87.3	83.7	4393.1	6.4
LG16	22	268	346	48	18.7	129	4	2.9	100.0	100.0	100.0	100.0	1425.9	17.8
STR2450	22	335	473	69	24.6	158.5	3	1.4	100.0	100.0	100.0	100.0	0	30.7
LOC600	25	260	299	69	32.8	223.2	3	1.0	100.0	100.0	100.0	100.0	0	50.9
LG5	27	337	468	85	20.2	119.6	4	2.5	100.0	100.0	100.0	100.0	881.1	2.7
Tirimbina	28	331	403	84	23.6	149.6	2	2.4	100.0	99.8	85.8	75.2	5078.3	8.2
Aceituno	36	286	366	105	46.7	311.6	3	2.5	100.0	100.0	100.0	100.0	4216.7	6.1
Manu	46	351	401	136	39.6	253.3	3	2.9	97.8	75.5	73.3	70.4	5000.2	20.9
Botarrama	48	306	351	101	38.1	251.3	3	3.1	67.5	63.0	53.3	56.4	5432.7	13.2
Selvaverde	old-growth	243	261	91	24.3	142.5	2	3.5	62.6	63.7	49.5	37.6	-	129.3
SHA50	old-growth	245	264	104	32.2	216.7	2	1.4	100.0	100.0	100.0	100.0	-	10197.6
SJ1700	old-growth	269	285	104	26.2	168.1	2	1.7	100.0	100.0	100.0	100.0	-	8158

Appendix 2. The error matrix of the species identification model based on the observations of the author (BH) of 2,383 1-minute recordings. The rows represent the direct observations and the columns represent the decision of the model.

	Detected	Not detected
Present	477 (true positives)	370 (false negatives)
Not present	77 (false positives)	1505 (true negatives)

Appendix 3. Monthly variation in a) total precipitation, b) mean relative humidity, and c) mean temperature during 2010 from the La Selva Biological Station.