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Ecology, conservation status, and phylogenetic placement of endemic *Pristimantis* frogs (Anura: Craugastoridae) in Trinidad and Tobago and genetic affinities to northern Venezuela

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Abstract

Trinidad and Tobago are home to three endemic species in the anuran genus *Pristimantis*, of which two (*Pristimantis charlottevillensis* and *Pristimantis turpinorum*) occur in Tobago alone and the third (*Pristimantis urichi*) is present on both islands. Earlier, the IUCN assessed the conservation status of these species as: *P. urichi*, Endangered (EN); *P. charlottevillensis*, Least Concern (LC); *P. turpinorum*, Vulnerable (VU). However, these assessments were based on very little field-based evidence. Here, we present survey results which contributed to reassessments as LC, VU and Data Deficient for these three species, respectively. Despite the close proximity of Trinidad to northern Venezuela, the islands do not share any *Pristimantis* species with the mainland, which holds a rich endemicity of *Pristimantis* regionally. In this study, we used genetic sequencing from several island populations and compared them to northern Venezuelan endemics to assess genetic divergence for the first time. The time tree analyses found that only the northern Tobago species *P. turpinorum* is closely related to mainland *Pristimantis*, with a genetic split dating to the Late Miocene, suggesting a vicariant event of mainland and island species. *Pristimantis urichi*, although identical between the two islands,

remains highly divergent from the mainland species. Similar results were found for *P. charlottevillensis*. In addition, there was a high level of divergence between *P. urichi* and *P. charlottevillensis*. These findings indicate different island colonization events by different lineages. Sequencing other Venezuelan species remains pivotal to unravel the complexity of the colonization episodes in the region, likely influenced by the changing topography and multiple connection and isolation episodes of the islands by eustatic sea-level changes.

KEYWORDS
amphibian, biodiversity, conservation, island colonization, IUCN Red List

1 | INTRODUCTION

It has been well known since Stuart et al. (2004) published the first Global Amphibian Assessment (GAA) that about one third of the world's amphibian species are threatened with extinction, being classified in one of the IUCN's highest threat categories, defined as being critically endangered (CR), endangered (EN), or vulnerable (VU). However, this picture is complicated by two problems. First, there is taxonomic uncertainty: many studies (e.g., Funk et al., 2012) have documented diversity within species previously thought to be widespread, leading to the description of new taxa and the need for a reassessment of conservation status. Second, and especially in the tropics, it is sometimes the case that assessments of population sizes and trends are based on expert judgments, rather than on field estimations (Smith et al., 2021). This is almost inevitably the case when new taxa are described, a frequent event in amphibians with about 150 new species described annually (Frost, 2021). In the work reported here, we address these problems for three species in the neotropical genus *Pristimantis*, which are endemic to the islands of Trinidad and Tobago, including an assessment of their relationships to species in the nearby Paria Peninsula of Venezuela.

Murphy (1997) listed four species of ground-living direct-developing frogs as occurring in Trinidad and Tobago, three of them native and one (*Eleutherodactylus johnstonei*) recently introduced. The spread of *E. johnstonei* across Trinidad and into Tobago has been documented by Downie et al. (2017). Following a major taxonomic revision (Hedges et al., 2008), the three native species were assigned to the genus *Pristimantis* in the family Craugastoridae (Frost, 2021). The nomenclature of some of these species has undergone substantial changes in the last decades. Hardy (1982) first applied the name *Eleutherodactylus* cf. *rozei* to a Tobago population. Following Hardy (1982), Murphy (1997) provided an account for the Tobago taxon using the same name. The Tobago population subsequently became *Eleutherodactylus turpinorum* Hardy, 2001. Heinicke et al. (2007) moved

South American mainland *Eleutherodactylus* to the genus *Pristimantis*, producing the combination *Pristimantis turpinorum*. Similarly, Hardy (1982) considered a larger Tobago *Eleutherodactylus* to be the same as the Venezuelan *Eleutherodactylus incertus* (as *terraebolivaris*). Kaiser et al. (1995) named the Tobago population *Eleutherodactylus charlottevillensis* and Murphy (1997) followed this arrangement. When Heinicke et al. (2007) moved the South American mainland *Eleutherodactylus* to the genus *Pristimantis*, this resulted in the combination *Pristimantis charlottevillensis*.

Pristimantis urichi is believed to be endemic to Trinidad and Tobago, whereas *P. charlottevillensis* and *P. turpinorum* occur only in northeast Tobago (Auguste, 2019). The IUCN Red List assessed the status of these three species as follows: *P. urichi*, EN; *P. charlottevillensis*, LC (IUCN SSC Amphibian Specialist Group, 2013); *P. turpinorum*, VU. Full details of these assessments are embedded in the revised species assessments published by IUCN in 2020.

The IUCN has recently undertaken a revision of the status of all amphibian species, including the Trinidad and Tobago *Pristimantis* species (IUCN SSC Amphibian Specialist Group, 2020a, 2020b, 2020c). A problem with the earlier assessments was that they were not based on the results of any published data and therefore relied on partial impressions and best guesses. For example, the IUCN Red List based the *P. urichi* assessment on recent substantial population declines in Tobago, none of them documented, but used no information at all from Trinidad. However, Kenny (1969) considered this species to be distributed "throughout Trinidad from sea level to the highest elevations and probably the most ubiquitous of Trinidad amphibians." An analogous situation was the marsupial tree frog *Flectonotus fitzgeraldi*, which occurs in Trinidad, Tobago, and Venezuela. It had been assessed by the IUCN as EN in 2004, but no field data had contributed to this view. Since that time, Smith et al. (2021) have published data based upon field surveys and reassessed the species as LC (IUCN SSC Amphibian Specialist Group, 2020d).

The relationships of the island populations and closely related species from the mainland (northern Venezuela, in the Paria region) have not been assessed. Only one individual each of *P. urichi*, *P. turpinorum* (as *rozei*), and *P. charlottevillensis* (as *terraebolivaris = incertus*) have sequences from the islands (Trinidad, Tobago, and Tobago, respectively) currently available in GenBank. Furthermore, no *P. urichi* samples from Tobago have been sequenced to assess the population divergence between Trinidad and Tobago. Phylogenetic analyses (Hedges et al., 2008; Rivera-Correa et al., 2017) have shown a close relationship between *P. urichi* and *P. turpinorum* (as *rozei*) and high divergence of these from *P. charlottevillensis*. These data suggest *Pristimantis* colonization of Tobago at different times. However, genetic divergence in *Pristimantis* included in those genetic analyses suggests that they are not closely related (Hedges et al., 2008; Rivera-Correa et al., 2017) to the studied populations and that mainland populations closely related to the insular species are still to be sampled and analyzed.

In this work, we mapped the detailed distribution of all three species of *Pristimantis* endemic to Trinidad and Tobago for the first time, with the overall aim to assess their population ranges and abundance in order to better evaluate their correct conservation status. A second aim of this paper was to present further evidence on the genetic relationships of these three species to each other and, for the first time, to northern Venezuelan species.

2 | MATERIALS AND METHODS

2.1 | Presence/absence surveys, population estimates, and habitat preferences

Pristimantis are found on the forest floor or on low vegetation and, since they do not spawn in water, cannot be surveyed by counting individuals at breeding ponds. Instead, they require extensive visual and acoustic surveys throughout their forest habitats. The calls of the *Pristimantis* species were learned by the observers listening to a tape recording (Read, 1990) and by watching and listening to frogs in the field in the company of an expert. Figure 1 shows all three species. We used calls, rather than visualization, as the main evidence of presence since *Pristimantis* are cryptic, and searching for them can cause disturbance. However, if frogs were seen, their presence was recorded, using the descriptions in Murphy (1997) for identification. For *P. urichi*, previous work (Smith et al., 2021) had established that calling occurs from dusk (18:30) until about 20:00, so this was the time period used
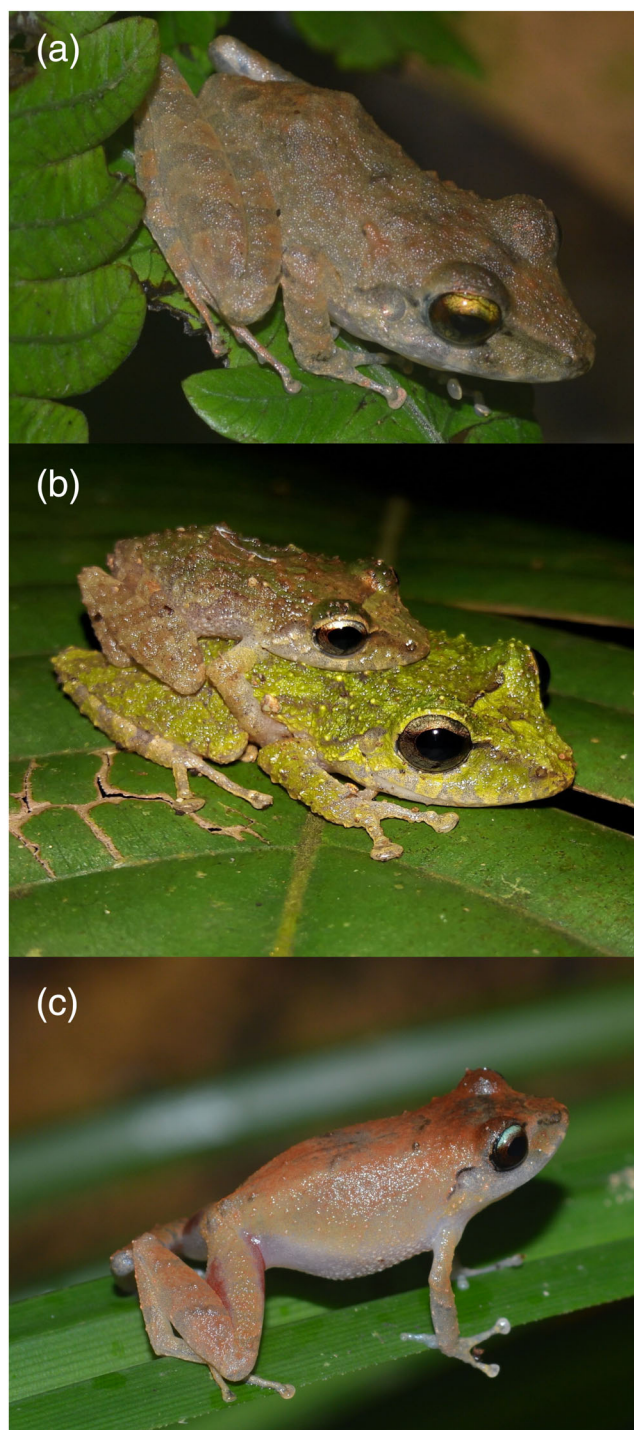
FIGURE 1 Three *Pristimantis* species from Trinidad and Tobago: (a) *Pristimantis charlottevillensis*, (b) *Pristimantis turpinorum*, (c) *Pristimantis urichi* (photographs by Renoir J. Auguste) [Color figure can be viewed at wileyonlinelibrary.com]

for surveys. To extend our knowledge of the current range of the species, road surveys were carried out in 2015 and 2016. For *P. charlottevillensis*, dusk to around 21:00 was found to be suitable for call counts. For *P. turpinorum*, previous work (Renoir Auguste) had

suggested that calling occurred during late afternoon, 15:00 to 17:00. Call surveys were made during that time period along forest trails in Tobago's Main Ridge Forest Reserve (MRFR).

Road surveys were made by vehicle, stopping every 500 m or so to listen for frogs with the engine off and all observers silent for 2 min before counting calls of the different species over 5 min. Forest path surveys were performed by setting up transects using tape tied to trees to measure a set of up to eight consecutive 25 m stretches. Transects were used not only to assess presence/absence but also to estimate relative abundance. The number of frogs heard calling along the length of the transect was noted on repeated occasions (generally five repeats) and the maximum number per 100 m used to give a relative abundance estimate. During all surveys, comments were noted on the habitats where *Pristimantis* were found. In Tobago, measurements were made in 2018 by recording where adult *P. urichi* and *P. charlottevillensis* were seen, either on the ground or in vegetation (including height above the ground measured using a tape measure accurate to 1 cm). Weather at the time (dry, wet, heavy rain) was also noted. Similar observations were made for *P. turpinorum* in 2018 and 2020. A linear model was run in R v1.2.5 with perching height, weather and site as the response variables, and species identifier as a fixed predictor variable, height ~ species. Neither weather nor site had any effect and were excluded from the model. A Tukey (HSD) post hoc comparison test on perch height was used to determine the significance of species pairwise comparisons.

Some of the survey work reported here was carried out by University of Glasgow students during research expeditions to Trinidad and Tobago. Other surveys were part of eight Bioblitzes organized by the University of the West Indies Zoology Museum and the Trinidad and Tobago Field Naturalists' Club (Smith et al., 2021). A Bioblitz aims to record every species encountered in a particular area over a period of 24 h. The herpetological surveys were forest walks at dusk organized by Renoir Auguste and John Murphy. In addition, any incidental findings of *Pristimantis* discovered during other work were noted and added to the maps produced. Survey points were recorded using a hand-held GPS device.

In Trinidad, transect surveys were carried out at seven Northern Range sites (Table S1) over 3 years (2012–2014), with each site visited five times between June and August. Trinidad road survey, Bioblitz and incidental sites are shown in Table S2. In Tobago, transect surveys were carried out at nine north-eastern forest sites (Table S3) over 7 years (2012–2018). Some sites were surveyed only in 2012 and a different set of sites in the period 2013–2018. Sites were visited five times between June and August. Tobago road survey sites are shown in Table S4. Simultaneous transect surveys for *P. urichi* and *P. charlottevillensis*, were done from 20:00 to 22:00. Surveys of the 100 m transect at Spring Trail in the Tobago MRFR from June to August 2017 and 2018, 20:00–22:00, were made to assess tentative numbers for *P. turpinorum*.

2.2 | Maps

We created maps based on GPS coordinates contributed by the authors, showing presence/absence in QGIS v3.0.2 software (QGIS Development Team, 2018) in WGS84 datum, using the Google Terrain Hybrid (Google, 2020) as a base layer. We determined the extent of occurrence of *P. urichi* and *P. charlottevillensis* in Trinidad and Tobago separately using QGIS (version 3.0.2-Girona). GPS coordinates where the species were present allowed the creation of convex hull polygons around the outermost coordinates within each island using the QGIS minimum bounding geometry tool. The convex hull polygons were clipped to their respective island's shapefile so that areas of the polygons which extended into the sea were excluded. The areas of the clipped polygons were then calculated to give the extent of occurrence of each species on each island in km^2. Because of data deficiency, we did not create a map for *P. turpinorum*.

2.3 | Specimen sampling and genetic analyses

Individuals of the three Trinidad and Tobago *Pristimantis* species were caught with hand nets. Identification in Trinidad was straightforward since *P. urichi* is the only *Pristimantis* species present. Specimens were euthanized following approved American Society of Ichthyologists and Herpetologists (ASIH) guidelines using MS-222 in

TABLE 1 Maximum number of calling *Pristimantis urichi* per 100 m at Northern Range sites in Trinidad (2012–2014)

Site	Year		
	2012	2013	2014
1. Caura 1	20	13	8
2. Caura 2	14	11	16
3. Mt St Benedict	13	18	14
4. Maracas Waterfall	–	4	8
5. Morne Bleu	9	17	13
6. Simla, lower	11	13	–
7. Simla, upper	5	4	–

TABLE 2 Maximum number of calling *Pristimantis urichi* and *Pristimantis charlottevillensis* individuals per 100 m at Tobago sites (2012–2017)

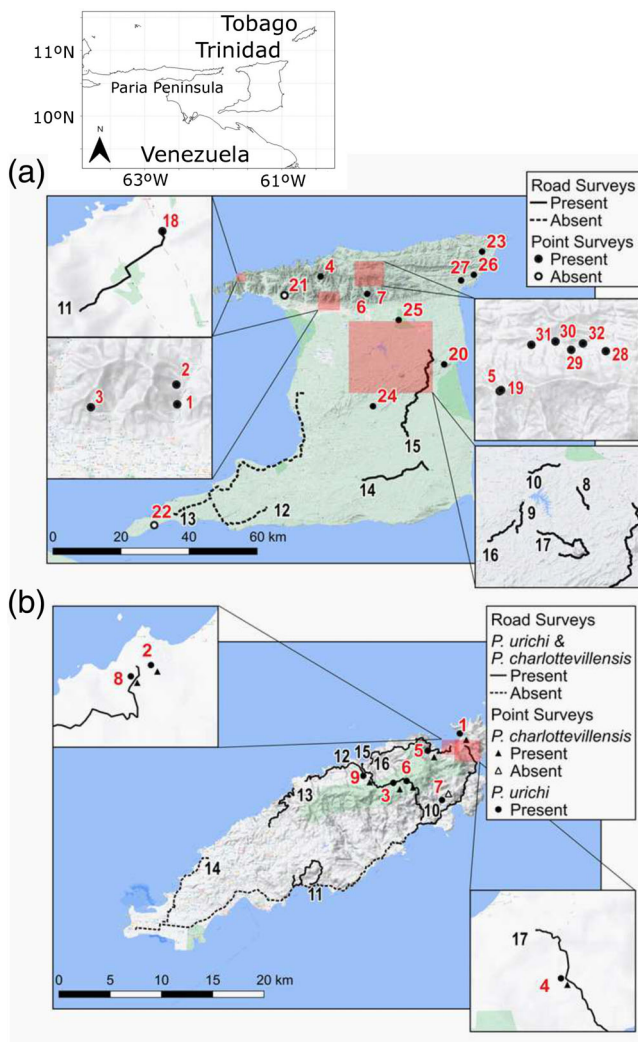| Site	Year(s)	*P. urichi*	*P. charlottevillensis*
1. Pirates Bay	2012	8	24
2. Charlotteville	2012	7	35
3. Gilpin Trace	2012	36	6
4. Doctor's	2015–2017	0, 3, 1	7, 4, 2
5. Hermitage	2016–2017	4, 0	12, 4
6. Spring Trail	2013–2017	6, 18, 1, 6, 3	6, 2, 3, 0, 1
7. Queen's River	2016	6	0
8. Cambleton	2013–2017	10, 21, 0, 3, 1	30, 15, 6, 6, 2
9. Dead Bay stream	2016–2017	3, 1	2, 3

FIGURE 2 Survey sites (points and roads) reported in this study: (a) Trinidad for *Pristimantis urichi*. (b) Tobago for *P. urichi* and *Pristimantis charlottevillensis*. Red numbers refer to point surveys and black numbers refer to road surveys [Color figure can be viewed at wileyonlinelibrary.com]

water, and tissues were preserved in 90% ethanol and deposited in the University of the West Indies Zoology Museum. In Tobago, the descriptions in Murphy

et al. (2018) were used to help distinguish the three species. Some specimens were deposited in the University of the West Indies Zoology Museum in Trinidad and others were transported to the University of Glasgow and deposited in the Hunterian Zoology Museum. Tissue samples (abdominal muscle) of all specimens were dissected for genetic analyses.

Whole genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. We sequenced one mitochondrial gene fragment, 16S rDNA, with primers 16SL and 16SH (Palumbi et al., 1991). Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous site base-calls in Sequencher v4.9 (Gene Codes Corporation, Ann Arbor, MI).

We conducted BLAST searches against GenBank for all individuals in order to identify closely related *Pristimantis* species (besides the three sequences in GenBank of *P. urichi*, *P. charlottevillensis*, and *P. turpinorum*). In this study, we aimed to unravel the closest insular species. For this purpose, we included in our analyses *Pristimantis nubisilva* and *Pristimantis pariagnomus* from northern Venezuela (Kaiser et al., 2015), which were collected by one of the authors in this study (Gilson Rivas) and recently identified through DNA barcoding (Rivas et al., 2021), but have never been included in comparative genetic analyses. The total dataset consisted of 25 individuals, including 6 *P. charlottevillensis*, 10 *P. urichi*, 2 *P. turpinorum*, 3 *P. pariagnomus*, and 4 *P. nubisilva*. These last two species were collected from the Paria region in northeastern Venezuela (Rivas et al., 2021). Sequences were aligned in Seaview v.4.2.11 (Gouy et al., 2010) with MAFFT (Katoh et al., 2002). The final alignment comprised 477 base pairs. Uncorrected *p*-distances were computed in MEGA (Kumar et al., 2016). We ran the Poisson Tree Processes (PTP) method as implemented in the bPTP web server for the BEAST tree (Zhang et al., 2013) to assess if *P. pariagnomus* and *P. nubisilva* were the same species.
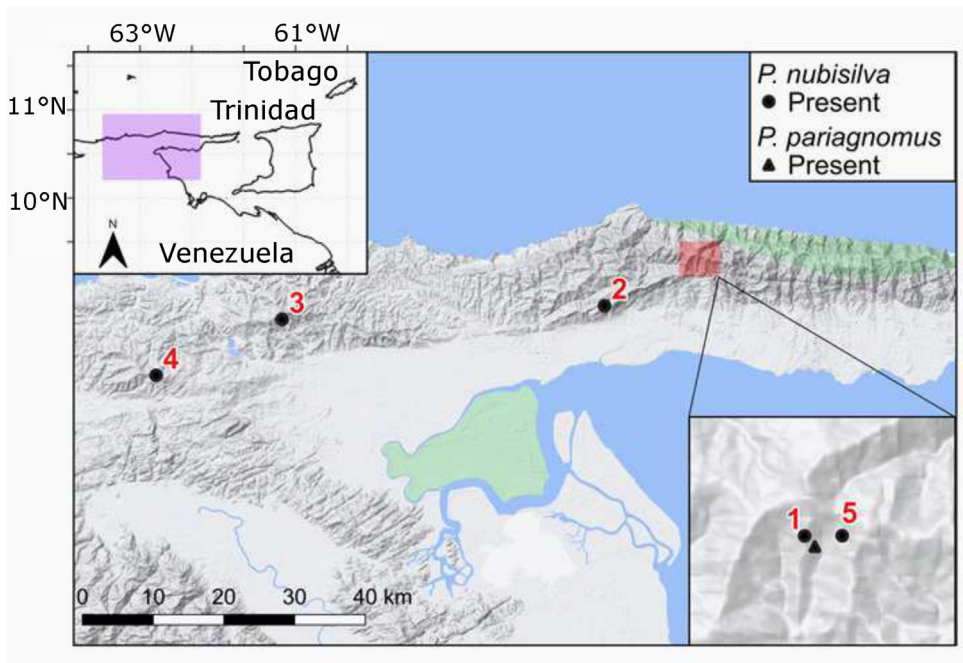
To obtain a time-calibrated tree that included closely related taxa we first identified mitochondrial 16S sequences deposited in GenBank that were within the first 100 BLAST matches after querying one representative sequence from each clade. We removed duplicated sequences by only taking unique accession numbers. We then downloaded the GenBank flat files using the script *acc2gb* (https://github.com/santiagosnchez/acc2gb). The GenBank records were then converted into FASTA sequences using *gb2fasta* (https://github.com/santiagosnchez/gb2fasta), extracting relevant information from the GenBank files. A total of 82 sequences were aligned together with our own sequences using default parameters in MAFFT (Katoh et al., 2002). The aligned file was loaded into BEAST v2.6.3 (Bouckaert et al., 2019), which we used to perform a divergence time analysis. As a calibration prior, we used the divergence time between *P. condor* and *P. bipunctatus* as estimated by TimeTree (http://timetree.org/; Hedges et al., 2015). The divergence time parameters, checked on May 28, 2021, were an estimated time of 22.2 Ma with a CI of 10.7–30.9 Ma. Both species were selected as taxon group in BEAST, but without enforcing monophyly, and the time to the last common ancestor (tMRCA) was calibrated with a normal distribution with a mean of 22.2 ($SD = 2$). The substitution model was co-estimated with the phylogeny by using the bModelTest package implemented in BEAST (Bouckaert & Drummond, 2017). We used a relaxed log-normally-distributed clock model parameterized using default settings. As tree prior we chose a constant coalescent prior to better represent branching times within species (i.e., Ritchie et al., 2017). Two independent MCMC runs with 10 million
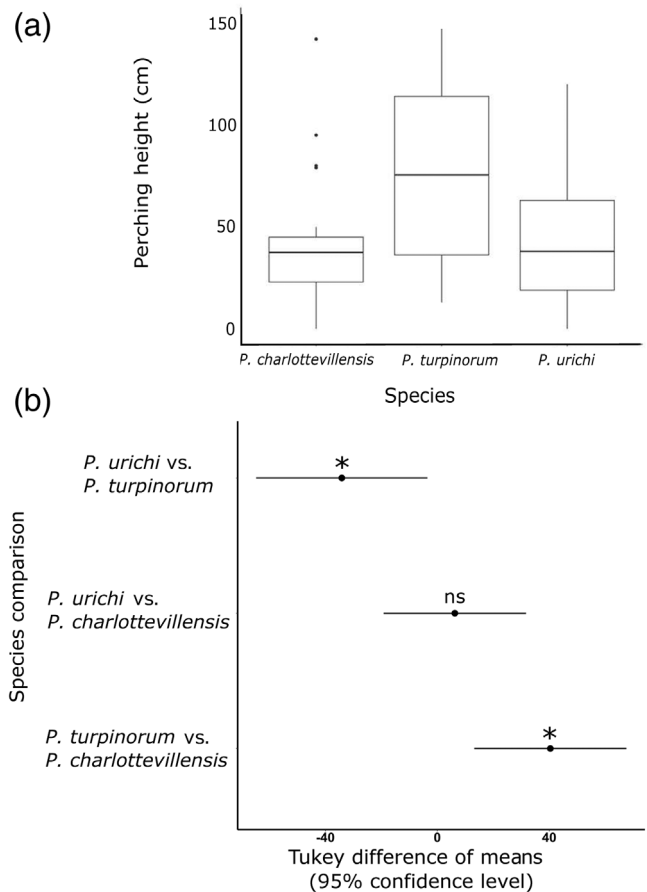

FIGURE 4 (a) Box plot of the perching height (cm) of the three *Pristimantis* species. (b) Tukey (HSD) pair-wise difference of median perching height (and 95% confidence level) in the three *Pristimantis* species. The associated significance level is displayed above each pair-wise comparison

TABLE 3 Uncorrected *P*-distances (mean and *SD*s) for the 16S rDNA partial sequence of *Pristimantis charlottevillensis*, *Pristimantis turpinorum*, *Pristimantis urichi*, *Pristimantis pariagnomus*, and *Pristimantis nubisilva*

	P. charlottevillensis	*P. turpinorum*	*P. urichi*	*P. pariagnomus*	*P. nubisilva*
P. charlottevillensis	0.026 (0.003)				
P. turpinorum	0.156 (0.004)	0.000 (0.000)			
P. urichi	0.157 (0.005)	0.143 (0.000)	0.000 (0.000)		
P. pariagnomus	0.150 (0.006)	0.043 (0.001)	0.150 (0.001)	0.005 (0.002)	
P. nubisilva	0.155 (0.005)	0.040 (0.000)	0.148 (0.000)	0.145 (0.000)	0.002 (0.001)

Note: Divergences were inferred from the alignment used for the minimum spanning network.

generations were completed and combined with LogCombiner after removing a 10% burnin. The MCMC chains were sampled every 1000 states, but were resampled at a lower rate (every 2000 states) to obtain a final posterior sample of about 10,000 states. Trees and parameters were summarized onto a maximum-clade-credibility (MCC) tree calculated by TreeAnnotator, keeping mean node heights. The MCC tree was then inspected in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

3 | RESULTS

3.1 | Surveys: *Pristimantis urichi* (Boettger, 1894)

In Trinidad, *P. urichi* was present at the seven Northern Range sites during each year and maximum numbers are shown in Table 1. Locations and results are shown in Table S2. Table S2 also shows the presence/absence results from eight Bioblitzes carried out from 2012 to 2019, and any incidental records made. Figure 2a shows all the Trinidad locations surveyed, including incidental findings. In Tobago, *P. urichi* was present during each year and at most sites, and maximum numbers are shown in Table 2. Road surveys were carried out over the same period. Locations are shown in Table S4, along with presence/absence findings. Figure 2b shows all the Tobago locations surveyed for *P. urichi*. This species occurred in forests and at forest edges on both islands, but was never found in towns or even close to sparse human habitations. Figure S1a,b shows the area of occurrence of the species on the two islands, 2624.4 and 170.9 km^2 on Trinidad and Tobago respectively, a total of 2795.3 km^2.

3.2 | Surveys: *Pristimantis charlottevillensis* (Kaiser et al., 1995)

Results are shown in Tables S3, S4, 2, and Figure 2b. Like *P. urichi*, *P. charlottevillensis* was mainly found in forests

and at forest edges and never in towns. However, *P. charlottevillensis* appeared to have more tolerance for human-modified habitats, such as quiet road edges, than *P. urichi*. The area of occurrence of the species in Tobago is shown in Figure S1b, an area of 170.9 km^2.

3.3 | Surveys: *Pristimantis turpinorum* (Hardy, 2001)

In 2017, a total of nine observations were made at Spring Trail over four visits. In 2018, 17 observations were made during eight visits, seven at Spring Trail and one at Cambleton. Three of the *P. turpinorum* observations were of small individuals, where identification can be questionable (as described below under Genetic analyses). As a direct comparison, in 2018 a total of 47 *P. charlottevillensis* and 18 *P. urichi* individuals were seen during the same surveys. In addition, we heard *P. turpinorum* calling from palm trees along the Roxborough-Parlatuvier road between 15:00 and 16:00, but we do not have sufficient numbers to report, nor do we have reliable information on locations for this species outside the MRFR. Due to the limited data, we do not show a distribution map for this species.

3.4 | Surveys: *Pristimantis nubisilva* (Kaiser et al., 2015)

Within the five *Pristimantis* species that inhabit Paria Range, *P. nubisilva* was the most common throughout the localities visited, being found in the three mountain chains that run across the Paria region in northeastern Venezuela (Figure 3). This species was observed seven times during the years 2014 (June), 2015 (July), 2016 (July, November), and 2017 (October). It can be encountered in a wide variety of habitats, including forests and plantations near forest, where it can be found in branches and leaves of trees and shrubs, within 1.5 m of the ground. Calling individuals were observed around 18:30–10:00.
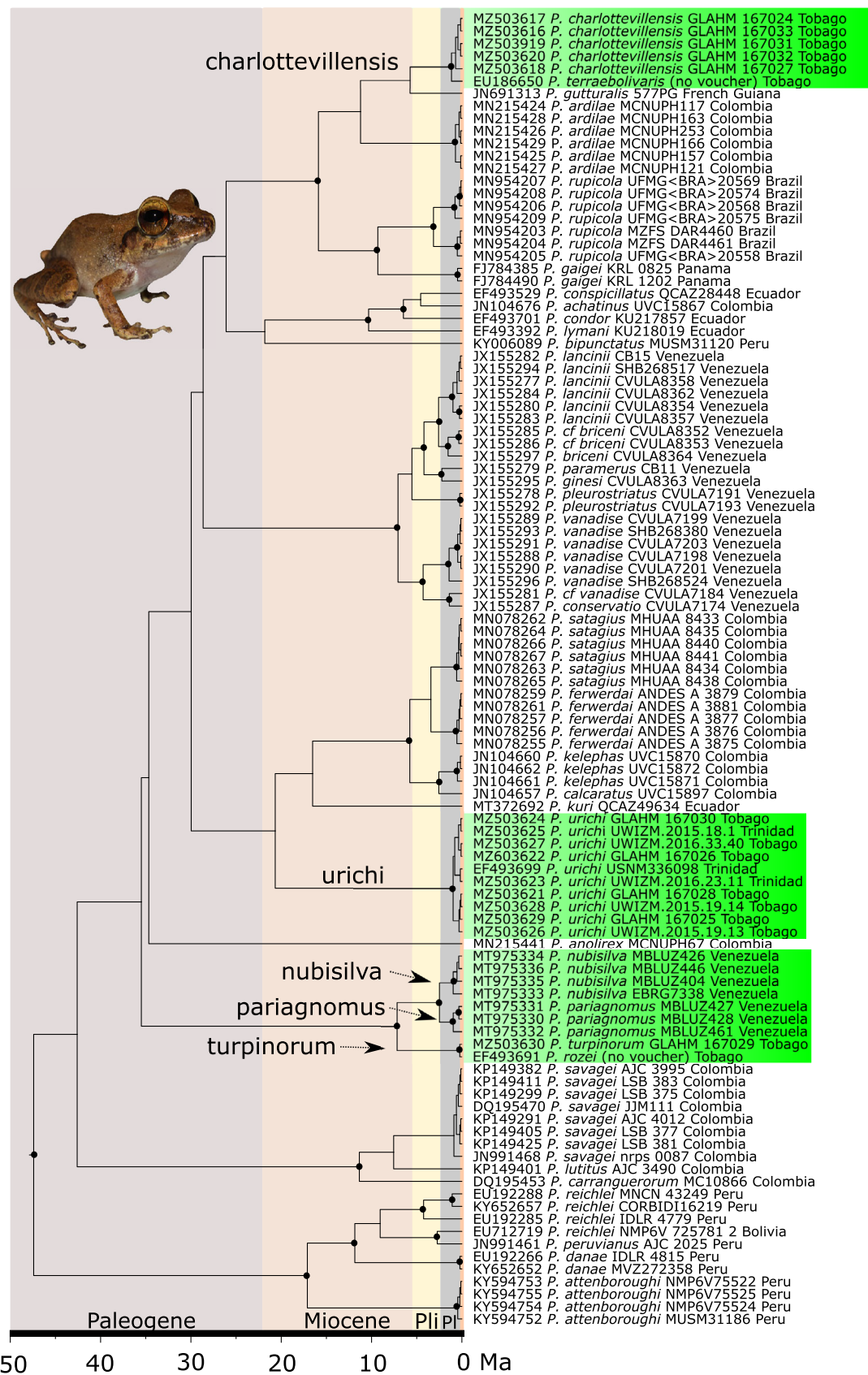
FIGURE 5 Bayesian time tree as inferred by BEAST 2.6.3 for the dataset of 16S rRNA of all *Pristimantis* species matched in the GenBank blasts. Black circles on nodes are posterior probabilities PP ≥0.98. The first orange line in tree terminals represents the Holocene. All names, vouchers and localities follow the GenBank titles of the sequences. The frog is *Pristimantis charlottevillensis* (photo credit: John C. Murphy). Pl, Pleistocene; Pli, Pliocene [Color figure can be viewed at wileyonlinelibrary.com]

3.5 | Surveys: *Pristimantis pariagnomus* (Kaiser et al., 2015)

This species was observed two times in June 2014 and July 2016 by one of us (GR) within the humid cloud forest leaf litter in the southern versant of Cerro Humo at 1000–1200 m elevation, between 13:00 and 15:00. The forest in the area presented a moderate closed canopy, and the soil was covered by abundant organic matter in decomposition.

3.6 | Microhabitat choice

In 2018, the positions of 17 *P. urichi*, 32 *P. charlottevillensis* and 14 *P. turpinorum* adults were determined in the MRFR or the Cambleton trail in Tobago. Most *P. urichi* were located on understorey vegetation, often ferns, at a mean ($\pm$ SD) height of 45.2 ± 35.3 cm above the ground (maximum 120 cm), with only 18% of individuals found on the ground. Similarly, *P. charlottevillensis* were on vegetation at a mean height of 43.1 ± 26.5 (maximum 142) cm, with only 9% of individuals located on the ground. Overall, *P. turpinorum* were found at higher locations on ferns, small trees, and palm leaves at 74.4 ± 49.2 (maximum 150) cm above the ground, with no individuals on the ground. The perch height differences between *P. turpinorum* and the other two species were significantly different, but not those between *P. urichi* and *P. charlottevillensis* (Figure 4 and Tables S5 and S6).

3.7 | Genetic analyses

Locations and museum accession details for all 25 specimens used in this study are shown in Table S7. Two of the Tobago specimens identified in 2018 as *P. turpinorum* turned out to be *P. charlottevillensis* from the sequencing results. Figure 3 shows the sample locations for the Venezuelan specimens and the relative positions of the three locations surveyed for this study.

BLAST searches resulted in highly divergent matches. Uncorrected *p*-distances of the dataset revealed high genetic divergence between taxa, with approximately 15% divergence between species, with the exception of the Venezuelan species (*P. pariagnonus*, *P. nubisilva*) and *P. turpinorum*, with approximately 4% divergence (Table 3). Results from the PTP analyses suggest that *P. pariagnomus* and *P. nubisilva* are the same species (0.83% node support). Genetic divergences within species were low and surprisingly absent between Trinidad and Tobago's *P. urichi* populations.

3.8 | Phylogenetic relationships and divergence times

Within the studied *Pristimantis* species, we recovered three highly divergent clades (Figure 5): (1) *P. charlottevillensis*, (2) *P. urichi*, and (3) *P. turpinorum* + *P. pariagnomus* + *P. nubisilva*. The *P. charlottevillensis* clade dates to 1 Ma (95% highest posterior density [HPD], 0.24–2.5), showing a mean genetic divergence split to *P. gutturalis* from French Guiana at 5.3 Ma (95% HPD, 1.6–11). *Pristimantis urichi*'s clade dates to 0.84 Ma (95% HPD, 0.17–2.14) and is highly divergent to all other *Pristimantis* species. *Pristimantis turpinorum* is basal to the sister clade composed of *P. pariagnomus* + *P. nubisilva*. This clade dates to 6.41 Ma (95% HPD, 1.8–14.7). The *P. pariagnomus* + *P. nubisilva* split dates to 2.22 Ma (95% HPD, 0.61–4.86), with the *P. pariagnomus* clade dating to 0.8 Ma (95% HPD, 0.11–2.08) and *P. nubisilva* to 0.81 Ma (95% HPD, 0.15–2.03). Based on this analysis, we estimated a median clock rate of 6×10^{-3} (95% HPD, 2.6×10^{-3}–1×10^{-2}) substitutions per site per year for this mitochondrial 16S rRNA fragment.

4 | DISCUSSION

4.1 | Species surveys

This study highlights the importance of conducting field surveys in the region of northern Venezuela and Trinidad and Tobago to assess and estimate the conservation status of some of its amphibians (Lehtinen et al., 2016). The initial part of this paper presents results from surveys made from 2012 to 2020 for the three *Pristimantis* species endemic to Trinidad and Tobago. Surveys comprised audio and visual encounters along forest trail transects, allowing estimations of numbers, and road surveys which recorded presence/absence. The work was labor intensive and only possible because of the willing participation of many volunteers, students, and members of the public participating in Bioblitzes. Others have commented on the value of such "citizen science" activities for the documentation of biodiversity and threats to it (Ellwood et al., 2017). A problem with such work can be a lack of consistency when multiple observers contribute over several years. We did our best to deal with this by pre-survey training, and having the same observers as much as possible.

Coverage of the northern part of Trinidad has been extensive, but we have limited data from the south of the island, and expect that the area of occurrence of *P. urichi* will be extended once more work is possible in the forested and less populated areas of that region.

In Tobago, we have survey data documenting the absence of the three *Pristimantis* species in the deforested agricultural and urban areas of the southwest, and evidence of presence over the forested northeast. However, large areas of that part of the island are unsurveyed because of the inaccessibility of much of the MRFR. Overall, the three species occur in forested regions, and are absent from urbanized areas and agricultural land.

The abundance data we present are not intended as actual population estimates, but more as numbers that can be compared with future data (there are no earlier estimates) in order to search for trends. This is because we cannot tell what proportion of the adult male population is calling on any particular night: this is why we use the maximum number heard on any of the survey nights, rather than the average, since it should more closely approach the actual number present. Lawrence (2017) has demonstrated the problem in using call counts to estimate population sizes in tropical frogs, especially where there is territoriality, a factor not established for our species. For *P. urichi*, the Trinidad counts were reasonably consistent year to year, with no obvious trends; Tobago numbers were somewhat lower than for Trinidad, and more variable, with the lowest counts in the most recently surveyed years, possibly a concerning trend. In Tobago, *P. charlottevillensis* counts were generally higher than for *P. urichi* except at the two Main Ridge sites, Gilpin Trace and Spring Trail. Again, there was high variability from year to year with the lowest counts most recent. As noted in the results, we do not have reliable counts for *P. turpinorum*, but do have some evidence on presence/absence.

In Tobago's Main Ridge Forest, the three species can be found along the same trails. There is some evidence for niche separation based on calls, with *P. turpinorum* found higher in vegetation, and calling late afternoon, while *P. urichi* calls in the early evening from intermediate heights, and *P. charlottevillensis* later and from the lowest heights. However, more work is needed to establish ecological differences between the three species, and reliable ways to distinguish small individuals of *P. charlottevillensis* and *P. turpinorum* need to be established.

4.2 | Conservation status

The IUCN's recent revision of the Red List of Threatened Species for amphibians has altered the assessment of *P. urichi* from EN to LC, *P. charlottevillensis* from LC to VU and *P. turpinorum* from VU to Data Deficient (DD; IUCN SSC Amphibian Specialist Group, 2020a, 2020b, 2020c) largely on the basis of the data presented in this paper. In our view, *P. urichi* was previously assessed as EN because of a lack of data from Trinidad; the

improvement in its assessed status does not imply improved numbers or distribution, or reduced threats in the field. However, our results show that this is an abundant and widespread species in Trinidad, with an additional substantial population in Tobago. Similarly, the altered status for *P. charlottevillensis* fully recognizes the species' very limited distribution, rather than any recent change in numbers or threats. The revised assessment of DD for *P. turpinorum* recognizes that, even almost 40 years after Hardy's (1982) original description, we know very little about this species. It is our hope that the baseline data we present here will form the basis for regular future updates which will provide reliable estimates of the status of these three species. In addition, the assessment of *P. turpinorum* as DD should provide a stimulus for focused studies on this species. The difficulties so far experienced in monitoring this species suggest that new techniques may be needed, such as environmental DNA analysis or automated audio monitoring.

In Trinidad and Tobago, habitat alteration is a major threat to all amphibians (Auguste et al., 2022). Although all three species of *Pristimantis* in Trinidad and Tobago do occur within protected areas (Auguste, 2019; Auguste & Hailey, 2018), there is a lack of enforcement and patrols are limited because of inadequate resources for biodiversity monitoring. The fact that all three of Trinidad and Tobago's *Pristimantis* species occur in Tobago's MRFR, highlights the importance of this protected area. In 2020, it was designated as part of a UNESCO Man and Biosphere Reserve, the first for the country. Indeed, a photograph of *P. turpinorum* was used as part of the proposal to designate northeast Tobago as a UNESCO site in order to highlight endemic species on the island (Environmental Research Institute Charlotteville, personal communication).

4.3 | Genetic analyses

The second part of this paper presents molecular data from the three Trinidad and Tobago *Pristimantis* species, as well as two *Pristimantis* species from north-eastern Venezuela, in order to supplement the sparse dataset available on GenBank from previous investigations. Our aim is to discuss possible speciation and colonization events which may have led to the current distribution of species.

The presence of endemic *Pristimantis* species in Trinidad and Tobago suggests that island populations diverged from those on the mainland, eventually leading to speciation. The continental island of Trinidad has six endemic reptile and amphibian species and Tobago has eight endemics, of which only three are frogs (Rivas et al., 2021). The lower number of endemic species on Trinidad than on the much smaller island of Tobago is likely due to its past geology.

Trinidad's Northern Range was a connected north-eastern extension of the Paria Peninsula Range until the Pliocene when subsidence associated with the Gulf of Paria separated these two landmasses (Erlich & Barrett, 1990; Liddle, 1946).

Interestingly, some of the Tobago endemics seem to be more closely related to species of the Venezuelan coastal montane complex than to Trinidad. Some examples include Tobago's dendrobatid *Mannophryne olmonae* (Manzanilla et al., 2009), the Tobago glass frog, *Hyalinobatrachium orientale* (Jowers et al., 2015), the Tobago stream snake, *Erythrolamprus pseudoreginae* (Murphy et al., 2019) and the fossorial Hallowell's ground snake *Atractus fuliginosus* (Jowers et al., 2021). Despite some of these species having members of the same genus in Trinidad, they are not closely related to them, suggesting that colonization of Tobago did not happen through a stepping-stone process from Trinidad. Until the work reported here, no other study had found that some mainland *Pristimantis* species are closely related to some insular species. Four scenarios support the presence of closely related species between northern Venezuela and Tobago: (1) when Tobago's position was much further west of its current position (Jowers et al., 2015), shallow marine depths (<50 m) in the region and sea-level falls during the Pliocene (Miller et al., 2005) would have facilitated land-bridge connections between northern Venezuela and Tobago (Jowers et al., 2015); (2) through stepping-stone connection of several Pliocene islands that once existed between Tobago and the mainland and Trinidad, and all of the islands were connected by very shallow and likely emergent coastal systems (Jowers et al., 2021; Samlal, 2016); (3) over-water dispersal with frogs rafting on vegetation, possibly from river mouths or an estuary such as the Orinoco Delta (Camargo et al., 2006; Yanek et al., 2006); and (4) a stepping-stone colonization from Trinidad with subsequent extinctions locally, which remains more unlikely due to the much larger area of suitable habitat present in Trinidad's Northern Range. Further sampling and sequencing of other loci are required to run accurate phylogenetic interpretations and models. The genetic distances detected between *P. pariagnomus* and *P. nubisilva* with *P. turpinorum* (~4%) do suggest recent land connections between the Paria Peninsula and Tobago, probably as far back as the Late Miocene. The time tree (Figure 5) suggests that the presence of *P. turpinorum* in Tobago is likely the result of a vicariant event roughly at the time of the detachment of Trinidad from northern Venezuela circa 4–5 Ma, followed by a stepping-stone colonization event to Tobago with extinctions of intermediate populations in Trinidad thereafter. A different scenario implies a direct colonization event from the mainland to Tobago, as is the case for other species such as the glass frog *Hyalinobatrachium orientalis*, which is only present in Tobago and northern Venezuela (Jowers et al., 2015) or the

Hallowell's ground snake *A. fuliginosus*, which is present in Tobago and central-western Venezuela (Jowers et al., 2021). Under this last scenario, Tobago would have been positioned several kilometers to the west of its current position and it could had been easier to colonize directly from the mainland, explaining the absence of the species in Trinidad, as has already been proposed for another anuran (Jowers et al., 2015). Similarly, the split between *P. charlottevillensis* and *P. gutturalis* from French Guiana corresponds to 5.3 Ma, suggesting a vicariant event between the mainland and Tobago.

The lack of genetic differentiation between populations of *P. urichi* on the islands of Trinidad and Tobago, suggests recent connectivity between the islands, possibly through Pleistocene land-bridges (Murphy, 1997) and/or human introductions. Lack of closely related species to *P. urichi* prevents us from proposing further hypotheses on the timing of colonization events in the region for this species. Future sequencing of other species of *Pristimantis* (*Pristimantis longicorpus*, *Pristimantis geminus*, *Pristimantis hoogmoedi*) from Venezuela's Paria Peninsula will be key to fully assess the phylogenetic relationships in the region, as well as genetic data from species that inhabit the Turimiquire Massif located further south-east. In this location, *Pristimantis turimiquirensis* and at least one undescribed species are present (Rivas et al., 2021). Similarly, in the central coastal range (Cordillera de la Costa Central), there are several species (*Pristimantis anotis*, *Pristimantis bicumulus*, *Pristimantis incertus*, *Pristimantis reticulatus*, *Pristimantis rozei*, *Pristimantis riveroi*, *Pristimantis stenodiscus*) that could bring light to key evolutionary relationships, although some of them are only known from their type specimens and their taxonomic status is unclear (i.e., *P. rozei*).

We found low genetic divergence between two species of *Pristimantis* from the Paria Peninsula, which seem to correspond to *P. nubisilva* and *P. pariagnomus*. These two taxa show consistent morphological differences, as well as apparent microhabitat segregation (this work, Kaiser et al., 2015) and different altitude preferences, perhaps with some parapatric overlap. These biological parameters could be useful to validate them as two distinct biological units. Nevertheless, the results from the PTP analyses suggest that these taxa might actually constitute populations of the same species. Further surveys in the region, as well as further sequencing will be needed to further clarify the taxonomy of these species.

5 | CONCLUSION

This study shows the importance of conducting field work for conservation status assessments, revealing distributional ranges and areas where future surveys should

be conducted, as well as giving key estimates of abundance. In the tropics, where resources are often limited, citizen science methods can facilitate this effort. In addition, this study indicates that increased molecular sampling, especially from the mainland, could reveal uncommon phylogeographic patterns in the region and contribute to our understanding of colonization processes on the islands.

AUTHOR CONTRIBUTIONS
Mark Greener: led the team which carried out *P. urichi* surveys in Trinidad. Lynsey Harper, Trudie Marshall, Robyn Thomson, Isabel Byrne, Ciara Loughrey, Leah Graham and Will McGhee led the teams which surveyed Tobago for *Pristimantis* species. In addition, Leah collected Tobago specimens for DNA work. John Murphy collected *P. urichi* specimens in Trinidad and helped train the teams in Tobago on species recognition. He also co-led the Bioblitzes in Trinidad and Tobago. Renoir Auguste co-led the Bioblitzes and carried out Tobago survey work for *P. turpinorum*. Cammy Beyts created the maps and did the statistical analysis of perch heights. Gilson Rivas collected specimens and contributed results from Venezuela. Michael Jowers carried out the molecular analyses and wrote the sections based on them and coordinated the manuscript preparation. Santiago Sánchez-Ramírez gathered sequences from GenBank, performed the molecular dating analysis in BEAST and wrote sections of the manuscript. Roger Downie coordinated the project, assembled the survey data and wrote the sections based on them.

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REFERENCES
Auguste, R. J. (2019). Herpetofaunal checklist for six pilot protected areas in Trinidad and Tobago. *Herpetology Notes*, *12*, 577–585.

Auguste, R. J., Downie, J. R., Jowers, M. J., Lehtinen, R. L., Mohammed, R. S., & Murphy, J. C. (2022). Amphibians and their conservation in Trinidad and Tobago. In H. Heatwole & N. Lopez-Rios (Eds.), *Amphibian biology* (Vol. 9/5, Chapter 25, In press). Exeter: Pelagic Publishing.

Auguste, R. J., & Hailey, A. (2018). Diversity and species composition of amphibians of the Aripo savannas scientific reserve, Trinidad, W.I. *Journal of Herpetology*, *52*, 86–93.

Boettger, O. (1894). Hylodes urichi, Bttgr, n.sp. In R. Mole & F.W. Urich (Eds.), *A preliminary list of the reptiles and batrachians of the island of Trinidad* (Vol. 2, pp. 77-90). Journal of the Trinidad Field Naturalists' Club.

Bouckaert, R., & Drummond, A. (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology*, *17*, 42. https://doi.org/10.1186/s12862-017-0890-6

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., de Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., ... Stadler, T. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Camargo, A., de Sa, R. O., & Heyer, W. R. (2006). Phylogenetic analyses of mtDNA sequences reveal three cryptic lineages in the widespread neotropical frog *Leptodactylus fuscus* (Schneider, 1799) (Anura, Leptodactylidae). *Biological Journal of the Linnean Society*, *87*, 325–341.

Downie, J. R., Greener, M. S., Geerah, P. A., & Auguste, R. J. (2017). Further expansion of the range of the frog *Eleutherodactylus johnstonei* (Anura: Eleutherodactylidae) in

Trinidad and Tobago, with a note on reproduction. *Living World: Journal of the Trinidad and Tobago Field Naturalists' Club*, 2017, 58–60.

Ellwood, E. R., Crimmins, T. M., & Miller-Rushing, A. J. (2017). Citizen science and conservation: Recommendations for a rapidly moving field. *Biological Conservation*, 208, 1–4.

Erlich, R. N., & Barrett, S. F. (1990). Cenozoic plate tectonic history of the northern Venezuela-Trinidad area. *Tectonics*, 9, 161–184.

Frost, D. R. (2021). *Amphibian species of the world: An online reference*. Version 6.0. Cenozoic Plate tectonic history of the northern Venezuela-Trinidad Area. American Museum of Natural History. Electronic database accessible at http://research.amnh.org/herpetology/amphibia/index.html

Funk, W. C., Caminer, M., & Ron, S. R. (2012). High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B*, 279, 1806–1814.

Google. (2020). *Royal Roads University*. http://mto.google.com/vt/lyrs=y&ht=en&x={x}&y={y}&z={z}

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4. A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27, 221–224.

Hardy, J. D., Jr. (1982). Biogeography of Tobago, West Indies, with special reference to amphibians and reptiles: A review. *Bulletin of the Maryland Herpetological Society*, 18(2), 38–143.

Hardy, J. D., Jr. (2001). A new frog of the genus *Eleutherodactylus* from the Island of Tobago, West Indies. *Bulletin of the Maryland Herpetological Society*, 37, 25–31.

Hedges, S. B., Duellman, W. E., & Heinicke, M. P. (2008). New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, 1737, 1–182.

Hedges, S. B., Marin, J., Suleski, M., Paymer, M., & Kumar, S. (2015). Tree of life reveals clock-like speciation and diversification. *Molecular Biology and Evolution*, 32(4), 835–845.

Heinicke, M. P., Duellman, W. E., & Hedges, S. B. (2007). Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 10092–10097.

IUCN SSC Amphibian Specialist Group. (2013). *Pristimantis charlottevillensis*. The IUCN Red List of Threatened Species. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T56507A174485078.en

IUCN SSC Amphibian Specialist Group. (2020a). *Pristimantis urichi*. The IUCN Red List of Threatened Species 2020. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T57027A175202942.en

IUCN SSC Amphibian Specialist Group. (2020b). *Pristimantis turpinorum*. The IUCN Red List of Threatened Species 2020. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T57018A175201064.en

IUCN SSC Amphibian Specialist Group. (2020c). *Pristimantis charlottevillensis*. The IUCN Red List of Threatened Species 2020. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T56507A174485078.en

IUCN SSC Amphibian Specialist Group (2020d). *Flectonotus fitzgeraldi*. The IUCN Red List of Threatened Species 2020. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T55316A109535175.en

Jowers, M. J., Lehtinen, R. M., Downie, R. J., Georgiadis, A. P., & Murphy, J. C. (2015). Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): Evidence for Pliocene connections between mainland Venezuela and the Island of Tobago. *Mitochondrial DNA*, 26, 613–618.

Jowers, M. J., Schargel, W. E., Muñoz-Mérida, A., Sánchez-Ramírez, S., Weber, J., Faria, J. F., Harris, D. J., & Murphy, J. C. (2021). The enigmatic biogeography of Tobago's marooned relics: The case study of a fossorial snake. *Journal of Zoological Systematics and Evolutionary Research*, 59, 1382–1389.

Kaiser, H., Barrio-Amorós, C. L., Rivas, G. A., Steinlein, C., & Schmid, M. (2015). Five new species of *Pristimantis* (Anura: Strabomantidae) from the coastal cloud forest of the Península de Paria, Venezuela. *Journal of Threatened Taxa*, 7, 7047–7088.

Kaiser, H., Dwyer, C. M., Feichtinger, W., & Schmid, M. (1995). A new species of *Eleutherodactylus* (Anura: Leptodactylidae) from Tobago, West Indies, and its morphometric and cytogenetic characterization. *Herpetological Natural History*, 3, 151–163.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059–3066.

Kenny, J. S. (1969). Amphibia of Trinidad. *Studies in the Fauna of Curaçao and other Caribbean Islands*, 29, 1–78.

Kumar, S., Stecher, G., & Tamura, H. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.

Lawrence, J. P. (2017). Ineffectiveness of call surveys for estimating population size in a widespread neotropical frog, *Oophaga pumilio*. *Journal of Herpetology*, 51, 52–57.

Lehtinen, R. M., Calkins, T. L., Novick, A. M., & McQuigg, J. L. (2016). Reassessing the conservation status of an Island endemic frog. *Journal of Herpetology*, 50, 249–255.

Liddle, R. A. (1946). *The geology of Venezuela and Trinidad*. Paleontological Research Institution.

Manzanilla, J., La Marca, E., & García-París, M. (2009). Phylogenetic patterns of diversification in a clade of Neotropical frogs (Anura: Aromobatidae: *Mannophryne*). *Biological Journal of the Linnean Society*, 97, 185–199.

Miller, K. G., Kominz, M. A., Browning, J. V., Wright, J. D., Mountain, G. S., Katz, M. E., Sugarman, P. J., Cramer, B. S., Christie-Blick, N., & Pekar, S. F. (2005). The Phanerozoic record of global sea-level change. *Science*, 310, 1293–1298.

Murphy, J. C. (1997). *Amphibians and reptiles of Trinidad and Tobago*. Krieger Publishing Company.

Murphy, J. C., Braswell, A. L., Charles, S. P., Auguste, R. J., Rivas, G. A., Borzee, A., Lehtinen, R. M., & Jowers, M. J. (2019). A new species of *Erythrolamprus* from the oceanic Island of Tobago (Squamata, Dipsadidae). *Zookeys*, 817, 131–157.

Murphy, J. C., Downie, J. R., Smith, J. M., Livingstone, S. R., Mohammed, R. S., Lehtinen, R. M., Eyre, M., Sewlal, J.-A., Noriega, N., Casper, G. S., Anton, T., Rutherford, M. G., Braswell, A. L., & Jowers, M. J. (2018). *A field guide to the amphibians and reptiles of Trinidad & Tobago*. Trinidad & Tobago Field Naturalists Club.

Palumbi, S. R., Martin, A. P., Romano, S., Mcmillan, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR*. University of Hawaii.

QGIS Development Team. (2018). *QGIS geographical information system*. Open Source Geospatial Foundation Project.

Read, M. (1990). *Frogs of Trinidad, Tobago and the Lesser Antilles, a guide to their calls*. Privately published.

Ritchie, A. M., Lo, N., & Ho, S. H. W. (2017). The impact of the tree prior on molecular dating of data sets containing a mixture of inter- and intraspecies sampling. *Systematic Biology*, *66*(3), 413–425. https://doi.org/10.1093/sysbio/syw095

Rivas, G. A., Lasso-Alcalá, O. M., Rodríguez-Olarte, D., De Freitas, M., Murphy, J. C., Pizzigalli, C., Weber, J. C., de Verteuil, L., & Jowers, M. J. (2021). Biogeographical patterns of amphibians and reptiles in the northernmost coastal montane complex of South America. *PLoS One*, *16*, e0246829. https://doi.org/10.1371/journal.pone.0246829

Rivera-Correa, M., Jimenez-Rivillas, C., & Daza, J. M. (2017). Phylogenetic analysis of the Neotropical *Pristimantis leptolophus* species group (Anura: Craugastoridae): Molecular approach and description of a new polymorphic species. *Zootaxa*, *4242*(2), 313–343.

Samlal, S. (2016). *Structural and depositional history of the early Pliocene M0 reservoir of the north coast marine area, Trinidad and Tobago* (M.Sc. thesis). University of Leeds.

Smith, J., Jowers, M. J., Auguste, R. J., Hoskisson, P., Beyts, C., Muir, G., Greener, M. S., Thornham, D., Byrne, I., Lehtinen, R., Eyre, M., Rutherford, M. G., Murphy, J. C., De Freitas, M., Rivas, G. A., & Downie, J. R. (2021). The distribution and conservation status of the dwarf marsupial frog (*Flectonotus fitzgeraldi*) in Trinidad, Tobago and Venezuela. *Amphibian and Reptile Conservation*, *15*(1), 36–56.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*, 1783–1786.

Yanek, K., Heyer, W. R., & De Sa, R. O. (2006). Genetic resolution of the enigmatic Lesser Antillean distribution of the frog *Leptodactylus validus* (Anura, Leptodactylidae). *South American Journal of Herpetology*, *1*, 192–201.

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation with applications to phylogenetic placements. *Bioinformatics*, *29*(22), 2869–2876.

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