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Recent evolutionary history of Lost World endemics: Population genetics, species delimitation, and phylogeography of sky-island treefrogs

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ABSTRACT

The tepuis of South America are massive flattop mountains with cliffs up to 1000 m and summits up to 3100 m. Tepuis hold enormous endemicity levels, but little is known about the origins of the endemic flora and fauna. Recently diverged lineages offer the possibility of understanding the origins of summit endemicity by examining population dynamics and dispersal. We examine species delimitation, clade relationships, and demographic patterns of three recently diverged lineages of *Tepuihyla*, an endemic treefrog clade. These three lineages represent two currently recognized species, *T. edelcae* and *T. rodriguezi*. Given the low divergences in both nuclear and mitochondrial genes among lineages, we find unexpectedly high numbers of unique nuclear haplotypes and moderate levels of lineage sorting. We also find support from multiple analyses for a cryptic, undescribed summit species within *T. edelcae*. We suggest that the genetic and distribution patterns of the four most recently diverged *Tepuihyla* lineages support a concurrent speciation event during the Pliocene, and suggest a biogeographic hypothesis in which a widespread climatic change made mid- and low-elevation habitat unsuitable for the common ancestor within the timeframe of their divergence.

1. Introduction

Montane regions harbor many of the global hotspots of diversity, yet the extent of diversity is not fully appreciated because many regions are inaccessible. The flattop mountains (tepuis) of northern South America form an endemicity hotspot that remains largely unexplored. Researchers traditionally believed that the summit biota of the tepuis—the "Lost World" that inspired Conan Doyle's book of the same name—has been isolated atop summits since their formation. However, molecular analyses indicate that the Lost World is not as isolated and "prehistoric" as popularly thought (Givnish et al., 1997; Rull, 2004; Kok et al., 2012; Salerno et al., 2012; Bonaccorso and Guayasamin, 2013), though much remains to be done to elucidate the evolutionary history of this fascinating sky-island ecosystem and the causes of its enormous endemicity.

The tepuis are remnants of the Precambrian Guiana Shield plateau, which encompasses a large area of South America east of the Andes and north of the Amazon River basin. These sandstone table mountains were formed approximately 60–90 mya after many cycles of erosion of the Guiana Shield plateau, starting around 300 mya. These remnants form hundreds of sky-islands that reach up to 3000 m, with walls up to 1000 m high. Hundreds of kilometers of drastically different lowlands separate most summits (Briceño et al., 1990; Briceño and Schubert, 1990; Gibbs and Barron, 1993). Thus, tepuis form a discontinuous ecosystem of sky-islands called Pantepui (Mayr and Phelps, 1955; Huber, 1988), similar to yet arguably much more extreme in topography than other well-known sky-island systems such as the Rocky Mountains and the Western Ghats (DeChaine and Martin, 2005; Smith and Farrell, 2005; Robin et al., 2010).

The Pantepui holds enormous endemicity of many taxa, particularly frogs (~77%; McDiarmid and Donnelly, 2005) and plants (~60%; Huber, 1988; Berry and Riina, 2005). Traditionally, the high endemism has been explained by the Lost World Hypothesis (Chapman, 1931; Maguire, 1970; Rull, 2004; McDiarmid and Donnelly, 2005). However, accumulating evidence strongly

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supports divergene times within the last 35–2 my, long after tepuis were formed (Mayr and Phelps, 1967; Givnish et al., 1997, 2011; Rull, 2004; Rull and Nogué, 2007; Salerno et al., 2012; Kok et al., 2012; Bonaccorso and Guayasamin, 2013).

Tepuihyla is a treefrog group of seven endemic Pantepui species. Recent phylogenetic analyses led to the addition of two species, *T. exophthalmus* and *T. warreni* (Salerno et al., 2012; Jungfer et al., 2013). These two species are the most deeply diverged within *Tepuihyla*, with the divergence of *T. exophthalmus* estimated to be 15 mya (Salerno et al., 2012); both are found in the mid-elevations of the eastern Pantepui (Fig. 1). The most recent divergences, occurring within the last 2–5 mya, represent four lineages: *T. rodriguezi* (mid-elevations of the eastern Pantepui), *T. aecii* (highlands of Cerro Duida in western Pantepui), *T. edelcae* Auyán (summit of Auyán-tepui in eastern Pantepui), and *T. edelcae* Chimantá (summits of several tepuis atop the Chimantá massif in eastern Pantepui; Salerno et al., 2012).

Several studies that estimated phylogenetic relationships for members of this group (Kok et al., 2012; Salerno et al., 2012; Jungfer et al., 2013) suggested that *Tepuihyla edelcae* may represent two allopatric species that are not sister-groups. Support for among-clade relationships was low in all studies, and sampling was not sufficient to test whether the apparent paraphyly of *T. edelcae* was due to sampling error, or if *T. edelcae* indeed represents two evolutionary lineages. Greater sampling of loci and use of coalescent species-delimitation methods should improve the estimates of relationships among these recently diverged lineages and clarify the evolutionary history and systematics of these Pantepui endemics.

To clarify the biogeographic history and speciation within *Tepuihyla*, we focus on three of the most recently diverged *Tepuihyla* lineages: *T. edelcae* Auyán, *T. edelcae* Chimantá, and *T. rodriguezi*. Using increased sampling of loci and taxa, we performed Bayesian concatenated-gene analyses, coalescent gene-tree reconstructions, and species-delimitation analyses to elucidate species relationships. Population genetic analyses were used to infer recent demographic history and to evaluate concordance of patterns across loci within the biogeographic context of the tepui landscape.

2. Materials and methods

2.1. Genetic samples and sequences

The ingroup includes 49 samples within *Tepuihyla*. The samples represent four recognized *Tepuihyla* species: 14 samples of *Tepuihyla edelcae* Auyán from a single locality (southern Auyán-tepui summit), 19 samples of *T. edelcae* Chimantá from three tepui summits on the Chimantá massif (six from Eruoda-tepui, six from Abakapá-tepui, and seven from Churí-tepui, all accessed by helicopter), 12 samples of *T. rodriguezi* from several localities in low and mid-elevations of Venezuela and Guyana, a single *T. aecii* from Cerro Duida, and three *T. exophthalmus* from two lowland localities.

Tepuihyla rodriguezi includes the recently synonymized names *Tepuihyla galani* and *Tepuihyla talbergae* (Jüngfer et al., 2013). The species *T. warreni*, which is outside our focal group, was excluded because of the paucity of GenBank data. The outgroup consists of four species of the sister-taxon *Osteocephalus* (*O. leprieurii, O. taurinus, O. deridens, and O. planiceps*).

We sequenced two mitochondrial segments (*12S* and *16S* ribosomal rDNA genes, 1170 bp; *ND1*, 1160 bp) and three nuclear loci (*POMC*, 480 bp; *RAG-1*, 460 bp; *Rhodopsin*; 835 bp). The combined dataset for mitochondrial and nuclear sequences included 4105 bp. The GenBank accession numbers for new and previously published sequences are provided in Appendix.

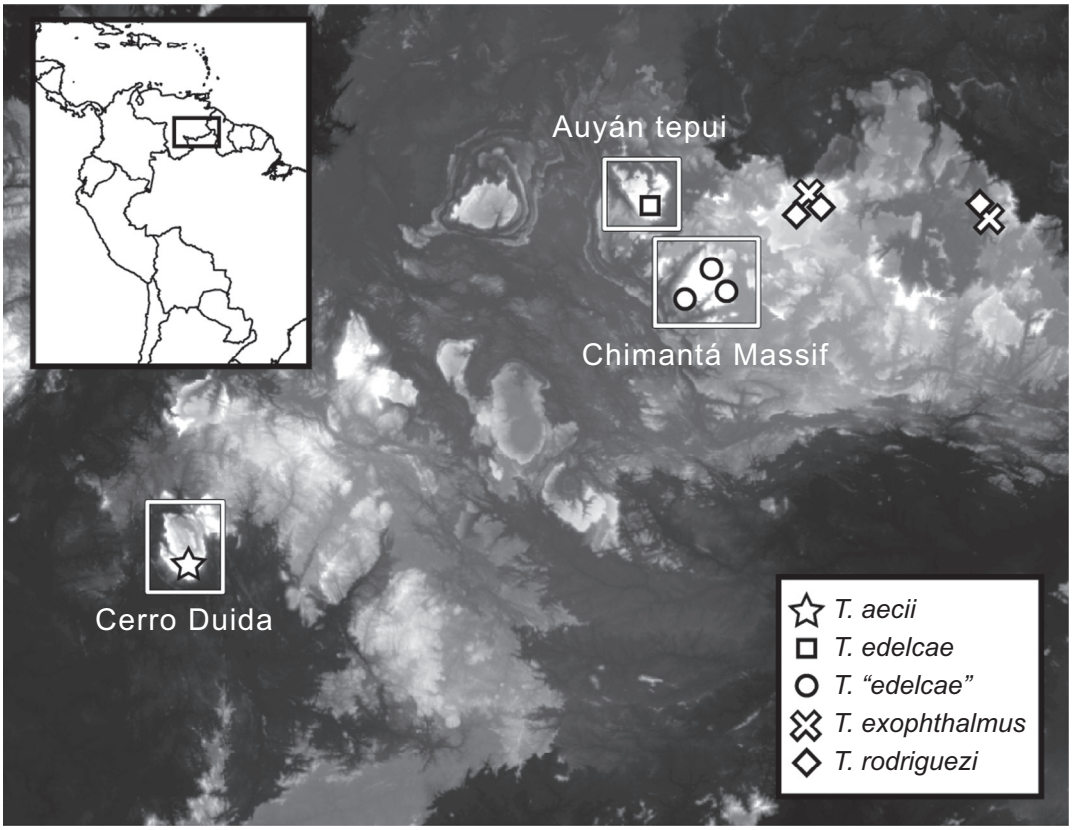

Fig. 1. Map of southeastern Venezuela, showing locations of genetic samples of *Tepuihyla*.

The protocols for DNA extraction, PCR amplification (including primers), and sequencing for *12S* and *POMC* are identical to those of Salerno et al. (2012). The thermocycler protocol for *RAG-1* and *Rhodopsin* were the same as for *POMC* (Salerno et al., 2012), and the protocol for *ND1* was the same as for *12S–16S* (Salerno et al., 2012). *RAG-1* was amplified using the primers from Faivovich et al. (2005), and *ND1* was amplified using primers from Moen and Wiens (2009). *Rhodopsin* was amplified with the primers Rhod1U (5′-AACGGAACAGAAGGCCCAAACTT-3′) and Rhod 1L (5′-GCCAAAGCCATGATCCAGGTGA-3′; Pauly, 2008).

2.2. Gene-tree and species-tree estimation

We estimated a Bayesian tree with MrBayes v3.2.2 (Ronquist et al., 2012), using 10 million generations, four chains, two replicate runs, and a 10% burnin. We evaluated convergence and stationarity in Tracer v1.6 (Rambaut and Drummond, 2013) using ESS values >200 as the criterion. Given that *Tepuihyla* species have very low sequence divergence (Kok et al., 2012; Salerno et al., 2012), the coalescent method implemented in *BEAST (Heled and Drummond, 2010) is appropriate. This coalescent method estimates species trees, taking into account different gene trees as well as population demographic parameters. Unlinked parameters were used for all loci except for the two mitochondrial genes (*12S* and *ND1*), which were treated as having linked topologies. We used the MrBayes topology as the input for *a priori* assignment of clade membership for the *BEAST analysis. Using BEAST 2 (Bouckaert et al., 2014) we performed three independent runs of 120 million generations with a 10% burnin to reach appropriate ESS values (>200). We also evaluated the parameters using TRACER v1.6 to assess stationarity and convergence of runs. For the *BEAST and the MrBayes analyses, we estimated the best model of evolution and partitioning scheme in PartitionFinder (Lanfear et al., 2012) using the Akaike Information Criterion; we treated branch lengths as unlinked across partitions. The best partitioning scheme and models of evolution were: *12S* (GTR + G), *ND1* (GTR + I), 1st + 2nd positions of *POMC* (HKY + G), 3rd position of *POMC* (GTR + G), 1st + 2nd positions of *RAG-1* (HKY + G), 3rd position of *RAG-1* (K80 + I), intron of *Rhodopsin* (HKY + I), 1st + 2nd positions of Rhodopsin exon (HKY), 3rd position of Rhodopsin exon (HKY).

We calculated pairwise uncorrected genetic distances (p-distances) with Mega 5.0 (Tamura et al., 2011) for the two mitochondrial loci separately (*12S* and *ND1*) using only the four most recently diverged clades (*T. aecii, T. edelcae* Auyán, *T. edelcae* Chimantá, and *T. rodriguezi*).

2.3. Species delimitation analyses

Species delimitation analyses were performed using BP&P v2.1 (Rannala and Yang, 2003; Yang and Rannala, 2010), which accommodates the species phylogeny as well as incomplete lineage sorting due to ancestral polymorphism. BP&P requires an input topology of species relationships. Because there is uncertainty of the relationships among the most recently diverged *Tepuihyla* species, and because the species inferences can be extremely sensitive to incorrect input topologies (Leaché and Fujita, 2010), we eliminated *T. aecii*, which is represented by one sample. We input two general hypotheses: (1) *T. edelcae* Chimantá and *T. edelcae* Auyán as sister-groups, and (2) *T. edelcae* as non-monophyletic with *T. rodriguezi* as the sister-group of *T. edelcae* Chimantá (Fig. 4). *Tepuihyla exophthalmus* is the outgroup in both cases. We also performed seven-taxon analyses and four-taxon analyses (Fig. 4) to evaluate the effect of *a priori* partitioning of clades, and to examine if the separate summit localities of *T. edelcae* Chimantá and geographically distant populations of *T. rodriguezi* were supported as separate species. We used both speciation algorithms (0 and 1) with different combinations of priors to confirm stability across runs as suggested by the authors (Yang and Rannala, 2010). Thus, we ran algorithm 0 with three different ε priors (2, 5, 20) and algorithm 1 with four different combinations of α and m ($\alpha = 1, 2$; $m = 0.5, 2$). This was done for each input topology. We also performed analyses manipulating the gamma prior G for the population sizes (thetas) to assume either a small (2, 2000) or large (1, 10) ancestral population size. The gamma prior of the age of the root in the species trees (tau) was assumed to have a shallow divergence G (2, 2000). The combination of large ancestral population sizes and shallow divergences is assumed to be the most conservative, leading to a lower number of speciation events (Yang and Rannala, 2010; Leaché and Fujita, 2010). All analyses were performed using a burnin of 500 samples and a total run length of 10,000 samples. We used an ESS value of >200 to determine whether the Markov chains reached stationarity.

2.4. Population genetics

The population genetic analyses were performed for each of the three putative species: *T. edelcae* Auyán (14 individuals from one summit locality), *T. edelcae* Chimantá (19 individuals from three separate summits atop the Chimantá massif; Fig. 1), and *T. rodriguezi* (12 individuals of *T. rodriguezi* and the recently synonymized species *T. galani* and *T. talbergae*). *Tepuihyla aecii* was excluded since only one specimen was sampled.

To screen loci for appropriateness as markers, we examined recombination within the loci using the GARD algorithm (Kosakovsky Pond et al., 2006) in DataMonkey (Delport et al., 2010); no loci showed evidence of recombination. To infer demographic history, we estimated number of polymorphic sites, number of haplotypes, nucleotide diversity, and theta using Arlequin 3.5 (Excoffier and Lischer, 2010). We also tested for neutrality of markers using Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) in Arlequin. These commonly used tests assume neutrality, but significant results do not distinguish between non-neutrality and shifts in demographic parameters (Fu, 1997; Excoffier and Lischer, 2010).

Nuclear haplotypes were estimated in PHASE 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003). Because missing data affect the success of haplotype phasing and detection of identical sequences, we reduced all the individual gene alignments to have complete datasets for all loci, which included deleting characters (base pairs) as well as individual samples. No more than six individuals were eliminated from any single gene matrix. Identical haplotypes were eliminated in COLLAPSE 1.2 (Posada, 2004) in order to calculate haplotypes per population for input in Arlequin. We estimated haplotype networks using the minimum spanning network algorithm in Arlequin v3.5, and we used HapStar v0.7 (Teacher and Griffiths, 2011) to edit the network figures.

3. Results

3.1. Phylogenetic and species-tree reconstructions

The concatenated MrBayes phylogeny was generally consistent with previous estimates (Fig. 2). *Tepuihyla rodriguezi*, *T. edelcae* Auyán, and *T. edelcae* Chimantá were each recovered as monophyletic (BPP = 1). For ease of discussion, we call these the three "putative species." Relationships among the three putative species and *T. aecii* are poorly supported (BPP = 0.51 and 0.55). *Tepuihyla edelcae* is not recovered as a monophyletic group based on the most favored topology. However, support for non-monophyly of *T. edelcae* sensu lato (*T. edelcae* Chimantá + *T. rodriguezi*) is low (BPP = 0.55), and thus the hypothesis of monophyly for *T. edelcae* is not statistically rejected.

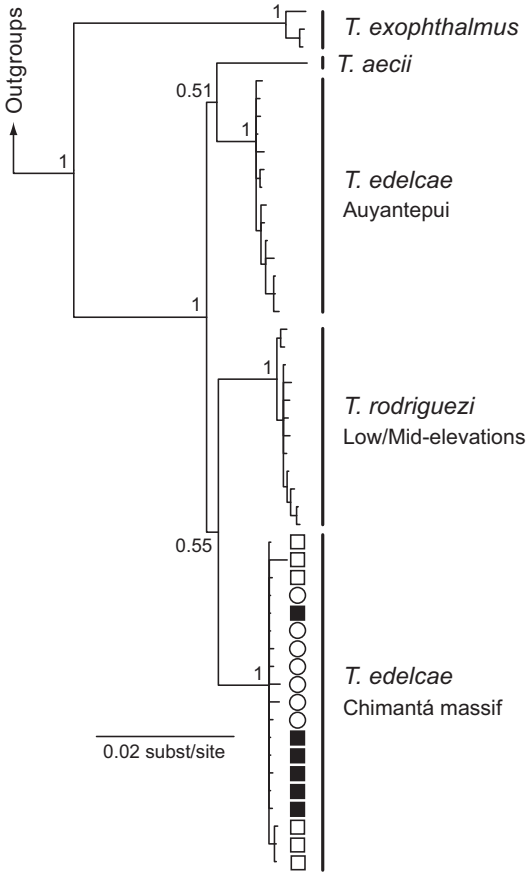

Fig. 2. Phylogenetic reconstruction of the concatenated dataset obtained in MrBayes. Bayesian posterior probabilities are shown at nodes. Shapes on terminal nodes represent localities atop Chimantá massif: Abakapá tepui (black squares), Churi tepui (circles), and Eruoda tepui (white squares). Outgroups not shown.

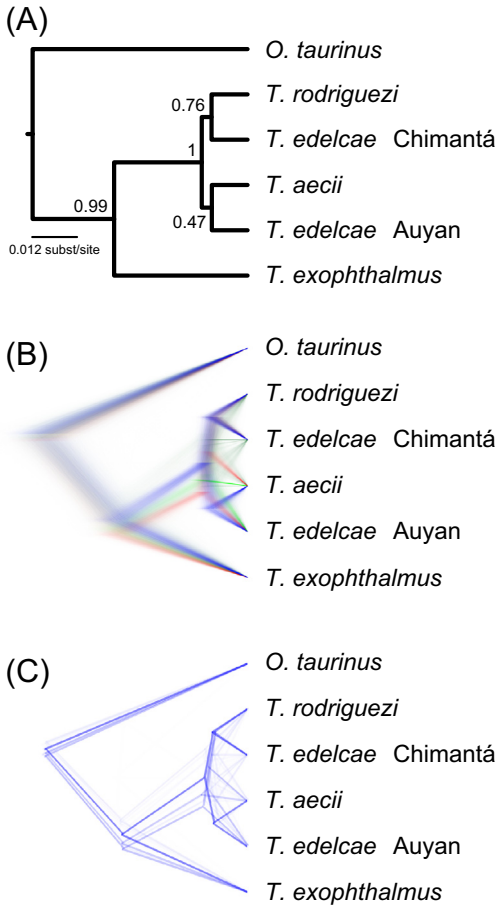

Fig. 3. Coalescent species reconstruction in *BEAST. (A) Species tree with posterior probabilities, (B) DensiTree visualization of all estimated gene trees, and (C) DensiTree visualization of possible consensus trees for the given loci.

The *BEAST species tree (Fig. 3A) recovered a sister-group relationship of *T. rodriguezi* and *T. edelcae* Chimantá with low support (BPP = 0.76), and a sister-group relationship for *T. aecii* + *T. edelcae* Auyán with low support (BPP = 0.47). *Tepuihyla exophthalmus* was always recovered (BPP = 0.99) as the sister lineage to all other *Tepuihyla*. All gene-tree reconstructions (Fig. 3B) show that even though some trees support monophyly of *T. edelcae*, this relationship is weak, and that the most common reconstruction of consensus trees (Fig. 3C) is *T. rodriguezi* + *T. edelcae* Chimantá.

All comparisons between pairs of individuals (uncorrected pairwise distances) from the three putative species were 1.3–3.1% for *12S* and between 1.7–3.2% for *ND1* (Table 2). The greatest among-clade distance among these three lineages (3.2% for *ND1*) was found for pairwise comparisons between *T. edelcae* Chimantá and *T. rodriguezi*. Distances with *T. aecii* and the three putative species were all between 1.7% and 2.5%. All pairwise distances within a putative species were less than 0.5%.

3.2. Species delimitation

With the exception of a single set of priors for algorithm 1 that did not reach appropriate ESS values for most parameters, all analyses for all three input topologies yielded 99–100% speciation probabilities for *T. edelcae* Auyán, *T. edelcae* Chimantá, and *T. rodriguezi*. This result was found regardless of the input topology of the four-taxon guide tree for the alternative hypotheses: monophyletic *T. edelcae*; (Fig. 4B) and non-monophyletic *T. edelcae* (Fig. 4C). Thus, we present a single representative result in Fig. 4, and an extensive list of results in Supplementary Figs. 1 and 2.

Speciation within *T. edelcae* Chimantá (among Eruoda, Abakapá, and Churí tepuis) and within *T. rodriguezi* (Venezuela and Guyana) was evaluated using the seven-taxon species tree (Fig. 4A). The speciation probabilities within Chimantá varied substantially with changing priors, particularly when changing the theta prior from G (1, 10) to G (2, 2000); they also varied depending on input topology (Supplementary Fig. 2). As expected, the most conservative prior combination for theta G (1, 10) and tau G (2, 2000) yielded the lowest speciation probabilities within Chimantá, regardless of speciation algorithm and priors. These speciation probabilities varied substantially between 0.37 and 0.99, and were always lower for the most recent speciation event within Chimantá, suggesting a strong influence of input topology. Speciation probabilities for *T. rodriguezi* were always moderate to very high (0.88–1.00) (see Fig. 4 and Supplementary Fig. 2).

3.3. Population genetics and demographics

The Chimantá population (all three tepuis combined) had the smallest number of polymorphic sites and number of haplotypes among the three nuclear loci (Table 1). The two mitochondrial genes had similar numbers of haplotypes and polymorphic sites for Chimantá and Auyán; *T. rodriguezi* had the fewest 12S haplotypes. All haplotypes found for each of the three putative species and for all five loci were unique to that population with the

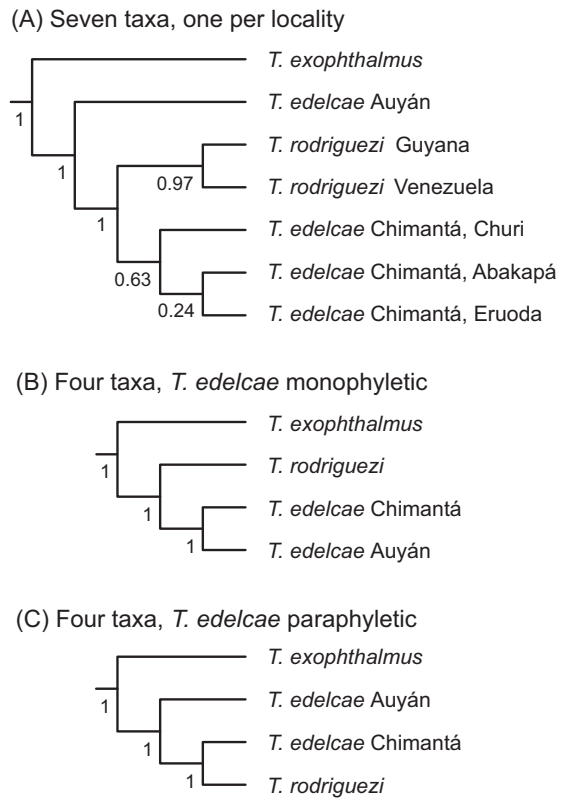

Fig. 4. Species delimitation analyses performed in BP&P. (A) Reconstruction given the seven-taxon input tree, and (B) and (C) are the four-taxon input tree, with the two alternative hypotheses (*Tepuihyla edelcae* as monophyletic and *T. edelcae* as non-monophyletic, respectively).

exception of a single *RAG-1* haplotype, which was the most common haplotype for all three populations. None of the individual tepuis atop the Chimantá massif had unique haplotypes (Fig. 5). When *T. rodriguezi* was analyzed as two populations (Venezuela and Guyana) some unique haplotypes were found in each locus.

Lineage sorting of haplotypes cannot be assessed using the Arlequin networks because these are not directly interpretable as trees. Thus, to assess lineage sorting we examined individual gene trees for *T. edelcae* Chimantá, *T. edelcae* Auyán, and *T. rodriguezi*. These trees (not shown) correspond well to the topology of the Arlequin haplotype trees. We tried various rooting positions among the three populations such that at least one population has completely sorted haplotypes (monophyly). At any rooting position the haplotypes are completely sorted among all three populations for the mitochondrial genes *ND1* and *16S*. The *POMC* tree can be rooted such that the *T. rodriguezi* haplotypes are sorted (form a monophyletic tree), but the Auyán haplotypes are paraphyletic with respect to those of Chimantá (incomplete sorting). In contrast, the *Rhodopsin* tree can be rooted so that the Auyán haplotypes are sorted, but the *T. rodriguezi* haplotypes are paraphyletic with respect to those of Chimantá. In the *RAG-1* tree, one allele is shared by several individuals in each population; therefore it is impossible to root the tree such that any population has completely sorted haplotypes. In summary, no nuclear gene trees can be rooted so that all three populations have sorted haplotypes.

Almost all test statistics were negative, but many were not statistically significant. Fu's Fs statistic showed several highly significant values, but the distribution of these across loci and populations showed no general pattern. Only two Tajima's D values were significant (*12S*; Table 1); *Rhodopsin* showed no significant results for Tajima's D or Fu's Fs.

4. Discussion

4.1. Phylogenetic reconstructions

The concatenated MrBayes tree was concordant in topology and degree of support with published phylogenies (Salerno et al., 2012; Kok et al., 2012; Jungfer et al., 2013). We found very strong support (BPP = 1) for three monophyletic putative species (Fig. 2): *T. edelcae* Chimantá, *T. edelcae* Auyán, and *T. rodriguezi*. The support for a clade comprising *T. edelcae* Chimantá + *T. rodriguezi* is low (BPP = 0.55). The *BEAST coalescent species reconstruction method, which is perhaps more appropriate given the shallow divergences, yielded results very similar those of the MrBayes tree (Figs. 2 and 3A). The DensiTree visualization and the consensus trees from *BEAST (Fig. 3B and C) suggest that the problematic reconstructions are due to the placement of *T. aecii* and *T. edelcae* Auyán. Even though the latter relationships are unresolved, the reconstruction of *T. rodriguezi* + *T. edelcae* Chimantá is the most commonly obtained, supporting the hypothesis that *T. edelcae* is paraphyletic regardless of other relationships in the group.

Increased sampling of loci and taxa did not significantly improve phylogenetic estimation of among-species relationships, even when using coalescent species-tree inference (*BEAST). It is possible that if these lineages diverged during near-simultaneous speciation events, then more genetic data will not yield dichotomous relationships (Karl et al., 2012).

4.2. Species delimitation and systematic implications

All recognized *Tepuihyla* species were described based on a combination of phenotypic and morphometric differences (Ayarzagüena et al., 1992). However, these are subtle; most highland species are of similar size, dorsum coloration (dark brown/gray), webbing, and general appearance, and differ only slightly from the low- and mid-elevation species, in which the most obvious difference is coloration pattern (Ayarzagüena et al., 1992). No morphological differences have been reported between the two putative species of *T. edelcae*, which were described as a single species with a disjunct distribution, atop Auyán-tepui and many tepuis atop the Chimantá Massif (Ayarzagüena et al., 1992).

In the seven-taxon species delimitation analysis we tested speciation within Chimantá Massif (atop the three individual tepui summits) and within *T. rodriguezi* (in the geographically distant populations in Venezuela and Guyana). The populations atop the three tepuis (Eruoda, Abakapá, and Churí) within the Chimantá massif were not supported as separate evolutionary lineages, particularly when using conservative speciation priors; speciation probabilities were largely variable but overall low (Supplementary Fig. 2). This result is consistent with the concatenated MrBayes tree (Fig. 2).

There is fairly high support for two lineages within the species *T. rodriguez*i, corresponding to two general localities of Venezuela and Guyana, even when using the conservative combination of priors (the support varies from 0.88 to 1.0; Supplementary Fig. 2). Frogs at each locality were considered distinct species (*T. rodriguezi* in Venezuela and *T. talbergae* in Guyana) until they were synonymized recently (Jungfer et al., 2013). Our molecular data suggest that the synonymy should be re-considered. More extensive sampling and consideration of other lines of evidence (i.e., integrative taxonomic approaches) are needed.

Regardless of the input topology, the Auyán and Chimantá lineages of *Tepuihyla edelcae* are supported as distinct (Fig. 4A and B). Given the body of evidence, such as monophyly of each lineage, lack of support for the monophyly of *T. edelcae* Chimantá + *T. edelcae* Auyán, distribution of unique haplotypes, and highly supported

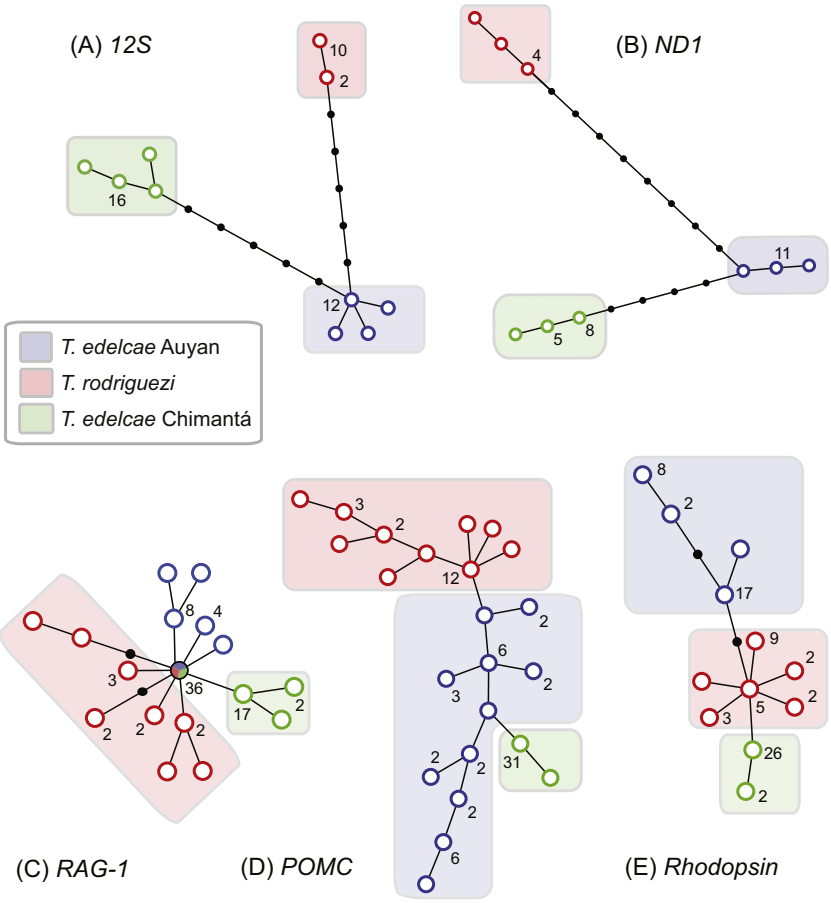

Fig. 5. Haplotype networks obtained in Arlequin for all five loci: (A) *12S*, (B) *ND1*, (C) *RAG-1*, (D) *POMC*, and (E) *Rhodopsin*. Black dots represent missing haplotypes and tic marks represent mutations. Colored circles represent haplotypes in populations; the number of individuals with that haplotype is represented as a number next to the circle. Colors identify the three populations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary statistics of population parameters obtained in Arlequin for five loci and three populations: *T. edelcae* Auyán, *T. edelcae* Chimantá, and *T. rodriguezi*. Single asterisk indicates significance at $p < 0.05$, and double asterisk significance at $p < 0.01$.

Locus	Population	Sample size	# Polymorphic sites	# Haplotypes	# Haplotypes/sample size	Nucleotide diversity (π) ± SD	Theta (S)	Tajima's D	Fu's Fs
12S (mtDNA)	*T. edelcae* Auyán	15	3	4	0.267	0.0006 ± 0.00066	0.923	−1.685*	−2.369**
	T. edelcae Chimantá	19	2	4	0.211	0.0004 ± 0.00062	0.572	−1.511*	−3.670**
	T. rodriguezi	12	1	2	0.166	0.0005 ± 0.00057	0.331	−0.194	0.297
ND1 (mtDNA)	*T. edelcae* Auyán	13	2	3	0.231	0.0011 ± 0.00141	0.644	−1.468	−1.402*
	T. edelcae Chimantá	11	2	3	0.273	0.0027 ± 0.00242	0.682	0.199	−0.019
	T. rodriguezi	7	2	3	0.429	0.0026 ± 0.00242	0.816	−0.275	−0.438
POMC (nDNA)	*T. edelcae* Auyán	28	5	11	0.393	0.0047 ± 0.00304	1.285	1.877	−4.120**
	T. edelcae himantá	32	1	2	0.063	0.0001 ± 0.00035	0.248	−1.142	−1.265*
	T. rodriguezi	24	5	10	0.417	0.0037 ± 0.00262	1.339	0.411	−5.259**
RAG1 (nDNA)	*T. edelcae* Auyán	28	4	6	0.214	0.0028 ± 0.00227	1.028	−0.226	−1.934
	T. edelcae Chimantá	32	3	4	0.125	0.0022 ± 0.00191	0.745	−0.241	−0.499
	T. rodriguezi	24	6	9	0.375	0.0044 ± 0.00311	1.607	−0.215	−4.024**
Rhodopsin (nDNA)	*T. edelcae* Auyán	28	4	4	0.143	0.0021 ± 0.00148	1.028	1.067	1.312
	T. edelcae Chimantá	28	1	2	0.071	0.0002 ± 0.00034	0.257	−0.740	−0.380
	T. rodriguezi	22	5	6	0.273	0.0017 ± 0.00129	1.372	−0.393	−1.581

speciation probabilities from the BP&P analysis, we argue that *T. edelcae* be considered two distinct species; this question is under further investigation (Salerno et al., in preparation).

4.3. Tepuis as islands is the sky: the case of Tepuihyla

Levels of genetic divergence on distinct tepuis have been shown to be surprisingly low among several species (Salerno et al., 2012; Kok et al., 2012; Bonaccorso and Guayasamin, 2013) indicating that populations have moved between summits long after tepui formation. Assuming that tepuis are sky-islands, whether topographical or ecological, one can make predictions about patterns of genetic diversity. For example, genetic diversity within and among populations should follow general island biogeography and population genetic predictions: smaller islands should hold smaller effective population sizes and will have lower genetic

Table 2
Uncorrected pairwise distances within (diagonal values) and among (off-diagonal values) putative species, *T. edelcae* Auyán, *T. edelcae* Chimantá, *T. rodriguezi*, and *T. aecii*. Numbers on top are range of *p*-distances for *12S*, and on bottom are *p*-distances for *ND1*.

	T. edelcae Auyán	*T. edelcae* Chimantá	*T. rodriguezi*
T. edelcae Auyán (*12S n* = 14, *ND1 n* = 12)	0.000–0.005 0.000–0.003	–	–
T. edelcae Chimantá (*12S n* = 19, *ND1 n* = 14)	0.013–0.019 0.020–0.028	0.000–0.003 0.000–0.011	–
T. rodriguezi (*12S n* = 12, *ND1 n* = 10)	0.017–0.023 0.017–0.027	0.010–0.031 0.017–0.032	0.000–0.003 0.000–0.005
T. aecii (*12S n* = 1, *ND1 n* = 0)	0.017–0.020	0.020	0.024–0.025

diversity due to genetic drift and higher fixation rates. Larger distances among islands should result in higher levels of divergence and a higher number of unique haplotypes.

Ecological distances or differences between these sky-islands may function similarly to geographic distances. For example, for species restricted to islands with elevations >2000 m it is likely that the lower elevations are currently unsuitable or at the very least not preferred, thus increasing the effective distance between these populations. On the other hand, mid- and low-elevation species should have a higher tolerance for lowland conditions, thus reducing the ecological distance between them, even if suitable habitat is patchy.

Because only a single locality on Auyán-tepui was sampled, we expected that the three Chimantá samples would in sum have much higher haplotype diversity than Auyán. However, we observed the opposite (Fig. 5); the single Auyán population has 2–5 times as many nuclear haplotypes as Chimantá has in its three combined populations (*POMC*: 11 vs 2, *RAG-1*: 6 vs 4, and *Rhodopsin*: 4 vs 2). Another expectation is some level of population structure among the Chimantá tepuis, though each population with lower diversity than Auyán (since the summit areas are individually smaller than Auyán). However, none of the three summit populations atop Chimantá has unique haplotypes for any gene. The most likely explanation for the low diversity and lack of structure across the Chimantá summits is that the populations experienced a bottleneck that decimated the populations (and thus the haplotype diversity) within the last ~100,000 years, and subsequently expanded atop the massif. This is consistent with the general pattern of low haplotype diversity (both nuclear and mitochondrial) of Chimantá and the lack of structure among populations, but is not consistent with neutrality tests, since only *12S* and *POMC* have significant Fs statistics. Also, this bottleneck scenario may explain the suggestion that Chimantá has a depauperate herpetofauna (McDiarmid and Donnelly, 2005; Señaris and MacCulloch, 2005). The smaller individual summits and the much more complex landscape of Chimantá may in general support much smaller effective population sizes, making Chimantá more prone to stochastic effects and local extinctions compared to Auyán.

4.4. Biogeographic scenarios and hypothesis

Given their very low levels of genetic divergence, it is clear that *Tepuihyla* species have overcome topographic barriers such as cliffs and dispersed across the highlands of the Pantepui. Thus it is likely that the cessation of gene flow among the three putative species is due to separation by unsuitable habitat and not extreme topography. Climatic and ecological conditions are therefore likely the main forces promoting divergence in a manner similar to other sky-island ecosystems (DeChaine and Martin, 2005; Smith and Farrell, 2005; Robin et al., 2010).

Auyán-tepui and the Chimantá massif, which are part of the Eastern Pantepui province, are separated by about 60 km of mid-altitude savannas. These formations, as well as the Venezuelan sampling localities of *T. rodriguezi*, are all found within the Gran Sabana, a mid-altitude plateau with an average elevation of ~1000 m (Fig. 1), and composed mostly of dry savannah-like vegetation and grasslands with seasonal flooding events (Huber et al., 2001; Huber, 2006). An important difference between Auyán-tepui and the Chimantá Massif is that the latter is a massif supporting ten tepuis. The three tepuis we sampled atop Chimantá are 15–30 km apart and have extreme intervening landscape features. Rather than dry lowland savannas, mid-elevation wet tropical forest separates the classic summit Pantepui habitat common to most sandstone tepuis of the Eastern District (Huber, 2006).

The single sample of *Tepuihyla aecii* is from Cerro Duida, a tepui located ~400 km southwest of Auyán and Chimantá (Fig. 1). Its genetic distances to other *Tepuihyla* completely overlap in range with those between *T. edelcae* Auyán and *T. edelcae* Chimantá, even though the geographic distance is almost seven times greater. For example, the distances between *T. aecii* and *T. edelcae* Auyán are 1.7–2.0%. The single *T. aecii* datum suggests a recent, pervasive dispersal of *Tepuihyla* species across the entire Eastern Pantepui region, followed by fragmentation into isolated species, which may have happened 3.2–5.3 mya (Salerno et al., 2012).

The unresolved dichotomous branching pattern among the four clades (*T. aecii*, *T. rodriguezi*, *T. edelcae* Auyán and *T. edelcae* Chimantá), together with the similar levels of genetic divergence regardless of geographic distance, can be used to generate a hypothesis for the evolution of these lineages. If the relationships are unresolved due to concurrent speciation events, then we can hypothesize that the common ancestor of all four may have been a widespread Pantepui lowland dweller, widespread in both the eastern and western Pantepui, that must have been present before the split of the common ancestor approximately 5.3 mya (Salerno et al., 2012). During that same timeframe low- to mid-elevation conditions may have become unsuitable, at which point most lowland populations became extinct, initiating concurrent speciation events due to isolation of populations on the tepui summits. This hypothesis is consistent with our analyses; for example, sequence divergences are very similar among the four lineages regardless of the geographic distance separating them. Because these divergences occur mostly during the Pliocene rather than the Pleistocene, it is possible that Pliocene climatic fluctuations may have promoted lowland dispersal either by slightly cooler climates or by less dry conditions, making the low- and mid-elevations of the Gran Sabana more similar to current wetter and cooler summit climates where *Tepuihyla* is most commonly found. Particularly, given that *Tepuihyla* seems to be strongly associated with *Brocchinia* plants regardless of whether the species are associated with summits (Ayarzagüena et al., 1992), it is likely that suitable habitat is due more to the availability of *Brocchinia* than by other climatic conditions, and that climatic changes affected the distributions of *Brocchinia*.

Although summit habitats, particularly those with *Brocchinia* peat bog habitats, seem to be preferred by *Tepuihyla* (Ayarzagüena et al., 1992), the intervening wet tropical forest

between Chimantá's tepuis is almost certainly more suitable for dispersal of *Tepuihyla* than are the dry tropical savannas separating Auyán-tepui from the Chimantá Massif. However, there is little evidence that *Tepuihyla* persists in these intervening wet forests between the tepui summits on Chimantá Massif. Thus, it is likely that these populations are currently not experiencing gene flow, but did so within the past ~100,000 years. This would be consistent with a hypothesis of Pleistocene glacial fluctuations and vertical displacement of summit biota (Rull, 2004; Rull and Nogué, 2007), in which the Chimantá Massif populations may have experienced gene flow as recently as 10,000 years ago during the last glacial maximum, but are currently not experiencing genetic exchange. This is also consistent with studies of pollen data that suggest as much as 1100 m of downward vertical displacement of the flora occurred during the last glacial maximum in the Pleistocene, allowing for lowland interchange and dispersal to other highlands (Rull, 2004; Rull and Nogué, 2007).

The case of *Tepuihyla* offers some insights on the recent evolutionary history atop the Lost World. For example, even though the three putative species are very recently diverged (3.2–5.3 mya; Salerno et al., 2012) and colonized summits long after tepui formation, they are currently evolving separately, as evidenced by almost complete lineage sorting and unique haplotypes. Thus, the complex topography alone does not seem to be the main barrier to gene flow. Local tepui conditions and extreme biotic and abiotic differences among highlands and lowlands may promote high endemicity atop the tepui summits, effectively making them an ecological archipelago of islands in the sky.

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Appendix A

Genetic samples, localities, and GenBank accession numbers. Numbers in bold were downloaded from GenBank, all others were obtained for this study.

Species	Field/museum code	Locality	Coordinates	Genbank sequences				
				12S	ND1	POMC	RAG1	Rhodopsin
T. aecii	MHNLS 12013	Cerro Duida, Amazonas, Vzla	3°18′N 65°37′W	**JQ868533**	–	**JQ868478**	–	–
T. exophthalmus	BPN 166	Guyana	5°37.30′N 60°14.42′W	**JQ868523**	KP010970	KP011101	KP011058	KP011014
T. exophthalmus	MHNLS 19583	Sierra de Lema, Canaima, Vzla	5°54.045′N 61°26.290′W	**JQ868524**	–	–	–	–
T. exophthalmus	MHNLS 19584	Sierra de Lema, Canaima, Vzla	5°54.045′N 61°26.290′W	**JQ868525**	KP010971	**JQ868483**	KP011059	–
T. edelcae	MHNLS16090	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	**JQ868534**	–	**JQ868477**	KP011029	KP010987
T. edelcae	PS002	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	**JQ868537**	KP010945	**JQ868475**	KP011028	KP010986
T. edelcae	MHNLS 05824	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	**JQ868535**	KP010944	KP011074	KP011027	KP010985
T. edelcae	MHNLS 05825	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011112	KP010943	KP011073	KP011026	KP010984
T. edelcae	MHNLS 05826	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011111	KP010942	KP011072	–	KP010983
T. edelcae	MHNLS 05827	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011110	KP010941	KP011071	KP011025	KP010982
T. edelcae	MHNLS 05828	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011109	KP010940	KP011070	KP011024	KP010981
T. edelcae	MHNLS 05829	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011108	–	–	KP011023	–
T. edelcae	MHNLS 05830	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011107	KP010939	KP011069	KP011022	KP010980
T. edelcae	MHNLS 05831	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011106	KP010938	KP011068	KP011021	KP010979
T. edelcae	MHNLS 05833	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011105	KP010937	KP011067	KP011020	KP010978
T. edelcae	MHNLS 05834	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011104	KP010936	KP011066	KP011019	KP010977
T. edelcae	MHNLS 05835	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011103	KP010935	KP011065	KP011018	KP010976
T. edelcae	MHNLS 05836	Auyán-tepui, Canaima, Vzla	5°46.599′N 62°32.251′W	KP011102	KP010934	KP011064	KP011017	KP010975

(*continued on next page*)

Appendix A (*continued*)

Species	Field/museum code	Locality	Coordinates	Genbank sequences				
				12S	ND1	POMC	RAG1	Rhodopsin
T. cf. "edelcae"	MHNLS 20817	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	**JQ868538**	–	KP011090	KP011045	KP011001
T. cf. "edelcae"	MHNLS 20483	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011130	KP010959	KP011089	KP011044	KP011000
T. cf. "edelcae"	MHNLS 20484	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011129	KP010958	KP011088	KP011043	KP010999
T. cf. "edelcae"	MHNLS 20485	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011128	KP010957	KP011087	KP011042	KP010998
T. cf. "edelcae"	MHNLS 20487	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011127	KP010956	KP011086	KP011041	KP010997
T. cf. "edelcae"	MHNLS 20490	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011121	KP010952	KP011082	KP011037	KP010993
T. cf. "edelcae"	MHNLS 20492	Churí-tepui, Chimantá massif, Canaima, Vzla	5°15.257′N 62°00.472′W	KP011120	KP010951	KP011081	KP011036	KP010992
T. cf. "edelcae"	MHNLS 20510	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011126	KP010955	KP011085	KP011040	KP010996
T. cf. "edelcae"	MHNLS 20511	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011125	KP010954	KP011084	KP011039	KP010995
T. cf. "edelcae"	MHNLS 20512	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011124	–	–	–	–
T. cf. "edelcae"	MHNLS 20513	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011123	KP010953	KP011083	KP011038	KP010994
T. cf. "edelcae"	MHNLS 20533	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011122	–	–	–	–
T. cf. "edelcae"	MHNLS 20534	Abakapá-tepui, Chimantá massif, Canaima, Vzla	5°11.497′N 62°18.939′W	KP011119	KP010950	KP011080	KP011035	KP010991
T. cf. "edelcae"	MHNLS 20545	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011116	–	KP011077	KP011032	KP010989
T. cf. "edelcae"	MHNLS 20580	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011115	–	–	–	–
T. cf. "edelcae"	MHNLS 20581	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011114	KP010947	KP011076	KP011031	–
T. cf. "edelcae"	MHNLS 20582	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011113	KP010946	KP011075	KP011030	KP010988
T. cf. "edelcae"	MHNLS 20583	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011118	KP010949	KP011079	KP011034	–
T. cf. "edelcae"	MHNLS 20584	Eruoda-tepui, Chimantá massif, Canaima, Vzla	5°22.525′N 62°05.674′W	KP011117	KP010948	KP011078	KP011033	KP010990
T. rodriguezi	BPN 1101	Mazaruni-Potaro, Guyana	5°43.389′N 60°16.087′W	**JQ868541**	KP010962	KP011092	KP011048	KP011004
T. rodriguezi	BPN 1218	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011138	–	KP011100	KP011057	KP011013
T. rodriguezi	BPN 1219	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	**JQ868542**	KP010963	**JQ868473**	KP011049	KP011005
T. rodriguezi	BPN 1220	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011137	KP010969	KP011099	KP011056	KP011012
T. rodriguezi	BPN 1221	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011136	KP010968	KP011098	KP011055	KP011011
T. rodriguezi	BPN 1222	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011135	KP010967	KP011097	KP011054	KP011010
T. rodriguezi	BPN 1223	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011134	KP010966	KP011096	KP011053	KP011009
T. rodriguezi	BPN 1224	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011133	–	KP011095	KP011052	KP011008
T. rodriguezi	BPN 1225	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011132	KP010965	KP011094	KP011051	KP011007
T. rodriguezi	BPN 1226	Mazaruni-Potaro, Guyana	5°72′N 60°27′W	KP011131	KP010964	KP011093	KP011050	KP011006
T. rodriguezi	PS 003	Luepa, Bolívar, VENEZUELA	5°44.46′N 61°31.02′W	**JQ868540**	KP010961	**JQ868474**	KP011047	KP011003
T. rodriguezi	MHNLS 19575	Sierra de Lema, Canaima, Vzla	5°49.228′N 61°25.473′W	**JQ868539**	KP010960	KP011091	KP011046	KP011002
O. taurinus	PS 004	Las Claritas, Bolívar, Venezuela	6°10.49′N 61°25.17′W	**JQ868512**	KP010972	**JQ868487**	KP011060	–
O. leprieurii	MHNLS 18689	Uey River, Bolívar State, Venezuela	–	**JQ868505**	–	**JQ868497**	KP011062	KP011015
O. planiceps	QCAZ 19195	Estación Científica Yasuní, Orellana, Ecuador	–	**JQ868521**	KP010973	**JQ868495**	KP011061	–
O. deridens	QCAZ 20868	Ecuador	–	**JQ868501**	KP010974	**JQ868484**	KP011063	KP011016

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.10.020.

References

Ayarzagüena, J., Señaris, J.C., Gorzula, S., 1992. The group *Osteocephalus rodriguezi* from the highlands of the Venezuelan Guayana: description of five new species. Mem. Soc. Cien. Nat. La Salle 52, 113–142.

Berry, P.E., Riina, R., 2005. Insights on the diversity of the Pantepui flora and the biogeographic complexity of the Guayana Shield. Biol. Skrif. 55, 145–167.

Bonaccorso, E., Guayasamin, J.M., 2013. On the origin of Pantepui montane biotas: a perspective based on the phylogeny of *Aulacorhynchus* toucanets. PLoS ONE 8, 0067321. http://dx.doi.org/10.1371/journal.pone.0067321.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T.G., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.

Briceño, H.O., Schubert, C., 1990. Geomorphology of the Gran Sabana, Guayana Shield, southeastern Venezuela. Geomorphology 3, 125–141.

Briceño, H.O., Schubert, C., Paolini, J., 1990. Table-mountain geology and surficial geochemistry: Chimantá Massif, Venezuelan Guayana shield. J. South Am. Earth Sci. 3, 179–194.

Chapman, F.M., 1931. The upper zonal bird-life of Mts. Roraima and Duida. Bull. Am. Mus. Nat. Hist. 63, 1–135.

DeChaine, E.G., Martin, A.P., 2005. Marked genetic divergence among sky island populations of *Sedum lanceolatum* (Crassulaceae) in the Rocky Mountains. Am. J. Bot. 92, 477–486.

Delport, W., Poon, A., Frost, S.D.W., Kosakovsky Pond, S.L., 2010. Datamonkey: a suite of phylogenetic analysis tools for evolutionary biology. Bioinformatics 26, 2455–2457. http://dx.doi.org/10.1093/bioinformatics/btq429.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.

Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Frost, D.R., Campbell, J.A., Wheeler, W.C., 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bull. Am. Mus. Nat. Hist. 294, 1–240.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Gibbs, A.K., Barron, C.N., 1993. The Geology of the Guiana Shield. Oxford University Press, Oxford, England.

Givnish, T.J., Sytsma, K., Smith, J.F., Hahn, W.J., Benzing, D.H., Burkhardt, E.M., 1997. Molecular evolution and adaptive radiation in *Brocchinia* (Bromeliaceae: Pitcairnioideae) atop tepuis of the Guayana Shield. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, pp. 259–312.

Givnish, T.J., Barfuss, M.H.J., Van Ee, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.

Huber, O., 1988. Guayana highlands versus Guayana lowlands, a reappraisal. Taxon 37, 595–614.

Huber, O., 2006. Herbaceous ecosystems on the Guayana Shield, a regional overview. J. Biogeogr. 33, 464–475.

Huber, O., Febres, G., Arnal, H., 2001. Ecological guide to the Gran Sabana. Ecograph Proyectos y Ediciones, Caracas, 192pp.

Jungfer, K.H., Faivovich, J., Padial, J., Castroviejo-Fisher, S., Lyra, M.M., Berneck, B., Iglesias, P.P., Kok, P.J.R., MacCulloch, R.D., Rodrigues, M.T., Verdade, V.K., Torres Gastello, C.P., Chaparro, J.C., Valdujo, P.H., Reichle, S., Moravec, J., Gvozdik, V., Gagliardi-Urrutia, G., Ernst, R., De la Riva, I., Means, D.B., Lima, A.P., Señaris, J.C., Wheeler, W.C., Haddad, C.F.B., 2013. Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle. Zool. Scr. 42, 351–380.

Karl, S.A., Toonen, R.J., Grant, W.S., Bowen, B.W., 2012. Common misconceptions in molecular ecology: echoes of the modern synthesis. Mol. Ecol. 21, 4171–4189.

Kok, P.J.R., MacCulloch, R.D., Means, D.B., Roelants, K., Van Boxclaer, I., Bossuyt, F., 2012. Low genetic diversity in tepui summit vertebrates. Curr. Biol. 22, 589–590.

Kosakovsky Pond, S.L., Posada, D., Gravenor, M.B., Woelk, C.H., Frost, S.D.W., 2006. Automated phylogenetic detection of recombination using a genetic algorithm. Mol. Biol. Evol. 23, 1891–1901.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. Partition Finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.

Leaché, A.D., Fujita, M.K., 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). Proc. Roy. Soc. B 277, 3071–3077.

Maguire, B., 1970. On the flora of the Guayana Highland. Biotropica 2, 85–100.

Mayr, E., Phelps, W.H., 1955. The origin of the bird fauna of South Venezuelan Highlands. Bull. Am. Mus. Nat. Hist. 136, 269–328.

Mayr, E., Phelps, W.H., 1967. The origin of the bird fauna of the south Venezuelan highlands. Bull. Am. Mus. Nat. Hist. 136, 273–327.

McDiarmid, R.W., Donnelly, M.A., 2005. The herpetofauna of the Guayana Highlands: amphibians and reptiles of the Lost World. In: Donnelly, M.A., Crother, B.I., Guyer, C., Wake, M.H., White, M.E. (Eds.), Ecology and Evolution in the Tropics: A Herpetological Perspective. University of Chicago Press, Chicago, Illinois, pp. 461–560.

Moen, D.S., Wiens, J.J., 2009. Phylogenetic evidence for competitively driven divergence. Body-size evolution in Caribbean treefrogs (Hylidae: *Osteopilus*). Evolution 63, 195–214.

Pauly, G.B., 2008. Phylogenetic Systematics, Historical Biogeography, and the Evolution of Vocalizations in the Nearctic toads (*Bufo*). PhD Dissertation, University of Texas, Austin.

Posada, D., 2004. COLLAPSE 1.2. <http://darwin.uvigo.es/software/collapse.html>.

Rambaut, A., Drummond, A.J., 2013. Tracer v1.6 [Online]. <http://tree.bio.ed.ac.uk/software/tracer/>.

Rannala, B., Yang, Z., 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. Genetics 164, 1645–1656.

Robin, V.V., Sinha, A., Ramakrishnan, U., 2010. Ancient geographical gaps and paleo-climate shape the phylogeography of an endemic bird in the sky islands of southern India. PLoS ONE 5, e11321. http://dx.doi.org/10.1371/journal.pone.0013321.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Rull, V., 2004. An evaluation of the Lost World and Vertical Displacement hypotheses in the Chimantá Massif, Venezuelan Guayana. Glob. Ecol. Biogeogr. 13, 141–148.

Rull, V., Nogué, S., 2007. Potential migration routes and barriers for vascular plants of the Neotropical Guyana Highlands during the Quaternary. J. Biogeogr. 34, 1327–1341.

Salerno, P.E., Ron, S.R., Señaris, J.C., Rojas-Runjaic, F.J.M., Noonan, B.P., Cannatella, D.C., 2012. Ancient tepui summits harbor young rather than old lineages of endemic frogs. Evolution 66, 3000–3013.

Salerno, P.E., Rojas-Runjaic, F.J.M., Cannatella, D.C., Señaris, J.C., in preparation. A new morphologically cryptic species of *Tepuihyla* from the sky islands of the Chimantá Massif in Venezuela. ZooKeys.

Señaris, J.C., MacCulloch, R.D., 2005. Amphibians. In: Hollowell, T., Reynolds, R.P. (Eds.), Checklist of the Terrestrial Vertebrates of the Guiana Shield. Bulletin of the Biological Society of Washington 13, pp. 8–23.

Smith, C., Farrell, B., 2005. Phylogeography of the longhorn cactus beetle *Moneilema appressum* LeConte (Coleoptera: Cerambycidae): was the differentiation of the Madrean sky islands driven by Pleistocene climate changes? Mol. Ecol. 14, 3049–3065.

Stephens, M., Donnelly, P., 2003. A comparison of Bayesian methods for haplotype reconstruction. Am. J. Human Gen. 73, 1162–1169.

Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Human Gen. 68, 978–989.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.

Tamura, K.D., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Teacher, A.G.F., Griffiths, D.J., 2011. HapStar: automated haplotype network layout and visualisation. Mol. Ecol. Res. 11, 151–153.

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci. U.S.A. 107, 9264–9269.