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Out of taxonomic limbo: a name for the species of *Tepuihyla* (Anura: Hylidae) from the Chimantá Massif, Pantepui region, northern South America

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Abstract. We describe a new hylid species of the genus *Tepuihyla* from Pantepui, northeastern South America. The new species inhabits the Chimantá Massif, Bolívar state, Venezuela. The new species is likely part of a recent non-adaptive radiation, and was confused for more than a decade with *T. edelcae*, a morphologically similar species occurring on the summit of Auyán-tepui, Bolívar state, Venezuela. The new species is mainly distinguished from known congeners by phylogenetic data, as well as a medium size (37.1 mm maximum SVL in males, 38.4 mm maximum SVL in females), diameter of eye greater than distance from nostril to eye, skin on dorsum smooth in females, with scattered, fine, white-tipped spicules in males, skin on flanks smooth to faintly granular, presence of a pale labial stripe and a dark band or stripe from nostril to eye, a dorsal ground colour from pale grey to dark brown, usually suffused with small to minute dark brown or black markings, no transverse bars on limbs, rear of thighs patternless, axillary membrane poorly developed, breeding males with conspicuous, usually black, nuptial pads extending beyond thenar tubercle, iris dark brown to copper with gold flecks and sometimes fine dark brown reticulation, and white limb bones. The new species inhabits open, mostly flat areas on tepui summits, between ca 1,800 and 2,600 m altitude, where it is intimately associated with carnivorous bromeliads of the genus *Brocchinia*. The species breeds in deep pools in marshy areas and small shallow rocky pools; its tadpole and advertisement call are described. The IUCN conservation status of the new species is considered Least Concern (LC) because population size still seems relatively large, the species occurs in a number of locations, and is apparently not declining fast enough to qualify for any of the threat categories. Differentiation in morphological, acoustic, and genetic traits of species endemic to tepui summits are briefly discussed. Finally, *Tepuihyla rimarum* is considered a junior synonym of *T. rodriguezi*.

Key words. Amphibia, cryptic species, genetic divergence, Guyana, morphology, tepui, new species, *T. rimarum*, Venezuela.

Introduction

Species delineation is a core problem in the study of biodiversity. The task is complicated by a lack of consensus among biologists on the exact definition of a species (e.g., Wheeler & Meier 2000). Delimiting species and species' distributions as accurately as possible is nevertheless critically important for conservation, particularly in mountainous areas that have been reported as highly sensitive to global warming and threatened with habitat loss by upward displacement (Rull & Vegas-Vilarrúbia 2006, Nogué et al. 2009). Even though the concept of species remains highly debated, the so-called "integrative taxonomy", i.e., the use of multiple lines of evidence to distinguish between species (reviewed in Padial et al. 2010), has become increasingly popular and been demonstrated to be effective in diverse challenging taxonomic groups (e.g., Vicente et al. 2013, Diaye et al. 2014, Soldati et al. 2014). Whatever the species concept being applied, it appears that the perceived ease of distinguishing between species based on external morphology strongly varies among lineages. New species are also more complex to identify in some geographic areas compared to others. This seems to be the case when allopatric speciation occurred "recent-

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ly" in comparable habitats that impose strong and similar ecological constraints. In any case, "cryptic species" are obviously more common than initially thought (Funk et al. 2012, Gehara et al. 2014), and the danger of leaving considerable parts of biodiversity unaddressed (Jörger & Schrödl 2013) is blatant.

The tepuis of the Pantepui biogeographic region of northern South America (Fig. 1) are among those areas where delineating species boundaries is often particularly difficult for reasons that have not yet been properly explained. The term tepui has been widely used to characterize the tabletop mountains made of Precambrian sandstone that rise above the savannah and tropical forest, mainly in the Guayana region of southern Venezuela (states of Bolívar and Amazonas), in west-central Guyana (district of Cuyuni-Mazaruni), and in extreme northern Brazil (states of Amazonas and Roraima). Because of their ancient origin and their physiographic, edaphic, and climatic isolation, tepui summits have for long been thought of harbouring old endemic lineages, with some even possibly predating the separation of Africa and South America (e.g., McDiarmid & Donnelly 2005). However, Kok et al. (2012), using a broad sampling of amphibian and reptile taxa, demonstrated that genetic diversity among most tepui summit species and populations is much lower than expected, suggesting that tepuis were only sporadically impermeable barriers to gene flow within the Pantepui region throughout history. Kok et al. (2012) also indicated that in spite of low genetic distances, a number of tepui summit-populations recognized as distinct species exhibit conspicuous phenotypic differences (in colouration for example), while some tepui summit-populations exhibit identical morphology in spite of substantial genetic divergences (see also Kok 2013). Both situations could potentially lead to taxonomic chaos.

The genus *Tepuihyla* was introduced by Ayarzagüena et al. (1993b) to accommodate six species these authors had previously included in the *Osteocephalus rodriguezi* group (Ayarzagüena et al. 1993a). *Tepuihyla* species, at that time all from the Venezuelan Guayana, were reported to differ morphologically from *Osteocephalus* sensu stricto mainly in osteological characters (Ayarzagüena et al. 1993b). Since then, *Tepuihyla* has been regarded as sister to *Osteocephalus* (Faivovich et al. 2005, Pyron & Wiens 2011), or more recently, to a clade composed of *Osteocephalus* and *Dryaderces* (Jungfer et al. 2013). The genus *Tepuihyla* is currently restricted to Pantepui, with seven recognized species occurring from eastern and southeastern Venezuela to western Guyana (Jungfer et al. 2013). Several populations of *Tepuihyla* have been reported as single tepui summit-endemics (see Gorzula & Señaris 1999, McDiarmid & Donnelly 2005). Kok et al. (2012) demonstrated that genetic divergence between geographically distant populations (some from different tepui summits) of *Tepuihyla galani*, *T. rodriguezi*, and *T. talbergae* was extremely low, even in a fragment of the fast-evolving protein-coding mitochondrial gene NADH dehydrogenase subunit 1 (ND1). The intent of Kok et al. (2012) was not to make any taxonomic decision, but this was done shortly thereafter by Jungfer et al. (2013) who considered *Tepuihyla galani* and *T. talbergae* to be junior synonyms of *T. rodriguezi* based on the absence of reliable diagnostic morphological characters and a very low genetic distance among populations in a fragment of the mitochondrial gene 16S rDNA (here-

Figure 1. Northern part of Auyán-tepui, Bolívar state, Venezuela, showing typical tepuian sheer cliffs and lower forested slopes. Photograph taken while flying by helicopter over the Devil's Canyon (17 June 2012). Photo: PJRK.

inafter 16S). Jungfer et al. (2013) placed *Osteocephalus exophthalmus*, *O. phasmatus*, and "*Hyla*" *warreni* in *Tepuihyla* to resolve the non-monophyly of *Osteocephalus* as suggested by their phylogenetic tree topology, and considered *T. phasmata* to be a junior synonym of *T. exophthalma*. Two single tepui summit-endemics, *Tepuihyla rimarum* and *T. luteolabris*, could not be included in Jungfer et al.'s (2013) molecular phylogenetic analysis due to the lack of tissue samples. To date, the phylogenetic position of these two microendemic species remains unknown.

An additional puzzling taxon is *Tepuihyla* "aff. *edelcae*" from the Chimantá Massif. Although *Tepuihyla* populations from that massif (more specifically from Amurí-tepui, Abakapá-tepui, Akopán-tepui, Apakará-tepui, Chimantá-tepui, Churí-tepui, and Murei-tepui, but also from Tereke-Yurén-tepui in the Los Testigos Massif) have been referred to as *T. edelcae* for more than a decade (Gorzula & Señaris 1999, McDiarmid & Donnelly 2005), all available comprehensive *Tepuihyla* phylogenies either based solely on mitochondrial DNA (e.g. Kok et al. 2012, Salerno et al. 2012, Jungfer et al. 2013) or on nuclear and mitochondrial DNA (Salerno et al. 2014) recover a non-monophyletic *Tepuihyla edelcae*. All studies, except Kok et al. 2012 (supplement) suggest a sister relationship between *T.* aff. *edelcae* from the Chimantá Massif and *T. rodriguezi*, but always with low statistical node support. Kok et al. 2012 (supplement) indicate a well-supported sister relationship between *T. edelcae* from Auyán-tepui and *T. rodriguezi*, with *T.* aff. *edelcae* falling sister to that clade. The phylogenetic and taxonomic status of the populations of *Tepuihyla* aff. *edelcae* from the Chimantá Massif compared to those of *T. edelcae* from the type locality (Auyán-tepui) and of *T. rodriguezi* (sensu Jungfer et al. 2013) therefore remain uncertain. Although the airline distance between tepuis of the Chimantá Massif and Auyán-tepui is only ca 50 km, these mountains are physically separated by deep and wide valleys more than 1,000 m lower in elevation, covered by different habitat, and have only very few species in common on their respective summits.

The purpose of this paper is to clarify the phylogenetic position and taxonomic identity of the populations currently assigned to *Tepuihyla* aff. *edelcae* from the Chimantá Massif, Bolívar state, Venezuela, using an integrative approach by including morphological (adult and tadpole) and molecular data (nuclear and mitochondrial DNA), as well as bioacoustics. We also include samples of *T. rimarum* from the type locality in our phylogenetic analyses and comment on the taxonomic status of that species.

Materials and methods

Fieldwork and deposition of specimens

The 31 specimens and 24 tadpoles of *Tepuihyla* aff. *edelcae* used in this study are from (1) the summit of Abakapá-tepui (05°11' N, 62°17' W, ca 2,200 m a.s.l.; Figs 2–3), where 11 adult individuals (seven males, four females), four subadults, and 18 tadpoles were secured; (2) from the summit of Amurí-tepui (05°08' N, 62°07' W, ca 2,200 m a.s.l.; Figs 2–3) where four adult males, and one subadult were collected; and (3) from the summit of Chimantá-tepui (05°19' N, 62°12' W, ca 2,200 m a.s.l.; Figs 2–3), where nine adult males, one subadult, one juvenile (not used in the morphological analyses), and six tadpoles (5 additional larvae were preserved in ethanol for DNA barcoding, and 1 additional, poorly preserved tadpole was not used in the morphological analyses) were secured. These individuals were compared in detail with the holotype of *T. edelcae* (MHNLS 10626), as well as with 19 freshly collected adult specimens (12 males, 7 females) from two geographically close locations on the summit of Auyán-tepui, the type locality of *T. edelcae* (05°45' N, 62°32' W, between 2,200–2,300 m a.s.l.; Fig. 2), and seven tadpoles collected on Cerro El Sol (06°06' N, 62°32' W, ca 1,800 m a.s.l.; Fig. 2), a small tepui located north of Auyán-tepui (conspecificity of these tadpoles with *T. edelcae* from Auyán-tepui was confirmed by DNA analyses). Comparisons of external character states are also based on original descriptions and examination of museum specimens, usually including the type series and/or topotypic specimens. A comprehensive list of additional specimens examined is provided in the Appendix.

Specimens were collected by hand (adults and juveniles) or fish nets (tadpoles), and euthanised by immersion in a 2% lidocaine solution (Linisol), fixed in 10% formalin for a few days, and then transferred to 70% ethanol (adults and juveniles) or preserved in 10% formalin (tadpoles) for permanent storage. A piece of liver and/or thigh muscle was taken from most individuals prior to fixation and preserved in 95% ethanol for later molecular analyses. Some tadpoles were preserved in 95% ethanol for the same purpose. Specimens were deposited in the collections of the Institut Royal des Sciences Naturelles de Belgique (IRSNB). Tissue samples were deposited in the Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB). Museum acronyms follow Frost (2015).

Morphology

All morphometric data were taken from the preserved specimens by the same person (SR), to the nearest 0.01 mm and rounded to the nearest 0.1 mm, under a Leica stereo dissecting microscope using electronic digital callipers (adults and juveniles), and/or a ruler and an ocular micrometer (tadpoles). Measurements were taken from the right side of the specimens. To improve accuracy, each measurement was taken three times and a mean value was used for the statistical analyses (Table 1).

Abbreviations and standard measurements for adults and juveniles are as follows: (1) snout–vent length (SVL); (2) head length from angle of jaw to tip of snout (HL); (3) head width at level of angles of jaws (HW); (4) snout length from anterior corner of eye to tip of snout (SL); (5) eye to naris distance from anterior corner of eye to posterior margin of naris (EN); (6) internarial distance

(IN); (7) eye length (EL); (8) interorbital distance (IO); (9) greatest length of tympanum from its anterior margin to its posterior margin (TYM); (10) forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow (FaL); (11) largest forearm breadth (FaB); (12) hand length from proximal edge of palmar tubercle to tip of Finger III (HaL); (13) width of disc on Finger III (WFD); (14) thigh length from vent to outer edge of flexed knee (ThL); (15) tibia length from outer edge of flexed knee to heel (TiL); (16) tarsus length from heel to proximal edge of inner metatarsal (TaL); (17) foot length from proximal edge of inner metatarsal tubercle to tip of Toe IV (FL); and (18) width of disc on Toe IV (WTD).

All tadpoles are from shallow pools in peat bogs or puddles in rocky areas. Developmental stages follow Gosner (1960); terminology and oral disc characters follow Altig & McDiarmid (1999). Abbreviations and standard measurements for tadpoles are as follows: (1) total length from tip of snout to tip of tail (TL); (2) body length from tip of snout to junction of posterior body and tail musculature (BL); (3) tail length from junction of body and tail musculature to tip of tail (TAL); (4) greatest body width (BW); (5) highest body height (BH); (6) head width at level of eyes (HW); (7) tail muscle height at base of tail (TMH); (8) tail muscle width at base of tail (TMW); (9) maximum height of tail (MTH); (10) maximum upper tail fin height (UTF); (11) maximum lower tail fin height (LTF); (12) spiracle–snout distance (SSD); (13) eye–naris distance (END); (14) naris–snout distance (NSD); (15) internarial distance (IND); (16) interorbital distance between the unpigmented skin covering the eyes, as the eyeball is not completely visible (IOD); and (17) eye diameter (ED). The oral disc was measured for its

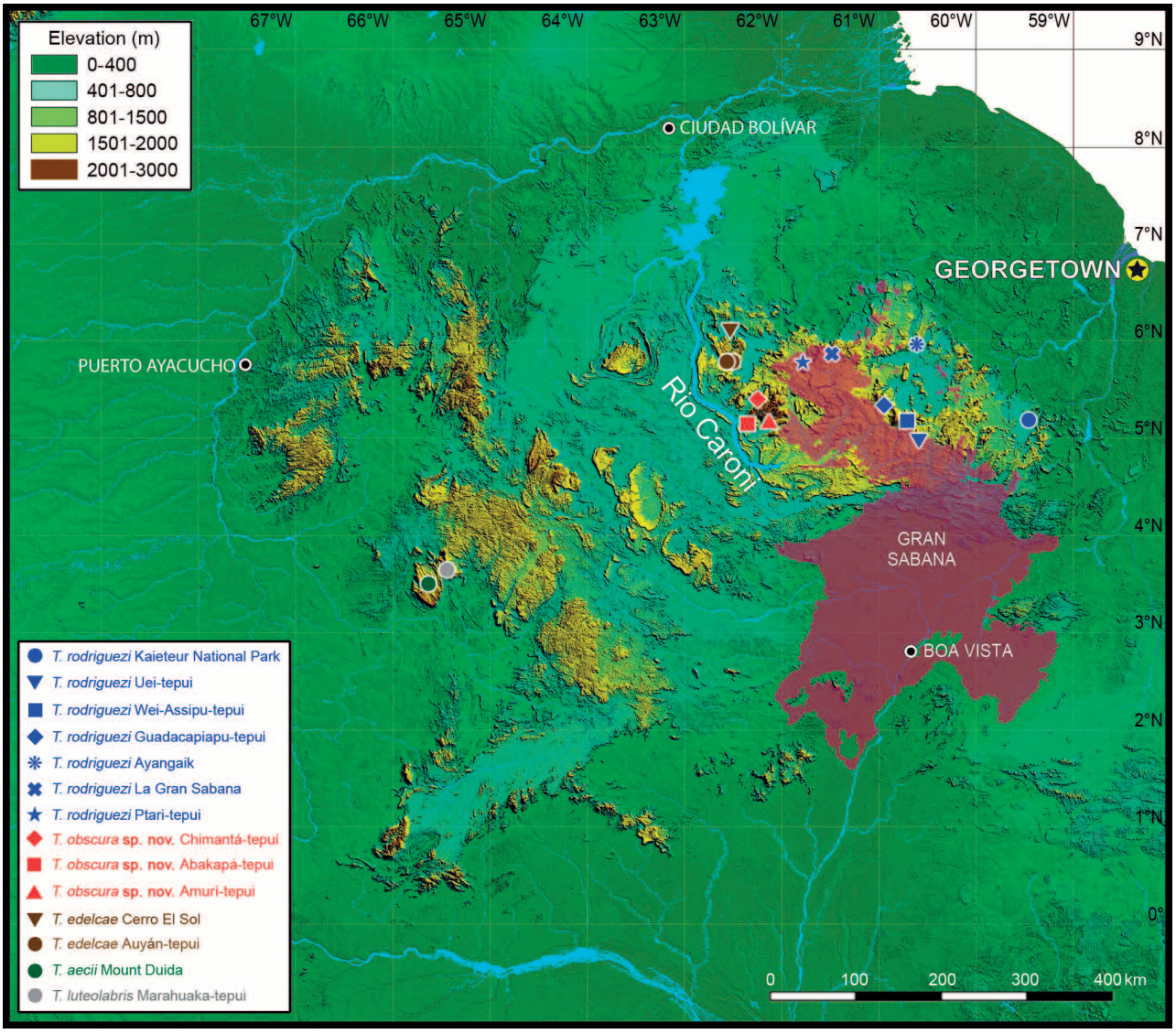

Figure 2. Map of Pantepui showing the known distribution of *Tepuihyla rodriguezi*, *T. obscura* sp. n., *T. edelcae*, *T. aecii*, and *T. luteolabris* (*T. rodriguezi* clade). Localities east of the Rio Caroní correspond to our sampling sites. The Gran Sabana is highlighted in pink. Map (modified) courtesy of Charles Brewer Carías.

maximum length and width, as well as for the length of the anterior gap between the marginal papillae (GAP); teeth rows on the oral disc were counted, and the labial tooth row formula (LTRF) was identified according to Altig & McDiarmid (1999). Ethanol-preserved tadpoles were excluded from the morphological analyses, because ethanol caused soft tissue to desiccate and led to body deformation.

Colour in life is described from digital photographs and field notes. Sex and maturity status were identified by the presence/absence of vocal slit(s) and nuptial pads, and confirmed by dissection and examination of gonads when sexing was doubtful. The internal soft anatomy was examined by dissection of preserved specimens; the number of teeth on the vomerine odontophores was estimated with the help of a fine needle.

Bioacoustics

Advertisement calls of one male from Abakapá-tepui (IRSNB 4170), and one male from Chimantá-tepui (IRSNB 4192, holotype) were recorded in the field by PJRK at a distance of ca 1.0 m from the specimens using a Sennheiser ME66/K6 microphone attached to a Marantz PMD661 solid-state recorder. One recording of a male of *T. edelcae* from the summit of Auyán-tepui was obtained from Renaud Boistel (University of Poitiers, France). Calls were analysed at a sampling rate of 44,100 Hz using Raven Pro 1.4, version 64 bit for Windows (Charif et al. 2010). Temporal variables were measured on the oscillogram, and included the following (see Kok & Kalamandeen 2008): call duration (beginning of the first to the end of the last note of a call); note duration (beginning of the note to the end of the note); number of notes per call (a call is here defined as a series of notes emitted in groups between longer silent intervals); and inter-note interval (end of one note to beginning of the next). Spectrogram parameters were set to Blackman window, with DFT size at 256 samples; other settings were left default. Peak of the dominant (emphasized) frequency of the note was measured from a spectral slice taken through the portion of the note with the highest amplitude (using the Blackman window function at a 3 dB filter bandwidth of 60 Hz, DFT size set at 1,206 samples).

The most accurate method to measure temporal variables is using the oscillogram because the spectrogram comes with a time/frequency trade-off (Charif et al. 2010). However, note length can be difficult to estimate due to background noise, and the decision of where the note exactly stops may vary between analysts. To circumvent this problem we performed the following procedure (1) we selected a 0.1-second segment of background noise shortly after each note; (2) we measured the amplitude peak of that segment; and (3) we used that amplitude peak as a threshold to discriminate between note and background noise.

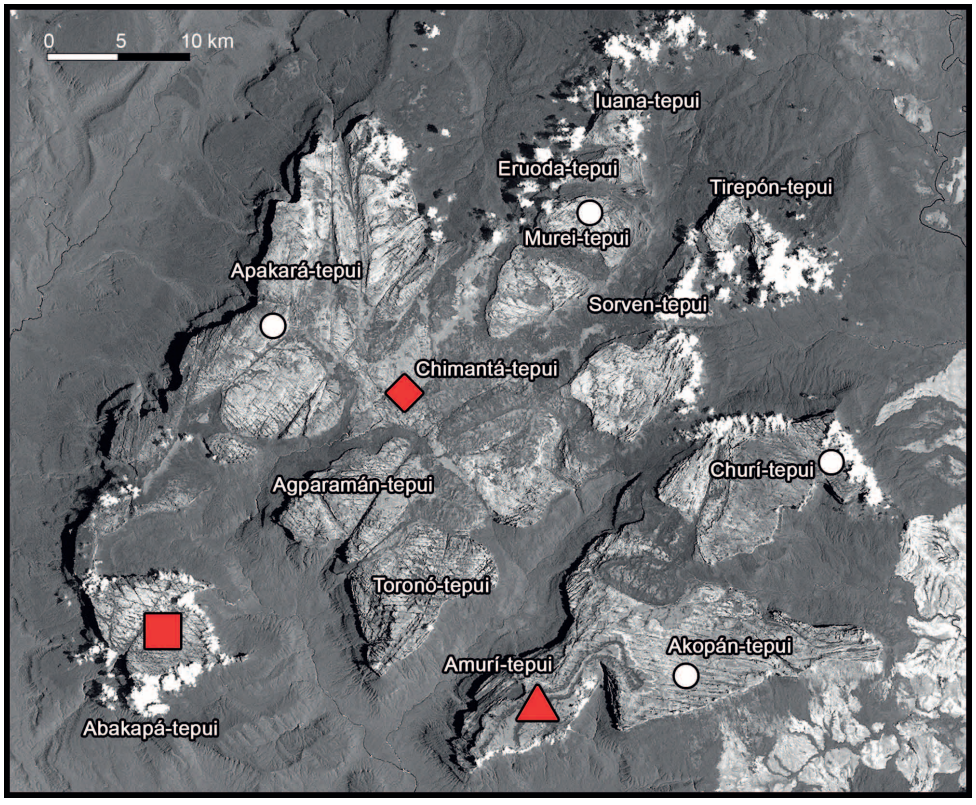

Figure 3. Map of the Chimantá Massif showing sampling localities of *Tepuihyla obscura* sp. n. (in red, diamond represents the type locality), and localities from the literature (white circles, as *T. edelcae* or *T.* aff. *edelcae*).

Table 1. Measurements of the type series of *Tepuihyla obscura* sp. n. Mean ± SD are followed by the range in parentheses. All measurements are in mm, except tooth counts.

Character	Holotype, Chimantá-tepui (male)	Males from Chimantá–tepui (paratypes) N = 8	Juvenile from Chimantá-tepui (paratype)	Males from Amurí–tepui (paratypes) N = 4	Subadult from Amurí-tepui (paratype)
SVL	34.56	35.05±1.62 (32.05–37.12)	21.67	30.24±3.18 (26.44–33.67)	23.56
HL	11.97	12.23±0.61 (11.21–13.31)	7.74	10.78±1 (9.62–11.89)	8.78
HW	12.15	12.17±0.45 (11.48–13.02)	7.73	10.56±0.92 (9.52–11.52)	8.46
EN	2.95	3.02±0.17 (2.63–3.16)	2.09	2.71±0.14 (2.53–2.86)	2.31
EL	4.06	3.96±0.21 (3.65–4.24)	2.80	3.73±0.33 (3.45–4.08)	3.22
TYM	2.68	2.82±0.23 (2.52–3.15)	1.43	2.46±0.27 (2.21–2.74)	1.65
IND	2.47	2.49±0.18 (2.14–2.7)	1.64	2.20±0.11 (2.12–2.35)	1.95
SL	4.85	5.00±0.31 (4.41–5.32)	3.30	4.45±0.36 (3.92–4.69)	3.77
HaL	10.25	11.31±0.84 (10.22–12.71)	6.76	9.32±0.97 (8.16–10.45)	7.61
FaL	6.84	6.77±0.15 (6.56–6.97)	4.29	6.12±0.37 (5.62–6.5)	4.57
FaB	3.08	2.88±0.23 (2.64–3.28)	1.35	2.15±0.29 (1.84–2.51)	1.77
THL	16.88	18.10±1 (16.68–19.64)	10.84	14.68±0.91 (13.4–15.55)	12.69
TiL	18.83	19.38±1.04 (17.85–20.91)	11.84	16.23±1.31 (14.74–17.75)	13.68
TaL	10.04	10.31±0.5 (9.63–10.94)	6.57	9.06±0.72 (8.07–9.6)	7.50
FL	13.59	14.25±0.76 (13.18–15.32)	8.91	10.99±1.26 (9.55–12.55)	9.30
WFD	1.72	1.77±0.15 (1.59–2.05)	1.08	1.24±0.18 (1.07–1.48)	1.03
WTD	1.49	1.52±0.13 (1.39–1.79)	0.89	1.01±0.17 (0.86–1.25)	0.81
Vomerine teeth left side	7	6.75±1.04 (5–8)	4	6.25±0.96 (5–7)	5
Vomerine teeth right side	5	7.25±1.39 (5–9)	4	6.75±0.5 (6–7)	5

Character	Females from Abakapá–tepui (paratypes) N = 4	Males from Abakapá–tepui (paratypes) N = 7	Subadults from Abakapá–tepui (paratypes) N = 4
SVL	34.55±2.77 (31.9–38.41)	29.49±2.26 (26.33–33.73)	21.47±1.84 (19.08–23.52)
HL	12.21±1.05 (11.4–13.67)	10.63±0.6 (9.78–11.6)	8.01±0.64 (7.19–8.65)
HW	12.08±1.14 (11.09–13.71)	10.68±0.53 (9.96–11.43)	7.94±0.52 (7.19–8.37)
EN	3.06±0.39 (2.72–3.54)	2.66±0.21 (2.37–2.99)	2.05±0.11 (1.9–2.15)
EL	4.00±0.17 (3.86–4.23)	3.58±0.25 (3.13–3.88)	2.71±0.25 (2.44–3.04)
TYM	2.44±0.19 (2.26–2.68)	2.31±0.16 (2.02–2.55)	1.43±0.14 (1.25–1.54)
IND	2.43±0.12 (2.26–2.55)	2.22±0.16 (2–2.39)	1.80±0.09 (1.72–1.92)
SL	5.02±0.54 (4.62–5.77)	4.45±0.28 (4.01–4.84)	3.38±0.22 (3.1–3.65)
HaL	10.77±1.06 (9.92–12.26)	9.29±0.88 (8.24–10.85)	6.64±0.6 (5.93–7.37)
FaL	6.72±0.54 (6.32–7.51)	5.82±0.5 (5.22–6.69)	4.29±0.31 (3.92–4.65)
FaB	2.18±0.34 (1.98–2.68)	2.29±0.21 (1.99–2.64)	1.36±0.15 (1.14–1.5)
THL	17.23±2.1 (15.62–20.32)	14.98±1.12 (13.7–16.56)	11.09±1.29 (9.21–12.1)
TiL	18.14±2.12 (16.56–21.25)	15.32±1.49 (13.48–17.87)	11.60±0.96 (10.45–12.64)
TaL	9.91±0.88 (9.41–11.22)	8.28±0.71 (7.31–9.55)	6.22±0.58 (5.53–6.82)
FL	13.47±1.82 (12–15.98)	11.36±1.15 (10.09–13.35)	8.10±0.77 (7.08–8.85)
WFD	1.66±0.27 (1.36–2.01)	1.31±0.06 (1.22–1.38)	0.96±0.11 (0.83–1.1)
WTD	1.38±0.24 (1.16–1.72)	1.16±0.07 (1.09–1.29)	0.83±0.09 (0.72–0.92)
Vomerine teeth left side	8±0.82 (7–9)	7±0.89 (6–8)	5±0.82 (4–6)
Vomerine teeth right side	8±2.58 (5–11)	7.29±1.25 (5–9)	6±1.41 (5–8)

Only calls that were not overlapping and clearly distinguishable from other calling males were analysed. Air temperature at the calling sites was measured with a Hanna digital pH/thermometer.

Statistical analyses

All statistical analyses were conducted in R 3.0.2 (R Core Team 2013). Normality and equal variances were tested with Shapiro-Wilk tests and Levene tests for each category separately. Because criteria for parametry of data were not fulfilled for a few variables, nonparametric Mann-Whitney Wilcoxon tests (aka Wilcoxon tests) were used for group comparisons. They were conducted for females and males separately. Subadults were excluded from the analyses, as the number of adults was sufficient and allometric growth likely.

A Principal Components Analysis (PCA) was performed using the package "ade4" for R (Chessel et al. 2004) in order to summarize the variation throughout males and females of *Tepuihyla edelcae*, *T*. aff. *edelcae*, and *T. rodriguezi*. Non-normality of a few variables was considered negligible because the PCA was used only for data summarisation and visualization and not for testing hypotheses. Also, the eigenvalues with PC scores were not used for further statistical tests. Because the dudi.pca function cannot handle missing data, the respective rows were excluded from PCA analyses. Compared to the full dataset, the number of *Tepuihyla* aff. *edelcae* was reduced by two and that of *T. edelcae* specimens by four. To exclude size bias, the residuals from a linear regression of the variables against SVL were used for group comparisons of the variables found to separate the species in the PCA. We considered the sample size of the female specimens too small to obtain reliable p-values, for which reason only male p-values are listed in Table 2. Tadpole measurements were summarized according to Gosner (1960) stages and only similar stages were compared to each other.

Molecular genetics

Choice of markers: For ease of comparison, the same mitochondrial gene fragments as used in Kok et al. (2012) were selected [i.e., 16S and subunit 1 of the NADH protein-coding gene (hereinafter ND1)]. We added two nuclear genes (RAG1 and CXCR4), totalling 2,404 base pairs (bp). New sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) under accession numbers (KT390931–KT391008).

DNA extraction, PCR, sequencing and sequence alignment: Tissue samples (thigh muscle, liver, tadpole fin) were taken in the field immediately after euthanisation and stored in 95% ethanol. Total genomic DNA was extracted and purified using the Qiagen DNeasy® Tissue Kit as per manufacturer's instructions. Fragments of the mitochondrial ribosomal gene 16S (ca 550 bp), the protein-coding mitochondrial gene NADH dehydrogenase subunit 1 (ND1, ca 650 bp), and the nuclear recombination activating gene 1 (RAG1, ca 550 bp) and C-X-C chemokine receptor type 4 gene (CXCR4, ca 625 bp) were amplified and sequenced using the primers listed in Kok et al. (2012) and Biju & Bossuyt (2003) under previously described PCR conditions (Biju & Bossuyt 2003, Roelants et al. 2007, Van Bocxlaer et al. 2010). PCR products were checked on a 1% agarose gel and either purified with the Qiagen PCR purification kit as per manufacturer's instructions and sequenced on both strands using the BigDye cycle sequencing kit (Applied Biosystems) on an ABI 3100 automated sequencer, or sent to BaseClear (Leyden, The Netherlands) for purification and sequencing. Chromatograms were read with CodonCode Aligner 5.0.2 (http://www.codoncode.com/index.htm) and a consensus sequence was assembled from the forward and reverse primer sequences. MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) was used to perform the preliminary alignment with G-INS-i and default parameters. Minor alignment corrections were made with MacClade 4.08 (Maddison & Maddison 2005). Protein-coding sequences were translated into amino-acid sequences to check for unexpected stop codons that would indicate the presence of pseudogenes. When present, ambiguous regions were excluded from subsequent analyses.

Molecular phylogenetic analyses: Uncorrected pairwise distances were estimated using PAUP* 4.0a136 for Macintosh (Swofford 2002) (Table 3). *Osteocephalus oophagus* was used as outgroup taxon. Our phylogenetic analysis includes all available species of *Tepuihyla* except *T. aecii*. We did not include *T. aecii* because (1) we could only use a small fragment of 16S in our alignment; (2) including *T. aecii* lowers the node support in multigene approaches (see Jungfer et al 2013, Salerno et al 2014); and (3) all *Tepuihyla* phylogenies (including *T. aecii* or not) show the non-monophyly of *T. edelcae* and *T.* aff. *edelcae*. Therefore, regardless of the putative phylogenetic position of *T. aecii*, *T. edelcae* and *T.* aff. *edelcae* would nevertheless remain paraphyletic (see Jungfer et al 2013, Salerno et al 2014). Maximum Likelihood (ML) analyses were conducted in RAxML 7.6.6 (Stamatakis 2006) on the CIPRES Science Gateway V 3.3 (https://www.phylo.org/, Miller et al. 2010) for the concatenated 4-gene dataset; nodal bootstrap values (Felsenstein 1985) for the ML analysis were calculated using 1,000 pseudoreplicates under the GTRCAT model (Stamatakis et al. 2008). Clade credibility was also estimated by Bayesian posterior probabilities (BPP) in MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway V 3.3. The Bayesian analyses implemented a mixed general time-reversible model (GTR + G + I) partitioned over the different gene fragments, flat Dirichlet priors for base frequencies and substitution rate matrices, and uniform priors for among-site rate parameters. Two parallel Markov chain Monte Carlo (MCMC) runs of four incrementally heated (temperature parameter = 0.2) chains were performed, with a length of 50,000,000 generations, a sampling frequency of 1 per 1,000 generations, and a burn-in corresponding to the first 5,000,000 generations. Convergence of the parallel runs

Table 2. Test statistics and p-values of the pairwise Wilcoxon tests on measurements of males. Significant values are indicated in **bold**.

Variable	*T. obscura* sp. n. vs. *T. edelcae*	*T. obscura* sp. n. vs. *T. rodriguezi*	*T. edelcae* vs. *T. rodriguezi*
SVL	**W = 22, P = 0.00039**	**W = 110, P = 0.0013**	W = 136, P = 0.85
EL	**W = 4, P = 3.3e-05**	W = 322, P = 0.087	**W = 253, P = 1.5e-05**
HL	**W = 19, P = 0.00019**	W = 164, P = 0.058	**W = 190.5, P = 0.034**
HW	**W = 23.5, P = 0.0011**	W = 188.5, P = 0.18	W = 185.5, P = 0.052
EN	**W = 35, P = 0.0063**	**W = 30.5, P = 6.9e-07**	**W = 36, P = 0.00096**
FAL	**W = 7, P = 4.5e-06**	W = 251, P = 0.94	**W = 237, P = 0.00017**
FAB	**W = 46.5, P = 0.028**	W = 326, P = 0.07	**W = 225, P= 0.00084**
WFD	W = 63.5, P = 0.16	W = 315.5, P = 0.12	**W = 211.5, P = 0.0042**
WTD	W = 73, P = 0.32	**W = 347, P = 0.022**	**W = 216.5, P = 0.0024**
IND	**W = 25.5, P = 0.0015**	W = 223, P = 0.59	**W = 191, P = 0.033**
TYM	**W = 36.5, P = 0.0078**	**W = 120, P = 0.0036**	W = 100.5, P = 0.31
HAL	**W = 23, P = 0.0010**	W = 167.5, P = 0.069	W = 183, P = 0.064
THL	**W = 31.5, P = 0.0038**	**W = 134, P = 0.0088**	W = 109, P = 0.48
TIL	**W = 40, P = 0.011**	**W = 156, P = 0.038**	W = 143.5, P = 0.65
TAL	**W = 26.5, P = 0.0018**	**W = 156, P = 0.038**	W = 180, P = 0.080
FL	W = 53.5, P = 0.06	**W = 148.5, P = 0.024**	W = 139, P = 0.76
	Residuals *T. obscura* sp. n. vs. *T. edelcae*	Residuals *T. obscura* sp. n. vs. *T. rodriguezi*	Residuals *T. edelcae* vs. *T. rodriguezi*
EL	**W = 7, P = 4.5e-06**	**W = 449, P = 4.0e-07**	**W = 260, P = 7.9e-09**
HL	W = 52, P = 0.05	**W = 402, P = 0.00022**	**W = 229, P = 0.00019**
HW	W = 70, P = 0.27	**W = 460, P = 5.3e-08**	**W = 239, P = 2.4e-05**
EN	W = 124, P = 0.19	**W = 52, P = 1.3e-06**	**W = 23, P = 3.8e-05**
FAL	**W = 44, P = 0.017**	W = 416, P = 4.6e-05	W = 244, P = 6.8e-06
FAB	W = 78, P = 0.46	**W = 465, P = 1.9e-08**	**W = 247, P = 2.9e-06**
WFD	W = 98, P = 0.91	**W = 413, P = 6.5e-05**	**W = 228, P = 0.00023**
WTD	W = 127, P = 0.15	**W = 440, P = 1.7e-06**	**W = 223, P = 0.00056**
IND	W = 67, P = 0.21	**W = 350, P = 0.017**	**W = 206, P = 0.0062**
TYM	W = 132, P = 0.094	W = 220, P = 0.55	W = 91, P = 0.18
HAL	W = 67, P = 0.21	**W = 340, P = 0.033**	**W = 202, P = 0.0099**
THL	W = 122, P = 0.23	W = 321, P = 0.091	W = 127, P = 0.93
TIL	W = 130, P = 0.11	**W = 392, P = 0.00060**	W = 166, P = 0.21
TAL	W = 72, P = 0.31	**W = 337, P = 0.039**	**W = 196, P = 0.019**
FL	W = 120, P = 0.27	**W = 335, P = 0.044**	W = 131, P = 0.99

was confirmed by split frequency SDs (< 0.01) and potential scale reduction factors (~ 1.0) for all model parameters, as reported by MrBayes. All analyses were checked for convergence by plotting the log-likelihood values against generation time for each run using Tracer 1.5 (Rambaut & Drummond 2009). RaxML and MrBayes trees were edited and manipulated with FigTree 1.3.1 (Rambaut 2009).

Species concept

The concept of species as metapopulations following separate evolutionary trajectories is – implicitly or explicitly – fundamental to all contemporary species concepts (De Queiroz 1998, 2007). Under this general lineage species concept, the only compulsory requirement of a population of individuals for being considered a species is to be part of a single evolving lineage, while secondary criteria like morphology, reproductive isolation, or ecological niche occupation can be part of a set of additional evidence factors for the recognition of such a lineage (De Queiroz 2007). In addition to this prerequisite, we concur that "good taxonomical practices" should employ multiple lines of evidence for species delineation, i.e., apply so-called "integrative taxonomy" (e.g., Dayrat 2005, DeSalle et al. 2005, Padial et al. 2010). Genetic evidence in-

Table 3. Uncorrected pairwise distances in 16S (above diagonal) and in ND1 (below diagonal) between *Tepuihyla* species/populations from tepui summits and uplands in the eastern Pantepui Region.

	T. edelcae Auyán-tepui	*T. edelcae* Cerro El Sol	*T. obscura* sp. n. Abakapá-tepui	*T. obscura* sp. n. Amurí-tepui	*T. obscura* sp. n. Chimantá-tepui	*T. "rimarum"* Ptari-tepui	*T. rodriguezi* Gran Sabana	*T. rodriguezi* Ayangaik	*T. rodriguezi* Kaieteur	*T. rodriguezi* Guadacapi-apu-tepui	*T. rodriguezi* Wei-Assi-pu-tepui	*T. rodriguezi* Uei-tepui	*T. exophthalma* Kaieteur	*T. warreni* Maringma-tepui	*O. oophagus*
T. edelcae Auyán-tepui		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.08
T. edelcae Cerro El Sol	0.00		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.06	0.08
T. obscura sp. n. bakapá-tepui	0.04	0.04		0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.08
T. obscura sp. n. murí-tepui	0.04	0.04	0.00		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.05	0.08
T. obscura sp. n. Chimantá-tepui	0.04	0.04	0.00	0.01		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.05	0.08
T. "rimarum" Ptari-tepui	0.05	0.05	0.05	0.05	0.05		0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.08
T. rodriguezi Gran Sabana	0.05	0.05	0.05	0.05	0.05	0.00		0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.08
T. rodriguezi Ayangaik	0.05	0.05	0.05	0.05	0.05	0.00	0.00		0.00	0.00	0.00	0.00	0.05	0.05	0.08
T. rodriguezi Kaieteur	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01		0.00	0.00	0.00	0.05	0.06	0.08
T. rodriguezi Guadacapiapu-tepui	0.05	0.05	0.05	0.05	0.05	0.01	0.00	0.01	0.01		0.00	0.00	0.05	0.05	0.08
T. rodriguezi Wei-Assipu-tepui	0.05	0.05	0.05	0.05	0.05	0.01	0.00	0.01	0.01	0.00		0.00	0.05	0.05	0.08
T. rodriguezi Uei-tepui	0.05	0.05	0.05	0.05	0.05	0.01	0.00	0.01	0.01	0.00	0.00		0.05	0.06	0.08
T. exophthalma Kaieteur	0.13	0.12	0.13	0.12	0.12	0.13	0.14	0.14	0.14	0.14	0.14	0.14		0.06	0.06
T. warreni Maringma-tepui	0.13	0.13	0.13	0.12	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.14		0.08
O. oophagus	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.19	0.18	0.19	0.19	0.19	0.19	0.19	

dicating candidate species status should be congruent with additional species delineation criteria such as morphology, bioacoustics, ecology, phylogeography, or any other indication of evolutionary distinctiveness. However, additional criteria other than molecular phylogenetic relationships and geographic range may be sometimes difficult to detect or even be absent (e.g., in non-adaptive radiations, see Discussion).

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: BD94A6FC-F648-444A-AE11-DCF89CC9E160. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: www.salamandrajournal.com.

Results

Our multidisciplinary analysis leads us to the conclusion that populations of *Tepuihyla* aff. *edelcae*, although morphologically almost identical to *T. edelcae*, form a distinct evolutionary unit, and should thus better be named as a new species, which is hereafter described. Lines of evidence supporting our hypothesis are provided following the description.

Tepuihyla obscura sp. n.
(Figs 5–7, 13–14; Tables 1, 6–7)

ZooBank LSID: urn:lsid:zoobank.org:act: 7243439C-B1C7-4A4C-9811-B7F92BEFB612.
Ololygon sp. – Gorzula 1992: 269.
Osteocephalus edelcae – Ayarzagüena et al. 1993a: 122.
Tepuihyla edelcae (partim) – Aubrecht et al. 2012: 141, Ayarzagüena et al. 1993b: 215, Barrio-Amorós 1998: 38, 2004: 18, Gorzula & Señaris 1998: 49, McDiarmid & Donnelly 2005: 490, Myers & Donnelly 2008: 60, Salerno et al. 2012: 3, 2014: 315, Señaris et al. 2014: 192.
Tepuihyla aff. "*edelcae*" – Kok et al. 2012: 14 (supplement), Jungfer et al. 2013: 7.
Tepuihyla cf. *edelcae* – Salerno et al. 2014: 322.

Holotype: IRSNB 4192, an adult male collected by PJRK on 17 Nov. 2013 at 21:00 h on the summit of Chimantá-tepui (5°19'27" N, 62°12'10" W, 2,224 m a.s.l.).

Paratypes (N = 29): Fifteen specimens from the summit of Abakapá-tepui (5°11'24" N, 62°17'49" W, ca 2,172 m a.s.l.) collected between 2 and 10 May 2011 by PJRK: IRSNB 4166, IRSNB 4170–71, IRSNB 4172–74, IRSNB 4176 (males); IRSNB 4169, IRSNB 4175, IRSNB 4179, IRSNB 4180 (females); IRSNB 4167–68, IRSNB 4177–78 (subadults); five specimens from the summit of Amurí-tepui (5°08'36" N, 62°07'10" W, ca 2,209 m a.s.l.) collected between 11 and 13 June 2012 by PJRK: IRSNB 4181, IRSNB 4182–83, IRSNB 4185 (males); IRSNB 4184 (subadult); and nine specimens from the type locality collected between 14 and 19 Nov. 2013 by PJRK and DBM: IRSNB 4187–90, IRSNB 4186, IRSNB 4191, IRSNB 4193, IRSNB 4195 (males); IRSNB 4194 (juvenile).

Etymology: The specific epithet derives from Latin "*obscurus*" meaning "hidden" or "indistinct" in reference to the cryptic nature of the new species.

Definition and diagnosis: A species of *Tepuihyla* characterized by the following combination of characters (details about colouration refer to specimens in life): (1) medium size, max SVL in males 37.1 (26.3–37.1) mm, max SVL in females 38.4 (31.9–38.4) mm; (2) head approximately as wide as long; (3) diameter of eye greater than distance from nostril to eye, ratio of EL/EN = 1.34 ± 0.09 (1.16–1.47); (4) diameter of tympanum 50–75% of the diameter of eye; (5) vomerine odontophores oblique, located between large choanae; (6) number of vomerine teeth on each odontophore 5–11; (7) skin on dorsum smooth in females, with scattered, fine, white-tipped spicules in males; (8) skin on flanks smooth to faintly granular; (9) skin on belly coarsely areolate; (10) pale labial stripe present; (11) dark band or stripe from nostril to eye usually conspicuous; (12) dorsal ground colour variable, from pale grey to dark brown, usually suffused with small to minute dark brown or black markings; (13) no transverse bars on limbs; (14) rear of thighs patternless; (15) heel extending to immediately before anterior edge of eye to between eye and nostril; (16) row of ulnar tubercles absent or inconspicuous; (17) toes approximately one-half webbed; (18) distal subarticular tubercle on fourth toe distinct, simple or bifid; palmar tubercle variable in size and shape, simple, bifid, or heart-shaped, its proximal edge usually poorly distinct; (19) outer metatarsal tubercle small, rounded, prominent; (20) axillary membrane present but poorly developed; (21) supratympanic fold present; (22) breeding males with conspicuous, usually black nuptial pads extending beyond thenar tubercle; (23) iris dark brown to copper with gold flecks and sometimes fine dark brown reticulation; (24) limb bones white.

Among the known *Tepuihyla* species distributed east of the Rio Caroní, *T. obscura* sp. n. is readily distinguished from *T. exophthalma*, *T. rodriguezi* (including the latter's junior synonym *T. rimarum*, see below), and *T. warreni* by lacking transverse bars on its limbs (always present in *T. exophthalma*, *T. rodriguezi*, and *T. warreni*, even if sometimes poorly marked, Figs 4a–b, g–h vs. Fig. 5). It also dif-

Figure 4. *Tepuihyla* species known to occur east of the Rio Caroní in Venezuela and Guyana. a) *T. warreni*, IRSNB 15863, male from the southeastern slope of Maringma-tepui, Guyana; b) *T. exophthalma*, IRSNB 14644, female, from Kaieteur National Park, Guyana; c) *T. edelcae*, IRSNB 16121, female, from Auyán-tepui, Venezuela; d) *T. edelcae*, IRSNB 16124, male, from Auyán-tepui, Venezuela; e) *T. edelcae*, IRSNB 16131, female, from Auyán-tepui, Venezuela; f) *T. edelcae*, IRSNB 16133, female, from Auyán-tepui, Venezuela; g) *T. rodriguezi*, IRSNB 15658, male, from La Gran Sabana, Venezuela; h) *T. rodriguezi*, IRSNB 16181, female, from La Gran Sabana, Venezuela. Photos: PJRK.

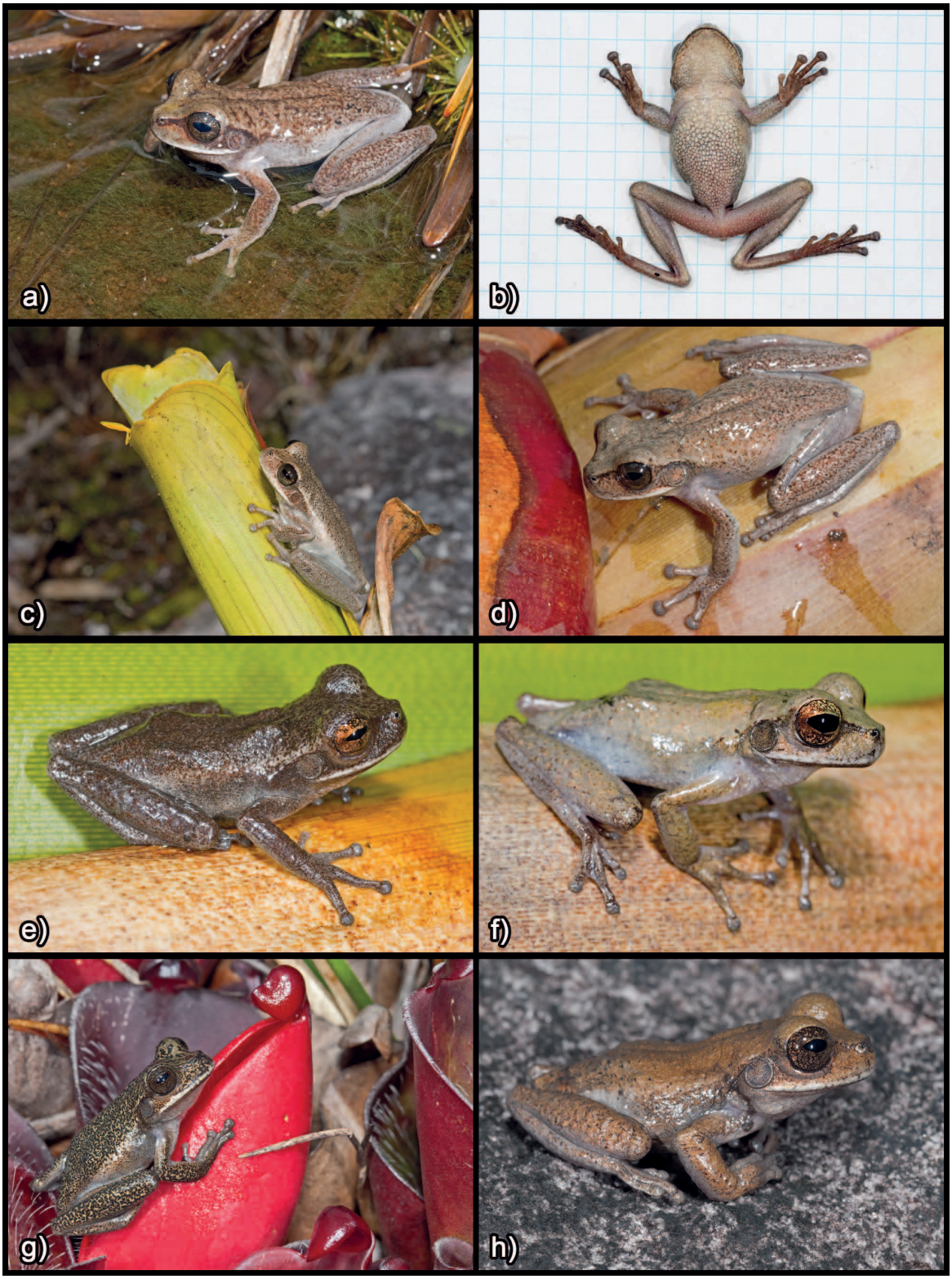

Figure 5. *Tepuihyla obscura* sp. n. a) IRSNB 4192, male holotype from Chimantá-tepui, Venezuela; b) ventral face of IRSNB 4191, male paratype from Chimantá-tepui, Venezuela; c) IRSNB 4187, male paratype from Chimantá-tepui, Venezuela; d) IRSNB 4191, male paratype from Chimantá-tepui, Venezuela; e) IRSNB 4181, male paratype from Amurí-tepui, Venezuela; f) IRSNB 4182, male paratype from Amurí-tepui, Venezuela; g) IRSNB 4170, male paratype from Abakapá-tepui, Venezuela; h) IRSNB 4174, male paratype from Abakapá-tepui, Venezuela. Photos: PJRK.

fers notably from *T. exophthalma* by its skin texture on flanks (smooth to faintly granular in *T. obscura* sp. n. vs. areolate in *T. exophthalma*, Fig. 4b vs. Fig. 5), and in having white limb bones (vs. green in *T. exophthalma*); from *T. rodriguezi* by having different head proportions, including larger eyes and a shorter eye–naris distance [EL/EN = 1.34 ± 0.09 (1.16–1.47; N = 30) in *T. obscura* sp. n. vs. EL/EN = 0.98 ± 0.14 (0.77–1.31; N = 35) in *T. rodriguezi*, Figs 4g–h vs. Fig. 5]; from *T. warreni* by the dorsal skin texture in males (finely spiculated in *T. obscura* sp. n. vs. strongly granular in *T. warreni*, Fig. 4a vs. Fig. 5), its dorsal colour pattern (pale grey to dark brown, usually suffused with small to minute dark brown or black markings in *T. obscura* sp. n. vs. greenish grey with brown blotches in *T. warreni*, Fig. 4a vs. Fig. 5), and the iris colouration in life (dark brown to copper with gold flecks and sometimes fine dark brown reticulation in *T. obscura* sp. n. vs. yellowish green in *T. warreni*, Fig. 4a vs. Fig. 5). The new species is morphologically most similar to *T. edelcae* (Figs 4c–f), from which it can be distinguished by rather subtle characters, such as a smaller SVL, especially in females (max. 37.1 mm SVL [26.3–37.1 mm] in males and max. 38.4 mm SVL [31.9–38.4 mm] in females vs. max. 41.5 mm SVL [32.0–41.5 mm] in males and 50.5 mm SVL [38.5–50.5 mm] in females *T. edelcae* [Myers & Donnelly 2008 and pers. obs.]), and by always lacking any yellow or pale orange colouration on its body and limbs (usually present in *T. edelcae*, Figs 4c–f vs. Fig. 5).

Tepuihyla aecii and *T. luteolabris* are distributed west of the Rio Caroní, and can be distinguished from *T. obscura* sp. n. mainly by lacking an axillary membrane (according to the original descriptions, Ayarzagüena et al. 1993a; axillary membrane poorly developed but present in *T. obscura* sp. n.). *Tepuihyla aecii* furthermore differs from the new species by having EL > SL (EL < SL in *T. obscura* sp. n.). *Tepuihyla luteolabris* furthermore differs from the new species in having a distinctly larger SVL [max. 37.1 mm SVL (26.3–37.1 mm) in males and max. 38.4 mm SVL (31.9–38.4 mm) in females *T. obscura* sp. n. vs. max. 42.8 mm SVL (36.8–42.8 mm) in males and 59.2 mm SVL (52.0–59.2 mm) in females of *T. luteolabris*], and by having a granular dorsal skin, especially on the head (smooth to finely spiculate in *T. obscura* sp. n.).

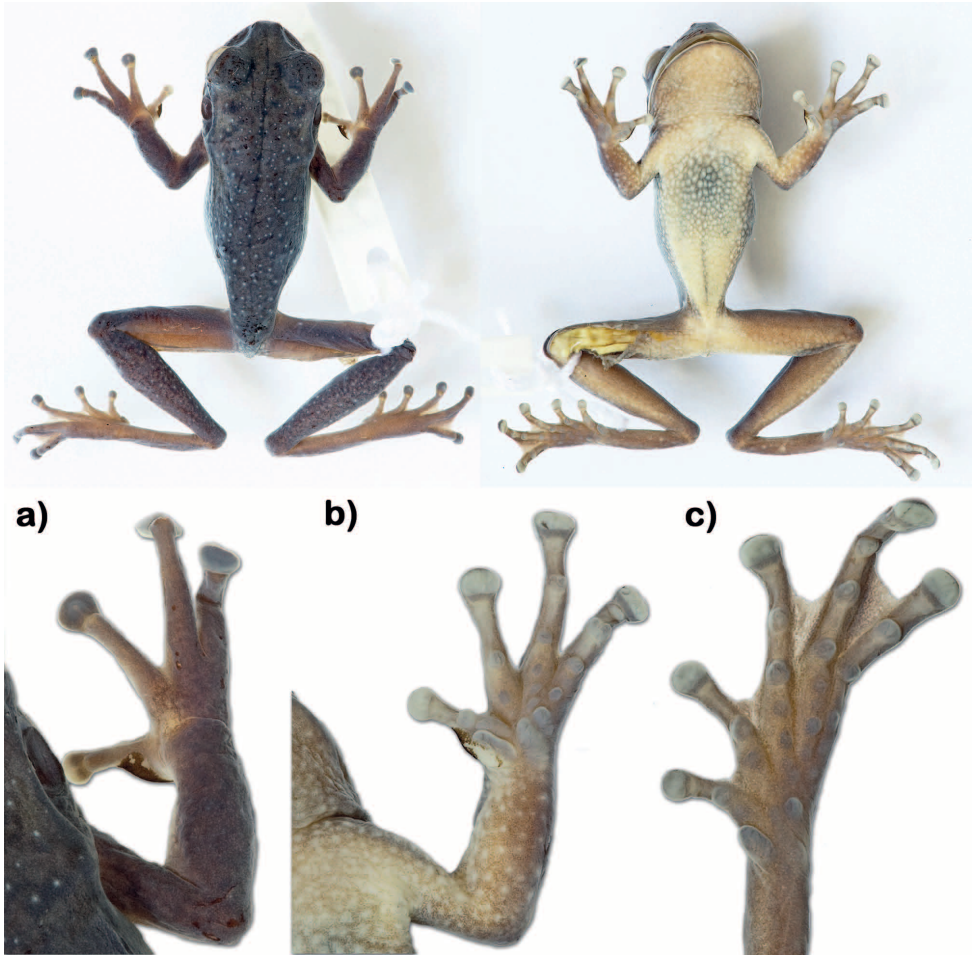

Figure 6. *Tepuihyla obscura* sp. n., preserved male holotype (IRSNB 4192). Upper left, dorsal face. Upper right, ventral face. a) dorsal view of right hand; b) ventral view of left hand; c) ventral view of left foot. Note enlarged forearm, black nuptial pads, and white-tipped spicules on back. Photos: PJRK.

Description of the holotype: An adult male (collected while calling, Figs 5a, 6), 34.6 mm SVL, in very good condition except for an incision in the ventral part of the right thigh where a piece of muscle was removed. Head slightly wider than long, wider than body; head width 35.2% of SVL; head length 34.6% of SVL; snout truncate in dorsal view, bluntly rounded in profile; canthus rostralis distinct, concave, rounded in section; loreal region concave, slightly granular; lips rounded; internarial region slightly depressed; nostrils protuberant, orientated posterolaterally. Top of head from nostrils to anterior third of eyeballs slightly concave, posteriorly slightly convex, lateral edges of frontoparietals inconspicuous; IOD 76% of width of upper eyelid; diameter of eye greater than distance from nostril to eye, ratio of EL/EN = 1.38; tympanum circular, 66% of ED, separated from eye by a distance of about one-third of the length of the tympanum; tympanic annulus distinct, smooth; supratympanic fold conspicuous, covering upper edge of tympanum, extending to a point above arm insertion. Granular skin fold anterodorsal to forelimb insertion.

Axillary membrane poorly developed, extending for about one-fifth of the length of the upper arm; forearm robust; breadth of forearm 45% of length of forearm; row of ulnar tubercles present, but inconspicuous; fingers moderately short, basally webbed between Fingers II and III; relative length of adpressed fingers III > IV > II > I; discs large, rounded to slightly truncate; width of disc on Finger III smaller than one-half the diameter of the tympanum. Subarticular tubercles moderately small, rounded, elevated; distal subarticular tubercle on Finger IV larger, simple; supernumerary tubercles small, indistinct, present only on proximal segments of Fingers II–IV; palmar tubercle bifid, roughly heart-shaped, its proximal edge poorly distinct; thenar tubercle large, kidney-shaped, pointing towards the middle of the palmar tubercle; black nuptial pads extending ventrally beyond the thenar tubercle; small flap-like dermal fold above wrist (Fig. 6).

Hind limb slender; tibia length 54% of SVL; foot length 39% of SVL; when hind limb is adpressed anteriorly along the side of body, the heel will extend to immediately before anterior edge of eye; heels distinctly overlap when hind limbs are flexed at right angles to sagittal plane. Inner tarsal fold absent; inner metatarsal tubercle large, ovoid, elevated and projecting; outer metatarsal tubercle rounded, much smaller, inconspicuous; relative length of toes IV > V > III > II > I; toes about one-half webbed; webbing formula **I**2^{-}–3^{-}**II**1$^{1/3}$–3**III**1^{½}– 3**IV**2–1^{+}**V**; subarticular tubercles moderately small, subconical; supernumerary tubercles small, rounded, moderately elevated (Fig. 6).

Skin on dorsum, posterior part of head, and limbs smooth with scattered, white-tipped, minute spicules; skin on flanks smooth; skin on throat, chest, belly, and ventral faces of thighs and forearms coarsely areolate. Cloacal opening directed ventrally at midlevel of thighs; cloacal sheath long; large conical tubercles below cloacal opening and on proximal posteroventral faces of thighs (Fig. 6).

Tongue broadly cordiform, shallowly notched posteriorly, barely free behind.

Vomerine odontophores large, elevated, with seven teeth on the left and five on the right side; odontophores oblique, located posteromedially to the elliptical choanae, separated by a gap of ca one third of their size and orientated towards each other in a wide angle of approximately 130°; lateral edges of odontophores in line with medial edge of the choanae.

Vocal slits elongated, located anteriorly to the insertion of the M. adductor mandibulae in a fold parallel to the dental bone. Vocal sac not apparent.

Colour of holotype in life (Fig. 5a): Dorsally light brown with irregular, small, dark brown markings and a fine dark brown median stripe. Dark brown markings more numerous posteriorly and on limbs (except upper thighs). Dark brown band extending from nostril to anterior edge of eye, and from posterior edge of eye to about midway before arm insertion (along supratympanic fold). White labial stripe. A fine black line marks the contours of the lower jaw ventrally. Tympanum brown, speckled with dark brown. Flanks, dorsal face of thighs, and cloacal region light silvery grey; a few minute, irregular, brown speckles on flanks and upper thighs. Rear face of thighs light grey, patternless. Ventral face of body creamy white. Ventral face of limbs light grey, reddish on lower thighs. Finger and toe discs dark grey; nuptial pads black. Limb bones white. Iris dark brown with gold flecks and some fine dark brown reticulation.

Colour of holotype in preservative (Fig. 6): Dorsal colour pattern brownish grey with irregular small dark brown markings and a very fine dark brown median stripe. Dark brown band extending from nostril to eye, and from tympanum to a point before arm insertion (along supratympanic fold). White labial stripe. A fine dark grey line marks the contours of the lower jaw ventrally. Tympanum dark brown and speckled with black. Flanks and cloacal region light grey. Ventral face creamy white. Dorsal face of limbs grey, irregularly suffused with dark speckles, rear face of thighs brown, patternless. Ventral face of limbs light brown, becoming cream proximally. Finger and toe discs grey; nuptial pads black.

Variation among paratypes, sexual dimorphism, and juvenile coloration (Figs 5–7): Snout–vent length in adult paratypes varies from 31.9–38.4 mm in females, and 26.3–37.1 mm in males. Head length is always about as long as wide, either slightly longer than wide, or slightly wider than long.

There is considerable variation in the shape and size of the vomerine odontophores. The gap between odontophores is small in some specimens, and the angle between them can reach 180° (e.g., in IRSNB 4180). The odontophores can laterally exceed the medial edge of the choanae up to their midpoint. The number of vomerine teeth in adult specimens varies from 5–11 per odontophore.

Sexual dimorphism is conspicuous in skin texture and forearm breadth, and usually in SVL: females are often larger, with smooth dorsal skin vs. heavily spiculate in males (although some males have smaller granules less densely packed); forearms are slightly more robust in males. Colour and shape of the nuptial pads varies among male para-

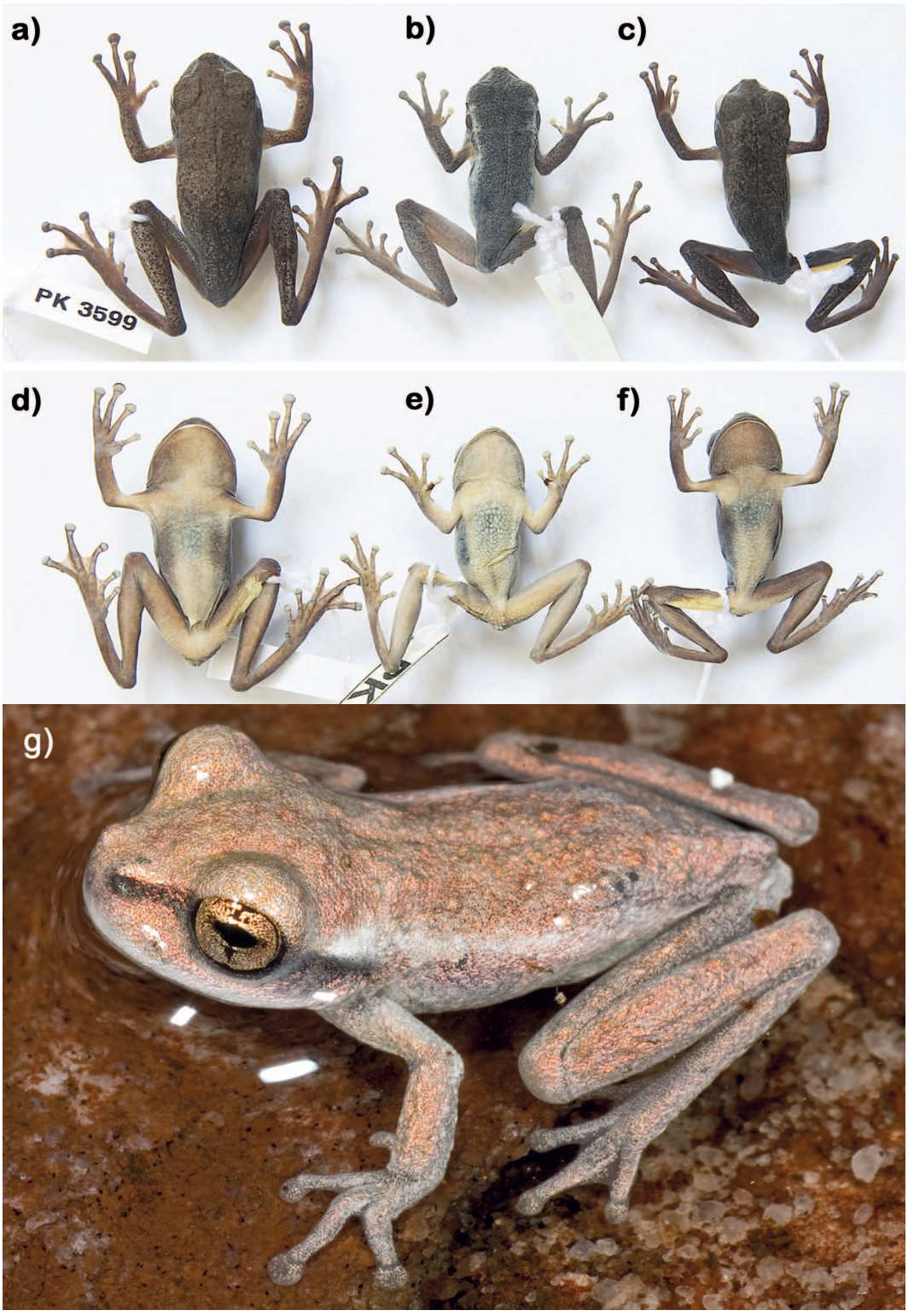

Figure 7. *Tepuihyla obscura* sp. n., variation in paratypes, and juvenile colour pattern. a) dorsal view of IRSNB 4180, female from Abakapá-tepui; b) dorsal view of IRSNB 4187, male from Chimantá-tepui; c) dorsal view of IRSNB 4175, female from Abakapá-tepui; d) ventral view of IRSNB 4180, female from Abakapá-tepui; e) ventral view of IRSNB 4187, male from Chimantá-tepui; f) ventral view of IRSNB 4175, female from Abakapá-tepui; g) IRSNB 16183, juvenile (ca 15 mm SVL) in life. Note enlarged forearm, black nuptial pads, and white-tipped spicules on the back in the male; the poorly developed axillary membrane (best visible in [a] and [c]); and the tubercular dorsal skin in the juvenile (g). Photos: PJRK.

Table 4. Eigenvalues of Principal Components for females and males of *Tepuihyla obscura* sp. n. and *T. edelcae*.

	Females of *T. obscura* sp. n., *T. edelcae*			Males of *T. obscura* sp. n., *T. edelcae*		
Variable	PC1	PC2	PC3	PC1	PC2	PC3
SVL	0.983	0.010	0.091	-0.969	-0.098	-0.02
HL	0.994	-0.080	0.014	-0.981	-0.064	-0.036
HW	0.995	0.057	-0.073	-0.973	-0.048	-0.072
EN	0.952	-0.243	-0.121	-0.899	-0.094	0.334
EL	0.837	0.362	0.393	-0.824	-0.393	-0.352
TYM	0.947	-0.244	0.170	-0.902	-0.081	-0.219
IND	0.929	0.213	-0.047	-0.890	-0.180	0.210
SL	0.976	-0.137	-0.065	-0.935	-0.056	0.165
HaL	0.991	-0.007	-0.105	-0.930	-0.089	-0.144
FaL	0.961	-0.204	0.127	-0.919	-0.182	0.084
FaB	0.930	0.222	-0.053	-0.883	0.119	0.051
THL	0.988	0.031	-0.016	-0.960	0.098	0.005
TiL	0.991	-0.044	0.076	-0.971	0.051	0.073
Tal	0.972	-0.152	0.100	-0.951	-0.043	0.076
FL	0.989	-0.019	-0.048	-0.951	0.189	-0.002
WFD	0.926	0.228	-0.263	-0.885	0.397	-0.066
WTD	0.983	0.075	-0.135	-0.831	0.496	-0.127

Table 5. Eigenvalues of Principal Components for females and males of *Tepuihyla obscura* sp. n., *T. edelcae* and *T. rodriguezi*.

	Females, complete dataset			Males, complete dataset		
Variable	PC1	PC2	PC3	PC1	PC2	PC3
SVL	-0.980	-0.135	-0.054	-0.915	0.284	-0.059
HL	-0.992	-0.043	-0.065	-0.978	0.029	0.047
HW	-0.994	-0.044	-0.003	-0.965	-0.086	-0.013
EN	-0.888	-0.388	0.066	-0.530	0.754	-0.026
EL	-0.598	0.722	-0.282	-0.704	-0.505	-0.223
TYM	-0.874	-0.089	-0.238	-0.836	0.328	0.076
IND	-0.939	0.070	-0.184	-0.744	-0.085	-0.546
SL	-0.976	-0.088	-0.038	-0.911	0.155	-0.186
HaL	-0.972	-0.187	0.019	-0.808	0.042	-0.261
FaL	-0.957	0.041	0.019	-0.858	-0.202	0.194
FaB	-0.840	0.412	0.006	-0.759	-0.498	-0.052
THL	-0.975	-0.186	-0.016	-0.863	0.195	0.291
TiL	-0.895	-0.070	0.175	-0.875	0.160	0.245
Tal	-0.969	-0.117	-0.090	-0.917	0.149	-0.063
FL	-0.979	-0.176	0.017	-0.931	0.191	0.096
WFD	-0.835	0.348	0.300	-0.732	-0.472	0.188
WTD	-0.845	0.312	0.337	-0.692	-0.520	0.218

types from black, brown, white to inconspicuous (e.g., in the specimens from Amurí-tepui). This might reflect seasonality in sexual activity.

The palmar tubercle is bifid in most paratypes, but sometimes simple (IRSNB 4166–67), or bifid on one and simple on the other hand (IRSNB 4172, IRSNB 4191). Distal subarticular tubercle on Finger III bifid in only one specimen (IRSNB 4186), distal subarticular tubercle on Finger IV slightly bifid on one hand in IRSNB 4189, and bifid on both hands in four of the 29 paratypes (IRSNB 4184, IRSNB 4190, IRSNB 4186, IRSNB 4194).

Webbing formula of paratypes **I**2^{-}–(3-3^{-})**II**$1^{1/2}$–(3-$2^{2/3}$)**III**($1^{2/3}$-$1^{1/2}$)–(3-$2^{1/2}$)**IV**(2^{+}-2)–($1^{1/2}$-1^{+})**V** in females, and **I**(2-$1^{2/3}$)–(3^{+}-$2^{1/3}$)**II**(2^{-}-1^{-})–($3^{1/3}$-$2^{1/2}$)**III**($2^{1/3}$-$1^{1/2}$)–(3^{-}-$2^{1/2}$)**IV**($2^{1/2}$-2^{-})–($1^{2/3}$-1^{-})**V** in males.

Colour pattern in life and in preservative varies among localities. Colour pattern of paratopotypes very similar to the holotype. The dark markings, including the facial band, are reduced in some individuals. In IRSNB 4187 and IRSNB 4194, the silvery grey colouration of the flanks extends dorsally and anteriorly beyond the tympanum to the posterior corner of the eyes, forming contrasting stripes in dorsal view. Paratypes from Abakapá-tepui are copper brown to greyish brown dorsally, with the black markings being numerous and distinct in most specimens and becoming more numerous posteriorly. Some specimens, e.g., IRSNB 4169–70, have an almost reticulated dorsal pattern. The dark facial band is sometimes less distinct and extends to the dark grey flanks. Many males (except IRSNB 4166, IRSNB 4173) and some subadults have contrasting light grey or pale cream stripes dorsal of the dark lateral band. Specimens from Amurí-tepui have brown dorsa and limbs; the dark markings are often less clearly distinct.

The colour pattern of juveniles is similar to that of adults, although usually lighter and less dense; juveniles have a tubercular dorsal skin (Fig. 7g).

Morphometric comparisons: The PCA including data from *Tepuihyla obscura* sp. n. and *T. edelcae* (Figs 8a, b) separates the sampled populations in different clusters. On PC1, all characters strongly covariate with SVL, while PC2 separates the male specimens from the type locality from the equally sized *T. edelcae* specimens from Auyán-tepui (Fig. 8b). PC3 shows no difference between the localities (not shown). When *T. rodriguezi* is added to the dataset, the separation between male *T. obscura* sp. n. and *T. edelcae* is less clear, but *T. rodriguezi* is clearly distinct. Again, PC1 mainly summarized covariation with SVL, while PC2 separated the more similar *T. obscura* sp. n. and *T. edelcae* from *T. rodriguezi* (Figs 8c, d). PC eigenvalues are shown in Tables 4–5.

Pairwise Wilcoxon tests between the type series and *Tepuihyla edelcae* specimens from Auyán-tepui revealed high similarities between these two morphologically variable species, but both are clearly distinct from *T. rodriguezi*. The most conspicuous difference between *T. obscura* sp. n. and *Tepuihyla edelcae* is SVL, where *T. obscura* sp. n. is significantly different from *T. edelcae* (females: p = 0.0061, males: p = 0.00076), as well as from *T. rodriguezi* (females: p = 0.0081, males: p = 0.0013; Figs 9a–d; Table 2). At population level, however, there is a strong overlap in size of

males of *T. obscura* sp. n. from the type locality and *T. edelcae* (p = 0.145; not significant, Fig. 9b).

After correcting for size differences, only EL and FAL residuals remained significantly different between *T. obscura* sp. n. and *T. edelcae* (EL resid.: p = 0.0000045, FAL resid.: p = 0.017, Table 2). After size, EL is the best diagnostic character to discriminate between the three species, especially between *T. obscura* sp. n. and *T. rodriguezi* (Figs 9e–h, Table 2). Although significant, FAL overlaps too much to be a reliable diagnostic character (Figs 10e–h). HL residuals are different between *T. obscura* sp. n. and *T. edelcae* (p = 0.05), showing a similar distribution as EL residuals, even though with greater overlap (Figs 11a–d, Table 2). Many more characters differ in their residuals between *T. rodriguezi* and *T. obscura* sp. n. + *T. edelcae*, such as HW, EN, FAB, WFD, WTD, IND, HAL, and TAL (Table 2, see also Fig. 12). TIL and FL residuals are significantly different only between *T. obscura* sp. n. and *T. rodriguezi*, but with considerable

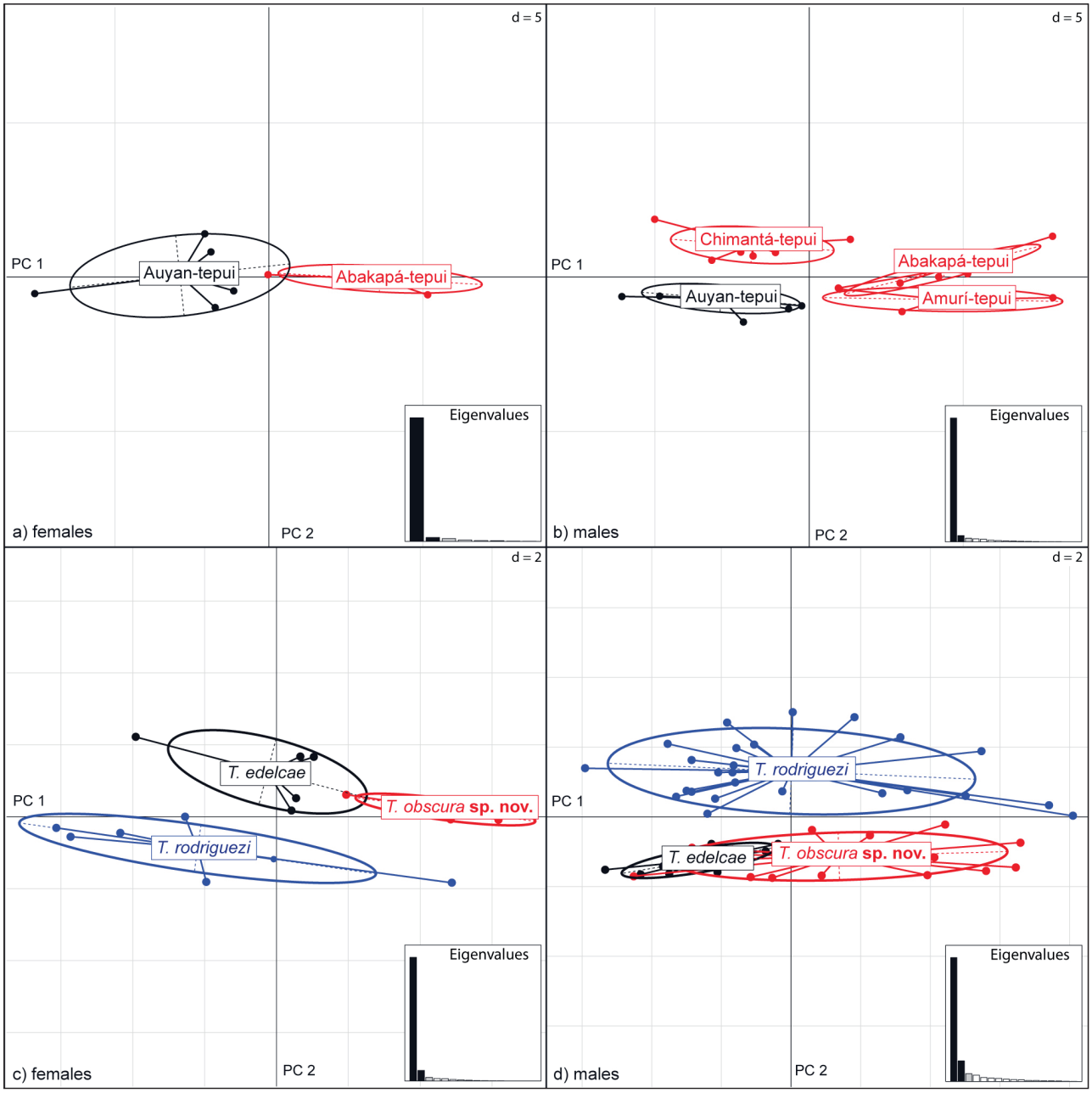

Figure 8. First two Principal Components (PCs) of the morphological measurements taken. a) females; and b) males of *Tepuihyla obscura* sp. n. (red) and *T. edelcae* (black), illustrating variation among populations. PCs of a) were multiplied by -1 to obtain the same orientation as in the male plot; c) females; and d) males of *T. obscura* sp. n. (red), *T. edelcae* (black) and *T. rodriguezi* (blue), illustrating variation between species. Note the small differences between *T. obscura* sp. n. and *T. edelcae* compared to *T. rodriguezi*. The first PCs are strongly correlated with differences in SVL.

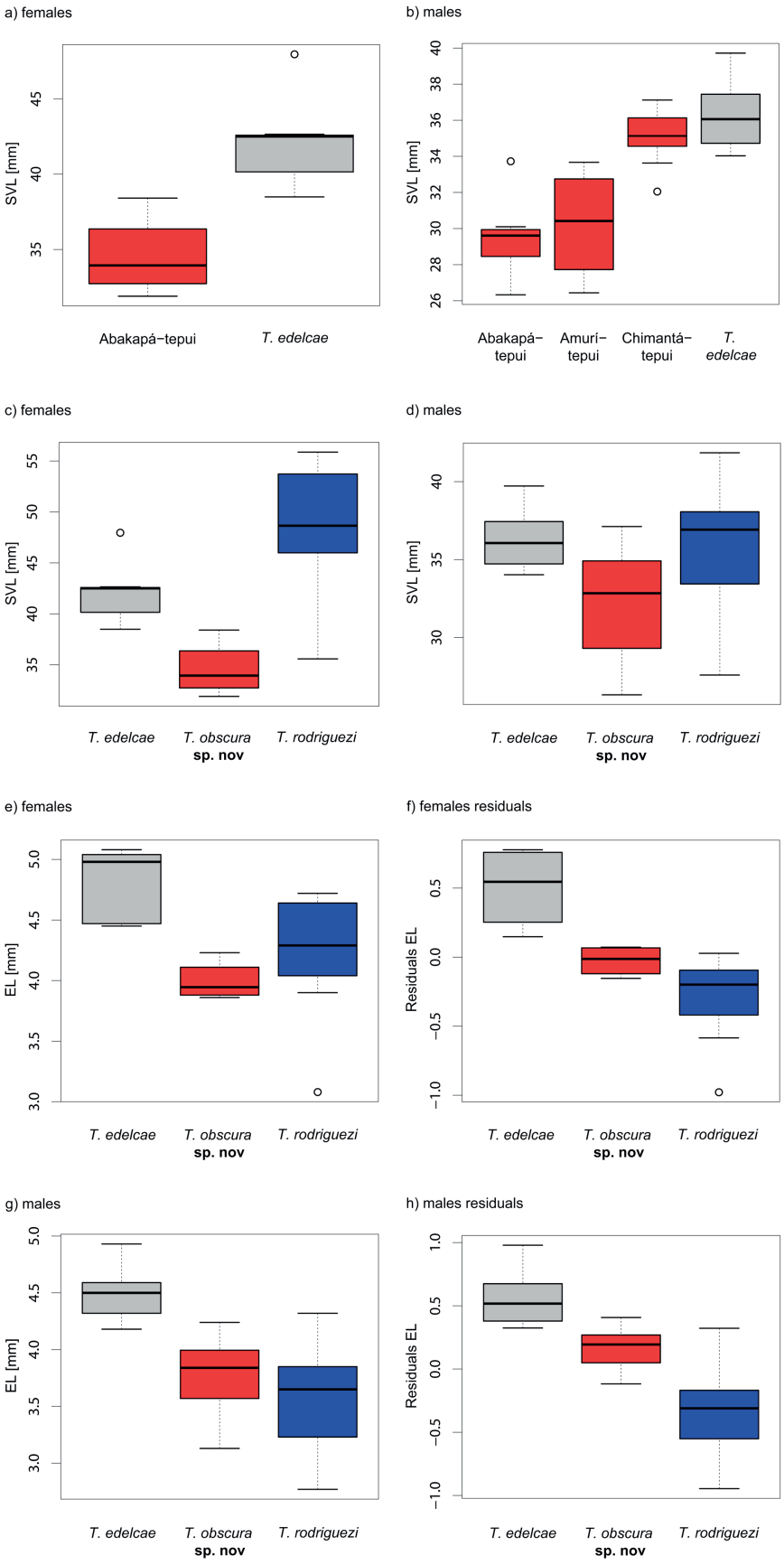

Figure 9. SVL of *T. obscura* sp. n. (red) and *T. edelcae* (grey). a) females, b) males at population level; c) females, d) males at species level, including *T. rodriguezi* (blue). Note that, except at the type locality, there is a large difference in SVL between *T. obscura* sp. n. and *T. edelcae*; d–g) comparison of EL among the three species; d) raw measurements of females; e) residuals in females; f) raw measurements of males; g) residuals in males. Differences among the species are presented in raw data as well as in residuals.

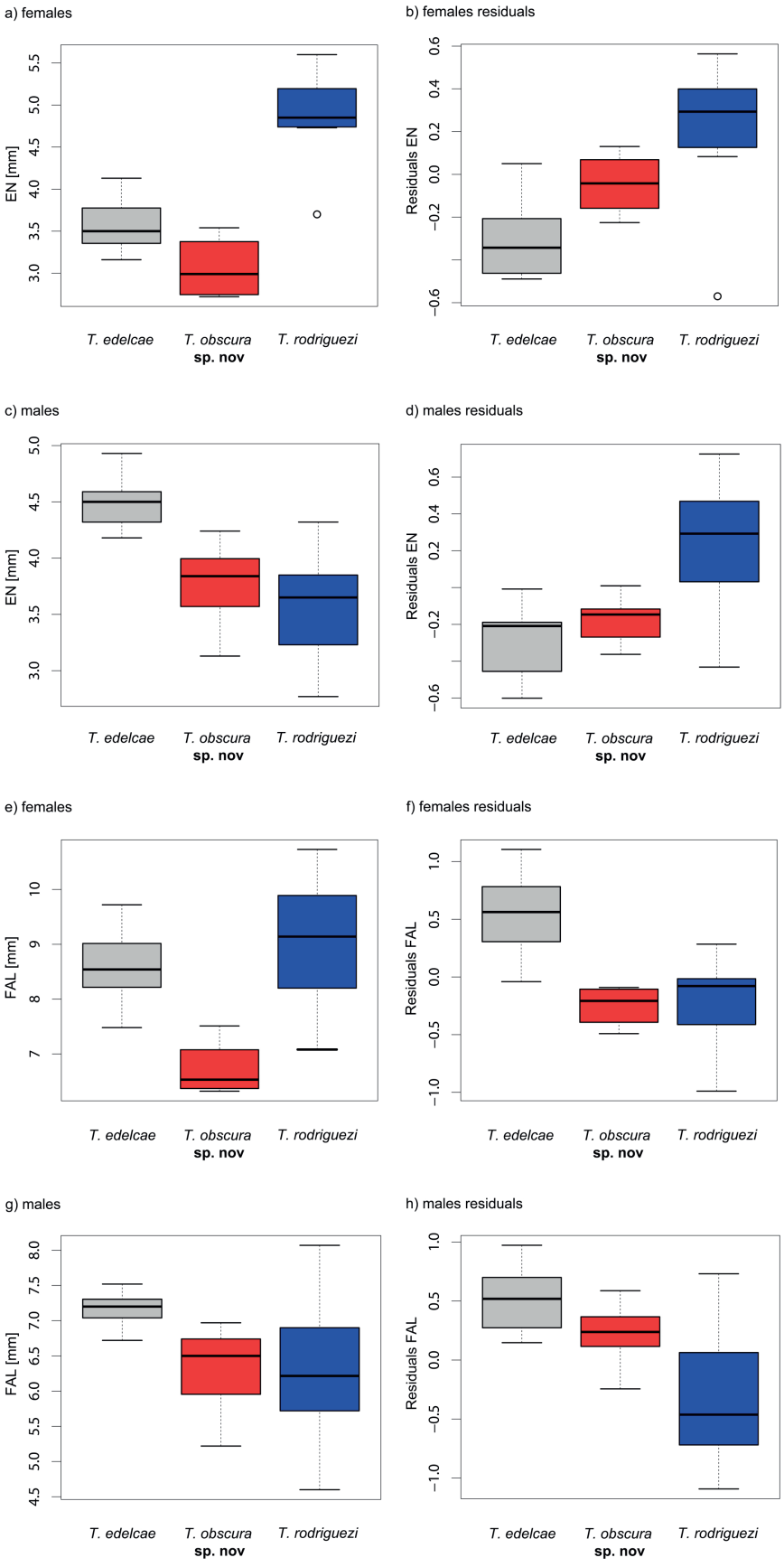

Figure 10. EN (a–d) and FAL (e–h) of *T. obscura* sp. n. (red), *T. edelcae* (grey), and *T. rodriguezi* (blue). a) and e) raw measurements of females; b) and f) residuals of a linear regression of pooled measurements against SVL for females; c) and g) raw measurements of males; d) and h) residuals in males.

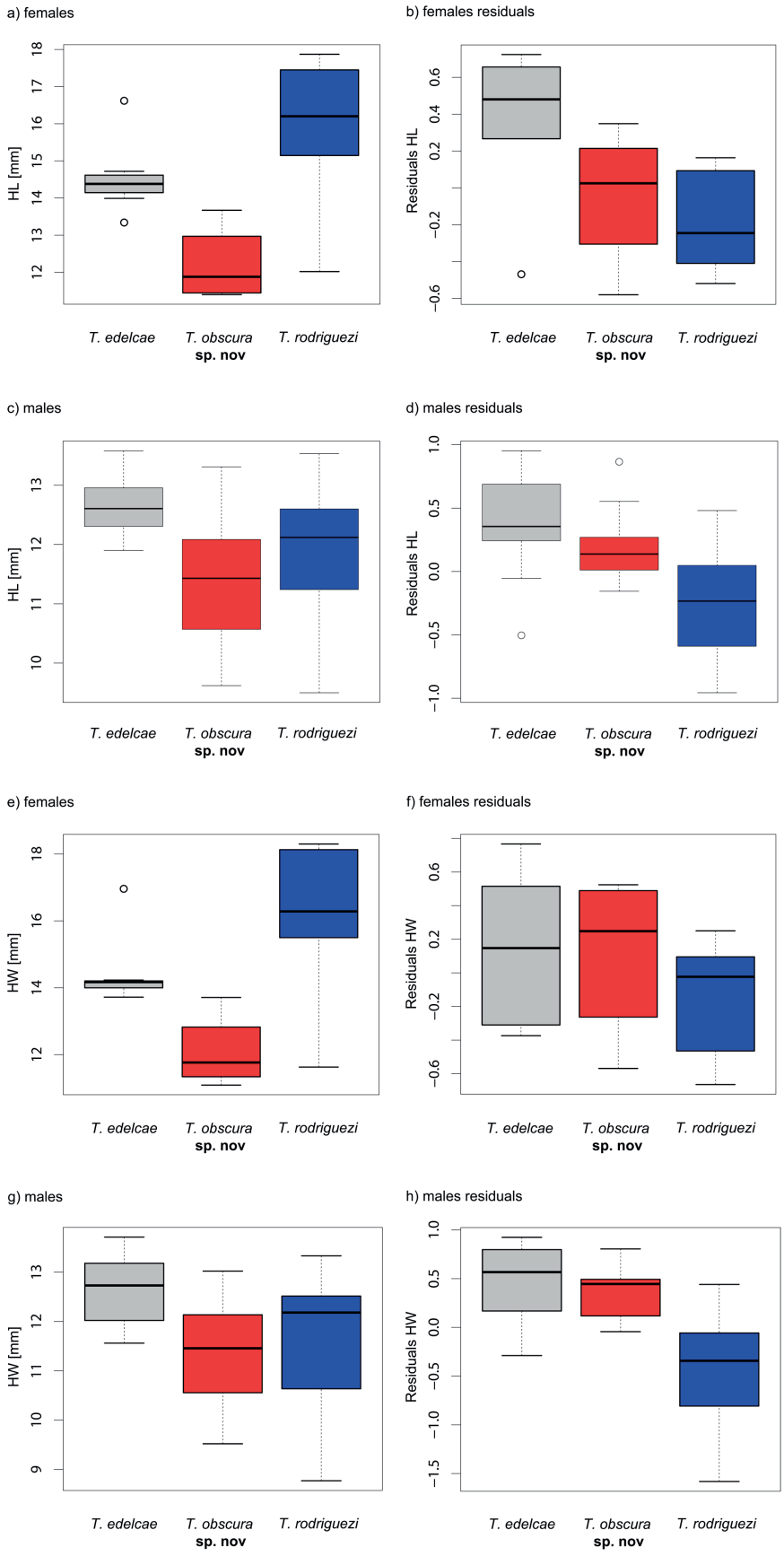

Figure 11. HL (a–d) and HW (e–h) of *T. obscura* sp. n. (red), *T. edelcae* (grey), and *T. rodriguezi* (blue). a) and e) raw measurements of females; b) and f) residuals of a linear regression of pooled measurements against SVL for females; c) and g) raw measurements of males; d) and h) residuals in males.

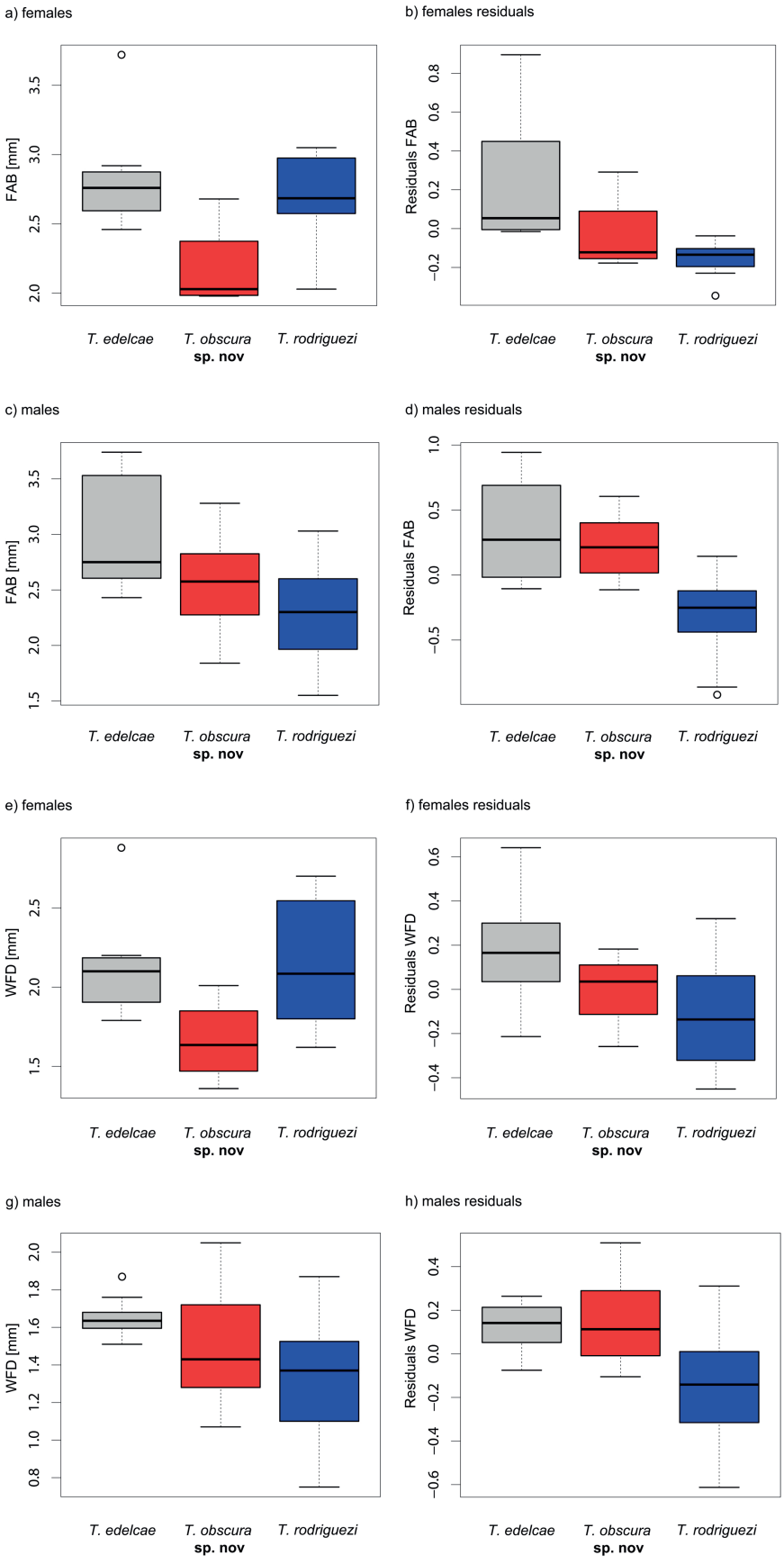

Figure 12. FAB (a–d) and WFD (e–h) of *T. obscura* sp. n. (red), *T. edelcae* (grey), and *T. rodriguezi* (blue). a) and e) raw measurements of females; b) and f) residuals of a linear regression of pooled measurements against SVL for females; c) and g) raw measurements of males; d) and h) residuals in males.

overlaps (not shown, Table 2). EN is remarkable in that it is the only character that differs from *T. rodriguezi* in both raw measurements and residuals (Figs 10a–c). Combined with the relatively larger EL of *T. obscura* sp. n. (Figs 4, 5, 9e–h), this gives *T. rodriguez*i conspicuously different head proportions compared to *T. obscura* sp. n., which can be used as a reliable diagnostic character.

Tadpole description: The following morphological description is based on a stage-32 tadpole (IRSNB 16177-C) of *T. obscura* sp. n. from Abakapá-tepui, collected by PJRK on 9 May 2011 at 19:40 h in a shallow rocky pool full of algae: Medium-sized tadpole, exotrophic, benthic ecomorphological guild (Altig & McDiarmid 1999). TL 43 mm, BL 14.8 mm (34.4% of TL). TAL 29 mm (67.4% of TL). Body ovoid in dorsal view, flattened dorsoventrally. BW 8.7 mm, BH 6.7 mm, HW 6.6 mm. Snout rounded, slightly acuminate towards tip near the oral disc in lateral view. Naris small, ovoid, directed dorsolaterally and positioned medially at the level of the medial margin of the eyes. NSD 1.6 mm, END 1.1 mm. IND 3.3 mm (50% of HW). Eyes situated dorsolaterally, not visible in ventral view. ED 1.7 mm, IOD 4.7 mm (71.2% of HW). Spiracle sinistral, tube medially attached to body, translucent with non-clustered dark chromatophores. SSD 9.2 mm (62.2% of BL). Vent tube dextrally attached to caudal fin. Caudal musculature robust, highest anteriorly, tapering towards end of the tail. TMH 3.6 mm, TMW 3.0 mm. Upper and lower tailfins originate at junction of body and tail. Upper tailfin straight towards end of the tail, lower tailfin slightly convex. MTH 6.9 mm, exceeding body height only slightly (103% of BH).

Lateral line system (Figs 13a–b) only partially visible (on the pigmented parts of the body). Supraorbital branch starting from snout and surrounding naris and eye medially, with a gap anteromedial to the eyes. Infraorbital branch splitting from supraorbital branch anteriorly to nostrils, surrounding naris and eye distally. Angular branch extending from eyes ventrally. Short posterior supraorbital branch posterodorsal to eyes, short posterior infraorbital branch posteroventral to eyes. Superior trunk branches located in the posterior third of the body, extending onto base of the tail muscle. Middle trunk branch originating anterodorsal to spiracle and extending onto the lateral line of the tail muscle. Lower trunk branch originating at the anterior edge of the spiracle, surrounding it dorsally and following an arc towards the ventral body side.

Oral disc (see also Fig. 13c illustrating IRSNB 16177-B) located anteroventrally, not emarginated. LTRF 2(2)–4(1) (with ontogenetic variation, see below). Rows A1 and A2 equal in length, A2 with medial gap, which holds a conspicuous cavity. P1 shorter than A1/A2 and slightly shorter than P2, medially interrupted. P2 the longest of the posterior rows. P3 of equal length as P1, P4 small, teeth smaller, less keratinised. Labial teeth slim, elongated, bent inwards, all very closely set, forming a dense comb. Tips of labial teeth blunt.

Upper jaw sheath broadly arched with pointed serrations, which become very small on the lateral processes. Lower jaw sheath smaller, fitting into the upper sheath. Jaws are closed in IRSNB 16177-C as in almost all other tadpoles examined, therefore the description of the lower jaw sheath is based on IRSNB 16178-D from Chimantá-tepui (jaws partially open), which has the lower jaw sheath V-shaped, with pointed serrations. Both jaw sheaths with a bronze-coloured, metallic shine.

Marginal papillae with large anterior gap, starting in one row anteriorly, and in multiple rows laterally and posteriorly. Approximately 107 papillae around outer fringe of oral disc. Papillae tapered, ending in a blunt tip.

Colour of tadpole in life (see Fig. 14): Dorsum dark brown, with – often inconspicuous – dark brown spots more visible at later Gosner (1960) stages. Tail light brown, heavily covered with dark chromatophores that usually cluster in larger dark brown spots on tail muscle. Lower part and sides of head reddish. Belly semi-translucent with black coiled gut visible. Iris silver to copper with fine dark brown reticulation.

Colour of tadpole in preservative: Dorsum dark brown, with – often inconspicuous – dark brown spots more visible at later Gosner (1960) stages (Fig. 13a–b). Clearly distinct

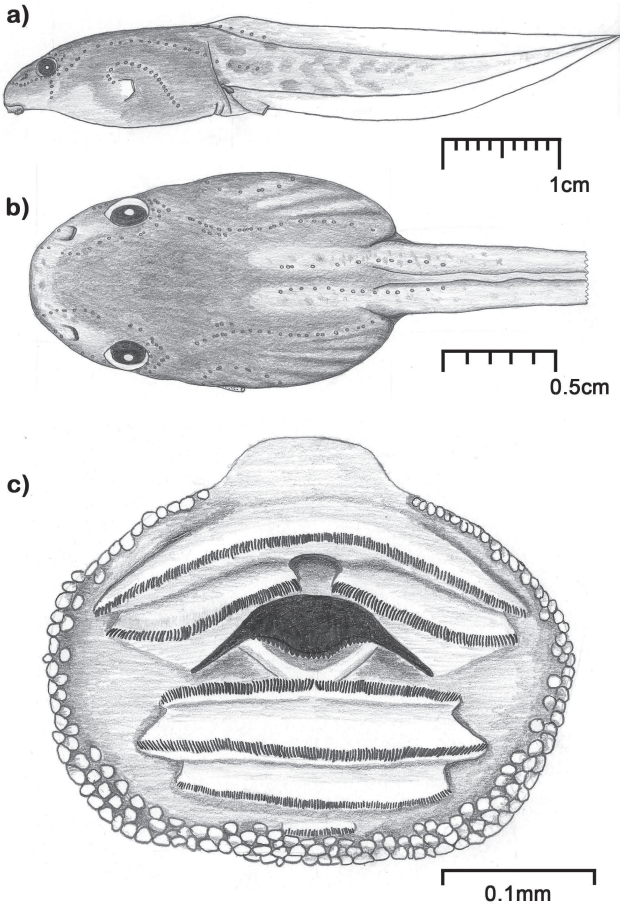

Figure 13. Drawings of *Tepuihyla obscura* sp. n. tadpole illustrating the lateral line system in IRSNB 16177-C (stage 32). a) Lateral and b) dorsal views (tail not completely shown); and c) oral disc in IRSNB 16177-B (stage 34). Drawings: SR.

spots are visible in IRSNB 16178-A. Tailfin semi-translucent, covered with large dark chromatophores. Chromatophores cluster in large brown spots on the tail muscle. The ventral side of the body is semi-translucent and patternless.

Ontogenetic changes and variation: Changes in body size are summarized in Tables 6–7. Pigmentation increases during ontogeny, as well as the discernability of the lateral line system. Shape of snout broad and bluntly rounded during earlier stages, but becomes more ovoid and elongated later. The fourth posterior labial tooth row (P4) develops at stages 26–27 and increases in width and keratinisation during later stages. P1 is usually medially interrupted, but not always (e.g., in IRSNB 16178-C, IRSNB 16179-B-C). LTRF therefore varies from 2(2)–3[1] to 2(2)–4[1].

Comparisons with other *Tepuihyla* tadpoles: We could only find minor differences when comparing the tadpole of *Tepuihyla obscura* sp. n. with that of *T. edelcae*. Even though the description provided by Myers & Donnelly (2008) suggests differences in body proportions, our own measurements based on additional tadpoles of *T. edelcae* (IRSNB 16180-A-G) revealed them to be very similar. The *Tepuihyla edelcae* tadpole exhibits, however, a tendency towards having a longer rostrum (naris–snout distance and eye–naris distance, see Fig. 15). The tadpoles of the other *Tepuihyla* species are currently undescribed.

Advertisement call: The following description is based on call sequences recorded from the holotype on 17 Nov. 2013 at 21:00 h (15 calls with 30 notes), 15.4°C air temperature (15.2°C water temperature), and of IRSNB 4170, a male from Abakapá-tepui recorded on 5 May 2011 at 19:20 h (24 calls with 156 notes), 18.5°C air temperature (18.2°C water temperature).

Call structure: The advertisement call of *T. obscura* sp. n. usually consists of paired notes (Fig. 16). The first note (hereafter called "pre-note") is shorter with a lower amplitude, and sometimes pulsed or scattered in up to three distinct short notes (Fig. 16). The dominant frequency of the pre-notes is often not well defined. The pre-note is usually followed by a note with a much higher amplitude and clearly defined harmonics (hereafter called "main note"). The maximum amplitude of pre-notes is on average 13.72% (2.87–53.22%) of the main note's maximum amplitude. The maximum amplitude of the main notes often decreases at the end of longer calls. The number of notes per call is highly variable; calls of the holotype consist on average of two notes (1–3, N = 15), while the calls from the specimen from Abakapá-tepui are longer with a mean of 6.5 notes per call (4–9, N = 24). Call duration of the holotype averages 0.18 s (0.02–0.23 s) vs. 0.54 s (0.05–0.83 s) in IRSNB 4170 from Abakapá-tepui. Call rate and intercall intervals irregular, with long periods of silence. Mean internote interval between pairs of notes (main note to following pre-note) in calls from the Abakapá-tepui specimen is 0.09 s (0.04–0.14 s). Mean internote interval within pairs of notes (pre- to following main note) of the holotype specimen is

Figure 14. Tadpole of *Tepuihyla obscura* sp. n. in life (IRSNB 16178-A, stage 40). a) dorsal view; b) lateral view; c) ventral view. Photos: PJRK.

Table 6. Measurements of *Tepuihyla obscura* sp. n. tadpoles from the type locality (Chimantá-tepui). Mean ± SD are followed by the range in parentheses. Measurements are in mm, except tooth counts.

Character	Stage 25 (N = 2)	Stage 36 (N = 1)	Stage 38 (N = 1)	Stage 40 (N = 1)	Stage 41 (N = 1)
TL	2.28±0.14 (2.18–2.38)	4.50	4.10	5.25	4.10
BL	0.82±0.01 (0.81–0.82)	1.49	1.44	1.75	0.74
TAL	1.49±0.07 (1.44–1.54)	3.10	2.70	3.70	2.70
BH	0.41±0.04 (0.38–0.44)	1.33	0.66	1.05	0.66
ED	0.12±0.01 (0.11–0.12)	0.18	0.18	0.22	0.23
TMH	0.19±0.01 (0.18–0.20)	0.41	0.33	0.48	0.33
MTH	0.52±0.01 (0.51–0.52)	0.66	0.66	1.07	0.66
UTF	0.2±0.01 (0.19–0.21)	0.23	0.25	0.34	0.23
LTF	0.19±0.01 (0.18–0.20)	0.18	0.20	0.31	0.2
SSD	0.56±NA	1.13	0.95	1.10	1.21
NSD	0.13±0	0.23	0.23	0.25	0.11
END	0.09±0.01 (0.08–0.10)	0.13	0.14	0.16	0.13
IND	0.23±0	0.34	0.33	0.38	0.16
IOD	0.34±0.02 (0.33–0.36)	0.49	0.49	0.62	0.44
HW	0.49±0.01 (0.48–0.50)	0.75	0.67	0.84	0.64
BW	0.53±0.01 (0.52–0.54)	0.89	0.82	0.98	0.82
TMW	0.16±0	0.31	0.34	0.46	0.33
ODW	0.21±0	0.30	0.30	0.33	0.23
ODH	0.09±0.01 (0.08–0.10)	0.11	0.11	0.16	0.08
LTRF	2(2)–3[1]	2(2)–4	2(2)–4(1)	2(2)–4(1)	NA

0.09 s (0.01–0.13 s) vs. 0.04 s (0.01–0.08 s) in IRSNB 4170 from Abakapá-tepui. Average main note duration of the holotype is 0.08 s (0.04–0.11 s) vs. 0.06 s (0.04–0.1 s) in IRSNB 4170 from Abakapá-tepui.

The main notes are usually composed of six harmonics. Mean peak frequency of the holotype's main notes is 1207.29 Hz (775.2–1,378.1 Hz). Mean peak frequency of the main notes of IRSNB 4170 from Abakapá-tepui is 1,357.34 Hz (796.7–1,571.9 Hz). These frequencies are usually in the second harmonic. Amplitude differences between the first (700–800 Hz) and the second harmonic are often small (Fig. 16).

Comparisons with other *Tepuihyla* calls: The call of *Tepuihyla edelcae* is very similar to that of *T. obscura* sp. n. in its structural, temporal, and spectral properties. Unlike the call described by Myers & Donnelly (2008: 68), we found *T. edelcae* calls to be usually composed of paired notes with the maximum amplitude of pre-notes averaging 10.29% (4.49–17.91%) of the main note maximum amplitude. The calls of *T. edelcae* consist on average of three notes (1–4, N = 9), with a call duration of 0.25 s (0.03–0.4 s) and a peak frequency of 1,458.85 Hz (1,382.8–1,523.4 Hz), which falls within the variation of *T. obscura* sp. n.

The *Tepuihyla edelcae* call we analysed differs in temporal aspects compared to the call of *T. obscura* sp. n. in having shorter notes (pre-note duration 0.02 s [0–0.04 s] in Chimantá, 0.03 s [0.01–0.05 s] in Abakapá; main note duration 0.08 s [0.04–0.11 s] in Chimantá, 0.06 s [0.04–0.1 s] in Abakapá vs. pre-note duration 0.01 s [0–0.02 s]; main note duration 0.04 s [0.03–0.06 s]) in *T. edelcae*). Additionally, the intervals between note pairs are shorter in *T. obscura* sp. n. (0.09 s [0.04–0.14 s] in Abakapá) than in *T. edelcae* (0.15 s [0.11–0.18 s]), while the interval among note pairs overlaps (*T. obscura* sp. nov: 0.09 s [0.01–0.13 s] in Chimantá, 0.04 s [0.01–0.08 s] in Abakapá; *T. edelcae:* 0.06 s [0.04–0.08 s]). We regard differences in temporal aspects as poorly reliable, because the recordings of *T. obscura* sp. n. and *T. edelcae* differ greatly in their relative intensity of background noise (likely due to different recording equipments and distances to the calling males), which probably masks parts of the calls of *T. edelcae*.

The call of *Tepuihyla rodriguezi* (Fig. 17) is clearly distinct, with 8–21 notes per call and call duration varying from 0.69–1.64 s (vs. 0.02–0.83 s in *T. obscura* sp. n.). The notes are also paired, but the pre-notes are more similar to the main notes than in *T. edelcae* or *T. obscura* sp. n. The maximum amplitude of pre-notes is 43.02% (9.98–83.06%) of the main note amplitude, which is much higher than in *T. obscura* sp. n. (13.72% [2.87–53.22%] of the main note amplitude). Main notes of *T. rodriguezi* are shorter on average, but more variable, with a mean duration of 0.041 s (0.013–0.420 s) (vs. 0.08 and 0.06 s in *T. obscura* sp. n.), with shorter intervals averaging 0.071 s (0.003–0.207 s) among pairs of notes, and 0.021 s (0.001–0.111 s) within pairs of notes (vs. 0.09 s [Chimantá], respectively 0.04 s [Abakapá], and 0.09 s among note pairs in *T. obscura* sp. n.). Compared to *T. obscura* sp. n., the pre-notes of *T. rodriguezi* have usually clearly defined harmonics (Fig. 16 vs. Fig. 17). The peak frequencies of the main notes average 1,489.2 Hz (624.5–2,627.1 Hz), which is similar to *T. obscura* sp. n.

Table 7. Measurements of *T. obscura* sp. n. tadpoles from Abakapá-tepui. Mean ± SD are followed by the range in parentheses. Measurements are in mm, except tooth counts. Asterisks indicate reduced sample sizes due to damaged tail fins.

Character	Stage 25 (N = 2)*	Stage 26 (N = 5)*	Stage 27 (N = 2)*	Stage 28 (N = 2)	Stage 29 (N = 1)	Stage 31 (N = 1)	Stage 32 (N = 1)	Stage 34 (N = 1)	Stage 35 (N = 3)
TL	1.96 (N = 1)	2.40±0.10 (2.30–2.50)(N = 3)	2.20 (N = 1)	3.70 (N = 1)	4.20	3.80	4.30	4.50	4.23±0.06 (4.20–4.30)
BL	0.62±0.01 (0.61–0.62)	0.80±0.04 (0.75–0.84)	0.95±0.28 (0.75–1.15)	1.24±0.06 (1.20–1.28)	1.44	1.33	1.48	1.56	1.45±0.03 (1.43–1.49)
TAL	1.34 (N = 1)	1.62±0.10 (1.56–1.74) (N = 3)	1.54 (N = 1)	2.50 (N = 1)	2.80	2.60	2.90	3.10	2.82±0.18 (2.65–3.00)
BH	0.34±0.01 (0.33–0.34)	0.38±0.03 (0.33–0.39)	0.46±0.18 (0.33–0.59)	0.57±0.02 (0.56–0.59)	0.66	0.63	0.67	0.75	0.72±0.04 (0.67–0.75)
ED	0.08±0	0.11±0 (0.11–0.11)	0.13±0.04 (0.10–0.16)	0.14±0.01 (0.13–0.15)	0.18	0.18	0.17	0.18	0.2±0.02 (0.18–0.21)
TMH	0.16±0.01 (0.15–0.16)	0.18±0.02 (0.16–0.20)	0.26±0.14 (0.16–0.36)	0.30±0.01 (0.30–0.31)	0.33	0.30	0.36	0.41	0.37±0.03 (0.34–0.39)
MTH	0.45±0.01 (0.44–0.46)	0.43±0.02 (0.41–0.46) (N = 4)	0.38 (N = 1)	0.66±0.01 (0.66–0.67)	0.70	0.66	0.69	0.74	0.78±0.06 (0.74–0.85)
UTF	0.16 (N = 1)	0.16±0.01 (0.15–0.18) (N = 4)	0.13 (N = 1)	0.22±0.01 (0.21–0.23)	0.23	0.25	0.23	0.25	0.29±0.01 (0.28–0.30)
LTF	0.16 (N = 1)	0.15±0.01 (0.13–0.16) (N = 4)	0.13 (N = 1)	0.20±0	0.20	0.21	0.18	0.21	0.23±0.03 (0.21–0.26)
SSD	0.49±0	0.57±0.09 (0.49–0.72)	0.66±0.22 (0.51–0.82)	0.81±0.01 (0.80–0.82)	0.95	0.85	0.92	0.97	0.97±0.04 (0.93–1.00)
NSD	0.10±0	0.10±0.01 (0.08–0.11)	0.14±0.04 (0.11–0.16)	0.16±0.01 (0.15–0.16)	0.21	0.20	0.16	0.21	0.18±0.03 (0.15–0.21)
END	0.08±0.01 (0.07–0.08)	0.07±0.01 (0.07–0.08)	0.09±0.03 (0.07–0.11)	0.12±0.01 (0.11–0.13)	0.14	0.12	0.11	0.11	0.11±0.01 (0.10–0.12)
IND	0.18±0	0.21±0.03 (0.18–0.26)	0.26±0.07 (0.21–0.31)	0.30±0.01 (0.30–0.31)	0.33	0.31	0.33	0.36	0.35±0.01 (0.34–0.36)
IOD	0.30±0.01 (0.3–0.31)	0.31±0.02 (0.27–0.33)	0.37±0.08 (0.31–0.43)	0.48±0.02 (0.46–0.49)	0.48	0.44	0.47	0.48	0.5±0.02 (0.48–0.52)
HW	0.41±0.03 (0.39–0.43)	0.44±0.02 (0.41–0.46)	0.49±0.14 (0.39–0.59)	0.64±0.04 (0.62–0.67)	0.67	0.64	0.66	0.70	0.72±0
BW	0.45±0.01 (0.44–0.46)	0.49±0.03 (0.43–0.51)	0.58±0.22 (0.43–0.74)	0.72±0.04 (0.69–0.75)	0.80	0.75	0.87	0.90	0.91±0.07 (0.84–0.97)
TMW	0.12±0.01 (0.11–0.13)	0.15±0.01 (0.15–0.16)	0.17±0.23 (0.01–0.33)	0.29±0.01 (0.28–0.30)	0.30	0.30	0.30	0.34	0.35±0.02 (0.34–0.38)
ODW	0.19±0.01 (0.18–0.20)	0.21±0.01 (0.20–0.22)	0.20±0.01 (0.19–0.20)	0.30±0.01 (0.29–0.30)	0.33	0.31	0.33	0.34	0.34±0
ODH	0.08±0.01 (0.08–0.09)	0.110 (0.10–0.11)	0.12±0.01 (0.11–0.13)	0.13±0	0.15	0.13	0.13	0.15	0.16±0.01 (0.15–0.16)
LTRF	2(2)–3(1)	2(2)–3(1) [N = 4] – 2(2)–4(1) [N = 1]	2(2)–3(1) [N = 1] – 2(2)–4(1) [N = 1]	2(2)–4(1)	2(2)–4(1)	2(2)–4(1)	2(2)–4(1)	2(2)–4(1)	2(2)–4(1)

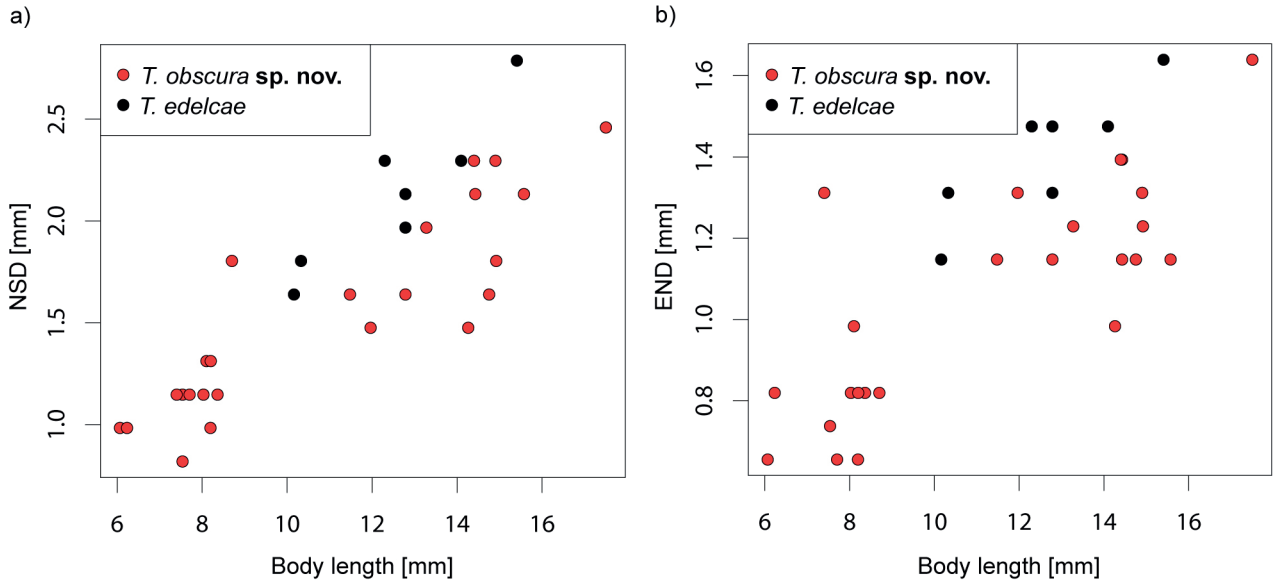

Figure 15. Relationship of naris-snout distance (a) and eye-naris distance (b) of tadpoles of *T. obscura* sp. n. (red) and *T. edelcae* (black) to body length (based on stages 25-32, 34, 35, 36, 38, 40, 41).

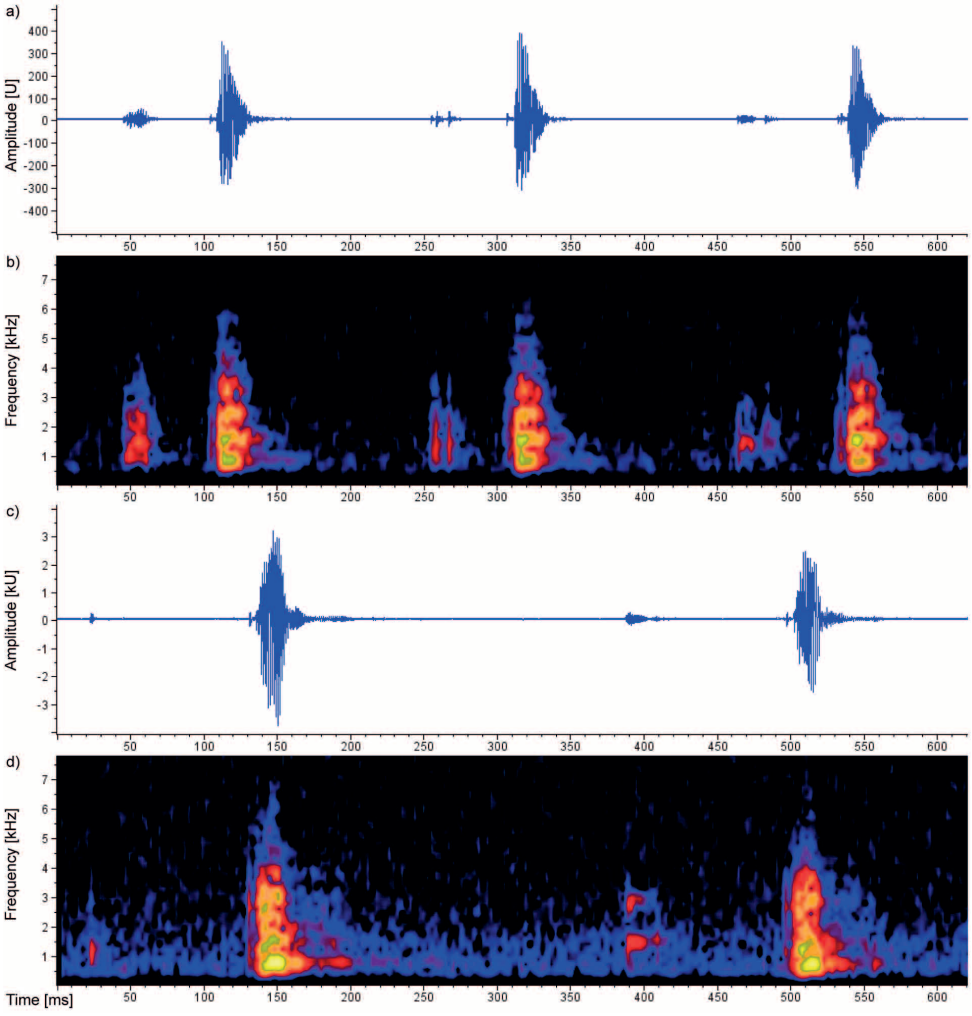

Figure 16. Call of *Tepuihyla obscura* sp. n. a) oscillogram, and b) spectrogram of a call of IRSNB 4170 from Abakapá-tepui consisting of three-note pairs. Male calling partially immersed in water, air temperature 18.5°C, water 18.2°C; note that the first pre-note shows traces of harmonics, while the second is pulsed and the third is scattered in two parts; c) oscillogram, and d) spectrogram of a call of the holotype IRSNB 4192 (calling partially immersed in water) consisting of two-note pairs. Blackman weighting, DFT = 265 samples, 3 dB filter bandwidth = 283 Hz. A bandpass filter was applied for frequencies below 500 and above 10,000 Hz. Air temperature 15.4°C, water 15.2°C.

Distribution and ecology: *Tepuihyla obscura* sp. n. is known with certainty only from the Chimantá Massif in Venezuela (Figs 2–3) where it has been reported (under the name *T. edelcae*) from Amurí-tepui, Abakapá-tepui, Akopán-tepui, Apakará-tepui, Chimantá-tepui (type locality), Churí-tepui, and Murei-tepui (sometimes named Eruoda-tepui, see Kok & Rivas 2011) between ca 1,800–2,600 m a.s.l. (Gorzula & Señaris 1999, McDiarmid & Donnelly 2005). The species is probably widespread in the Chimantá Massif.

Tepuihyla obscura sp. n. is nocturnal and inhabits open, mostly flat areas on tepui summits (Fig. 18). During the day, specimens were mostly collected in terrestrial bromeliads (genus *Brocchinia*), and more specifically in the carnivorous bromeliads *Brocchinia hechtioides* and *B. reducta* (Fig. 5C) where individuals often hide when inactive. A

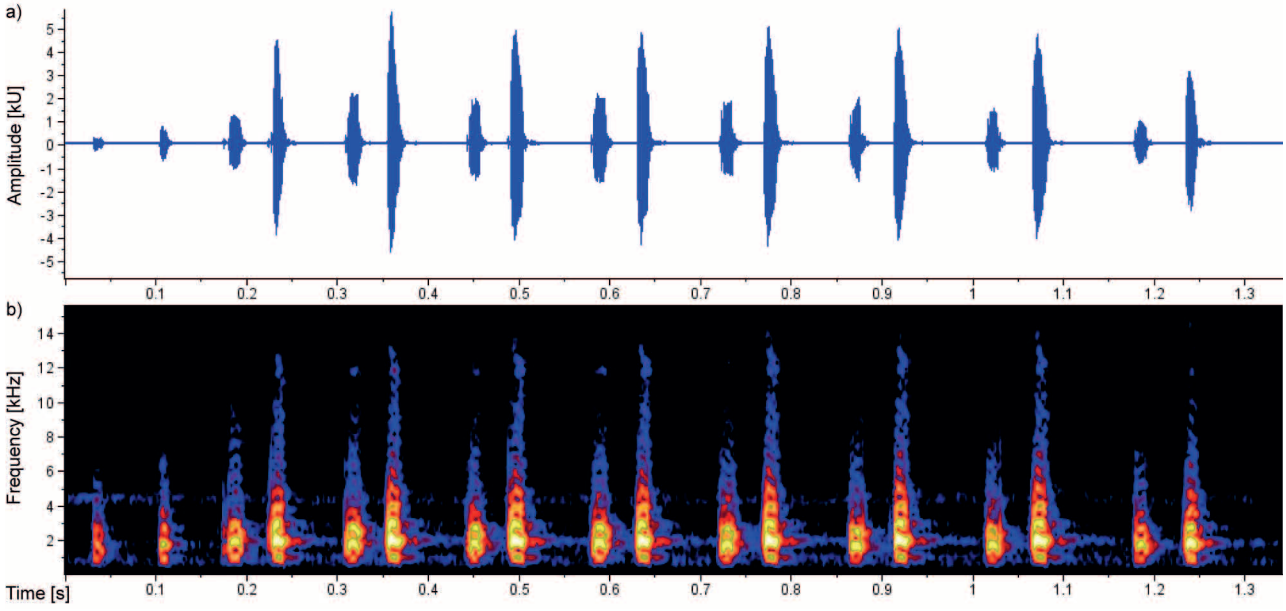

Figure 17. Call of *Tepuihyla rodriguezi* from La Gran Sabana, Venezuela (IRSNB 15673, calling partially immersed in water). a) oscillogram and b) spectrogram. Blackman weighting, DFT = 265 samples, 3 dB filter bandwidth = 283 Hz. A bandpass filter was applied for frequencies below 500 and above 18,000 Hz. Air temperature 20.1°C, water 20.0°C.

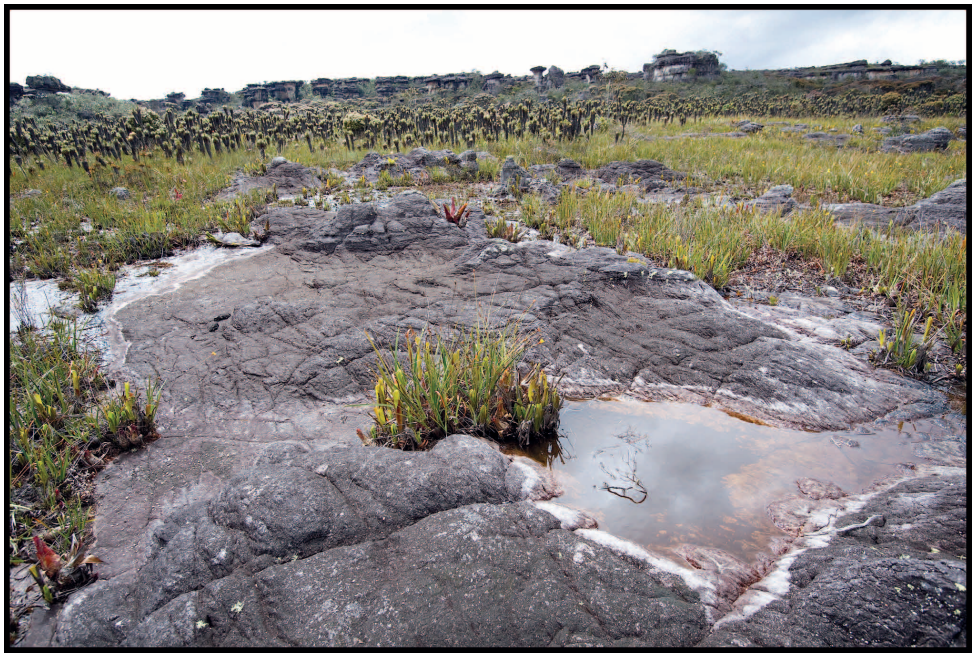

Figure 18. Habitat at the type locality (Chimantá-tepui, Venezuela, 17 November 2013). Photo: PJRK.

few specimens were collected on the ground among *Stegolepis ligulata*. At night, specimens were collected active in the vegetation, or in/along deep pools in marshy areas and small shallow rocky pools. Males call from the shallow edges of pools and puddles (partially immersed), or, more rarely, from low vegetation surrounding pools and puddles, where they often congregate. Amplexus is axillary, and eggs are deposited in the water as gelatinous masses. Tadpoles can tolerate acidic water (pH values ca 4) and are opportunistic feeders.

Phylogenetic relationships

Our phylogenetic hypothesis is congruent with previous results (Kok et al. 2012, Salerno et al. 2012, 2014, Jungfer et al. 2013) in recovering a non-monophyletic *Tepuihyla edelcae* (Fig. 19). It agrees with Kok et al. (2012) in showing *T.* aff. *edelcae* (here described as *T. obscura* sp. n.) as sister to a statistically well-supported clade composed of *T. edelcae* sensu stricto and *T. rodriguezi* (*T.* aff. *edelcae* is recovered as sister to *T. rodriguezi* in Salerno et al. 2012, 2014, and Jungfer et al. 2013). We have more confidence in our current hypothesis because the sister relationship between *T. edelcae* and *T. rodriguezi* is better supported (BPP = 97%; bootstrap = 92%) than in Salerno et al. (2012, 2014) and Jungfer et al. (2013).

Surprisingly, but concordant with the results of Kok et al. (2012), the genetic structure within *T. obscura* sp. n. is very shallow, although the three populations included in this study occur on three different tepui summits. These tepui summits are part of the same massif, but isolated from each other by deep fractures (Fig. 3), which probably harbour sub-optimal habitat for the species. The inter-population genetic structure in *T. rodriguezi* is slightly deeper, in particular between specimens from Kaieteur National Park (previously known as *T. talbergae*) and all other populations. It is noteworthy that specimens from Kaieteur are genetically closer to those from the type locality (La Escalera, Venezuela) than to specimens from tepuis that are geographically closer. A general pattern of shallow genetic divergence in the *T. rodriguezi* clade (i.e., *T. obscura* sp. n. + *T. edelcae* + *T. rodriguezi*) suggests that these populations were probably never isolated for a substantial length of time (see Discussion). Genetic distances are very low in 16S (0.2–0.7% among populations of *T. rodriguezi*; 0.7–

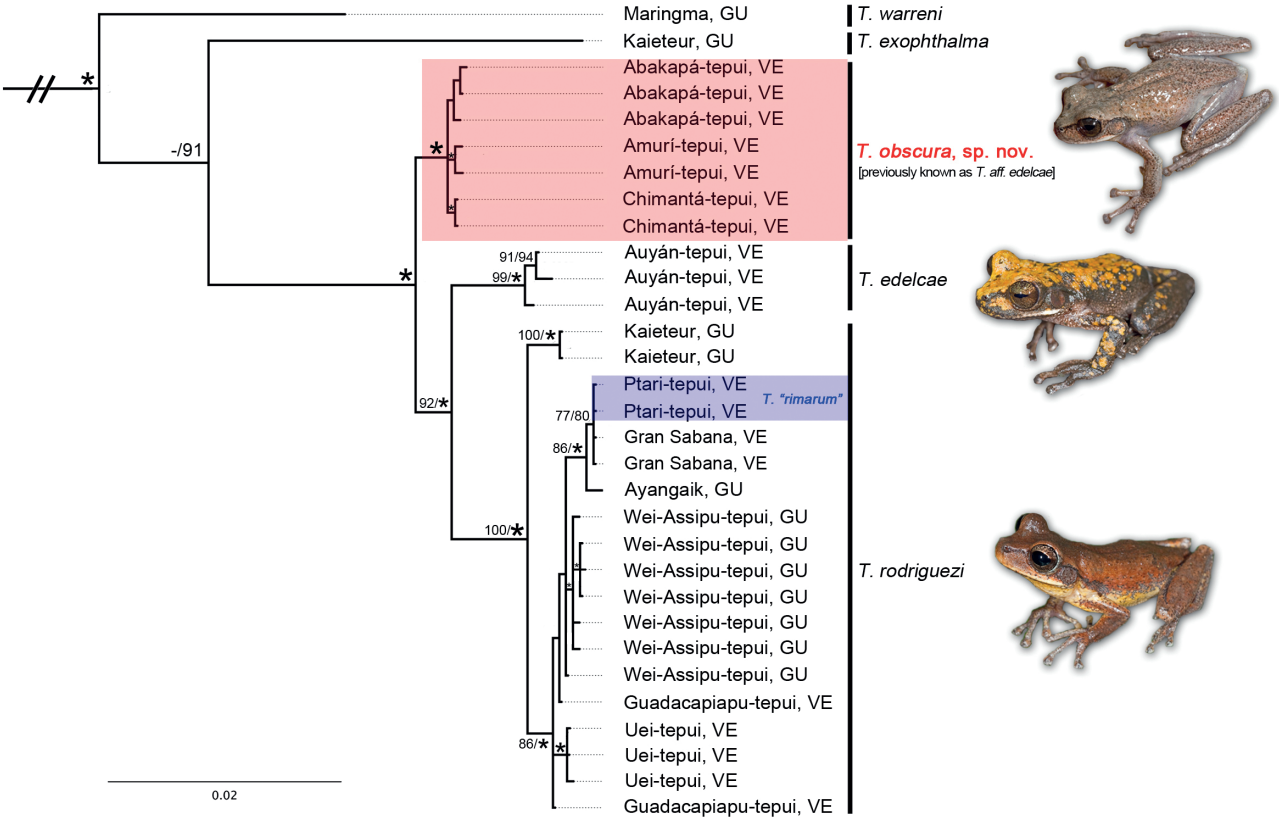

Figure 19. Bayesian phylogenetic tree based on our *Tepuihyla* concatenated dataset (16S + ND1 + RAG1 + CXCR4, totalling 2,404 bp). Numbers at the nodes represent statistical supports for ML and BA, respectively. BPP above 95% are represented by an asterisk, BPP lower than 75% and bootstrap supports lower than 50% are not shown, or are represented by a dash. Some bootstrap supports between terminals are not shown for clarity purposes. The new species is highlighted in red, and the two *Tepuihyla rimarum* samples are highlighted in blue.

1.2% among populations of *T. obscura* sp. n. and *T. edelcae*). In the faster-evolving gene ND1, genetic distances remain low among populations of *T. rodriguezi* (0.5–1.6%), but are significantly higher among populations of *T. obscura* sp. n. and *T. edelcae* (3.8–4.4%), which tends to confirm our taxonomic decision (see Discussion).

The taxonomic status of *Tepuihyla rimarum* (Ayarzagüena, Señaris & Gorzula, 1993a)

Tepuihyla rimarum (Fig. 20) was described in 1993 (as *Osteocephalus rimarum*, Ayarzagüena et al. 1993a) on the basis of 17 specimens collected on the summit of Ptari-tepui, 2,400 m a.s.l., Bolívar state, Venezuela. No clear comparison with congeneric species is provided in the original description, but according to the key to the genus (at that time considered the *Osteocephalus rodriguezi* species group), the authors used the relative size of the tympanum and the presence/absence of ulnar tubercles to discriminate between *T. rimarum* and *T. rodriguezi*. Examination of 78 specimens of *T. rodriguezi* from throughout its known distribution range (including the holotype of *T. galani*, see Appendix), and eight additional topotypic specimens of *T. rimarum* (including the holotype, see Appendix) reveals that these characters are not diagnostic for the species due to intraspecific variability. Furthermore, we obtained tissue samples of *T. rimarum* from the type locality for the first time, and included the species in our phylogenetic analyses. *Tepuihyla rimarum* falls in the same clade as specimens of *T. rodriguezi* with high support (BPP = 100%; bootstrap = 100%) and clusters (with good support, BPP = 100%; bootstrap = 86%) with specimens of *T. rodriguezi* from the type locality and from Ayangaik, Guyana (Fig. 19). The taxonomic validity of *T. rimarum* is thus supported neither by morphological data nor phylogenetic evidence, and we therefore consider *T. rimarum* a junior synonym of *T. rodriguezi*.

Figure 20. *Tepuihyla* "*rimarum*" from Ptari-tepui, Venezuela (IRSNB 16140, male), here synonymised with *T. rodriguezi*. Photo: PJRK.

Discussion

Tepuihyla obscura sp. n. is likely part of a recent non-adaptive radiation (i.e., lineage diversification with minimal ecological diversification; Rundell & Price 2009). As speciation is a gradual process, the limits between species and populations can remain diffuse, hence the debate about the recognition of young species lacking clearly distinct morphological traits (i.e., De Queiroz 1998, 2007, Portillo & Greenbaum 2014). Fouquet et al. (2007) proposed a genetic divergence of 3% in 16S as a threshold for the recognition of candidate species of Neotropical anurans. Divergence in 16S between *T. obscura* sp. n. and *T. edelcae* is max. 1.2% and thus far lower (see Table 3). 16S has been proposed as a standard barcoding marker for vertebrates because of its universality and high amplification success rate, but 16S can fail in detecting recent entities (Vences et al. 2005). ND1 is a faster evolving mitochondrial gene, and our preliminary results (unpubl.) suggest that a divergence of ca 4% and above in that gene allows discriminating between species with a high success rate. Divergence in ND1 between *T. obscura* sp. n. and *T. edelcae* is 3.8–4.4%; in contrast, genetic divergence in the same gene among populations of *T. rodriguezi* is 0.0–1.5%, despite the fact that these populations occupy a much wider range (Fig. 2) and occur from uplands (ca 400 m) to tepui summits (ca 2,400 m). However, taxonomic decisions should not solely depend on genetic divergence, but rather incorporate a pluralistic approach (Padial et al. 2009, Barley et al. 2013), including common sense. There are several examples of recent radiations having similarly low genetic divergences in 16S between recognized valid anuran species, such as in the bufonid genus *Osornophryne* (Páez-Moscoso & Guayasamin 2012), the arthroleptid genus *Leptopelis* (Portillo & Greenbaum 2014), and the dendrobatid genus *Ranitomeya* (Perez-Peña et al. 2010). In addition to the small genetic divergence between *T. obscura* sp. n., *T. edelcae* and *T. rodriguezi*, we only found subtle morphological differences among these species. Although colour pattern may apparently evolve fast, a highly conservative general morphology seems to be common in tepui summit-anurans, even in deeply diverged lineages (PJRK, unpubl. data). Environmental conditions on tepui summits are very similar and selective forces affecting species morphology, especially within short timeframes, are probably minimal. It is therefore not surprising that most morphological characters overlap strongly between *T. obscura* sp. n. and its closest relatives, *T. edelcae* and *T. rodriguezi* (see Figs 8–12). In the same vein, the similarity between the advertisement calls of *T. edelcae* and *T. obscura* sp. n. is probably due to a lack of contrasting selection in very similar habitats. Minimal acoustic divergence is also observed in other tepui summit species, such as *Oreophrynella*, *Pristimantis*, and *Stefania* (PJRK, unpubl.). Differentiation in morphological and acoustic traits in tepui summit-endemic *Tepuihyla* species would therefore mostly depend on genetic drift, which is likely opposed by a strong selection towards an optimal "tepui summit-morphotype". By con-

trast, *Tepuihyla rodriguezi* inhabits a much wider range of habitats, extending from the Venezuelan uplands of the Gran Sabana, Venezuela, and Kaieteur National Park, Guyana, to several tepui summits, e.g., Wei-Assipu-tepui, Guadacapiapu-tepui, Uei-tepui, and Ptari-tepui – all far above 1,000 m a.s.l. (Duellman & Yoshpa 1996, Kok et al. 2013, PJRK, unpubl. data). This might explain the more marked morphological differences between *T. rodriguezi* and the tepui summit-endemic *Tepuihyla* species.

Our concatenated dataset gained high support for a sister relationship between *T. obscura* sp. n. and a clade consisting of *T. edelcae* and *T. rodriguezi*, as did the mitochondrial marker ND1 in a single-locus tree (unpubl. data). 16S and the nuclear genes RAG1 and CXCR4 single-locus trees contain substantial polytomies. Nevertheless, our analyses confirm the non-monophyly of *T. edelcae* recovered by previous studies (Kok et al. 2012, Salerno et al. 2012, 2014, Jungfer et al. 2013). The only options to remedy the non-monophyly of *Tepuihyla edelcae* are either to synonymise *T. edelcae* with *T. rodriguezi* or to describe the paraphyletic taxon as a new species. We chose the latter because *T. rodriguezi* is clearly distinct morphologically (head proportion and colour pattern, see above), bioacoustically (see above, Figs 16–17, and Myers & Donnelly 2008, suggesting pre-zygotic isolation), and ecologically (distributed in highlands as well as uplands) from *T. edelcae* and *T. obscura* sp. n.

Hybridisation might have occurred between some *Tepuihyla* species of the *rodriguezi* clade in a "recent" past, and genetic introgression or incomplete lineage sorting could explain our genetic results (see also Salerno et al. 2014). It is known that genetic introgression is often driven by climatic change and shifting habitats (Rheindt & Edwards 2011), a scenario proposed for diversification in Pantepui (see for instance Rull 2005, Kok 2013). In any case, this should not affect our taxonomic decision since available data converge to indicate that *Tepuihyla obscura* sp. n. as described here represents a distinct evolutionary unit.

The IUCN conservation status of the new species is considered Least Concern (LC) because of its apparently large population size and relatively high numbers of locations. Although likely at risk due to global warming and threatened with habitat loss by upward displacement, the species is apparently not declining fast enough to qualify for any of the threat categories (IUCN 2013).

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Appendix
Additional material examined

Tepuihyla aecii: Venezuela, Estado Amazonas, Mount Duida, MHNLS 12014 (holotype). *Tepuihyla edelcae*: Venezuela, Estado Bolívar, Auyán-tepui, MHNLS 10626 (holotype), IRSNB 16121–39; Cerro El Sol, IRSNB 16180-A-G (tadpoles).

Tepuihyla exophthalma: Guyana, Potaro-Siparuni District, Kaieteur National Park, IRSNB 14644, IRSNB 14662, IRSNB 14664–65, IRSNB 14673. *Tepuihyla obscura* sp. n.: Venezuela, Estado Bolívar, Abakapá-tepui, IRSNB IRSNB 16174-A-B, IRSNB 16175-A-E, IRSNB 16176-A-C, IRSNB 16177-A-G (tadpoles); Chimantá-tepui, IRSNB IRSNB 16178-A-D, IRSNB 16179-A-C (tadpoles), IRSNB 16183 (juvenile). *Tepuihyla rodriguezi*: Guyana, Cuyuni-Mazaruni District, Wei-Assipu-tepui, IRSNB 15856–62, IRSNB 16150–72; Potaro-Siparuni District, Kaieteur National Park, IRSNB 13692–99, IRSNB 13700–01, IRSNB 13703, IRSNB 13705–11, IRSNB 13713–16, IRSNB 13718, IRSNB 13720, IRSNB 14750–54. Venezuela, Estado Bolívar, Gran Sabana, IRSNB 15655, IRSNB 15658–61, IRSNB 15673, IRSNB 16180; Guadacapiapu-tepui (slopes), MHNLS 10608 (holotype *T. galani*), IRSNB 15701–02, IRSNB 15712; Ptari-tepui, MHNLS 10646 (holotype *T. rimarum*), IRSNB 16140–46; Uei-tepui, IRSNB 15768–70, IRSNB 15774–75, IRSNB 16147–49.

Tepuihyla luteolabris: Venezuela, Estado Amazonas, Marahuaka-tepui, MHNLS 9376 (holotype). *Tepuihyla warreni*: Guyana, Cuyuni-Mazaruni District, Maringma-tepui (slopes), IRSNB 15863, IRSNB 16182.