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ORIGINAL ARTICLE

Unraveling unique island colonization events in *Elachistocleis* frogs: phylogeography, cryptic divergence, and taxonomical implications

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Abstract

Over the last two decades, molecular work on the reptile and amphibian fauna of the island of Trinidad (West Indies) has revealed the presence of cryptic species and island endemics resulting in the re-description of species for northern South America. In this study, we assess the taxonomy of a population of *Elachistocleis* sp. formerly assigned to the invalid species *E. ovalis*, and *E. surinamensis*, both present in Trinidad and throughout South America. We conduct phylogenetic, morphological, and call characteristic analyses to assess their taxonomy and discuss the phylogeography and evolutionary history of the genus. We find an Andean origin of the genus dating to the Oligocene and two sister clades within *Elachistocleis* with a Mid-Miocene divergence, coinciding with marine incursions at the time. We discuss their likely dispersal throughout South America following the dry-out of the Pebas mega-wetland system and attempt to find the routes of expansions, probably aided through the Amazon basin and hydrological systems towards the north and east. We find that each of the species is highly divergent and recovered in both sister clades, and we time the colonization of each Trinidad population at 2.1 and 0.5 million years ago, arriving from different regions (Venezuela and Guyana) facilitated by sea-level drops at the time. Lastly, we select a neotype for *E. surinamensis* from Trinidad to stabilize the nomenclature and describe the frog formerly considered *E. ovalis* in Trinidad as *E. nigrogularis* sp. nov.

Keywords South America · Trinidad · dispersal · phylogeography · diversity · Microhylids

Introduction

Widespread taxa that span biogeographic barriers pose a particular challenge for taxonomists as barriers limit gene flow allowing opportunities for speciation and often hide overlooked genetic diversity, lineages, and cryptic species. Species that are widely distributed also disperse or colonize from distant locations and individuals from such populations

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can converge at unlikely geographic localities with the assistance of abiotic factors such as dispersal through estuarine and marine water currents or through riverine systems. These species often are pivotal to understand how diverse ecological factors may drive regional patterns of species divergence and speciation (Card et al. 2016). Occasionally, cryptic species with widespread inland or mainland distribution ranges are found in islands, which conform as outliers within their distribution. Isolation on marine islands often leads to the presence of population structure, lineage divergence, and new species (Card et al. 2016; Murphy et al. 2019a, b). Consequently, assessing such species taxonomic identity is key to understanding the ecology and the evolutionary biology of likely distinct endangered populations and/or species on islands to ensure needed conservation strategies (Young 2000; Spielman et al. 2004). Fortunately, the use of molecular phylogenetics provides resolution to our understanding of the evolutionary history of even rarely studied species and has dramatically changed the way we look at patterns and processes of species diversity, having profound implications in its applicability to conservation genetics.

The original descriptions of *Elachistocleis ovalis* (Schneider 1799), *E. bicolor* (Guérin-Méneville 1838) and *E. surinamensis* (Daudin 1802), the first three species of *Elachistocleis* to be described, have resulted in considerable nomenclatural confusion. *Rana ovalis* (Schneider 1799) is invalid because there was no holotype or type locality given and the original description lacks detail (Caramaschi 2010). The combination *E. ovalis* has been applied to many populations across South America, which are now undescribed taxa and here referered as *E.* sp.). Lavilla et al. (2003) and Caramaschi (2010) used the two ventral color morph groups in *Elachistocleis* to resolve some confusion and re-defined some members of the genus. Their two groups were based on an immaculate venter (*E. ovalis* and *E. bicolor*) and a blotched or spotted pattern that may or may not have vermiculations (Toledo et al. 2010; Toledo et al. 2010; Frost 2020). The taxonomic history of *Rana ovalis* (Schneider 1799) is long and tortuous. The reader interested can refer to Lavilla et al. (2003) and Caramaschi (2010).

The first report of *Elachistocleis* on the continental island of Trinidad (Republic of Trinidad and Tobago) came from Mole and Urich (1894); they reported it as *Engystoma ovale*. Lutz (1927) discussed its presence on Trinidad while later, Murphy (1997) commented on different color patterns. Daudin's (1802) original description of *Bufo surinamensis* is not associated with an extant name-bearing type. However, the description was based upon a specimen from M. de Bèze, which was collected in Suriname. Daudin's (1802) description includes mention of a spotted ventral coloration, and the Suriname location suggests the specimen came from northern South America. The multi-colored, spotted ventral was mentioned by numerous workers, discussing the name *Elachistocleis surinamensis* (Kenny 1969; Rivero et al. 1986; Lavilla et al. 2003; Caramaschi 2010).

The island of Trinidad was formerly attached to Venezuela and formed by a pull-apart basin in the Late Miocene when a downwarping event separated both land masses (Liddle 1946; Erlich and Barrett 1990). Consequently, Trinidad's geological past connections to the mainland make it a unique model to study the evolutionary processes in the Eastern Caribbean. Findings from previous phylogenetic studies on the Trinidad herpetofauna reflect a pattern of colonization and diversification of species in congruence with their ecological requirements, dispersal capability, and the area from where the founder mainland populations likely originated. Such studies have yielded contrasting results on the timings and possible modes of speciation in such a complex topographical terrain (Jowers et al. 2008, 2011, 2015; Murphy et al. 2016, 2019a, b). The presence of widespread South American species such as *Elachistocleis* populations in Trinidad offers a suitable scenario to address the presence of cryptic species and assess the factors and timing involved in the colonization processes (Jowers et al. 2011, 2019). Today, the microhylid frog genus *Elachistocleis* is widely distributed across *cis*-Andean (east of the Andes) South America and the genus ranges from Panama and Colombia southward to southern Brazil, Argentina, and Uruguay (Frost 2020; Fig. 1). With the exception of *E.* sp. (formerly assigned to *E. ovalis* and here described as a new species) and *E. surinamensis* in Trinidad, no other species within the genus was known to be present in islands. The two species show contrasting distributions, but both are found in the coastal regions proximal to Trinidad. *E. surinamensis* has a more restricted coastal distribution ranging from Guyana to Suriname, Trinidad and Tobago, and Venezuela. On the other hand the literature suggests that *E.* sp. (formerly *E. ovalis*) exhibits a vast distribution across inland South America, ranging from the whole of Brazil, to Paraguay, Bolivia, Peru, Colombian Venezuela, Guyana, Suriname, French Guyana, and Trinidad (in the Republic of Trinidad and Tobago, from here-on referred to as Trinidad). Taxonomic arrangements within *Elachistocleis* are tentative, given that the genus contains considerable cryptic diversity (Nelson 1973; Lavilla et al. 2003; Caramaschi 2010; Toledo et al. 2010).

Here, we assess the taxonomy of the Trinidadian *Elachistocleis* sp. population (formerly *E. ovalis*, in order to contribute to the correct species designation), and *E. surinamensis,* and we compare them to counterpart populations in Guyana and Venezuela. We assess their phylogenetic relationships to all other available sequences using three mitochondrial and two nuclear loci; use a time tree to infer colonization events; and use morphological characters for comparing them to other populations. As we neared completion of this paper, we discovered a third species on Trinidad. We have no morphological or molecular data on

Fig. 1 Map of known species of *Elachistocleis* and their approximate ranges. Specimens sequenced in GenBank with no species identification are also included in the map. Note that *E. ovalis* is not included in the map. Species areas are made by joining presence of locality points and are therefore an estimation of likely or possible distribution ranges

this population, but we do have photographs and a recording of a call.

Materials and methods

Molecular methods

DNA was extracted from tissue samples, and target gene fragments were amplified by polymerase chain reaction using the DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) following the manufacturers' instructions. We sequenced a total of 20 *Elachistocleis* sp. (previously *E. ovalis*) and *E. surinamensis* specimens from Trinidad and Guyana (Supplementary material Table 1). Primers used are reported in supplementary material Table 2. We amplified the complete mitochondrial small and large ribosomal subunits and transfer RNAs (12S rDNA, 16S rDNA, respectively), and fragments of the cytochrome oxidase (*COI*), the nuclear tyrosinase (*TYR*) and the recombination-activating protein 1 gene (*RAG-1*) (Supplementary material Table 2). These gene fragments are especially informative in interspecific and intraspecific studies on frogs (Faivovich et al. 2005).

Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous basecalls in Sequencher v4.9 (Gene Codes Corp., Ann Arbor, MI). The lengths of the amplified fragments were approximately (some lengths varied): 2450 bp for 12S, 16S rDNA, and tRNAs, 664 bp for *COI*, 531 bp for *TYR*, and 791 for *RAG-1*. *COI*, *TYR*, and *RAG-1* were translated to amino acids for presence of stop codons. Following de Sá et al. (2012) we used two genera that were sisters to *Elachistocleis* (*Hypopachus variolosus* and *Gastrophryne olivacea*) as outgroups (Supplementary material Table 1). Sequences were

aligned in Seaviewv4.2.11 (Gouy etto the.2010) under ClustalW2 with default settings (Larkin et al. 2007) and the 12S and 16S rDNA with MAFFT (Katoh et al. 2002). The total concatenated alignment was 4436 bp long. Uncorrected genetic *p*-distances were calculated using MEGA 7 (Kumar et al. 2016).

The complete DNA matrix included 91 taxa and encompassed all *Elachistocleis* sequences for the aforementioned genes. Analyses were performed using Bayesian inference and maximum likelihood (ML) methods. We implemented the most appropriate substitution model for each gene fragment as determined by the Bayesian Information Criterion in PartitionFinder v2 (Lanfear et al. 2017) to choose the optimal partitioning strategy for both phylogenetic analyses (Supplementary material Table 3). MrBayes v3.2 (Ronquist and Huelsenbeck 2003) was used to construct a concatenated Bayesian tree under the best-fitting substitution model for each gene partition. We used default priors and Markov chain settings, and searches were performed with random starting trees. Each run consisted of four chains of 30,000,000 generations, sampled every 3000 generations. ML searches were conducted in RAxML v7.0.4 (Silvestro and Michalak 2012) using partition data sets under default settings and support was assessed by using 1000 bootstrapped replicates (GTRGAMMAI). Phylogenetic analyeses were performed through the CIPRES platform (Miller et al. 2010).

We used the BEAST v2.5.0 (Bouckaert et al. 2019) suite together with the bModelTest package (Bouckaert and Drummond 2017) to simultaneously estimate the phylogeny, substitution model, and divergence times. For this analysis, the data set was much reduced and only taxa with multiple available loci were included in the alignment. Per-gene alignments were imported into BEAUti, setting clock and substitution models independently for each partition, but linking the tree model (i.e., concatenation). We used a relaxed lognormally distributed clock model, choosing an uninformative uniform prior (0,Inf) for the mean rate parameter. As calibration points, we applied normal distributions (all with one standard deviation) to three secondary calibration points based on mean estimates from de Sá et al. (2012) (Table 3, nodes 19, 17, 15): *Hypopachus-Gastrophryne* 20.97 Mya (highest posterior density (HPD: 13.09–31.50), *Elachistocleis-Hypopachus-Gastrophryne*, 30.30 Mya (HPD: 19.51–41.58), and all the *Elachistocleis* ingroup 23.24 Mya (HPD: 13.30–33.79). The *Elachistocleis* ingroup calibration did not include the newly described species' divergence *E. araios* (Sanchez-Nivicela et al. 2020), but monophyly was not enforced in order to infer its placement. All other parameters were set to default values. Two BEAST MCMC chains were run independently with 20 million generations each, sampling every 2000 states. Convergence and parameter mixing were verified with Tracer v1.7 (Rambaut et al. 2018), ensuring consistency across runs and that most parameters had sufficient effective sample sizes (> 200). Trees and logfiles of both runs were then combined using LogCombiner v2.5.0, and TreeAnnotator v2.5.0 was used to summarize estimates into a maximum-clade-credibility (MCC) tree. Trees were visualized and edited in FigTree v.1.4 (Rambaut 2010). All analyses were performed on the Niagara Supercomputer hosted by the SciNet Consortium at the University of Toronto (Ponce et al. 2019).

Morphological methods

Measurements were taken on preserved specimens using digital calipers under a dissecting microscope to the nearest 0.1 mm. Data collection follows Watters et al. (2016) and Caramaschi (2010). Abbreviations of the measurements are as follows: SUL (snout-urostyle length); HL (head length); HW (head width); IND (internarial distance); END (eye to nostril distance); ED (eye diameter); IOD (interorbital distance); HAL (hand length); THL (thigh length); TL (tibia length); FL (foot length); LL (leg length). Specimens were examined at the EBRG (Museo de la Estación Biologica de Rancho Grande, Ministerio del Ambiente y de los Recursos Naturales Renovables, Maracay, Venezuela) and UWIZM (The University of the West Indies Zoology Museum, St. Augustine, Trinidad). Note that the acronym CAREC is the Caribbean Epidemiology Centre collection which is now incorporated into the UWIZM collection.

Morphological data was taken from 30 specimens (15 from Trinidad and 15 from Venezuela) but only 27 were used in a discrimination analysis (DA) in Xlstats and Excel. The DA included nine specimens assigned to species 1 *Elachistocleis* sp. (formerly *E. ovalis*) and six specimens assigned to species 2 (*surinamensis*) from Trinidad; six specimens that had ventral patterns similar to Trinidad *E.* sp. populations from Venezuela (formerly *ovalis*); and eight specimens that had ventral patterns similar to *surinamensis* from Venezuela. Most of the localities for the Venezuelan specimens were within 300 km of Trinidad (Morphological measurements are in the Supplementary material Table 4 and details of the DA are in Supplementary material Table 5).

Material examined. *E. nigrogularis*-Trinidad—CAREC.A.22, CAREC.A.33, UWIZM.2017.5.4, UWIZM.2011.20.7, UWIZM.2016.22.62, UWIZM.2016.22.55, UWIZM.2016.22.56, UWIZM.2015.18.14. *E. surinamensis*—UWIZM.2012.27.8, UWIZM.2016.22.58, UWIZM.2016.22.57, UWIZM.2012.27.7, UWIZM.2016.22.59, UWIZM.2014.27, UWIZM.2011.20.8. *Elachistocleis* sp.-Venezuela - Monagas: La Inglesa, Tabanal river, Aguasay municipality EBRG4976, EBRG4993. Bolivar: Nichare river mouth, Cauca river — EBRG5023, EBRG5026: Santa María Camp, Arrau Tortuga Wildlife Refuge EBRG5353; Caruachi Dam, Island 15-4

EBRG5029; EBRG6353, EBRG6355, EBRG6356; Carabobo: University of Carabobo, Naguanagua EBRG5620; Montalban, EBRG6405; Miranda: Caño Arena Tourist Camp, EBRG6183; Falcón: SE Mene Mauroa EBRG6958, EBRG7037, EBRG7039.

Call properties

Calls of *Elachistocleis* sp. were recorded with a Sony TC-1535D cassette recorder and a Sony ECM 99 microphone, and *E.* spp. (formerly *E. ovalis*) were recorded with a Sony TC-D5M cassette recorder and an ECM 969 microphone. No temperature data were collected. For this analysis, the wording used to describe the calls is based on the work describing the call properties of *Elachistocleis* sp. (Nelson 1973; De La Riva et al. 1996; Nunes et al. 2010). The calls of *Elachistocleis* are relatively simple in terms of structure, with a single prolonged note lasting up to several seconds and with a harmonic interval of 100–250 Hz. We recorded five call characteristics: call duration (s), peak frequency (Hz), number of pulses per call, pulse rate (pulses per second), and inter-call interval (time in s between calls = notes here). We did not discriminate between the dominant frequency at the beginning and at the end of the note (see De La Riva et al. 1996) as there was no visible variation within the note. Call properties were extracted from a 44.4-s long recording attributed to *E.* sp. and from a 1-min 6.4-s long recording attributed to the population formerly called *E. ovalis*.

Prior to data extraction, background noises were filtered out at 2.4 kHz, and spectrogram configuration was set at Hann window of 256-sample window size, 128-sample hop size with 50% frame overlap, and 172-Hz frequency grid spacing. Each call was analyzed for both temporal and spectral domains (Raven Pro 1.4; Cornell Lab of Ornithology, New York, USA), following the recommendations of Köhler et al. (2017). We then analyzed variations between the two species for the five variables recorded through analyses of variance. The biostatistical analyses were conducted in SPSS (SPSS, Inc., Chicago, USA).

Results

Molecular results

No stop codons were found in the protein-coding genes. The best-fitting models and partitions are shown in Supplementary material Table 3. The Peruvian *E. araios* is the most basal of all species in the genus and *E. panamensis* is the most recent common ancestor to all other *Elachistocleis* (Fig. 2). We recovered two strongly supported clades, highly divergent with *E.* sp. (formerly *E. ovalis*—hereafter "*ovalis*" refers to *E.* sp. and geographical origin is indicated for clarity) and *E. surinamensis* from Trinidad strongly supported in both clades. The top clade consists of *E. helianneae*, sister clade to *E.* sp. (former *E. ovalis*) from Trinidad and Venezuela, which form a monophyletic clade. This clade (*heliannaea*+ *E.* sp.—former *ovalis*) is sister clade to *E. bicolor*, showing two divergent clades, one of them is sister clade to an undescribed species (*E.* sp.) from Bolivia. The bottom clade consists of *E. piauiensis* and *E. mauriquitan*, but their support remains weak. The *E. cesarii* clade is strongly supported. *E. pearsei* from Colombia and *E.* sp. (the former invalid *E. ovalis* from Panama) are monophyletic with low support and sister clade to a strongly supported clade of *E.* sp., another former *E. ovalis* population from Colombia. This clade (*E. pearsei*+*E.* sp.—former *ovalis*) is sister clade to the *surinamensis* clade composed of individuals from Trinidad and Guyana sequenced from this study. This well-supported clade has specimens named after the former invalid species *E. ovalis* and a Venezuelan *surinamensis*. The recovery of individuals named under the former invalid species *E. ovalis* from French Guiana and Brazil in the *E. surinamensis* clade (and are therefore *E. surinamensis*) confirms a much larger distribution for this species towards the southeast (not shown in the map, Fig. 1). Our results are not the first to demonstrate the paraphyly of the former *E. ovalis* populations (corrected to *E.* sp.), as it is clear that a thorough taxonomical revision is needed (Caramaschi 2010). *E. bicolor* E47 (Fig. 2) is labeled *E. ovalis* in de Sá et al. (2012), but it is named *E. bicolor* in GenBank. Here, we use the GenBank name in accordance with the rest of the taxonomic tree; however, this implies paraphyly of *E. bicolor* and may suggest that this taxon is *E.* sp.

The BEAST time tree recovered *Elachistocleis araios* as basal to all *Elachistocleis* spp. having a mean divergence of 28 Mya (24.7–32.2, 95% HPD) (Fig. 3). *Elachistocleis panamensis* also resulted somewhat basal to the rest of the ingroup, diverging 22.6 Mya (20.8–24.4, 95% HPD). The time to the most recent common ancestor (TMRCA) for both sister clades dates to 14.4 Mya (11.5–17.1, 95% HPD). *Elachistocleis helianneae* is a sister clade to Trinidad and Venezuelan populations formerly assigned to the invalid species *E. ovalis* and named *E. nigrogularis* in Fig. 3 to avoid confusion and following the new species description (see results below), dating at 3.8 Mya (2.5–5.2, 95% HPD), and Trinidad and Venezuela populations have a divergence dating at 2.17 Mya (0.9–3.6, 95% HPD). Divergence between *E. helianneae* + *E.* sp. from Venezuela and Trinidad to the remaining sister clade (*E. bicolor* + *E.* sp. A from Bolivia) dates at 6 Mya (4.4–8.0, 95% HPD). The weak support for *E. bicolor* + *E.* sp A makes phylogenetic relationships inconclusive but suggests a split at 4.8 Mya (2.8–6.8, 95% HPD). *Elachistocleis muiraquitan* is basal to the second clade at 4.3 Mya (2.9–6.7, 95% HPD). *Elachistocleis piauiensis* is sister clade to

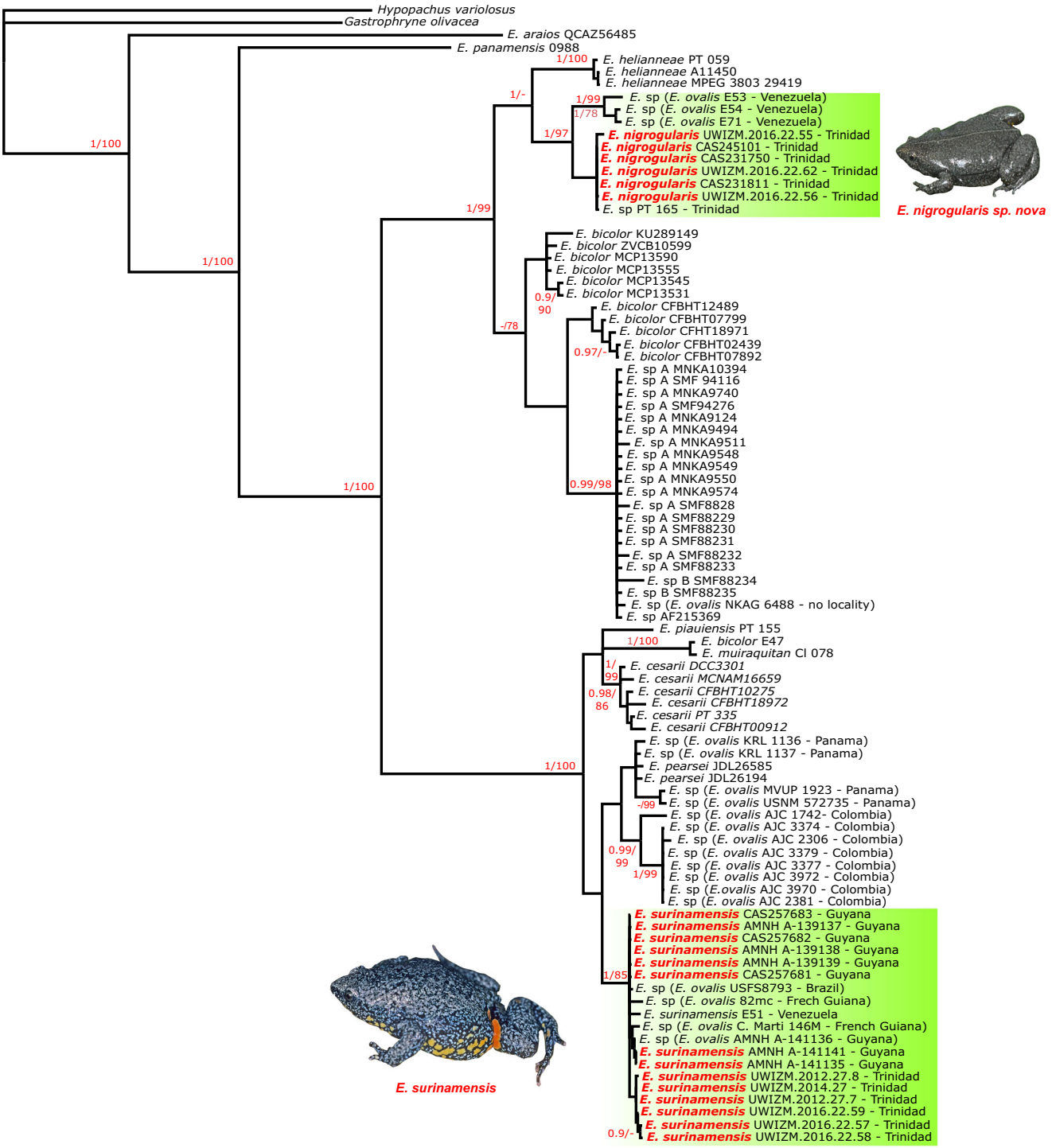

Fig. 2 Bayesian tree inferred by Mr. Bayes based on the data set ($n = 91$) of concatenated 12S, 16S rDNA, *COI*, *RAG-1*, and *TYR* sequences. Values in red by nodes at each side (left and right) or top and bottom of the dash (/) represent Bayesian posterior probabilities (>90%) and Maximum Likelihood bootstrap values (>75%), respectively. Individuals sequenced in this study are shown in bold red and their clades are represented in light green color. Note: the former populations of *E. ovalis* are named *E.* sp. and in between brackets the GenBank names and population locality. All taxa in green clades have localities

E. cesarii but weakly supported dating to 2.5 Mya (1.7–3.4, 95% HPD). The sister clade relationship *E. piauiensis* + *E. cesarii* to *E. surinamensis* + *E. pearsei* + the Colombian *E.* sp. (former *ovalis*) is weakly supported at 2.9 Mya (2.7–4.6, 95% HPD). *E. pearsei* + *E.* sp. (former *ovalis* from Colombia) date to 1.9 Mya (1.1–2.9, 95% HPD), and *E. surinamensis* from these at 2.9 Mya (2–3.9, 95% HPD) (Fig. 3).

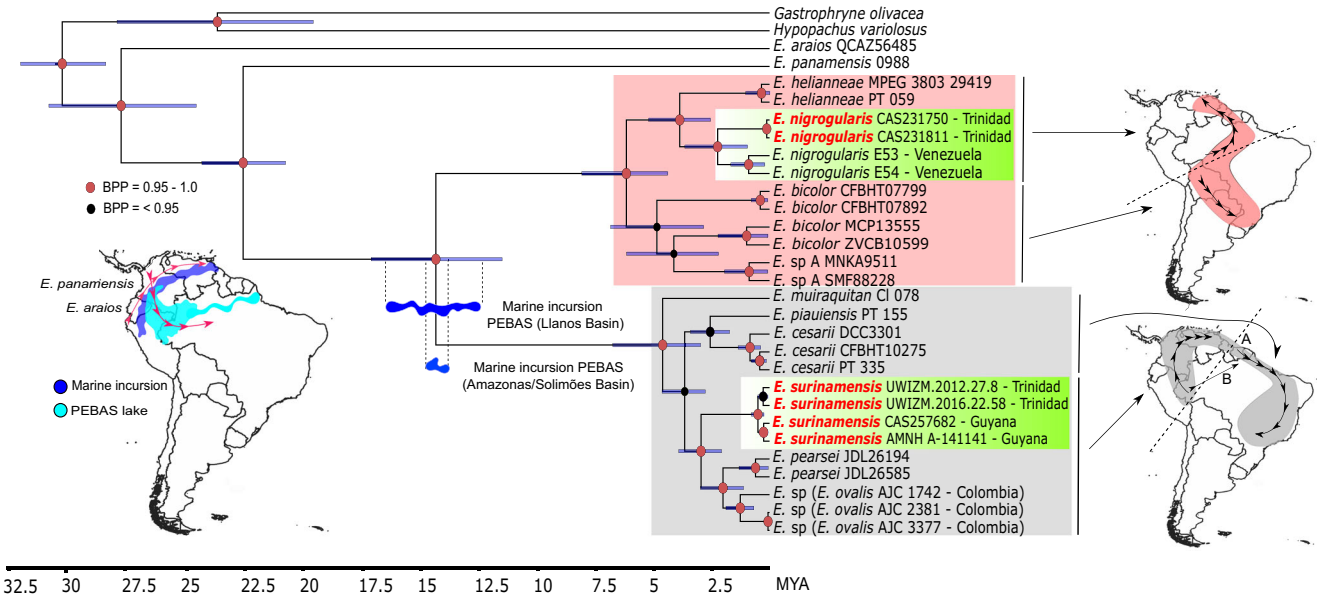

Fig. 3 MCC discrete coalescent tree of 12S rDNA, 16S rDNA, *COI*, *RAG-1*, and *TYR* sequences. Node circles in red represent posterior probabilities (>0.95) and black nodes (<0.95) and their sizes indicate lower support. Blue bars by nodes denote 95% highest posterior density ranges. The map depicted at the lower left represents the marine incursions in northwestern South America (in dark blue) and the PEBAS wetland lake (light blue) and the red arrows point to likely dispersal routes. The drawing of water in the tree depicts the timing of marine incursions in the Llanos Basin and in the Amazonas/Solimões Basin. The two maps on the right depict each sister clade with its subclades and the likely routes of dispersal (in black arrows) throughout South America. In the gray clade, A and B represent different scenarios of routes of dispersal; see text for details. Specimens sequenced in this study are in bold red and their clades are represented in light green color. Note: the former populations of *E. ovalis* are named *E.* sp. and in between brackets the GenBank names and population locality. All taxa in green clades have localities. Former *E. ovalis* from Venezuela are renamed *E. nigrogularis*

Genetic divergence (*p*-uncorrected distances) between the *E.* sp. populations (former *E. ovalis*) from Venezuela (E53, E54, E71) and Trinidad was low for the 16S rDNA (range 0.4–0.7%, mean 0.6%) but moderate for the TYR gene fraction (0.2–0.9%, mean 0.5%). Exclusion of specimen E53 from the latter analyses resulted in much lower *p*-distances (range 0.2–0.5%, mean 0.3%).

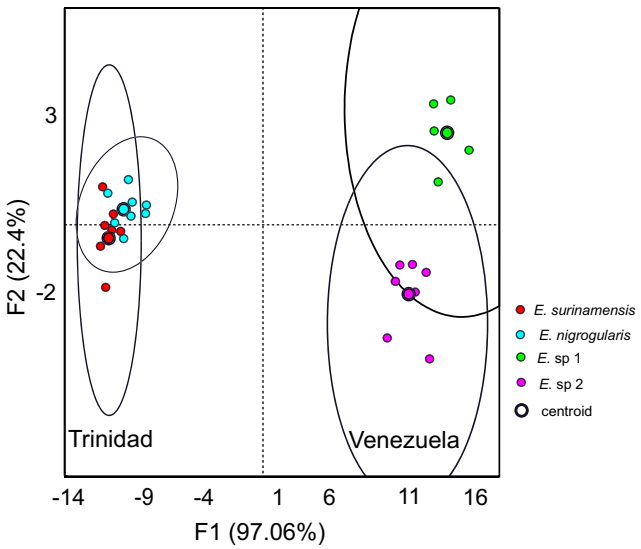

Fig. 4 Results of the discrimination analysis. The DA results suggested Trinidad and Venezuela *Elachistocleis* with similar coloration and pattern phenotypes are not always the same species. The DA compared morphometrics from the island and mainland populations. The Venezuelan populations labeled sp. 1 are similar in appearance to *E. nigrogularis* and sp. 2. are similar in appearance to *E. surinamensis*

Taxonomic—systematic results

Despite similar ventral patterns, the DA resulted in non-overlapping morphometric space between the *Elachistocleis* sp. formerly assigned to the invalid species *E. ovalis*, and *E. surinamensis* in Trinidad and Venezuela, suggesting these samples might represent distinct species (Fig. 4, Supplementary material Table 5). The DA's Wilk's lambda test, Pillai's trace, Hotelling-Lawley trace, and Roy's greatest root all had *p* values below 0.0001 rejecting the null hypothesis that the specimens were all the same species.

Therefore, the Venezuelan taxa labeled *E.* sp. (previously *ovalis*) and *surinamensis* represented in our molecular sample were not represented in our samples used for morphological analysis. The similarity in ventral patterns therefore does not always indicate conspecific status. The body proportions of the Trinidad populations and the Venezuela populations in our sample were quite different.

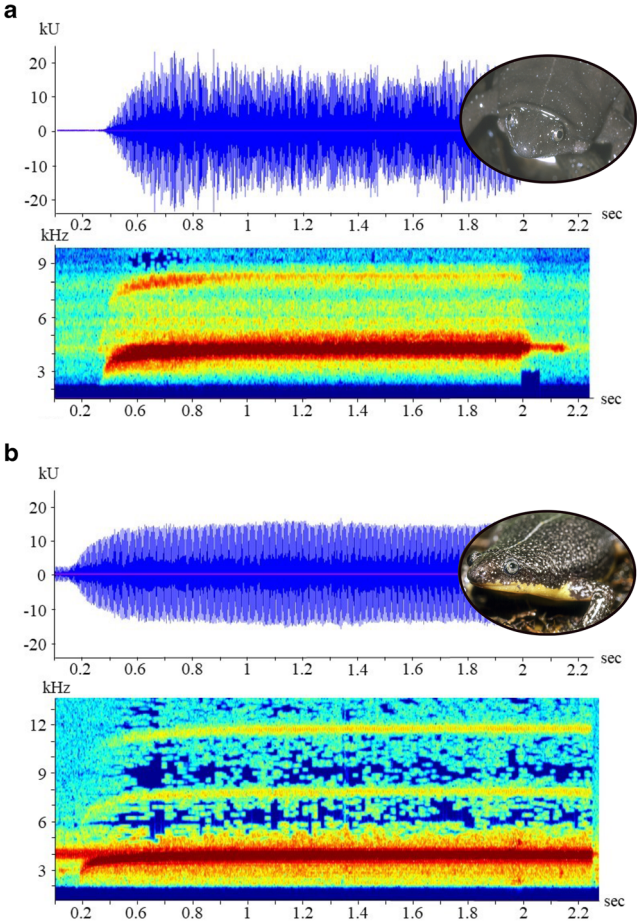

Fig. 5 Calls. **a** *Elachistocleis nigrogularis* without Orthoptera calls at 7000 Hz (bandpass filter: 6800–7200 Hz). **b** *Elachistocleis* sp. (undescribed species) after removing the calls of the other amphibians to be able to count pulses. The beginning of the call is clearly visible despite a second individual calling on the background and two harmonics are visible for this call from this individual. Pictures of frogs by the spectrograms are actual male individuals recorded calling

Call characteristics results

Elachistocleis sp. (former *ovalis*): Calling from a shallow pool partially filled with leaf litter in closed-canopy forest near the edge of the Arena Reservoir, Trinidad, the night of 20 February 1989. Approximate location 10.5490° N; 61.2528° W. Sympatric calling species: *Dendropsophus goughi*, *Phyllomedusa trinitatis*, *Leptodactylus validus*. The photograph (Fig. 5a), taken in situ, is of an individual encountered shortly after the recording was made, floating on the water surface partially concealed in leaf litter and was probably the closest individual calling in the recording.

Elachistocleis sp.: Calling from waterlogged grass beside a road in a belt of coconut plantation separating the east coast from the Nariva Swamp, Manzanilla/Mayaro road near the mouth of Nariva River, Trinidad, 6 August 1980, about one hour after dark. Approximate location 10.3911° N; 61.0198° W, about three males calling, distance of closest individual about one meter, *Pseudis paradoxa* and *Scinax ruber* calling in the background. The photograph (Fig. 5b) is of an individual captured at the same site shortly after the recording was made and suggests this is a third species of *Elachistocleis* on Trinidad.

In total, we extracted call property data for seven notes for *E.* sp. and six notes for *E.* sp. (former Trinidad *ovalis*). Each call is a single periodic pulse train (Fig. 5), with a non-significantly different number of pulses per call for the two species (Table 1, ANOVA: $\chi^2 = 17{,}760.07$, $F_{1,12} = 2.54$, $p = 0.139$). The call duration (ANOVA: $\chi^2 = 3.80$, $F_{1,12} = 6.29$, $p = 0.029$) and inter-call-interval (ANOVA: $\chi^2 = 55.49$, $F_{1,12} = 34.01$, $p < 0.005$) were significantly higher in *E.* sp. (the former Trinidad *ovalis*) than in *E.* sp. (Table 1). The peak frequency, however, follows the opposite pattern and is significantly higher (ANOVA: $\chi^2 = 110{,}030.1$, $F_{1,12} = 7.82$, $p = 0.017$) in *E.* sp. than in *E.* sp. (former *E. ovalis*) (Table 1). Finally, the pulse rate is not significantly different between the two species (ANOVA: $\chi^2 = 1324.88$, $F_{1,12} = 4.06$, $p = 0.069$). Based on available data from the literature (Table 1), it is not possible to statistically assess for variations with our dataset; however, the means for *E.* sp. (former *E. ovalis*) continental populations remain closer to that of *E.* sp. from our study (former *E. ovalis*), than to the new species.

Taxonomy—species description

The two species long considered present on Trinidad have been called *E. ovalis* and *E. surinamensis* (Kenny 1969; Murphy 1997). Evidence connecting the Trinidad species called *E. ovalis* and the original description of *R. ovalis* by Schneider (1799) is absent. Hence, we are describing that species as a new taxon. The geography and ventral coloration associated with Daudin's description of *Bufo surinamensis* link it to the Trinidad population; that population has been called *E. surinamensis* since 1969. Thus, we have selected a neotype for *E. surinamensis* from Trinidad to stabilize the nomenclature.

Elachistocleis nigrogularis sp. nova
Bufo ovalis—Daudin 1802:92
Engystoma ovale—Boulenger 1882:163
Engystoma ovalis—Nieden 1926:66
Elachistocleis ovale ovale—Parker 1927:4
Elachistocleis ovalis—Kenny 1969:68

Holotype: UWIZM 2016.22.56, a female from Trinidad, West Indies. 7.7 km SE of Arima; along Cumuto Tamana Road, at construction site (10.58664° N, 61.23144° W) collected 1 June 2016 by Alvin Braswell, John Murphy, Mike Rutherford. In amplexus with UWIZM 2016.22.55. Photographs taken and tissues collected.

Table 1 Descriptive statistics of call properties for *Elachistocleis nigrogularis* and *E.* sp. and published call characteristics for *E.* sp. (former *E. ovalis*)

	Elachistocleis sp. (former *ovalis*, Trinidad)			*Elachistocleis* sp., Trinidad			*E.* sp. (former *ovalis*, Central Llanos, Venezuela), Tárano (2010)	*E .* sp. (former *ovalis*, Panama, Colombia, SA east of Andes), De la Riva et al. (1996)	*E.* sp. (former *ovalis*, Dubulay Ranch, Guyana), Bourne and York (2001)
	N	Mean	SD	*N*	Mean	SD			
Note duration (s)	15	2.71	0.49	6	3.41	0.69	-	-	2.85 ± 0.13
Inter-call interval l (s)	13	4.85	1.91	5	8.28	1.66	-	-	3.84 ± 5.18
Peak frequency (Hz)	15	4141.93	155.91	6	3875.98	94.35	5020 ± 210	3779.9	-
Number of pulses per note	15	320.58	58.57	6	410.00	97.68	345.64 ± 86.86	-	-
Pulses rate (/s)	15	110.31	5.24	6	132.99	26.21	-	-	232.4 ± 3.01

Referred material: Trinidad: Aripo Savanna UWIZM.2011.20.7, UWIZM.2015.18.14, Arena Forest, Cumoto Rd. UWIZM.2016.22.62, UWIZM.2017.5.4; Nariva Swamp CAREC.A.22, CAREC.A.33.

Diagnosis: A medium-sized *Elachistocleis*, SUL 28.3–32.8 mm in four males, 34.8–41.9 mm in four females, characterized by head length about equal to head width, HW about 0.99 (r = 0.89–1.12, n = 4) of length in females and HW 1.09 (r = 1.02–1.11, n = 4) of HL in males (x = 8.62; SD = 5.6; n = 4); the fold of skin on the back of the head extends to the shoulder and then runs along the ventrolateral surface to just in front of the hind leg (may not be present in preserved material) the postcommisural gland is present and obvious in live specimens; tympanum indistinct; dorsum smooth; in preservative black with few minutes scattered white to gray spots; an indistinct, pale vertebral line, from snout to vent; ventral surface is brown-gray with minute pale stippling. There is an abrupt transition from dorsal coloration to ventral coloration ventrolaterally; no spots or coloration in the axillae or groin region; a narrow well-defined red or orange (cream in preserved material) band on the posterior surface of the thighs; the gular region is jet black, the chest and abdomen are gray with fine yellow to cream stippling that extends to the ventral surface of the thighs but not to the tibia or foot which are black. The tips of the digits on the hands tend to be cream, and the toes tend to have cream tips plus off-white marking at the joints of the phalanges. Rear legs 1.02–1.17× the body length.

Holotype measurements: SUL 32.77, HL 10.86, HW 9.12, IND 1.96, END 2.88, IOD 4.48, HAL 6.95, THL 10.49, TL 8.85, FL 17.23. Measurements for referred material are given in Supplementary material Table 4.

Description of holotype: Body ovoid, head small, triangular, slightly longer than broad, head length 119% of head width and 0.33 of SUL. Snout subelliptical in dorsal view, protruding in profile. Nostrils small, not protuberant, directed anterolaterally, closer to tip of snout than to eye, internarial distance is 1.96 mm, eye orbit diameter in two females 2.14 mm. Canthus rostralis rounded; loreal region flat, sloping and rounded to the upper lip; lips not flared. Eyes small, dorsolateral, only slightly protruding. Interorbital space slightly convex, about half the head width. No cranial crests. A well-defined transverse skinfold across back of the head, joints fold around body bending downwards slightly behind the eyes to the shoulder and extends posteriorly to the femoral axial region. Tympanum concealed, supratympanic fold absent. Lower jaw with truncate, fold on posterior edge. Tongue large, oval, heart-shaped with a slight notch on posterior border. Choanae large, subcircular, widely separated. Premaxillary, maxillary, and vomerine odontophores absent. A weak skinfold crossing the chest between axillae. Arms moderately robust, no tubercles, or crests on forearm; a pair of palmar tubercles on base of palm; fingers slender, free, with subarticular tubercles developed, rounded; fingers round not flattened or expanded; terminal grooves absent. Relative lengths of fingers, 1<2>4>3. Prepollex not evident. Legs short, robust. Thigh length longer than tibia, tibia about 0.84 of the femur, and about 0.51 of the foot. Back leg length longer (112% of the SUL). Toes slender, free, not fringed; supranumerary tubercles absent; tips of toes rounded, not flattened, or expanded; terminal grooves absent. Relative lengths of toes, 2<3<1<4<5. (See Table 2 for morphological comparison to all other 19 *Elachistocleis* species.)

Skin smooth above and beneath. Anal opening not modified, para-anal tubercles absent. In life (Fig. 7), dorsum and dorsal surfaces of limbs grayish brown, pale medial stripe; distinctive mid-dorsal light cream stripe, from between the eyes to the urostyle; a gradation of color and pattern laterally between the dorsal and ventral regions is mostly black with some gray reticulations; venter dark gray, mottled with light gray and yellow; throat dark gray to black with fine white spots; axial region have no flash colors; tiny orange flecks on base of thigh and groin spots on axillae and groin; a well-defined red stripe on the posterior surface of thighs.

Variation in referred material: The variations in body measurements are in Supplementary material 4. The variation in *E.*

Table 2 A comparison of the 19 described species of *Elachistocleis*. Note that *E. ovalis* is not included given that its identity is unknown. We have also excluded speculation about the distribution of the various species

Species	Habitat	Size m/f, mm	Ventral pattern	Dorsolateral fold present	Postcommisural, gland development	Mid-dorsal stripe	Pattern on femur	Distribution
E. magnus	Wet fields, gallery forests, tropical dry forests, flooded pasture	37/44	Blotches	Yes	Good	White	Broad irregular	South Amazonas, and the western Mato Grosso to eastern Pará, Brazil
E. bumbameuboi	Restingas	29/43	Blotches	Yes	Good	None	Broad irregular	NE Brazil
E. surinamensis	Forest edge, savanna	32/42	Mottled	Yes	Good	Absent or black	Broad irregular	Trinidad WI, Venezuela, Guyana to Suriname, French Guiana
E. pearsei	Tropical dry forest	39/45	Mottled	Yes	Good	White	Well defined	Caribbean Colombia and Panama, NE Venezuela
E. carvalhoi	Savanna, forest	32/37	Spots	Yes	Good	None	Broad irregular	Northwestern Tocantins and SE Pará, Brazil
E. surumu	Forest edge, calling grassy pools	27/27	Spots	Yes	Good	Absent	Broad irregular	Roraima, Brazil; likely into adjacent Venezuela and Guyana
E. panamensis	Tropical dry forest	24/26	Spots	Yes	Good	White	Well defined	Central Panama to Magdalena, Cundinamarca, and Chocó, Colombia
E. corumbaensis	Pantanal, seasonal flooded forest	29/40	Spots	Yes	Poor	White	Broad irregular	West Pantanal, Mato Grosso do Sul, north into SW Mato Grosso, Brazil
E. skotogaster	Edge moist tropical forest	29/34	Spots vermiculations	Yes	Absent	None	Broad irregular	Provincia de Formosa, Argentina, and South Bolivia
E. nigrogularis	Savanna	35/48	Finely spotted	Yes	Good	White	Well defined	Trinidad, Venezuela
E. erythrogaster	Wet grassland and savanna	32/38	Uniform	Yes	Good	None	Absent	Southestern border of the Planalto das Araucárias, Serra Geral, Rio Grande do Sul, Brazil
E. cesarii	Savanna. closed field, Campoija	28/36	Uniform	Yes	Good	White	Well defined	Southeast to Central Brazil
E. piauiensis	Caatinga. Cerrado, urbanized areas	23/–	Uniform	Yes	Good	None	Broad irregular	Northeastern Brazil
E. helianneae	Savanna, pools urban area	29/36	Uniform	Yes	Good	White	Broad irregular	Amazonian Brazil, and in northeast Bolivia
E. araios	Forest leaf litter	–/26	Uniform	No	Poor	Yes	Narrow line	Azuay and Guayas provinces of Ecuador
E. haroi	Wet Chaco	30/28	Uniform	Yes	Poor	White	Well defined	North Salta, West Formosa, East Chaco, and Southeast Santa Fe provinces, Argentina, North into Tarija Province, Bolivia
E. matogrosso	Cerrado, pools in urban areas	25/34	Uniform	Yes	Poor	White	Broad irregular	Central Brazil in Southwest Mato Grosso and Northwest Mato Grosso do Sul, South Paraguay

Table 2 (continued)

Species	Habitat	Size m/f, mm	Ventral pattern	Dorsolateral fold present	Postcommisural gland development	Mid-dorsal stripe	Pattern on femur	Distribution
E. muiraquitan	Tropical forests, flooded grassy areas	33/40	Uniform	Yes	Poor	White	Broad irregular	Southeast Peru, Northwest Bolivia, and Acre, Brazil
E. bicolor	Pine, eucalyptus unmodified	29/31	Uniform	Yes	Poor	None	Broad irregular	Central Argentina and Uruguay through Paraguay to South Brazil

nigrogularis dorsal patterns ranges from solid black to black with scattered small white spots. The ventral pattern variation is much greater, and males have large yellow or orange-red blotches that are surrounded by finely stippled gray and yellow pigment. The large blotches may be restricted to the thorax or the abdomen or may not be present. Figure 6 shows dorsal and ventral views for museum material.

Etymology: *E. nigrogularis* is from the Latin *nigro* for black and *gula* for gular region and refers to the black vocal sac of the male and the black pigmented gular region of the female.

Natural history: The holotype was collected at pools in a construction site; large chorus of several other species were present (*Elachistocleis sp.*, *Scinax ruber*, *Dendropsophus microcephalus*, *D. goughi*, *Engystomops pustulosus*, *Boana xerophylla*, *Leptodactylus fuscus*, *L. validus*).

Designation of a neotype for *Elachistocleis surinamensis* (Daudin)

Neotype. UWIZM 2016.22.58 from Trinidad, St. George, Arena, Cumuto Rd. (10.5866° N 4–61.23144° W). A female. Collected by Alvin Braswell, Renoir Auguste, John Murphy on June 1, 2016. Figs. 6 and 7.

Referred material. Trinidad: Aripo Savanna UWIZM.2011.20.8, UWIZM.2014.27; Arena Forest, Cumuto Rd UWIZM.2016.22.57, UWIZM.2016.22.59; Lopinot UWIZM.2012.27.7–8.

Diagnosis. A medium-sized species, SUL 26.5–34.4 mm in males, 33.8 35.1 mm in females. Kenny (1969) reports males to 35 mm and females to 48 mm. The postcommisural gland obvious in live animals; tympanum indistinct; dorsum smooth; in preservative black with extensive gray reticulations and an indistinct black vertebral line, from snout to vent in some specimens; in others, there is a large patch of black reticulations centered on the vertebral line; ventral surface is highly variable but has brown to gray mottling with large patches of yellow. The yellow patches are irregular and vary in size. There is a transition from dorsal coloration to ventral coloration laterally, black vermiculations with white pigment that become yellow ventrally; yellow and orange patches in the axillae and groin regions; a broad irregular yellow to orange band on the posterior surface of the thighs, this may be broken into blotches. In males, the gular region is mottled with black and yellow-orange, the chest and abdomen are brown-black with yellow-orange blotches that extend to the ventral surface of the thighs and onto the tibia and foot. The tips of the digits on the hands tend to be cream, and the toes tend to have cream tips plus off-white marking at the joints of the phalanges. Rear legs 1.07–1.24× the body length.

Neotype measurements. SUL 33.09; HL 11.87; HW 9.86; IND 2.18; END 2.73; ED 2.05; IOD 4.52; HAL 6.62; THL

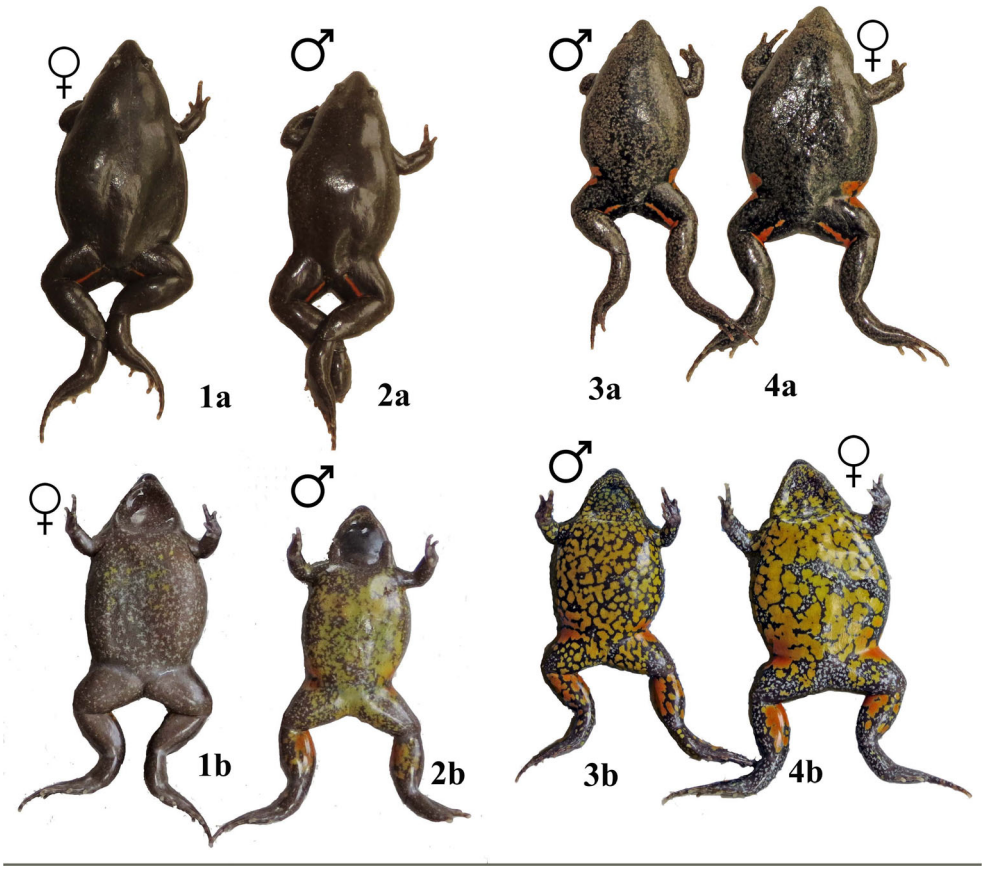

Fig. 6 A comparison of *Elachistocleis nigrogularis* (**1** and **2**) and *E. surinamensis* (**3** and **4**). **1a** and **1b** are dorsal and ventral views of the female *E. nigrogularis* holotype, UWIZM 2016.22.56. **2a** and **2b** show UWIZM 2016.22.55, the male collected with the holotype. **4a** and **4b** are dorsal and ventral views of the *E. surinamensis* neotype UWIZM 2016.22.58. **3a** and **3b** are dorsal and ventral views of the male she was collected with. Photography ALB

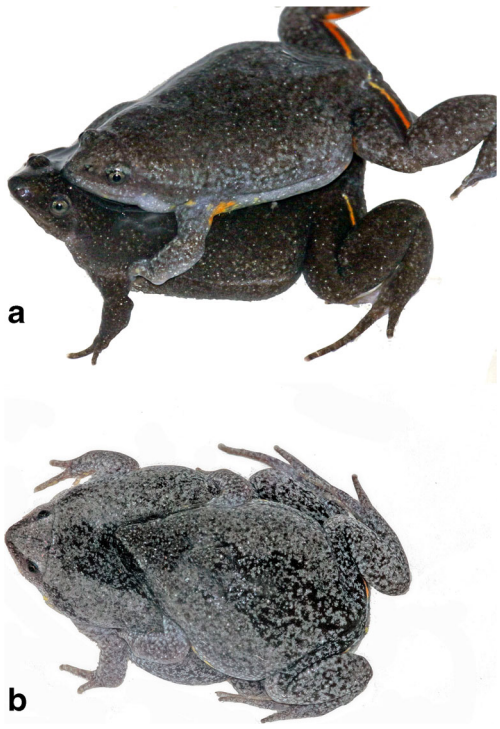

Fig. 7 **a** An *Elachistocleis nigrogularis* in amplexus. **b** *Elachistocleis surinamensis* in amplexus. Photography JCM

11.98; TL 8.97; FL 15.51. Measurements for referred material are given in Supplementary material Table 4.

Description of neotype. Body ovoid, head small, triangular, about as broader as long; head length 35.0% of SUL head width and 29% of SUL. SUL 33.93 mm; Snout subelliptical in dorsal view, protruding in profile. Nostrils small, not protuberant, directed anterolaterally, closer to tip of snout than to eye; internarial distance (2.18 mm) greater than the eye to nostril (1.33 mm), and less than interorbital distances (4.52 mm). Canthus rostralis rounded; loreal region rounded; lips not flared. Postcommisural gland well developed and marked with yellow pigment less obvious in preserved state. Eyes small (2.05 mm), dorsolateral, only slightly protruding. Interorbital space slightly convex, almost three times the upper eyelid width. No cranial crests. A well-defined transverse skinfold across back of the head, bending ventrally and posteriorly slightly behind the eyes to the shoulder. Tympanum concealed, supratympanic fold absent. Lower jaw with small fold at rictus.

Tongue large, heart-shaped. Choanae large, subcircular, widely separated. Vocal slits present. A weak skinfold crossing the chest between axillae. Arms moderately robust, three tubercles on the wrist and forearm. Two palmar tubercles are large, divided longitudinally, the size of the thenar tubercle;

fingers slender, free, with subarticular tubercles developed, fingers not flattened or expanded; terminal grooves absent. Relative lengths of fingers, 2<1<3<4. Prepollex not evident.

Nuptial pads or asperities absent. Legs 1.07 times the SUL. Thigh length (11.9 mm) tibia (8.97 mm) and both shorter than foot length (15.5 mm). Knee and heel with a transverse skinfold; no tibial or tarsal ridges; no metatarsal tubercle; plantar tubercles small and number six. Toes slender, free, weakly fringed; subarticular tubercles developed, rounded; tips of toes rounded; terminal grooves absent. Relative lengths of toes, 2<3<1<4<5. Skin smooth above and beneath; Anal opening not modified, no para-anal tubercles.

In life (Figs. 6 and 7) dorsum and dorsal surfaces grayish with darker gray to black reticulations, no dark vertebral stripe but a patch of black reticulations on the mid-dorsum. Small transition zone between dorsal and ventral colors; venter mottling of black, yellow-orange, and white flecks that vary in intensity and dominance gular region has less yellow-orange and more black mottling than the chest and belly which are mostly yellow-orange; black areas have small areas of yellow-orange spotting surrounded with black. The pattern covers the ventral surface of the limbs and there are large patches of yellow-orange on the tibia, and in the axilla and in groin regions. The posterior surface of thighs has a distinctive, thick, poorly defined orange-red stripe.

Variation in referred material. The variations in body measurements are in Supplementary material Table 4. The variation in *E. surinamensis* dorsal pattern is a vermiculated pattern of pale and dark grays. The ventral pattern variation is much greater, both sexes appear to have mottled gular regions, the thorax and abdomen patterns are quite variable with large yellow or orange-red blotches that are surrounded dark gray, brown, or black pigments. Figure 6 shows dorsal and ventral views for museum material.

Natural history. The neotype was collected from shallow pools in a construction site; large chorus of several other species was present (*Elachistocleis nigrogularis* sp. nova, *Scinax ruber*, *Dendropsophus microcephalus*, *D. goughi*, *Boana xerophylla*, *Engystomops pustulosus*, *Leptodactylus fuscus*, *L. validus*).

Discussion

Elachistocleis phylogeography

Elachistocleis araios is the only species restricted to the Pacific versant of the Andes while *E. panamensis* and *E. pearsei* are found up to the Caribbean Sea. Our data suggest a northern expansion from Ecuador towards Colombia in the Late Oligocene to Early Miocene, a timing in agreement with new world microhylid radiations, believed to have started in the Oligocene in northern South America (van der Meijden et al. 2007; de Sá et al. 2012). Until the recent discovery of *E. araios* (Sanchez-Nivicela et al. 2020), the basal positioning of *E. panamensis* pointed towards an Atlantic to Caribbean Colombian origin (de Sá et al. 2012). Still, the ancestral position of both species suggests an Andean origin, in congruence with molecular studies to account for the Amazonian amphibian diversity (Santos et al. 2014). The high genetic divergence of both ancestral species likely reflects allopatric events in the region, by the uplift of the Andes between ~40 and ~23 Mya, affecting the isolation of *E. araios* to the west. Also, *E. panamensis* expansion may have been constrained throughout the Late Miocene by a deep marine incursion draining to the west of their distribution, ending in the Caribbean region (Santos et al. 2009; Hoorn et al. 2010; Kay 2015; Cordeiro Bicudo et al. 2019) (Fig. 3).

The divergence between both major *Elachistocleis* clades corresponds to the Mid-Miocene. In the Llanos Basin, the first marine transgression covered the basin during the Early Miocene, between 19.1 to 17.2 Mya (lasting 0.9 My) and again in northwestern Amazonas between 18.0 and 17.8 Mya (lasting 0.2 My). A second much more prolonged transgression occurred in the Llanos Basin between 16.1 and 12.4 Mya (lasting 3.7 My) and its continuation towards the Amazonas/Solimões Basin between 14.1 and 13.7 Mya (continuing 0.4 My; Jaramillo et al. 2017). The timing of the last two marine transgressions fall exactly within our divergence time estimates of both clades and may suggest that during this period, the ancestors of the recovered clades were separated through vicariant events in the region. In accordance, the distribution of *E. surinamensis* as far west as the Maracaibo basin and the presence of *E. pearsei* and *E. panamensis* to the west suggests that the northern Andean cordillera, the Sierra del Perijá continues to be a barrier to further dispersal for some species. The Pebas mega-wetland system is however also believed to have been a fluvial system of low salinity (Boeger and Kritsky 2003; Gross et al. 2015; Gross and Piller 2020) and may have facilitated the dispersal route for extinct turtles by the connectivity between major and minor drainages (Cadena and Jaramillo 2014). By the Late Miocene to Pliocene, this system had branched extensively to form the drainage systems of the present-day Magdalena, Maracaibo, Orinoco, and Amazonas rivers (Hoorn et al. 2010). Between 10 and 7 Mya, the Acre system was a series of fluvial connections from the Pebas lake to the Amazon River northeast to the Guianas and may have facilitated dispersal events towards the Amazon (Hoorn et al. 2010; Albert et al. 2018). More recent evidence suggests that the Pebas system gradually dried out at about 10.5 Mya (Jaramillo et al. 2017) and after the draining of the wetlands (Late Miocene), diversification in western Amazonia must have been particularly rapid, as the diversity of this area greatly outnumbers the diversity in the cratonic areas. The presence of the Amazon River still today suggests

connecting corridors through fluvial systems across the Amazon. It is, therefore, reasonable to assume that the availability of savanna after the Pebas dried out and branching of rivers towards the Amazon River tributaries opened a route of dispersion towards the east.

The colonization routes and events of the *Elachistocleis* clades present today remain partially tentative as key species are missing from the analyses that could elucidate alternative scenarios. The timing of both clades corresponds well with the transcontinental Amazon River that was formed over a period of about 4.9–5.6 million years, via several river capture events (Albert et al. 2018) and likely facilitated the dispersal towards the west in the Late Miocene. Our analyses suggest the likely split of *E. helianneae*+*E. nigrogularis* from the Amazon Basin region towards a northward expansion to the Guianas about 6 Mya (4.4–8.0, 95% HPD) and a southward expansion of *E. bicolor*+*E.* sp. (from Bolivia) towards Brazil at 4.8 Mya (2.8–6.8, 95% HPD). The northern populations (i.e., Guyana) may have originated following the Branco-Essequibo drainage towards the Guianas from northern Amazonas (Albert et al. 2018).

The phylogeography of the second clade is more intricate and suggests two likely dispersal events. The basal position of *E. muiraquitan* suggests a western Amazon origin at circa 4.3 Mya (2.9–6.7, 95% HPD) with a northern expansion consisting of species from Panama and Colombia (e.g., *E. pearsei*) following the closure of the isthmus of Panama circa 2.5 Mya. In accordance with this timing is the formation of the isthmus *sensu stricto*, dating to approximately 2.8 Mya (O'Dea et al. 2016), coinciding with the Great American Biotic Interchange (Stehli and Webb 1985) over dry land. The divergence of the Colombian and Panamanian species in our analyses agrees with these time estimates. A likely northern migration circa 2.9 Mya (2–3.9, 95% HPD) through the hydrological connections flowing towards the Caribbean-draining Andean foreland, from Los Llanos to Amacuro (Albert et al. 2018), leading towards the Guianas to what today is the *E. surinamensis* clade. The relationship of *E. piauiensis* + *E. cesarii* can be accounted for either by a southern migration from the Guianas (scenario A, Fig. 3) or by an eastern dispersal following the Amazon Basin followed by a coastal southern route (Scenario B, Fig. 3) at approximately 2.5 Mya (1.7–3.4, 95% HPD). Molecular sampling of *E. carvalhoi* and *E. bumbameuboi* from northern Brazil and *E. magnus* and *E. matogrosso* in southern Brazil, proximal to Bolivia, will elucidate important information to ascertain dispersal routes (Fig. 3).

Phylogeography of Trinidad *Elachistocleis*

The presence of *E. surinamensis* and *E. nigrogularis* in Trinidad not only represents the only two species within the genus found on an island but is also the result of two independent colonization events from different epochs and regions (Fig. 8). The TMRCA of *E. nigrogularis* from Venezuela and Trinidad dates to the Pleistocene (2.17 Mya) and suggests prolonged isolation between mainland and island populations. *E. surinamensis* shows a much more recent divergence between Guyana and Trinidad conspecifics at approx. 0.5 Mya (0.25–0.79, 95% HPD). Interestingly, their presence in Trinidad is not the result of vicariance when Venezuela's Paria Peninsula and Trinidad's Northern Range detached by a downwarping event that separated both land masses in the Late Miocene, circa 4 Mya (Liddle 1946; Erlich and Barrett 1990). Therefore, *Elachistocleis* colonized Trinidad after its separation from Venezuela. This colonization pattern is somewhat like the New World coral snakes present in Trinidad, with *Micrurus circinalis* present in Venezuela and *M. lemniscatus* in Guyana, the latter with moderate genetic divergence between regions (Jowers et al. 2019). In fact, low sea level stands since the Pleistocene have facilitated the colonization of Trinidad from northern Venezuela, especially from the Bocas del Dragón and the Paria Peninsula (Kaiser et al. 2015; Murphy et al. 2018; Jowers et al. 2019). Comparably, low genetic divergence of species between Guyana, French Guiana, and Trinidad also suggests Pleistocene colonization events from the eastern coastal region, most likely through eustatic sea-level falls (Murphy et al. 2016, 2019c; Jowers et al. 2019). Reconstruction of Pleistocene habitat and vegetation suggests that northern Venezuela and Trinidad were dry forests in that period, bordered by savannah to the south. Similarly, Guyana and all coastal Venezuela were covered by dry forests, surrounded by savanna throughout (Melo França et al. 2015). In accordance, *E. nigrogularis* and *E. surinamensis* in Trinidad today are found in similar habitats, such as tropical evergreen forest, marsh forests, and savannas, including anthropogenic savannas (Murphy et al. 2018).

Surprisingly, the Venezuelan individuals in our analyses that showed close genetic affinities to *Elachistocleis nigrogularis* from Trinidad were from the geographically most distant localities for *Elachistocleis* in Venezuela. One of these specimens (E54) is from the southern Maracaibo lake basin, a region located between Cordillera de Merida and Serrania de Perija (western versant Cordillera de Mérida), a locality for *E. pearsei*. A second individual (E71) is from Barinas, in the eastern versant of the Cordillera de Mérida. Pictures of *E.* sp. (former *E. ovalis*), taken close to the aforementioned localities (Barrio-Amorós et al. 2019, plates 216B and 216E), show considerable differences to *E. nigrogularis*, which suggest the possible presence of cryptic species in the region. Furthermore, all pictures of *E.* sp. (previous *E. ovalis*, 6 in total) from Barrio-Amorós et al. (2019) look to be distinct species, which suggests the need for a taxonomic revision in Venezuela.

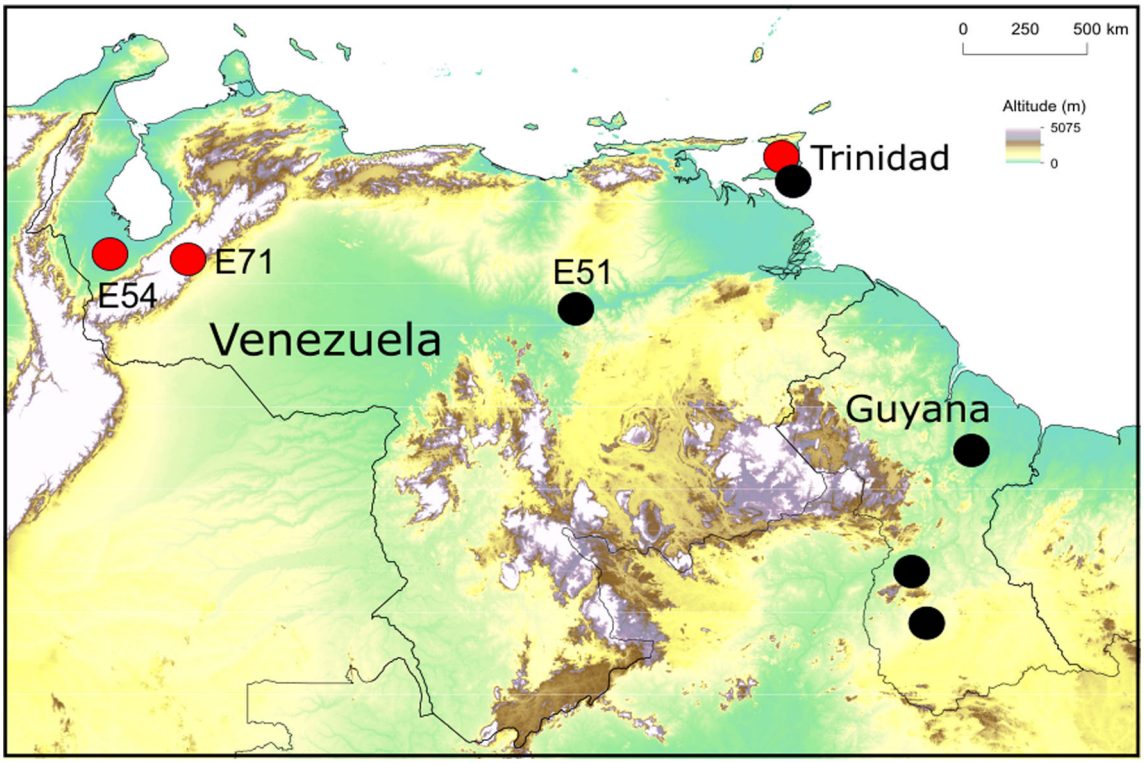

Fig. 8 Map showing localities for *Elachistocleis nigrogularis* (red dots) and *E. surinamensis* (black dots) sequenced for this study and included in the phylogenetic analyses. Venezuelan specimens not sequenced in this study are also included (E54 and E71 are refereed as *E. nigrogularis* and E51 as *E. surinamensis*)

Future research

Most recently, Barrio-Amorós et al. (2019) wrote, "Current authors agree that a complex of species is hidden under that name, and even knowing that the *nomen ovalis* is not valid, but must be used while awaiting a definitive solution and new available names for this long-standing taxonomic conundrum." Caramaschi (2010) considers the name *Rana ovalis* (Schneider 1799) to be a *nomen inquirenda*. Future authors should consider using *Elachistocleis* sp. or doing the research needed to describe the population as new, as done in this study.

Fig. 9 Photograph of the supposed new species for Trinidad found in 1980 by M. Read

The description of *Elachistocleis nigrogularis* in Trinidad and the discovery of a new undescribed species call for additional surveys to assess the exact distribution of the species in Trinidad. The specimens from Venezuela (named *E. nigrogularis* E53, E54, and E71, Figs. 8 and 9) likely represent populations of *E. nigrogularis* in Venezuela and deserve further investigation and additional sequencing, as only a small fraction of the 16S rDNA and *TYR* genes were available. Unfortunately, the economic and political turnover in Venezuela at present time makes fieldwork extremely dangerous and even access to local museum collections remains challenging. It is therefore unlikely that new material will become available within the near future and any identification will rely on morphological identification of vouchered specimens from international museums. Furthermore, the presence of two undescribed species in Venezuela, as seen from our morphological analyses, strongly suggests the need for a thorough taxonomic revision of the genus in Venezuela. Today, all three species in Trinidad are believed to inhabit the same sites, but no specific surveys have been carried out to map the distribution of these three species. The restricted distribution of *E. nigrogularis* and *Elachistocleis* sp. likely will require conservation assessment. The widespread distribution of the former invalid *E. ovalis* has prompted a Least Concern (LC) label which is clearly inadequate for the Trinidad

species and most likely for some or all the populations in northern Venezuela.

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Data availability The molecular data generated during the current study are available in the GenBank repository [https://www.ncbi.nlm.nih.gov/genbank/].

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