



## A new *Chiasmocleis* (Anura: Microhylidae) from the eastern Guiana Shield with an amended definition of *C. haddadi* Peloso, Sturaro, Forlani, Gaucher, Motta, & Wheeler, 2014

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### Abstract

The existence of an unnamed (candidate) species from French Guiana and the state of Amapá (Brazil) closely related to *Chiasmocleis haddadi* has been hypothesized in previous DNA-based studies. With an integrative use of genetic, morphological and acoustic data we confirm that these populations are indeed distinct from *C. haddadi* and all other known congeners. We herein describe and name this new species, and amend the definition of *C. haddadi* since the type-series and its original diagnosis included specimens of the new species. Geographically, the new species occurs eastward of its sister species *C. haddadi* from which it can be distinguished by having a larger body, a proportionally smaller eye and a distinct dorsal colouration. We suspect that the new species breeds in the leaf litter and that tadpoles undergo endotrophic development whereas co-occurring *C. haddadi* and *C. hudsoni* lay clutches in the leaf litter nearby standing water of temporary ponds where exotrophic tadpoles complete their development. This new species is a new addition to the already long list of animals being endemic to the easternmost part of the Guiana Shield, a region identified as a singular bioregion within Amazonia by previous research.

**Key words:** Acoustics, Amazonia, Amphibia, DNA, Morphology, Neotropics, Taxonomy

### Introduction

The diversity of frogs inhabiting Amazonia remains vastly underestimated as suggested by DNA-based estimations (e.g., Vacher *et al.* 2020) and by the fast pace of species description (e.g., Guerra *et al.* 2020; Rivera-Correa *et al.* 2021). This is notably the case of *Chiasmocleis* with nine of the 36 currently valid nominal species having been described since 2010 (Frost 2022) and with the existence of many additional putatively unnamed species (Peloso *et al.* 2014; de Sá *et al.* 2019; Vacher *et al.* 2020).

*Chiasmocleis* Méhely, 1904 is composed of terrestrial, usually semi-fossorial, species distributed in most of South America, especially diverse in Amazonia, the Atlantic Forest and the Cerrado. Several clades within *Chiasmocleis* were identified by Peloso *et al.* (2014) based on a phylogenetic study of DNA sequences (*Chiasmocleis albopunctata* clade, *Chiasmocleis bassleri* clade, *Chiasmocleis hudsoni* clade, *Chiasmocleis shudikarensis* clade, *Chiasmocleis ventrimaculata* clade). These clades were corroborated by subsequent phylogenetic studies (Pyron and Jetz 2018; de Sa *et al.* 2019), although alternative taxonomic arrangements proposed for the genus does not entirely match these clades (i.e., available names usually include more than one of the clades). De Sá *et al.* (2019) put forth an alternative arrangement for the group, proposing that the genus should be divided into

three subgenera: *Chiasmocleis*, *Syncope* Walker, 1973 and *Relictocleis* Dubois *et al.*, 2021 (*Relictocleis* was established as an alternative name to the invalid nonima *Relictus* de Sá *et al.*, 2019 and *Unicus* de Sá *et al.*, 2019, originally suggested as the subgeneric names for *C. gnomia*).

The *Chiasmocleis bassleri* and *C. hudsoni* clades are exclusively Amazonian and together compose a lineage which includes mainly small species with reduced fingers (with noticeable exceptions in some populations of *C. bassleri*). These lineages (*C. bassleri* and *C. hudsoni* clades) have been recognized as either a separate genus (*Syncope*), or as a subgenus of *Chiasmocleis*. Some of the species in this group are bromeliculous and have (or are suspected to have) endotrophic larval development (*C. antenori* Krügel and Richter, 1995, *C. magnova* Moravec and Köhler, 2007; *C. carvalhoi* Nelson, 1975).

This second large lineage includes members of the *Chiasmocleis albopunctata*, *Chiasmocleis shudikarensis*, and *Chiasmocleis ventrimaculata* clades—collectively, these clades are often recognized as a subgenus (*Chiasmocleis*). The lineage is widely distributed in Amazonia, Cerrado, Chaco, and in the Atlantic Forest. It is formed by larger species with well-developed fingers and toes, and always bearing exotrophic larval development.

The last lineage is currently represented by a single species *Chiasmocleis gnomia* with a very restricted range in the Atlantic Forest. This taxon is the sister group of all the other *Chiasmocleis* and has been recognized as a subgenus (*Relictocleis*) of *Chiasmocleis* (De Sá *et al.* 2019) or as a distinct genus (Dubois *et al.* 2021; Segalla *et al.* 2021).

Within the *Chiasmocleis hudsoni* clade (the focus of the present paper), a subgroup formed by *Chiasmocleis hudsoni* Parker, 1940, *C. jimi* Caramaschi and Cruz, 2001, and *C. haddadi* Peloso, Sturaro, Forlani, Gaucher, Motta, and Wheeler, 2014 has been recurrently recovered in all recent phylogenetic analyses (Peloso *et al.* 2014; Pyron and Jetz 2018; de Sá *et al.* 2019). The taxonomy of these species has been in flux in the last decade. Peloso *et al.*, (2014) named *C. haddadi* and considered *C. jimi* and *C. hudsoni* as synonyms, based on the lack of reciprocal monophyly and of phenotypic (morphological and acoustic characters) diagnosability between specimens assignable to each of the two species—*C. haddadi* was recovered as the sister species of *C. hudsoni*. De Sá *et al.* (2019), based exclusively on genetic data and leaning heavily on the results of a DNA-based species delimitation analyses, considered *C. jimi* and *C. hudsoni* as distinct and revalidated *C. jimi*. The clade including *C. jimi*, *C. hudsoni* and suggested unnamed taxa was again recovered as the sister group of *C. haddadi*.

*Chiasmocleis haddadi* was described to designate populations from French Guiana and the Brazilian state of Amapá, which were previously identified as *C. hudsoni* (Peloso *et al.*, 2014). In the original description of *Chiasmocleis haddadi*, Peloso *et al.* (2014) noted that a specimen from Mont Bakra (southeastern French Guiana) had over 4% genetic distance on 16S (uncorrected) from the specimens of Mont Kotika, the type locality (western French Guiana). Nevertheless, two other populations further east, in the Brazilian state of Amapá (Igarapé Santo Antônio, Floresta Nacional do Amapá; Rio Anacuí, Parque Nacional Montanhas do Tumucumaque) were included in the type series of *C. haddadi*, without associated genetic data (i.e., based solely on morphological similarity). Subsequently, *C. haddadi* was documented from several localities in French Guiana (Waki, Itoupe) and further west in Suriname (Sipaliwini, Bakhuis) while other populations from French Guiana (including Mont Bakra) and Amapá (Brazil) were considered to belong to a putatively distinct but related species, *C. aff. haddadi*, based on mtDNA divergence (Fouquet *et al.* 2015; 2019a; Vacher *et al.* 2020).

After an integrative examination of additional molecular, morphological and acoustic data we were able to confirm that these other populations in French Guiana and Amapá (Brazil) are distinct from *C. haddadi*. Moreover, *C. haddadi* has an exotrophic free living larvae while field observations suggest that the new species may have terrestrial, endotrophic, tadpoles. We herein describe and name this new species and redefine the diagnosis for *C. haddadi* based on our new findings.

## Material and methods

**Field work and deposition of specimens.** We undertook extensive field work in French Guiana, Suriname, and the Brazilian states of Amapá, Amazonas and Pará, collecting specimens, tissue samples, recording advertisement calls and ecological data of numerous *Chiasmocleis* populations. Frogs were collected by hand and killed with an intraperitoneal injection of Xylocaine® (lidocaine hydrochloride). All specimens were individually tagged, fixed in 10 % formalin, and then transferred to a solution of 70 % ethanol for permanent storage. Newly collected

specimens were deposited in the Museum National d'Histoire Naturelle (Paris, France) (MNHN-RA-2022.0016–33). Other specimens examined are deposited in various zoological collections (Appendix 1 & 2).

**Molecular analyses.** We gathered all 16S sequences of *Chiasmocleis* available in GenBank (376) which were collated with 14 newly generated sequences of *Chiasmocleis haddadi* (n=1), of *C. hudsoni* (n=4) and of the new species named herein (previously referred in the literature as *C. aff. haddadi*) (n=9). We undertook a preliminary Neighbour Joining analysis including all the newly generated data and the ones from GenBank, and selected representatives of major *Chiasmocleis* lineages. We finally kept 123 sequences, including 73 sequences of the *C. hudsoni* clade (Appendix 1) that were collated with five other genera of Gastrophryninae that were used as outgroups.

Newly generated sequences were obtained after extraction of the genomic DNA from tissue samples (muscle or liver) using either the Wizard® Genomic DNA Purification Kit (Promega; Madison, WI, USA) or the DNeasy® Blood & Tissue Kit (Qiagen; Hilden, Germany) following the manufacturer's protocols. We amplified targeted loci by standard PCR protocols and then used Sanger sequencing (primers 16RF: TATCCCTAGGGTAACTTG; 16FR: TTACCAAAAACATCGCCT from Salducci *et al.* 2005).

Sequences were aligned on the MAFFT7 online server under default parameters except the E-INS-i strategy, which is designed for sequences with multiple conserved domains and long gaps (Katoh *et al.* 2017). We trimmed the alignment to keep only regions where sequences overlap, resulting in a final alignment of 566 base pairs (bp). We investigated the phylogenetic relationships among a total of 128 terminals under Maximum Likelihood (ML), applying a GTRCAT model with RAxML v.8.2.4 (Stamatakis 2014). All RAxML analyses were performed on the CIPRES Science Gateway online server (Miller *et al.* 2010). Nonparametric bootstrapping values (Felsenstein 1985) were estimated using 1,000 pseudoreplicates.

**Morphometric analysis.** We measured nine adult specimens of *Chiasmocleis aff. haddadi* (five males, four females, seven of which were included in our genetic dataset), 14 adult specimens of *C. haddadi* (13 males, one female, among which three were included in our molecular dataset and four are paratypes) and 14 adult specimens of *C. hudsoni* (10 males, four females, four being included in our genetic dataset and three coming from Guyana—ca. 200 km from the type locality) all from the lowlands of the Eastern Guiana Shield (Appendix 2). Sex was determined either in the field via calling activity, or in the laboratory by examining the presence of vocal slits and gonads. We measured 13 morphometric variables (adapted from Peloso *et al.* 2014) as follows: SVL = snout-vent length; HL = head length; HW = head width; ED = horizontal eye diameter; IOD = interorbital distance; IND = internarial distance; EN = eye-nostril distance (straight line distance between anterior corner of eye and nostrils); FeL = femur length; TiL = tibia length; FL = foot length (from tibia-tarsal junction to the tip of toe IV); 4TD = disc width of Toe IV; 3FD = disc width of Finger III; FAM = forearm length. Tympanum was not measured because ill-defined in most specimens.

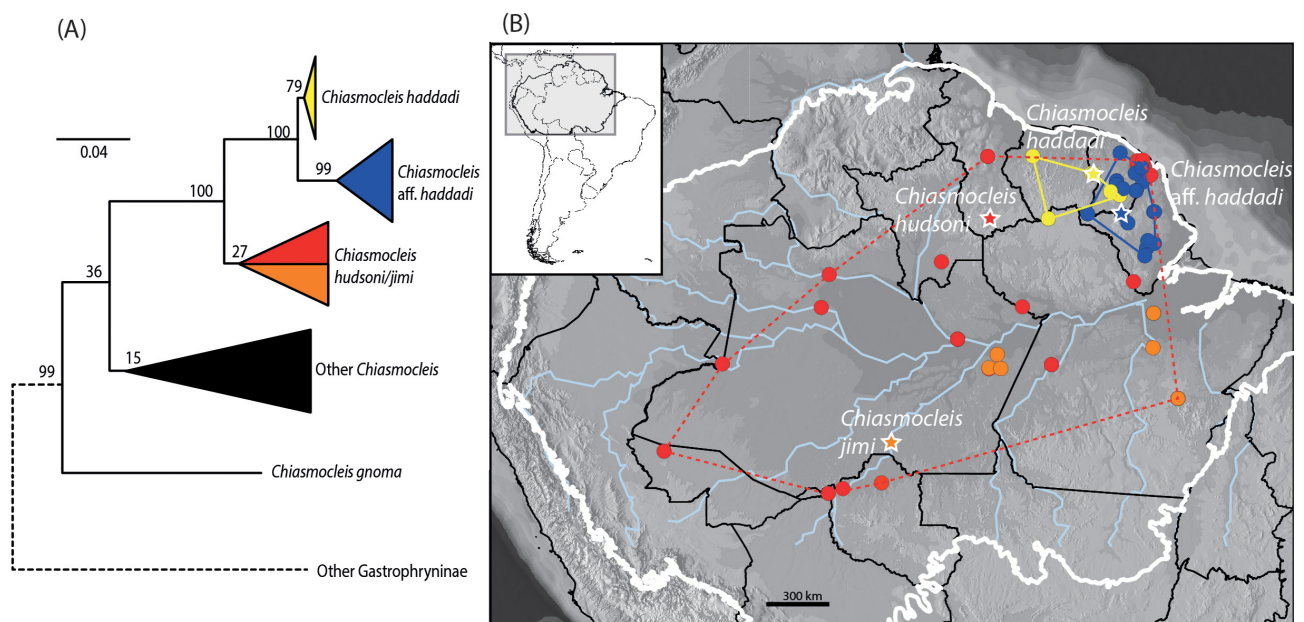
Measurements were taken using a digital caliper to the nearest 0.01 mm and rounded to the nearest 0.1 mm to avoid pseudo-precision (all measurements by AF or PP). We examined the variation of morphometric data among adult males through a principal component analysis (PCA) via FactoMineR package in R v.3.2.4 (Lê *et al.* 2008; R Development Core Team 2016). To control for variation in body-size among individuals we performed additional analyses on a size-corrected dataset (residuals) obtained by linear-regressing the original morphometric measures of each variable on SVL (Strauss 1985).

Additionally, the holotypes of *C. hudsoni* and *C. jimi* were examined from photographs (including for collection of SVL and qualitative characters).

**Acoustic analysis.** We gathered six call recordings of males of *Chiasmocleis haddadi*, four call recordings of males of *C. hudsoni* and one call of the new species. They consist in long series of clusters (delimited by upward—downward amplitude modulations) of pulsed notes (single or fused), we thus considered these clusters as “calls” following a call-centered definition of Köhler *et al.* (2017) and measured the following six variables using Audacity v.2.1.1: call length (CL), silence between calls (SC), note length (3<sup>rd</sup>) (NL), silence between notes (between the 2<sup>nd</sup> and the 3<sup>rd</sup> note) (SN), number of notes (NN), dominant frequency (taken with a spectral slice over the entire call) (DF). Whenever possible, up to four measurements of each variable were taken per recorded male, and the average was considered as a single measurement. We also undertook PCA on these bioacoustic data. Call recordings are deposited at [www.sonotheque.mnhn](http://www.sonotheque.mnhn) (Appendix 3).

## Results

**Phylogenetic relationships.** The deepest relationships among *Chiasmocleis* species inferred from the ML analysis are poorly supported (Fig. 1; full tree available as Appendix 4). However, sequences from individuals corresponding to *C. hudsoni*, *C. jimi*, *C. haddadi* and of the new species form a strongly supported (100% bootstrap) clade nested within the genus. The new species and *C. haddadi* are recovered as sister species with strong support (100% bootstrap; mean p-dist = 2.85%), each forming, respectively, moderately and strongly supported clades. *Chiasmocleis jimi* and *C. hudsoni* are not reciprocally monophyletic and together form a poorly supported clade sister to *C. haddadi* + *C. aff. haddadi* (mean p-dist = 4.33 and 6.08% respectively). The distribution of the samples identified as *C. jimi* are embedded within the larger range of *C. hudsoni* and genetic distances are low among populations assigned to these two taxa. Peloso *et al.* (2014) examined type specimens and specimens of several populations assigned to these two taxa and concluded that they are indistinguishable morphologically. Cassunde *et al.* (2022) suggested that “these two taxa should not be treated as separate species until more convincing evidence is available”. Nevertheless, the taxonomic status of these populations and the putative need to synonymise *C. jimi* with *C. hudsoni* is out of the scope of the present paper but, for the aforementioned reasons, we will consider only *C. hudsoni* further in this manuscript.



**FIGURE 1.** Maximum Likelihood phylogram (A) with collapsed branches and map (B) showing the distribution of the samples included in the phylogenetic analysis and type localities (stars) for each species.

The range of *Chiasmocleis haddadi* extends in Suriname and in southern French Guiana where it marginally overlaps with the range of *C. aff. haddadi*, that extends throughout Amapá (Brazil) and French Guiana. However, the two species were not found in syntopy. *Chiasmocleis hudsoni/jimi* occur throughout Amazonia with the exception of the westernmost portions. In French Guiana, it seems circumscribed to the coastal strip and co-occurs with *C. aff. haddadi* in at least one location in northeastern FG (Saut Maripa).

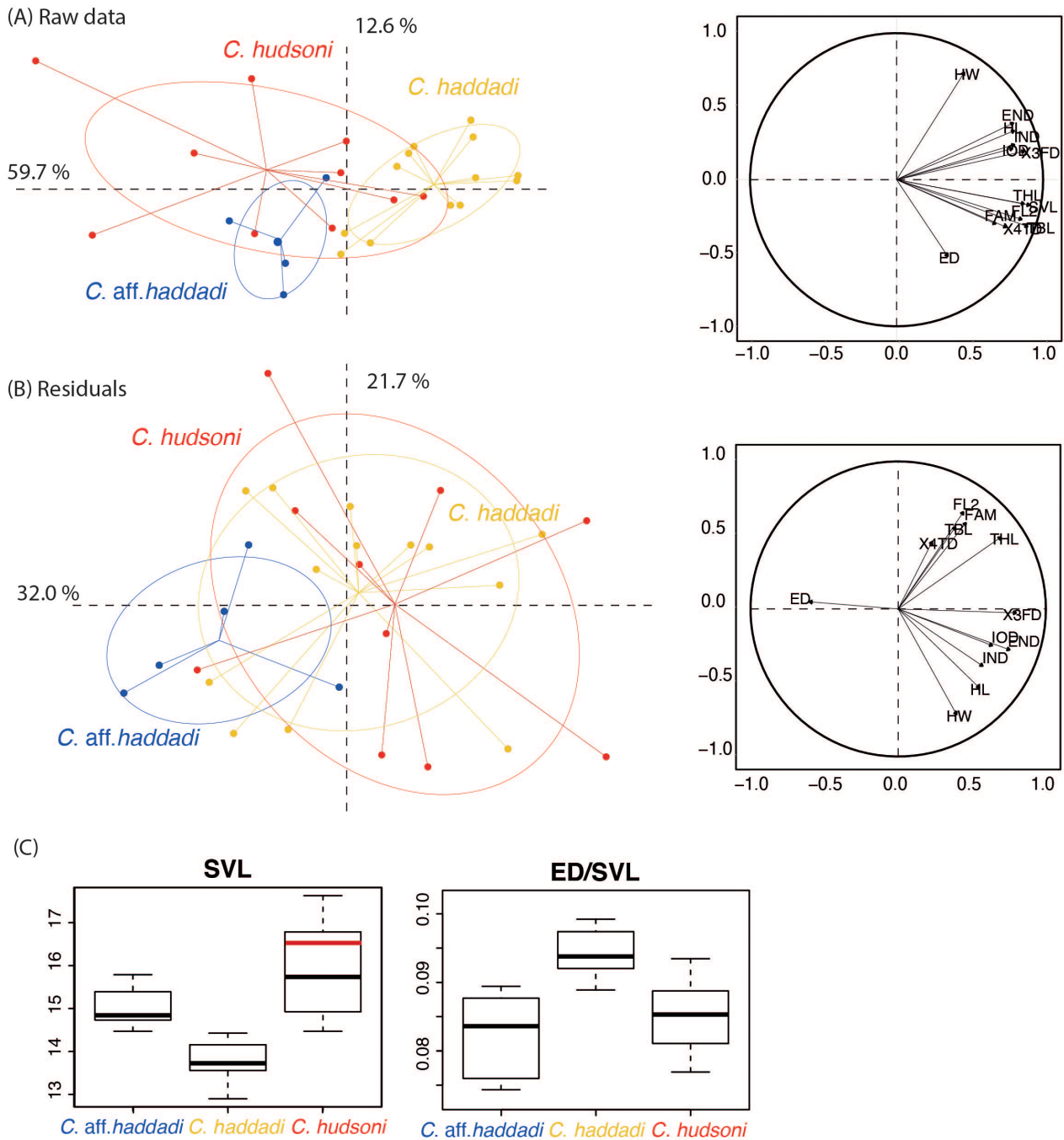
**Morphometric analyses.** The PCA based on raw morphometric measurements provided two first components accounting for 72.3 % of the total variation (Fig. 2a). The first component explains 59.7 % of the variation, and variable coefficients are all highly and positively correlated. Overall, the variation along the first principal component (PC) axis is related to body size which segregates *C. haddadi* being smaller from the two others (Table 1; Fig. 2c). The PCA based on size-corrected morphometric measurements (residuals) provided two first components accounting for 53.7 % of the total variation (Fig. 2b). The three species largely overlap along the first two axes although the contribution ED along the first axis is noteworthy and ED/SVL segregates *C. haddadi* from the new species (Fig. 2c).

**Acoustic analysis.** The calls of the three species analyzed differ markedly although they share a temporal structure of short clusters of 5–8 notes with amplitude modulation (Fig. 3a). The PCA based on the measured acoustic variables provided two first components accounting for 88.7 % of the total variation (Fig. 3b). The first



component explains 60.3 % of the variation and segregates *C. hudsoni* from the two others mostly from having shorter intervals among calls and among notes within them (Table 2). The second component explains 28.4 % of the variation and segregates the new species from the two others mostly from having longer intervals among calls and shorter intervals among notes (Table 2).

**Taxonomy.** Considering that part of the specimens used in the original diagnosis of *Chiasmocleis haddadi* in fact belong to the new species described herein, we present below an updated diagnosis of *C. haddadi* before proceeding with the description of the new species.

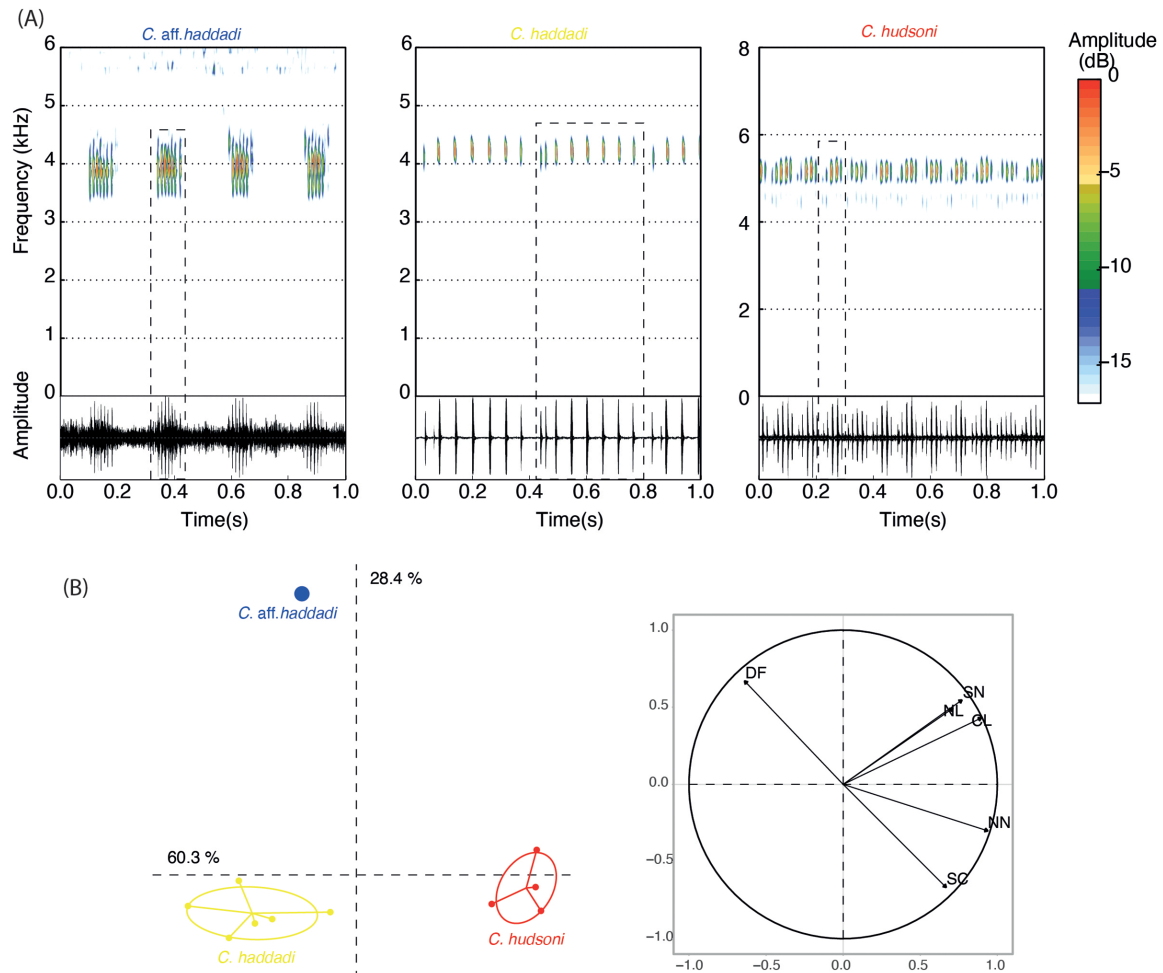


**FIGURE 2.** Morphometric analyses. (A) PCA on morphometric measurements; (B) PCA on residuals of the linear regression of measurements with SLV; (C) boxplot for SVL, and ratio of selected variables with SVL. SVL of the holotype of *C. hudsoni* is indicated with a red bar in the boxplot

Holotype: MNHNP 2011.0139; Paratypes: MNHNP 2011.0140, 2011.0143–2011.0145, Mont Kotika, French Guiana. Specimens removed from the type series: IEPA (FL 453–455, FL 511–513), IEPA (TQ 1036).

**Referred specimens examined.** 13 males including four paratypes, one female (Appendix 2)

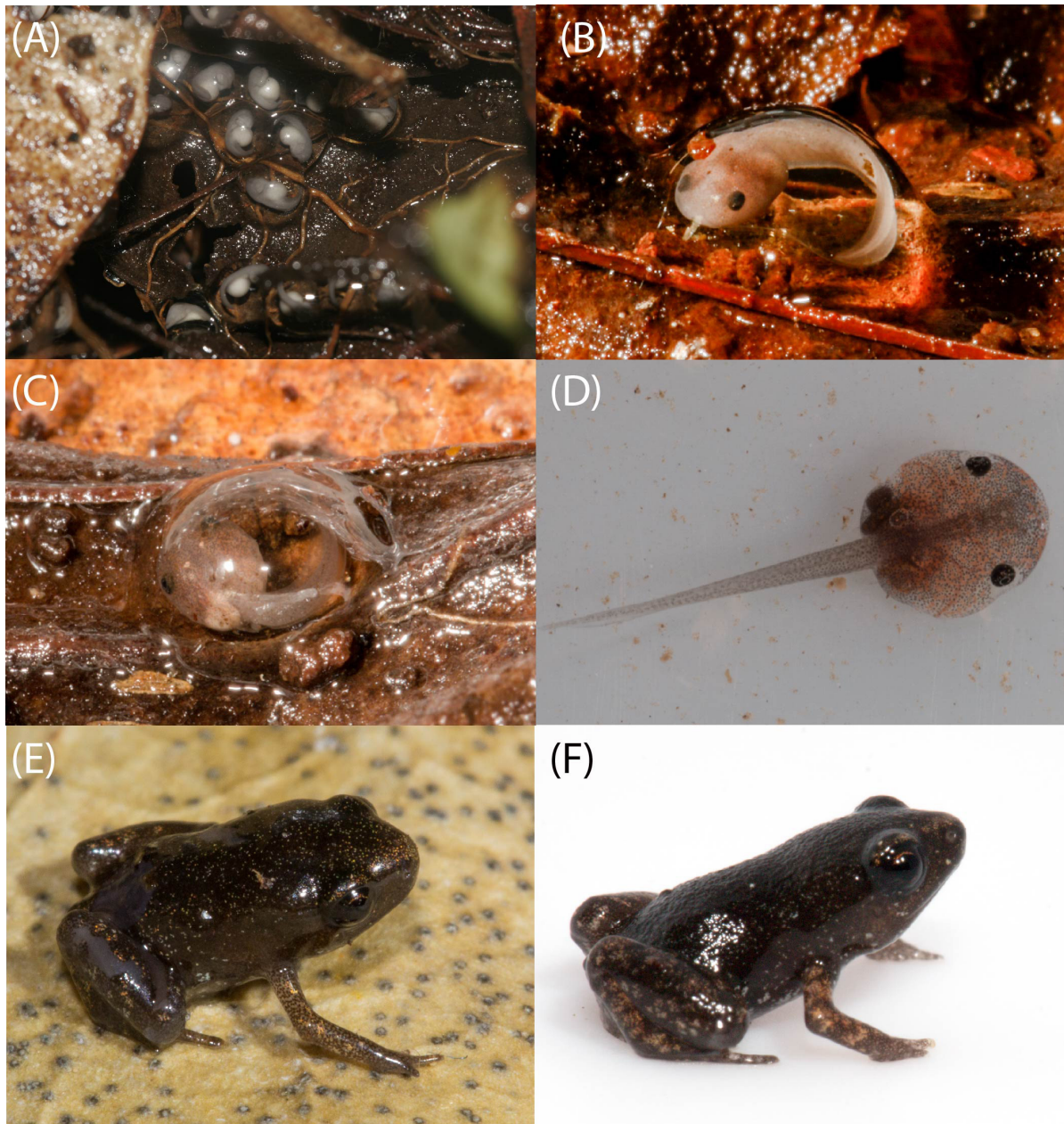
**Definition.** A small sized species for the genus; SVL in males 12.9–14.5 mm (N = 9); in females 17.2 mm (N = 1). Body ovoid, robust; head much narrower than body, snout rounded in dorsal and lateral views. Four distinctive fingers; all fingers slightly fringed on both sides, less pronounced distally on FIII, FIV and FI, fingers unwebbed; FI reduced with a rounded or slightly pointed tip, subarticular tubercle large and well-defined; finger tips of FII–IV rounded or slightly pointed, tips of FII and III slightly swollen; adpressed FI reached the middle of subarticular tubercle of FII; adpressed FIV reaches middle or distal margin of distal tubercle of FIII; thenar tubercle present; relative finger lengths I<II<IV<III. Well-defined dorsolateral stripes enlarging posteriorly. Eyes with red iris. Upper surface of arms with brick-coloured blotches in life, grey in preservative. Five distinctive toes, first very small; toes slightly fringed, less pronounced distally on all toes, with small spicules on the fringes, toes not webbed; all toes with conspicuous subarticular tubercle; adpressed TI does not reach subarticular tubercle of TII; adpressed TV reaches the base of the middle subarticular tubercle of TIV; TII–IV with terminal discs. Thigh with ventral surface dark brown with blueish speckles in life, whitish in preservative. Some males with few small dermal spines on chin. Advertisement call with 6–8 notes, internote silences 0.035 s and dominant frequency 4.57 kHz (Fig. 3).



**FIGURE 3.** Examples of sonograms (A) for each species and PCA (B) on the variables measured from the available records. Dotted frames depict the pattern that we considered to represent a “call” for each species.

**Comparisons with related species.** *Chiasmocleis haddadi* can be distinguished from *C. hudsoni* (data in parentheses) by its smaller body size (SVL range = 12.9–14.5 vs. 15.2–18.5 in males); well-defined dorsolateral stripes enlarging posteriorly (absent or poorly defined); eyes with a red iris (copper, bronze or silverfish); upper surface of arms with brick-coloured blotches (dark brown with clear speckles); larger eyes (8.9–10.2% vs. 7.7–9.3% of SVL); thigh with small light speckles over a dark background (large dark blotches on the periphery over light in preservative to orange in life background); an advertisement call with more notes (6–8 vs 5 notes), longer silences among notes (0.035 vs 0.007 s) and lower dominant frequency (4.57 vs 4.94 kHz). For comparisons with more distantly related species of *Chiasmocleis* see Peloso *et al.* (2014).

**Natural history and distribution.** All the observations of this species have been made during breeding events, near temporary pools on hilltops (as opposed to riverbeds pools). The males were found calling under the leaves of the leaf litter at the periphery of the water. The clutch (8–12 eggs) is deposited in the litter near the waterline. Tadpoles are exotrophic and pelagic (Fig 4).



**FIGURE 4.** (A) clutches of *C. haddadi*, each with about 8–12 eggs, found nearby calling males on the bank of a temporary pond in Itoupé, French Guiana; (B) embryo after 7 days; (C) embryo after 10 days; (D) tadpole found in small temporary puddles in Sipaliwini, Suriname (genotyped AF2231); (E–F) two imagos of *C. jacki* **sp. nov.** found in the leaf litter distant from water, AF2595 from St Eugene and AF2880 (both genotyped) from Mitaraka collected nearby the recorded calling male (not collected).



**TABLE 1.** Mean, minimum and maximum values of morphometric measurements.

	Sex	SVL	HL	HW	ED	IOD	IND	END	THL	TBL	FL2	4TD	3FD
<i>C. jacki</i> sp. nov. (n=5)	M	15.44	3.98	4.8	1.27	2.88	1.47	1.44	7.26	7.13	10.7	0.58	0.34
	Min	14.8	3.8	4.5	1.1	2.7	1.4	1.3	7.2	6.6	10.2	0.5	0.3
	Max	16.3	4.1	5.0	1.4	3.1	1.5	1.5	7.3	7.5	11.8	0.7	0.4
<i>C. jacki</i> sp. nov. (n=4)	F	19.17	4.71	5.58	1.57	3.3	1.6	1.57	8.74	8.49	12.7	0.74	0.4
	Min	18.5	4.4	5.1	1.4	2.9	1.5	1.4	8.3	7.8	12.2	0.7	0.4
	Max	20.2	5.5	6.5	1.8	3.7	1.7	1.9	9.5	8.9	13.0	0.8	0.5
<i>C. haddadi</i> (n=13)	M	13.9	3.73	4.42	1.31	2.58	1.38	1.19	6.43	6.52	9.36	0.56	0.26
	Min	12.9	3.5	4.1	1.2	2.2	1.2	1.0	5.9	6.0	8.5	0.5	0.2
	Max	14.7	4.2	4.8	1.4	2.9	1.6	1.4	6.9	7.0	10.5	0.6	0.3
<i>C. haddadi</i> (n=1)	F	17.2	4.3	5.0	1.5	2.8	1.5	1.3	7.7	7.7	11.1	0.7	0.3
	Min	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Max	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>C. hudsoni</i> (n=10)	M	16.37	4.02	4.59	1.39	2.84	1.5	1.34	7.07	7.76	11.2	0.65	0.31
	Min	14.8	3.6	4.3	1.3	2.4	1.3	1.2	6.0	6.5	9.0	0.5	0.2
	Max	18.5	4.3	5.1	1.5	3.2	1.8	1.6	8.6	9.7	13.6	0.8	0.4
<i>C. hudsoni</i> (n=4)	F	21.42	4.64	5.3	1.6	3.31	1.58	1.51	9.46	10.4	15.5	0.82	0.42
	Min	17.2	4.4	5.1	1.3	3.0	1.4	1.1	7.6	7.9	12.4	0.7	0.4
	Max	23.4	4.9	5.5	1.7	3.4	1.7	1.7	10.5	11.7	17.4	1	0.5

***Chiasmocleis jacki* sp. nov.***Chiasmocleis hudsoni* Lescure & Marty 2000*Chiasmocleis hudsoni* Fouquet *et al.* 2007*Chiasmocleis haddadi* Peloso *et al.* 2014 (part)*Chiasmocleis* aff. *haddadi* Fouquet *et al.* 2019*Chiasmocleis* cf. *haddadi* Vacher *et al.* 2020

**Holotype.** MNHN-RA-2020.0026 (field n° AF2716), an adult male collected at Alikéné, French Guiana (3.21873, -52.3964) by J.P. Vacher and S. Cally on the 17/02/2015 (Fig. 5, 6).

**Paratypes.** MNHN-RA-2020.0030–31 (field n° AF2714–5), two males and MNHN-RA-2020.0029 (field n° AF2669) a female collected with the holotype; MNHN-RA-2020.0033 (field n° AF5405) a male collected at Savane roche Dachine, French Guiana (3.469583, -53.229783) by M. Dewynter and E. Courtois on the 03/06/2019; MNHN-RA-2020.0028 (field n° AF1547) a female collected at Saul, French Guiana (3.615576, -53.227093) by M. Berroneau, M. Berroneau, L. Barthe and P.O. Cochard on the 22/01/2014; MNHN-RA-2020.0027 (field n° PG717) a female collected at Saut Maripa, French Guiana (3.806111, -51.893389) by M. Dewynter and K. Pineau on the 01/02/2012; MNHN-RA-2020.0032 (field n° AF5489), an adult male, collected at Montagne Takulu, French Guiana (2.20963, -52.98903) by Elodie Courtois and Maël Dewynter on the 30 April 2019; MNHN 1997.2272 a female collected at Saint Eugene, French Guiana (4.82167, -53.06766) by J.-C. De Massary on the 31 March 1997.

**Etymology.** The specific epithet, a patronym formed as a noun in the genitive case, refers to Jack Dalton, the second tallest brother of the Dalton gang, the outlaw characters of Morris and Goscinny comic books Lucky Luke, reminding the different in size of the four species of *Chiasmocleis* co-occurring in the Guiana Shield, the new species being intermediary in body size.

**Definition.** The new species is characterized by the following unique combination of characters: SVL small, mean SVL in adult males 15.4 mm (range 14.8–16.3 mm, n = 5) and mean SVL in adult females 18.8 mm (range 19.2–20.2 mm, n = 4) (Table 1); body ovoid, robust; head much narrower than body, snout rounded in dorsal and lateral views; four distinctive fingers; Fingers I, II and III fringed proximally, particularly Finger III; fingers not webbed; FI developed but small with a rounded or slightly pointed tip, subarticular tubercle large two tubercles



on FIII and FIV and only one on FI and II; rounded or slightly pointed and slightly swollen tips of FIII and IV; adpressed FI reaches the base of subarticular tubercle of FII; adpressed FIV reaches the base of distal tubercle of FIII; thenar and palmar tubercle present in all individuals; relative finger lengths  $I < II < IV < III$ . Upper surface of arms with brick-coloured blotches in life, grey in preservative. Dorsolateral stripes enlarging posteriorly and ill-defined dorsally. Eyes with a copper iris. Five distinctive toes, first very small; toes slightly fringed except Toe V and distally on FIV, more pronounced proximally on TI–III; toes not webbed; TI with without subarticular tubercle; adpressed TI does not reach subarticular tubercle of TII; adpressed TV does not reach or reaches only the middle of the middle subarticular tubercle of TIV; TII–IV with terminal discs. Thigh with ventral surface dark brown with blueish speckles in life, whitish in preservative. Some males with few small dermal spines on chin. Advertisement call with 7–8 notes, intercall silences 0.14 s and dominant frequency 3.84 kHz (Fig. 3).

**Morphological comparisons with other *Chiasmocleis*.** Phylogenetically the new species is part of the *Chiasmocleis hudsoni* species group, as defined by Peloso *et al.* (2014). We focus our comparisons to the known species in the group. *Chiasmocleis jacki* is most similar to *C. haddadi* and *C. hudsoni*, three species that co-occur in the Guiana Shield, for which we provide more detailed comparisons.

*Chiasmocleis jacki* **sp. nov.** can mainly be distinguished from its closest relative *C. haddadi* (in parentheses) by its larger body size (SVL range = 14.8–16.3 mm vs. 12.9–14.7 in males; 18.5–20.2 mm vs. 17.2 in the female); dorsolateral stripes ill-defined dorsally (well defined); smaller eyes (7.4–8.9 % vs. 8.9–9.7% of SVL) (Fig. 6); its advertisement call with shorter calls (0.09 vs 0.32s), longer silences among calls (0.14 vs 0.06 s) and lower dominant frequency (3.84 vs 4.57 kHz) (Fig. 3).

*Chiasmocleis jacki* **sp. nov.** can mainly be distinguished from *C. hudsoni* (in parentheses) by its dorsolateral stripes ill-defined dorsally (absent or poorly defined); upper surface of arms with brick-coloured blotches sometimes entirely brick coloured (dark brown sometimes with clear speckles); ventral surface of thigh with blueish speckles cream in preservative over a dark brown background (no speckles and light brown background in preservative to orange in life); an advertisement call with more notes (7–8 vs 5 notes), longer silences among calls (0.14 vs 0.013 s) and lower dominant frequency (3.84 vs 4.94 kHz).

*Chiasmocleis jacki* differs from *C. antenori*, *C. carvalhoi*, *C. magnova*, *C. parkeri* *C. tridactyla* in having four fully developed fingers, although FI is small (FI not evident externally in *C. antenori*, *C. carvalhoi*, *C. tridactyla*; FI and FIV much reduced in *C. parkeri*; FVI much reduced in *C. magnova*) and by its reticulated ventral colour pattern (dark with scattered light spots in *C. antenori*, *C. magnova* and *C. parkeri*; beige with white spots or blotches in *C. carvalhoi*).

**Description of the holotype.** An adult male, SVL 15.9 mm. Body ovoid, relatively robust. Head wider than long (1.2X), much narrower than body trunk; nostrils not protuberant, positioned anterolaterally, directed laterally; snout rounded in dorsal and in lateral views; *canthus rostralis* indistinct; interorbital region flat. Eyes small; tympanum not visible; pupil round; supratympanic fold indistinct. Tongue large, elongate, with free lateral and posterior edges, extended beyond the extent of the jaw; vocal slits present, one on each side of the tongue; choanae small, rounded, widely separated, anterior to eye; vomerine teeth absent. Vocal sac not externally evident, many small white dermal spines on the chin. Arms slender, FI reduced, FII, FIII, and FIV well developed; tips of FII and FIII rounded and of FI and FIV slightly pointed; no webbing between fingers; Fingers I, II and III fringed proximally, particularly Finger III; relative finger lengths  $I < II < IV < III$ . Subarticular tubercles well developed on all fingers; one subarticular tubercle on FI and FII, two on FIII and FIV; no supernumerary tubercles; thenar tubercle present, rounded; palmar (metacarpal) tubercle divided, not prominent. No dermal spines visible on arms, hands, or fingers. Legs short (combined THL, TBL, and FL 1.7 X the SVL), relatively robust, lacking tubercles, tibial and tarsal ridges; toes not webbed; TI weakly developed (tip fails to reach subarticular tubercle on TII); relative toe lengths  $I < II < V < III < IV$ ; toes slightly fringed except Toe V and distally on FIV, more pronounced proximally on TI–III; toe tips rounded with small discs on all except TI. Inner metatarsal tubercle present. Subarticular tubercles present on all toes except TI; no outer metatarsal tubercle; toes without dermal spines. Skin smooth dorsally and ventrally.

**Colour of holotype in life.** Dorsum uniform dark brown or mostly dark brown with a few scattered small white dots. Poorly defined dorsolateral stripes clearer than dorsum enlarging posteriorly; *canthus rostralis* and border of eyelid yellowish-cream. Upper surface of arms brick-coloured with brown blotches; upper surface of legs from thighs to feet reddish-brown with dark brown vermiculations; eye iris copper; throat, chest, ventral sides of flanks and ventral surfaces of limbs dark brown with blueish coloured speckles denser on the belly and forming a marbled pattern.



*C. jacki* sp. nov.  
MNHN-RA-2022.0032  
(AF5489) paratype  
Mont. Takulu, FG



*C. haddadi*  
MNHN-RA-2022.0022  
(AF3406)  
Itoupe, FG



*C. hudsoni*  
not collected  
Patawa, FG



10 mm



1 mm

*C. jacki* sp. nov.  
MNHN-RA-2022.0026  
(AF2716) holotype  
Mont Alikéné, FG



1 mm

**FIGURE 5.** Pictures of in life specimens of the three species of the *Chiasmocleis hudsoni* clade of the Guiana Shield; and dorsal, ventral, palmar and plantar view of preserved holotype of *C. jacki* **sp. nov.**





**FIGURE 6.** Comparison of living specimens of the three species of the *Chiasmocleis hudsoni* clade occurring in the Guiana Shield (left: *C. jacki* sp. nov.; middle: *C. haddadi*; right: *C. hudsoni*).

**Colour of holotype in preservative.** After seven years in 70 % ethanol, colours of the specimen faded, dorsum became brown, arms became light brown and ventral speckles became cream coloured; the throat appears darker than the other ventral surface (Fig. 5).

**Variation in the type series.** Proportions vary little among the types. Males are smaller than females (14.8–16.3 mm vs. 18.5–20.2). Dermal spines on the throat can be completely absent. Dorsal colour pattern varies little except the number and position of the small white dots and the extent of brick colouration on the arms. Ventral colouration varies extensively from large blotches to a dense marbled pattern (Fig. 6).

**Advertisement call.** A single specimen calling from the under the leaf litter was recorded from about 2 m away at air temperatures between 23–25°C and 90–100% relative humidity. Descriptive statistics of call parameters are presented in Table 2. *Chiasmocleis jacki* emits series of short calls of 7–8 pulsed notes. These calls last 0.092 s on average (range 0.078–0.105) between silence lasting 0.138 s on average (range 0.109–0.166 s). The notes are very short and fused (note length mean = 0.010 s; range 0.009–0.011 s). These notes are emitted with an upward and then downward amplitude within the call. The dominant frequency is 3.84 kHz on average (range 3.82–3.86 kHz) (Fig. 3, Table 2).

**TABLE 2.** Mean, minimum and maximum values of acoustic variables.

	CL (s)	SC (s)	NL (s)	SL (s)	NN	DF (kHz)
<i>C. haddadi</i> (n=6)	0.320	0.060	0.015	0.035	6.8	4.573
Min	0.259	0.046	0.013	0.031	6.0	4.167
Max	0.353	0.091	0.019	0.040	7.3	4.748
<i>C. aff. haddadi</i> (n=1)	0.093	0.138	0.01	0	7.5	3.845
<i>C. hudsoni</i> (n=4)	0.083	0.013	0.011	0.007	5.0	4.943
Min	0.077	0.009	0.008	0.005	5.0	4.829
Max	0.093	0.018	0.014	0.012	5.0	5.127

**Natural history and distribution.** *Chiasmocleis jacki* is found in *terra firme* primary forest and seemingly calls far from any water body. An imago (AF2880 Mitaraka) has been found nearby the unique calling male (not collected) observed so far, but other imagos have been found (AF2595—St Eugene) at considerable distance from water as well as nearby water (Saut Richard). Moreover, gravid females with large sized eggs have been examined. Its sister species, *C. haddadi*, lays large terrestrial eggs that hatch nearby the water that the exotrophic tadpoles reach after hatching, which is similar to what has been observed in *C. hudsoni* (Lima *et al.* 2006; pers. obs. in FG). We therefore suspect that its mode of development is endotrophic, like several closely related species in western Amazonia such as *C. antenori* (Krugel & Richter 1995).

This species is only known from a few localities east of the Approuague river, French Guiana (Savane Virginie, Noussiri, Armontabo, Alikéné, Mont Bakra, Roche Dachine, Trois Saut) and in Amapá, Brazil (Lourenço, Porto Grande, Rio Vila Nova) but also in Saül, in the Mitaraka massif and in the northern half of French Guiana as far as Saint-Eugène (Fig. 1). It is probably more widely distributed in the interior of the Guianas region but most likely remains endemic to the easternmost part of the Guiana region. It probably coexists in syntopy with *C. haddadi* since their ranges overlap at least in central French Guiana, although they have not yet been observed in syntopy.

## Discussion

We herein described and named another species endemic to the eastern Guiana Shield which adds another brick to the understanding of the biological diversity and distribution of taxa in the region. A similar pattern of distribution (circumscribed to the easternmost part of the Guiana Shield) has been recurrently highlighted in recent frog species descriptions notably *Anomaloglossus baeobatrachus* (Fouquet *et al.* 2019b), *Dendropsophus counani* (Fouquet *et al.* 2015), *Synapturanus zombie* (Fouquet *et al.* 2021), *Amazophrynella teko* (Rojas *et al.* 2018), *Pristimantis crepitaculus* (Fouquet *et al.* 2022), as well as squamate reptiles (e.g., *Alopoglossus theodorusi* Ribeiro-Junior *et al.* 2020). The recurrence of this pattern led Vacher *et al.* (2020) to delimit a bioregion in the easternmost part of the Guiana Shield. They based their bioregionalisation on the distribution of mtDNA-delimited lineages. Some



corresponded to nominal species and some to unnamed lineages, among which *Chiasmocleis jacki* was already identified and is now confirmed by the present study. In fact, the range of both *C. haddadi* and *C. jacki* lie within the bioregion delimited by Vacher *et al.* (2020). Among codistributed endemic species in the easternmost part of the Guiana Shield only a few such as *Anomaloglossus baeobatrachus*, *Pristimantis* sp. “Guianas” (Fouquet *et al.* 2022) and *Atelopus flavescens* have, like *C. jacki* and *C. haddadi*, their closest relative westward within the Guiana Shield. This pattern suggests that speciation occurred within the eastern lowlands of the Guiana Shield. Conversely, most other endemics of the eastern Guiana Shield such as *Dendropsophus counani* (Fouquet *et al.* 2015) or *Boana diabolica* (Fouquet *et al.* 2016) have their closest relatives elsewhere in Amazonia and are examples of a panamazonian diversification.

This endemism in the easternmost subregion of the lowlands of the Guiana Shield, which comprises the Brazilian state of Amapá, French Guiana and eastern Suriname may be partly explained by the current and historical precipitation regime that is driven by the proximity of the Atlantic Ocean. These abundant precipitations may have been more stable over time during the Pleistocene climatic fluctuations (Silva *et al.*, 2018; Vacher *et al.* 2020). The emergence of endotrophy in *Anomaloglossus* has been suggested to have favoured by wet and stable climatic conditions in the eastern part of the Guiana (Fouquet *et al.* 2019c). Based on the observation of a calling male and an imago of *C. jacki* far from any water point and the large size of ovaries, we assume that the new species is probably also endotrophic. If confirmed, this would represent another example of similarly recent and spatially congruent acquisition of this mode of reproduction that deserves further investigation.

The three species of *Chiasmocleis* of the region (*C. jacki*, *C. haddadi* and *C. hudsoni*) do not seem to widely co-occur (there is a single known exception). We can therefore hypothesize that at least some level of ecological exclusion exists, despite putatively distinct breeding modes (hypothesized endotrophy of *C. jacki*) and ecological differences in breeding sites between *C. haddadi* and *C. hudsoni*.

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## Declarations

**Conflict of Interests/Competing Interests.** The authors have no competing interests to declare that are relevant to the content of this article.

**Availability of data and material.** All new DNA sequences, call recordings will be deposited upon manuscript acceptance.

**Ethics approval.** Experiments have been conducted in accordance with relevant national legislation on the use of animals for research.

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**APPENDIX 1.** List of accession numbers and other information for the molecular data of the *Chiasmocleis hudsoni* clade used in the phylogenetic analysis.

GB Accession	species	Voucher specimens	tissue voucher	Tissue collection	Locality	State / Country	Lat	Lon
KDQF01000129	<i>jacki</i> sp. nov.		AF0526	EDB	Saul	French Guiana	3.6376	-53.2137
KDQF01000463	<i>jacki</i> sp. nov.		AF1237	EDB	Noussiri	French Guiana	3.5864	-52.1134
KDQF01000585	<i>jacki</i> sp. nov.	MNHN-RA-2022.0028	AF1547	EDB	Saul	French Guiana	3.6156	-53.2271
KDQF01001008	<i>jacki</i> sp. nov.		AF2595	EDB	Saint-Eugène	French Guiana	4.8217	-53.0677
<b>OP476731</b>	<i>jacki</i> sp. nov.	MNHN-RA-2022.0029	AF2669	EDB	Alikéné	French Guiana	3.2187	-52.3964
<b>OP476732</b>	<i>jacki</i> sp. nov.	MNHN-RA-2022.0030	AF2714	EDB	Alikéné	French Guiana	3.2187	-52.3964
KDQF01001073	<i>jacki</i> sp. nov.	MNHN-RA-2022.0031	AF2715	EDB	Alikéné	French Guiana	3.2187	-52.3964
KDQF01001074	<i>jacki</i> sp. nov.	MNHN-RA-2022.0026	AF2716	EDB	Alikéné	French Guiana	3.2187	-52.3964
KDQF01001128	<i>jacki</i> sp. nov.		AF2880	EDB	Mitaraka	French Guiana	2.2358	-54.4493
<b>OP476733</b>	<i>jacki</i> sp. nov.		AF3296	EDB	Mitaraka	French Guiana	2.2358	-54.4493
<b>OP476734</b>	<i>jacki</i> sp. nov.		AF5395	EDB	Saut Richard	French Guiana	3.4736	-53.2086
<b>OP476735</b>	<i>jacki</i> sp. nov.	MNHN-RA-2022.0033	AF5405	EDB	Savane roche dachine	French Guiana	3.4696	-53.2298
<b>OP476736</b>	<i>jacki</i> sp. nov.	MNHN-RA-2022.0032	AF5489	EDB	Trois-Saut	French Guiana	2.2096	-52.9890
EU201099	<i>jacki</i> sp. nov.		AG085	EDB	Monts Bakra	French Guiana	3.3005	-52.9493
<b>OP476737</b>	<i>jacki</i> sp. nov.		CEC3248	UFAP	Pedra Branca do Amapari	Amapá, Brazil	0.7713	-51.9466
<b>OP476738</b>	<i>jacki</i> sp. nov.		CEC3249	UFAP	Pedra Branca do Amapari	Amapá, Brazil	0.7713	-51.9466
KDQF01002507	<i>jacki</i> sp. nov.		FL16	IEPA	Plote PPBIO	Amapá, Brazil	0.9793	-51.6149
<b>OP476739</b>	<i>jacki</i> sp. nov.		FTA79	IEPA	Rio Vila Nova	Amapá, Brazil	0.4493	-52.0221
KDQF01003292	<i>jacki</i> sp. nov.		MTR13928	MTR	Lourenço	Amapá, Brazil	2.3236	-51.6453
KDQF01003602	<i>jacki</i> sp. nov.		MTR24274	MTR	Lourenço	Amapá, Brazil	2.3216	-51.6109
KDQF01003833	<i>jacki</i> sp. nov.		PG159	EDB	Armontabo	French Guiana	3.9611	-52.4286
KDQF01004114	<i>jacki</i> sp. nov.		QM0457	EDB	Savane Virginie	French Guiana	4.1959	-52.1490
KF621181	cf. <i>jimi</i>	MPEG3803-23283		MPEG	Rio Xingú, Fazenda Caracol	Pará, Brazil	-3.4528	-51.6753
KF621182/MH919949	cf. <i>jimi</i>	MPEG3803-23285		MPEG	Rio Xingú, Fazenda Caracol	Pará, Brazil	-3.4528	-51.6753
KF621179/MH919946	cf. <i>jimi</i>	MPEG3803-24527		MPEG	Floresta Nacional de Caxuanã	Pará, Brazil	-1.9833	-51.6500
MH919930	cf. <i>jimi</i>	T275		MPEG	Tapirapé	Pará, Brazil	-5.6089	-50.6153
MH919877	cf. <i>jimi</i>	T81		MPEG	Tapirapé	Pará, Brazil	-5.6089	-50.6153
KDQF01000884	<i>haddadi</i>		AF2231	EDB	Sipaliwini	Suriname	2.0325	-56.1145

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**APPENDIX 1. (Continued)**

GB Accession	species	Voucher specimens	tissue voucher	Tissue collection	Locality	State / Country	Lat	Lon
KDQF01000886	<i>haddadi</i>	NZCS A1056	AF2236	EDB	Sipaliwini	Suriname	2.0325	-56.1145
KDQF01001401	<i>haddadi</i>	MNHN-RA-2022.0020	AF3403	EDB	Bakhuis	Suriname	4.6837	-56.7721
KDQF01001442	<i>haddadi</i>	MNHN-RA-2022.0024	AF3585	EDB	Itoupe	French Guiana	3.0265	-53.0798
<b>OP476740</b>	<i>haddadi</i>		AF3992	EDB	Itoupé	French Guiana	3.0250	-53.0800
KF621170	<i>haddadi</i>	MNHN2011.0139	PG403	EDB	Mont Kotika	French Guiana	3.9564	-54.2786
KF621171	<i>haddadi</i>	MNHN2011.0140	PG443	EDB	Mont Kotika	French Guiana	3.9564	-54.2786
EU201100	<i>haddadi</i>		PG439	EDB	Mont Kotika	French Guiana	3.9347	-54.2047
KF621172	<i>haddadi</i>		PG445	EDB	Mont Kotika	French Guiana	3.9333	-54.1972
KF621173	<i>haddadi</i>		PG446	EDB	Mont Kotika	French Guiana	3.9333	-54.1972
KDQF01004460	<i>haddadi</i>		WAAM009	EDB	Flat de la waki	French Guiana	3.1834	-53.4775
MG806095	<i>hudsoni</i>		5517	?	Experimental farm UFAM	Amazonas, Brazil	-3.0899	-59.9632
MG806093	<i>hudsoni</i>		5539	?	Experimental farm UFAM	Amazonas, Brazil	-3.0899	-59.9632
MG806094	<i>hudsoni</i>		5556	?	Experimental farm UFAM	Amazonas, Brazil	-3.0899	-59.9632
MG806091	<i>hudsoni</i>		5592	?	Experimental farm UFAM	Amazonas, Brazil	-3.0899	-59.9632
MG806092	<i>hudsoni</i>		5593	?	Experimental farm UFAM	Amazonas, Brazil	-3.0899	-59.9632
KR811107	<i>hudsoni</i>		AF0758	EDB	St Georges	French Guiana	3.8112	-51.8896
KDQF01000506	<i>hudsoni</i>		AF1361	EDB	Montagne tortue	French Guiana	4.2927	-52.3495
KDQF01000988	<i>hudsoni</i>		AF2565	EDB	RN2 PK65	French Guiana	4.4874	-52.3478
KR811108	<i>hudsoni</i>		AG396	EDB	Kourouaie	French Guiana	4.2379	-52.0382
KR811106	<i>hudsoni</i>		AG397	EDB	Kourouaie	French Guiana	4.2379	-52.0382
MH919910	<i>hudsoni</i>	CTMZ7043		MZUSP	?	Rondonia, Brazil	NA	NA
KDQF01002596	<i>hudsoni</i>		H2320	MTR	UHE Jirau	Rondonia, Brazil	-9.4378	-64.8328
KDQF01002602	<i>hudsoni</i>		H2555	MTR	UHE Jirau	Rondonia, Brazil	-9.4378	-64.8328
KF621174/MH919849	<i>hudsoni</i>		INPA2593-31276	INPA	ca 3 Km de casa del Sr Domingo	Roraima, Brazil	0.1960	-60.6485
KF621175	<i>hudsoni</i>		JMP2286	?	Senda Zafire	Colombia	-4.1363	-69.9406
KC180060	<i>hudsoni</i>		MAD116	?	Iwokrama	Guyana	4.6714	-58.6850
MH919969	<i>hudsoni</i>	MPEG18456		MPEG	?		NA	NA

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**APPENDIX 1. (Continued)**

GB Accession	species	Voucher specimens	tissue voucher	Tissue collection	Locality	State / Country	Lat	Lon
KM509111/MH919977/ KF621178/KM509111	<i>hudsoni</i>	MPEG18547		MPEG	Itaituba, PARNA Amazonia	Pará, Brazil	-4.1693	-55.9811
MH919963	<i>hudsoni</i>	MPEG18900		MPEG	?		NA	NA
MH919967/MH919967	<i>hudsoni</i>	MPEG3803-18545		MPEG	Itaituba, PARNA Amazonia	Pará, Brazil	-4.1693	-55.9811
KF621176/MH919960	<i>hudsoni</i>	MPEG3803-23206		MPEG	FLOTA Faro	Pará, Brazil	-1.7140	-57.2133
KF621180	<i>hudsoni</i>	MPEG3803-27763		MPEG	Maués, FLONA Pau-Rosa	Amazonas, Brazil	-3.7411	-58.2856
KDQF01003052	<i>hudsoni</i>	MTD47898		MTD	Iwokrama	Guyana	4.6714	-58.6850
KR811109	<i>hudsoni</i>		MTR24296	MTR	Oiapoque	Amapá, Brazil	3.8794	-51.7710
KDQF01003697	<i>hudsoni</i>		MTR33601	MTR	Maraã	Amazonas, Brazil	-1.7429	-65.7496
<b>OP476741</b>	<i>hudsoni</i>		MTR37760	MTR	Itapuã do Oeste	Rondonia, Brazil	-9.1923	-63.1916
<b>OP476742</b>	<i>hudsoni</i>		MTR41188	MTR	Boa Vista	Amazonas, Brazil	-0.3434	-65.4083
KDQF01003881	<i>hudsoni</i>		PG493	EDB	Kaw, Patawa	French Guiana	4.5161	-52.1005
<b>OP476743</b>	<i>hudsoni</i>		PRMS760	MTR	Programa de Assentamento	Acre, Brazil	-7.8472	-72.4167
<b>OP476744</b>	<i>hudsoni</i>		UHE163	IEPA	UHE Santo Antônio	Amapá, Brazil	-0.6456	-52.5006
MH919962	<i>jimi</i>	MPEG27763	FPR96	MPEG	Maués	Amazonas, Brazil	-3.7411	-58.2856
MH919880	<i>jimi</i>		MTR12728	MTR	Igarapé Açú, Rio Abacaxis	Amazonas, Brazil	-4.3442	-58.6350
MH919958	<i>jimi</i>		MTR12941	MTR	São Sebastião, Rio Abacaxis	Amazonas, Brazil	-4.3089	-58.6364
MH919922	<i>jimi</i>		MTR13195	MTR	Pacamiri, Rio Abacaxis	Amazonas, Brazil	-4.3333	-58.1333
MH919968	<i>jimi</i>		MTR13197	MTR	Pacamiri, Rio Abacaxis	Amazonas, Brazil	-4.3333	-58.1333

**APPENDIX 2. Material examined.**

species	Voucher	Field N	Locality	Country	Latitude	Longitude	Sex	Collector	Date
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0028	AF1547	Saul	French Guiana	3.615576	-53.22709	F	M. Berroneau - M. Berroneau - L. Barthe - P.O. Cochard	22_01_2014
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0029	AF2669	Alikéné	French Guiana	3.21873	-52.3964	F	J.P. Vacher - S. Cally	13_02_2015
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0030	AF2714	Alikéné	French Guiana	3.21873	-52.3964	M	J.P. Vacher - S. Cally	17_02_2015
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0031	AF2715	Alikéné	French Guiana	3.21873	-52.3964	M	J.P. Vacher - S. Cally	17_02_2015
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0026	AF2716	Alikéné	French Guiana	3.21873	-52.3964	M	J.P. Vacher - S. Cally	17_02_2015
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0033	AF5405	Savane roche Dachine	French Guiana	3.46958	-53.22978	M	M. Dewynter E. Courtois	03_06_2019
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0032	AF5489	Trois-Saut	French Guiana	2.20963	-52.98903	M	M. Dewynter E. Courtois	30_04_2019
<i>C. jacksi</i> sp. nov.	MNHN-RA-2022.0027	PG717	St Georges	French Guiana	3.80611	-51.89339	F	M Dewynter K Pineau	01_02_2012
<i>C. jacksi</i> sp. nov.	MNHN1997.2272	591 G	Saint Eugene	French Guiana	4.82167	-53.06766	F	J.-C deMassary	31_03_1997
<i>C. haddadi</i>	MNHN-RA-2022.0016	AF2269	Sipaliwini	Suriname	2.03253	-56.11449	M	A. Fouquet - J.P. Vacher	27_04_2014
<i>C. haddadi</i>	MNHN-RA-2022.0017	AF2270	Sipaliwini	Suriname	2.03253	-56.11449	M	A. Fouquet - J.P. Vacher	27_04_2014
<i>C. haddadi</i>	MNHN-RA-2022.0018	AF2271	Sipaliwini	Suriname	2.03253	-56.11449	M	A. Fouquet - J.P. Vacher	27_04_2014
<i>C. haddadi</i>	MNHN-RA-2022.0019	AF2275	Sipaliwini	Suriname	2.03253	-56.11449	M	A. Fouquet - J.P. Vacher	27_04_2014
<i>C. haddadi</i>	MNHN-RA-2022.0020	AF3403	Bakhuis	Suriname	4.68367	-56.77214	M	A. Fouquet - R. Jairam - S. Cally	29_04_2015
<i>C. haddadi</i>	MNHN-RA-2022.0021	AF3405	Bakhuis	Suriname	4.68367	-56.77214	F	A. Fouquet - R. Jairam - S. Cally	29_04_2015

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**APPENDIX 2. (Continued)**

species	Voucher	Field N	Locality	Country	Latitude	Longitude	Sex	Collector	Date
<i>C. haddadi</i>	MNHN-RA-2022.0022	AF3406	Bakhuis	Suriname	4.68367	-56.77214	M	A. Fouquet - R. Jairam - S. Cally	29_04_2015
<i>C. haddadi</i>	MNHN-RA-2022.0023	AF3581	Itoupé	French Guiana	3.02647	-53.07983	M	A Fouquet - E. Courtois - B Villette - M Dewynter	07_01_2016
<i>C. haddadi</i>	MNHN-RA-2022.0024	AF3585	Itoupé	French Guiana	3.02647	-53.07983	M	A Fouquet - E. Courtois - B Villette - M Dewynter	07_01_2016
<i>C. haddadi</i>	MNHN-RA-2022.0025	AF3995	Itoupé	French Guiana	3.025	-53.08	M	E. courtois M. Dewynter	17_11_2018
<i>C. haddadi</i>	MNHN 2011.0140	PG443	Kotika	French Guiana	3.93333	-54.19722	M	P. Gaucher	21_02_2005
<i>C. haddadi</i>	MNHN 2011.0143	PG440	Kotika	French Guiana	3.93333	-54.19722	M	P. Gaucher	21_02_2005
<i>C. haddadi</i>	MNHN 2011.0144	PG441	Kotika	French Guiana	3.93333	-54.19722	M	P. Gaucher	21_02_2005
<i>C. haddadi</i>	MNHN 2011.0145	PG442	Kotika	French Guiana	3.93333	-54.19722	M	P. Gaucher	21_02_2005
<i>C. hudsoni</i>	no Voucher	AF0758	St Georges	French Guiana	3.81116	-51.88964	M	A. Fouquet - D. Baudain - A. Cristinoi	03_05_2012
<i>C. hudsoni</i>	no Voucher	AF1361	Montagne tortue	French Guiana	4.29266	-52.34954	M	A. Fouquet - B. Villette - Q. Martinez	26_12_2013
<i>C. hudsoni</i>	no Voucher	AF2565	RN2 PK65	French Guiana	4.48737	-52.34781	M	A. Fouquet - E. Courtois - G. Robert	01_01_2015
<i>C. hudsoni</i>	MNHN 2001.0837	PG050	Patawa	French Guiana	4.51610	-52.10053	M	P. Gaucher	21_12_2001
<i>C. hudsoni</i>	MNHN 2001.0838	PG051	Patawa	French Guiana	4.51610	-52.10053	M	P. Gaucher	21_12_2001

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**APPENDIX 2. (Continued)**

species	Voucher	Field N	Locality	Country	Latitude	Longitude	Sex	Collector	Date
<i>C. hudsoni</i>	MNHN 2001.0839	PG066	Patawa	French Guiana	4.51610	-52.10053	M	P. Gaucher	21_12_2001
<i>C. hudsoni</i>	MNHN 2001.0322	AG023	CD 6 pk 52	French Guiana	4.59028	-52.24783	M	M. Blanc	04_12_2000
<i>C. hudsoni</i>	AMNH166437	JC7326	Berbice River	Guyana	5.08500	-58.23722	M		
<i>C. hudsoni</i>	AMNH166438	JC7507	Berbice River	Guyana	5.08500	-58.23722	M		
<i>C. hudsoni</i>	AMNH166439	JC7621	Berbice River	Guyana	5.08500	-58.23722	M		
<i>C. hudsoni</i>	no Voucher	MTR24296	Oiapoque	Amapá, Brazil	3.87942	-51.77097	F	S.M. Sousa A. Fouquet	09_12_2012
<i>C. hudsoni</i>	MZUSP64565		Reserva INPA-WWF	Amazonas, Brazil			F		
<i>C. hudsoni</i>	MZUSP64567		Reserva INPA-WWF	Amazonas, Brazil			F		
<i>C. hudsoni</i>	MZUSP64568		Reserva INPA-WWF	Amazonas, Brazil			F		
<i>C. hudsoni</i>	BMNH 1939.1.1.3		New River	Guyana					
<i>C. jimi</i>	MNRJ 14549		Humaitá	Amazonas, Brazil					
<i>C. antenori</i>	KU 124004		Rio Aguarico Napo	Ecuador					
<i>C. antenori</i>	KU 124005		Rio Aguarico Napo	Ecuador					
<i>C. antenori</i>	KU 124006		Rio Aguarico Napo	Ecuador					
<i>C. carvalhoi</i>	MZUSP 36429		Loreto: Estirón, Rio Ampiyacú	Peru					
<i>C. carvalhoi</i>	AMNH 88065		Loreto: Estirón, Rio Ampiyacú	Peru					
<i>C. magna</i>	AMNH 96398		Loreto: 3 km SSW of Mishana, Rio Nanay	Peru					
<i>C. magna</i>	AMNH 103550		Loreto: 3 km SSW of Mishana, Rio Nanay	Peru					

**APPENDIX 3.** Details (accession numbers, localities and collectors) regarding the acoustic data analysed in this study.

Accession	Species	locality	collector	file name
MNHN-SO-2022-631	<i>C. haddadi</i>	Itoupe	E. Courtois	160107_0699 Chiasmocleis haddadi Itoupe
MNHN-SO-2022-633	<i>C. haddadi</i>	Kotika	P. Gaucher	DAT 12 prg 15 Kotika
MNHN-SO-2022-634	<i>C. haddadi</i>	Kotika	P. Gaucher	DAT 12 prg 20 Kotika
MNHN-SO-2022-635	<i>C. haddadi</i>	Bakhuis	A. Fouquet	LS110025 Chiasmocleis haddadi Bakhuis
MNHN-SO-2022-636	<i>C. haddadi</i>	Sipaliwini	J.P. Vacher	STE-102_Chiasmocleis_haddadi_#1_27_4_2014_Sipaliwini_AF2270
MNHN-SO-2022-630	<i>C. jacki</i> <b>sp. nov.</b>	Mitaraka	M. Dewynter	Chiasmocleis aff haddadi_Mitaraka_LS110228
MNHN-SO-2022-637	<i>C. hudsoni</i>	Mont. Tortue	A. Fouquet	201218_01 Gde Montagne Tortue
MNHN-SO-2022-640	<i>C. hudsoni</i>	Lower Jari	J. Lima	Chiasmoleis hudsoni Jucivaldo Amapa
MNHN-SO-2022-638	<i>C. hudsoni</i>	Patawa	P. Gaucher	DAT 06 prg 15 mare Patawa
MNHN-SO-2022-639	<i>C. hudsoni</i>	Saut Maripa	A. Fouquet	LS100655 Chiasmocleis hudsoni_Saut Maripa

**APPENDIX 4.** Full Maximum Likelihood phylogram with collapsed branches for the focal clade.

