



Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with the description of a new species from the eastern Guiana Shield

ANTOINE FOUQUET^{1,7}, QUENTIN MARTINEZ¹, LAUREN ZEIDLER², ELODIE A. COURTOIS¹,
PHILIPPE GAUCHER¹, MICHEL BLANC³, JUCIVALDO DIAS LIMA⁴, SERGIO MARQUES SOUZA⁵,
MIGUEL T. RODRIGUES⁵ & PHILIPPE J. R. KOK⁶

¹CNRS Guyane USR3456, Immeuble Le Relais, 2 Avenue Gustave Charlery, 97300, Cayenne, French Guiana

²Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA

³Pointe Maripa, RN2/PK35, 97311, Roura, French Guiana

⁴Centro de Pesquisas Zoobotânicas e Geológicas (CPZG), Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA), Macapá, AP, Brazil

⁵Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, Caixa Postal 11.461, CEP 05508-090, São Paulo, SP, Brazil

⁶Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, 2 Pleinlaan, B-1050 Brussels, Belgium

⁷Corresponding author: e-mail: fouquet.antoine@gmail.com

Abstract

We used molecular and morphological data to investigate the hidden diversity within the *Hypsiboas semilineatus* species group, and more specifically within *H. geographicus*, an allegedly widespread species in northern South America. As a result, the identity of *H. geographicus* was clarified, several candidate species were detected and one of them, from the eastern Guiana Shield, is described herein as a preliminary step to resolve the taxonomy of the group. *Hypsiboas diabolicus* **sp. nov.** is mainly distinguished from closely-related species by an acuminate snout in lateral view, well-developed webbing between fingers and toes, and unspotted carmine/crimson colouration on the concealed surfaces of legs, feet and hands in life. The tadpole of the new species is described and is characterized by a large A-2 gap, a mostly single row of large marginal papillae, and a dark brown to black colouration. We also describe the advertisement call of the new species, which is defined as a soft call consisting of short clusters of 2–3 chuckles with a dominant frequency ranging between 1.11–1.19 kHz. *Hypsiboas diabolicus* **sp. nov.** is currently known only from the eastern Guiana Shield, and is probably endemic to that region. The new species' range overlaps broadly with another candidate species referred to as *H. aff. semilineatus* 1. Our preliminary results stress out a high cryptic diversity in that species group and the need for a formal redescription of *Hypsiboas geographicus* based on more topotypic material than what is currently available to properly sort out the taxonomic status of several lineages in that clade.

Key words: Anura, conservation, endemism, Guiana Shield, taxonomy

Introduction

Hypsiboas Wagler 1830 is a speciose genus of hyliid frogs currently including 90 nominal species (Frost 2015). These so called “gladiator frogs” form a well-supported monophyletic clade (*e.g.* Wiens *et al.* 2010), but without established morphological synapomorphies (Faivovich *et al.* 2005). Seven species groups are currently recognized within the genus (Faivovich *et al.* 2005). Among these, the *H. semilineatus* group is strongly supported by molecular evidence (see Faivovich *et al.* 2005; Pyron & Wiens 2011), and the presence of a reticulated lower eyelid represents a putative morphological synapomorphy (Faivovich *et al.* 2005). The *H. semilineatus* group comprises six nominal species: *H. semilineatus* (Spix, 1824) and *H. pombali* (Caramaschi, Feio & Pimenta, 2004), which occur in the Atlantic Forest; *H. geographicus* (Spix, 1824), which is widely distributed in northern South America; *H. hutchinsi* (Pyburn & Hall, 1984), which occurs in western Amazonia; *H. boans* (Linnaeus, 1758), which occurs in Amazonia, extending to trans-Andean lowlands of Colombia, Ecuador and Panama; and *H. wavrini* (Parker,

1936), which occurs in central and northern Amazonia. Among these species, a subgroup containing *H. semilineatus*, *H. geographicus* and *H. hutchinsi* is characterized by a smaller size, a prepollex not modified into a projecting spine, the presence of pigmented nuptial excrescences, and a finely granular dorsal skin. Two species of that subgroup, *H. geographicus* and *H. semilineatus*, are taxonomically challenging because morphologically very similar both in adult and larval stage (d'Heursel & de Sá 1999). Both taxa have large tadpoles unpalatable to fishes that display schooling behaviour (Duellman & Lescure 1973; Caldwell 1989).

The exact identity of *Hypsiboas geographicus* remains ambiguous. The holotype, from Tefé, Amazonas, Brazil (western Amazonia), is lost (Hoogmoed & Gruber 1983), and the only testimonies of it are the succinct description and illustration in Spix (1824). Recent molecular analyses revealed the existence of distantly related lineages attributed to *H. geographicus* in the Guiana Shield (Fouquet *et al.* 2007) and in Bolivia (Jansen *et al.* 2011). Gans (1960) and later Duellman (1973) have already reported a striking geographical variation in the colour of the webbing of *H. geographicus*. Duellman (1973) also noted that morphometrics and bioacoustics discriminate populations displaying differences in webbing colouration in western Amazonia. Additionally, several studies found conspicuous variation in *H. geographicus* tadpole teeth rows that were, at the time, partly interpreted as ontogenetic and only partly considered as of taxonomic value (Bokermann 1963; Kenny 1969; Duellman & Lescure 1973; d'Heursel & de Sá 1999); differences in tadpole body size were found as well (Caldwell 1989). All these results converge to suggest the occurrence of several unnamed species erroneously identified as *H. geographicus* in Amazonia.

We compared molecular, morphological, and acoustic data attributed to that species, and our analysis reveals multiple lineages suggestive of the existence of no less than nine confirmed and unconfirmed candidate species in that group, including two species in the Guiana Shield currently referred to as *Hypsiboas geographicus*. Based on a comprehensive sampling we describe one of them as a new species, and closely compare it to the original description of *H. geographicus* from Tefé, Brazil, to one recently collected topotypic specimen, and to other specimens attributable to *H. geographicus sensu stricto* according to our phylogenetic hypothesis. Pending its formal description, the second species is herein referred to as *H. aff. semilineatus* 1 because of its closer relationship to *H. semilineatus* than to *H. geographicus sensu stricto* (see results).

Materials and methods

Molecular analysis. Genomic DNA was extracted using Promega DNA extraction kits from 48 tissue samples, including 14 of the new species (six from individual tadpoles collected from different schools), all preserved in 95% ethanol. We targeted one mitochondrial locus (16S) that was already available for twelve specimens of the *H. semilineatus* group, as well as eight congeneric species used as outgroup (Table 1). Fragments were amplified by standard PCR techniques using previously described primers (16Sar-5' and 16Sbr-3') and PCR conditions (Hillis *et al.* 1996). Sequencing was performed using ABI Big Dye V3.1 (ABI Foster City USA) and resolved on an automated sequencer at Genomic Engenharia corp. (São Paulo Brazil) and Macrogen (Korea). Sequences were edited and aligned using CodonCode Aligner v.3.5.2 (<http://www.codoncode.com/aligner/download.htm>). Novel sequences were deposited in Genbank (Table 1).

The 48 newly generated sequences were collated to 20 available additional sequences from Genbank (totaling 68 sequences), and aligned using MAFFT v6 (Kato *et al.* 2013) under default parameters except the use the E-INS-i strategy, which is adapted to sequences with conserved domains and long gaps. We obtained a final 850 base pairs (bp) alignment that remained incomplete for the first 290 bp for 42 terminals (all the major lineages except *H. aff. semilineatus* 3 & 4 have complete representatives) and the last *ca.* 100 bp for 24 terminals.

We used the software jModeltest version 0.1.1 (Guindon & Gascuel 2003; Posada 2008) to select the substitution model that best fits the data under the Bayesian Information Criterion. The resulting model was employed in a Bayesian analysis (BA) with MrBayes 3.1 (Huelsenbeck & Ronquist 2001). The BA consisted of a 10×10^6 generations run starting with random trees and 10 Markov chains (one cold) sampled every 1000 generations. Adequate burn-in (the first 1000 sampled generations) was determined by examining likelihood scores of the heated chains for convergence on stationarity as well as the effective sample size of values in Tracer 1.5 (Rambaut & Drummond 2007). We considered relationships to be strongly supported when posterior probabilities were equal to or higher than 0.95.

TABLE 1. List of the specimens included in the phylogenetic analysis and GenBank accession numbers. Numbers between parentheses correspond to field numbers; data in bold face correspond to the new species.

Species	Locality	Country/state	Voucher	Accession number	Latitude	Longitude
<i>H. aff. semilineatus</i> 1	Road to Apura	Suriname	R 140 (AF0107)	KU168866	5.18333	-55.616667
<i>H. aff. semilineatus</i> 1	Igarapé Camaipi	Brazil/Amapá	(MTR6216)	KU168905	-0.024167	-51.897222
<i>H. aff. semilineatus</i> 1	Road to Apura	Suriname	R 139 (AF0106)	KU168865	5.18333	-55.616667
<i>H. aff. semilineatus</i> 1	Pidima	French Guiana	(CM335)	KU168886	3.293856	-54.103300
<i>H. aff. semilineatus</i> 1	Igarapé Camaipi	Brazil/Amapá	(MTR6214)	KU168904	-0.024167	-51.897222
<i>H. aff. semilineatus</i> 1	REBIO Maicuru	Brazil/Pará	MPEG30285	KU168894	0.828619	-53.931200
<i>H. aff. semilineatus</i> 1	Lourenço	Brazil/Amapá	(MTR13933)	KU168896	2.323611	-51.645278
<i>H. aff. semilineatus</i> 1	Warnabo Creek, SW Dubulay Ranch house	Guyana	AMNH-A 141054	AY843628	5.620769	-57.856865
<i>H. aff. semilineatus</i> 1	<i>Crique Margot</i>	French Guiana	(CM140)	KU168885	5.466667	-53.950000
<i>H. aff. semilineatus</i> 1	Piste Risquetout	French Guiana	(BM101)	KU168882	4.916731	-52.500064
<i>H. aff. semilineatus</i> 1	Piste Risquetout (tadpole)	French Guiana	(AF175)	KU168867	4.916731	-52.500064
<i>H. aff. semilineatus</i> 1	Serra do Navio	Brazil/Amapá	(MTR13841)	KU168895	0.918056	-52.002778
<i>H. aff. semilineatus</i> 1	Igarapé Camaipi	Brazil/Amapá	(MTR6217)	KU168906	-0.024167	-51.897222
<i>H. aff. semilineatus</i> 1	Nouragues	French Guiana	R 143 (AF1285)	KU168875	4.084790	-52.680630
<i>H. aff. semilineatus</i> 1	Crique Bon Espoir, Mana River (tadpole)	French Guiana	(AF1112B)	KU168873	5.099493	-53.800571
<i>H. aff. semilineatus</i> 1	Grand Santi	French Guiana	(CM033)	EF376054	4.333333	-54.250000
<i>H. aff. semilineatus</i> 1	Bagne des Annamites (tadpole)	French Guiana	(AF1373)	KU168876	4.818210	-52.522123
<i>H. aff. semilineatus</i> 1	Mont. Cacao	French Guiana	(CAAM29)	KU168884	2.349156	-53.216013
<i>H. semilineatus</i>	Duque de Caxias	Brazil/R. Janeiro	CFBH5424	AY843779	-22.791375	-43.317947
<i>H. semilineatus</i>	unknown	Brazil/R. Janeiro	unknown	KM390786	unknown	unknown
<i>H. aff. semilineatus</i> 2	Altamira, UHE Belo Monte	Brazil/Pará	(BM334)	KU168883	-2.950584	-51.936607
<i>H. aff. semilineatus</i> 2	Guaraí	Brazil/Tocantins	(MRT7584)	KU168907	-8.816943	-48.383300
<i>H. aff. semilineatus</i> 3	Juruena	Brazil/M. Grosso	(AF252)	KU168880	-12.849000	-58.925000
<i>H. aff. semilineatus</i> 3	Apiacás	Brazil/M. Grosso	(968365)	KU168863	-9.650846	-57.39366
<i>H. aff. semilineatus</i> 3	Gatúcha do Norte	Brazil/M. Grosso	(976482)	KU168864	-13.235935	-53.15208
<i>H. aff. semilineatus</i> 3	Tapajos, Tocantinzinho	Brazil/Pará	(JOG179)	KU168889	-6.061700	-56.296620
<i>H. aff. semilineatus</i> 3	Itaituba, Mina Palito	Brazil/Pará	(JOG737)	KU168890	-6.317317	-55.798644
<i>H. aff. semilineatus</i> 4	Campo Catuquira	Brazil/Pará	(SMS153)	KU168909	-4.9136111	-61.109160
<i>H. aff. semilineatus</i> 5	<i>Rio Içá, comm. cachoeirinha</i>	Brazil/Amazonas	(MTR36136)	KU168899	-2.891870	-68.355180
<i>H. aff. semilineatus</i> 5	Rio Içá, comm. Cuiaúá	Brazil/Amazonas	(MTR36149)	KU168900	-2.891870	-68.369280
<i>H. aff. semilineatus</i> 5	Fonte Boa	Brazil/Amazonas	(MTR36821)	KU168902	-2.532115	-66.162602
<i>H. cf. geographicus</i> 1	UHE Jirau, Rio Madeira	Brazil/Rondonia	(HJ0048)	KU168888	-9.576376	-64.923134
<i>H. cf. geographicus</i> 1	FLONA Caxiuanã, Plote PPBIO	Brazil/Pará	MPEG24822	KU168893	-1.792306	-51.434028
<i>H. cf. geographicus</i> 1	FLONA Caxiuanã, Plote PPBIO	Brazil/Pará	MPEG24821	KU168892	-1.792306	-51.434028

... continued on the next page

TABLE 1. (Continued)

Species	Locality	Country/state	Voucher	Accession number	Latitude	Longitude
<i>H. cf. geographicus</i> l	Rio Ica, São Pedro	Brazil/Amazonas	(MTR36719)	KU168901	-3.028560	-68.882600
<i>H. geographicus</i>	Lago Chaviana, Rio Purus	Brazil/Amazonas	MZUSP157083 (MTR18569)	KU168897	-4.307750	-61.813833
<i>H. geographicus</i>	Concepcion	Bolivia	MNKA9347	JF790119	-16.351768	-61.975708
<i>H. geographicus</i>	Concepcion	Bolivia	MNKA9343	JF790118	-16.351768	-61.975708
<i>H. geographicus</i>	Concepcion (tadpole)	Bolivia	SMF94088	KF723057	-16.359600	-62.000100
<i>H. geographicus</i>	Concepcion (tadpole)	Bolivia	MNKA9497	KF723060	-16.359600	-62.000100
<i>H. geographicus</i>	Concepcion (tadpole)	Bolivia	MNKA9501	KF723061	-16.359600	-62.000100
<i>H. geographicus</i>	Concepcion (tadpole)	Bolivia	SMF94091	KF723059	-16.359600	-62.000100
<i>H. geographicus</i>	Serra do Divisor	Brazil/Acre	MZUSP157090 (MTR28250)	KU168898	-7.434348	-73.660390
<i>H. geographicus</i>	Teifé	Brazil/Amazonas	MZUSP157060 (MTR36895)	KU168903	-2.890867	-68.361287
<i>H. diabolicus</i> sp. nov	Trinite	French Guiana	R158 (AG196)	EU01106	4.608465	-53.415191
<i>H. diabolicus</i> sp. nov	Mont Saint Marcel	French Guiana	(PG111)	KU168908	2.385833	-53.018889
<i>H. diabolicus</i> sp. nov	Nouragues (Holotype)	French Guiana	R157 (AF1284)	KU168874	4.084790	-52.680630
<i>H. diabolicus</i> sp. nov	Kaw	French Guiana	R152 (AF667)	KU168869	4.627158	-52.307289
<i>H. diabolicus</i> sp. nov	Crique Bon Espoir, Mana River (tadpole)	French Guiana	(AF111C)	KU168872	5.099493	-53.800571
<i>H. diabolicus</i> sp. nov	Gaa Kaba (tadpole)	French Guiana	(MBT136)	KU168891	4.462112	-54.392738
<i>H. diabolicus</i> sp. nov	St Elie	French Guiana	R151 (AF0595)	KU168868	5.341557	-53.038820
<i>H. diabolicus</i> sp. nov	Paracou	French Guiana	R153 (AF0701)	KU168870	5.272546	-52.923596
<i>H. diabolicus</i> sp. nov	Sparouine (tadpole)	French Guiana	(AG406)	KU168881	4.816926	-54.281875
<i>H. diabolicus</i> sp. nov	Apatou (tadpole)	French Guiana	(AF1788)	KU168877	5.249830	-54.206730
<i>H. diabolicus</i> sp. nov	Apatou (tadpole)	French Guiana	(AF1800)	KU168878	5.249830	-54.206730
<i>H. diabolicus</i> sp. nov	Montagne des Singes (tadpole)	French Guiana	(AF1827)	KU168879	5.069670	-52.696800
<i>H. diabolicus</i> sp. nov	Rio Vila Nova	Brazil/Amapá	IEPA5028 (FTA114)	KU168887	0.449335	-52.022137
<i>H. diabolicus</i> sp. nov	Serra Macacoara	Brazil/Amapá	IEPA3099 (ST290)	KU168910	0.909122	-53.228479
<i>H. diabolicus</i> sp. nov	Oiaoque	Brazil/Amapá	R149 (AF1092)	KU168871	3.904047	-51.772270
<i>H. boans</i>	Cano Agua Blanca,	Venezuela	RWM 17746	AY843610		
<i>H. polytaenius</i>	Itatiaia, Maringa	Brazil/R. Janeiro	CFBH 5752	AY843655		
<i>H. pulchellus</i>	Entre Rios: Dio. Uruguay: Basavilbaso	Argentina	MACN 37664	AY549351		
<i>H. pardalis</i>	Estação Biológica de Boraceia	Brazil/Sao Paulo	USNM 303046	AY843651		
<i>H. siblezi</i>	Mount Ayanganna	Guyana	ROM 39561	AY843667		
<i>H. lanciformis</i>	Pastaza; 5,6 km N Puyo; 1150 m	Ecuador	WED 54081	AY326054		
<i>H. raniceps</i>	Santa Fe, Vera, Ea. 'Las Gamas'	Argentina	MACN 37795	AY843657		
<i>H. benitezi</i>	Roraima, Vila Pacaraima,	Brazil/Roraima	USNM 302435	AY843606		
<i>H. rufiellus</i>	Parque Nacional 'Omar Torrijos'	Panama	KRL 798	AY843662		

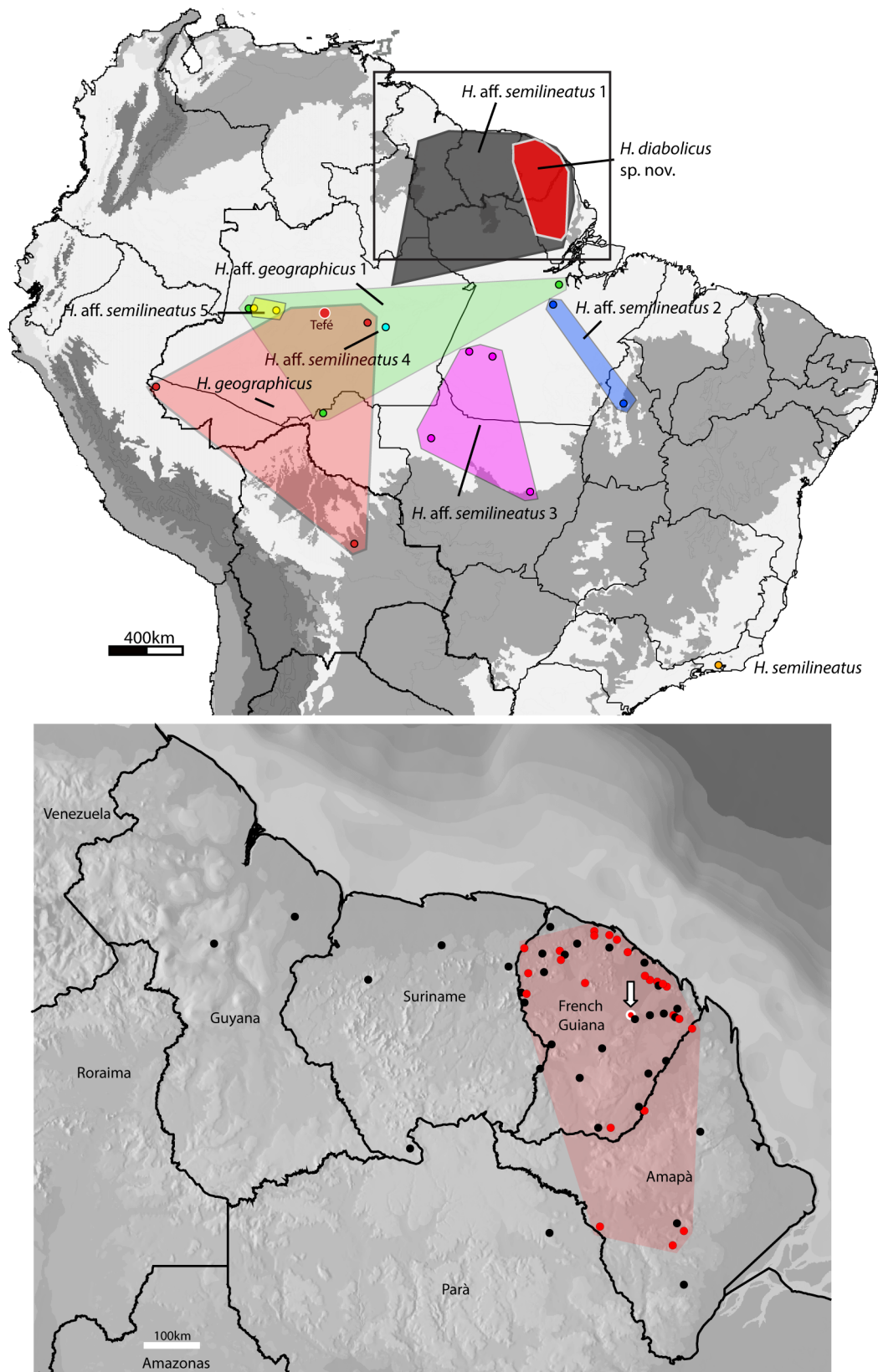


FIGURE 1. (A) Map of northern South America showing the distribution of the major lineages recovered from the phylogenetic analysis. (B) Distribution records of *H. diabolicus* sp. nov. (red dots) and *H. aff. semilineatus* 1 (black dots) in the Eastern Guiana Shield (from specimens, DNA sequences, as well as unambiguously identified individuals from the literature and various herpetological surveys; other records are ignored). The type locality of *Hypsiboas diabolicus* sp. nov. is indicated with a white arrow.

We also calculated p-distances among major groups recovered from the phylogenetic analysis using a 400 bp region, where all the sequences overlap, using MEGA 5 (Tamura *et al.* 2011).

Fieldwork and deposition of specimens. Twenty-two adult specimens of the new species were collected by hand at night. Nineteen are from eight localities in French Guiana, and three are from distinct localities in Amapá, Brazil (see below). Nineteen tadpoles from five localities in French Guiana were also collected (six specimens from five schools were genotyped). Thirteen additional localities of occurrence are based on available molecular data and direct field observations. In total, the new species is reported from 25 localities in the eastern Guiana Shield (Appendix 1; Fig. 1).

This material was compared with 22 adult specimens and seventeen tadpoles (three specimens from three schools were genotyped) of the sympatric *H. aff. semilineatus* 1, from diverse localities in French Guiana (see Appendix 2). Additional occurrence records were also gathered from available molecular data and direct field observations. In total, we provide 33 occurrence records in the Eastern Guiana Shield for that species (Fig. 1). *Hypsiboas aff. semilineatus* 1 probably also occurs in Reserva Adolfo Ducke, north of Manaus, Amazonas, Brazil (Lima *et al.* 2006). The description of adults, call, and pictures provided by Lima *et al.* (2006) match with *Hypsiboas aff. semilineatus* 1 and none of the other lineages has been recorded north of the Amazon River. Nevertheless, the status of this population was not assessed in our study, and thus remains tentative.

The new taxon was also compared to one topotypic adult specimen of *Hypsiboas geographicus* from Tefé (Amazonas, Brazil). Nine adult specimens from Rio Purus (Amazonas state, Brazil) and Serra do Divisor (Acre state, Brazil) (Appendix 2) were also unambiguously attributed to that species according to molecular data and morphology (see results).

Frogs were killed by intraperitoneal injection of Xylocaïne® (lidocaine chlorhydrate). Tissue samples (a piece of liver) were removed and stored in 95% ethanol before specimen preservation. All specimens were tagged and fixed in 10% formalin before being transferred to a solution of 70% ethanol for permanent storage. Type specimens were deposited in the collections JAGUARS IPG/Kwata FR973A (Cayenne, French Guiana), in the Instituto de Pesquisas Científicas e Tecnológicas, Estado do Amapá, (Macapá, Brazil), and in the Museum National d'Histoire Naturelle de Paris (Paris, France).

Morphology. Webbing formulae follow Savage and Heyer (1967), as subsequently modified by Myers and Duellman (1982). All other terminology follows Duellman (1970). Abbreviations for morphological characters used throughout the text are: SVL, Snout-Vent Length; TiL, Tibia Length; CL, Calcar Length; ThL, Thigh Length (from vent to outer edge of flexed knee); TaL, Tarsus Length; FL, Foot Length (from proximal edge of inner metatarsal tubercle to tip of fourth toe); 4TD, Disc width on Toe IV; FD, Disc width on Finger III; 1FiL, Length of Finger I (from proximal edge of palmar tubercle to tip of Finger I); 2FiL, Length of Finger II (from proximal edge of palmar tubercle to tip of Finger II); HaL, Hand Length (from proximal edge of palmar tubercle to tip of Finger III); ETS, Eye-Tip of Snout distance (straight line between anterior corner of eye and tip of snout); EN, Eye to Nostril distance (straight line between anterior corner of eye and posterior edge of nostril); HL, Head Length (straight line from posterior corner of mouth to the tip of snout); HW, Head Width (at widest point); IN, Internarial Distance; ED, horizontal Eye Diameter; IOD, Interorbital Distance (between the anterior margins of the orbits), and TYD, Tympanum Diameter. All measurements (rounded to the nearest 0.1 mm) were taken by the same person (Quentin Martinez, hereafter QM) using electronic digital callipers under a stereomicroscope.

Sex was determined either by the presence or absence of secondary sexual characters (*i.e.* pigmented nuptial pads, vocal slits) or by calling activity at the time of collection. Relative length of digits was estimated by adpressing adjacent digits to one another. Colour pattern in life was taken from field notes and colour photographs that were compared to the colour definitions proposed by Köhler (2012).

Available tadpoles of the new species range from Gosner stage 27–39 (Gosner 1960), and were closely compared with tadpoles of the sympatric *Hypsiboas aff. semilineatus* 1. Gosner stage, Labial Tooth Row Formula (LTRF) and Total Length were taken on 19 tadpoles of the new species and 16 tadpoles of *H. aff. semilineatus* 1. The following measurements were taken on the best preserved larvae (ten tadpoles of the new species and five tadpoles of *H. aff. semilineatus* 1) by the same person (Antoine Fouquet, hereafter AF) as summarized in Kok and Kalamandeen (2008) using calibrated pictures from a Stemi 2000C Zeiss stereomicroscope connected to an Axiocam ERC 5S: TL, Total Length (from tip of the snout to tip of the tail); BL, Body Length (from tip of snout to junction of posterior body and tail musculature); TAL, Tail Length (from junction of posterior body and tail musculature to tip of tail); BW, Body Width (highest width of body); BH, Body Height (highest height of body); HW, Head Width (at level of eyes); TMH, Tail Muscle Height (at base of tail); UTF, Upper Tail Fin Height (highest

height of upper fin, from upper margin of tail musculature to upper margin of upper fin); LTF, Lower Tail Fin Height (highest height of lower fin, from lower margin of lower fin to lower margin of tail musculature); TMW, Tail Muscle Width (at base of tail); MTH, Maximum Height of Tail; END, Eye-Naris Distance (from anterior corner of eye to posterior margin of nostril); NSD, Naris-Snout Distance (from anterior margin of naris to tip of snout); SSD, Snout-Spiracle Distance (from tip of snout to posterior margin of spiracle); IND, Internarial Distance (distance between the median margins of nares); IOD, Interorbital Distance; ED, Eye Diameter (greatest length of orbit from anterior margin to posterior margin of eye).

Bioacoustics. Call recordings of the new species were obtained from a voucher specimen at the Nouragues biological station (AF1283/MNHN 2014.0109), and from an uncollected specimen at Camp Patawa, Kaw Mountains, French Guiana; recorded using SONY PCM-M10 and a microphone SENNHEISER ME66 on 15 March 2014 at night (21:20; ~24°C). Unfortunately the quality of the voucher recording was too low to be properly analysed. Therefore, we used the unvouchered call, which is unambiguously attributed to a specimen of the new species because of its characteristic leg colouration (see below). In addition, both recordings display similar temporal and spectral structures. This recording (four calls analyzed) was compared with a recording of *H. aff. semilineatus* 1 (four calls analyzed) from an uncollected specimen from Camp Arataï, Nouragues biological station, French Guiana, which was recorded using a tape recorder SONY WM-D6C (tape: UX-Pro90) and a microphone SENNHEISER MKH60 on 21 January 1996 at night (20:32; ~23°C). This individual was unambiguously identified in the field because of its colouration. Two other recordings of *H. aff. semilineatus* 1 were obtained from voucher specimens at the Nouragues biological station and at Chutes Voltaire (AF1288/R145, and AF1755/MNHN 2014.0119, respectively). These two recordings display similar temporal and spectral structures, but their quality was too low to be properly analysed. Despite the absence of voucher specimens for the calls we analysed, we consider that these recordings can be unambiguously attributed to each species. Calls were also compared to the call of *H. geographicus* from Bolivia available from Márquez *et al.* (2002).

Calls were analysed at a sampling rate of 44.1 kHz using Audacity (<http://audacity.sourceforge.net/>). We measured call duration (beginning of the first to the end of the last note of a call); number of notes per call (a call is here defined as a series of notes emitted in groups between longer silent intervals); note duration (beginning of the note to the end of the note); pulses per second (mean number of pulses/second calculated in 3 notes); internote silent interval (end of one note to beginning of the next); intercall silent interval (end of one call to the beginning of the next; as summarized in Kok & Kalamandeen 2008). Peak of the dominant frequency of the note was measured from a spectral slice taken through each entire call (using the Blackman window function at a 3 dB filter bandwidth of 120 Hz). Call characteristics of the new species compared to those of *H. aff. semilineatus* 1 and *H. geographicus* are illustrated as spectrograms using the R package SEEWAVE (Sueur *et al.* 2008).

Results

Phylogenetic analysis. *Hypsiboas boans* is recovered as the sister species of a clade formed by all the other representatives of the *H. semilineatus* group included in this study (*i.e.* nine main lineages). These lineages display poorly supported relationships among them (Fig. 2, one of them corresponds to the new species described herein). *Hypsiboas geographicus sensu stricto* includes samples from southwestern and central Amazonia: Tefé (Amazonas, Brazil), Bolivia, Serra do Divisor (Acre, Brazil) and Rio Purus (Amazonas, Brazil); and *H. cf. geographicus* 1 includes samples from central and eastern Amazonia: Caxiuanã (Pará, Brazil), Jirau (Rondônia, Brazil) and Rio Içá (Amazonas, Brazil). Genetic distances among these lineages are substantial: > 4.8% in the targeted fragment of 16S (Table 2). The other six lineages display lower genetic distances (> 2.1% among them, minimum found between *H. semilineatus* and *H. aff. semilineatus* 3) and form a strongly supported clade hereafter referred to as the *H. semilineatus* clade (Fig. 2). This clade comprises *H. semilineatus* from the Atlantic Forest, *H. aff. semilineatus* 1 from the Guiana Shield, *H. aff. semilineatus* 2 from Eastern Amazonia, *H. aff. semilineatus* 3 from Southeastern Amazonia, *H. aff. semilineatus* 4 from central Amazonia, and *H. aff. semilineatus* 5 from central Amazonia.

***Hypsiboas geographicus* identity.** Considering that several species might be involved under the name *Hypsiboas geographicus*, the only unambiguous diagnostic characters that can be used to differentiate species similar to *H. geographicus* rely so far on the succinct description and the illustration of Spix (1824). We attempted in vain to find museum specimens from Tefé, and the curator of the Berlin Museum confirmed that the holotype is lost (M.O. Rödel pers. com.).

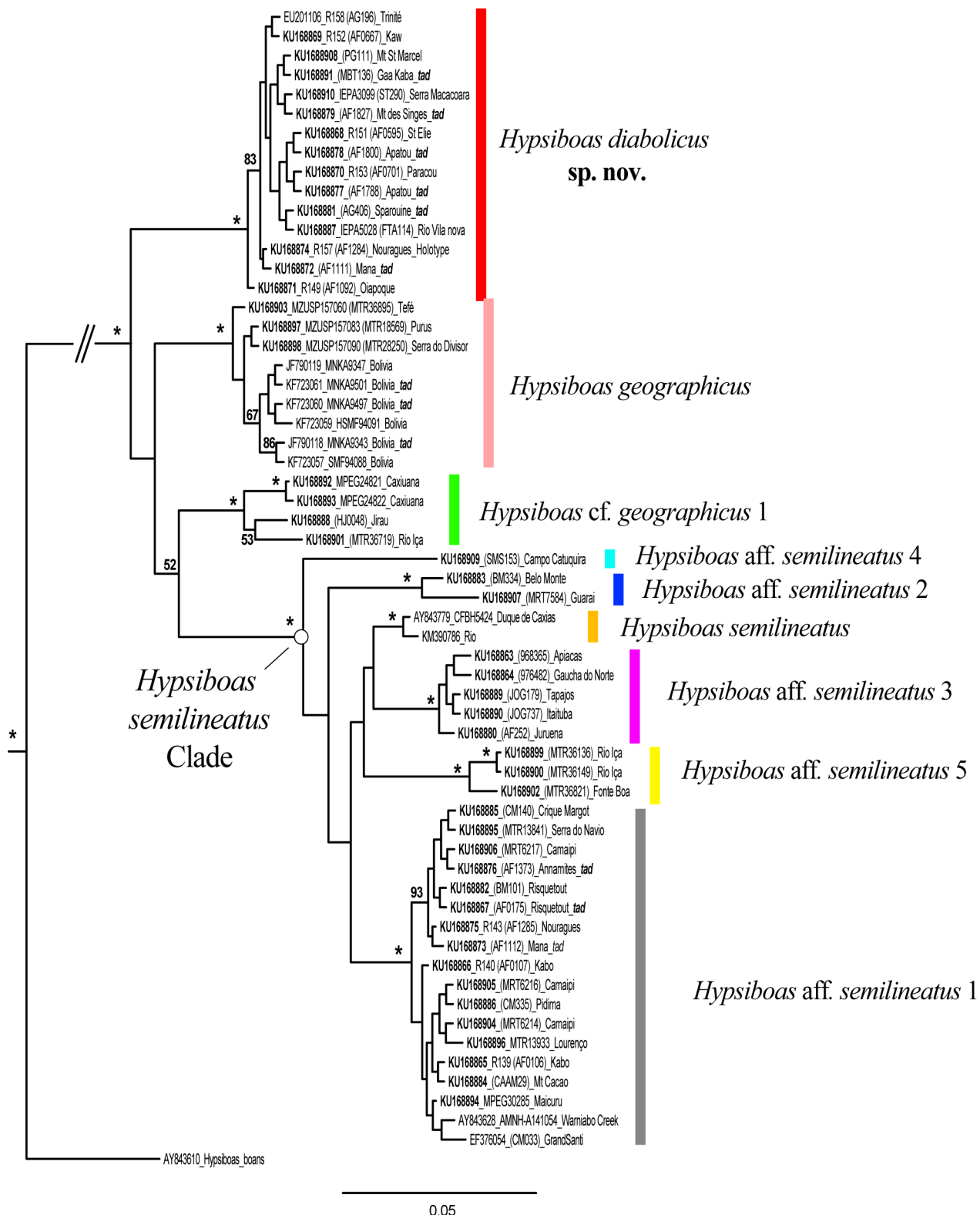


FIGURE 2. Phylogram (50% majority rule with frequencies of all observed bipartition) inferred from Bayesian Analysis of 850 bp of the 16S gene. Posterior probabilities (*100) are indicated on the upper left of the nodes (*=0.99 or 1; not indicated when <0.5).

TABLE 2. Mean genetic distances (p-distance, lower diagonal) and sd (upper diagonal, estimated using 100 bootstraps replicates) among major lineages using pairwise deletion.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. <i>H. aff. semilineatus</i> 1		0.006	0.008	0.008	0.01	0.011	0.01	0.012	0.013
2. <i>H. semilineatus</i>	0.023		0.008	0.006	0.009	0.012	0.009	0.012	0.014
3. <i>H. aff. semilineatus</i> 2	0.035	0.031		0.009	0.011	0.012	0.011	0.013	0.014
4. <i>H. aff. semilineatus</i> 3	0.034	0.021	0.046		0.011	0.012	0.01	0.013	0.014
5. <i>H. aff. semilineatus</i> 4	0.04	0.033	0.054	0.044		0.012	0.011	0.012	0.013
6. <i>H. cf. geographicus</i> 1	0.068	0.065	0.069	0.065	0.067		0.014	0.01	0.011
7. <i>H. aff. semilineatus</i> 5	0.051	0.035	0.057	0.038	0.051	0.08		0.013	0.015
8. <i>H. geographicus</i>	0.059	0.06	0.068	0.06	0.062	0.05	0.072		0.01
9. <i>H. diabolicus</i> sp. nov.	0.079	0.083	0.083	0.084	0.084	0.066	0.087	0.048	

However, a specimen recently collected in Tefé and the specimens from Purus and Serra do Divisor, which form a clade altogether, are morphologically similar and match well the pattern described by Spix in the original description of *H. geographicus*. These specimens are characterized by (1) numerous (8–10) dark grey transversal bands on the granular dorsal surface of thigh and tibia, extending towards the ventral face of the leg (best visible in preservative); (2) granular skin on upper thigh covering a broad surface (as broad as the inner and outer smooth faces of thigh); (3) inner and outer faces of thigh, tibia and flank from dark grey with transversal stripes to black (in life and in preservative); (4) dark spots on the belly in life and in preservative; (5) reduced hand webbing between Fingers II–IV; and (6) hand and foot webbing red except between Fingers III–IV and Toes IV–V (grey), fingers and toes dorsally grey except Finger I and Toes I–II (red). We consider these characters as diagnostic for *H. geographicus sensu stricto*, and consider all the specimens belonging to this clade as *H. geographicus*. The call from Bolivia provided by Marquez *et al.* (2002) corresponds to the call of the specimens genotyped by Jansen *et al.* (2011; Jansen com. pers.). Sequences from these specimens and from the tadpole described by Schulze *et al.* (2015) from Bolivia are included in our phylogenetic analysis. Both call and tadpole can thus be unambiguously identified as those of *H. geographicus sensu stricto*. A formal redescription of this taxon is clearly needed, but the available material from the type locality (one specimen only) is currently insufficient for an accurate description and analysis of variation. Therefore, we refrain to redescribe this taxon until additional topotypic material is available (see below).

Candidate species within the *H. semilineatus* species group. Except the new species described hereafter, all the other major lineages identified in this study are considered to be candidate species, and their taxonomic status needs to be clarified. Phylogeography, genetic distances, and overall morphology indicate that some clades unambiguously correspond to undescribed species whereas the taxonomic status of some lineages remains less clear (e.g. within the *H. semilineatus* clade) and deserve further investigation, which is beyond the scope of this paper. We tentatively grouped samples under the names *H. cf. geographicus* 1 (given its ambiguous relationship with any nominal taxon) and *H. aff. semilineatus* 1–5 (for the lineages related to *H. semilineatus*), pending additional data.

Known junior synonyms of *Hypsiboas geographicus* are all from the Atlantic Forest, except *H. appendiculata* (Boulenger, 1882), for which a lectotype from Canelos, Ecuador (BMNH 1947.2.13.1) was designated by Parker (1933). *Hypsiboas appendiculata* has vertical blackish lines on flanks, which immediately distinguishes it from the two species occurring in the Guiana Shield. We conclude that the new species described hereafter unambiguously differs from both *Hypsiboas geographicus* and its junior synonym *H. appendiculata*.

New species description

Hypsiboas diabolicus sp. nov.

Hypsiboas geographicus Fouquet *et al.*, 2007

Hypsiboas aff. geographicus Dewynter *et al.*, 2008

Holotype. R157 (AF1284), an adult male collected by AF and QM at Nouragues biological station near “Pont Ficelle” on Crique Nouragues, (4.08479, -52.68063, 100 m elevation) in the Reserve Naturelle des Nouragues, municipality of Régina, French Guiana, on 1 June 2013 (Fig. 3).

Paratypes (n=18; numbers between parentheses correspond to field numbers) (Fig. 3). R150 and 151 (AF0594–5), two adult males collected by AF at Piste St Elie (5.341557, -53.03882; 10 m elevation) municipality of Sinnamary, French Guiana on 1 March 2012; R152 (AF0667) an adult male collected by AF at Chutes Fourgassié (4.627158, -52.307289, 30 m elevation) municipality of Roura, French Guiana on 7 April 2012; MNHN 2014.0103 (QM459) an adult male collected by QM at « Arlésienne » pond (4.5787333, -52.01965, 200 m elevation) municipality of Roura; R153 (AF0701), an adult male collected by AF at Paracou (5.272546, -52.923596, 10 m elevation) municipality of Sinnamary, French Guiana on 29 April 2012; R154 (AF1115), an adult male collected by AF at “Crique Bon Espoir” near the left margin of the Mana River (5.099493, -53.800571, 50 m elevation) municipality of St Laurent du Maroni, French Guiana on 1 April 2013; R158, 159 and 155 (AG196, AG294, AF1185), three adult males, and R160 (AG290) an adult female, collected by Michel Blanc, AF, and Elodie A. Courtois (EAC) at Aya camp in the Reserve Naturelle de la Trinité (4.608465, -53.415191, 150 m elevation) municipality of St Elie, French Guiana on 15 January 2007 and 1 May 2013; R156 and MNHN 2014.0109 (AF1283, AF1304), two adult males collected by AF, QM and EAC with the holotype; R161 (AG064), an adult female collected by Benoît de Thoisy on the Counamy river (5.403682, -53.274515, 10m elevation) municipality of Iracoubo, French Guiana on 16 July 2001 (this specimen was not measured); MNHN 2014.0104-8 (AF1829, 1828, QM293, 307, 437) an adult female and an adult male collected by Jean-Pierre Vacher and Andy Lorenzini on 20 March 2014, and three adult males collected by QM on 13 January 2014 at Montagne des singes (5.06967, -52.6968, 20 m elevation) municipality of Kourou, French Guiana; R149 (AF1092), an adult male collected by AF near Vila Vitoria (3.904047, -51.77227, 10 m elevation) municipality of Oiapoque, Amapá, Brazil on 1 March 2013; IEPA5028 (FTA114), an adult male collected by Jucivaldo Dias Lima at Rio Vila Nova within FLOTA, (0.46966, -52.020493, 100 m elevation) municipality of Mazagão Amapá, Brazil (this specimen was not measured); IEPA3099 (STD290), an adult male collected by Jucivaldo Dias Lima at Serra Macacoara within Parque Nacional das Montanhas do Tumucumaque (0.909122, -53.228479, 150 m elevation), municipality of Laranjal do Jari, Amapá, Brazil (this specimen was not measured).

Etymology. The specific name is a noun in apposition and refers to the “Diables Rouges” (Red Devils), traditional characters of the carnival in French Guiana who dress in red and black, reminiscent of the black flanks and the carmine/crimson legs and webbing of the new species (colours 62, 64 in Köhler 2012).

Definition and diagnosis. The new species is morphologically characterized by the following unique combination of characters: (1) medium size, adult males $X=43.5$ mm (38.5–48.0 mm, n=17), adult females $X=56.3$ mm (55.9–56.7 mm, n=2; Table 3); (2) thighs long (ThL/SVL 0.50–0.58); (3) dorsal skin finely granular; (4) dorsal colour pattern generally consisting of a X-shaped mark on the scapular region; (5) typically 6–8 large dark brown bands on the granular dorsal surface of tibia and thighs, not extending towards the ventral face of the leg; (6) granular skin on upper thigh covering a narrow surface (narrower than inner and outer smooth faces of thigh); (7) flanks black with white speckles, well delimited ventrally; (8) ventral surface immaculate; (9) lower eyelid translucent with thin brownish reticulations; (10) snout long ETS/EN 0.67–0.89 and slightly acuminate in lateral view; (11) superelliptical pupils when contracted; (12) hands, feet and concealed surfaces of legs carmine/crimson (in life) to light grey (in preservative) without white spots; (13) large and rugous dark P-shaped nuptial pad on the medial surface of Finger I extending onto the dorsal surface in males; (14) prepollex not modified into a projecting spine; (15) concealed surface of upper arm, axillary region, flanks, and groin black with numerous small bluish white speckles; (16) fingers fully webbed; (17) feet fully webbed; (18) small calcar on heel, sometimes barely visible and often coloured with a cream spot.

Morphological comparison with congeners of the *semilineatus* group. The new species is unambiguously assigned to the *Hypsiboas semilineatus* species group based on molecular analyses (Fouquet *et al.* 2007; this work) as well as by the presence of a reticulated lower eyelid. The new species is closely related to a subgroup containing *H. semilineatus*, *H. geographicus* and *H. hutchinsi* that can all be distinguished from other species of the *semilineatus* group (*H. boans*, *H. wavrini* and *H. pombali*) by smaller size, prepollex not modified into a projecting spine, presence of pigmented nuptial excrescences, and finely granular dorsal skin.

We closely compared the new species with the six nominal species of the *Hypsiboas semilineatus* group (*H. boans*, *H. geographicus*, *H. hutchinsi*, *H. pombali*, *H. semilineatus*, and *H. wavrini*), and with *H. aff. semilineatus* 1 since that candidate species occurs in sympatry (other candidate species are phylogenetically distinct and allopatric). The presence of immaculate red webbing on fingers and toes, and the red concealed surfaces of hind

limbs immediately distinguish *Hypsiboas diabolicus* **sp. nov.** from *H. semilineatus* and *H. aff. semilineatus* 1 (webbing brown, dorsoposterior concealed surface of hindlimbs dark grey with white spots, and anterior concealed surface of hindlimbs cream with dark stripes in *H. semilineatus* and *H. aff. semilineatus* 1), from *H. geographicus* (red webbing except between Fingers III–IV and Toes IV–V, fingers and toes grey dorsally except Finger I and Toes I–II, and dorsoposterior and anterior concealed surface of hindlimbs grey with black stripes in *H. geographicus*), and from *H. hutchinsi* (webbing and concealed surfaces of hindlimbs black in *H. hutchinsi*). *Hypsiboas diabolicus* **sp. nov.** can be further distinguished from *H. geographicus* by its immaculate venter (vs. with dark spots in *H. geographicus*), by its longer snout (ETS/EN 0.67–0.89 vs. 0.53–0.69 in *H. geographicus*), by its longer thighs (ThL/SVL 0.50–0.58 vs. 0.44–0.49 in *H. geographicus*), by its less acuminate snout, and by its more developed hand and foot webbing. *Hypsiboas diabolicus* **sp. nov.** can be further distinguished from *H. aff. semilineatus* 1 by a sharp delineation between the dorsal and the flank patterns (vs. dark stripes in *H. aff. semilineatus* 1), by its smaller SVL (43.5 ± 2.6 vs. 48.1 ± 2.5 mm in *H. aff. semilineatus* 1; Table 3, Fig. 5A), and by its slightly acuminate snout in lateral view (truncate in *H. aff. semilineatus* 1). The new species is easily distinguished from *H. boans*, *H. wavrini* and *H. pombali* by its smaller size, red colouration of concealed surface of hindlimbs and webbing (never red in *H. boans* and *H. wavrini*), and absence of a prepollex modified in a projecting spine.

Description of holotype (Fig. 3). An adult male (48.0 mm SVL). Dorsal skin finely granular, less granular on head except on its sides. Head as long as wide (HW/HL 0.97), cranial crests absent. Snout slightly acuminate in lateral view, much longer than eye length (ETS/ED 1.62); canthus rostralis curved and loreal region strongly concave; eye-naris distance longer than eye length (EN/ED 1.13). Nares directed laterally, visible from frontal and dorsal views. Lower eyelid finely reticulated, pupil superelliptical. Tympanum round, 3.4 mm long (TD/EL 0.73). Supratympanic fold inconspicuous, post-riotal tubercles absent. Vocal sac single and subgular, visible. Throat smooth anteriorly without mental gland, posterior surface of throat, belly, cloacal region, and undersurface of thighs areolate, undersurface of tibia and tarsus smooth. Dentigerous processes of the vomers oblique, closely set, each bearing 13 teeth. Vocal slits present. Tongue slightly cordiform.

Hand length/SVL 0.30. Relative length of fingers: I < II < IV < III; Finger I/Finger II 0.78; adpressed Finger I fails to reach proximal edge of finger disc of Finger II; adpressed Finger IV reaches base of disc of Finger III on both sides. Large and rugose dark P-shaped nuptial pad on medial and dorsal surface of Finger I, prepollex not modified into a projecting spine. Two lateral fringes on hand and arm, one extending latero-dorsally from the second segment of Finger IV to the anterior edge of the elbow and one extending latero-ventrally from the disc of Finger IV to the posterior edge of the elbow forming a metacarpal and an ulnar fold. Fingers fully webbed, webbing formula I 2⁺–2 II 1[–]–2 III 1[–]–1[–] IV. Finger discs broadly expanded, round, as wide as long; disc of Finger III (1.9 mm) 1.5 times wider than the distal end of the adjacent phalanx. Palmar tubercle inconspicuous; thenar tubercle indistinct; supernumerary tubercles absent; subarticular tubercles large, round and protuberant, one tubercle on Finger I and Finger II, two tubercles on Finger III and Finger IV (with the second one much smaller); many small round accessory palmar tubercles.

Hind limbs long, heels slightly overlap when bent limbs are held at right angles to sagittal plane; TiL/SVL 0.55; FL/SVL 0.39 Relative length of toes I < II < III < V < IV; tip of Toe V extends to the distal edge of the distal subarticular tubercle on Toe IV; tip of Toe III extends to the proximal edge of the distal subarticular tubercle on Toe IV. Two lateral fringes along foot and leg, one extending from the proximal edge of the inner metatarsal tubercle to knee and even further delimiting the concealed part of the leg forming a tarsal fringe, and one extending laterally from the base of the disc of Toe V to the tarsus, forming a tarsal fold interrupted before the calcar. Toes fully webbed, webbing formula I 0[–]–1[–] II 0[–]–1^{1/3} III 0[–]–1[–] IV 1[–]–0[–] V. Toe discs slightly smaller than finger discs, broadly expanded, round. Inner metatarsal tubercle elongate, oval; outer metatarsal tubercle absent; subarticular tubercles round, large and protuberant; supernumerary plantar tubercles small, round.

Colour of the holotype in preservative (Fig. 3). After two years in 70% ethanol, the dorsal background colour faded to light brown, with fine dark brown irregular transversal reticulations also present on the dorsal surface of arms and legs. Five small cream round spots (colour 1 in Köhler 2012) on the posterior half of the body. A large light cream spot (colour 1 in Köhler 2012) at the tibio-tarsal articulation, extending to the calcar. Hands, feet and concealed surfaces of legs pale pinkish (colour 3 in Köhler 2012). Lips, throat and chest cream (colour 1 in Köhler 2012). Belly, ventral surface of arms and thighs, and ventral surface of hands, tarsi and feet pale pinkish (colour 3 in Köhler 2012). Concealed surface of upper arms, axillary region, flanks, and groin black with numerous white speckles.

TABLE 3. Mean and sd of measurements of specimens of *H. diabolica* sp. nov., *H. aff. semilineatus* 1 and *H. geographica*; abbreviations are explained in the text.

	SVL	TiL	CL	ThL	TaL	FL	4TD	FD	IFiL	2FiL	HaL	ETS	EN	HL	HW	IN	ED	IOD	TYD
<i>H. aff. semilineatus</i> 1 M	n=20	48.40	25.20	1.33	25.50	14.50	18.00	1.79	2.12	7.61	10.40	13.90	7.92	5.95	17.29	3.35	5.28	5.72	4.06
		2.23	1.41	0.48	1.42	1.67	1.58	0.27	0.34	0.47	0.55	0.73	0.60	0.47	1.18	1.42	0.26	0.96	0.28
<i>H. aff. semilineatus</i> 1 F	n=3	68.30	34.80	1.93	35.90	20.50	26.20	3.13	3.60	10.60	14.90	19.70	16.30	9.63	23.87	4.43	6.07	7.40	5.13
		3.03	0.78	0.67	0.93	0.66	0.53	0.42	0.26	0.67	0.75	0.64	8.52	2.57	1.76	1.79	0.06	0.59	0.26
<i>H. diabolica</i> sp. nov. M	n=17	43.50	23.20	0.91	23.50	13.30	16.30	1.61	1.92	6.79	9.20	12.30	7.26	5.39	15.75	2.98	5.28	5.41	3.55
		2.56	1.56	0.25	1.75	1.05	1.40	0.24	0.23	0.63	0.79	1.21	0.76	0.59	1.63	1.03	0.26	0.50	0.22
<i>H. diabolica</i> sp. nov. F	n=2	56.30	30.30	1.05	29.60	18.10	21.60	2.05	2.35	9.10	12.20	16.50	9.70	6.40	20.50	20.90	3.85	5.60	4.45
		0.57	0.49	0.78	0.57	0.35	1.63	0.21	0.64	0.57	0.35	1.63	0.71	0.57	1.697	1.84	0.64	0.14	0.42
<i>H. geographica</i> M	n=5	43.00	23.10	0.84	19.70	13.40	17.00	1.96	2.18	6.73	9.45	12.40	7.52	4.68	14.82	15.59	3.11	4.42	3.11
		1.62	0.48	0.16	0.47	0.59	0.90	0.12	0.18	0.53	0.64	0.65	0.41	0.50	0.56	0.88	0.03	0.13	0.04
<i>H. geographica</i> F	n=5	48.60	26.90	1.03	22.40	15.80	19.50	2.18	2.44	7.65	10.80	14.4	8.29	5.48	16.99	17.84	3.50	4.89	10.00
		2.62	1.68	0.33	1.61	1.42	1.49	0.18	0.22	0.57	0.69	0.93	0.56	0.38	1.136	1.573	0.2	0.29	0.49

TABLE 4: List of measured tadpoles. A = LTRF on anterior labium, P = LTRF on posterior labium, other abbreviations are explained in the text.

Species	Locality	Voucher	Gosner	A	P	TL	BL	TAL	BW	BH	HW	TMH	UTF	LTF	TMW	MTH	END	NSD	SSD	IND	IOD	ED	Date Coll.
<i>H. diabolica</i> sp. nov.	GaaKaba	MBT136D	27	2(2)	4(1)	20.4	8.3	12.3	4.8	4.3	4.2	1.3	1.3	1.3	1.4	4.0	0.9	1.0	5.4	1.7	3	0.9	14 09 2010
<i>H. diabolica</i> sp. nov.	GaaKaba	MBT136C	27	2(2)	4(1)	21.4	9.2	12.2	5.5	4.7	4.3	1.6	1.3	1.2	1.6	4.1	0.8	1.0	5.9	1.6	2.6	0.9	14 09 2010
<i>H. diabolica</i> sp. nov.	GaaKaba	MBT136B	28	2(2)	4(1)	23.9	9.9	14.0	5.9	4.6	4.6	1.8	1.5	1.4	1.9	4.7	1.0	1.2	6.4	1.8	3	0.9	14 09 2010
<i>H. diabolica</i> sp. nov.	Mont. des singes	QM474B	29	2(2)	4(1)	25.2	11.1	14.0	6.2	3.9	5.2	2.0	1.6	1.5	1.3	4.6	1.0	1.1	7.0	1.9	3.1	1.5	20 06 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362I	31	3(1,3)	5(1,5)	35.0	13.8	21.3	7.7	6.8	6.5	2.9	2.5	2.7	2.7	7.0	1.5	1.1	8.9	1.9	3.9	1.5	19 07 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362B	32	3(1,3)	5(1,5)	37.4	13.9	26.7	9.2	8.0	6.8	3.1	2.9	2.8	2.9	8.0	1.4	1.5	9.6	2.3	4.1	1.5	19 07 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362D	36	3(1,3)	5(1,5)	43	14.5	27.7	11.1	10.2	8.1	4.3	3.2	2.9	3.6	9.1	1.5	1.7	10.9	2.6	5.7	1.8	19 07 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362F	34	3(1,3)	5(1,5)	38.8	15.2	25.2	9.4	7.1	6.9	2.9	2.8	2.7	3.0	8.3	1.5	1.6	9.5	2.4	4.4	1.8	19 07 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362C	38	3(1,3)	5(1)	46.4	17.5	29.8	10.8	9.5	8.5	4.1	3.6	3.5	3.3	10.7	1.7	2.0	10.1	2.7	5.5	1.8	19 07 2014
<i>H. diabolica</i> sp. nov.	Patawa	AF2362E	38	3(1,3)	5(1)	43.7	18.0	26.1	11.9	9.3	9.1	4.5	3.9	3.0	3.9	10.7	1.9	2.0	11.7	2.9	5.9	2.1	19 07 2014
<i>H. aff. semilineatus</i> 1	Sipaliwini	AF1996A	29	3(1,3)	5(1,5)	24.8	9.5	15.0	6.0	4.8	4.7	2.1	2.0	1.8	1.7	5.1	0.9	1.3	6.4	1.9	3.2	1.2	18 04 2014
<i>H. aff. semilineatus</i> 1	Crique mirat	AF1303C	34	4(1,2,4)	6(1,6)	45.2	16.9	28.5	10.9	9.5	7.0	4.1	3.1	2.8	2.8	9.0	1.2	1.9	11.1	2.7	5.5	1.7	06 01 2005
<i>H. aff. semilineatus</i> 1	Crique mirat	AF1303B	35	4(1,2,4)	6(1,6)	48.7	17.0	31.4	10.9	7.9	7.5	3.6	3.0	3.0	2.8	9.4	1.7	1.8	8.1	2.6	4.6	2.1	06 01 2005
<i>H. aff. semilineatus</i> 1	Crique mirat	AF1303D	37	4(1,2,4)	6(1,6)	52.7	19.0	37.7	12.6	10	8.0	4.5	2.8	3.6	2.9	10.1	1.9	1.8	11.7	2.8	6.2	2.0	06 01 2005
<i>H. aff. semilineatus</i> 1	Crique Bon Espoir	AF1112	40	4(1,2,4)	6(1,5)	53.2	20.8	32.4	12.7	10.5	9.5	3.8	4.1	4.3	4.1	12.1	2.2	2.1	12.7	2.9	7.0	2.4	04 04 2013

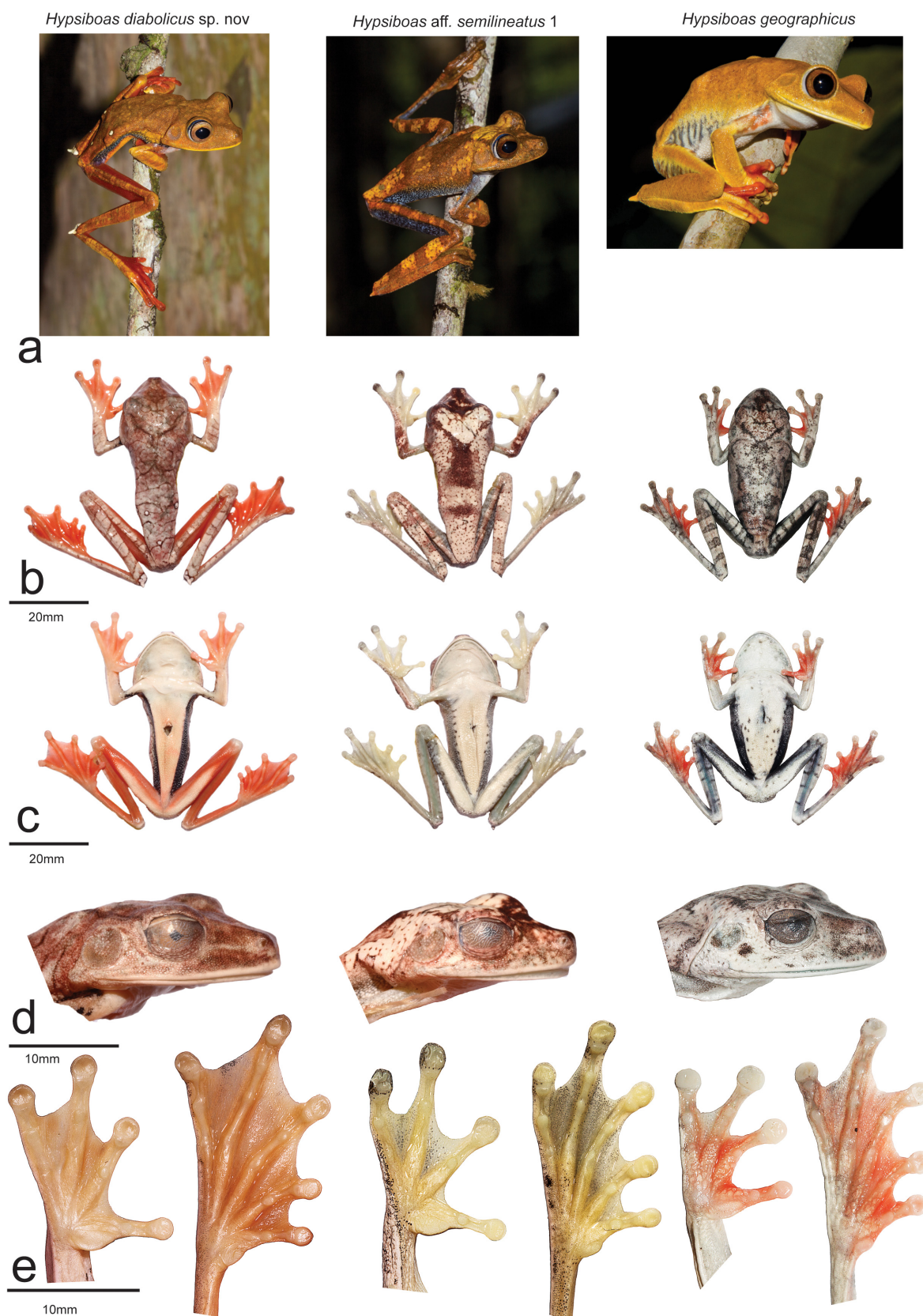


FIGURE 3. Holotype R157 (AF1284) of *H. diabolicus* sp. nov. compared to R143 (AF1285), a specimen of *H. aff. semilineatus* 1 (both recently preserved) collected along the same stream at the same time, and *H. geographicus* [preserved specimen MZUSP157060 (MTR36895) from Tefé]. a: in life (*H. geographicus* from Rio Purus active at night); b: dorsal view; c: ventral view; d: lateral view of the head; e: ventral face of right hand and foot.



FIGURE 4. Intraspecific variation in *Hypsiboas diabolicus* **sp. nov.** as shown by dorsal and ventral views of nine specimens of the type series. Numbers between parentheses correspond to field numbers.

Colour of the holotype in life at night (Fig. 3). Dorsal background colour dark orange rufous (colour 56 in Köhler 2012), with ill-defined lighter brownish orange spots and ill-defined darker reticulations. Transverse brown bars on arms and legs. Dorsal surface of tarsi and distal half of feet orange with poorly defined brown bars. Five small round cream spots (colour 1 in Köhler 2012) on the posterior half of the body. A large cream spot (colour 1 in Köhler 2012) at the tibio-tarsal articulation, extending to the calcar. Hands, feet and concealed surfaces of legs carmine/crimson. Lips orange. Throat and chest white. Belly and ventral surface of thighs bright orange. Ventral surface of hands, tarsi and feet carmine/crimson. Concealed surface of upper arms, axillary region, flanks, and groin black with numerous bluish white speckles. Iris brownish orange, lighter dorsally, surrounded by a black circle; eyeball white with a dark blue region posterodorsally, bones white.

Variation (Fig. 4). The most noticeable variation occurs in the dorsal colouration and in the shape of the calcars. *Hypsiboas diabolicus* **sp. nov.** is highly variable in colour and pattern. Background colouration ranges from dark brown to light brown, and dorsal pattern varies from fine reticulations to large transverse brown chevrons. A X-shaped brown mark is often present in the scapular region and is generally thin, rarely thick.

Markings on the dorsal part of the legs range from small dark reticulations to chevrons, which range from cream to light brown. Individuals may display asymmetrically distributed small to large cream spots on the dorsum. The bluish white speckles on the black flanks vary in size and number among individuals; some individuals have small dark spots extending on the belly. In preservative, the red colouration first fades to orange then turn into pale pinkish (colour 3 in Köhler 2012). Females have similar colouration than males, but are larger. Calcar size ranges from inconspicuous to prominent, which does not seem to be associated with any sexual dimorphism; calcars are generally coloured with a cream spot.

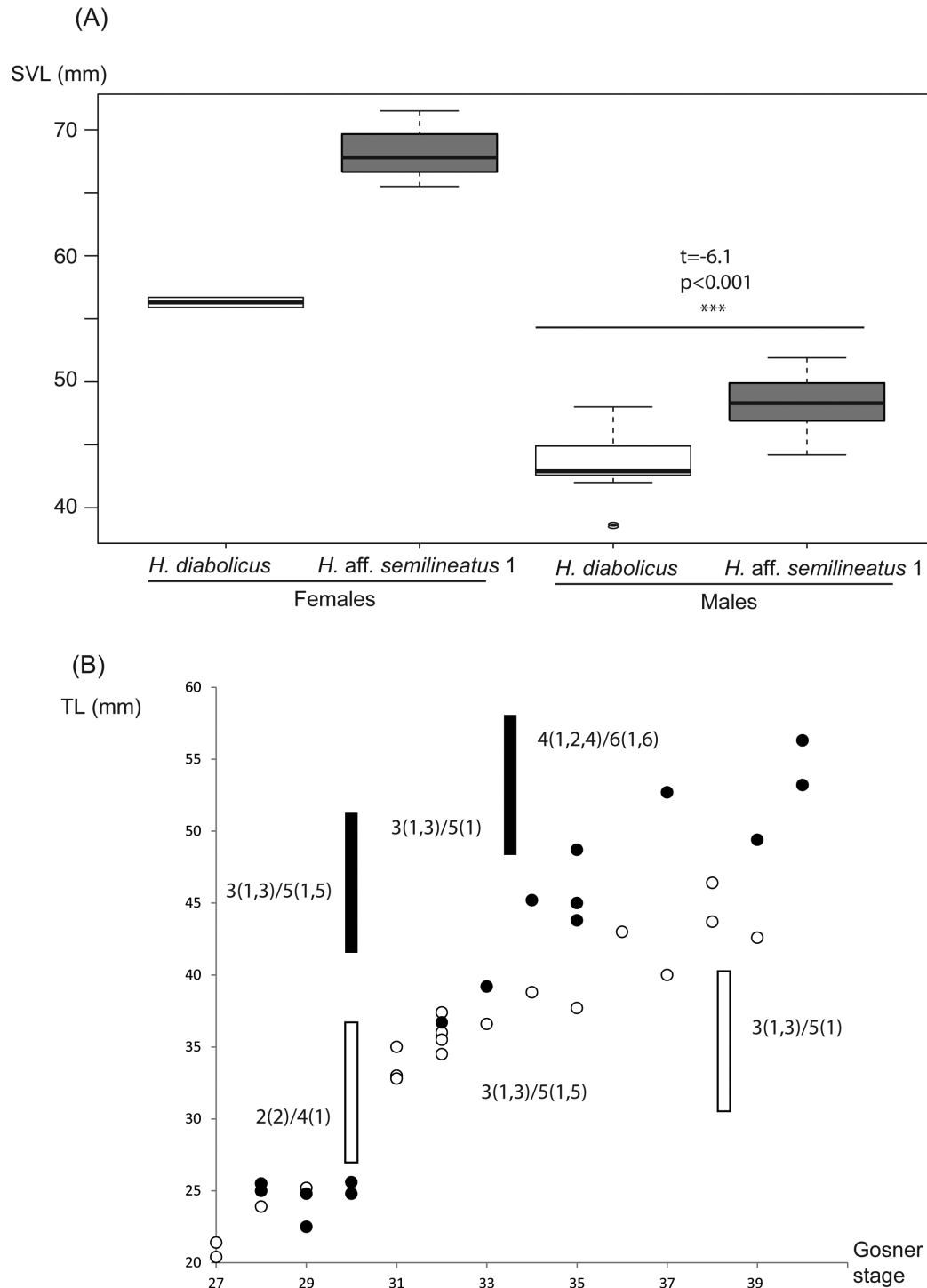


FIGURE 5. (A) Boxplots of adult SVL of *H. diabolica* sp. nov. and *H. aff. semilineatus* 1 for each sex. A Student *t*-test was performed on males and shows statistically significant differences between species ($p<0.001$). (B) Tadpole total length as a function of Gosner stage for both species (*H. diabolica* in white *H. aff. semilineatus* 1 in black). Ontogenetic changes of LTRF are also indicated.

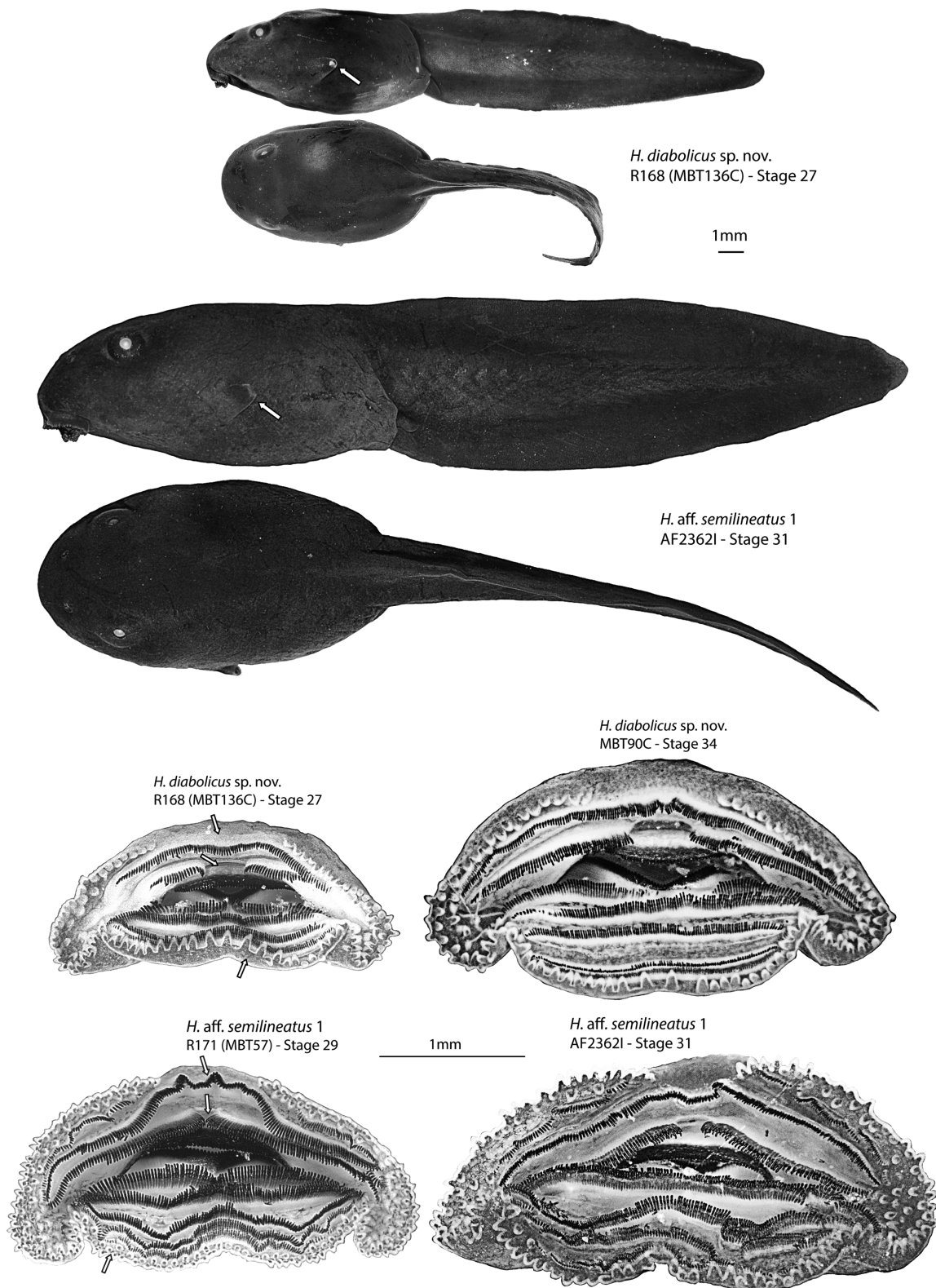


FIGURE 6. External and buccal morphology of tadpoles of *Hypsiboas diabolicus* sp. nov. and *H. aff. semilineatus* 1. Arrows highlight some diagnostic characters (upper labium and A2 gaps, marginal papillae).

Description of the tadpole. The following description is based on one individual in Gosner stage 27 (R168 Fig. 5B, Fig. 6, Table 4). Type 4 tadpole (Orton 1953); exotrophic; body skin smooth; TL 21.4 mm; BL/TL 0.43, BL/BW 1.7, BL/BH 2.0, BW/BH 1.2. Body depressed, oval in dorsal view; snout round in dorsal and lateral views; eyes positioned and directed dorsolaterally; ED 0.9 mm, ED/IOD 0.35; IOD less than IND; nares positioned and

directed anterodorsally; narial opening reniform in dorsal view; END 0.8 mm, less than NSD (1.0 mm). Spiracular tube sinistral, projecting posterodorsally, its base located 64 % of BL from snout. Vent tube not visible, concealed in a dermal fold. Lateral line system conspicuous. Caudal musculature highest at body-tail junction, tapering posteriorly, terminating anterior to tail tip; tail tip tapered, rounded; upper fin originating anterior to junction of body and tail, gradually increasing in height to about midlength of tail, decreasing to tail tip; UTF/TMH 0.81; comparable ULF/TMH 0.75; lower fin gradually similar in height along the 2/3 of the tail and decreasing to terminus; MTH/TL 0.34.

Mouth ventral, oral disc strongly emarginated posteriorly, width 2.4 mm. Labial teeth long, in single rows, LTRF 2(2)/4(1). A-2 consisting of two short rows, separated by a large and deep gap, A-1 slightly shorter than posterior rows that are of equal length; P-1 medially interrupted by a small gap. Marginal papillae moderately long, tapered, blunt-tipped, in a single row; broad median gap on upper labium approximately half the length of A-1. A few submarginal papillae present laterally; jaw sheaths typical, large, serrated, lower jaw sheath broadly V-shaped.

Variation of 17 meristic characters from tadpoles in stages 27–39 is given in Table 4. Considerable variation occurs in the number of posterior labial teeth rows, ranging from 2(2)/4(1), 3(1,3)/5(1,5) and 3(1,3)/5(1) and in the number of submarginal papillae laterally; apparently this variation is largely ontogenetic. Body and tail are black to dark brown.

The tadpole of *Hypsiboas diabolicus* **sp. nov.** can be distinguished from that of *H. geographicus* (described by Schulze *et al.* 2015), *H. aff. semilineatus* 1 [from examined material and previously described by Duellman and Lescure (1973) from French Guiana] and from that of *H. semilineatus* [described by Bokermann (1963) and d’Heursel & de Sá (1999)] by: (1) smaller size [TL 46.4 mm maximum at stage 38 in *H. diabolicus* vs. TL 48.6 mm at stage 35 and 59.7 mm at stage 41 in *H. geographicus*/TL 46.4 mm maximum at stage 38 and >56.3 mm at stage 40 in *H. aff. semilineatus* 1/TL >65 mm in *H. semilineatus* (Bokermann 1963)] (Fig. 5B, Table 4); (2) larger upper labium gap in marginal papillae than in *H. aff. semilineatus* 1 (0.85 mm in *H. diabolicus* R168 vs. 0.75 mm in *H. aff. semilineatus* 1 AF23621) (Fig. 6 ; but of similar proportion in *H. geographicus*); (3) large and mostly single-rowed marginal papillae (vs. irregularly biserial laterally and on lower labium in *H. geographicus*, small and double-rowed in *H. aff. semilineatus* 1 and *H. semilineatus*); and (4) larger A-2 gap (only slightly larger than in *H. geographicus* and clearly larger than in *H. aff. semilineatus* 1; 0.25 mm in *H. diabolicus* R168 vs. 0.12 mm in *H. aff. semilineatus* 1 AF23621). The tadpole of *H. diabolicus* can also be distinguished in having more teeth rows at similar stage than in *H. geographicus*: 3(1,3)/5(1,5) at stage 30–40 in *H. diabolicus* vs. 2(2)/4(1) a stage 35 in *H. geographicus*, but fewer teeth rows at similar stage than *H. aff. semilineatus* 1 and *H. semilineatus*: at stage 27–29: 2(2)/4(1) vs. 3(1,3)/5(1,5) in *H. aff. semilineatus* 1 and at stage 30–40 3(1,3)/5(1,5) vs. 3(1,3)/5(1) to 4(1,2,4)/6(1,6) in *H. aff. semilineatus* 1 (present data, Table 4); 2(2)/4 in *H. semilineatus* at stage 36 (Bokermann 1963).

The tadpole of the new species differs from that of *Hypsiboas hutchinsi* by its black to dark brown tail (creamish pink in life, sometimes with a dark spot in *H. hutchinsi*), and lower number of teeth rows (3/5 in *H. diabolicus* vs. 4/7 in *H. hutchinsi*; Pyburn & Hall 1984).

The tadpole of the new species differs from that of *Hypsiboas boans* and *H. pombali* by its black to dark brown colouration [body beige with reddish brown spots and dark brown specks in *H. boans* and *H. pombali* (Junca *et al.* 2012)], upper and lower fins of similar size (upper fin larger in *H. boans* and *H. pombali*) and a larger A-2 gap].

The tadpole of *Hypsiboas diabolicus* differs from that described and attributed to *H. geographicus* from Trinidad by Kenny (1969) by smaller body size and LTRF (reported to reach 75.0 mm and to have small and numerous marginal papillae and lacking an A2 gap in Trinidad), and from that of the species studied by Caldwell (1989) from Rondônia (Brazil) by smaller size (reported to be >50 mm from stage 37, reaching >90 mm in Rondônia).

The tadpoles from Reserva Adolfo Ducke examined by d’Heursel and de Sá (1999) and identified as *Hypsiboas geographicus* match with the tadpoles of *H. aff. semilineatus* 1. As mentioned previously, the species reported by Lima *et al.* (2006) from this location matches with *H. aff. semilineatus* 1, none of the other lineages have been recorded north of the Amazon River and the tadpoles of d’Heursel and de Sá (1999) differ from *H. diabolicus* and *H. geographicus* (see above). Nevertheless, the status of this population was not assessed in our study, and thus remains tentative.

Advertisement call (Fig. 7). Calling specimens, perched ~1m above ground on vegetation near standing water, were recorded at a distance of about 1 m. The call of the new species is very soft and consists of short clusters ($X=0.33$ s; range=0.24–0.39 s) of 2–3 chuckles ($X=0.05$ s; range=0.04–0.07 s) with an internote interval of

$X=0.11$ s (range=0.10–0.13 s) emitted between silent intervals of $X=6.01$ s (range=3.06–10.72 s; Fig. 7, Table 5). Each note consists of pulses emitted at $X=186.8$ pulses/s (range=175.5–200). The call has a fundamental frequency dominating ($X=1.16$ kHz, range=1.11–1.19 kHz).

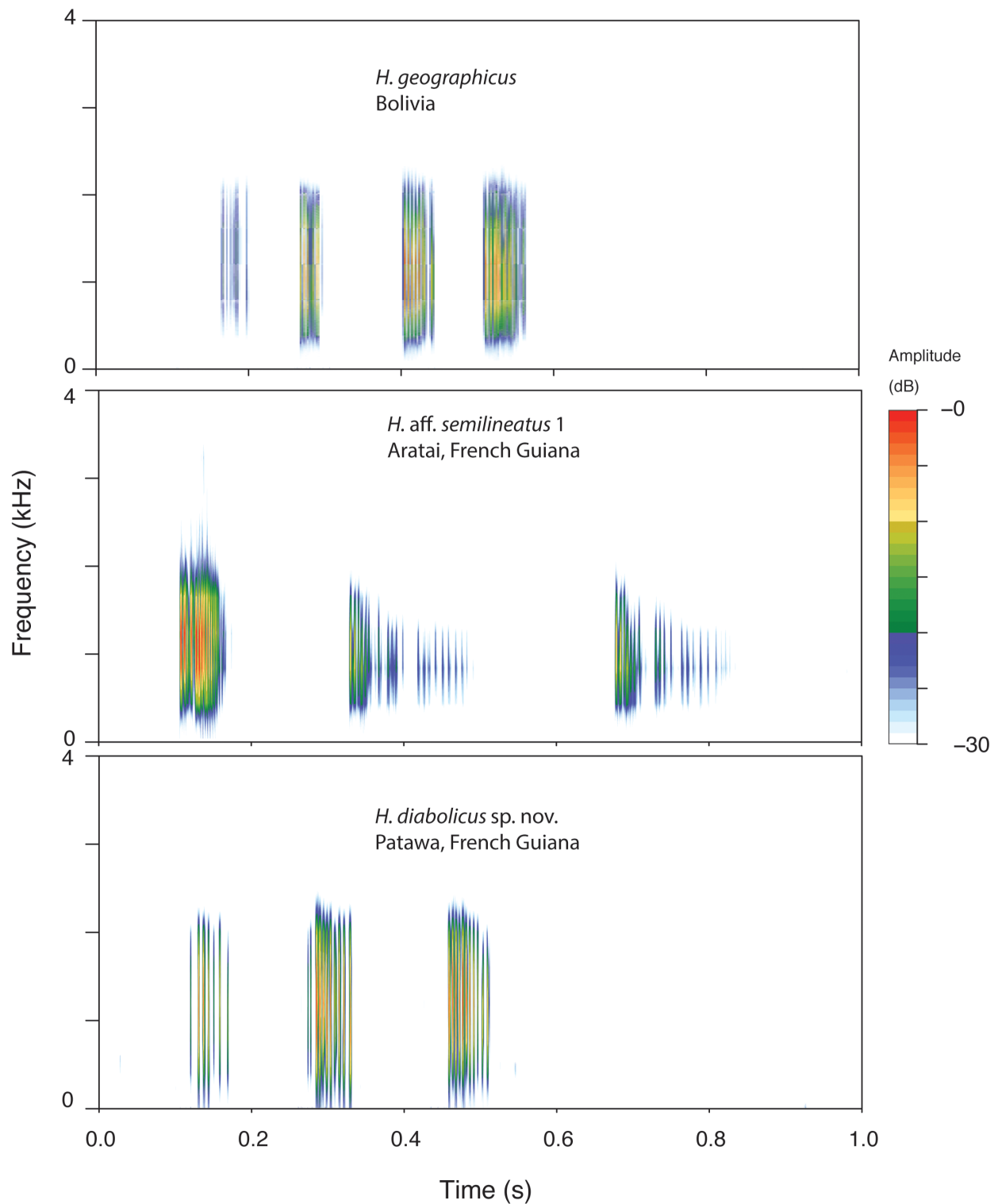


FIGURE 7. Spectrograms of calls of *H. diabolicus* sp. nov. (bottom), *H. aff. semilineatus* 1 (middle) and *H. geographicus* (top, from Marquez *et al.* 2002).

TABLE 5. Call variables of four calls of *Hypsiboas diabolicus* **sp. nov.** compared to *H. aff. semilineatus* 1 and *H. geographicus* (recording in Marquez *et al.* 2002).

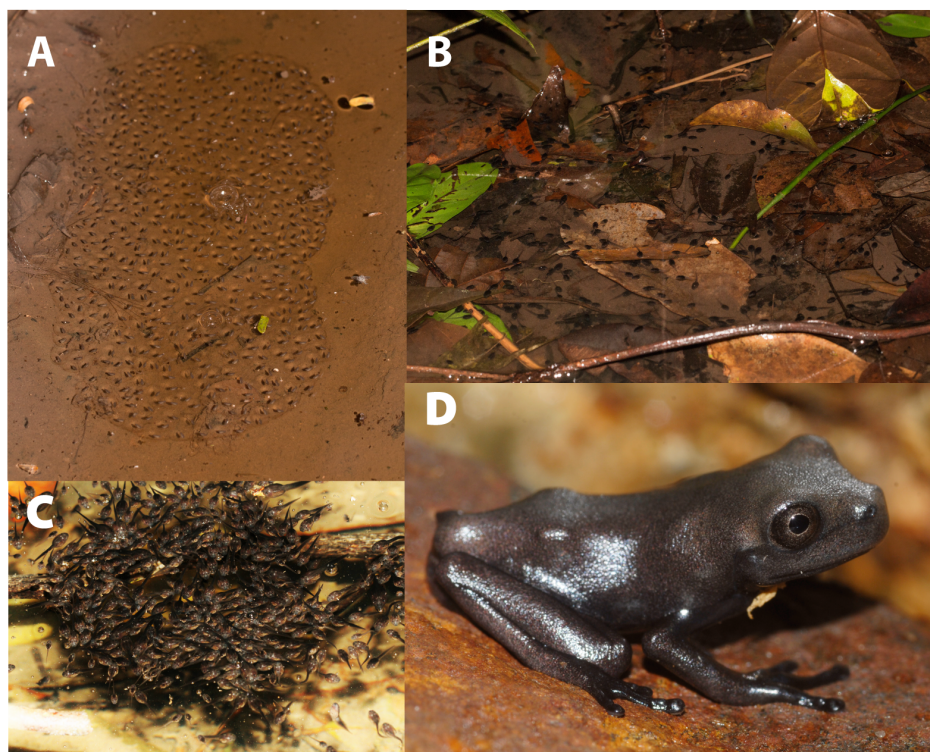
	<i>H. diabolicus</i> sp. nov.					<i>H. aff. semilineatus</i> 1				
	call 1	call 2	call 3	call 4	Mean	call 1	call 2	call 3	call 4	Mean
Call duration (s)	0.34	0.24	0.37	0.39	0.33	1.88	1.50	0.64	0.52	1.13
notes/call	3	2	3	3	2.75	7	5	3	3	4.50
Note duration (s)	0.05	0.05	0.06	0.05	0.05	0.13	0.13	0.11	0.07	0.11
Pulses/s	NA	200.00	185.00	175.60	186.90	154.40	151.90	177.80	200.00	171.00
Internote silence (s)	0.10	0.13	0.10	0.11	0.11	0.18	0.17	0.14	0.15	0.16
Note dom freq (Hz)	1189	1189	1108	1149	1159	905	870	830	905	877
Intercall silence (s)	3.06	4.24	10.72	NA	6.01	1.60	0.50	0.40	0.60	0.77

<i>H. geographicus</i>					
	call 1	call 2	call 3	call 4	Mean
Call duration (s)	0.40	0.42	0.36	0.40	0.39
notes/call	3	4	4	3	3.50
Note duration (s)	0.05	0.05	0.04	0.05	0.05
Pulses/s	218	226	206	214	216.10
Internote silence (s)	0.10	0.07	0.07	0.10	0.08
Note dom freq (Hz)	883	1023	983	885	943.50
Intercall silence (s)	5.20	6.40	2.90	NA	4.83

The call of *Hypsiboas diabolicus* **sp. nov.** is distinguished from that of *H. aff. semilineatus* 1 (Fig. 7; Table 5) by shorter notes ($X=0.05$ s; range=0.04–0.07 s vs. $X=0.11$ s; range=0.07–0.13 s in *H. aff. semilineatus* 1), shorter internote intervals ($X=0.11$ s; range=0.10–0.13 s vs. $X=0.16$ s; range=0.14–0.18 s in *H. aff. semilineatus* 1), and higher dominant frequency ($X=1.16$ kHz, range=1.11–1.19 kHz vs. $X=0.88$ kHz, range=0.81–0.91 kHz in *H. aff. semilineatus* 1). *Hypsiboas aff. semilineatus* 1 also generally produces more notes per call ($X=4.5$; range 3–7) than *H. diabolicus* ($X=2.75$; range 2–3), and a first note louder than the following ones whereas the notes contain the same energy across the call of *H. diabolicus*. A call recorded in Bolivia is available from Marquez *et al.* (2002) and corresponds to *H. geographicus* (same lineage as identified as *H. geographicus* in this study, Jansen pers. com.). That call shares many similarities with the call of *H. diabolicus*, and also matches the call described by Duellman (1973) from Santa Cecilia, Ecuador. However, the dominant frequency of the call of *H. geographicus* is lower (0.94 in *H. geographicus* vs. 1.16 kHz in *H. diabolicus*), more notes per call are produced (3–4 in *H. geographicus* vs. 2–3 in *H. diabolicus*), notes are emitted at a faster rate (internote intervals = 0.08 in *H. geographicus* vs. 0.11 s in *H. diabolicus*), and the pulses are emitted faster (216/s in *H. geographicus* vs. 187/s in *H. diabolicus*). The new species' call is also clearly distinct from the one of *H. hutchinsi* that consists of more notes (5–7 in *H. hutchinsi* vs. 2–3 in *H. diabolicus*), each note being longer (0.10 s in *H. hutchinsi* vs. 0.04–0.07 s in *H. diabolicus*), and with a decrease of intensity of the notes within the call. It also clearly differs from the calls of *H. geographicus* described by Duellman (2005) from Pilcopata (dominant frequency = 1.75 kHz) and Tambopata, Peru (dominant frequency = 2.3 kHz).

Distribution and Ecology. To date *Hypsiboas diabolicus* **sp. nov.** has been found throughout French Guiana (except in the southeast) and in Amapá, Brazil, between 10–150 m elevation. It occurs in sympatry with *H. aff. semilineatus* 1 over its entire distribution area, but the latter species seems more widely distributed over the Guiana Shield.

Both species have been found active at night, sometimes at the very same time and locality. Nevertheless, the two species seem to display slight differences in reproduction sites. *Hypsiboas diabolicus* generally breeds in pools formed at the margin of small streams whereas *H. aff. semilineatus* 1 usually breeds in larger rivers, generally in marginal pools formed in regularly flooded parts of the bed. Males of *H. diabolicus*, were generally found



H. diabolicus sp. nov.

H. aff. semilineatus 1



FIGURE 8. (A) Egg clutch of *Hypsiboas diabolicus* containing 585 eggs (photo by AF from Mitán, AP, Brazil), (B) Tadpoles of *H. diabolicus* at early Gosner stage (26) not forming a school (photo by AF from Mitán, AP, Brazil), (C) Tadpoles of *H. diabolicus* at a later stage forming a school (photo by D. Baudain from Pedra Branca, AP, Brazil) and (D) Postmetamorphic juvenile of *H. diabolicus* (photo by MB from Gaa Kaba, French Guiana). (E) Tadpoles of *H. aff. semilineatus* 1 at early Gosner stage (26) forming a loose school (photo by AF from Mitán, AP, Brazil), (F) Older tadpoles of *H. aff. semilineatus* 1 forming a tight school (photo by D. Baudain from Crique Gabaret, French Guiana) and (G) Juvenile of *H. aff. semilineatus* 1 (photo by AF from Crique Bon Espoir, French Guiana).

perched on small branches over the water of shallow streams or pools. Reproductively active males were found during the rainy season (January–June) and at the beginning of the dry season (July), suggesting an extended breeding period. Tadpoles were found during the end of the rainy season (April) and during the dry season (July–September). Tadpoles of *H. diabolicus* stay in group, but do not seem to form schools as large and as tight as in *H. aff. semilineatus* 1 (Fig. 8). Moreover, they are generally found in shallow stagnant water, often in pools along the margins of stream, while the tadpoles of *H. aff. semilineatus* 1 are generally found in the main bed of rivers, where its large tadpoles can be observed in deep waters. One recently laid clutch contained 585 eggs (Fig. 8). Significant differences in buccal morphology between the tadpoles of these two species suggest different larval ecology. Postmetamorphic juveniles of both species are dorsally grey brown; hands and concealed parts of legs are black, therefore that character does not allow to discriminate between *H. diabolicus* and *H. aff. semilineatus* 1 in postmetamorphic juveniles. *Hypsiboas diabolicus* does not seem to be rare, but the species is never found in abundance (1–3 individuals per site).

Discussion

Amphibian diversity in Amazonia. The inaccuracy of our perception of species boundaries and distributions can dramatically hamper inferences about biodiversity structure and evolution as well as the adequacy of conservation strategies (Bickford *et al.* 2007; Angulo & Icochea 2010; Peloso 2010). This is particularly true in Amazonia where a growing amount of studies reveals that some widely distributed species are in fact diverse species complexes (Fouquet *et al.* 2007; Jansen *et al.* 2011; Funk *et al.* 2012; Nunes *et al.* 2012; Fouquet *et al.* 2014; Peloso *et al.* 2014). This is obviously the case in the *H. semilineatus* species group, which currently contains seven described taxa (including *H. diabolicus*) and six candidate species. However, our molecular sampling is far from being exhaustive because no material from Peru, Ecuador or Colombia was included. Therefore, it is plausible that several additional putative species could be added to that group. Given the numerous threats faced by tropical forests in general (Laurance *et al.* 2002; da Silva *et al.* 2005), and by amphibians in particular (Blaustein & Dobson 2006; Peloso 2010), it is necessary and urgent to describe this diversity.

Neotype designations and species redescrptions. Recurrent problems that thwart the efforts to describe new species are the lack of precision of many old original descriptions, the frequent poor quality and difficult access to type-material, and the fact that several morphologically similar species often occur at a same locality (leading to some ambiguity about the species name-bearers). Redescrptions of these poorly described species, and designation of neotypes when necessary, are important steps towards a stable taxonomy. However, this task cannot be taken lightly in order to avoid adding confusion instead of solving issues. Two recent examples exemplify this problem: the cases of *Rhinella margaritifera* (Bufonidae) and *Scinax x-signatus* (Hylidae), two widely recognized species complexes (Fouquet *et al.* 2007a,b; Sturaro & Peloso 2014). In the case of the bufonid taxon, a neotype was designated by Lavilla *et al.* (2013), but the choice of the type locality was rather arbitrary and the lack of acoustic and molecular data for the neotypical material impedes further comparisons, especially because several morphologically similar species may occur sympatrically at the designated type locality. The case of *Scinax x-signatus* is rather similar. New species continue to be described when sufficiently diagnosable from Spix's original drawings and descriptions (*e.g.* Sturaro & Peloso 2014), but several undescribed species are more difficult to diagnose from Spix's description and, thus, remain unnamed. A neotype for *Scinax x-signatus* is needed as well as a thorough revision of the group, but the same precaution should be taken *i.e.* combine molecular, acoustic and morphological evidence and make sure that diagnostic characters are provided, especially in comparison with sympatric species. We advise to tackle this task only when adequate sampling is available from—or as close as possible from—the type locality (reason why we refrain to redescribe *Hypsiboas geographicus* at this stage).

In the present case, although *Hypsiboas diabolicus* could be distinguished from *H. geographicus* by examination of the original description (and illustration), the recent collection of new topotypical material helped to provide decisive evidence (morphological and genetic) to discriminate between the two taxa. As noted by Duellman (1973), striking variations in colouration of the flanks and concealed parts of the legs and arms exist across species in that group, which will probably prove to be taxonomically useful.

Hypsiboas semilineatus is another species described by Spix (1824), its description consisting in twelve lines in Latin. The species was synonymised with *H. geographicus* by Duellman (1973) then revalidated by Silveira and

Caramaschi (1989) in a congress abstract. Given the actual diversity within this group, thorough redescrptions of *Hypsiboas geographicus* and *H. semilineatus* are necessary. This can only be adequately accomplished by examining additional material from the type localities of these two taxa. These redescrptions should come before, or be part of, a revision of the entire species group.

Biogeography and distribution. From a biogeographic point of view, the *Hypsiboas semilineatus* clade displays a striking pattern of allopatry throughout Amazonia. This distribution pattern matches well that of other species groups investigated at this scale (e.g. Nunes *et al.* 2012; Fouquet *et al.* 2014). However, the nested position of the Atlantic Forest *H. semilineatus* within Amazonian representatives and low genetic distance separating them is surprising. The origin of this species seems to be the result of a recent dispersal from Amazonia. A similar pattern is found within the *Rhinella margaritifera* species group with *R. hoogmoedi* displaying a nested position within Amazonian relatives with shallow genetic distances (Fouquet *et al.* 2007a).

Unlike the pattern of allopatry found within the *H. semilineatus* clade, the geographic ranges of the lineages displaying deeper divergence (*H. geographicus*, *H. cf. geographicus* 1, *H. diabolicus* and the *H. semilineatus* clade *sensu lato*) broadly overlap, leaving few doubts about the reproductive isolation of these taxa

Hypsiboas diabolicus seems to have a limited range, likely restricted to the easternmost part of the Guiana Shield. A total of 25 localities of occurrence have been reported in French Guiana and adjacent Amapá State. Such a distribution pattern matches that of species endemic to the easternmost part of the Guiana Shield such as *Pristimantis chiastonotus*, *P. gutturalis*, *Anomaloglossus baobatrachus*, but also other *Hypsiboas* such as *H. dentei*. This pattern likely originates from historical isolation (Fouquet *et al.* 2012) and current climatic parameters such as the distribution of rainfall that concentrates on that part of the Guiana Shield.

Competing interests. We disclose any financial or non-financial competing interests.

Acknowledgements

This work has benefited from an “Investissement d’Avenir” grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01), France, from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, and partially by the Dimensions of Biodiversity Program [FAPESP (BIOTA, 2013/50297-0), NSF (DOB 1343578), and NASA]. We thank Marc Gayot (ONF, Reserve Naturelle Trinité), Marguerite Delaval (ONF, Reserve Naturelle Nouragues). The Nouragues station (CNRS), DEAL Guyane, the DEDD (ONF). We also thank Kévin Pineau and Christine Poixblanc for sharing thoughts about the choice of the name of the new species. Molecular data were produced thanks to Institut Pasteur de Guyane (Vincent Lacoste and his team), University of Canterbury New Zealand (Neil Gemmell) and Université de Provence (André Gilles). We thank the Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG) and in particular T.C. Avila-Pires, M.S. Hoogmoed and J. Gomes for the loan of material, R. Montesinos for helping with specimens at USP and M. Jansen and A. Schulze for sharing information about Bolivian specimens. We thank Paul Ouboter, Rawien Jairam, Maël Dewynter, Antoine Baglan, Kévin Pineau, Vincent Pelletier, Olivier Chaline, Vincent Rufay, Jean-Pierre Vacher, Agathe Chen, Benoît Villette, Daniel Baudain, Renato Recoder, José Ghellere, Francisco dal Vechio, Marco Sena, Mauro Teixeira, José Cassimiro, Francisco Dal Vechio, Ivan Prates and Anthony Cochard for the loan of material or for contributing to field work during which we collected material. PJRK’s work was granted by a postdoctoral fellowship from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO12A7614N). Specimens from Brazil were collected under permit 30309-1 of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio - Brazil) provided to Miguel T. Rodrigues.

Literature cited

- Angulo, A. & Icochea, J. (2010) Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). *Systematics and Biodiversity*, 8, 357–370.
<http://dx.doi.org/10.1080/14772000.2010.507264>
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22, 148–155.
<http://dx.doi.org/10.1016/j.tree.2006.11.004>

- Blaustein, A.R. & Dobson, A. (2006) Extinctions: a message from the frogs. *Nature*, 439, 143–144.
<http://dx.doi.org/10.1038/439143a>
- Bokermann, W.C. (1963) Girinos de anfíbios brasileiros I (Amphibia, Salientia). *Anais da Academia Brasileira de Ciências*, 35, 465–474.
- Boulenger, G. (1882) *Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum*. 2nd Edition. Printed by order of the Trustees, London, 600 pp.
- Caldwell, J.P. (1989) Structure and behavior of *Hyla geographica* tadpole schools, with comments on classification of group behavior in tadpoles. *Copeia*, 1989 (4), 938–948.
<http://dx.doi.org/10.2307/1445980>
- Caramaschi, U., Feio, R.N. & Pimenta, B.V.S. (2004) Nova espécie do grupo *Hyla geographica* Spix, 1824 da Floresta Atlântica, Brasil (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional. Nova Serie, Zoologia*, 518, 1–14.
- da Silva, J.M., Rylands, A.B. & da Fonseca, G.A. (2005) The fate of the amazonian areas of endemism. *Conservation Biology*, 19, 689–694.
<http://dx.doi.org/10.1111/j.1523-1739.2005.00705.x>
- Dewynter, M., Marty, C., Blanc, M., Gaucher, P., Vidal, N., Frétey, T., de Massary, J.-C. & Fouquet, A. (2008) Liste des Amphibiens et des Reptiles de Guyane. Available online: <http://www.chelidae.com/pdf/dewynter2008.pdf/> (accessed 16 August 2013)
- Duellman, W.E. (1970) *The Hylid Frogs of Middle America*. 2 Vols. Monographs of the Museum of Natural History, University of Kansas, Lawrence, 753 pp.
- Duellman, W.E. (1973) Frogs of the *Hyla geographica* group. *Copeia*, 1973 (3), 515–533.
<http://dx.doi.org/10.2307/1443117>
- Duellman, W.E. (2005) *Cusco Amazónico: the lives of amphibians and reptiles in an Amazonian rainforest*. Comstock Pub, Cornell University Press, Ithaca & London, 433 pp.
- Duellman, W.E. & Lescure, J. (1973) Life history and ecology of the hylid frog *Osteocephalus taurinus*, with observations on larval behavior. *Occasional Papers of the Museum of Natural History, University of Kansas*, 13, 1–12.
- Faivovich, J., Haddad, C.F., Garcia, P.C., Frost, D.R., Campbell, J.A. & Wheeler, W.C. (2005) Systematic review of the frog family Hylidae, with special reference to Hyalinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History*, 294, 1–240.
[http://dx.doi.org/10.1206/0003-0090\(2005\)294\[0001:SR0TFF\]2.0.CO;2](http://dx.doi.org/10.1206/0003-0090(2005)294[0001:SR0TFF]2.0.CO;2)
- Fouquet, A., Cassini, C., Haddad, C.F.B., Pech, N. & Rodrigues, M.T. (2014) Species delimitation, patterns of diversification and historical biogeography of a Neotropical frog genus; *Adenomera* (Anura, Leptodactylidae). *Journal of Biogeography*, 41 (5), 855–870.
<http://dx.doi.org/10.1111/jbi.12250>
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007a) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS one*, 2, e1109.
- Fouquet, A., Noonan, B.P., Rodrigues, M.T., Pech, N., Gilles, A. & Gemmell, N.J. (2012) Multiple quaternary refugia in the Eastern Guiana Shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology*, 61, 461–489.
<http://dx.doi.org/10.1093/sysbio/syr130>
- Fouquet, A., Vences, M., Salducci, M.D., Meyer, A., Marty, C., Blanc, M. & Gilles, A. (2007b) Revealing cryptic diversity using molecular phylogenetics and phylogeography in frogs of the *Scinax ruber* and *Rhinella margaritifera* species groups. *Molecular Phylogenetics and Evolution*, 43 (2), 567–582.
- Frost, D.R. (2015) *Amphibian Species of the World: an Online Reference*. Version 6.0. Electronic Database. American Museum of Natural History, New York, USA. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 1 June 15)
- Funk, W.C., Caminer, M. & Ron, S.R. (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1806–1814.
<http://dx.doi.org/10.1098/rspb.2011.1653>
- Gans, C. (1960) Notes on a herpetological collecting trip through the southeastern lowlands of Bolivia. *Annals Carnegie Museum*, 283–314.
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.
<http://dx.doi.org/10.1080/10635150390235520>
- d’Heursel, A. & de Sá, R.O. (1999) Comparing the tadpoles of *Hyla geographica* and *Hyla semilineata*. *Journal of Herpetology*, 33, 353–361.
<http://dx.doi.org/10.2307/1565631>
- Hillis, D.M., Moritz, C. & Mable, B.K. (1996) *Molecular Systematics*. Second edition. Sinauer Associates, Sunderland, MA, 636 pp.
- Hoogmoed, M.S. & Gruber, U. (1983) Spix and Wagler type specimens of reptiles and amphibians in the Natural History Museum in Munich (Germany) and Leiden (The Netherlands). *Spixiana Supplement*, 9, 319–415.

- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Jansen, M., Bloch, R., Schulze, A. & Pfenninger, M. (2011) Integrative inventory of Bolivia's lowland anurans reveals hidden diversity. *Zoologica Scripta*, 40, 567–583.
<http://dx.doi.org/10.1111/j.1463-6409.2011.00498.x>
- Junca, F.A., Camurugi, F. & Mercés, E.D.A. (2012) The tadpole of *Hypsiboas pombali* (Caramaschi, Pimenta & Feio, 2004) (Anura, Hylidae). *Zootaxa*, 3184, 64–66.
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30 (4), 772–780.
<http://dx.doi.org/10.1093/molbev/mst010>
- Kenny, J.S. (1969) The Amphibia of Trinidad. *Studies of the Fauna of Curaçao and other Caribbean Islands*, 108, 1–78.
- Köhler, G. (2012) *Color catalogue for field biologists*. Herpeton, Offenbach, 49 pp.
- Kok, P.J.R. & Kalamandeen, M. (2008) *Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana*. Belgian Development Cooperation, 278 pp.
- Laurance, W.F., Albernaz, A.K., Schroth, G., Fearnside, P.M., Bergen, S., Venticinque, E.M. & Da Costa, C. (2002) Predictors of deforestation in the Brazilian Amazon. *Journal of biogeography*, 29, 737–748.
<http://dx.doi.org/10.1046/j.1365-2699.2002.00721.x>
- Lavilla, E.O., Caramaschi, U., Langone, J.A., Pombal, J.P. Jr. & De Sa, R.O. (2013) The identity of *Rana margaritifera* Laurenti, 1768 (Anura, Bufonidae). *Zootaxa*, 3646 (3), 251–264.
<http://dx.doi.org/10.11646/zootaxa.3646.3.4>
- Lima, A.P., Magnusson, W.E., Menin, M., Erdtmann, L.K., Rodrigue, D.J., Keller, C. & Hödl, W. (2006) *Guia de sapos da Reserva Adolpho Ducke – Amazônia Central / Guide to the frogs of Reserva Adolpho Ducke – Central Amazonia*. Attema, Manaus, 176 pp.
- Linnaeus, C. von (1758) 1 *Systema naturae: per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Impensis Direct. Laurentii Salvii, Holmiae, 847 pp.
- Márquez, R., De la Riva, I., Bosch, J. & Matheu, E. (2002) *Sounds of frogs and toads of Bolivia*. [CD audio]
- Myers, C.W. & Duellman, W.E. (1982) A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 2752, 1–32.
- Nunes, P.M., Fouquet, A., Curcio, F.F., Kok, P.J. & Rodrigues, M.T. (2012) Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. *Zoological Journal of the Linnean Society*, 166, 361–376.
<http://dx.doi.org/10.1111/j.1096-3642.2012.00846.x>
- Orton, G.L. (1953) The systematics of vertebrate larvae. *Systematic Biology*, 2, 63–75.
<http://dx.doi.org/10.2307/sysbio/2.2.63>
- Parker, H. (1936) A collection of reptiles and amphibians from the Upper Orinoco. *Bulletin du Musée Royal d'Histoire Naturelle de Belgique*, 12, 1–4.
- Parker, H.W. (1933) *A list of the frogs and toads of Trinidad*. US Government Printing Office.
- Peloso, P.L.V. (2010) A safe place for amphibians? A cautionary tale on the taxonomy and conservation of frogs, caecilians, and salamanders in the Brazilian Amazonia. *Zoologia*, 27 (5), 667–673.
<http://dx.doi.org/10.1590/S1984-46702010000500001>
- Peloso, P.L.V., Sturaro, M.J., Forlani, M.C., Gaucher, P., Motta, A.P. & Wheeler, W.C. (2014) Phylogeny, taxonomic revision, and character evolution of the genera *Chiasmocleis* and *Syncope* (Anura, Microhylidae) in Amazonia, with descriptions of three new species. *Bulletin of the American Museum of Natural History*, 386, 1–112.
<http://dx.doi.org/10.1206/834.1>
- Posada, D. (2008) JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253–1256.
<http://dx.doi.org/10.1093/molbev/msn083>
- Pyburn, W.F. & Hall, D.H. (1984) A new stream-inhabiting treefrog (Anura: Hylidae) from southeastern Colombia. *Herpetologica*, 40, 366–372.
- Pyron, A.R. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
<http://dx.doi.org/10.1016/j.ympev.2011.06.012>
- Rambaut, A. & Drummond, A.J. (2007) Tracer Version 1.5. Available from: <http://tree.bio.ed.ac.uk/software/tracer/> (accessed 20 May 15)
- Savage, J.M. & Heyer, W.R. (1967) Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, central America. *Studies on Neotropical Fauna and Environment*, 5, 111–131.
- Schulze, A., Jansen, M. & Köhler, G. (2015) Tadpole diversity of Bolivia's lowland anuran communities: molecular identification, morphological characterisation, and ecological assignment. *Zootaxa*, 4016 (1), 1–111.
<http://dx.doi.org/10.11646/zootaxa.4016.1.1>
- Silveira, S.R. & Caramaschi, U. (1989) *Revalidação de Hyla semilineata Spix, 1824 (Anura, Hylidae)*. Resumos XVI Congresso Brasileiro de Zoologia, Universidade Federal da Paraíba, João Pessoa, Paraíba. [unknown pagination]
- Spix, J.B. (1824) *Animalia Nova sive species novae testudinum et ranarum, quas in itinere per Brasiliam, annis 1817-1820*

jussu et auspiciis Maximiliani Josephi I. typ. Franc. Seraph. Hübschmanni, 53 pp.

- Sturaro, M.J. & Peloso, P.L.V. (2014) A new species of *Scinax* Wagler, 1830 (Anura: Hylidae) from the Middle Amazon River Basin, Brazil. *Papéis Avulsos de Zoologia São Paulo*, 54, 9–23.
<http://dx.doi.org/10.1590/0031-1049.2014.54.02>
- Sueur, J., Aubin, T. & Simonis, C. (2008) Equipment review: seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18, 213–226.
<http://dx.doi.org/10.1080/09524622.2008.9753600>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
<http://dx.doi.org/10.1093/molbev/msr121>
- Wagler, J.G. (1830) *Natürliches System der Amphibien: mit vorangehender Classification der Säugethiere und Vögel*. In der J.G. Cotta'schen Buchhandlung, München, Stuttgart, Tübingen, 382 pp.
- Wiens, J.J., Kuczyński, C.A., Hua, X. & Moen, D.S. (2010) An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution*, 55, 871–882.
<http://dx.doi.org/10.1016/j.ympev.2010.03.013>

APPENDIX 1. Occurrence records of *H. diabolicus* sp. nov.

Locality	State	Lat	Long
St Elie carbet	French Guiana	5.341557	-53.038820
Kaw Fourgassié	French Guiana	4.627158	-52.307290
Paracou	French Guiana	5.272546	-52.923600
Oiapoque	Brazil/Amapá	3.904047	-51.772270
Crique Bon Espoir	French Guiana	5.099493	-53.800510
Trinite Aya	French Guiana	4.608465	-53.415190
Nouragues Camp Inselberg	French Guiana	4.084790	-52.680630
Apatou	French Guiana	5.249830	-54.206730
Montagne des singes	French Guiana	5.069670	-52.696800
Kaw Patawa	French Guiana	4.574621	-52.226140
Pedra Branca	Brazil/Amapá	0.772970	-51.945700
Mitan D	Brazil/Amapá	2.627640	-52.541950
Counamy	French Guiana	5.403682	-53.274510
Montagnes Sparouine	French Guiana	4.808075	-54.168830
Rio Vila Nova	Brazil/Amapá	0.449335	-52.022140
Monts Gaa kaba	French Guiana	4.462112	-54.392740
Mont Saint Marcel	French Guiana	2.385830	-53.018890
Parna Tumucumaque	Brazil/Amapá	0.909122	-53.228480
Réserve Trésor	French Guiana	4.548060	-52.151940
Route Nationale 2	French Guiana	4.105350	-52.049440
Pointe Maripa	French Guiana	4.676256	-52.354120
Riviere Kourouai	French Guiana	4.114492	-52.060250
Carrière Crique Biche	French Guiana	4.772883	52.431900
Crique Belle Etoile	French Guiana	5.231226	-53.663490
Piste Patagai	French Guiana	5.393000	-53.18600

APPENDIX 2. Additional material examined.

Hypsiboas aff. *semilineatus* 1 (field numbers between parentheses)

R138 (AG335) a male from Crique Camopi-Crique Jalbot, French Guiana (3.15, -52.5667) collected by M. Blanc on 26 April 2009, R139-40 (AF0106-7) two males from Road to Apura, Suriname (5.1833333, -55.6166667) collected by A. Fouquet and M. Blanc on 10 January 2006, R141 (AF0842) a male from Chutes Voltaire camp, French Guiana (05.0526389, -54.0884444) collected by A. Fouquet, V. Rufray and O. Chaline on 29 December 2012, R142 (AF0939) a male from Chutes Grégoire, French Guiana (05.0974722, -53.0506667) collected by A. Fouquet and A. Chen on 10 February 2013; R143-5 (AF1285-6, AF1288) three males from Nouragues reserve station "Inselberg", French Guiana (4.08479, -52.68063) collected by A. Fouquet and Q. Martinez on 01 June 2013, R146 (AG370) a male from Monts Gaa Kaba, French Guiana (4.462112, -54.39273) collected by M. Blanc et A. Cochard on 13 September 2010; R147 (AM027) a male from Inini Tolenga, French Guiana (3.663159, -53.928308) collected by M. Dewynter and K. Pineau on 10 February 2012; R148 (CAAM029) a female from Montagne Cacao, French Guiana (2.349156, -53.216013) collected by K. Pineau and V. Rufray on 10 July 2012. MNHN 2014.0110 (QM377) a male from Belizon, French Guiana (4.347832, -52.336203) collected by Q. Martinez on 27 May 2014; MNHN 2014.0111 (AF1825) a male from Mana road, French Guiana (5.55614, 53.59496) collected by J.P. Vacher on 17 March 2014; MNHN 2014.0112-5 (QM310, 403, 417, 428) a female and three males from Montagne de fer, French Guiana (5.318094, -53.598318) collected by Q. Martinez on 15 January 2014; MNHN 2014.0116 (AF1556) a female from Saül, French Guiana (3.60654, -53.17625) collected by M. Berroneau, M. Berroneau, L. Barth, P.O. Cochard on 22 January 2014; MNHN 2014.0117-9 (AF1752, 1753, 1755) three males from Camp Voltaire, French Guiana (5.05226, -54.08909) collected by A. Fouquet and J.P. Vacher on 05 March 2014; MNHN 2014.0120 (AF1861) a male from Waki flat, French Guiana (3.0895, -53.39846) collected by J.P. Vacher on 01 April 2014.

Hypsiboas geographicus

MZUSP 157083, 157086, 157088, 157089, 157082, 157084, 157087 (MTR 18569, 18763, 18940, 18828, 18771, 18866, 18650) four males and three females from Lago Chaviana, Rio Purus, Municipality of Beruri, Amazonas, Brazil (-4.307750, -61.813833) collected by M. T. Rodrigues, S. Marques de Souza, A. Fouquet, M. Teixeira Jr, R. Recoder, M.A. Sena, F. Dal Vecchio, J.M.B. Ghellere on 14-24 October 2010; MZUSP 157090, 157091 (MTR 28250, 28249) two females from Serra do Divisor, Municipality of Mâncio Lima, Acre, Brazil (-7.434348, -73.660390) collected by F. Dal Vecchio, I. Prates, P. Peloso on 25 January 2013; MTR36895) a male from Flutuante Mamirauá, municipality of Tefé, Amazonas, Brazil (-2.890867 -68.361287) collected by M.T. Rodrigues, S.M. Souza, F. Dal Vecchio, R. Recoder, J.M.B. Ghellere, M. Sena, M. Teixeira Jr, I. Prates, P. Dias on 28 April 2015.