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Source: Journal of Vertebrate Biology, 72(23026)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.23026>

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Exceptional diversity of *Pristimantis* Landfrogs (Anura: Terraranae) on the Wokomung Massif, Guyana, with descriptions of three new species

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► Received 8 March 2023; Accepted 5 May 2023; Published online 28 June 2023

Abstract. We describe three new species of landfrogs, genus *Pristimantis*, from near the summit of Mt. Kopinang, one of the several high points of the Wokomung Massif, a large horseshoe-shaped tepui (= mesa) in west-central Guyana. *Pristimantis koki* n. sp. is known from 1,067 to 1,525 m elevation. It is characterized by small-sized adults averaging 12.4 mm SVL (snout-vent length) in males and 18.4 mm in females; a pointed, depressed, elongated snout; lack of an obvious tympanum, vocal slit, or sac; and diagnostically black pigment prominently arranged around the anus fringed by light pigment. When handled, *P. koki* seems to emit volatile organic compounds and leaves a slightly numbing taste at the base of the human tongue. *Pristimantis kopinangae* n. sp. is known from three specimens collected at approx. 1,385 m elevation on the Wokomung Massif and two specimens from slightly higher in elevation on Mt. Ayanganna. About the size of most *Pristimantis* inhabiting the Guyana uplands and highlands (20-30 mm SVL), it is characterized by 2-3 light yellow inguinal flash-mark blotches, short broadly round snout, large eye with a blue iris, white skin of chin and areolate belly with dark brown vermiculations; and absence of a tympanum. *Pristimantis kalamandeenae* n. sp. is known from three specimens collected on the Wokomung Massif including an amplexing pair at approx. 1,550 m elevation. Similar in size to *P. kopinangae*, it is characterized by an acuminate snout, black iris, obvious tympanum, and uniform tan pigmentation dorsally after dark that becomes uniformly dark brown in daytime. Phylogenetic results show that *P. koki* and *P. kopinangae* are sister species and are members of a larger assemblage of related species endemic to the Pantepui Region within the *P. unistrigatus* species group. *Pristimantis kalamandeenae* is not closely related to these species, instead forming a clade with the *P. lacrimosus* species group. The three new species occur in sympatry with at least five other *Pristimantis* species on the Wokomung Massif, the greatest known *Pristimantis* species richness on a single tepui of the Guiana Shield.

Key words: Brachycephaloidea, Craugastoridae, Pantepui, South America, Strabomantidae, systematics, tepui, volatile skin secretions

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Introduction

A poorly explored biodiversity hotspot, the Pakaraima Mountains of west-central Guyana (Fig. 1a) has already yielded more than 25 species of frogs new to science in the first two decades of the 21st century (Noonan & Harvey 2000, Smith & Noonan 2000, MacCulloch & Lathrop 2002, 2005, Noonan & Bonett 2003, Señaris et al. 2005, Kok 2006, 2009, Kok et al. 2006, 2010, 2011, 2018a, Lathrop & MacCulloch 2007, Means & Savage 2007, MacCulloch et al. 2008) including an entirely new family of frogs (Heinicke et al. 2009). Aligned in a southeast to northwest direction along the borders of Guyana, Brazil, and Venezuela, the Pakaraima Mountains are a line of flat-topped table mountains called tepuis. These classic mesas are erosional remnants of Roraima Supergroup sandstones laid down 1.8 to 1.6 billion years ago (Santos et al. 2003) on top of the Guiana Shield, part of the ancient Gondwanan heartland of South America (Krooneberg et al. 2016). Eons later, the shield began rising tectonically (Schubert & Briceño 1987), causing a once-contiguous landscape to be fragmented into isolated flat-topped summits, some of which today reach over 2,500 m in height (McDiarmid & Donnelly 2005, Rull et al. 2019). Mayr & Phelps (1967) coined the term Pantepui to represent these tepui summits which spread westward from the Pakaraima Mountains into the Venezuelan states of Bolívar and Amazonas.

With more than 595 species already named, *Pristimantis* is the most species-rich genus of vertebrates (Frost 2023). By far the greatest number of these are found in the Andes from Colombia to Peru (Duellman & Lehr 2009), but a secondary area of endemism is emerging as herpetological exploration takes place on the remote tepuis of the geologically ancient Pantepui, i.e. the western Guiana Shield highlands in Venezuela, Brazil, and Guyana. For instance, the eight species of pebble toads (family Bufonidae) of the genus *Oreophrynella* are restricted to Pantepui with most of them endemic on one or two tepui summits (Kok et al. 2018b, 2020). An ancient clade of Microhylidae frogs in the genera *Adelastes*, *Otophryne*, and *Synapturanus* is distributed mostly across the Guiana Shield, likely originating from Pantepui (Fouquet et al. 2021). At least 21 species of carrying frogs of the family Hemiphractidae in the genus *Stefania* are exclusively confined to Pantepui and primarily endemic on individual tepuis (Duellman 2015, Kok et al. 2016, 2017). In fact, MacCulloch & Lathrop (2002) described “exceptional diversity of *Stefanias*” on Mt. Ayanganna and its

sister tepui complex, the Wokomung Massif in Guyana (MacCulloch & Lathrop 2006).

Among the Pakaraima Mountains of Guyana in particular – and the Pantepui biogeographical region in general – the Wokomung Massif is revealing exceptional diversity of *Pristimantis* frogs. Following four expeditions to the Wokomung Massif, a large tepui about 31 km long by 7.5 km wide, three species of frogs of the genus *Pristimantis* were described that had malodorous and distasteful skin secretions (Means & Savage 2007). The first molecular phylogenetic analysis to include these taxa (Hedges et al. 2008) showed that one of these – *P. dendrobatoides* – lacks close relatives while the other two, *P. jester* and *P. saltissimus*, are closely related to each other within the large *P. unistrigatus* species group, but a lack of comparative material from other Guiana Shield species precluded determination of finer-scale patterns. More recently, integrative studies have greatly expanded knowledge of the diversity and evolutionary relationships of *Pristimantis* from the Guiana Shield. These include phylogeographic studies (Fouquet et al. 2012, Kok et al. 2012), twelve additional new species descriptions (Barrio-Amorós & Brewer-Carias 2008, Myers & Donnelly 2008, Barrio-Amorós et al. 2010, Kok et al. 2011, Fouquet et al. 2013, 2022, Kok 2013, Mônico et al. 2022, Rödder & Jungfer 2008, Rojas-Runjaic et al. 2013) and a redescription of *P. marmoratus* that was previously a catch-all taxon used to designate many Guiana Shield *Pristimantis* populations but has now been restricted to populations in the Pantepui Region surrounding the Brazil/Guyana/Venezuela border triple point including the Wokomung Massif (Kok et al. 2018c). From this burst of recent work, sequence data are now available for more than 20 out of 32 named species of Guiana Shield *Pristimantis*, and it is clear that *P. jester* and *P. saltissimus* belong to a broader clade of mostly Guiana Shield species within the *unistrigatus* group (Fouquet et al. 2022). This new context allows us to revisit a set of *Pristimantis* specimens from the Wokomung Massif that were previously left unidentified, tentatively identified, or simply misidentified. Here we describe a fourth species of malodorous and distasteful *Pristimantis* from the Wokomung Massif as well as two other species in the genus. The Wokomung Massif is home to at least eight species in the genus, the largest number of sympatric, with some occurring syntopically, *Pristimantis* yet recorded from any tepui, followed by seven sympatric undescribed species reported from Cerro Neblina (McDiarmid & Donnelly 2005).

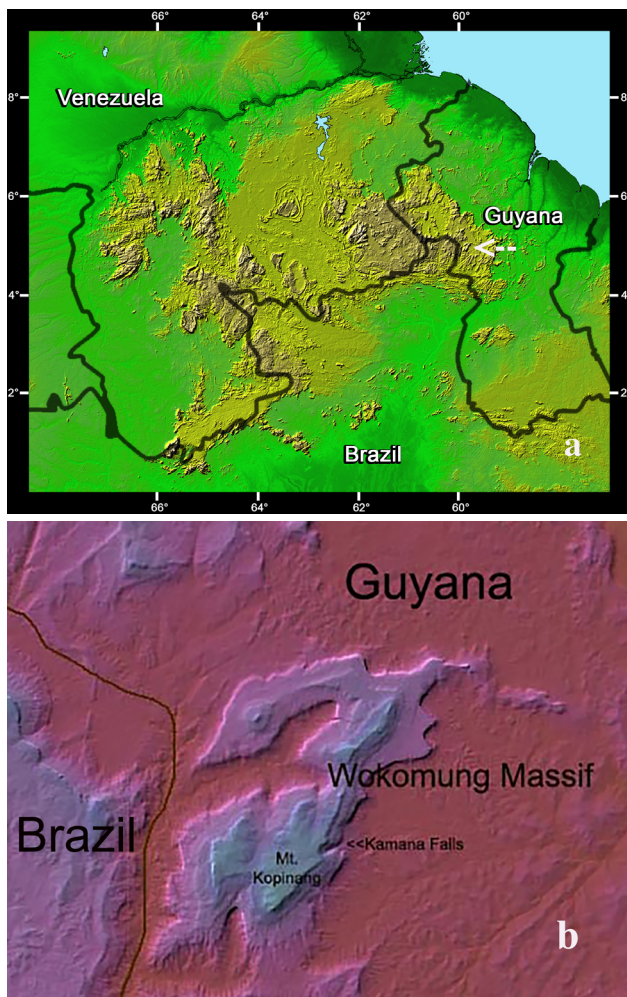


Fig. 1. a) Guiana Shield Highlands (Pantepui); the Pakaraima Mountains include all the mountainous terrain along the mid-western border of Guyana from 4° to about 7° N latitude. b) Wokomung Massif in Guyana at the tip of the arrow in 1a. Images courtesy of P.J.R. Kok.

Material and Methods

General

The Wokomung Massif, entirely in Guyana, is a large horse-shoe-shaped table-mountain that drains west into the River Ireng, a tributary of the Amazon, and east into the River Potaro, a tributary of the north-flowing River Essequibo. It rises from surrounding seasonal rainforest lying at about 600 m elevation along the two rivers to a cloud-forested plateau between 1,200 and 1,800 m elevation (Fig. 1b). The summit elevations are fringed all around by steep but densely forested sandstone cliffs of the Roraima Supergroup (Gibbs & Barron 1993). Rising above 1,500 m are five higher eminences, some with local names such as Wokomung, Kopinang, and Little Ayanganna.

From measurements from Guyana Survey Department 1:50,000 maps (42 SW, 49 NW 1975,

edition 1), the Wokomung Massif is about 31 km long by 7.5 km wide. Among its high points, Little Ayanganna and Mt. Wokomung possess only about 1 km² of summit area above 1,500 m, but the much larger Mt. Kopinang has about 12 km² above 1,500 m. These high points are not connected at that elevation, but all are connected above the 1,200 m contour. This larger area is approx. 100 km² in size (Fig. 1b). Wokomung and Kopinang are densely vegetated by a moss- and epiphyte-laden cloud forest, while Little Ayanganna is primarily vegetated by patches of gnarled shrub forest in a matrix of dense herbaceous vegetation. The Wokomung Massif is so large that numerous streams of varying size (up to 2.5 cubic meters per second) drain the higher eminences, meander through the plateau, and cascade down the steeper slopes within the tepui and plunge as waterfalls or cascades down the fringing cliffs. Above 1,200 m elevation, most of the landscape is technically a wetland of deep, squishy organic soil kept wet by constant mists and rain. The ground as well as the boles, branches, and even the leaves of trees and shrubs are densely covered with epiphytes, especially a dense, olive-green moss and numerous other plants including aroids, orchids, and bromeliads (Fig. 2).

Specimens were collected by hand after dark, most often following or during rainfall. They were euthanized 1) in 20% benzocaine dissolved in water or 2) using a mixture of clove oil, water, and 95% ethanol. A piece of liver and thigh muscle was removed from each frog and preserved in 95% ethanol for DNA study and then specimens were fixed 10 min later in 90% ethanol. For long-term storage, specimens were transferred to 70% ethanol. The skin of live specimens was licked and smelled by one of us (D.B. Means). Specimens were deposited in the herpetological collections of the United States National Museum (USNM), Royal Ontario Museum (ROM), and Florida Museum of Natural History (UF). Coordinates and elevations were obtained using a Garmin eTrex Vista HCx global positioning system unit referenced to map datum WGS84.

Morphology

Morphometric data to the nearest 0.1 mm were taken from each preserved specimen using a dial calliper. Description of morphology follows the standards for the genus in Savage (1987), Lynch & Duellman (1997), and Duellman & Lehr (2009) with a modification of the terminology of the digital discs as detailed in Savage et al. (2004). Measurements were taken as described by Campbell (1994), Lynch & Duellman (1997), Means & Savage (2007), Duellman & Lehr 2009, and Kok et



Fig. 2. Cloud forest with abundant epiphytic growth of mosses, ferns, aroids, and bromeliads from ground to treetop.

al. (2011) and are abbreviated as follows: snout-vent length from tip of the snout to the posterior-most part of the dorsal midline (SVL); head length from the posterior-most part of the upturned commissure of the lips to the tip of snout (HL); greatest width of the head (HW); snout length from the anterior corner of the orbit to tip of snout (SL); eye to naris distance from anterior corner of the orbit to the posterior corner of the naris (EN); internarial distance between median margins of the nares (IN); eye length from anterior to posterior corners of the orbit (EL); interorbital distance between the median margins of the orbits (IOD); forearm length from elbow to the proximal edge of the thenar tubercle (FOR); length of finger I from proximal edge of thenar tubercle to tip of finger (HAND I); length of finger II from the proximal edge of the thenar tubercle to the tip of finger II (HAND II); length of finger III from the proximal edge of the thenar tubercle to the tip of finger (HAND III); length of finger IV from the proximal edge of the thenar tubercle to the tip of finger (HAND IV); width of disc on finger III (WFD); foot length from proximal edge of inner metatarsal tubercle to the tip of toe IV (FL); shank (or tibia) length from knee to heel (TIL); tarsus length from heel to proximal edge of inner metatarsal tubercle (TAR); thigh length from knee to heel (THI); width of disc on toe IV (WTD); width of tympanum (TYM).

Colour pattern of live individuals was taken from field notes and digital colour photographs. Sex was

determined by observing amplexant pairs and examination of the dissected gonads. We compared specimens of our three new species with specimens of other live *Pristimantis* species that we have collected from the Wokomung Massif (*marmoratus*, *dendrobatoides*, *jester*, *saltissimus*, *pulvinatus*), from other tepuis in the Pakaraima Mountains (*P. aureoventris*, *P. yuruaniensis*), and from published descriptions.

Molecular analyses

We generated new sequences of the mitochondrial region consisting of the complete 12S/tRNAV/16S genes for 24 Guyana *Pristimantis* samples representing eight species, including the three taxa being described herein. We obtained comparative data from GenBank for a comprehensive selection of *Pristimantis* species for which the complete 12S/tRNAV/16S gene region was available. Shorter 16S sequences were also included for species occurring in the Guiana Shield if longer sequences were not available. Comparative sequence data were sampled at a depth of one sequence per species; GenBank sequences not identified to species were included if they were genetically distinct enough to represent separate species. We included one additional representative of each of the Terraranan families (Brachycephalidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, Strabomantidae) to serve as outgroups. We apply the family level taxonomy used in a primary database for amphibians



(AmphibiaWeb 2023, <https://amphibiaweb.org>) and a recent phylogenomic study (Heinicke et al. 2018) but acknowledge alternative interpretations (Barrientos et al. 2021, Frost 2023). The complete molecular data set includes 205 sequences (Table S1). For specimens sequenced in this study, genomic DNA was extracted from frozen or ethanol-preserved tissue samples using a Qiagen DNeasy Blood and Tissue kit. Polymerase chain reactions were performed at 50 μ L volume using AmpliTaq DNA polymerase and ThermoPol buffer (NEB). Primer sequences and reaction conditions are as in Heinicke et al. (2007). Amplified PCR products were purified via gel filtration (Millipore). Sequencing was performed in forward and reverse directions for all samples at the Pennsylvania State University Genomics Core Facility. MEGA 11 (Tamura et al. 2021) was used to inspect chromatograms and generate contigs. New sequences are deposited under GenBank accession numbers OQ884602-OQ884622.

Sequences were aligned using MUSCLE 3.6 under default parameters (Edgar 2004), producing an alignment of 3,111 bases including 1,819 informative sites. A maximum likelihood phylogenetic analysis was performed using IQ-TREE 2.2 (Minh et al. 2020). The alignment was treated as a single partition because the genes assort as a single locus and are not protein-coding. Alignment gaps were treated as missing data. ModelFinder was used to identify the best fitting model of evolution under the Bayesian Information Criterion (Kalyaanamoorthy et al. 2017), with the tree search employing this model. Branch support was assessed using 1,000 ultrafast bootstrap replicates (Hoang et al. 2018).

Results

Phylogeny

The phylogeny (Fig. 3) shows that *Pristimantis* from the Guiana Shield belong to at least five distinct clades. Most of the new samples belong to a well-supported (100% bootstrap support) subclade containing species assigned to the large *unistrigatus* group (Hedges et al. 2008). This subclade includes the long misidentified species *P. marmoratus* along with 16 other species from Pantepui, four from the Guiana Shield lowlands, and five species from upper Amazonia or the Andes. Phylogenetic patterns hint that this subclade originated in the greater Guiana Shield region with some subsequent dispersal, but no genetic data exist for approximately half of the described species of *Pristimantis* so alternate biogeographic interpretations cannot be ruled out. However, we refer to this set of

species as the Guiana Shield clade for convenience. Among the 12 species from the Guiana Shield clade endemic to the Pantepui Region, two are previously undescribed species from the Wokomung Massif. A set of five newly sequenced samples (UF-191300-1, -191306-8) are genetically identical to one another and represent a heretofore unidentified lineage. A further three new samples (UF-193779-81) form a monophyletic group with a specimen (ROM-40164) that was originally identified as *P. inguinalis* (Hedges et al. 2008) but the availability of additional genetic material has since shown that it is a distinct species from *P. inguinalis* labelled as *P. sp.* “Ayanganna” in the phylogeny included in Fouquet et al. (2022). These two previously undescribed species are notable in that they are sister species but occur on the Wokomung Massif. This implies that they probably evolved phenotypic and genetic differences (5% 12S/16S divergence) within a narrowly circumscribed geographic area. Other new sequences generated for this study belong to the Guiana Shield clade species *P. aureoventris*, *P. gutturalis*, *P. jamescameroni*, *P. koehleri*, *P. marmoratus*, *P. pulvinatus* and *P. saltissimus*.

Most sampled Pantepui species belong to the Guiana Shield clade, but two *Pristimantis* occurring in the Wokomung Massif region are not closely related to the Guiana Shield clade or to one another. One is *P. dendrobatoides*, the other a previously undescribed species. The undescribed species, represented by specimen ROM-43978 and by newly sequenced sample UF-193778, was labelled as *P. zeuctotylus* in Hedges et al. (2008) and as *P. sp.* “Wokomung” in Fouquet et al. (2022). It is phylogenetically nested within the *lacrimosus* species group, most species of which occur in Amazonia and inhabit bromeliads (Hedges et al. 2008). Within this group the undescribed Wokomung species is closely related to *P. zimmermanae* and *P. cf. pluvialis*, which occur in the Guiana Shield lowlands. True *P. zeuctotylus* is found elsewhere in the tree, as are the remaining Guiana Shield species *P. chiastonotus*, *P. guianensis*, *P. gutturalis*, *P. koehleri*, *P. vilarsi*, and *P. cf. fenestratus*. These six species belong to the *conspicillatus* group and have Amazonian affinities, occurring in lowlands rather than the Pantepui Region.

Systematics

Pristimantis koki n. sp. (Figs. 4-10, Table 1), Guyanan Black-rumped Landfrog

Holotype: USNM-573463 (field number CPI-10552), an adult female (Fig. 4a, b) sitting on a small leaf about 50 cm off the ground next to a narrow footpath



about 100 m west of the top of Kamana Falls on the south side of Mt. Kopinang of the Wokomung Massif, Potaro-Siparuni District, west-central Guyana: 04°04'50" N, 59°52'43" W, approx. 1,538 m elevation; collected in amplexus with male paratype (USNM-573464), collected 19 July 2007 by D. Bruce Means and Michelle Kalamandeen.

Paratypes: n = 7, USNM-573464, an adult male (CPI-10553) found in amplexus with the holotype (Fig. 4a), same data as for holotype. USNM-573462 (CPI-10508) adult male, USNM-573465 (CPI-10554) adult female, USNM-573466 (CPI-10555) adult male, all three collected from along 100 m-footpath west of the top of Kamana Falls, Wokomung Massif, Potaro-Siparuni District, Guyana: 04°04'50" N, 59°52'43" W, approx. 1538 m elevation, 15-21 July 2007 by D. Bruce Means. UF-191299 (CPI-10404) male, UF-191300 (CPI-10405) female, UF-191301 (CPI-10406) female, all three in cloud forest sitting on mossy roots on the ground of the southern side of Mt. Kopinang, Potaro-Siparuni District, Guyana: 04°04'50" N, 59°52'43" W at approx. 1,538 m elevation, collected 11 December 2006 by D. Bruce Means and Michelle Kalamandeen; field collection number DBM-3378.

Referred specimens: n = 25, USNM-573467 (CPI-10556) adult female and USNM-573468 (CPI-10557) adult male: two carcasses whose skins were removed for skin secretion study, from along 100 m-footpath west of the top of Kamana Falls, Wokomung Massif, Potaro-Siparuni District, Guyana: 04°04'50" N, 59°52'43" W, 1,538 m elevation, collected 15-21 July 2007 by D. Bruce Means.

UF-191302 (CPI-10627) male in amplexus with UF-191303 (CPI-10628) female; UF-191304 (CPI-10912) a single egg laid by UF-191303 (CPI-10628), Potaro-Siparuni District, Guyana, in cloud forest of the southern side of Mt. Kopinang: 04°04'50" N, 59°52'43" W at approx. 1,538 m elevation, collected 19 June 2012 by D. Bruce Means.

UF-191305 (CPI-10424) adult female. Potaro-Siparuni District, Guyana, southern side of Mt. Kopinang, the westernmost high point on the Wokomung Massif, at 04°03'50" N, 59°52'56" W, approx. 1,067 m elevation, collected 5 December 2006 by D. Bruce Means and Michelle Kalamandeen; field collection DBM-3370.

UF-191306 (CPI-10425) adult female, Potaro-Siparuni District, Guyana, in summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the

vicinity of the top of Kamana Creek Falls: 05°00'08" N, 59°52'47" W, approx. 1,524 m elevation, collected 7 December 2006 by D. Bruce Means and Michelle Kalamandeen; field collection DBM-3372.

UF-191307 (CPI-10426) adult female found on vegetation 15 cm above ground, Potaro-Siparuni District, Guyana, cloud forest summit of Mt. Kopinang (southwestern high point of the Wokomung Massif) along a trail paralleling Kamana Creek for about 300 yards west of its cascade off the summit at the E end of the trail: 05°00'08" N, 59°52'47" W, approx. 1,555 m elevation, collected 10 December 2006 by D. Bruce Means and Michelle Kalamandeen; field collection DBM-3376.

UF-191308 (CPI-10427) adult female, Potaro-Siparuni District, Guyana, summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the vicinity of the top of Kamana Creek Falls: 05°00'08" N, 59°52'47" W, approx. 1,524 m elevation, collected 10 December 2006 by D. Bruce Means and Michelle Kalamandeen; field collection DBM-3377.

UF-191309 to -191312 (CPI-10623 to -10626). Series of four, Potaro-Siparuni District, Guyana, summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the vicinity of the top of Kamana Creek Falls: 05°00'01" N, 59°50'50" W, approx. 1,575 m elevation, collected 18 June 2012 by D. Bruce Means. Preserved whole body in 70% ethanol and stored in it.

UF-191313 to -191316 (CPI-10688 to -10691). Series of four, Potaro-Siparuni District, Guyana, summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the vicinity of the top of Kamana Creek Falls: 05°00'01" N, 59°50'50" W, approx. 1,575 m elevation, collected 26 June 2012 by D. Bruce Means. Whole body preserved in 95% ethanol and stored in it.

UF-191317 to -191319 (CPI-10787 to -10789). Series of three, Potaro-Siparuni District, Guyana, summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the vicinity of the top of Kamana Creek Falls: 05°00'01" N, 59°50'50" W, approx. 1,575 m elevation, collected 19 June 2012 by D. Bruce Means.

UF-191320 to -191324 (CPI-10834 to -10838). Series of five, Potaro-Siparuni District, Guyana, summit cloud forest of Mt. Kopinang, part of the Wokomung Massif, in the vicinity of the top of Kamana Creek Falls: 05°00'01" N, 59°50'50" W, approx. 1,575 m elevation, collected 28 June 2012 by D. Bruce Means.



Fig. 4. a) *Pristimantis koki* holotype female (USNM-573463) in amplexus with paratype male (USNM-573464) as first seen in the field sitting on a leaf; b) ventral view of live gravid female; c) typical perianal black pigment fringed with lighter colour; d) side view showing dorsoventrally compressed and acuminate snout.

Diagnosis: A very small *Pristimantis* less than approx. 20 mm long (mean SVL in males = 12.4 ± 0.7 mm, $n = 5$; mean SVL in females = 18.4 ± 1.7 mm, $n = 5$) with the following diagnostic characters: skin of dorsum granular with abundant larger pustules/blisters; eyelid with two tubercles. Choanae small, round; vomerine odontophores not evident. Legs stouter than in other species of *Pristimantis* on the Wokomung Massif with more robust thighs and shanks (Figs. 4, 5, 8, 9). Elongate snout tapering to a sharp point in dorsal and dorsolateral view (Fig. 4a, d) and dorsoventrally compressed in lateral view; posterior commissure of the lips upturned (Fig. 6). Tympanum, vocal slits, and vocal sac not evident. Nuptial pads absent. Finger I much shorter than finger II; enlarged oval discs on all digits except finger I. Digital fringes and interdigital webbing absent in live and preserved specimens. Undersurfaces of manus and pes relatively smooth with thenar tubercle most prominent in females (Fig. 7). In all specimens examined ($n = 49$), colouration includes a black bar angling forward from the anterior lower eyelid to the upper lip (Figs. 4a, d, 8), and an elongated half-moon of black pigment encircles the cloaca fringed dorsally by a narrow, unpigmented (whitish) line (Figs.

4c, 5a). Other diagnostic characters often present: dense black pigment on the inside of the heels (Fig. 4b, c); black bar often between orbits (Figs. 4a, 9a); thin black scapular bar or dark spots across the back associated with a transverse line of two or more raised tubercles (Figs. 4a, c, 9b); cream-coloured sides strongly contrasting with a dark, middorsal saddle (Figs. 4a, d, 5a, 9a). Currently not associated with any species group but morphologically similar to the polyphyletic *unistrigatus* group (Hedges et al. 2008).

Etymology: The species name *koki* is a patronym honouring Belgian evolutionary biologist/ecologist, Philippe J.R. Kok, our friend and colleague who has emerged as a leading authority in tepui herpetology. The suggested common name, Guyanan Black-rumped Landfrog, reflects the diagnostic character of a black perianal area. Our use of common names follows established guidelines (Hedges et al. 2019).

Description of the holotype: A gravid female 18 mm SVL (Fig. 4a); HW 39.5% of SVL; head length (6.7 mm) slightly shorter than wide (6.9 mm), not wider than body (8.3 mm); cranial crests absent; snout pointed in dorsal and lateral aspect (Fig. 4a) and depressed

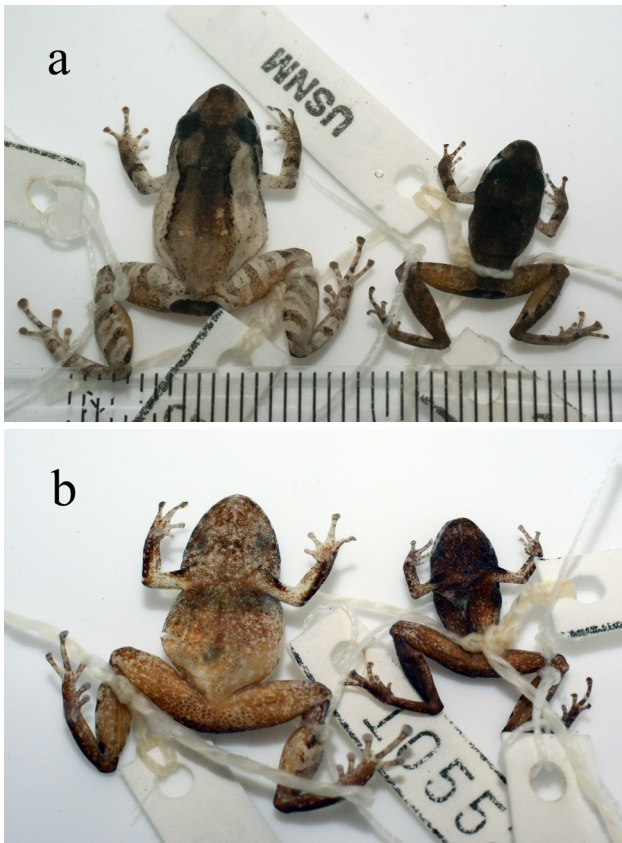


Fig. 5. a) *Pristimantis koki* holotype female and b) paratype male, a – dorsal and b – ventral views after five years in 70% ethanol. Scale = 1.0 mm.

dorsoventrally as in Fig. 4d; canthus rostralis forming a prominent ridge between the top of the orbit and pointed tip of the snout, passing over the top of the naris; loreal region slightly concave, sloping outward onto the upper lip; nares slightly protuberant, directed laterally; eye relatively small (EL 2.6 mm/IOD 2.5 mm = 1.04); upper eyelid granular and adorned with three or more enlarged tubercles (deflated and nearly invisible in preservative); iris colour densely-spaced minute flecks of grey with a thin, reddish-brown ring around the pupil; tympanum absent; lip commissure roundly upturned posteriorly (as in Fig. 6); choanae small, round, not concealed under palatal shelf of maxillae; vomerine odontophores not apparent; tongue round, slightly notched posteriorly, posterior one-half free; dorsal skin densely granular with tiny round, raised, rust-coloured bumps or tiny blisters arranged in weakly parallel lines, especially on the light-coloured flanks; belly skin and undersurfaces of thighs granular; throat skin smooth to slightly shagreened; larger bumps or blisters aligned on top of the black bar across the top of the head, on the dorsolateral pair of lines of black pigment, and on top of the darker bars on the legs; very large (half the size of toepads), round, liquid-filled pustules or blisters present on the dorsal surface of the arms (Fig. 4a); a



Fig. 6. *Pristimantis koki* upturned lip commissure posterior to the inverted V.

line of 3-5 raised tubercles present on lateral edges of the shank, leg, and heel; scapular fold or ridge absent; finger I 56% length of finger II; relative length of fingers III > IV > II > I; discs on fingers II, III, IV expanded and twice as wide as long; disc of finger I not or very slightly expanded (Fig. 7a); hind limbs relatively stout; TIL 55% of SVL; FL 36% of SVL; relative length of toes IV > V > III > II > I; toe discs oval to sub-round, slightly larger in size than finger discs; digital webbing and fringes absent; when grasped, the holotype and most other *P. koki* exhibited a faintly pungent smell, and a slightly bitter taste when their dorsal skin was licked. Indeed, within 5-10 secs, the back of the collector's (D.B. Means') tongue felt slightly numb for about 120 secs thereafter.

Colouration of holotype in life: A wide, light grey, middorsal saddle originates from a wavy-edged dark brown bar on the midline between the orbits and proceeds posteriad, narrowing slightly on the neck, widening over the belly, and terminating on the top of the end of the urostyle (Fig. 4a). The snout is light bluish grey with a thin irregular wash of rusty pigment, concentrated slightly on top of a weak canthus rostralis and the top of the sharply pointed tip of the snout. The saddle is bordered on both sides

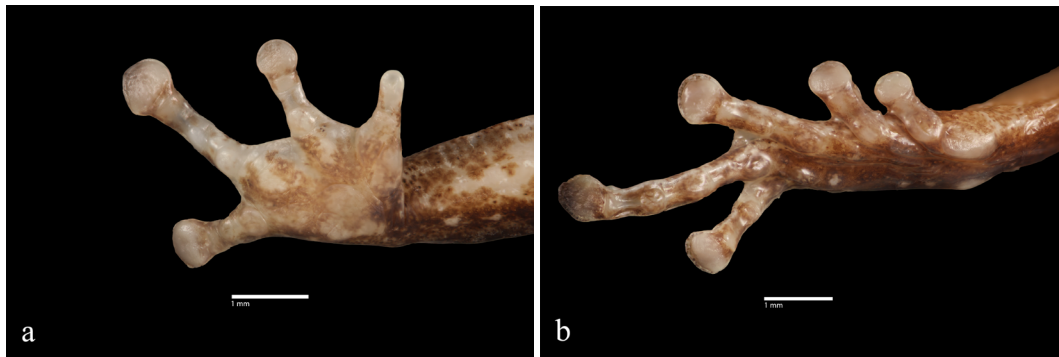


Fig. 7. Undersurfaces of hand a) and foot b) of *Pristimantis koki*.

by lines of dark brown to black pigment originating at the sides of the dark bar between the eyes. Cream-coloured sides below the dark dorsolateral lines contrast strongly with the middorsal saddle and are patchily overwashed with faint rusty colour. A line of dark brown pigment originates at the posterior edge of the orbit and runs downward to terminate at the armpit. A prominent, wide, black bar, diagnostic for the species, angles forward from the forward margin of the eyelid to the lip. Two weak dark bars occur on the upper lip below the eye. Upper half of iris copper to grey with a thin line of brighter rust colour fringing the pupil; lower half of iris dark brown. Four crossbars of weakly darker grey colour on the dorsolateral aspects of the femur, shank, and tarsus/hind-foot line up in apposition when the legs are drawn up next to the body. One slightly darker grey crossbar is visible on the forearm. The spaces between the crossbars are about twice as wide and of a light, bluish grey hue. A thin, irregular wash of rusty colour overwashes the entire dorsal and lateral aspects of the frog, most prominently on the raised warts, arms, legs, and the tops of the toes. All ventral surfaces are dark grey or black and peppered with tiny white specks (Fig. 4b). An elongated half-moon patch of black pigment extends laterally on both sides from the cloacal opening, fringed dorsally by a narrow zone of cream colour, immediately below the end of the urostyle.

Colouration of holotype in preservative: All of the overwashed rusty colour of the live specimen was removed by ethanol. All of the light grey, brown, or black pigment of the live specimen is chocolate brown of varying intensity including a chocolate brown middorsal saddle bordered dorsolaterally by lines of darker brown colour, crossbands on the arms and legs, dark bars between the orbits and on the lips, and all the darker pigment of the undersurfaces. All the remaining areas between the crossbands and along the sides below the middorsal dark brown saddle are dirty white in colour (Fig. 5a).

Variation: See Table 1 for measurements and Figs. 4, 5, 8, 9 for intraspecific variation in colour pattern. The most obvious variation is in dorsal colour and pattern. In live specimens, the top of head (tip of snout to a scapular line across the back over the armpits) can be orangish tan, light grey, or sometimes covered by a forward extension of a wide, middorsal, elongated saddle of middorsal pigment. An irregular-edged or incomplete bar of dark brown to black pigment lies between the eyes and very commonly an incomplete scapular bar or spots of black pigment are found across the shoulders. Scapular tubercles are always associated with the black scapular spots or bar, but both may be absent. Unless the dorsum is dominated by the dark grey to brown elongated saddle, these dark scapular spots are almost a diagnostic feature of the species. The back is variable from plain tan to light grey with a few small spots of dark pigment or sometimes, with a broad longitudinal stripe saddle of dark grey pigment bordered by a darker line of brown to black dorsolateral pigment. Sides are set off from the dorsum below the dorsolateral dark line by contrasting light tan to dirty cream colour, terminating ventrolaterally in another broken line of dark pigment that runs from the back of the eye over the top of the arm and thence downward and backward along edge of the belly to terminate posteriad at the insertion of the hind limbs. In life, this species is subject to metachrosis. Colour patterns at night are often soft pastels (Figs. 5, 9b), but in daylight the underlying melanophores often become dominant (Figs. 4d, 8, 9a). Note also how the underlying melanophores become faded in preserved versus living individuals (Fig. 4 vs. 5). Undersurfaces of the hands and feet as shown in Fig. 7. Choanae are round in males, bean-shaped in females. Tubercles and small, rust-coloured “blisters” on the eyelids, back, arms, and legs are present and obvious in live individuals (Figs. 4a, 9), but are absent to very faint in alcohol preserved specimens (Figs. 5, 6). The hygroscopic nature of 70-90% ethanol in which most specimens were preserved suggests that such



Fig. 8. *Pristimantis koki* gravid female with single large egg she laid overnight in collecting bag. One other large egg remained visible through her abdomen.

tubercles, blisters, and bumps probably are filled with liquids of high-water content, which collapses these structures when the water is gone.

Measurements of holotype (in mm): SVL 18.0; TIL 8.43; FL 6.97; HL 6.0; HW 7.10; IOD 2.66; EW 1.34; IN 1.40; EL 2.60; EN 2.50; TYM 0.0; HANDI 0.88; HANDII 1.32; HAND III 2.05; HAND IV 1.01; WFD 0.69; WTD 0.98; SL 2.80; TAR 4.96; THI 8.35; FOR 3.95.

Reproductive characteristics: A gravid female (UF-191303) collected in amplexus laid a single large egg (UF-191304) overnight in the moss of the plastic collecting bag (Fig. 8). Another large egg was visible through the belly skin on the other side of the female, so clutch size in this specimen = two. The relatively large egg measured 3.6 mm in diameter after three months in 95% ethanol and was 18% of the female's 20.0 mm SVL. The egg was probably not so large *in vivo* because once oviposited, outer egg capsules and membranes swell with the uptake of water, but it was probably larger before immersed in ethanol. Gravid females in amplexus were found after dark

on 19 June 2012, 19 July 2007, and 11 December 2006 following or during rain. It is presumed, therefore, that oviposition in this species can take place year-round following or during rainfall. Amplexus was axillary in two pairs and the male held the humerus of the female's arm in the third pair (Fig. 4a). Obvious tympana are not visible in this species. It is tempting to speculate that the sacs or granules present on the dorsal skin of the female (Fig. 4a) produce a skin secretion whose odour has a reproductive function, possibly attracting males to the female when she is ready to oviposit. An anti-predator function is also likely.

Advertisement call: Tympanum absent or unrecognizable. Vocal slit and sac not evident. No individuals were heard vocalizing, but vocal communication cannot be ruled out.

Habitat and habits: The amplexing holotype and paratype were found three hours after dark at approx. 9:30 p.m. Specimens were found and photographed (n > 35 times) within 1.5 m of the ground (approx. 85%)



Fig. 9. Variation in dorsal colour patterns of live *Pristimantis koki*. Note the black perianal area in b), obscured by faeces in a); both gravid females.



Fig. 10. Habitat of *Pristimantis koki*, cloud forest with abundant epiphytic growth from ground to treetop. All specimens collected from the ground to no more than 1 m.

or on logs or sticks on or near the ground (approx. 15% of the time). Among the eight species of *Pristimantis* on the Wokomung Massif, *P. koki* is the most terrestrial.

Distribution: Known only from the Wokomung Massif (Fig. 1b), but likely to be present at similar elevations on Mt. Ayanganna 40 km to the north because of connecting highlands between these tepuis. On the

other hand, no specimens are known from the chain of tepuis running west of the River Ireng along Guyana's border with Brazil (Maringma, Yakontipu, Apokilang, Weiassipu, Roraima). One might expect *P. koki* on these tepui slopes because it is so common on Wokomung. Possibly the warm lowlands along the master stream, the River Ireng, have been a barrier to its dispersal.



Table 1. Measurements (in mm) of adults of three new species of *Pristimantis* landfrogs; averages followed by one standard deviation and range (in parentheses).

Character	Acronym	<i>P. koki</i>		<i>P. koki</i>		<i>Pristimantis kopinangae</i>		<i>Pristimantis kopinangae</i>		<i>Pristimantis kalamandeenae</i>	
		Males n = 5	Females n = 5	Males n = 1	Females n = 1	Males n = 1	Females n = 1	Males n = 1	Females n = 1	Males n = 1	Females n = 1
Snout-vent length	SVL	12.4 ± 0.7 (11.8-13.7)	18.4 ± 1.7 (16.0-20.0)	22.2	25.1	22.1	25.7	22.1	25.1	22.1	25.7
Shank length	TIL	6.7 ± 0.5 (6.0-7.3)	9.5 ± 0.7 (8.4-10.0)	11.9	13	12.4	14.7	12.4	13	12.4	14.7
Foot length	FL	4.4 ± 0.3 (4.2-4.9)	6.2 ± 1.1 (5.0-7.2)	10.5	11	9.4	11.5	9.4	11	9.4	11.5
Head length	HL	4.1 ± 1.3 (2.3-5.6)	5.7 ± 1.6 (3.0-7.0)	7.5	10.3	8.8	11.2	8.8	10.3	8.8	11.2
Head width	HW	4.9 ± 0.6 (4.3-6.0)	7.2 ± 0.6 (6.8-8.2)	8.8	10	8.5	10.4	8.5	10	8.5	10.4
Interorbital distance	IOD	1.6 ± 0.4 (1.2-2.0)	2.5 ± 0.2 (2.2-2.7)	2.2	2.4	2.3	3.7	2.3	2.4	2.3	3.7
Eyelid width	EW	1.0 ± 0.1 (0.9-1.1)	1.3 ± 0.1 (1.1-1.5)	2.3	3.1	1.8	2.6	1.8	3.1	1.8	2.6
Internarial distance	IN	1.0 ± 0.1 (0.9-1.2)	1.4 ± 0.2 (1.3-1.8)	3	3.2	2.3	2.4	2.3	3.2	2.3	2.4
Eye length	EL	2.2 ± 0.3 (1.9-2.6)	2.6 ± 0.3 (2.3-3.0)	3.8	4.7	3.8	4.5	3.8	4.7	3.8	4.5
Eye-nostril	EN	1.4 ± 0.3 (1.1-1.8)	2.1 ± 0.3 (1.8-2.5)	1	1.3	2.6	3	2.6	1.3	2.6	3
Hand I		1.2 ± 0.3 (1.0-1.6)	1.7 ± 0.1 (1.5-1.8)	3.8	2.8	3	3.5	3	2.8	3	3.5
Hand II		1.8 ± 0.1 (1.8-1.9)	2.4 ± 0.3 (2.2-2.8)	4.5	7.4	4.3	5	4.3	5.1	4.3	5
Hand III		2.7 ± 0.2 (2.4-2.9)	3.9 ± 0.2 (3.7-4.3)	6.6	6.5	6.5	7.8	6.5	7.4	6.5	7.8
Hand IV		2. ± 0.1 (2.0-2.2)	3.1 ± 0.4 (2.7-3.8)	5.8	6.5	5	6.3	5	6.5	5	6.3
Width finger III disc	WFD	0.5 ± 0.1 (0.4-0.6)	0.8 ± 0.1 (0.7-0.8)	1.2	1.9	1.4	1.4	1.4	1.9	1.4	1.4
Width toe IV disc	WTD	0.5 ± 0.1 (0.4-0.6)	0.8 ± 0.1 (0.7-1.0)	1	1.7	1.4	1.6	1.4	1.7	1.4	1.6
Snout length	SL	2.3 ± 0.4 (1.6-2.8)	3.0 ± 0.3 (2.6-3.3)	6.3	8	3.2	3.5	3.2	8	3.2	3.5
Tarsus length	TAR	3.8 ± 0.3 (3.3-4.2)	5.3 ± 0.5 (4.8-5.8)	7	7.1	6	8.1	6	7.1	6	8.1
Thigh length	THI	6.5 ± 0.4 (6.0-7.0)	8.3 ± 0.8 (7.3-9.1)	10.8	13.7	11.5	13.4	11.5	13.7	11.5	13.4
Forearm length	FOR	2.5 ± 0.2 (2.5-2.7)	3.4 ± 0.6 (3.0-4.0)	5.6	6.9	5.4	6.8	5.4	6.9	5.4	6.8
Tympanum width	TYM	not visible	not visible	not visible	not visible	not visible	1.6	not visible	not visible	1.2	1.6
Vocal slits	VS	not visible	not applicable	present	not applicable	not visible	not applicable	not visible	not applicable	not visible	not applicable
Museum Number		not applicable	not applicable	UF193780	UF193779	UF193777	UF193778	UF193777	UF193779	UF193777	UF193778



Pristimantis kopinangae n. sp. (Figs. 11, 12, 13, Table 1),
Kopinang Landfrog

Pristimantis inguinalis Hedges et al. 2008

Pristimantis inguinalis MacCulloch & Lathrop 2009

Pristimantis inguinalis Cole et al. 2013

Pristimantis sp. "Ayanganna" Jablonski et al. 2017

Pristimantis sp. "Ayanganna" Kok et al. 2018c

Pristimantis sp. "Ayanganna" Fouquet et al. 2022

Holotype: UF-193779 (field number CPI-10408), an adult female (Fig. 11a, c, e, f) from the south side of Mt. Kopinang of the Wokomung Massif, Potaro-Siparuni District, west-central Guyana: 04°49'50" N, 59°52'43" W, approx. 1,385 m elevation; collected three hours after dark at approx. 9:30 p.m. in a heavy rain on 11 December 2006 by D. Bruce Means and Michelle Kalamandeen. Field collection DBM-3378.

Paratopotype: (n = 1) UF-193780, an adult male (CPI-10409) found within 25 m of the holotype (Fig. 11d); same data as for holotype.

Referred specimens: (n = 3) UF-193781, an adult male (CPI-10622) from along 100 m-footpath west of the top of Kamana Falls, Wokomung Massif, Potaro-Siparuni District, Guyana: 04°04'50" N, 59°52'43" W, approx. 1,538 m elevation, collected 22 June 2012 by Talesha Nurse and D. Bruce Means.

ROM-40163, male, Mt. Ayanganna, vicinity of camp, northeast plateau, District 7, Guyana: 05°21'43" N, 59°57'24" W, approx. 1,490 m elevation, collected 28 October 2000 by Ross MacCulloch and Amy Lathrop.

ROM-40164, female, Mt. Ayanganna, vicinity of camp, northeast plateau, District 7, Guyana: 05°21'43" N, 59°57'24" W, 1,550 m elevation, collected 31 October 2000 by Ross MacCulloch and Amy Lathrop.

Diagnosis: A medium-sized *Pristimantis* (SVL one male = 22.2 mm; one female = 25.1 mm) with the following diagnostic characters: snout short and broadly rounded with relatively large eye, (Fig. 11a, b); texture of belly areolate but undersurface of the chin and thighs shagreened (Fig. 11f); eyelid with 1-2 tubercles. Tympanic membrane absent or not visible externally; vocal slit present but vocal sac not evident in the paratype male; tongue round; choanae small, round; vomerine odontophores not apparent. Toe and finger pads fairly large, about 2 × width of the digit (Figs. 11a, b, 12a); relative length of fingers: III > IV > II > I with finger discs broadly expanded (Fig. 12a); relative length of toes IV > V > III > II > I with toe discs broadly expanded (Fig. 12b). Neither

digital webbing, fringes, nor nuptial pad visible on preserved specimens. Diagnostic colour pattern elements include 2-3 large, light yellow, flash-mark spots on the inguinal region hidden by the thigh (femur) but revealed when the leg is extended (Fig. 11a, b, e); belly colour and undersurface of chin white with dark brown vermiculations (Fig. 11f); iris blue.

Etymology: The species name *kopinangae* refers to the Patamona Amerindian village Kopinang and adjacent Mt. Kopinang, the southwestern-most high peak of the Wokomung Massif. The suggested common name, Kopinang Landfrog, refers to the village and the mountain.

Description of the holotype: An adult female 25.1 mm SVL (Figs. 11a, c, e, f, 12, 13); HW 39.8% SVL; head length (10 mm) about the same as head width (10.3 mm); not wider than body in life; head width approx. 70% of body width in live specimen (Fig. 11c, f); cranial crests absent; snout round and snout length about as long as eye length in live specimen (Fig. 11a, b); canthus rostralis not ridged; loreal region round and slightly convex; nares slightly protuberant and directed anterolaterally; eye relatively large (EL 4.7 mm/IOD 2.4 mm = 1.96); upper eyelid with one or two small tubercles; iris blue; tympanic membrane absent or invisible; lip commissure not upturned posteriorly; choanae small, round, not concealed under palatal shelf of maxilla; vomerine odontophores not apparent; tongue round, anteriorly attached to the jaw; dorsal skin smooth with scattered large tubercles; belly skin areolate; throat skin and undersurface of thighs shagreened; tubercles scattered on snout, eyelids, dorsum, and legs, with a row of tubercles along the posterior margin of both forearm and tarsus (Fig. 11a); scapular area not ridged but with a prominent, light brown "W", the tips of the two lower inflections each with a prominent tubercle; finger I 65% length of finger II; relative length of fingers: III > II > IV > I (Fig. 12a); discs on fingers II, III, IV expanded and about twice as wide as long (Fig. 12a); disc on finger I slightly expanded; TIL 52% of SVL; FL 44% of SVL; relative length of toes IV > V > III > II > I with toe discs broadly expanded (Fig. 12b); toe discs oval to subround, slightly larger than finger discs; digital webbing, fringes, and nuptial pads not evident in preserved specimens; top of short humerus unpatterned; top of forearm with two wide and one narrow, dark brown crossbands; dorsal aspect of thigh, shank and tarsus with three prominent dark brown crossbands that match up when either leg is held against the sides (Fig. 11a, b); dorsal tubercles unremarkable on live specimens except at inflection points of the scapular

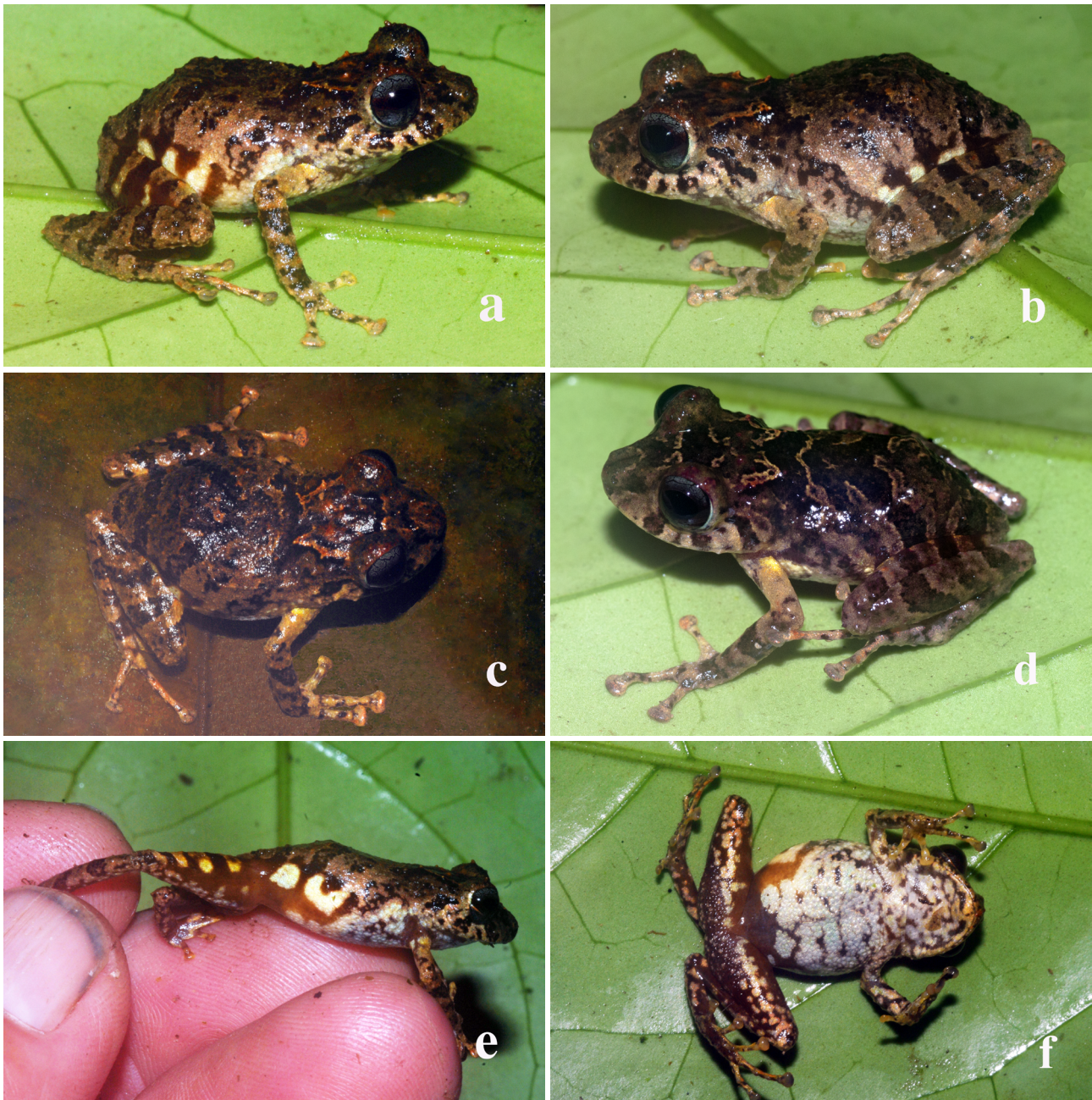


Fig. 11. *Pristimantis kopinangae* n. sp. (a, b, c, e, f) = holotype female (UF193779); d) = paratype male (UF193780).

“W” (Fig. 11a, b, c) and one or two small ones on the dorsal eyelids, all less conspicuous in preservation.

Colouration of holotype in life: Across the back, three black bands transversely cross a dark-spotted, medium brown background (Fig. 11a, c); upper sides of the body are lighter brown and less spotted with black pigment but transitioning abruptly into three bold light yellow flash-marks (Fig. 11a, b, e) that are hidden when the frog is sitting; three or four light yellow blotches appear on the anterior side of the thigh when the leg is extended (Fig. 11e); a reddish brown scapular “W” is visible and a broken, reddish brown, transverse ridge lies at the front of the black

interorbital area (Fig. 11c); belly and undersurface of the chin have dark brown vermiculations starkly set off from the prominent white colour (Fig. 11f); on the lower sides, the flash marks are separated from the white belly by a longitudinal zone tapering anteriorly of light reddish brown colour devoid of darker brown melanin spots. The undersurfaces of the thighs are dark brown to black with a marbling of light brown colour anteriorly, and paralleling a black band with tiny tubercles (Fig. 11f); iris blue (Fig. 11a, b).

Colouration of holotype in preservative: After 17 years in preservative, the reddish brown colour has disappeared, revealing the pattern formed by



Fig. 12. *Pristimantis kopinangae* undersurfaces of hand a) and foot b) while alive.

the denser melanin, although it, too, has become considerably weaker (Fig. 13). The black bands are more evident, with the middle band forming a chevron pointing forward; the scapular “W” and the dark bars across the dorsal surfaces of the arms and legs are more prominent (Fig. 13a). Ventrally, the belly skin has become translucent so that the internal organs show through (Fig. 13b). The dark vermiculations under the chin are weaker in intensity, but appear slightly wider than during life and the dark undersurfaces of the thigh- have very little of the dark pigment left (Fig. 13b).

Variation: Based on the two specimens from Mt. Kopinang, *P. kopinangae* is sexually dimorphic in size, as is usual in most frogs. Slight variation in colour pattern is evident in Figs. 11b, d.

Measurements of holotype (in mm): SVL 25.1; TIL 13.0; FL 11.0; HL 10.3; HW 10.0; IOD 2.4; EW 3.1; IN 3.2; EL

4.7; EN 1.3; HANDI 2.8; HANDII 5.1; HAND III 7.4; HAND IV 6.5; WFD 1.9; WTD 1.7; SL 8.0; TAR 7.1; THI 13.7; FOR 6.9; TYM 0.0.

Reproductive characteristics: The adult female holotype had no enlarged ova on 11 December 2006. No other reproductive characteristics are known in this species.

Advertisement call: Tympanum absent or unrecognizable. No individuals were heard vocalizing, but vocal communication cannot be ruled out.

Habitat and habits: The holotype and paratype were found about three hours after dark during a heavy rain as each was sitting about 2 m above ground on different green leaves about 25 m apart. The habitat was a closed canopy lower montane tropical forest (cloud forest), approx. 1,385 m elevation, with subcanopy shrubs and epiphytes over deep leaf litter



Fig. 13. Holotype of *Pristimantis kopinangae* after 10 years in 70% ethanol. Scale: tag is 27 mm long; a) dorsum, b) venter.



on the forest floor. A small, first-order cascading creek was nearby. No behaviours were observed. The two specimens were caught late in the evening during a hard rain as the collectors were struggling to find their way to a temporary camp. We did not smell nor taste the skin for noxious secretions.

Distribution: Known from the Wokomung Massif (Fig. 1b), and present at similar elevations on Mt. Ayanganna 40 km to the north based on the genetic affinity of specimen ROM-40164, which was collected from Mt. Ayanganna. No specimens are known, but might be expected, from the chain of tepuis running west of the River Ireng along Guyana's border with Brazil (Maringma, Yakontipu, Apokilang, Weiassipu, Roraima).

Pristimantis kalamandeenae n. sp. (Figs. 14, 15, 16, Table 1), Guyanan Black-eyed Landfrog

Pristimantis zeuctotylus Hedges et al. 2008

Pristimantis sp. (ROM-43978) Padial et al. 2014

Pristimantis sp. "Wokomung" Kok et al. 2018c

Pristimantis sp. "Wokomung" Fouquet et al. 2022

Holotype: UF-193778 (field number CPI-10393), an adult female (Fig. 14a, b, c, d) collected in amplexus with male paratopotype UF-193777 (CPI-10392) at night, from the south side of Mt. Kopinang of the Wokomung Massif, Potaro-Siparuni District, west-central Guyana: 05°00'08" N, 59°52'47" W, approx. 1,550 m elevation, on 10 December 2006 by D. Bruce Means and Michelle Kalamandeen. Field collection DBM-3376.

Paratopotype: (n = 1), UF-193777 (field number CPI-10392), adult male found in amplexus with the holotype (Fig. 14a, d), same data as for holotype.

Referred specimens: (n = 1), ROM-43978, male, Wokomung, vicinity of camp 2, District 8, Guyana; 05°06'35" N, 59°49'16" W, 1,234 m elevation, collected 31 October 2004 by Ross MacCulloch and Amy Lathrop.

Diagnosis: A medium-size *Pristimantis* (SVL one male = 22.1 mm, one female = 25.7 mm) with the following diagnostic characters: dorsal skin texture smooth to shagreen, non-tuberculate, including upper eyelids; snout long acuminate (Fig. 14a, b); a prominent canthus rostralis (Fig. 14a); tongue ovoid, attached to front of lower jaw; dentigerous process of vomers in transverse row, widely separated, posterior to large choanae not concealed by the palatal shelf of the maxilla; vomerine odontophores minute to

invisible; tympanic annulus and membrane present; vocal slit and vocal sac not recognizable in preserved paratopotype. Iris dark brown to black so that overall eye colour is completely black as in the sympatric hylid frog, *Nesorohyla kanaima* (Pinheiro et al. 2018). Relative length of fingers: III > IV > I > II with finger discs broadly expanded (Fig. 15a); relative length of toes IV > V > III > II > I with toe discs broadly expanded (Fig. 15b). Digital webbing or fringes not evident on preserved specimens. Nuptial pads not evident on preserved paratopotype male. Diagnostic colouration elements include iris dark brown or black such that eye appears completely black (Fig. 14a, b); top of head, back, arms and legs completely devoid of a pigmentary pattern, but skin colour uniformly light brown at night (Fig. 14a) changing in daytime (Fig. 14b, d) to dark reddish brown on head, face, arms and legs but slightly lighter over the posterior part of the back and sides (Fig. 14b).

Etymology: The species name *kalamandeenae* is a patronym honouring Guyanese ecologist, Michelle Kalamandeen, our friend and colleague, for her scientific research contributions to climate change reduction, sustainable and equitable development and conservation, remote sensing, and Guyana's herpetology. The suggested common name, Guyanan Black-eyed Landfrog, calls attention to the black iris that renders the entire eye colour black.

Description of the holotype: A gravid female 25.7 mm SVL; HW 40.5% of SVL; head length (11.2 mm) slightly longer than wide (10.4 mm); not wider than body; cranial crests absent; snout acuminate (Fig. 14a, b, c); canthus rostralis prominent (Fig. 14a); loreal region slightly convex; nares not protuberant and directed forward; eye relatively small (EL 4.5 mm/IOD 3.7 mm = 1.22); upper eyelid tubercles absent; iris dark brown to black so that overall eye colour is completely black (Fig. 14a, b); tympanic annulus and membrane present (Fig. 14a); tympanic membrane width approx. 35% that of eye length (TYM/EL); lip commissure not upturned; choanae relatively large and not concealed by the palatal shelf of the maxilla; dentigerous process of vomers in transverse row, widely separated, posterior to choanae; vomerine odontophores minute to invisible; tongue ovoid, attached to front of lower jaw; dorsal skin smooth to shagreened with no tubercles, including upper eyelid (in contrast with most *Pristimantis*); belly skin areolate; throat skin and undersurface of thighs weakly shagreened; tubercles, blisters, water-filled pustules absent from entire body; scapular fold or ridge absent; finger I 57% of finger II; relative length of fingers III



Fig. 14. *Pristimantis kalamandeenae* n. sp. holotype female (UF193778) in amplexus with paratype male (UF193777) at night-time moment of discovery a). Holotype female 12 hours later in daytime b) and her belly c). Male paratype and female holotype (bottom) in daytime d).

> IV > I > II with finger discs broadly expanded (Fig. 15); discs on fingers III and IV expanded and about twice as wide as long, but on finger II only about half the size of III and IV (Fig. 15); disc on finger I slightly expanded but only half the size of disc on finger II (Fig. 15); TIL 57% length of SVL; FL 45% length of SVL; relative length of toes IV > V > III > II > I with toe discs broadly expanded (Fig. 15); toe discs about $1.5 \times$ wider than long except rounder toe I disc that is about $0.5 \times$ the others (Fig. 15); digital webbing or fringes not evident on preserved specimens, nor skin folds on arms/legs.

Colouration of holotype in life: Colour pattern of all dorsal surfaces is uniformly light brown at night (Fig. 14a) or dark brown in daytime (Fig. 14b, d) with no spotting, flecking, banding, or other accumulations of pigment. Belly is uniformly light tan with a thin line of brown pigment down the midline but the chin is slightly darker with tiny flecks of brown (Fig. 14c). Palmar, plantar surfaces, and undersurfaces of the arms very dark brown, similar to the upper back, but undersurface of the thigh, shank, and tarsus similar to the chin (Fig. 14c). Iris very dark brown such that eye appears completely black (Fig. 14a, b).

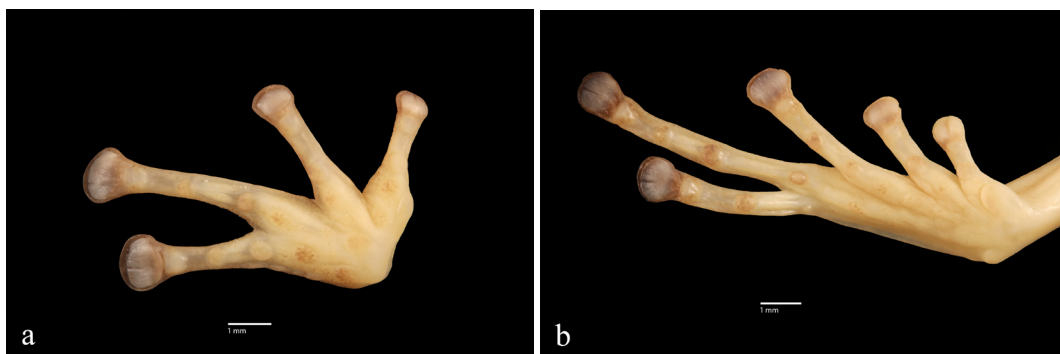


Fig. 15. Hand a) and foot b) of preserved *Pristimantis kalamandeenae*.

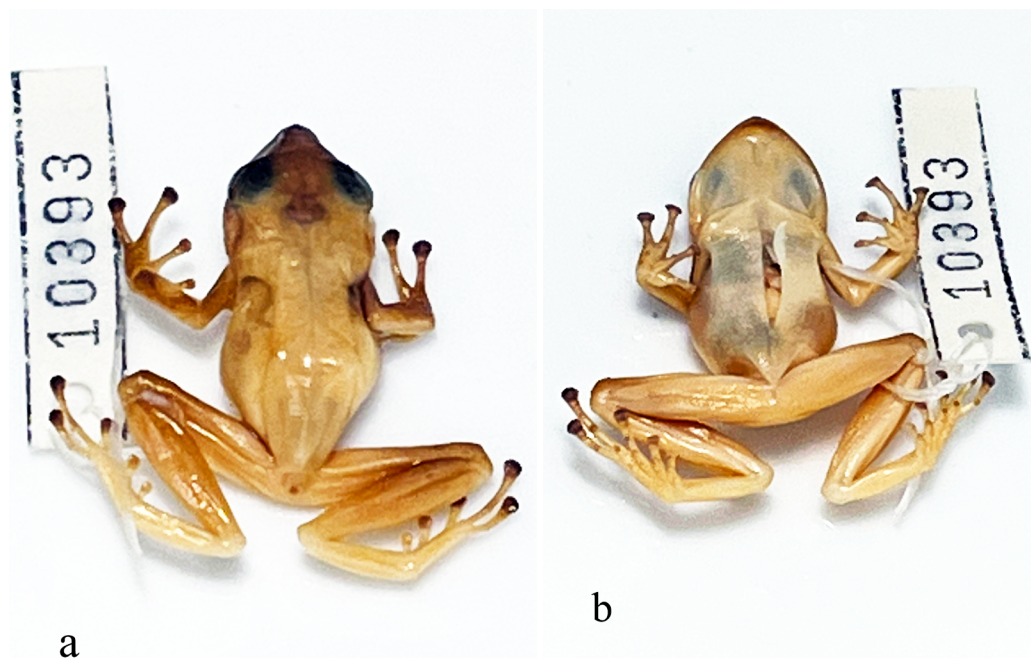


Fig. 16. *Pristimantis kalamandeenae* holotype female (UF193778) after 17 years in 70% ethanol. Scale: tag is 27 mm long; a) dorsum, b) venter.

Colouration of holotype in preservative: After 17 years in 70% ethanol, the dark dorsal pigments have lightened to a plain, light brown colour with the snout, fingers, and toe tips darker (Fig. 16a); all undersurfaces are tan (Fig. 16b).

Variation: Other than the obvious sexual size dimorphism visible in the amplexing holotype and paratopotype (Fig. 14a, d), no variation in pigmentary patterns is known.

Measurements of holotype (in mm): SVL 25.7; TIL 14.7; FL 11.5; HL 11.2; HW 10.4; IOD 3.7; EW 2.6; IN 2.4; EL 4.5; EN 3.0; HANDI 3.5; HANDII 2.0; HAND III 7.8; HAND IV 6.3; WFD 1.4; WTD 1.6; SL 3.5; TAR 8.1; THI 13.4; FOR 6.8; TYM 1.6.

Reproductive characteristics: The holotype is a gravid female in amplexus on 10 December 2006. Presumably, she would have oviposited shortly after. Amplexus was axillary (Fig. 14a). No other reproductive information is known.

Advertisement call: Distinct tympanic annulus and membrane present. Vocal slit not found in paratype. No individuals were heard vocalizing.

Habitat and habits: The amplexing holotype and paratype were found about three hours after dark on a misty night approx. 1,550 m elevation in a dense cloud forest on the NE slopes of Mt. Kopinang. The closed canopy forest had a dense subcanopy of shrubs

and small trees covered in moss and other epiphytes from the ground to the treetops. The ground was covered in decomposing leaf litter with moss-covered twisting roots, trunks, and downfallen logs. The pair was perched about 1 m above ground on the frond of a fern. Neither offensive smell nor bitter taste was detected.

Distribution: Known only from the Wokomung Massif (Fig. 1b), but likely to be present at similar elevations on Mt. Ayanganna to the north because the two montane areas are connected by a low ridge and both are drained by the River Potaro drainage system. No specimens are known from the chain of tepuis running west of the River Ireng along Guyana's border with Brazil (Maringma, Yakontipu, Apokilang, Weiassipu, Roraima).

Comparisons among *Pristimantis* of the Wokomung Massif

Comparisons are made among the following taxa, representing the *Pristimantis* species known to occur on the Wokomung Massif (Boulenger 1900, Rivero 1968, Means & Savage 2007, Kok et al. 2018c): *P. dendrobatoides*, *P. jester*, *P. kalamandeenae*, *P. koki*, *P. kopinangae*, *P. marmoratus*, *P. pulvinatus*, and *P. saltissimus*. *Pristimantis koki* can be distinguished from all other *Pristimantis* on the Wokomung Massif by its black rump (Figs. 4c, 5a). Also helpful in its field identification are its tiny size, absence of a tympanum (present in all other species except *P. jester* and *P. kopinangae*), lack of distinct vomerine teeth (present



Fig. 17. Exceptional diversity of *Pristimantis* (family Strabomantidae) on a single tepui; a) *koki*, b) *kopinangae*, c) *kalamandeenae*, d) *dendrobatoides*, e) *jester*, f) *saltissimus*, g) *marmoratus*, h) *pulvinatus*, i) *Ceuthomantis smaragdinus* (family Ceuthomantidae) sympatric with all the above and similar in size and general appearance.

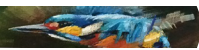
in all other species except *P. kopinangae*), granular skin with pustules (different from others except *P. marmoratus*), lack of vocal slits in males (slits present in *P. kopinangae*, *P. marmoratus*, and *P. pulvinatus*), noxious skin secretions (seemingly absent in *P. kalamandeenae*, *P. kopinangae*, *P. marmoratus*, and *P. pulvinatus*), relatively sharp-pointed snout (similar to Fig. 44a, c in Duellman & Lehr 2009), and an upturned posterior commissure. Additionally, many specimens instantly can be recognized when the dark, middorsal saddle is present contrasting with the light cream-coloured sides (Figs. 4d, 5a). On live specimens a dark brown or black bar between the eyes and/or a broken line of dark spots over the shoulders associated with two or more raised tubercles can also be helpful.

Pristimantis kopinangae is distinguished from other *Pristimantis* on the Wokomung Massif by its relatively large, blue iris (iris gold, brown, or black in other species), absence of a tympanum (present in all other species except *P. jester* and *P. koki*), lack of distinct vomerine teeth (present in all other species except *P. koki*), presence of vocal slits in males (absent in all other species except *P. marmoratus* and *P. pulvinatus*),

tuberculate dorsal skin (differs from others except *P. dendrobatoides*), short round snout, and light yellow inguinal flash marks, hidden by the adpressed legs until the species jumps. Its white areolate belly with dark brown vermiculations is distinctive. It is most easily confused with *P. marmoratus* and *P. pulvinatus* (Fig. 17g, h, respectively), but unlike these species, lacks an obvious vocal sac and has a blue iris.

Pristimantis kalamandeenae is easily distinguished from other *Pristimantis* on the Wokomung Massif by its unusual uniform dorsal colouration with no pattern, dark brown irises rendering the eye black in appearance, and acuminate snout. It further differs from other *Pristimantis* of the Wokomung Massif by possessing a smooth dorsum (shagreen to granular or tuberculate in other species except *P. jester*), seemingly lacking malodorous skin secretions (present in four other syntopic congeners, *P. dendrobatoides*, *P. saltissimus*, *P. jester*, *P. koki*), and possessing a distinct tympanum (tympanum absent or indistinct in *P. jester*, *P. koki*, *P. kopinangae*, and *P. saltissimus*).

Among the Pakaraima Mountains from Maringma to Yuruani, all three species we describe here can be



distinguished from *P. aureoventris*, known from Mt. Roraima and Weiassipu tepui, by the latter's gold-flecked belly (Kok et al. 2011); and from Yuruani's and Kukenan's *P. yuruaniensis* by the latter's uniform dorsal colouration combined with its round, non-acuminate, snout (Rödler & Jungfer 2008).

Discussion

Frogs of the genus *Pristimantis* were once thought to be part of a large vertebrate group loosely termed "eleutherodactyline" frogs (Lynch & Duellman 1997). Hedges et al. (2008) analysed DNA sequences from mitochondrial and nuclear genes to estimate the phylogenetic relationships among the "eleutherodactyline" frogs. These authors reclassified this large vertebrate group into the unranked clade Terraranae and identified four families: Brachycephalidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae. A fifth family, Ceuthomantidae, was subsequently identified (Heinicke et al. 2009). *Pristimantis* is part of the large family Strabomantidae, which some authors synonymize with Craugastoridae (e.g. Barrientos et al. 2021). *Pristimantis* and other Strabomantidae are largely South American, with a large representation in the Andes (Duellman & Lehr 2009). As Pantepui – part of the ancient heartland of South America – is explored, it is emerging as an important centre of species richness, endemism, and possibly antiquity. *Pristimantis dendrobatoides*, for instance, is one of the basalmost *Pristimantis* (Fig. 3). Other terraranan genera with distributions centred on the Guiana Shield include *Adelophryne*, *Ceuthomantis*, and *Dischidodactylus*.

It is interesting that *P. dendrobatoides* has noxious skin secretions and is aposematic. Until recently, bioactive skin secretions had not been found in the large Strabomantidae radiation. Alkaloids were found to be lacking in skin extracts from nine species (*Craugastor fitzingeri*, *C. gollmeri*, *C. longirostris*, *Eleutherodactylus jamaicensis*, *Ischnocnema guentheri*, *I. parva*, *Pristimantis caryophyllaceus*, *P. gaigeae*, and *P. vilasi*) examined by Daly et al. (1987) and J.W. Daly (pers. comm.). Also, Roseghini et al. (1986) reported that neither serotonin, histamine, nor peptides active on smooth muscle preparations were detected in skin from four species (*Craugastor ranoides*, *C. rugulosus*, *Strabomantis bufoniformis*, and *S. cornutus*) and Daly & Heatwole (1966) reported that serotonin and histamine could not be detected in skin extracts from seven species (*Eleutherodactylus eneidae*, *gryllus*, *karlschmidti*, *locustus*, *portoricensis*, *richmondi*, and

wightmanae), nor were the extracts toxic to mice or active on smooth muscle preparations. A dipeptide carnosine was present, but a possible function for this dipeptide was not apparent.

Means & Savage (2007) first reported disagreeably smelly and bad tasting skin secretions from three species of *Pristimantis* from the Wokomung Massif. *Pristimantis koki* is the fourth species of landfrog described from the massif having distasteful and malodorous skin secretions. Noxious skin secretions often occur with aposematic colouration. While the colour pattern of *P. koki* is probably cryptic, providing camouflage against the multicoloured cloud forest vegetation, at least two other *Pristimantis* on the Wokomung Massif (*P. dendrobatoides*, *P. jester*) are clearly aposematic (see Fig. 17d, e). Future research on these frogs, the Wokomung Massif, and potential arthropod sources of the disagreeable or noxious skin chemicals could be crucial for understanding the evolution of aposematism.

The three new species of *Pristimantis* described here (*P. koki*, *P. kopinangae*, *P. kalamandeenae*) join three others previously described from the Wokomung massif (*P. dendrobatoides*, *P. jester*, *P. saltissimus*, Means & Savage 2007), and two others (*P. marmoratus*, *P. pulvinatus*) also known from Wokomung (Fig. 17, Kok et al. 2018c). Summing up, these eight species of *Pristimantis* (Fig. 17) all live in the same cloud forest environments of the same tepui between about 1,250 m and 1,650 m in elevation. Looking very similar, and living in the same habitat and locality with all these *Pristimantis*, is the related terraranan *Ceuthomantis smaragdinus* (Fig. 17i). The Wokomung Massif harbours more species of landfrogs than is known from any other tepui in the Guiana Shield Highlands (i.e. Pantepui) and more species are likely to be recognized on the large massif. It is not surprising that at least one of the three new species described here is also found on Mt. Ayanganna because Mt. Ayanganna and the Wokomung Massif are physically close and both are partially drained by the River Potaro. One might also expect them to be found to the west across the River Ireng in cloud forest habitats on the chain of Guyana tepuis (Maringma, Yakontipu, Appokilang, Weiassipu) leading to and including Mt. Roraima. However, in spite of two herpetological expeditions to Maringma, four to Weiassipu, and numerous others to Mt. Roraima (MacCulloch et al. 2007, Kok et al. 2011, Means 2023), it is significant that *P. koki* has not been found west of the River Ireng but is so common across the river on the Wokomung Massif. The other two species (*P. kopinangae*, *P. kalamandeenae*) were so

difficult to find on the Wokomung Massif (only one pair of adults in five expeditions), it is not surprising they may have been missed.

Acknowledgements

Expeditions were funded in 2003 by the Smithsonian Institution, Conservation International, and National Geographic Society Research Grant #7498-03; in 2004 by ROM Reproductions Acquisitions Fund and ROM Department of Natural History; in 2006 by D.B. Means; in 2007 by NIH to Jerrold Meinwald and Valerie Clark of Cornell University; in 2012 by National Geographic Expeditions Council grant EC0504-11. We thank the Guyana Environmental Protection Agency for permission to conduct biodiversity research (permits 100703 BR 009;

011206 BR 065; 120707 BR 075; 061212 BR 020) and to export specimens for taxonomic analysis (permits 030310 SP 001; 151206 SP 030; 230707 SP 008; 072412 SP 054).

Author Contributions

D.B. Means, R.D. Macculloch, and A. Lathrop conducted the fieldwork; M.P. Heinicke and S.B. Hedges did the DNA sequence collection and analyses; and all five participated in writing the manuscript.

Data Availability Statement

New sequence data supporting this study's findings are available on Genbank: accession numbers OQ884602-OQ884622 at <https://www.ncbi.nlm.nih.gov/genbank/>.



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Supplementary online material

Table S1. GenBank accession numbers of samples used in this study. Specimen numbers are institutional catalogue numbers when available, specimen numbers in italics are field or collector numbers reported in the studies where these sequences were published. Note that sequence accession numbers beginning with “KDQF” are subsamples from a targeted locus study accessible through GenBank accession number KDQF00000000. Institutional acronyms: AMNH (American Museum of Natural History), BWMC (Bobby Witcher Memorial Collection, Avila University), CORBIDI (Center of Ornithology and Biodiversity), CPI (Coastal Plains Institute), CVULA (Collection of Vertebrates, University of the Andes), ICN (Institute of Natural Sciences, National University of Colombia), KU (University of Kansas Biodiversity Institute), LSUMZ (Louisiana State University Museum of Zoology), MHNSM (Museum of Natural History, San Marcos National University, Lima, Peru), MTD (Senckenburg Museum of Zoology), MVZ (University of California Museum of Vertebrate Zoology), QCAZ (Zoology Museum, Pontifical Catholic University of Ecuador), ROM (Royal Ontario Museum), UF (University of Florida Museum of Natural History), USNM (Smithsonian National Museum of Natural History), VUB (Free University of Brussels) (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-72-2023-Means-D.B.-et-al.Table-S1-1.pdf>)

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