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A new species of bamboo-dwelling *Ranitomeya* (Anura: Dendrobatidae) from the Upper Purus River Basin of Brazil and Peru

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Abstract

A new species of *Ranitomeya* from Amazonian lowland forests in western Brazil and southeastern Peru is described and named. This species was formerly considered to be an outlying population of *R. sirensis* on the far eastern periphery of its geographic distribution. We analyze new and existing phylogenomic data and infer that the new species is not part of, or closely related to *R. sirensis,* but is sister to a clade including *R. aetherea*, *R. aquamarina*, *R. cyanovittata*, *R. flavovittata*, and *R. yavaricola.* The new species can be distinguished from species in its sister clade by its color pattern (yellow dorsal stripes, finely spotted ventral pattern, and a distinctive black band separating the gular and belly regions), and from *R. sirensis* by the presence/absence of a ventral color patch (absent in the new species, present in *R. sirensis*). Calls of the new species are longer in duration, with more pulses per call, and a slightly higher pulse rate, than any of the species in its sister clade for which call data are available. The new species is strongly associated with native *Guadua* bamboo, which it uses for reproduction. Based on museum records the new species also occurs in northern Bolivia. Unlike other close relatives, which are mostly monogamous, males of the new species appear to be polygynous, recruiting multiple females per breeding site.

Key words: Anura, poison dart frog, ultraconserved elements, phylogenomics, bioacoustics

Introduction

Ranitomeya Bauer, 1986, of the family Dendrobatidae, contains 18 species, all of which have predominantly (if not exclusively) Amazonian distributions. Within this genus, eight species are members of the so-called *vanzolinii*

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group: *Ranitomeya aetherea* Koch *et al.*, 2025, *R. aquamarina* Mônico *et al.*, 2025, *R. cyanovittata* Perez-Peña *et al.,* 2010, *R. flavovittata* (Schulte, 1999), *R. imitator* (Schulte, 1986), *R. sirensis* (Aichinger, 1991), *R. vanzolinii* (Myers, 1982), and *R. yavaricola* Perez-Peña *et al.*, 2010. Species in this group are clearly distinguishable from other *Ranitomeya* species on the basis of their advertisement call, consisting of a tonal trill (versus short, atonal buzz in other *Ranitomeya*), as well as aspects of life history, with at least three species (*R. imitator, R. flavovittata,* and *R. vanzolinii*) exhibiting biparental care and facultative egg-feeding (Brown *et al.* 2010; Caldwell & de Oliveira 1999; Tegnér 2014).

Within the *vanzolinii* group, species delimitation has been hampered by two main challenges. First, many of the known species occur in remote areas across vast distances, impeding specimen collection as well as an understanding of intraspecific variation across their ranges. This is particularly the case for species occurring near the Peru-Brazil border and western Brazil. Of the five species known from this area (*R. aetherea, R. aquamarina, R. cyanovittata*, *R. vanzolinii*, and *R. yavaricola*), all except *R. vanzolinii* were originally described from a single locality. Second, the continued reliance on mitochondrial DNA sequences for species delimitation in this group is problematic, as studies using nuclear markers have documented substantial conflict between mitochondrial and nuclear phylogenies in this group (Muell *et al.* 2022).

In the last major revisionary work on *Ranitomeya* (Brown & Twomey *et al.* 2011), the definition of *R. sirensis* was expanded, having subsumed *R. lamasi* and *R. biolat* (Morales, 1992) as junior synonyms. In the same publication, an outlying population of *Ranitomeya* occurring near Rio Branco, Acre, Brazil, was allocated to *R. sirensis* based on morphology, although this population was not placed in a phylogeny. Later, Twomey *et al.* (2023), sequencing nuclear ultraconserved elements (UCEs), included this population in a phylogeny for the first time ("*Ranitomeya* sp. Catuaba") and found that it was not closely related to *R. sirensis*, but rather more closely related to a clade containing *R. yavaricola, R. cyanovittata*, and *R. flavovittata*. In the year prior, Muell *et al.* (2022) published a UCE-based phylogeny with greater sampling within the *vanzolinii* group, but which lacked "*Ranitomeya* sp. Catuaba"; thus, the taxonomic implications of the two studies in isolation, each of which both included and lacked key samples, were unclear. In this study, we analyze the phylogenomic data from these two studies and add sequence data from two additional specimens of "*Ranitomeya* sp. Catuaba", including one from the Sepahua River, Peru, 540 km to the west of the Brazilian locality near Rio Branco. Our analyses provide a clear phylogenetic framework for the recognition of these populations of *Ranitomeya* as a new species, which we describe herein.

Materials and methods

Specimen collection

Museum abbreviations follow Sabaj (2020): Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (MCP), Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), Universidade Federal do Acre, Rio Branco, Brazil (UFAC-RB), Carnegie Museum of Natural History, Pittsburgh, USA (CM), Museo de História Natural Noel Kempff Mercado, Santa Cruz, Bolivia (MNK), and Museo de Biodiversidad del Perú, Cusco, Peru (MUBI).

Ten individuals of the new species were collected from Fazenda Experimental Catuaba, Acre state, Brazil on 6 March 2016 by ET and PRMS. Seven of these specimens were skinned post-mortem for skin pigment analysis but two (MCP 13635 and MNRJ 91674) were vouchered whole. Another two specimens were collected from a site 66 km E from Sepahua, Ucayali department, Peru, on 22 February 2014 by JMP, LAG, JCC, and RG. Frogs were euthanized with a lidocaine injection. Carcasses of skinned specimens were preserved in 100% ethanol and tissue samples were taken from the preserved carcass immediately prior to DNA extraction. Whole specimens were preserved in 70% ethanol with a separate tissue sample stored in 100% ethanol. Specimens were collected under the permit numbers Sisbio 7876-1, Sisbio 7486-1, and ICMBio nº. 19347-1 issued by the Brazilian government, #004-2014-SERNANP-PNAP issued by the Peruvian National Service of Natural protected Areas (SERNANP), and #192-2015-SERFOR-DGGSPFFS issued by the Peruvian National Forest and Wildlife Service (SERFOR). Tissues were exported for sequencing under the permit numbers Ibama/Siscites 136579 issued by the Brazilian government and AI 060-2016-SERFOR issued by SERFOR, and with CITES #16 PE 000768. Additionally, we reviewed the collections of UFAC-RB and MNRJ and found 15 specimens that could be allocated to the new species (specimen details given below).

Phylogenetic data and analysis

Of the 25 samples included in our phylogenetic analysis, 23 came from two previous studies (Muell *et al.* 2022; Twomey *et al.* 2023), both of which targeted UCEs. This dataset included one outgroup terminal (*Ranitomeya ventrimaculata*) and at least one terminal from every recognized species in the *vanzolinii* group: seven *R. sirensis*, two *R. vanzolinii*, four *R. imitator*, two *R. yavaricola*, one *R. cyanovittata*, and three *R. flavovittata.* We included one sample from Envira, Amazonas, Brazil, which we can assign with confidence to *R. aquamarina* based on relative proximity to the type locality (approx. 60 km, same side of Jurua River) and color pattern similarity. Mônico *et al.* (2025) reached the same conclusion regarding the Envira population. We also included one sample from Eirunepe, Amazonas, Brazil, which we can assign to *R. aetherea* based on proximity to the type locality (approx. 52 km, same side of Jurua River) and color pattern similarity, a conclusion reached by Koch *et al.* (2025) in the description of this species.

For the two samples new to this study, tissue samples consisted of a piece of thigh muscle preserved in 100% ethanol. Genomic DNA was extracted from MCP 13506 with the Promega Wizard extraction kit and from MUBI 14866 with the Qiagen Dneasy extraction kit. Extracted DNA was processed following the same protocols as Twomey *et al.* (2023), and library preparation and Illumina sequencing of UCEs was done following Faircloth *et al.* (2012). The Tetrapod UCE 5Kv1 probe set was used to target 5060 UCE loci, and roughly 1–2 million paired-end 150 bp reads were sequenced per sample. Accession numbers for UCE sequencing reads are listed in Table 1.

TABLE 1. Vouchers and localities of samples included in the phylogenetic analysis. Acession numbers are for raw sequencing reads. Accession prefixes "SRX" and "ERS" indicate Genbank and European Nucleotide Archive, respectively.

Sample	Locality	Lat	Long	Accession	Data source
Ranitomeya aetherea Eirunepe BR MCP 13495	10 km N Eirunepe, Amazonas, Brazil	-6.5829	-69.9042	ERS16172551	Twomey *et al.* 2023
Ranitomeya aquamarina Envira BR MCP 13492	10 km SE Envira, Amazonas, Brazil	-7.5080	-69.9563	ERS16173478	Twomey *et al.* 2023
Ranitomeya cyanovittata N Cruzeiro do Sul BR MCP 13502	50 km NW Cruzeiro do Sul, Amazonas, Brazil	-7.3867	-73.0585	ERS16172553	Twomey *et al.* 2023
Ranitomeya flavovittata Jenaro Herrera PE 0456	Jenaro Herrera, Loreto, Peru	-4.9039	-73.6681	SRX13129406	Muell *et al.* 2022
Ranitomeya flavovittata Requena PE ET16144	Requena, Loreto, Peru	-5.0460	-73.8254	ERS16172552	Twomey *et al.* 2023
Ranitomeya flavovittata Rio Tahuayo PE 0076	Quebrada Blanco, Loreto, Peru	-4.3584	-73.1844	SRX13129405	Muell *et al.* 2022
Ranitomeya hwata **sp. nov.** Catuaba BR MCP 13485	24 km E Rio Branco, Acre, Brazil	-10.0727	-67.6239	ERS16173477	Twomey *et al.* 2023
Ranitomeya hwata **sp. nov.** Catuaba BR MCP 13506	24 km E Rio Branco, Acre, Brazil	-10.0727	-67.6239	ERS26514762	This study
Ranitomeya hwata **sp. nov.** E Sepahua PE MUBI 14866	66 km E Sepahua, Ucayali, Peru	-11.0527	-72.4565	ERS26514763	This study
Ranitomeya imitator Micaela Bastidas PE ET16006	Micaela Bastidas, Loreto, Peru	-5.9554	-76.2424	ERS16172550	Twomey *et al.* 2023
Ranitomeya imitator San Jose PE ET16038	Cainarachi valley, San Martin, Peru	-6.4242	-76.2850	ERS16172549	Twomey *et al.* 2023
Ranitomeya imitator Sauce PE ET16051	Sauce, San Martin, Peru	-6.7073	-76.1965	ERS16172548	Twomey *et al.* 2023
Ranitomeya imitator Varadero PE ET16062	Vardero, Loreto, Peru	-5.6821	-76.4171	ERS16172547	Twomey *et al.* 2023

.....continued on the next page

TABLE 1. (Continued)

Sample	Locality	Lat	Long	Accession	Data source
Ranitomeya sirensis Alto Chivas PE 0089	17 km S Puerto Bermudez, Pasco, Peru	-10.4463	-74.9393	SRX13129416	Muell *et al.* 2022
Ranitomeya sirensis Codo del Pozuzo PE 0086	Codo del Pozuzo, Huanuco, Peru	-9.7349	-75.5104	SRX13129417	Muell *et al.* 2022
Ranitomeya sirensis Contamana PE 0091	16 km N Contamana, Loreto, Peru	-7.2160	-74.9490	SRX13129419	Muell *et al.* 2022
Ranitomeya sirensis Cordillera El Sira PE 031	Cordillera El Sira, Huanuco, Peru	-9.4636	-74.8175	SRX13129414	Muell *et al.* 2022
Ranitomeya sirensis Puerto Inca PE 0093	Puerto Inca, Huanuco, Peru	-9.3672	-74.9379	SRX13129415	Muell *et al.* 2022
Ranitomeya sirensis "biolat" Pagoreni PE 0465	Rio Urubamba, Cusco, Peru	-11.6954	-72.9531	SRX13129421	Muell *et al.* 2022
Ranitomeya sirensis "biolat" Rio Los Amigos PE 0053	Los Amigos Biological Station, Madre de Dios, Peru	-12.5670	-70.1000	SRX13129420	Muell *et al.* 2022
Ranitomeya vanzolinii Porto Walter BR LSUMZ-13658	Porto Walter, Acre, Brazil	-8.2587	-72.7770	SRX13129422	Muell *et al.* 2022
Ranitomeya vanzolinii unknown locality 0826	unknown			SRX13129423	Muell *et al.* 2022
Ranitomeya ventrimaculata Rio Momon PE 0827	Rio Momon, Loreto, Peru	-3.6250	-73.3276	ERS16172538	Twomey *et al.* 2023
Ranitomeya yavaricola Rio Yavari PE 0173	Rio Yavari, Loreto, Peru	-4.4597	-71.7510	SRX13129409	Muell *et al.* 2022
Ranitomeya yavaricola Rio Yavari PE 0174	Rio Yavari, Loreto, Peru	-4.4597	-71.7510	SRX13129408	Muell *et al.* 2022

For both new and published sequence data, raw sequencing reads were processed with Illumiprocessor version 2.10 (Faircloth 2013) to remove adapter contamination and low quality bases. We used Phyluce 1.7.3 (Faircloth 2016) to assemble cleaned reads into contigs, extract UCE loci from the pools of contigs, and to identify, align, and trim homologous UCE loci for phylogenetic analysis. Assembly within Phyluce was done with Spades using default parameters (Bankevich *et al.* 2012; Prjibelski *et al.* 2020). Contigs were matched to UCE loci using the *Tetrapods-UCE-5Kv1.fasta* probe set. UCE loci were aligned with MAFFT (Katoh & Standley 2013), edge-trimmed, and further trimmed with Gblocks, all done within Phyluce under default settings. For phylogenetic analysis we used a 75% complete matrix (i.e., a UCE locus must be present in at least 75% of taxa to be retained), which resulted in a set of 2202 UCE loci for analysis.

We used ModelFinder (Kalyaanamoorthy *et al.* 2017) to search for the best partition scheme and models of nucleotide evolution for the UCE loci. This was run with *-m TESTNEWMERGEONLY* and *-rcluster 30* options. ModelFinder selected 47 partitions for the dataset. In total, there were 17,184 parsimony-informative sites and 28,921 singleton sites in our final phlogenetic dataset. A maximum likelihood tree search was performed with IQTREE2 (Minh *et al.* 2020) using the partition scheme from ModelFinder. The tree search was done with the following search parameters: *-allnni -nstop 1000 -ninit 1000 -ntop 50 -nbest 10.* Gaps were treated as missing data. Branch support was assessed with 1000 replicates of the ultrafast bootstrap (*-bb 1000*) and 1000 replicates of the SH-like approximate likelihood ratio test (*-alrt 1000*).

Measurements

Measurements of the new species were taken with a digital caliper and from standardized high-resolution scaled photographs of specimens using the "Measure" tool of GIMP 2.10.0. Following Brown & Twomey *et al.* (2011)

and Mônico *et al.* (2025), we measured to the nearest 0.1 mm snout-vent length (SVL), femur length from middle of vent to lateral surface of knee (FL), tibia length from heel to lateral surface of knee (TL), upper arm length measured ventrally from insertion of arm to outer corner of elbow (UAL), forearm length measured ventrally from base of hand to corner of the elbow (FAL), foot length from proximal edge of outer metatarsal tubercle to tip of toe IV (FOL), hand length from proximal edge of palmar tubercle to tip of longest finger (HAL), head length from tip of snout to angle of jaw (HL), head width between tympana (HW), body width under axilla (BW), upper eyelid width (UEW), interorbital distance (IOD), internarial distance (IND), tympanum diameter (TD), eye length (EL), distance from outer corner of eye to tympanum (DET), length of finger I from proximal edge of palmar tubercle to tip of finger disc (L1F), length of finger II from proximal edge of palmar tubercle to tip of finger disc (L2F), width of disc of finger III (W3D), and width of finger III just below disc (W3F). Sex was determined through inspection of vocal slits and through field observations. For one specimen (UFAC-RB 4585) which was transporting a tadpole but vocal slits were concealed, we verified the sex by direct inspection of the gonads.

Bioacoustics

Advertisement calls of three males of the new species were recorded with a Sony ICX-50 digital recorder by PRMS on 24 January 2009 and 29 November 2009 at the type locality. Temperature was not recorded. Additionally, three videos of calling *R. flavovittata* from Rio Tahuayo were available from F. Tegnér (available at https://www.youtube.com/@teggner; date of access 6 May 2022); audio was extracted from the videos and calls were analyzed. We measured four bioacoustic variables that have been commonly used in this group (Brown & Twomey *et al.* 2011; Mônico *et al.* 2025; Twomey *et al.* 2015): call length (length of a call, measured from the start of the first pulse to the end of the last pulse; s), pulses per call, pulse repetition rate (pulses/s, calculated by dividing the number of pulses in a call by its length), and dominant frequency (frequency at which peak amplitude is reached; Hz). This terminology follows the note-centered approach sensu Köhler *et al.* (2017), for consistency with previous research on this group. Calls of the new species were compared to closely related species based on previously published works (Brown & Twomey *et al.* 2011; Koch *et al.* 2025; Mônico *et al.* 2025; Perez-Peña *et al.* 2010; Twomey *et al.* 2015).

Data availability

Raw sequencing reads from the two samples new to this study are available on the European Nucleotide Archive with the study accession PRJEB96489. Other data from this study are available on Zenodo with the DOI: 10.5281/zenodo.16909701. This repository includes adapter sequences for the sequencing reads, DNA alignment and partition scheme, phylogenetic tree, raw call recordings of the new species, and raw call measurements taken from these recordings.

Results

Our phylogenetic analysis found that the three terminals of the new species form a clade that is sister to a clade containing *R. flavovittata*, *R. yavaricola, R. cyanovittata*, *R. aquamarina*, and *R. aetherea* (Fig. 1). Based on this topology and the values of associated support indices (= 100 for all relevant nodes), we can reject the arrangement that considered the new species as part of *R. sirensis* (sensu Brown & Twomey *et al.* 2011). *Ranitomeya sirensis* is paraphyletic in our phylogeny, with the populations from central Peru showing a sister relationship with *R. vanzolinii*, and populations from southern Peru sister to *vanzolinii* + *sirensis.* One of these southern samples (Rio Los Amigos, PE) originates from close to the type locality of *R. biolat*, a species currently in synonymy with *R. sirensis,* which suggests that the taxonomy of the *sirensis/vanzolinii* clade is in need of revision. In the meantime, our results also confirm that the name *biolat* does not apply to these populations, suggesting instead that it is a new and undescribed species.

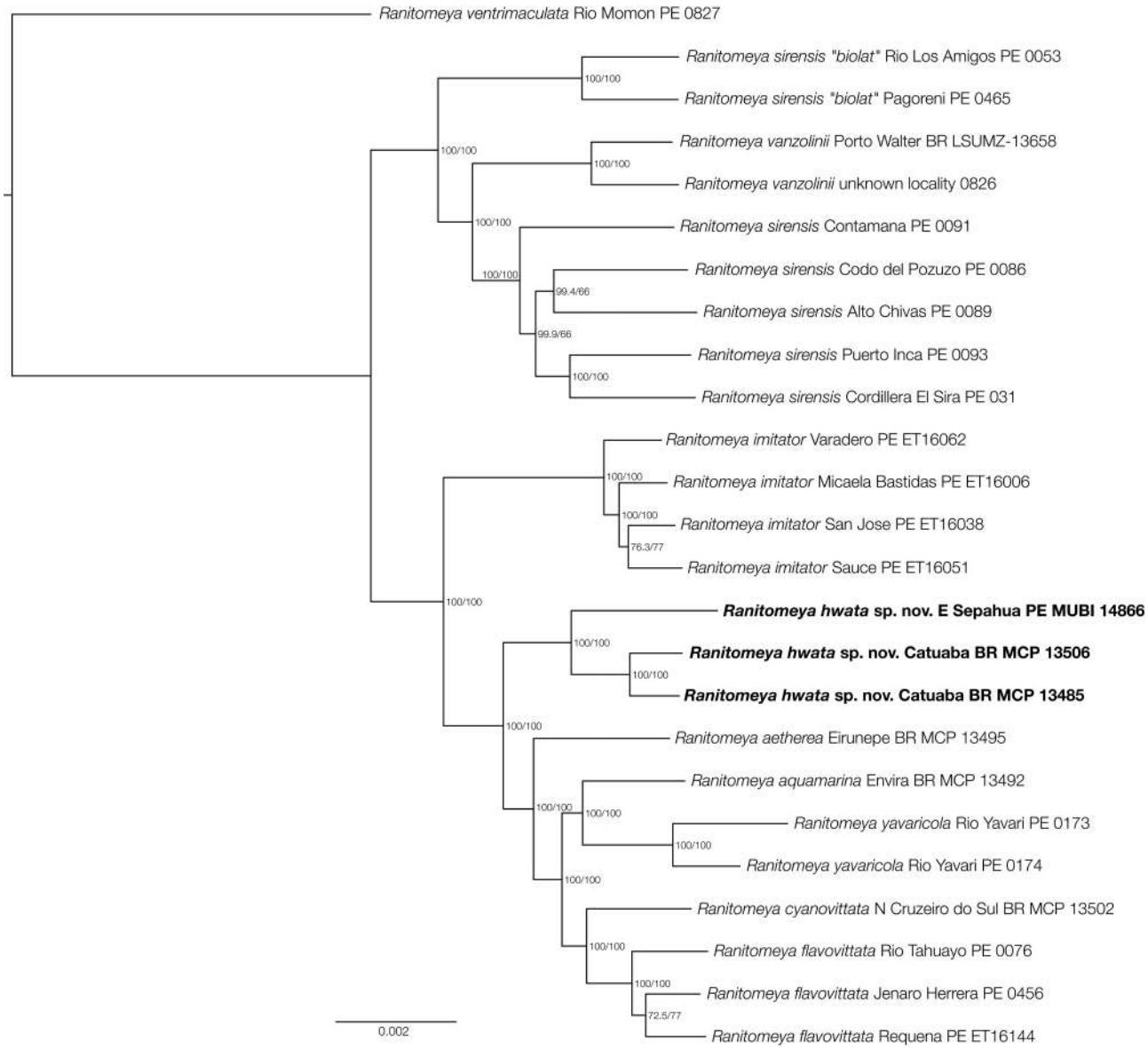

FIGURE 1. Maximum likelihood phylogeny of the *vanzolinii* group based on 2202 UCEs. Samples of the new species shown in bold. Numbers at nodes are SH-aLRT support and ultrafast bootstrap support, respectively.

Ranitomeya hwata **new species**

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Figures 2, 3, 4 & 6

Ranitomeya biolat—Maldonado & Reichle, 2007 (MNK 8251; Fig. 1); Melo-Sampaio & Souza, 2009; Melo-Sampaio, 2010
Dendrobates quinquevittatus—Souza, 2010 (UFAC-RB field series 2994; Figure 3.16E)
Ranitomeya sirensis—Brown, Twomey, Amézquita, Souza, Caldwell, Lötters, von May, Melo-Sampaio, Mejía-Vargas, Perez-Peña, Pepper, Poelman, Sanchez-Rodriguez, and Summers, 2011 (part: referred specimens from Acre, Brazil and Pando, Bolivia, e.g. Fig. 25 M)
Ranitomeya sp. Catuaba—Twomey, Melo-Sampaio, Schulte, Bossuyt, Brown, and Castroviejo-Fisher, 2023 (specimen MCP 13485 placed in phylogeny, Fig. 1); Melo-Sampaio, 2023

Holotype. MNRJ 91674 (Fig. 2), an adult male collected on 16 March 2016 by Paulo Melo-Sampaio and Evan Twomey at Fazenda Experimental Catuaba, Senador Guiomard, state of Acre, Brazil (10.0727°S, 67.6239°W), 200 m elevation.

FIGURE 2. Holotype of *Ranitomeya hwata* sp. nov., MNRJ 91674. Dorsal (left) and ventral (right) views. SVL = 15.3 mm. Photos courtesy of Jose Pombal Jr. and Manoela Woitovicz Cardoso.

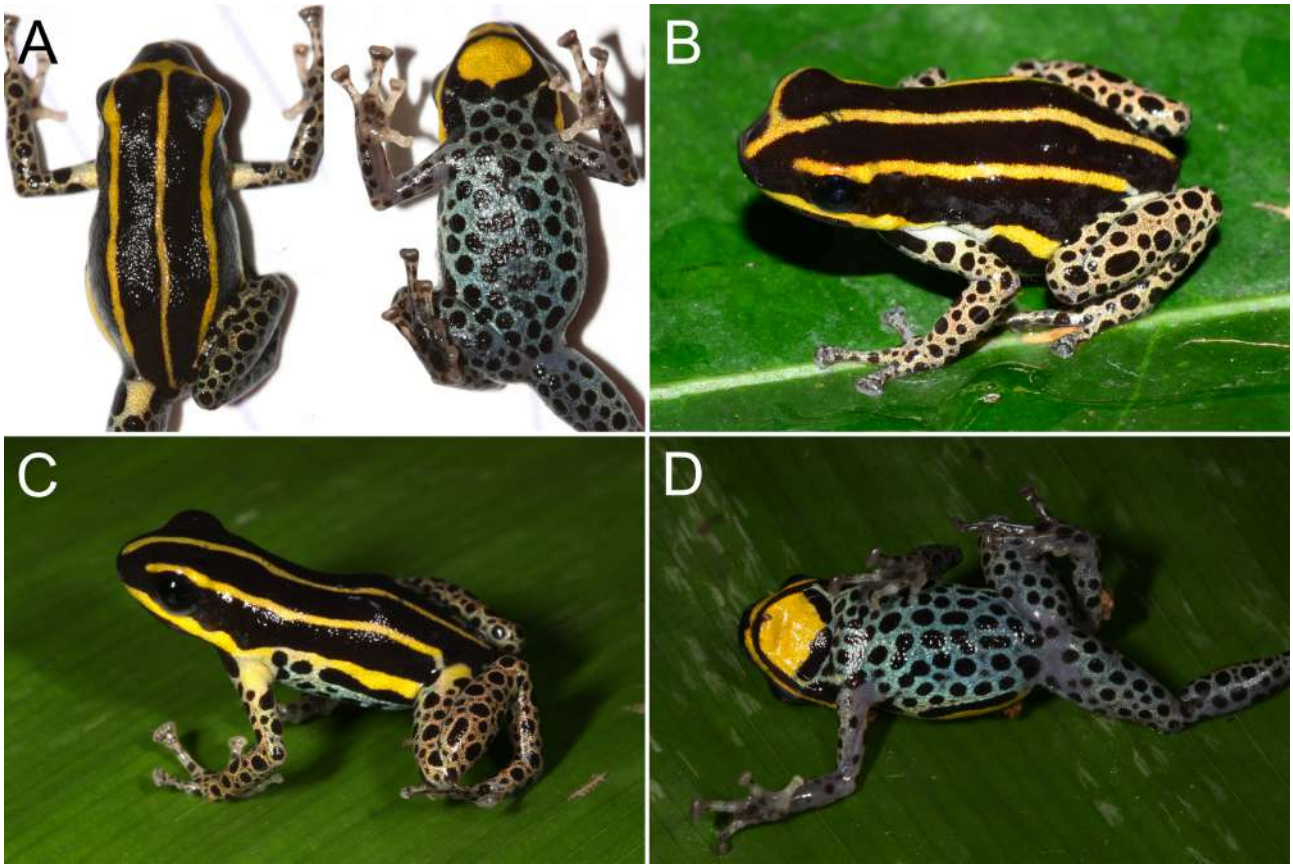

FIGURE 3. *Ranitomeya hwata* sp. nov. in life. (A) Dorsal and ventral view of MCP 13506 from the type locality, Fazenda Experimental Catuaba, Acre, Brazil (ET). (B) Dorsal view of MNRJ 95832 from Reserva Extrativista Arapixi, Amazonas, Brazil (PRMS). (C & D) Dorsal and ventral view respectively of MUBI 14866, of 66 km E from Sepahua, Ucayali, Peru (JMP).

TABLE 2. Measurements of *Ranitomeya hwata* **sp. nov.** type series. Abbreviations are defined in text. Measurements are given in millimeters.

	SVL	FL	TL	UAL	FAL	FOL	HAL	HL	HW	BW	UEW	IOD	IND	TD	EL	DET	L1F	L2F	W3D	W3F	SEX
MNRJ 91674 (holotype)	15.3	6.6	6.3	2.9	3.1	5.7	4.1	4.2	4.9	5.0	1.3	2.3	1.6	0.5	1.8	0.5	2.2	2.8	0.9	0.4	M
UFAC-RB 0419	14.6	6.8	6.4	3.7	3.7	5.7	4.2	4.0	5.1	5.0	1.1	1.8	1.7	0.9	1.8	0.5	2.2	2.7	0.9	0.4	M
UFAC-RB 3678	15.2	6.5	6.3	3.1	3.5	6.3	4.1	5.1	5.4	5.0	1.1	2.2	1.7	0.9	2.0	0.6	2.5	2.6	1.0	0.4	M
UFAC-RB 4157	15.3	6.7	6.2	3.8	3.8	6.0	4.0	4.4	4.5	4.9	1.4	2.3	1.6	1.0	1.8	0.6	2.1	2.6	1.0	0.4	F
UFAC-RB 4160	14.0	6.4	6.3	3.7	3.4	5.8	4.0	3.7	4.5	4.1	1.1	1.7	1.5	0.7	1.6	0.6	2.1	2.8	0.7	0.4	F
UFAC-RB 4585	14.9	6.5	6.1	3.5	3.3	5.8	4.0	4.3	5.0	5.0	1.3	2.0	1.7	0.7	2.0	0.6	2.1	2.7	0.9	0.4	M
UFAC-RB 4592	14.6	6.7	6.3	4.4	3.3	5.9	3.9	4.1	4.3	4.0	1.1	1.9	1.6	0.9	2.0	0.6	2.1	2.6	0.7	0.4	F
UFAC-RB 4595	14.5	6.1	6.1	3.5	3.8	5.8	4.0	4.4	4.7	4.4	1.1	2.4	1.8	0.6	1.8	0.5	1.9	2.5	0.8	0.4	M
UFAC-RB 4596	16.6	6.6	6.5	3.1	3.2	6.0	4.3	4.7	4.9	4.7	1.3	2.5	1.8	1.0	2.1	0.5	1.9	2.7	0.8	0.3	F
UFAC-RB 4597	16.8	6.7	6.4	3.7	3.0	6.1	4.1	4.4	5.1	4.5	1.3	2.2	1.5	0.8	2.1	0.5	2.2	2.6	1.1	0.4	F
UFAC-RB 6531	16.7	7.6	6.7	3.7	3.3	7.1	4.7	4.5	5.2	5.2	1.3	2.2	1.6	0.9	2.0	0.6	2.7	3.3	1.1	0.3	F
UFAC-RB 9699	16.5	7.6	6.6	3.4	3.6	7.1	4.6	4.7	5.5	5.9	1.4	2.2	1.9	1.0	2.2	0.7	2.5	2.9	1.0	0.3	M
MCP 13635	15.2	6.2	6.2	3.2	3.4	5.6	4.0	4.3	5.3	5.7	1.3	2.1	1.8	0.8	1.6	0.5	2.0	2.8	1.0	0.5	-
MNRJ 95832	17.5	7.5	7.3	3.9	3.9	6.4	4.3	4.5	5.8	6.2	1.3	2.5	2.0	0.8	1.9	0.5	2.3	3.2	1.1	0.4	F
MUBI 14866	16.0	7.7	6.8	4.0	3.7	6.8	4.7	4.6	5.7	5.6	1.5	2.8	1.9	0.8	1.7	0.5	2.2	3.2	1.1	0.4	M
UFAC-RB 9700	15.5	6.4	6.4	3.9	3.5	5.5	3.8	4.0	4.8	4.5	1.3	2.0	1.6	0.7	1.7	0.5	1.8	2.1	0.8	0.3	M
CM 158616	17.6	7.8	7.2	4.0	4.0	6.7	4.7	5.0	5.8	7.2	1.5	2.3	2.1	1.1	1.7	0.7	2.6	3.3	1	0.6	F

Paratypes. Sixteen specimens in total. From Brazil: UFAC-RB 0419, an adult male collected on 19 February 1988 by M. B. Souza, F. N. Castro, and A. J. Cardoso at type locality. UFAC-RB 4157, an adult female collected on 28 March 2009 by PRMS at type locality. UFAC-RB 4160, an adult female collected on 2 April 2009 by PRMS and T. R. B. Silva at type locality. UFAC-RB 4592, an adult female collected on 15 August 2009 by PRMS and W. C. Silva at type locality. UFAC-RB 4585, an adult male collected on 1 November 2009 by PRMS at type locality. UFAC-RB 4595 (male) and UFAC-RB 4596 (female) collected on 28 November 2009 by PRMS at type locality. UFAC-RB 4597, an adult female collected on 20 January 2010 by PRMS and T. R. B. Silva at type locality. MCP 13635 (sex undetermined), collected on 16 March 2016 by PRMS and ET at type locality. UFAC-RB 3678, an adult male collected on 23 to 25 November 2000 by M. B. Souza, and R. Guerra at "Foz do Rio Tejo" ("mouth of Tejo River"), Reserva Extrativista Alto Juruá, Marechal Thaumaturgo, Acre (approx. 9.05°S, 72.73°W, 250 m elevation). UFAC-RB 9699, an adult male collected in 2005 by J. R. D. Souza at Estação Ecológica Rio Acre, Assis Brasil (11.0012°S, 70.2132°W), 345 m elevation. UFAC-RB 9700, an adult male collected on 14 January 2019 by I. Prates, V. Prates, and PRMS, Reserva Florestal Humaitá, Porto Acre, Acre (9.7509°S, 67.6724°W), 190 m elevation. UFAC-RB 6531, an adult female collected on 13 September 2014 by D. P. Silva and D. C. Machado at Parque Estadual Chandless, Manoel Urbano, Acre (9.3804°S, 69.9230°W), 220 m elevation. MNRJ 95832, an adult female collected on 22 January 2016 by PRMS and J. Costa, Comunidade São José, Boca do Acre, Amazonas (8.9747°S, 67.8606°W), 135 m elevation. From Peru: MUBI 14866 (male) and CM 158616 (female), a breeding pair collected on 22 February 2014 by JMP, LAGG, JCC, and RG from the Sepahua River, in the buffer zone of Alto Purús National Park, Provincia Atalaya, Ucayali department (11.0527°S, 72.4565°W), 356 m elevation (Fig. 3). Measurements of the type series are provided in Table 2.

FIGURE 4. Hand (left) and foot (right) of *Ranitomeya hwata* sp. nov. The specimen depicted is CM 158616 from 66 km E Sepahua, Ucayali, Peru. Photos courtesy of Mariana Marques.

Additional referred material. MCP 13485–13489, MCP 13506 (Fig. 3), and MCP 13511, all skinned carcasses, collected on 16 March 2016 by PRMS and ET at type locality. UFAC-RB 4158, a juvenile collected on 28 March 2009 by PRMS at type locality. MUBI 14867, an egg clutch from the Sepahua breeding pair. MNK 8251, and adult frog of undetermined sex from Pando department, Bolivia (Maldonado & Reichle 2007).

Etymology. Among the indigenous peoples inhabiting the Upper Purus River drainage are the Manxineru, who use several terms to refer to native spiny bamboos, known in Portuguese as *taboca*. One of these words, *hwata*, is

a generic term for the native *Guadua* Kunth, 1822 bamboos (Virtanen *et al.* 2022), of which there are at least nine species in the region, including the locally dominant *G. sarcocarpa* and *G. weberbaueri* (Carvalho *et al.* 2013). As the new species appears to be strongly associated with *Guadua* bamboo, we chose the specific epithet *hwata* to reflect this connection and to honor the indigenous peoples who inhabit the same region as the new species. The specific epithet is used as a noun in apposition.

Diagnosis. Twomey *et al.* (2023) retrieved the new species unambiguously nested within the *vanzolinii* group of *Ranitomeya*. Besides this phylogenetic inference, the new species is assigned to the genus *Ranitomeya* due to the combination of the following characteristics: small adult size (< 18 mm SVL), conspicuous and bright dorsal coloration, with pale limb reticulation, first finger distinctly shorter than the second, inner metacarpal tubercle present (Fig. 4), maxillary teeth absent. Further assigned to the *vanzolinii* group of *Ranitomeya* based on its advertisement call, which is a high pitched, tonal trill of approximately 1–2 s in length (see below for further details and comparisons of calls). The following characteristics in combination distinguish *Ranitomeya hwata* from all other members of the *vanzolinii* group: (1) dorsal stripes fine, yellow in life, and largely unbroken along the length of the body, (2) ventral color patch absent in life, (3) gular region yellow in life and lacking spots, with a distinct black band delimiting gular and belly coloration, and (4) belly with fine and regular black spotting and pale blue reticulation in life.

Comparisons. Based on morphology, advertisement call, and our phylogenetic analysis (see also Twomey *et al.* 2023), *Ranitomeya hwata* is nested within the *vanzolinii* species group and thus we focus our comparisons on only members of this clade. Diagnostic characters of the new species given in parentheses. (Note that call comparisons are given in the next section.) *Ranitomeya vanzolinii* has a dorsal color pattern consisting of yellow dots or irregular dashes (vs. yellow stripes) on a black background. Furthermore, the ventral color pattern in *R. vanzolinii* consists of yellow and black marbling (vs. pale blue with fine black spots). *Ranitomeya sirensis* is variable in coloration but can consistently be distinguished by the presence of a "ventral color patch", that is, a patch on the belly of the same color as the dorsal coloration (vs. patch absent in *R. hwata*). This ventral color patch is also present in southern populations of *R. sirensis,* including those near the type locality of the former *R. biolat,* allowing for a simple diagnosis between the two species, barring the occasional aberrant individual (see Brown & Twomey *et al.* 2011, Fig. 25N for an example). *Ranitomeya imitator* is also variable, and striped populations occurring in the lowlands of northern San Martin and Loreto, Peru can most reliably be distinguished from *R. hwata* by ventral color pattern, where *R. imitator* lacks the distinctive black band delimiting gular and belly regions (vs. black band present and regular, occasionally broken in the middle in *R. hwata*). There are also differences in breeding biology, with *R. imitator* demonstrating pair-bonding and monogamy (vs. polygyny in *R. hwata*), as well as call differences (see next section). *Ranitomeya aetherea, R. aquamarina*, *R. cyanovittata,* and *R. yavaricola* all have blue, greenish-blue, or yellowish-green dorsal markings in the form of dots, dashes, or stripes (vs. yellow stripes). Additionally, all of these species can have bluish belly coloration similar to *R. hwata*, but have black spots that are generally much coarser, irregular, and occasionally sparse (vs. spots fine, black, and mostly round in *R. hwata*), and with a gular color that matches the belly color (vs. yellow gular color and pale blue belly in *R. hwata*). In the case of *R. aquamarina,* the gular region and belly can be of subtly different color, fading from bluish-green on the gular region to yellowish-green on the belly, but the distinctive two-toned color scheme of *R. hwata* (yellow gular region and pale blue belly, separated by a black band, with no fading) is not observed. *Ranitomeya flavovittata* has yellow dots or dashes on the dorsum (vs. continuous yellow stripes that are rarely broken in *R. hwata*). The ventral coloration between the two species is similar (both with a yellow gular color and pale blue belly), but the black patterning in *R. flavovittata* is more marbled and irregular, with larger black patches (vs. black spots fine and mostly round in *R. hwata*). The two species can also be distinguished by their calls (next section). Most notably, *R. aetherea, R. aquamarina, R. cyanovittata*, *R. flavovittata*, and *R. yavaricola* all lack the distinctive black band of *R. hwata* that delimits the gular and belly regions.

Bioacoustics. Three male *Ranitomeya hwata* were recorded during 2009 at the type locality. The advertisement call is typical of the *vanzolinii* group, consisting of a high-pitched, tonal trill (Fig. 5). The following values are based on average values for each calling male. Call length 1.25–1.78 s, with 48–66 pulses per call, pulse rate 36–49 pulses/s. Dominant frequency 5884–6064 Hz.

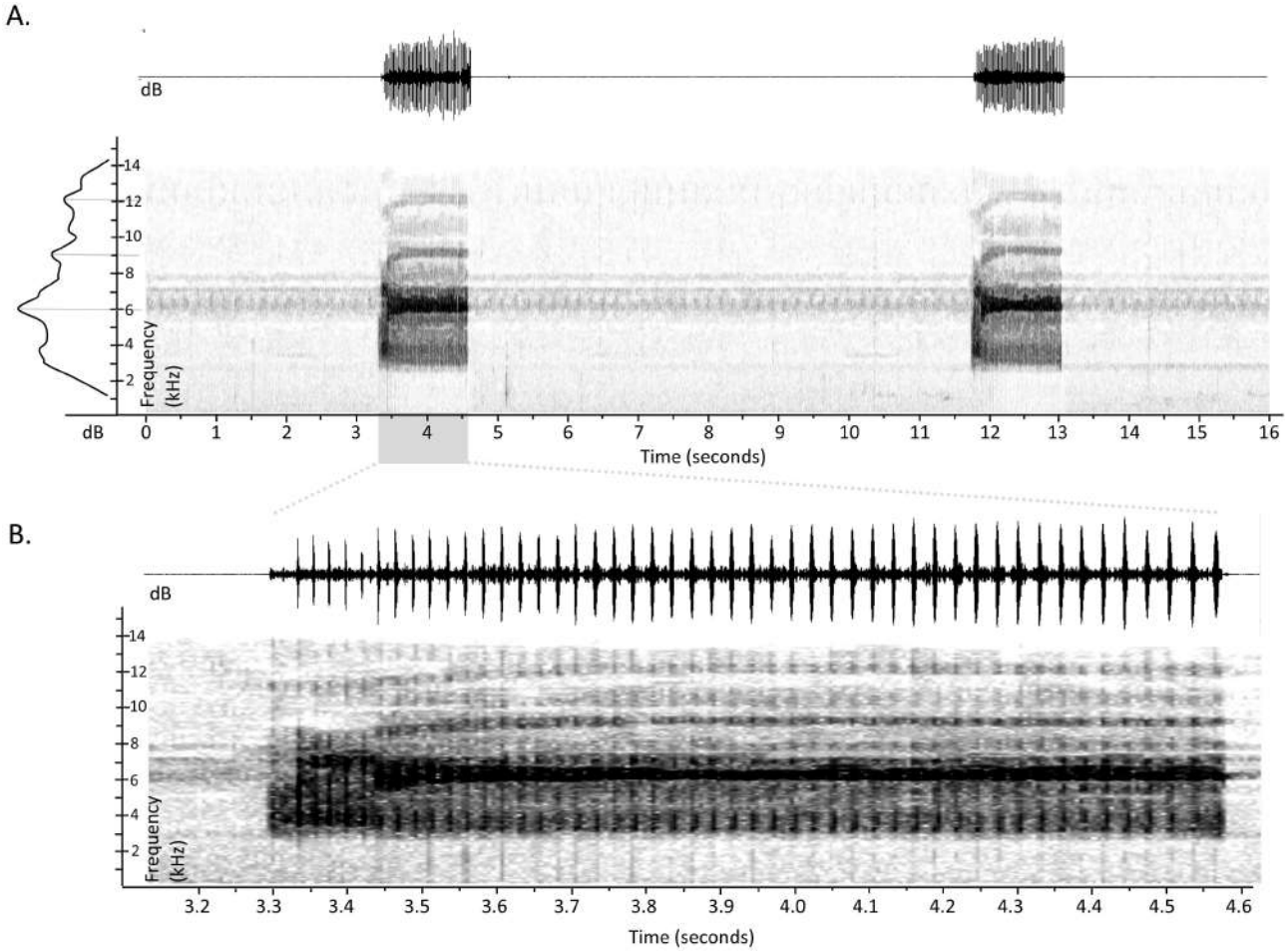

FIGURE 5. Advertisement call of *Ranitomeya hwata* sp. nov. Recorded at type locality on 29 November 2009 (unvouchered individual). (A) Series of two advertisement calls, with oscillogram (above), spectrogram (below), and power spectrum (left). (B) Expanded view of the first call, showing individual pulses comprising the call. Oscillogram (above) and spectrogram (below).

The combination of a relatively long call length with a high pulse rate yields an exceptionally high number of pulses per call. This is diagnostic against all other members of the *vanzolinii* group, except for *R. sirensis*, which can produce up to 55 pulses per call, and *R. cyanovittata*, for which no call data exist. Compared to the species in its sister clade (*R. aetherea, R. aquamarina, R. flavovittata,* and *R. yavaricola*)*, Ranitomeya hwata* has a longer call length, with more pulses per call, and a higher pulse rate (Table 3). Compared to the other (non-sister) species in the *vanzolinii* group, *Ranitomeya vanzolinii* has a shorter call length, fewer pulses per call, a lower pulse rate, and a lower dominant frequency than *R. hwata* (Table 3). *Ranitomeya sirensis* can produce a call as long as or exceeding the length of *R. hwata*, but with a lower pulse rate and dominant frequency (Table 3). *Ranitomeya imitator* overlaps with *R. hwata* with respect to call length, pulse rate, and dominant frequency, but has a lower number of pulses per call (9–46), which is noteworthy given the large sample size for this species ($N = 132$; Twomey *et al.*, 2015).

Description of holotype. The holotype is an adult male, 15.3 mm SVL. The skin is nearly smooth on the dorsum and weakly granular on the venter. Snout weakly rounded in dorsal and ventral view, sloping and truncate in lateral view. Canthus rostralis rounded, loreal region vertical. Upper eyelid width is half interorbital distance. Eye 1.8 mm in length; tympanum 0.5 mm in diameter.

Forearm and upper arm of nearly equal length, 20% of SVL. Hand length 27% of SVL. Relative lengths of fingers III > IV ≈ II > I. First finger 79% length of second. Finger discs moderately expanded on fingers II, III, and IV, weakly expanded on finger I. Unpigmented palmar and inner metacarpal tubercles present; unpigmented proximal subarticular tubercles present on all four fingers; finger III also has distal subarticular tubercle. Fingers lack fringes and webbing.

TABLE 3. Call comparisons between *Ranitomeya hwata* **sp. nov.** and other members of the *vanzolinii* group.

species	Call length (s)	Pulses per call	Pulse rate (pulses/s)	Dominant frequency (Hz)	Data source
R. hwata **sp. nov.**	1.25–1.78	48–66	36–50	5884–6064	This study
R. aetherea	0.54–1.13	18–34	30–36	5471–6029	Koch *et al.* 2025
R. aquamarina	0.68–1.21	23–39	30–36	5394–6288	Mônico *et al.* 2025
R. flavovittata	0.67–1.15	19–37	25–32	5488–5945	Brown & Twomey *et al.* 2011; F. Tegnér
R. yavaricola	0.63–0.88	20–27	31–32	5400–6000	Perez-Peña *et al.* 2010
R. imitator	0.26–1.38	9–46	29–47	4703–5976	Twomey *et al.* 2015
R. vanzolinii	0.57–0.64	16–17	26–28	5350–5440	Brown & Twomey *et al.* 2011
R. sirensis	0.88–2.2	21–55	24–30	5010–5690	Brown & Twomey *et al.* 2011

Femur and tibia length nearly equal, each roughly 40% of SVL. Relative lengths of toes IV > V ≈ III > II > I. First toe with no appreciable disc, disc weakly expanded on toe II, moderately expanded on toes III, IV, and V. Two unpigmented metatarsal tubercles present at base of foot, one medial to base of toe I, the other at the base of the fifth metatarsal. Proximal subarticular tubercles at base of toes I, II, and V. Toes III and V each have a single distal subarticular tubercle while toe IV has two. Toes lack fringes and webbing.

In preservative, there are three complete dorsal stripes, pale grey in color, of equal width, extending from the head to the base of the dorsum. The medial stripe extends to the tip of the snout. Between the eyes and the groin, all three stripes are unbroken and form no perpendicular ramifications. On the head, the two lateral stripes branch medially and the left stripe joins the medial stripe. Two pale grey lateral stripes are present, running from the tip of the snout, under the nostrils, eye, and tympanum, extending posteriorly to groin. The ventral edge of the lateral stripes blends into the coloration of the venter while the dorsal edge is well-defined. The arms and legs are pale bronze in preservative, with fine black spotting. The spotting is sparser on the ventral surface of the limbs. Dorsally, the spotting is interrupted at the insertion of the arms and legs, giving the impression of weakly defined pale grey axillary and inguinal patches. On the ventral side, the gular region is bare and silvery-grey, framed with melanic pigmentation that is fine anteriorly along the mouth and thick along the posterior margin below the eyes. This latter pigmentation is also thickened laterally on the underside of the head, between the eyes and arms. The belly is pale grey with black spots; spots mostly round; larger than spots on limbs.

Coloration in life and variation. Among the type series, the snout shape varies from rounded to truncate in dorsal view. In life, the dorsal stripes are typically complete between the eyes and the groin, but in rare cases one of the lateral dorsal stripes may be broken. Between the eyes and the groin, perpendicular ramifications that connect medial and lateral stripes are seldom observed, found only in a single adult (MCP 13488) and occasionally in metamorphs. Between the eye and snout, stripe variation is much more pronounced. Commonly, a partial or complete stripe is seen running across the head just anterior to the eyes, forming a cross pattern on the nose. Dorsal and ventral limb coloration varies from silvery grey to pale blue, the former being more common. Pale yellow patches, weakly defined, are present dorsally at the limb insertions. Ventrally, the gular region is always yellow and strongly delimited from the belly coloration by a black band. In some individuals this band is medially broken or irregular. Variation in belly coloration is minimal. Reticulation is always pale blue; some individuals have finer or coarser black spotting but relatively speaking (i.e., compared to the sister clade), the black spots on the belly are fine and mostly round. Overall, *Ranitomeya hwata* exhibits remarkably little variation in color pattern across its range.

Tadpole. Although we are unaware of any preserved material that would allow a detailed tadpole description, several live tadpoles were extracted from bamboo culms and photographed between 2009 and 2010, allowing for a brief description of their external morphology and development. Upon hatching (Fig. 6a), tadpoles are pale grey with two round black eyes located on top of the head. As they develop (e.g. at a total length of approximately 26 mm), coloration darkens, with the body appearing uniform brownish-grey, fading towards translucent grey along the length of the tail. By approximately Gosner stage 40, the yellow dorsal stripes are faintly visible (Fig. 6g). This is also the point where maximum size was reached, with a total length of 28 mm. Froglets are generally 12 mm long after metamorphosis.

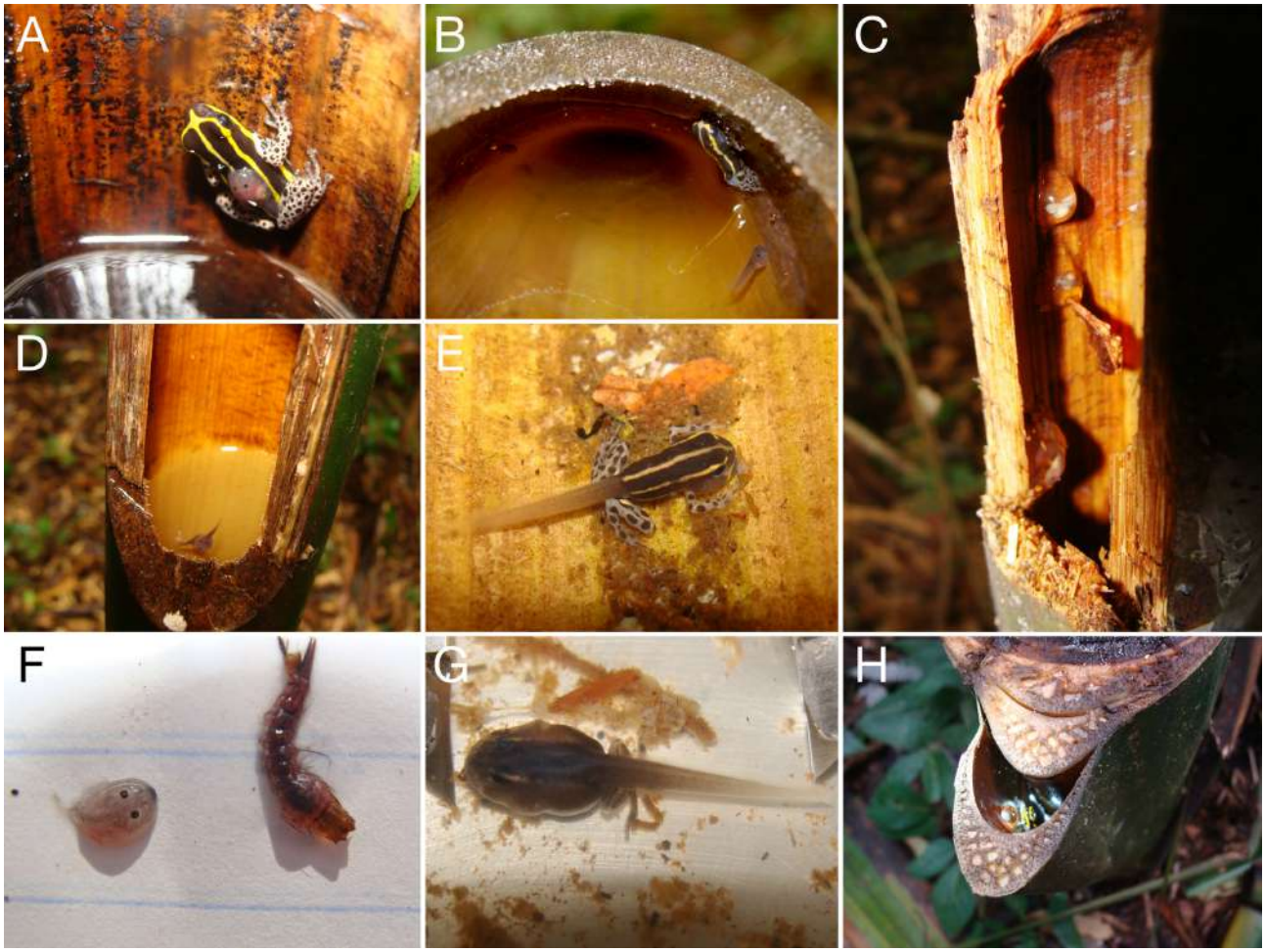

FIGURE 6. *Ranitomeya hwata* sp. nov. natural history. All photos taken by PRMS at type locality. (A) Adult frog in *Guadua* bamboo transporting tadpole at night. (B) Metamorph sharing bamboo culm with young tadpole. (C) Egg clutches in bamboo internode. Top two clutches are a single egg each, bottom clutch has two eggs. (D) Two young tadpoles of similar age sharing bamboo internode. (E) Late-stage tadpole just prior to metamorphosis. (F) Injured tadpole (left) with *Toxorhynchites* larva (right) extracted from the same phytotelm. (G) Tadpole with total length 28 mm, stripes just beginning to become apparent. (H) Adult frog with a single egg inside a cut *Phenakospermum guyannense* leaf sheath.

Distribution. *Ranitomeya hwata* is a lowland species, distributed throughout much of the state of Acre, Brazil, and extends into southeastern Peru in Ucayali department near the village of Sepahua (Fig. 7). Additionally, it is known from one site in Amazonas, Brazil (Reserva Extrativista Arapixi), Pando department in northern Bolivia, and from Rio Acre (which forms the border of Acre and Madre de Dios) (Freitas *et al.* 2020), making its presence in Madre de Dios department of Peru a virtual certainty. The species occurs at elevations ranging from 190 to 356 m, with the most distant known localities separated by approximately 575 kilometers. Drawing a minimum convex polygon around all known localities, the extent of occurrence of *R. hwata* is approximately 121,000 km^2. As much of this habitat is protected (e.g. Alto Purús National Park, Chandless State Park, Reserva Extrativista Chico Mendes, Reserva Extrativista do Alto Jurua), we suggest this species be listed as Least Concern following the IUCN Red List criteria (IUCN 2024).

Natural history. Throughout its range, *Ranitomeya hwata* is found in close association with *Guadua* bamboo. This is true not only for the populations in Brazil, but for the Peruvian population as well. The two Peruvian paratypes were found while clearing a patch of *Guadua* for a campsite, together inside a culm guarding a clutch of eggs. Other species in the *vanzolinii* group are also known to use *Guadua* in certain locations, such as *Ranitomeya vanzolinii* (Souza 2009), *Ranitomeya sirensis* (as *biolat*) (von May *et al.* 2009; Waldram 2008), and *Ranitomeya aquamarina* (personal observation), as well as non-native bamboo such as *Dendrocalamus asper (*e.g. *R. sirensis*

near Tingo Maria, Peru). All three of these latter species exhibit some flexibility in their choice of phytotelmata, using tree holes, *Xanthosoma* spp. (as well as non-native *Alocasia* spp.), small bromeliads, *Ischnosiphon* spp. (Marantaceae), and *Phenakospermum guyannense* (Streliziaceae) when available. In the case of *Ranitomeya hwata*, reproductive behavior was monitored intensively at the type locality over a span of two years (Melo-Sampaio 2010) and we have only one observation of this species breeding outside of *Guadua*, which was in *Phenakospermum guyannense*, known in Portuguese as *bananeira brava* and in Spanish as *patujú gigante* (Fig. 6h). In the publication documenting *R. hwata* in Bolivia (Maldonado & Reichle 2007), it is mentioned that the specimen was collected in a pitfall trap located within a patch of *P. guyannense,* but that this species is more frequently associated with *Guadua weberbaueri.*

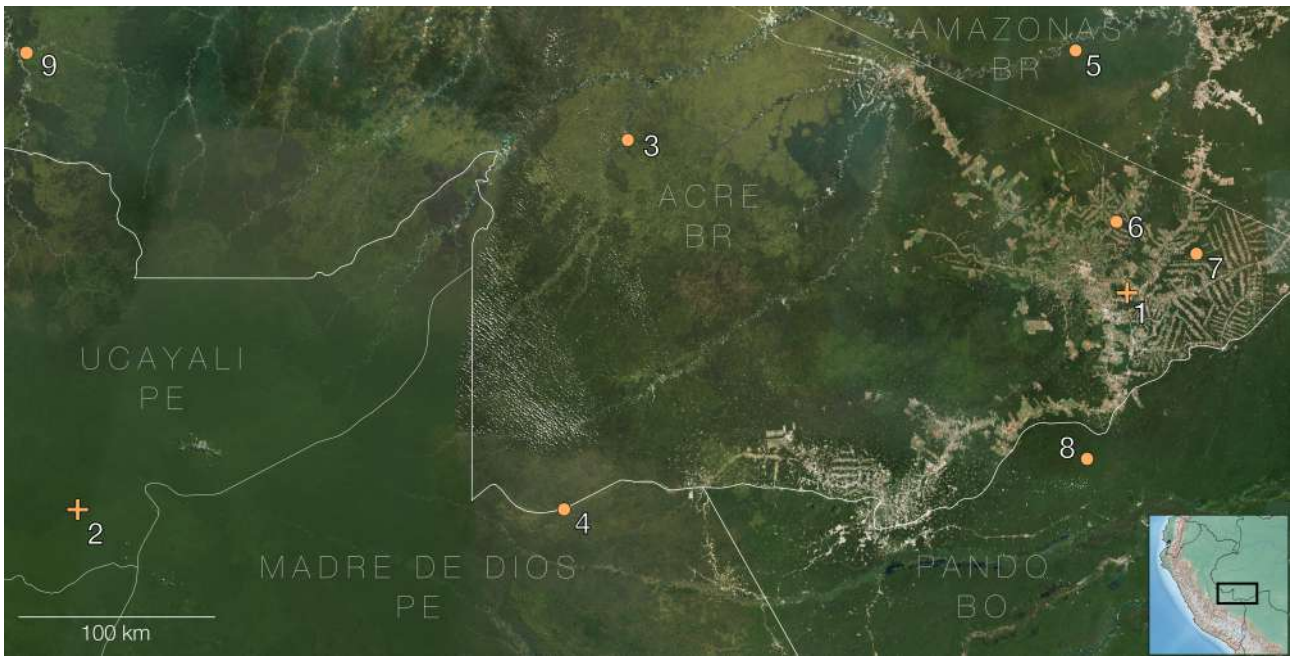

FIGURE 7. Distribution of *Ranitomeya hwata* sp. nov. Crosses indicate localities where at least one individual has been sequenced and placed in the phylogeny. Dots indicate localities where identification is based on morphology. (1) **Type locality,** Fazenda Experimental Catuaba, 23 km SE Rio Branco, Acre, Brazil. (2) 66 km E Sepahua, Ucayali, Peru. (3) Parque Estadual Chandless, Acre, Brazil. (4) Rio Acre Ecological Station, Acre, Brazil. (5) Reserva Extrativista Arapixi, Amazonas, Brazil. (6) Reserva Florestal Humaitá, Acre, Brazil. (7) Fazenda Bonal, Acre, Brazil. (8) Provincia Nicolás Suárez, Pando, Bolivia. (9) Mouth of Rio Tejo, Acre, Brazil.

At the type locality, between 2009 and 2010, PRMS studied the reproductive ecology of *Ranitomeya hwata*. Water-filled bamboo culms were installed to observe preferences in tadpole deposition height (0.5 to 4 m) during weekly visits in the rainy season (January to April) and bi-weekly visits in the dry season (May to September), and again weekly from October to December. The highest deposition occurred during the rainy season, and even in the dry season, when culms were available, tadpole deposition still occurred. These observations also indicate that nurse frogs transport tadpoles away from the site of oviposition into new culms. One of the paratypes (UFAC-RB 4585) was transporting a tadpole at the time of collection. Although this specimen lacked vocal slits, direct inspection of the gonads confirmed that it was a male. Males call in the early morning hours (around 06:00h), sporadically decreasing frequency during the warming part of the day (10:00h to 14:00h), and ceasing around 17:00h. Males were not heard vocalizing at night, although tadpole transport activity has been observed until 20:00h (Fig. 6a). Males are polygynous and can recruit up to three females in the same culm, achieving multiple matings and optimizing the spawning site, which usually contains one or two eggs (Fig. 6c). Cannibalism among tadpoles was not observed, and up to four tadpoles were found sharing the same phytotelm (Fig. 6d). The main enemies of the tadpoles in the phytotelmata are damselfly naiads (*Microstigma* spp. and *Mecistogaster* spp.) and mosquito larvae (*Toxorhynchites* sp.) (Fig. 6f). In one case we observed a mosquito pupa (10.5 mm total length) and young tadpole (13.1 mm total length) in the same bamboo culm, suggesting that the tadpoles do not prey on the mosquito larvae.

Discussion

The lack of significant color pattern variation in *Ranitomeya hwata* is noteworthy and unusual for members of the *vanzolinii* group, and *Ranitomeya* in general. Most variation in *R. hwata* is subtle, typically involving minor variations of the stripes on the head, and the species appears to be more variable within a population (e.g. the type locality, where a large sample has been obtained) than between populations, even over distances of several hundred kilometers. The only other species in the *vanzolinii* group that is similar in this regard is *Ranitomeya vanzolinii*, which has a large distribution in central Peru and extreme western Brazil, but maintains its characteristic spotted dorsal pattern over large distances (Brown & Twomey *et al.* 2011; Myers 1982). Other cases of apparent monomorphism in this group are an artifact of limited sampling. In their original descriptions, most species in the group (*Ranitomeya aetherea, R. aquamarina*, *R. cyanovittata*, *R. flavovittata*, *R. imitator, R. sirensis,* and *R. yavaricola*) were described from a single locality. Given the remote areas where many of these species occur, this leads to a situation where high polymorphism, combined with large sampling gaps, gives the impression of every distant population appearing to be unique. Although some of these species have withstood increased sampling scrutiny over the years (e.g., *R. imitator, R. flavovittata*), others have not (e.g., *R. lamasi*). As the key aspect of species delimitation is distinguishing whether observed variation is due to speciation versus other processes or biases (Chambers *et al.* 2025; Padial & De la Riva 2021), we urge future researchers to strongly consider how sampling gaps may be driving observed variation and how this impacts any proposed taxonomic actions.

In a similar vein, Chambers *et al.* (2025) recommend that "*all species delimitation studies (especially of geographically variable groups) clarify what new evidence would be sufficient to change the taxonomic recommendations*". We will attempt to do that here. First, an extant sister species of *Ranitomeya hwata* is not yet known. Our phylogenetic analyses recovered *R. hwata* as the sister to a clade including *R. aetherea*, *R. aquamarina*, *R. cyanovittata*, *R. flavovittata*, and *R. yavaricola*. If these relationships are refuted with additional data (for example, resulting in synonymies within the sister clade or novel phylogenetic relationships), the taxonomic arrangement could change such that *R. hwata* is sister to single extant species, potentially challenging species limits inferred herein. For example, the northern edge of the known geographic range of *R. hwata* has a sampling gap of approximately 120–160 km between the southern range limit of populations currently allocated to *R. aquamarina* (e.g. Envira and Rio Liberdade, a site just east of Cruzeiro do Sul). The town of Feijó, in Acre, Brazil, is located directly in the middle of this gap, and although we (PRMS and ET) spent two days here in 2016 trying to find *Ranitomeya,* we were unsuccessful. Locating *Ranitomeya* here would narrow this gap considerably and could generate the kind of data needed to test the new species hypothesis as rigorously as possible. For example, sufficient sampling of individuals and genetic loci would allow one to test if the inferred relationships persist and would support the new species hypothesis. If populations in this gap were genetically or phenotypically admixed between *R. aquamarina* to the north and *R. hwata* to the south, the phylogenetic relationships inferred herein may be refuted, rejecting the taxonomic conclusions here. Alternatively, if the inferred relationships hold in spite of admixture, or if gene flow is low despite close physical proximity, the new species would be further supported. Additional samples, both from this gap and elsewhere within the range of *R. hwata*, as well as new samples from other species, may also reject our hypothesis if the monophyly of *R. hwata* is no longer supported. In this case, mitochondrial DNA may not be sufficient to perform a strong test. A number of processes and biases causing mito-nuclear discordance (see DeRaad *et al.* 2023; Wüster *et al.* 2024 and references therein) warn us to be cautious about overreliance on mtDNA in species delimitation, especially if sampling is scattered, does not cover zones of contact among species, and there is not additional evidence such as direct or indirect evidence of reproductive isolation. Indeed, there is substantial mito-nuclear discordance in this group as well as other dendrobatid frogs (Brown *et al.* 2019; Brown & Twomey 2009; Muell *et al.* 2022). For example, previous phylogenetic studies based largely or exclusively on mtDNA have found *R. vanzolinii* to be sister to or nested within *R. flavovittata* (Brown & Twomey *et al.* 2011; Perez-Peña *et al.* 2010), whereas studies based on nuclear loci have found a sister relationship with *R. sirensis* (Muell *et al.* 2022; Twomey *et al.* 2023). In two recent papers using mtDNA, *R. aquamarina* was found to be sister to *R. vanzolinii* + *R. flavovittata* (Mônico *et al.* 2025), and *R. aetherea* was found to be sister to *R. cyanovittata* (Koch *et al.* 2025), whereas our results place *R. aquamarina* sister to *R. yavaricola*, and *R. aetherea* sister to a clade containing four species (Fig. 1). Ultimately, it would be desirable to obtain additional evidence on the variation of the call of the new species, as well as other phenotypic evidence such as mating preferences, reproductive ecology, or anatomical traits that may help us support or reject hypotheses of species under the light of genetic evidence.

In two recent papers describing species in the *vanzolinii* group (Koch *et al.* 2025; Mônico *et al.* 2025), the authors argued that most species descriptions in this group to date are based on color pattern. For example, Koch *et al.* (2025: 25), referring to the genus *Ranitomeya*, stated that "*all the descriptions up to date are based mostly on the comparisons of the species color patterns*". We think this a mischaracterization of the taxonomic progress that has been made in this genus in the past two decades, not to mention exemplary earlier work that was integrative far ahead of its time. Examples include Myers & Daly (1980) and Myers (1982), who used the presence of a novel alkaloid and bioacoustics to justify the resurrection of *Ranitomeya reticulata.* Schulte (1986) used natural history and bioacoustics to recognize *R. imitator* as a new species, surmise that the color pattern similarity with *R. variabilis* was due to Müllerian mimicry, and used this hypothesis to uncover subtle but important diagnostic traits among these co-mimics. Later work routinely used bioacoustics and morphometrics to work out species limits (Brown *et al.* 2008, Twomey & Brown 2009, Brown & Twomey *et al.* 2011). This fact was unintentionally proven by Koch *et al.* (2025) and Mônico *et al.* (2025), who were only able to include comparisons on morphometry and bioacoustics with other species of the genus by relying on published information on these topics.

In retrospect, it is true that the *diagnoses* typically focus on color pattern, but there is nothing intrinsically wrong about a phenotypic diagnosis based on coloration. First, it would be more appropriate to say that the more recent species descriptions were motivated by phylogenetic analyses of DNA sequences. However, providing a list of molecular diagnostic traits is of little practical use, and further (in the case of phylogenomic datasets) would result in a long list of potentially hundreds (or more) of unambiguous synapomorphies. Second, within this phylogenetic framework, the principle of reciprocal illumination (Hennig 1966) allows for an appraisal (and re-appraisal) of the phenotypic traits that are genuinely useful for species diagnosis versus those that are simply variable. Such a process of inquiry allows for an appreciation of subtle but critically important diagnostic characters even among incredibly accurate co-mimics such as *R. imitator* and *R. summersi,* or among the many similar-looking cryptic species that are now widely accepted, phylogenetically distinct, and easily recognized by a discerning eye (e.g. *R. ventrimaculata* vs. *R. uakarii; R. toraro* vs. *R. amazonica).* In the case of *Ranitomeya hwata,* the black band delimiting gular and belly regions and absence of the ventral color patch and allows for an easy and reliable diagnosis against other close relatives occurring in the same region.

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