

Supplementary Materials

Supplementary Notes

A) Molecular laboratory work, phylogenetic inference and haplotype network construction

The final 16S alignment comprised 11 sequences obtained in this study and 34 sequences from GenBank consisting of 540 bp, of which 428 sites were variable, 387 parsimony-informative, and 41 singletons; the final alignment for the *COI* gene comprised 8 sequences from our material and 30 ones from GenBank, consisting of 567 bp, of which 293 sites were variable, 282 parsimony-informative, and 11 singletons. A maximum likelihood (ML) analysis was conducted for both genetic markers using IQ-TREE (Nguyen *et al.*, 2015; Trifinopoulos *et al.*, 2016). The substitution model was not defined a priori; instead, the implemented ModelFinder (Kalyaanamoorthy *et al.*, 2017) was applied to find the best models during analysis under the Bayesian information criterion. For estimates of support, 5000 replicates of ultrafast bootstrapping (Minh *et al.*, 2013) were performed.

To infer a Bayesian (BA) phylogenetic tree from a concatenated 2-gene dataset, we conducted the analysis by applying the site-heterogeneous mixture model CAT + GTR + Γ 4 implemented in the Phylobayes MPI software (Lartillot *et al.*, 2013). Two independent Monte Carlo Markov chains (MCMC) were run. We checked for the convergence in the tree space with bpcomp program and generated output of the largest (maxdiff = 0.062493) and mean (meandiff = 0.00214141) discrepancy observed across all bipartitions and generated majority-rule consensus tree using a burn-in of 10 000 trees and sub-sampling every 10 trees. Additionally, we used program Tracecomp to check for convergence of the continuous parameters of the model.

We constructed a haplotype network using the 95% limit of parsimony as implemented in TCS 1.21 (Clement *et al.*, 2002) for the available eastern Panama samples and the geographically closest GenBank sample of *Atelopus spurreli* (DQ502895.1) from Colombia, for 16S and COI separately. The raw output of TCS was visualized in PopART version 1.7 (Leigh & Bryant, 2015). The descriptive statistics for nucleotide diversity was produced also in PopART version 1.7.

B) Bioacoustics

Male advertisement calls of *Atelopus fronterizo* and *A. certus* were recorded from frogs sitting on rocks, using Panasonic RRXS410 digital recorder, and Sennheiser ME66 microphone with

K6 powering module. The microphone was held at a distance 1–1.5 m from the calling male. *Atelopus glyphus* was recorded using an Android phone, with the RecForge II App. Recordings were made at the sampling rate of 44 kHz and 16 bits resolution in uncompressed PCM format and saved as wav files. Call recordings of *A. varius* from Panama highlands were provided by E. Griffith, recorded in captivity at El Valle Amphibian Conservation Center (EVACC), the call of *A. zeteki* was recorded by A. Batista using a cellphone with the RecForge II App in October 11th 2019 at EVACC, the call of *A. limosus* was provided by R. Ibañez,. For each call, we analyzed call duration, pulse duration, pulses per second, call interval, and minimum, maximum, and dominant frequencies. The spectral and temporal parameters analysis follow Batista *et al.* (2016). Temporal measurements of calls such as call duration (msec), call interval (msec), pulse rate (pulses/sec), number of pulses and calls/bouts were taken on the waveforms view of Raven. Terminology used in the advertisement call description follows Zimmermann (1990) and Erdtmann & Amézquita (2009). The call repetition rate was calculated as the number of calls per call bout duration. The environmental temperature and humidity were measured using a digital thermo-hygrometer Garden HighPro Medium.

C) Synonymy

Atelopus fronterizo sp. nov.

Atelopus glyphus – Puerto Obaldia: Guna Yala: Panama (USNM 48594-5 adult) Dunn (1931)

Atelopus varius glyphus Rio Sucubti, Chalichiman's Cr. – Breder (1946)

Atelopus sp. – Rio Sucubti, Chalichiman's Cr., Tacarcuna Village: Darien; Puerto Obaldia: Armila: Guna Yala – Savage (1972)

Atelopus sp. – Sasardi camp, Guna Yala – Cocroft *et al.* (1990)

Atelopus sp. – Lötters (1996)

Atelopus spurrelli – Rivera_Correa (2005)

Atelopus aff. *limosus* – Capurganá: Colombia – Flechas *et al.* (2017a)

Atelopus aff. *limosus* – Cerro Tarcacuna, Darien National Park: Darién – Lewis *et al.* (2019)

Atelopus sp. Puerto Obaldía – Capurganá – Ramírez *et al.* (2020)

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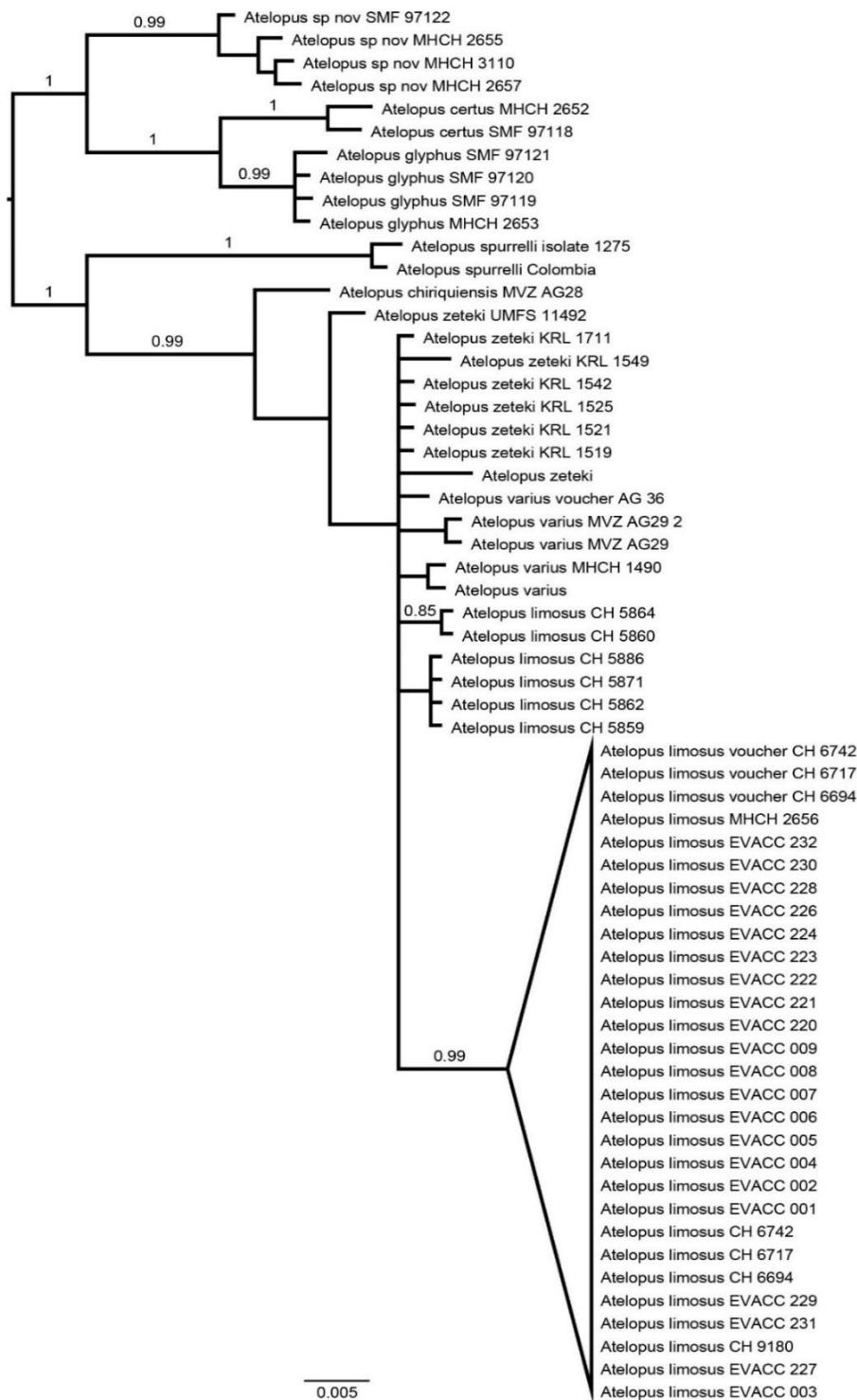
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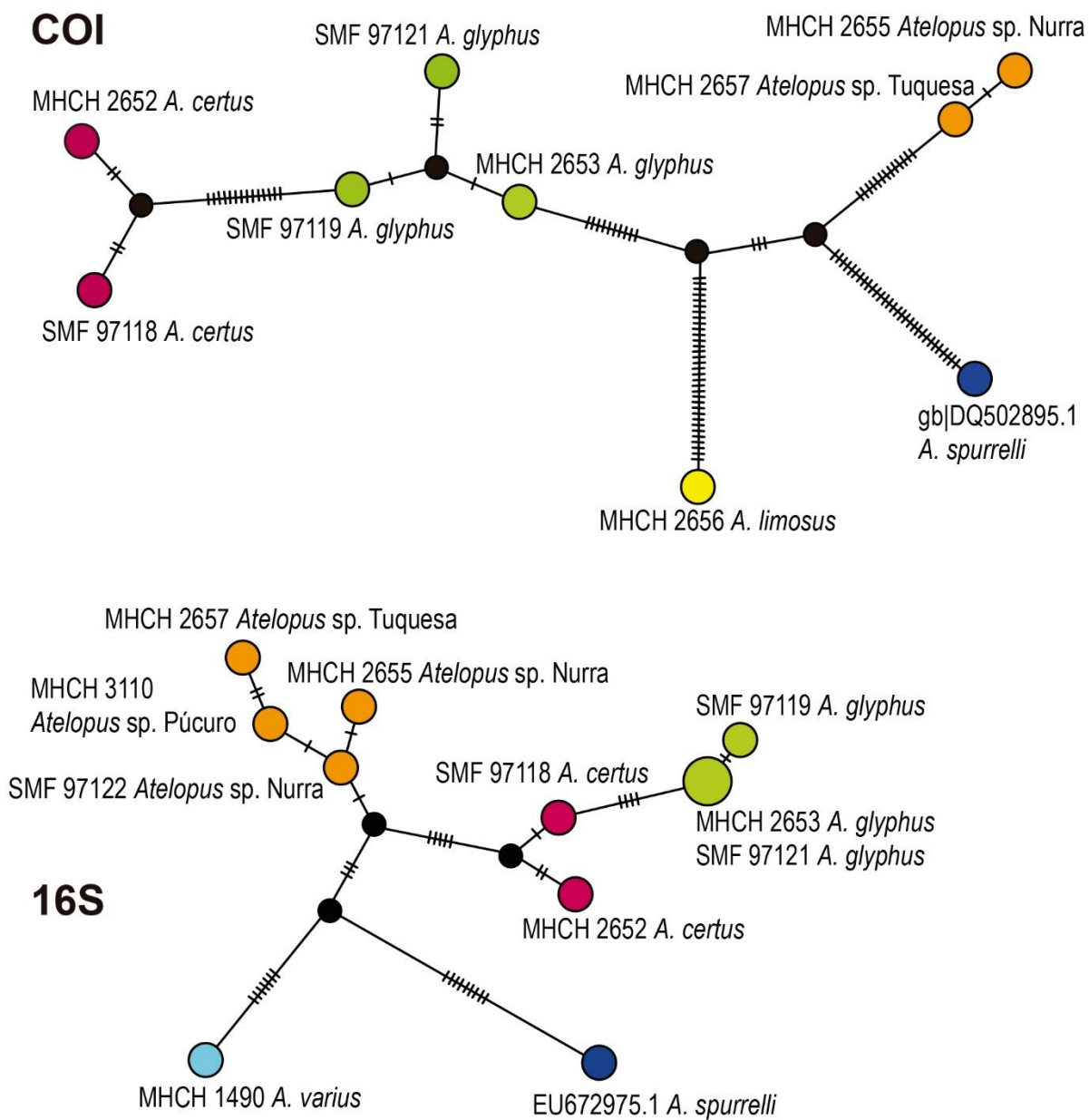
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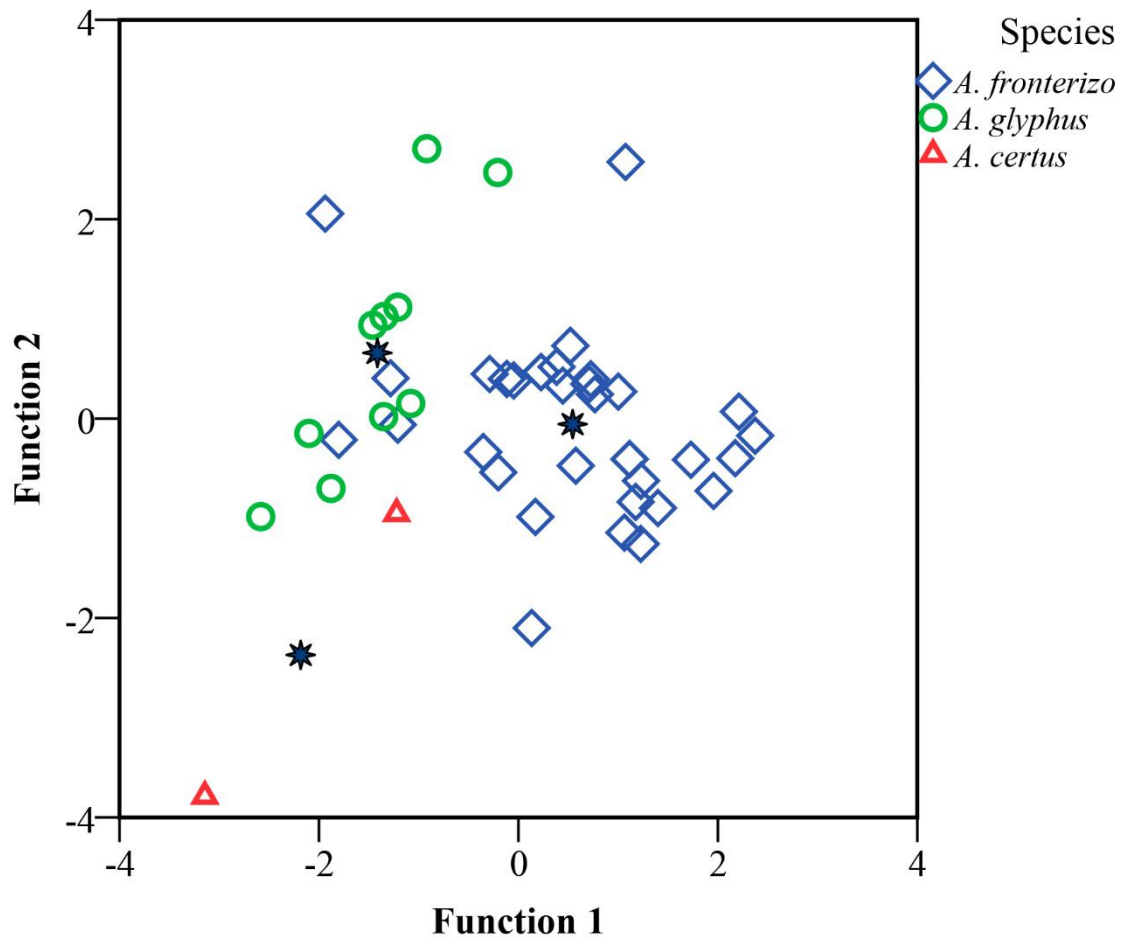
Supplementary Figures



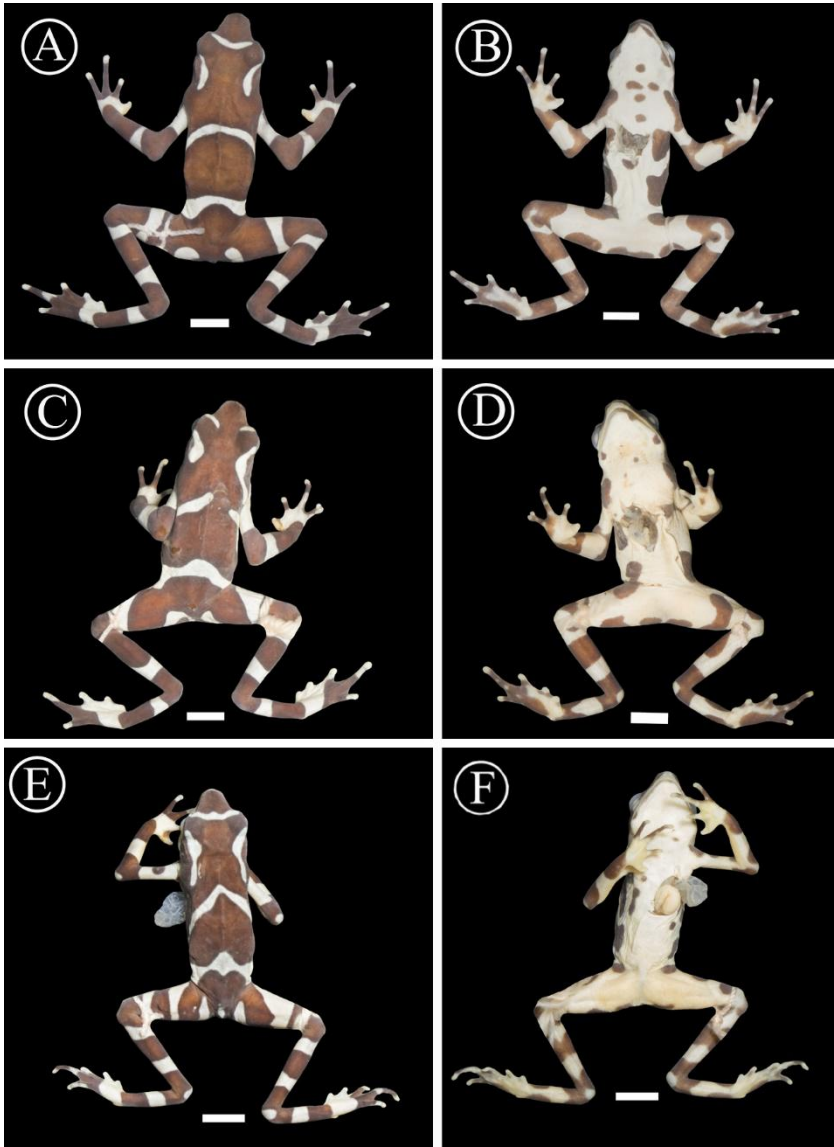
Supplementary Figure S1. Bayesian tree based on concatenated sequences of 16S and COI of *Atelopus* from Panamá and adjacent Colombia (*A. spurrelli*). Posterior probabilities >0.80 are shown above branches. Specimens are indicated by their museum voucher numbers. Scale represents estimated substitutions per site.



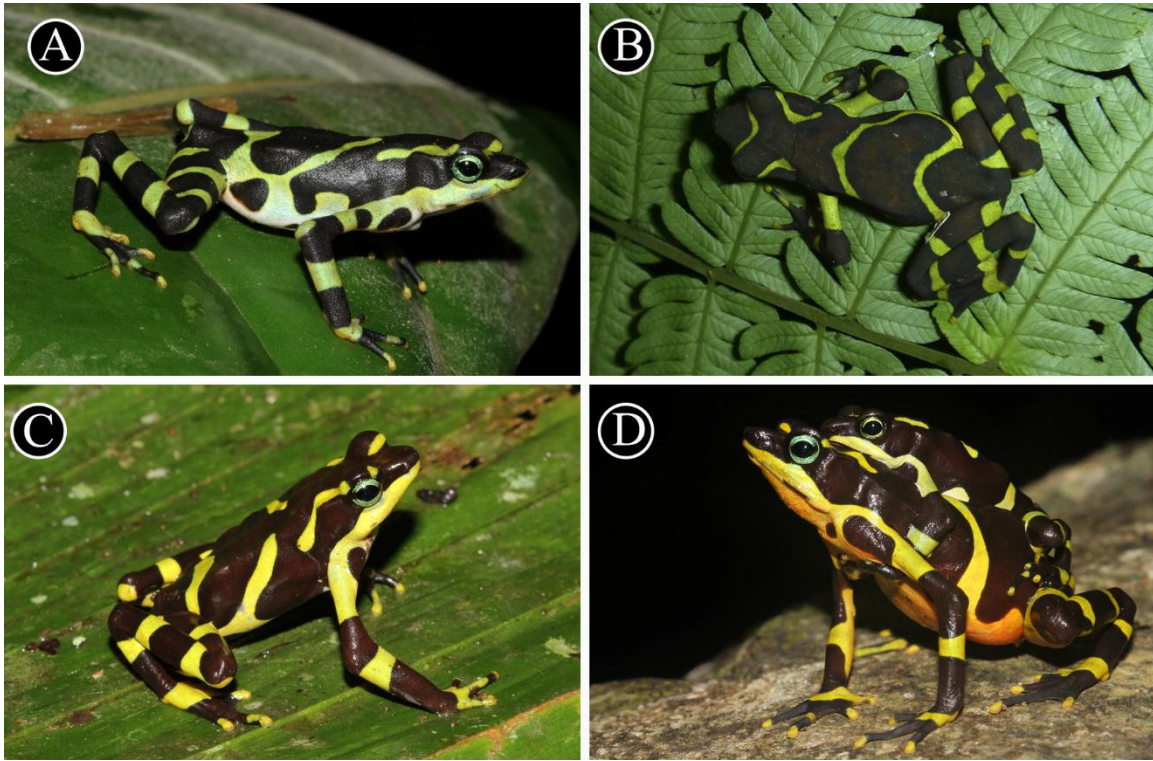
Supplementary Figure S2. Haplotype networks of mitochondrial COI and 16S genes. Species are presented by colors. Sizes of circles are proportional to sample size. Each hatch mark on lines connecting haplotypes represents a single nucleotide substitution. Small black circle represents inferred intermediate haplotype.



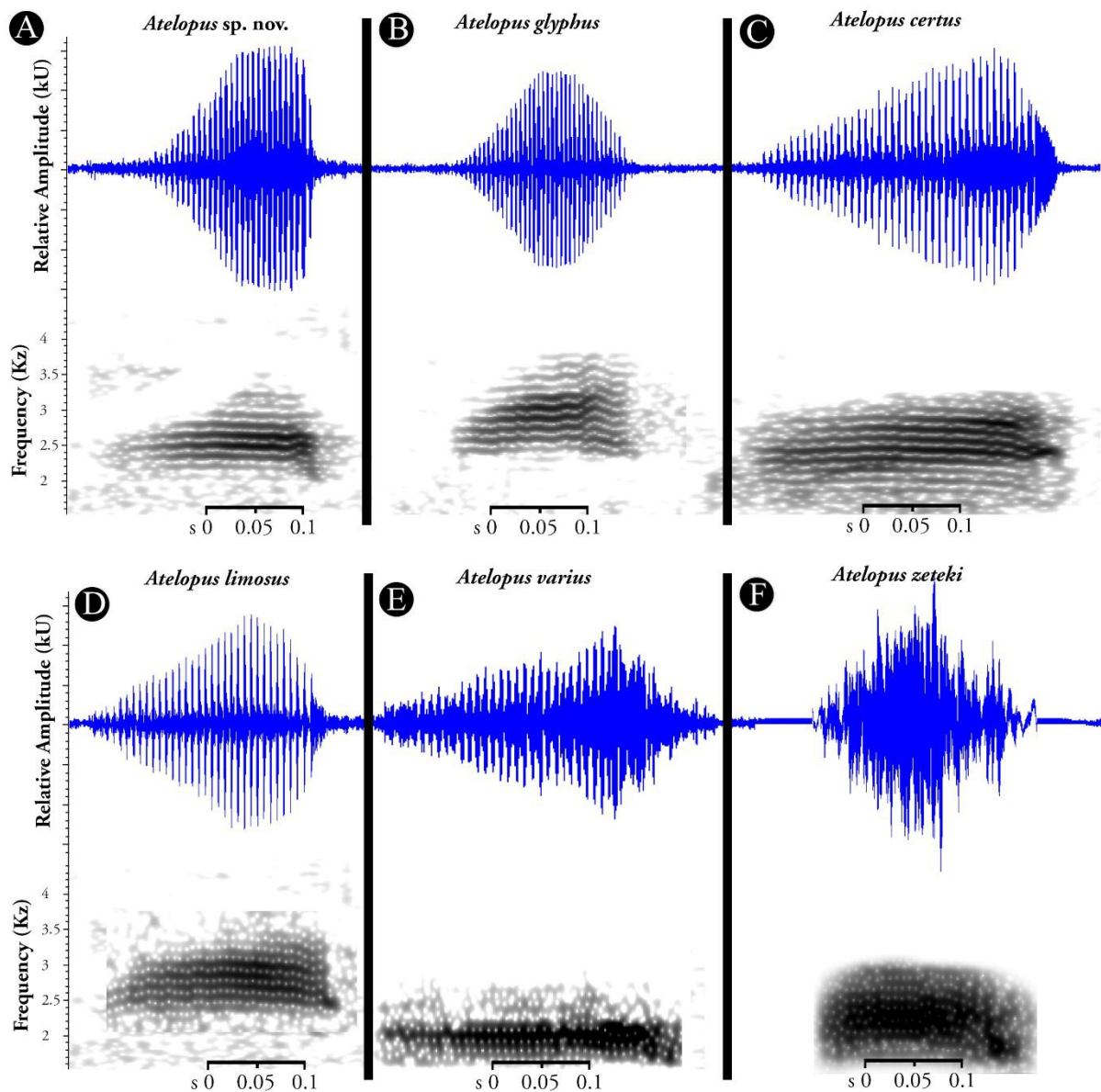
Supplementary Figure S3. Discriminant function analysis of morphometric characters of *Atelopus* species from eastern Panama. Variables included in analysis: HW/SVL, EYND/HW, TIBL/SVL, HAL/SVL, THBL/SVL, IND/IOD, HL/HW, FTL/SVL, FTL/ TIBL, THBL/HAL.



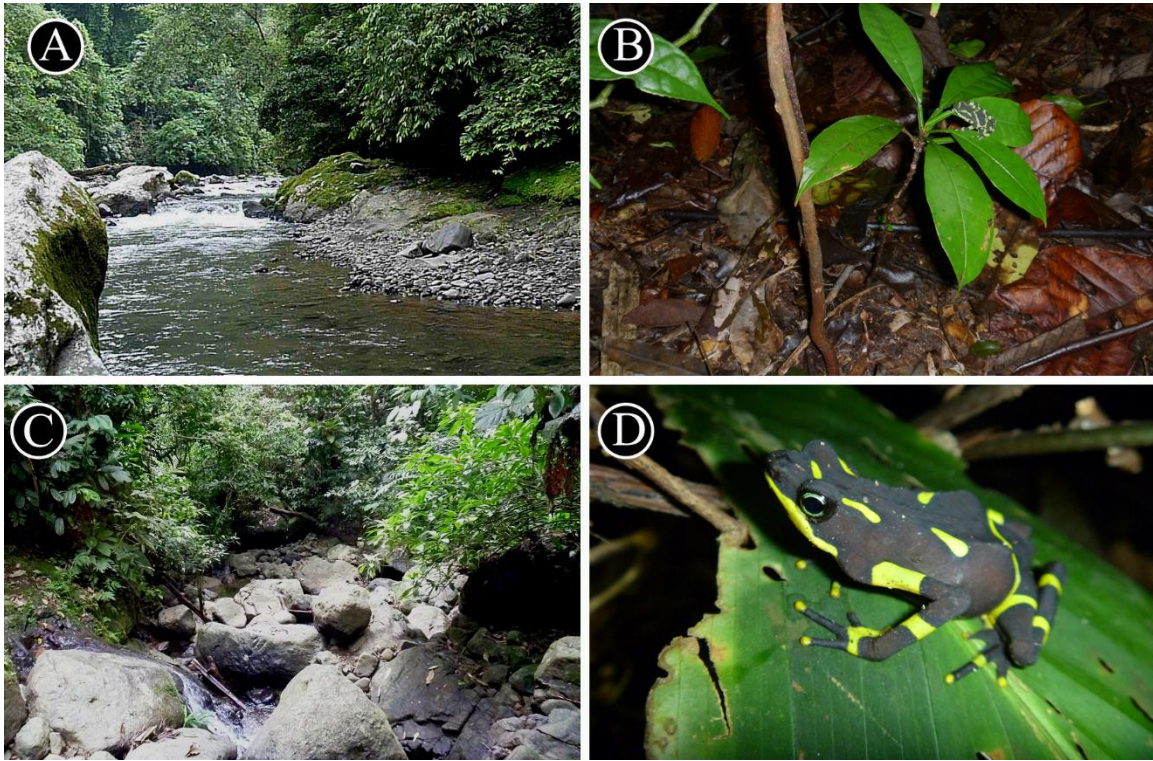
Supplementary Figure S4. Paratype specimens of *Atelopus fronterizo* **sp. nov.** in preservative **A-B)** SMF 97122; **C-D)** MHCH 2655; **E-F)** MHCH 2657.



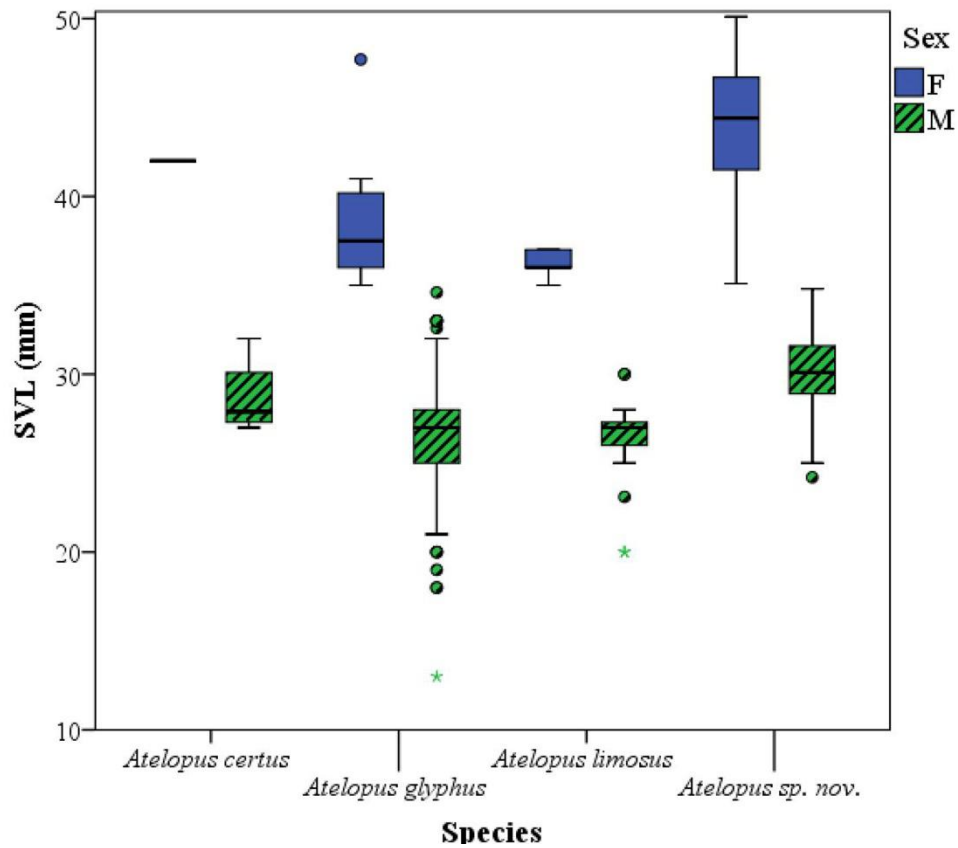
Supplementary Figure S5. Color variation of *Atelopus fronterizo* sp. nov. in life: A) Paratype, Pechito Parao near Tuquesa river (MHCH 2657); B) Male, from Nurra Caribe (not collected); C) Paratype, Nurra Caribe (MHCH 2655); D) Amplexus from Nurra Caribe (not collected).



Supplementary Figure S6. Oscillograms (above) and spectrograms (below) of advertisement calls of six species of *Atelopus* from Panama. A) *Atelopus fronterizo* **sp. nov.** (from San Blas Mountain range, 3 October 2021, 09:49 h, 339 m asl, 25.5 °C; B) *A. glyphus* (from Darien National Park, 250 m asl, 12 December 2019, ↑ ↑ 11:13 h); C) *A. certus* (from Garachiné, Chepigana, Darién, Panamá, 200 m asl, 10 April 2014, 18:00 h, 28.1 °C; D) *A. limosus*, (from Panama Central, provided by R. Ibañez); E) *A. varius* (from Panama highlands, provided by E. Griffith); F) *A. zeteki* (in captivity at El Valle Amphibian Conservation Center, EVACC). See Supplementary Notes 1 for more details.



Supplementary Figure S7. Habitat of *Atelopus fronterizo* **sp. nov.** A) Type locality at Pucuro River; B) Pechito Parado Mount, at Rio Tuquesa, male found at night in understory, at least 1 km from nearest stream; C) Nurra Caribe, Taintidu stream; D) Female from Nurra Caribe, photographed at night near Taintidu stream.



Supplementary Figure S8. Differences in snout-vent length (SVL) of *Atelopus* species from eastern Panama, separated by sex. Bottom and top of box are first and third percentiles respectively, and band inside box represents median, whiskers show extreme values; circles and asterisk above or below boxes represent outliers. *Atelopus fronterizo sp. nov.*: females=13, males=37; *A. glyphus*, *n*: females=10, males=124; *A. certus*, *n*: females=1, males=4; *A. limosus*, *n*: females=5, males=22.

Supplementary Tables

Supplementary Table S1. Numbers of voucher specimens included in morphological analysis or comparison. Specimens not collected were captured, measured, and released under a monitoring project. Those with field numbers were documented photographically.

Museum number	Field number	Species	Province	Locality
Not collected		<i>Atelopus certus</i>	Darién	Qda. Perresenico
SMF97118	AB401	<i>Atelopus certus</i>	Darién	Río San Antonio, Cerro Sapo.
Not collected		<i>Atelopus certus</i>	Darién	Cerro Sapo
Not collected		<i>Atelopus certus</i>	Darién	Cerro Sapo
MHCH 2652		<i>Atelopus certus</i>	Darién	Pavarando
MHCH 3110	AB 543	<i>Atelopus fronterizo</i>	Darién	Río Púcuro
SMF97122	AB 720	<i>Atelopus fronterizo</i>	Darién	Nurra
MHCH2655	AB 733	<i>Atelopus fronterizo</i>	Darién	Nurra
MHCH2657	AB 830	<i>Atelopus fronterizo</i>	Darién	Río Tuquesa
SMF 97125	AB 911	<i>Atelopus fronterizo</i>	Darién	Río Tuquesa
AMNH 104422		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104427		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104428		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104429		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104430		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104431		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104432		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104433		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104434		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104421		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104423		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104424		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104425		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104426		<i>Atelopus fronterizo</i>	Darién	Malí
AMNH 104435		<i>Atelopus fronterizo</i>	Darién	Río Púcuro
AMNH 104436		<i>Atelopus fronterizo</i>	Darién	Río Púcuro
AMNH 104437		<i>Atelopus fronterizo</i>	Darién	Río Púcuro
AMNH 104438		<i>Atelopus fronterizo</i>	Darién	Río Púcuro
AMNH 104438		<i>Atelopus fronterizo</i>	Darién	Río Púcuro
AMNH 39764		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40901		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 39762		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 39763		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40506		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40520		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40521		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40535		<i>Atelopus fronterizo</i>	Darién	Sucubtí
AMNH 40889		<i>Atelopus fronterizo</i>	Darién	Sucubtí

AMNH 40890		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40891		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40892		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40893		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40894		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40898		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40899		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40900		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40902		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40903		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40904		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40905		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40906		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40907		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40908		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40909		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40910		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
AMNH 40912		<i>Atelopus fronterizo</i>	Darién	Sucubtí	
Not collected	1	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	2	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	3	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	4	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	5	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	6	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	7	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	8	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	9	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	10	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	11	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	13	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	14	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	15	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	16	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	17	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	18	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	19	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
Not collected	20	<i>Atelopus glyphus</i>	Darién	Qda Uruseca	PND
SMF 97119	AB 0126	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico	
SMF 97120	AB 0153	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico	
SMF 97121	AB 1174	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico	
	AB 1186	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico	
	AB0146	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca	
	AB0180	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca	
	AB0337	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca	
	AB0346	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca	
	AB1173	<i>Atelopus glyphus</i>	Darién	Cerro Pirre	

SMF97121	AB1174	<i>Atelopus glyphus</i>	Darién	Cerro Pirre
	AB1174	<i>Atelopus glyphus</i>	Darién	Cerro Pirre
	AB1185	<i>Atelopus glyphus</i>	Darién	Cerro Pirre
MHCH2654	AB1186	<i>Atelopus glyphus</i>	Darién	Cerro Pirre
	AB1186	<i>Atelopus glyphus</i>	Darién	Cerro Pirre
SMF97119	AB126	<i>Atelopus glyphus</i>	Darién	Qda Perresenico
MHCH2653	AB127	<i>Atelopus glyphus</i>	Darién	Qda Perresenico
SMF97120	AB153	<i>Atelopus glyphus</i>	Darién	Qda Perresenico
	AB776	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	M1	<i>Atelopus glyphus</i>	Darién	Qda. Almendro
Not collected	M11	<i>Atelopus glyphus</i>	Darién	Qda. Almendro
Not collected	M32	<i>Atelopus glyphus</i>	Darién	Qda. Almendro
Not collected	PA04	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA07	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA08	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA09	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA10	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA13	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA14	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA16	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA16	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA17	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA19	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA20	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA21	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA22	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA23	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PA32	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA34	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA35	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA36	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA38	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA38	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA40	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA42	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA43	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA44	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA45	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA46	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA47	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA48	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA50	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA51	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA53	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA54	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
Not collected	PA55	<i>Atelopus glyphus</i>	Darién	Qda. Almendro

Not collected	PP82	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PP83	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PP84	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PP86	<i>Atelopus glyphus</i>	Darién	Qda. Perresenico
Not collected	PP94	<i>Atelopus glyphus</i>	Darién	Qda Uruseca PND
Not collected	PP95	<i>Atelopus glyphus</i>	Darién	Qda Uruseca PND
Not collected	PP98	<i>Atelopus glyphus</i>	Darién	Qda Uruseca PND
Not collected	PP99	<i>Atelopus glyphus</i>	Darién	Qda Uruseca PND
	AB1375	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
	AB1376	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
	AB1376	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
	AB1377	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
	AB1377	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
	AB1378	<i>Atelopus glyphus</i>	Darién	Qda. Uruseca
MHCH2656	AB 984	<i>Atelopus limosus</i>	Panama	Río Terable Cocobolo Nature Private Reserve
Not collected	ALI-038	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-069	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-101	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-114	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 01	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 02	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 03	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 04	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 05	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 06	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 125	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 138	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 184	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 217	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 242	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 243	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 247	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 110	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve

Not collected	MP 111	<i>Atelopus limosus</i>	Panama	Reserve Cocobolo Nature Private Reserve
Not collected	MP 160	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 161	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 165	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 176	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 178	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 201	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve
Not collected	MP 205	<i>Atelopus limosus</i>	Panama	Cocobolo Nature Private Reserve

Supplementary Table S2. Tissue samples and sequences (16S, COI) of Panamanian *Atelopus* species used in this study.

Field No.	Mus. No.	Locality	species	Coordinates		Source	GenBank accession number	
				N	E		16S	COI
AB126	SMF97119	Qda Perresenico	<i>Atelopus glyphus</i>	7,99764	-77,71086	This study		MT540972
AB127	MHCH2653	Qda Perresenico	<i>Atelopus glyphus</i>	7,99764	-77,71086	This study	MT560720	MT540973
AB153	SMF97120	Serrania de Pirre.	<i>Atelopus glyphus</i>	7,98746	-77,70778	This study	MT560719	
		Rio San Antonio,				This study	MT560713	
AB401	SMF97118	Cerro Sapo.	<i>Atelopus certus</i>	8,00447	-78,34854			
			<i>Atelopus fronterizo</i>			This study	MT560718	MT540974
AB543	MHCH 3110	Río Púculo	sp. nov.	8,02908	-77,41318			
			<i>Atelopus fronterizo</i>			This study	MT560717	
AB720	SMF97122	Serrania San Blas	sp. nov.	9,06105	-77,97971			
			<i>Atelopus fronterizo</i>			This study	MT560715	
AB733	MHCH2655	Serrania San Blas	sp. nov.	9,06105	-77,97971			
		Río Tuquesa, Bajo	<i>Atelopus fronterizo</i>			This study	MT560716	MT540975
AB830	MHCH2657	pequeño	sp. nov.	8,47553	-77,54883			
		Pavarandó, Garra				This study	MT560721	MT540976
AB939	MHCH2652	garra	<i>Atelopus certus</i>	7,76310	-78,10156			
		Pirre top (1400);				This study	MT560712	MT540977
AB1174	SMF97121	Rancho Frío	<i>Atelopus glyphus</i>	7,94194	-77,70252			
		Donoso, Colón,				This study	MT560714	MT540979
	MHCH 1490	Panama	<i>Atelopus varius</i>					
AB984	MHCH2656	Río Terable, Burbayar	<i>Atelopus limosus</i>	9,28298	-78,98370	This study		MT540978

Supplementary Table S3. List of GenBank sequences (with GenBank accession numbers and their localities) used for phylogenetic analyses.

GenBank Accession Number COI	species 16S	Locality	Departamento	Authors	Reference	
KC129184.1		<i>Atelopus limosus</i> voucher_CH_9180	Chagres PN,Cerro	Chepo	Crawford et al.	Mol Ecol Resour
KC129183.1	KC129291.1	<i>Atelopus limosus</i> voucher_CH_6742	Brewster			13 (6), 1005-1018
KC129182.1	KC129290.1	<i>Atelopus limosus</i> voucher_CH_6717				(2013)
KC129181.1	KC129289.1	<i>Atelopus limosus</i> voucher_CH_6694				
KC014635.1	KC014780.1	<i>Atelopus limosus</i> voucher_EVACC_223	Chagres PN,Sierra	Chepo	Crawford et al.	Mol Ecol Resour
KC014634.1	KC014779.1	<i>Atelopus limosus</i> voucher_EVACC_222	Lorona			13 (6), 1005-1018
KC014633.1		<i>Atelopus limosus</i> voucher_EVACC_221				(2013)
KC014632.1		<i>Atelopus limosus</i> voucher_EVACC_220				
KC014631.1	KC014778.1	<i>Atelopus limosus</i> voucher_EVACC_009				
KC014630.1	KC014777.1	<i>Atelopus limosus</i> voucher_EVACC_008				
KC014629.1	KC014776.1	<i>Atelopus limosus</i> voucher_EVACC_007				
KC014628.1	KC014775.1	<i>Atelopus limosus</i> voucher_EVACC_006				
KC014627.1	KC014774.1	<i>Atelopus limosus</i> voucher_EVACC_005	Mamoni, Madroño	Chepo		
KC014626.1	KC014773.1	<i>Atelopus limosus</i> voucher_EVACC_004	Chagres PN,Cerro	Chepo	Crawford et al.	Mol Ecol Resour
KC014625.1	KC014772.1	<i>Atelopus limosus</i> voucher_EVACC_003	Brewster			13 (6), 1005-1018
	KC014771.1	<i>Atelopus limosus</i> voucher_EVACC_002				(2013)
KC014624.1	KC014770.1	<i>Atelopus limosus</i> voucher_EVACC_001				
KC014623.1	KC014768.1	<i>Atelopus limosus</i> voucher_EVACC_231				
KC014622.1	KC014767.1	<i>Atelopus limosus</i> voucher_EVACC_229				
KC014621.1	KC014766.1	<i>Atelopus limosus</i> voucher_EVACC_228				
KC014620.1	KC014765.1	<i>Atelopus limosus</i> voucher_EVACC_227				
KC014619.1	KC014764.1	<i>Atelopus limosus</i> voucher_EVACC_226				
KC014618.1	KC014763.1	<i>Atelopus limosus</i> voucher_EVACC_224				
	KC014762.1	<i>Atelopus limosus</i> voucher_EVACC_230				
	KC014769.1	<i>Atelopus limosus</i> voucher_EVACC_232				
FJ766577.1		<i>Atelopus zeteki</i> voucher_CH_5859	Omar Torrijos PN	Coelá	Crawford et al.	Proc. Natl. Acad.
FJ766576.1		<i>Atelopus zeteki</i> voucher_CH_5860				Sci. U.S.A. 107
FJ766575.1		<i>Atelopus zeteki</i> voucher_CH_5862				(31), 13777-13782
FJ766574.1		<i>Atelopus zeteki</i> voucher_CH_5886				(2010)
FJ766573.1		<i>Atelopus zeteki</i> voucher_CH_5864				

FJ766572.1 DQ502895.1	<i>Atelopus zeteki</i> voucher CH 5871 <i>Atelopus spurrelli</i> isolate_1275	Bahía Solano	Chocó, Colombia	Grant et al.	Bull. Am. Mus. Nat. Hist. 299, 1-261 (2006)
DQ502857.1	<i>Atelopus zeteki</i>	Las Filipinas Near Sora	Coclé	Frost et al.	Bull. Am. Mus. Nat. Hist. 297, 1-370 (2006)
U52780.1	<i>Atelopus chiriquiensis</i> MVZ_AG28		Chiriquí	Graybeal, A.	Mol Ecol Resour 13 (6), 1005-1018 (2013)

Supplementary Table S4. Male intraspecific variation in basic morphometric characters and ratios among three populations of *Atelopus fronterizo* **sp. nov.** from eastern Panama. Data follow the format Minimum-Maximum (Average±Standard Deviation (SD)). All measurements are in mm, all ratios are in %. For abbreviations, please see Methods section.

Population	Púcuro (n=3)	Malí (n=5)	Sucubtí/ Nurra/ Tuquesa (n=29)
Measurements			
SVL	24.2 – 31.6 (28.4 ± 3.8)	31.9 – 34.8 (33.8 ± 1.2)	25.6 – 34.1 (30.0 ± 2.1)
HW	7.2 – 8.8 (8.0 ± 0.8)	9.3 – 10.0 (9.7 ± 0.3)	7.5 – 9.4 (8.7 ± 0.4)
HL	7.2 – 8.5 (7.8 ± 0.7)	8.4 – 9.5 (8.9 ± 0.4)	6.2 – 9.3 (7.8 ± 0.7)
IND	2.1 – 2.8 (2.5 ± 0.3)	2.8 – 3.6 (3.1 ± 0.3)	2.4 – 3.5 (2.9 ± 0.2)
IOID	2.3 – 2.9 (2.6 ± 0.3)	2.7 – 3.5 (3.3 ± 0.3)	2.3 – 3.4 (2.8 ± 0.2)
EYDM	2.3 – 3.0 (2.7 ± 0.3)	2.5 – 3.8 (3.2 ± 0.4)	2.1 – 3.6 (2.7 ± 0.3)
EYND	2.5 – 3.0 (2.7 ± 0.2)	2.6 – 3.8 (3.1 ± 0.3)	2.3 – 3.2 (2.7 ± 0.2)
TIBL	12.1 – 17.0 (14.0 ± 2.7)	14.5 – 17.0 (16.3 ± 0.9)	12.3 – 15.7 (13.8 ± 0.7)
FTL	10.3 – 14.0 (12.0 ± 1.9)	12.3 – 15.4 (14.0 ± 1.1)	9.4 – 13.8 (11.0 ± 0.9)
FAL	7.4 – 10.4 (8.5 ± 1.7)	9.3 – 10.6 (10.3 ± 0.5)	7.8 – 9.5 (8.5 ± 0.4)
THBL	1.8 – 2.8 (2.3 ± 0.5)	2.7 – 3.2 (3.0 ± 0.2)	1.9 – 2.8 (2.3 ± 0.3)
HAL	6.4 – 8.8 (7.3 ± 1.1)	8.2 – 9.4 (9.0 ± 0.4)	6.6 – 8.5 (7.2 ± 0.4)
Ratios			
HW/SVL	27.1 – 29.7 (28.2 ± 1.3)	27.9 – 29.4 (28.7 ± 0.6)	27.2 – 34.0 (29.1 ± 1.5)
EYND/HW	31.3 – 35.9 (33.8 ± 2.4)	28.2 – 31.0 (29.9 ± 1.1)	26.5 – 36.0 (30.9 ± 2.6)
TIBL/SVL	41.0 – 54.0 (49.4 ± 7.3)	45.3 – 49.6 (47.7 ± 1.6)	41.5 – 55.6 (46.4 ± 3.3)
HAL/SVL	22.0 – 27.7 (25.3 ± 2.9)	25.8 – 26.8 (26.4 ± 0.5)	21.9 – 27.9 (23.9 ± 1.6)
THBL/SVL	6.1 – 9.0 (8.0 ± 1.7)	8.4 – 9.5 (8.9 ± 0.4)	6.3 – 10.7 (8.0 ± 1.1)
IND/IOID	89.7 – 103.8 (95.5 ± 7.4)	82.4 – 108.6 (96.8 ± 10.8)	85.5 – 121.6 (103.0 ± 9.4)
HL/HW	95.2 – 106.3 (100.5 ± 5.6)	85.5 – 95.7 (92.4 ± 4.0)	70.7 – 106.5 (90.2 ± 9.4)
FTL/SVL	34.9 – 42.7 (39.9 ± 4.4)	37.5 – 45.0 (41.2 ± 3.1)	32.1 – 44.2 (36.9 ± 3.1)
HAL/SVL	25.8 – 32.9 (29.8 ± 3.7)	29.3 – 31.3 (30.4 ± 0.8)	25.0 – 35.6 (29.0 ± 2.3)

Supplementary Table S5. Female intraspecific variation in basic morphometric characters and ratios among three populations of *Atelopus fronterizo* **sp. nov.** (no female was available for populations from Nurra and Río Tuquesa). Data follow the format Minimum-Maximum (Average \pm SD). All measurements are in mm, all ratios are in %. For abbreviations, please see Methods section.

Population	Púcuro (n=2)	Mali (n=9)	Sucubtí (n=2)
<i>Measurements</i>			
SVL	43.2– 44.4 (43.8 \pm 0.9)	41.4 – 50.1 (45.4 \pm 2.9)	35.1 – 38.3 (36.7 \pm 2.2)
HW	10.5– 11.2 (10.8 \pm 0.5)	11.0 – 12.0 (11.5 \pm 0.4)	10.1 – 11.1 (10.6 \pm 0.7)
HL	8.7 – 10.8 (9.8 \pm 1.5)	10.0 – 11.4 (10.6 \pm 0.5)	10.0 – 10.7 (10.4 \pm 0.5)
IND	3.3 – 3.3 (3.3 \pm 0.0)	3.2 – 4.0 (3.6 \pm 0.3)	3.4 – 3.6 (3.5 \pm 0.1)
IOID	4.4 – 4.7 (4.6 \pm 0.2)	3.6 – 4.5 (4.2 \pm 0.3)	3.0 – 3.6 (3.3 \pm 0.5)
EYDM	2.8 – 3.5 (3.2 \pm 0.5)	3.1 – 4.0 (3.6 \pm 0.3)	2.7 – 3.4 (3.1 \pm 0.5)
EYND	3.0 – 3.9 (3.5 \pm 0.7)	3.4 – 4.5 (3.9 \pm 0.3)	3.0 – 3.6 (3.3 \pm 0.4)
TIBL	19.0 – 23.6 (21.3 \pm 3.3)	18.9 – 23.0 (20.6 \pm 1.4)	19.4 – 19.7 (19.5 \pm 0.2)
FTL	16.4 – 20.4 (18.4 \pm 2.8)	16.9 – 21.2 (18.8 \pm 1.5)	16.1 – 19.8 (18.0 \pm 2.6)
FAL	13.8 – 14.1 (14.0 \pm 0.2)	12.3 – 14.8 (13.4 \pm 0.8)	11.9 – 11.9 (11.9 \pm 0.0)
THBL	3.5 – 4.6 (4.0 \pm 0.8)	3.5 – 5.0 (4.0 \pm 0.5)	3.7
HAL	10.2 – 12.5 (11.4 \pm 1.6)	10.2 – 12.6 (11.7 \pm 0.8)	not available
<i>Ratios</i>			
HW/SVL	24.3 – 25.2 (24.7 \pm 0.6)	23.4 – 26.8 (25.4 \pm 1.1)	28.8 – 29.0 (28.9 \pm 0.1)
EYND/HW	28.6 – 35.2 (31.9 \pm 4.7)	28.5 – 38.8 (33.5 \pm 2.8)	29.9 – 32.0 (31.0 \pm 1.5)
TIBL/SVL	44.0 – 53.2 (48.6 \pm 6.5)	40.5 – 49.2 (45.4 \pm 2.8)	50.7 – 56.0 (53.4 \pm 3.7)
HAL/SVL	23.6 – 28.2 (25.9 \pm 3.2)	22.7 – 27.2 (25.7 \pm 1.5)	not available
THBL/SVL	8.0 – 10.4 (9.2 \pm 1.6)	8.1 – 9.9 (8.8 \pm 0.6)	9.6
IND/IOID	83.0 – 96.5 (89.8 \pm 9.6)	73.3 – 104.0 (85.4 \pm 8.8)	99.5 – 115.3 (107.4 \pm 11.2)
HL/HW	83.0 – 96.5 (89.8 \pm 9.6)	86.1 – 97.1 (92.0 \pm 3.2)	96.5 – 98.9 (97.7 \pm 1.7)
FTL/SVL	46.0 – 46.0 (46.0 \pm 0.0)	37.7 – 44.5 (41.4 \pm 2.2)	42.1 – 56.4 (49.2 \pm 10.1)
HAL/SVL	31.1 – 32.7 (31.9 \pm 1.2)	27.3 – 31.6 (29.4 \pm 1.6)	31.2 – 33.8 (32.5 \pm 1.9)

Supplementary Table S6. Spectral and temporal parameters of advertisement call of *Atelopus* spp. from eastern Panama. No specimen was measured or collected. Measurements are: Mean±SD (range; n). *data from Ibáñez et al. (1995). ** data from a single call (see Methods for details on specimens).

Trait	<i>A. fronterizo</i> sp. nov. (25.5 °C)	<i>A. certus</i> (28.1 °C)
Call duration (msec)	209.8 ± 20.3 (176–235; 8)	296.7 ± 46.1 (163–351; 14)
Call interval (msec)	3036.3 ± 521.3 (2504–3878; 7)	2602.2 ± 734.5 (1519–4052; 13)
Pulse rate (pulses/sec)	131.7 ± 16.4 (97.4–144.7; 7)	132.3 ± 3.3 (122.7–136.8; 14)
Pulses #	28 ± 5.5 (19–34; 7)	39.6 ± 6.7 (20–48; 14)
Low Freq (Hz)	2367.1 ± 134.5 (2114.2–2547.9; 8)	2153.2 ± 44.6 (2076.2–2222; 15)
High Freq (Hz)	2685.7 ± 137.6 (2541.5–3006.2; 8)	2863.6 ± 196.3 (2498.3–3010.4; 15)
Dom Freq (Hz)	2505.9 ± 66.1 (2422.5–2605.5; 8)	2506.5 ± 195.3 (2314.8–2820.8; 15)
Bandwidth (Hz)	318.6 ± 254.4 (116.3–892; 8)	710.4 ± 195.9 (354.5–915.2; 15)
Calls/bouts	5–8 n: 3	2–14; n: 3
Trait	<i>A. glyphus</i> (25.7 °C)	<i>A. limosus</i> (26.0 °C) *
Call duration (msec)	250.1 ± 13.2 (225–272; 14)	260 ± 30 (205–295; 13)
Call interval (msec)	2714.8 ± 663.3 (2140–4606; 13)	
Pulse rate (pulses/sec)	139.4 ± 4.4 (128.9–143.4; 13)	151.6 ± 3.1 (146.4–156.3; 13)
Pulses #	34.9 ± 2.7 (29–38; 13)	40 ± 5 (31–45; 13)
Low Freq (Hz)	2548.9 ± 46.3 (2463.7–2630.7; 14)	2160 ± 49 (2080–2200; 13)
High Freq (Hz)	3172.1 ± 34.7 (3074.5–3200.2; 14)	3409 ± 174 (3080–3760; 13)
Max Freq (Hz)	2988.1 ± 41.8 (2874.7–3025.4; 14)	2689 ± 93 (2600–2800; 13)
Bandwidth (Hz)	623.2 ± 50.1 (528.4–670; 14)	729.1**
Calls/bouts	7–13; n: 2	