Evolutionary Biology

Model-based analyses suggest Pleistocene refugia over ancient divergence as main diversification driver for a Neotropical open-habitat treefrog

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Online Resource

Sampling

We obtained 68 tissue samples of *Scinax fuscomarginatus* from 43 localities representing populations from Cerrado, Humid Chaco, Chiquitano dry forest, Pantanal, Guianan savannas, and Amazon forest (Appendix S1).

Appendix S1: Geographical information and GenBank accessions of sequences of *Scinax fuscomarginatus*. Voucher acronyms follow Sabaj (2016) except for CAB (C. Barrio-Amorós field number) and FPM (deposited in tissue collection of UFMT). Map codes follow Figure 1. COI, Cytochrome c oxidase subunit I gene; RPL3, Ribosomal protein L3 (intron 5) gene; BFIB, B–fibrinogen (intron 7) gene; MVZ27-28, Lactose Dehydrogenase Chain Beta (Intron 3) gene. (GenBank accession of sequences generated for this article upon acceptance)

voucher	map code	country	department/ province/state	locality	lat	long	COI	RPL3	BFIB	MVZ27-28
CAB5751	1	Venezuela	Bolívar	Santa Elena Uairén	4.603	-61.111	KJ004192	OR450880	OR450823	
CHUNB38024	2	Brazil	Tocantins	Paranã	-12.616	-47.884	MT313243		OR450824	OR453622
CHUNB51008	3	Brazil	Bahia	Jaborandi	-14.430	-45.890	OR432592	OR450881	OR450825	OR453623
CFBH20548_L	3	Brazil	Bahia	Jaborandi	-14.430	-45.890	KJ004205		OR450826	OR453624
CFBH20548_S	3	Brazil	Bahia	Jaborandi	-14.430	-45.890				OR453625
CFBH22156	4	Brazil	Bahia	Caetité	-14.055	-42.474	OR432593	OR450882	OR450827	
CFBH23345	5	Brazil	Bahia	Caetité	-14.130	-42.509	KJ004210	OR450883	OR450828	
ZUFG4760	6	Brazil	Distrito Federal	Brazlândia	-15.678	-48.199	KJ004262	OR450884	OR450829	OR453626
ZUFG4761	6	Brazil	Distrito Federal	Brazlândia	-15.678	-48.199	OR432594	OR450885	OR450830	
CHUNB49560	7	Brazil	Goiás	Alto Paraíso de Goiás	-14.134	-47.522	KJ004232		OR450831	OR453627

ZUFG4445	8	Brazil	Goiás	Jataí	-17.885	-51.723	KJ004261	OR450886		OR453628
CHUNB36466	9	Brazil	Goiás	São Domingos	-13.261	-46.733	OR432595		OR450832	OR453629
CFBH24359	10	Brazil	Minas Gerais	Lagoa Santa	-19.535	-43.959	KJ004212	OR450887	OR450833	OR453630
CFBH24360	10	Brazil	Minas Gerais	Lagoa Santa	-19.535	-43.959	KJ004213	OR450888	OR450834	OR453631
CHUNB43485	11	Brazil	Minas Gerais	Buritizeiro	-17.414	-45.150	KJ004230		OR450835	OR453632
CFBHT1949	12	Brazil	Minas Gerais	Serra do Cipó	-19.115	-43.680	OR432596	OR450889	OR450836	OR453633
CFBHT1950	12	Brazil	Minas Gerais	Serra do Cipó	-19.115	-43.680	KJ004223		OR450837	OR453634
CFBH18855	13	Brazil	São Paulo	Estação Ecológica Assis	-22.606	-50.388	KJ004203	OR450890	OR450838	OR453635
CFBH18856	13	Brazil	São Paulo	Estação Ecológica Assis	-22.606	-50.388	OR432597	OR450891	OR450839	OR453636
CFBH6527	14	Brazil	São Paulo	Estação Ecológica Itirapina	-22.226	-47.894	KJ004217	OR450892	OR450840	OR453637
CFBH6288	15	Brazil	São Paulo	Pindamonhangaba	-22.924	-45.463	OR432598	OR450893	OR450841	
CFBH19354_L	16	Brazil	São Paulo	Monte Alegre, Itapetininga	-23.595	-48.054	KJ004204	OR450894	OR450842	OR453638
CFBH19354_S	16	Brazil	São Paulo	Monte Alegre, Itapetininga	-23.595	-48.054				OR453639
MNKA9624	17	Bolivia	Santa Cruz	Velasco, Caparu	-14.910	-61.072	KJ004257	OR450895		
CFBH10049	18	Brazil	São Paulo	P. E. Morro do Diabo	-22.541	-52.298	KJ004194	OR450896	OR450843	OR453640
CFBH18678	18	Brazil	São Paulo	P. E. Morro do Diabo	-22.541	-52.298	KJ004201		OR450844	OR453641
IIBPH1593	19	Paraguay	Guairá	Coronel Martínez	-25.677	-56.688	KJ004248	OR450897	OR450845	OR453642
IIBPH1594	19	Paraguay	Guairá	Coronel Martínez	-25.677	-56.688	OR432599	OR450898	OR450846	OR453643
IIBPH1576	20	Paraguay	Itapúa	Reserva San Rafael	-26.497	-55.786	KJ004247		OR450847	OR453644
IIBPH436	21	Paraguay	Cordillera	Emboscada	-25.140	-57.358	KJ004250		OR450848	OR453645
IIBPH1558_L	22	Paraguay	Presidente Hayes	La Golondrina	-24.933	-57.700	OR432600	OR450899		OR453646
IIBPH1558_S	22	Paraguay	Presidente Hayes	La Golondrina	-24.933	-57.700				OR453647
IIBPH265	23	Paraguay	Ñeembucú	Estancia San José	-27.202	-58.449	OR432601		OR450849	OR453648
IIBPH269	23	Paraguay	Ñeembucú	Estancia San José	-27.202	-58.449	KJ004249		OR450850	OR453649
LGE16118	24	Argentina	Corrientes	Rincón Santa María	-27.539	-56.582	KJ004251		OR450851	OR453650
LGE16148_L	25	Argentina	Corrientes	Rincón Santa María	-27.517	-56.600	OR432602	OR450900	OR450852	
LGE16148_S	25	Argentina	Corrientes	Rincón Santa María	-27.517	-56.600		OR450901		
ZUFMS482_L	26	Brazil	Mato Grosso do Sul	Porto Murtinho	-21.713	-57.726	KJ004268		OR450853	OR453651
ZUFMS482_S	26	Brazil	Mato Grosso do Sul	Porto Murtinho	-21.713	-57.726				OR453652
ZUFMS483	26	Brazil	Mato Grosso do Sul	Porto Murtinho	-21.713	-57.726	OR432603	OR450902	OR450854	OR453653
FPM61	27	Brazil	Mato Grosso do Sul	Nioaque	-21.126	-55.833	OR432604	OR450903	OR450855	
CFBH21857	28	Brazil	Mato Grosso	E. E. Serra das Araras	-15.187	-56.864	KJ004208	OR450904	OR450856	OR453654
CFBH21858	28	Brazil	Mato Grosso	E. E. Serra das Araras	-15.187	-56.864	OR432605	OR450905	OR450857	
CFBH14302	29	Brazil	Mato Grosso	Chapada dos Guimarães	-15.176	-55.756	KJ004199	OR450906	OR450858	OR453655
CFBH14303	29	Brazil	Mato Grosso	Chapada dos Guimarães	-15.176	-55.756	MT313228	OR450907	OR450859	OR453656

CFBH14335	30	Brazil	Mato Grosso	Dom Aquino	-15.678	-55.056	KJ004200		OR450860	OR453657
CTMZ5731	31	Brazil	Mato Grosso	Guiratinga	-16.336	-53.765	KJ004239		OR450861	OR453658
CTMZ5178_L	32	Brazil	Mato Grosso	Sapezal	-12.985	-58.760	KJ004237	OR450908	OR450862	
CTMZ5178_S	32	Brazil	Mato Grosso	Sapezal	-12.985	-58.760		OR450909		
CTUFMT193	33	Brazil	Mato Grosso	Lucas do Rio Verde	-13.061	-55.921	OR432606		OR450863	OR453659
CTUFMT199	33	Brazil	Mato Grosso	Lucas do Rio Verde	-13.061	-55.921	OR432607		OR450864	OR453660
CTMZ6223	34	Brazil	Mato Grosso	Itaúba	-11.095	-55.304	KJ004241		OR450865	OR453661
CTMZ6225	34	Brazil	Mato Grosso	Itaúba	-11.095	-55.304	OR432608		OR450866	OR453662
CTMZ6684	35	Brazil	Mato Grosso	Paranaíta	-9.067	-56.779	KJ004242	OR450910	OR450867	OR453663
CTMZ2886	36	Brazil	Tocantins	U. H. Peixe Angical	-12.242	-48.404	KJ004236		OR450868	OR453664
CTMZ2887	36	Brazil	Tocantins	U. H. Peixe Angical	-12.242	-48.404	OR432609		OR450869	OR453665
CHUNB46078	37	Brazil	Tocantins	Caseara	-9.316	-49.957	MT313237		OR450870	OR453666
CHUNB43180	38	Brazil	Tocantins	Pedro Afonso	-8.972	-48.175	KJ004229		OR450871	OR453667
CFBH13240	39	Brazil	Tocantins	Lizarda	-9.590	-46.675	OR432610		OR450872	OR453668
CFBH13241_L	39	Brazil	Tocantins	Lizarda	-9.590	-46.675	OR432611	OR450911	OR450873	OR453669
CFBH13241_S	39	Brazil	Tocantins	Lizarda	-9.590	-46.675		OR450912		
CHUNB51596	40	Brazil	Maranhão	Carolina	-7.377	-47.426	KJ004235	OR450913	OR450874	OR453670
CFBH7349_L	41	Brazil	Alagoas	Passo de Camarajibe	-9.241	-35.491	KJ004219	OR450914	OR450875	OR453671
CFBH7349_S	41	Brazil	Alagoas	Passo de Camarajibe	-9.241	-35.491		OR450915		
CFBH7351	41	Brazil	Alagoas	Passo de Camarajibe	-9.241	-35.491	MT313218	OR450916	OR450876	OR453672
CFBH39905	42	Brazil	Ceará	E. E. Pecém, Caucaia	-3.736	-38.653	KJ004193		OR450877	OR453673
CFBH39906	42	Brazil	Ceará	E. E. Pecém, Caucaia	-3.736	-38.653	OR432612	OR450917	OR450878	OR453674
CFBHT10947	43	Brazil	Piauí	Baixa Grande	-5.865	-42.184	KJ004221		OR450879	OR453675

Table S1: Primers and annealing temperature used to amplify each locus. The amplification of Cytochrome c oxidase subunit 1 (CO1) was performed by a step-up reaction (UP) following Lyra et al. (2017).

Locus ID (base pair number)	**Primer sequence 5'- 3'**	**Annealing temperature (ºC)**	**Reference**
CO1 (637 bp) Cytochrome c oxidase subunit 1	ANF1 ACHAAYCAYAAAGAYATYGG ANR1 CCGGTCTGAACTCAGATCACGT		Jungfer et al., 2013
RPL3 (418 bp) Ribosomal Protein L3 (Intron 5)	RPL35F AAGAAGTCYCACCTCATGGAGAT RPL36RA AGTTTCTTTGTGTGCCAACGGCTAG	50	Pinho et al., 2009
BFIB (380 bp) B-fibrinogen (intron 7)	FIB-B17L TCCCCAGTAGTATCTGCCATTAGGGTT FIB-B17U GGAGAAAACAGGACAATGACAATTCAC	47; 48; 50; 52; 54	Prychitko & Moore, 1997

MVZ 27-28 (356 bp) Lactose Dehydrogenase Chain Beta (Intron 3)	MVZ27 ATTATTCCGTAACAGCAAACTC MVZ28 GTAACCATGGCAACTGGTAG	53	Bell et al., 2011

Population assignment with BAPS

BAPS assignment subdivided GENELAND groups (Fig. S1). Group A of GENELAND includes two clusters, a northern cluster (A1) and a southern cluster (A2). GENELAND group B was separated in three distinct clusters, a central-southeastern (B1), a southern (B2), and a northern cluster (B3). BAPS also breaks GENELAND group C into three separate clusters, one for each locality, that is, cluster C1 from Guianan Savanna, C2 from western Cerrado and C3 from Chiquitano dry forest. Cluster D is maintained in BAPS assignment the same as in GENELAND.

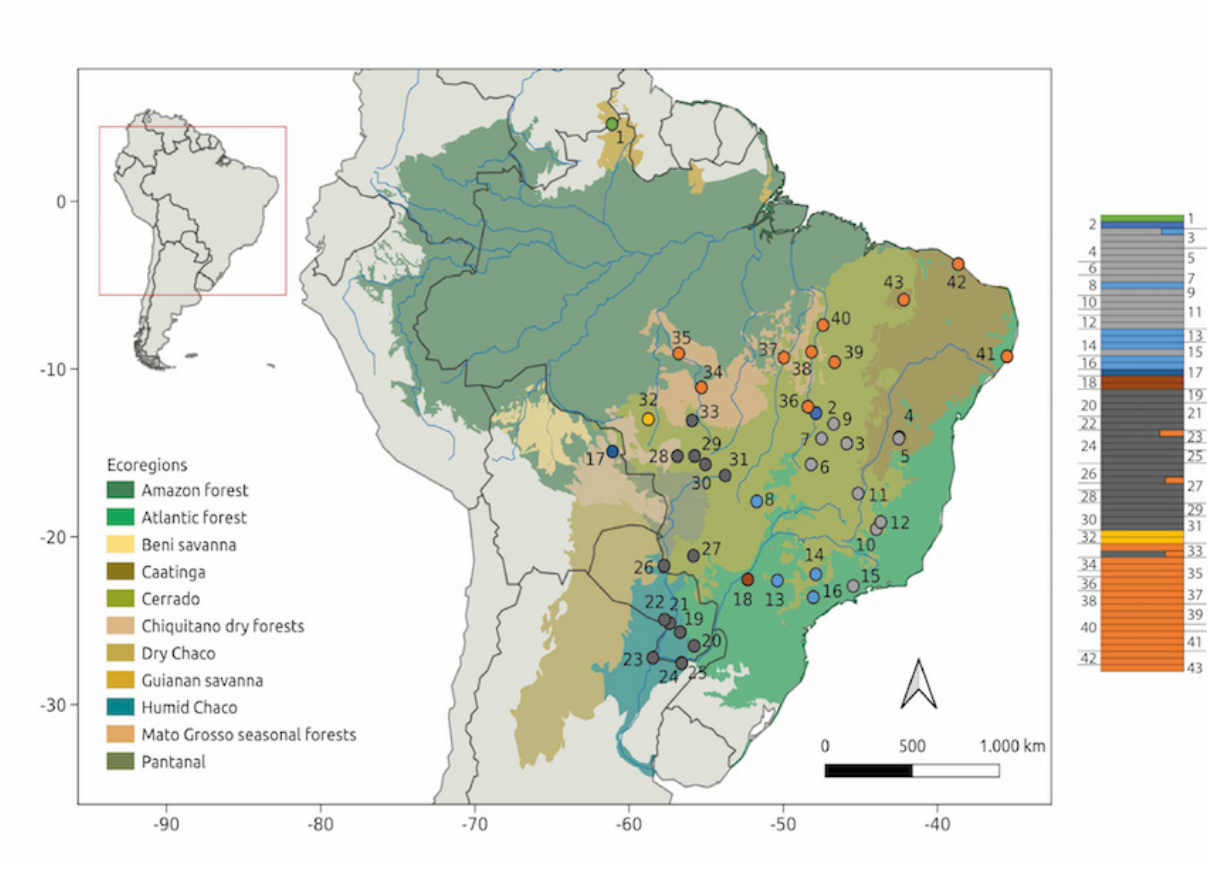

Fig. S1 Sampled localities and population assignment of *Scinax fuscomarginatus*. Horizontal bars (right) correspond to each specimen analyzed in BAPS, genetic clusters (groups) are represented by colors: group A1 = orange; group A2 = dark gray; group B1 = light gray; group B2 = turquoise; group B3 = blue; group C1 = green; group C2 = yellow; group C3 =

dark blue; group D = red. Numbers in the map and horizontal bars correspond to map codes in Appendix S1 in Online Resource. Blue lines represent main rivers, and black lines represent country borders. Ecoregions were modified from Olson et al. (2001)

Species trees

In *BEAST run effective samples sizes (ESS) were >200 for all parameters denoting a high confidence of the estimates. The resulting topology recovered group B at the base of the tree, sister of group D and the clade with the groups A + C. The relationship between group D and the clade with the groups A + C is not fully supported, with 0.7 of posterior probability.

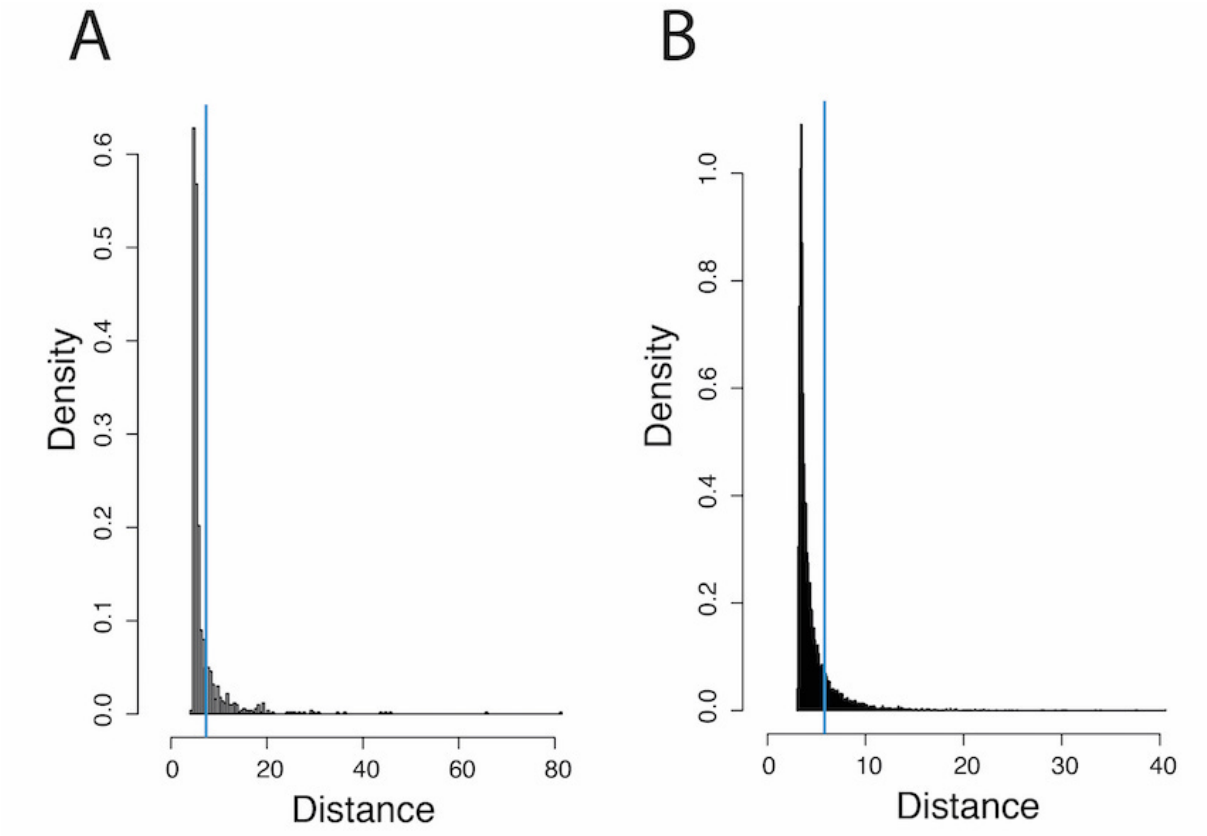

Fig. S2 Goodness-of-fit of the data simulated from the best-supported model (model 3: isolation by instability 1) in relation to empirical data. Gray bars correspond to the density of the simulated data and blue bars to empirical data. (a) Represent the data set transformed by PCA, and (b) represent the untransformed data set

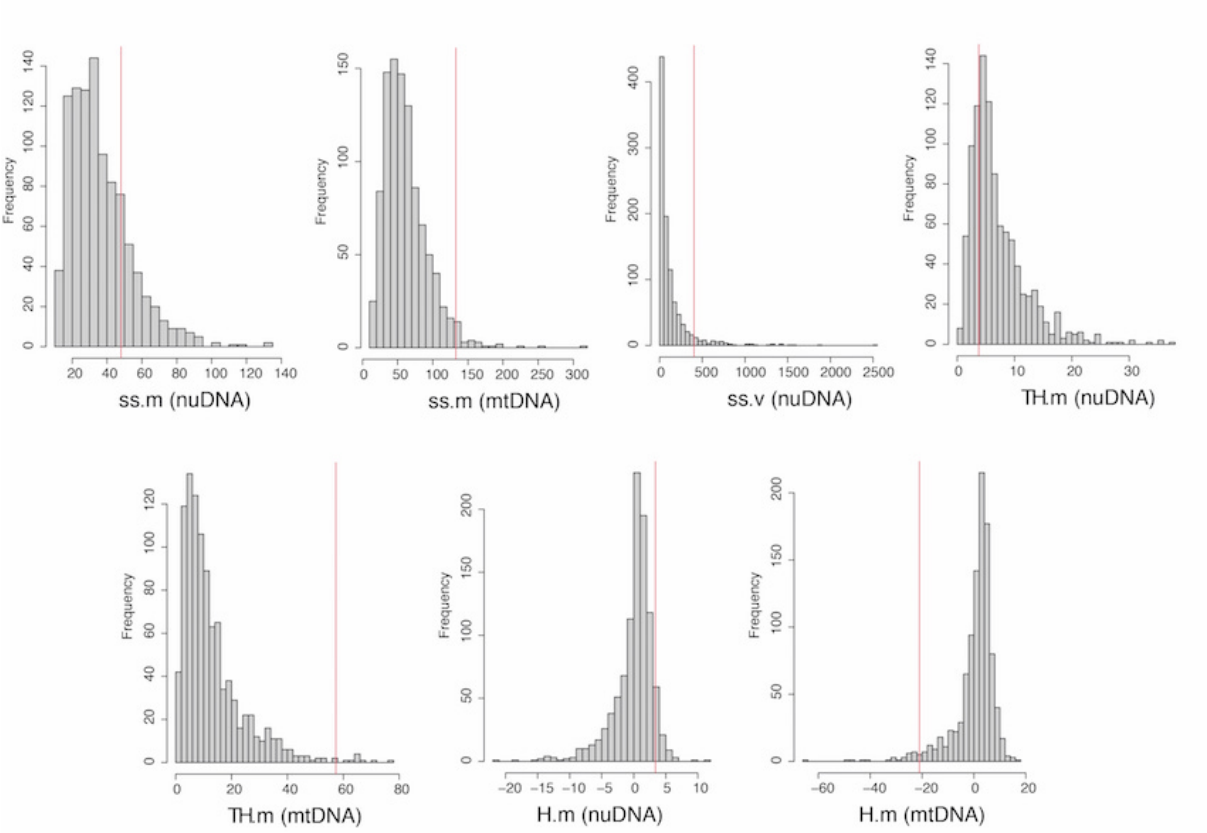

Fig. S3 Posterior predictive check of each summary statistic simulated from the best-supported model (model 3: isolation by instability 1) in relation to empirical data. Gray bars correspond to simulated summary statistics and red lines to empirical data. Summary statistic abbreviations are as follow: ss.m = media of number of segregating sites; ss.v = variance of number of segregating sites; TH.m = median of Fay and Wu's thetaH; H.m = median of the difference between thetaH and pi (pi is the average number of pairwise differences between sequences)

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