Supplemental Online Material

Historical Amphibian Declines in Brazil Linked to Chytridiomycosis

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Supplemental Text

Material and Methods

List of collections and museums from which samples were obtained.

- Coleção de Anuros Célio F. B. Haddad, Universidade Estadual Paulista, Rio Claro (CFBH)
- Coleção Científica de Anfíbios do Departamento de Zoologia e Botânica, Universidade Estadual Paulista, São José do Rio Preto (DZSJRP)
- Coleção de Anfíbios e Répteis, Instituto Nacional de Pesquisa da Amazônia, Manaus (INPA)
- Museu Nacional, Rio de Janeiro (MNRJ)
- Museu Paraense Emílio Goeldi, Belém (MPEG)
- Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP)
- Museu de Ciência e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP)
- Coleção Zoológica de Vertebrados, Universidade Federal de Mato Grosso, Cuiabá (CZV-UFMT)
- Coleção Científica do Laboratório de Herpetologia, Universidade Federal de Santa Maria, Santa Maria (ZUFSM)
- Museu de Zoologia "Prof. Adão José Cardoso", Universidade Estadual de Campinas, Campinas (ZUEC)

Table S1. Museum-preserved tadpoles analyzed visually and histologically for the presence of zoosporangia of *Batrachochytrium dendrobatidis*.

Specimen	Visual inspection	Histological Result
ZUEC 11546	*Depigmented*	*Bd Zoosporangia present*
ZUEC 15827	*Depigmented*	*Bd Zoosporangia not found*[*]
ZUEC 15859	*Depigmented*	*Bd Zoosporangia present*
ZUEC 17257	*Depigmented*	*Bd Zoosporangia present*
ZUEC 17257	*Depigmented*	*Bd Zoosporangia present*
ZUEC 19203	*Depigmented*	*Bd Zoosporangia present*
ZUEC 19203	*Depigmented*	*Bd Zoosporangia present*
ZUEC 15843	*Depigmented*	*Bd Zoosporangia present*
ZUEC 15843	*Depigmented*	*Bd Zoosporangia present*
ZUEC 20515	*Depigmented*	*Bd Zoosporangia present*
ZUEC 1083	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15201	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15320	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15402	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15404	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15417	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15474	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15642	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 15821	*Pigmented*	*Bd Zoosporangia not found*
ZUEC 17811	*Pigmented*	*Bd Zoosporangia not found*

* Tadpole with fully depigmented mouthpart.

Table S2. Proportion of *Bd*-positive or *Bd*-undetected tadpoles from three screening methods.

Pigmentation	**Method**	***Bd*$^{+}$ n (%)**	***Bd undetected* n (%)**
Normal	Histological Sections	1 (10%)	9 (90%)
Depigmented	Histological Sections	10 (100%)	0
Normal	qPCR test *	2 (14.3%)	12 (85.7%)
Depigmented	qPCR test **	10 (100%)	0
Normal	*Bd* isolation success	6 (11%)	48 (89%)
Depigmented	*Bd* isolation success	120 (100%)	0

**Bd* infection loads (zoospore genomic equivalents; g.e.) of samples 1-12=0, 13=2, 14=2.

** *Bd* infection loads (g.e.) of samples 15=742, 16=570, 17=404, 18=388, 19=294, 20=566, 21=2604, 22=62, 23=1990, 24=1426.

Table S3. Species, site (number in parenthesis stands for the number of the site in Figure 2), probable period of population decline or extinction (some data refers to the last specimen collected and deposited in museums; though we did not have access to all collected specimens), suggested causes of threat, and references.

Family / Species	Site, State (Reference to Figures 2)	Date	Decline / Extinction	Suggested Cause	Reference
Aromobatidae					
Allobates capixaba	Santa Teresa, ES (10)	1988	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Allobates olfersioides	Engenheiro Paulo de Frontin, RJ (8)	1987	Extinct	Unknown	Izecksohn & Carvalho e Silva 2001 DOU 2014; data based on the museum record MNRJ 40580
Bufonidae					
Melanophryniscus moreirae	Itatiaia, RJ (7)	1975-1994	Decline	Ultraviolet radiation and infectious diseases	Guix *et al.* 1998
Brachycephalidae					
Ischnocnema oea	Santa Teresa, ES (10)	1942-1984	Decline	Unknown	JL Gasparini, person. comm.; Heye 1984
Ischnocnema paranaensis	Antonina, PR (3)	1988-1999	Extinct	Unknown	Eterovick *et al.* 2005; Bornschein *e al.* 2015
Ischnocnema parva	Boraceia, SP (5)	1979-1983	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Ischnocnema sp. (aff. *guentheri*)	Boraceia, SP (5)	1979-1983	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Centrolenidae					
Vitreorana eurygnatha	Santa Teresa, ES (10)	1981-1987	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Vitreorana eurygnatha	Boraceia, SP (5)	1979-1982	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Vitreorana uranoscopa	Rio de Janeiro, RJ (8)	1951	Decline	Unknown	Izecksohn & Carvalho e Silva 2001 between 1951 (MNRJ) and 2011

					(MNRJ) no individuals have been collected
Craugastoridae					
Holoaden bradei	Itamonte, MG (7)	1978	Extinct	Habitat degradation and heavy frost	Stuart *et al.* 2008; DOU 2014; data based on the museum record ZUEC 8138-45
Cycloramphidae					
Cycloramphus boraceiensis	Boraceia, SP (5)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988
Cycloramphus duseni	Morretes, PR (3)	1982	Extinct	Pollution and heavy frost	Eterovick *et al.* 2005
Cycloramphus eleutherodactylus	Rio de Janeiro, RJ (8)	1949	Extinct	Unknown	Izecksohn & Carvalho e Silva 2001 Last specimens collected in 1949: MNRJ 60503
Cycloramphus fuliginosus	Santa Teresa, ES (10)	1981-1987	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Cycloramphus fuliginosus	Rio de Janeiro, RJ (8)	1985	Extinct	Unknown	Izecksohn & Carvalho e Silva 2001 Last specimens collected in 1985: MNRJ 76112-13
Cycloramphus granulosus	Itatiaia, RJ (7)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988
Cycloramphus mirandaribeiroi	São João da Graciosa, PR (3)	1978-1986	Extinct	Unknown	Eterovick *et al.* 2005
Cycloramphus ohausi	Serra dos Órgãos, RJ (8)	1942	Extinct	Unknown	DOU 2014; Last specimens collecte in 1942: MNRJ 78796-97, MNRJ 78800-01
Cycloramphus semipalmatus	Boraceia, SP (5)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988
Cycloramphus stejnegeri	Serra dos Órgãos, RJ (8)	1979	Extinct	Unknown	Garcia *et al.* 2010; DOU 2014; Last specimens collected in 1979: MNR 57052
Cycloramphus valae	Lauro Müller, SC (2)	1982	Extinct	Unknown	Heyer 1983
Cycloramphus valae	Cambará do Sul, RS (1)	1976	Extinct	Unknown	Garcia & Vinciprova 1998

Thoropa taophora	Boraceia, SP (5)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988
Thoropa petropolitana	Serra dos Órgãos, RJ (8)	1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988; DOU 2014; MNRJ 61403-4
Thoropa lutzii	Serra dos Órgãos, RJ (8)	1951	Extinct	Unknown	Izecksohn & Carvalho e Silva 2001 MNRJ 23484-5
Dendrobatidae					
Ameerega flavopicta	Serra do Cipó, MG (9)	1974-1996	Decline	Fire, human settlement and habitat fragmentation	Eterovick *et al.* 2005
Eleutherodactylidae					
*Adelophryne baturitensis**	Pacoti, CE	1993-1994	Extinct	Unknown	Eterovick *et al.* 2005
Hemiphractidae					
Fritziana ohausi	Boraceia, SP (5)	1979-1982	Decline or Extinct	Lack of breeding site, due to Bamboo reproductive cycle	Heyer *et al.* 1988
Hylidae					
Aplastodiscus flumineus	Serra dos Órgãos, RJ (8)	2003	Decline	Unknown	SP Carvalho e Silva, persn. comm.; UNIRIO 1589; 1736
Aplastodiscus musicus	Serra dos Órgãos, RJ (8)	1986	Extinct	Unknown	SP Carvalho e Silva, persn. comm.
Bokermannohyla circumdata	Rio de Janeiro, RJ (8)	n/a	Decline	Unknown	Izecksohn & Carvalho e Silva 2001
Bokermannohyla clepsydra	São José do Barreiro, SP (7)	1968	Extinct	Unknown	Garcia *et al.* 2010; DOU 2014; Last specimens collected in 1968: ZUEC 15937
Bokermannohyla izecksohni	Botucatu, SP (4)	1993	Extinct	Habitat destruction, pollution	Machado *et al.* 2005; Last specimen collected in 1993: MNRJ 61401, 79116
Bokermannohyla langei	Morretes, PR (3)	1946-1986	Extinct	Unknown	Eterovick *et al.* 2005
Dendropsophus ruschii	Santa Teresa, ES (10)	1982-1987	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Hypsiboas cymbalum	Campo Grande da Serra,	1963***	Extinct	Habitat loss and	Stuart *et al.* 2008; Garcia *et al.* 201

	SP (5)			chytridiomycosis	DOU 2014
Hypsiboas prasinus	Boraceia, SP (5)	1965	Extinct	Competition with *H. albopunctatus*	Heyer *et al.* 1988
Ololygon cf. *perpusillus*	Boraceia, SP (5)	1979-1982	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Ololygon peixotoi	Queimada Grande Island, SP (5)	2007***	Decline/ Extinct	Unknown	DOU 2014
Ololygon heyeri	Santa Teresa, ES (10)	1982-1987	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
*Phyllodytes luteolus***	Linhares, ES	1993-1995	Extinct	Fire	Eterovick (1999); Papp & Papp (2000)
Xenohyla truncate	Itaguaí, RJ (8)	1967	Decline	Unknown	Stuart *et al.* 2008; DOU 2014; Last specimens collected in 1967: MNR. 74019-20
Hylodidae					
Crossodactylus dispar	Boraceia, SP (5)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988; Last specimens collected in 1977: USNM 318225, 318227, 318230
Crossodactylus cf. *gaudichaudii*	Boraceia, SP (5)	1979-1994	Extinct	Progressive aridity of the climate	Heyer *et al.* (1988), Bertoluci & Heyer (1995)
Crossodactylus cf. *gaudichaudii*	Santa Teresa, ES (10)	1982-1988	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Crossodactylus timbuhy	Santa Teresa, ES (10)	1982-1988	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Crossodactylus trachystomus	Serra do Cipó, MG (9)	1974-1996	Decline	Habitat reduction and fragmentation	Eterovick *et al.* 2005
Crossodactylus werneri	Itatiaia, RJ (7)	1978	Extinct	Unknown	Pimenta *et al.* 2014
Hylodes asper	Boraceia, SP (5)	1979-1982	Extinct	Pollution and heavy frost	Heyer *et al.* 1988

Hylodes babax	Santa Teresa, ES (10)	1982-1987	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Hylodes babax	Santa Teresa, ES (10)	1988	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Hylodes glaber	Brejo da Lapa, MG/RJ (7)	1979-1984	Extinct	Pollution and heavy frost	Heyer *et al.* 1988
Hylodes lateristrigatus	Santa Teresa, ES (10)	1982-1988	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Hylodes mertensi	Old road between São Paulo and Santos, SP (5)	1956	Extinct	Unknown	Bokermann 1956
Hylodes phyllodes	Boraceia, SP (5)	1979-1982	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Megaelosia bocainensis	São José do Barreiro, SP (7)	1968 ***	Extinct	Unknown	Giaretta *et al.* 1993; Holotype: MNRJ 15900.
Leptodactylidae					
Adenomera marmorata	Boraceia, SP (5)	1979-1983	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Paratelmatobius lutzii	Itatiaia, RJ (7)	1978	Extinct	Unknown	Pombal & Haddad 1999; DOU 201
Paratelmatobius gaigeae	Paranapiacaba, SP (5) / Boraceia, SP (5)	1931	Extinct	Habitat degradation	Pombal & Haddad 1999; Zaher *et a* 2005
Paratelmatobius mantiqueira	Campos do Jordão, SP (6)	1953	Extinct	Unknown	Pombal & Haddad 1999; Vrcibradi *et al.* 2010
Physalaemus jordanensis	Campos do Jordão, SP (6)	2007	Extinct	Unknown	IA Martins, persn. comm.
Odontophrynidae					
Proceratophrys moratoi	Botucatu, SP (4)	n/a	Extinct	Habitat destruction, pollution	Machado *et al.* 2005
Phyllomedusidae					
Phasmahyla guttata	Teresópolis, RJ (8)	1979-1984	Decline	Pollution and heavy frost	Heyer *et al.* 1988
Phasmahyla guttata	Rio de Janeiro, RJ (8)	1969-2010	Decline	Unknown	Izecksohn & Carvalho e Silva 2001 Between 1969 (MNRJ 49235-36)

					and 2010 (MNRJ 78409-10) specimens were collected only in 1985 (MNRJ 81341-42)
Phasmahyla exilis	Santa Teresa, ES (10)	1982-1987	Decline	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Phasmahyla exilis	Santa Teresa, ES (10)	1988	Extinct	Pollution, climatic changes and epidemic diseases	Weygoldt 1989
Phrynomedusa appendiculata	Paranapiacaba, SP (5)	1966	Extinct	Unknown	Garcia *el al.* 2004
Phrynomedusa bokermanni	Mongaguá, SP (5)	1978***	Extinct	Unknown	Present study
Phrynomedusa fimbriata	Paranapiacaba, SP (5)	1923***	Extinct	Unknown	Machado *et al.* 2005; Stuart *et al.* 2008; Garcia *et al.* 2010; DOU 201
Phrynomedusa marginata	Santa Teresa, ES (10)	1974***	Extinct	Unknown	Present study
Phrynomedusa vanzolinii	Boraceia, SP (5)	1929***	Extinct	Unknown	Cruz 1991; Garcia *et al.* 2010; DOU 2014
Phrynomedusa vanzolinii	Serra dos Órgãos, RJ (8)	1929***	Extinct	Unknown	Cruz 1991; Garcia *et al.* 2010; DOU 2014

Both species indicated with asterisks were not mapped, either because it does not occurs in the Atlantic Forest "*" or the causes of decline are well known and do not relate to *Bd* "**". UNIRIO: Universidade Federal do Rio de Janeiro; USNM: National Museum of Natural History; SP: state of São Paulo, RJ: state of Rio de Janeiro, MG: state of Minas Gerais, ES: state of Espírito Santo, PR: state of Paraná, SC: state of Santa Catarina, RS: state of Rio Grande do Sul. n/a = not available. Three asterisks "***" indicates the year of description, and that no other individual was found after that.

Table S4. Spatiotemporal clusters of *Bd*-infected tadpoles obtained with 21-year temporal aggregations. Abbreviations as follows: temporal aggregation in years (AG), cluster radius in km (Radius), number of locations (NL), log-likelihood ratio (LLR), observed number of positive samples (O), expected positive cases (E), relative risk (RR).

Cluster	AG	Latitude	Longitude	Radius	Period	NL	LLR	*P* value	O	E	RR
Bd-C1	21	23.895 S	46.425 W	368.69	1974-2015	6207	336.5	< 0.001	1630	1028.09	2.52
Bd-C2	21	29.242 S	50.449 W	-	1974-2015	772	167.9	< 0.001	338	127.87	2.88
Bd-C3	21	27.243 S	53.953 W	-	1995-2015	30	29.3	< 0.001	24	4.97	4.86
Bd-C4	21	26.564 S	52.556 W	33.44	1995-2015	13	23.4	< 0.001	13	2.15	6.06
Bd-C5	21	27.737 S	51.442 W	44.63	1995-2015	14	13.0	< 0.001	11	2.32	4.76

Table S5. *Bd*-C1 obtained with 20-, 18-, 16-, 14-, 12-, and 10-year temporal aggregations. Abbreviations as follows: temporal aggregation in years (AG), cluster radius in km (Radius), number of locations (NL), log-likelihood ratio (LLR), observed number of positive samples (O), expected positive cases (E), relative risk (RR).

AG	Latitude	Longitude	Radius	Period	NL	LLR	*P* value	O	E	RR
10	23.895 S	46.425 W	368.69	1976-2015	6047	366.2	< 0.001	1628	1001.59	2.62
12	23.895 S	46.425 W	368.69	1980-2015	5844	382.6	< 0.001	1606	967.97	2.68
14	23.895 S	46.425 W	368.69	1974-2015	6207	336.5	< 0.001	1630	1028.09	2.52
16	23.895 S	46.425 W	368.69	1984-2015	5776	387.6	< 0.001	1598	956.70	2.69
18	23.895 S	46.425 W	368.69	1980-2015	5844	382.6	< 0.001	1606	967.97	2.68
20	23.895 S	46.425 W	368.69	1976-2015	6047	366.2	< 0.001	1628	1001.59	2.62

* Centroid location and radius coincide among six alternative *Bd*-C1.

Table S6. AIC model averaging results for *Bd* occurrence (temporally detrended) in Brazilian tadpoles.

Data source	Parameter	Estimate	Std Error
Without error	Intercept	-0.415	.
	Human footprint	0.159	0.474
	Vegetation density	0.065	0.474
	Topographic complexity	0.224	0.022
	Rainfall	0.083	0.022
	Temperature	-0.125	0.030
	Elevation	0.322	0.031
10% screening error			
	Intercept	-0.411	.
	Human footprint	0.228	0.022
	Vegetation density	0.047	0.022
	Topographic complexity	0.202	0.020
	Rainfall	0.056	0.021
	Temperature	-0.120	0.029
	Elevation	0.290	0.029

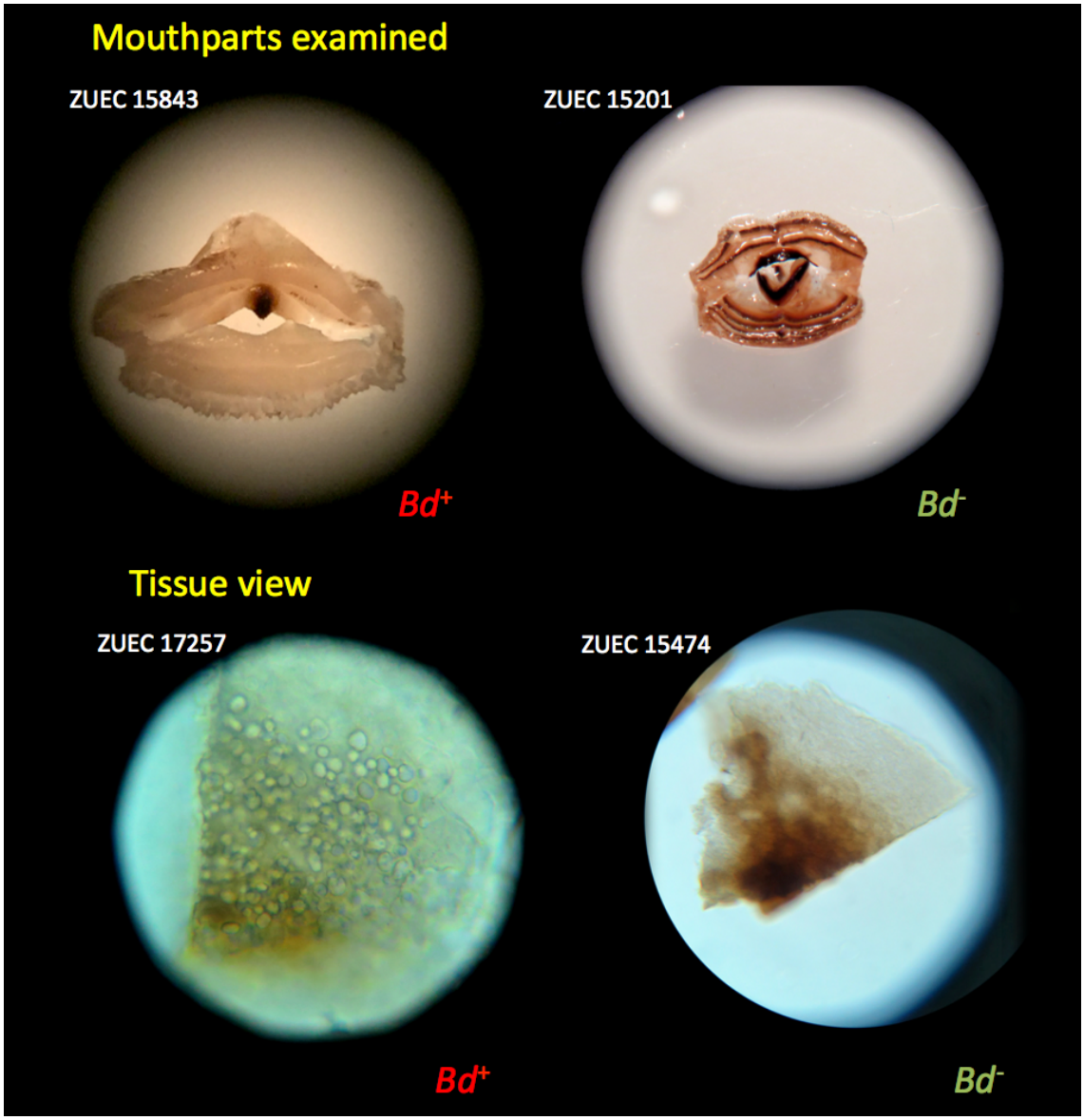

Figure S1. Mouthparts and tissue view of one *Bd*-infected and one uninfected tadpole analyzed histologically.

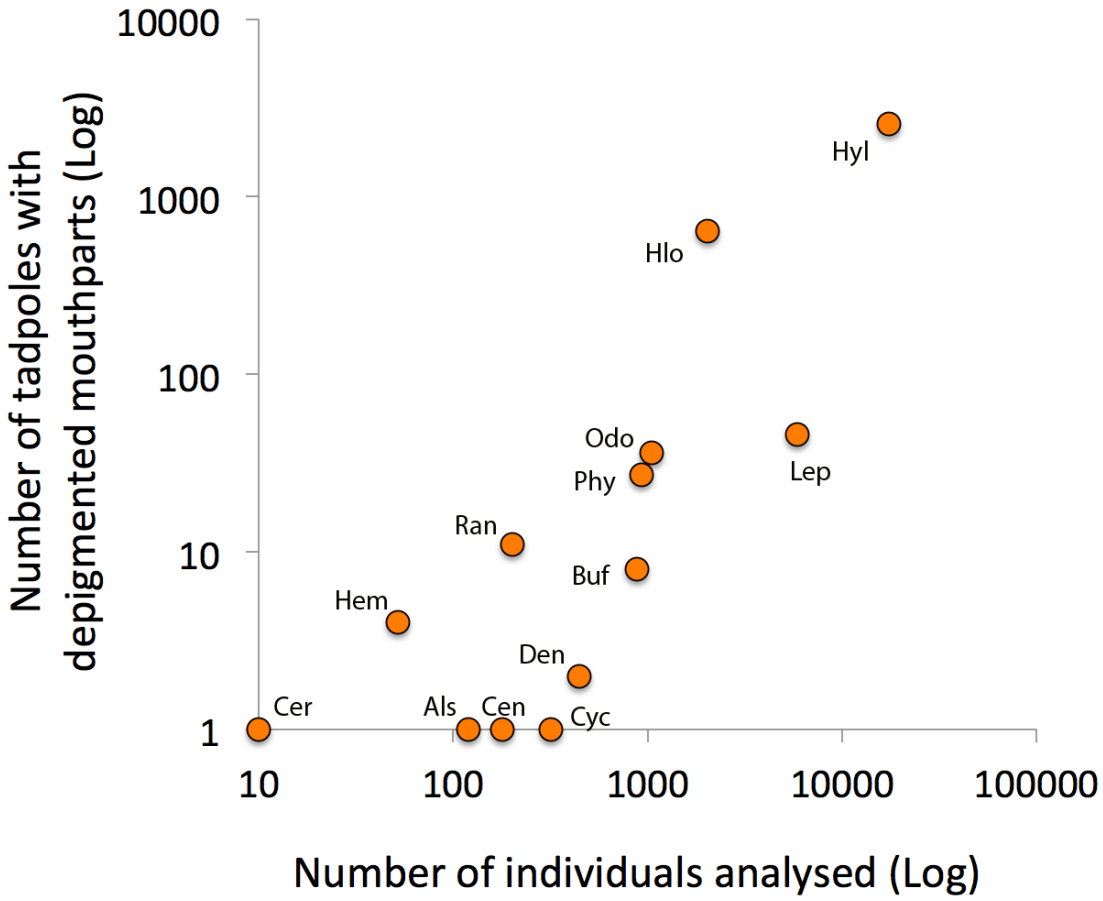

Figure S2. Correlation between number of tadpoles with depigmented mouthparts and number of specimens analyzed per family. The families are presented by the abbreviations: Als (Alsodidae), Buf (Bufonidae), Cen (Centrolenidae), Cer (Ceratophryidae), Cyc (Cycloramphidae), Den (Dendrobatidae), Hem (Hemiphractidae), Hyl (Hylidae), Hlo (Hylodidae), Lep (Leptodactylidae), Odo (Odontophrynidae), Phy (Phyllomedusidae), and Ran (Ranidae).

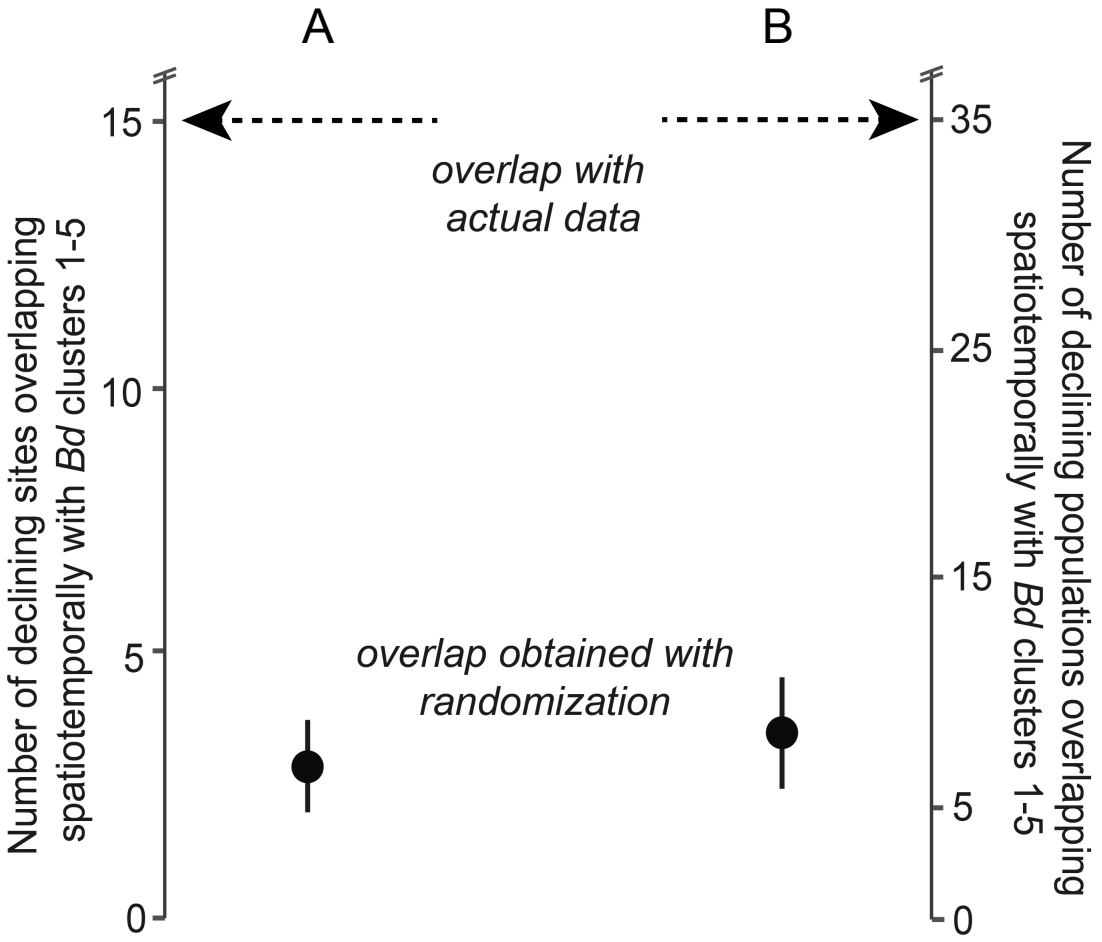

Figure S3. Number of declining sites (A) and amphibian populations (B) spatiotemporally overlapping with the actual data (*Bd* clusters 1-5; arrows). Number of declining sites and populations overlapping with randomly located *Bd* clusters 1-5 obtained with 100 randomizations of clusters' centroid locations; averages +/- 95% CI are shown.

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