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Supplementary Materials for

Recent introduction of a chytrid fungus endangers Western Palearctic salamanders

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Materials and Methods

Animal infections

The infection experiments, approved by the ethical committee of the Faculty of Veterinary Medicine (Ghent University), were performed as described before (*3*). In total, 48 anurans, belonging to 10 species, 112 urodelans, belonging to 24 species and a single caecilian were used (Table S1). All animals were healthy and negative for *B. salamandrivorans*, *B. dendrobatidis* and Ranavirus. After infection, monitoring for clinical signs was performed daily and the microbiological status was assessed by weekly swabbing and qPCR analysis (*6*). Positive animals were kept until they died or returned negative. Negative animals were euthanized after three negative qPCR results obtained with one week intervals. Chytridiomycosis was diagnosed using histological examination on haematoxylin eosin staining of transverse sections through the body: behind the forelegs, before the hind legs and in the middle of the tail (only for urodela). Since most specimens were obtained from single source populations, in order to avoid lineage specific biases, conclusions were drawn on higher taxonomic levels rather than at the species level.

To determine *B. salamandrivorans*' invasive ability amphibians belonging to 7 anuran and 10 urodelan species (one animal per species) (Table S1) were exposed to a high amount (10,000) of zoospores for 24 hours and euthanized immediately after exposure. A piece of abdominal skin was taken and stained immunohistochemically to detect skin invasion (*9*). The experiment was performed in duplicate.

To determine interspecies *B. salamandrivorans* transmission between susceptible European urodelan species, six infected *Salamandra salamandra* (mean log(10) GE load per swab: 1.74 +/-0.12) were co-housed (1:1) with four uninfected *Ichthyosaura alpestris* or two uninfected *Pleurodeles waltl.* To determine transmission from a presumed reservoir species to a susceptible European species, three infected *Cynops pyrrhogaster* (mean log(10) GE load per swab: 1.68 +/-0.14) were co-housed (1:1) with three uninfected *Salamandra salamandra*. Co-housing lasted 8 hours at 5°C (two *Ichthyosaura alpestris*) and 15°C (two *Ichthyosaura alpestris*, two *Pleurodeles waltl* and three *Salamandra salamandra*). After co-housing, monitoring for clinical signs was performed daily during 10 days and the microbiological status was assessed by weekly swabbing and qPCR analysis (*6*).

Screening

Toe clips from museum specimens (Supplementary Table S4) were taken from specimens deposited at in the Museum for Natural History - Naturalis, Leiden, The Netherlands. DNA was extracted using the QIAamp FFPE Tissue kit (Qiagen). Previously collected samples from amphibian assemblages, trade (pet shops in Europe, exporter in Hong Kong and Heathrow Airport) and captive kept amphibians (*10-26*) (Supplementary Table S2, S5, S7) were tested for the presence of *B. salamandrivorans* using qPCR (*6*).

Bayesian divergence time estimates for the origin of *B. salamandrivorans*

We combined protein sequences from three nuclear genes, RPB1, RPB2 and EF1a for a representative set of 12 ingroup fungal species and one fungal outgroup (*Rozella allomycis*). Alignment of the protein sequences was done with MAFFT (*27*) using the L-

INS-i method and resulted in a data matrix of 1973 reliably aligned amino acids. Maximum Likelihood (ML) analyses were run in PAUP* (*28*), using a LG amino-acid rate matrix with empirical frequencies, estimated proportion of invariable sites (0.235325) and distribution of rates at variable sites following a gamma distribution with four categories and estimated shape parameter 0.69288. This resulted in a single ML tree with likelihood score -Ln L = 20836.93. Support was calculated as Bayesian posterior probabilities in MrBayes (*29*). To estimate the age of the divergence of *B. dendrobatidis* from *B. salamandrivorans*, we used a Bayesian relaxed molecular clock model implemented in Beast v1.7.5 (*30*). As a calibration point, we used the divergence of *Hyaloraphidium curvatum* from its sister clade, a relationship that is strongly supported by the literature (*31*) and in our ML tree. We implemented a broad range for this calibration based on Berney et al. (*32)*, who estimated this divergence at 651.2 million years ago, with an upper bound of 860.0 and a lower bound of 477.4 for the 95% confidence interval. We used this information to set the prior distribution for our calibration point to a normal distribution with the same mean of 651.2 mya, and a standard deviation of 106.6, corresponding to a 95% highest posterior density (HPD) ranging from 860.1 till 442.3 mya. These settings for our calibration thus completely encompass the broad range of Berney et al. (*32*). The MCMC chain was run for 10 million generations and trees were sampled every 1,000 generations. Convergence of parameters and a burn-in of 2,000 were determined with Tracer v1.3. The median and 95% HPD for fungal time estimates (Fig. S3) were therefore calculated from 8,000 sampled trees.

Amphibian timetree construction

The amphibian timescale was constructed using all but one species (i.e., 34 operational taxonomic units) that had been experimentally tested for their susceptibility against *B. salamandrivorans*. One *Ambystoma* species was excluded from the analyses because a divergence estimate between *Ambystoma opacum* and *Ambystoma maculatum* was not available. This however does not influence any of the results obtained.

Dating estimates for amphibian diversification were based on previous work (*7, 31, 33-37*).

Ancestral reconstruction of amphibian susceptibility

We used the results of our infection experiments as a proxy to reconstruct the evolution of susceptibility against *B. salamandrivorans* in the three amphibian orders.

The results of the experimentally infected amphibians were classified into four categories:

1. Resistant (no infection, no disease), 2. Tolerant (infection in the absence of disease), 3. Susceptible (infection resulting in clinical disease with subsequent recovery) or 4. Lethal. The classification per species is listed in Table S9.

Ancestral state reconstructions were performed using Maximum Parsimony (MP) and Maximum Likelihood (ML) approaches on the timetree (see amphibian timetree construction) with Mesquite v2.6 (*38*). MP reconstructions where done with characters unordered and equally weighted. ML reconstructions where done under a single rate Mk likelihood model (mk1) for discrete characters (*38*), and the likelihood decision threshold was set to 2.0 (default). The results are listed in Table S10. Corresponding node numbers can be found in Fig. S5. Both analyses gave similar results.

Supplementary figures

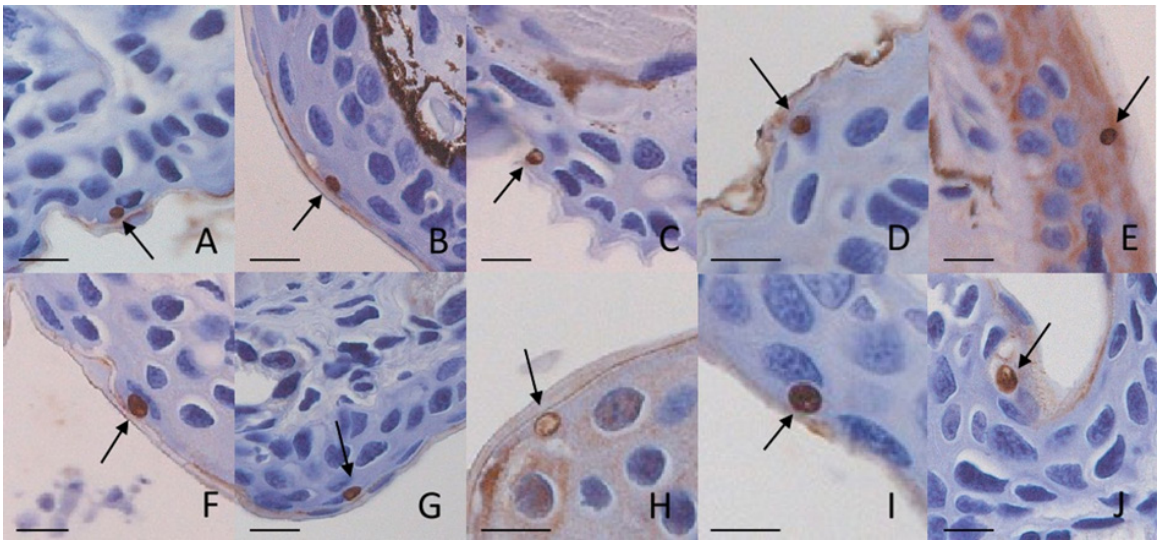

Fig. S1. Invasion of *B. salamandrivorans* in the urodelan abdominal skin after 24 hours exposure. Immunohistochemical staining. Arrows point at *B. salamandrivorans* organisms. A. *Ichthyosaura alpestris*, B. *Triturus cristatus*, C. *Lissotriton helveticus*, D. *Notophthalmus viridescens*, E. *Pleurodeles waltl*, F. *Neurergus crocatus*, G. *Euproctus platycephalus*, H. *Salamandrella keyserlingii*, I. *Plethodon glutinosus*, J. *Salamandra salamandra*. Scale bar = 10 µm.

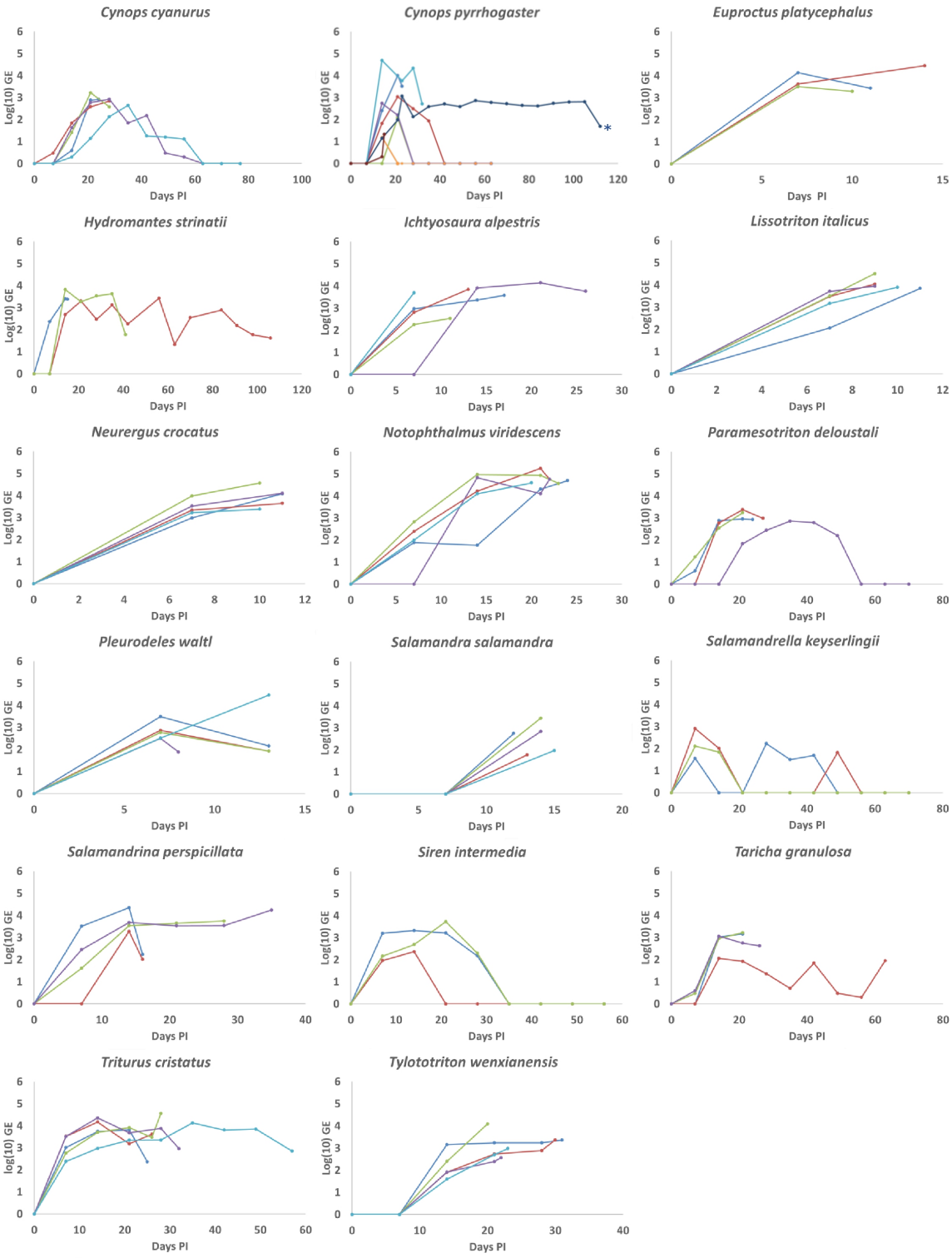

Fig. S2. *B. salamandrivorans* infection course in infected amphibian species. Log(10) genomic equivalent (GE) values expressed per swab.

***** End of experiment with animal showing no clinical anomalies

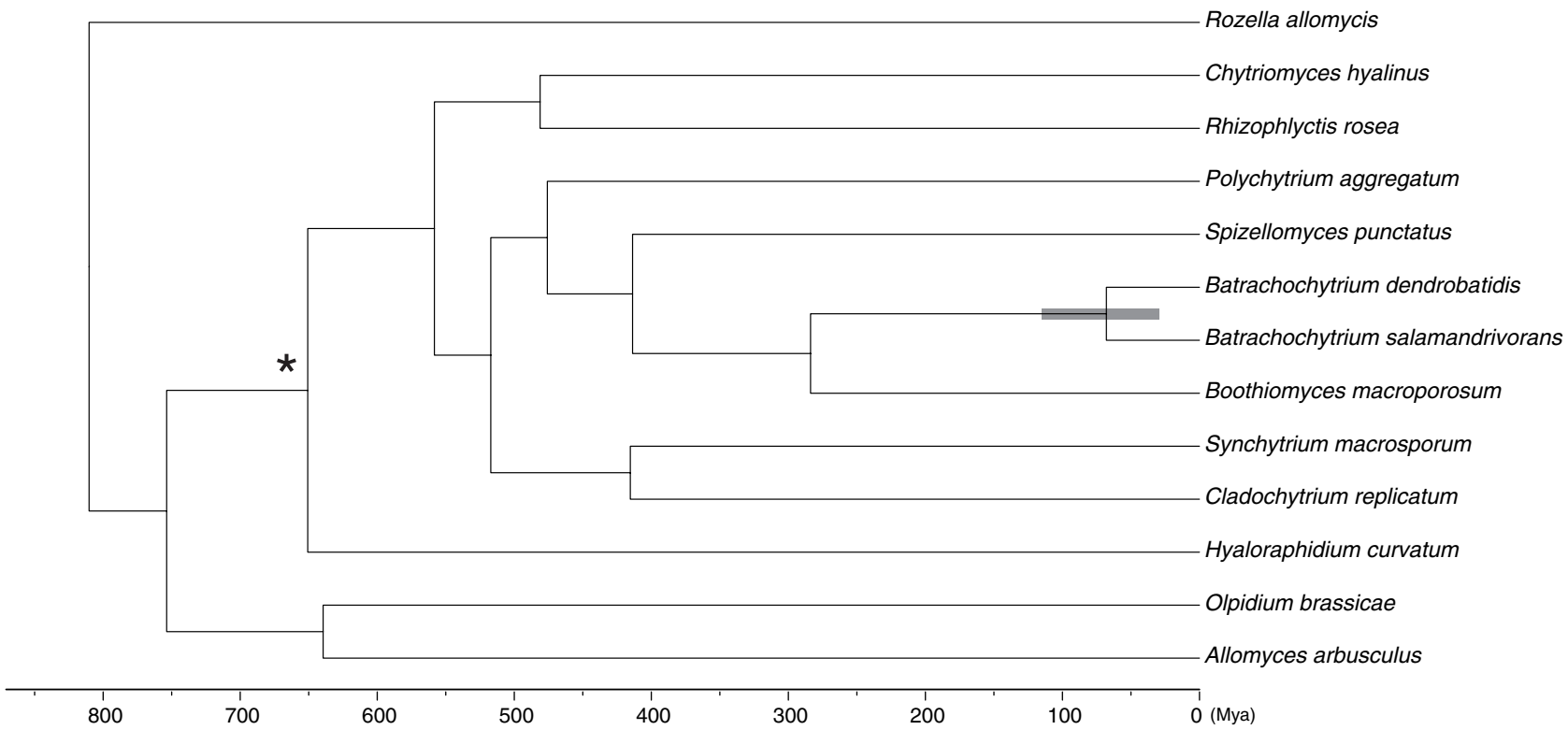

Fig. S3. Divergence time estimates for fungi. The asterisk denotes the calibration point, the grey bar indicates the 95% HPD for the divergence of *B. salamandrivorans* from its sister species.

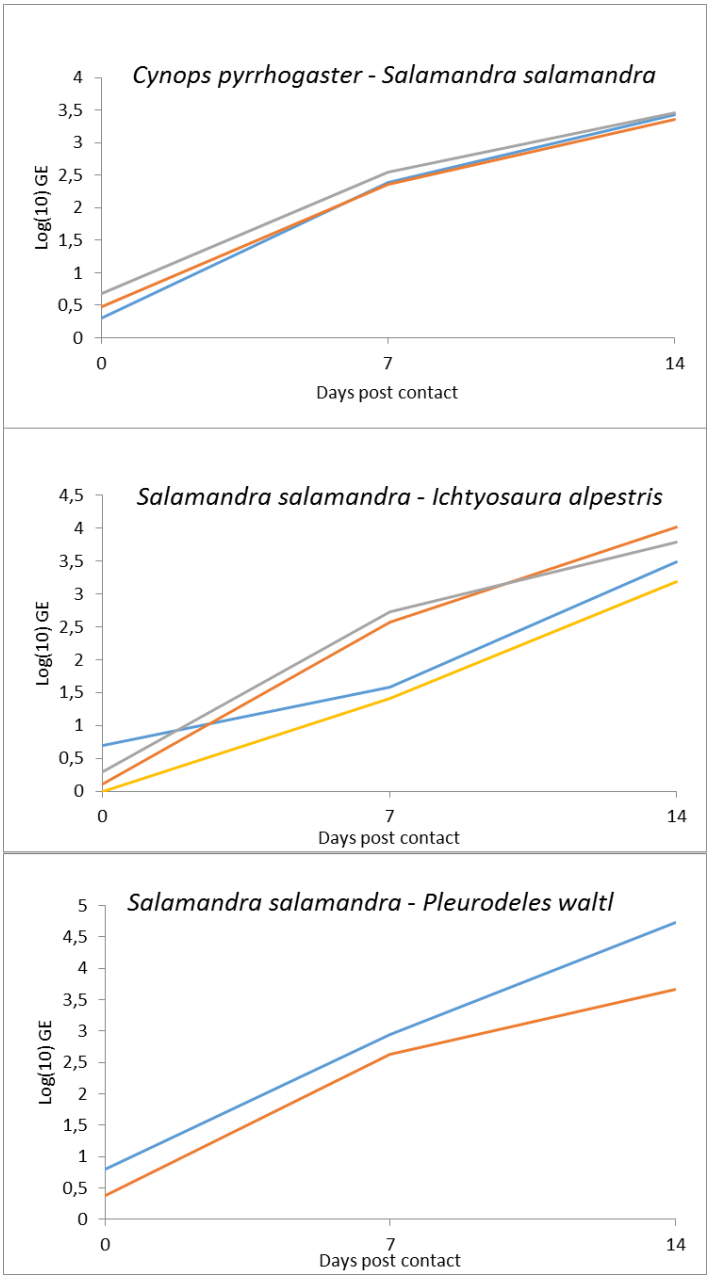

Fig. S4. Interspecies transmission of *B. salamandrivorans*. *B. salamandrivorans* infected *Cynops pyrrhogaster* and *Salamandra salamandra* were co-housed with *B. salamandrivorans* negative *Salamandra salamandra* and *Ichthyosaura alpestris* or *Pleurodeles waltl*, respectively, for 8 hours. Immediately after the co-housing and one and two weeks later, the animals were swabbed (time point 0). Log (10) GE values expressed per swab.

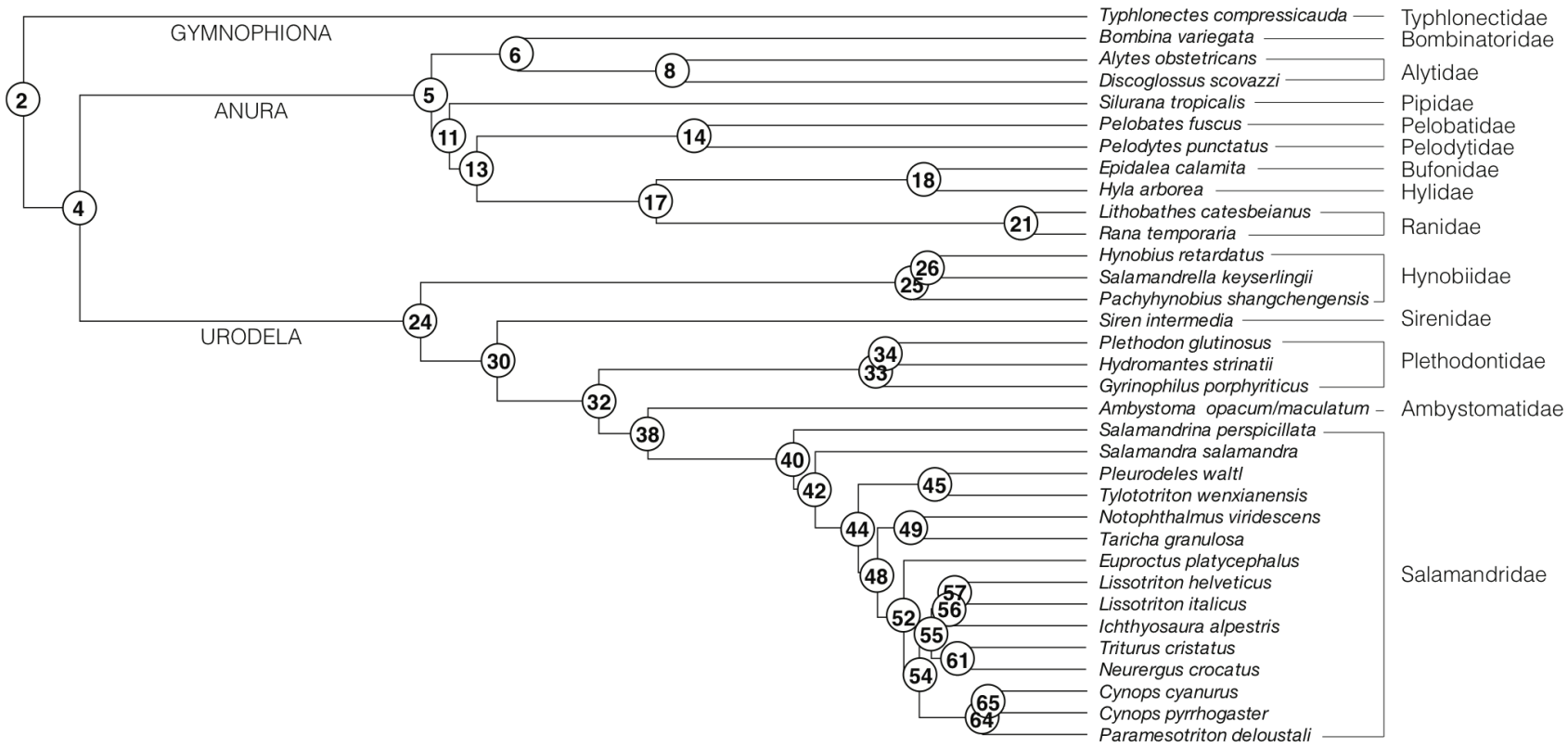

Fig. S5. Tree of 34 amphibian species with node numbers that are cross-referenced in the ancestral state reconstructions of Table S10.

Supplementary tables

Table S1. Summary of the infection experiments. Responses to infection (in terms of presence or absence of *B. salamandrivorans* colonization after exposure) were always highly consistent within a given taxon, even if multiple sources of animals were used.

Amphibian species	Family	Age	Origin	Continent of origin	No used[a]	No Infected[b]	No Sick[c]	No Dead[d]	Average days to mortality (min – max)	Invasion[e]	Category[h]
Anura											
Alytes obstetricans	Alytidae	< 1 year	C1[f]	Europe	5	0	0	0	-	-	R
Bombina variegata	Bombinatoridae	> 1 year	C1	Europe	4	0	0	0	-	-	R
Discoglossus scovazzi	Alytidae	< 1 year	C1	Africa	5	0	0	0	-	-	R
Epidalea calamita	Bufonidae	< 1 year	C1	Europe	4	0	0	0	-	-	R
Hyla arborea	Hylidae	< 1 year	C1	Europe	5	0	0	0	-	-	R
Lithobathes catesbeianus	Ranidae	> 1 year	C1	North America	5	0	0	0	-	-	R
Pelobates fuscus	Pleobatidae	< 1 year	C1	Europe	5	0	0	0	-	Nt[g]	R
Pelodytes punctatus	Pelodytidae	< 1 year	C1	Europe	5	0	0	0	-	Nt	R
Rana temporaria	Ranidae	< 1 year	C1	Europe	5	0	0	0	-	Nt	R
Silurana tropicalis	Pipidae	> 1 year	C1	Africa	5	0	0	0	-	-	R

Amphibian species	Family	Age	Origin	Continent of origin	No used[a]	No Infected[b]	No Sick[c]	No Dead[d]	Average days to mortality (min – max)	Invasion[e]	Category[h]
Urodela											
Ambystoma maculatum	Ambystomatidae	< 1 year	C1	North America	5	0	0	0	-	-	R
Ambystoma opacum	Ambystomatidae	< 1 year	W1	North America	5	0	0	0	-	Nt	R
Cynops cyanurus	Salamandridae	> 1 year	W1	Asia	5	5	5	3	27 (24 – 28)	Nt	S
Cynops pyrrhogaster	Salamandridae	< 1 year	C2	Asia	8	8	8	4	44 (15 – 105)	Nt	S
Euproctus platycephalus	Salamandridae	> 1 year	C1	Europe	3	3	3	3	12 (10 – 15)	+	L
Hydromantes strinatii	Plethodontidae	> 1 year	W1	Europe	3	3	3	3	54 (15 – 106)	Nt	L
Gyrinophilus porphyriticus	Plethodontidae	> 1 year	C1	North America	5	0	0	0	-	Nt	R
Hynobius retardatus	Hynobiidae	> 1 year	C1	Asia	3	0	0	0	-	Nt	R
Ichthyosaura alpestris	Salamandridae	< 1 year	C2	Europe	5	5	5	5	15 (8 – 26)	+	L
Lissotriton helveticus	Salamandridae	< 1 year	C2	Europe	3	3	0	0	-	+	R
Lissotriton italicus	Salamandridae	< 1 year	C1	Europe	5	5	5	5	10 (9-11)	Nt	L
Neurergus crocatus	Salamandridae	> 1 year	C1	Europe	5	5	5	5	11 (10-11)	+	L
Nothophthalmus viridescens	Salamandridae	> 1 year	W1	North America	5	5	5	5	22 (20 – 34)	+	L

Amphibian species	Family	Age	Origin	Continent of origin	No used[a]	No Infected[b]	No Sick[c]	No Dead[d]	Average days to mortality (min – max)	Invasion[e]	Category[h]
Pachyhynobius shangchengensis	Hynobiidae	>1 year	C1	Asia	3	0	0	0	-	Nt	R
Paramesotriton deloustali	Salamandridae	> 1 year	C1	Asia	4	4	4	3	24 (21 – 27)	Nt	S
Plethodon glutinosus	Plethodontidae	> 1 year	W1	North America	5	0	0	0	-	+	R
Pleurodeles waltl	Salamandridae	< 1 year	C1	Europe	5	5	5	5	11 (8 – 13)	+	L
Salamandra salamandra	Salamandridae	< 1 year	C3	Europe	5	5	5	5	14 (12 – 15)	+	L
Salamandrella keyserlingii	Hynobiidae	> 1 year	C1	Asia	3	3	0	0	-	Nt	T
Salamandrina perspicillata	Salamandridae	< 1 year	C1	Europe	5	5	5	5	24 (16 – 35)	Nt	L
Siren intermedia	Sirenidae	> 1 year	C1	North America	3	3	3	0	-	Nt	T
Taricha granulosa	Salamandridae	< 1 year	C1	North America	4	4	4	4	28 (21 – 42)	Nt	L
Triturus cristatus	Salamandridae	< 1 year	C1	Europe	5	5	5	5	34 (25 -57)	+	L
Tylototriton wenxianensis	Salamandridae	< 1 year	C1	Asia	5	5	5	5	25 (20 – 31)	Nt	L
Gymnophiona											
Typhlonectes compressicauda	Typhlonectidae	> 1 year	C1	South America	2	0	0	0	-	Nt	R

[a] number of animals exposed to *B. salamandrivorans*
[b] number of animals infected by *B. salamandrivorans*
[c] number of animals that developed clinical signs: anorexia, apathy and/or abnormal behaviour (preferring dry places)

[d] number of animals that died
[e] determined using skin from two animals per species, after 24h of exposure to *B. salamandrivorans*
[f] C captive bred; W wild caught animals, number reflects the number of source populations
[g] Nt not tested
[h] R = resistant (no infection, no disease), T = tolerant (infection in the absence of disease and mortality), S = susceptible (infection resulting in clinical disease with possibility of subsequent clinical recovery), L = lethal (infection resulting in lethal disease in all infected animals)

Table S2. Screening of amphibian assemblages for the presence of *B. salamandrivorans*.

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
WESTERN PALEARCTIC					
Anura					
Alytidae	*Alytes obstetricans*	The Netherlands	15	2009	0
Bombinatoridae	*Bombina variegata*	The Netherlands	8	2009	0
Bufonidae	*Bufo bufo*	Belgium	100	2011	0
Bufonidae	*Bufo bufo*	The Netherlands*	19	2013	0
Bufonidae	*Epidalea calamita*	The Netherlands	24	2009	0
Hylidae	*Hyla arborea*	The Netherlands	22	2009	0
Ranidae	*Lithobates catesbeianus*	Belgium	100	2010-2011	0
Ranidae	*Rana temporaria*	Belgium	7	2010	0
Ranidae	*Rana temporaria*	The Netherlands	73	2013	0
Urodela					
Salamandridae	*Calotriton arnoldi*	Spain	5	2009-2012	0
Salamandridae	*Ichthyosaura alpestris*	Belgium	35	2009	0
Salamandridae	*Ichthyosaura alpestris*	France	5	2009	0
Salamandridae	*Ichthyosaura alpestris*	Switzerland	1239	2008-2013	0
Salamandridae	*Ichthyosaura alpestris*	The Netherlands*	44	2013	1 (13)
Salamandridae	*Lissotriton boscai*	Spain	5	2009-2012	0
Salamandridae	*Lissotriton helveticus*	Belgium	33	2009	0
Salamandridae	*Lissotriton helveticus*	Spain	6	2009-2012	0
Salamandridae	*Lissotriton helveticus*	Switzerland	323	2008-2009	0
Salamandridae	*Lissotriton vulgaris*	Belgium	6	2008	0
Salamandridae	*Lissotriton vulgaris*	Switzerland	62	2008-2013	0
Salamandridae	*Lissotriton vulgaris*	The Netherlands*	2	2013	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Salamandridae	*Ichthyosaura alpestris*	Spain	5	2009-2012	0
Salamandridae	*Mertensiella caucasica*	Turkey	8	2010	0
Salamandridae	*Ommatotriton ophryticus*	Turkey	3	2010	0
Salamandridae	*Salamandra algira*	Morocco	10	2011	0
Salamandridae	*Salamandra atra*	Austria	122	2010	0
Salamandridae	*Salamandra atra*	Germany	120	2011-2013	0
Salamandridae	*Salamandra atra*	Switzerland	120	2008-2013	0
Salamandridae	*Salamandra salamandra*	Belgium	233**	2012-2014	1*** (5) 24**** (14(3.7-60))
Salamandridae	*Salamandra salamandra*	France	9	2011	0
Salamandridae	*Salamandra salamandra*	Spain	132	2009-2013	0
Salamandridae	*Salamandra salamandra*	Switzerland	26	2008-2013	0
Salamandridae	*Salamandra salamandra*	The Netherlands	39	2010-2013	13 (17(4-139))
Plethodontidae	*Hydromantes imperialis*	Italy	79	2009-2012	0
Plethodontidae	*Hydromantes supramontis*	Italy	42	2009-2012	0
Plethodontidae	*Hydromantes flavus*	Italy	25	2004-2012	0
Plethodontidae	*Hydromantes genei*	Italy	173	2009-2012	0
Plethodontidae	*Hydromantes sarrabusensis*	Italy	5	2004-2012	0
Plethodontidae	*Hydromantes strinatii*	Italy	70	2012-2013	0
Salamandridae	*Pleurodeles waltl*	Spain	11	2009-2012	0
Salamandridae	*Triturus cristatus*	Belgium	2	2010	0
Salamandridae	*Triturus cristatus*	Switzerland	38	2008-2009	0
Salamandridae	*Triturus carnifex*	Switzerland	9	2008-2009	0
Salamandridae	*Triturus marmoratus*	Spain	54	2009-2012	0
Salamandridae	*Triturus pygmaeus*	Spain	5	2009-2012	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
EASTERN ASIA					
Anura					
Bombinatoridae	*Bombina maxima*	Vietnam	4	2010	0
Dicroglossidae	*Fejervarya limnocharis*	Vietnam	1	2010	0
Dicroglossidae	*Limnonectes* sp.	Vietnam	4	2010	0
Dicroglossidae	*Quasipaa* sp.	Vietnam	2	2010	0
Megophryidae	*Leptobrachium* sp.	Vietnam	2	2010	0
Megophryidae	*Leptolalax sungi*	Vietnam	3	2010	0
Megophryidae	*Megrophrys* sp.	Vietnam	2	2010	0
Megophryidae	*Ophryophryne* sp.	Vietnam	3	2010	0
Megophryidae	*Xenophrys major*	Vietnam	2	2010	0
Microhylidae	*Microhyla heymonsi*	Vietnam	3	2010	0
Microhylidae	*Microhyla pulchra*	Vietnam	2	2010	0
Microhylidae	*Micryletta ornate*	Vietnam	4	2010	0
Ranidae	*Amolops* sp.	Vietnam	3	2010	0
Ranidae	*Hylarana* sp.	Vietnam	6	2010	0
Ranidae	*Odorrana* sp.	Vietnam	8	2010	0
Rhacophoridae	*Kurixalus verrucosus*	Vietnam	1	2010	0
Rhacophoridae	*Polypedates leucomystax*	Vietnam	3	2010	0
Rhacophoridae	*Rhacophorus dennysi*	Vietnam	2	2010	0
Rhacophoridae	*Rhacophorus orlovi*	Vietnam	1	2010	0
Rhacophoridae	*Rhacophorus kio*	Vietnam	1	2010	0
Rhacoporidae	*Theloderma* sp.	Vietnam	1	2010	0
Urodela					
Salamandridae	*Cynops cyanurus chuxiogensis*	China	6	2013	0
Salamandridae	*Cynops ensicauda*	Japan	76	2009	8 (12 (2-27))
Salamandridae	*Cynops pyrrhogaster*	Japan	116	2009	1 (10)

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Salamandridae	*Echinotriton andersoni*	Japan	3	2009	0
Salamandridae	*Paramesotriton deloustali*	Vietnam	30	2010-2013	2 (13 (9-16))
Salamandridae	*Paramesotriton chinensis*	China	3	2008	0
Salamandridae	*Paramesotriton hongkongensis*	China	1	2014	0
Salamandridae	*Paramesotriton granulosus*	Chinae	3	2008	0
Salamandridae	*Paramesotriton longliensis*	China	3	2011	0
Salamandridae	*Paramesotriton* sp.	China	3	2014	0
Salamandridae	*Paramesotriton yunwuensis*	China	2	2014	0
Salamandridae	*Tylototriton panhai*	Thailand	9	2003-2006	0
Salamandridae	*Tylototriton uyenoi*	Thailand	9	2001-2006	1 (13)
Salamandridae	*Tylototriton vietnamensis*	Vietnam	60	2010	0
Salamandridae	*Tylototriton verrucosus*	Laos	1	2013	0
Salamandridae	*Tylototriton ziegleri*	Vietnam	9	2010	1 (11)
Salamandridae	*Tylototriton* cf. shanjing	Thailand	3	2006	0
Salamandridae	*Tylototriton* sp.	Vietnam	3	2012	0
Hynobiidae	*Hynobius kimurae*	Japan	5	2009	0
Hynobiidae	*Hynobius lichenatus*	Japan	3	2009	0
Hynobiidae	*Hynobius naevius*	Japan	2	2009	0
Hynobiidae	*Hynobius nebulosus*	Japan	15	2009	1 (2)
Hynobiidae	*Hynobius nigrescens*	Japan	15	2009	0
Hynobiidae	*Hynobius retardatus*	Japan	3	2009	0
Hynobiidae	*Onychodactylus japonicas*	Japan	19	2009	1 (12)
Hynobiidae	*Salamandrella keyserlingii*	Japan	4	2009	2 (2 (2-2))
Cryptobranchidae	*Andrias japonicas*	Japan	26	2009	0
NEOTROPICS					
Anura					
Bufonidae	*Atelopus glyphus*	Panama	6	2007	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Bufonidae	*Atelopus limosus*	Panama	1	2007	0
Bufonidae	*Incilius coniferus*	Panama	4	2007	0
Bufonidae	*Rhaebo haematiticus*	Panama	8	2007	0
Bufonidae	*Rhinella alata*	Panama	14	2007	0
Bufonidae	*Rhinella marina*	Panama	15	2008	0
Centrolenidae	*Centrolene ilex*	Panama	4	2007	0
Centrolenidae	*Cochranella albomaculata*	Panama	10	2007	0
Centrolenidae	*Cochranella euknemos*	Panama	5	2007	0
Centrolenidae	*Cochranella spinosa*	Panama	12	2007	0
Centrolenidae	*Cochranella pulverata*	Panama	1	2007	0
Centrolenidae	*Espadarana prosoblepon*	Panama	9	2007	0
Centrolenidae	*Hyalinobatrachium colymbiphyllum*	Panama	11	2007	0
Centrolenidae	*Hyalinobatrachium fleischmanni*	Panama	9	2008	0
Craugastoridae	*Craugastor bransfordii*	Panama	2	2007	0
Craugastoridae	*Craugastor crassidigitus*	Panama	10	2007	0
Craugastoridae	*Craugastor fitzingeri*	Panama	26	2007-2008	0
Craugastoridae	*Craugastor gollmeri*	Panama	3	2007	0
Craugastoridae	*Craugastor megacephalus*	Panama	1	2007	0
Craugastoridae	*Craugastor noblei*	Panama	3	2007	0
Craugastoridae	*Craugastor raniformis*	Panama	9	2008	0
Craugastoridae	*Craugastor tabasarae*	Panama	1	2007	0
Craugastoridae	*Craugastor talamancae*	Panama	22	2007	0
Dendrobatidae	*Allobates talamancae*	Panama	1	2007	0
Dendrobatidae	*Colestethus flotator*	Panama	1	2007	0
Dendrobatidae	*Colestethus panamensis*	Panama	4	2007	0
Dendrobatidae	*Colostethus pratti*	Panama	5	2007	0
Dendrobatidae	*Colostethus* sp.	Panama	2	2007	0
Dendrobatidae	*Dendrobates auratus*	Panama	8	2007	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Dendrobatidae	*Silverstoneia nubicola*	Panama	5	2007	0
Eleutherodactylidae	*Diasporus diastema*	Panama	18	2007	0
Eleutherodactylidae	*Diasporus orange*	Panama	3	2007	0
Eleutherodactylidae	*Diasporus quidditus*	Panama	7	2007	0
Eleutherodactylidae	*Eleutherodactylus sp.*	Panama	1	2007	0
Hemiphractidae	*Hemiphractus fasciatus*	Panama	1	2007	0
Hemiphractidae	*Gastrotheca cornuta*	Panama	2	2007	0
Hylidae	*Agalychnis callidryas*	Panama	7	2007-2008	0
Hylidae	*Dendropsophus* sp.	Panama	2	2008	0
Hylidae	*Hyloscirtus colymba*	Panama	1	2007	0
Hylidae	*Hypsiboas boans*	Panama	1	2007	0
Hylidae	*Hypsiboas rufitelus*	Panama	1	2007	0
Hylidae	*Hypsiboas rosenbergi*	Panama	2	2007	0
Hylidae	*Scinax rostratus*	Panama	1	2008	0
Hylidae	*Scinax ruber*	Panama	7	2008	0
Hylidae	*Smilisca phaeota*	Panama	19	2007	0
Hylidae	*Smilisca sila*	Panama	2	2007	0
Hylidae	*Trachycephalus typhonius*	Panama	15	2008	0
Hylinae	*Hyloscirtus palmeri*	Panama	1	2007	0
Leptodactylidae	*Engystomops pustulosus*	Panama	30	2007	0
Leptodactylidae	*Leptodacytlus bolivianus*	Panama	1	2008	0
Leptodactylidae	*Leptodactylus fragilis*	Panama	1	2007	0
Leptodactylidae	*Leptodactylus labialis*	Panama	6	2007	0
Leptodactylidae	*Leptodactylus poecilochilus*	Panama	3	2008	0
Leptodactylidae	*Leptodactylus savagei*	Panama	2	2008	0
Microhylidae	*Elachistocleis panamensis*	Panama	9	2008	0
Ranidae	*Lithobates warszewitschii*	Panama	2	2007	0
Strabomantidae	*Pristimantis achatinus*	Panama	2	2008	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Strabomantidae	*Pristimantis caryophyllaceus*	Panama	24	2007	0
Strabomantidae	*Pristimantis cerasinus*	Panama	23	2007	0
Strabomantidae	*Pristimantis cruentus*	Panama	24	2007-2008	0
Strabomantidae	*Pristimantis gaigei*	Panama	3	2007	0
Strabomantidae	*Pristimantis museosus*	Panama	1	2007	0
Strabomantidae	*Pristimantis pardalis*	Panama	8	2007	0
Strabomantidae	*Pristimantis pirrensis*	Panama	1	2007	0
Strabomantidae	*Pristimantis taeniatus*	Panama	2	2007	0
Strabomantidae	*Strabomantis bufoniformis*	Panama	27	2007	0
Urodela					
Plethodontidae	*Bolitoglossa* sp.	Panama	8	2007	0
NEARCTIC					
Anura					
Bufonidae	*Anaxyrus americanus*	Illinois	8	2008-2009	0
Hylidae	*Acris crepitans*	Illinois	8	2008-2009	0
Hylidae	*Hyla avivoca*	Illinois	8	2008-2009	0
Hylidae	*Hyla chrysoscelis*	Illinois	8	2008-2009	0
Hylidae	*Hyla cinerea*	Illinois	8	2008-2009	0
Hylidae	*Hyla versicolor*	New York	16	2011	0
Hylidae	*Pseudarcis crucifer*	Illinois	8	2008-2009	0
Hylidae	*Pseudacris crucifer*	New York	47	2011	0
Hylidae	*Pseudarcis feriarum*	Illinois	5	2008-2009	0
Hylidae	*Pseudarcis triseriata*	Illinois	8	2008-2009	0
Ranidae	*Lithobates areolatus*	Illinois	22	2008-2009	0
Ranidae	*Lithobates catesbeianus*	Illinois	8	2008-2009	0
Ranidae	*Lithobates catesbeianus*	New York	7	2011	0
Ranidae	*Lithobates clamitans*	Illinois	8	2008-2009	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Ranidae	*Lithobates clamitans*	New York	8	2011	0
Ranidae	*Lithobates pipiens*	Illinois	16	2008-2009	0
Ranidae	*Lithobates pipiens*	New York	1	2011	0
Ranidae	*Lithobates septentriona*	New York	1	2011	0
Ranidae	*Lithobates sphenocephalus*	Illinois	8	2008-2009	0
Ranidae	*Lithobates yavapaiensis*	Arizona	38	2009	0
Urodela					
Ambystomatidae	*Ambystoma jeffersonianum*	New York	3	2011	0
Ambystomatidae	*Ambystoma maculatum*	New York	8	2011	0
Ambystomatidae	*Ambystoma texanum*	Illinois	9	2008	0
Plethodontidae	*Demognathus imitator*	Appalachians	24	2009-2011	0
Plethodontidae	*Desmognathus ocoee*	Appalachians	20	2009-2011	0
Plethodontidae	*Desmognathus wrighti*	Appalachians	26	2009-2011	0
Plethodontidae	*Eurycea wilderae*	Appalachians	11	2009-2011	0
Plethodontidae	*Plethodon cinereus*	Appalachians	343	2009-2011	0
Plethodontidae	*Plethodon cinereus*	New York	3	2011	0
Plethodontidae	*Plethodon cylindraceus*	Appalachians	15	2009-2011	0
Plethodontidae	*Plethodon glutinosus*	Appalachians	54	2009-2011	0
Plethodontidae	*Plethodon jordani*	Appalachians	35	2009-2011	0
Plethodontidae	*Plethodon jor x met*	Appalachians	47	2009-2011	0
Plethodontidae	*Plethodon jor x tey*	Appalachians	6	2009-2011	0
Plethodontidae	*Plethodon metcalfi*	Tennessee	8	2009-2011	0
Plethodontidae	*Plethodon raceus x glutinosus*	Appalachians	1	2009-2011	0
Plethodontidae	*Plethodon richmondi*	Appalachians	12	2009-2011	0
Plethodontidae	*Plethodon serratus*	Appalachians	14	2009-2011	0
Plethodontidae	*Plethodon teyahalee*	Appalachians	21	2009-2011	0
Plethodontidae	*Plethodon welleri*	Appalachians	8	2009-2011	0

Family	Species	Country	No tested	Years sampled	No positive (GE load)[a]
Plethodontidae	*Plethodon yonahlossee*	Appalachians	8	2009-2011	0
Salamandridae	*Notophthalmus viridescens*	Illinois	19	2009-2011	0
Salamandridae	*Notophthalmus viridescens*	New York	12	2011	0

[a] (GE number per qPCR reaction (minimum GE number – maximum GE number); all positive samples were sequenced in triplicate and showed a 100% identity with Genbank accession number KC762295

[*] sampled in the outbreak area Bunderbos

[**] three samples from outbreak area in Eupen, Belgium, 60 samples from outbreak area in Robertville, Belgium.

[***] from outbreak area Eupen, Belgium.

[****] from outbreak area Robertville, Belgium

Table S3. Summary of the *B. salamandrivorans* survey data, for each area and taxonomic group based on the data of Supplementary Table 2. The table lists the number of individuals that were tested, the number that were *B. salamandrivorans*-positive, *B. salamandrivorans* prevalence (proportion infected), the Clopper-Pearson 95% confidence interval (CI) for prevalence and the probability of detecting at least one positive individual (assuming a prevalence of 1%).

Area	Taxonomic group	Number tested	Number positive	Proportion infected	Clopper-Pearson 95% CI	Probability of detecting at least 1 positive individual (assuming prevalence = 0.01)
Netherlands, Belgium	urodelans	394	39	0.0901	0.0648 – 0.1211	0.9871
Western Palaearctic (Netherlands and Belgium excluded)	urodelans	2711	0	0.0000	0.0000 - 0.0014	1.0000
Western Palaearctic	anurans	368	0	0.0000	0.0000 - 0.0100	0.9752
Eastern Asia	anurans	58	0	0.0000	0.0000 - 0.0616	0.4417
Eastern Asia	urodelans	432	17	0.0379	0.0222 – 0.0599	0.9890
Neotropics	anurans	472	0	0.0000	0.0000 - 0.0078	0.9913
Neotropics	urodelans	8	0	0.0000	0.0000 - 0.3694	0.0773
Nearctic	anurans	241	0	0.0000	0.0000 - 0.0152	0.9113
Nearctic	urodelans	707	0	0.0000	0.0000 - 0.0052	0.9992

The probability of detecting at least 1 positive individual, D, (assuming prevalence = 0.01) was calculated using the equation: $D = 1 – (1-p)^n$ where p is prevalence and n is the number of individuals tested (*39*).

Table S4. Presence of *B. salamandrivorans* in toe clips from Asian archived specimens.

Family	Species	Year of deposition	No tested	No positive
Salamandridae	*Cynops ensicauda*[*]	1861	10	1
Salamandridae	*Cynops pyrrhogaster*	unknown	3	0
Salamandridae	*Cynops pyrrhogaster*	1967	1	0
Salamandridae	*Cynops pyrrhogaster*	2009	1	0
Salamandridae	*Pachytriton sp.*	1928	1	0
Salamandridae	*Paramesotriton hongkongensis*	1957	1	0
Salamandridae	*Tylototriton verrucosus*	1965	2	0
Salamandridae	*Tylototriton verrucosus*	1966	2	0
Salamandridae	*Tylototriton verrucosus*	1967	2	0

***** all animals were kept in one container, 2 animals showed ulcerations.

Table S5. Presence of *B. salamandrivorans* in skins swabs from amphibians in trade.

Family	Species	Continent of origin	Trade origin[a]	Number of samples tested	Number of samples positive
Anura					
Alytidae	*Alytes obstetricans*	Europe	Pet shop	3	0
Arthroleptidae	*Leptopelis argenteus*	Africa	Airport	28	0
Bombinatoridae	*Bombina orientalis*	Asia	Pet shop	20	0
Bombinatoridae	*Bombina orientalis*	Asia	Airport	38	0
Bombinatoridae	*Bombina variegata*	Europe	Pet shop	9	0
Brevicipitidae	*Breviceps adspersus*	Africa	Airport	3	0
Bufonidae	*Anaxyrus debilis*	North America	Pet shop	4	0
Bufonidae	*Amietophrynus rangeri*	South America	Airport	12	0
Bufonidae	*Amietophrynus regularis*	Africa	Airport	61	0
Bufonidae	*Bufotes viridis*	Africa	Pet shop	2	0
Bufonidae	*Bufotes viridis*	Africa	Airport	37	0
Bufonidae	*Duttaphrynus melanostictus*	Asia	Pet shop	10	0
Bufonidae	*Incilius alvarius*	North America	Pet shop	4	0
Bufonidae	*Melanophryniscus stelzneri*	South America	Pet shop	15	0
Bufonidae	*Rhinella marina*	Americas	Airport	2	0
Bufonidae	*Schismaderma carens*	Africa	Airport	1	0
Ceratophryidae	*Ceratophrys cornuta*	South America	Airport	6	0
Ceratophryidae	*Ceratophrys cranwelli*	South America	Airport	81	0
Ceratophryidae	*Ceratophrys ornata*	South America	Airport	15	0
Ceratophryidae	*Lepidobatrachus laevis*	South America	Airport	15	0
Dendrobatidae	*Dendrobates tinctorius*	South America	Pet shop	5	0
Dendrobatidae	*Dendrobates tinctorius*	South America	Airport	2	0
Hemisotidae	*Hemisus marmoratus*	Africa	Airport	2	0
Hylidae	*Agalychnis callidryas*	South America	Pet shop	13	0

Family	Species	Continent of origin	Trade origin[a]	Number of samples tested	Number of samples positive
Hylidae	*Agalychnis callidryas*	North America	Airport	7	0
Hylidae	*Dendropsophus leucophyllatus*	South America	Airport	2	0
Hylidae	*Hyla chrysoscelis*	North America	Airport	7	0
Hylidae	*Hyla cinerea*	North America	Pet shop	37	0
Hylidae	*Hyla cinerea*	North America	Airport	34	0
Hylidae	*Hyla gratiosa*	North America	Airport	6	0
Hylidae	*Hyla versicolor*	North America	Pet shop	19	0
Hylidae	*Hyla* sp.	unknown	Airport	14	0
Hylidae	*Hypsiboas calcaratus*	South America	Airport	2	0
Hylidae	*Litoria caerulea*	Australia/Asia	Airport	19	0
Hylidae	*Litoria infrafrenata*	Australia/Asia	Airport	8	0
Hylidae	*Litoria* sp.	unknown	Airport	29	0
Hylidae	*Phyllomedusa bicolor*	South America	Airport	2	0
Hylidae	*Phyllomedusa hypochondrialis*	South America	Airport	2	0
Hylidae	*Pseudacris crucifer*	North America	Airport	2	0
Hylidae	*Tachycephalus resinifinctrix*	South America	Airport	3	0
Hylidae	*Trachycepahlus resinifictrix*	South America	Pet shop	10	0
Hyperoliidae	*Afrixalus fornasini*	Africa	Airport	6	0
Hyperoliidae	*Heterixalus alboguttatus*	Africa	Airport	3	0
Hyperoliidae	*Heterixalus madagascariensis*	Africa	Airport	3	0
Hyperoliidae	*Heterixalus punctatus*	Africa	Airport	3	0
Hyperoliidae	*Hyperolius argus*	Africa	Airport	23	0
Hyperoliidae	*Hyperolius concolor*	Africa	Airport	17	0
Hyperoliidae	*Hyperolius guttulatus*	Africa	Pet shop	2	0
Hyperoliidae	*Hyperolius marmoratus*	Africa	Airport	26	0
Hyperoliidae	*Hyperolius parkeri*	Africa	Pet shop	3	0
Hyperoliidae	*Hyperolius picturatus*	Africa	Airport	6	0
Hyperoliidae	*Hyperolius puncticulatus*	Africa	Airport	20	0

Family	Species	Continent of origin	Trade origin[a]	Number of samples tested	Number of samples positive
Hyperoliidae	*Hyperolius tuberilingus*	Africa	Airport	5	0
Hyperoliidae	*Hyperolius viridiflavus*	Africa	Airport	19	0
Hyperoliidae	*Hyperolius* sp.	unknown	Airport	79	0
Hyperoliidae	*Kassina maculate*	South America	Pet shop	2	0
Hyperoliidae	*Kassina maculata*	South America	Airport	22	0
Hyperoliidae	*Kassina senegalensis*	Africa	Pet shop	2	0
Hyperoliidae	*Kassina senegalensis*	Africa	Airport	13	0
Mantellidae	*Mantella aurantiaca*	Africa	Airport	2	0
Mantellidae	*Mantella baroni*	Africa	Airport	8	0
Mantellidae	*Mantella betsileo*	Africa	Pet shop	4	0
Mantellidae	*Mantella betsileo*	Africa	Airport	4	0
Mantellidae	*Mantella laevigata*	Africa	Airport	1	0
Mantellidae	*Mantella madagascarensis*	Africa	Airport	4	0
Mantellidae	*Mantella nigricans*	Africa	Airport	4	0
Mantellidae	*Mantella pulchra*	Africa	Airport	6	0
Megophryidae	*Megophrys montana*	Asia	Airport	3	0
Megophryidae	*Megophrys nasuta*	Asia	Pet shop	9	0
Megophryidae	*Megophrys nasuta*	Asia	Airport	8	0
Microhylidae	*Dyscophus guineti*	Africa	Pet shop	10	0
Microhylidae	*Dyscophus guineti*	Africa	Airport	31	0
Microhylidae	*Dyscophus insularis*	Africa	Airport	4	0
Microhylidae	*Kaloula pulchra*	Asia	Pet shop	6	0
Microhylidae	*Kaloula pulchra*	Asia	Airport	9	0
Microhylidae	*Phrynomantis bifasciatus*	Africa	Airport	57	0
Microhylidae	*Scaphiophryne gottlebei*	Africa	Airport	2	0
Microhylidae	*Scaphiophryne madagascariensis*	Africa	Airport	2	0
Microhylidae	*Scaphiophryne marmorata*	Africa	Airport	2	0
Pipidae	*Hymenochirus boettgeri*	Africa	Pet shop	15	0

Family	Species	Continent of origin	Trade origin[a]	Number of samples tested	Number of samples positive
Pipidae	*Xenopus laevis*	Africa	Airport	63	0
Ptychadenidae	*Ptychadena mascareniensis*	Africa	Airport	4	0
Pyxicephalidae	*Pyxicephalus adspersus*	Africa	Pet shop	13	0
Pyxicephalidae	*Pyxicephalus adspersus*	Africa	Airport	37	0
Pyxicephalidae	*Tomopterna marmorata*	Africa	Airport	4	0
Rhacophoridae	*Chiromantis xerampelina*	Africa	Pet shop	8	0
Rhacophoridae	*Chiromantis xerampelina*	Africa	Airport	3	0
Rhacophoridae	*Polypedates leucomystax*	Asia	Pet shop	10	0
Rhacophoridae	*Polypedates otilopus*	Asia	Airport	9	0
Rhacophoridae	*Rhacophorus dennysi*	Asia	Pet shop	1	0
Rhacophoridae	*Rhacophorus dennysi*	Asia	Airport	6	0
Rhacophoridae	*Rhacophorus nigropalmatus*	Asia	Airport	2	0
Rhacophoridae	*Rhacophorus prominanus*	Asia	Airport	10	0
Rhacophoridae	*Theloderma asperum*	Asia	Pet shop	7	0
Rhacophoridae	*Theloderma corticale*	Asia	Airport	6	0
Rhacophoridae	*Theloderma corticale*	Asia	Pet shop	4	0
Urodela					
Ambystomatidae	*Ambystoma maculatum*	North America	Pet shop	11	0
Ambystomatidae	*Ambystoma maculatum*	North America	Airport	9	0
Ambystomatidae	*Ambystoma mexicanum*	South America	Pet shop	10	0
Ambystomatidae	*Ambystoma opacum*	North America	Pet shop	14	0
Ambystomatidae	*Ambystoma opacum*	North America	Airport	3	0
Ambystomatidae	*Ambytoma tigrinum*	North America	Pet shop	25	0
Plethodontidae	*Desmognathus auriculatus*	North America	Airport	4	0
Proteidae	*Necturus maculosus*	North America	Pet shop	7	0
Salamandridae	*Cynops cyanurus*	Asia	Pet shop	24	0
Salamandridae	*Cynops cyanurus*	Asia	Airport	5	0

Family	Species	Continent of origin	Trade origin[a]	Number of samples tested	Number of samples positive
Salamandridae	*Cynops orientalis*	Asia	Pet shop	68	0
Salamandridae	*Cynops orientalis*	Asia	Airport	5	0
Salamandridae	*Cynops orientalis*	Asia	Exporter	72	0
Salamandridae	*Cynops pyrrhogaster*	Asia	Pet shop	11	0
Salamandridae	*Ichthyosaura alpestris*	Europe	Pet shop	12	0
Salamandridae	*Notophthalmus viridescens*	North America	Pet shop	14	0
Salamandridae	*Paramesotriton hongkongensis*	Asia	Pet shop	11	0
Salamandridae	*Paramesotriton hongkongensis*	Asia	Exporter	72	0
Salamandridae	*Paramesotriton labiatus*	Asia	Pet shop	16	0
Salamandridae	*Paramesotriton labiatus*	Asia	Airport	2	0
Salamandridae	*Pleurodeles waltl*	Europe	Pet shop	41	0
Salamandridae	*Salamandra salamandra*	Europe	Pet shop	21	0
Salamandridae	*Salamandra salamandra*	Europe	Airport	12	0
Salamandridae	*Triturus marmoratus*	Europe	Pet shop	4	0
Salamandridae	*Tylototriton asperrimus*	Asia	Pet shop	8	0
Salamandridae	*Tylototriton kweichowensis*	Asia	Pet shop	11	0
Salamandridae	*Tylototriton shanjing*	Asia	Pet shop	45	0
Sirenidae	*Siren intermedia*	North America	Pet shop	1	0
Sirenidae	*Siren lacertina*	North America	Airport	2	0
Sirenidae	*Siren lacertina*	North America	Pet shop	2	0
Gymnophiona					
Dermophiidae	*Geotrypetes seraphini*	Africa	Pet shop	2	0

Table S6. Summary of the *B. salamandrivorans* survey of amphibians in the trade. The table lists the number of individuals that were tested, the number that were *B. salamandrivorans*-positive, *B. salamandrivorans* prevalence (proportion infected) and the Clopper-Pearson 95% confidence interval (CI) for prevalence.

Taxonomic group	Number tested	Number positive	Proportion infected	Clopper-Pearson 95% CI	Probability of detecting at least 1 positive individual (assuming prevalence = 0.01)
urodelans	542	0	0	0.0000-0.0068	0.9681
anurans	1221	0	0	0.0000-0.0030	0.9765

Table S7. Presence of *B. salamandrivorans* in skin swabs from captive kept amphibians.

Family	Species	Continent of origin	No tested	No positive
Anura				
Bufonidae	*Atelopus hoogmoedi*	South America	1	0
Bufonidae	*Barbarophryne brongersmai*	Africa	1	0
Bombinatoridae	*Bombina variegata*	Europe	17	0
Dendrobatidae	*Dendrobates auratus*	South America	6	0
Dendrobatidae	*Dendrobates leucomelas*	South America	2	0
Dendrobatidae	*Dendrobates tinctorius*	South America	33	0
Dendrobatidae	*Oophaga pumilio*	South America	14	0
Dendrobatidae	*Phyllobates terribilis*	South America	16	0
Dendrobatidae	*Ranitomeya reticulata*	South America	2	0
Discoglossidae	*Discoglossus pictus*	Africa	6	0
Hemiphractidae	*Gastrotheca riobambae*	South America	1	0
Hylidae	*Hyla eximia*	North America	2	0
Hylidae	*Agalychnis callidryas*	South America	1	0
Hylidae	*Hyla arborea*	Europe	19	0
Hylidae	*Litoria aurea*	Asia	3	0
Hylidae	*Litoria caerulea*	Asia	1	0
Hylidae	*Litoria infrafrenata*	Asia	1	0
Hylidae	*Trachycephalus resinifictrix*	South America	2	0
Hyperoliidae	*Hyperolius puncticulatus*	Africa	5	0
Leptodactylidae	*Eleutherodactylus montanus*	South America	2	0
Leptodactylidae	*Leptodactylus fallax*	South America	1	0
Microhylidae	*Dyscophus guineti*	Africa	1	0
Pipidae	*Silurana tropicalis*	Africa	5	0
Pipidae	*Xenopus laevis*	Africa	16	0
Rhacophoridae	*Rhacophorus dennysi*	Asia	1	0
Urodela				
Ambystomatidae	*Ambystoma andersoni*	North America	1	0
Ambystomatidae	*Ambystoma californiense*	North America	1	0
Ambystomatidae	*Ambystoma gracile*	North America	1	0
Ambystomatidae	*Ambystoma jeffersonianum*	North America	2	0
Ambystomatidae	*Ambystoma laterale*	North America	1	0
Ambystomatidae	*Ambystoma mexicanum*	North America	2	0
Ambystomatidae	*Ambystoma macrodactylum*	North America	4	0
Ambystomatidae	*Ambystoma maculatum*	North America	9	0
Ambystomatidae	*Ambystoma mavortium*	North America	5	0
Ambystomatidae	*Ambystoma opacum*	North America	2	0
Ambystomatidae	*Ambystoma ordinarium*	North America	3	0
Ambystomatidae	*Ambystoma rivulare*	North America	1	0
Ambystomatidae	*Ambystoma tigrinum*	North America	3	0
Ambystomatidae	*Ambystoma velasci*	North America	1	0
Cryptobranchidae	*Andrias davidianus*	North America	3	0
Cryptobranchidae	*Andrias japonicas*	North America	3	0
Dicamptodontidae	*Dicamptodon tenebrosus*	North America	3	0

Family	Species	Continent of origin	No tested	No positive
Hynobiidae	*Hynobius boulengeri*	Asia	1	0
Hynobiidae	*Hynobius dunni*	Asia	3	0
Hynobiidae	*Hynobius naevius*	Asia	1	0
Hynobiidae	*Hynobius quelpartensis*	Asia	1	0
Hynobiidae	*Hynobius retardatus*	Asia	8	0
Hynobiidae	*Hynobius tokyoensis*	Asia	1	0
Hynobiidae	*Pachyhynobius shangchengensis*	Asia	10	0
Hynobiidae	*Paradactylodon gorganensis*	Asia	4	0
Plethodontidae	*Aneides lugubris*	North America	2	0
Plethodontidae	*Aneides flavipunctus*	North America	1	0
Plethodontidae	*Bolitoglossa platydactyla*	North America	5	0
Plethodontidae	*Bolitoglossa rufescens*	North America	8	0
Plethodontidae	*Chiropterotriton multidentatus*	North America	1	0
Plethodontidae	*Chiropterotriton sp.*	North America	6	0
Plethodontidae	*Chiropterotriton sp.*	North America	8	0
Plethodontidae	*Desmognathus carolinensis*	North America	3	0
Plethodontidae	*Desmognathus fuscus*	North America	1	0
Plethodontidae	*Desmognathus marmoratus*	North America	2	0
Plethodontidae	*Desmognathus ochrophaeus*	North America	1	0
Plethodontidae	*Desmognathus quadramaculatus*	North America	2	0
Plethodontidae	*Ensatina eschscholtzii*	North America	2	0
Plethodontidae	*Eurycea bislineata*	North America	2	0
Plethodontidae	*Gyrinophilus porphyriticus*	North America	1	0
Plethodontidae	*Plethodon cylindraceus*	North America	1	0
Plethodontidae	*Plethodon glorobrionus*	North America	1	0
Plethodontidae	*Plethodon glutinosus*	North America	8	0
Plethodontidae	*Plethodon shermani*	North America	1	0
Plethodontidae	*Pseudoeurycea bellii*	Central America	7	0
Plethodontidae	*Pseudoeurycea cephalica*	Central America	5	0
Plethodontidae	*Pseudoerycea leprosa*	Central America	18	0
Plethodontidae	*Pseudoeurycea longicauda*	Central America	5	0
Plethodontidae	*Pseudoeurycea nigromaculata*	Central America	1	0
Plethodontidae	*Pseudoeurycea robertsi*	Central America	3	0
Plethodontidae	*Pseudotriton ruber*	North America	2	0
Plethodontidae	*Thorius troglodytes*	Central America	1	0
Salamandridae	*Calotriton asper*	Europe	1	0
Salamandridae	*Cynops cyanurus*	Asia	3	0
Salamandridae	*Cynops ensicauda*	Asia	6	0
Salamandridae	*Cynops pyrrhogaster*	Asia	17	0
Salamandridae	*Cynops orientalis*	Asia	3	0
Salamandridae	*Euproctus platycephalus*	Europe	11	0
Salamandridae	*Ichthyosaura alpestris*	Europe	16	0
Salamandridae	*Laotriton laoensis*	Asia	4	0
Salamandridae	*Lissotriton boscai*	Europe	1	0
Salamandridae	*Lissotriton montandoni*	Europe	1	0
Salamandridae	*Lyciasalamandra billae*	Asia	4	0
Salamandridae	*Lyciasalamandra fazilae*	Asia	2	0
Salamandridae	*Mertensiella caucasica*	Asia	8	0

Family	Species	Continent of origin	No tested	No positive
Salamandridae	*Neurergus crocatus*	Asia	1	0
Salamandridae	*Neurergus kaiseri*	Asia	20	0
Salamandridae	*Neurergus strauchii*	Asia	6	0
Salamandridae	*Ommatotriton ophryticus*	Asia	3	0
Salamandridae	*Pachytriton* sp.	Asia	2	0
Salamandridae	*Paramesotriton caudopunctatus*	Asia	4	0
Salamandridae	*Paramesotriton chinensis*	Asia	12	0
Salamandridae	*Paramesotriton deloustali*	Asia	4	0
Salamandridae	*Paramesotriton fuzhongensis*	Asia	1	0
Salamandridae	*Paramesotriton hongkongensis*	Asia	7	0
Salamandridae	*Pleurodeles nebulosus*	Africa	2	0
Salamandridae	*Pleurodeles waltl*	Europe	14	0
Salamandridae	*Pleurodeles poireti*	Africa	1	0
Salamandridae	*Salamandra algira*	Africa	7	0
Salamandridae	*Salamandra corsica*	Europe	1	0
Salamandridae	*Salamandra infraimmaculata*	Asia	7	0
Salamandridae	*Salamandra salamandra*	Europe	5	0
Salamandridae	*Taricha granulosa*	North America	2	0
Salamandridae	*Taricha rivularis*	North America	1	0
Salamandridae	*Taricha sierrae*	North America	1	0
Salamandridae	*Taricha torosa*	North America	1	0
Salamandridae	*Triturus carnifex*	Europe	6	0
Salamandridae	*Triturus cristatus*	Europe	1	0
Salamandridae	*Triturus dobrogicus*	Europe	1	0
Salamandridae	*Triturus karelinii*	Europe	1	0
Salamandridae	*Triturus marmoratus*	Europe	1	0
Salamandridae	*Tylototriton asperrimus*	Asia	1	0
Salamandridae	*Tylototriton kweichowensis*	Asia	12	0
Salamandridae	*Tylototriton shanjing*	Asia	6	0
Salamandridae	*Tylototriton taliangensis*	Asia	1	0
Salamandridae	*Tylototriton vietnamensis*	Asia	18	3
Salamandridae	*Tylototriton verrucosus*	Asia	4	0
Salamandridae	*Tylototriton wenxianensis*	Asia	3	0
Salamandridae	*Tylototriton ziegleri*	Asia	1	0
Sirenidae	*Siren intermedia*	North America	1	0
Gymnophiona				
Dermophiidae	*Geotrypetes seraphini*	Africa	1	0
Herpelidae	*Herpele* sp.	Africa	1	0

Table S8. Summary of the *B. salamandrivorans* survey of amphibians in captivity. The table lists the number of individuals that were tested, the number that were *B. salamandrivorans*-positive, *B. salamandrivorans* prevalence (proportion infected) and the Clopper-Pearson 95% confidence interval for prevalence.

Taxonomic group	Number tested	Number positive	Proportion infected	Clopper-Pearson 95% CI	Probability of detecting at least 1 positive individual (assuming prevalence = 0.01)
urodelans	408	3	0.0073	0.0015 - 0.0212	0.9834
anurans	159	0	0.0000	0.0000 - 0.0229	0.7977

Table S9. Species susceptibility category used for ancestral state reconstruction.

Species	Category
Typhlonectes compressicauda	Resistant
Bombina variegata	Resistant
Alytes obstetricans	Resistant
Discoglossus scovazzi	Resistant
Silurana tropicalis	Resistant
Pelobates fuscus	Resistant
Pelodytes punctatus	Resistant
Epidalea calamita	Resistant
Hyla arborea	Resistant
Lithobathes catesbeianus	Resistant
Rana temporaria	Resistant
Hynobius retardatus	Resistant
Salamandrella keyserlingii	Tolerant
Pachyhynobius shangchengensis	Resistant
Siren intermedia	Tolerant
Plethodon glutinosus	Resistant
Hydromantes strinatii	Lethal
Gyrinophilus porphyriticus	Resistant
Ambystoma opacum/maculatum	Resistant
Salamandrina perspicillata	Lethal
Salamandra salamandra	Lethal
Pleurodeles waltl	Lethal
Tylototriton wenxianensis	Lethal
Nothophthalmus viridescens	Lethal
Taricha granulosa	Lethal
Euproctus platycephalus	Lethal
Lissotriton helveticus	Resistant
Lissotriton italicus	Lethal
Ichthyosaura alpestris	Lethal
Triturus cristatus	Lethal
Neurergus crocatus	Lethal
Cynops cyanurus	Susceptible
Cynops pyrrhogaster	Susceptible
Paramesotriton deloustali	Susceptible

Table S10. Results of the Maximum Parsimony (MP) and Maximum Likelihood ancestral state reconstructions of amphibian susceptibility to *B. salamandrivorans*. Node numbers are cross-referenced on the tree in Figure S5.

Node N°	MP	Maximum Likelihood Probabilities			
		Resistant	Tolerant	Susceptible	Lethal
2	Resistant	0.988270	0.004081	0.003628	0.004021
4	Resistant	0.995242	0.001770	0.001282	0.001706
5	Resistant	0.999975	8.48E-06	7.91E-06	8.40E-06
6	Resistant	0.999792	6.94E-05	6.93E-05	6.94E-05
8	Resistant	0.999366	2.11E-04	2.11E-04	2.11E-04
11	Resistant	0.999986	4.87E-06	4.75E-06	4.85E-06
13	Resistant	0.999968	1.07E-05	1.07E-05	1.07E-05
14	Resistant	0.999248	2.51E-04	2.51E-04	2.51E-04
17	Resistant	0.999606	1.31E-04	1.31E-04	1.31E-04
18	Resistant	0.999846	5.12E-05	5.12E-05	5.12E-05
21	Resistant	0.999968	1.08E-05	1.08E-05	1.08E-05
24	Resistant	0.993609	0.003138	4.68E-04	0.002785
25	Resistant	0.995580	0.004139	1.28E-04	1.53E-04
26	Resistant	0.992494	0.007093	1.97E-04	2.17E-04
30	Resistant	0.985569	0.006714	7.29E-04	0.006988
32	Resistant	0.984866	0.001610	3.23E-04	0.013201
33	Resistant	0.991893	1.49E-04	1.13E-04	0.007845
34	Resistant	0.990246	1.98E-04	1.65E-04	0.009392
38	Resistant	0.956639	0.001879	9.90E-04	0.040492
40	Lethal	0.008573	2.55E-04	2.47E-04	0.990926
42	Lethal	8.28E-04	2.96E-05	2.89E-05	0.999113
44	Lethal	5.86E-06	6.66E-07	6.70E-07	0.999993
45	Lethal	1.21E-05	1.18E-05	1.18E-05	0.999964
48	Lethal	2.00E-07	1.42E-07	3.21E-07	0.999999
49	Lethal	7.10E-06	7.09E-06	7.12E-06	0.999979
52	Lethal	9.92E-07	9.66E-07	3.33E-05	0.999965
54	Lethal	7.13E-06	6.72E-06	5.65E-04	0.999421
55	Lethal	9.35E-07	1.34E-07	5.85E-06	0.999993
56	Lethal	8.93E-05	2.37E-06	3.31E-06	0.999905
57	Lethal	8.71E-04	2.18E-05	2.27E-05	0.999085
61	Lethal	3.01E-06	2.93E-06	3.53E-06	0.999991
64	Susceptible	2.52E-05	2.52E-05	0.997828	0.002122
65	Susceptible	7.54E-06	7.54E-06	0.999422	5.63E-04

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