

1 **Title:** Recent Asian origin of chytrid fungi causing global amphibian declines*

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89 **One Sentence Summary:** East Asia is the source of amphibian panzootic chytrid fungi
90 causing global amphibian declines that have emerged during the 20th century
91

92 **Abstract:**

93 Globalized infectious diseases are causing species declines worldwide but their source often
94 remains elusive. We use whole-genome sequencing to solve the spatiotemporal origins of the
95 most devastating panzootic to date, caused by the fungus *Batrachochytrium dendrobatidis*, a
96 proximate driver of global amphibian declines. We trace the source of *B. dendrobatidis* to the
97 Korean peninsula where one lineage, *BdASIA-1*, exhibits the genetic hallmarks of an
98 ancestral population that seeded the panzootic. We date the emergence of this pathogen to the
99 early 20th century coinciding with the global expansion of commercial trade in amphibians
100 and show that intercontinental transmission is ongoing. Our findings point to East Asia as a
101 geographic hotspot for *B. dendrobatidis* biodiversity, and the original source of these lineages
102 that now parasitize amphibians worldwide.

103 Main Text:

104 Discovery of the amphibian-killing fungus *Batrachochytrium dendrobatidis* (1, 2) was a
105 turning point in understanding why amphibian species worldwide are in steep decline.
106 Amphibian declines and extinctions had been recorded by herpetologists as early as the
107 1970s, but were only recognized at a landmark meeting in 1990 as a global phenomenon
108 which could not be explained by environmental changes and anthropogenic factors alone (3).
109 The emergence of *B. dendrobatidis* and the disease that it causes, amphibian
110 chytridiomycosis, as a causative agent of declines has been documented across six different
111 regions: Australia (~1970s and 1990s) (4), Central America (~1970s) (5), South America
112 (~1970s and 1980s) (6, 7), the Caribbean islands (~2000s) (8), the North American Sierra
113 Nevada (~1980s and 1990s) (9), and the Iberian Peninsula (~1990s) (10). The panzootic has
114 been attributed to the emergence of a single *B. dendrobatidis* lineage, known as *BdGPL*
115 (Global Panzootic Lineage) (11). However, twenty years after identification of the disease,
116 the timing of its worldwide expansion remains unknown and previous estimates for time to
117 most recent common ancestor (TMRCA) for *BdGPL* span two orders of magnitude, from 100
118 ybp (11) to 26,000 ybp (12). The geographic origin of the pathogen is similarly contested,
119 with the source of the disease variously suggested to be Africa (13), North America (14),
120 South America (15), Japan (16) and East Asia (17).

121 Global diversity of *B. dendrobatidis*

122 To resolve these inconsistencies, we isolated *B. dendrobatidis* from all the candidate source
123 continents and sequenced the genomes of 177 isolates to high depth then combined our data
124 with published genomes from three prior studies (11, 12, 18) to generate a globally
125 representative panel of 234 isolates (Fig. 1A). This dataset covers all continents from which
126 *B. dendrobatidis* has been detected to date, and spans infections of all three extant orders of

127 Amphibia (Fig. S1 and Table S1). Mapped against the *B. dendrobatidis* reference genome
128 JEL423, our sequencing recovered 586,005 segregating single nucleotide polymorphisms
129 (SNPs). Phylogenetic analysis recovered all previously detected divergent lineages (Fig. 1B
130 and Fig. S2). The previously accepted lineages *BdGPL* (global), *BdCAPE* (African), *BdCH*
131 (European) and *BdBRAZIL* (Brazilian), were all detected (19), but our discovery of a new
132 hyperdiverse lineage in amphibians native to the Korean peninsula (*BdASIA-1*) redefined
133 these lineages and their relationships. The *BdCH* lineage, which was previously thought to be
134 enzootic to Switzerland (11) now groups with the *BdASIA-1* lineage. A second Asian-
135 associated lineage (*BdASIA-2*) was recovered from invasive North American bullfrogs in
136 Korea and is closely related to the lineage that is enzootic to the Brazilian Atlantic forest
137 (*BdBRAZIL*) (20). It was not possible to infer the direction of intercontinental spread
138 between isolates within this lineage so it was named *BdASIA-2/BdBRAZIL*. Conditional on
139 the midpoint rooting of the phylogeny in Fig. 1B, we now define the main diverged lineages
140 as *BdGPL*, *BdCAPE*, *BdASIA-1* (which includes the single *BdCH* isolate) and *BdASIA-*
141 *2/BdBRAZIL*. Previous phylogenetic relationships developed using the widely used
142 ribosomal intragenic spacer *ITS-1* region do not accurately distinguish *B. dendrobatidis*
143 lineages (Fig. S3) and this likely explains much of the place-of-origin conflict in the literature
144 (15-17).

145 Pairwise comparisons among isolates within each lineage show that the average number of
146 segregating sites is three-fold greater for *BdASIA-1* than for any other lineage (Fig. 1A and
147 Table 1) and that nucleotide diversity (π ; Fig. S4) is two to four-fold greater. Seven of our
148 eight *BdASIA-1* isolates were recently cultured from wild South Korean frogs while the other
149 came from the pet-trade in Belgium, all of which were aclinical infections. These isolates
150 show that the Korean peninsula is a global centre of *B. dendrobatidis* diversity and that East
151 Asia may contain the ancestral population of *B. dendrobatidis*, as suggested by Bataille *et al*

152 (17). We investigated this hypothesis further using Bayesian-based haplotype clustering (21)
153 and found the greatest haplotype sharing among isolates within *BdASIA-1* and between
154 *BdASIA-1* and all other lineages. This provides direct genetic evidence that *BdASIA-1* shares
155 more diversity with the global population of *B. dendrobatidis* than any other lineage (Fig.
156 S5). In an independent test of ancestry, we used OrthoMCL (22) to root a *B. dendrobatidis*
157 phylogeny to its closest known relative *B. salamandrivorans* which currently threatens
158 salamanders (23). This tree indicates that the Asian and Brazilian isolates of *B. dendrobatidis*
159 lie outside a clade comprising all other isolates (Fig. S6 and Table S2). To identify the
160 signature of demographic histories across lineages we used Tajima's *D* (24). Genome scans
161 of most lineages showed highly variable positive and negative values of *D* with maxima
162 exhibited by *BdGPL* (-2.6 to +6.2; Fig. 2F), indicating that these lineages (*BdASIA-*
163 *2/BdBRAZIL*, *BdCAPE* and *BdGPL*) have undergone episodes of population fluctuation,
164 strong natural selection, or both, that are consistent with a history of spatial and host
165 radiations. In striking contrast, *BdASIA-1* shows a flat profile for Tajima's *D* (Fig. 2F)
166 indicating mutation-drift equilibrium likely reflective of pathogen endemism in this region.

167 **Dating the emergence of *BdGPL***

168 The broad range of previous estimates for the TMRCA of *BdGPL* spanning 26,000 years (11,
169 12) can be explained by two sources of inaccuracy: (1) unaccounted recombination and (2)
170 the application of unrealistic evolutionary rates. To address these, we first interrogated the
171 178,280 kbp mitochondrial genome (mtDNA), which has high copy number and low rates of
172 recombination compared to the nuclear genome. To resolve the structure of the mtDNA
173 genome we resorted to long-read sequencing using a MinION device (Oxford Nanopore
174 Technologies, Cambridge, UK), which allowed us to describe this molecules unusual
175 configuration; *Batrachochytrium dendrobatidis* carries three linear mitochondrial segments,
176 each having inverted repeats at the termini with conserved mitochondrial genes spread over

177 two of the segments (Fig. S7). Additionally, we sought regions of the autosomal genome with
178 low rates of recombination to obtain an independent estimate of the TMRCA of *BdGPL*.

179 Detection of crossover events in the *B. dendrobatidis* autosomal genome (18) using a subset
180 of the isolates in this study revealed a large (1.66Mbp) region of Supercontig_1.2 in *BdGPL*
181 that exhibits several features that identified it as a recombination ‘coldspot’: (1) a continuous
182 region of reduced Tajima’s *D* (Fig. 2D); (2) sustained high values of F_{ST} when compared
183 with all other lineages (Fig. 3A); (3) a continuous region of reduced nucleotide diversity (π ,
184 Fig. S4) and (4) shared loss-of-heterozygosity (Fig. S8). We expanded sampling to infer the
185 temporal range of pathogen introductions using a broad panel of isolates with known date of
186 isolation ($n = 184$, ranging from 1998 to 2016) and whole-genome RNA-baiting to obtain
187 reads from preserved amphibians that had died of chytridiomycosis. We then investigated
188 whether our dataset contained sufficient signal to perform tip-dating inferences by building
189 phylogenetic trees using PhyML (25) (Fig. 2A and 2C) then fitting root-to-tip distances to
190 collection dates both at the whole-tree and within-lineage scales. We observed a positive and
191 significant correlation within *BdGPL* only, for both the mitochondrial and nuclear genomes,
192 demonstrating sufficient temporal signal to perform thorough tip-dating inferences at this
193 evolutionary scale (Fig. 2B and 2D).

194 Tip-dating in BEAST was used to co-estimate ancestral divergence times and the rate at
195 which mutations accumulate within the *BdGPL* lineage. The mean mitochondrial substitution
196 rate was 1.01×10^{-6} substitutions/site/year (95% highest posterior density (HPD) $4.29 \times 10^{-7} -$
197 1.62×10^{-6}). The mean nuclear substitution rate was 7.29×10^{-7} substitutions/site/year (95%
198 HPD $3.41 \times 10^{-7} - 1.14 \times 10^{-6}$), which is comparable to a recent report of an evolutionary rate
199 of $2.4 - 2.6 \times 10^{-6}$ substitutions/site/year for another unicellular yeast, *Saccharomyces*
200 *cerevisiae* beer strains (26). These estimates are over 300-fold faster than the rate used in a

201 previous study (12) to obtain a TMRCA of 26,400 years for *BdGPL*. Accordingly, we
202 estimate the ancestor of the amphibian panzootic *BdGPL* originated between 120 and 50
203 years ago (Fig. 2E), with HPD estimates of 1898 [95% HPD 1809-1941] and 1962 [95%
204 HPD 1859-1988] for the nuclear and mitochondrial dating analyses respectively (Fig. 2F).

205 We considered an additional calibration approach for the TMRCA of the mitochondrial
206 genome where we included informative priors on nodes around the dates for the first
207 historical descriptions of *BdGPL* detection in Australia (1978), Central America (1972),
208 Sierra de Guadarrama (Europe) (1997), and the Pyrenees (Europe) (2000). We did not
209 include priors for nodes where observed declines have been reported, but where the lineage
210 responsible for those declines is unknown. This mixed dating method based on tips and nodes
211 calibration yielded very similar estimates (TMRCA estimates of 1975 [95% HPD 1939 –
212 1989] (Fig. S9)), further strengthening our confidence in a recent date of emergence for
213 *BdGPL*. An expansion of *BdGPL* in the 20th century coincides with the global expansion in
214 amphibians traded for exotic pets, medical and food purposes (27, 28). Within our phylogeny,
215 we found representatives from all lineages among traded animals (Figs. S10-14), and
216 identified ten events where traded amphibians were infected with non-enzootic isolates (Fig.
217 4). This finding demonstrates the ongoing failure of international biosecurity despite the
218 listing of *B. dendrobatidis* by the World Organisation for Animal Health (the OIE) in 2008.

219 **Hybridisation between recontacting lineages of *B. dendrobatidis***

220 To determine the extent to which the four main lineages of *B. dendrobatidis* have undergone
221 recent genetic exchange, we used the site-by-site based approach implemented in
222 STRUCTURE (29). Although most isolates could be assigned unambiguously to one of the
223 four main lineages, we identified three hybrid genotypes (Fig. 3B), including one previously
224 reported hybrid (isolate CLFT024/2) (20), and discovered two newly identified hybrids of

225 *BdGPL* and *BdCAPE* in South Africa. Furthermore, *BdCH* (isolate 0739) appears to be a
226 chimera of multiple lineages that may represent unsampled genomic diversity that resides in
227 East Asia, rather than true hybridisation. These hybrid genomes demonstrate that *B.*
228 *dendrobatidis* is continuing to exchange haplotypes among lineages when they interact
229 following continental invasions, generating novel genomic diversity. We analysed isolate
230 clustering using principle components analysis on a filtered subset of 3,900 SNPs in linkage
231 equilibrium, revealing an overall population structure that is consistent with our phylogenetic
232 analyses (Fig 3C). In addition, the putatively identified hybrid isolates of *B. dendrobatidis*
233 were shown to fall between main lineage clusters (Fig. 3C) further strengthening our
234 hypothesis of haplotype exchange occurring during secondary contact between lineages.

235 **Associations among lineage, virulence and declines**

236 Genotypic diversification of pathogens is commonly associated with diversification of traits
237 associated with host exploitation (30), and is most commonly measured as the ability to infect
238 a host and to cause disease post-infection. We tested for variation of these two phenotypic
239 traits across four *B. dendrobatidis* lineages by exposing larval and post-metamorphic
240 common toads (*Bufo bufo*). Larvae are highly susceptible to infection but do not die before
241 metamorphosis, in contrast to post-metamorphic juveniles, which are susceptible to infection
242 and fatal chytridiomycosis (31). In tadpoles, both *BdGPL* and *BdASIA-1* were significantly
243 more infectious than *BdCAPE* and *BdCH* (Fig. S15 and Tables S3 & S4). In metamorphs,
244 *BdGPL* was significantly more infectious than the other treatments, compared to the control
245 group, and significantly more lethal in experimental challenge, than the geographically more
246 restricted *BdCAPE*, *BdASIA-1* and *BdCH* (Fig. 2G). We further tested for differences in
247 virulence among lineages by using our global dataset to examine whether chytridiomycosis
248 was non-randomly associated with *B. dendrobatidis* lineage. We detected a significant

249 difference ($p < 0.001$) in the proportion of isolates associated with chytridiomycosis among
250 the three parental lineages (*BdASIA-1* and *BdASIA-2/BdBRAZIL* were grouped due to low
251 sample sizes), and *post hoc* tests indicated significant excess in virulence in both *BdGPL* and
252 *BdCAPE* lineages relative to the combined *BdASIA-1* and *BdASIA-2/BdBRAZIL* (all $p <$
253 0.05). However, we did not detect a significant difference between *BdGPL* and *BdCAPE*
254 (Fig. S16 and Table S5). These data suggest that although *BdGPL* is highly virulent,
255 population-level outcomes are also context dependent (32); under some conditions other
256 lineages can also be responsible for lethal amphibian disease and population declines (33).

257 **Historical and contemporary implications of panzootic chytridiomycosis**

258 Our results point to endemism of *B. dendrobatidis* in Asia, out of which multiple panzootic
259 lineages have emerged. These emergent diasporas include the virulent and highly
260 transmissible *BdGPL* which spread during the early 20th century via a yet unknown route to
261 infect close to 700 amphibian species out of ~1300 thus far tested (34). With over 7800
262 amphibian species currently described, the number of affected species is likely to rise. The
263 international trade in amphibians has undoubtedly contributed directly to vectoring this
264 pathogen worldwide (Fig. 4; 35,36), and within our phylogeny we identified many highly
265 supported ($\geq 90\%$ bootstrap support) clades on short branches that linked isolates collected
266 from wild amphibian populations across different continents (Fig. 4; Fig. S10-S14).
267 However, the role of globalised trade in passively contributing to the spread of this disease
268 cannot be ruled out. It is likely no coincidence that our estimated dates for the emergence of
269 *BdGPL* span the globalisation ‘big bang’, the rapid proliferation in intercontinental trade,
270 capital, and technology that started in the 1820s (37). The recent invasion of Madagascar by
271 Asian common toads hidden within mining equipment (38) demonstrates the capacity for
272 amphibians to escape detection at borders and exemplifies how the unintended anthropogenic

273 dispersal of amphibians has also likely contributed to the worldwide spread of pathogenic
274 chytrids.

275 The hyperdiverse hotspot identified in Korea likely represents a fraction of the
276 *Batrachochytrium* genetic diversity in Asia and further sampling across this region is
277 urgently needed because the substantial global trade in Asian amphibians (39) presents a risk
278 of seeding future outbreak lineages. Unique ribosomal DNA haplotypes of *B. dendrobatidis*
279 have been detected in native amphibian species in India (40, 41), Japan (16) and China (42).
280 Although caution should be observed when drawing conclusions about lineages based on
281 short sequence alignments (Fig. S3), other endemic lineages probably remain undetected
282 within Asia. Significantly, the northern European countryside is witnessing the emergence of
283 *B. salamandrivorans*, which also has its origin in Asia. The emergence of *B.*
284 *salamandrivorans* is linked to the amphibian pet trade (43), and the broad expansion of
285 virulence factors that are found in the genomes of these two pathogens are testament to the
286 evolutionary innovation that has occurred in these Asian *Batrachochytrium* fungi (23). Our
287 findings show that the global trade in amphibians continues to be associated with the
288 translocation of chytrid lineages with panzootic potential. Ultimately, our work confirms that
289 panzootics of emerging fungal diseases in amphibians are caused by ancient patterns of
290 pathogen phylogeography being redrawn as largely unrestricted global trade moves
291 pathogens into new regions, infecting new hosts and igniting disease outbreaks. Within this
292 context, the continued strengthening of transcontinental biosecurity is critical to the survival
293 of amphibian species in the wild (44).

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550

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591 authors.

592

593 **Competing interests:** KAM sits on an expert panel at the European Food Safety Authority
594 addressing the risks of importation and spread of the salamander chytrid *Batrachochytrium*
595 *salamandrivorans*, a species of fungus that is the closest known relative to the pathogen
596 addressed in this manuscript.

597

598 **Data availability:** Sequences have been deposited in the National Center for Biotechnology
599 Information (NCBI) Sequence Read Archive (SRA). All sequences are available from NCBI
600 BioProject accession PRJNA413876
601 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA413876>). The supplementary materials
602 contain additional data. Phylogenetic trees are available from TreeBASE, project accession
603 url: <http://purl.org/phylo/treebase/phyloids/study/TB2:S22286>. A browsable version of the
604 phylogeny and metadata in Fig. 1B is accessible at: <https://microreact.org/project/GlobalBd>

605 **List of supplementary materials:**

606 Materials and Methods

607 Figs. S1 to S15

608 Tables S1 to S5

609 Data S1 to S3

610 References (45-92)

611 **Tables:**

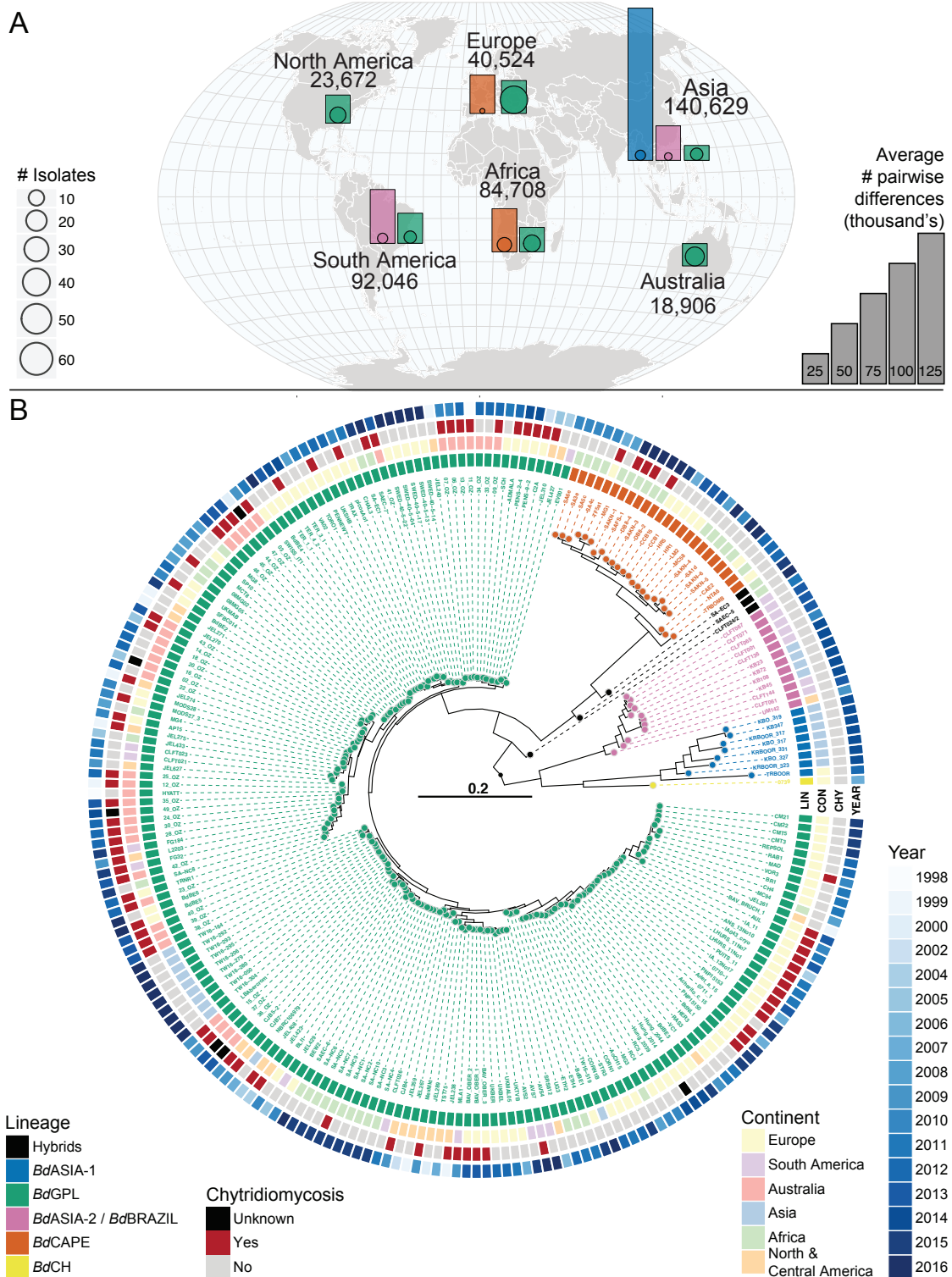
Lineage	Number of Isolates	Total segregating sites	Average pairwise-segregating sites	Total homozygous segregating sites	Average pairwise-homozygous segregating sites	π	Tajima's D
<i>Bd</i> ASIA-1	8	327,996	142,437	108,353	21,716	0.0044	0.2540
<i>Bd</i> ASIA-2 / <i>Bd</i> BRAZIL	12	148,021	51,069	48,722	6,216	0.0018	0.9825
<i>Bd</i> CAPE	24	146,466	38,881	53,884	4,977	0.0016	0.3143
<i>Bd</i> GPL	187	127,770	26,546	68,493	3,101	0.0009	0.9792

612

613 **Table 1.** Comparison of common genetic diversity measures among *Batrachochytrium*
614 *dendrobatidis* lineages. Total segregating sites for each lineage include all segregating sites
615 where genotype calls were made in at least half of the isolates. Average pairwise-segregating
616 sites is the average number of sites with different genotypes between all pairs of isolates
617 within a lineage. Total homozygous segregating sites includes all sites within a lineage where
618 there is at least one homozygous difference between isolates. Average pairwise homozygous
619 segregating sites is the average number of sites with different homozygous genotypes
620 between all pairs of isolates within a lineage. Nucleotide diversity (π) is the mean of the per-
621 site nucleotide diversity. Tajima's D is reported as the mean over 1 kbp bins.

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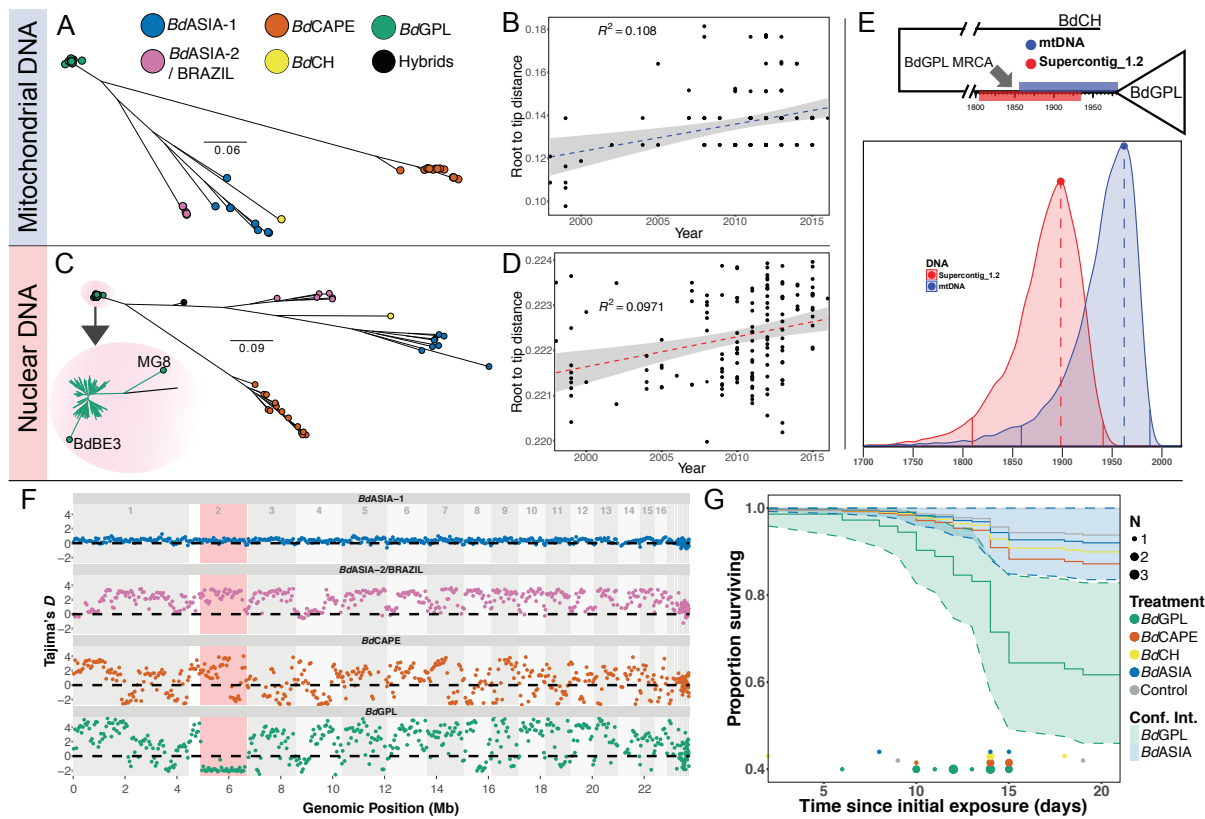
623 **Figures:**



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625 **Fig. 1:** Genetic diversity and phylogenetic tree of a global panel of 234 *Batrachochytrium*
 626 *dendrobatidis* isolates. **A.** Map overlaid with bar charts showing the relative diversity of
 627 isolates found in each continent and by each major lineage (excluding isolates from traded
 628 animals). The bar heights are the average number of segregating sites between all pairwise

629 combinations of isolates of each lineage in each continent (therefore only lineages with two
630 or more isolates from a continent are shown). Outlined points at the base of each bar are
631 scaled by the number of isolates for each lineage in that continent. The numbers around the
632 outside of the globe are the average number of segregating sites between all pairwise
633 combinations of isolates grouped by continent. Colours denote lineage as given by the legend
634 in Fig 1B. **B.** Midpoint rooted radial phylogeny supports four deeply diverged lineages of *B.*
635 *dendrobatidis*: *BdASIA-1*; *BdASIA-2/BdBRAZIL*; *BdCAPE* and *BdGPL*. All major splits
636 within the phylogeny are supported by 100% of 500 bootstrap replicates. See Fig. S2 for tree
637 with full bootstrap support values on all internal branches.
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641 **Fig. 2:** Dating the emergence of *BdGPL*. **A.** Maximum likelihood (ML) tree constructed from642 1,150 high quality SNPs found within the 178 kbp mitochondrial genome. **B.** Linear643 regression of root-to-tip distance against year of isolation for *BdGPL* isolates in

644 mitochondrial DNA phylogeny in panel A, showing significant temporal trend (F-statistic =

645 14.35, $p = 0.00024$). **C.** ML tree constructed from a 1.66 Mbp region of low recombination in646 Supercontig_1.2. Two *BdGPL* isolates, BdBE3 and MG8 fall on long branches away from647 the rest of the *BdGPL* isolates (see inset zoom), due to introgression from another lineage648 (*BdCAPE*; see Fig. 3B) and were excluded from the dating analysis. **D.** Linear regression of649 root-to-tip distance against year of isolation for *BdGPL* isolates from phylogeny in panel C,650 with significant temporal trend (F-statistic = 15.92, p -value = 0.0001). **E.** Top figure shows651 *BdGPL* and outgroup *BdCH*, with the 95% HPD estimates for MRCA for *BdGPL* from

652 mtDNA dating (blue) and nuclear DNA dating (red). Lower figure shows full posterior

653 distributions from tip dating models for mtDNA (blue) and partial nuclear DNA (red)

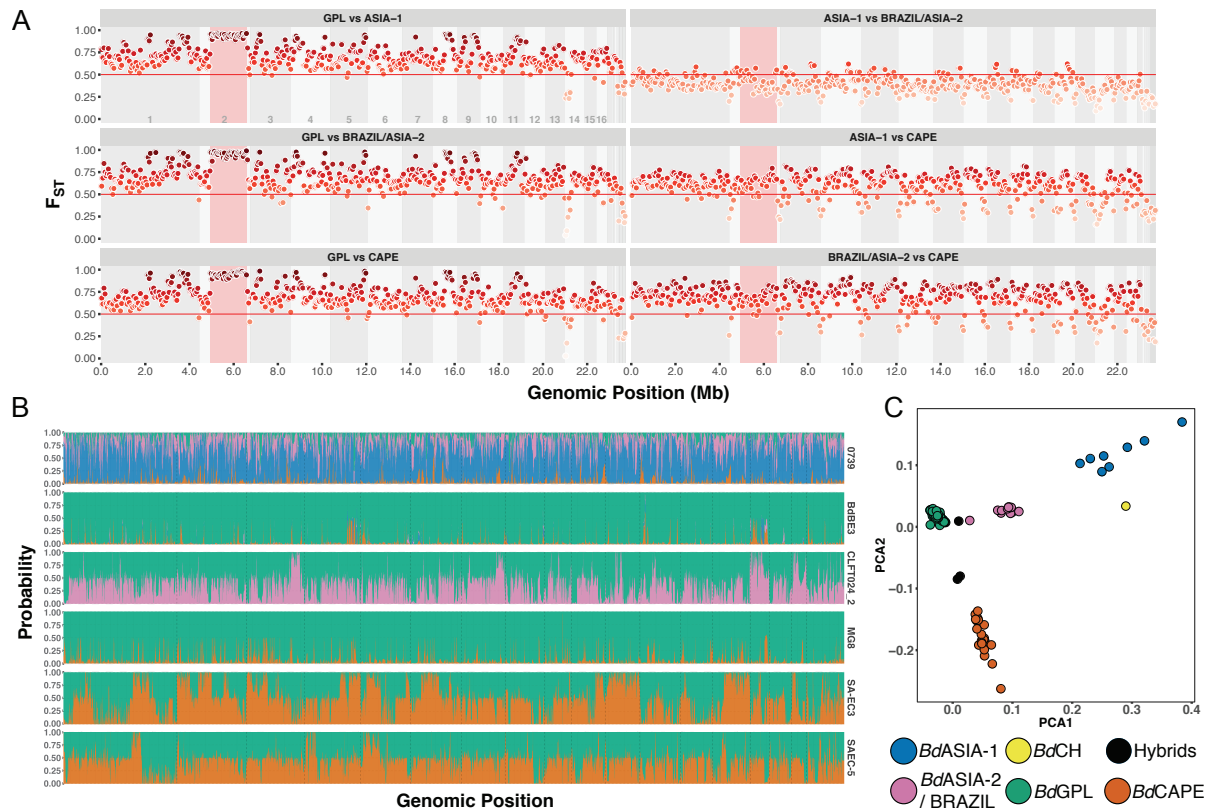
654 genomes. Solid vertical lines are limits of the 95% HPD. Dashed vertical lines denote the

655 maximal density of the posterior distributions. **F.** Sliding 10 kb, non-overlapping window656 estimates of Tajima's D for each of the main *B. dendrobatidis* lineages. The region657 highlighted in red is the low recombination segment of Supercontig_1.2. **G.** Survival curves658 for *Bufo bufo* metamorphs for different *B. dendrobatidis* treatment groups: *BdASIA*-1 (blue);659 *BdCAPE* (orange); *BdCH* (yellow); *BdGPL* (green) and Control (grey). Confidence intervals

660 are shown for *Bd*GPL and *Bd*ASIA-1, showing no overlap by the end of the experiment.
661 Instances of mortalities in each treatment group are plotted along the x-axis, with points
662 scaled by number of mortalities at each interval (day).

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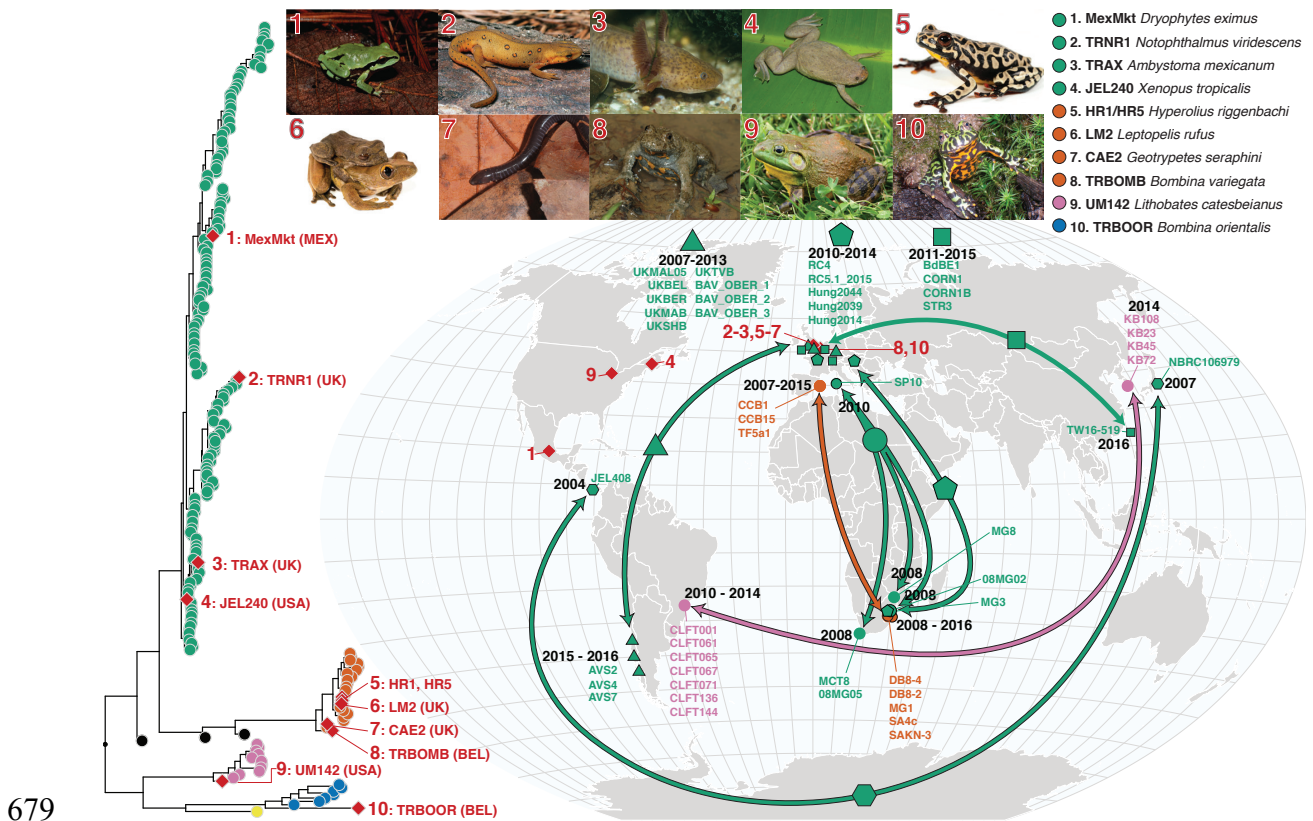
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Fig. 3: F_{ST} and site-by-site STRUCTURE analysis. **A.** Non-overlapping, 10 kb sliding window of F_{ST} between lineages. The region highlighted in red is Supercontig_1.2:500,000-2,160,000 low recombination region. **B.** Site-by-site analysis of population ancestry for a random selection of 9,905 SNPs. Results show those isolates found to be either hybrid (SA-EC3, SA-EC5 and CLFT024/2), or with significant introgression from non-parental lineages (isolates BdBE3 and MG8) or a chimera of un-sampled diversity, likely originating from East Asia (0739, the *BdCH* isolate). Each column represents a bi-allelic SNP position. The column is coloured according to the joint-probability of either allele copy arising from one of four distinct populations. Colours represent assumed parental lineages as given in Fig. 3C. **C.** Principle Components Analysis (PCA) of 3,900 SNPs in linkage equilibrium. Each point represents an isolate, coloured by phylogenetic lineage. The isolates separate into clearly defined clusters. The axes plot the first and second principle components.



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680 **Fig. 4:** Genotypes of *Bd* isolated from infected amphibians in the international trade and
 681 phylogenetically linked genotypes from segregated geographic localities. The red diamonds
 682 on the phylogeny indicate isolates recovered from traded animals. Their geographic location
 683 is displayed by the red diamonds on the map. The red numbers link each trade isolate to the
 684 relevant picture of the donor host species atop the figure panel and their placement in the
 685 phylogeny. The arrows on the map link geographically separated isolates which form closely
 686 related phylogenetic clades with high bootstrap support ($\geq 90\%$). Each clade is denoted by a
 687 different shape point on the map with the names of isolates within each clade displayed on
 688 the map. The dates displayed indicate the sampling time-frame for each clade. The
 689 phylogenetic position of each clade is displayed in Figs S10-14. The colours of points and
 690 arrows on the map indicate lineage according to the legend in Fig 1. A browsable version of
 691 this phylogeny can be accessed at <https://microreact.org/project/GlobalBd>. Photo credits: (1)
 692 *Hyla eximia* Ricardo Chaparro, (2) *Notophthalmus viridescens* Patrick Coin / CC-BY-SA 2.5,
 693 (3) *Ambystoma mexicanum* Henk Wallays, (4) *Xenopus tropicalis* Daniel Portik, (5)
 694 *Hyperolius riggenbachi* and (6) *Leptopelis rufus* Brian Freiermuth, (7) *Geotrypetes seraphini*
 695 Peter Janzen, (8) *Bombina variegata* and (9) *Rana catesbeiana* and (10) *Bombina orientalis*
 696 Frank Pasmans