Recent Asian origin of chytrid fungi causing global amphibian declines

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

Although amphibian declines and extinctions had been recorded by herpetologists as early as the 1970s, they were only recognized in 1990 as a global phenomenon that could not be explained by environmental changes and anthropogenic factors alone (3). The emergence of B. dendrobatidis and the disease that it causes, amphibian chytridiomycosis, as a causative agent of declines has been documented across six different regions: Australia (~1970s and 1990s) (4), Central America (~1970s) (5), South America (~1970s and 1980s) (6, 7), the Caribbean islands (~2000s) (8), the North American Sierra Nevada (~1980s and 1990s) (9), and the Iberian Peninsula (~1990s) (10). The panzooptic has been attributed to the emergence of a single B. dendrobatidis lineage, known as BdGPL (Global Panzooptic Lineage) (11). However, 20 years after identification of the disease, the timing of its worldwide expansion remains unknown and previous estimates for time to most recent common ancestor (MRCA) for BdGPL span two orders of magnitude, from 100 years before the present (11) to 26,000 years before the present (12). The geographic origin of the pathogen is similarly contested, with the source of the disease variously suggested to be Africa (13), North America (14), South America (15), Japan (16), and East Asia (17).

Global diversity of B. dendrobatidis

To resolve these inconsistencies, we isolated B. dendrobatidis from all the candidate source continents and sequenced the genomes of 177 isolates to high depth, then combined our data with published genomes from three prior studies (11, 12, 18) to generate a globally representative panel of 234 isolates (Fig. 1A and fig. S1). This data set covers all continents from which B. dendrobatidis has been detected to date, and spans infections of all three extant orders of Amphibia (fig. S1 and table S1). Mapped against the B. dendrobatidis reference genome JELA423, our sequencing recovered 586,005 segregating single-nucleotide polymorphisms (SNPs). Phylogenetic analysis recovered all previously detected divergent lineages (Fig. 1B and fig. S2). The previously accepted lineages BdGPL (global), BdCAPE (African), BdCH (European), and BdBRAZIL (Brazilian) were all detected (19), but our discovery of a new hyperdiverse lineage in amphibians native to the Korean peninsula (BdASIA-1) redefined these lineages and their relationships. The BdCH lineage, which was previously thought to be enzootic to Switzerland (17), now groups with the BdASIA-1 lineage. A
Fig. 1. Genetic diversity and phylogenetic tree of a global panel of 234 B. dendrobatidis isolates. (A) Map overlaid with bar charts showing the relative diversity of isolates found in each continent and by each major lineage (excluding isolates from traded animals). The bar heights represent the average numbers of segregating sites between all pairwise combinations of isolates of each lineage in each continent (therefore, only lineages with two or more isolates from a continent are shown). Outlined points at the base of each bar are scaled by the number of isolates for each lineage in that continent. The numbers around the outside of the globe are the average number of segregating sites between all pairwise combinations of isolates grouped by continent. Colors denote lineage as shown in (B). (B) Midpoint rooted radial phylogeny supports four deeply diverged lineages of B. dendrobatidis: BdASIA-1, BdASIA-2/BdBRAZIL, BdCAPE, and BdGPL. All major splits within the phylogeny are supported by 100% of 500 bootstrap replicates. See fig. S2 for tree with full bootstrap support values on all internal branches.

second Asian-associated lineage (BdASIA-2) was recovered from invasive North American bullfrogs in Korea and is closely related to the lineage that is enzootic to the Brazilian Atlantic forest (BdBRAZIL) (20). It was not possible to infer the direction of intercontinental spread between isolates within this lineage, so it was named BdASIA-2/BdBRAZIL. Conditional on the midpoint rooting of the phylogeny in Fig. 1B, we now define the main diverged lineages as BdGPL, BdCAPE, BdASIA-1 (which includes the single BdCH isolate), and BdASIA-2/BdBRAZIL. Previous phylogenetic relationships developed using the widely used ribosomal intragenic spacer ITS-1 region do not accurately distinguish B. dendrobatidis lineages (fig. S3), and this likely explains much of the place-of-origin conflict in the literature (15–17).

Pairwise comparisons among isolates within each lineage show that the average number of segregating sites is greater for BdASIA-1 than for any other lineage by a factor of 3 (Fig. 1A and Table 1) and that nucleotide diversity (n; fig. S4) is greater by a factor of 2 to 4. Seven of our eight BdASIA-1 isolates were recently cultured from wild South Korean frogs, and the other came from the pet trade in Belgium; all
eight were acinal infections. These isolates show that the Korean peninsula is a global center of *B. dendrobatidis* diversity and that East Asia may contain the ancestral population of *B. dendrobatidis*, as suggested by Bataille et al. (17).

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

**Dating the emergence of BdGPL**

The broad range of previous estimates for the TMRCA of BdGPL spanning 26,000 years (11, 12) can be explained by two sources of inaccuracy: (i) unaccounted recombination and (ii) the application of unrealistic evolutionary rates. To address these, we first interrogated the 178,280-kbp mitochondrial genome (mtDNA), which has high copy number and low rates of recombination relative to the nuclear genome. To resolve the structure of the mtDNA genome, we resorted to long-read sequencing using a MinION device (Oxford Nanopore Technologies, Cambridge, UK), which allowed us to describe this molecule’s unusual configuration; *B. dendrobatidis* carries three linear mitochondrial segments, each having inverted repeats at the termini with conserved mitochondrial genes spread over two of the segments (fig. S7). Additionally, we sought regions of the autosomal genome with low rates of recombination to obtain an independent estimate of the TMRCA of BdGPL.

Detection of crossover events in the *B. dendrobatidis* autosomal genome (18) using a subset of the isolates in this study revealed a large (1.66 Mbp) region of Supercontig_1.2 in BdGPL that exhibits several features that identified it as a recombination “coldspot”: (i) a continuous region of reduced Tajima’s D (Fig. 2F), (ii) sustained high values of population differentiation as measured by the fixation index (F~ST~) when compared with all other lineages (Fig. 3A), (iii) a continuous region of reduced nucleotide diversity (pi; fig. S4), and (iv) shared loss of heterozygosity (fig. S8).

We expanded sampling to infer the temporal range of pathogen introductions using a broad panel of isolates with known date of isolation (*n* = 184, ranging from 1998 to 2016) and whole-genome RNA baiting to obtain reads from preserved amphibians that had died of chytridiomycosis. To determine the extent to which the four main lineages of *B. dendrobatidis* contributed to those declines is unknown. This mixed dating method based on tip and node calibration yielded very similar estimates [TMRCA estimates of 1975 (95% HPD, 1939 to 1989) (fig. S9)], further strengthening our confidence in a recent date of emergence for BdGPL. An expansion of BdGPL in the 20th century coincides with the global expansion in amphibians traded for exotic pet, medical, and food purposes (27, 28). Within our phylogeny, we found representatives from all lineages among traded animals (figs. S10 to S14) and identified 10 events where traded amphibians were infected with non-enzootic isolates (Fig. 4). This finding demonstrates the ongoing failure of international biosecurity despite the listing of *B. dendrobatidis* by the World Organisation for Animal Health (OIE) in 2008.

**Hybridization between recontacting lineages of *B. dendrobatidis***

To determine the extent to which the four main lineages of *B. dendrobatidis* have undergone recent genetic exchange, we used the site-by-site-based approach implemented in STRUCTURE (29). Although most isolates could be assigned unambiguously to one of the four main lineages, we identified three hybrid genotypes (Fig. 3B), including one previously reported hybrid (isolate

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**Table 1. Comparison of common genetic diversity measures among *B. dendrobatidis* lineages**. Total segregating sites for each lineage include all segregating sites where genotype calls were made in at least half of the isolates. Average pairwise-segregating sites are the average numbers of sites with different genotypes between all pairs of isolates within a lineage. Total homozygous segregating sites include all sites within a lineage where there is at least one homozygous difference between isolates. Average pairwise-homozygous segregating sites are the average numbers of sites with different homozygous genotypes between all pairs of isolates within a lineage. Nucleotide diversity (π) is the mean of the per-site nucleotide diversity. Tajima’s D is reported as the mean over 1-kbp bins.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Number of isolates</th>
<th>Total segregating sites</th>
<th>Average pairwise segregating sites</th>
<th>Total homozygous segregating sites</th>
<th>Average pairwise-homozygous segregating sites</th>
<th>π</th>
<th>Tajima’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>BdASIA-1</td>
<td>8</td>
<td>327,996</td>
<td>142,437</td>
<td>108,353</td>
<td>21.716</td>
<td>0.0044</td>
<td>0.2540</td>
</tr>
<tr>
<td>BdASIA-2/BdBRAZIL</td>
<td>12</td>
<td>148,021</td>
<td>51,069</td>
<td>48,722</td>
<td>6.216</td>
<td>0.0018</td>
<td>0.9825</td>
</tr>
<tr>
<td>BdCAPE</td>
<td>24</td>
<td>146,466</td>
<td>38,881</td>
<td>53,884</td>
<td>4,977</td>
<td>0.0016</td>
<td>0.3143</td>
</tr>
<tr>
<td>BdGPL</td>
<td>187</td>
<td>127,770</td>
<td>26,546</td>
<td>68,493</td>
<td>3,101</td>
<td>0.0009</td>
<td>0.9792</td>
</tr>
</tbody>
</table>
CLFT024/2) (20), and discovered two newly identified hybrids of \textit{BdGPL} and \textit{BdCAPE} in South Africa. Furthermore, \textit{BdCH} (isolate 0739) appears to be a chimera of multiple lineages that may represent unsampled genomic diversity residing in East Asia, rather than true hybridization. These hybrid genomes demonstrate that \textit{B. dendrobatidis} is continuing to exchange haplotypes among lineages when they interact after continental invasions, generating novel genomic diversity. We analyzed isolate clustering using principal components analysis on a filtered subset of 3900 SNPs in linkage equilibrium, revealing an overall population structure that is consistent with our phylogenetic analyses (Fig. 3C). In addition, the putatively identified hybrid isolates of \textit{B. dendrobatidis} were shown to fall between main lineage clusters (Fig. 3C), further strengthening our hypothesis of haplotype exchange occurring during secondary contact between lineages.

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

Genotypic diversification of pathogens is commonly associated with diversification of traits associated with host exploitation (30) and is most commonly measured as the ability to infect a host and to cause disease post-infection. We tested for variation of these two phenotypic traits across four \textit{B. dendrobatidis} lineages by exposing larval and postmetamorphic common toads (\textit{Bufo bufo}). Larvae are highly susceptible to infection but do not die before metamorphosis, in contrast to postmetamorphic juveniles, which are susceptible to infection and fatal chytridiomycosis (31). In tadpoles, both \textit{BdGPL} and \textit{BdASIA-1} were significantly more infectious than \textit{BdCAPE} and \textit{BdCH} (fig. S15 and tables S3 and S4). In metamorphs, \textit{BdGPL} was significantly more infectious than the other treatments, relative to the control group, and was significantly more lethal in experimental challenge than the geographically more restricted \textit{BdCAPE}, \textit{BdASIA-1}, and \textit{BdCH} (Fig. 2G). We further tested for differences in virulence among lineages by using our global data set to examine whether chytridiomycosis was nonrandomly associated with \textit{B. dendrobatidis} lineage. We detected a significant difference ($P < 0.001$) in the

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**Fig. 2. Dating the emergence of \textit{BdGPL}**

(A) Maximum likelihood (ML) tree constructed from 1150 high-quality SNPs found within the 178-kbp mitochondrial genome. (B) Linear regression of root-to-tip distance against year of isolation for \textit{BdGPL} isolates in mitochondrial DNA phylogeny in (A), showing a significant temporal trend ($F = 14.35$, $P = 0.00024$). (C) ML tree constructed from a 1.66-Mbp region of low recombination in Supercontig_1.2. Two \textit{BdGPL} isolates, \textit{BdBE3} and \textit{MGB}, fall on long branches away from the rest of the \textit{BdGPL} isolates (see inset zoom) as a result of introgression from another lineage (\textit{BdCAPE}; see Fig. 3B) and were excluded from the dating analysis. (D) Linear regression of root-to-tip distance against year of isolation for \textit{BdGPL} isolates from phylogeny in (C), with a significant temporal trend ($F = 15.92$, $P = 0.0001$). (E) Top: \textit{BdGPL} and outgroup \textit{BdCH}, with the 95% HPD estimates for MRCA for \textit{BdGPL} from mtDNA dating (blue) and nuclear DNA dating (red). Bottom: Full posterior distributions from tip-dating models for mtDNA (blue) and partial nuclear DNA (red) genomes. Solid vertical lines are limits of the 95% HPD. Dashed vertical lines denote the maximal density of the posterior distributions. (F) Sliding 10-kb, nonoverlapping window estimates of Tajima's $D$ for each of the main \textit{B. dendrobatidis} lineages. The region highlighted in red is the low-recombination segment of Supercontig_1.2. (G) Survival curves for \textit{Bufo bufo} metamorphs for different \textit{B. dendrobatidis} treatment groups: \textit{BdASIA-1} (blue), \textit{BdCAPE} (orange), \textit{BdCH} (yellow), \textit{BdGPL} (green), and control (gray). Confidence intervals are shown for \textit{BdGPL} and \textit{BdASIA-1}, showing no overlap by the end of the experiment. Instances of mortalities in each treatment group are plotted along the x axis, with points scaled by number of mortalities at each interval (day).
though S16 and table S5). These data suggest that al-
P < 0.05). However, we did not detect a signifi-
cant difference between

sponsible for lethal amphibian disease and pop-
pulations declines (33). Some conditions, other lineages can also be re-
covered because of low sample sizes), and post hoc tests
indicated significant excess in virulence in both

BdGPL and BdCAPE lineages relative to the com-
bined BdASIA-1 and BdASIA-2/BdBRAZIL (all
P < 0.05). However, we did not detect a signifi-
cant difference between BdGPL and BdCAPE (fig.
S16 and table S5). These data suggest that al-
though BdGPL is highly virulent, population-level outcomes are also context-dependent (32); under some conditions, other lineages can also be re-
sponsible for lethal amphibian disease and pop-
ulation declines (33).

Historical and contemporary implications of panzootic
chytridiomycosis

Our results point to endemism of B. dendrobatidis
in Asia, out of which multiple panzootic lineages
have emerged. These emergent diasporas include

the virulent and highly transmissible BdGPL,
which spread during the early 20th century via
a yet unknown route to infect close to 700 am-
phibian species out of ~1300 thus far tested (34).
With more than 7800 amphibian species cur-
rently described, the number of affected species
is likely to rise. The international trade in amphib-
ians has undoubtedly contributed directly to vector-
ing this pathogen worldwide (Fig. 4) (35, 36),
and within our phylogeny we identified many highly
supported (≥90% bootstrap support) clades on
short branches that linked isolates collected from
wild amphibian populations across different con-
tinents (Fig. 4 and figs. S10 to S14). However, the
role of globalized trade in passively contributing
to the spread of this disease cannot be ruled out.
It is likely no coincidence that our estimated dates
for the emergence of BdGPL span the globaliza-
tion “big bang”—the rapid proliferation in inter-
continental trade, capital, and technology that
started in the 1820s (37). The recent invasion of
Madagascar by Asian common toads hidden
within mining equipment (38) demonstrates the
capacity for amphibians to escape detection at
borders and exemplifies how the unintended an-
thropogenic dispersal of amphibians has also
likely contributed to the worldwide spread of
pathogenic chytrids.

The hyperdiverse hotspot identified in Korea
likely represents a fraction of the Batrachochytrium
genetic diversity in Asia, and further sampling
across this region is urgently needed because the
substantial global trade in Asian amphibians (39)
presents a risk of seeding future outbreak lin-
eages. Unique ribosomal DNA haplotypes of B.
dendrobatidis have been detected in native am-
phibian species in India (40, 41), Japan (16),
and China (42). Although caution should be observed
when drawing conclusions about lineages based
on short sequence alignments (fig. S3), other en-
demic lineages probably remain undetected within
Asia. It is noteworthy that the northern European
countryside is witnessing the emergence of B.
salamandrivorans, which also has its origin in
Asia. The emergence of *B. salamandrivoras* is linked to the amphibian pet trade (43), and the broad expansion of virulence factors that are found in the genomes of these two pathogens is testament to the evolutionary innovation that has occurred in these *Batrachochytrium* fungi (23).

Our findings show that the global trade in amphibians continues to be associated with the translocation of chytrid lineages with panzootic potential. Ultimately, our work confirms that panzootics of emerging fungal diseases in amphibians are caused by ancient patterns of pathogen phylogeography being redefined as largely unrestricted global trade moves pathogens into new regions, infecting new hosts and igniting disease outbreaks. Within this context, the continued strengthening of transcontinental biosecurity is critical to the survival of amphibian species in the wild (44).

**REFERENCES AND NOTES**

We thank J. Rhodes for the provision of flow cells and reagents for MinION sequencing, the staff at Oxford Nanopore Technologies for admission to the MinION Early Access Programme, and three anonymous reviewers for constructive comments and suggestions. **Funding:** S.J.O., T.W.J.G., L.Br., A.Lo., A.A.C., D.S.S., E.A.C., C.M., J.B., D.M.A., F.C., and M.C.F. were supported through NERC (standard grant NE/K014455/1). S.J.O. acknowledges a Microsoft Azure for Research Sponsorship (subscription ID: ab7c6d95-49ef-4a83-910a-ef7f603c708b).

**Acknowledgments**

DNA sequencing was carried out in the NBAF GenePool (grant number FT100100375, DP120100811). A.A.C. was supported by a Royal Society Wolfson Research Merit award. J.H., A.La., and S.M. were funded by the Swedish Research Council Formas (grant no. 2013-1389/26445-20). C.W. was funded by the National Research Foundation, South Africa. T.Y.J. and T.S.J. acknowledge NSF grant DEB-1601259. W.E.H. was funded by the NSERC Strategic and Discovery grant programs. **Author contributions:** All authors contributed ideas, data, and editorial advice. S.J.O., A.R., R.A.F., K.A.M., B.B., and M.C.F. conducted analyses. G.M.R., T.W.J.G., and M.C.F. wrote the paper with input from all authors. **Competing interests:** K.A.M. sits on an expert panel at the European Food Safety Authority addressing the risks of importation and spread of the salamander chytrid Batrachochytrium salamandrivorans, a species of fungus that is the closest known relative to the pathogen addressed in this manuscript. **Data and materials availability:** Sequences have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). All sequences are available from NCBI BioProject accession PRJNA413876 (www.ncbi.nlm.nih.gov/bioproject/PRJNA413876). The supplementary materials contain additional data. Phylogenetic trees are available from TreeBASE, project accession URL http://purl.org/phylo/treebase/ph2Publish/study/TB2:S22286. A browsable version of the phylogeny and metadata in Fig. 1B is accessible at https://micreareact.org/project/GlobalBD.

**SUPPLEMENTARY MATERIALS**

www.sciencemag.org/content/360/6389/621/suppl/DC1

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14 October 2017; accepted 29 March 2018

10.1126/science.aar1985
Recent Asian origin of chytrid fungi causing global amphibian declines


Science 360 (6389), 621-627.
DOI: 10.1126/science.aar1965

Panzootic chytrid fungus out of Asia

Species in the fungal genus Batrachochytrium are responsible for severe declines in the populations of amphibians globally. The sources of these pathogens have been uncertain. O’Hanlon et al. used genomics on a panel of more than 200 isolates to trace the source of the frog pathogen B. dendrobatidis to a hyperdiverse hotspot in the Korean peninsula (see the Perspective by Lips). Over the past century, the trade in amphibian species has accelerated, and now all lineages of B. dendrobatidis occur in traded amphibians; the fungus has become ubiquitous and is diversifying rapidly.

Science, this issue p. 621; see also p. 604