Title: Host Species is Linked to Pathogen Genotype for the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*)

Authors: Allison Q. Byrne[1*], Anthony W. Waddle[2,3], Veronica Saenz[4], Michel Ohmer[4,5], Jef R. Jaeger[3], Corinne L. Richards-Zawacki[4], Jamie Voyles[6], Erica Bree Rosenblum[1]

Affiliations:

1. Department of Environmental Science, Policy, and Management, University of California Berkeley, Berkeley, CA, USA
2. One Health Research Group, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Victoria, Australia
3. School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV, USA
4. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA
5. Department of Biology, University of Mississippi, University, MS, USA
6. Department of Biology, University of Nevada Reno, Reno, NV, USA

*Corresponding author

Email: allie128@berkeley.edu (AQB)

Abstract

Host-pathogen specificity can arise from certain selective environments mediated by both the host and pathogen. Therefore, understanding the degree to which host species identity is correlated with pathogen genotype can help reveal historical host-pathogen dynamics. One animal disease of particular concern is chytridiomycosis, typically caused by the global panzootic lineage of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd), termed the Bd-GPL. This pathogen lineage has caused devastating declines in amphibian communities around the world. However, the origin of Bd-GPL and the fine-scale transmission dynamics of this lineage have remained a mystery. This is especially the case in North America where Bd-GPL is widespread, but disease outbreaks occur sporadically. Herein, we use Bd genetic data collected throughout the United States from amphibian skin swab and cultured isolate samples to investigate Bd genetic patterns. We highlight two case studies in Pennsylvania and Nevada where Bd-GPL genotypes are strongly correlated with host species identity. Specifically, in some localities bullfrogs (*Rana catesbeiana*) are infected with Bd-GPL lineages that are distinct from those infecting other sympatric amphibian species. Overall, we reveal a previously unknown association of Bd genotype with host species and identify the eastern United States as a *Bd* diversity hotspot and potential ancestral population for Bd-GPL.

38 **Introduction**

39 When host-pathogen specificity exists within a system it can serve to reveal historical
40 disease dynamics and has consequences for the evolution of virulence. Theoretical work predicts
41 host-pathogen specificity will evolve, as a pathogen consistently exposed to a particular host
42 would be subject to stronger selection from that host and therefore evolve faster than a generalist
43 pathogen (1). However, the high frequency of multi-host pathogens in nature implies that
44 interacting factors present in complex natural systems may tip the scales in favor of generalist
45 pathogens (2). When host-specificity is high, it is predicted that host-pathogen feedback is
46 predicted to favor selection towards decreased pathogen virulence because an increase in
47 virulence would be disadvantageous for the preferred host (3). Similarly, low levels of specificity
48 favor pathogens with high virulence and often lead to the competitive exclusion of other less fit
49 pathogen strains (3). Therefore, understanding the degree to which host-pathogen specificity
50 exists in a system can shed light on the underlying evolutionary processes and disease dynamics
51 at play.

52 Advances in genetic monitoring tools have allowed us to investigate fine scale disease
53 dynamics in natural systems and uncover previously unknown host-pathogen genetic
54 associations. The utility of DNA sequence data in understanding pathogen evolution and genetic
55 diversity has been evidenced by genetic monitoring efforts during the Covid-19 pandemic (e.g.,
56 (4). By applying technologies used in human pathogen systems to wildlife diseases we can
57 investigate the epidemiology of diseases that are of conservation concern. Of particular interest is
58 the amphibian chytrid fungus [*Batrachochytrium dendrobatidis* (Bd)] (5) because of its
59 association with amphibian die offs and extinctions in many disparate areas of the world (6–10).
60 For Bd, there are at least five distinct genetic lineages, but the Global Panzootic Lineage (Bd-

61 GPL) has been associated with almost all disease outbreaks around the world (11–13). Recent
62 DNA sequencing of Bd samples collected in North America has confirmed previous reports of
63 two distinct sub-lineages within the Bd-GPL, termed "Bd-GPL1" and "Bd-GPL2" (14–16).
64 While it has become increasingly clear that there is deep genetic structure within the Bd-GPL,
65 and that both Bd-GPL1 and Bd-GPL2 often co-occur, what remains unclear is how these patterns
66 arose and are maintained across fine spatial scales.

67 A growing number of studies have shown that Bd, and specifically the Bd-GPL, has
68 patterns of genetic diversity that are linked to geography, but none have tested the hypothesis
69 that there is a link between Bd genetics and host species remains untested (13,15,16). In this
70 study, we investigated the relationship between host species and Bd genotype in two distinct
71 amphibian communities within the United States. One community was sampled in northwestern
72 Pennsylvania near the Pymatuning Lab of Ecology (PA). Sampling in this temperate region took
73 place at eight sites (including permanent and ephemeral ponds and one forested stream site). The
74 second amphibian community was sampled in southern Nevada (NV) within the eastern Mojave
75 Desert in an area characterized by permanent water bodies separated by larger distances. While
76 most amphibian species encountered in this study were found in only one of these regions, the
77 American bullfrog [*Rana catesbeiana* (Shaw 1802), hereafter "bullfrogs"] occurred across both
78 regions in either its native (PA) or introduced (NV) range. By comparing the relationship
79 between Bd genotype and host species in these two localities we not only reveal more about the
80 evolutionary history of Bd in the United States (US), but can also begin to understand the
81 repeatability of these patterns across systems and the role certain amphibian species may have in
82 shaping Bd genetic diversity.

83 **Materials and Methods**

84 *Sample Collection in Northwestern Pennsylvania and Southern Nevada*

85 We collected Bd samples in PA between 2017–2019 and in NV between 2016–2018. In
86 both areas, we captured frogs by hand using clean gloves. In PA we also used nets with
87 recommended field hygiene protocols followed (17). We kept captured amphibians individually
88 in clean plastic bags during processing. To collect skin cell samples, we swabbed frogs in PA 5
89 times each on dorsal, ventral, right, and left sides, and feet with a sterile swab. In NV, we
90 swabbed frogs 10 times on each ventral side and 5 times on each foot (18). We initially stored all
91 swabs in tubes on ice and promptly transferred them to a freezer at -20 °C until processing. We
92 extracted DNA from swabs in PA using the Qiagen DNEasy Blood and Tissue kit
93 (Qiagen,Valencia, USA), and from NV using PrepMan Ultra (19), following both manufacturer's
94 protocols.

95 To generate pure cultures of Bd from the relict leopard frog (*Rana onca*) we used a non-
96 lethal method in the field (20). We excised a small piece of webbing tissue (1 mm^2) that was
97 cleaned and embedded into TGhL agar plates containing a cocktail of antibiotics (kanamycin,
98 ciprofloxacin, streptomycin, and penicillin) (21). For the other species in NV from which
99 cultures were derived, we brought the animals into the laboratory and humanely euthanized them
100 prior to excising 1 mm^2 pieces of skin tissue from the ventral abdomen, legs, and rear feet. Each
101 tissue sample was then wiped through and embedded into a TGhL agar plate containing
102 antibiotics. We established pure cultures by transferring Bd growth to H-broth liquid medium.
103 We used a modified phenol-chloroform extraction to isolate DNA from the Bd cultures (22).

104 *DNA Sequencing and Preprocessing*

105 We genotyped Bd from PA and NV using a custom genotyping assay (23). For the PA
106 samples, we initially sequenced 115 skin swabs and for the NV samples, we initially sequenced

107 28 pure isolates and 36 skin swabs. After downstream filtering for missing data (see below) our
108 final dataset included 71 samples from PA, and 52 samples from NV. For some of our analyses
109 we also included previously published Bd sequence data from samples collected from around the
110 world (13).

111 We used the Fluidigm Access Array platform to perform microfluidic multiplex PCR on
112 191 regions of the Bd genome and one diagnostic locus for the closely related *Batrachochytrium*
113 *salamandrivorans* (24). Each target locus is 150-200 base pairs long, with the Bd targets
114 distributed across the Bd nuclear and mitochondrial genomes (23). Extracted DNA from swab
115 samples was cleaned and concentrated using an isopropanol precipitation. Isolate samples were
116 diluted to a concentration of 3ng/µl using PCR-grade water. All samples were preamplified in
117 two separate PCR reactions, each containing 96 primer pairs at a final concentration of 500nM.
118 For each preamplification PCR reaction we used the FastStart High Fidelity PCR System
119 (Roche) at the following concentrations: 1x FastStart High Fidelity Reaction Buffer with $MgCl_2$,
120 4.5mM $MgCl_2$, 5% DMSO, 200µM PCR Grade Nucleotide Mix, 0.1 U/µl FastStart High Fidelity
121 Enzyme Blend. We added 1µl of cleaned DNA to each preamplification reaction and used the
122 following thermocycling profile: 95˚C for 10 min, 15 cycles of 95˚C for 15 sec and 60˚C for 4
123 min. Preamplified products were treated with 4µl of 1:2 diluted ExoSAP-it (Affymetrix Inc.) and
124 incubated for 30 min at 30˚C, then 15 min at 80˚C. Treated products were diluted 1:5 in PCR-
125 grade water. The diluted products from each of the two preamplification reactions were
126 combined in equal proportions and used for downstream amplification and sequencing. Each
127 preamplified sample was then loaded onto a Juno™ LP 192.24 IFC (Fluidigm, Inc.) for library
128 preparation. This flow cell performs microfluidic multiplex PCR to amplify all samples using 24
129 separate pools each containing 8 primer pairs. Barcoded samples were then pooled and

130 sequenced on an Illumina MiSeq lane using the 300bp paired-end kits at the University of Idaho
131 IBEST Genomics Resources Core.

132 *Sequence Data Analysis*

133 We pre-processed all sequencing data as described in (23) and generated consensus
134 sequences for all variants present for each sample at each locus using IUPAC ambiguity codes
135 for multiple alleles. We filtered reads by selecting sequence variants that were present in at least
136 5 reads and represented at least 5% of the total number of reads for that sample/locus using
137 dbcamplicons (https://github.com/msettles/dbcAmplicons).

138 We used a gene-tree to species-tree approach to construct phylogenies separately for the
139 PA and NV sequence datasets. These trees allow us to explore the relationship of the Bd
140 collected for this study to previously-published Bd samples representing all known Bd lineages
141 (N=13; 13,25). First, we trimmed our consensus sequence dataset to eliminate loci that had more
142 than 50% missing data for either the NV or PA datasets, resulting in 174 loci. Next, for each
143 dataset we individually aligned all loci using the MUSCLE package (26) in R (v.3.4.3), checked
144 the alignments for errors in Geneious (v.10.2.3) (27), and used the RAxML plugin (28) in
145 Geneious to search for the best scoring maximum likelihood tree for each locus using rapid
146 bootstrapping (100 replicates). We then collapsed all branches in all trees with <10 bootstrap
147 support and used Astral-III to generate a consensus tree (29). Astral generates an unrooted
148 species tree given a set of unrooted gene trees.

149 To further explore the genetic variation of Bd collected at our sites, we generated a
150 dataset of single nucleotide polymorphisms (SNPs). First, we aligned all raw sample reads (PA
151 and NV) and reads from 26 previously published Bd-GPL reference samples (13,25) using bwa
152 mem (30) to a reference fasta containing target sequences for each amplicon extracted from

153 reference genome JEL423 (Broad Institute). We then used Freebayes (v.1.1.0) to call variants
154 based on haplotypes (31) stipulating that variants only be called on the 174 amplicons that
155 passed the 50% missing data cutoff described above. Variants were then filtered using vcftools
156 (v.4.2) (32) to only include variants with a minor allele frequency > 0.01, quality > 30, less than
157 10% missing data, and minimum depth of 5. The final variant set included 129 binary SNPs with
158 3.8% missing data across all samples.

159 We then imported our SNPs into R using the vcfR package (v.1.12.0) (33) and converted
160 the variants to a genlight object. To determine the optimal number of clusters in our data we used
161 a Discriminant Analysis of Principal Components (DAPC) (34). First, we ran the find.clusters
162 function from the R package adegenet (v.2.1.1) with a maximum number of clusters = 10 (35).
163 This function implemented the clustering procedure used in DAPC by running successive K-
164 means with clusters from K = 1 to K = 10 after transforming the data using a principal
165 component analysis (PCA). For each model BIC was computed as a measure of statistical
166 goodness of fit. The best K was chosen as the value for K after which increasing K no longer
167 leads to an appreciable improvement of fit (measured as substantial decrease in BIC) (34). We
168 then ran the DAPC analysis specifying the optimal number of clusters. To determine the optimal
169 number of PCs to use for this analysis we ran the adegenet function optim.a.score which
170 calculates the alpha score as (P_t-P_r), where P_t is probability of reassignment to the true cluster
171 and P_r is the probability of reassignment to randomly permuted clusters. We used a minimum of
172 2 PCs in the DAPC calculations. Finally, we conducted a PCA using the glPCA function in
173 adegenet and plotted the PCA using the DAPC clusters to visually group samples together.
174 Samples with posterior probability of inclusion in one cluster < 0.99 were labeled "unassigned."
175 We plotted the first two PCs which together explain 68.2% of the variation in our data. We then

176 computed a PCA separately for each locality to show within locality diversity across species and
177 sites.
178 To evaluate the relative contribution of sampling site, host species, and individual
179 variation, we ran an analysis of molecular variance (AMOVA) (36) using our SNP data. This
180 analysis was conducted separately for the PA and NV datasets and determined the relative
181 influence of within individual variation, host species identity, and individual sampling locality on
182 Bd genetic structure. We ran this analysis using the poppr package in R (37) and only used SNPs
183 with <5% missing data. This resulted in 40 high quality SNPS for PA and 126 high quality SNPs
184 for NV. We then tested for statistical significance using the "randtest" function in the package
185 ade4, randomly permuting our data 999 times.
186 To evaluate the frequency of mixed infections on individuals, we searched our set of
187 amplicon sequences for those that had diagnostic alleles that discriminate between the Bd-GPL1
188 and Bd-GPL2 lineages. First, we searched the amplicon sequences from the set of 26 pure
189 culture, reference samples. We identified amplicons that had at least 1 SNP that distinguished
190 GPL1 samples from GPL2 (as defined by both the DAPC and phylogenetic analyses described
191 above). The GPL1 Bd isolates in this reference set were CJB4, JEL238, JEL267, JEL359,
192 MexMkt, MLA1, SRS812, TST75. The GPL-2 Bd isolates were Campana, CLB5-2, CJB7,
193 EV001, JAM81, JEL271, JEL275, JEL310, JEL427, JEL429, JEL433, JEL627, Lb_Aber,
194 NBRC106979, Pc_CN_JLV, and RioMaria. These samples were all sequenced from pure
195 cultures grown in the lab, and previously-published whole genome data indicates these are pure
196 Bd cultures representing a single lineage (25,38). From these samples we identified 24 loci that
197 occur on five separate Bd chromosomes that have specific Bd-GPL1 or Bd-GPL2 alleles. For
198 the diagnostic alleles on chromosome 1, heterozygotes (those having both alleles present) are

199 characteristic of Bd-GPL2 isolates (see Figure S3). We then manually scored each sample from
200 PA and NV at each as having either the Bd-GPL1, Bd-GPL2, or both alleles present
201 (heterozygote).

202 **Results**

203 We found a repeated pattern of Bd genetic structure that is most closely associated with
204 amphibian host species in both PA and NV. The DAPC analysis of the combined PA and NV Bd
205 genetic data show that two clusters (K=2) was the most likely grouping for our data (as inferred
206 from ΔBIC, Figure S1B). In the combined PCA, we see a clear division of Bd genotypes into
207 two clusters, with the presence of intermediate "unassigned" samples (Figure 1C). By evaluating
208 each site separately, we see that PA harbors much more genetic variability within samples
209 (accounting for all "unassigned" Bd genotypes, Figure 1). We confirmed that "unassigned"
210 samples were not simply the product of missing data by showing that each genotype group (Bd-
211 GPL1, Bd-GPL2, and unassigned) had the same distribution of missing data (Figure S1 A,C).
212

213 *Figure 1:* Map of sampling localities for Bd in southern Nevada (A) and northwestern
214 Pennsylvania (B). Individual sampling localities are numbered and represented with a pie chart
215 that shows the proportion of samples belonging to each Bd-GPL sub-lineage as determined using
216 the DAPC clustering analysis. Unassigned samples (in grey) are those with a posterior
217 probability of assignment to either group < 0.99. Pie charts are scaled in size according to the
218 number of samples collected at that site. (C) PCA calculated from 129 SNPS using the combined
219 dataset of southern Nevada (N=52, squares), northwestern Pennsylvania (N=71, circles), and
220 global reference samples (N=26, triangles). Symbols and ellipses colored as in maps above.
221

Further phylogenetic analyses incorporating previously published Bd isolates shows that that all Bd samples sequenced for this study belong to the Bd-GPL lineage (Figures 2,3) and that the two clusters identified in our dataset correspond to the previously published Bd-GPL subclades Bd-GPL1 and Bd-GPL2 (14,39). The difference between Bd-GPL1 and Bd-GPL2 samples is shown clearly in the combined PCA (Figure 1C), where PC1 (which explains 58.4% of the variation in our genetic data) separates samples into each of these subclades. In NV, 20 samples were assigned to Bd-GPL1 and 32 samples were assigned to Bd-GPL2 (Figure 2). In PA, 27 samples were assigned to Bd-GPL1, 23 samples were assigned to GPL-2, and 21 samples we unassigned (Figure 3).

Figure 2: (A) Phylogenetic tree and (B) PCA for 52 Bd samples collected in southern Nevada. Colors represent the locality where the Bd was sampled (numbered as in Figure 2) and symbols represent host species (as in Figure 2). Tree is calculated using Astral-III and includes and additional 13 reference samples representing all known clades of Bd. Nodes are labeled with posterior probability and those with posterior < 0.7 have been collapsed.

Figure 3: (A) Phylogenetic tree and (B) PCA for 71 Bd samples collected in northwestern Pennsylvania. Colors represent the locality where the Bd was sampled (numbered as in Figure 2) and symbols represent host species (as in Figure 2). Tree is calculated using Astral-III and includes and additional 13 reference samples representing all known clades of Bd. Nodes are labeled with posterior probability and those with posterior < 0.7 have been collapsed.

244 The factors that may explain Bd genetic structure at each site were determined using an
245 AMOVA. We found that most of the variation in Bd genotypes (64.3% in PA, 58.4% in NV) can
246 be explained by variation between host species, rather than within an individual (34.7% in PA,
247 8.8% in NV) or between sampling sites (1.0% in PA, 32.8% in NV). Comparisons of variation
248 within individuals and variation between host species were statistically significant using
249 permutation tests to randomly permute samples between groups (999 times, $p < 0.001$ for each
250 test, Figure S2).
251 We can evaluate each individual sample for signatures of coinfections or the presence of
252 a hybrid lineage (i.e., the presence of both Bd-GPL1 and Bd-GPL2) by looking at Bd-GPL1/2
253 diagnostic alleles. The allelic patterns show that the "unassigned" samples in PA appear to either
254 be from a mixed infection or infection with a hybrid lineage – that is, they show a high frequency
255 of heterozygosity and/or the presence of alleles from different Bd-GPL subclades at genomically
256 proximate loci. In PA, one site (PA Site 1) accounts for most of the unassigned samples (67%,
257 14/21); additionally, 71% (15/21) of unassigned Bd samples were collected from green frogs
258 (*Rana clamitans*). Furthermore, all 18 samples collected from bullfrogs in PA were assigned to
259 GPL-1, despite being collected at five different localities, each with multiple Bd-GPL genotypes
260 present (Figure 3, Figure 4).
261
262 *Figure 4*: Alleles for PA Site 1 (left top), PA Site 3 (left middle), PA Site 4 (left bottom), NV
263 Site 1 (right top), and NV Site 4 (right bottom). Each row represents an individual sample, and
264 each numbered column represents a nuclear locus that has an informative GPL1/GPL2 allele
265 (N=24). Loci are organized according to position along the Bd genome, with the yellow dashed
266 indicating a change to a new chromosome and the chromosome number labeled along the top.

267 Samples are grouped by host species ("species" column) and labeled with the DAPC assigned
268 lineage ("lineage" column). A red box indicates a Bd-GPL2 allele for that sample at that locus, a
269 blue box represented the Bd-GPL1 allele, a gray box indicates both alleles are present, and a
270 white box indicates missing data. Representative sequences for each allele are included in the
271 supplemental materials.
272
273 In NV we see very little Bd-GPL1/Bd-GPL2 mixing within samples and a strong
274 association of the Bd-GPL1 lineage with bullfrogs at one site (NV Site 4), and *R. onca* at another
275 (NV Site 3). While *R. onca* was the only species caught at NV Site 3, bullfrogs were caught
276 alongside Pacific tree frogs (*Pseudacris regilla*) at NV Site 4 and these two species were host to
277 distinct Bd-GPL genotypes with virtually no allele mixing (Figure 4). At another site (NV Site
278 1), however, bullfrogs were host to Bd-GPL2 as were all other amphibian hosts at that locality.
279 Additionally, the Bd-GPL2 lineage found at NV Site 1 shows a pattern of high heterozygosity at
280 diagnostic alleles found on Bd Chromosome 1, a pattern similar to the reference Bd-GPL2
281 isolates JEL627, JEL271, Lb_Aber, CJB7, and JAM81 (Figure S3).
282 **Discussion**
283 Herein, we show a clear, repeated pattern of Bd genetic diversity associated with host
284 species at single geographic sites. While previous studies have shown some broad geographic
285 patterning in Bd genetic structure (15,16), this is the first study to link fine-scale Bd genetic
286 variation to host species identity. Furthermore, this pattern is repeated across individual sites in
287 PA, and in two separate regions of the US. Remarkably, the same association of Bd-GPL1 with
288 bullfrog hosts was seen in both PA and NV. Below, we explore how this pattern may have arisen

289 in each region, discuss what this may mean for the origin of the Bd-GPL, and explore the
290 potential importance of genetically diverse Bd infections.

291 *How did host-pathogen specificity arise in each region?*

292 Processes occurring at different timescales could produce the patterns of Bd genotype and
293 host species association observed in each region. One possibility is that long-term coevolutionary
294 relationships between Bd and its host species likely triggered *in situ* evolution and subsequent
295 pathogen divergence over time in the ancestral Bd-GPL population. This process could have
296 been mediated by many factors including host immune systems, skin microbial communities, and
297 the environment. Furthermore, mutational changes in the pathogen that have consequences for
298 infectiousness across different hosts could contribute to evolution towards host specificity (40).
299 Bd can suppress host immune responses and some susceptible species show an over-activation of
300 the immune system that may worsen disease outcomes (41). At the same time, immunization
301 against Bd has been successful in some species, indicating the relationship between adaptive
302 immunity and Bd varies between hosts (42). Amphibian species identity is often the strongest
303 predictor of skin microbial community composition (43,44) and these microbes can be associated
304 with Bd disease outcomes (7,45,46), providing another possible mechanism for facilitating the
305 evolution of host-pathogen specificity. Finally, Bd isolates in the lab can show rapid
306 evolutionary change over short time periods (47,48). Therefore, differences between Bd
307 genotypes in the way they interact with amphibian skin microbes or immune systems, in
308 combination with Bd's evolutionary lability, could allow for the formation of the host-pathogen
309 genetic correlations that we detected.

310 In PA, we observed a ubiquitous pattern of association between Bd-GPL1 and bullfrogs
311 in all individual sites. Moreover, not a single bullfrog sample (N=18) in the PA region was

312 infected with Bd-GPL2, or included in the "unassigned" group. The finding that host species was
313 more important than geography in explaining Bd genetic patterns in PA has not been
314 documented in other regions and may indicate that Bd-GPL has been present for a long time in
315 this region and has coevolved with native amphibians, including bullfrogs. We can see additional
316 evidence for this hypothesis at individual sites; at PA Site 4 bullfrogs and the closely related
317 green frog (*Rana clamitans*) were both preferentially infected with Bd-GPL1, while the more
318 distantly related northern leopard frog (*Rana pipiens*) was infected with Bd-GPL2 (49). This
319 pattern follows other studies showing that specialized pathogens are more likely to infect closely
320 related host species (50). Overall, when comparing patterns of Bd genotypes and host species in
321 PA to those documented in other areas of the world, we see evidence for a coevolutionary
322 mechanism shaping Bd-GPL genetic diversity.

323 An alternative possibility that could produce patterns of host species-Bd genotype
324 correlations is a recent introduction of a novel Bd-GPL lineage into an amphibian community.
325 Existing differences in the ability of the novel Bd-GPL lineage to infect different hosts could
326 maintain the stratification of Bd genotypes along species boundaries. In NV the association
327 between Bd genotypes and host species is not as consistent as in PA, indicating the coexistence
328 of different Bd genotypes in the NV region may be a more recent phenomenon. For example, at
329 NV Site 4 Bd genotypes were strictly partitioned by species, with Bd-GPL1 only present on
330 bullfrogs; however, at NV Site 1 all individuals sampled across species were infected with the
331 same Bd-GPL2 genotype, including bullfrogs. Bullfrogs are not native to Nevada and have been
332 present since at least 1933, likely introduced during fish stocking or from the food trade (51).
333 Therefore, in this region, bullfrog colonization may have provided for a more recent introduction
334 of Bd-GPL1. At NV Site 1 the Bd-GPL2 present may have outcompeted other Bd lineages that

335 could have been present when bullfrogs were first introduced. Supporting this hypothesis, one
336 study found that bullfrogs farmed in Idaho and used for an infection experiment initially
337 harbored cryptic, low intensity infections of Bd-GPL1 that were eventually outcompeted by an
338 inoculation with a high dose of a Bd-GPL2 isolate (52). What host and environmental factors
339 allow for the replacement of one Bd lineage by another is not altogether clear but is a key
340 question going forward to better understand the role of potential disease vectors such the
341 bullfrog.

342 *What does our data tell us about the origin of Bd-GPL?*

343 The implied coexistence of Bd and amphibians at evolutionary time scales in PA could
344 indicate that the ancestral population of Bd-GPL existed in this part of the world. This possibility
345 has been previously proposed, following reports of higher diversity in Bd samples collected from
346 bullfrog populations (53,54), and evidence that bullfrogs may have facilitated Bd spread from
347 east to west in the US (55). Adding to evidence that North America may be the ancestral source
348 of Bd-GPL, a recent study found as much genetic variation in the Bd-GPL collected from the
349 Sierra Nevada as was present in a large global sample of Bd-GPL genotypes (15). In other
350 regions, such as Panama and Mexico, genetic diversity of Bd-GPL is either spatially structured
351 across the region (Mexico) or genetically homogenous (Panama), the latter indicating a more
352 recent introduction and spread (15,16). Studies using swabs collected from museum specimens
353 have found Bd in Illinois as early as 1888 (56) and in bullfrogs collected in California in 1928
354 (57). Altogether, a growing body of evidence suggests North America, and perhaps the eastern
355 US (within the native range of the bullfrog) may have been the ancestral population for Bd-GPL.

356 *What can the "unassigned" samples tell us about Bd dynamics?*

357

358 One key question raised from our data concerns the Bd genotypes that were "unassigned"
359 and lie in between the Bd-GPL1 and Bd-GPL2 genotypes in our PCA. These samples, all of
360 which were from swabs collected in PA, show high levels of heterozygosity across the entire
361 panel of diagnostic alleles. There are two possible explanations for the occurrence of a highly
362 heterozygous, intermediate Bd genotype. The first is that these individuals were coinfected with
363 both Bd-GPL1 and Bd-GPL2. The second is that they were infected with a hybrid lineage
364 resulting from recombination between Bd-GPL1 and Bd-GPL2. Indeed, if we look at the
365 reference Bd isolates in Figure S3 we see that some Bd-GPL2 pure isolates are heterozygous at
366 certain alleles, indicating that high heterozygosity could result from a single infection. Herein,
367 we are unable to confidently say whether these ambiguous samples represent a mixed infection
368 or an infection with a single hybrid lineage.
369 Our data show not only the coexistence of multiple Bd-GPL lineages at a single site, but
370 also highlights areas where Bd lineage mixing on individuals is rare (in NV, and PA Sites 3 and
371 4), or common (PA Site 1). The most common frog species caught at PA Site 1 was the green
372 frog (*R. clamitans*), in fact this species accounts for 71% of all unassigned samples in this study.
373 At this point it is unclear whether there is something unique about PA Site 1, or *R. clamitans*,
374 that may allow for such consistent lineage mixing or the potential presence of hybrid lineages.
375 To our knowledge no chytridiomycosis outbreaks have been reported in *R. clamitans*, and
376 individuals from the same site in PA showed no increase in stress hormones when
377 experimentally infected with Bd (58). Probably, this species is a tolerant carrier of Bd, like the
378 closely related bullfrog. This host-pathogen relationship is worth further exploration as the
379 potential for Bd hybridization could lead to novel pathogen phenotypes.

380 The frequency and circumstances under which recombination occurs in Bd is still
381 unclear. There is, however, plenty of genetic evidence that recombination has occurred between
382 divergent Bd lineages (11,14,59). A likely mechanism for genomic recombination in Bd is
383 parasexual reproduction – where diploid spores fuse to form tetraploid progeny, after which
384 chromosomes are lost as the organism returns back to a diploid state (60). This process has been
385 documented in the human fungal pathogen *Candida albicans*, although the use of this
386 reproductive mode seems to be restricted in nature, possibly to preserve complex gene sets
387 needed to exploit hosts (61). Parasexual reproduction often results in aneuploidy, which is a
388 common phenomenon in Bd (25,62). Furthermore, elevated chromosomal copy number has been
389 linked to increased virulence in Bd (62), and hybrids of Bd have been more deadly than parent
390 lineages when paired with certain host species (63). Indeed, it was originally proposed that Bd-
391 GPL could have originated from recombination between closely related Bd lineages (12), but this
392 hypothesis has been a topic of much debate (64). Most researchers on the subject, however,
393 would agree that precautions should be taken to reduce unintended spread of Bd to limit the
394 possibility of recombination that could produce more deadly lineages.

395 **Conclusions**

396 Herein we document an association between Bd-GPL genotypes and host species in two
397 regions of the US. We believe the patterns seen in PA may be the result of a long-term
398 coevolutionary relationship between Bd-GPL1 and bullfrogs (and perhaps other closely related
399 species such as the green frog). We observed that the association of Bd-GPL1 with bullfrogs is
400 consistent in NV where bullfrogs have been more recently introduced, and that the patterns in
401 this region are consistent with more recent introductions of novel Bd lineages. We also found
402 evidence of either mixed Bd infections or a potential hybrid lineage that was common at a single

403 site in PA and on green frogs across all PA sites. Given the high genetic diversity and evidence
404 of evolutionary relationships between Bd-GPL lineages and particular host species, we posit that
405 North America may be the ancestral origin of Bd-GPL. Overall, this study presents evidence that
406 Bd-GPL has a long history of coexistence with amphibians in the US, reveals more about the
407 relationship of the bullfrog to Bd, and points to a possible cradle of Bd-GPL genetic diversity in
408 the eastern US.

409 **Acknowledgements**

410 We thank Frank van Breukelen, Mark Slaughter, and Jonathan Smith for their assistance with
411 funding acquisition and management for the Nevada study. We thank the following people for
412 their assistance with field and laboratory work in Nevada: Rebeca Rivera, Daniel Villanueva,
413 Yesenia Vasquez, David Miller, Joshua Levy, Kevin Guadalupe, Marlai Sai, Ghazal Rezaei,
414 Alexandra Zmuda, Jessica Hill, Shaylene Scarlett, and Alexa Krauss. We thank the following
415 people for assistance with field and laboratory work in Pennsylvania: Laura Brannelly, Karie
416 Altman, Talisin Hammond, Jeffery Bednark, Paradyse Blackwood, Robert Campion, Lauren
417 Chronister, Jordan Coscia, Nina Dunnell, Conor Harrington, Ally Hartman, Marcus Hough,
418 Jennifer Kassimer, Stephanie Kubik, Sadie Parker, Natalie Popielski, Phoebe Reuben, Ayla
419 Ross, Samantha Shablin, Samantha Skerlec, Trina Wantman, Lydia Zimmerman, and Jakub
420 Zegar, Aimee Danley.

421 **Reproducibility Statement**

422 All data used for this study and R code used for the analyses are available on github:
423 (https://github.com/allie128/Bd_NV_PA).

424 **References**

425 1. Kawecki TJ. Red Queen Meets Santa Rosalia: Arms Races and the Evolution of Host

426 Specialization in Organisms with Parasitic Lifestyles. Am Nat [Internet]. 1998 Oct
427 1;152(4):635–51. Available from: https://doi.org/10.1086/286195

428 2. Rigaud T, Perrot-Minnot M-J, Brown MJF. Parasite and host assemblages: embracing the
429 reality will improve our knowledge of parasite transmission and virulence. Proc R Soc B
430 Biol Sci [Internet]. 2010 Dec 22;277(1701):3693–702. Available from:
431 https://doi.org/10.1098/rspb.2010.1163

432 3. Kirchner JW, Roy BA. Evolutionary implications of host–pathogen specificity: fitness
433 consequences of pathogen virulence traits. Evol Ecol Res. 2002;4(1):27–48.

434 4. Deng X, Gu W, Federman S, du Plessis L, Pybus OG, Faria NR, et al. Genomic
435 surveillance reveals multiple introductions of SARS-CoV-2 into Northern California.
436 Science (80-) [Internet]. 2020 Jul 31;369(6503):582 LP – 587. Available from:
437 http://science.sciencemag.org/content/369/6503/582.abstract

438 5. Longcore JE, Pessier AP, Nichols DK. Batrachochytrium Dendrobatidis gen. et sp. nov., a
439 Chytrid Pathogenic to Amphibians. Mycologia [Internet]. 1999;91(2):219–27. Available
440 from: http://www.jstor.org/stable/3761366

441 6. Lips KR, Diffendorfer J, Mendelson III JR, Sears MW. Riding the Wave: Reconciling the
442 Roles of Disease and Climate Change in Amphibian Declines. PLoS Biol [Internet]. 2008
443 Mar 25;6(3):e72. Available from: http://dx.doi.org/10.1371%2Fjournal.pbio.0060072

444 7. Woodhams DC, Voyles J, Lips KR, Carey C, Rollins-Smith LA. PREDICTED DISEASE
445 SUSCEPTIBILITY IN A PANAMANIAN AMPHIBIAN ASSEMBLAGE BASED ON
446 SKIN PEPTIDE DEFENSES. J Wildl Dis [Internet]. 2006 Apr 1;42(2):207–18. Available
447 from: https://doi.org/10.7589/0090-3558-42.2.207

448 8. Ron SR, Merino A. Amphibian declines in Ecuador: overview and first report of
449 chytridiomycosis from South America. Froglog. 2000;42:2–3.

450 9. Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ. Investigating the Population-Level
451 effects of Chytridiomycosis: An Emerging Infectious Disease of Amphibians. Ecology
452 [Internet]. 2005 Dec 1;86(12):3149–59. Available from: http://dx.doi.org/10.1890/04-
453 1428

454 10. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al.
455 Chytridiomycosis causes amphibian mortality associated with population declines in the
456 rain forests of Australia and Central America. Proc Natl Acad Sci [Internet]. 1998 Jul
457 21;95(15):9031–6. Available from: http://www.pnas.org/content/95/15/9031.abstract

458 11. O'Hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Bataille A, et al. Recent Asian
459 origin of chytrid fungi causing global amphibian declines. Science (80-) [Internet]. 2018
460 May 11;360(6389):621 LP – 627. Available from:
461 http://science.sciencemag.org/content/360/6389/621.abstract

462 12. Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, et al. Multiple
463 emergences of genetically diverse amphibian-infecting chytrids include a globalized
464 hypervirulent recombinant lineage. Proc Natl Acad Sci [Internet]. 2011 Nov
465 15;108(46):18732–6. Available from: http://www.pnas.org/content/108/46/18732.abstract

466 13. Byrne AQ, Vredenburg VT, Martel A, Pasmans F, Bell RC, Blackburn DC, et al. Cryptic
467 diversity of a widespread global pathogen reveals expanded threats to amphibian
468 conservation. Proc Natl Acad Sci [Internet]. 2019 Oct 8;116(41):20382 LP – 20387.
469 Available from: http://www.pnas.org/content/116/41/20382.abstract

470 14. Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira CA, Lee M, et al. Novel,
471 panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the
472 bullfrog trade. Mol Ecol [Internet]. 2012;21(21):5162–77. Available from:
473 http://www.ncbi.nlm.nih.gov/pubmed/22857789

474 15. Rothstein AP, Byrne AQ, Knapp RA, Briggs CJ, Voyles J, Richards-Zawacki CL, et al.
475 Divergent regional evolutionary histories of a devastating global amphibian pathogen.
476 Proc R Soc B. 2021;288(1953):20210782.

477 16. Basanta MD, Byrne AQ, Rosenblum EB, Piovia-Scott J, Parra-Olea G. Early presence of
478 Batrachochytrium dendrobatidis in Mexico with a contemporary dominance of the global
479 panzootic lineage. Mol Ecol. 2021;30(2):424–37.

480 17. Phillott AD, Speare R, Hines HB, Skerratt LF, Meyer E, McDonald KR, et al. Minimising
481 exposure of amphibians to pathogens during field studies. Dis Aquat Organ. 2010;92(2–
482 3):175–85.

483 18. Jaeger JR, Waddle AW, Rivera R, Harrison DT, Ellison S, Forrest MJ, et al.
484 Batrachochytrium dendrobatidis and the Decline and Survival of the Relict Leopard Frog.
485 Ecohealth. 2017;14(2):285–95.

486 19. Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. Rapid quantitative detection of
487 chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-
488 time Taqman PCR assay. 2004;60:141–8. Available from:
489 https://dx.doi.org/10.3354/dao060141

490 20. Waddle AW, Levy JE, Rivera R, van Breukelen F, Nash M, Jaeger JR. Population-level
491 resistance to chytridiomycosis is life-stage dependent in an imperiled anuran. Ecohealth.

492 2019;16(4):701–11.

493 21. Waddle AW, Sai M, Levy JE, Rezaei G, van Breukelen F, Jaeger JR. Systematic approach
494 to isolating Batrachochytrium dendrobatidis. Dis Aquat Organ. 2018;127(3):243–7.

495 22. Zolan ME, Pukkila PJ. Inheritance of DNA methylation in Coprinus cinereus. Mol Cell
496 Biol [Internet]. 1986 Jan 1;6(1):195–200. Available from:
497 http://mcb.asm.org/content/6/1/195.abstract

498 23. Byrne AQ, Rothstein AP, Poorten TJ, Erens J, Settles ML, Rosenblum EB. Unlocking the
499 story in the swab: A new genotyping assay for the amphibian chytrid fungus
500 *Batrachochytrium dendrobatidis*. Mol Ecol Resour [Internet]. 2017 May 3 [cited 2017 Jul
501 21]; Available from: http://doi.wiley.com/10.1111/1755-0998.12675

502 24. Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, et al.
503 Batrachochytrium salamandrivorans sp. nov. causes lethal chytridiomycosis in
504 amphibians. Proc Natl Acad Sci [Internet]. 2013 Sep 17;110(38):15325–9. Available
505 from: http://www.pnas.org/content/110/38/15325.abstract

506 25. Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, et al. Complex
507 history of the amphibian-killing chytrid fungus revealed with genome resequencing data.
508 Proc Natl Acad Sci USA [Internet]. 2013;110(23):9385–90. Available from:
509 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3677446&tool=pmcentrez&re
510 ndertype=abstract

511 26. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high
512 throughput. Nucleic Acids Res [Internet]. 2004 Jan 19 [cited 2016 Sep 2];32(5):1792–7.
513 Available from: http://nar.oxfordjournals.org/cgi/content/long/32/5/1792

514 27. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious
515 Basic: An integrated and extendable desktop software platform for the organization and
516 analysis of sequence data. Bioinforma [Internet]. 2012 Jun 15;28(12):1647–9. Available
517 from: http://bioinformatics.oxfordjournals.org/content/28/12/1647.abstract

518 28. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
519 large phylogenies. Bioinformatics [Internet]. 2014 May 1;30(9):1312–3. Available from:
520 http://dx.doi.org/10.1093/bioinformatics/btu033

521 29. Zhang C, Rabiee M, Sayyari E, Mirarab S. ASTRAL-III: polynomial time species tree
522 reconstruction from partially resolved gene trees. BMC Bioinformatics [Internet].
523 2018;19(6):153. Available from: https://doi.org/10.1186/s12859-018-2129-y

524 30. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform.
525 Bioinformatics [Internet]. 2009 May 18;25(14):1754–60. Available from:
526 https://doi.org/10.1093/bioinformatics/btp324

527 31. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing.
528 arXiv Prepr arXiv12073907. 2012;

529 32. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant
530 call format and VCFtools. Bioinformatics [Internet]. 2011 Aug 1;27(15):2156–8.
531 Available from: https://doi.org/10.1093/bioinformatics/btr330

532 33. Knaus BJ, Grünwald NJ. vcfr: a package to manipulate and visualize variant call format
533 data in R. Mol Ecol Resour [Internet]. 2017 Jan 1;17(1):44–53. Available from:
534 https://doi.org/10.1111/1755-0998.12549

535 34. Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new
536 method for the analysis of genetically structured populations. BMC Genet [Internet].
537 2010;11(1):94. Available from: https://doi.org/10.1186/1471-2156-11-94

538 35. Jombart T. adegenet: a R package for the multivariate analysis of genetic markers.
539 Bioinformatics. 2008;24(11):1403–5.

540 36. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric
541 distances among DNA haplotypes: application to human mitochondrial DNA restriction
542 data. Genetics. 1992;131(2):479–91.

543 37. Kamvar ZN, Tabima JF, Grünwald NJ. Poppr: an R package for genetic analysis of
544 populations with clonal, partially clonal, and/or sexual reproduction. PeerJ. 2014;2:e281.

545 38. Voyles J, Woodhams DC, Saenz V, Byrne AQ, Perez R, Rios-Sotelo G, et al. Shifts in
546 disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation.
547 Science (80-) [Internet]. 2018 Mar 30;359(6383):1517 LP – 1519. Available from:
548 http://science.sciencemag.org/content/359/6383/1517.abstract

549 39. James TY, Toledo LF, Rödder D, da Silva Leite D, Belasen AM, Betancourt-Román CM,
550 et al. Disentangling host, pathogen, and environmental determinants of a recently emerged
551 wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research.
552 Ecol Evol [Internet]. 2015;n/a-n/a. Available from:
553 http://doi.wiley.com/10.1002/ece3.1672

554 40. Woolhouse MEJ, Taylor LH, Haydon DT. Population Biology of Multihost Pathogens.
555 Science (80-) [Internet]. 2001 May 11;292(5519):1109–12. Available from:
556 https://doi.org/10.1126/science.1059026

557 41. Ellison AR, Savage AE, DiRenzo G V, Langhammer P, Lips KR, Zamudio KR. Fighting a
558 Losing Battle: Vigorous Immune Response Countered by Pathogen Suppression of Host
559 Defenses in the Chytridiomycosis-Susceptible Frog Atelopus zeteki. G3 (Bethesda)
560 [Internet]. 2014;4(July):1275–89. Available from:
561 http://www.ncbi.nlm.nih.gov/pubmed/24841130

562 42. Waddle AW, Rivera R, Rice H, Keenan EC, Rezaei G, Levy JE, et al. Amphibian
563 resistance to chytridiomycosis increases following low-virulence chytrid fungal infection
564 or drug-mediated clearance. J Appl Ecol. 2021;1–12.

565 43. Kueneman JG, Parfrey LW, Woodhams DC, Archer HM, Knight R, McKenzie VJ. The
566 amphibian skin-associated microbiome across species, space and life history stages. Mol
567 Ecol [Internet]. 2014 Mar 1;23(6):1238–50. Available from:
568 https://doi.org/10.1111/mec.12510

569 44. McKenzie VJ, Bowers RM, Fierer N, Knight R, Lauber CL. Co-habiting amphibian
570 species harbor unique skin bacterial communities in wild populations. ISME J [Internet].
571 2012;6(3):588–96. Available from: https://doi.org/10.1038/ismej.2011.129

572 45. Lam BA, Walke JB, Vredenburg VT, Harris RN. Proportion of individuals with anti-
573 Batrachochytrium dendrobatidis skin bacteria is associated with population persistence in
574 the frog Rana muscosa. Biol Conserv [Internet]. 2010;143(2):529–31. Available from:
575 http://www.sciencedirect.com/science/article/pii/S0006320709004807

576 46. Piovia-Scott J, Rejmanek D, Woodhams DC, Worth SJ, Kenny H, McKenzie V, et al.
577 Greater Species Richness of Bacterial Skin Symbionts Better Suppresses the Amphibian
578 Fungal Pathogen Batrachochytrium Dendrobatidis. Microb Ecol [Internet].

579 2017;74(1):217–26. Available from: https://doi.org/10.1007/s00248-016-0916-4

580 47. Langhammer PF, Lips KR, Burrowes PA, Tunstall T, Palmer CM, Collins JP. A Fungal
581 Pathogen of Amphibians, Batrachochytrium dendrobatidis, Attenuates in Pathogenicity
582 with In Vitro Passages. PLoS One [Internet]. 2013 Oct 10;8(10):e77630. Available from:
583 http://dx.doi.org/10.1371%2Fjournal.pone.0077630

584 48. Refsnider JM, Poorten TJ, Langhammer PF, Burrowes PA, Rosenblum EB. Genomic
585 Correlates of Virulence Attenuation in the Deadly Amphibian Chytrid Fungus ,
586 Batrachochytrium dendrobatidis. 2015;5(November):2291–8. Available from:
587 https://doi.org/10.1534/g3.115.021808

588 49. Yuan Z-Y, Zhou W-W, Chen X, Poyarkov Nikolay A. J, Chen H-M, Jang-Liaw N-H, et
589 al. Spatiotemporal Diversification of the True Frogs (Genus Rana): A Historical
590 Framework for a Widely Studied Group of Model Organisms. Syst Biol [Internet]. 2016
591 Sep 1;65(5):824–42. Available from: http://dx.doi.org/10.1093/sysbio/syw055

592 50. Davies TJ, Pedersen AB. Phylogeny and geography predict pathogen community
593 similarity in wild primates and humans. Proc R Soc B Biol Sci [Internet]. 2008 Jul
594 22;275(1643):1695–701. Available from: https://doi.org/10.1098/rspb.2008.0284

595 51. Linsdale JM. Amphibians and Reptiles in Nevada. Proc Am Acad Arts Sci [Internet].
596 1940 Oct 22;73(8):197–257. Available from: http://www.jstor.org/stable/25130182

597 52. Byrne AQ, Poorten TJ, Voyles J, Willis CKR, Rosenblum EB. Opening the file drawer:
598 Unexpected insights from a chytrid infection experiment. PLoS One.
599 2018;13(5):e0196851.

600 53. James TY, Litvintseva AP, Vilgalys R, Morgan JAT, Taylor JW, Fisher MC, et al. Rapid
601 Global Expansion of the Fungal Disease Chytridiomycosis into Declining and Healthy
602 Amphibian Populations. PLOS Pathog [Internet]. 2009 May 29;5(5):e1000458. Available
603 from: https://doi.org/10.1371/journal.ppat.1000458

604 54. Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, et al. Amphibian
605 chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan.
606 Mol Ecol [Internet]. 2009 Dec [cited 2016 Aug 22];18(23):4757–74. Available from:
607 http://www.ncbi.nlm.nih.gov/pubmed/19840263

608 55. Yap TA, Koo MS, Ambrose RF, Vredenburg VT. Introduced bullfrog facilitates pathogen
609 invasion in the western United States. PLoS One [Internet]. 2018 Apr 16;13(4):e0188384.
610 Available from: https://doi.org/10.1371/journal.pone.0188384

611 56. Talley BL, Muletz CR, Vredenburg VT, Fleischer RC, Lips KR. A century of
612 Batrachochytrium dendrobatidis in Illinois amphibians (1888–1989). Biol Conserv
613 [Internet]. 2015;182(October):254–61. Available from:
614 http://linkinghub.elsevier.com/retrieve/pii/S0006320714004807

615 57. Huss M, Huntley L, Vredenburg V, Johns J, Green S. Prevalence of Batrachochytrium
616 dendrobatidis in 120 archived specimens of Lithobates catesbeianus (American bullfrog)
617 collected in California, 1924-2007. Ecohealth. 2013;10(4):339–43.

618 58. Hammond TT, Blackwood PE, Shablin SA, Richards-Zawacki CL. Relationships between
619 glucocorticoids and infection with Batrachochytrium dendrobatidis in three amphibian
620 species. Gen Comp Endocrinol [Internet]. 2020;285:113269. Available from:
621 https://www.sciencedirect.com/science/article/pii/S0016648019302953

622 59. Jenkinson TS, Betancourt Román CM, Lambertini C, Valencia-Aguilar A, Rodriguez D,
623 Nunes-de-Almeida CHL, et al. Amphibian-killing chytrid in Brazil comprises both locally
624 endemic and globally expanding populations. Mol Ecol [Internet]. 2016 Jul [cited 2016
625 Aug 26];25(13):2978–96. Available from:
626 http://www.ncbi.nlm.nih.gov/pubmed/26939017

627 60. Samarasinghe H, You M, Jenkinson TS, Xu J, James TY. Hybridization Facilitates
628 Adaptive Evolution in Two Major Fungal Pathogens. Vol. 11, Genes . 2020.

629 61. Nielsen K, Heitman JBT-A in G. Sex and Virulence of Human Pathogenic Fungi. In:
630 Fungal Genomics [Internet]. Academic Press; 2007. p. 143–73. Available from:
631 https://www.sciencedirect.com/science/article/pii/S006526600657004X

632 62. Farrer RA, Henk DA, Garner TWJ, Balloux F, Woodhams DC, Fisher MC. Chromosomal
633 copy number variation, selection and uneven rates of recombination reveal cryptic genome
634 diversity linked to pathogenicity. PLoS Genet. 2013;9(8):e1003703.

635 63. Greenspan SE, Lambertini C, Carvalho T, James TY, Toledo LF, Haddad CFB, et al.
636 Hybrids of amphibian chytrid show high virulence in native hosts. Sci Rep [Internet].
637 2018;8(1):9600. Available from: https://doi.org/10.1038/s41598-018-27828-w

638 64. Fisher MC, Garner TWJ. Chytrid fungi and global amphibian declines. Nat Rev Microbiol
639 [Internet]. 2020;18(6):332–43. Available from: https://doi.org/10.1038/s41579-020-0335-
640 x

641

642 **Supplementary Figure Legends**

Figure S1: (A) PCA as shown in Figure 1C colored by proportion missing data. (B) BIC chart for DAPC analysis showing K = 2 clusters as the optimal number of clusters (or "elbow" in the chart). (C) Density plot showing that unassigned, Bd-GPL1, and Bd-GPL2 samples all have the same distribution as samples with missing data.

Figure S2: Results from AMOVA testing the relative importance of variation within samples (top), variation between host species (middle), and variation between sites (bottom) in explaining Bd genetic variance for northwestern Pennsylvania (left) and southern Nevada (right). Percentage after each category reflects the relative proportion of variation explained by each category calculated from the components of covariance. Each histogram shows the results from randomly permuting the sample matrices 999 times using the rand.test function in the R package ade4. The black dot indicates the value obtained in this study with a red asterisk indicating statistical significance.

Figure S3: (A) Astral phylogenetic tree and (B) allele plot for 26 pure-isolate Bd-GPL reference samples used to identify the 24 Bd-GPL1 and Bd-GPL2 diagnostic alleles shown in Figures 6 and 7. Tree displayed as in Figures 3 and 4, allele plot displayed as in Figures 6 and 7. A frog icon indicates that isolate was collected from a bullfrog.

Figure 1

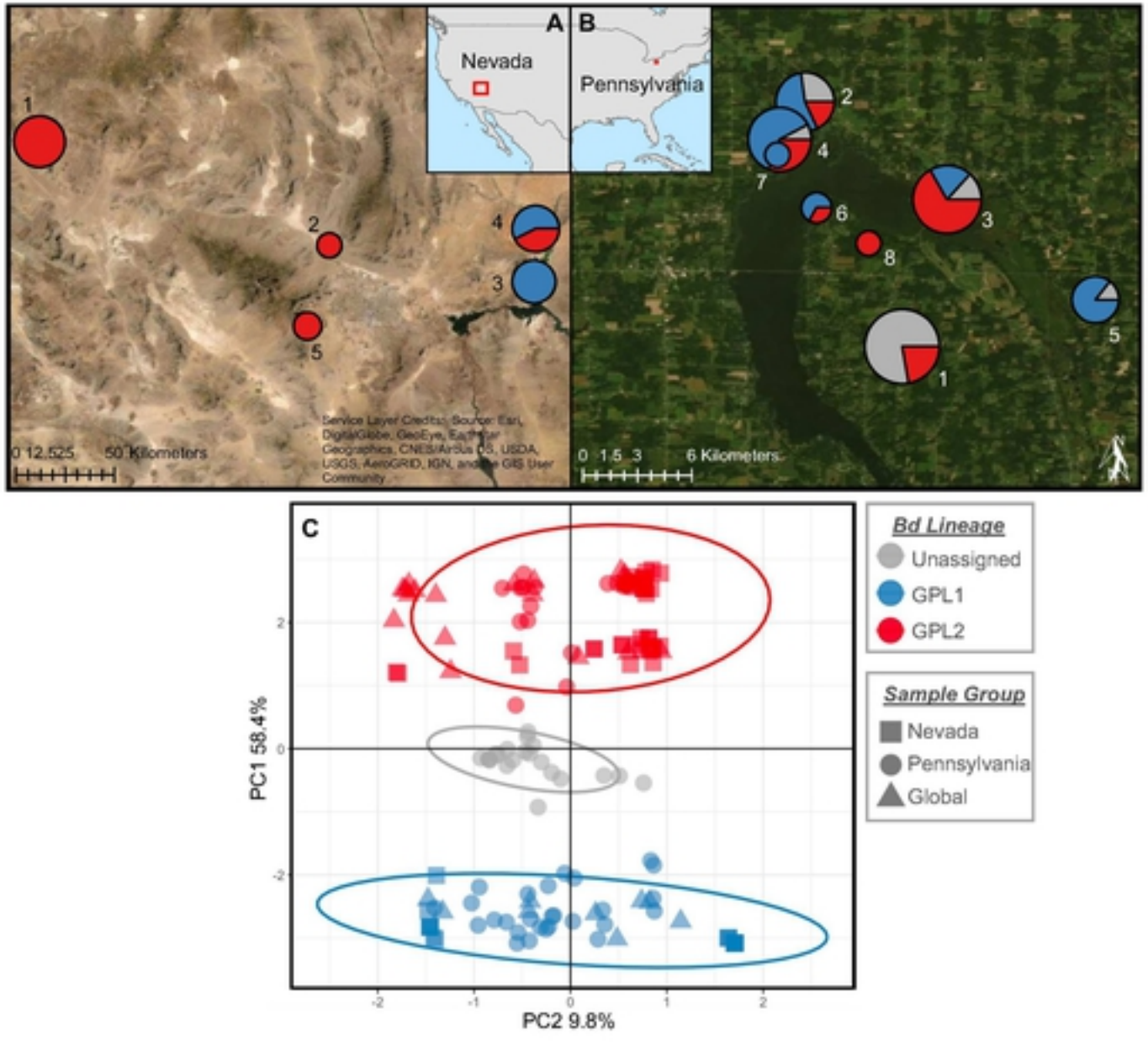

A
Nevada
B
Pennsylvania
1
2
3
4
5
0 12.525 50 Kilometers
0 1.5 3 6 Kilometers
C
PC1 58.4%
PC2 9.8%
Bd Lineage
Unassigned
GPL1
GPL2
Sample Group
Nevada
Pennsylvania
Global

Figure 2

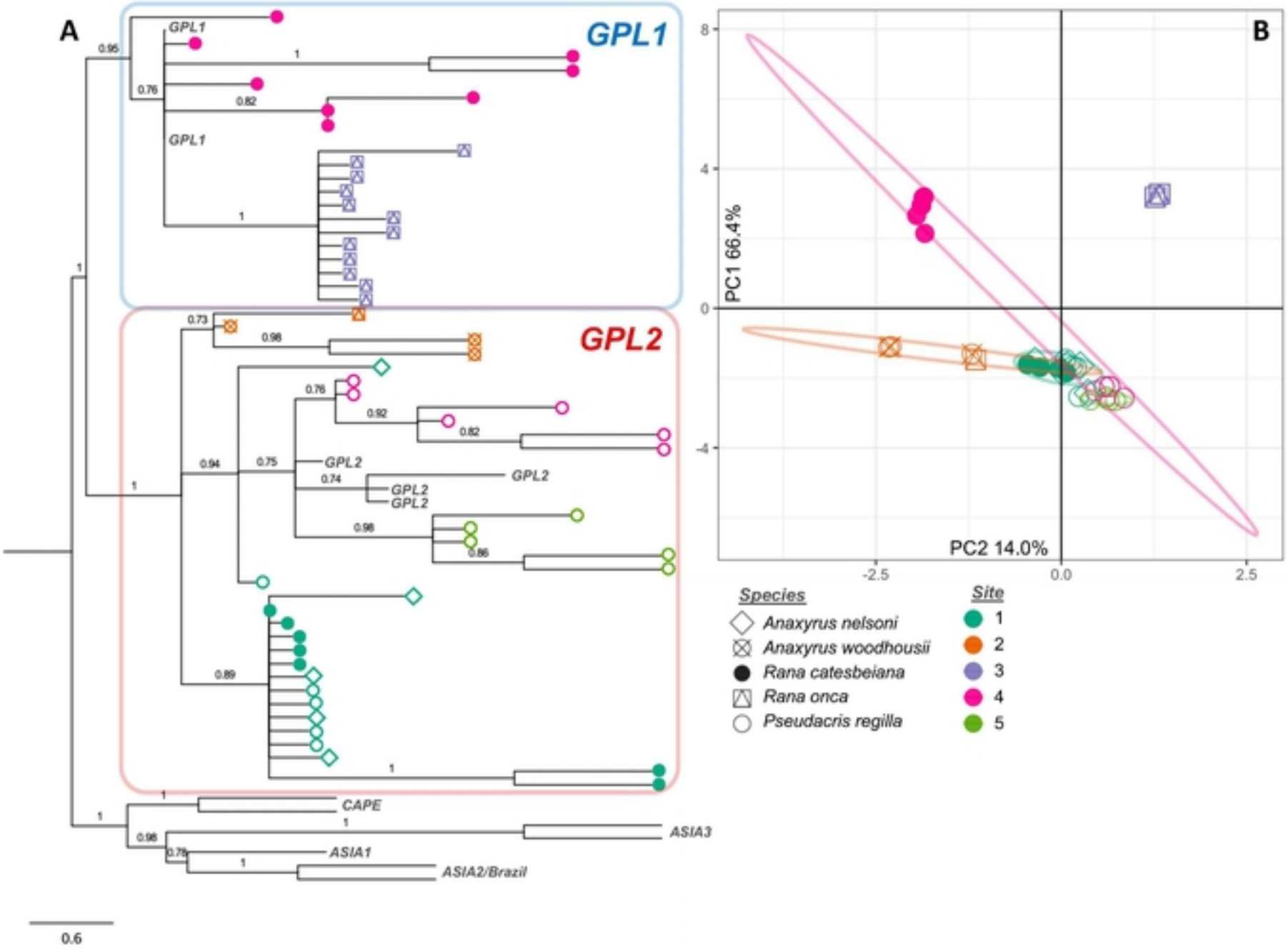

A
GPL1
GPL2
GPL1
GPL1
GPL2
GPL2
GPL2
GPL2
CAPE
ASIA3
ASIA1
ASIA2/Brazil
0.6
B
PC1 66.4%
PC2 14.0%
Species
Anaxyrus nelsoni
Anaxyrus woodhousii
Rana catesbeiana
Rana onca
Pseudacris regilla
Site
1
2
3
4
5

Figure 3

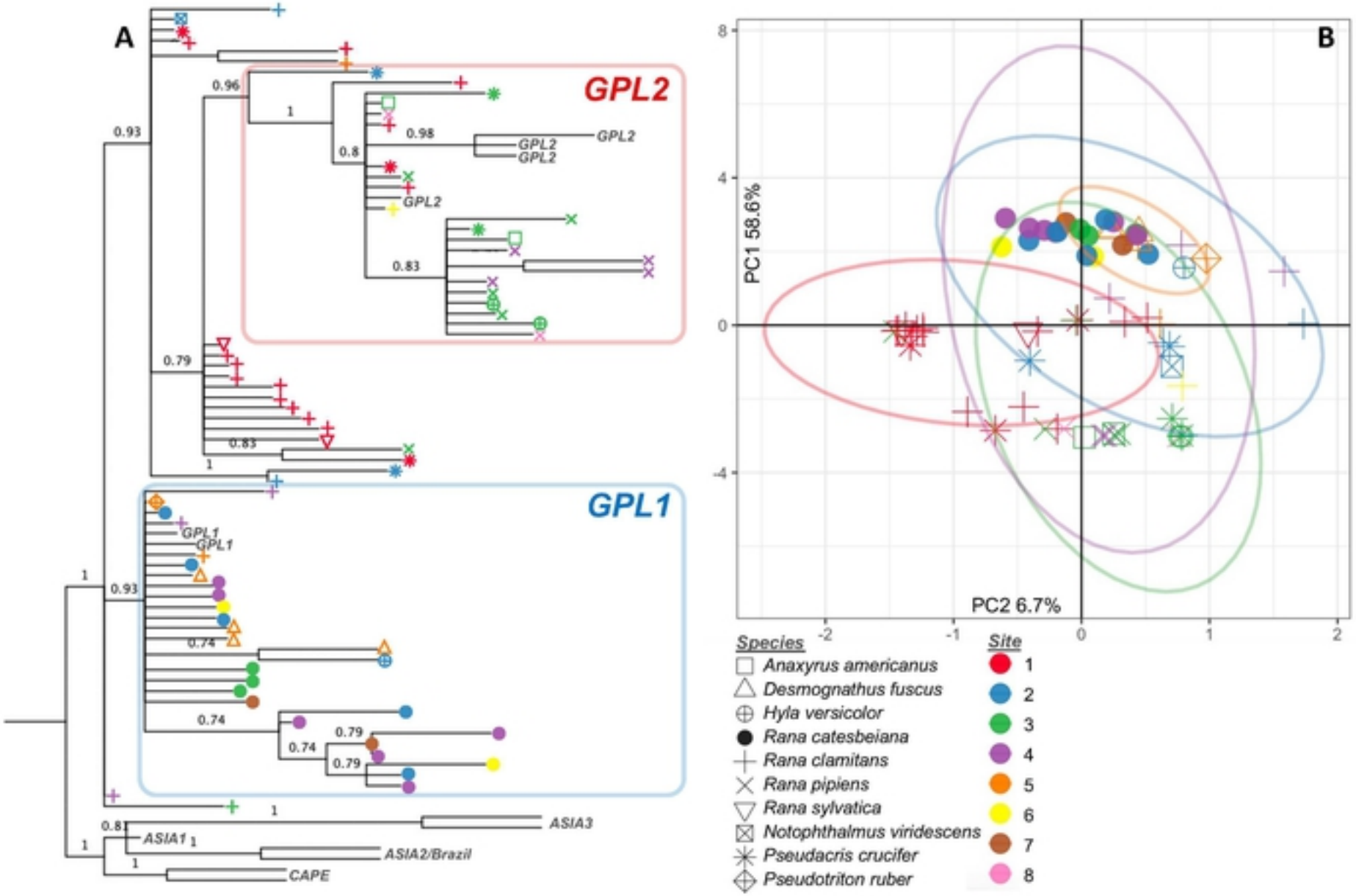

A
GPL2
GPL1
GPL2
GPL2
GPL2
GPL2
GPL1
GPL1
ASIA3
ASIA1
ASIA2/Brazil
CAPE
0.6
B
PC1 58.6%
PC2 6.7%
Species
Anaxyrus americanus
Desmognathus fuscus
Hyla versicolor
Rana catesbeiana
Rana clamitans
Rana pipiens
Rana sylvatica
Notophthalmus viridescens
Pseudacris crucifer
Pseudotriton ruber
Site
1
2
3
4
5
6
7
8

Figure 4

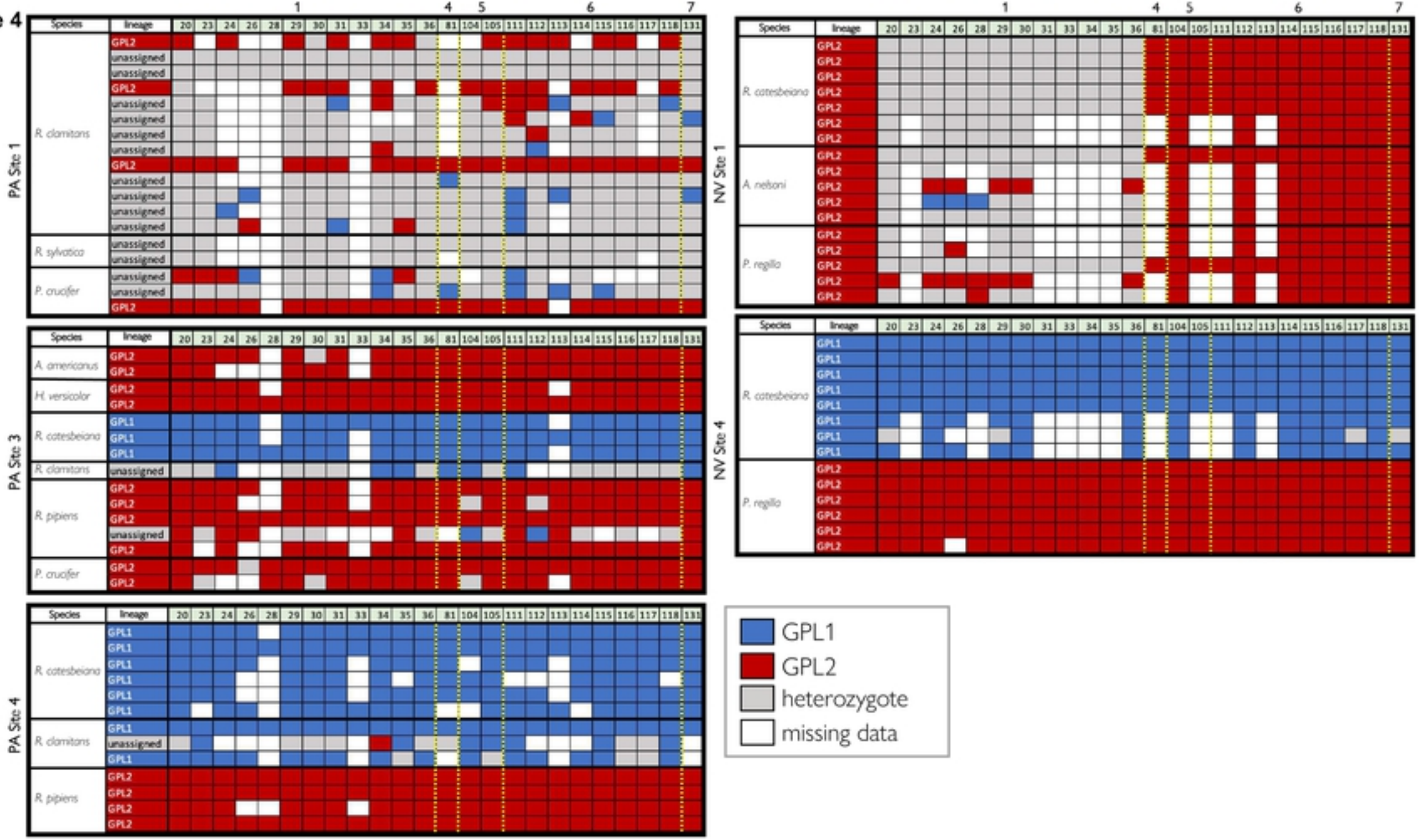

PA Site 1
PA Site 3
PA Site 4
NV Site 1
NV Site 4
Species
lineage
20 23 24 26 28 29 30 31 33 34 35 36 81 104 105 111 112 113 114 115 116 117 118 131
1 4 5 6 7
R. clamitans
R. sylvatica
P. crucifer
A. americanus
H. versicolor
R. catesbeiana
R. pipiens
A. nelsoni
P. regilla
GPL1
GPL2
unassigned
GPL1
GPL2
heterozygote
missing data

Figure S1

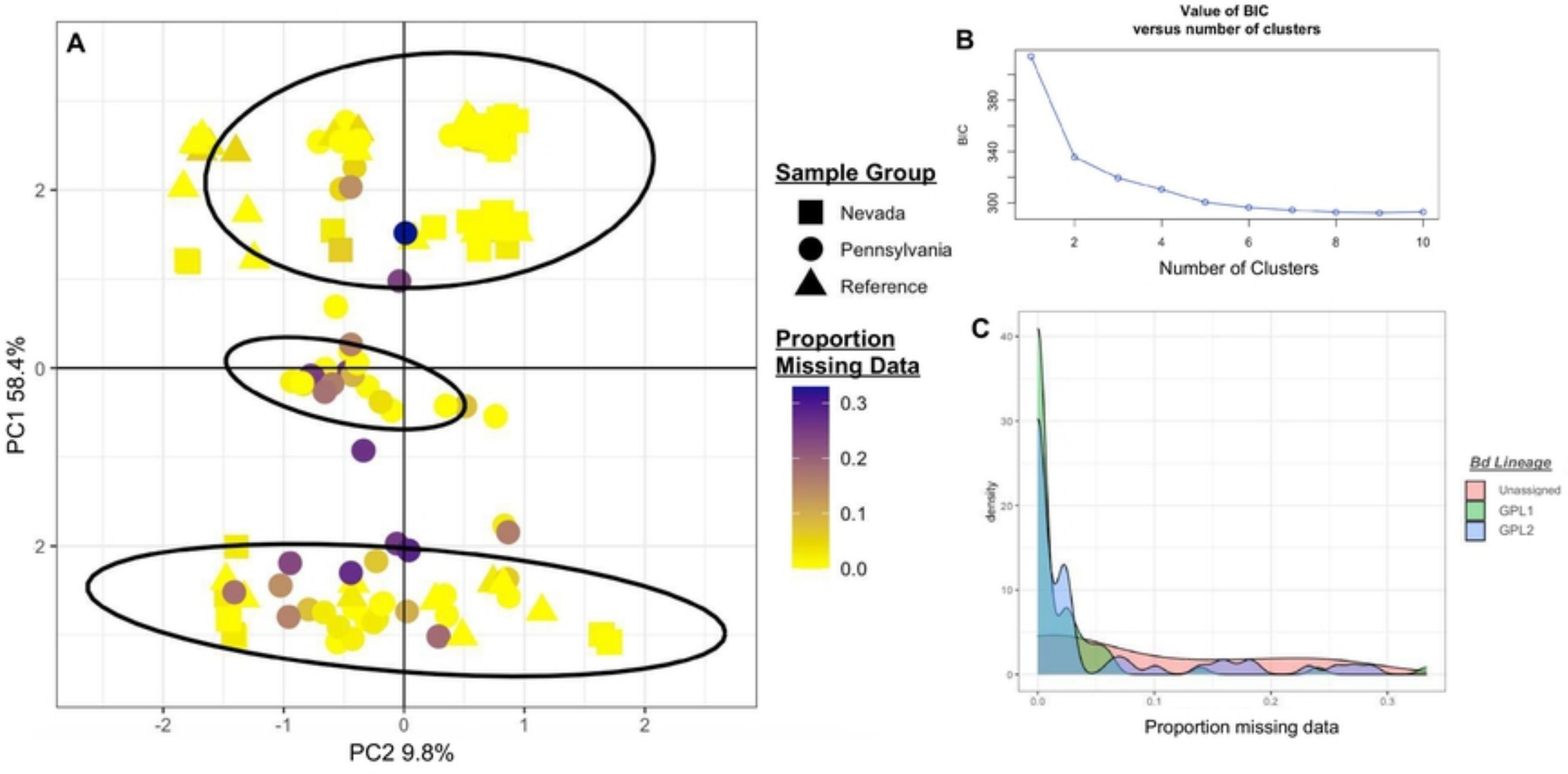

A
PC1 58.4%
PC2 9.8%
Sample Group
Nevada
Pennsylvania
Reference
Proportion Missing Data
0.3
0.2
0.1
0.0
B
Value of BIC versus number of clusters
BIC
Number of Clusters
C
density
Proportion missing data
Bd Lineage
Unassigned
GPL1
GPL2

Figure S2

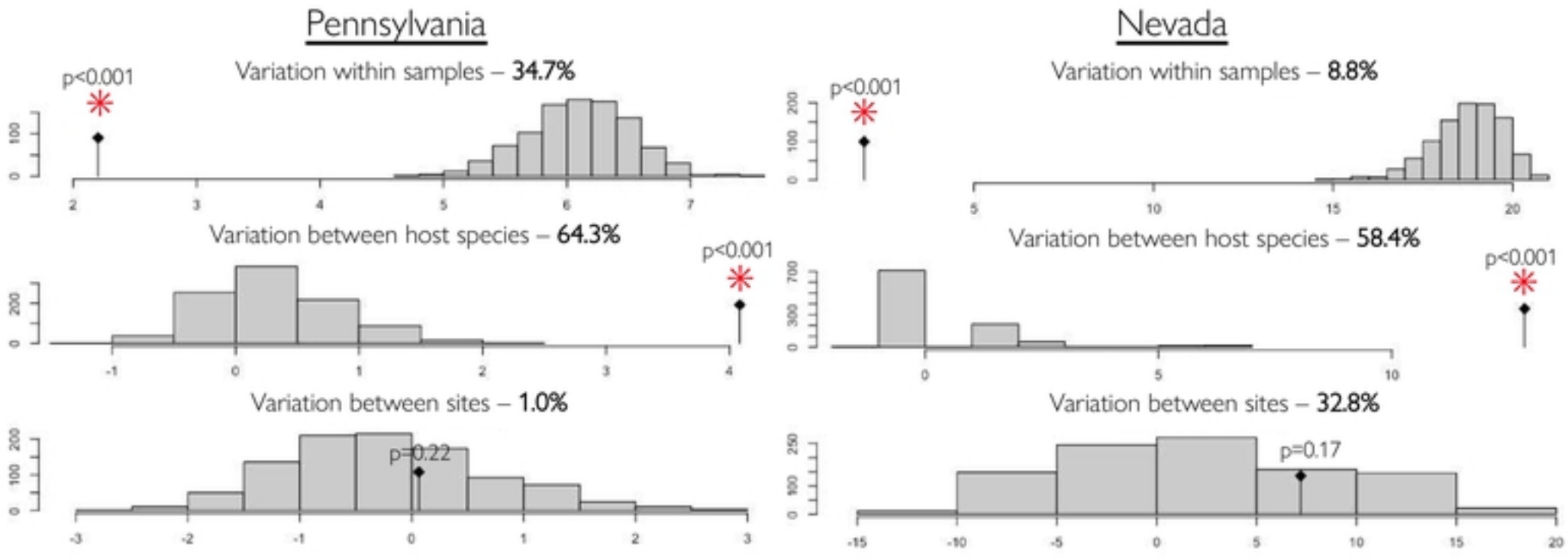
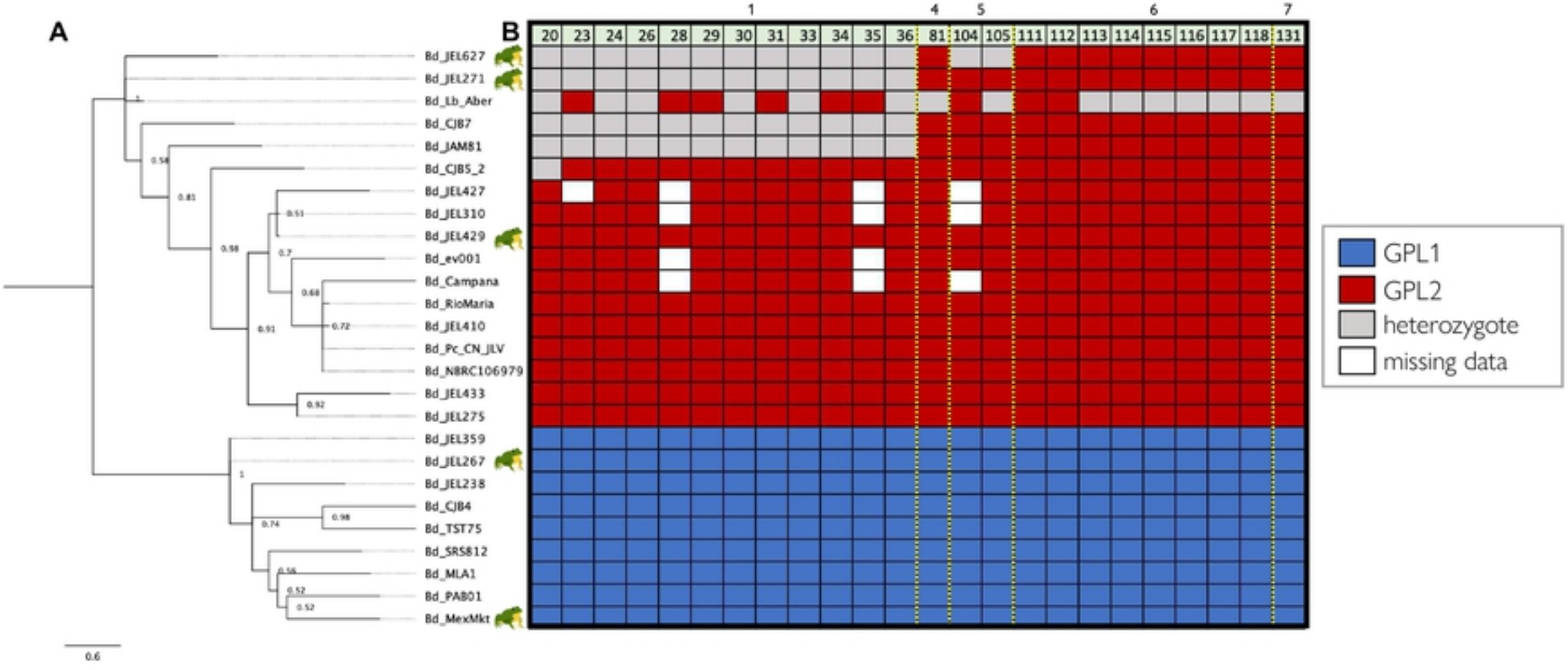

Pennsylvania
Variation within samples – 34.7%
p<0.001
Variation between host species – 64.3%
p<0.001
Variation between sites – 1.0%
p=0.22
Nevada
Variation within samples – 8.8%
p<0.001
Variation between host species – 58.4%
p<0.001
Variation between sites – 32.8%
p=0.17

Figure S3


A
B
Bd_JEL627
Bd_JEL271
Bd_Lb_Aber
Bd_CJB7
Bd_JAM81
Bd_CJB5_2
Bd_JEL427
Bd_JEL310
Bd_JEL429
Bd_ev001
Bd_Campana
Bd_RioMaria
Bd_JEL410
Bd_Pc_CN_JLV
Bd_NBRC106979
Bd_JEL433
Bd_JEL275
Bd_JEL359
Bd_JEL267
Bd_JEL238
Bd_CJB4
Bd_TST75
Bd_SRS812
Bd_MLA1
Bd_PAB01
Bd_MexMkt
0.6
1
4
5
6
7
20 23 24 26 28 29 30 31 33 34 35 36 81 104 105 111 112 113 114 115 116 117 118 131
GPL1
GPL2
heterozygote
missing data