



Endemic Lineages of *Batrachochytrium dendrobatidis* Are Associated With Reduced Chytridiomycosis-Induced Mortality in Amphibians: Evidence From a Meta-Analysis of Experimental Infection Studies

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Emerging infectious wildlife diseases have caused devastating declines, particularly when pathogens have been introduced in naïve host populations. The outcome of disease emergence in any host population will be dictated by a series of factors including pathogen virulence, host susceptibility, and prior opportunity for coevolution between hosts and pathogens. Historical coevolution can lead to increased resistance in hosts and/or reduced virulence in endemic pathogens that allows stable persistence of host and pathogen populations. Adaptive coevolution may also occur on relatively short time scales following introduction of a novel pathogen. Here, we performed a meta-analysis of multi-strain *Batrachochytrium dendrobatidis* (Bd) infection experiments to test whether: (1) amphibian hosts exhibit lower mortality rates when infected with strains belonging to endemic Bd lineages relative to the Global Panzootic Lineage (Bd-GPL), hypothetically owing to long co-evolutionary histories between endemic Bd lineages and their amphibian hosts; and (2) amphibians exhibit lower mortality rates when infected with local Bd-GPL strains compared with non-local Bd-GPL strains, hypothetically owing to recent selection for tolerance or resistance to local Bd-GPL strains. We found that in a majority of cases, amphibians in endemic Bd treatments experienced reduced mortality relative to those in Bd-GPL treatments. Hosts presumed to have historically coexisted with endemic Bd did not show reduced mortality to Bd-GPL compared with hosts that have not historically coexisted with endemic Bd. Finally, we detected no overall difference in amphibian mortality between local and non-local Bd-GPL treatments. Taken together, our results suggest that long-term historical coexistence is associated with less disease-induced mortality potentially due to hypovirulence in endemic Bd lineages, and

that more recent coexistence between amphibians and Bd-GPL has not yet resulted in reduced host susceptibility or pathogen virulence. This corroborates previous findings that Bd-GPL introduced via the global amphibian trade has a high capacity for causing disease-induced mortality.

Keywords: *Batrachochytrium dendrobatidis* (Bd), meta-analysis, pathogen genotypes, experimental infection, amphibian, virulence, chytridiomycosis

INTRODUCTION

Emerging wildlife diseases pose significant threats to global biodiversity, but the mechanisms by which some hosts are able to survive recently emerged diseases are not yet fully understood (1). To aid in assessment of disease risk in host populations and species of concern, it is critical to understand the mechanisms that determine the outcome of infection. Hosts may be *susceptible* to infection and/or disease-induced mortality, or may survive infection due to *resistance* (i.e., the ability to prevent infection or reduce infection load) and/or *tolerance* (i.e., the ability to sustain infection without experiencing substantial negative impacts). Likewise, infection outcomes can also depend on variation in pathogen virulence, which can result from genotypic differences among pathogen strains or genotypes (2–4). While contemporary conditions (i.e., local abiotic and biotic environments) may significantly influence host susceptibility [e.g., (5)] and/or pathogen virulence [reviewed in Turner et al. (6)], prior interactions between host and pathogen populations may also contribute to significant variability in disease outcomes. For example, a pathogen introduced to naïve host populations may cause severe outbreaks (i.e., epizootic disease), while recently or historically co-occurring hosts and pathogens may show disease dynamics that are tempered with mild or no disease despite pathogen presence [i.e., enzootic disease or absent disease; (7, 8)]. When the co-evolutionary trajectory between host and pathogen takes the form of a classical oscillating arms race between host resistance and pathogen virulence, different host and pathogen populations may exhibit different levels of resistance and virulence at the same point in time (9). In these cases, current infection outcomes can vary according to host-pathogen co-evolutionary history, leading to a “geographic mosaic” of disease threat (10).

Amphibian chytridiomycosis caused by *Batrachochytrium dendrobatidis* (Bd) is one of the most devastating wildlife diseases documented in the scientific literature to date (11–13). Bd is believed to have originated in East Asia (14), and one hypervirulent lineage, the Global Panzootic Lineage (hereafter Bd-GPL) spread globally by the transcontinental amphibian trade (15). Bd-GPL has been implicated in die-offs around the world (12), and the prevailing hypothesis is that introductions of Bd-GPL were particularly damaging in naïve amphibian communities where many or all hosts lacked any effective form of Bd resistance (16, 17). In East Asia, Brazil, South Africa, and Switzerland, additional geographically restricted and early-diverged genotypic lineages of Bd have been identified that may have much longer coevolutionary histories with amphibians

compared to Bd-GPL (14). These lineages named *Bd-Asia*, *Bd-Brazil*, *Bd-Cape*, and *Bd-CH*, are referred to as endemic Bd lineages. Studies have shown that at least some endemic Bd lineages were associated with low mortality rates in susceptible species (14) and that amphibian species that have coexisted historically with endemic Bd lineages appear to exhibit Bd resistance and/or tolerance [e.g. (18)]. These studies support the hypotheses that due to a long history of host-pathogen coevolution, endemic Bd lineages are broadly hypovirulent, and communities of amphibians in Bd-endemic areas could be protected from Bd outbreaks (16, 19).

However, as studies of Bd have increased, we have found surprising diversity in Bd lineages and their distributions, and also unpredicted variability in disease outcomes. Experimental results have varied widely according to focal host: for instance, initial experiments did not use hosts that had locally adapted to presumed hypovirulent genotypes [e.g. (14)], and later experiments showed host species-level variation in disease susceptibility even when infected by the same endemic Bd lineage (18). Retrospective studies of Bd infections in museum specimens revealed that Bd-GPL may have been present for longer than previously thought (i.e., predating amphibian trade) in areas such as southeastern Brazil (20) and the United States (21, 22), providing the opportunity for local adaptation to Bd-GPL. Furthermore, field studies have shown that multiple genotypes (endemic Bd and Bd-GPL) and multiple Bd-GPL strains can be present in sympatry in wild populations [e.g. (23)], with the potential to produce differing disease outcomes across amphibian communities. For example, local Bd-GPL strains, defined here as the only or predominant Bd-GPL strain in a host community, may protect hosts from more virulent non-local Bd-GPL strains found in surrounding communities (24). Given that in some cases Bd-GPL has been introduced through several independent events to disparate amphibian communities (25), we may expect variable levels of local, shorter-term co-adaptation between amphibian hosts and the Bd-GPL strains found in different host communities, climates, and geographies. Because Bd is globally distributed and is a highly generalist pathogen capable of infecting at least 500 amphibian species (12, 13), understanding how coevolution and potential co-adaptation of amphibians and Bd impact infection outcomes is critical to predicting and mitigating future chytridiomycosis declines.

Here, we perform meta-analyses of host mortality in multi-strain, controlled Bd infection experiments to determine whether opportunity for historical or recent selection alters disease outcomes in amphibian hosts that are susceptible to disease-induced mortality. Multiple factors may contribute to

experimental disease outcomes, including characteristics of the host source population; therefore we restricted our meta-analysis to controlled experiments and calculated pairwise effect sizes for Bd infection treatments involving hosts from the same source population or locality. This approach allows us to summarize the impact of Bd infection across experimental studies published to date using comparable quantitative effect sizes. We test two hypotheses: (1) the historical adaptation (HA) hypothesis posits that endemic Bd lineages show hypovirulence relative to Bd-GPL as a result of long coevolutionary histories with their amphibian hosts; and (2) the recent adaptation (RA) hypothesis posits that amphibians show lower susceptibility to local Bd-GPL strains relative to non-local Bd-GPL strains due to recent and rapid adaptation or co-adaptation of amphibian hosts and Bd. To test these hypotheses, we performed two meta-analyses to evaluate whether Bd genotype and/or locality impacts mortality due to standardized Bd infection. First, to test the HA hypothesis, we analyzed the effect of Bd genotypic lineage to determine whether endemic Bd strains result in lower mortality than Bd-GPL strains, regardless of host and Bd origin (i.e., collection locality). Second, to test the RA hypothesis, we analyzed the effect of recent geographic co-occurrence of host and pathogen to determine whether local Bd-GPL strains (collected from the same locality as the focal host) result in lower mortality relative to non-local Bd-GPL strains. Our results provide insight into the significance of host-pathogen adaptation at two temporal scales in chytridiomycosis outcomes, and suggest future directions for research that would aid conservation in the face of devastating wildlife disease.

METHODS

Data Compilation

To test the HA and RA hypotheses, we performed two meta-analyses of experimental Bd studies on susceptible amphibians, here defined as hosts that can experience chytridiomycosis-induced mortality. As a starting point for compiling experimental Bd studies, we used the systematic review of experimental studies performed by Blaustein et al. (26). This study utilized a Web of Science search supplemented with a Google Scholar search for studies between 1999 and 2017 using the search terms “*Batrachochytrium dendrobatidis* + amphibians + experiments (26).” We retained 14 studies identified by Blaustein et al. (26) that involved multi-strain infection experiments. We replicated this search to compile recent studies published between 2017 and 2021 using Web of Science (searched on April 21, 2021), supplemented with a Google Scholar search [October 7, 2021 and collated in Publish or Perish (27)], and included one unpublished study from the authorship team’s collaborator (28).

Our supplemental search resulted in 3,177 publications total, of which four met our criteria of being multi-strain experiments that reported mortality in susceptible host species (i.e., species that exhibited mortality in at least one infection treatment; **Supplementary Figure 1**). Study units were excluded from analyses if: (i) mortality in control treatments significantly exceeded mortality in experimental infection treatments; (ii) Bd genotype (i.e., belonging to GPL or an endemic lineage) and/or

collection locality information were not available; and/or (iii) hosts were fully resistant to chytridiomycosis (i.e., experienced zero mortality in all treatments). Study units were defined as a single study x species x treatment combination.

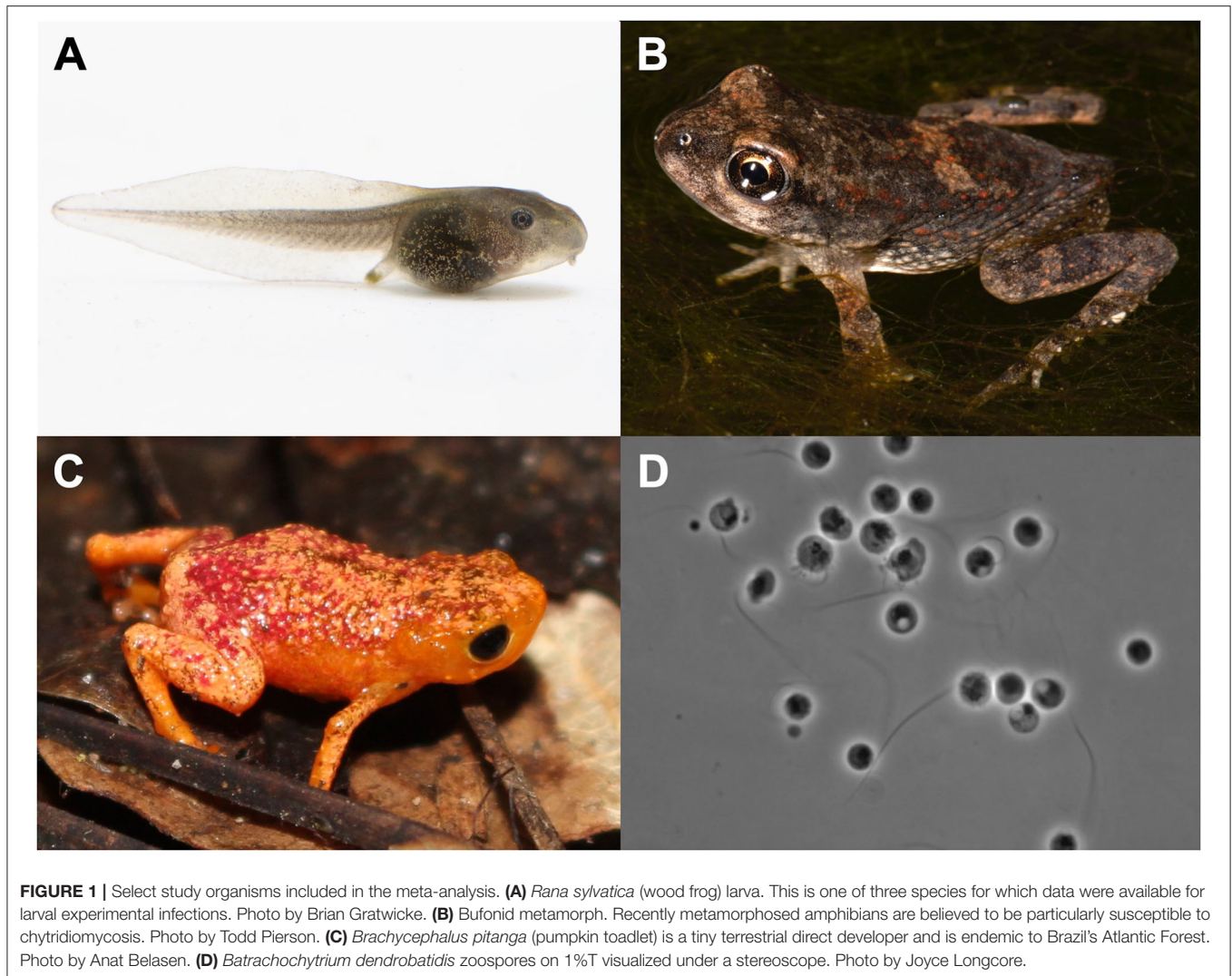
Between Blaustein et al. (26) and our supplemental search, we recovered 30 study units across 12 publications, covering 19 ecologically diverse species of amphibians and three amphibian life stages (**Figure 1** and **Supplementary Figure 1**). Studies used in the HA analysis ($n = 21$ study units, **Table 1**) included either wild-collected or captive-reared amphibians, and were required to include at least two genotypes of Bd (Bd-GPL plus one endemic genotype, i.e., Bd-Brazil, Bd-Asia, Bd-Cape, or Bd-CH) that were separately inoculated on test animals. If experimental Bd strains were not genotyped by the authors of the study, genotype information was sourced from papers that genotyped these strains using DNA sequencing and phylogenetic analyses (**Supplementary Table 1**). In one case, Bd genotype was inferred by the current authors to be Bd-GPL [isolate LC63 from a captive Australian frog (38)]. Studies used in the RA analysis ($n = 17$ study units, **Table 1**) were required to include wild-collected amphibians and at least two strains of Bd-GPL, at least one of which was collected locally (where focal hosts were collected) and at least one of which was collected non-locally.

Data Analysis and Visualization

Data extraction, analyses, and visualizations were performed in the R environment (39). For each study unit, we extracted the sample sizes for control and experimental infection treatments, and percent mortality for each genotype/strain of Bd. If mortality values were not directly present in publication text, we used the metaDigitise package in R (40) to extract numeric data from published survival curves. Mortality values were averaged in cases where there were multiple isolates from a given genotype x locality combination tested on the same amphibian host species in different treatments within a given study. We also compiled metadata for each study unit, including host adult ecology, host reproductive mode, host life stage at infection, and historical coexistence status with endemic Bd (i.e., whether the focal host species historically coexisted with the endemic Bd lineage used in the experiment).

In experiments using sensitive amphibian hosts, background mortality can be common. While we excluded studies that contained significantly higher mortality in controls relative to infection treatments, we included 24 study units with low levels of control mortality that did not significantly exceed treatment mortality. In these cases, the control mortality rate was subtracted from each treatment mortality rate to obtain a “background mortality-corrected” rate for each treatment. In cases where control mortality marginally exceeded treatment mortality ($n = 3$ studies in the HA analysis, $n = 1$ study in the RA analysis), the corrected treatment mortality value was rounded up to zero. In all of these studies, mortality was significantly higher in at least one infection treatment than in the control treatment, indicating Bd-induced mortality above potential background mortality due to captive conditions.

Unless otherwise stated, all calculations and analyses were performed in the R metafor package (41). To estimate the



effect size of Bd genotype (HA analysis) or source locality (RA analysis) on mortality, log Risk Ratio was calculated for each study unit. Subsequently, linear mixed models were constructed to analyze effect sizes weighted by variance for each analysis. Models constructed for each analysis were fitted using a restricted maximum likelihood (REML) approach. A generalized, weighted least squares extension of Cochran's Q test (41) and I^2 (42) were calculated to test whether the heterogeneity among effect sizes was greater than expected. Outliers or influential cases were identified by calculating Cook's distances (≥ 0.45), Hat values [$\geq 3*(1/k)$], and $dfbetas$ [≥ 1 ; (41, 43)], and removed if at least two metrics identified the outlier.

To test whether variation among effect sizes was associated with any subgroup variables, three models were constructed for each analysis: (1) intercept-only (base) models; (2) multifactor (full) models that included all subgroup terms (HA only); and (3) single-factor models that consisted of separate models for each subgroup term. Subgroup variables included life stage at infection (both HA and RA) and historical coexistence (HA only). Host adult ecology and host reproductive mode were not

included as subgroup moderators due to a lack of sufficient statistical power (little or no replication for some factor levels). In all models (including base models), study (publication) ID was included as a random term to account for non-independence among study units within publications (44). AIC was used to determine model ranking within each analysis. Average log Risk Ratios were exponentiated to calculate the Risk Ratio values for subgroup terms that were significant in single-factor models. Fligner-Killeen non-parametric tests for homogeneity of variance were used to evaluate variance among subgroup variables of interest. Results were visualized in metafor (41) and ggplot2 (45).

RESULTS

Historical Adaptation Analysis

In the HA analysis, Bd genotype was associated with a significantly positive log Risk Ratio according to the intercept-only model, indicating that Bd-GPL infections resulted in higher mortality relative to endemic lineages of Bd (RR = 1.79, $Z = 3.99$, $p < 0.0001$; **Figure 2**). The HA dataset showed a high degree of

TABLE 1 | Study units included in the two meta-analyses.

	Host species	Study number	Life stage at Bd exposure	Exposure dose	Experiment duration	Endemic Bd used	Historical Bd coexistence	Citation	
Historical Adaptation (HA) Analysis	<i>Alytes muletensis</i>	1	larva*†	Cumulative: 2.3 k zsp over 2w	160 days	Bd-Cape	Yes	Doddington et al. (29)	
	<i>Bombina orientalis</i>	6a	adult	8 × 10 ⁵ zsp	3 months	Bd-Asia1	Yes	Fu and Waldman (18)	
	<i>Brachycephalus ephippium</i>	7a	adult	1.8 × 10 ⁶ zsp	60 days	Bd-Brazil	Yes	Greenspan et al. (30)	
	<i>Brachycephalus pitanga</i>	9	adult	3.9 × 10 ⁶ zsp	15 days	Bd-Brazil	Yes	McDonald (28)	
	<i>Bufo bufo</i>	2a-b	larva*†	Cumulative: 190 or 19k zsp every 4d over 2w (treatments averaged for analysis) Max total—5.7 × 10 ⁴ zsp	80 days	Bd-Cape	No	Fisher et al. (2)	
		3a-b	larva*	Cumulative: 3–17k zsp every 4d × 8 reps; Max total—1.36 × 10 ⁵ zsp	122 days	Bd-Cape	No	Farrer et al. (31)	
		4a-c	larva*†	Cumulative: 7.5–37.5k zsp every 4d × 8 reps; Max total = 3 × 10 ⁵	42+ days	Bd-Asia1, Bd-Cape, Bd-CH	No	O'Hanlon et al. (14)	
		4d-f	metamorph	Cumulative: 10–36k zsp every 4d × 5 reps; Max total = 8.5 × 10 ⁴ zsp	22 days	Bd-Asia1, Bd-Cape, Bd-CH	No	O'Hanlon et al. (14)	
		<i>Dendropsophus minutus</i>	7b	adult	1.8 × 10 ⁶ zsp	60 days	Bd-Brazil	Yes	Greenspan et al. (30)
		<i>Hymenochirus curtipes</i>	8	adult	1.25 × 10 ⁶ zsp	25 days	Bd-Brazil	No	Jenkinson et al. (32)
		<i>Ischnocnema parva</i>	7c	adult	1.8 × 10 ⁶ zsp	60 days	Bd-Brazil	Yes	Greenspan et al. (30)
Recent Adaptation (RA) Analysis	<i>Alytes obstetricans</i>	10a-d	metamorph	1 × 10 ⁶ fo 24 h	13 weeks			Greener et al. (24)	
	<i>Anaxyrus americanus</i>	11a-c	metamorph	3.33 × 10 ⁵ zsp	73 days			Burrow et al. (33)	
	<i>Anaxyrus boreas</i>	12a	metamorph	10 ⁶⁻⁷ zsp + 10 ⁵⁻⁶ spg	40 days			Gahl et al. (34)	
	<i>Bufo bufo</i>	13a-b	larva†	10 ⁵ zsp	20 days			Dang et al. (35)	
		2c	larva*†	Cumulative: 190 or 19k zsp every 4d over 2w (treatments averaged for analysis)	80 days			Fisher et al. (2)	
		14	metamorph	4 ml of 4 × 10 ⁶ zsp/ml inoculum for consecutive days	30 days			Meurling et al. (36)	
		<i>Physalaemus fernandezae</i>	15	metamorph	6 × 10 ⁴ for 5 h	14 days		Arellano et al. (37)	
		<i>Pseudacris regilla</i>	13c-d	larva†	10 ⁵ zsp	20 days		Dang et al. (35)	
		<i>Rana clamitans</i>	12b	metamorph	10 ⁶⁻⁷ zsp + 10 ⁵⁻⁶ spg	40 days		Gahl et al. (34)	
		<i>Rana pipiens</i>	12c	metamorph	10 ⁶⁻⁷ zsp + 10 ⁵⁻⁶ spg	40 days		Gahl et al. (34)	
		<i>Rana sylvatica</i>	12d	larva*†	10 ⁶⁻⁷ zsp + 10 ⁵⁻⁶ spg	40 days		Gahl et al. (34)	
			5b	larva*	10 ⁶ zsp/tank	70 days		Becker et al. (4)	
		<i>Rana cascadae</i>	13e-f	larva†	10 ⁵ zsp	20 days		Dang et al. (35)	
		<i>Rana onca</i>	16a-b	larva* & metamorph	3 × 10 ⁶ zsp over 3d	18 weeks		Waddle et al. (38)	

Studies in the top half of the table were included in the Historical Adaptation (HA) analysis, while those in the bottom half were included in the Recent Adaptation (RA) analysis. Study number corresponds with superscript notations in **Figures 2, 4**. In studies where hosts were exposed to Bd as larvae, asterisks (*) denote that mortality values were reported post-metamorphosis, and crosses (†) denote that mortality values were reported pre-metamorphosis. Use of both symbols denotes mortality was recorded across both life stages. Abbreviations in the exposure dose column are as follows: zsp, zoospore; spg, zoosporangia; d, days; w, weeks; reps, repetitions. For HA analysis studies, the endemic Bd treatment column indicates the genotype of the endemic Bd lineage used in the experiment. Historical Bd coexistence is reported based on known or hypothesized historical coexistence of the focal host with the endemic Bd used in the experiment. Citation is provided with corresponding literature cited reference number in parentheses. See **Supplementary Table 1** for strain-specific genotyping information.

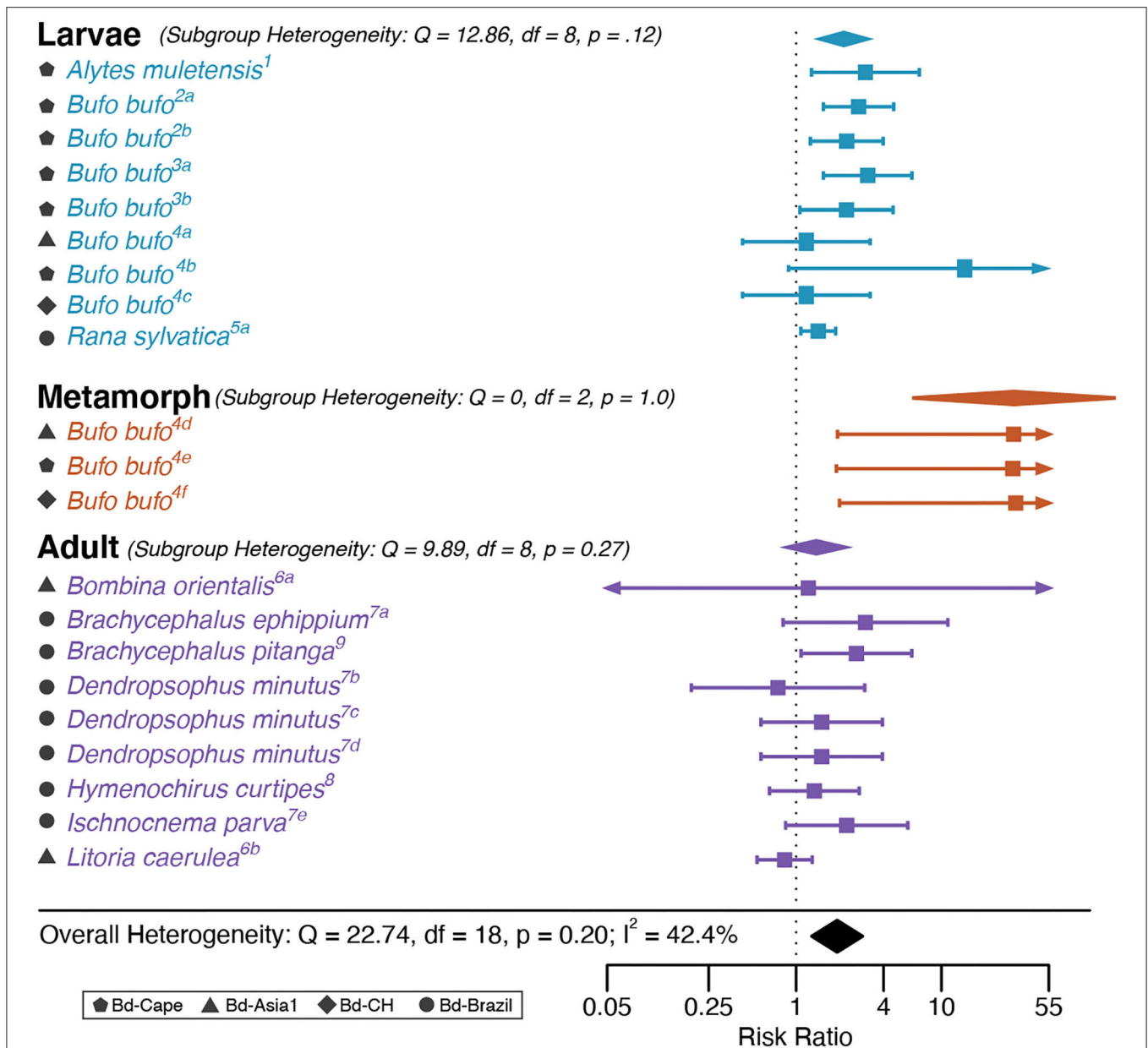


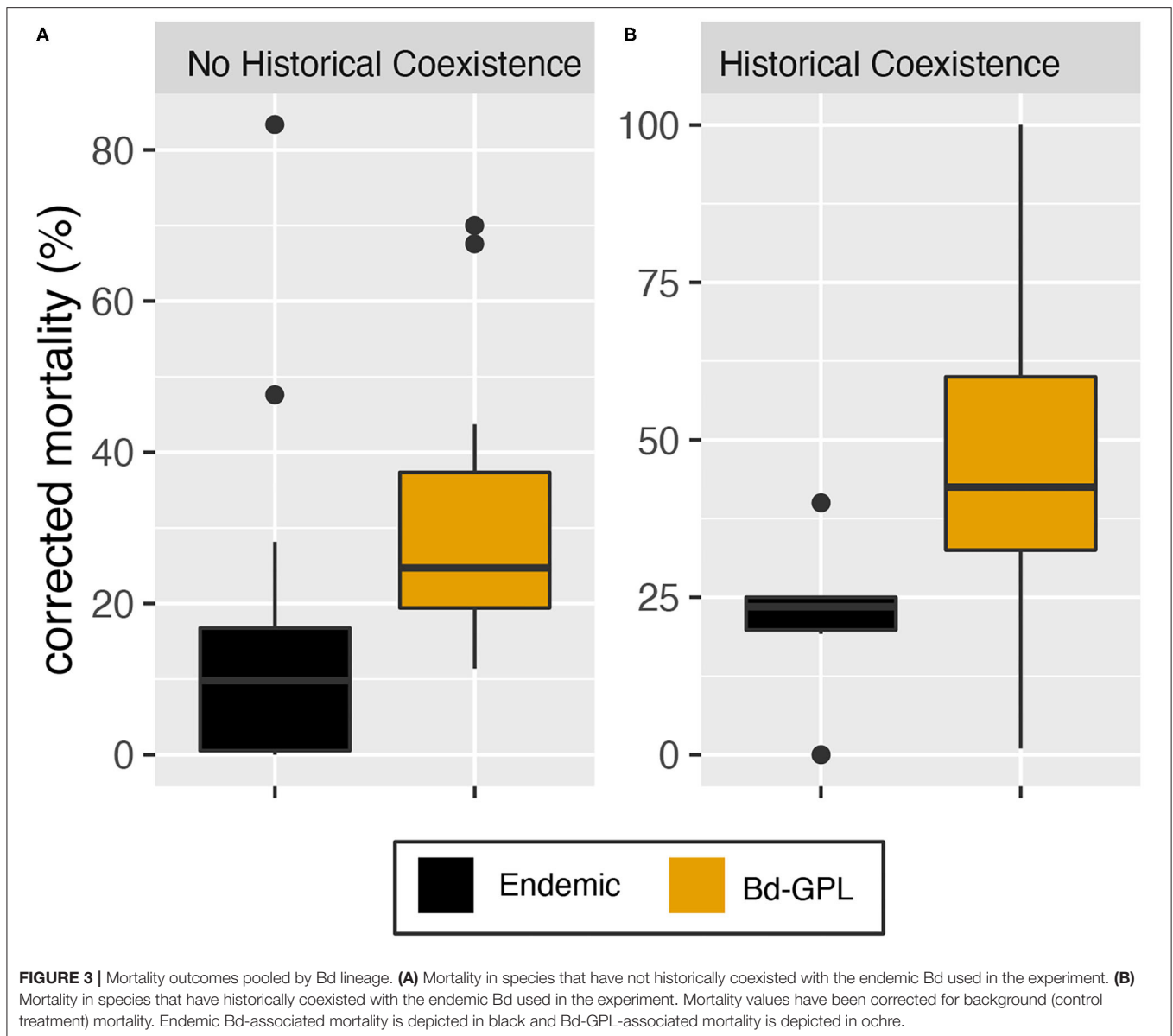
FIGURE 2 | Historical Adaptation analysis forest plot depicting Risk Ratio effect sizes for each life stage category. Squares indicate mean effect size for each study unit and error bars depict 95% Confidence Interval (CI). The meta-analytic mean effect size across study units (black diamond) was calculated without any subgroup moderator terms. Arrows on error bars indicate that the CI exceeds the provided x-axis scale. Study unit ID numbers correspond to **Table 1**. Heterogeneity test statistics (Q), degrees of freedom (df), and p-values for Cochran’s Q test of heterogeneity are reported for each subgroup and the overall model.

heterogeneity ($I^2 = 42.38\%$; $Q = 40.104$, $p = 0.0048$). No outliers or influential cases were detected (Cook’s $D < 0.45$; $\text{Hat} < 0.14$; $\text{dfbetas} < 1$).

The multifactor (full) HA model exhibited better fit than the intercept-only (base) model (AIC = 38.77 for the full model vs. 51.68 for the base model), and reduced heterogeneity in the dataset such that it was not significant ($Q = 16.12$, $p = 0.516$). The HA single-factor model that included host life stage was significant ($QM = 14.24$, $p = 0.0008$; **Figure 2**). Risk Ratio was

higher in individuals infected as metamorphs compared with those infected as larvae or adults, and higher in individuals infected as larvae than as adults ($RR_{\text{metamorph}} = 6.9$, $RR_{\text{larvae}} = 1.99$, $RR_{\text{adults}} = 1.36$; **Figure 2**).

The single-factor model for historical coexistence was not statistically significant. However, data visualization showed evidence of greater variability in mortality due to infection with endemic Bd among hosts that had not historically coexisted with endemic Bd (**Figure 3** and **Supplementary Figure 2**). We further



explored this relationship by testing for statistical differences in mortality variance in study units with historical coexistence vs. those without historical coexistence for endemic vs. panzootic (Bd-GPL) Bd genotypes. We found no statistically significant differences in variance for either Bd genotype group (Fligner-Killeen test: Bd-GPL- $X^2 = 1.57$, $p = 0.211$; endemic Bd- $X^2 = 0.46$, $p = 0.498$).

Recent Adaptation Analysis

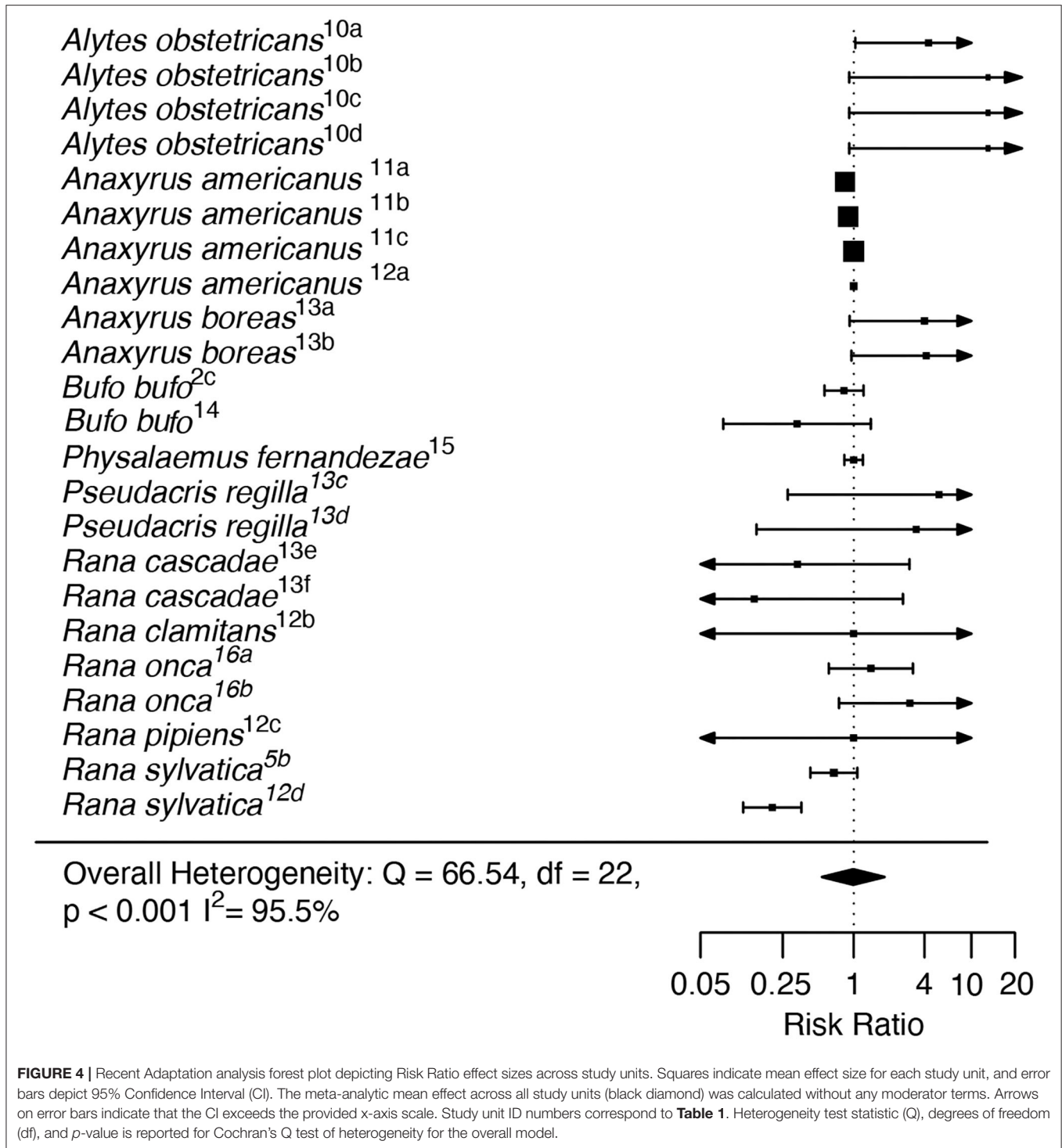
In the RA analysis, the intercept-only model showed no significant effect of Bd-GPL collection locality on Risk Ratio (RR = -0.996 , $Z = -0.024$, $p = 0.98$; **Figure 4**). The RA dataset showed a high degree of heterogeneity ($I^2 = 95.5\%$; $Q = 66.54$, $p = 0.001$). The moderator model including life stage at exposure did not exhibit better fit than the intercept-only model (AIC =

65.06 for the full model vs. 64.55 for the intercept-only model), and reduced but did not eliminate heterogeneity ($Q = 64.27$, $p < 0.001$). No outliers or influential cases were detected (Cook's $D < 0.45$; Hat < 0.18 ; $dfbetas < 1$).

DISCUSSION

Endemic Bd Lineages Are Associated With Reduced Chytridiomycosis Mortality

We performed meta-analyses of multi-strain Bd infection experiments to evaluate support for the hypotheses that the opportunity for historical or recent adaptation reduces chytridiomycosis-induced mortality. We found strong support for potential historical adaptation: we detected a significant effect of Bd genotype on Risk Ratio such that endemic Bd



lineages on average resulted in reduced mortality relative to Bd-GPL, regardless of whether hosts have historically coexisted with endemic Bd lineages. This apparent difference in virulence of Bd genotypes could have resulted through a number of mechanisms, including: historical coevolution between Bd and local amphibian hosts has led to a stable state of hypovirulence

in endemic Bd lineages; genomic changes have resulted in hypervirulence in Bd-GPL; and/or, as a recently emerged lineage of Bd, Bd-GPL has escaped host defense mechanisms such that even hosts that have a long co-evolutionary history with endemic Bd are functionally naïve to Bd-GPL infections. These mechanisms are not mutually exclusive. For instance, hosts that

have historically coexisted with endemic Bd exhibited relatively consistent and generally low mortality rates when exposed to endemic Bd. This may indicate selection for reduced pathogen virulence and/or increased host resistance or tolerance in these systems. At the same time, ample phylogenetic and genomic evidence, the global spread of Bd-GPL, and its association with large-scale amphibian declines, support Bd-GPL as a recently emerged and widely introduced hypervirulent lineage (19, 31, 46). Independent of the mechanisms, our results confirm a strong difference in infection outcomes between endemic Bd lineages and Bd-GPL.

A notable exception to this overall pattern was Fu and Waldman (18), in which *L. caerulea* experienced high mortality from both Bd-Asia1 and Bd-GPL. The *L. caerulea* frogs used in this study were collected in New Guinea, which as of 2020 was entirely Bd-free (47). When compared with the other species in this study (*Hyla japonica*, *Bufo gargarizans*, and *Bombina orientalis*), which were collected from South Korea where Bd-Asia1 is historically endemic and widely distributed today (48), the pattern of disparity in outcomes due to differences in historical host adaptation opportunity is exemplified: Korean amphibians showed low mortality in both Bd-Asia1 and Bd-GPL treatments, suggesting that a long history with Bd-Asia1 may have led to evolution of broad Bd tolerance or resistance in these species. In contrast, entirely Bd-naïve *L. caerulea* frogs showed high chytridiomycosis mortality, even when infected with Bd-Asia1, which was previously reported to be hypovirulent (14). These findings suggest that Bd-Asia1 can be as virulent as Bd-GPL in naïve hosts, and that host defenses may be responsible for the apparent lack of epizootics due to Bd-Asia1 in Korean amphibian communities. Additional work is needed to assess the role of historical coexistence with endemic Bd in providing broad protection against lethal chytridiomycosis in other systems.

Individuals infected as metamorphs showed the highest Risk Ratios compared to all other life stages in the HA analysis. This may be due to the lag in the full development of the adaptive immune system following metamorphosis (49) and increased stress during this developmental transition (50, 51). Bd infects keratinized tissues in amphibians, which are restricted to mouthparts in larvae and become more widespread in post-metamorphic amphibian skin. In individuals infected as larvae, infection can shift through metamorphosis as more keratinized tissue becomes available on the body (52). Our results indicate that in the included studies, individuals infected at, rather than before, the metamorph stage, show the greatest increase in mortality due to Bd-GPL relative to endemic Bd. Metamorphosis is associated with immunosuppression, which functions to avoid a self-destructive autoimmune response as new adult-specific molecules develop but can also increase disease susceptibility in the vulnerable metamorph life stage (50). Full maturation of adult-like adaptive immunity takes a minimum of 2 months in amphibian species with fast development. Likewise, a full complement and mature levels of AMPs can be delayed until multiple months post-metamorphosis (53), although this is host species-dependent (49). Overall, our finding that individuals infected as larvae and metamorphs exhibit the highest Risk Ratios

supports the idea that Bd-GPL poses a significant threat to these relatively poorly-surveyed life stages in the wild.

We note that the individuals that were infected as metamorphs in our dataset only represent a single study and species, *Bufo bufo* (14). However, this species was also exposed to comparable cumulative zoospore loads in two additional studies [57,000–136,000 zoospores over 2–4 weeks (2, 31); Table 1], and all three studies included the endemic lineage Bd-Cape. Comparing these three studies, *B. bufo* metamorphs show a higher Risk Ratio than larvae, indicating that metamorphs show the greatest difference in mortality between Bd-GPL and endemic Bd. Research on strain-specific and life stage-specific susceptibility in additional species is needed to determine whether these differences among larvae and metamorphs are common.

Recent Coexistence Is Insufficient to Reduce Lethal Chytridiomycosis

Our Recent Adaptation analysis comparing mortality due to infection by local vs. non-local Bd-GPL showed no overall effect on disease outcomes and no significant subgroup factors. We note that there were no available studies that were performed on adults, thus interpretation of our findings should potentially be limited to larval and metamorphic life stages. The relatively high variance in the results of the RA analysis may be explained by a number of factors that can influence disease outcomes. One important factor may be the host species from which Bd strains were isolated. Due to a lack of statistical power, we were unable to test the effect of host identity from which a Bd strain was isolated. However, we note one case study with disparities based on fine-scale origin of local Bd isolate. In Waddle et al. (38), the local Bd-GPL (LBP) was isolated from a *R. onca* frog collected from the same source population used for the experiment, while the non-local Bd-GPL (LC63) was isolated from a very sick captive White's tree frog (*Litoria caerulea*). It is noteworthy that the Bd-GPL strain isolated from a stable (non-epizootic) population of the focal host resulted in functionally zero mortality (no different from control), while the Bd-GPL strain isolated from a susceptible and sick host resulted in high mortality. In another treatment, *R. onca* were exposed to a relatively local Bd-GPL (SMR) that was instead isolated from *Pseudacris regilla* located ~65 km from the nearest *R. onca* population. *P. regilla* is hypothesized to be Bd-tolerant and an important reservoir species in the western United States (54). Bd isolates from a stable population of a focal host, a susceptible species, or a reservoir species may have different consequences on the focal host as a result of selection for different virulence phenotypes. To aid in understanding the relationship between isolate origin and Bd virulence, it is important that future studies provide as much information as possible about the isolation history of Bd strains used in experiments.

In addition, local adaptation may be obscured by factors such as variation in Bd's phenotypic expression. Bd is known to exhibit phenotypic plasticity in response to temperature (55), and recent work has shown that Bd can be highly plastic over short time scales owing to functional variation in the pathogen's gene

expression (56). Although amphibians can exhibit relatively fast adaptive change in immune responses (57, 58), host evolution is still expected to lag behind that of a plastic pathogen. Nonetheless, in field studies, local recovery and persistence have been documented in post-epizootic amphibian communities (59) even where local Bd pathogenicity remains high (60). Our negative RA analysis results combined with findings from wild amphibian communities suggest that recovery following Bd epizootics may not necessarily be due to short-term adaptation to local Bd strains, but rather due to broad defense against or tolerance of Bd, or avoidance of infections altogether [e.g., see (61)].

Future Research Directions

Taken together, our results suggest that long periods of coexistence may be required for stable enzootic disease dynamics in the amphibian-chytridiomycosis system. We detected strong evidence across studies for hypovirulence in endemic Bd that may have arisen from historical coexistence with amphibian hosts. It remains unclear at what time scale adaptation of pathogen and/or host takes place, but our results do suggest that the relatively short time periods of coexistence with Bd-GPL in the geographic regions represented by the included studies (North America and Europe) have not yet been sufficient to protect many susceptible amphibian species from lethal chytridiomycosis. Further studies of the variation in disease outcomes resulting from endemic Bd lineages may provide a basis for identifying the mechanisms that reduce chytridiomycosis-induced mortality, and identifying cases where endemic Bd lineages retain virulence in naïve hosts. Similarly, host populations in which local adaptation may be beginning or in progress [e.g., in *Rana onca* (38)] may hold important insights into the potential for host adaptation to the highly successful and widely introduced Bd-GPL.

Our findings support the hypothesis that in many systems, endemic Bd lineages pose a lower threat to amphibian species than Bd-GPL. Recent studies have shown that previous infection with hypovirulent Bd-GPL strains can protect against severe chytridiomycosis due to hypervirulent Bd-GPL strains (24, 38). However, the question of whether historical coexistence with hypovirulent endemic Bd strains provide protection against Bd-GPL in the wild and/or in hosts that have not previously experienced Bd infection in their lifetimes remains to be adequately tested. Future research should focus on identifying the mechanisms that reduce lethal chytridiomycosis, which may include innate or adaptive immune mechanisms in the host, or reductions in virulence mechanisms in the pathogen. In addition, evaluating competitive dynamics between Bd-GPL and diverse endemic Bd strains may be critically important to determining the threat that Bd poses to local amphibian communities. To our

knowledge, strain competition has only been assessed for Bd-GPL vs. Bd-Brazil to date (32). It remains unclear to what extent other endemic Bd lineages show similar competitive dynamics. Deepening our understanding of the relationships between co-evolutionary history, virulence, and disease outcomes will help predict and mitigate future hotspots of chytridiomycosis and aid in amphibian conservation efforts.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: <https://github.com/m-bletz/Frontiers-meta-analysis-2021>.

AUTHOR CONTRIBUTIONS

AB, MB, IR, and KZ conceived of the study. AB, IR, and MB compiled the data and created the figures. MB performed the analyses and visualized results. AB led manuscript writing. AB and MB co-led revisions. All authors contributed to writing and revising the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2022.756686/full#supplementary-material>

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