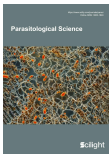
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Review

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Ribeiroia ondatrae-Induced Amphibian Malformations: Patterns, Host-Parasite Interactions and Ecological Consequences

Héctor A. Castro-Bastidas [1], Maribel Méndez-Tepepa [2] and David R. Aguillón-Gutiérrez [3,*]

[1] Posgrado en Ciencias Aplicadas al Aprovechamiento de los Recursos Naturales, Centro de Estudios "Justo Sierra" (CEJUS), Badiraguato 80600, Sinaloa, Mexico
[2] Centro de Investigación en Genética y Ambiente, Universidad Autónoma de Tlaxcala, Autopista San Martín-Tlaxcala Km 10.5, Ixtacuixtla 90120, Tlaxcala, Mexico
[3] Laboratorio de Bioindicadores, Centro de Investigación y Jardín Etnobiológico, Universidad Autónoma de Coahuila, Dr. Francisco González 37, Viesca 27480, Coahuila, Mexico
* Correspondence: david_aguillon@uadec.edu.mx

How To Cite: Castro-Bastidas, H.A.; Méndez-Tepepa, M.; Aguillón-Gutiérrez, D.R. *Ribeiroia ondatrae*-Induced Amphibian Malformations: Patterns, Host-Parasite Interactions and Ecological Consequences. *Parasitological Science* **2026**, *1*(1), 8. https://doi.org/10.53941/parsci.2026.100008

Received: 18 June 2026
Revised: 1 September 2026
Accepted: 29 October 2026
Published: 12 November 2026

Abstract: This review synthesizes the current knowledge on the host-parasite system formed by the trematode *Ribeiroia ondatrae* and amphibians, integrating information from the cellular mechanisms of parasitic teratogenesis to its ecological consequences at the landscape scale. It describes how the parasite's metacercariae alter the normal morphological development of amphibians during critical windows of organogenesis, inducing severe structural malformations in the limbs through synergistic processes of mechanical disruption and alteration of retinoic acid signaling. The analysis shows that susceptibility to infection varies significantly among host species, where the presence of resistant organisms can mitigate transmission through a dilution effect in the aquatic community. Additionally, an adaptive strategy of phenotypic manipulation by the parasite is highlighted, which impairs host locomotion and modifies escape behavior to facilitate trophic transmission to birds, the definitive hosts. Finally, anthropogenic factors such as eutrophication, the use of agrochemicals, and the effects of climate change are identified as powerful amplifiers of parasitic outbreaks under natural conditions. The congenital malformations induced by *R. ondatrae* represent a critical and irreversible biological stressor that compromises the survival of amphibian populations. Understanding the complex interactions of this system within the context of global change is essential for predicting disease emergence and designing effective biological conservation strategies in aquatic ecosystems.

Keywords: anthropogenic stressors; Anura; Digenea; dilution effect; teratogenesis; trophic transmission

1. Introduction

For more than two decades of research, fundamental questions persist regarding the exact mechanisms of malformation induction, the variability in susceptibility among host species, the synergistic interaction with anthropogenic environmental factors, and the long-term implications for the persistence of amphibian populations, which as a group are among the most threatened vertebrates on the planet [1]. Infection by the digenean trematode *Ribeiroia ondatrae* represents one of the best-documented cases of parasitism that induces structural malformations in vertebrates, particularly in amphibians of the orders Anura and Caudata. Since the late 1990s, multiple outbreaks of frogs and salamanders with severe anomalies in the western United States have generated

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intense research that linked these anomalies to infection by this complex life-cycle parasite [2–4]. The relevance of this review lies in the fact that the phenomenon of malformations in amphibians is neither an isolated nor a historically recent event. Therefore, the purpose of this review is to synthesize the current knowledge on the *Ribeiroia*-amphibian system, integrating information from the molecular and cellular mechanisms of parasitic teratogenesis to the ecological and evolutionary consequences at the levels of communities and landscapes, while providing a conceptual framework to guide future research and conservation strategies.

Ribeiroia ondatrae is a trematode of the family Psilostomatidae whose obligatory life cycle includes three hosts: freshwater snails of the family Planorbidae as the first intermediate host, amphibian larvae or fish as the second intermediate host, and piscivorous birds or mammals as definitive hosts, where the parasite reaches sexual maturity (Figure 1) [5,6]. Infective cercariae emerge from the snails and actively penetrate the tissues of tadpoles, forming metacercarial cysts primarily around the developing limb buds, the cloacal region, and the lower jaw [7,8]. Unlike other trematodes that cause inflammatory or metabolic pathologies, *R. ondatrae* has the unique ability to alter host morphological development during critical windows of organogenesis, generating a spectrum of anomalies ranging from protuberances to structural deviations (including supernumerary limbs, fusions, and reductions) [3,9,10]. This specificity in the induction of structural malformations, combined with the parasite's dependence on multiple host species to complete its life cycle, makes the *Ribeiroia*-amphibian system an exceptional model for studying disease ecology, the evolution of parasite virulence, and comparative teratology.

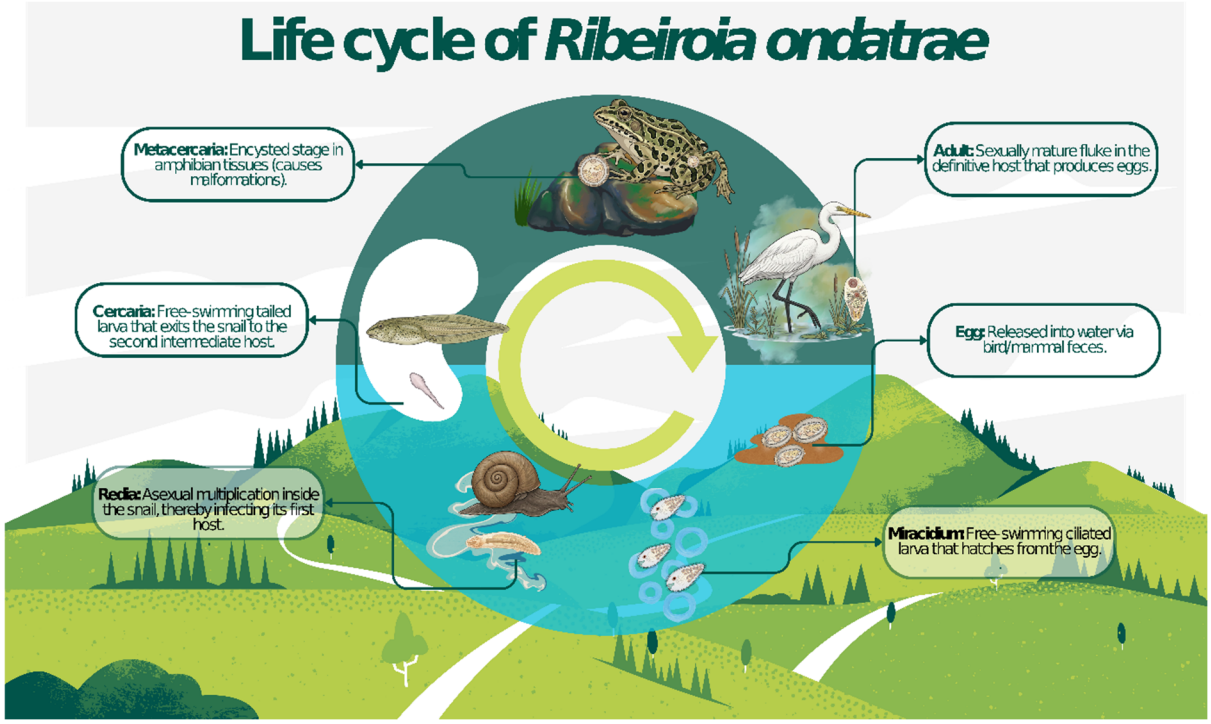


Figure 1. Life cycle of the digenean trematode *Ribeiroia ondatrae*. The parasite requires three hosts to complete its life cycle: planorbid snails (first intermediate host), amphibians (second intermediate host), and piscivorous birds or mammals (definitive host). The metacercariae encysted in amphibian tissues are responsible for the limb malformations. Adapted from Johnson, et al. [6], Roberts, et al. [11], and Hernandez-Caballero, et al. [12]. Note: The graphic elements and vector illustrations in this diagram were generated using artificial intelligence with Gemini (v3.6 Flash, Google) and subsequently assembled and edited using the Canva design platform.

Museum specimen studies have shown that *R. ondatrae* was infecting amphibians since at least the 1940s; however, the frequency and severity of outbreaks have increased dramatically in recent decades, reaching prevalences of 50–90% in some western North American populations [13,14]. This increase has been correlated with the eutrophication of water bodies, extensive use of agrochemicals, the creation of artificial ponds that favor intermediate hosts, and climate changes that disrupt the phenological synchrony between parasites and hosts [15–17]. Understanding how these environmental factors interact with the parasite's biology is essential not only to explain the emergence of the disease, but also to predict its evolution under global change scenarios and to design management interventions that do not compromise the integrity of aquatic ecosystems.

From a theoretical perspective, the *Ribeiroia*-amphibian system challenges traditional conceptions of parasitism by illustrating how a parasite can act as a biological teratogen, actively manipulating host development

to produce a phenotype that enhances its own transmission [18,19]. The induced malformations reduce the host's locomotion, foraging, and escape abilities, thereby increasing its vulnerability to predation by aquatic birds, which serve as the parasite's definitive hosts [19]. This manipulation of the extended phenotype raises fundamental questions about the evolution of virulence, host-parasite coevolution, and the boundaries between pathology and adaptation [20,21]. Furthermore, comparisons of *R. ondatrae* with other teratogenic trematodes (such as *Strigea robusta* in Europe, *Acanthostomum burminis* in Sri Lanka, and *Clinostomum* spp. in North America) reveal patterns of evolutionary convergence across different geographic regions and host groups (fish and amphibians), enriching our understanding of evolutionary developmental biology (evo-devo) and comparative teratology [12,22].

2. Anatomical Patterns of Malformations Induced by *Ribeiroia ondatrae*

The morphological anomalies induced by *R. ondatrae* represent a spectrum of developmental alterations that range from molecular and cellular disturbances to visible structural malformations (Figure 2). Unlike post-developmental acquired deformities (such as traumatic lesions caused by sublethal predation in already-developed tadpoles, which produce irreversible damage but do not alter developmental programs), malformations caused by *R. ondatrae* originate during embryonic or larval organogenesis and require the presence of the parasite during critical windows of limb development. Once metamorphosis is complete, exposure no longer induces structural alterations of the limbs, although it can still cause infection in other tissues [2,6,7,10]. This temporal specificity fundamentally distinguishes *R. ondatrae* from other agents that cause anomalies in amphibians, such as dragonfly nymphs that provoke limb loss through sublethal predation on already-developed tadpoles [23].

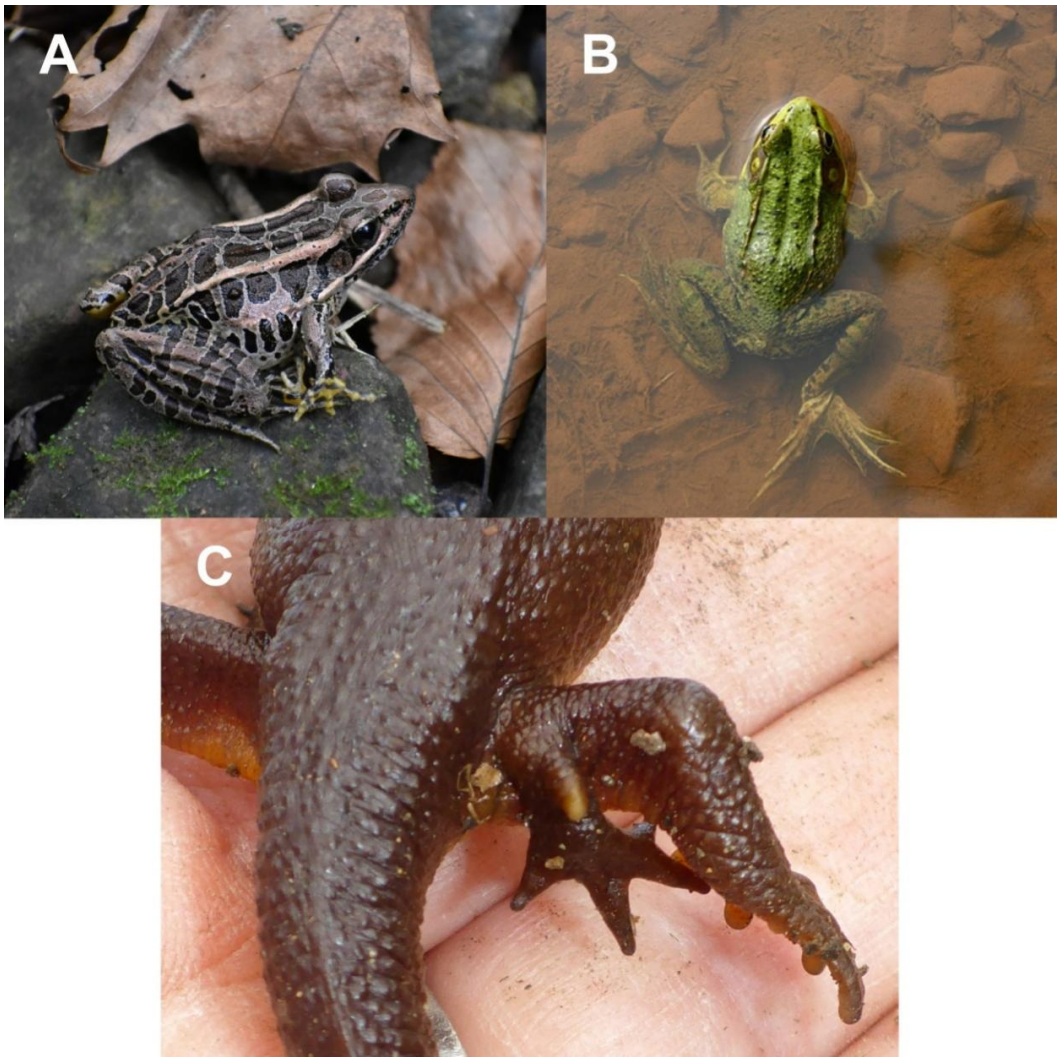


Figure 2. Macroscopic evidence and taxonomic variability of malformations observed under wild conditions, recorded on the iNaturalist platform. (**A**) Unilateral polymely in a pickerel frog (*Lithobates palustris*; Photo: Brenna Farrell, observation 59932345). (**B**) Severe unilateral polymely in a northern leopard frog (*L. pipiens*; Photo: Rick Koval, observation 198101774). (**C**) Polymely in the hind limbs of a California newt (*Taricha torosa*; Photo: Robin Gwen Agarwal, observation 152169396).

The spectrum of macroscopic anomalies induced by *R. ondatrae* has been quantified in multiple field and experimental studies, revealing consistent patterns in their anatomical distribution and relative frequency (Table 1). In anurans, approximately 94% of the anomalies affect the hind limbs, which is explained by the proximity of these limb buds to the aquatic environment during the larval phase, whereas the forelimbs develop protected within the branchial cavity. In urodeles, the pattern differs, with 41.9% of anomalies in the forelimbs and 56.9% in the hind limbs, because caudates develop all four limbs externally and use them actively in locomotion [3,9,24]. Head anomalies (anophthalmia, mandibular hypoplasia) and generalized anomalies (wounds, edema) represent less than 5% of the total and may be related to other factors, although their presence in infected animals suggests that the parasite can affect multiple tissues when the infective load is high or infection occurs at very early stages [24,25]. Anomalies of the autopod are more difficult to detect than those affecting the zeugopod or stylopod, but are equally indicative of distal developmental disruption [3,9,26]. Anomalies of the zeugopod and stylopod are the most conspicuous and have been extensively documented; the severity index, defined as the average number of anomalies per affected frog, increases directly with the dose of cercariae, rising from 1.77 in light treatments to 2.54 in heavy treatments, demonstrating a clear dose-response relationship [9,10].

Table 1. Description, anatomical location, and reported host species for the morphological anomalies associated with *Ribeiroia ondatrae* infection in amphibians.

Anomaly	Description	Typical Location	Relative Frequency	Host Species
Anophthalmia	Absence of one or both eyes	Head	<5%	*Pseudacris regilla*, *Lithobates pipiens* [24].
Mandibular hypoplasia	Underdevelopment of the lower jaw	Head	<5%	
Wounds	Lesion where the skin or other tissue is broken, cut, torn, or damaged	Variable	<5%	Anurans of Western North America (Commonly reported in the field) [24].
Edema	Swelling caused by excess fluid trapped in body tissues	Generalized	<5%	
Ectrodactyly	Absence or malformation of one or more central digits	Autopodium	Variable	As described by Henle et al. [26], these malformations generally affect the studied anurans (*P. regilla*, *L. pipiens*, *A. boreas*) and are particularly notable in the forelimbs of the long-toed salamander (*Ambystoma macrodactylum*).
Syndactyly	Condition in which two or more digits are webbed or fused	Autopodium	Variable	
Polydactyly	Presence of extra digits	Autopodium	Variable	
Brachydactyly	Abnormally short digits due to underdevelopment of the bones (phalanges, metacarpals, or metatarsals)	Autopodium	Variable	
Polyphalangy	Presence of multiple phalanges	Autopodium	Variable	
Supernumerary bones	Additional bone pieces that are not part of the standard skeletal anatomy	Variable	Variable	
Amely	Complete absence of one or more limbs	Zeugopodium/ Stylopodium	Variable	Reported by Schotthoefer, et al. [10] in *L. pipiens*.
Micromely	Abnormally small, short, or underdeveloped arms or legs	Zeugopodium/ Stylopodium	Variable	
Apody	Congenital absence of feet	Autopodium	Variable	
Ectromely	Absence or severe imperfection of one or more limbs	Zeugopodium/ Stylopodium	4.7% (exp.)	*Anaxyrus boreas* [9].
Hemimely	Partial or total absence of the distal (lower) half of a limb	Zeugopodium/ Stylopodium	4.7% (exp.)	
Polymely	Presence of one or more supernumerary (extra) limbs attached to a body segment	Zeugopodium/ Stylopodium	15.5% (exp.)	
Taumelia (bony triangle)	Long bone in the limbs of tadpoles or frogs that bends backward on itself, frequently creating a sharp angle	Zeugopodium/ Stylopodium	14.7% (exp.)	
Polypody	Presence of extra feet	Autopodium	4.4% (exp.)	
Femoral projection	Presence of a finger-like projection	Zeugopodium/ Stylopodium	Variable	Reported in multispecific field complexes by Johnson et al. [3] and Johnson et al. [27], affecting *P. regilla* and *L. pipiens*.
Limb hyperextension	When a joint (such as the knee or elbow) bends beyond its normal range in the opposite direction	Zeugopodium/ Stylopodium	Variable	

Note: Relative frequencies marked as "(exp.)" correspond to the percentages obtained under controlled experimental conditions by Johnson, et al. [9] using *A. boreas* larvae. Anomalies categorized as "Variable" derive from ecological studies and multispecies field surveys, in which quantification varies depending on parasite burden and locality. The embryological and cellular classification criteria are based on Kumar, et al. [28] and Langman [29].

The mechanisms underlying these anomalies have been investigated at multiple levels of biological organization. The mechanical disruption hypothesis, proposed by Sessions and Ruth [4], is based on experimental evidence in which the implantation of small 200 μm beads into the limb bud tissues of long-toed salamander and Pacific treefrog larvae (*Ambystoma macrodactylum* and *Pseudacris regilla*) induced deformities similar to those caused by the parasite, including supernumerary limbs, cutaneous webbing, and bifurcations. This demonstrated that physical obstruction within the limb bud can reorganize dividing cells and cause them to lose their spatial organization [4]. The cercariae of *R. ondatrae* penetrate host tissues using proteolytic enzymes (serine proteases, collagenases, elastases, and hyaluronidases) and preferentially encyst in the regions of developing limb buds, where the metacercariae form cysts approximately 200 μm in diameter that act as physical obstructions interfering with normal morphogenesis [6,7].

The second mechanistic hypothesis, proposed by Szuroczki et al. [8], posits that *R. ondatrae* alters retinoic acid signaling during limb development. Using high-performance liquid chromatography and mass spectrometry, these authors demonstrated that limb bud tissues of parasitized wood frogs (*L. sylvaticus*) contained 70% more retinoic acid than uninfected controls, and that free-living parasite stages and recently infected individuals contained approximately 56% more retinoic acid than encysted organisms. Given that retinoic acid is a critical morphogen for the anteroposterior patterning and differentiation of limbs during embryonic development, a localized source of this compound could disrupt normal concentrations during the critical period of organogenesis, generating the observed duplications, fusions, and absences [8,22]. These two hypotheses are not mutually exclusive and likely act synergistically: mechanical disruption alters cellular spatial organization, while retinoic acid alteration perturbs molecular developmental signaling, with both effects converging to generate structural malformations [6].

At the cellular and tissue levels, infection triggers a localized inflammatory response that contributes to disruption of the limb bud microenvironment. In second intermediate fish hosts such as Lepomis macrochirus, infection is associated with acute hemorrhages in the lateral line and dermis at 36 h post-exposure, followed by progressive granulomatous inflammation that leads to the destruction of encysted metacercariae. Amphibians, however, do not achieve this effective clearance, and the persistent inflammatory response disrupts normal development [6]. Histologically, the metacercariae measure approximately 200 μm in diameter with a 10 μm thick eosinophilic tegument, and their mechanical and biochemical presence in the bud tissue alters cell proliferation and differentiation [6]. Although the retinoic acid results of Szuroczki, et al. [8] are robust, it remains intriguing to consider how the parasite affects other key limb patterning genes such as *Hox*, *shh*, *fgf8*, and *Wnt*—a molecular research line that is still to be explored.

The dependence of these anomalies on a critical developmental window has profound implications for infection dynamics under natural conditions. Tadpoles of *P. regilla* and *Lithobates pipiens* exposed to *R. ondatrae* cercariae during early stages, before or during limb organogenesis, are much more prone to severe malformations or mortality, whereas exposure at later stages mainly results in sublethal infection without structural alterations [2,10]. In laboratory trials, exposure during early stages caused 72.5% mortality, while exposure during limb growth led to the development of structural limb malformations in 16% of surviving individuals, demonstrating that the timing of infection determines the type and severity of the pathology [10]. This critical window also explains why the induced anomalies are permanent and irreversible once metamorphosis is complete, as limb tissues no longer possess regenerative capacity or structural remodeling ability [26,30]. Consequently, congenital malformations caused by *Ribeiroia* represent a developmental debt that the host carries throughout its post-metamorphic life, irreversibly compromising its survival and reproduction.

3. Host-Parasite Interactions: Susceptibility, Resistance, and Phenotypic Manipulation

One of the most revealing findings in the study of the *Ribeiroia*-amphibian system is that not all host species respond equivalently to infection, exhibiting patterns of susceptibility and resistance that reflect millions of years of coevolution. Johnson and Hartson [24] demonstrated that while nearly 60% of emerging American Toads (*Anaxyrus americanus*) exhibited severe malformations, less than 4% of Gray Treefrogs (*Dryophytes versicolor*) from the same pond presented anomalies, despite the fact that in some cases they harbored the same number of cysts. On average, toads harbored 75 times more metacercariae than metamorphosing treefrogs, indicating that the resistance of *D. versicolor* operates primarily at the level of preventing infection rather than through pathological tolerance [24].

The immunological mechanisms of this resistance have been elucidated through studies combining pharmacological immunosuppression and in vivo tracking of marked parasites. LaFonte and Johnson [31] used an in vivo tracking assay with fluorescently labeled parasites over 72 h, finding that cercariae were approximately

25% less successful at penetrating larvae of three hylid species (*D. versicolor*, *D. gratiosus*, and *D. cinereus*), and were cleared more than 45 times faster than in the susceptible species *P. regilla*. Median parasite survival was 10.3 to 14.0 h in hylids, compared with 26.9 days in *P. regilla*. Immunosuppression through chronic exposure to corticosterone dramatically reduced this resistance and increased infection (from 67% to 190%), correlating with a 62% reduction in circulating eosinophils, suggesting that these white blood cells play a central role in defense against the parasite [31].

The specificity of *D. versicolor* resistance is particularly notable because it is not generalized immunity. Johnson and Hartson [24] observed that gray treefrogs did not show the same resistance against echinostomes, which were more abundant in their kidneys than in those of toads from the same pond. LaFonte and Johnson [31] confirmed that *D. versicolor* exhibits significantly greater resistance to *Ribeiroia* than to other trematodes such as *Echinostoma* and *Alaria*. This pattern suggests the existence of specific defensive mechanisms against *R. ondatrae* (possibly associated with the skin or subcutaneous tissues, given that *R. ondatrae* encysts subcutaneously while other trematodes use different infection routes). This is supported by the finding that cercariae were 25% less successful at penetrating hylids and were cleared 45 times faster than in *P. regilla*, with median parasite survival of only 10.3–14.0 h versus 26.9 days in the susceptible species. The initial hypothesis that this resistance might be due to its tetraploid genome was partially ruled out when comparably strong resistance was documented in other non-tetraploid hylid species (*D. gratiosus* and *D. cinereus*), indicating that the explanation lies in specific immunological mechanisms or dermal defenses [24,31]. Calhoun et al. [32] investigated the role of antimicrobial peptides and found that although *D. versicolor* acquired fewer metacercariae than *P. regilla*, treatment with norepinephrine increased infection in *D. versicolor*, with a smaller magnitude of damage compared to the overall difference between the two species. This suggests that antimicrobial peptides are probably not the sole mechanism of resistance and that additional dermal defenses are involved, possibly associated with unique compounds such as the peptide hylaseptin P1 [31,32].

The differential resistance of host species has profound ecological consequences that extend beyond the individual level. Johnson, et al. [33] experimentally demonstrated the dilution effect in this system: American Toad tadpoles (*Anaxyrus americanus*) raised together with *D. versicolor* tadpoles (a resistant species) exhibited 37% lower infection and fewer malformations than when raised alone. This occurs because cercariae have a substantially lower capacity to locate and infect *D. versicolor* larvae and waste infection attempts on non-competent hosts [24,33]. This effect, originally proposed by Ostfeld and Keesing [34] for vector-borne diseases, operates here by reducing the probability of effective contact between the parasite and susceptible hosts. It has important implications for conservation: the loss of biodiversity that eliminates resistant species from aquatic ecosystems can indirectly increase transmission to more susceptible species, creating a link between biodiversity decline and disease emergence [13,33].

In parallel with interspecific variation in susceptibility, *R. ondatrae* has evolved the ability to alter the phenotype of its hosts in ways that increase the probability of transmission to the definitive host, representing one of the clearest examples of parasitic behavioral manipulation in vertebrates. Goodman and Johnson [19] documented that infected frogs exhibited shorter jumps (41%), slower swimming speeds (37%), reduced endurance (66%), and lower foraging success than uninfected frogs. In natural populations, deformed frogs had lower biweekly survival (22%) and rarely recruited into the adult population over a two-year period. Beyond these physical limitations, the parasite induces specific behavioral changes that increase vulnerability to predation: infected frogs waited longer before escaping simulated predation, allowed the artificial predator to approach more closely, used terrestrial microhabitats more frequently than vertical refuges, and perched closer to the ground representing more open habitats with higher risk of predation by snakes and aquatic birds such as herons [19]. These behavioral changes are not merely mechanical consequences of the malformations but appear to constitute an adaptive strategy of the parasite to increase its transmission, meeting the criteria of the parasite manipulation hypothesis: they are complex, show signs of purposeful strategy, have arisen in multiple trematode lineages, and increase parasite fitness by facilitating trophic transmission to the definitive host [12,19,21].

Phenotypic manipulation by *R. ondatrae* extends beyond immediate escape behavior to include alterations in thermoregulation and habitat use that have cascading effects on host metabolism and immune response capacity. Infected frogs experienced greater difficulty occupying thermal refuges, maintaining higher body temperatures that compromise locomotor performance and escape ability. This creates a vicious cycle in which infection reduces physical fitness, limiting access to optimal microhabitats, which in turn increases vulnerability to predation and reduces investment in immune defenses [19]. This phenomenon fits within the extended phenotype framework [18], where parasite genes are expressed through the host's phenotype to maximize their own transmission. From an evolutionary perspective, host manipulation raises the question of why amphibians have not evolved more effective defenses against this strategy. The answer likely lies in the evolutionary costs of maintaining constant

immunological resistance against a parasite whose presence is spatially and temporally variable, combined with fluctuating selection pressure in which rare genotypes have a fitness advantage [31,35].

Finally, the interaction among differential susceptibility, the dilution effect, and parasitic manipulation generates a complex ecological landscape in which amphibian community structure, the diversity of resistant and competent species, and predation pressure act synergistically to determine the prevalence and severity of malformations at the landscape scale. The presence of resistant species such as *D. versicolor* can indirectly protect susceptible species by reducing effective transmission, but this protection erodes when biodiversity declines or when environmental conditions favor massive cercarial production that exceeds the community's dilution capacity [13,33].

4. Environmental Factors and Disease Ecology: The Ecosystem Context of *Ribeiroia ondatrae*

The emergence and amplification of *R. ondatrae* infections cannot be understood in isolation from the environmental conditions that modulate each link in its complex life cycle. Johnson and Chase [13] established one of the most robust links between environmental disturbance and parasitic disease by demonstrating that the eutrophication of freshwater bodies is directly correlated with the emergence of *R. ondatrae* and the subsequent induction of malformations in amphibians. The underlying mechanism is indirect but powerful: enrichment with nitrogen and phosphorus (derived primarily from agricultural runoff, urban effluents, and atmospheric deposition) stimulates the proliferation of algae and aquatic macrophytes, which in turn provide abundant food and shelter for populations of planorbid snails, particularly genera such as *Helisoma* and *Planorbella*, which serve as the first intermediate hosts of the parasite [13,15]. In a field experimental study in Montana, Johnson et al. [15] directly manipulated nutrient availability in natural ponds and found that nitrogen and phosphorus enrichment increased the density of infected snails and cercarial production per snail by approximately one order of magnitude, resulting in malformation prevalences exceeding 50% in Pacific treefrogs (*P. regilla*), compared to less than 5% in non-enriched ponds. This finding establishes causally that eutrophication of aquatic ecosystems acts as an ecological amplifier of parasitic disease in amphibians.

The interaction between *Ribeiroia* infection and chemical contamination, particularly agricultural pesticides and herbicides, represents a paradigmatic example of synergy between biological and chemical stressors that exceeds the additive effects of each factor alone. Kiesecker [16] reported one of the first documented cases of synergism in environmental ecotoxicology: tadpoles exposed to both *Ribeiroia* cercariae and sublethal levels of pesticides showed higher infection rates and reduced immune responses compared to amphibians exposed to the parasite alone. Field experiments indicated a direct link between agricultural runoff and increased infection, while laboratory studies demonstrated that pesticide exposure reduced host immunocompetence as the underlying biological mechanism. Rohr et al. [17] extended these findings by showing that nutrient addition and pesticide contamination can jointly lead to increased infections by simultaneously increasing exposure to cercariae (through higher densities of snail hosts) and suppressing host immune systems (through immunomodulatory toxicity), creating a "double-hit" effect that dramatically amplifies the impact of the parasite. The herbicide atrazine has been particularly implicated in this synergism due to its well-documented ability to weaken amphibian immune systems and alter endocrine regulation, making them more susceptible to *R. ondatrae* infections while simultaneously favoring eutrophic conditions [16,17].

Ultraviolet-B (UV-B) radiation constitutes another environmental factor that, although it does not directly cause malformations, modulates susceptibility to infection through well-documented immunosuppressive effects in amphibians. UV-B radiation, which has increased in intensity at temperate latitudes due to partial degradation of the stratospheric ozone layer, is known to cause direct DNA damage, induce oxidative stress, and suppress both innate and adaptive immune responses in amphibian larvae [36]. However, in large-scale field studies covering parts of California, Oregon, Washington, Idaho, and Montana, Johnson et al. [3] found that none of the measured water quality variables (including water transparency as an indirect proxy for UV-B exposure) were directly associated with malformed amphibians. Instead, the presence of snail hosts (*Planorbella* spp.) was the strongest predictor of infection presence and abundance. This finding suggests that, although UV-B may be a relevant cofactor in specific systems with high water clarity and low vegetation cover, its effect is probably subordinate to eutrophication and agrochemical contamination in most field scenarios [3,36].

Global climate change emerges as a transversal driver that affects multiple components of the *Ribeiroia*-amphibian system simultaneously but unevenly, altering phenological synchrony between hosts and parasites. Higher temperatures could cause *R. ondatrae* cercariae to be released earlier in the breeding season, which would have critical implications because tadpoles exposed at earlier developmental stages are much more prone to severe pathology [10,37]. Although tadpoles are also likely to accelerate their growth in response to warming, parasites are generally expected to increase their development rates even more rapidly due to their smaller body sizes and

higher metabolic rates, following the rules of metabolic biology [37]. Additionally, amphibian breeding timing is typically co-controlled by temperature and precipitation, suggesting that they may be less plastic in their ability to shift developmental timing than parasites with shorter generation times [37]. A documented outbreak in August 2022 in Colorado (the most severe case reported in the state in over 70 years, with approximately 68% of *L. pipiens* malformed) was potentially attributed to unusual drought conditions that may have driven migratory birds eastward in search of more stable water sources, increasing the activity of infected birds in small ponds where snail hosts proliferated under conditions of high productivity [27]. This case illustrates how extreme climatic events can alter the distribution and abundance of definitive hosts, creating infection hotspots in regions historically free of severe outbreaks (Figure 3).

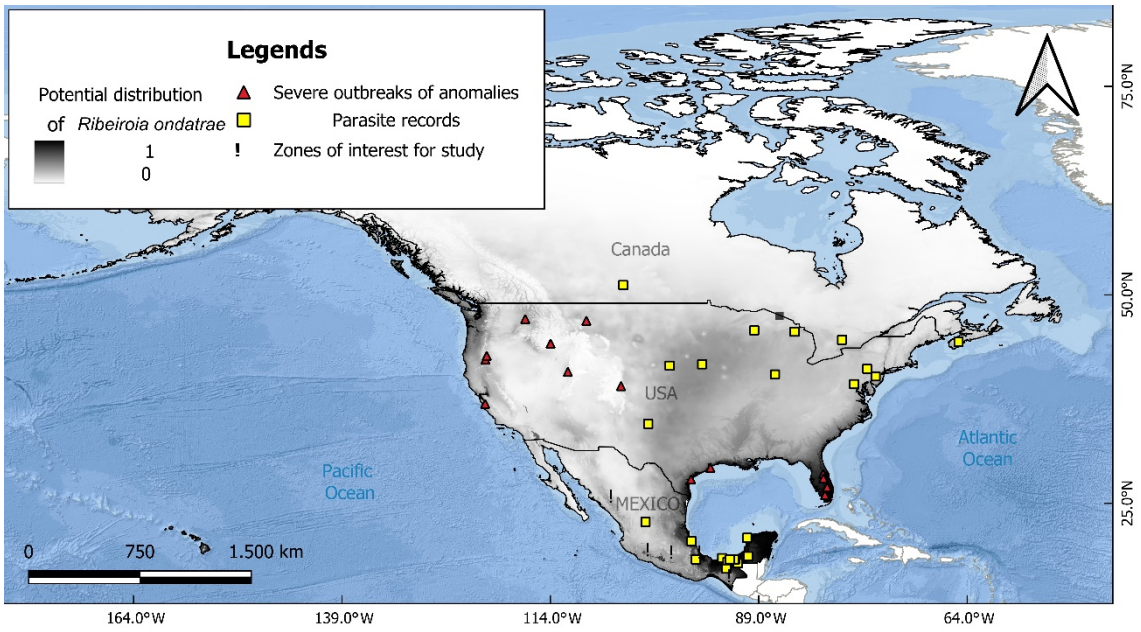


Figure 3. Geographic records and potential distribution of *Ribeiroia ondatrae* in North America. Dots indicate localities with confirmed presence of the parasite and sites where malformation outbreaks coincide with those records [3,27,38]. The gray shading represents the potential distribution model of the trematode generated with Maxent [39] using the following variables extracted from WorldClim [40]: Elevation, BIO1 (Annual mean temperature), BIO4 (Temperature seasonality), BIO12 (Annual precipitation), and BIO15 (Precipitation seasonality).

The activity of definitive host birds constitutes a crucial determinant of the parasite's continental distribution. Johnson et al. [6] noted that the distribution of *R. ondatrae* and amphibian malformations is positively correlated with the major bird migratory routes in North America. The relative rarity of infection in the Intermountain West of the United States may derive from the relatively low abundance of migratory birds in this arid region, compared to the Pacific, Mississippi, and Atlantic flyways where the concentration of waterbirds is higher [37]. This dependence on bird mobility introduces an element of spatial stochasticity into disease dynamics: a pond may remain free of *Ribeiroia* for years until a flock of infected birds temporarily lands, initiating an outbreak that can persist as long as local snail hosts maintain the infection [6,7].

The structure of the aquatic community modulates the transmission of *R. ondatrae* through multiple pathways, including competition, predation, and facilitation among species. Infected planorbid snails exhibit a phenomenon of "parasitic gigantism," growing at rates twice as high as uninfected snails at 26 °C. This confers a competitive advantage under conditions of high productivity, but also makes them more visible to predators [15]. Lunde et al. [7] conducted an ecosystem-scale manipulation by dividing a 1600 m^2 pond with an impermeable barrier and adding more than 500 infected snails to one side. They observed sharp increases in infection and severe malformations in *P. regilla* only on the manipulated side during the year of the intervention; however, this effect disappeared the following year. This indicates that natural ponds have a resilience capacity that small-scale experiments do not fully capture. This finding has critical implications for extrapolating experimental results to real-world conservation scenarios: effects observed in mesocosms may overestimate the severity and persistence of outbreaks in more complex and connected natural ecosystems.

Finally, the synergistic interaction among multiple environmental factors (eutrophication, chemical contamination, climate change, community structure, and migratory bird dynamics) creates a "multiple stressor

syndrome" scenario in which predicting outbreaks requires the simultaneous consideration of all these components. Rohr et al. [17] demonstrated that nutrient addition and pesticide exposure act synergistically to increase *Ribeiroia* infection, with a combined effect greater than the sum of their individual effects. This greatly complicates environmental management efforts because reducing a single factor may be insufficient to control the disease. Habitat degradation, the introduction of invasive species that alter the trophic structure of wetlands, and the loss of biodiversity of snail predators can all contribute indirectly to the emergence of *R. ondatrae* as a dominant pathogen in disturbed ecosystems [37,41]. Understanding these complex ecosystem interactions is indispensable for designing mitigation strategies that go beyond direct control of the parasite or its intermediate hosts, focusing instead on the restoration of wetland ecological integrity as the most sustainable long-term intervention.

5. Synthesis: Evolutionary Implications, Conservation, and Future Directions

The *Ribeiroia*-amphibian system illuminates fundamental processes in the evolution of parasitism that transcend its relevance as a case study. The ability of *R. ondatrae* to induce structural malformations that increase host vulnerability to predation constitutes one of the most robust examples of the extended phenotype [18,19]. However, its theoretical importance lies in how this manipulation integrates with variation in susceptibility among hosts and the environmental factors that modulate transmission. Comparison with other teratogenic trematodes (*Strigea robusta* in Europe, *Acanthostomum burminis* in Sri Lanka, and *Clinostomum* spp. in North America) reveals evolutionary convergence, in which independent lineages have developed the capacity to alter limb development in vertebrates. This suggests convergent selection toward host manipulation as a transmission strategy [6,12]. This convergence enriches the field of comparative teratology and provides a window for studying how external perturbations can interact with conserved developmental regulatory networks (retinoic acid pathways, *Hox* genes) to produce phenotypes that benefit the inducing agent [8,22].

The coevolutionary dynamics between *R. ondatrae* and its hosts challenge simplified models of a pure arms race. Stutz et al. [35] applied the theoretical resistance-tolerance framework to animal-parasite interactions and found that parasite infection success varied from 8% to 100% among genetic families of California newts (*Taricha torosa*), but with no evidence of heritable variation in resistance or tolerance. This finding suggests that opportunities for the evolution of these traits are limited, likely requiring significant increases in mutation, gene flow, or environmental heterogeneity [35]. The specific resistance of *D. versicolor* (mediated by eosinophils and possible dermal defenses [31]) represents a host adaptation that imposes evolutionary costs and is maintained under parasitic selection pressure. However, fluctuating selection (where rare genotypes have a fitness advantage and frequencies oscillate over time) may be more relevant than a directional arms race, particularly given that parasitic selection pressure varies spatially and temporally with the distribution of snail hosts and the activity of migratory birds [6,35]. The evolution of virulence in *R. ondatrae*, a macroparasite that does not reproduce within the amphibian host, is linked to host life history and infection intensity: the probability of mortality or malformation increases monotonically with the number of cercariae, but transmission to the definitive host depends on the amphibian surviving long enough to be predated [2,42]. This tension between virulence and transmission constitutes a fertile ground for theoretical models that integrate host community structure as a modulating variable.

The implications for amphibian conservation are immediate. *Ribeiroia* infections can strongly affect individual performance and population viability: although parasite-induced malformations can affect a large fraction (20–100%) of newly metamorphosed frog populations, few malformations are observed in adult frogs returning to breed. This suggests that such anomalies are highly detrimental to survival, creating a "malformation debt" that manifests as massive post-metamorphic mortality [6,19]. At Hog Lake, multi-year sampling indicated that malformed frogs were unlikely to return as reproductive adults [7]. The 2022 outbreak in Colorado (~68% of *L. pipiens* malformed, a species in the highest conservation priority category) provides compelling evidence that parasitic infection can impact already declining populations [27]. Given that the distribution of *Ribeiroia* is spatially variable, impacts at a single wetland could be alleviated by migration from nearby parasite-free sites i.e., a "rescue effect" that critically depends on landscape connectivity [6,7].

Wetland management emerges as the most practical front where scientific knowledge can be translated into conservation actions, but also as a terrain full of ecological dilemmas. Constructed wetlands (stormwater ponds, livestock ponds) can function as "ecological traps" if they provide reproductive habitat for amphibians but impose a high *Ribeiroia* burden due to eutrophic conditions that favor planorbid snails [7]. These ponds often present conditions of high productivity that indirectly increase the risk of infection, creating a paradoxical conflict between habitat creation and disease emergence. Mitigation strategies that reduce eutrophication (vegetated buffer zones that filter nutrients, restoration of riparian vegetation that provides shade and reduces water temperature, and

maintenance of habitat corridors) can be more effective and sustainable than direct interventions against the parasite or its intermediate hosts [13,15]. The induced malformations have potential as bioindicators of ecosystem health, but their use requires standardization: a robust approach would combine malformation monitoring with detection of *R. ondatrae* in snails via PCR, eDNA analysis of water samples, and evaluation of water quality variables that reflect the trophic status of the ecosystem [3,43].

Despite more than two decades of intensive research, critical gaps persist that limit our ability to predict and mitigate the impacts of *R. ondatrae*. At the mechanistic level, the exact molecular pathway of malformation induction remains uncertain: although the mechanical disruption and retinoic acid alteration hypotheses are consistent with experimental evidence, it has not been causally demonstrated how these perturbations translate into the specific patterns of duplication, fusion, and absence observed, nor has the role of other key developmental genes such as *Hox*, *shh*, *fgf8*, and *Wnt* been systematically evaluated [6,8]. At the geographic level, the almost exclusive restriction of severe outbreaks to North America, despite the presence of related parasites in the Caribbean, South America, and Africa, remains an enigma: is it due to differences in parasite species, susceptibility of local hosts, the structure of aquatic communities, or simply a bias in research effort? [6,42]. At the ecological level, the real long-term impact on the population dynamics of threatened species has not been rigorously quantified: although malformations clearly reduce individual survival, it is unknown how this mortality compares with other sources of recruitment loss such as predation, competition, and habitat loss, and whether populations can compensate for the loss through increased fecundity or improved adult survival [27,35].

Finally, at the applied level, there is an urgent need for mitigation strategies that balance parasite control with the conservation of parasitic biodiversity and ecosystem integrity. Parasites, including *R. ondatrae*, play important ecological roles as population regulators, indicators of environmental health, and essential components of biodiversity; therefore, their indiscriminate elimination is neither desirable nor feasible [37]. Instead, interventions should focus on reducing the environmental factors that amplify transmission, particularly eutrophication and agrochemical contamination, through watershed management practices that minimize nutrient and pesticide runoff, restoration of riparian zones that filter contaminants and provide habitat for natural snail predators, and the design of constructed wetlands that avoid high-productivity conditions [15,17]. The integration of molecular, ecological, and evolutionary approaches will be indispensable for addressing these questions and for translating scientific knowledge into effective conservation strategies for amphibians a taxonomic group that faces multiple converging threats in the Anthropocene and serves as a sensitive indicator of the health of aquatic ecosystems worldwide [1].

Author Contributions

H.A.C.-B. and D.R.A.-G.: Conceptualization; D.R.A.-G. and M.M.-T.: Data curation and analysis; H.A.C.-B. and D.R.A.-G.: Investigation and methodology; D.R.A.-G.: Original draft preparation; H.A.C.-B., M.M.-T. and D.R.A.-G.: Review and editing of the draft. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Acknowledgments

We thank the users of the iNaturalist citizen science platform (Brenna Farrell, Rick Koval, and Robin Gwen Agarwal) for allowing us to use their valuable photographs of malformed amphibians. Their observations have been fundamental for illustrating the macroscopic impacts discussed in this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used Claude (Sonnet 5.5, Anthropic, San Francisco, CA, USA) to improve the writing style, syntax, and clarity of the text and to eliminate redundancies, and Grok (4.5, xAI, Palo Alto, CA, USA) for the translation of the manuscript into English, which was verified by all authors. The graphic elements and vector illustrations of Figure 1 were generated using Gemini (v3.6 Flash, Google, Mountain View, CA, USA). AI tools were not involved in the conceptual development of the study, the analysis, or the interpretation of results. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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