# Pathogens in anurans from a seasonal tropical environment in Guanajuato, Mexico

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Leyte–Manrique, A., Alejo–Iturvide, F., Aguillón–Gutiérrez, D. R., Ochoa–Ochoa, L. M., Rodríguez–Gutiérrez, M. F., 2023. Pathogens in anurans from a seasonal tropical environment in Guanajuato, Mexico. *Animal Biodiversity and Conservation*, 46.1: 35–46, Doi: https://doi.org/10.32800/abc.2023.46.0035

**Abstract**

*Pathogens in anurans from a seasonal tropical environment in Guanajuato*, *Mexico*. In the present study, we characterized species of bacteria and fungi across a frog community in a seasonal tropical environment in the Natural Protected Area (NPA) Las Musas, Guanajuato, Mexico, using morphological and biochemical protocols. Our three main objectives were to: i) identify the species of microorganisms present in the frog community, ii) determine microorganism prevalence and how it changes throughout the year, and iii) detect diseases and injuries caused by pathogens (bacteria and fungi). Across 150 anurans analyzed, seven species of anurans especies (*Anaxyrus compactilis*, *Incilius occidentalis*, *Dryophytes arenicolor*, *D. eximius*, *Hypopachus variolosus*, *Lithobates neovolcanicus* y *Spea multiplicata*) hosted at least four genera of pathogenic fungi (*Penicillium*, *Rhizopus*, *Aspergillus* y *Batrachochytrium*) and two genera of pathogenic bacteria (*Aeromonas hydrophila* and *Pseudomonas* sp.). We found the prevalence of positive cases was higher in autumn (0.51) than in summer (0.125). Lesions such as peeling of the skin on the belly and swelling of the extremities (red leg) were observed in four of the seven species. This study confirms the presence of bacterial and fungal pathogenic agents in this frog community, and serves as a precedent for future work on monitoring the state of amphibian population health in Guanajuato. It also provides support for the implementation of conservation strategies for this group of vertebrates.

Key words: Amphibians, Amphibian population health, Bacteria, Conservation, Fungi, Prevalence

**Resumen**

*Patógenos en los anuros de un ambiente tropical estacional en Guanajuato (México).* En el presente estudio se caracterizaron las especies de bacterias y hongos de una comunidad de ranas de un ambiente tropical estacional en el Área Natural Protegida (ANP) Las Musas, en Guanajuato (México), usando protocolos morfológicos y bioquímico–microbianos. Los objetivos principales fueron tres: identificar las especies de microorganismos presentes en la comunidad de ranas, determinar la prevalencia de microorganismos y cómo cambia a lo largo del año y detectar enfermedades y lesiones ocasionadas por patógenos (bacterias y hogos). De los 150 anuros analizados, siete especies (*Anaxyrus compactilis, Incilius occidentalis, Dryophytes arenicolor, D. eximius, Hypopachus variolosus, Lithobates neovolcanicus y Spea multiplicata*) hospedaron al menos cuatro géneros de hongos patógenos (*Penicillium, Rhizopus, Aspergillus y Batrachochytrium*) y dos géneros de bacterias patógenas (*Aeromonas hydrophila y Pseudomonas sp*). La prevalencia de casos positivos fue mayor en otoño (0,51) y menor en verano (0,125). En cuatro de las siete especies se observaron lesiones como la descamación en el vientre y la hinchazón en las extremidades (pata roja). Este estudio permite confirmar la presencia de bacterias y hongos patógenos en esta comunidad de anuros y sirve de precedente para trabajos futuros sobre el estado de salud de la población de anfibios en Guanajuato, así como para la implementación de estrategias de conservación para este grupo de vertebrados.

Palabras clave: Anfibios, Salud de las poblaciones de anfibios, Bacterias, Conservación, Hongos, Prevalencia

ISSN: 1578–665 X
eISSN: 2014–928 X

*Received: 23 II 22; Conditional acceptance: 20 V 22; Final acceptance: 19 XII 22*

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## Introduction

Alterations in environmental patterns and landscape elements cause microclimatic changes in temperature and precipitation that can give rise to emerging diseases such as chytridiomycosis caused by *Batrachochytrium dendrobatidis* in anurans, or *B. salamadrivorans* in salamanders (Lips et al., 2004; López–Velázquez, 2018). These fungi have had serious consequences on amphibian populations, with the former species being associated with massive mortality and population declines worldwide in tropical and temperate ecosystems (Johnson, 2006; Skerratt et al., 2007; Wake and Vredenburg, 2008; Vásquez–Ochoa et al., 2012; Scheele et al., 2020). What's more, bacterial diseases such as the well–known 'red leg' disease in anurans, caused by the bacterium *Aeromonas hydrophila*, are also causing mortality (Bosch, 2003).

Both pathogens can lead to the alteration of reproductive, ecological, and behavioral processes in amphibians (Lips et al., 2004; Luja et al., 2012; Vitt and Caldwell, 2014). In Mexico, the effects of landscape alterations combined with the onset of amphibian diseases undoubtedly have had repercussions on population sizes, species distributions, and diversity, considering that Mexico has 420 amphibian species, of which 257 are anurans (frogs and toads, both with high endemism; Frost, 2021).

Although pathogens pose a serious threat to amphibian biodiversity, knowledge regarding how they may affect species diversity is lacking, not only in tropical environments but worldwide (Biaggini et al., 2018; Scheele et al., 2020). Mexico is no exception to this general pattern. In an attempt to fill this gap, studies have characterized the morphological, biochemical, and molecular properties of bacterial and fungal pathogens in various amphibian populations and species throughout tropical and temperate Mexico (López–Velázquez, 2018). Most recent surveys have focused on fungal pathogens, specifically the detection of *B. dendrobatidis* and monitoring for the occurrence of *B. salamadrivorans* (Lips et al., 2004; Frías–Alvarez et al., 2008; Luja et al., 2012; Mendoza–Almeralla et al., 2015; López–Velázquez, 2018; Basanta et al., 2020).

In Mexico, *B. dendrobatidis* has been recorded in Baja California Sur, Baja California Norte, Jalisco, Michoacán, Guerrero, Oaxaca, Chiapas, Veracruz, Tabasco, Tamaulipas, Nuevo León, Sonora, the Federal District, the State of Mexico, Puebla, Querétaro, Hidalgo, Aguascalientes, Zacatecas and Durango (López–Velázquez, 2018). To date, *B. salamadrivorans* has not been detected in Mexico.

In the central Mexican state of Guanajuato, few studies have been performed on pathogens of in nurans. Rodríguez–Gutiérrez et al. (2017), however, recorded the presence of the bacteria *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas* sp., and *Salmonella typhimurium* in various reptile species from the lower basin of the Temascatio River, Irapuato, Gto. The presence of these pathogens in reptiles serves as a warning for the health status of amphibian species and the potential for fungal–type pathogens in the state, especially considering the presence of *B. dendrobatidis* in the neighboring states of Jalisco and Michoacán (López–Velázquez, 2018). Furthermore, amphibians are more sensitive than reptiles to microclimatic changes resulting from habitat alteration and contamination of water bodies that can promote the emergence of bacterial and fungal pathogens (Bosch, 2003; Lips et al., 2004). These changes have likely influenced the decline of populations whose causes of decline are often described as 'largely unknown'. Additionally, over time these amphibian mortalities modify species' distributions and result in a lack of data when researchers cannot find populations to survey while performing fieldwork.

Considering the reported distribution of *B. dendrobatidis* in seasonal tropical environments in several regions of Mexico (Mendoza–Almeralla et al., 2015), we hypothesized that within the Natural Protected Area (NPA) of Las Musas (a predominantly tropical deciduous forest environment) *B. dendrobatidis* and other potentially pathogenic fungi and bacteria may be present. The present work thus has three main aims: i) to identify the species of microorganisms present in the frog community; ii) to determine their prevalence and how it changes throughout the year; and iii) to detect diseases and injuries caused by bacterial or fungal pathogens.

## Material and methods

The Natural Protected Area (NPA) of Las Musas is located in the municipality of Manuel Doblado, Guanajuato (20º 37' 18'' N, 101º 54' 2'' W). Spanning 3,174.76 ha, the area has elevations ranging from 1,740–1,810 m. The climate is dry, and sub–humid, and has an annual average temperature of 20 ºC. Precipitation fluctuates from 700–800 mm per year on average (García, 2004). The most important water body is the Colorado River that runs 37.8 km (Walter and Brooks, 2009). The primary vegetation is tropical dry forest, gallery forest (along the riparian zone of the Colorado River), xerophytic scrub, induced grassland, and agricultural crops (Rzedowski, 1978).

### Specimen and data collection

Amphibian surveys and data collection were performed between December 2016 and October 2017 during 12 monthly field trips of three days each. We established 10 x 3,000 m transects across four vegetation types and included four replicate transects per vegetation type. To ensure data independency, transects were established at least 50 m away from one another (Vite–Silva et al., 2010). Target species were surveyed according to their habits and activity during two sampling times, diurnal (09:00–16:00 h) and nocturnal (20:00–24:00 h). Anurans were surveyed in water bodies, surrounding vegetation, and underneath tree trunks and rocks (Leyte–Manrique et al., 2018a, 2018b; Luria–Manzano et al., 2019). Encountered animals were captured with individual sterilized mesh fish nets, in accordance with Lips (1999), or directly

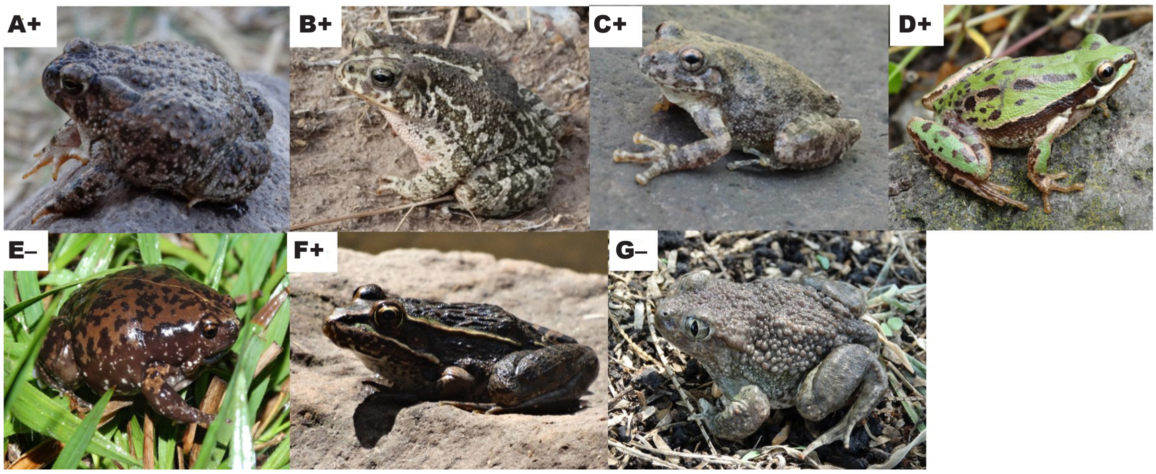


Fig. 1. Sampled species: A, *Anaxyrus compactilis;* B, *Incilius occidentalis*; C, *Dryophytes arenicolor*; D, *Dryophytes eximius*; E, *Hypopachus variolosus*; F, *Lithobates neovolcanicus*; G, *Spea multiplicata*. (The symbol following each letter indicates the presence (+) or absence (–) of microorganisms found in this study).

*Fig. 1. Especies estudiadas: A,* Anaxyrus compactilis*; B,* Incilius occidentalis*; C,* Dryophytes arenicolor*; D,* Dryophytes eximius*; E,* Hypopachus variolosus*; F,* Lithobates neovolcanicus*; G,* Spea multiplicata*. (El símbolo después de cada letra indica la presencia (+) o la ausencia (–) de los microorganismos encontrados en este estudio).*

by hand. Body temperatures of collected animals were recorded with a digital thermometer (capacity –10 to 80 ºC) to relate with their activity schedule. Specimens were identified in situ using amphibian identification guidebooks, and photographs were taken of each animal to generate an image bank of local species.

### Microbiological samples

We followed the sampling and handling protocols detailed in Lips (1999) and Vredenburg et al. (2010). Nitrile gloves were used while handling animals, with gloves being changed between each animal to avoid cross–contamination. Subjects were visually examined for lesions or signs of disease (e.g., excessive shedding, hyperkeratosis, hyperplasia, abnormal posture, lethargy, loss of reflexes, or anorexia) (Lips et al., 2005). Samples were taken from each specimen by using a sterile swab on the hands, thighs, and inguinal region. The samples were stored in vials at 4 ºC with 300 µL of 70 % ethanol. We assigned an identification acronym to each vial and added data regarding the species, life stage, sex, date, time, and microhabitat (Vázquez–Ochoa et al., 2012). After collecting data and swabbing samples, we released the specimens at the location where they were collected.

### Processing and characterization of bacteria and fungi

Microorganisms from the swab samples were isolated and characterized at the morphological (Berger et al., 1998; Daszak et al., 1999) and biochemical level. Samples were processed at the Laboratorio de Biología de los Institutos Tecnológicos Superior de Salvatierra, and at the Laboratorio de Química del Instituto Tecnológico Superior de Irapuato. For bacterial identification, swab samples were inoculated on blood–based agar and MacConkey agar plates and incubated at 37 ºC (Darling and Jones, 2012). Biochemical tests for *Aeromonas* bacteria were carried out using the Voges Proskauer test, Cromocen ® AE medium base, and gelatin agar following the technique proposed by Chinchilla–Magaña et al. (2014) and Aguillón–Gutiérrez et al. (2007). For fungal samples, swabs were inoculated on Sabouraud dextrose agar and incubated at 29 ºC. All isolates were identified using a conventional biochemical test identification system. For the isolation of *B. dendrobatidis*, 100 µl of samples were suspended in physiological solution and transferred to potato dextrose agar plates, Luria Bertani (LB) agar plates, and tryptone glucose (TG) liquid medium tubes (1 % tryptone, 0.3 % glucose), and incubated for 24 hours at 23 ºC before being transferred to a solid medium of TGhL agar (1.6 % tryptone, 4 % hydrolyzed gelatin, 0.5 % lactose, 1 % agar) supplemented with penicillin G and streptomycin sulfate. The fungal isolates obtained were morphologically characterized to genus level using conventional techniques, by referencing guidelines detailed in Bosch (2003), veterinary manuals (The Merck Veterinary Manual, 2018), and descriptions of zoospores detailed in Berger et al. (2005). Finally, prevalence was calculated following the protocol by Mendoza–Almerella et al. (2015), where the number of *B. dendrobatidis*

Table 1. Presence and prevalence of pathogens in five species of the anuran community: I, individuals analyzed; Po, positive; Pre, prevalence.

*Tabla 1. Presencia y prevalencia de patógenos en cinco especies de la comunidad de anuros: I, individuos analizados; Po, positivo; Pre, prevalencia.*

| Species | I | Po | Pre | Microorganisms |
|---|---|---|---|---|
| *Anaxyrus compactilis* | 40 | 5 | 0.12 | *Penicillium* sp., *Aeromonas hydrophila, Pseudomonas* sp. |
| *Incilius occidentalis* | 9 | 1 | 0.11 | *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp. |
| *Dryophytes arenicolo*r | 18 | 6 | 0.33 | *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp., *Aeromonas hydrophila, Pseudomonas* sp. |
| *Dryophytes eximius* | 47 | 20 | 0.42 | *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp., *Batrachochytrium dendrobatidis, Aeromonas hydrophila, Pseudomonas* sp. |
| *Hypopachus variolosus* | 1 | 0 | 0 | None |
| *Lhitobates neovolcanicus* | 34 | 10 | 0.29 | *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp. *Aeromonas hydrophila*, *Pseudomonas* sp. |
| *Spea multiplicata* | 1 | 0 | 0 | None |
| Total | 150 | 42 | | |

positive individuals of each species is divided by the total number of individuals sampled, with a range of potential values from 0–1, and 1 indicating 100% prevalence (Mendoza–Almeralla et al., 2015).

## Results

One hundred and fifty anurans pertaining to seven species were sampled in Las Musas NPA (*Incilius occidentalis*, *Anaxyrus compactilis*, *Dryophytes arenicolor*, *D. eximius*, *Hypopachus variolosus*, *Lithobates neovolcanicus*, and *Spea multiplicata*; fig. 1). The presence of fungal and bacterial pathogens was confirmed by morphological and microbiological characterization in 38 individuals from five species. We found prevalence values ranging from 0.11 in *Incilius occidentalis* to 0.43 in *Dryophytes eximius*. For *Hypopachus variolosus* and *Spea multiplicata*, analyses of bacteria and fungi were negative (table 1). The presence of *B. dendrobatidis* was detected in a single specimen of *Dryophytes eximius* based on microbiological isolations, morphology of sporangia, and zoospore observations. The distribution of detected fungal and bacterial species in five of seven species within Las Musas NPA is shown in figure 2. Table 2 presents the individual results for each anuran species and their respective morphologically and biochemically characterized fungal and bacterial agents. We observed a higher incidence of infection across the Hylidae and Ranidae families. Sampled individuals of both *Hypopachus variolosus* and *Spea multiplicata* were negative for fungi or bacteria of interest (fig. 3, table 1).

### Year round variation

The prevalence of positive cases of bacterial or fungal microbes found on individuals was highest in autumn, with a prevalence of 0.51. In summer, the percentage of positive cases was at its lowest, with a prevalence of 0.125 (table 3). Figure 4 shows the contrast between negative and positive cases in fall.

### Injuries and illnesses

The presence of *Pseudomonas* sp. and *Aeromonas hydrophila* was confirmed in four species: *Anaxyrus compactilis, Dryophytes arenicolor, D. eximius,* and *Lithobates neovolcanicus*. Visible symptoms of *A. hydrophila* included redness and swelling in limbs, consistent with the pathogen's association with red leg disease, as observed in an individual of *D. arenicolor* (fig. 5). The fungal species *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. were identified in five species, and *B. dendrobatidis* in one *D. eximius* specimen, which presented skin lesions on the ventral area and extremities.

## Discussion

The causal factors of the decline in amphibian species have been discussed from various approaches, but all attribute this decrease to three main factors: the alteration and loss of habitat, climate change, and emerging infectious diseases (Lips et al., 2005; Basanta et al., 2020). In Mexico, the incidence of *Batrachochytrium dendrobatidis* has increased to 46.1%, higher than in

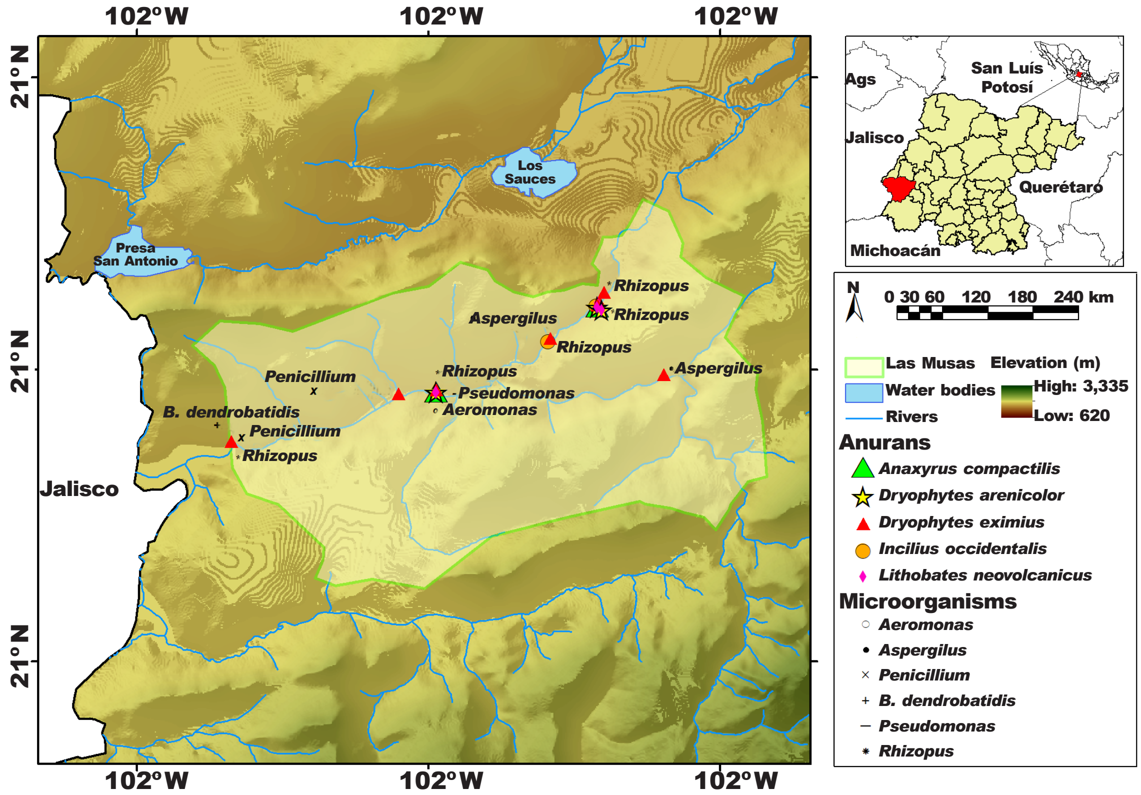


Fig. 2. Distribution map of positive records for bacteria and fungi in five species of anurans of the Las Musas Natural Protected Area, Guanajuato, Mexico.

*Fig. 2. Mapa de la distribución de los registros positivos para bacterias y hongos en cinco especies de anuros del Área Natural Protegida Las Musas, en Guanajuato, México.*

other countries in the Americas such as Costa Rica (20.1%) and Brazil (32.7%). The only country in the Americas with a higher overall prevalence than that in Mexico is Venezuela (49.7%; Zhongle et al., 2021).

Using morphological and biochemical characterization methods, we identified fungal and bacterial microbes from skin swabs obtained from anurans in Las Musas NPA. Among the anuran families surveyed, we found a consistent pattern that agreed with other studies in that the presence of fungi, including *B. dendrobatidis*, was detected on species in the Hylidae and Ranidae families (Frías–Alvarez et al., 2008; García–Feria et al., 2017; López–Velázquez, 2018). The presence of *B. dendrobatidis* could be confirmed within Las Musas NPA; the fungal pathogen was morphologically characterized in a specimen of *Dryophytes eximius*, a species that has previously been reported as susceptible to the fungus (Rosenblum et al., 2013; Garcia–Feria et al., 2017). Regarding the other fungal pathogens detected, mycotoxins in *Aspergillus* can cause hyperkeratosis in amphibians, affecting gas exchange and respiration in the host (Bosch, 2003). *Rhizopus* can cause dermatitis, lethargy, inflamed nodules, and growths on amphibians that can cause host mortality within two weeks (Brent and Whitaker, 2017; Juan–Sallés et al., 2020). *Penicillium* was recorded in all species surveyed in this study. However, its effect on anurans in the wild is not known, and data on its pathogenicity Mexican species is lacking (Juan–Sallés et al., 2020).

## Prevalence

For *Dryophytes eximius* and *Lithobates neovolcanicus*, we found a higher percentage of individuals testing positive for both fungi and bacteria during the fall, with most of the positive records occurring during the months of October to December, when precipitation and temperature are low. Woodhams et al. (2003) showed that the increase in temperature and humidity can trigger the emergence of opportunistic fungi. Regarding habitat quality and its effect on infectious bacteria in anurans, Ellison et al. (2019) found that two aspects influence the presence and abundance of infectious bacteria on anurans: phylogeny, which at high taxonomic levels (orders) has a relationship between hosts and pathogens; and environmental factors of the habitat, which influence the bacterial composition at the species level. However, data in

Table 2. Incidence of fungi and bacteria in the anuran community. Also shown are vegetation types, life habits, and season of the year in which records were created: vegetation (TDF, tropical deciduous forest; GF, gallery forest; and CF, cornfields); habits (Roc, rocks; Aquat, aquatic; Arb, arboreal); and season (S, spring; SUM, summer; A, autumn; W, winter).

*Tabla 2. Incidencia de hongos y bacterias en la comunidad de anuros. También se muestran los tipos de vegetación, los hábitos de vida y la estación del año en la que se hicieron los registros: vegetación (TDF, bosque caducifolio tropical; GF, bosque de ribera; CF, maizales); hábitos (Roc, rupícolas; Aquat, acuáticos; Arb, arbóreos); y estacion (S, primavera; SUM, verano; A, otoño; W, invierno).*

| Species | Microorganisms | Vegetation | Habits | Season |
|---|---|---|---|---|
| *Anaxyrus compactilis* | *Penicillium* sp. | GF–TDF | Roc | SUM |
| *Anaxyrus compactilis* | *Aeromonas hydrophila* | TDF | Roc | SUM |
| *Anaxyrus compactilis* | *Pseudomonas* sp. | TDF | Aquat | SUM |
| *Incilius occidentalis* | *Penicillium* sp. | CF | Roc | SUM |
| *Incilius occidentalis* | *Rhizopus* sp. | GF | Roc | S |
| *Dryophytes arenicolor* | *Penicillium* sp. | GF–TDF | Aquat | SUM |
| *Dryophytes arenicolor* | *Rhizopus* sp. | GF | Aquat | SUM |
| *Dryophytes arenicolor* | *Rhizopus* sp. | GF–TDF | Aquat | A |
| *Dryophytes arenicolor* | *Aspergillus* sp. | GF | Aquat | A |
| *Dryophytes arenicolor* | *Aeromonas hydrophila* | GF | Aquat | A |
| *Dryophytes arenicolor* | *Pseudomonas* sp. | GF–TDF | Aquat | W |
| *Dryophytes arenicolor* | *Pseudomonas* sp. | GF–TDF | Aquat | W |
| *Dryophytes eximius* | *Penicillium* sp. | GF–TDF | Arb | SUM |
| *Dryophytes eximius* | *Penicillium* sp. | GF | Arb | A |
| *Dryophytes eximius* | *Penicillium* sp. | GF | Arb | W |
| *Dryophytes eximius* | *Penicillium* sp. | TDF | Arb | S |
| *Dryophytes eximius* | *Rhizopus* sp. | GF | Arb | A |
| *Dryophytes eximius* | *Rhizopus* sp. | GF | Arb | A |
| *Dryophytes eximius* | *Rhizopus* sp. | GF | Arb | W |
| *Dryophytes eximius* | *Aspergillus* sp. | TDF | Arb | SUM |
| *Dryophytes eximius* | *Aspergillus* sp. | GF | Arb | SUM |
| *Dryophytes eximius* | *Batrachochytrium dendrobatidis* | GF | Arb | W |
| *Dryophytes eximius* | *Pseudomonas* sp. | GF | Arb | W |
| *Dryophytes eximius* | *Pseudomonas* sp. | GF | Arb | W |
| *Dryophytes eximius* | *Aeromonas hydrophila* | GF | Arb | W |
| *Dryophytes eximius* | *Aeromonas hydrophila* | GF | Arb | W |
| *Dryophytes eximius* | *Pseudomonas* sp. | GF | Arb | W |
| *Lithobates neovolcanicus* | *Penicillium* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Rhizopus* sp. | GF | Aquat | SUM |
| *Lithobates neovolcanicus* | *Rhizopus* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Aspergillus* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Aspergillus* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Pseudomonas* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Aeromonas hydrophila* | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Aeromonas hydrophila* | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Pseudomonas* sp. | GF | Aquat | A |
| *Lithobates neovolcanicus* | *Aeromonas hydrophila* | GF | Aquat | W |
| *Lithobates neovolcanicus* | *Aeromonas hydrophila* | GF | Aquat | W |

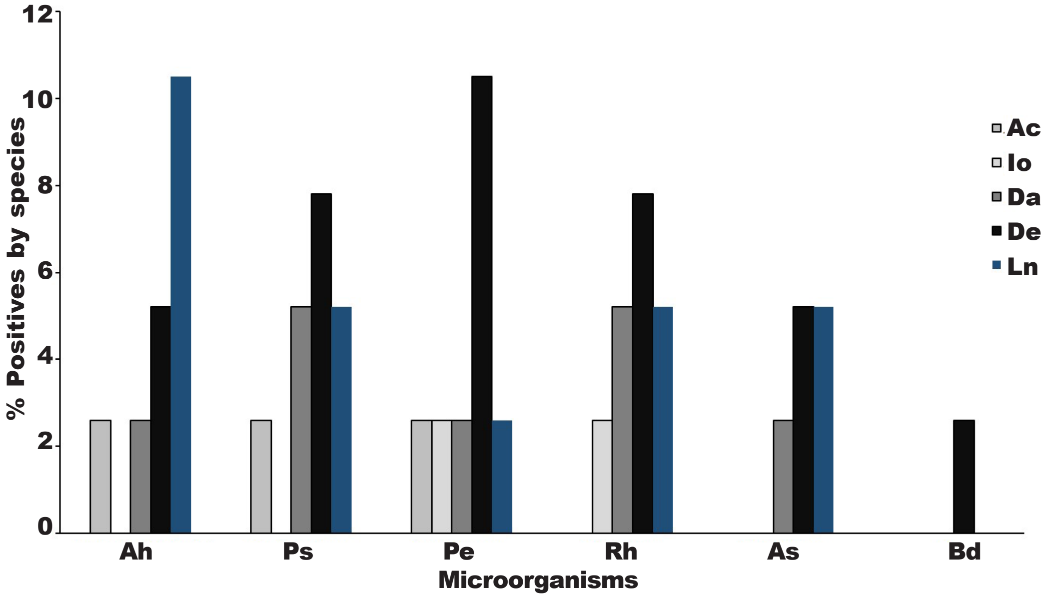


Fig. 3. Positive cases of bacteria and fungi per species; Ac, *Anaxyrus compactilis;* Io, *Incilius occidentalis*; Da, *Dryophytes arenicolor*; De, *Dryophytes arenicolor eximius*; Ln, *Lhitobates neovolcanicus.* Microorganisms: Ah, *A. hidrophilus; Ps, Pseudomonas* sp.; Pe, *Penicillium* sp.; Rh, *Rhizopus* sp.; As, *Aspergilus* sp.; Bd, *B. dendrobatidis.*

*Fig. 3. Casos positivos de bacterias y hongos por especies; Ac,* Anaxyrus compactilis*; Io,* Incilius occidentalis*; Da,* Dryophytes arenicolor*; De,* Dryophytes eximius*; Ln,* Lhitobates neovolcanicus. *Microorganismos: Ah,* A. hidrophilus*; Ps,* Pseudomonas *sp.; Pe,* Penicillium *sp.; Rh,* Rhizopus *sp.; As,* Aspergilus *sp.; Bd,* B. dendrobatidis.

other publications such as that of Hernández–López et al. (2018) differ regarding this aspect, alluding that the presence of these fungi is not related to environmental factors because they are present all year round in tropical environments. Hernández–López (2018) asserts that infection dynamics depend on the susceptibility and predisposition of each species and host specificity. Unfortunately, for the case of anurans within Las Musas NPA, we cannot confirm the effects of these pathogens on populations. Nevertheless, microorganisms were present in all species of anurans. We therefore, consider that symbiotic relationships and ecological factors should be analyzed in greater depth to understand this relationship between pathogens and hosts (Kolby et al., 2015).

Table 3. Year–round variation in percentage of anurans positive and negative to bacteria and fungi.

*Tabla 3. Variación anual del porcentaje de los anuros positivos y negativos a bacterias y hongos.*

| Season | % Positive | % Negative |
|---|---|---|
| Spring | 22.2 | 77.8 |
| Summer | 12.5 | 87.5 |
| Autumn | 51.1 | 40.4 |
| Winter | 17.6 | 79.4 |

## Injuries and illnesses

Of the microorganisms characterized, we found that five of the seven recorded individuals of the genus *Dryophytes* presented symptoms of red leg disease likely caused by *Aeromonas hydrophila*. According to Brent and Whitaker (2017), the presence and incidence of this bacterium is the result of poor water quality in rivers, streams, and pools. Indeed, one of the problems faced by Las Musas NPA is contamination from the discharge of wastewater from domestic use, and leachates of chemical products from crop areas adjacent to water bodies (Leyte–Manrique et al., 2018a, 2018b). The former could explain the presence of *A. hydrophila* throughout the year, unlike fungi such as *Rhizopus*, *Aspergillus*, and *B. dendrobatidis*, which are more prevalent with increased humidity during the rainy season. Our results

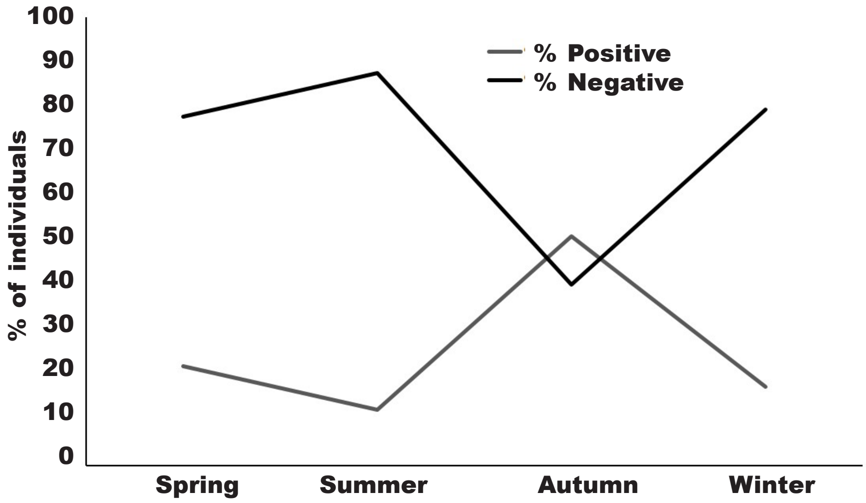


Fig. 4. Year–round variation of incidence, positive and negative cases.

*Fig. 4. Variación anual de la incidencia, casos positivos y negativos.*

confirm that the prevalence was lowest in the summer rainy season. Interestingly, the presence of *B. dendrobatidis* was recorded in winter. According to authors such as Brent and Whitaker (2017), the pathogenicity of *B. dendrobatidis* decreases at temperatures of 25 to 27 ºC. Winter temperatures during our surveys never exceeded 25 ºC, thus allowing for the viability and development of the pathogenic fungus in aquatic environments (Hernández–López et al., 2018).

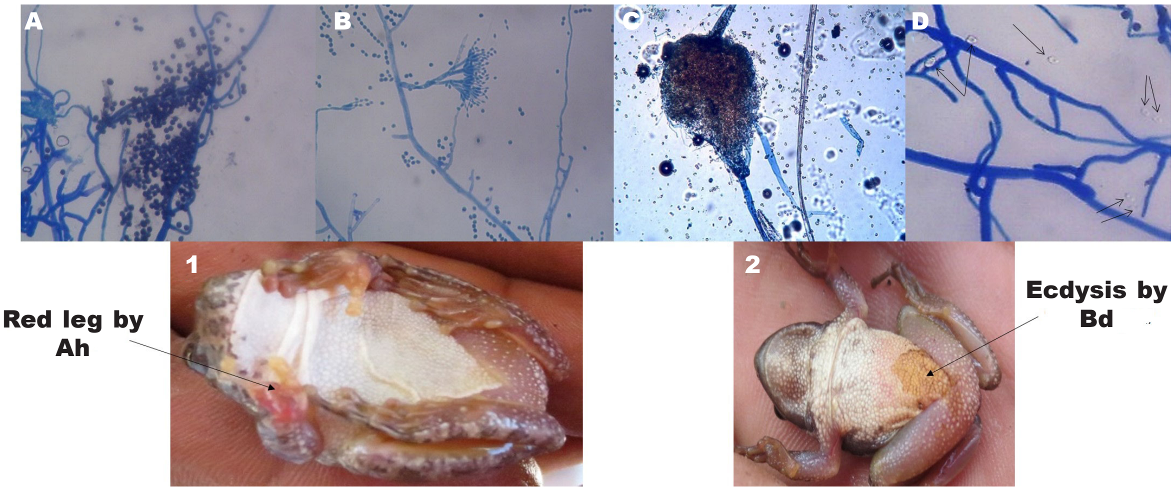


Fig. 5. Characterization of microorganisms and lesions. Fungi: A, *Aspergillus* sp.; B, *Penicillium* sp.; C, *Rhizopus* sp.; D, *Batrachochytrium dendrobatidis*; arrows indicate the spores of *B. dendrobatidis*. Lesions: 1, *Dryophytes arenicolor* infected by *Areomonas hydrophila* (*Ah*), and characteristic lesions of the "red leg" disease, and 2, *D. eximius* showing lesions from *B. dendrobatidis* (Bd).

*Fig. 5. Caracterización de los microorganismos y las lesiones. Hongos: A,* Aspergillus *sp.; B,* Penicillium *sp.; C,* Rhizopus *sp.; D,* Batrachochytrium dendrobatidis*; las flechas indican las esporas de* B. dendrobatidis*. Lesiones: 1,* Dryophytes arenicolor *infectado por* Areomonas hydrophila *(Ah) y lesiones características de la enfermedad de la pata roja; 2,* D. eximius *con lesiones causadas por* B. dendrobatidis *(Bd).*

From among the species recorded, the only one listed as under any category of risk of extinction NOM–059–ECOL–2010 (D.O.F., 2019) was *Lithobates neovolcanicus*, which is listed as a threatened species. However, unlisted species such as *Dryophytes arenicolor*, *D. eximius*, *Anaxyrus compactilis*, and *Incilius occidentalis* presented a high prevalence of bacteria and fungi, which could put their populations at risk. In this context, frogs of the genera *Lithobates* and *Dryophytes* are most susceptible to infection, as their aquatic habits (during at least one stage of development or life cycle) make them very good hosts. Bufonids, on the other hand, seem to be more resistant to bacteria and fungi, despite their initial stage of development in aquatic environments (Lips et al., 2005).

We believe that future studies of these frog communities should incorporate histological and PCR analyses for the characterization of microorganisms, in addition to those carried out herein (morphological and biochemical methods). These additional data would help to understand the factors that mediate pathogen dynamics with greater accuracy. Together with the environmental and ecological factors, ad hoc conservation strategies could be established to mitigate the diseases faced by anurans in Las Musas NPA.

## Acknowledgements

We thank the Tecnológico Nacional de México, Campus Salvatierra (ITESS) for funding the Project "Identification and characterization of fungal pathogens in the anuran community of a seasonal tropical environment in Guanajuato, Mexico" with code 429.16–PD, which was approved in the call for Basic Scientific and Technological Research Projects 2016 by the National Technological Institute of Mexico.

## References

Aguillón–Gutiérrez, D. R., Lazcano–Villareal, D., Ramírez–Romero, R., Aguirre–Ramos, A., Zárate–Ramos, J. J., Wong–González, A., 2007. Bacterias cloacales y evaluación física del herpetofauna del Parque Ecológico Chipinque. *CIENCIA U.A.N.L.*, 10(2): 168–173, http://eprints.uanl.mx/1761/1/VIBORAS.pdf

Basanta, M. D., Byrne, A. Q., Rosenblum, E. B., Piovia–Scott, J., Parra–Olea, G., 2020. Early presence of *Batrachochytrium dendrobatidis* in Mexico with a contemporary dominance of the global panzootic lineage. *Molecular Ecology*, 30(2): 424–437, Doi: 10.1111/mec.15733

Biaggini, M., Campetti, I., Corti, C., 2018. Breeding activity of the agile frog *Rana dalmatina* in a rural area. *Animal Biodiversity and Conservation*, 41(2): 405–413, Doi: 10.32800/abc.2018.41.0405

Berger, L. R., Speare, P., Daszak, D. E., Green, A. A., Cunningham, C. L., Goggin, R., Slocombe, M. A., Ragan, A. D., Hyatt, K. R., McDonald, H. B., Hines, K. R., Lips, G., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America*, 95(15): 9031–9036, Doi: 10.1073/pnas.95.15.9031

Berger, L., Hyatt, A., Speare, R., Longcore. J. E. 2005. Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of aquatic organisms*, 68(1): 51–63, Doi: 10.3354/dao068051

Brent. R., Whitaker, M. S., 2017. *Infectious diseases of amphibians: exotic and laboratory animals*. Veterinary Manual, Merck Manual, http://www.merckvetmanual.com/exoticandlaboratoryanimals/amphibians/infectiousdiseasesofamphibians#v3300972

Bosch, J., 2003. Enfermedades emergentes en anfibios. *El Ecologista*, 37: 28–30, https://www.ecologistasenaccion.org/8124/enfermedades-emergentes-en-anfibios/

Chinchilla Magaña, A. M., Henríquez Garcíaaguirre, M. X., Martinez Menjivar, N. J., 2014. Procedimientos de laboratorio y microbiología de las bacterias. In: *Determinación de la flora bacteriana en cavidad oral de serpientes de la familia Boidae y Colubridae en el parque zoológico Nacional del Salvador*: 18–31. Doctoral dissertation, Universidad de El Salvador, http://ri.ues.edu.sv/5916/1/13101577.pdf

Darling, K. T., Jones, B., 2011. Microbiology Review. In: *Veterinary Infection Prevention and Control*: 21–29 (L. Caveney, B, Jones, K. Ellis, Eds.). John Wiley & Sons, Inc.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., Speare, R., 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases*, 5(6): 735–748, Doi: 10.3201/eid0506.990601

D.O.F. (Diario Oficial de la Federación), 2019. MODIFICACIÓN del Anexo Normativo III, Lista de especies en riesgo de la Norma Oficial Mexicana NOM–059–SEMARNAT–2010, *Protección ambiental–Especies nativas de México de flora y fauna silvestres–Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio–Lista de especies en riesgo* (14/11/2019), https://www.dof.gob.mx/nota_detalle.php?codigo=5578808&fecha=14/11/2019#gsc.tab=0

Ellison, S., Rovito, S., Parra–Olea, G., Vásquez–Almazán, C., Flechas, S. V., Bi, K., Vredenburg, V. T., 2019. The Influence of habitat and phylogeny on the skin microbiome of amphibians in Guatemala and Mexico. *Microbial Ecology*, 78: 257–267, Doi: 10.1007/s00248-018-1288-8

Frías–Álvarez, P., Vredenburg, V. T., Familiar–López, M., Longcore, J. E., González–Bernal, E., Santos–Barrera, G., 2008. Chytridiomycosis survey in wild and captive Mexican amphibians. *EcoHealth*, 5(1): 18–26, Doi: 10.1007/s10393-008-0155-3

Frost, D. R., 2021. *Amphibian Species of the World,* version 6, https://amphibiansoftheworld.amnh.org/

García, E., 2004. *Modificaciones al sistema de clasificación climática de Köppen: para adaptarlo a las condiciones de la República Mexicana*. 5ta edición, Universidad Nacional Autónoma de México, México D.F.

García–Feria, L. M., Brousset Hernández–Jáuregui, D. M., Bravo, D. V., Cervantes–Olivares, R. A., 2017. El comercio de anfibios y la presencia de *Batrachochytrium dendrobatidis* en vida libre: ¿dispersión en círculo vicioso? *Neotropical Biology and Conservation*, 12(1): 30–36, Doi: 10.4013/nbc.2017.121.04

Hernández–López, P., Santiago–Alarcón, D., López–Velázquez, A., 2018. Factores ecológicos que afectan la infección por *Batrachochytrium dendrobatidis* en anfibios de México. In: *Quitridiomicosis en México*: 53–78 (A. López–Velázquez, M. Delia Basanta, L. Ochoa–Ochoa, Eds.). Sociedad Herpetológica Mexicana, A.C. Publicaciones de la Sociedad Herpetológica Mexicana No. 5, Ciudad de México, México.

Juan–Sallés, C., Almagro, V., Carbonell, L., Valls, X., Montesinos, A., Fernández–Bellon, H., 2020. Enfermedades infecciosas y parasitarias en anfibios en cautividad: estudio retrospectivo de 131 pacientes. *Clínica Veterinaria de Pequeños Animales*, 40(1): 15–27.

Johnson, P. T. L., 2006. Amphibian diversity: Decimation by disease. *Proceedings of the National Academy of Sciences of the United States of America*, 3(9): 3011–3012, Doi: 10.1073/pnas.0600293103

Kolby, J. E., Ramirez, S. D., Berger, L., Richards–Hrdlicka, K. L., Jocque, M., Skerratt, L. F., 2015. Terrestrial dispersal and potential environmental transmission of the amphibian Chytrid fungus (*Batrachochytrium dendrobatidis*). *Plos One,* 10(4): 1–13, Doi: 10.1371/journal.pone.0125386

Leyte–Manrique, A., Berriozabal–Islas, C., Mata–Silva, V., Morales–Castorena, J. P., 2018a. Herpetofaunal diversity in Área Natural Protegida Las Musas, Guanajuato. *Mesoamerican Herpetology*, (5)1: 121–136.

Leyte–Manrique, A., González–García, R. L. E., Quintero–Díaz, G. E., Alejo–Iturvide, F., Berriozabal–Islas, C., 2018b. Aspectos ecológicos de una comunidad de anuros en un ambiente tropical estacional en Guanajuato, México. *Acta Zoológica Mexicana (nueva serie)*, 34: 1–14.

Lips, K. R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology,* 13(1): 117–125.

Lips, K. R., Burrowes, P. A., Parra–Olea, G., 2005. Amphibian population declines in Latin America: A synthesis. *Biotropica*, 37(2): 222–226, http://www.jstor.org/stable/30043174

Lips, K. R., Mendelson, J. R., Muñoz–Alonso, A., Canseco–Márquez, L., Mulcahy, D. G., 2004. Amphibian population declines in montane southern México: Resurveys of historical localities. *Biological Conservation*, 119: 555–564, Doi: 10.1016/j.biocon.2004.01.017

López–Velázquez, A., 2018. *Ocurrencia de la Quitridiomicosis en México*. In: Quitridiomicosis en México: 35–52 (A. López–Velázquez, M. D. Basanta, L. Ochoa–Ochoa, Eds.). Sociedad Herpetológica Mexicana A. C., Publicaciones de la Sociedad Herpetológica Mexicana No. 5, Ciudad de México, México.

Luja, V. H., Rodríguez–Estrella, R., Ratzlaff, K., Parra–Olea, G., Ramírez–Bautista, A., 2012. The chytrid fungus *Batrachochytrium dendrobatidis* in isolated populations of the Baja California tree frog *Pseudacris hypochondriaca* curta in Baja California Sur, Mexico. *The Southwestern Naturalist*, 57: 323–327, Doi: 10.1894/0038-4909-57.3.323

Luria–Manzano, R., Oropeza–Sánchez, M. T., Aguilar–López, J. L., Díaz–García, J. M., Pineda, E., 2019. Dieta de la rana de hojarasca *Craugastor rhodophis* (Anura: Craugastoridae): una especie abundante en la región montañosa del este de México. *Revista de Biología Tropical*, 67(1): 196–205, Doi: 10.15517/RBT.V67I1.33135

Mendoza–Almeralla, C., Burrowesb, P., Parra–Olea, G., 2015. La quitridiomicosis en los anfibios de México: una revisión. *Revista Mexicana de Biodiversidad*, 86: 238–248. Merk Veterinary Manual. 2018, https://www.merckvetmanual.com/news/editorial/2018/01/30/18/04/msd-veterinary-manual-content-available-free-in-an-app

Rodríguez–Gutiérrez, M. F., Leyte–Manrique, A., Alejo–Iturvide, F., 2017. *Presencia de enterobacterias en la cuenca baja del río Temascatio, Guanajuato, México*. Congreso Nacional sobre la Investigación y Conservación de Anfibios y Reptiles. Del 2 al 5 de octubre de 2017, https://aicaroficial.com/congresos/

Rzedowski, J., 1978. *Vegetación de México*. Limusa, México D.F.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M. C., Flechas, S. V., Foster, C. N., Frías–Álvarez, P., Garner, T. W. J., Gratwicke, B., Guayasamin, J. M., Hirschfeld, M., Kolby, J. E., Kosch, T. A., E. La Marca, D., Lindenmayer, B., K. R. Lips, K. L., Longo, A. V., Maneyro, R., McDonald, C. A., Mendelson, J., P. Palacios–Rodríguez, P., Parra–Olea, G., Richards–Zawacki, C. L., Mark–Oliver, R., Rovito, S. M., Soto–Azat, C., Toledo, L. F., Voyles, J., Weldon, C., Whitfield, S. M., Wilkinson, M., Zamudio, K. R., S. Canessa, S., 2020. Response to Comment on "Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity". *Science*, 367 (6484): 1–4, Doi: 10.1126/science.aay2905

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Dawn Phillott, A., Hines, H. B., Kenyon, N., 2007. Spread of chytridiomycosis has acused the rapid global decline and extinction of frogs. *EcoHealth*, 4: 125–134, Doi: 10.1007/s10393-007-0093-5

The Merck Veterinary Manual, 2018. Merck & Co. Inc., Whitehouse Station, NJ.

Vásquez–Ochoa, A., Bahamón–Carmona, P., Prada–Salcedo, L. D., Franco–Correal, M., 2012. Detección y cuantificación de *Batrachochytrium dendrobatidis* en anfibios de las regiones andina central, oriental, Orinoquia y Amazonia de Colombia. *Herpetropicos*, 8(12): 13–21.

Vredenburg, V. T., Knappb, R. A., Tunstall, T. S., Brisggs, C. J., 2010. Dynamics of an emerging disease drive large–scale amphibian population extinctions. *Proceedings of the National Academy of Sciences of the United States of America*, 107(21):

9689–9694, Doi: 10.1073/pnas.0914111107

Vite–Silva, V. D., A. Ramírez–Bautista, A., Hernández–Salinas, U., 2010. Diversidad de anfibios y reptiles de la Reserva de la Biosfera Barranca de Metztitlán, Hidalgo, México. *Revista Mexicana de Biodiversidad*, 81: 473–485.

Vitt, J. L., Caldwell, J. P., 2014. *Herpetology: An Introductory Biology of Amphibians and Reptiles,* 4th edition. Academic Press Elsevier, San Diego.

Wake, D. B., Vredenburg, V. T., 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, 105:11466–11473, Doi: 10.1073/pnas.0801921105

Walter, F., Brooks, B., 2009. *Diagnóstico pesquero y acuícola de Guanajuato.* Gobierno del Estado de Guanajuato, SAGARPA y Universidad Michoacana de San Nicolás de Hidalgo.

Woodhams, D. C., Alford, R. A., Marantelli, G., 2003. Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms*, 55(1): 65–67, Doi: 10.3354/dao055065

Zhongle, L., Qi, W., Keping, S., Jiang, F., 2021. Prevalence of *Batrachochytrium dendrobatidis* in amphibians from 2000 to 2021: A global systematic review and meta–analysis. *Frontiers in Veterinary Science*, 8: 791237, Doi: 10.3389/fvets.2021.791237