



Anomalies in Three Species of Anurans in Chiapas, Mexico

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Amphibians are the vertebrates facing the highest risk of extinction (Hoffmann et al. 2010). The primary threats they face are the loss and transformation of habitat, climate change, introduced species, pollution, infectious diseases caused by agents such as the chytrid fungus (*Batrachochytrium dendrobatidis*), and interactions between these factors (Alford and Richards 1999; Collins and Storfer 2003; Wake and Vredenburg 2008). Alarming rates of morphological abnormalities have been recorded in amphibian populations around the world (e.g., Hoppe 2000; McCallum and Trauth 2003; Lannoo 2008). The physiological characteristics of amphibians, such as a permeable skin and, in most species, dependence on an aquatic environment in the early stages of their development and during reproduction, make them vulnerable to contaminants in that environment.

Malformations occur early in development at the cellular level with errors in communication or the transcription of genetic information, whereas a deformity is the result of the influence of mechanical factors that alter the shape or anatomy of a structure that has developed normally (Meteyer 2000). Being able to distinguish one from the other is not always possible (Johnson et al. 2001). To avoid confusion, misinterpretation, or simply not knowing its origin without a detailed study of each case, Henle et al. (2017a) referred to both abnormal phenotypes as “anomalies,” which he defined as any deviation of the phenotype (morphological and non-morphological), regardless of cause, from the range of phenotypes considered to be normal.

Most of the investigations that have verified some of the causes of malformations have been carried out in laboratory conditions and have been shown to cause alterations in development, behavior, and even mortality. Among the agents related to the presence of anomalies are the chemical products used in agriculture (Ouellet et al. 1997; Taylor et al. 2005; Lannoo 2008; Lajmanovich et al. 2012), ultraviolet radiation (Ankley et al. 2000, 2002), parasitic infections caused by the trematode *Ribeiroia ondatrae* (Sessions and Ruth 1990;

Johnson et al. 1999, 2002), predator attacks (Ballengée and Sessions 2009; Bowerman et al. 2010), hybridization (Arntzen and Wallis 1991; Mecham 1965), and inbreeding (Williams et al. 2008). However, in many cases, basic knowledge about the causes of deformities and their role in amphibian declines remain unclear (Johnson et al. 1999; Blaustein and Johnson 2003).

In Chiapas, Mexico, Lynch (1965) described the first record of a morphological anomaly in an amphibian, an apparent union of the third and fourth toes (syndactyly) of the left hind limb of a *Rana palmipes* (most likely *Lithobates forreri*); however, x-rays showed that only the skin was joined (cutaneous fusion) and not the bones of different digits as this malformation was described by Henle et al. (2017a). Recently, polymelia (the presence of extra limbs) was recorded in an individual *Plectrohyla acanthodes* (López-Méndez and Aranda-Coello 2021) and a *Leptodactylus fragilis* with bump-like tumors on the body (Mendoza-Velázquez et al. 2022) in



Figure 1. An *Agalychnis taylori* with a black eye (heterochromia) in the Zona Sujeta a Conservación Ecológica Laguna Bélgica, Ocozocoautla de Espinosa, Chiapas. Photograph by Romeo Montejo-Hernández/SEMAHN.

the municipalities of San Cristóbal de las Casas and Marqués de Comillas, respectively. Both cases were near human settlements and the bodies of water where the frogs were found receive contaminants from surrounding areas. Herein we report six anomalies in three species of anurans in Chiapas, Mexico.

At 1556 h on 10 November 2019, RMH, as part of a routine tour in the Zona Sujeta a Conservación Ecológica Laguna Bélgica in the municipality of Ocozocoautla de Espinosa (16.879194 N, -93.456778 W; elev. 930 m asl), found an adult *Agalychnis taylori* perched on the underside of a leaf on a bush about 2 m from the lagoon and approximately 80 cm off the ground. The left eye of the frog was completely black (heterochromia) (Fig. 1). No other physical or behavioral abnormalities were evident and, after taking photographs, the frog was left where it was found. The unilateral black-eye is a subcategory of heterochromia and is likely to be due to a recessive mutation (Henle et al. 2017a; Vershinin 2004). In Mexico this anomaly has previously been reported in *Lithobates forreri* and *Tripurion spinosus* (Monroy-Vilchis et al. 2015; Cerón-de la Luz and Vásquez-Cruz 2021), and in Costa Rica has been observed in *A. callidryas* (Güell et al. 2021). A possible cause to consider in this case is inbreeding, due to the isolation and resulting low gene flow at the lagoon where the frog was found. In Brazil, about 20% of adult *Rhinella jimi* on the island Fernando de Noronha had ocular malformations including eye discoloration, whereas this number was lower on the mainland. Such anomalies could be the result of inbreeding caused by founder effects or as a consequence of chemical pollutants in the environment (Toledo and Ribeiro 2009; Bacon et al. 2013; Toledo and Toledo 2015).

At 1120 h on 13 March 2020, MEHV found a tadpole of *Lithobates maculatus* (72.70 mm total length) at Gosner stage 39 (Gosner 1960), in the Centro Ecoturístico Cascada El Chorreadero, municipality of Chiapa de Corzo (16.755722 N, -92.970833 W; elev. 613 m asl). The tad-

pole had an extremely enlarged right eye, the iris and pupil were not visible, and there was redness inside (Fig. 2); no other abnormality or swimming problems were noted. None of three additional tadpoles captured in the same pond had any anomalies. All tadpoles were released back into the pond where they were initially found. The place where the anomalous tadpole was found is a canyon and the water that flows there comes from small streams from surrounding areas with livestock and crops. Areas devoted to these activities have increased in recent years and ecotourism might also increase the vulnerability of this population. Similar cases have been reported in adult *Hyla chrysoscelis* (McAllister et al. 2008) and *Rhinella marina* (Tipantiza-Tuguminago et al. 2020).

At 2251 h on 14 March 2021, in the Cañón Río La Venta between the municipalities of Ocozocoautla de Espinosa and Jiquipilas (16.743056 N, -93.529083 W; elev. 450 m asl), we found an individual of *Smilisca baudinii* perched on a bush (*Lindenia rivalis*) approximately 1.4 m above the ground and 1 m from the river bank. We noticed that the frog was missing its right eye (anophthalmia), and the upper eyelid covered the orbit. The frog had no other abnormalities, scars, or signs of injury and behaved normally. After a few minutes of observation and taking photographs, the frog jumped onto nearby branches and disappeared. Throughout the night we found three more conspecifics and a *Leptodactylus melanonotus*, none of which had any apparent anomalies. The location was in a canyon that can receive runoff from agricultural areas that are carried out in the upper parts of the confluence. In other parts of the river, anthropogenic activities have effects on the river (González-Díaz et al. 2017) and these also could affect amphibians in the area. Anophthalmia is characterized by the absence of one or both eyes (Johnson et al. 2001). Its origin in anurans is unknown, but some studies have shown the most likely causes to be exposure to chemical contaminants (Bacon et al. 2006), hybridization (Watson 1972; Mable and Rye 1992; Christiansen et al. 2005), ultraviolet radiation (Ankley et al. 2002), and selective predation (Ballengée and Sessions

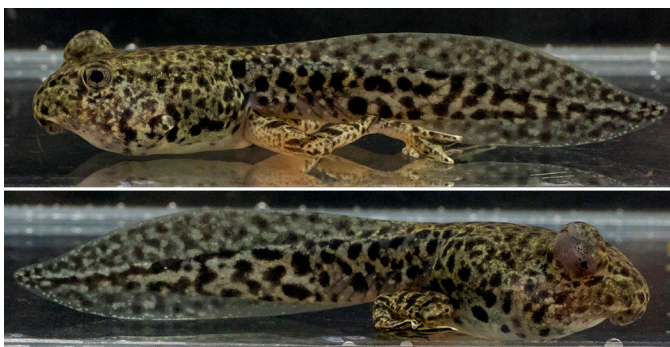


Figure 2. Tadpole of *Lithobates maculatus* at Gosner stage 39 in the Centro Ecoturístico Cascada El Chorreadero, Chiapa de Corzo, Chiapas: Left lateral view showing the prominent eye on the right side (top) and a right lateral view of the grossly enlarged eye, in which the iris and pupil are not present (bottom). Photographs by Miguel E. Hernández-Vázquez.



Figure 3. *Smilisca baudinii* with anophthalmia in the Cañón Río La Venta, Ocozocoautla de Espinosa, Chiapas. Photograph by Marco Antonio García-Jiménez.

2009). This is the third report of unilateral anophthalmia for the species, previously reported in a metamorph and an adult, both in Mexico (Hernández-Jauregui et al. 2021; Aréchaga-Ocampo and Roa-Mata 2022).

MEHV found a juvenile *Smilisca baudinii* at 1200 h on 16 August 2019 in ejido Copoya, municipality of Tuxtla Gutiérrez (16.717278 N, -93.128278 W; elev. 866 m asl) in the axil of a bromeliad (*Tillandsia fasciculata*) that had fallen from a large tree and was in a small stream. The frog had a pale blue (axanthism) dorsum and limbs and a dark spotted pattern but the colors of the venter and iris were normal (Fig. 4A). The dorsal coloration of the species can vary from light green or olive to brown, with dark olive green or brown spots, respectively; the venter is white and sometimes yellowish, flanks are cream with brown or dark mottling, especially evident in the groin (Duellman and Trueb 1966; Savage 2002). The lower jaw also had abnormal forward growth (prognathism) and did not close completely (Fig. 4B), and the urostyle had a lateral deviation (Fig. 4C). The frog's movements did not seem to be affected. The frog was not collected, and therefore the types of skeletal anomalies were not specified. Fort et al. (2006)

conducted laboratory tests by exposing embryonic (to the first larval stages) *Xenopus laevis* and *Rhinella marinus* to petroleum hydrocarbons and metals, which resulted in craniofacial and other malformations in most individuals. Brunelli et al. (2009) found highly toxic effects in *Bufo bufo* tadpoles exposed to endosulfan (a broad-spectrum organochlorine insecticide used in agriculture, which is capable of producing neurotoxic effects in both humans and laboratory animals), causing oral and skeletal malformations, as well as developmental and behavioral problems and even mortality. Other possible causes of oral and spinal malformations could be related to hybridization (Miranda 1989), parasitic infections (Johnson et al. 1999, 2002; Rajakaruna et al. 2008; Jayawardena et al. 2010; Pathirana et al. 2019), and nutritional problems (Martínez et al. 1992; Gagliardo et al. 2010).

Henle et al. (2017a) described lower-jaw anomalies as “underdeveloped or completely missing lower jaw” and considered mandibular dysplasia, hypognathia, and micrognathia synonymous with mandibular hypoplasia, an anomaly classified within the category of absence and reduction of skeletal elements (Henle et al. 2017b). However, none of these refers to



Figure 4. A juvenile *Smilisca baudinii* in ejido Copoya, Tuxtla Gutiérrez, Chiapas, with a pale blue dorsum (axanthism), a normal pattern of dark blotches, and the venter and iris with normal coloration (A); a frontal view showing the elongation of the lower jaw (prognathism) (B); and a dorsal view showing the urostyle with a lateral deviation (indicated by the arrow) (C); and an adult with the typical green phenotype (D). Photographs by Miguel E. Hernández-Vázquez.

the elongation of the lower jaw. Mandibular elongation or prognathism is a congenital malformation in which the lower jaw grows abnormally forward and does not align with the upper jaw. Gollmann et al. (1984) described a metamorph of *Bombina* sp. with a swollen dorsum. In the accompanying photograph they depicted a tadpole in which the length of the lower jaw exceeds the upper, but the authors did not comment on this.

Axanthism is the reduction or absence of yellow pigments (xanthophores), which typically cause underlying iridophores (blue pigments) to reflect as green coloration; in their absence, skin can appear to be blue, gray, or dark, depending on the color that the individual would have been (Berns and Narayan 1970; Bagnara and Matsumoto 2006; Martínez-Silvestre et al. 2016). According to Jablonski et al. (2014), axanthism is a rare color aberration and the least known, whereas albinism and leucism are more common in amphibians (Rivera et al. 2001; Jablonski et al. 2014; Henle et al. 2017b) and are associated with genetic mutations (Duellman and Trueb 1994; Bechtel 1995; Jablonski et al. 2014; Henle et al. 2017b; Miura et al. 2017), although other causes such as contamination should not be overlooked, since populations with several axanthic individuals have been found near places with high environmental contamination (Jablonski et al. 2014; Miura 2018; Bringsøe 2020; Marushchak et al. 2021), as well as other color aberrations (Severtsova et al. 2012; Maslova et al. 2018; Henle et al. 2017b and references therein; Marushchak et al. 2021). Conspicuous coloration can compromise the survival of less cryptic individuals, which are more vulnerable to predators in both larval and adult stages (Busack and Donaire 2014; Rojas 2017). This is the second report of axanthism in *S. baudinii*. It was previously reported in an adult in Veracruz (Vásquez-Cruz and Fuentes-Moreno 2020).

The causes of the anomalies presented herein were not determined as inferring their origins is difficult without detailed studies of the individuals and the places where the frogs were found, particularly since several factors could be involved, such as genetic, environmental, or both (Kiesecker 2002; Johnson et al. 2006; Fort et al. 2006). Even though we did not detect contamination in the locations where the frogs were found, nearby agricultural areas likely represent a risk, since the chemical products used in agriculture are considered the main causative agent of malformations (Ouellet et al. 1997; Marco et al. 1999; Koleska and Jablonski 2016). Regional studies of amphibian populations are needed to delve into this subject, because some herbicides used in the state can cause lethal effects on amphibian populations (Morales 2013). Also places that are under various anthropogenic pressures can elicit negative effects (Mendoza-Velázquez et al. 2022).

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