Morphological abnormalities in anurans from central Mexico: A case study
(Anura: Ranidae, Hylidae)

Morphologische Anomalien bei Anuren aus dem mittleren Mexiko: Ein Fallbericht
(Anura: Ranidae, Hylidae)

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KURZFASSUNG

Hohe Raten an morphologischen Anomalien (Mißbildungen) werden bei Amphibien auf die Einwirkung von Parasiten, chemischen Substanzen, UV-Strahlung und Beutegreifern zurückgeführt. Ziele der vorliegenden Untersuchung waren die quantitative und qualitative Erfassung grobmorphologischer äußerer Mißbildungen an Anuren des Sierra Nanchititla Naturreservats (Mexiko) sowie die Identifizierung möglicher Ursachen.

Sechs (6.23 %) der 95 gefangenen Individuen von *Lithobates forreri* (BOULENGER, 1883) sowie je ein „Beifangexemplar“ von *Lithobates zweifeli* (HILLIS, FROST & WEBB, 1984) und *Hyla arenicolor* COPE, 1866 zeigten insgesamt acht Typen morphologischer Mißbildungen. Die beobachtete Mißbildungsrate lag somit geringfügig über dem mit fünf Prozent angenommenen Hintergrundwert einer Population. An Makroparasiten wurden Nematoda (*Ozwaldocruzi*a sp. und *Rhabdias savagei*) und Trematoda (*Haematoloechus* sp. und *Gorgoderina tarascae*) an inneren Organen sowie Milben der Gattung *Hannemania* auf der Körperoberfläche festgestellt. In den Muskelgewebsproben, waren die Metalle Blei (Pb) und Kupfer (Cu) nicht nachweis- oder quantifzierbar, während Zink (Zn) in niedrigen (physiologischen) Konzentrationen vorlag. In den Wasserproben wurde Pb nicht nachgewiesen, die Zn and Cu Konzentrationen lagen innerhalb der in Mexiko zulässigen Grenzwerte für Fließgewässer. Die Autoren schließen als Ursache der beobachteten, erhöhten Mißbildungsrate aus: (1) die Tätigkeit von Makroparasiten, aufgrund des Fehlens von Trematoda der Gattung *Riberoia*, von denen man weiß, das sie Mißbildungen verursachen können und (2) die Einwirkung von Pb, Cu and Zn. Mögliche andere Ursachen werden angesprochen.

ABSTRACT

High rates of morphological abnormalities (malformations) in amphibians are attributed to parasites, chemical pollution, UV radiation and selective predation. The objectives of this study were to quantify and qualify the external, gross-morphological abnormalities in anurans of the Sierra Nanchititla Natural Reserve (Mexico) and to identify possible causes.

Six specimens (6.23 %) out of 95 *Lithobates forreri* (BOULENGER, 1883), as well as two “bycatch“ specimens of *Lithobates zweifeli* (HILLIS, FROST & WEBB, 1984), and *Hyla arenicolor* COPE, 1866, showed a total of eight types of morphological abnormalities. The observed abnormality rate thus slightly exceeded the assumed background rate of five percent in a population. Among macroparasites, Nematoda (*Ozwaldocruzi*a sp. and *Rhabdias savagei*) and Trematoda (*Haematoloechus* sp. and *Gorgoderina tarascae*) were present in internal organs, and chigger mites (*Hannemania* sp.) on the skin. In the muscle samples, lead (Pb) and copper (Cu) were not detected nor quantified, whereas low (physiological) concentrations of zinc (Zn) were found. In the water samples, Pb was not detected and Zn and Cu was within the level for rivers as accepted in Mexico. The authors reject as possible causes for the observed, increased rate of abnormalities: (1) the effect of macroparasites, due to the absence of Trematoda of the genus *Riberoia* that are known to develop anatomical abnormalities; and, (2) chemical pollution by Pb, Cu and Zn. Possible causes are discussed.

KEY WORDS

Amphibia: Anura: *Hyla arenicolor*, *Lithobates forreri*, *Lithobates zweifeli*, morphological abnormalities, malformations; heavy metals; parasites, veterinary medicine, ecotoxicology; Sierra Nanchititla Natural Reserve, Mexico

INTRODUCTION

In various ecosystems, amphibians constitute the largest fraction of vertebrate biomass (BLAUSTEIN et al. 1994). Biological diversity is particularly high in this vertebrate class since its representatives inhabit both aquatic and terrestrial habitats (COHEN 2001; BLAUSTEIN & JOHNSON 2003). Due to these traits and, among others, the permeable and unprotected skin these animals are considered particularly susceptible to environmental changes (COHEN 2001), and thus, appropriate indicators of environmental disturbances such as chemical pollution and changes in temperature, precipitation, and ultraviolet radiation (COHEN 2001; BLAUSTEIN & JOHNSON 2003).

In several zones of the world, the number of amphibians featuring anatomical abnormalities is reported to be on the rise (GARDINER et al. 2003). In this vertebrate class, the normal frequency of abnormalities (gross morphological malformations attributed to failures in embryonic development caused by genetic alterations or traumata) was estimated to range from zero to five percent (STOCUM 2000; JOHNSON et al. 2002). Frequencies above normal can result from environmental factors such as pollution, UV radiation, presence of parasites or selective predation, all of which potentially pose threat to the affected amphibian population (COHEN 2001; GRAY et al. 2002; BLAUSTEIN & JOHNSON 2003; BEEBEE & GRIFFITHS 2005; PIHA et al. 2006; STOLYAR et al. 2007; LANNOO 2008; BALLENGEE & SESSIONS 2009).

The present study quantifies the gross morphological abnormalities observed in anurans of the Mexican Sierra Nanchititla Natural Reserve (SNNR), and assesses metals (copper, lead, and zinc) and parasites as possible causes.

MATERIALS AND METHODS

Sierra Nanchititla Natural Reserve (SNNR) is located in central Mexico, Sierra Madre del Sur Province, and extends across 663,887 km^2 at 19°36'46" - 18°45'38" N and 100°15'54" - 100°36'28" W, in the physiographic region of the Balsas River Basin. The vegetal cover comprises pine-oak forest, broadleaf forest, low deciduous forest, induced grassland and cultivation. However, these latter two increased their extension due to forest extraction, which favored the creation of new cultivation and livestock breeding zones (MONROY-VILCHIS et al. 2008). For the reserve, the presence of 20 amphibian species is reported (CASAS-ANDREU & AGUILAR-MIGUEL 2005). According to the Institute for the Promotion of Mining and Geological Studies of the State of Mexico, there are mining activities of economic importance in the SNNR area, aiming on the exploitation of non-ferrous and precious metals. The registered localities are La Candelaria (gold and copper [Cu]), Cañadas de Nanchititla (silver, lead [Pb] and zinc [Zn]), Luvianos (disseminated copper) and Agua Colorada (copper) (IFOMEGEM 2008).

The authors performed monthly field trips to SNNR from June 2009 to July 2010 to conduct nonsystematic sampling of frogs which was done manually and with the help of nets at various points along the river. Sampling was restricted to 140 person hours and specimens of Forrer's Grass Frog, *Lithobates forreri* (BOULENGER, 1883), a species in which abnormalities had been registered earlier in the study area. The randomly captured individuals (95 adults with and without visible abnormalities) were taken to the lab, where the abnormalities of each individual were identified according to the literature (METEYER 2000; U. S. FISH AND WILDLIFE SERVICE 2007; LANNOO 2008). The frogs were killed by inhalation of chloroform; permission was not needed for this purpose. Voucher photographs were taken from the anomalies with a 4.0 megapixel Minolta camera. The frequency of abnormalities observed in *L. forreri* was calculated as the percentage of individuals with abnormalities (%) relative to the captured total of the species.

By means of a stereo microscope, in all the captured individuals an external revision was made to identify the presence of ectoparasites and a ventral incision to look for endoparasites in the visceral organs. Macroscopic parasites found were preserved in 10 % formol. For the preparation of Trematoda, Mayer's paracarmine staining was applied, whereas Nematoda were clarified in glycerin. The parasites were later examined under a microscope to taxonomically identify them with the help of identification keys (SLADKY et al. 2000; LEÓN- RÈGAGNON & PAREDES-CALDERÓN

2002; Bursey & Goldberg 2006; Mata-López 2006).

Leg muscle tissue samples were taken from all collected frogs and stored in cryovials at -4 °C for later analysis of their metal contents. For metal analysis, digestion of the samples was made with nitric acid (HNO_3) in two steps. Wet tissue samples of 0.07 g each were added to 10 ml of HNO_3 overnight for pre-digestion at room temperature. The following day, the digestion of the samples was continued in a microwave oven (CEM brand, model MARS-5) for 10 minutes at 1200 W and a pressure of 150 psi (U. S. Environmental Protection Agency 2007). The samples were then cooled to room temperature and filtered (Whatman 5). The retained matter was placed in 25 ml of distilled water. The reading of heavy metals (Pb, Zn and Cu) in tissue was performed by means of an atomic absorption spectrophotometer (Perkin Elmer brand, model 3110). A Wilcoxon nonparametric test (Statistica 8.0 software) was run to identify whether there were differences between the metal levels observed in the tissues from individuals with and without abnormalities (Lindgren 1993).

During frog sampling, water samples were taken directly from the water flow (Bautista 2004) in three random points along the river; water sampling was done with plastic bottles, previously washed and rinsed with a solution of 10 % HNO_3. The water samples were fixed with a solution of 10 % HNO_3 until pH < 2 was obtained; the bottles were labeled and stored at a temperature below 4 °C to be transported to the laboratory. The analysis of heavy metals in the water samples was done immediately thereafter, twice for each sample. The measured chemical elements were Pb, Zn and Cu, which are found in the mines of the study area. Limits of detection in the study were 0.015 ppm (Cu), 0.0073 ppm (Pb), 0.031 ppm (Zn); limits of quantification were 0.046 ppm (Cu), 0.35 ppm (Pb), and 0.091 (Zn). The measured metal concentrations were dry mass based.

RESULTS

Six individuals out of 95 *Lithobates forreri* (6.32 %) presented visible abnormalities. Apart from these, the two "bycatch" individuals of *Lithobates zweifeli* (Hillis, Frost & Webb, 1984), and *Hyla arenicolor* Cope, 1866, were affected by abnormalities. The individual of *L. zweifeli* showed hemimelia or micromelia (Fig. 1.1) in the left posterior limb, with the shank and foot clearly shortened and their muscles poorly developed. The *H. arenicolor* individual exhibited polyphalangy in the 4th finger of the left hand (Fig. 1.2); the additional terminal bone in this digit, produced a bifurcated finger tip.

The individuals of *L. forreri* presented six types of abnormalities:

(i) Brachydactyly in the anterior left limb of two individuals (Figs. 1.3a and 1.3b); the 4th digit was affected in one individual, the 3rd digit in the other.

(ii) Ectrodactyly in the left posterior limb (one individual, Fig. 1.3c); besides, the inter-digital membrane between 4th and 5th toes is absent.

(iii) Hemimelia (one individual, Fig. 1.3d) in the anterior right limb, which is malformed with complete bones.

(iv) Brachygnathia in the lower jaw (one individual, Fig. 1.3e), resulting in the inability to close the mouth properly.

(v) Abnormal coloration of the iris in one individual (Fig. 1.3f), which also shows incomplete interdigital membranes between 4th and 5th toe of the right posterior limb.

All of the six abnormal *L. forreri* individuals and 16 without externally visible abnormalities were infested by chigger mites of the genus *Hannemania* in various body regions and Nematoda of the species *Rhabdias savagei* and the genus *Oswaldocruzia* in the digestive tract and lungs, respectively. Besides, five of the individuals without abnormalities presented Trematoda of the species *Gorgoderina tarascae* in the digestive tract, two contained Trematoda of the genus *Haematoloechus* in the lungs; one individual was found with nematode larvae in the visceral cavity.

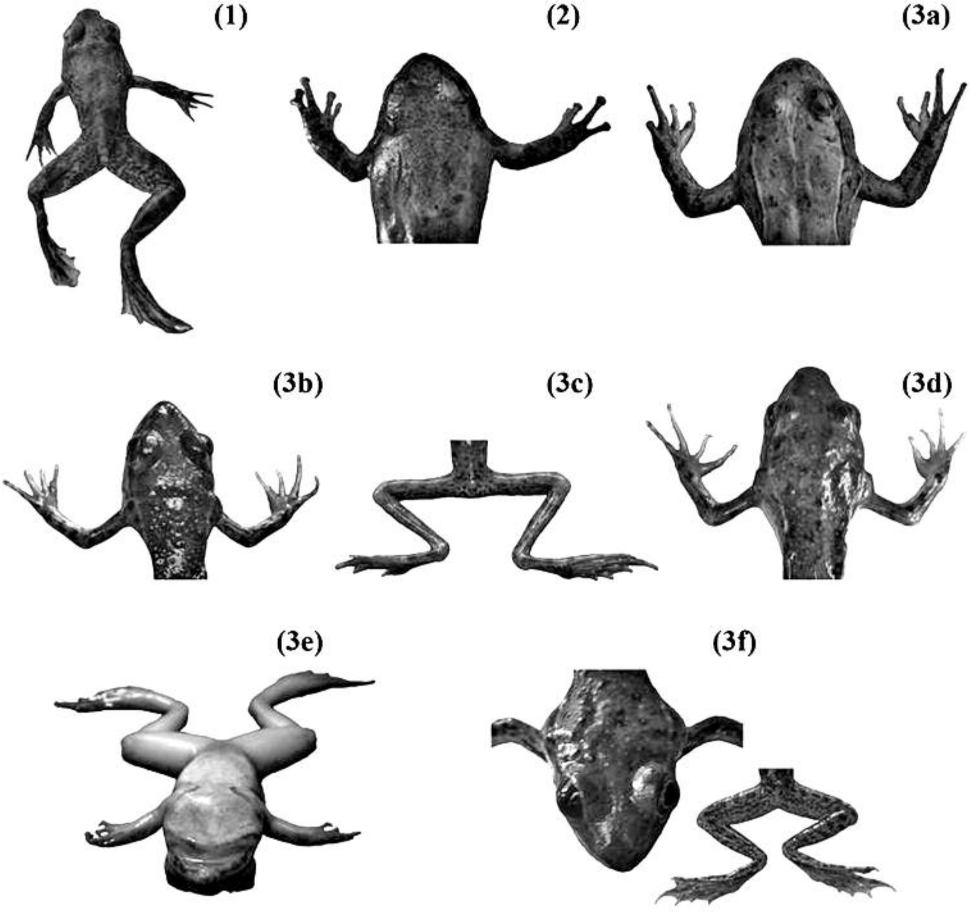

Fig. 1: Anatomical abnormalities in anurans of the Sierra Nanchititla Natural Reserve, central Mexico.
1 - *Lithobates zweifeli* with micromelia; 2 - *Hyla arenicolor* with polyphalangy;
3 - *Lithobates forreri* with brachydactyly (3a, 3b), ectrodactyly (3c), hemimelia (3d), brachygnathia (3e), and eye discoloration and incomplete interdigital membrane (3f).

Abb. 1: Anatomische Anomalien bei Anuren des Sierra Nanchititla Naturreservats, mittleres Mexiko.
1 - *Lithobates zweifeli* mit Micromelie; 2 - *Hyla arenicolor* mit Polyphalangie;
3 - *Lithobates forreri* mit Brachydactylie (3a, 3b), Ectrodactylie (3c), Hemimelie (3d), Brachygnathie (3e), sowie Fehlfarbigkeit des Auges und Unvollständigkeit der Zehenspannhäute (3f).

Table 1: Heavy metal concentrations [ppm] in anuran muscle tissue and water samples of the Sierra Nanchititla Natural Reserve, central Mexico. Range in parentheses. n. d. - not detected, n. q. - not quantified.

Tab. 1: Schwermetallkonzentrationen [ppm] in Muskelgewebsproben von Fröschen und Wasserproben des Sierra Nanchititla Naturreservats, mittleres Mexiko. Spannweiten in Klammern. n. d. - nicht nachweisbar, n. q. - Konzentration nicht quantifizierbar.

Samples	Average body mass (g)	Pb [ppm]	Zn [ppm]	Cu [ppm]
Individuals with abnormalities (n = 8)	0.0705	n.d.	61.7 (38.6-106)	n.q.
Individuals without abnormalities (n =10)	0.0706	n.d.	95.7 (36.8-123)	n.d.
Water (n = 3)	——	n.d.	< 0.1	< 0.06

Lead and copper were not detected in the muscles of either the individuals with or those without abnormalities (Table 1). The concentration of zinc was 61.7 ± 20.8 ppm in individuals with abnormalities and 104 ± 79.9 ppm in individuals without abnormalities; this difference was not significant ($W = 38$, $P > 0.05$; $n = 24$). The water samples revealed detectable levels of Zn (< 0.1 ppm) and Cu (< 0.06 ppm) (Table 1).

DISCUSSION

The percentage of abnormalities in the studied *L. forreri* individuals (6.32 %) was slightly above normal suggesting that the species was exposed to some noxious external agent (Stocum 2000; Johnson et al. 2002; Piha et al. 2006). For the present study, a sampling bias may be excluded because the total number of captured individuals (95) was larger or similar to the numbers in Sower et al. (2000) and Cohen (2001), who captured between 50 and 150 individuals per sampling event / amphibian community; however, in these studies there was no registration of the sampling effort. Suárez-Badillo & Ramírez-Pinilla (2004) and Gross (2009) indicated sampling efforts of 243.07 and 281.13 person hours, in which they captured 119 and 80 individuals, respectively. This turns into sampling efforts of 2.04 and 3.51 hours per individual, which is a lower value than of the present study with 95 individuals captured in 140 person hours, i. e., 1.47 hours per captured individual. In all aforementioned studies, the samlpe size is larger than that reported by Johnson et al. (2002), who mention the size of 25 individuals to be sufficient. In fact, the minimum sample size required varies depending on population size, confidence interval and sampling error accepted.

In the course of the sampling campaign, the number of captured *L. forreri* decreased gradually, may be due to the presence of cattle, which remained for several months while grazing in the vicinity of the water bodies. This agrees with observations by Schmutzer et al. (2008) and Burton et al (2009), who found that the presence of livestock in the distribution zones of anurans can negatively affect amphibian abundance (larvae, juveniles or adults) and species diversity by reduction of vegetation and increase of aquatic turbidity, nitrogen and phosphorus.

The parasites which account for amphibian malformations are mainly Trematoda of the genus *Ribeiroia*, hosted in the tissue of the pelvic region and hind limbs of anurans (Johnson et al. 1999; Cohen 2001). However, in the present study focusing on *L. forreri*, only Nematoda (*Oswaldocruzia* sp., *Rhabdias savagei*) were collected which have not been reported to cause anatomical abnormalities in amphibians. The sorts of abnormalities developed by the presence of *Ribeiroia* sp. are mainly extra members and/or phalanges (Sessions & Ruth 1990), and since these sorts of abnormalities were not found in the present study, helmiths were probably not the cause of the abnormalities observed in the captured anurans.

Lead was not detected in the muscles of both individuals with and without abnormalities whereas copper was detected but could not be quantified. The concentration of Zn varied between 36.8 and 123 ppm in the sampled individuals. However, Zn is an essential micronutrient for organisms, acts as a co-factor in about 300 enzymes and is found in all body tissues, with the highest levels in bones, muscles, prostate, liver and kidney (U. S. Environmental Protection Agency 2005). The levels detected in this anuran species are similar to those mentioned for amphibian species in unpolluted places, which range from 33 to 150 ppm of Zn (U. S. Department of the Interior 1998) but higher than reported by Linzey et al. (2003) who registered dry mass based levels of 11-28.3 ppm of Zn as harmless to anurans.

The water samples presented non-detectable levels of Pb as well as levels of Zn and Cu within the quality range accepted in (i) drinking water according to Mexican regulation NOM-127-SSA1-1994, and (ii) rivers for the protection of aquatic life according to NOM-001-ECOL-1996. It was reported that Zn or Cu concentrations exceeding 1.5 ppm in the water provoke malformations in amphibians (U. S. Depart-

MENT OF THE INTERIOR 1998). In the present study, Zn attained levels below 0.1 ppm; thereby revealing the studied water bodies in SNNR to be free from pollution by these three metals, and thus nontoxic for aquatic life in this region.

The presence of Trematoda of the genus *Ribeiroia* and critical concentrations of the heavy metals analyzed were excluded as the causes of the observed anatomical malformations in SNNR anurans. However, other causes such as agrochemical pollution, acidification, increasing UV radiation or selective predation cannot be excluded to have provoked the abnormalities as was shown for anuran species in different parts of the world (COHEN 2001; GRAY et al. 2002; BLAUSTEIN & JOHNSON 2003; BEEBEE & GRIFFITHS 2005; LANNOO 2008; BALLENGEE & SESSIONS 2009; BOWERMAN et al. 2010).

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REFERENCES

BALLENGEE, B. & SESSIONS, S. (2009): Explanation for missing limbs in deformed amphibians.- Journal of Experimental Zoology, New York etc.; (Ser. A) 312: 1-10.

BAUTISTA, Z. F. & DELFÍN, G. H. & PALACIO, P. J. & DELGADO, C. M. (2004): Técnica de muestreo para manejadores de recursos naturales. México, D.F. (UNAM- Universidad Nacional Autónoma de México), pp. 509.

BEEBEE, T. J. C. & GRIFFITHS, R. A. (2005): The amphibian decline crisis: A watershed for conservation biology?- Biological Conservation, Barking; 125: 271-285.

BLAUSTEIN, A. R. & WAKE, D. B. & SOUSA, W. P. (1994): Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions.- Conservation Biology, Malden; 8: 60-71.

BLAUSTEIN, A. R. & JOHNSON, P. T. J. (2003): Explaining frog deformities.- Scientific American, New York; 288: 60-65.

BOWERMAN, J. & JOHNSON, P. T. J. & BOWERMAN, T. (2010): Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians.- Ecology, New York; 91: 242-251.

BURSEY, C. R. & GOLDBERG, S. R. (2005): New species of *Oswaldocruzia* (Nematoda: Molineoidae), new species of *Rhabdias* (Nematoda: Rhabdiasidae), and other helminths in *Rana* cf. *forreri* (Anura: Ranidae) from Costa Rica.- Journal of Parasitology, Lawrence; 91: 600-605.

BURTON, E. C. & GRAY, M. J. & SCHMUTZER, A. C. (2009): Differential responses of postmetamorphic amphibians to cattle grazing in wetlands.- Journal of Wildlife Management, Oxford; 73: 269-277.

CASAS-ANDREU, G. & AGUILAR-MIGUEL, X. (1997): Claves de identificación de los anfibios del Estado de México.- Revista Siyan Can, Toluca; 1: 39-46.

CASAS-ANDREU, G. & AGUILAR-MIGUEL, X. (2005): Herpetofauna del Parque Sierra de Nanchititla, Estado de México, México: Lista, distribución y conservación.- Ciencia Ergo Sum, Toluca; 12: 44-53.

COHEN, M. M. J. (2001): Frog decline, frog malformations, and a comparison of frog and human health.- American Journal of Medical Genetics, Hoboken; 104: 101-109.

GARDINER, D. & NDAYIBAGIRA, A. & GRÜN, F. & BLUMBERG, B. (2003): Deformed frogs and environmental retinoids.- Pure and Applied Chemistry; Research Triangle Park, NC; 75: 2263-2273.

GRAY, H. M. & OUELLET, M. & GREEN, D. M. (2002): Traumatic injuries in two Neotropical frogs, *Dendrobates auratus* and *Physalaemus pustulosus*.- Journal of Herpetology, Lawrence; 36: 117-121.

GROSS, J. (2009): Declive en poblaciones de seis especies de anfibios anuros del Páramo de Mucubají, Estado Mérida, Venezuela.- Herpetotropicos, Bogotá; 5: 09-20.

IFOMEGEM (2008): Carta de Recursos Minerales. Secretaría de Desarrollo Económico, Gobierno del Estado de México, Toluca, México.

JOHNSON, P. T. J. & LUNDE, K. B. & RITCHIE, E. G. & LAUNER, A. E. (1999): The effect to trematode infection on amphibian limb development and survivorship.- Science, Washington; 284: 802-804.

JOHNSON, P. T. J. & LUNDE, K. B. & THURMAN, E. M. & RITCHIE, E. G. & WRAY, S. N. & STHERLAND, D. R. & KAPFER, J. M. &. FREST, T. J. & BOWERMAN, J. & BLAUSTEIN, A. R. (2002): Parasite (*Ribeiroia ondatrae*) infection linked to amphibians malformations in the Western United States.- Ecological Monographs, Washington; 72: 151-168.

KAM, Y. & CHEN, T. (2000): Abundance and movement of a riparian frog (*Rana swinhoana*) in a subtropical forest of Guandau Stream, Taiwan.- Zoological Studies, Taipei; 39: 67-76.

LANNOO, M. (2008): Malformed frogs: The collapse of aquatic ecosystems. Berkeley, Los Angeles (University of California Press), pp. 44.

LEÓN-RÈGAGNON, V. & PAREDES-CALDERÓN, E. L. (2002): *Haematoloechus dandrooksi* n. sp. (Digenea: Plagiorchioidea) from *Rana vaillanti* from Los Tuxtlas, Veracruz, Mexico.- Journal of Parasitology, Lawrence; 88: 1215-1221.

LINDGREN, B. W. (1993): Statistical theory. 4th edition. New York (Chapman & Hall), pp. XII, 633.

Linzey, D. & Burroughs, J. & Hudson, L. & Marini, M. & Robertson, J. & Bacon, J. & Nagarkatti, M. & Nagarkatti, P. (2003): Role of environmental pollutants on immune functions, parasitic infection and limb malformations in marine toads and whistling frogs from Bermuda.- International Journal of Environmental Health Research, Abingdon; 13: 125-148.

Mata-López, R. (2006): A new gorgoderid species of the urinary bladder of *Rana zweifeli* from Michoacán, Mexico.- Revista Mexicana de Biodiversidad, México, D. F; 77: 191-198.

Menin, M. & Waldez, F. & Lima, A. P. (2008): Temporal variation in the abundance and number of species of frogs in 10,000 ha of a forest in Central Amazonia, Brazil.- South American Journal of Herpetology, Washington [electronic resource available at < http:// www.bioone.org/toc/sajh/9/1 >]; 3: 68-81.

Meteyer, C. U. (2000): Field guide to malformations of frogs and toads with radiographic interpretations.- U. S. Department of the Interior, U. S. Geological Survey, Biological Science Report 2000-0005, Washington.

Monroy-Vilchis, O. & Cabrera, L. & Suárez, P. & Zarco-González, M. & Rodríguez-Soto, C. & Urios, V. (2008): Uso tradicional de vertebrados silvestres en la Sierra Nanchititla, México.- Interciencia, Caracas; 33: 308-313.

NOM-127-SSA1-1994 (1994): Salud ambiental, agua para uso y consumo humano: límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización. Secretaría de Salud, México, D. F.

NOM-001-ECOL-1996 (1996): Límites máximos permisibles de contaminantes en las descargas residuales en aguas y bienes nacionales. Diario Oficial de la Federación, México, D. F.

Piha, H. & Pekkonen, M. & Merila, J. (2006): Morphological abnormalities in amphibians in agricultural habitats: A case study of the common frog *Rana temporaria*.- Copeia, Washington; 2006 (4): 810-817.

Ramírez-Bautista, A. & Hernández-Salinas, U. & García-Vázquez, U. O. & Leyte-Manrique, A. & Canseco-Márquez, L. (2009): Herpetofauna del Valle de México: Diversidad y conservación. México, D.F. (UAEH - Universidad Autónoma del Estado de Hidalgo, CONABIO - Comisión nacional para el conocimiento y uso de la biodiversidad), pp. 213.

Sessions, S. K. & Ruth, S. B. (1990): Explanation for naturally occurring supernumerary limbs in amphibians.- Journal of Experimental Zoology, New York etc.; 254: 38-47.

Schmutzer, A. C. & Gray, M. J. & Burton, E. C. & Miller, D. L. (2008): Impact of cattle on amphibian larvae and the aquatic environment.- Freshwater Biology, Oxford; 53: 2613-2625.

Sladky, K. K. & Norton, T. M. & Loomis, M. R. (2000): Trombiculid mites (*Hannemania* sp.) in canyon tree frogs (*Hyla arenicolor*).- Journal of Zoo and Wildlife Medicine, Lawrence; 31: 570-575.

Sower, S. A. & Reed, K. L. & Babbitt, K. J. (2000): Limb malformations and abnormal sex hormone concentrations in frogs.- Environmental Health Perspectives, Research Triangle Park, NC ; 108: 1085-1090.

Stocum, D. L. (2000): Frog limb deformities: An “Eco-Devo” riddle wrapped in multiple hypotheses surrounded by insufficient data.- Teratology, New York; 62: 147-150.

Stolyar, O. B. & Loumbourdis, N. S. & Falfushinska, H. I. & Romanchuk, L. D. (2007): Comparison of metal bioavailability in frogs from urban and rural sites of Western Ukraine.- Archives of Environmental Contamination and Toxicology, Heidelberg; 54: 107-113.

Suárez-Badillo, H. A. & Ramírez-Pinilla, M. P. (2004). Anuros del gradiente altitudinal de la Estación experimental y demostrativa El Rasgón (Santander, Colombia).- Caldasia, Bogotá; 26: 395-416.

U. S. Department of the Interior (1998): Guidelines for interpretation of the biological effects of selected constituents in biota, water and sediments. National Irrigation Water Quality Program, Information Report No. 3. Washington D. C.

U. S. Environmental Protection Agency (2005): Toxicological review of zinc and compounds: in support of summary information on the Integrated Risk Information System. U. S. Environmental Protection Agency. Washington D.C. EPA/635/R-05/002.

U. S. Environmental Protection Agency (2007): Method 3051A: Microwave assisted acid digestion of sediments, sludge, soils and oils. Washington, D.C.

U.S. Fish and Wildlife Service (2007): Standard Operated Procedures for abnormal amphibian monitoring: Abnormality classification SOP 2007. WWW document available at < http://www.fws.gov/contaminants/anphibian/abnormalSOP.html > [last accessed on May 5, 2014].

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