In Mesoamerica, hylid frogs represent one of the most diverse groups of anurans and their study is imperative to develop more accurate conservation plans. This image shows a *Plectrohyla thorectes* found near Juquila, Oaxaca, Mexico. This frog is one of the smallest species of *Plectrohyla*, one of the most diverse genera of amphibians in Mexico. © Luis F. Vázquez-Vega

Mesoamerican Herpetology
www.mesoamericanherpetology.com
Eagle Mountain Publishing, LC
www.eaglemountainpublishing.com
OPEN ACCESS

Everything is not lost: recent records, rediscoveries, and range extensions of Mexican hylid frogs

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Abstract: Amphibians are threatened by multiple factors, including climate change, habitat loss, and infectious diseases. In Mexico, amphibian declines have been attributed mainly to habitat loss, disease, pollution, and in some cases illegal trafficking of species. Despite evidence of amphibian declines, recent studies have recorded species that had not been found in the wild for many years. We report on the results of fieldwork conducted within individual amphibian-focused projects in the Mexican highlands. Our findings include 26 frog species, 23 of them endemic to Mexico, the discovery of five new populations that expand the restricted distributions of three microendemic and one endemic species (*Plectrohyla ameibothalame, P. cembra, P. cyclada*, and *P. hazelae*, respectively), and the rediscovery of five species that were considered possibly extinct in the wild (*P. celata*, *P. cembra*, *P. chryses, P. crassa,* and *P. robertsorum*). For each species we report the conservation status (NOM-059 and IUCN Red List), the date of the most recent field observations, and the most recent reference that suggests species declines and/or possible extinctions. Our results show that while some frog species persist in the face of multiple threats, additional fieldwork and conservation efforts should be conducted to monitor these species and help ensure their future persistence in the wild.

Key Words: Amphibians, endangered species, Hylidae, Mexico, range extensions, rediscoveries

Resumen: Los anfibios son un grupo que se encuentra amenazado por múltiples factores, incluyendo el cambio climático, la pérdida de hábitat, y las enfermedades infecciosas. En México, el declive de los anfibios se ha atribuido principalmente a la pérdida de hábitat, enfermedades, contaminación y en algunos casos el tráfico ilegal de especies. A pesar del evidente declive de anfibios, recientes estudios han registrado especies que no se habían encontrado en el campo hace muchos años. El presente estudio es el conjunto del trabajo de campo correspondiente a proyectos individuales enfocados a anfibios en las zonas montañosas de México. Nuestros resultados incluyen 26 especies de ranas de la familia Hylidae, 23 de estas endémicas de México, el descubrimiento de cinco nuevas poblaciones que expanden el área de distribución de tres especies micro endémicas y una especie endémica (*Plectrohyla ameibothalame, P. cembra, P. cyclada,* y *P. hazelae* respectivamente) y el redescubrimiento de cinco especies que se consideraban posiblemente extintas en estado silvestre (*P. celata*, *P. cembra*, *P. chryses*, *P. crassa* y *P. robertsorum*). Para cada especie encontrada, registramos su estado de conservación (NOM-059 e IUCN Red List), la fecha más reciente en que se había registrado en campo y la referencia mas reciente que sugiere el declive y/o la posible extinción de esta especie. Nuestros resultados muestran que algunas especies de ranas persisten a pesar de las múltiples amenazas, y trabajo de campo adicional así como mayores esfuerzos de conservación hacen falta para monitorear estas especies y ayudar a asegurar su futura persistencia en el campo.

Palabras Claves: Anfibios, especies en peligro, extensiónes de distribución, Hylidae, México, redescubrimientos

Citation: Caviedes-Solis, I. W., L. F. Vázquez-Vega, I. Solano-Zavaleta, E. Pérez-Ramos, S. M. Rovito, T. J. Devitt, P. Heimes, O. A. Flores-Villela, J. A. Campbell, and A. Nieto Montes de Oca. 2015. Everything is not lost: recent records, rediscoveries, and range extensions of Mexican hylid frogs. Mesoamerican Herpetology 2: 230–241.

Received: 11 August 2015; **Accepted:** 23 August 2015; **Published:** 25 September 2015.

INTRODUCTION

A total of 377 species of amphibians have been recorded from Mexico, which ranks the country fifth in amphibian diversity worldwide (Parra-Olea et al., 2014) and second among countries with the highest number of endangered species (Stuart et al., 2008). The largest number of Alliance for Zero Extinction Sites (AZE) also are found in Mexico; these sites are designated based on areas with a large "number of species that are likely to go extinct without immediate conservation action" (Lamoreaux et al., 2015). Amphibian declines in Mexico mainly are caused by habitat loss and fragmentation, especially in cloud and pine-oak forest habitats where perturbations reduce the survival and dispersal of some species (Frías-Alvarez et al., 2010; Ochoa-Ochoa et al., 2009). Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), also has contributed to dramatic amphibian declines (Collins and Storfer, 2003, Lötters et al., 2009).

Within the amphibians, the Hylidae is the most diverse family of anurans in Mexico, where it occurs mainly in highland areas and is composed of 97 species, 66 of them endemic (88.8%; Parra-Olea et al., 2014). Campbell and Duellman (2000) described a number of new species of hylids from Oaxaca and stated that "Sadly, we suspect that history will confirm our notions that the last several decades, and perhaps the first decade of the new millennium, will stand out as the period when the highlands of Oaxaca, as well as many other places around the planet, lost their greatest wealth of amphibian biodiversity." Young et al. (2001) reported 16 species of amphibians with population declines in the highlands of southern Mexico, mainly in Guerrero, Oaxaca, Chiapas, and Veracruz. Lips et al. (2004) reported that 27 species have declined and 11 of them might be extinct. Baena et al. (2008) reported 29 species of amphibians, 13 of them hylid frogs, as possibly extinct, all of them with restricted distributions associated with cloud forest; however, they did not search for them throughout most of their ranges, and did not reject the possibility of finding them in future surveys. Rovito et al. (2009) assessed plethodontid salamander populations at different elevations in southern Mexico by comparing historical records to recent survey efforts, and reported major declines in cloud and pine-oak forests at high elevations. The latest summary by Frías-Alvarez et al. (2010) reported that 69.5% of the endemic amphibians are threatened and 64.29% are in decline. All these studies were conducted during specific seasons for short periods of time; only a small number of studies involved long-term surveys to determine the status of populations (Parra-Olea et al., 1999, Muñoz-Alonso, 2013; Hernández-Ordóñez, 2014).

Surveying amphibians is challenging, in part because of their small distributions and short breeding seasons (Young et al., 2001). The lack of tissues for molecular work in herpetological collections from most of the microendemic Mexican highland species has led to an intensification of fieldwork to find them. According to the IUCN, 12 species of the family Hylidae are listed as possibly extinct. Recent surveys rediscovered four species of hylid frogs from the Oaxacan highlands (Heimes and Aguilar, 2011; Delia et al., 2013; Lamoreaux et al., 2015); however, some of the rediscovered populations tend to be small and still sensitive to disappearance (Lamoreaux et al., 2015). The present compilation includes information on important recent distribution records and the rediscovery of species. Rediscoveries reinforce the fact that more fieldwork is necessary, including more time effort and the visiting of remote localities; in addition, demographic studies are needed to clearly assess species decline and extinction, and therefore improve the development of effective conservation strategies and the accurate assessment of threatened species (Frías-Alvarez et al., 2010).

MATERIALS AND METHODS

From 2007 to 2014, we conducted 10 surveys at 22 localities in the Mexican highlands to search for amphibians. The field sites are characterized as semi-deciduous, cloud, and pine-oak forests, located in seven montane systems: Sierra Madre Oriental (SMO), Sierra Madre Occidental (SMOcc), Sierra Mixteca (SMixt), Sierra Mixe (SM), Cerro Piedra Larga (CP), Sierra de Juárez (SJ), and Sierra Madre del Sur (SMS) (Fig. 1). We provide the survey dates and site locations in Table 1. Each trip included three to five herpetologists who conducted searches both day and night.

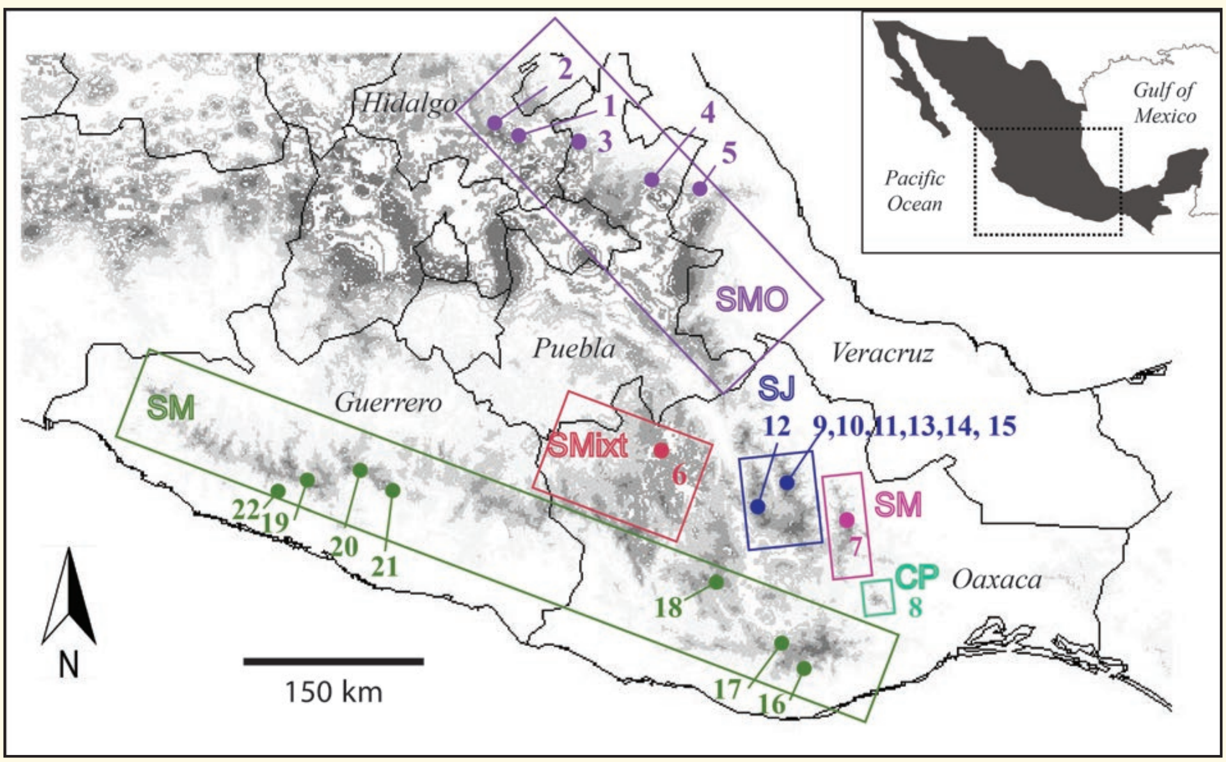

Fig. 1. Survey localities included in this study, including mountain systems and municipalities (see Table 1 for abbreviations).

Table 1. Mountain system, state, municipality, year, and dates of sampled localities.

Mountain System State	Municipality	Year	Dates
Sierra Madre Oriental (SMO)			
Hidalgo Puebla	1. Zacualtipan 2. Zoquizoquipan 3. Huahuchinango	2011	July 4–8
Puebla	4. Zacapoaxtla	2012	September 20–21
Veracruz	5. Altotonga	2012	September 22–23
Sierra Mixteca (SMixt)			
Oaxaca	6. San Cristobal Suchixtlahuaca	2011	August 23
Sierra Mixe (SM)			
Oaxaca	7. Totontepec Villa de Morelos	2011	September 24–25
		2014	August 24–26
Cerro Piedra Larga (CP)			
Oaxaca	8. Santa Maria Nizaviguiti	2011	September 19–23
Sierra de Juárez (SJ)			
Oaxaca	9. Santiago Comaltepec 10. San Pedro Yolox	2010	July 19–26
	9, 10 11. San Pablo Macuiltianguis 12. San Miguel Aloapam 13. La Esperanza	2011	April 25–May 1
	9, 11 14. San Juan Luvina 15. Ixtlán de Juárez	2014	August 27–30
Sierra Madre del Sur (SMS)			
Oaxaca	16. Pluma Hidalgo	2007	January, April, July, October (one week each)
	17. San Miguel Suchixtepec 18. San Vicente Laxichio	2011	November 3–10
	18.	2011	August 25
Guerrero	19. Puerto del Gallo 20. Carrizal de Bravo 21. El Tejocote 22. Atoyac de Álvarez	2010	September 24–28

The field sites are located on communal lands in each municipality, and permission to conduct the studies was approved by the municipal authorities. The Mexican wildlife agency Dirección Nacional de Vida Silvestre, Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT), México, granted ethical approval (handling and sacrifice) for scientific collecting purposes; the permit was granted to Adrián Nieto Montes de Oca (License FAUT 093). The specimens were collected by hand, sacrificed by applying an overdose of the anesthetic Pentobarbital, then fixed with 10% formalin and preserved in 70% ethanol and deposited at the Museo de Zoología Alfonso L Herrera, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC, UNAM).

Each specimen was identified to species by morphology and/or a thorough molecular phylogenetic analysis based on a Bayesian approach (I. Caviedes-Solis and A. Nieto Montes de Oca, unpublished). Cryptic species were identified based on molecular data alone. The species rediscoveries and range extensions were based on comparisons with historical records (Lips et al., 2004; Delia et al., 2013, Frost, 2014). For each species, we recorded the dates and localities where they were found, their conservation status according to national and international endangered species lists (Norma Oficial Mexicana NOM-059-SEMARNAT-2010 and IUCN Red List 2013, respectively), previous reports of species decline (Lips et al., 2004; Baena et al., 2008; Frías-Alvarez et al., 2010; Delia et al., 2013), and habitat loss within potential ranges in protected areas they inhabit according to Ochoa-Ochoa et al. (2009). These species were placed into the following categories according to the percentage of habitat loss: low reduced (LR; ≤ 20%), medium reduced (MR; 21%–50%), very threatened (VR; 51%–80%), and severely reduced (SR; > 80%).

The primary purpose of this fieldwork was not to evaluate the status of amphibian populations, but rather to make a species inventory (Caviedes-Solis, 2009) and obtain tissues for three molecular phylogenetic studies (Rovito et al., 2009). In addition, we include personal communications from several researchers who found the species during the same years we conducted our surveys. These new records constitute an important contribution to our knowledge of the distribution and persistence of Mexican amphibians.

RESULTS

We found 26 species of hylid frogs classified in eight genera (Fig 2, Table 2). Twenty-three species are endemic to Mexico, and the distributions of three species (*Smilisca baudinii*, *Agalychnis moreletii*, and *Hyla arenicolor*) extend to other countries. We rediscovered five species of *Plectrohyla* that have not been seen in the field for 10 years or more (*P. celata*, *P. cembra*, *P. chryses*, *P. crassa*, and *P. robertsorum*). Of these species, all but *P. chryses*, and *Exerodonta juanitae*, previously were reported as possibly Extinct in the Wild (Lips et al., 2004; Baena et al., 2008; Frías-Alvarez et al., 2010; Delia et al., 2013). We found our specimens of *P. robertsorum* near a previous locality for this species ca. 2 km NE (by road) from Zoquizoquipan, Hidalgo. Although our specimens are similar to other specimens previously collected from the same river (Río Canela), they differ slightly morphologically from topotypical specimens of *P. robertsorum*. Thus, we consider the assignment of our specimens to *P. robertsorum* as tentative. We could not corroborate their identity with molecular data.

We found five populations that represent range extensions for four species. (1) *Plectrohyla ameibothalame* previously was known only from its type-locality in the SMixt (Yosocuno, Oaxaca) and the surrounding areas; the new population lies in the municipality of San Cristóbal Suchixtlahuaca, ca. 28 km SW (airline) from the former locality. (2) *Plectrohyla cyclada* previously was known from several localities in the SJ and the SM, Oaxaca; the new record lies on La Cofradia, San Vicente Laxichio SMS, ca. 71 km SE (airline) from its nearest locality Cerro San Felipe (SJ). (3) *Plectrohyla hazelae* was known only from the SJ; we noted two populations located in SMS, San Vicente Laxichio and Santa Catarina Juquila ca. 70 and 128 km SE (airline), respectively, from the previously nearest known locality of El Punto (SJ). The distribution of the last two species extends into a different mountain system (SMS) than previously known, across Central Valley of Oaxaca. Finally, (4) *P. cembra* was known from the type locality at Río Molino in San Miguel Suchixtepec, SMS, Oaxaca, and from 7.5 km SE of Llano de Guadalupe, 172 km NE (airline) of the type locality (Mendelson and Canseco-Márquez, 2002); the last specimen was deposited at MZFC, UNAM, but was lost while on loan and thus could not be examined to confirm its identity. The new population was found ca. 10 km N from the type-locality.

The mountain systems with the highest numbers of species found in our surveys were the SMS and SJ, with 13 and nine species, respectively. Both of these mountain systems are notable for their large number of hylid species (Duellman, 2001). According to the IUCN Red List, 65% of the species found are at risk: eight are Critically Endangered, six Endangered, and three Vulnerable (Table 2). Only 46% of these species, however, are at risk according to the Mexican threatened species list NOM-059 (five species threatened and seven under special protection). According to Ochoa-Ochoa et al. (2009), the status of the potential distribution in protected natural areas inhabited by these species ranged from low to severely reduced.

DISCUSSION

Current studies on the natural history, biodiversity, and conservation of Mexican amphibians are more important than ever, given the multiple and severe threats that these species are facing. The development and implementation of accurate conservation plans are imperative. More studies are needed to discover and describe species-level diversity and its evolutionary history, document amphibian declines, and reassess the conservation status of listed species.

Most studies of amphibian declines in Mexico are based on short-term surveys at specific localities; only a few include several localities within the range of a particular species, and for more extensive periods of time. Skelly et al. (2003) suggested that short surveys could infer an erroneous high rate of species decline, while multiyear resurveys would estimate more accurate rates of decline. In addition, Delia et al. (2013) pointed out that studies

Fig. 2. Species found in the field. Letters a–z correspond to the first column in Table 2.

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Table 2. Conservation and previous threat status, habitat loss in potential distribution within protected areas, survey year, and site locations for hylid species recorded in this study.

Fig 2	Species	IUCN	NOM 059	Previous Threat Status	Loss of Range	Survey Year	Mountain Systems (Municipalities)
a	*Agalychnis moreletii*	CR		a,c	**	2007	SMS (16)
b	*Charadrahyla nephila*	VU			*	2010 2011 2011 2014	SJ (9) SJ (9, 13) SM (7) SM (7)
c	*Charadrahyla taeniopus*	VU	A		***	2011 2012	SMO (2) SMO (4, 5)
d	*Ecnomiohyla miotympanum*	NT			**	2011 2012	SMO (1, 2) SMO (4,5)
e	*Exerodonta abdivita*	DD			****	2011	SJ (13)
f	*Exerodonta juanitae*	VU	A	d	**	2007	SMS (16) SMS (Guerrero)*
g	*Exerodonta sumichrasti*	LC			**	2007	SMS (15)
h	*Hyla arenicolor*	LC				2011	SMO (3)
i	*Plectrohyla ameibothalame*	DD			**	2011	**SMixt (6)**
j	*Plectrohyla bistincta*	LC	Pr		**	2011 2014 ? ?	SJ (9, 10), CP (8), SMS (18) SJ (15) SMOcc (Michoacan)* SMOcc (Jalisco, Nayarit)*
k	*Plectrohyla calthula*	CR			****	2012	SM (7)*
l	***Plectrohyla celata*** (1984)	CR		a,b,c,d	*	2011 2012	SJ (11) SJ (9)*
m	***Plectrohyla cembra*** (1993)	CR	A	a,b,c	****	2011 2012	SMS (17) SMS (San Jose del Pacífico)*
n	***Plectrohyla chryses*** (2000)	CR	Pr		*	2010	SMS (20)
o	***Plectrohyla crassa*** (1978)	CR	Pr		**	2011	SJ (12)
p	*Plectrohyla cyclada*	EN			**	2010 2011 2014	SJ (9,10) SJ (9,10,11), **SMS (18)** SM (7)
q	*Plectrohyla hazelae*	CR	Pr	a,b,c	**	2011 2012 2014	**SMS (18)** **SMS (Sta. Catarina Juquila, Oax)*** SJ (15)
r	*Plectrohyla mykter*	EN	A		*	2010	SMS (21)
s	*Plectrohyla pentheter*	EN			*	2007	SMS (16)
t	***Plectrohyla robertsorum*** (1996)	EN	A		***	2011	SMO (2)
u	*Plectrohyla thorectes*	CR	Pr	a,b,c	**	2012	SMS (Sta Ma. Juquila, Oax)*
v	*Ptychohyla achrocorda*	DD			**	2010	SJ (9)
w	*Ptychohyla erythromma*	EN	Pr		**	2010	SMS (22)
x	*Ptychohyla leonhardschultzei*	EN	Pr	c	*	2007	SMS (16), CP (8)
y	*Ptychohyla zophodes*	DD			**	2011	SJ (13)
z	*Smilisca baudinii*	LC				2011	SJ (13)

Species in bold represent rediscoveries, followed by the year of the last previous record in parentheses. Mountain ranges and localities in bold face represent range extensions, and localities with an asterisk represent surveys conducted after our fieldwork (E. Pérez-Ramos, J. Campbell, J. Orozco, and V. Luja, pers. comm.). Conservation status: IUCN Red List: CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened; NOM-059: A = threatened, Pr = special protection. Previous threat status: a = evidence of local extinction (Lips et al., 2004), b = species presumably extinct (Baena et al., 2008); c = lack of recent observations (Frías-Alvarez et al., 2010); d = species not found (Delia et al., 2013). Habitat loss in potential distribution within protected areas: PE = Possible extinctions, **** = SR severely reduced, *** = VR very threatened, ** = MR medium reduced, * = LR low reduced (Ochoa-Ochoa et al., 2009; see text for abbreviations). Mountain system and numbers in parentheses correspond to specific localities in Table 1.

conducted over only a few nights are not sufficient to accurately assess changes in populations, and short resurveys at the same localities might generate contradictory results. In the SMS, our findings support these suggestions. In this region, our multiple short visits to the same localities allowed us to find species that were not seen in previous short expeditions by other workers, e.g., *Exerodonta juanitae* (Delia et al., 2013).

In Central America, the rate of amphibian decline in upland areas above 500 m is higher than that below this elevation (Young et al., 2001), and species with small ranges (microendemics) are subject to a greater risk of extinction (Stuart et al., 2004). The rediscovery of amphibian species increases over time mainly in tropical and subtropical forests in South America, due to a rise in the number of expeditions and the large number of endangered species distributed in the area that have not being seen in long periods of time. Some of the species are known only from the type-series and some also inhabit restricted distributions above 1,000 m, remarking the fact that they can be found even though the rate of decline is high under that distribution or related to the species rareness (Scheffers et al., 2011). The rediscovery of species in Mexico is possible and relatively common, as there are several example cases among hylid frogs (Heimes and Aguilar, 2011; Delia et al., 2013; Lamoreaux et al., 2015), the genus *Lithobates* (Ranidae; Campos-Rodríguez et al., 2012), and salamanders (Sandoval-Comte et al., 2012). We discovered several species at their type localities that had not been found in years. Our findings, therefore, reaffirm that conducting surveys in different seasons in new and previously known sites might lead to a higher detection of species.

The number of individuals of the species reported herein varied with respect to factors like season, weather, and species behavior, and thus studies that account for the variability in such factors are necessary to inform overall population trends at any site. Although the goal herein was not to present a demographic study, a notable reduction in the abundance of species is apparent when compared to surveys conducted in the 1970s and 1980s (Duellman, 2001; Caldwell, 1974); this pattern also was suggested by Lips et al. (2004) and Lamoreaux et al. (2015). Habitat loss has been suggested as one of the main reasons for amphibian declines. Ochoa-Ochoa et al. (2009) concluded that the potential distribution of the species reported herein in natural protected areas has not been reduced; in these cases, habitat loss could be a factor behind declines, but likely is not the main cause. An increase in the protection of montane forest, which holds the highest rate of microendemisms in Mexico, is imperative.

Chitridiomycosis is a major threat to amphibians and has caused large declines in some Mexican species (Cheng et al., 2011). Even though the evidence suggests that some anuran populations susceptible to *Bd* are recovering and increasing their numbers, e.g., *L. tarahumarae* in northern Mexico (Hale et al., 2005), chytrid fungus still is the main cause for the decline of more susceptible species and more actions are needed to reduce the effect of the disease (Lamoreaux et al., 2015). Some of our results also agree with those of Lips et al. (2003), who suggested that declines are more correlated with tolerance to environmental change and altitudinal distribution, with high elevation and microendemic species being more sensitive. Another reason could be the increase of temperatures and inconsistent rainy seasons, since hylid frogs depend on riparian habitats for breeding, and the lack of rain and water affects their life cycles and reduces the survival rate of the larvae. We concur with studies such as those of Baena et al. (2008) and Frías-Alvarez et al. (2010), which have assessed threatened species; long term studies focused on population dynamics, as well as studies that distinguish among the possible causes of declines, are needed to avoid the possible overestimation of declines and extinction rates, and help to understand what factors need to be considered in amphibian conservation plans.

Acknowledgments.—Field and laboratory work were funded by grants from the DGAPA, Universidad Nacional Autónoma de Mexico (PAPIIT no. IN 224009) and CONACYT (no 154093) to Adrián Nieto Montes de Oca. Fieldwork was supported in part by National Science Foundation grant No. DEB- 0613802 to Jonathan A. Campbell. Itzue W. Caviedes-Solis was funded by a CONACyT Master's degree fellowship. Sean M. Rovito and Tom J. Devitt were funded by UC MEXUS-CONACyT postdoctoral fellowships. Oscar Flores-Villela was supported by DGAPA, UNAM during a sabbatical year. We thank Rodrigo Villegas-Garcia, Oscar Rivera-Huerta, Andrea J. Roth-Monzon, Darrel Frost, Carl Franklin, Joseph Mendelson, D. Aportela, Cesar Gaona-Gaona, Andres A. Mendoza-Hernández, Karlo A. Soto Huerta, Uri O. García-Vázquez, Carlos A. Hernández-Jiménez, Jaziel M. Loaeza, Miguel A. Negreros-Amaya, Manuel M. Acosta Sánchez, and Walter Schmidt for their help with fieldwork. We are grateful to Joseph Mendelson and Larry David Wilson for providing valuable comments that helped improve this manuscript. We also thank the municipal authorities that granted us the permission to conduct fieldwork, and the local people for helping us find localities.

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