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Amphibian Diversity and Conservation in the Ejido Tierra y Libertad, Southwestern Chiapas, Mexico

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Abstract.—**Amphibians constitute one of the richest and most endangered groups in Mexico; however, their diversity and threat factors at the regional level remain little known in many parts of the country, including in protected areas. We analyzed amphibian diversity of the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico, 40% of which occurs within the La Sepultura Biosphere Reserve (Reserva de la Biósfera La Sepultura, or REBISE). We conducted fieldwork from January 2016 to April 2020, including 980 person-hours of systematic sampling across 11 transects spanning different habitat types. We documented 20 amphibian species, including the first record of the Yellowbelly Mushroomtongue Salamander (*Bolitoglossa flaviventris*) for the REBISE. Seven of these amphibian species are included in some risk category: three in the NOM-059-SEMARNAT-2010 of the Mexican federal government, two in the Red List of Threatened Species of the International Union for Conservation of Nature, and two categorized in both. The Common Leaf-litter Frog (*Craugastor loki*) was the most abundant species, and together with four other anuran species, constituted 82.1% of the total abundance observed. Inter-transect faunal similarity was generally associated with proximity, forest corridor connectivity, and the presence of water. Conserved Semi-Deciduous Forest had the highest alpha diversity, but somewhat surprisingly, urban and some disturbed habitats had the highest species richness. Pasture was the least rich and diverse habitat, however, so we discuss the consequences of ongoing expansion of pastures on amphibian populations. We propose conservation measures intended to promote both amphibian diversity and human health.**

Key Words.—abundance; Central Depression; Jiquipilas; land-use change; richness; sampling; Sierra Madre de Chiapas

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Resumen.—Los anfibios constituyen uno de los grupos más ricos y amenazados de México; sin embargo, su diversidad y factores de amenaza a nivel regional siguen siendo poco conocidos en muchas partes del país, incluidas las áreas **naturales protegidas. Analizamos la diversidad de anfibios del Ejido Tierra y Libertad, Municipio de Jiquipilas, Chiapas, México, el cual se ubica, en más del 40% de su superficie, dentro de la Reserva de la Biosfera La Sepultura (REBISE). Realizamos el trabajo de campo del período de enero de 2016 a abril de 2020, incluidas 980 horas-persona de muestreo sistemático en 11 transectos que abarcan diferentes tipos de hábitat. Documentamos un total de 20 especies de anfibios, incluido el primer registro de la Salamandra Lengua de Hongo de Vientre Amarillo (*Bolitoglossa flaviventris*) para la REBISE. Siete especies de anfibios están incluidas en alguna categoría de riesgo: tres en la NOM-059-SEMARNAT-2010 del gobierno federal mexicano, dos en la Lista Roja de Especies Amenazadas de la Unión Internacional para la Conservación de la Naturaleza y dos categorizadas en ambas. La Rana Común de Hojarasca (*Craugastor loki*) fue la especie más abundante y, junto con otras cuatro especies de anuros, constituyó el 82,1% de la abundancia total observada. La similitud faunística entre transectos se asoció generalmente con la proximidad, la conectividad del corredor forestal y la presencia de agua. La Selva Mediana Subcaducifolia sin disturbio presentó la mayor diversidad alfa, sorprendentemente, los hábitats urbanos y algunos perturbados tuvieron la mayor riqueza de especies. El potrero fue el hábitat menos rico y diverso, por lo que discutimos las consecuencias de la continua expansión de los potreros en las poblaciones de anfibios. Proponemos medidas de conservación destinadas a promover tanto la diversidad de anfibios como la salud humana.**

Palabras clave.—abundancia; cambio de uso de suelo; Depresión Central; Jiquipilas; muestreo; riqueza; Sierra Madre de Chiapas

Traducción al español del artículo: http://www.herpconbio.org/Volume_17/Issue_3/ Javier-Vázquez _etal_2022_Español

Introduction

Mexico has among the greatest geological and climatic variability of any country, and thus hosts one of the richest biotas on the planet (Ochoa-Ochoa and Flores-Villela 2006). Among the most important elements of Mexican biodiversity are the amphibians (Flores-Villela 1993; Flores-Villela and Gérez 1994). There are 424 species documented from the country, of which over half are endemic, positioning Mexico as the seventh richest country in amphibian species globally (https://herpetologiamexicana.org/; https://amphibiaweb.org/). Amphibians are exceptional indicators of the quality and health of natural ecosystems, thanks to their high sensitivity to environmental changes at different scales; however, they are also among the most threatened groups of vertebrates (Ramírez-Bautista 2004; Stuart et al. 2008). Worldwide, amphibians are currently suffering from a severe extinction crisis due to many factors that include habitat destruction, the exploitation of species, introduction of exotic species, global climate change, emerging infectious diseases, and contamination by pesticides and other chemicals (Skelly et al. 2002; Collins and Storfer 2003; Sodhi et al. 2008; Wake and Vredenburg 2008). According to the International Union for Conservation of Nature (IUCN), approximately 41% of the amphibian species on the planet are threatened (IUCN 2022). In an analysis carried out by the same organization, the most important factors in Mexican amphibian population declines are deforestation and transformation of vegetation (Frías-Álvarez et al. 2010).

Fortunately, the protected natural area network in Mexico (known as the Sistema Nacional de Áreas Naturales Protegidas, or SINANP) offers some level of protection to numerous ecosystems, in addition to promoting sustainable development among local communities (García-Amado et al. 2013; Caballero 2020; Godínez-Gómez et al. 2020). The SINANP network is particularly well developed in the southernmost state of Chiapas, which also contains a remarkably high diversity of 108 amphibian species (Johnson et al. 2015; Hernández-Ordoñéz et al. 2017). The La Sepultura Biosphere Reserve (Reserva de la Biósfera La Sepultura, REBISE), is the second largest protected area in Chiapas, encompassing an area of 167,300 ha, of which 8.2% corresponds to five core zones and the remainder to a broad buffer zone. The amphibian diversity within REBISE remains poorly studied and this area, although protected, faces serious environmental problems that include an increase in human population density, forest fires, and deforestation due to expanding livestock practices and agriculture (Figueroa and Sánchez-Cordero 2008; Godínez-Gómez et al. 2020). Understanding the effects of these threats to the diversity of amphibians in this area is vital so that informed management can be promoted.

The Ejido Tierra y Libertad (ETL) is highly suitable for studies on this topic, because more than 40% of its territory is in the buffer zone of REBISE, and because it contains a high diversity of habitats. Given that ETL supports > 11.5% of the human population that exists within REBISE (Instituto Nacional de Ecología1999; Comisión Nacional de Áreas Naturales Protegidas 2013), it also exemplifies the major environmental problems mentioned above for this Reserve. Núñez-Orantes and Muñoz-Alonso et al. (2000) indicated the presence of 39 amphibian species within REBISE, but the authors did not sample within the ETL. Furthermore, there are no records of amphibian specimens from ETL in the online database VertNet (https://blog.vertnet.org/), and there are fewer than five amphibian records from ETL in iNaturalist (https://www.inaturalist.org/), demonstrating the need for a local-level accounting of this fauna. The objective of this study was to investigate the richness, relative abundance, alpha diversity, and beta diversity of amphibian communities in the ETL across the high diversity of habitats that exist in the area. Based on our results, we propose actions for the conservation of the group and of the respective ecosystems of the study area.

Materials and Methods

Study site.—The ETL, also known as Ortiz, is a community located in the southwestern region of the state of Chiapas, between coordinates 16.32/16.43 and -93.91/-93.77 (Fig. 1). The Government of the State of Chiapas established the ETL in 1929, as a response to the demand for land from laborers and low-income workers from various rancherias in the Municipalities of Cintalapa and Jiquipilas (Vázquez-López et al. 1977). The ETL today encompasses 6,501 ha, which include the original endowment plus two extensions made in 1939 and 1980, respectively (https://phina.ran.gob.mx/consultaPhina.php). A total of 5,793 ha lie in the Municipality of Jiquipilas, while an additional small non-contiguous plot of 708 ha (which is one of the later extensions) is located in the adjacent Municipality of Arriaga (https://idegeo.centrogeo.org.mx/layers/geonode:ran_nucleosagrarios_chiapas).

The ETL ranges from 620–1,220 m elevation and is located in a transitional area between two physiographic regions, the Sierra Madre de Chiapas and the Central Depression (Müllerried 1982). According to the Köeppen climate classification system modified by García (2004), the ETL is of two climate types. First, a warm sub-humid climate with rains and heat waves in summer, winter rain < 5% of the annual total, and an average annual temperature of 24°–28º C. This climate extends from the lowest zone of the ETL (620 m elevation) up to 1,000 m elevation. Second, a semi-warm sub-humid climate with similar seasonality of heat

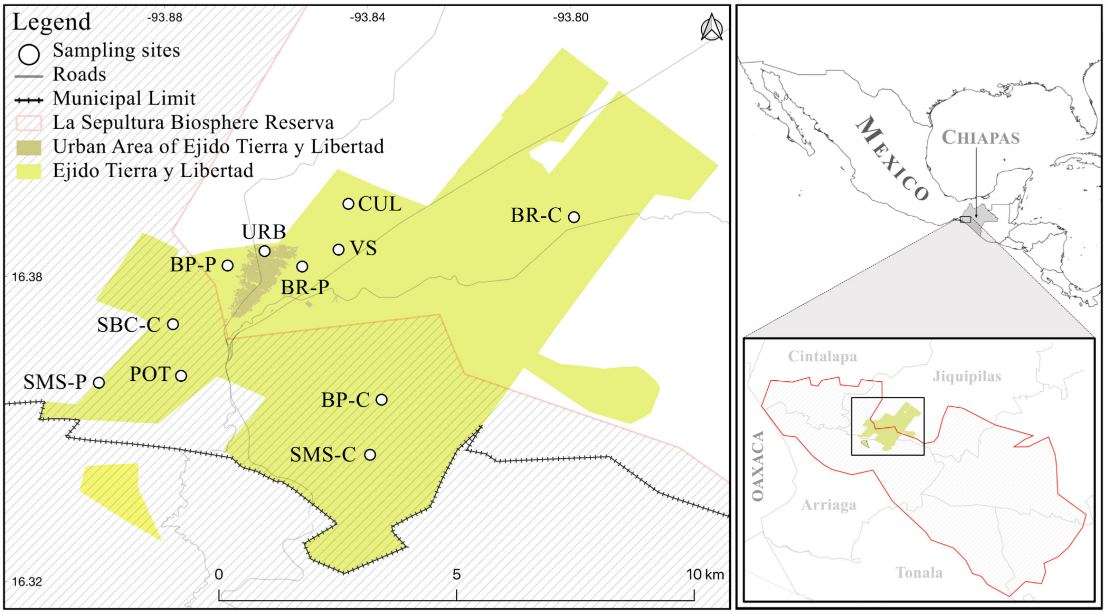

Figure 1. Geographic location of study area and survey transects in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico. Transect abbreviations are SMS-C = undisturbed mid-elevation Semi-Deciduous Forest, SMS-P = mid-elevation Semi-Deciduous Forest disturbed with coffee plantations, SBC-C = undisturbed Lowland Deciduous Forest, VS = secondary-growth lowland deciduous scrubland/grassland, BP-C = undisturbed Pine Forest, BP-P = disturbed Pine Forest, BR-C = undisturbed Riparian Forest, BR-P = disturbed Riparian Forest, CUL = cultivated cropland, excluding coffee plantations, POT = pastureland, excluding secondary vegetation, and URB = urban area.

waves and precipitation but average annual temperature of 18°–22º C occurs above 1,000 m elevation. Based on the classification by the Instituto Nacional de Estadística y Geografía (2015), the following types of land use and vegetation exist in the ETL: Montane Cloud Forest, 1,150–1,220 m elevation; mid-elevation Semi-Deciduous Forest, 720–1,150 m elevation; Pine-Oak Forest, 680–830 m elevation; Lowland Deciduous Forest, 650–1,080 m elevation; Pine Forest, 650–1,030 m elevation; urban areas, 650–720 m elevation; Riparian Forest, 620–1,050 m elevation; secondary-growth lowland deciduous scrubland/grassland, 620–1,000 m elevation; and agricultural areas, 620–900 m elevation.

Data collection.—We gathered field data from January 2016 to April 2020. From January 2016 to April 2019, we carried out non-systematic sampling throughout the study area both by day and at night in the dry and rainy seasons. From May 2019 to April 2020, we shifted to a systematic sampling strategy focused on 11 well-defined survey transects. Each transect measured 500 m long by 3 m wide and was selected for close proximity to a stream or other body of water to maximize detections of amphibians (Gaviño de la Torre et al. 1977). We sampled these transects 6 d per month, with surveys taking place from 0900 to 1200 and from 1900 to 2200 (Casas-Andreu et al. 1991). A single person (EJV) carried out most of the systematic surveys, but two people participated from May to September 2019, summing to a total systematic effort of 980 person-hours. Each of our 11 transects sampled distinct environments (Fig. 1): undisturbed mid-elevation Semi-Deciduous Forest; mid-elevation Semi-Deciduous Forest disturbed with coffee plantations; undisturbed Lowland Deciduous Forest; secondary-growth lowland deciduous scrubland/grassland; undisturbed Pine Forest; disturbed Pine Forest; undisturbed Riparian Forest; disturbed Riparian Forest; cultivated cropland, excluding coffee plantations; pastureland, excluding secondary vegetation; and an urban area. Cultivated crops and pasture are subsets of agricultural land use, and four pairs of transects compare undisturbed and disturbed patches of mid-elevation Semi-Deciduous Forest, Lowland Deciduous Forest, Riparian Forest, and Pine Forest, respectively.

During surveys, we used the visual encounter technique (Crump and Scott 1994; Lips et al. 2001) and examined potential microhabitats from ground level up to 3 m in height (Manzanilla and Péfaur 2000). For each individual amphibian observed, we recorded the date, time, vegetation type, microhabitat, latitude, longitude, and elevation at its site. We classified microhabitat into eight categories following criteria proposed by previous authors (Canseco-Márquez 1996; Ramírez-Bautista and Nieto-Montes de Oca 1997; Vitt et al. 2000): (1) fossorial (inhabiting holes in the ground or in earthen walls); (2) semifossorial (under leaf litter, logs, or rocks); (3) terrestrial (on the surface of the ground, with or without leaf litter); (4) saxicolous (found among or on rocks); (5) arboreal (above ground level on shrubs or trees, including the forest canopy, under bark of standing trees, or among epiphytic vegetation); (6) anthropogenic (artificial water reservoirs, storm drains, among human rubble or on houses, or among planted crops); (7) riparian vegetation (on plants in families such as Araceae and Liliaceae growing on the banks of natural bodies of water); and (8) freshwater (in seasonal or permanent natural bodies of water). We did not mark any individuals due to the low recapture rate of herpetofauna in neotropical environments (Zabala-Forero and Urbina-Cardona 2021). For most amphibian species observed, we deposited voucher photographs in the digital collection of the Natural History Museum of Los Angeles County (LACM PC), Los Angeles, California, USA. For most species that we documented both within and outside the boundaries of the REBISE, we deposited voucher photographs supporting the presence of the species in both areas.

Data analysis.—We identified observed amphibian species using the checklist for Chiapas available in Johnson et al. (2015), supplemented with the dichotomous keys available in Köhler (2011), and the original descriptions for certain species. We follow the taxonomy of AmphibiaWeb (https://amphibiaweb.org/) and use the genus name *Rana* instead of *Lithobates* in accordance with Yuan et al. (2016). For common names, we followed (Frost, D. 2022. Amphibian Species of the World: an Online Reference. Version 6.1. Available from https://amphibiansoftheworld.amnh.org/ [Accessed 28 July 2022]) and Crother et al. (2017). We categorized the risk status of each species by referencing the Norma Oficial Mexicana NOM-059-SEMARNAT-2010 of the Mexican federal government (Secretaría de Medio Ambiente y Recursos Naturales 2010), the IUCN Red List of Threatened Species (IUCN 2022), and the Environmental Vulnerability Score (EVS) system proposed by Wilson et al. (2013).

We calculated expected species richness (Moreno 2001), using the EstimateS 9.1 program (https://www.robertkcolwell.org/pages/estimates). For this analysis, we used four estimators: (1) Jackknife 1 and 2, which assume habitat heterogeneity between the samples and perform well with small samples (Magurran, 2004; Zabala-Forero and Urbina-Cardona, 2021); (2) Bootstrap, which considers the estimated richness of amphibians derived from the different habitats and states of human intervention (Magurran 2004); and (3) Chao 1, which is based on abundance and assumes homogeneity of the habitat in the samples (Jiménez-Valverde and Hortal 2003; Magurran 2004). For the general species accumulation curve, we used all estimators in the same way, with the exception of Chao 1.

We estimated Alpha and Beta Diversity based only on the records we obtained from systematic fieldwork. For Alpha Diversity, we used the Shannon-Wiener index:

$$H' = -\Sigma\, p_i \ln p_i$$

Where ln is the natural logarithm, and p_i is the proportional abundance of i species, that is, the number of individuals of species i divided by the total number of individuals in the sample. We applied this index because it is an easy-to-use standard method (Vite-Silva et al. 2010) that assumes random selection of individuals and assumes that all species are represented in the sample (Magurran 2004). To quantify the similarity of the amphibian assemblage between pairs of transects with similar vegetation types, we performed a cluster analysis using a presence-absence matrix and Jaccard's Similarity Coefficient (JSC):

$$JSC = (100 \times S) / (n_1 + n_2 - S)$$

Where S = number of shared species, n_1 = number of species at site 1, and n_2 = number of species at site 2 (Moreno 2001). We performed these statistical analyses using Past4 software (https://www.nhm.uio.no/english/research/infrastructure/past/).

Results

We recorded a richness of 20 amphibian species in the ETL, from 15 genera and 10 families. Hylidae and Bufonidae were the most well-represented families, with five and four species detected, respectively. The order with the most species was Anura (85% of the total richness), followed by Caudata (10%) and Gymnophiona (5%; Table 1). Of the total number of species, we documented four only within REBISE, three only outside REBISE, and 13 both within and outside REBISE (Table 1). Five species are categorized as Subject to Special Protection (Sujeta a Protección Especial, Pr, on the NOM-059-SEMARNAT-2010 list of the Mexican federal government): the Schmidt's Mountain Brook Frog (*Duellmanohyla schmidtorum*), the Forrer's Grass Frog (*Rana forreri*), the Burrowing Toad (*Rhinophrynus dorsalis*), the Southern Banana Salamander (*Bolitoglossa occidentalis*), and the Mexican Caecilian (*Dermophis mexicanus*). Four species are considered threatened to some degree (IUCN 2022), with *Duellmanohyla schmidtorum*, the Matuda's Spikethumb Frog (*Plectrohyla matudai*), and *Dermophis mexicanus* categorized as Vulnerable (VU), while the Yellowbelly Mushroomtongue Salamander (*Bolitoglossa flaviventris*) is categorized as Endangered (EN). Of the remaining species, 16 are categorized as

Table 1. Amphibians of Ejido Tierra y Libertad, Jiquipilas, Chiapas, with conservation status of the species documented in and outside La Sepultura Biosphere Reserve. Digital photographic vouchers on deposit with the Natural History Museum of Los Angeles County (see Appendix 1 for Voucher numbers). Microhabitat abbreviations are A = arboreal, B = buildings, F = fossorial, FW = freshwater, R = Riparian, S= Saxicoulous, SF = semifossorial, T = terrestrial. An asterisk (*) indicates an endemic species to Mexico. Conservation statuses are: NM = NOM-059-SEMARNAT-2010 (Mexico) with Pr = subject to special protection and NS = no status; IUCN = International Union for Conservation of Nature Red List of Threatened Species 2015 with NE = not evaluated, LC = Least Concern, NT = Near Threatened, VU = Vulnerable, and EN = Endangered; EVS = Environmental Vulnerability Score with L = Low (3–9) and M = Medium (10–13).

	Conservation Status			La Sepultura		
Taxon	NM[1]	IUCN[2]	EVS[3]	In	Out	Micro-habitat
Anura						
Bufonidae						
Dwarf Toad (*Incilius canaliferus*)	NS	LC	L(8)	Yes	No	T,R
Wiegmann's Toad (*I. marmoreus*)*	NS	LC	M(11)	No	Yes	T
Southern Gulf Coast Toad (*I. valliceps*)	NS	LC	L(6)	Yes	Yes	T,R,FW
Mesoamerican Cane Toad (*Rhinella horribilis*)	NS	LC	L(3)	Yes	Yes	F,SF,T,R,FW
Craugastoridae						
Common Leaf-litter Frog (*Craugastor loki*)	NS	LC	M(10)	Yes	Yes	SF,T,R,FW
Eleutherodactylidae						
Whistling Chirping Frog (*Eleutherodactylus pipilans*)	NS	LC	M(11)	Yes	Yes	T
Hylidae						
Mertens' Yellow Tree Frog (*Dendropsophus robertmertensi*)	NS	LC	L(9)	Yes	Yes	A,R
Schmidt's Mountain Brook Frog (*Duellmanohyla schmidtorum*)	Pr	VU	L(8)	Yes	No	S,A,R,FW
Matuda's Spikethumb Frog (*Plectrohyla matudai*)	NS	VU	M(11)	Yes	No	S,R,F
Stauffer's Treefrog (*Scinax staufferi*)	NS	LC	L(4)	Yes	Yes	A,R,F
Mexican Treefrog (*Smilisca baudinii*)	NS	LC	L(3)	Yes	Yes	T,A,B,R,FW
Leptodactylidae						
Mexican White-lipped Frog (*Leptodactylus fragilis*)	NS	LC	L(5)	No	Yes	T
Sabinal Frog (*L. melanonotus*)	NS	LC	L(5)	Yes	Yes	
Microhylidae						
Mexican Narrow-mouthed Toad (*Hypopachus variolosus*)	NS	LC	L(4)	Yes	Yes	F,S,T
Ranidae						
Forrer's Grass Frog (*Rana forreri*)	Pr	LC	L(3)	Yes	Yes	T,R,FW
Masked Mountain Frog (*R. maculata*)	Pr	LC	L(3)	Yes	Yes	T
Rhinophrynidae						
Burrowing Toad (*Rhinophrynus dorsalis*)	Pr	LC	L(8)	No	Yes	F,T,FW
Caudata: Plethodontidae						
Yellowbelly Mushroomtongue Salamander (*Bolitoglossa flaviventris*)	NS	EN	M(13)	Yes	Yes	A,R
Southern Banana Salamander (*B. occidentalis*)	Pr	LC	M(11)	Yes	No	A
Gymnophiidae: Dermophiidae						
Mexican Cecilian (*Dermophis mexicanus*)	Pr	VU	M(11)	Yes	Yes	T

Least Concern (LC; Table 1). In contrast, no species that we found had high values of the Environmental Vulnerability Score; all species fell in the medium and low categories. The Wiegmann's Toad (*Incilius marmoreus*) is the only amphibian species we documented in the ETL that is endemic to Mexico.

The total species accumulation curve for our study area indicated that the amphibian assemblage recorded during the field phase was highly representative, varying from 80.41% with Jacknife 2, 91.2% with Jacknife 1, and 100% with Bootstrap. Based on the Jacknife 2 estimator, detection of up to five additional species is necessary to reach the asymptote and completeness of the inventory (Fig. 2). The values of representativeness for each transect ranged from 50–90% (Table 2). The five most frequently encountered species in the ETL were

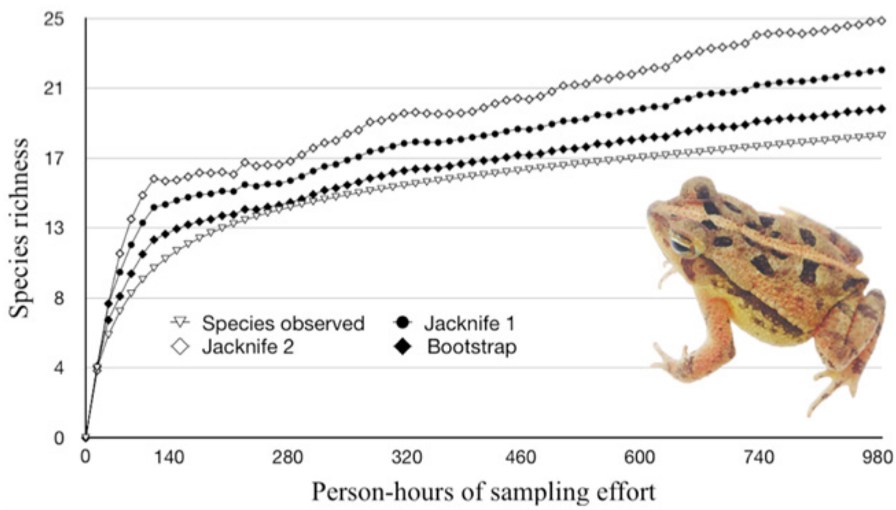

Figure 2. Accumulated general richness of amphibian species from the total number of samples in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico. Inset illustrates the Dwarf Toad (*Incilius canaliferus*). (Photographed by Emmanuel Javier-Vázquez).

the Common Leaf-litter Frog (*Craugastor loki*) with 1,038 observations, the Mesoamerican Cane Toad (*Rhinella horribilis*) with 364 observations, the Forrer's Grass Frog (*Rana forreri*) with 195 observations, the Sabinal Frog (*Leptodactylus melanonotus*) with 135 observations, and *Duellmanohyla schmidtorum* with 87 observations (Fig. 3). Survey transects with the highest documented species richness were the urban areas with 10 species and the disturbed Riparian Forest with eight species, while the pastureland and the secondary-growth lowland deciduous scrubland/grassland had the lowest richness, with only four and two species, respectively (Table 2).

The three types of vegetation with the highest Alpha Diversity were undisturbed mid-elevation Semi-deciduous Forest (H' = 2.39), followed by disturbed Riparian Forest (H' = 2.36) and undisturbed Riparian Forest (H' = 2.24), while the vegetation types with the lowest Alpha Diversity were undisturbed Pine Forest (H' = 1.44) and pastureland (H' = 0.96; Table 2). The Jaccard Similarity Coefficient analysis generated two major groups, one comprised of undisturbed Lowland Deciduous Forest; cultivated cropland; undisturbed Riparian Forest; disturbed Riparian Forest; urban area; pastureland; undisturbed Pine Forest and disturbed Pine

Table 2. Values of amphibian abundance, alpha diversity (H'), richness, and richness estimators for transects in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico. The abbreviation N/A indicates estimators that were not calculated because the assumptions of environmental homogeneity were unmet. In the case of secondary-growth lowland Deciduous scrubland/grassland, the low number of species we observed prevented the calculation of any estimator. Anthropogenic disturbance statuses are U = undisturbed and D = disturbed.

Vegetation	Abundance	H'	Richness	Jack 1	Jack 2	Chao 1	Chao 2
Mid-elevation Semi-Deciduous Forest (U)	485	2.39	5	5.96	6	5	5
Mid-elevation Semi-Deciduous Forest (D, Coffee Plantations)	264	2.13	4	4	4	4.48	4
Lowland Deciduous Forest (U)	179	1.94	5	6.92	7.87	5.99	5.48
Secondary-growth Lowland Deciduous Scrubland/Grassland	44	1.45	2	N/A	N/A	N/A	N/A
Pine Forest (U)	51	1.44	5	5	N/A	5	5
Pine Forest (D)	86	1.46	7	N/A	7.87	N/A	N/A
Riparian Forest (U)	274	2.24	7	7.96	8.88	7	7
Riparian Forest (D)	283	2.36	8	9.92	10.87	8	8.48
Cultivated Crops	39	1.98	7	10.83	13.62	9.92	9.88
Pastureland	9	0.96	4	4	4	N/A	N/A
Urban Area	498	1.94	10	12.88	12.99	10.5	10.72

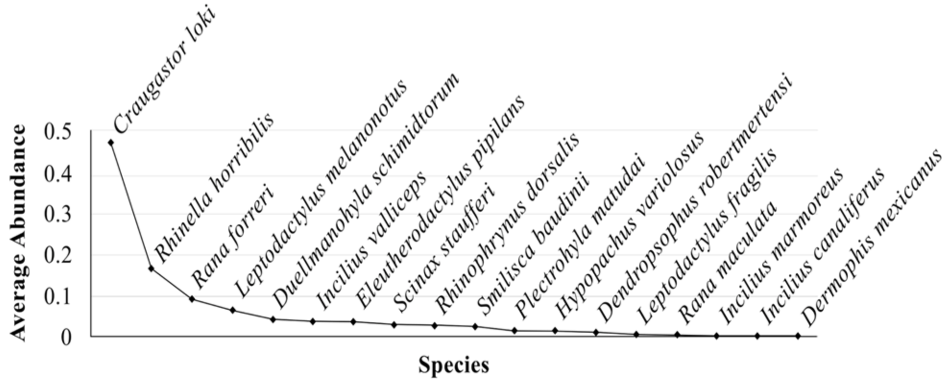

Figure 3. Relative abundance of amphibians as a proportion of total individuals observed in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico. Species are Common Leaf-litter Frog (*Craugastor loki*), Mesoamerican Cane Toad (*Rhinella horribilis*), Forrer's Grass Frog (*Rana forreri*), Sabinal Frog (*Leptodactylus melanonotus*), Schmidt's Mountain Brook Frog (*Duellmanohyla schmidtorum*), Southern Gulf Coast Toad (*Incilius valliceps*), Whistling Chirping Frog (*Eleutherodactylus pipilans*), Stauffer's Treefrog (*Scinax staufferi*), Burrowing Toad (*Rhinophrynus dorsalis*), Mexican Treefrog (*Smilisca baudinii*), Matuda's Spikethumb Frog (*Plectrohyla matudai*), Mexican Narrow-mouthed Toad (*Hypopachus variolosus*), Mertens' Yellow Tree Frog (*Dendropsophus robertmertensi*), Mexican White-lipped Frog (*Leptodactylus fragilis*), Masked Mountain Frog (*Rana maculata*), Wiegmann's Toad (*Incilius marmoreus*), Dwarf Toad (*Incilius canaliferus*) and Mexican Cecilian (*Dermophis mexicanus*).

Forest. The second group of similar transects included undisturbed mid-elevation Semi-deciduous Forest; mid-elevation Semi-deciduous Forest disturbed with coffee plantations and secondary-growth lowland deciduous scrubland/grassland). The greatest similarity, however, was found between the transect pair undisturbed Lowland Deciduous Forest and pastureland, followed by the pairs undisturbed Riparian Forest/disturbed Riparian Forest and cultivated cropland/disturbed Riparian Forest (Fig. 4). With respect to microhabitat, in the ETL, we found the most amphibian species in the terrestrial microhabitats (13 species), followed by the riparian microhabitats (11 species). We found only one species in anthropogenic sites (i.e., in buildings; Fig. 5).

Discussion

Amphibian species richness in the ETL represents 18.5% of the 108 species reported for the state of Chiapas (Johnson et al. 2015; Hernández-Ordoñéz et al. 2017). At the national level, the ETL amphibian assemblage includes 4.7% of the total species reported for Mexico (https://amphibiaweb.org/). Although we recorded the salamander species *Bolitoglossa flaviventris* and *B. occidentalis* only outside of our systematic sample transects, they are important additions to the amphibian richness of the ETL. In the case of *B. flaviventris*, this is the first record of the species for the ETL and REBISE and represents a major range extension. The

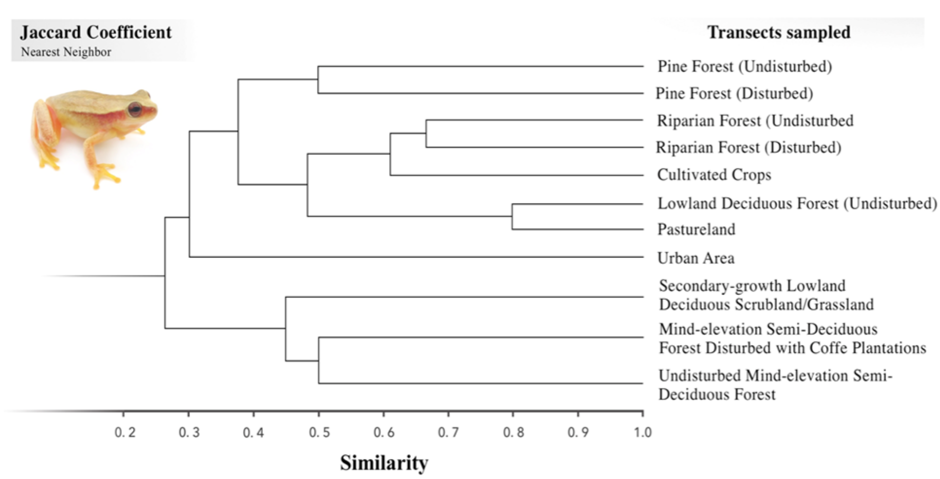

Figure 4. Dendrogram of amphibian similarity between standardized survey transects in the Ejido Tierra and Libertad, Municipality of Jiquipilas, Chiapas, Mexico, based on the Jaccard Coefficient. Inset is the Mertens' Yellow Tree Frog (*Dendropsophus robertmertensi*). (Photographed by Emmanuel Javier-Vázquez).

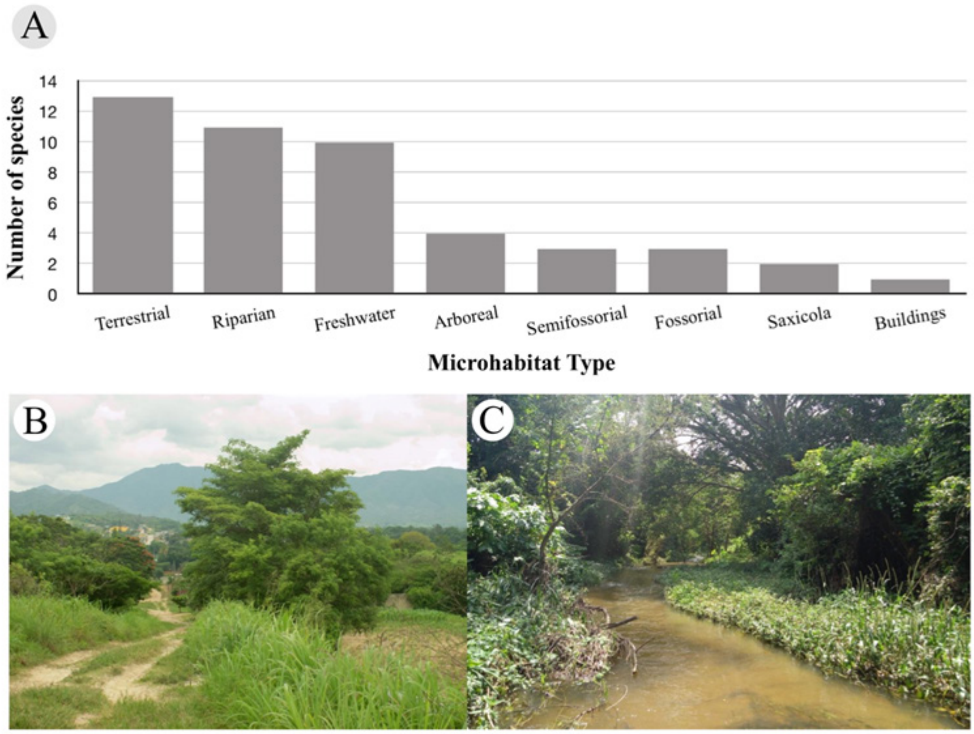

Figure 5. (A) Richness of amphibian species by microhabitat in the Ejido Tierra y Libertad (ETL), Municipality of Jiquipilas, Chiapas, Mexico. (B) Typical terrestrial habitat on the edges of the urban area and (C) Typical riparian habitat along the main river of ETL. (Photographed by Emmanuel Javier Vázquez).

ETL has a higher amphibian species richness than other similarly sized areas of Chiapas such as the Iglesia Vieja Archaeological Site in the Municipality of Tonalá (12 species; López-Villa 2018), or Polygon I of the El Triunfo Biosphere Reserve (17 species; Luna-Reyes 1997). According to the REBISE management program (Instituto Nacional de Ecología 1999), we documented 70.9% of the amphibians reported from the Reserve (24 species) but based on (Núñez-Orantes and Muñoz-Alonso 2000), the percentage is lower (43.6%) because they reported 39 species from the Reserve (Table 1). The high amphibian richness of the ETL is likely attributable both to its location at the junction of two different physiographic regions, and to its diversity of habitat types across a wide elevational gradient (Johnson et al. 2010).

The species assemblage that we detected comprised a high percentage of the total species richness present (91.2% and 100%, respectively), suggesting that our sampling effort was adequate; however, the Jacknife 2 estimator indicates that an additional four to five species might be expected to occur in the area. According to the maps of Köhler (2011), the Large-crested Toad (*Incilius macrocristatus*), the Chimalapas Toad (*Incilius tutelarius*), the Veined Treefrog (*Trachycephalus typhonius*), the Northern Glassfrog (*Hyalinobatrachium viridissimum*), the Polymorphic Robber Frog (*Craugastor rhodophis*), and the Sumichrast's Treefrog (*Exerodonta sumichrasti*) are the additional species most likely to be found at the site. Sampling in high mountain areas with cloud forest-type vegetation, in addition to some sites located in the Central Depression, could reveal the presence of these missing species, as long as the expanded sampling effort uses appropriate techniques (Urbina-Cardona and Reynoso 2005).

Remarkably, disturbed areas supported among the highest amphibian diversity across our study area. In fact, the urban area transect possessed the greatest number of species (10), followed by the transects disturbed Riparian Forest (eight species), undisturbed Riparian Forest (seven), cultivated cropland (seven), and disturbed Pine Forest (seven). Most of the dominant species in these transects are widely distributed and considered to be generalists (e.g., *Smilisca baudinii*, *Rana forreri*, *Incilius valliceps*, *Hypopachus variolosus*, and *Eleutherodactylus pipilans*), with apparent tolerance to anthropogenic activities that cause vegetation fragmentation and degradation as long as suitable breeding sites remain (Duellman 1966; Johnson 1989; Jellineck et al. 2004; Gonzáles-García et al. 2009). *Rhinella horribilis* was particularly abundant in the urban area. One likely contributing factor to the persistence of amphibians in urban and disturbed areas of the ETL is the presence of silvopastoral systems such as living fences and narrow corridors of native trees, which generate functional landscape connectivity that allows amphibian movement and dispersal (Arroyo-Rodríguez et al. 2020). Additionally, urban areas can have permanent water sources (in the form of storage tanks, artificial pools, etc.), thus providing moist refugia and even breeding sites for many amphibian species (Pedroza-Banda and Angarita-Sierra 2011).

The most frequently observed amphibian species in the ETL was *Craugastor loki*, with almost three times as many observations as the next-most commonly seen species. This species numerically dominated the majority of transects, including both undisturbed and disturbed vegetation types: undisturbed mid-elevation Semi-deciduous Forest, mid-elevation Semi-deciduous Forest disturbed with coffee plantations, undisturbed Riparian Forest, secondary-growth lowland deciduous scrubland/grassland, undisturbed Pine Forest, disturbed Riparian Forest and cultivated cropland. The abundance of *C. loki* may be explained by its being one of a few anuran species documented in the ETL that undergoes direct development, with no aquatic larval phase (Savage 2002).

When we group *C. loki* with *Rhinella horribilis*, *Rana forreri*, *Leptodactylus melanonotus* and *Duellmanohyla schmidtorum*, these five species represented 82.1% of the total number of individual amphibians we recorded in the ETL. In contrast, we rarely saw species such as the *Leptodactylus fragilis*, *Incilius canaliferus*, *Scinax staufferi*, *Rhinophrynus dorsalis*, or *Dermophis mexicanus* in the ETL. Their scarcity is either because of their secretive habits or because their populations are naturally smaller than other species, or both. We mostly observed these species active during the rainy season, with only chance encounters in the dry season. *Rhinophrynus dorsalis* represented 10.8% of the total amphibian abundance in the urban area transect in the month of May, which is consistent with its natural history as an explosive breeder. Adults emerge from underground after the first intense rains to gather in temporary ponds and reproduce during a time window known to be one of the shortest among amphibians, with a duration ranging from one night to a few nights (Leenders 2001; Sandova et al. 2015). Similarly, we only observed the largely fossorial species *Dermophis mexicanus* alive at night moving on roads between the moist soil with grass and muddy sites, and by day only as dead specimens on roads.

We found the highest amphibian diversity (H' = 2.39) in undisturbed mid-elevation Semi-deciduous Forest, followed by undisturbed Riparian Forest (H' = 2.36) and disturbed Riparian Forest (H' = 2. 24). The high amphibian diversity in these sites is closely associated with the existence of water sources, which offer breeding sites and vegetation that provide favorable environmental conditions for many amphibian species (Ray 1995). This makes the mid-elevation Semi-deciduous Forest and the Riparian Forest important habitats within the ETL for the protection of amphibians (Granados-Sánchez et al. 2006). Despite experiencing serious local environmental problems due to drought, contaminants from agrochemicals, urban runoff, and runoff from Pig (*Sus scrofa*) farms, these two habitat types remain important areas for maintenance of local amphibian diversity in the long term.

Between-site comparisons using the Jaccard Coefficient showed the existence of two main groups of transects with cohesive amphibian assemblages: those of transects below 800 m elevation (undisturbed Lowland Deciduous Forest, cultivated cropland, undisturbed Riparian Forest, disturbed Riparian Forest, urban area, pastureland, undisturbed Pine Forest, disturbed Pine Forest), and those of transects of higher-elevation, more temperate areas > 800 m elevation (undisturbed mid-elevation Semi-deciduous Forest, mid-elevation Semi-deciduous Forest disturbed with coffee plantations), except for the secondary-growth lowland deciduous scrubland/grassland. This demonstrates that most amphibian species in the ETL are widely distributed between localities in low-lying areas of the Sierra Madre de Chiapas and the Central Depression.

The localities of undisturbed Lowland Deciduous Forest/pastureland presented the greatest similarity of amphibians, which was probably associated with the fact that both the undisturbed Lowland Deciduous Forest and the pastureland present a relatively dry environment most of the year, supporting species with direct development such as *Craugastor loki* and *Eleutherodactylus pipilans* in the driest months of the year, due to their relative reproductive independence from temporary or permanent bodies of water. The similarity of amphibians between the undisturbed Riparian Forest and disturbed Riparian Forest could be explained because these forests (despite the disturbed areas) form part of a continuous habitat and certain fragments share part of the structure and composition of the vegetation, as well as similar conditions regarding the availability of water in both areas. Likewise, the amphibian similarity between the crop localities/disturbed Riparian Forest pair was probably due to the proximity between the two transects, and the existence of a heterogeneous matrix formed by crops such as Corn (*Zea mays*) and Common Beans (*Phaseolus vulgaris*) and some original vegetation elements such as the trees *Madrecacao* (*Gliricidia sepium*), Pencil Willow (*Salix humboldtiana*), and *Amates* (*Ficus insipida*).

The high species richness in the terrestrial microhabitat (13) is due to nearly all amphibian species using the ground at some point in their lives, as they pass between and among vegetation and water (Wells 2007). Riparian and freshwater microhabitats, including the banks of streams and rivers, were the second- and third-most used, and supported a fauna that relies on water for reproduction (Hewlett and Helvey 1970). In the arboreal habitat we found mainly hylid frogs. In the semifossorial microhabitat we found direct developing species such as *C. loki* and *E. pipilans,* consistent with the findings of Urbina-Cardona and Reynoso (2005), who stated that this is a preferred microhabitat for species with direct development.

Among our most important conservation results is that the pasture transect has the lowest diversity of amphibians in the ETL (H ́= 0.96), in addition to having the lowest number of observations, and the second-lowest species richness of all survey transects. Pastures lack the trees and shrubs that create important microhabitat heterogeneity for amphibians. Unfortunately, such pastures are a common and growing feature on the landscape in the ETL, due to a variety of factors that include historical tree extraction by private sawmills, expansion of cattle grazing, meteorological phenomena such as Hurricane Barbara in 2013 that caused forest loss, the use of fire by hunters, and passers-by that intentionally or unintentionally cause fires on the main thoroughfares (Ocozocoautla-Arriaga highway and the Villaflores-Santa Isabel state highway; Braasch et al. 2018; Caballero-Salinas 2020). These factors, coupled with minimal local societal emphasis on maintaining natural areas, have resulted in the establishment of substantial pastureland to the clear detriment of amphibian populations, consistent with findings from other researchers (Lara-Tufiño et al. 2019; Zabala-Forero and Urbina-Cardona 2021).

There have been numerous efforts to reduce the loss of forest cover in the ETL. Such activities include the Payment for Hydrological Environmental Services program in 2005–2009 and 2012–2016 financed by the Mexican government, the productive transition towards extensive cattle ranching in wild pastures that maintain some natural canopy, the adoption of agrosilvopastoral systems, the implementation of controlled burning, and active reforestation (Caballero-Salinas 2020). These activities, however, generally remain unconsolidated and only adopted piecemeal, and thus have generated limited benefit in the protection and maintenance of the forest areas of the study area (CONANP 2013; Caballero-Salinas 2020). Most local inhabitants do not choose to carry out conservation activities unless there is a payment, because those activities usually involve substantial effort to implement. Additionally, some residents remain noncompliant with the prohibition against use of fire, due to long held beliefs that burning is an indispensable practice for livestock husbandry (Gutiérrez-Navarro et al. 2017; Caballero-Salinas 2020). These facts have contributed to the well-documented history of regular, severe fires that occur in the REBISE, which progressively cause replacement of forest with grassland and scrubland (Román-Cuesta and Martínez-Vilalta 2006).

The predictions of different regional climate models (MPI-ESM-LR, CNRMCM5, HADGEM2-ES and GFDL-CM3) and two representative concentration pathways (RCP), indicate for the Sierra Madre de Chiapas an increase in temperature of 3°–4° C (RCP 4.5 and 8.5, respectively) is expected by the years 2045–2069. Similarly, annual precipitation is expected to decrease by 60–100 mm (RCP 4.5 and 8.5, respectively; Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Instituto de Biología de la Universidad Nacional Autónoma de México (IB-UNAM), Comisión Nacional de Áreas Naturales Protegidas (CONANP), Programa de las Naciones Unidas para el Desarrollo (PNUD) and Instituto Nacional de Ecología (INE) y Cambio Climático (INECC). 2020. Explorador de cambio climático y biodiversidad. https://www.biodiversidad.gob.mx/pais/cambio-climatico ([Accessed 23 November 2021]). These climatic changes threaten to further increase the frequency and magnitude of fires in the ETL because it is located in one of the two fire hotspot zones and in one of the three areas with the most human pressure in the Sierra Madre de Chiapas (Godínez-Gómez et al. 2020).

Recommendations.—To maximize the persistence of amphibian species in the ETL, along with other taxonomic groups, we consider basic environmental education of local residents to be a necessity. Without clear, targeted communication about the ecological problems that exist and the effects of those problems on local biodiversity (such as the poor habitat value of pastures for amphibians, as documented in this study), conservation action will stall. In the dissemination processes, we consider photographs and other visual materials to be valuable tools for sharing knowledge with a wide array of people, creating a foundation of societal environmental literacy that is critical before effective conservation processes can be developed.

After, or concurrent with, this education strategy, we propose implementing activities that, according to Aguilar-López et al. (2020), have proven to be effective in the conservation of amphibians in modified landscapes, such as: (1) increasing vegetation and green spaces in urban areas; (2) promoting silvopastoral systems to establish greater landscape connectivity; (3) ecological restoration of forests that were transformed into induced grasslands and other modified environments (Smith et al. 2015; Díaz-García et al. 2017); (4) long-term protection of existing forests, particularly Riparian Forests and Pine

Forests (Granados-Sánchez et al. 2006; Rodríguez-Mendoza and Pineda 2010; Méndez-Arcadio 2011); (5) maintenance of certain agroecosystems (Lara-Tufiño et al. 2019); and (6) phased substitution of glyphosate as a herbicide and improved management of cattle grazing in conserved mountain areas to prevent damage to water resources (Gómez-Baggethun and Groot 2007; Douterlungne and Ferguson 2012). Ultimately the discipline of agroforestry, which is a land use system that modulates land degradation and supports the conservation of biodiversity, and for which there exists a wide diversity of management strategies and possible spatial arrangements, can help to maximize the heterogeneity and quality of native habitats for many organisms, including amphibians (Rice and Greenberg 2000; Zabala-Forero and Urbina-Cardona 2021). Such actions would not only protect the ecosystems on which amphibian species depend but can also promote human health and welfare within the ETL and in surrounding communities. In addition, several of these actions would cushion the effects of climate change in the region. Despite the great challenge that their compliance in the ETL represents, we hope that, as in other REBISE locations, they will be gradually accepted, and good use can be made of these spaces responsibly (Caballero-Salinas 2020).

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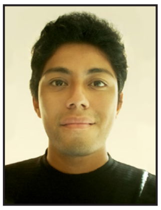

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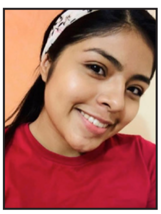

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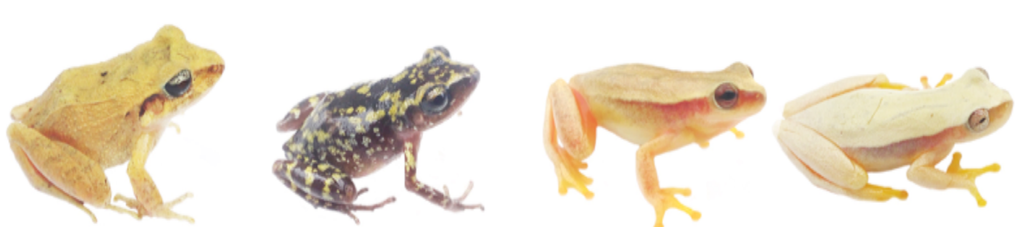
Appendix Figure. Photographic record of amphibian species documented in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico, with selected corresponding voucher numbers deposited in the digital collection of the Natural History Museum of Los Angeles County, Los Angeles, California, USA. For standardization, some photographs are intentionally mirrored horizontally. Images are not to scale. (Photographed by Emmanuel Javier-Vázquez).

LACM PC 2850 — *Incilius canaliferus*

No number in LACM PC — *Incilius marmoreus*

LACM PC 2855 — *Incilius valliceps*

LACM PC 2857 — *Rhinella horribilis*



LACM PC 2858 — *Craugastor loki*

LACM PC 2860 — *Eleutherodactylus pipilans*

LACM PC 2862 (individual at left) — *Dendropsophus robertmertensi*

Appendix Figure (Continued). Photographic record of amphibian species documented in the Ejido Tierra y Libertad, Municipality of Jiquipilas, Chiapas, Mexico, with selected corresponding voucher numbers deposited in the digital collection of the Natural History Museum of Los Angeles County, Los Angeles, California, USA. For standardization, some photographs are intentionally mirrored horizontally. Images are not to scale. (Photographed by Emmanuel Javier-Vázquez).

LACM PC 2864 (individual at left) LACM PC 2865 LACM PC 2867

Duellmanohyla schmidtorum *Plectrohyla matudai* *Scinax staufferi*

LACM PC 2868 LACM PC 2869 LACM PC 2870 LACM PC 2871

Smilisca baudinii *Leptodactylus fragilis* *Leptodactylus melanonotus*

LACM PC 2873 LACM PC 2875 No number in LACM PC No number in LACM PC

Hypopachus variolosus *Rana forreri* *Rana maculata* *Rhinophrynus dorsalis*

No number in LACM PC LACM PC 2883 No number in LACM PC

Bolitoglossa flaviventris *Bolitoglossa occidentalis* *Dermophis mexicanus*