



# Climatic niche, natural history, and conservation status of the Porthole Treefrog, *Charadrahyla taeniopus* (Günther, 1901) (Anura: Hylidae) in Mexico

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**Abstract.**—Amphibian species of the family Hylidae exhibit a high degree of endemism in Mexico. To better understand ongoing declines of many amphibian populations, especially for endemic species that are particularly vulnerable to extinction, information on diverse aspects of their biological makeup is required, including their ecology. This study provides an analysis of the distribution, natural history, feeding habits, reproduction, morphology, and conservation status of *Charadrahyla taeniopus*, a species endemic to central Mexico. The distribution of this species extends along the Sierra Madre Oriental, primarily in cloud forest. Based on changes in climatic niche, decreases of 14.14% and 37% of its distributional range are predicted to occur by the years 2050 and 2070, respectively. An examination of the stomach contents from 31 adults and two juveniles revealed plant materials and arthropods as major parts of their diet. *Charadrahyla taeniopus* is sexually dimorphic in size. Females were larger than males, and after correcting for body size, females had larger jaws than males. Based on guidelines proposed by national legislation (NOM-059), we propose that this species should continue to be classified as Threatened. Further studies are necessary to classify it in a high conservation category by international legislation (IUCN) guidelines, due to the high vulnerability indicated by the Environmental Vulnerability Score, which is caused by an accelerated loss of habitat. *Charadrahyla taeniopus* is a good model for analyzing the conservation status of hylid frogs from temperate areas and in highly transformed environments, as this species exemplifies the conservation status of endemic amphibians in central Mexico.

**Keywords.** Amphibians, Central America, cloud forest, diet, morphology, reproduction

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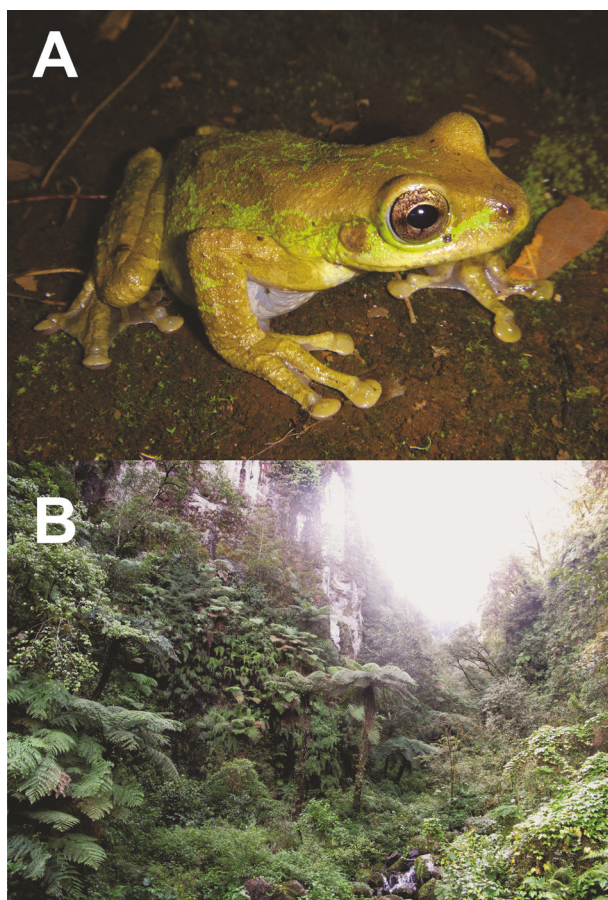
## Introduction

Scientists are concerned about the worldwide decline of amphibians associated with habitat degradation (Delia et al. 2013; Wilson et al. 2013), which is caused by a variety of factors, including shifts in land use, increased pollution, and the splitting and fragmentation of habitat (Becker et al. 2007; Ochoa-Ochoa et al. 2009; Cruz-Elizalde et al. 2015). As a result, many species are globally threatened (Lips et al. 2004; Ochoa-Ochoa et al. 2009) by human activities that are destroying habitat, leading to population decline, extirpation, or even species extinction (Delia et al. 2013). The creation of

regulations and laws at national and international levels, and resources such as the Red List of the International Union for the Conservation of Nature (IUCN), have been important tools for the conservation and management of biodiversity (Wilson et al. 2013).

In Mexico, flora and fauna are protected by the NOM-059, a regulation that provides a way to evaluate the threat level or conservation status of species through a nationally-recognized method: the *Método de Evaluación del Riesgo de Extinción de las Especies Silvestres en México* (method for evaluation of the extinction risk of wild species, MER; DOF 2010). MER has been used to evaluate the conservation status of diverse plant taxa,

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**Fig. 1.** Female individual of *Charadrahyla taeniopus* (A) in a cloud forest and (B) at Tenango de Doria, Hidalgo, Mexico. Photos by Uriel Hernández-Salinas (A) and Raciél Cruz-Elizalde (B).

but major groups of animals, including many species of amphibians, have not yet been evaluated (DOF 2010). Moreover, the lack of knowledge of the distribution and natural history of amphibian species populations impedes the proper application and evaluation of the MER. For example, *Lithobates johni* (Blair, 1965) is an endemic species that is considered endangered, and was reported as extirpated at the type locality in Palictla, San Luis Potosí, Mexico (DOF 2010; Campos-Rodríguez et al. 2012). However, Hernández-Austria et al. (2015) found several robust populations of the species in the state of Hidalgo.

According to MER, conservation assessments should be updated to include current data and to focus on such critical factors as population density, reproductive period, and natural history (Wilson et al. 2013). One method for assessing conservation status that takes these factors into consideration is the Environmental Vulnerability Score (EVS). It is based on an algorithm proposed by Wilson et al. (2013) for amphibian species inhabiting Mexico, and has been accepted for biodiversity and conservation studies of this group at a variety of spatial scales (Johnson et al. 2015; Mata-Silva et al. 2015; Lemos-Espinal et al. 2018a,b). Use of this index has enabled better estimates of the conservation status of amphibian species at regional (Cruz-Elizalde et al. 2015, 2016) and state levels

(Johnson et al. 2015; Mata-Silva et al. 2015) by utilizing information on ecological distribution and reproductive modes (Wilson et al. 2013).

Approximately 99 species of the family Hylidae occur in Mexico (Wilson et al. 2013; Parra-Olea et al. 2014; Canseco-Márquez et al. 2017; Johnson et al. 2017; Jiménez-Arcos et al. 2019), and 68 of these are endemic to the country (Parra-Olea et al. 2014; Caviedes-Solis et al. 2015; Canseco-Márquez et al. 2017; Johnson et al. 2017). Many of the endemic species are distributed in temperate and tropical environments (Flores-Villela et al. 2010; Delia et al. 2013). The family Hylidae exhibits a remarkable species richness and diversity in montane regions (Duellman 2001; Flores-Villela et al. 2010), which are at high risk because of a shift in land use from forests to agroecosystems such as shade coffee plantations and grazing areas (Ochoa-Ochoa et al. 2009; Santos-Barrera and Urbina-Cardona 2011; Murrieta-Galindo et al. 2013). Furthermore, the temperate environments of Mexico are expected to be affected by climate change, leading to a decrease in species richness and diversity (Lips et al. 2004; Urbina-Cardona and Flores-Villela 2010).

The genus *Charadrahyla* (Faivovich et al. 2005) is composed of ten species (Frost 2019) which inhabit the highlands of the Sierra Madre Oriental, Sierra Madre Occidental, and the sierras of Oaxaca and Chiapas (Duellman 2001; Campbell et al. 2009; Frost 2019). Little is known about their ecology and natural history, so most of the species are assigned to categories of high extinction risk by the IUCN. Four of the species, *C. juanita* (Synder, 1972), *C. nephila* (Mendelson and Campbell, 1999), *C. pinorum* (Taylor, 1937), and *C. taeniopus* (Günther, 1901) are in the Vulnerable category; *C. chaneque* (Duellman, 1961) is Endangered; *C. altipotens* (Duellman, 1968) and *C. trux* (Adler and Dennis, 1972) are Critically Endangered; and *C. sakbah* (Jiménez-Arcos, Calzada-Arciniega, Alfaro-Juantorena, Vázquez-Reyes, Blair, and Parra-Olea, 2019, *C. esperancensis* (Canseco-Márquez, Ramírez-González, and González-Bernal, 2017, and *C. tecuani* (Campbell, Blancas-Hernández, and Smith, 2009) have not been evaluated (IUCN 2019). According to NOM-059-SEMARNAT-2010, *C. altipotens*, *C. chaneque*, and *C. pinorum* are in the Subject to Special Protection (Pr) category; *C. juanita*, *C. taeniopus*, and *C. trux* are Threatened (A); and the remaining four have not been evaluated (DOF 2010).

*Charadrahyla taeniopus* (Fig. 1A) inhabits undisturbed cloud forests (Fig. 1B) and pine-oak forests in the Sierra Madre Oriental (Duellman 2001; Kaplan and Heimes 2015), at elevations from 1,100 to 1,200 m in the states of Hidalgo, Puebla, and Veracruz (Duellman 2001). To date, knowledge about the potential distribution, ecology, reproduction, and natural history of this species is limited (Duellman 2001; Kaplan and Heimes 2015). The primary goal of this study is to assess changes in

the current and future (2050 and 2070) climatic niche of this species in Mexico. Secondary goals include characterizing the feeding habits, basic reproductive parameters, morphological variation, and conservation status of this species. This information can be used to develop future conservation strategies in environments with high species numbers and endemism, such as the cloud forests of the Sierra Madre Oriental (Ponce-Reyes et al. 2012).

## Materials and Methods

### Data Collection

Occurrence data for *C. taeniopus* were obtained from (i) the databases of the Global Biodiversity Information Facility (GBIF), the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and HerpNet; (ii) records from publications by Duellman (2001) and Campbell et al. (2009); and (iii) specimens from field work (sporadic collecting in the state of Hidalgo from 2008 to 2016) deposited in the Colección Herpetológica, Centro de Investigaciones Biológicas at the Universidad Autónoma del Estado de Hidalgo (Appendix 1).

### Climatic Niche Modelling

The 37 unique occurrence records of *C. taeniopus* were used to generate climatic niche models. For this study, climate information was obtained from the 19 current climate layers available in the WorldClim database version 1.4 (Hijmans et al. 2005). These climate layers contain the averages of meteorological conditions recorded from North America, with a spatial resolution of 2.5 arc-min. To avoid overrepresentation of environmental variables, a bivariate correlation analysis was carried out with the aim of reducing multicollinearity among the input variables (Merow et al. 2013; Varela et al. 2014). For variables that were highly correlated ( $r > 0.7$ ), the variable was chosen that exhibited the greatest variation or that represented the greatest biological meaning for the actual distribution of the species (e.g., temperature or precipitation). After this procedure, eight climate variables were retained: annual mean temperature (BIO 1), temperature seasonality (standard deviation  $\times$  100, BIO 4), maximum temperature of the warmest month (BIO 5), minimum temperature of the coldest month (BIO 6), annual temperature range (BIO 5–BIO 6, BIO 7), mean temperature of the wettest quarter (BIO 8), mean temperature of the coldest quarter (BIO 11), and precipitation in the driest month (BIO 14; Hijmans et al. 2005). Subsequently, MAXENT was used, the selected climate variables were projected onto a map of Mexico and climate change scenarios were estimated (Phillips and Dudik 2008). Each model was replicated 100 times, the maximum number of repetitions allowed by available

computing power (Dambach and Rödder 2011). Average models for the present and for the future years 2050 and 2070 were then obtained. Current and future projections were estimated using the CCSM-GCM model under the greenhouse concentration scenario RCP8.5, which represents a pessimistic scenario (RCP8.5 = +8.5 W/m<sup>2</sup>).

Finally, to assess the impact of climate change on habitat suitability for the species, the percent change between current and future potential distribution areas was estimated. To determine habitat suitability, the formula % change =  $[(S_1 - S_0)/S_0] \times 100\%$  was used, where  $S_0$  is the total area that the species occupies in the country according to the current distribution scenario, and  $S_1$  is the total area that the species could occupy in the country under future climate change conditions (Gutiérrez and Trejo 2014).

### Diet

Stomach contents were removed from 31 adults and two juveniles (Appendix 1), and a stereomicroscope was used to classify all identifiable organisms to order, including plant material. The number of prey items in each stomach was tallied (n), the number of stomachs with each prey category (i) was determined ( $F_i$ ), and the percentage of stomachs with prey category i (%F) was calculated. Also, the number of prey items belonging to each prey category (N) and the numerical percentage of total abundance (%N) represented by each prey category were determined. Then the volume of each prey item (mm<sup>3</sup>) was calculated using the formula for an ellipsoid (Selby 1965; Duré and Kehr 2004; Duré et al. 2009):  $V = 4/3 \pi (\text{length}/2) (\text{width}/2)^2$ . The food importance index (I) was calculated using the formula of Biavati et al. (2004), which is  $I = (F\% + N\% + V\%)/3$ , where F% is the percentage of occurrence, N% is numerical percentage, and V% is volumetric percentage. The trophic niche amplitude was measured with Levin's standardized index using the formula  $B_A = ((1/\sum p_i^2) - 1)/n - 1$ , where  $p_i$  is the proportion of each prey category with respect to the total number of prey found in each sex, and  $n$  is the number of prey categories in the diet of individuals (Hurlbert 1978). The overlap in dietary composition between sexes was analyzed with Pianka's index (1986):  $O_{jk} = \sum p_{ij} P_{ik} / \sqrt{\sum p_{ij}^2 \sum p_{ik}^2}$ , where  $p_{ij}$  and  $P_{ik}$  are the numerical proportions of prey belonging to the  $i^{\text{th}}$  category that was used by organisms (sexes)  $j$  and  $k$  (Pianka 1986; Gadsden and Palacios-Orona 1997). Analyses of amplitude and overlap for males, females, and juveniles were carried out in the Ecological Methodology v. 6.1.1.1. program (<http://www.exetersoftware.com> [Accessed: 26 January 2016]).

### Reproduction

Thirty-one preserved adults (11 females and 20 males; Appendix 1) were examined to assess reproductive parameters. Fat bodies and livers from all individuals,

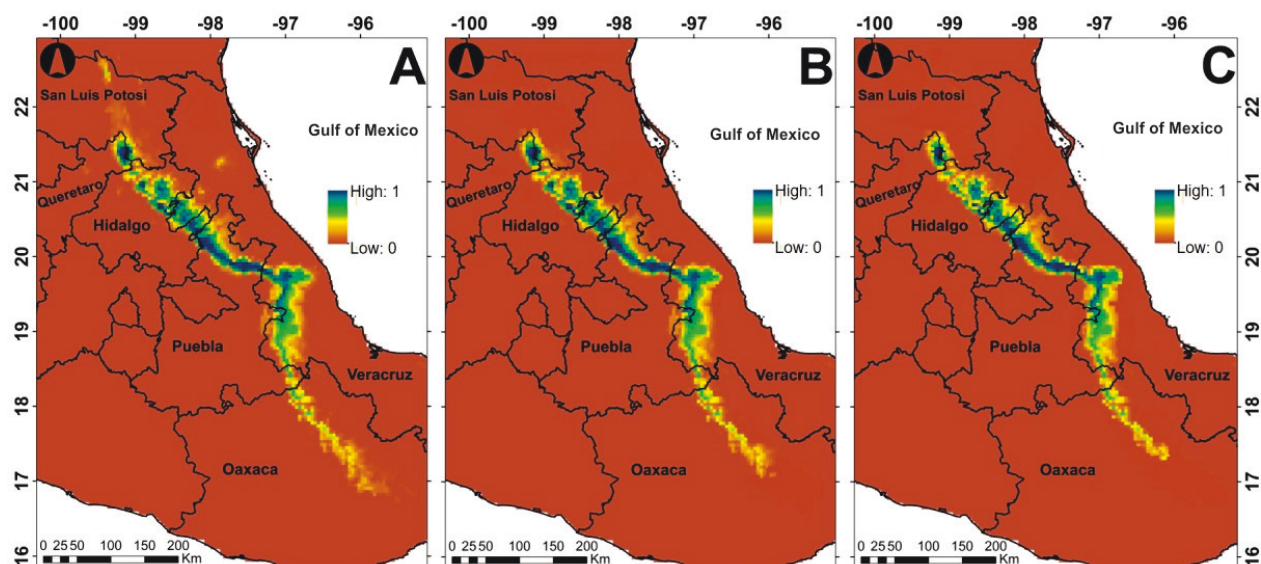


Fig. 2. Current (A), and future 2050 (B) and 2070 (C) scenarios of the climatic niche of *Charadrahyla taeniopus* in Sierra Madre Oriental province (degraded area). The color scale indicates the probability of occupancy for the species.

and egg mass in females or testes mass in males were weighed to the nearest 0.0001 g using an analytical balance. For males, the length and width of testes were measured to the nearest 0.01 mm with a digital caliper, and testicular volume was calculated using the formula for an ellipsoid (Selby 1965; Duré and Kehr 2004). In females, 10% of the total egg mass was excised from the oviducts, and the eggs contained therein were counted. The eggs were counted in a Petri dish with water using a stereomicroscope. Since the data were not normally distributed, Spearman's correlation test was used to evaluate correlations between body size and either egg mass or testicular mass. A Mann-Whitney U test was performed to evaluate the differences in mass, volume, length, and width of the testes.

### Morphology

For all adult specimens (11 females and 20 males), digital calipers were used to measure the following (each  $\pm 0.1$  mm): snout-vent length (SVL), internarial distance (IND), eye diameter (ED), interorbital diameter (IOD), tympanum diameter (TD), head length (HL), head width (HW), head height (HH), jaw length (JL), jaw width (JW), length from forearm to the fourth finger (LFFT), forearm length (FOL), length of the humerus (LHU), thigh length (THL), tibia length (TL), and foot length (FL; Watters et al. 2016). The normality of the data was checked with a Kolmogorov-Smirnov test. Since the data were normal, an analysis of covariance (ANCOVA) was conducted to analyze sexual dimorphism. The function of the ANCOVA was to eliminate, through linear regressions, the effect of SVL (covariate) on the dependent variables (IND, ED, IOD, TD, HL, HW, HH, JL, JW, LFFT, FOL, LHU, THL, TL, and FL), and to check whether the regression slopes were different between the sexes (factor; Zar 2009). The data are presented as mean  $\pm 1$  SE.

### Conservation Status

Conservation status was summarized by consulting Mexican regulations (NOM-059-SEMARNAT-2010; DOF 2010) and the IUCN (2019) Red List, and an Environmental Vulnerability Score (EVS; Wilson et al. 2013) was generated. The EVS recognizes three categories of risk: low (3–9 points), medium (10–13), and high (14–19). The score is the result of adding points assigned to features of a species based on three criteria: (i) extent of the geographic distribution, (ii) extent of ecological distribution (vegetation types used), and (iii) type of reproductive mode (Wilson et al. 2013).

### Results

#### Areas of Occupation and Exchange Rates of Climate Availability

The distribution of *C. taeniopus* was restricted to cloud forests in the central region of the Sierra Madre Oriental (Fig. 2A–C) in the states of Hidalgo, Puebla, Veracruz, and the northern portion of Oaxaca, Mexico. The analysis of habitat occupancy under current conditions and in the future (to 2050 and 2070), showed a general loss of climatic niche in much of the range of *C. taeniopus*. This loss occurred in temperate areas, where the current area of occupation (18,262.23 km<sup>2</sup>) will decrease to 15,678.45 km<sup>2</sup> by 2050, which represents a habitat availability decrease of 14.14% (Fig. 2B); and to an area of 11,032.93 km<sup>2</sup> by the year 2070, which represents a habitat availability decrease of 37.08% (Fig. 2C).

#### Diet

Fourteen taxa were identified in the stomachs, with 11 taxa present in both males and females (Table 1). The

**Table 1.** Diet of *Charadrahyla taeniopus*, for the species and by sex. I = Food Importance Index, N = number of prey, %N = percentage number of prey, F = number of stomachs with prey category indicated, %F = percentage of stomachs with prey category indicated, V = volume of prey category, %V = percentage of volume of prey category.

Prey category	Total (n = 33)						Female (n = 13)						Male (n = 20)									
	N	%N	F	%F	V	%V	I	N	%N	F	%F	V	%V	I	N	%N	F	%F	V	%V	I	
Arthropoda																						
Arachnida																						
Araneae	5	6.94	5	7.81	1821.6	6.33	7.03	1	3.85	1	3.85	301.59	3.26	3.65	4	8.70	4	10.53	1,520.01	7.78	9.00	
Acari	4	5.56	4	6.25	80.11	0.28	4.03	3	11.54	3	11.54	79.59	0.86	7.98	1	2.17	1	2.63	0.52	<0.01	1.60	
Solifugae	1	1.39	1	1.56	8,616.36	29.93	10.96							1	2.17	1	2.63	8,616.36	44.12	16.31		
Insecta																						
Coleoptera	5	6.94	4	6.25	631.99	2.20	5.13	1	3.85	1	3.85	6.28	0.07	2.59	4	8.70	3	7.89	625.70	3.20	6.60	
Coleoptera larva	1	1.39	1	1.56	980.18	3.40	2.12	1	3.85	1	3.85	980.18	10.59	6.09								
Dermaptera	3	4.17	3	4.69	28.27	0.10	2.98	2	7.69	2	7.69	27.23	0.29	5.23	1	2.17	1	2.63	1.05	0.01	1.60	
Diptera	1	1.39	1	1.56	0.52	<0.01	0.98	1	3.85	1	3.85	0.52	0.01	2.57								
Hemiptera	3	4.17	3	4.69	28.27	0.10	2.98	1	3.85	1	3.85	18.85	0.20	2.63	2	4.35	2	5.26	9.42	0.05	3.22	
Hymenoptera	2	2.78	2	3.13	97.39	0.34	2.08	2	7.69	2	7.69	97.39	1.05	5.48								
Formicidae	10	13.89	6	9.38	114.14	0.40	7.89	1	3.85	1	3.85	0.52	0.01	2.57	9	19.57	5	13.16	113.62	0.58	11.10	
Lepidoptera larva	3	4.17	1	1.56	502.66	1.75	2.49							3	6.52	1	2.63	502.66	2.57	3.91		
Orthoptera	20	27.78	19	29.69	9,655.18	33.54	30.33	8	30.77	8	30.77	4,780.47	51.64	37.73	12	26.09	11	28.95	4,874.72	24.96	26.66	
Diplopoda	1	1.39	1	1.56	466.53	1.62	1.52							1	2.17	1	2.63	466.53	2.39	2.40		
Plant material (leaves)	13	18.06	13	20.31	5,764.31	20.02	19.46	5	19.23	5	19.23	2,964.10	32.02	23.49	8	17.39	8	21.05	2,800.21	14.34	17.59	
<b>Totals</b>	<b>72</b>	<b>100</b>	<b>64</b>	<b>100</b>	<b>28,787.51</b>	<b>100</b>		<b>26</b>	<b>100</b>	<b>26</b>	<b>100</b>	<b>9,256.72</b>	<b>100</b>	<b>46</b>	<b>100</b>	<b>38</b>	<b>100</b>	<b>19,530.80</b>	<b>100</b>			

**Table 2.** Mean values  $\pm$  1 SE, and range in parentheses, of morphological characteristics of adult *Charadrahyla taeniopus* (SVL = snout-vent length, IND = internarial distance, ED = eye diameter, IOD = interorbital diameter, TD = tympanum diameter, HL = head length, HW = head width, HH = head height, JL = jaw length, JW = jaw width, LFFT = length from forearm to the fourth finger, FOL = forearm length, LHU = length of the humerus, THL = thigh length, TL = tibia length, FL = foot length). Comparisons were made with ANCOVA, with SVL as the covariate.

Characteristics	Female ( $n = 11$ )	Male ( $n = 20$ )	ANCOVA		
			<i>F</i>	<i>df</i>	<i>P</i>
IND	5.58 $\pm$ 0.24 (3.87–7.08)	4.94 $\pm$ 0.18 (2.91–6.72)	1.133	1, 28	0.296
ED	6.22 $\pm$ 0.23 (5.06–7.91)	5.86 $\pm$ 0.21 (4.86–8.80)	0.065	1, 28	0.800
IOD	7.02 $\pm$ 0.36 (4.24–8.74)	5.21 $\pm$ 0.39 (1.57–8.18)	5.396	1, 28	0.027
TD	3.75 $\pm$ 0.17 (2.64–4.62)	3.29 $\pm$ 0.26 (1.99–6.85)	0.457	1, 28	0.504
HL	19.82 $\pm$ 0.97 (12.60–24.09)	18.46 $\pm$ 0.38 (15.81–22.91)	0.002	1, 28	0.966
HW	19.99 $\pm$ 0.70 (13.94–22.51)	16.45 $\pm$ 0.32 (14.01–19.41)	24.521	1, 28	<0.001
HH	8.14 $\pm$ 0.36 (5.56–9.19)	6.83 $\pm$ 0.32 (4.95–9.08)	3.553	1, 28	0.069
JL	17.28 $\pm$ 0.66 (12.33–20.68)	15.33 $\pm$ 0.26 (13.57–17.45)	6.433	1, 28	0.017
JW	19.91 $\pm$ 0.80 (13.05–22.99)	17.22 $\pm$ 0.31 (15.20–20.87)	19.166	1, 28	<0.001
LFFT	16.83 $\pm$ 1.00 (12.03–21.05)	15.60 $\pm$ 0.53 (11.58–20.37)	0.003	1, 28	0.959
FOL	13.78 $\pm$ 0.65 (9.44–16.91)	13.12 $\pm$ 0.29 (11.08–15.58)	1.387	1, 28	0.249
LHU	16.38 $\pm$ 0.84 (10.77–20.80)	16.07 $\pm$ 0.51 (10.48–19.26)	2.365	1, 28	0.135
THL	31.32 $\pm$ 1.44 (20.21–38.05)	30.17 $\pm$ 0.65 (25.19–37.96)	0.927	1, 28	0.344
TL	32.68 $\pm$ 1.24 (22.02–37.08)	30.68 $\pm$ 0.54 (27.84–37.26)	0.016	1, 28	0.900
FL	43.06 $\pm$ 1.77 (30.21–51.14)	37.92 $\pm$ 2.17 (34.38–49.26)	0.035	1, 27	0.853

most important prey categories, according to the values of food importance for the species and for each sex, were orthopterans, plant material (leaves), and ants (Table 1). The overlap in diet between the sexes was high ( $O_{jk} = 0.822$ ; 63.21–100%), with males presenting a slightly higher value ( $B = 0.526$ ) of diet niche breadth than females ( $B = 0.504$ ).

### Reproduction

The mean number of eggs was  $722 \pm 277.53$  (range 426–1,138,  $n = 11$ ). There was no correlation between female SVL and either number of eggs ( $r_s = 0.09$ ,  $P = 0.79$ ,  $n = 11$ ) or egg mass in females ( $r_s = 0.09$ ,  $P = 0.79$ ,  $n = 11$ ). There were no differences among weights, lengths, widths, or volumes of the testes ( $P > 0.05$  in all cases). The average weight, length, width, and volume of the right testis was 0.198 g, 14.78 mm, 6.05 mm, and 305.57 mm<sup>3</sup>, respectively; and for the left testis the averages were 0.204 g, 14.63 mm, 6.0 mm, and 293.25 mm<sup>3</sup>, respectively. There was no correlation between SVL and testicular volume ( $r_s = 0.31$ ,  $P = 0.17$ ,  $n = 20$ ), but there was a positive correlation between SVL and testicular mass in males ( $r_s = 0.49$ ,  $P = 0.02$ ,  $n = 20$ ).

### Morphology

Five of the 15 characteristics measured exhibited sexual dimorphism, with females higher than males in SVL,

IOD, HW, JL, and JW (Table 2). Females (mean SVL =  $63.94 \pm 2.35$  mm; range 45.27–74.31,  $n = 11$ ) were larger than males (mean SVL =  $59.70 \pm 1.09$  mm, range 52.90–71.05,  $n = 20$ ;  $U = 60$ ,  $P = 0.04$ ).

### Conservation Status

*Charadrahyla taeniopus* is listed in conservation standards (DOF 2010; IUCN 2019) as being in high risk categories. According to the Mexican Standard NOM-059-SEMARNAT-2010 (DOF 2010), the species is considered to be Threatened. The IUCN Red List of Threatened Species places the species in the Vulnerable category, with a distributional area less than 20,000 km<sup>2</sup> in fragmented environments and with declining populations (status B1ab[iii]; IUCN 2019). In the EVS, it was classified as a medium environmental vulnerability species, with a value of 13 points. This EVS category was calculated from: (i) its distribution in Mexico, but not exclusive to the type locality (5 points), (ii) its occurrence in two vegetation types (pine-oak and cloud forest, 7 points), and (iii) a reproductive mode with egg laying in lentic or lotic water bodies (1 point; Wilson et al. 2013).

### Discussion

Conserving native populations of tree frogs at a local scale requires information on their ecological distribution,

feeding habits, reproduction, and morphology (Delia et al. 2013; Toledo et al. 2014). The analyses reported here suggest that the distribution of *C. taeniopus* will potentially decrease during the next 50 years. Microhabitats in currently occupied habitats (montane environments) are subject to change because of temperature and moisture shifts, and also because of changes in vegetation cover associated with high deforestation rates (Kaplan and Heimes 2015) and potential climate change, including shifts in temperature and moisture (Ponce-Reyes et al. 2012). For example, several authors including Pineda and Halfpeter (2004), Pineda et al. (2005), and Murrieta-Galindo et al. (2013), have suggested that the existence of abundant vegetation and native shrub cover provide appropriate humidity and temperature conditions for the permanence of hylid frogs in temperate environments such as cloud forests. If the abiotic and biotic conditions change in the forests inhabited by *C. taeniopus*, this species could be negatively affected. Loss of climatic niche in our models is consistent with that reported by Roxburgh et al. (2004). These authors mentioned that the expected changes could generate ecological scenarios that will delimit the overall distribution of arboreal species from cloud forests (Roxburgh et al. 2004; Pineda et al. 2005), and therefore could affect their associations with their environment (Urbina-Cardona and Flores-Villela 2010; Ponce-Reyes et al. 2012).

In addition to the above considerations, the thermal tolerances of anurans in high elevation or low temperature environments can determine the presence and distribution of their populations (Wells 2007). The hylid frogs are an example of this, as their limits of distribution are in high latitude regions such as the arid and semi-arid climates of northern Mexico (Wiens et al. 2006). This may be the result of the thermal tolerances that hylid species show in temperate environments, which are different from those of species that occur in tropical environments (Navas 2006; Wells 2007). To date, there are no studies of thermal tolerances or maximum/minimum temperature limits for *C. taeniopus*; therefore, it is very difficult to know the behavior of individuals and/or populations of this species in their distribution area. Future field studies, and *in situ* and laboratory experiments on thermal preferences are therefore necessary for this species. They could complement the results obtained in the potential distribution model of the species, enabling the analysis of variables that could be interacting to a greater degree with the biology of the organism, and improving determinations of the distribution range of the species (Gross and Price 2000; Wiens et al. 2006).

Ochoa-Ochoa et al. (2009), stated that in addition to the loss of vegetation, climate change is a determining factor in the loss of amphibian species in conserved environments, mainly in sites outside of natural protected areas (NPAs). The Sierra Madre Oriental Corridor occupies large areas of cloud forest, a type of environment that is highly threatened by the effects of

climate change (Ponce-Reyes et al. 2012), and in which the known distribution of the species is not included in any NPA (IUCN 2019). This shows the importance of evaluating the distribution of highly vulnerable hylid frogs throughout the potential distribution range based on climatic niche models and climate change scenarios. The results are worrisome, because despite the fact that amphibian richness in Mexico is high (Johnson et al. 2015, 2017), more than 50% of the species are listed in high vulnerability categories by the IUCN (Delia et al. 2013; Caviedes-Solis et al. 2015; IUCN 2019; Johnson et al. 2017). For example, recent studies have found that some mountain hylid species have not been recorded over prolonged periods of time (Delia et al. 2013; Caviedes-Solis et al. 2015). Due to multiple factors, such as vegetation loss, pollution, and in particular climate change, populations of these species tend to occur in highly vulnerable sites (Lips et al. 2004; Stuart et al. 2004). Therefore, the species that inhabit this type of environment (cloud forest, pine-oak) are highly threatened (Ochoa-Ochoa et al. 2009; Caviedes-Solis et al. 2015).

In addition to habitat fragmentation and climate change, the presence of the pathogenic fungus *Batrachochytrium dendrobatidis* Longcore, Pessier, and Nichols, 1999 (*Bd*) has contributed to amphibian population and species losses in Mexico (Mendoza-Almeralla et al. 2015, 2016) and other regions of the world (Lips et al. 2003; Fisher et al. 2009). However, *Bd* has not been detected thus far in *C. taeniopus* (Murrieta-Galindo et al. 2014; Hernández-Austria 2017). Therefore, further studies are needed to examine the potential presence of *Bd* in *C. taeniopus* populations through their distribution area (Hernández-Austria 2017).

The lack of information on the natural history of this species inhibits the development of strategies for its conservation (Toledo et al. 2014). The data presented here on diet provide valuable information on the basic ecology of *C. taeniopus*. The diet of this species consists of orthopterans, plant material, and ants, and there is a high degree of overlap in diet between the sexes. In *C. taeniopus*, plant material is the second most important food item. This is particularly notable since the diet of most anuran species in Mexico consists primarily of arthropods (Ramírez-Bautista and Lemos-Espinal 2004; Suazo-Ortuño et al. 2007), and the ingestion of plant material, such as leaves and flowers, is usually considered to be accidental (Evans and Lampo 1996). In the case of *C. taeniopus*, further studies are necessary to determine if consumption of plant material (leaves) is accidental or part of their diet, which would be unusual, but not unprecedented. For example, some species of tree frogs, such as *Ptychohyla zophodes* Campbell and Duellman, 2000 (Luría-Manzano 2012) and *Xenohyla truncata* (Izecksohn, 1959) do consume large quantities of plant material, and the latter (*X. truncata*) has been reported as entirely omnivorous, consuming fruits, seeds,

and flowers (da Silva and Britto-Pereira 2006).

Egg number and the relative sizes of eggs vary greatly in amphibians (Vitt and Caldwell 2009), and they are often related to female body size (Jorgensen 1992; Hartmann et al. 2010). The data presented here show that egg number is not related to female body size in *C. taeniopus*. This may be due to the fact that its reproductive period may have a longer duration, and the sample size obtained from the collections only reflects the behavior of the females in the first part of the year (March–April), not in the entire reproductive period. Females with eggs were found throughout the year, and aggregations of individuals of both sexes and amplexus were observed in the field in August. This seasonal variation in the correlation between egg size and size of females has been reported for other anuran species such as *Leptodactylus fuscus* (Schneider, 1799), *L. podicipinus* (Cope, 1862), and *Dendropsophus nanus* (Boulenger, 1889) [Prado and Haddad 2005]. Furthermore, testicular mass, but not testicular volume, increases with larger SVL. These data suggest that larger males invest more energy in sperm production to have greater reproductive success (Byrne et al. 2002).

Most species of frogs (nearly 90%) are sexually dimorphic, with females being larger than males (Wells 2007), and *C. taeniopus* is no exception. The larger size of females compared to males is presumably associated with the potential to produce more eggs. However, no correlation was found between SVL of females and egg number. Another explanation for sexual size dimorphism could be differences in growth rates (Kupfer 2007), in which the growth rate of males is faster than that of females in order to reach sexual maturity at a smaller size and compete with other males for access to calling sites, thereby maximizing the number of matings (Kupfer 2007; Wells 2007). Also, considering the ecological hypothesis to explain the sexual dimorphism, the larger jaw size in females compared to the males might indicate a larger gape in females, which could allow for partitioning of food resources in terms of prey size (Luría-Manzano 2012). However, additional studies on microhabitat use, behavior, and reproduction are required before the ecological significance of the sexual dimorphism in *C. taeniopus* can be determined.

Based on the information about climatic niche, feeding habits, reproduction, and morphology, *C. taeniopus* is highly threatened because it is distributed in environments (i.e., cloud, oak, and pine-oak forests) that are currently being dismantled by fragmentation and climate change (Ponce-Reyes et al. 2012). As with other hylid frogs (Caviedes-Solis et al. 2015), *C. taeniopus* could face a rapid rate of population decline, as has occurred in other species inhabiting the temperate areas of cloud forest in Oaxaca (Delia et al. 2013; Mata-Silva et al. 2015), Chiapas (Johnson et al. 2015), and areas of the Sierra Madre Oriental (Flores-Villela et al. 2010). To add to the information presented in this study, additional

studies on demography, ecology, physiological tolerances to temperature, length of the reproductive period, effect of fragmentation on populations, and population dynamics of this species should be conducted in order to devise efficient conservation strategies for *C. taeniopus*, and other species of anurans that inhabit the temperate and tropical montane environments of central and southern Mexico (Delia et al. 2013; Caviedes-Solis et al. 2015).

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**Appendix 1.** Voucher numbers of *Charadrahyla taeniopus* specimens analyzed in this study from Colección Herpetológica del Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, México.

Year **2008**, September: CIB 5422–5425 (males), December: CIB 5426 (female); **2009**, April: CIB 5427 (female), June: CIB 5428 (male), CIB 5429–5430 (females), July: CIB 5430 (female), August CIB 5431 (male), CIB 5432 (female), October: CIB 5433–5435 (males), CIB 5436–5437 (juveniles, females), November CIB 5438 (female), CIB 5439 (juvenile, female); **2010**, June: CIB 5440 (male); **2011**, April CIB 5441 (female), CIB 5442–5443 (males), CIB 5444 (female), CIB 5445 (juvenile, female), CIB 5446 (male), May CIB 5447–5449 (males), June CIB 5450 (male); **2012**, March CIB 5451–5452 (females), CIB 5453 (male); **2015**, April CIB 5454–5455 (males), CIB 5456 (female).



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