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Distribution and population structure of *Ambystoma altamirani* from the Llano de Lobos, state of México, Mexico

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Abstract.—*Ambystoma altamirani* is an endangered salamander found in high mountain streams in the vicinity of México City, and its populations might be threatened by urban growth. Here we report our research on a previously unstudied population of *A. altamirani* at the Llano de Lobos in the Sierra de las Cruces. The study ran from September 2018 to September 2019. The number of observed individuals was relatively constant across months. The only months when we observed gilled adults were February and July, and the only months when we saw juveniles without gills were January, February, and August, suggesting that the transformation of juveniles to adults takes place twice a year, once in January–February and once in July–August. We observed egg masses in December and January, and larvae from March to May and also in July. The sex ratio was significantly female-biased (about 2:1). We observed *A. altamirani* at 3 of the 25 permanent sites during the study period. Our comparison of the characteristics of occupied sites with those of unoccupied sites suggests that availability of sunlight (i.e., less cover) and low levels of human impact are important for occupancy by *A. altamirani*. These findings are consistent with observations of other populations of *A. altamirani*, suggesting that these characteristics might be important in their management and conservation.

Resumen.—*Ambystoma altamirani* es una salamandra en peligro de extinción que habita arroyos de alta montaña en las cercanías de la Ciudad de México y sus poblaciones podrían verse amenazadas por el crecimiento de la zona urbana. Aquí reportamos los resultados de un estudio de una población de *A. altamirani* que no había sido previamente estudiada, en el Llano de Lobos, Sierra de las Cruces, desarrollado de septiembre 2018 a septiembre 2019. El número de individuos observados fue relativamente constante a lo largo del periodo estudiado. Los únicos meses en los que observamos adultos con branquias fueron febrero y julio, y los únicos meses que observamos juveniles sin branquias fueron enero, febrero y agosto, lo que sugiere que la transformación de juveniles en adultos tiene lugar dos veces al año, una vez en enero y febrero y una vez más en julio y agosto. Observamos masas de huevos en diciembre y enero y larvas de marzo a mayo y julio. La proporción de sexos estuvo significativamente sesgada hacia las hembras (aproximadamente 2:1). Observamos individuos de *A. altamirani* sólo en 3 de los 25 sitios muestreados durante el período de estudio. La comparación de las características de los sitios ocupados con las de los sitios no ocupados sugiere que la disponibilidad de luz solar (es decir, sitios más abiertos y expuestos a rayos solares) y los niveles bajos de impacto humano son importantes para la ocupación de sitios por *A. altamirani.* Estos resultados son consistentes con observaciones de otras poblaciones de *A. altamirani*, sugiriendo que estas características pueden ser importantes en su manejo y conservación.

The Mountain Stream Siredon, *Ambystoma altamirani*, is found in high mountain streams in the states of México, Morelos, and México City (Lemos-Espinal et al. 1999, Woolrich-Piña et al. 2017). These locations are near México City and these populations might be threatened by the increased development and habitat loss associated with the outward growth of México City (see García-Romero 2001, 2002, Merlín-Uribe et al. 2013). Indeed, *A. altamirani* is listed as Endangered by the IUCN (Shaffer et al. 2008) and is categorized as Amenazada, or Threatened, in Mexico (SEMARNAT 2010). Other threats specifically confronting *A. altamirani* include introduced Rainbow Trout (*Oncorhynchus mykiss*) (Estrella Zamora et al. 2018) as well as genetic bottlenecks and reduced genetic variation due to the isolated nature of its populations in different streams or watersheds (Parra-Olea et al. 2012, Heredia-Bobadilla

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et al. 2017, Monroy-Vilchis et al. 2019). In addition, *A. altamirani* is subject to many of the same threats affecting all ambystomatid salamanders in central Mexico, including habitat loss, urban development, expanding agriculture, pollution, emerging diseases, and climate change (Frías-Alvarez et al. 2008, Soto-Rojas et al. 2017, Escalera-Vázquez et al. 2018, Basanta et al. 2019, Hernández-Guzmán et al. 2019).

Populations of *A. altamirani* appear to be concentrated in the Sierra de las Cruces in the state of México (Lemos-Espinal et al. 1999, Woolrich-Piña et al. 2017). In the Sierra de las Cruces, the population along the Arroyo los Axolotes has been relatively well studied (Lemos-Espinal et al. 2015, 2016, Villarreal Hernández et al. 2019, V. Villarreal Hernández et al. unpublished data). Based on these studies of other populations of *A. altamirani*, this salamander species appears to use deeper and wider sections of streams that have more oxygen, faster currents, grassy vegetation, and sand or mud substrates (Lemos-Espinal et al. 2016). *Ambystoma altamirani* consumes ostracods, gastropods, and insects (Lemos-Espinal et al. 2015), and their predators include *Thamnophis scaliger* (Villarreal Hernández et al. 2019). However, less is known about populations in other streams in the Sierra de las Cruces that are closer to México City. We studied *A. altamirani* from an unnamed stream on the Llano de Lobos in the Sierra de las Cruces, a site 11.25 km away (straight-line distance) from and in a different watershed than the Arroyo los Axolotes population. In contrast to other localities where *A. altamirani* has been studied primarily in grassland (e.g., the Arroyo de los Axolotes), this population was located primarily in a forest with only limited grassland habitat, and it is at a lower elevation. In particular, we examined the distribution, abundance, stage structure, sex ratio, and proportion of gilled individuals of *A. altamirani* at the Llano de Lobos in an effort to understand whether there is variation in these characteristics among populations.

The Sierra de las Cruces occurs in the extreme northwestern part of the state of México and has the Cerro de las Navajas as its highest peak (3710 m altitude). The runoff from this summit drains into a series of permanent streams that run through meadows and canyons surrounded by stands of coniferous and mixed deciduous-coniferous forests. These streams occur from 2830 m to 3460 m above sea level.

Our study stream is on the Llano de Lobos (2870 m elevation, exact location withheld for conservation purposes), located in Ejido San Jerónimo Zacapexco, municipality of Villa del Carbón, state of México. This locality is in the northwestern corner of the Sierra de las Cruces and the state of México, west of the Cumbres de la Sierra Nevada National Park. This plain is surrounded by a dense pine-oak mixed forest composed mainly of *Quercus* spp. The stream is winding with year-round water flow. It contains both pools and sections with reduced width or steeper slopes along its length, thus presenting sites with various water flow conditions along its length. The Llano de Lobos is also an ecotourist site that contains a small dam surrounded by cabins; the stream channel below the dam is controlled.

The mean monthly precipitation is highest in June (170 mm), July (235 mm), August (234 mm), and September (176 mm), with mean precipitation of <80 mm in all other months (Estación Meteorológica Villa del Carbón 2019). Mean monthly temperature is relatively constant, fluctuating between 12.32 °C in December and 17.44 °C in May (Estación Meteorológica Villa del Carbón 2019).

Using a mobile GPS unit, we established 25 permanent sites, each 5 m long and 40 m apart, along the Llano de Lobos stream (Fig. 1; see Table 1 for site descriptions). We visited the study sites (usually weekly) up to 4 times per month (range 1 to 4) from September 2018 to September 2019. During each visit, we visually searched the established sites along the stream for *A. altamirani*, using a herpetological hook to check the bottom of the stream and lateral cavities to induce movement by salamanders to make them more apparent. In addition, we searched under rocks and other objects in the stream at each site. We captured salamanders with a dip net, and we measured snout–vent length (SVL) to the nearest mm using a plastic ruler. We also noted whether an individual possessed gills or not. We determined the sex of adults based on the presence of a bulge in the region of the cloaca on both sides of the tail in the case of males, and on the absence of this bulge in the case of females (Brandon and Altig 1973). We

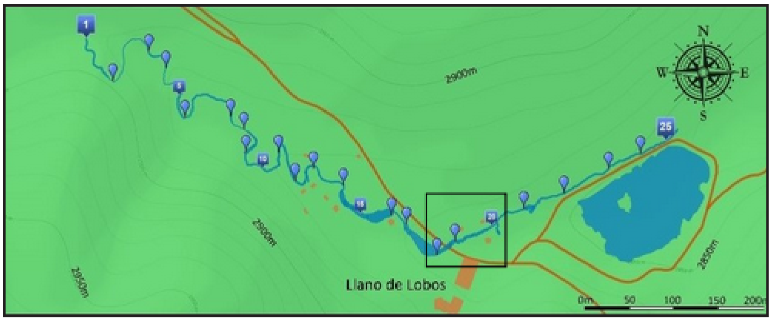

Fig. 1. Map of the study area showing the 25 sampling sites on the Llano de Lobos, Ejido San Jerónimo Zacapexco, municipality Villa del Carbón, state of México. The sites are separated from each other by 40 m (contiguous sites), making a total of 1000 m of stream. The box encompasses the 3 sites where we observed *Ambystoma altamirani*. The scale bar is in meters.

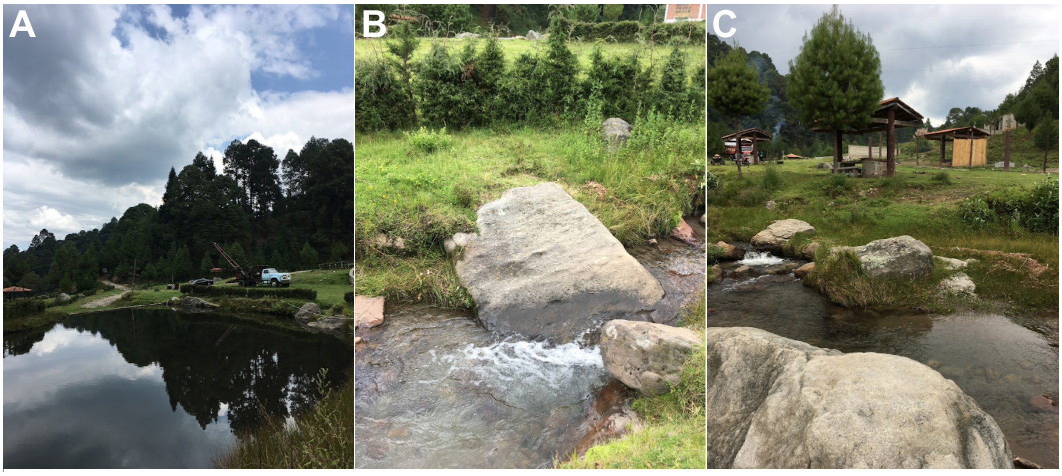

Fig. 2. Photographs of the 3 sites on the Llano de Lobos at which we observed *Ambystoma altamirani*. ***A,*** Site 18; ***B,*** Site 19; ***C,*** Site 20.

recorded the substrate where the salamanders were originally observed and released each individual at the point of capture.

We used the program PRESENCE (version 2.12.26; www.usgs.gov/software/presence) to estimate occupancy, detection probability, colonization probability, and extinction probability (see MacKenzie et al. 2003 and Mazerolle et al. 2007 regarding these methods). We used nonparametric Kruskal–Wallis analyses to compare the mean number of observed individuals, mean SVL, and mean proportion of individuals with gills among months. We used a chi-square test to determine whether the overall sex ratio differed from 1:1. We used JMP Pro 14 (SAS Institute, Cary, NC) for statistical analyses. For all statistical tests we used an α value of 0.05.

We observed *A. altamirani* at least once during the study period at 3 of the 25 permanent sites (Fig. 2). We found this salamander in every month at site 18, in 5 of 12 months at site 19, and in 7 of 12 months at site 20. Our analysis in PRESENCE confirmed that it is highly unlikely that we missed the presence of *A. altamirani* at the sites where we did not see them (estimated occupancy = 0).

The mean number of observed individuals was relatively constant across months (Table 2; $F_{12, 28} = 1.07$, $P = 0.42$; $H^2_{12} = 12.9$, $P = 0.37$). This consistency contrasts with fluctuations in the number of observed individuals in

TABLE 1. Qualitative descriptions of established permanent sites on the Llano de Lobos. Note that we only observed *Ambystoma altamirani* at sites 18, 19, and 20.

Site	Dominant substrate type(s)	Immediate surrounding habitat	Proximity to forest (m)	Width (m)	Water depth (cm)	Estimated relative current speed
1	Rock/Sand	Mixed *Quercus rugosa* and *Pinus hartwegii* forest	<2	<2	<30	Fast
2	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
3	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
4	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
5	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
6	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
7	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
8	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
9	Rock	Mixed *Q. rugosa* and *P. hartwegii* forest	<2	<2	<30	Fast
10	Rock	Woody shrubs, herbaceous plants, and short grassland	3–5	2–3	20–40	Medium
11	Rock	Woody shrubs, herbaceous plants, and short grassland	3–5	2–3	20–40	Medium
12	Rock	Woody shrubs, herbaceous plants, and short grassland	3–5	2–3	20–40	Medium
13	Rock/Sand	Woody shrubs, herbaceous plants, and short grassland	3–5	2–3	20–40	Medium
14	Rock	Woody shrubs, herbaceous plants, and short grassland	3–5	2–3	20–40	Medium
15	Rock	Grassland with tourist amenities (organic waste and garbage present)	10–20	1.5–2	<40	Very slow
16	Sand/Clay	Grassland with tourist amenities (organic waste and garbage present)	10–20	1.5–2	<40	Very slow
17	Rock	Grassland with tourist amenities (organic waste and garbage present)	10–20	1.5–2	<40	Very slow
18	Sand/Clay	Short grassland (Fig. 2A)	50	5–7	20–45	Pool (dam at downstream end)
19	Sand/Rock	Short grassland (Fig. 2B)	35–50	3–4	35–50	Very slow
20	Rock/Sand	Short grassland (Fig. 2C)	35–50	2.5–3	35–50	Very slow
21	Rock	Dense woody shrubs, short grasses, mixed *Q. rugosa* and *P. hartwegii* forest	<2	1.5	<40	Very fast
22	Rock	Dense woody shrubs, short grasses, mixed *Q. rugosa* and *P. hartwegii* forest	<2	1.5	<40	Very fast
23	Sand/Clay	Dense woody shrubs, short grasses, mixed *Q. rugosa* and *P. hartwegii* forest	<2	1.5	<30	Very fast
24	Sand/Clay	Dense woody shrubs, short grasses, mixed *Q. rugosa* and *P. hartwegii* forest	<2	1.5	<30	Very fast
25	Rock	Dense woody shrubs, short grasses, mixed *Q. rugosa* and *P. hartwegii* forest	<2	1.5	<30	Very fast

Table 2. Mean (±1 SE) number of individuals (adults and juveniles) observed per visit, mean (±1 SE) snout–vent length (SVL), and proportion of all individuals, adults, and juveniles with gills (pooled within month) of *Ambystoma atlamirani* from September 2018 to September 2019, Llano de Lobos, State of México, Mexico. The number of visits each month is given in parentheses after the month, and the number of individuals measured is given in parentheses in the mean SVL column.

Month	Mean number of individuals	Mean SVL (mm)	Proportion with gills		
			All	Adults	Juveniles
September (4)	5.8 ± 0.9	104.1 ± 5.4 (23)	0.17	0	1.0
October (4)	6.0 ± 0.9	104.4 ± 5.2 (24)	0.25	0	1.0
November (4)	5.0 ± 0.9	93.2 ± 5.8 (20)	0.20	0	1.0
December (3)	4.7 ± 1.0	90.8 ± 6.9 (14)	0.28	0	1.0
January (3)	3.3 ± 1.0	88.5 ± 8.1 (10)	0.50	0	0.83
February (3)	3.7 ± 1.0	79.1 ± 7.8 (11)	0.64	0.25	0.86
March (3)	5.3 ± 1.0	95.3 ± 6.4 (16)	0.37	0	1.0
April (3)	4.0 ± 1.0	63.3 ± 7.8 (11)	0.83	0	1.0
May (3)	3.3 ± 1.0	79.7 ± 8.1 (10)	0.60	0	1.0
June (3)	5.0 ± 1.0	73.1 ± 6.9 (14)	0.28	0	1.0
July (3)	3.7 ± 1.0	76.5 ± 7.8 (11)	0.64	0.33	1.0
August (4)	5.8 ± 0.9	85.5 ± 5.4 (23)	0.39	0	0.82
September (1)	3.0	110 ± 14.9 (3)	0	0	1.0

Table 3. Monthly variation in population structure (number of individuals at each stage and the adult sex ratio) of *Ambystoma altamirani* at the Llano de Lobos from September 2018 to September 2019. Proportions are given in parentheses. Data are pooled within months.

Month	Stage			Adult sex ratio (M:F)
	Larvae	Juveniles	Adults	
September	0 (0)	4 (0.17)	19 (0.83)	7:12
October	0 (0)	6 (0.25)	18 (0.75)	6:12
November	0 (0)	4 (0.20)	16 (0.80)	4:12
December	0 (0)	4 (0.29)	10 (0.71)	4:6
January	0 (0)	6 (0.60)	4 (0.40)	1:3
February	0 (0)	7 (0.64)	4 (0.36)	0:4
March	3 (0.16)	4 (0.21)	12 (0.63)	5:7
April	4 (0.33)	6 (0.50)	2 (0.17)	0:2
May	1 (0.1)	5 (0.50)	4 (0.4)	1:3
June	0 (0)	4 (0.29)	10 (0.71)	4:6
July	2 (0.18)	3 (0.27)	6 (0.55)	2:4
August	0 (0)	11 (0.48)	12 (0.52)	5:7
September	0 (0)	0 (0)	3 (1.0)	2:1

the Arroyo los Axolotes population, where the number of observed individuals peaked in July, with lows in February, March, and August through December (Lemos-Espinal et al. 2016, V. Villarreal Hernández et al. unpublished data). However, several sites at the Arroyo los Axolotes population dried up during some months of the year, and the variation in abundance appeared to coincide with variations in precipitation (Villarreal Hernández et al. 2020). The difference observed between these 2 populations might be due to more permanent sites, and perhaps even to the much smaller population, at the Llano de Lobos than at the Arroyo los Axolotes (see Lemos-Espinal et al. 2016, Villlarreal Hernández et al. 2020). However, more detailed demographic studies at these localities will be needed to address these differences in population abundance and variation.

Body size (SVL) varied among months, with a general trend for SVL to decline from September to April and then increase from April to September (Table 2; H^2_{12} = 38.5, P = 0.0001). In the Arroyo los Axolotes population, mean SVL varied from month to month with a peak in February (Villarreal Hernández et al. 2020). In addition, the size (age) structure of the Arroyo los Axolotes population was relatively consistent across months; however, small larvae (<25 mm SVL) appeared in May, June, July, and December (Villarreal Hernández

TABLE 4. Characteristics of egg masses of *Ambystoma altamirani* observed along the stream on the Llano de Lobos.

Date	Number of egg masses	Eggs per mass	Site water depth (cm)	Substrate
22 Dec 2018	>30	15–20	60	Rocks, branches, trash
2 Jan 2019	5	<10	40	Branches
5 Jan 2019	>25	15–20	53	Rocks and branches
12 Jan 2019	>25	9–14	55	Rocks and branches
19 Jan 2019	>10	7–9	60	Rocks and branches

et al. 2019, 2020). We observed larvae in a similar range of months (from March to May, and July) but not in December (Table 3).

The relative frequency of gilled individuals differed among months, with the lowest proportion of individuals without gills occurring from September to December (Table 2; $H^2{}_8$ = 23.7, P = 0.0026). In the Arroyo los Axolotes population, the proportion of individuals with gills peaked from March to May, with very low proportions from August to October (Villarreal Hernández et al. unpublished data). However, these analyses pooled adults and juveniles. When we considered adults and juveniles separately in the Llano de Lobos population, the only months when we found any gilled adults were February and July, and the only months when we observed juveniles without gills were January, February, and August (Table 2). It should be noted that we detected adults and juveniles in all months with >1 sampling visit (Table 3). These results indicate that transformation of juveniles to adults typically takes place twice a year, once in January and February and once in July and August. We reached this conclusion because, in months in which the transformation is taking place, one would expect to see juvenile-sized individuals without gills as they begin to transform, and also adult-sized individuals that might have gills as the transformation is occurring. Our observations also suggest that the vast majority of, if not all, adults in this population are transformed individuals (i.e., lacking gills and larval traits).

We found egg masses in December and January (Table 4). Most egg masses contained 10–20 eggs (Table 4), and all egg masses were found at a depth of 3–15 cm. The sites with egg masses frequently had a rock substrate, but also branches or roots, and in one site trash (Table 4). Egg clusters of *A. altamirani* have been reported from late April to early August in the Arroyo los Axolotes (Lemos-Espinal et al. 2016). Egg clusters of *A. altamirani* in the Arroyo los Axolotes population have been found under rocks and on branches at a depth of 10–20 cm and in the mud at the bottom of the stream at a depth of 25–30 cm (Villarreal Hernández et al. 2019).

Over the entire study period, the pooled sex ratio was significantly female biased (81 females : 39 males, $\chi^2{}_1$ = 14.7, P = 0.0001), and this trend appeared to be consistent (Table 3), but sample sizes for most months were too small for statistical testing. The sex ratio in the Arroyo los Axolotes population varied from a male bias in February, July, August, and November to a female bias in the other months (Villarreal Hernández et al. 2020). It therefore appears that these 2 populations might differ in sex ratio. Why this might be merits further study.

Given that we observed *A. altamirani* at only 3 of our 25 established sites at Llano de Lobos, we were not able to quantitatively compare sites that contained *A. altamirani* with those that did not. However, a comparison of the characteristics of these sites based on qualitative descriptions (Table 1) might be enlightening. Descriptions of sites 18, 19, and 20 reveal that they differed from unoccupied sites in several potentially key traits. These 3 sites were the farthest away from the forest and surrounded by short grassland with no shrubs. The only other sites resembling these sites were sites 15–17, but they were closer to the forest, and, perhaps more importantly, they were close to tourist amenities with obvious organic waste and garbage. These observations suggest that stream sites with more sunlight (i.e., more open) are important for *A. altamirani*, and that human visitation limits the use of stream sites by these salamanders. Our observations were consistent with those of the larger population of *A. altamirani* at the Arroyo los Axolotes because that site is much more open and free from human impacts than other sites are (Lemos-Espinal et al. 2016, Villarreal Hernández et al. 2020). At the Llano de Lobos, another factor that appeared to explain the distribution of *A. altamirani* was water velocity: sites 19 and 20 had very slow water velocity, and site 18 is a pool; other sites not impacted by humans had higher water velocity. It might be that *A. altamirani* needs pools or slower moving water. However, this distribution is not consistent with that observed at Arroyo los Axolotes, where *A. altamirani* was found at sites with faster moving water (Lemos-Espinal et al. 2016). Further research on thermal requirements and the response of these salamanders is needed to assess which stream characteristics are critical and which are helpful in maintaining *A. altamirani* populations.

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