

First records of occurrence and predation of *Pseudoeurycea jaguar* (Caudata: Plethodontidae) in the state of Puebla, Mexico

Mexico is the second most salamander-diverse country globally, boasting 159 species (AmphibiaWeb 2024). Plethodontids represent the most varied family, with the state of Puebla hosting 23 species (Woolrich-Piña et al. 2017, Parra-Olea et al. 2020, Fernández-Badillo 2020). The genus *Pseudoeurycea* stands out as the most diverse in Mexico, with seven recorded species in Puebla. *Pseudoeurycea jaguar* was recently described in the Sierra de Zongolica, Veracruz (Cázares-Hernández et al. 2022), near the Puebla border, and Peralta-Hernández (2023) reported new sightings in Veracruz. Despite the proximity of the localities to Puebla, no previous records of this species exist in the state.

Pseudoeurycea jaguar is a relatively large salamander (maximum snout-vent length 7.1 cm in females and 5.9 cm in males) with long limbs and wide feet. It exhibits two variations of dorsal coloration: with irregular yellow specks on a dark brown, almost black background (Cázares-Hernández et al. 2022), or uniform dark brown, almost black (Peralta-Hernández 2023). This species inhabits mountain cloud forests, coniferous forests, and pine-oak forests at elevations ranging from 2181 to 2676 m above sea level. While primarily arboreal, it can also be found on the ground and in caves (Cázares-Hernández et al. 2022, Peralta-Hernández 2023).

In this paper, we report the presence of *P. jaguar* in a new locality in Puebla. The species was identified based on morphology and molecular data.

Two field trips were carried out to Cerro Tsitstintepetl, located in the municipality of Coyomeapan, Puebla, México (Fig. 1) on August 4, 2023, and January 8, 2024, with the aim of increasing the limited knowledge about the salamanders that inhabit this and other neighboring municipalities. All

surveys were performed during daylight hours, with visual searches for salamanders under rocks, logs, leaf litter, and on epiphytic vegetation. Each salamander found was photographed. A single adult salamander was collected, and its tissue was preserved for molecular analysis. The specimen was deposited in the Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM (CNAR, IBH36062). We performed molecular analysis to corroborate the identity of the population reported here due to the great distance of this population concerning the populations of Veracruz (for example, the distance from the locality reported here to type locality is 2.9 times greater than the distance from Cerro Petlalcala to the type locality and 2 times greater than the distance from Cerro El Gentil to the type locality), and because we found variation in some morphological measurements in the collected individual.

Genomic DNA was extracted from the sample collected in Cerro Tsitstintepetl and a previously collected individual from Cerro El Gentil (Peralta-Hernández 2023); both samples were morphologically identified as *P. jaguar*. We used the modified protocol of phenol-chloroform (Sambrook & Russell 2006) and amplified the mitochondrial fragment L2 using primers LX12SN1 and LX16S1R (Zhang et al. 2008) with reaction at 35 cycles at 96 °C (2 min), 55 °C (1 min) and 72 °C (5 min). The sequences were assembled and exported to fasta files in Sequencher 5.0.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned using Mesquite v3.40 (Maddison & Maddison 2018). We reconstruct a phylogeny to corroborate the identity of Cerro Tsitstintepetl and Cerro El Gentil populations, including all species of the genus *Pseudoeurycea* obtained from GenBank (Table 1). We ran a Maximum Likelihood analysis in RAxML v8.2 (Stamatakis 2014) through the CIPRES data portal (Miller et al. 2010), with 1,000 bootstrap replicates as nodal support. We also calculated Kimura 2-parameter (K2P) corrected genetic distances with Mesquite v3.40. We used *Aquiloerycea galeanae*, *Ixalotriton niger*, and *I. parvus* as the outgroups for phylogenetic analyses.

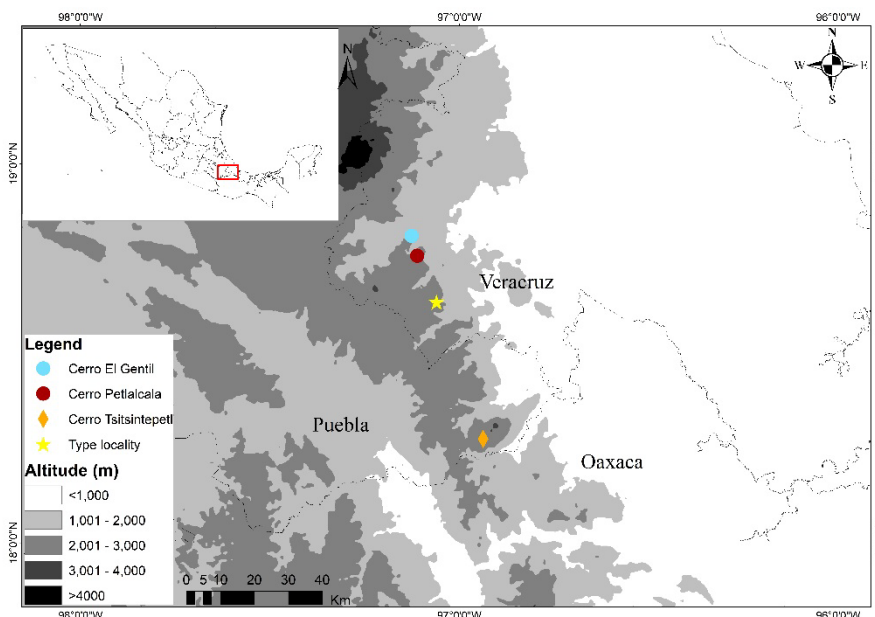


Figure 1. Map of the localities of *Pseudoeurycea jaguar* reported in this study (orange rhombus) and previously published: type locality (yellow star), Cerro El Gentil locality (blue circle), Cerro Petlalcala locality (red circle).

Table 1. Voucher information and GenBank accession numbers for sequences used in phylogenetic analysis.

Species	Voucher number	GenBank 16S
<i>P. ahuitzotl</i>	IBH 30211	MT303858
<i>P. altamontana</i>	IBH 22220	KP886861
<i>P. anitae</i>	MVZ 137939	AF451227
<i>P. aurantia</i>	IBH 20370	KP886844
<i>P. brunnata</i>	MVZ 137947	AF451232
<i>P. cochranae</i>	IBH 23064	KP886864
<i>P. conanti</i>	MVZ 146786	AF451241
<i>P. exspectata</i>	MVZ 160919	AF451234
<i>P. firscheini</i>	IBH 30995	MT303859
<i>P. jaguar</i>	MZFC-HE 35855	OP605487
<i>P. jaguar</i>	IBH36061	PQ012680
<i>P. jaguar</i>	IBH36062	PQ013681
<i>P. gadovii</i>	IBH 22982	KP886846
<i>P. goebeli</i>	CRVA1017	MT303860
<i>P. juarezi</i>	IBH 29718	KP886848
<i>P. leprosa</i>	IBH 22406	KP886866
<i>P. lineola</i>	IBH 29719	KP886867
<i>P. longicauda</i>	IBH 22247	KP886849
<i>P. lynchi</i>	GP160	AF451225
<i>P. melanomolga</i>	IBH 22784	KP886868
<i>P. mixcoatl</i>	IBH 14194	KP886869
<i>P. mixteca</i>	GP0289	AF380829
<i>P. mystax</i>	GP372	AF380795
<i>P. nigromaculata</i>	MVZ 185977	AF451238
<i>P. obesa</i>	MVZ 241574	KP886870
<i>P. orchileucos</i>	IBH 22562	KP886858
<i>P. orchimelas</i>	IBH 22999	KP886860
<i>P. papenfussi</i>	IBH 14198	KP886850
<i>P. rex</i>	MVZ 263590	KP886852
<i>P. robertsi</i>	IBH 22232	KP886853
<i>P. ruficauda</i>	IBH 21646	KP886871
<i>P. saltator</i>	IBH 22895	KP886854
<i>P. smithi</i>	IBH 29720	KP886855
<i>P. tenchalli</i>	IBH 29721	KP886856
<i>P. tlahcuiloh</i>	IBH 30233	MT303865
<i>P. unguidentis</i>	MVZ 117432	MT303866
<i>P. werleri</i>	IBH 22294	KP886872
<i>Ixalotriton niger</i>	IBH 29715	KP886874
<i>I. parvus</i>	AMA2534	KP886873
<i>Aquiloerycea galeanae</i>	IBH 24595	KP886847

The following measurements were obtained from the Cerro Tsitintepetl individual collected: Distance from snout to posterior angle of vent (snout-vent length, SVL), distance from posterior angle of vent to tip of tail (TL), forelimb length (FLL), hind limb length (HLL), distance from tip of snout to gular fold (head length, HL), width of head at angle of jaw (HW), foot width (FW). Finally, we consulted the review on salamander prey and predators (Jobe et al. 2019) and the review of predation of vertebrates by arthropods (Valdez 2020).

We conducted a complementary search on cases of amphibian predation by scorpions from 2018 until 2024 in search engines such as Google Scholar and specialized journals such as Herpetological Review (2018–2023) and Herpetology Notes (2018–2024). We set the year 2018 as the beginning of our search because Jobe et al. (2019) conducted

an exhaustive search in different sources up to that year.

Three individuals were found during the first day (Fig. 2A–C) in a pine-oak forest (18.274196° N, 96.936999° W; 2613 m asl) under the bark of fallen decaying logs. One of the individuals was being eaten by a scorpion of the genus *Megacormus* (Fig. 2A). During the second trip, near the site where we found the individuals photographed during the first trip, we found another individual (Fig. 2D) under the bark of a rotted fallen log (18.302223° N, 96.922501° W; 2775 m asl). We collected an adult male (IBH36062; Fig. 2C) with the following measurements: SVL= 60.7 mm, TL=63.6 mm, FLL=18.0 mm, HLL=18.9 mm, HL=14.4 mm, HW=9.1 mm and FW=6.7 mm.

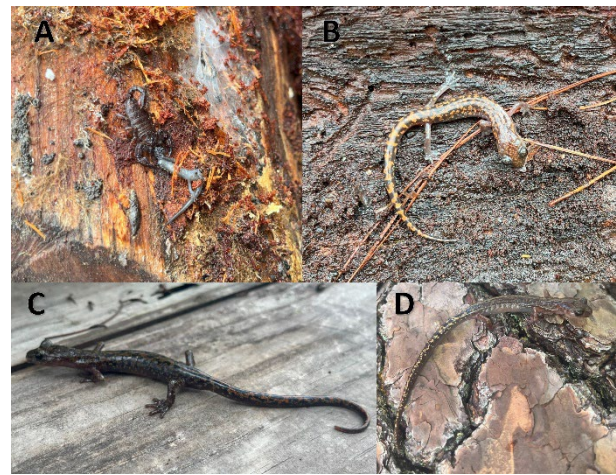


Figure 2. Individuals of *Pseudoeurycea jaguar* found during the field trips of this study: A) A juvenile individual being eaten by a scorpion. B) An individual found during the first field trip. C) An adult male collected (IBH36062) during the first field trip at Cerro Tsitintepetl, Coyomepan, Puebla. D) An individual found during the second field trip to the same locality.

Phylogenetic analysis provided strong support for placing the Puebla specimen (Fig. 3) with the individual from Cerro El Gentil and the individual from the type locality of *P. jaguar* (bootstrap support of the ML [BS] = 0.97) (Fig. 3). Kimura 2-parameter (K2P) corrected genetic distances between the Puebla specimen and the one from the type locality was 0.78% and 1.37% concerning the Cerro El Gentil individuals.

In this study, we report a new locality for the species *P. jaguar*, which had only been reported previously from three localities in the state of Veracruz. The new locality is 41.6 km southwest of the type locality in El Mirador, Texhuacan Municipality, Veracruz, Mexico (Cázares-Hernández et al. 2022), which was also the closest known location (Fig. 1). The new location also increases the species' highest altitude by 99 m to 2775 m asl (Peralta Hernández 2023). This record increases the number of *Pseudoeurycea* species registered in the state of Puebla to eight (Woolrich-Piña et al. 2017) and also represents the only species belonging to the *P. juarezi* group that is distributed in Puebla, filling a gap between the distribution of the populations of *P. jaguar* in Veracruz and the distribution of the other species of the group in Oaxaca.

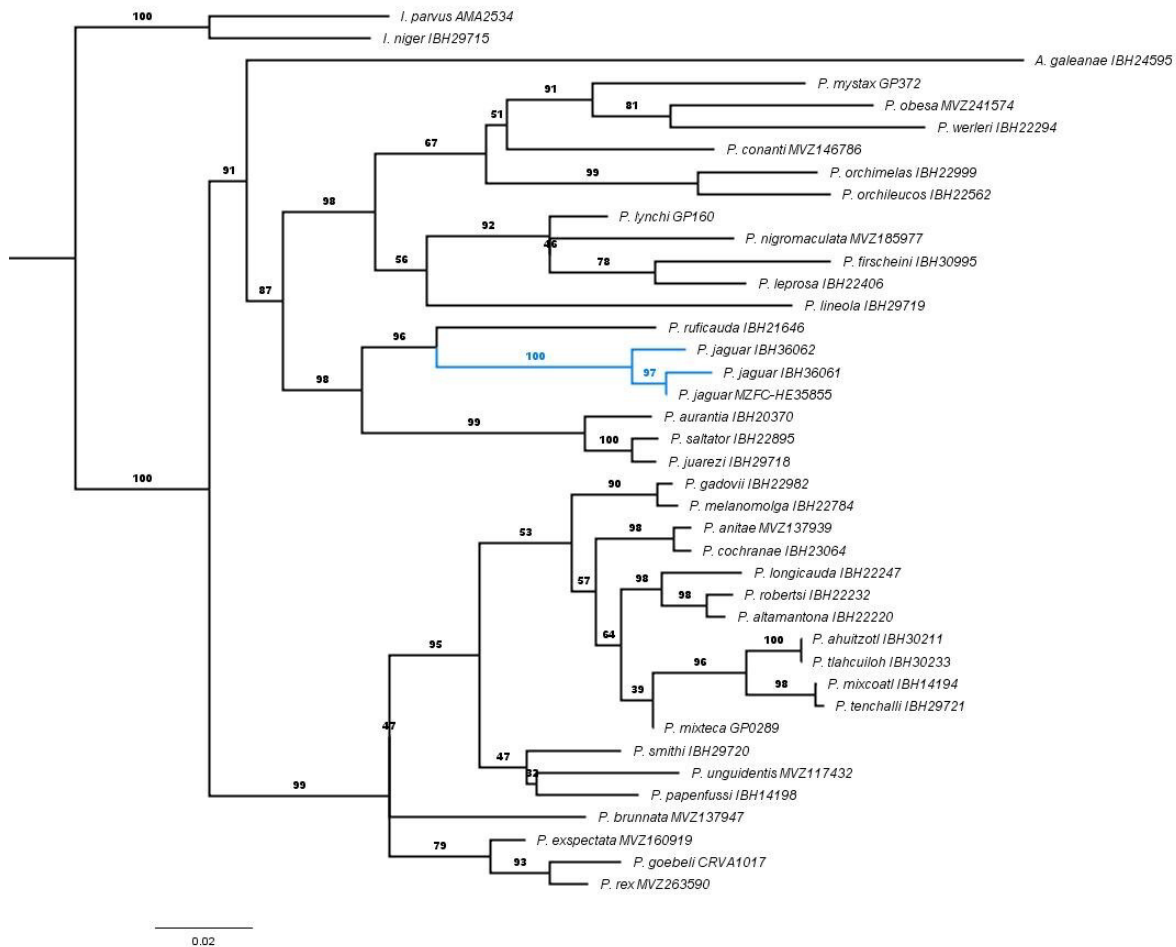


Figure 3. Phylogeny estimated from maximum likelihood analysis of L2 mtDNA sequence data. The numbers on the branches are bootstrap supports from ML analysis. *Pseudoeurycea jaguar* individuals sequenced during this study are indicated in blue.

Additionally, we discovered that there is genetic diversity among *P. jaguar* populations. The genetic distance between the Puebla locality and other known localities is within the range of genetic distances commonly seen in Neotropical salamander mitochondrial DNA (García-Paris et al. 2000). On the other hand, we recorded size variation in *P. jaguar*. The collected specimen corresponds to the largest male of this species (MZFC-HE 28694 SVL= 58.7 mm), with shorter HW (MZFC-HE 28694 HW= 10.3 mm) and FW (MZFC-HE 28694 FW= 8.0 mm) (Cázares-Hernández et al. 2022), and represents the third male with morphological data available.

Furthermore, we report the first predation documented for *Pseudoeurycea jaguar*; our record of predation on *P. jaguar* by *Megacormus* increases the knowledge of the life history of this species. Importantly, we did not find other published records of scorpion predation on salamanders, only for lizards and frogs (De Armas 2001) and caecilians (Auguste et al. 2019).

As in other areas where *P. jaguar* lives, logging occurs in the locality reported here. For this reason, it is recommended to conduct further sampling in this area and neighboring regions where *P. jaguar* might be found (for example, localities located within the elevation range where this species occurs, between the type locality and the population reported here, where we believe the species may be present but has not been reported due to the limited study of the area). This will

aid in gaining a better understanding of the distribution, life history, and population status of this species and would allow the development of strategies for its conservation if necessary.

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References

- AmphibiaWeb (2024): <<https://amphibiaweb.org>> University of California, Berkeley, CA, USA. Accessed on 18 Feb 2024.
- Auguste, R.J., Deo, R., Finnell, B., Ali, H. (2019): First report of a caecilian amphibian (Siphonopidae: *Microcaecilia* sp.) being preyed upon by a scorpion (Chactidae: *Brotheas* sp.). Herpetology Notes 12: 661-662.
- Cázares-Hernández, E., Jimeno-Sevilla, H.D., Rovito, S.M., López-Luna, M.A., Canseco-Márquez, L. (2022): A new arboreal *Pseudoeurycea* (Caudata: Plethodontidae) from the Sierra de Zongolica, Veracruz, Mexico. Vertebrate Zoology 72: 937-950.
- De Armas, L.F. (2001): Frogs and lizards as prey of some Greater Antillean Arachnids. Revista Iberoamericana de Aracnología 3: 87-88.
- Fernández-Badillo, L. (2020): On the Critically Endangered Cofre de Perote

- Salamander (*Isthmura naucampatepetl*): discovery of a new population in Puebla, Mexico, and update of its known distribution. *Amphibian and Reptile Conservation* 14 (3): 200-205.
- García-Paris, M., Good, D.A., Parra-Olea, G., Wake, D.B. (2000): Biodiversity of Costa Rican salamanders: implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proceedings of the National Academy of Sciences of the United States of America* 97: 1640-1647.
- Jobe, K.L., Montaña, C.G., Schalk, C.M. (2019): Emergent patterns between salamander prey and their predators. *Food Webs* 21: e00128.
- Maddison, W.P., Maddison, D.R. (2018): Mesquite: a modular system for evolutionary analysis. Version 3.40.
- Miller, M.A., Pfeiffer, W., Schwartz, T. (2010): Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Gateway Computing Environments Workshop, GCE 2010*.
- Parra-Olea, G., García-Castillo, M.G., Rovito, S.M., Maisano, J.A., Hanken, J., Wake, D.B. (2020): Descriptions of five new species of the salamander genus *Chiropoterotriton* (Caudata: Plethodontidae) from eastern Mexico and the status of three currently recognized taxa. *PeerJ* 8(2): e8800.
- Peralta-Hernández, R. (2023): New data on occurrence, ecology, and color variation of *Pseudoeurycea jaguar* (Caudata: Plethodontidae), an endemic salamander from Mexico. *Phyllomedusa Journal of Herpetology* 22 (1): 43-47.
- Sambrook, J., Russell, D.W. (2006): Purification of nucleic acids by extraction with phenol: chloroform. *Cold Spring Harbor Protocols* 2006. pdb.prot4455.
- Stamatakis, A. (2014): RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.
- Valdez, J.W. (2020): Arthropods as vertebrate predators: A review of global patterns. *Global Ecology and Biogeography* 29 (10): 1691-1703.
- Woolrich-Piña, G.A., García-Padilla, E., DeSantis, D.L., Johnson, J.D., Mata-Silva, V., Wilson, L.D. (2017): The herpetofauna of Puebla, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4 (4): 790-884.
- Zhang, P., Papenfuss, T.J., Wake, M.H., Qu, L., Wake, D.B. (2008): Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution* 49 (2): 586-597.

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