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Thorius narismagnus (Amphibia: Plethodontidae): rediscovery at the type locality and detection of a new population

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***Abstract.*—Of the 42 Critically Endangered species of plethodontid salamanders that occur in Mexico, thirteen have not been reported in more than ten years. Given the lack of reports since 1976, the minute plethodontid salamander *Thorius narismagnus* is widely considered as missing. However, this report describes the rediscovery of this minute salamander at the type locality (Volcán San Martín), as well as a new locality on Volcán Santa Marta, 28 km southeast of its previously known distribution, both in the Los Tuxtlas region of Veracruz, Mexico. The localities where *T. narismagnus* has been found are mature forests in a community reserve on Volcán San Martín and a private reserve on Volcán Santa Marta. The presence of maxillary teeth, generally absent in *Thorius*, are reported here in some *T. narismagnus* females. Two efforts which may contribute to the conservation of *Thorius narismagnus* are the preservation of the cloud forests where this species persists, as well as the determination of the presence and possible effect of chytrid fungus in these populations.**

Keywords. Conservation, ecological reserve, Los Tuxtlas, missing species, minute salamander, molecular analysis, Mexico

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Introduction

In Mexico, 132 species of plethodontid salamanders have been recorded (AmphibiaWeb 2019), 42 of which are Critically Endangered (CR) according to the International Union for the Conservation of Nature (IUCN 2019). This conservation status may be assigned due to various combinations of biological characteristics (e.g., restricted distribution or specific environmental requirements) and risk factors (e.g., habitat modification or climate change; Stuart et al. 2008). In recent decades, population declines have been observed or estimated for several species (Frias-Alvarez et al. 2010) and in some cases, despite intensive search efforts, finding them has not been possible.

According to the IUCN (2019), 13 Critically Endangered plethodontid species in Mexico have not been reported in more than 10 years. Since 2010, six of these species have been rediscovered: *Isthmura naucampatepetl* (Naturalista 2019), *Chiropterotriton magnipes*, *C. mosaueri, Pseudoeurycea ahuitzotl*, *P. tlahcuiloh,* and *Thorius munificus* (AmphibiaWeb 2019). However, so far there have been no subsequent records of the remaining seven species, including *Thorius narismagnus*. The unknown status of these species highlights the need to carry out sampling efforts in their historical localities and to explore those areas with favorable environmental conditions, in order to locate new populations (Sandoval-Comte et al. 2012).

Thorius narismagnus (Shannon and Werler 1955) is a minute salamander with a known distribution that comprises only four localities, not more than 8 km apart, on Volcán San Martín in the Los Tuxtlas region, Veracruz, Mexico (Fig. 1), in an elevational range between 890 and 1,200 m asl (Hanken and Wake 1998). *Thorius narismagnus* is considered as CR because the extent of its occurrence is < 17 km^2 with reductions in the extent and quality of its habitat, and due to a continuing decline in the number of mature individuals (IUCN 2016). The historical records of *T. narismagnus* include 55 specimens collected between 1953 and 1976, and since

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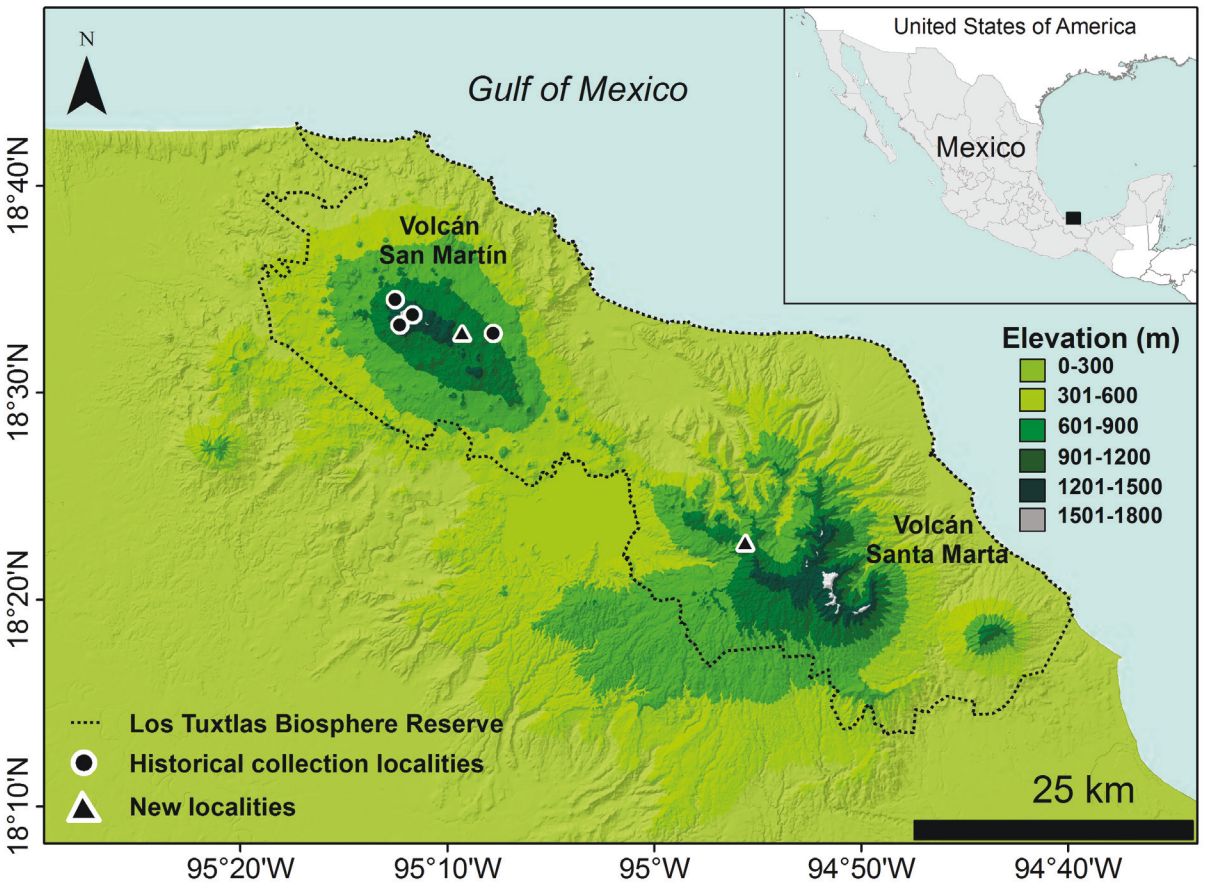

Fig. 1. Locations of historical collection localities and the new localities of *Thorius narismagnus* in Los Tuxtlas region, Mexico.

then this species has not been reported (IUCN 2016).

Rediscovery

As part of a study on the diversity and conservation of amphibians in Veracruz, fieldwork was carried out in a cloud forest in the community reserve Ejido Ruiz Cortines, San Andrés Tuxtla, Veracruz (18º32'53"N, 95º09'16"W; 1,136 m asl) on Volcán San Martín (Fig. 1; Fig. 2A) and in the private Ecological Reserve "La Otra Opción" Catemaco, Veracruz (18º22'32"N, 94º55'28"W; 1,075 m asl) on Volcán Santa Marta (Fig. 1; Fig. 2B). At both sites searches for amphibians (08:00–12:00 and 20:00–00:00 h) were conducted in all terrestrial microhabitats commonly used by these organisms (Crump and Scott 1994).

In the Ejido Ruiz Cortines community reserve, with a cumulative search effort of 72 person-hours in September 2012, three individuals of the genus *Thorius* were detected, two of which were collected (from which a tissue sample was taken and the individuals were subsequently preserved) and deposited in the Colección de Anfibios y Reptiles del Instituto de Ecología A.C. (CARIE 0857, 1137; Fig. 2C). A sampling effort of 24 person-hours was carried out in July 2015 at this locality, but there were no sightings of any minute salamanders. The two collected specimens measured 17.6 and 11.4 mm in SVL, the ratios between the length and width of the nostrils were 1.14 and 1.09, with four and five free intercostal grooves separating the adpressed fore and hind limbs, respectively.

In the private ecological reserve La Otra Opción, with cumulative search efforts of 96 person-hours in July 2015 and 66 person-hours in July 2017, three individuals of *Thorius* were detected in well-preserved forests in each year. The three individuals detected in 2015 were captured (each one was measured and a sample of tail tissue was taken) and subsequently released; these specimens had SVL measurements of 15.4, 15.9, and 19.4 mm. The three individuals found in 2017 were collected (CARIE 1251, 1258, 1259; Fig. 2D) and a sample of tissue was taken from each; they measured 19.9, 22.5, and 21.2 mm SVL, respectively. The proportions between the length and width of the nostrils were 1, 1, and 1.15, respectively, with 5.5 free intercostal grooves separating adpressed limbs. Two females from La Otra Opción (CARIE 1251 and 1259) had eight and five maxillary teeth, respectively, while the adult male (CARIE 1258) lacked maxillary teeth.

Identification of Specimens

The coloration in life of the specimens collected in the Volcán San Martín and Volcán Santa Marta localities

Fig. 2. Habitat in the Volcán San Martín locality **(A)** and a specimen **(C)** collected from it in life (CARIE 0857). Habitat in the Volcán Santa Marta locality **(B)** and a specimen **(D)** collected from it in life (CARIE 1251).

was light brown with a dark brown spike pattern in the dorsum, dark brown color on the sides and a dark venter with small white spots (Fig. 2C, 2D). With the exception of the presence of maxillary teeth in two females, the morphological characters and the coloration coincide with the diagnosis proposed by Shannon and Werler (1955) and Rovito et al. (2013) for *Thorius narismagnus*. Although maxillary teeth are absent in most species of *Thorius*, they are present in several species, including *T. smithi*, which is relatively closely related to *T. narismagnus*. Furthermore, at least two species (*T. grandis* and *T. omiltemi*) have maxillary teeth present only in females, and maxillary teeth are more common in females of *T. minydemus* than in males, which rarely have them (Hanken and Wake 1998; Hanken et al. 1999). Maxillary teeth were absent in a total of 18 specimens from the type locality of *T. narismagnus* on Volcán San Martín as reported by Shannon and Werler (1955) and Hanken and Wake (1998), suggesting that they do not occur in either sex at that locality.

In order to confirm that both populations belong to *Thorius narismagnus,* DNA was extracted from liver tissue of one specimen from Volcán San Martín (the type locality of *T. narismagnus*) and two specimens from Volcán Santa Marta using a salt extraction protocol. A 784 bp fragment of the cytochrome b gene was amplified using primers MVZ15 and MVZ16 (Moritz et al. 1992). PCR consisted of an initial denaturation step of 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. PCR products were purified using ExoSAP IT (USB Corporation, Cleveland, Ohio, USA) and sequenced using the BigDye v3.1 terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) on an ABI 3730 capillary sequencer. Sequences were edited using Geneious v8.1.8 (BioMatters, Auckland, New Zealand), and sequences used in analysis were 750 bp long after removing low-quality bases. Sequences for other species of *Thorius* were obtained from GenBank and sequences were aligned using Muscle v.3.8 (Edgar 2004). The pairwise GTR distance between sequences from the two populations was calculated using PAUP v4.165 (Swofford 2003). Sequences are deposited in GenBank (Table 1).

The average pairwise divergence for cytochrome b (cytb) between the two populations was 1.4% (Table 1). This level of divergence is comparable to, or lower than, that seen between conspecific populations of various species of *Thorius*. Several species of *Thorius*

Table 1. Percentage of average pairwise divergence for cytochrome b between the populations of *Thorius* species in GenBank.

	Species	Voucher	GenBank accession number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	*T. arboreus*	IBH22720	KC884119	-																															
2	*T. aureus*	IBH22356	KC884065	11.2	-																														
3	*T. boreas*	IBH22339	KC884066	11.4	8.7	-																													
4	*T. dubitus*	MCZA137386	KC884068	16.6	16.0	18.7	-																												
5	*T. grandis*	MZFC27550	KC884115	10.6	11.4	12.3	16.7	-																											
6	*T. insperatus*	IBH22901	KC884120	19.1	18.2	17.2	15.3	16.9	-																										
7	*T. longicaudus*	MCZA148744	KC884096	7.9	8.5	10.0	14.8	7.3	15.3	-																									
8	*T. lunaris*	IBH22341	KC884069	18.8	19.9	18.7	18.8	20.8	20.6	19.6	-																								
9	*T. magdougalli*	IBH22890	KC884072	8.1	8.8	11.6	14.5	9.5	17.5	8.9	20.8	-																							
10	*T. magnipes*	IBH22918	KC884122	18.5	18.4	17.8	13.7	17.6	15.2	18.8	18.2	16.9	-																						
11	*T. maxillabrochus*	MCZA148743	KC884105	18.1	20.7	21.2	15.3	19.2	18.2	19.6	21.8	16.0	15.7	-																					
12	*T. minutissimus*	IBH23011	DQ640021	13.1	12.4	13.5	17.5	15.1	18.4	12.5	18.1	12.8	17.1	22.8	-																				
13	*T. minydemus*	MVZ229269	KC884100	23.3	28.4	28.7	17.5	22.9	19.1	24.7	22.2	23.0	16.3	8.4	24.0	-																			
14	*T. munificus*	IBH29716	KC884073	19.1	19.2	19.3	19.8	23.1	21.8	18.6	5.6	20.6	19.8	19.2	20.6	21.6	-																		
15	*T. narismagnus*	CARIE1251	MK761019	19.4	18.7	20.0	16.3	19.3	19.1	18.3	20.9	18.1	17.7	19.9	20.7	22.8	22.2	-																	
16	*T. narismagnus*	CARIE1258	MK761018	19.4	18.7	20.0	16.3	19.3	19.1	18.3	20.9	18.1	17.7	19.9	20.7	22.8	22.2	0.0	-																
17	*T. narismagnus*	CARIE0857	MK761017	19.8	19.4	21.2	17.2	19.5	19.4	18.2	20.9	18.8	17.6	19.8	21.7	22.2	22.1	1.4	1.4	-															
18	*T. narisovalis*	IBH26500	KC884077	12.2	10.3	11.0	18.6	14.6	15.6	9.4	19.1	11.4	17.3	19.1	12.1	22.7	19.8	18.9	18.9	18.6	-														
19	*T. omiltemi*	MVZ269309	KC884079	11.2	11.2	12.6	17.5	7.3	17.9	7.3	22.9	10.2	18.0	18.3	16.5	21.9	22.1	18.6	18.6	18.5	14.1	-													
20	*T. papaloae*	IBH22355	KC884081	10.7	10.0	11.1	17.4	11.4	16.5	9.0	19.7	9.3	17.2	18.8	16.1	22.4	20.0	18.8	18.8	18.5	10.8	10.1	-												
21	*T. pennatulus*	IBH26499	KC884082	20.4	20.7	20.5	18.5	19.9	20.2	18.5	20.1	20.0	18.0	18.9	21.1	18.8	19.8	8.8	8.8	8.6	22.1	18.4	19.9	-											
22	*T. pinicola*	IBH13995	KC884094	11.9	8.5	10.9	15.8	8.3	16.8	6.0	19.4	11.5	16.9	18.9	13.6	21.9	18.9	18.5	18.5	18.4	11.3	6.3	10.9	18.5	-										
23	*T. pulmonaris*	MCZA148742	KC884101	8.8	10.6	12.5	18.8	6.6	14.8	6.5	15.5	11.4	18.9	18.9	14.4	20.4	15.0	20.1	20.1	20.1	10.7	7.2	7.9	20.3	4.6	-									
24	*T. schmidti*	MVZ269312	KC884085	19.3	21.0	20.0	12.1	19.8	18.3	19.7	22.6	19.8	9.2	15.6	20.5	17.3	22.4	17.7	17.7	18.6	19.5	21.2	18.9	18.9	18.7	20.9	-								
25	*T. smithi*	IBH26615	KC884121	19.6	18.6	18.7	15.8	20.9	13.6	16.9	21.2	18.2	17.0	14.9	19.8	16.6	20.0	10.3	10.3	10.2	17.5	18.5	16.6	11.8	17.7	18.5	17.4	-							
26	*T.* sp. 1	GP0099	KC884086	21.1	21.6	22.7	15.6	18.7	15.2	19.4	25.1	17.8	17.7	6.0	21.2	4.5	24.1	19.3	19.3	19.1	18.6	18.2	18.0	17.6	17.9	18.2	18.0	15.4	-						
27	*T.* sp. 2	MCZA148757	KC884118	9.6	9.4	10.4	17.2	8.1	16.2	6.2	17.9	9.6	18.6	19.9	13.1	22.3	18.3	18.4	18.4	18.3	12.4	7.5	9.9	17.7	6.9	6.6	22.6	18.1	18.8	-					
28	*T.* sp. 3	MCZA148759	KC884116	9.3	7.9	12.7	14.0	8.7	16.8	5.7	22.9	8.0	16.7	22.8	11.5	31.3	24.6	18.4	18.4	18.4	10.7	7.8	8.7	17.7	6.2	9.0	17.3	19.9	15.9	7.8	-				
29	*T.* sp. 7	MCZA148754	KC884111	13.2	11.7	13.9	19.7	14.4	18.5	11.3	22.4	9.8	22.4	19.7	18.1	25.3	22.0	20.6	20.6	21.0	13.7	13.2	9.3	22.5	12.7	13.2	21.9	19.2	17.7	12.4	10.2	-			
30	*T. spilogaster*	IBH22975	KC884087	18.5	20.3	21.1	14.2	18.2	14.4	18.8	20.5	17.5	14.8	7.4	20.0	2.3	20.8	17.7	17.7	18.1	18.5	18.0	17.0	16.2	17.2	17.3	15.6	14.5	4.5	18.0	14.2	17.6	-		
31	*T. tlaxiacus*	MCZA148745	KC884098	9.8	10.6	12.0	16.0	7.3	16.0	5.0	19.5	9.0	16.2	18.6	13.7	23.2	19.0	17.7	17.7	17.7	12.5	6.5	10.3	17.3	5.9	6.8	18.8	19.0	19.0	6.6	5.1	13.3	17.3	-	
32	*T. troglodytes*	IBH22981	KC614433	18.7	19.7	19.6	12.9	17.4	17.8	16.4	20.4	16.2	13.9	16.7	19.0	17.0	20.4	15.4	15.4	15.8	18.7	16.4	18.3	16.4	16.0	19.0	14.8	14.3	16.8	17.4	17.2	20.5	14.2	16.6	-

from Oaxaca, including *T. boreas, T. macdougalli,* and *T. narisovalis*, have substantially higher divergence than that seen between the two populations from Los Tuxtlas that were sequenced here. For example, two populations of *Thorius boreas* separated by only 17 km are 5% divergent for cytb (Rovito et al. 2013). The close genetic similarity between the populations from Volcán Santa Marta and from the type locality of Volcán San Martín strongly suggests that these two populations are conspecific.

Conservation Implications

The record from the Ejido Ruiz Cortines community reserve locality represents the rediscovery of *T. narismagnus*, 36 years after the last reported record (Hanken 1976: MVZ183028–183035) at the type locality on Volcán San Martín (Hanken and Wake 1998). Additionally, the record from Volcán Santa Marta extends the distribution range of this species 28 km southeast of the closest known locality (Fig. 1). Because the specimens reported here were found in primary vegetation, and Díaz-García et al. (2017) recorded *T. narismagnus* in mature forest but not in restoration areas and cattle pasture in "La Otra Opción" this species is probably not able to survive in disturbed forest or even in moderately disturbed forest. Of the other missing species that the authors have found recently, only one (*T. munificus*) was found in small, highly disturbed forest fragments and within San Juan del Monte state reserve (Juárez-Ramírez et al. 2016), while *Chiropterotriton magnipes* and *C. mosaueri* were found in a cave in a national park with only light to moderate habitat disturbance, and *Pseudoeurycea ahuitzotl* and *P. tlahcuiloh* were found in intact montane forest (AmphibiaWeb 2019).

To more fully understand the conservation status of *T. narismagnus,* an exhaustive sampling effort through time is needed to determine how the encounter rate of this species varies throughout the year and whether it is currently an uncommon species, and to obtain a more accurate estimate of the population size. Extensive fieldwork is also necessary in those areas with favorable environmental conditions on both volcanoes to determine the full distribution of this species. The extent of well-preserved forest on Volcán San Martín is ~100 km^2, while on Volcán Santa Marta it is ~185 km^2 (INEGI 2016).

In addition, determining the presence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and its possible effect on the survival of these *T. narismagnus* populations is critical, because the presence of *Bd* in Los Tuxtlas region has been confirmed (Mendoza-Almeralla et al. 2015) and this pathogen is suspected of being linked to the population declines of this species (IUCN 2016). The presence of *Bd* has been reported in geographically close species from Central Veracruz, such as *Bolitoglossa rufescens*, *Aquiloeurycea cephalica*, and *P. firscheini* (Van Rooij et al. 2011), as well as *P. nigromaculata* and *Thorius pennatulus*, for which the presence of the pathogen has been associated with declines in their populations (Cheng et al. 2011).

Finally, the finding of two populations of a Critically Endangered salamander species that has gone unrecorded for almost four decades highlights the importance of community and private reserves for harboring species in imminent danger of extinction. Although both reserves are relatively small (less than 200 ha) compared to the Los Tuxtlas Biosphere Reserve in which they are located, most of the land of these reserves is conserved forest surrounded by modified environments. In that sense, this work shows the complementarity between the governmental and non-governmental reserves for the protection of species at risk of extinction (see García-Bañuelos et al. 2019), particularly those species with restricted distributions and sensitivity to environmental disturbances.

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