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ORIGINAL ARTICLE

# Interpopulational genetic diversity in the medically important scorpion *Tityus obscurus* (Scorpiones: Buthidae) from northeastern Brazilian Amazonia

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## ABSTRACT

Scorpion envenoming is considered a public health problem in Brazil. A recent study described a variation in the clinical outcome of envenoming by *Tityus obscurus* in two populations separated by 850 km in the northeastern Amazon region. Our aim was to evaluate whether such clinical and toxinological variations are associated with underlying differences in genetic diversity between these two *T. obscurus* populations. We obtained DNA from five individuals of each population, in the municipalities of Belém and Santarém, located east and west of the state of Pará, Brazil, respectively. Gene regions encoding mitochondrial DNA (mtDNA) markers cytochrome oxidase subunit I (COI) and ribosomal 16S RNA (16S) were amplified and sequenced. Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inferences (BA) for both molecular data (COI and 16S). The sampled *T. obscurus* populations corresponded to two distinct mtDNA lineages (genetic distance $\text{COI}_{K2}\text{P} = 0.08$ to 0.13; $16\text{S}_{K2}\text{P} = 0.10$ to 0.11) with no shared mutations between groups and well supported by ML and BA inferences. Based on the divergence values found between eastern and western populations (COI, 0.07 to 0.12; 16S, 0.10), our study confirms the genetic heterogeneity of *T. obscurus* populations within the state of Pará, which correlates with observed venom and clinical differences, and reinforces the need for mapping the distribution of haplotypes throughout the geographic range of *T. obscurus*, to aid in future epidemiological, toxinological, and evolutionary studies.

**KEYWORDS:** mitochondrial DNA, phylogenetic analyses, cytochrome oxidase subunit I, 16S RNA

# Diversidade genética interpopulacional no escorpião de importância médica *Tityus obscurus* (Scorpiones: Buthidae) do nordeste da Amazônia brasileira

## RESUMO

O envenenamento por escorpiões é considerado um problema de saúde pública no Brasil. Um estudo recente descreve a variação do quadro clínico de envenenamento por *Tityus obscurus* em duas populações separadas por uma distância de 850 km no nordeste da Amazônia. O objetivo deste estudo foi avaliar se tais variações clínicas e toxicológicas estão associadas a diferenças subjacentes na diversidade genética entre essas duas populações de *T. obscurus*. Obtivemos DNA de cinco indivíduos de cada população nos municípios de Belém e Santarém, localizados a leste e oeste do estado do Pará, Brasil, respectivamente. Regiões gênicas que codificam marcadores de DNA mitocondrial (mtDNA) citocromo oxidase subunidade I (COI) e RNA ribossômico 16S (16S) foram amplificadas e sequenciadas. As análises filogenéticas foram realizadas por máxima verossimilhança (ML) e inferência Bayesiana (BA) para ambos os dados moleculares (COI e 16S). As populações de *T. obscurus* amostradas corresponderam a duas linhagens distintas de mtDNA (distância genética $\text{COI}_{K2}\text{P} = 0{,}08$ a 0,13; $16\text{S}_{K2}\text{P} = 0{,}10$ a 0,11) sem mutações compartilhadas entre os grupos, e bem corroboradas por inferências ML e BA. Com base nos valores de divergência encontrados entre as populações oriental e ocidental (COI, 0,07 a 0,12; 16S, 0,10), nosso estudo confirma a heterogeneidade genética das populações de *T. obscurus* no estado do Pará. Os resultados são congruentes com as diferenças observadas no quadro clínico dos acidentes e toxicidade do veneno, e reforçam a necessidade de mapear a distribuição de haplótipos em toda a distribuição geográfica de *T. obscurus*, para auxiliar em futuros estudos epidemiológicos, toxinológicos e evolutivos.

**PALAVRAS-CHAVE:** DNA mitocondrial, analise filogenética, citocromo oxidase subunidade I, RNA 16S

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## INTRODUCTION

Up to January 2021, 2433 known scorpion species have been reported worldwide (Dupré 2021). In Brazil, the scorpion diversity is high, with about 131 known species belonging to 23 genera and four families: Bothriuridae, Buthidae, Chactidae and Hormuridae (Lourenço 2002; 2017). The genus *Tityus* represents the most widely spread buthid group, responsible for the majority of severe and fatal scorpion envenoming cases in South America (Chippaux and Goyffon 2008; Borges *et al.* 2021).

In 2021, a total of 149,677 scorpion envenoming cases were registered in Brazil, with 228 accidents leading to death, of which 71 were reported from the Amazon region (Brasil 2022). Scorpionism is considered a health problem in Brazil associated with poverty and the lack of access to effective antivenoms in remote regions, together with the ability of some medically significant species to invade urban areas, being children and the elderly the most vulnerable groups (Chippaux and Goyffon 2008; Amado *et al.* 2021).

*Tityus obscurus*, Gervais 1843 is an 8 to 10 cm-long, dark scorpion frequently associated with envenomings in the Brazilian Amazon region, especially in the states of Pará and Amapá (Lourenço and Leguin 2008). The species is widely distributed in South America, from French Guiana to northern Brazil (states of Pará and Amapá) (Lourenço *et al.* 2019; Martins *et al.* 2021). The type and magnitude of clinical manifestations associated with scorpion envenoming are related, among other factors, to the amount of venom injected and inflammatory mediators released thereof (Abroug *et al.* 2020). Current classification of envenoming severity includes three groups of cases: mild, moderate and severe (Brasil 2001). A recent study involving scorpion envenoming cases by two populations of *T. obscurus* from eastern and western Pará showed that clinical manifestations and severity differed significantly between these areas, suggesting an underlying geographical variation in *T. obscurus* venom toxicity and composition across Pará (Pardal *et al.* 2014; Torrez *et al.* 2015). Further investigation of these variations between *T. obscurus* populations should ideally be based on the evaluation of their genetic divergence, to explore the existence of a putative *T. obscurus* species complex, as has been suggested previously based on morphological evidence (Lourenço *et al.* 2019). Other species complexes have been described for similarly amply distributed scorpions, such as the Australian *Urodacus yaschenko* (Luna-Ramirez *et al.* 2017) and the Afro-Mediterranean *Buthus occitanus* (Klesser *et al.* 2021). Significantly, variation in scorpion venom composition has been shown to generally increase with geographical distance in the case of amply distributed scorpion species in the African genera *Parabuthus*, *Uroplectes* and *Opistophthalmus* (Schaffrath *et al.* 2018). A study to evaluate genetic variability of toxinologically divergent populations of Amazonian scorpions of medical importance, such as *T. obscurus*, would clearly benefit the understanding of their public health importance as risk maps could be defined using a multidisciplinary approach.

Though mtDNA markers have been used previously in systematic studies of scorpions (Gantenbein *et al.* 2001; Borges *et al.* 2010; Ojanguren-Affilastro *et al.* 2017), their potential for elucidating genetic diversity in Amazonian scorpion species still remains unclear, particularly in the case of *Tityus* species associated with severe/lethal cases (Borges *et al.* 2021). Therefore, this study was designed as the first attempt to evaluate the genetic diversity among medically significant *T. obscurus* populations inhabiting Pará state, Brazil, and relationship with the regional clinical and toxinological variations observed in previous studies.

## MATERIAL AND METHODS

### Study Area

Samples (n = 5 individuals per site) were collected in two different sites located in the state of Pará, Brazil: two females, one male and two juveniles from Belém (01°27'21"S, 48°30'14"W), and five males from Santarém (2º24'52"S, 54º42'36"W). Both sites are 850 km apart from each other and are separated by the Xingu and Tocantins rivers (Figure 1).

### Species identification

All individuals were identified as *T. obscurus* at the Laboratory of Medical Entomology and Venomous Arthropods at the Nucleus Tropical Medicine of Federal University of Pará (LEMAP/NMT/UFPA), based on their

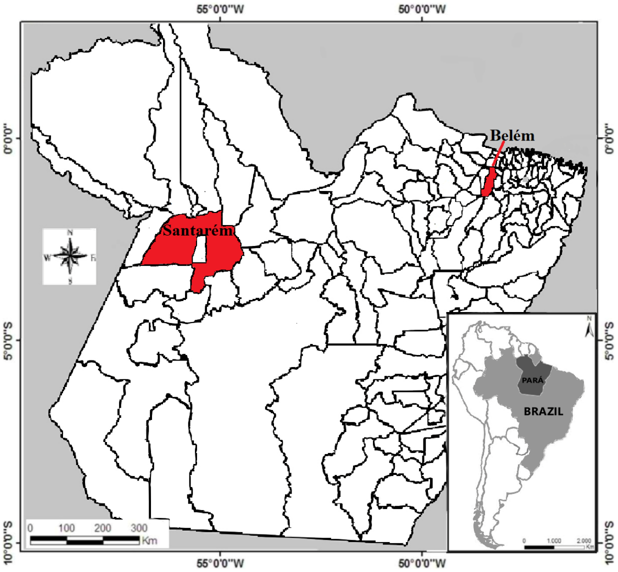

**Figure 1.** Location of Pará state in Brazil (small map), and outline of Pará state showing the location of the two sampling sites: municipality of Santarém (west) and municipality of Belém (east) where *Tityus obscurus* were collected. This figure is in color in the electronic version.

morphological characteristics (Lourenço 2002; Lourenço and Leguin 2008). To confirm their identity further, samples were sent to Instituto Butantan (São Paulo), where their classification as *Tityus obscurus* was assertained based on current taxonomic keys (Lourenço and Leguin 2008) (Figure 2). All samples were preserved in 70% ethanol, and are deposited at the UFPA Arachnological Collection, with the numbers 381, 359, 360, 362, 391 LEMAP/NMT/UFPA (eastern scorpions) and 393, 364, 377, 378 and 379 LEMAP/NMT/UFPA (western scorpions).

This study was conducted in strict accordance with the ethical recommendations for the use and management of animals in research, and was approved by the ethics committee on research involving human beings (medical ethics committee) of the Nucleus of Tropical Medicine at Universidade Federal do Pará-UFPA (license # 038/2007) and by the Brazilian Environmental Institute (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA) (SISBIO license # 11727-2).

**Figure 2.** *Tityus obscurus* collected from the eastern population (A – male; B – female) and the western population (C – male; D – female) in Pará state, Brazil. This figure is in color in the electronic version.

## DNA extraction, amplification and sequencing

DNA was extracted from tissue obtained from the pedipalps of each sampled specimen using Qiagen Kit (GENTRA® - Generation® Capture Column™ Kit) according to the manufacturer's recommendations. Fragments of mtDNA were amplified using primers 16SF (5'- CGATTTGAACTCAGATCA-3' designed by Gantenbein *et al.* 1999) and LR-J-12887 (5'-GTGCAAAGGTAGCATAATCA-3'; Simon *et al.* 1994) for the 16S rRNA gene. For COI amplification the following primers were used: C1-J-2183 (5'-CAACATTTATTTTGATTTTTTGG-3'; Simon *et al.* 1994) and COIKG-R2 (5'-GATATTAATCCTAAAAAATGTTGAGG-3'; Tanaka *et al.* 2001). All primers were synthesized by Operon Technologies (USA).

PCR conditions for 16S consisted of an initial denaturation step at 94°C for 5 min; 30 cycles of 45 sec at 94°C, 45 sec at 48°C and 45 sec at 72°C, followed by a final extension at 72°C for 10 min. COI amplification was performed with the following amplification cycles: denaturation at 94 $^0$C for 5 min; four cycles of denaturing at 94 $^0$C for 30 s, annealing at 48 $^0$C for 30 s, and extension at 72 $^0$C for 1 min; 29 cycles of denaturation at 94 $^0$C for 30 s, annealing at 52 $^0$C for 30 s, extension at 72 $^0$C for 1 min; a final extension at 72 $^0$C for 5 min (Borges *et al.* 2010).

Amplified PCR products were purified using ExoSAP-IT® (Amersham Pharmacia Biotech. Inc., Piscataway, NJ, USA). Sequencing was performed on a MegaBace 750 automated sequencing platform using BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's protocols.

Sequence alignment and edition of COI and 16S fragments were performed with BioEdit 7 (Hall 1999) and ClustalX 1,82 (Thompson *et al.* 1997). Nucleotide sequences obtained in this study were deposited in GenBank under accession numbers JX196960 – JX196969. The species *Centruroides vittatus* (Say, 1821) (GenBank sequences COI - EU404118.1; 16S - EU499946.1) was used as outgroup. GenBank sequences of *T. obscurus* Morona (COI - MF466178.1), *T. asthenes* (COI - MF466177.1), *T. perijanensis* (COI - AY586787.1; 16S - AY586754.1) *and T. clathratus* (COI - KY982266.1; 16S - KY981980.1) were used to improve the robustness of the phylogenetic arrangement. A saturation test was performed using the software DAMBE 4.2.13 (Xia and Xie 2001). Haplotype and nucleotide diversity were identified by DnaSP 4.0 (Rozas *et al.* 2003) and levels of genetic distance (Kimura Two-Parameters – K2P and *P* distances) were estimated by the software MEGA 6.0.6 (Tamura *et al.* 2013).

Phylogenetic analyses were performed with PhyML 3.1 (Guindon and Gascuel 2003) for the maximum likelihood (ML) method, and MrBayes 3.2 (Ronquist *et al.* 2012) for a Bayesian analysis (BA). The ML analysis was performed using 1000 bootstrap replicates, and using frequencies, gamma shape and transition/transversion rates set by user according to the parameters of the Akaike Information Criterion correction (AICc). For the Bayesian analysis, Monte Carlo Markov Chains were used with a parameter of $10^6$ generations, the initial heated chain set to 0.2 and a burn-in of 3000, determined by preliminary runs. The software MODELTEST 3.07 (Posada and Crandall 1998) was used for choosing the best mutation model applied in ML and BA analyses, using the AICc criterion and Bayesian Information Criterion (BIC), respectively. The best choice of model was the Hasegawa–Kishino–Yano, HKY (Hasegawa *et al.* 1985) for ML and for BA. A haplotype network was performed with Haploviewer (Salzburger *et al.* 2011), in order to establish the relationship between the different haplotypes, taking into account only variable sites using parsimony algorithms.

## RESULTS

521-bp and 364-bp long fragments were obtained after partial sequencing of amplified COI and 16S genes, respectively, in the sampled populations of *T. obscurus*. Sample ToW5 from western Pará did not amplify for COI (Table 1). After analysis of the COI sequences, the western population exhibited four haplotypes, with two polymorphic sites and values of 0.8000 and 0.0019 for haplotype and nucleotide diversity, respectively. The eastern population presented five haplotypes, with 24 polymorphic sites and values of 1.000 and 0.0255 for haplotype and nucleotide diversity, respectively. After analysis of the 16S sequences, the western population presented four haplotypes, with three polymorphic sites and values of 0.7000 and 0.0033 for haplotype and nucleotide diversity, respectively. The eastern population exhibited five haplotypes, with five polymorphic sites and values of 1.000 and 0.0071 for haplotype and nucleotide diversity, respectively. No evidence of shared mutations was observed between eastern and western *T. obscurus* populations regarding these genes. The network based on COI and 16S genetic markers showed that the haplotypes are divided in two haplogroups, with no shared haplotypes, and separated by 38 and 34 mutations for COI and 16S sequences, respectively. H7 was the only 16S haplotype observed in two individuals from the western group (Figure 3).

The K2P distance matrix showed the existence of two groups, with COI values across individuals ranging from 0.00 to 0.04 within populations, and from 0.08 to 0.13 between the eastern and western groups (Table 1). Similarly, the K2P distance for 16S across individuals ranged from 0.00 to 0.01 observed within each population and from 0.10 to 0.11 between the eastern and western groups (Table 1). The *P* distance matrix exhibited COI values across individuals ranging from 0.01 to 0.04 inside populations and from 0.07 to 0.12 between the eastern and western groups, also equivalent to *P* distance for 16S across individuals varying from 0.00 to 0.03 inside populations and exhibiting 0.10 between the eastern and western groups (Table 2). Tree topologies obtained by maximum likelihood (ML) and Bayesian (BA) methods with COI and 16S mitochondrial fragments were similar, and clearly showed the existence of divergence between the eastern and western *T. obscurus* groups (Figure 4 and 5) forming two independent groups.

## DISCUSSION

The genetic divergence between the eastern and western *T. obscurus* populations in Pará state, Brazil, uncovered in this study offers further proof of the existence of a *T. obscurus* species complex in Amazonia. According to Almeida *et al.* (2017), chromosomal diversity among *T. obscurus* populations inhabiting the region analyzed in our study may

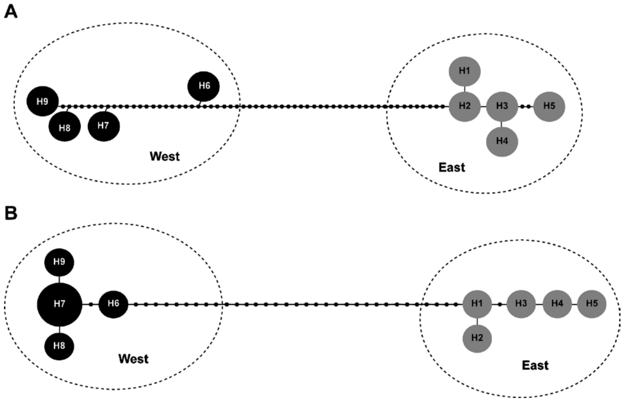

**Figure 3.** Haplotype network for the two populations of *Tityus obscurus*, performed for COI (A) and 16S (B) sequences obtained from the east and west of Pará state, Brazil.

**Table 1.** K2P genetic distance between two populations of *Tityus obscurus* (*T.o.*) from the west and east of Pará state (Brazil) and *Centruroides vittatus* (*C.v.*) obtained from mitochondrial 16S (below the diagonal) and COI (above the diagonal) sequences. NA – non-amplified sample.

| | ***T.o.* East ToE1** | ***T.o.* East ToE2** | ***T.o.* East ToE3** | ***T.o.* East ToE4** | ***T.o.* East ToE5** | ***T.o.* West ToW1** | ***T.o.* West ToW2** | ***T.o.* West ToW3** | ***T.o.* West ToW4** | ***T.o.* West ToW5** | ***C.v.*** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. obscurus* East ToE1 | ------- | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.11 | 0.13 | 0.08 | NA | 0.21 |
| *T. obscurus* East ToE2 | 0.00 | -------- | 0.00 | 0.00 | 0.00 | 0.13 | 0.11 | 0.13 | 0.08 | NA | 0.21 |
| *T. obscurus* East ToE3 | 0.01 | 0.01 | -------- | 0.00 | 0.00 | 0.13 | 0.11 | 0.13 | 0.08 | NA | 0.21 |
| *T. obscurus* East ToE4 | 0.00 | 0.01 | 0.01 | -------- | 0.00 | 0.13 | 0.11 | 0.13 | 0.08 | NA | 0.21 |
| *T. obscurus* East ToE5 | 0.01 | 0.01 | 0.00 | 0.01 | -------- | 0.13 | 0.11 | 0.13 | 0.08 | NA | 0.21 |
| *T. obscurus* West ToW1 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | -------- | 0.02 | 0.01 | 0.04 | NA | 0.27 |
| *T. obscurus* West ToW2 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.00 | -------- | 0.02 | 0.03 | NA | 0.26 |
| *T. obscurus* West ToW3 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.00 | 0.00 | -------- | 0.04 | NA | 0.27 |
| *T. obscurus* West ToW4 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.01 | 0.01 | 0.01 | -------- | NA | 0.26 |
| *T. obscurus* West ToW5 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.00 | 0.00 | 0.00 | 0.01 | -------- | NA |
| *C. vittatus* | 0.27 | 0.27 | 0.27 | 0.27 | 0.28 | 0.30 | 0.30 | 0.31 | 0.30 | 0.30 | -------- |

have contributed to their reproductive isolation. The extent of mtDNA divergence between the eastern and western *T. obscurus* lineages found by us exceeds estimates of interspecific divergence reported for other scorpion groups. In this sense, pairwise distances based on K2P for COI and 16S between the *T. obscurus* eastern and western groups ($COI_{K2P}$ = 0.08 to 0.13; $16S_{K2P}$ = 0.10 to 0.11) are higher than those observed between species of the genus *Mesobuthus* ($COI_{K2P}$ = 0.05 to 0.08) (Mirshamsi *et al.* 2010), and similar to those observed in different species of the genus *Euscorpius* ($16S_{K2P}$ = 0.04 to 0.12) (Soleglad and Fet 2003). The values of 16S pairwise distances (*P* distance) across different lineages of *Centruroides exilicauda* varyed from 0.01 to 0.15 (Gantenbein *et al.* 2001), resembling our values for *T. obscurus* ($16S_{P\ \text{distance}}$ = 0.10). The same was observed for different species of the genus *Euscorpius*, with 16S divergence varying from 0.04 to 0.12 (Soleglad and Fet 2003), similarly to our results. COI and 16S in both pairwise distances (K2P and *P* distance) between the eastern and western groups exhibited half of the divergence observed when compared to the far related *Centruroides vittatus*. This species was chosen as outgroup because *Centruroides*, together with *Zabius*, is paraphyletic with respect to *Tityus* (Esposito *et al.* 2018). Taken together, the above observations suggest the existence of a complex of species or subspecies related to *T. obscurus*, which deserves further research.

The mtDNA divergence observed between the regional lineages of *T. obscurus*, evidenced in our tree topologies and haplotype network may be a consequence of isolation by geographic or ecological barriers. The existence of 34 - 38 mutations (16S and COI, respectively) separating these groups without shared haplotypes supports this hypothesis. A priori,

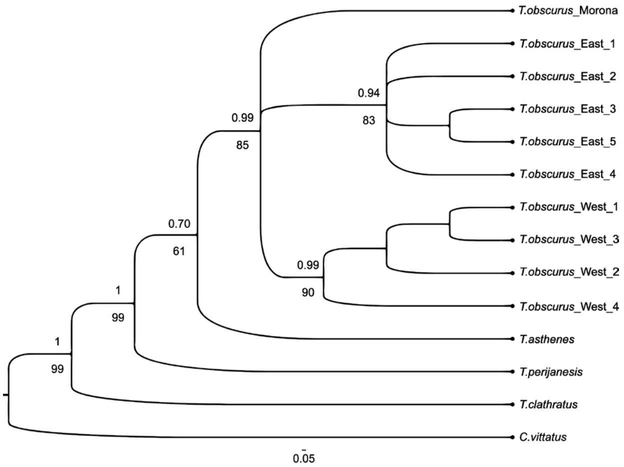

**Figure 4.** Phylogenetic tree constructed through maximum likelihood (ML) for *Tityus obscurus* from the west (*T. obscurus* West 1 to 4) and east (*T. obscurus* East 1 to 5) of Pará state (Brazil) and sequences from *T. obscurus* and species from GenBank based on COI. Bayesian posterior probabilities and bootstrap percentages are shown above and below the branches, respectively.

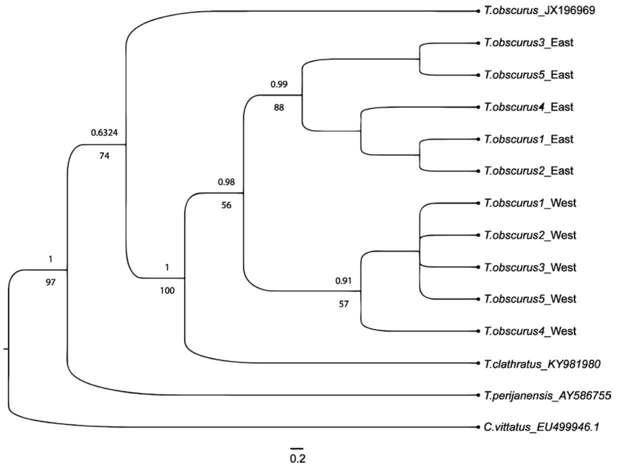

**Figure 5.** Phylogenetic tree performed by maximum likelihood (ML) for *Tityus obscurus* from the west (*T. obscurus* West 1 to 4) and east (*T. obscurus* East 1 to 5) of Pará state (Brazil) and sequences from *T. obscurus* and species from GenBank based on 16S. Bayesian posterior probabilities and bootstrap percentages are shown above and below the branches, respectively.

**Table 2.** *P* genetic distance between two populations of *Tityus obscurus* (*T.o.*) from the west and east of Pará state (Brazil) and *Centruroides vittatus* (*C.v.*) obtained from mitochondrial 16S (below the diagonal) and COI (above the diagonal) sequences. NA – non-amplified sample.

| | ***T.o.* East ToE1** | ***T.o.* East ToE2** | ***T.o.* East ToE3** | ***T.o.* East ToE4** | ***T.o.* East ToE5** | ***T.o.* West ToW1** | ***T.o.* West ToW2** | ***T.o.* West ToW3** | ***T.o.* West ToW4** | ***T.o.* West ToW5** | ***C.v.*** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. obscurus* East ToE1 | -------- | 0.00 | 0.02 | 0.02 | 0.02 | 0.12 | 0.10 | 0.12 | 0.08 | NA | 0.18 |
| *T. obscurus* East ToE2 | 0.00 | -------- | 0.02 | 0.02 | 0.02 | 0.12 | 0.10 | 0.12 | 0.08 | NA | 0.18 |
| *T. obscurus* East ToE3 | 0.01 | 0.01 | -------- | 0.04 | 0.00 | 0.11 | 0.10 | 0.11 | 0.11 | NA | 0.18 |
| *T. obscurus* East ToE4 | 0.00 | 0.01 | 0.01 | -------- | 0.00 | 0.12 | 0.10 | 0.12 | 0.08 | NA | 0.18 |
| *T. obscurus* East ToE5 | 0.01 | 0.01 | 0.00 | 0.01 | -------- | 0.11 | 0.10 | 0.11 | 0.07 | NA | 0.18 |
| *T. obscurus* West ToW1 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | -------- | 0.02 | 0.01 | 0.04 | NA | 0.22 |
| *T. obscurus* West ToW2 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 | -------- | 0.02 | 0.03 | NA | 0.22 |
| *T. obscurus* West ToW3 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.03 | 0.00 | -------- | 0.04 | NA | 0.22 |
| *T. obscurus* West ToW4 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.01 | 0.01 | 0.01 | -------- | NA | 0.22 |
| *T. obscurus* West ToW5 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.01 | -------- | NA |
| *C. vittatus* | 0.23 | 0.23 | 0.23 | 0.22 | 0.23 | 0.25 | 0.25 | 0.25 | 0.24 | 0.25 | -------- |

genetically differentiated populations of *T. obscurus* could have originated by vicariance, through the fragmentation of its ancestral distribution by barriers represented by the Xingu and Tocantins rivers, promoting geographic isolation and reducing gene flow between scorpion populations (Ceccarelli *et al.* 2017). The process of genetic differentiation in *T. obscurus* may also have been influenced by other biogeographic factors such as environmental heterogeneity and isolation by distance, (Santorelli *et al.* 2018; Dambros *et al.* 2020) since our two populations are separated by 850 km. *Tityus obscurus* is widely distributed throughout the Amazon region, and both populations analyzed in this study occupy several microhabitats, such as canopy in floodplain (*igapó*) forests, leaf sheaths of Arecaceae palms, and litter in *terra firme* forests (Martins *et al.* 2021). In Alter do Chão, municipality of Santarém (western population), the forest vegetation (especially floodplain forests) forms a mosaic with savannah areas, which is not observed in Belém (eastern population), increasing the environmental heterogeneity of this region (Magnusson *et al.* 2008). This habitat disruption limits the dispersal of scorpions adapted to forest environments (Lourenço 2002). This mosaic pattern may reflect historical events, such as processes of contraction and expansion of the vegetation cover of the Amazon forest during climate changes in the last ice ages, which resulted in small forest fragments, for which it is suggested that a certain rate of speciation/extinction occurred (Rocha and Kaefer 2019). This phenomenon contributed to the emergence of inter- and intrapopulational morphological variations in some *Tityus* species inthe Amazon (Lourenço 2018). These ecological and biogeographical factors, associated with the low vagility of scorpions (Lourenço 2015), may have played an important role in the cryptic genetic differentiation of *T. obscurus*.

The genetic divergence observed at the molecular level correlates with the previous clinical findings reported by Pardal *et al.* (2014). The symptom referred to by patients as "electric body shock" was reported as related to accidents with *T. obscurus* in northern Brazil and this clinical manifestation was associated to envenoming cases occurring solely in western Pará (Santarém). Symptoms such as myoclonus, difficulty in ambulation, and strong muscular contractions, are all totally absent in envenoming cases from eastern Pará (Belém), which suggests compositional and physio-pathological variations in the toxicity of *T. obscurus* venom from different areas of the Brazilian Amazon basin (Pardal *et al.* 2014; Borges *et al.* 2021). Similar cases of association between genetic differentiation and venom variation have been reported for other scorpion species (Abdel-Rahman *et al.* 2009; Borges *et al.* 2020). Once identified and mapped using genetic tools, knowledge of the distribution of these two groups could lead to more specific therapeutic interventions using region-specific strategies in future envenoming cases.

As suggested by Torrez *et al.* (2015), further characterization of the unique venom activity at the cerebellar level associated with western populations of *T. obscurus* is necessary, especially because the severity of this manifestation requires the use of benzodiazepines even after the antivenom administration. The antivenom produced in Brazil against *Tityus serrulatus* (Lutz & Melo, 1922), the species responsible for the most severe accidents in southeast Brazil, does not recognize the low molecular mass of the neurotoxic fraction of *T. obscurus* nor neutralizes significantly its hyaluronidase activity (Guerra-Duarte *et al.* 2019). The synergistic action of low molecular mass neurotoxins (which alter the gating mechanism of various ion channels) and the connective-tissue degrading hyaluronidase activity are responsible for the systemic effects of *Tityus* venoms (Abroug *et al.* 2020). The approach proposed here could be instrumental for evaluating the actual distribution of the medically important western *T. obscurus* population, and for estimating the overall genetic structure of the *T. obscurus* complex, which possibly underlies the existence of diversity gradients for venom toxic and immunogenic components. In this sense, the recent finding that *Tityus cisandinus* (Lourenço and Ythier 2017) from Amazonian Ecuador is genetically related to *T. obscurus* from Brazilian Amazonia, as its envenoming syndrome also includes neurological alterations (Román *et al.* 2018; Borges *et al.* 2021). This indicates that a combined approach using clinical, phylogenetic, and toxinological tools should prove rewarding in the study of *Tityus* diversity in the Amazon Basin.

## CONCLUSIONS

The results obtained in the present study based on mitochondrial DNA correlate with previous findings, demonstrating clinical and toxinological heterogeneity between Amazonian *T. obscurus* populations. Our findings reinforce the need for mapping the distribution area of *T. obscurus* haplotypes in Pará, which should help the design of region-specific therapeutic tools to treat scorpionism in the area.

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## DATA AVAILABILITY

The nucleotide sequences obtained in this study were deposited in GeneBank under accession numbers JX196960 – JX196969, and can be accessed at https://www.ncbi.nlm.nih.gov/nucleotide/.