Acta Tropica 178 (2018) 1–9

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Scorpion envenoming in Morona Santiago, Amazonian Ecuador: Molecular phylogenetics confirms involvement of the *Tityus obscurus* group

Juan P. Román[a], Fernanda García[a], Doris Medina[a], Manolo Vásquez[a], José García[a], Matthew R. Graham[b], Daniel Romero-Alvarez[c], Pedro P. de Oliveira Pardal[d], Edna A.Y. Ishikawa[d], Adolfo Borges[e,f,*]

[a] Hospital General de Macas, Macas, Morona Santiago, Ecuador
[b] Department of Biology, Eastern Connecticut State University, 83 Windham St., Willimantic, CT 06226, USA
[c] Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA
[d] Laboratory of Medical Entomology and Venomous Animals, Center of Tropical Medicine, Pará Federal University, Av. Generalíssimo Deodoro 92, Umarizal, 66055-240 Belém, Pará State, Brazil
[e] Carrera de Biotecnología, Facultad de Ingenierías y Ciencias Agropecuarias, Universidad de Las Américas, Quito, Ecuador
[f] Laboratorio de Biología Molecular de Toxinas y Receptores, Instituto de Medicina Experimental, Facultad de Medicina, Universidad Central de Venezuela, Caracas 1051, Venezuela

ARTICLE INFO

Keywords:
Amazonia
Cytochrome oxidase subunit I. Ecuador
Molecular phylogenetics
Scorpionism
Tityus
Tityus obscurus

ABSTRACT

Scorpion envenoming by species in the genus *Tityus* is hereby reported from rural locations in the Amazonian province of Morona Santiago, southeastern Ecuador. Twenty envenoming cases (18 patients under 15 years of age) including one death (a 4-year-old male) were recorded at the Macas General Hospital, Morona Santiago, between January 2015 and December 2016 from the counties of Taisha (n = 17), Huamboyo (n = 1), Palora (n = 1), and Logroño (n = 1). An additional fatality from 2014 (a 3-year-old female from Nayantza, Taisha county) is also reported. Leukocytosis and low serum potassium levels were detected in most patients. We observed a significant negative correlation between leukocytosis and hypokalemia. Scorpions involved in three accidents from Macuma, Taisha County, were identified as genetically related to *Tityus obscurus* from the Brazilian Amazonian region based on comparison of mitochondrial DNA sequences encoding cytochrome oxidase subunit I. These cases, along with previously reported envenoming from northern Manabí, reinforce the notion that scorpionism is a health hazard for children in Ecuador and emphasizes the need to supply effective antivenoms against local species, which are not currently available. The genetic affinity of the Ecuadorian specimens with *T. obscurus* may underlay toxinological, clinical, and venom antigenic relationships among Amazonian scorpions that deserves further exploration for designing therapeutic strategies to treat scorpionism in the region.

1. Introduction

Envenoming by scorpions can be life threatening, particularly in children under 10 years of age depending, among other factors, on the species involved (Amitai, 2005). Scorpion stings are common in tropical and sub-tropical regions, with an estimated 1.2 million stings per year and over 3500 deaths, mainly in children (Chippaux and Goyffon, 2008). Lethality is associated with the rapid distribution of low molecular mass scorpion toxins, which mostly affect voltage-gated ion channels located in excitable and immune cells (Amitai, 2005).

The Amazon region, with an area *ca.* 7 million km^2, has been identified as the main endemic area of scorpionism in South America (Chippaux and Alagón, 2008). Estimates for the region range from 30 to 60 cases per 100,000 inhabitants in French Guiana to over 200 cases per 100,000 inhabitants in scorpionism hotspots in the Pará state of western Brazil, and southwestern Amazonas, Mato Grosso, Tocantins, and Maranhão states (Costa et al., 2016; Chippaux and Goyffon, 2008; Hui Wen et al., 2015; Pardal et al., 2014b). Compared to eastern Amazonia, where scorpionism has been documented clinically, taxonomically, and epidemiologically (Benmosbah et al., 2013; Pardal et al., 2014b; Torrez et al., 2015), little is known about scorpion sting incidence and the species responsible for scorpionism in northwestern

* Corresponding author at: Laboratorio de Biología Molecular de Toxinas y Receptores, Instituto de Medicina Experimental, Facultad de Medicina, Universidad Central de Venezuela, Caracas 1051, Venezuela.
E-mail address: borges.adolfo@gmail.com (A. Borges).

http://dx.doi.org/10.1016/j.actatropica.2017.10.014
Received 21 July 2017; Received in revised form 15 October 2017; Accepted 16 October 2017
Available online 25 October 2017
0001-706X/

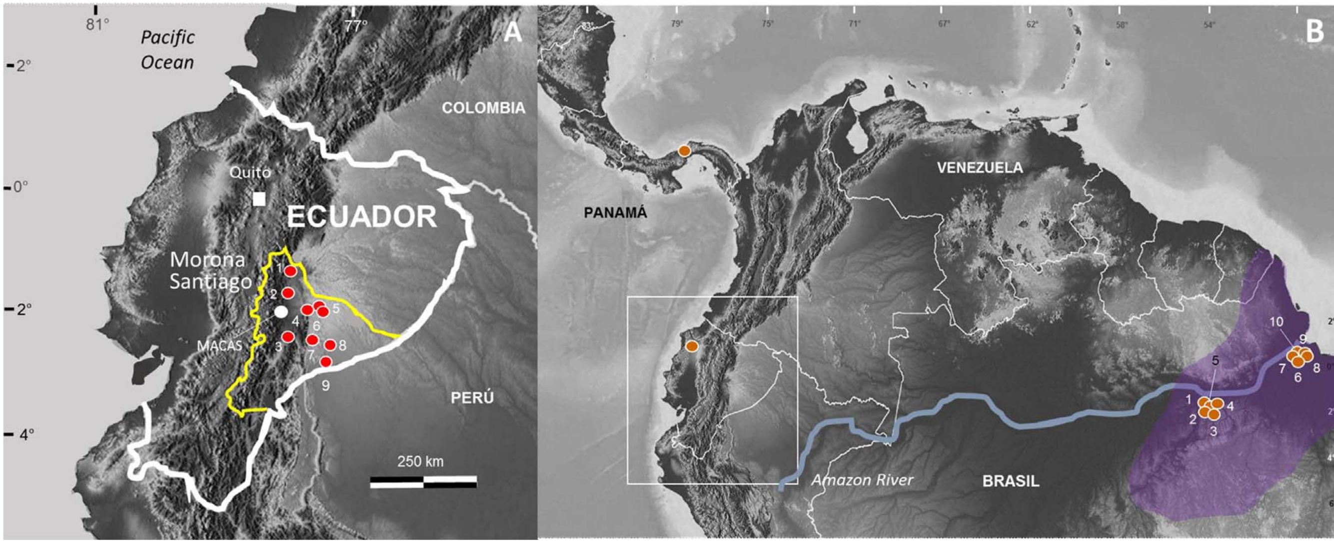

Fig. 1. Map of Ecuador and the Amazonian region of South America indicating scorpion envenoming localities described in this work and sampling sites for phylogenetic studies. (A) Localities in the Ecuadorian province of Morona Santiago (red dots, map locations in brackets): (1) Palora (01°42′03.9″S, 77°58′05.3″W), (2) Huamboya (01°56′45.1′S, 077°59′22.4′), (3) Tayuntsa (02°58′24.0″S, 77°55′20.0″), (4) Cuchaentza (02°07′16.3″, 077°51′50.0″W), (5) Kutsuka (02°30′27.9′S, 77°31′06.9″W), (6) Macuma (02°09′53.6″S, 077°39′32.8″W), (7) Taisha (02°20′22.0″S, 77°27′37″W), (8) Pumpuentza (02°28′39.7′S, 77°20′00.9″W), (9) Nayantza (02°51′48.9″S, 77°29′02.8″W). Provincial capital of Macas is shown as a white dot. (B) Localities elsewhere in Ecuador and Latin America where samples were obtained for phylogenetic analyses (orange dots): Panama (*T. asthenes*: Barro Colorado Island), Ecuador (*T. asthenes*: Viche, Esmeraldas), Brazil (*T. obscurus* (map locations in brackets): (1) ToW6 (Santarém, Curuá-Una): 02°62″99.2″S, 54°59′86.1′W); (2) ToW7 (Santarém, Santarém): 02°42′58.5′S, 54°71′77.4′W; (3) ToW8 (Santarém, Planalto): 02°35′69.2″S, 54°43′18.2″W; (4) ToW9 (Santarém, Terra Preta): 02°41′54.5″S, 54°38′45.1″W; (5) ToW10 (Santarém, Tabocal): 02°34′48.5″S, 54°43′56.4″W; (6) ToE1 (Belém, Outeiro): 01°16′38.2″N, 48°26′27.0″W; (7) ToE2 (Belém, Icoaraci): 01°19′0.02″S, 48° 27′24.4″W; (8) ToE3 (Belém, Maracangalha): 01°25′13.2″S, 48°28′81.2″W; (9) ToE4 (Belém, Val-de-Cans): 01°23′55.6″S, 48°29′0.30″W; (10) ToE5 (Belém, Terra Firme): 01°27′15.0″S, 48°26′58.7″W. Purple area: approximate distribution of *T. obscurus* in Brazil and French Guiana. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Amazonia, particularly in the countries sharing this area, which comprises 14% of the Amazon Basin (i.e. Ecuador, Colombia, and Peru).

Most medically important species in South America belong to the buthid genus *Tityus* (Borges and Graham, 2016). The genus is the most diverse group of scorpions in South America (Lourenço, 2002a). In eastern Amazonia, *T. obscurus*, *T. metuendus*, and *T. silvestris* have been attributed to human casualties (Hui Wen et al., 2015; Monteiro et al., 2016; Pardal et al., 2014a; Queiroz et al., 2015). Our team described scorpionism cases attributed to *Tityus asthenes* from the western Ecuadorian province of Manabí (Borges et al., 2015). Recent health reports, however, suggest that the Amazonian province of Morona Santiago (which represents 9% of the area of Ecuador, i.e. 25.690 km^2) has the highest scorpion sting incidence in the country, with 180 cases recorded in 2016 (Ministerio de Salud Pública de Ecuador, 2016).

In this work, we provide the first report on the severity of scorpionism cases from Morona Santiago. We document scorpion sting accidents, including two deaths, from victims representing four counties that received care at the Macas General Hospital (Macas, Morona Santiago) during 2015 and 2016. Most of the individuals stung were members of the Shuar, an ethnic group indigenous to Ecuador and Peru. Phylogenetic analyses of mitochondrial cytochrome oxidase one (COI) sequences suggest that the scorpions responsible for severe stings in Morona Santiago belong to the *Tityus obscurus* group, which contains species responsible for severe scorpionism elsewhere in the Amazon Basin. These findings will help elucidate the geographical distribution of scorpionism and noxious scorpions in the Amazon.

2. Patients and methods

2.1. Patients

Our study was conducted in the emergency department (ED) of Macas General Hospital, Macas, Morona Santiago, Ecuador, over a two-year period from January 2015 to December 2016. The hospital is a 70-bed second level center that serves the southeastern region of Ecuador, particularly Taisha County of the Morona Santiago province.

We retrospectively studied all charts of patients presented to the ED during the study period for scorpion stings and recorded epidemiological and clinical manifestations at admission. Epidemiological data included the patient's age and sex, the date of the sting, and site of the sting on the body. Clinical manifestations included presence of local pain, tachycardia, vomiting, tachypnea, fever, paraesthesia, nausea, swelling, somnolence, salivation, dysarthria, sweating, dyspnoea, headache, muscle fasciculation, myalgia, convulsions, cyanosis, and bradycardia. After clinical examination upon admission, blood was sampled for determination of paraclinical parameters comprising glucose, urea, creatinine, potassium and sodium serum levels, leukocyte and neutrophil counts (see Appendix A for individual case characteristics).

The envenoming severity was initially classified according to the Scorpion Consensus Expert Group (Khattabi et al., 2011). Briefly, mild envenoming cases (Class I) are characterized by local symptomatology including pain at the sting site, edema, paresthesia, emesis, and moderate tachycardia. Moderate envenoming cases (Class II) show manifestations that are not life threatening such as sialorrhea, diaphoresis, somnolence, hypo- and hypertension, tachycardia, tachypnea, and abdominal pain. Severe envenoming (Class III) includes life-threatening manifestations as cardiac insufficiency, respiratory and neurological failure.

The scorpions involved in three accidents in the village of Macuma (cases B, H, and P; 9-, 3- and 5-year-old children respectively) (see Appendix A) were brought to the hospital and preserved in absolute ethanol for molecular identification (see molecular methods section).

2.2. Statistical methods

Single and multiple regression analyses were performed to identify relationships between symptoms and signs, severity, and altered laboratory parameters using the R software package (https://www.r-project.org/). For all analyses, we recorded R^2 with $\alpha = 0.05$. Outliers

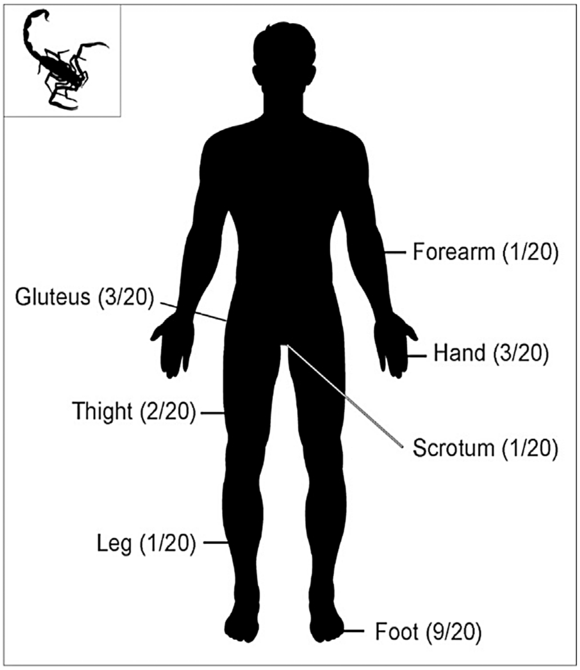

Fig. 2. Frequency of anatomical location of scorpion stings in the 20 cases recorded from Morona Santiago in this work.

in regression analyses were identified using the Tukey's honest significance test implemented in R.

2.3. Molecular methods

DNA was extracted from the three specimens responsible for accidents in Macuma, two *T. obscurus* individuals collected at different localities in the state of Pará, Brazil [classified according to Lourenço and Leguin (2008)], and from *T. asthenes* individuals collected at Barro Colorado Island, Panama (09°09′28″N, 79°50′49″W) and Viche, Esmeraldas province, Ecuador (00°38′57″N, 79°32′31″W) that were identified according to Lourenço (2000) (see Fig. 1 and Appendix B for localities). DNA was obtained from muscle tissue derived from pedipalps according to Borges et al. (2010). Amplification and sequencing of the nucleotide sequence encoding the C-terminal portion of cytochrome oxidase subunit I (COI hereafter) was performed according to Borges et al. (2010) using primers C1-J-2183 (5′-CAACATTTATTTTGATTTTTTGG-3′), and COIKG-R2 (5′-GATATTAATCCTAAAAAATGTTGAGG-3′). Amplification and sequencing of 16S ribosomal RNA (16S hereinafter) sequences was performed using primers LR-J-12887 (5′-CGATTTGAACTCAGATCA-3′) (Simon et al., 1994), and a scorpion-specific reverse primer (5′-GTGCAAAGGTAGCATAATCA-3′) (Gantenbein et al., 1999). Amplified fragments were bidirectionally sequenced using an Applied Biosystems 3130 Genetic Analyzer DNA sequencer as previously described (Borges et al., 2010). Sequences generated for this study were deposited at GenBank under the accession numbers MF466176–MF466180 and are listed, together with other sequences utilized in this work, in Appendix B.

2.4. Phylogenetic analyses

We inspected the phylogenetic position of the *Tityus* specimens involved in the accidents from Macuma, Morona Santiago, by generating a mitochondrial phylogeny using Bayesian inference (BI). We combined 16S and COI sequence data from previous studies (species from Panama, Brazil, and Venezuela) with new sequence data generated in this study (see Appendix B). This resulted in 51 different *Tityus* samples representing all major *Tityus* subgroups (*sensu* Fet and Lowe, 2000; Lourenço, 2006). A single *Zabius fuscus* sample (Ojanguren-Affilastro et al., 2017) was included as an outgroup. Consensus sequences were aligned independently for both loci in Geneious v. 7.1.7 (Biomatters Ltd., Auckland, New Zealand) using MUSCLE (Edgar, 2004), checked for accuracy by eye, and trimmed to minimize missing characters. The resulting alignments were 330 bp in length for 16S and 542 bp for COI. We determined the best-fit model of nucleotide substitution for each alignment (gene partition) with MEGA v. 7.0.21 (Kumar et al., 2016) using the Bayesian Information Criterion (GTR + G for 16S and HKY + G for COI). We did not use I + G models because they have been demonstrated to be highly correlated (Jia et al., 2014). We concatenated the alignments in Geneious and uploaded them to the CIPRES Science Gateway with a Bayes block specifying the appropriate substitution models and partitioning scheme (by gene). We conducted BI analyses using MrBayes 3.2.2 (Ronquist et al., 2012) ran for 40 million generations with four chains (one cold, three heated). Model parameters were unlinked across character partitions with sampling every 4000 generations. We adjusted the heating parameter until state-swap frequencies fell between 10% and 70% (temp = 0.05), and discarded the first 25% of trees as burn-in. Convergence of Markov chains was verified using Tracer 1.6 (Rambaut et al., 2014).

3. Results

3.1. Epidemiological data

Twenty patients between 1 and 40 years old were admitted to the ED with a history of a scorpion sting from different localities within the northeastern section of Morona Santiago from the following counties: Taisha (n = 17), Huamboya (n = 1), Palora (n = 1), and Logroño (n = 1) (Appendix A). Cases from Taisha county were from the villages of Macuma (11 cases), Taisha (2 cases), Kusutka (1 case), Cuchaentza (1 case), Nayantza (1 case), and Pumpuentza (1 case). Remaining cases were from Tayuntza, Logroño county (1 case), Huamboya, Huamboya county (1 case), and Palora, Palora county (1 case). These localities are shown in Fig. 1 (see legend for geographical coordinates). None of the patients presented previous medical history. In our case series, one resulted in death (a 4-year-old male from Nayantza) (see below for description of the case). An additional fatal case (a 3-year-old female also from Nayantza), recently identified by us as dating from 2014, is also presented (see below) but was not included in our analyses.

According to the age range, the number of patients examined was as follows: 15 (1–15 years old), 2 (16–30 years old), and 3 (31–45 years old). Most patients were males (13/20). The majority of stings took place within the home (13/20). The mean time elapsed between the sting and hospital admission was 8.3 ± 6.2 h (range 2–30 h). Fig. 2 shows the anatomical location of the stings, with a moderate predominance of feet (9/20) as the main affected site, followed by the gluteal region (3/20) and hands (3/20).

Of the total number of patients, one was classified as Class I, 18 patients as Class II (including 14 children, median = 5.4 ± 3.9 years old), and one case as Class III (a 4-year-old male) which proved fatal. As most of our cases were classified as Class II on the scale of Khattabi et al. (2011), and since we sought to determine whether a correlation existed between severity, age, and clinical parameters, an alternative scale of severity (local severity score hereinafter) was explored. The local severity score is based on the sum of signs and symptoms presented by each case at admission (see Appendix A). Thus, a patient presenting 9 signs and symptoms was classified as more severe than a patient with a score of 2.

3.2. Clinical and paraclinical data

Frequency of signs and symptoms presented by the 20 patients is shown in Fig. 3. All patients presented with local pain. Tachycardia (15/20) and vomiting (12/20) were the second most frequent

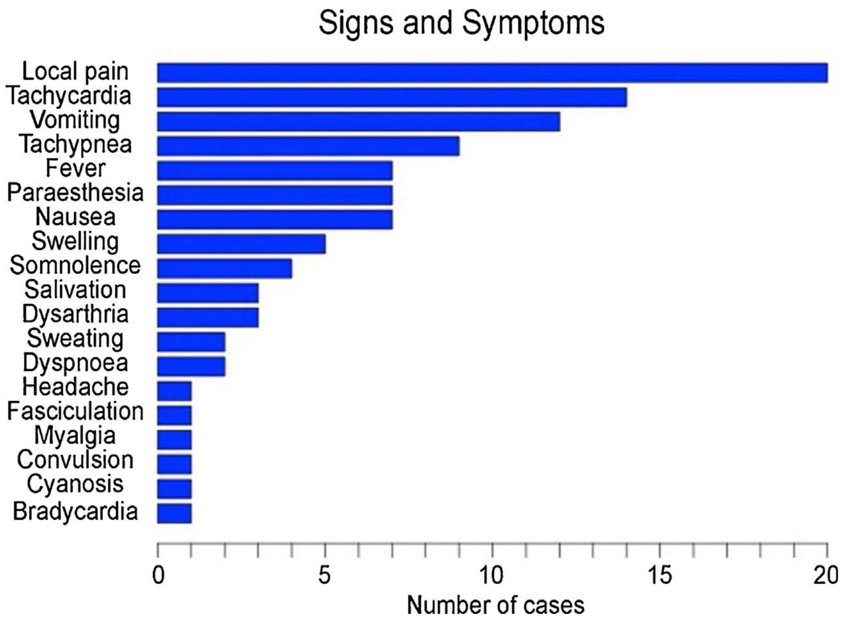

Fig. 3. Frequency of signs and symptoms presented by the 20 cases of scorpion sting recorded from Morona Santiago.

Table 1
Paraclinical parameters presented on admission by patients stung by *Tityus* sp. in Morona Santiago, Ecuador. *Individuals with available serum glucose and potassium levels were 15/20 and 14/20, respectively.

Parameter	n/N	Median	Range
Glycemia > 110 mg/dL	10/15*	172.6	117–309
Leukocyte count > 10,000 per mm3	19/20	17,560	10,350–32,460
Neutrophils (%) > 70%	14/20	83.1	75.6–89.6
Serum Potassium < 3.5 mEq/L	9/14*	2.95	2.50–3.25

manifestations. The paraclinical data presented on admission are summarized in Table 1. Leukocyte count was elevated in most cases (19/20), with significant neutrophilia (above 70%) in 14 patients. Hyperglycemia (> 110 mg/dL) was detected in 10 patients (serum glucose data were available for 15 patients), whereas hypokalemia (< 3.5 mEq/L) was detected in nine patients (serum potassium were data available for 14 patients). Serum creatinine, sodium, and urea levels were unremarkable in all individuals. A negative correlation was found between age of patients and their degree of severity (evaluated by the local severity score) ($R^2 = 0.29$, $p < 0.05$) (outlier = case C, see Appendix A). A negative correlation was also found between leukocyte levels and hypokalemia for the 14 patients from whom serum potassium values were available ($R^2 = 0.38$, $p < 0.05$; outlier = case J, see Appendix A). Although glucose levels were altered in 10 out of 15 patients, we did not find a significant correlation between leukocytosis and hyperglycemia (Appendix A).

At admission, all patients were hydrated with saline solution (0.9%), and treated with paracetamol or tramadol as analgesic, and metoclopramide hydrochloride as antiemetic. No patients received scorpion antivenom, as it is not available in Ecuador (Borges et al., 2015).

3.3. Fatal envenoming case 1

A 4-year-old male from Nayantza, Taisha County, Morona Santiago, with no previous medical history, was stung by a scorpion on the dorsum of right foot 8 h before being admitted to the Nayantza Health Center on 01/22/2016 (patient T, Appendix A). The patient complained of pain at the sting site (intensity 7 on the 1–10 Visual analog scale for pain) and experienced multiple episodes of emesis. He was treated with Paracetamol (150 mg orally) and transferred by air to Macas General Hospital where was admitted at 13H00. On physical examination, the patient was restless and presented algic facies, tachycardia (138 bpm), blood pressure of 98/60 mmHg, tachypnea (33 rpm), 99% blood oxygen saturation, and normal breath sounds. The abdomen was soft, depressible, and painless to palpation. Neurological examination was unremarkable. Laboratory parameters indicated hyperglycemia (309 mg/dL), hypokalemia (2.86 mEq/L), and leukocytosis (31,710 cells/mm^3), with 68.2% neutrophils. Serum urea, uric acid, creatinine, bilirubin, AST, ALT, and alkaline phosphatase levels were within normal range. The patient was treated with potassium chloride (25 mEq), Paracetamol (150 mg orally), Tramadol (25 mg intravenously), and diazepam (4.5 mg intravenously). Shortly after admission and considering the respiratory insufficiency (Class III envenoming), the patient was transferred to the intensive care unit presenting irritability, 90% blood oxygen saturation, peripheral cyanosis, and cough with pink, frothy sputum. Despite sustained oxygen supply, the patient underwent cardiorespiratory failure; advanced cardiopulmonary resuscitation maneuvers were then performed but the patient was pronounced dead at 23H00.

3.4. Fatal envenoming case 2

A 3-year-old female from Nayantza, Taisha County, Morona Santiago, with no previous medical history, was stung by a scorpion in right gluteus 6 h before being admitted to the Nayantza Health Center on 03/31/2014. The patient presented with intense pain at the sting site, multiple episodes of emesis of bilious aspect, and paresthesia in both legs. She was transported by air to Macas General Hospital where she was admitted at 18H30. On physical examination, the patient presented erythema of 3 cm diameter at the site of sting, was drowsy and irritable, presented algic facies, dry skin, tachycardia (126 bpm), tachypnea (34 rpm), and 98% blood oxygen saturation. Laboratory parameters indicated hyperglycemia (253 mg/dL), thrombocytosis (656,000 cells/mm^3), leukocytosis (24,500 cells/mm3), with 81.6% neutrophils. Patient was hydrated with saline solution (0.9%), and given ampicillin (700 mg), tramadol (25 mg intravenously), and metoclopramide hydrochloride (10 mg). Six hours after admission the patient presented sialorrhea, multiple episodes of emesis, fluctuating blood oxygen saturation (60–90%), dyspnea, peripheral cyanosis, and generalized tonic-clonic seizures. She underwent cardio-respiratory failure. Advanced cardiopulmonary resuscitation maneuvers were performed including endotracheal intubation, which resulted in abundant pink, frothy sputum, but the patient was pronounced dead at 01H00 on 04/01/2014.

3.5. Identification of scorpions associated with accidents in Macuma, Taisha County, Morona Santiago

Specimens associated with human stings in Macuma, Morona Santiago (male scorpion responsible for case P is shown in Fig. 4), were initially classified as *Tityus* sp. based on the presence of 15 oblique rows of granules on the dentate margins of the pedipalp-chela fixed and movable fingers (Lourenço, 2002b; Stahnke, 1972). Given the lack of synapomorphies in this genus (Fet and Lowe, 2000), and the considerable diversity of the Ecuadorian scorpion fauna (Brito and Borges, 2015), we resorted to a molecular study to objectively identify this new medically important *Tityus* population. Amplified DNA fragments encoding COI from all three analyzed specimens from Macuma rendered an identical 542 bp-long nucleotide sequence which was deposited in GenBank with the accession number MF466178. COI sequences were also obtained from *T. asthenes* populations from Panama and Ecuador and from *T. obscurus* populations inhabiting northwestern Brazil (see Fig. 1 and Appendix B). BI analyses were performed with these sequences, together with COI and 16S sequences retrieved from GenBank that represent other *Tityus* groups in Latin America (Borges et al., 2010; Ojanguren-Affilastro et al., 2017). This allowed generation of the phylogeny depicted in Fig. 5.

The BI analysis revealed a highly structured phylogeny for genus *Tityus* (Fig. 5). The majority of species were strongly supported as monophyletic, but *T. nororientalis*, *T. zulianus* (both from Venezuela), and *T. asthenes* (from Panama and Ecuador) were paraphyletic. The COI sequence retrieved from the Macuma specimens involved in human envenoming fell within a clade consisting of *T. obscurus* populations from Brazil. Support for the *T. obscurus* clade was 100% (1.0 posterior probability), indicating that the Macuma specimens clearly belongs to this group, which is genetically divergent from the clade containing *T. asthenes* and *T. pachyurus* from the Pacific coastline rainforests of Ecuador, Colombia, and Panama. Within clade uncorrected p-distances were 0.070 for the *T. asthenes/T. pachyurus* clade and 0.061 for the *T. obscurus* clade, whereas the between clade distance was 0.153.

Fig. 4. Male *Tityus* sp. from Macuma, Morona Santiago, responsible for envenoming case P described in this work.

4. Discussion

Ecuador is part of the region exhibiting the highest alpha-diversity for scorpions in the world, which also encompasses Southern Colombia, the Northeast region of Peru, and the Upper Amazon region of Brazil (Lourenço and Ythier, 2013). Despite this diversity, epidemiology and clinical consequences of scorpion stings have been largely overlooked in Ecuador and northwestern Amazonia in general. As previously mentioned, preliminary efforts have been made to correlate human incidents and the precise geographical distribution of the scorpion taxa involved, particularly *Tityus* (Brito and Borges, 2015; Lourenço, 2011). This study contributes to these efforts to map the risk areas for scorpionism in the Amazon Basin by describing the first envenoming cases from Amazonian Ecuador and assessing genetic relationships among *Tityus* populations in the basin responsible for severe envenoming.

Our results confirm previous findings by Ecuadorian health authorities that scorpion envenoming is prevalent in the Amazonian province of Morona Santiago (Ministerio de Salud Pública de Ecuador, 2016), and demonstrates that scorpionism is a health hazard for children within the Shuar community, the main ethnic group inhabiting the province. The majority (85%) of envenoming cases admitted to Macas General Hospital, the main health center in Morona, come from Taisha County. However, we suspect that scorpionism cases are significantly underreported in Taisha County considering that the Shuar believe that stings by the local “black” scorpion (identified by us as belonging to the *T. obscurus* group, see below) are irredeemably fatal, and thus few cases make it to local health centers. This belief, together with the difficult local topography (access to Macas is almost exclusively by plane), has probably contributed to the significant delay in hospital admission (8.3 ± 6.2 h). Therefore, we believe that there is an urgent need for appropriate scorpion antivenoms to the remote communities of Morona Santiago.

Based on our local severity scale, the significant negative correlation found between the age of patients and the degree of severity (albeit with a weak association, $R^2 = 0.29$) is reminiscent of previous studies elsewhere, which suggest that scorpionism is most common in children younger than 10 (Bouaziz et al., 2006). The two deaths from Taisha County clearly demonstrate the lethality of the region’s scorpion fauna.

General manifestations in scorpion sting victims from Morona Santiago are characteristic of stimulation of the autonomic nervous system, including adrenergic (e.g. tachycardia) and cholinergic (e.g. gastrointestinal alterations) manifestations. These effects are similar to those previously described for *Tityus* envenoming from Ecuador and elsewhere in South America (Borges et al., 2015; De Sousa et al., 1995; Gómez et al., 2010; Izquierdo and Rodríguez Buitrago, 2012; Pucca et al., 2014). Facilitation of cholinergic and cathecolaminergic neurotransmitter release is a known consequence of the depolarizing action of low molecular mass scorpion neurotoxins (Amitai, 2005). Hyperglycemia (> 110 mg/dL) was detected in 10 cases (out of 14 patients), and blood glucose levels were two to three times higher in the described fatal cases. This result was probably due to the sympathetic nervous system activation and release of catecholamines, which can directly stimulate glycogenolysis by the liver (Murthy and Hase, 1994). Leukocytosis (> 10,000 cells/mm^3) was also present in the majority of cases (19/20) and usually associated with neutrophilia. This could result from an inflammatory syndrome elicited by the release of inflammatory mediators from both targeted tissues and immune cells affected by scorpion toxins, and is associated with poor prognoses (Bouaziz et al., 2006). Interestingly, the thrombocytosis detected in our fatal envenoming case 2, is a condition that has been previously associated with scorpion envenoming severity in children from Turkey, and is possibly explained through the action of cathecolamines on α-adrenergic receptors of platelets. Alternatively, thrombocytosis could be a result of myocardial ischemia (Çağlar et al., 2015). In our particular case, however, a possible pre-existing essential thrombocythemia cannot be ruled out.

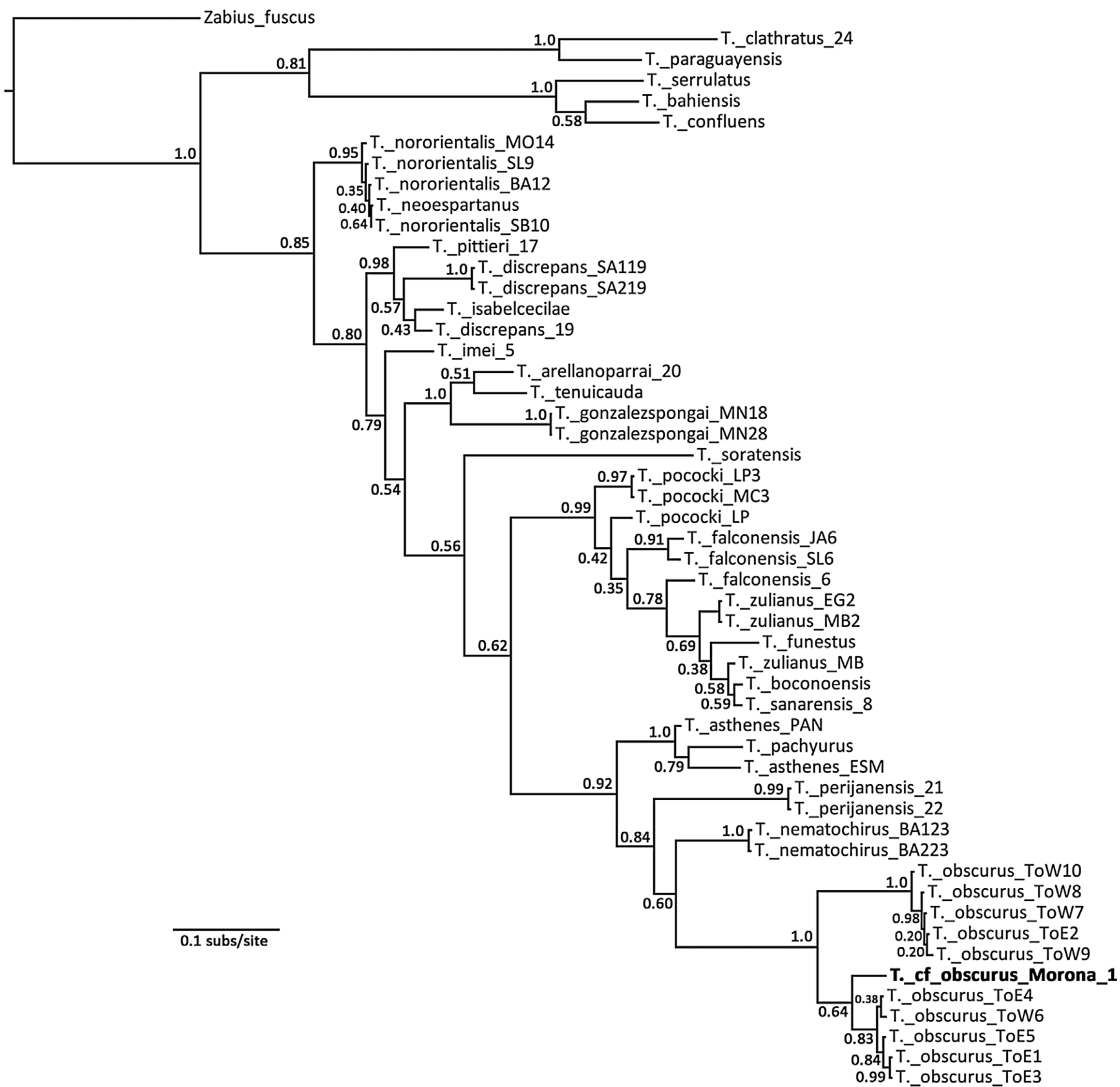

Fig. 5. Consensus tree depicting the results of a phylogenetic analysis of concatenated 16S and COI sequence data generated using MrBayes 3.2.2 (Ronquist et al., 2012). Values at nodes indicate posterior probabilities. *Tityus* cf. *obscurus* from Morona Santiago is in boldface type.

The negative correlation found between leukocyte levels (a known predictor of scorpion envenoming severity) and serum potassium levels in 14 of our patients was statistically significant (although weakly associated, $R^2 = 0.38$). This suggests that hypokalemia is also related to envenoming severity in these patients, confirming previous findings from children stung by *Centruroides* spp. in Mexico (Osnaya-Romero et al., 2016). In this study, the percentage of patients with hypokalemia at admission was statistically greater in symptomatic individuals, and hypokalemia correlated with elongation of the QT interval in electrocardiograms (Osnaya-Romero et al., 2016). The effect of the scorpion venom on electrolytes has been proposed to be associated with transient changes on sodium and potassium pumps (Bucaretchi et al., 2014). As hypokalemia increases the vulnerability to ventricular fibrillation (Pezhouman et al., 2015), these data agree with previous reports on abnormalities found in scorpion-envenomed children, such as arrhythmias and electrolyte alterations in the absence of hemodynamic compromise (Bucaretchi et al., 2014). Additional studies are needed to determine whether hypokalemia is related to plasma concentrations of potassium channel-specific toxins present in soluble *Tityus* venoms from Morona Santiago.

A major finding of this work is the significant phylogenetic affinity of scorpions involved in accidents from Macuma (Taisha County) with known populations of *T. obscurus* located approximately 6,600 km west in the Amazon Basin of northwestern Brazil (Figs. 1 and 5). This result suggests that species related to the medically important *T. obscurus* may be distributed across the basin. This finding was not anticipated given the supposedly *cis/trans* Andean distribution of *T. asthenes* in Ecuador (Lourenço, 1995), a species that has been involved in scorpionism cases from northern Manabí, west of the Andes (Borges et al., 2015). However, the high endemicity of scorpions in Ecuador (Brito and Borges, 2015) led us to examine the phylogenetic placement of scorpions associated with these accidents among other *Tityus* spp., particularly *bona fide* populations of *T. asthenes* from Central America (Panama) and South America (Esmeraldas province, Ecuador). Our results confirmed the Morona population as divergent from *T. asthenes* and the related species *T. pachyurus* (uncorrected p-distance = 0.153). Additional phylogenetic structure within the *T. obscurus* clade suggests that the scorpions from Macuma represent a new species. A formal taxonomic description of this new taxon is in preparation, which we treat in this work as *Tityus* cf. *obscurus*.

Our BI analysis revealed a highly structured phylogeny for the genus *Tityus*, containing taxa that represent all subgenera associated with accidents in South and Central America; i.e. *Tityus* (*Tityus*), *Tityus* (*Archaeotityus*), and *Tityus* (*Atreus*) (Lourenço, 2006) (Fig. 5). Taxa within the *Atreus* subgenus (*Tityus* spp. from Venezuela, Panama, and Brazil) strongly grouped according to their geographic origin, confirming previous results that suggested that geography through vicariance had a major role to play in diversification of the genus (Borges et al., 2010). The *T. obscurus* clade in particular (including the new species from Ecuador) was highly structured, hinting at a possible species complex. Previous taxonomic assessments have indicated the grouping of Amazonian scorpions with males exhibiting extremely long and slender pedipalps and elongated and thin chelae into a single, "*Tityus asthenes*" species complex (i.e. *T. asthenes*, *T. obscurus*, *T. nematochirus*, *T. antioquensis*, *T. apiacas*, *T. asthenes*, *T. brazilae*, *T. dedoslargos*, *T. matthieseni*, *T. nematochirus*, *T. obscurus*, *T. oteroi*, *T. unus*, and *T. vaissadei*) (Kovařík et al., 2013). We now provide genetic evidence that *T. obscurus* does not belong to a "*Tityus asthenes*" complex, as the presumed complex actually represents at least two divergent lineages that should not be treated as a single monophyletic group.

Future work should be directed at elucidating the evolutionary relationships and the possibility of shared venom components among *Tityus* spp. in the putative *T. obscurus* species group across the Amazon Basin. Venom gland transcriptomic studies should prove useful in identifying peptide sequences from the venom of Ecuadorian populations and related Amazonian species, including even the atypical venom molecules difficult to characterize by conventional methods, as in other venoms from the genus *Tityus* (Almeida et al., 2012; de Oliveira et al., 2015). Neurological manifestations are common in stings by *T. obscurus* inhabiting western Pará state, Brazil, with scarce autonomic effects (Pardal et al., 2014b). Cerebellar-muscular manifestations in envenoming cases from this region are not significantly reduced by the scorpion antivenom manufactured against *T. serrulatus*, a species inhabiting southeastern Brazil (Torrez et al., 2015). In our patient series, albeit small, ataxia was detected in 3/20 patients, a neurological manifestation that warrants further analysis. Given our results, antigenic affinities among the venoms of Amazonian scorpions in the *T. obscurus* group deserve to be explored further using transcriptomic and proteomic approaches. These data could then be used for designing therapeutic strategies to treat scorpionism across Amazonia. Antivenom therapy is the central treatment for severe scorpion envenoming, and wider availability in Amazonia could save lives, especially those of children in rural communities like the villages in Morona Santiago.

Acknowledgments

We would like to thank the people from the village of Macuma, Morona Santiago, for their help. Scorpion collection and genetic analysis in Ecuador was performed according to permit MAE-DNB-CM-2016-0040 by the Ministry of Environment of Ecuador. Scorpion collection and genetic analyses of specimens from Brazil was performed according to permit 11727-2 by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). Financial support of Fondo Nacional de Ciencia, Tecnología e Innovación (Venezuela), grant S1-2001000674 (to A.B.) is acknowledged.

Appendix A

See Table A1.

Table A1
Individual characteristics of the 20 cases of scorpion envenoming from Morona Santiago, Ecuador, presented in this study. *Cases are ordered chronologically as they were admitted. **Local severity score is defined as the sum of symptoms and signs recorded at admission. **Envenoming severity according to Khattabi et al. (2011). N/A, Data not available.

Cases*	Location	Age (years)	Time elapsed between accident and admission (hours)	Leukocytes (cells/ mm^3)	Serum potassium (mEq/L)	Glucose (mg/dl)	Local severity score**	Envenoming severity***
A	Taisha	1	4	11,850	3.78	108	6	II
B	Macuma	9	5	28,700	2.74	N/A	4	II
C	Macuma	40	6	14,280	2.98	122.1	5	II
D	Tayuntsa	38	11	11,440	3.25	132	3	II
E	Macuma	5	30	13,170	N/A	88	6	II
F	Kutsuka	4	4	14,800	3.13	178	6	II
G	Macuma	12	20	11,100	N/A	96	7	II
H	Macuma	3	6	16,080	2.95	164	8	II
I	Taisha	20	6	12,600	3.04	117	4	II
J	Macuma	2	2	16,900	5.05	83	4	II
K	Macuma	3	7	10,350	3.84	138	7	II
L	Pupuentza	2	7	23,200	3.11	140	7	II
M	Macuma	33	12	8,580	N/A	94	2	II
N	Macuma	22	7	10,490	3.77	N/A	3	I
O	Palora	14	12	24,700	3.74	N/A	6	II
P	Macuma	5	8	31,170	N/A	309	5	II
Q	Macuma	3	8	14,870	N/A	N/A	3	II
R	Cuchaentza	6	3	32,460	2.5	N/A	3	II
S	Huamboya	6	2	10,690	N/A	N/A	3	II
T	Nayantza	4	6	24,800	2.86	253	9	III

Appendix B

See Table B1.

Table B1
Locality data and GenBank accession numbers for taxa used in Fig. 5. Accession numbers beginning with "AY" were from Borges et al. (2010), with "KY" were from (Ojanguren-Affilastro et al., 2017), "DQ" (*T. sanarensis*) from Borges et al. (2006), and "KC" (*T. tenuicauda*) from (Cornejo-Escobar et al., 2013). Numbers MF466176 to MF466180 represent new data generated for this study. See legend of Fig. 1 for geographical coordinates of *T. obscurus* populations in Brazil.

Species	Sample ID	16S	COI
Tityus arellanoparrai (Venezuela)	T._arellanoparrai_20	AY586777.1	AY586805.1
Tityus asthenes (Panama)	T._asthenes_PAN	–	MF466176
Tityus asthenes (Ecuador)	T._asthenes_ESM	–	MF466177
Tityus bahiensis (Brazil)	T._bahiensis	KY674453.1	–
Tityus boconoensis (Venezuela)	T._boconoensis	AY586763.1	AY586791.1
Tityus clathratus (Venezuela)	T._clathratus_24	AY586782.1	AY586782.1
Tityus confluens (Argentina)	T._confluens	KY674456.1	–
Tityus discrepans (Venezuela)	T._discrepans_SA219	AY586770.1	–
Tityus discrepans (Venezuela)	T._discrepans_SA119	AY586769.1	–
Tityus discrepans (Venezuela)	T._discrepans_19	–	AY586796.1
Tityus falconensis (Venezuela)	T._falconensis_6	–	AY586792.1
Tityus falconensis (Venezuela)	T._falconensis_JA6	AY586765.1	–
Tityus falconensis (Venezuela)	T._falconensis_SL6	AY586764.1	–
Tityus funestus (Venezuela)	T._funestus	AY586758.1	–
Tityus gonzalespongai (Venezuela)	T._gonzalezspongai_MN18	AY586776.1	AY586803.1
Tityus gonzalespongai (Venezuela)	T._gonzalezspongai_MN28	–	AY586804.1
Tityus imei (Venezuela)	T._imei_5	AY586766.1	AY586793.1
Tityus isabelceciliae (Venezuela)	T._isabelceciliae	AY586768.1	AY586795.1
Tityus nematochirus (Venezuela)	T._nematochirus_BA123	AY586756.1	AY586788.1
Tityus nematochirus (Venezuela)	T._nematochirus_BA223	AY586757.1	–
Tityus neoespartanus (Venezuela)	T._neoespartanus	AY586771.1	AY586797.1
Tityus nororientalis (Venezuela)	T._nororientalis_SB10	AY586772.1	AY586798.1
Tityus nororientalis (Venezuela)	T._nororientalis_MO14	AY586773.1	AY586799.1
Tityus nororientalis (Venezuela)	T._nororientalis_BA12	AY586774.1	AY586800.1
Tityus nororientalis (Venezuela)	T._nororientalis_SL9	AY586775.1	–
Tityus cf. *obscurus* (Ecuador)	T._cf_obscurus_Morona_1	–	MF466178
Tityus obscurus (Brazil)	T._obscurus_ToE1	JX196960.1	MF466179
Tityus obscurus (Brazil)	T._obscurus_ToE2	JX196969.1	–
Tityus obscurus (Brazil)	T._obscurus_ToE3	JX196961.1	–
Tityus obscurus (Brazil)	T._obscurus_ToE4	JX196962.1	–
Tityus obscurus (Brazil)	T._obscurus_ToE5	JX196963.1	–
Tityus obscurus (Brazil)	T._obscurus_ToW6	JX196964.1	–
Tityus obscurus (Brazil)	T._obscurus_ToW7	JX196965.1	–
Tityus obscurus (Brazil)	T._obscurus_ToW8	JX196966.1	–
Tityus obscurus (Brazil)	T._obscurus_ToW9	JX196967.1	MF466180
Tityus obscurus (Brazil)	T._obscurus_ToW10	JX196968.1	–
Tityus pachyurus (Panama)	T._pachyurus	AY586753.1	AY586786.1
Tityus paraguayensis (Argentina)	T._paraguayensis	KY674459.1	KY674499
Tityus perijanensis (Venezuela)	T._perijanensis_21	AY586754.1	AY586787.1
Tityus perijanensis (Venezuela)	T._perijanensis_22	AY586755.1	–
Tityus pittieri (Venezuela 17)	T._pitttieri_17	AY586767.1	AY586794.1
Tityus pococki (Venezuela)	T._pococki_LP	–	AY586790.1
Tityus pococki (Venezuela)	T._pococki_LP3	AY586761.1	–
Tityus pococki (Venezuela)	T._pococki_MC3	AY586762.1	–
Tityus sanarensis (Venezuela)	T._sanarensis_8	–	DQ117483.1
Tityus serrulatus (Brazil)	T._serrulatus	AY586780.1	JN018155.1
Tityus soratensis (Bolivia)	T._soratensis	KY674460.1	–
Tityus tenuicauda (Venezuela)	T._tenuicauda	–	KC846138.1
Tityus zulianus (Venezuela)	T._zulianus_EG2	AY586759.1	–
Tityus zulianus (Venezuela)	T._zulianus_MB	–	AY586789.1
Tityus zulianus (Venezuela)	T._zulianus_MB2	AY586760.1	–
Zabius fuscus (Argentina)	Zabius_fuscus	KY674464.1	–

References

Çağlar, A., Köse, H., Babayiğit, A., Öner, T., Duman, M., 2015. Predictive factors for determining the clinical severity of pediatric scorpion envenomation cases in Southeastern Turkey. Wild. Environ. Med. 26, 451–458.

Almeida, D.D., Scortecci, K.C., Kobashi, L.S., Agnez-Lima, L.F., Medeiros, S.R., Silva-Junior, A.A., Junqueira-de-Azevedo, I., Fernandes-Pedrosa, M.F., 2012. Profiling the resting venom gland of the scorpion *Tityus stigmurus* through a transcriptomic survey. BMC Genom. 13, 362.

Amitai, Y., 2005. Scorpions. In: Brent, J., Wallace, K.L., Burkhart, K.K., Phillips, S.D., Donovan, J.W. (Eds.), Critical Care Toxicology: Diagnosis and Management of the Critically Poisoned Patient. Elsevier Mosby, Maryland Heights, pp. 1213–1220.

Benmosbah, M., Gueguenitat, P., Mayence, C., Egmann, G., Narcisse, E., Gonon, S., Hommel, D., Kallel, H., 2013. Epidemiological and clinical study on scorpionism in French Guiana. Toxicon 73, 56–62.

Borges, A., Graham, M.J., 2016. Phylogenetics of scorpions of medical importance. In: Gompalanokrishnanone, P., Calvete, J.J. (Eds.), Venom Genomics and Proteomics. Springer Netherlands, Dordrecht, pp. 81–103.

Borges, A., De Sousa, L., Manzanilla, J., 2006. Description of a new *Tityus* species (Scorpiones: Buthidae) from Sierra de Portuguesa, western Venezuela, based on morphological and mitochondrial DNA evidence. Zootaxa 1107, 49–68.

Borges, A., Bermingham, E., Herrera, N., Alfonzo, M.J., Sanjur, O.I., 2010. Molecular systematics of the neotropical scorpion genus *Tityus* (Buthidae): the historical biogeography and venom antigenic diversity of toxic Venezuelan species. Toxicon 55, 436–454.

Borges, A., Morales, M., Loor, W., Delgado, M., 2015. Scorpionism in Ecuador: first report of severe and fatal envenomings in northern Manabí by *Tityus asthenes* Pocock. Toxicon 105, 56–61.

Bouaziz, M., Bahloul, M., Hergafi, L., Kallel, H., Chaari, L., Hamida, C.B., Chaari, A., Chelly, H., Rekik, N., 2006. Factors associated with pulmonary edema in severe scorpion sting patients – a multivariate analysis of 428 cases. Clin. Toxicol. 44, 293–300.

Brito, G., Borges, A., 2015. A checklist of the scorpions of Ecuador (Arachnida: Scorpiones) with notes on the geographical distribution and medical significance of some species. J. Venom. Animals Tox. Inc. Trop. Dis. 21, 23.

Bucaretchi, F., Fernandes, L.C.R., Fernandes, C.B., Branco, M.M., Prado, C.C., Vieira, R.J., De Capitani, E.M., Hyslop, S., 2014. Clinical consequences of *Tityus bahiensis* and *Tityus serrulatus* scorpion stings in the region of Campinas, southeastern Brazil. Toxicon 89, 17–25.

Chippaux, J.P., Alagón, A.C., 2008. Envenimations et Empoisonnements par les Animaux Venimeux ou Vénéneux VII: L'Arachnidisme du Nouveau Monde. Med. Trop. 68, 215–221.

Chippaux, J.P., Goyffon, M., 2008. Epidemiology of scorpionism: a global appraisal. Acta Trop. 107, 71–79.

Cornejo-Escobar, P., Borges, A., Bónoli, S., Vásquez-Suárez, A., Gregoriani, T., De Sousa, L., 2013. *Tityus tenuicauda* Prendini, 2001 (Scorpiones, Buthidae) en Venezuela. Notas taxonómicas con sinonimia, distribución e historia natural. Saber (Universidad de Oriente) 25, 57–72.

Costa, C.L., Fé, N.F., Sampaio, I., Tadei, W.P., 2016. A profile of scorpionism, including the species of scorpions involved, in the State of Amazonas, Brazil. Rev. Soc. Bras. Med. Trop. 49, 376–379.

de Oliveira, U.C., Candido, D.M., Dorce, V.A., Junqueira-de-Azevedo, I., 2015. The transcriptome recipe for the venom cocktail of *Tityus bahiensis* scorpion. Toxicon 95, 52–61.

De Sousa, L., Kiriakos, D., Jiménez, J., Michieli, D., Rodríguez, C., Mirabal, J., Quiroga, M., 1995. Accidente cerebro-vascular isquémico por emponzoñamiento escorpiónico: observación clínica. Saber (Universidad de Oriente) 7, 7–14.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.

Fet, V., Lowe, G., 2000. Buthidae. In: Fet, V., Sissom, W.D., Lowe, G., Braunwalder, M.E. (Eds.), Catalog of the Scorpions of the World (1758–1998). New York Entomological Society, New York, pp. 54–286.

Gómez, J.P., Quintana, J.C., Arbeláez, P., Fernández, J., Silva, J., Barona, J., Gutiérrez, J., Abel, D., Otero, R., 2010. Picaduras por escorpión *Tityus asthenes* en Mutatá, Colombia: aspectos epidemiológicos, clínicos y toxinológicos. Biomédica 30, 126–139.

Gantenbein, B., Fet, V., Largiadér, C.R., Scholl, A., 1999. First DNA phylogeny of the genus *Euscorpius* Thorell, 1876 (Scorpiones, Euscorpiidae) and its bearing on the taxonomy and biogeography of this genus. Biogeographica (Paris) 75, 59–72.

Hui Wen, F., Monteiro, W.M., Moura da Silva, A.M., Tambourgi, D.V., Mendonça da Silva, I., Sampaio, V.S., dos Santos, M.C., Sachett, J., Ferreira, L.C.L., Kalil, J., Lacerda, M., 2015. Snakebites and scorpion stings in the Brazilian Amazon: identifying research priorities for a largely neglected problem. PLoS Negl. Trop. Dis. 9.

Izquierdo, L.M., Rodríguez Buitrago, J.R., 2012. Cardiovascular dysfunction and pulmonary edema secondary to severe envenoming by *Tityus pachyurus* sting. Case report. Toxicon 60, 603–606.

Jia, F., Lo, N., Ho, S.Y., 2014. The impact of modelling rate heterogeneity among sites on phylogenetic estimates of intraspecific evolutionary rates and timescales. PLoS One 9.

Khattabi, A., Soulaymani-Bencheikh, R., Salmi, L., 2011. Classification of clinical consequences of scorpion stings: consensus development. Trans. R. Soc. Trop. Med. Hyg. 105, 364–369.

Kovařík, F., Teruel, R., Cozijn, M.A.C., Seiter, M., 2013. *Tityus carolineae* sp. n. from Suriname and Guyana (Scorpiones: Buthidae). Euscorpius 178, 1–9.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lourenço, W.R., Leguin, E.A., 2008. The true identity of *Scorpio* (*Atreus*) *obscurus* Gervais, 1843 (Scorpiones, Buthidae). Euscorpius 75, 1–9.

Lourenço, W.R., Ythier, E., 2013. The remarkable scorpion diversity in the Ecuadorian Andes and description of a new species of *Tityus* C.L. Koch, 1836 (Scorpiones, Buthidae). ZooKeys 307, 1–13.

Lourenço, W.R., 1995. Les Scorpions (Chelicerata, Scorpiones) de l´Equateur avec quelques considérations sur le biogéographie et la diversié des espéces. Rev. Suisse Zool. 102, 61–88.

Lourenço, W.R., 2000. Synopsis of the Colombian species of *Tityus* Koch (Chelicerata, Scorpiones, Buthidae), with descriptions of three new species. J. Nat. Hist. 34, 449–461.

Lourenço, W.R., 2002a. Scorpiones. In: Adis, J. (Ed.), Amazonian Arachnida and Myriapoda. Pensoft Publishers, Sofia-Moscow, pp. 399–438.

Lourenço, W.R., 2002b. Scorpions of Brazil. Editions de ÍIf, Paris.

Lourenço, W.R., 2006. Nouvelle proposition de découpage sous-générique du genre *Tityus* C.L. Koch, 1836 (Scorpiones, Buthidae). Bol. Soc. Entomol. Arag. 39, 55–67.

Lourenço, W.R., 2011. The distribution of noxious species of scorpions in Brazilian Amazonia: the genus *Tityus* C.L. Koch, 1836, subgenus *Atreus* Gervais, (Scorpiones, Buthidae). Entomol. Mitt. Zool. Mus. Hamburg 15, 287–301.

Ministerio de Salud Pública de Ecuador, 2016. Dirección Nacional de Vigilancia Epidemiológica. Picadura por Escorpión en Ecuador. Weeks 01–41.

Monteiro, W.M., Oliveira, S.S.d., Pivoto, G., Alves, E.C., Sachett, J.d.A.G., Alexandre, C. N., Fé, N.F., Guerra, M.d.G.V.B., Mendonça da Silva, I., Tavares, A.M., Ferreira, L.C.d. L., Guimarães, M.V., Lacerda, M.V., 2016. Scorpion envenoming caused by *Tityus* cf. *silvestris* evolving with severe muscle spasms in the Brazilian Amazon. Toxicon 119, 266–269.

Murthy, K.R.K., Hase, N.K., 1994. Scorpion envenoming and the role of insulin. Toxicon 32, 1041–1044.

Ojanguren-Affilastro, A.A., Adilardi, R.S., Mattoni, C.I., Ramírez, M.J., Ceccarelli, F.S., 2017. Dated phylogenetic studies of the southernmost American buthids (Scorpiones; Buthidae). Mol. Phylogenet. Evol. 110, 39–49.

Osnaya-Romero, N., Acosta-Saavedra, L.C., Goytia-Acevedo, R., Lares-Asseff, I., Basurto-Celaya, G., Perez-Guille, G., Possani, L.D., Calderón-Aranda, E.S., 2016. Serum level of scorpion toxins, electrolytes and electrocardiogram alterations in Mexican children envenomed by scorpion sting. Toxicon 122, 103–108.

Pardal, P.P., Gadelha, M.A.d.C., Ortiz Meneses, M.M., Malheiros, R.S., Ishikawa, E.A., Gabriel, M.D.G., 2014a. Envenenamento grave pelo escorpião *Tityus obscurus* Gervais, 1843. Rev. Pan-Amazônica Saúde 5, 65–70.

Pardal, P.P., Ishikawa, E.A., Vieira, J.L., Coelho, J.S., Dórea, R.C., Abati, P.A., Quiroga, M.M., Chalkidis, H.M., 2014b. Clinical aspects of envenomation caused by *Tityus obscurus* (Gervais, 1843) in two distinct regions of Para state, Brazilian Amazon basin: a prospective case series. J. Venom. Animals Tox. Inc. Trop. Dis. 20, 3.

Pezhouman, A., Singh, N., Song, Z., Nivala, M., Eskandari, A., Cao, H., Bapat, A., Ko, C.Y., Nguyen, T.P., Qu, Z., Karagueuzian, H.S., Weiss, J.N., 2015. Molecular basis of hypokalemia-induced ventricular fibrillation. Circulation 132, 1528–1537.

Pucca, M.B., Neves Oliveira, F., Schwartz, E.F., Arantes, E.C., Lira-da-Silva, R.M., 2014. Scorpionism and dangerous species of Brazil. In: Gopalakrishnakone, P., Ferroni Schwartz, E., Possani, L.D., Rodriguez de la Vega, R.V. (Eds.), Scorpion Venoms. Springer Netherlands, Dordretch, pp. 299–324.

Queiroz, A.M., Sampaio, V.S., Mendonça, I., Fé, N.F., Sachett, J., Ferreira, L.C.L., Esaú, F., Wang, F.H., 2015. Severity of scorpion stings in the Western Brazilian Amazon: a surveillance-based study. PLoS One 10.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, Available at: http://beast.bio.ed.ac.uk/Tracer.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entom. Soc. Am. 87, 651–701.

Stahnke, H.L., 1972. A key to the genera of Buthidae (Scorpionida). Entomol. News 83, 121–133.

Torrez, P.P., Quiroga, M.M., Abati, P.A., Mascheretti, M., Costa, W.S., Campos, L.P., França, F.O., 2015. Acute cerebellar dysfunction with neuromuscular manifestations after scorpionism presumably caused by *Tityus obscurus* in Santarem, Pará/Brazil. Toxicon 96, 68–73.