


Original paper

VENEZUELAN ARACHNIDS. TWO NEW SPECIES OF THE *Tityus* GENUS (SCORPIONIDA: BUTHIDAE) AND THE CHROMATOGRAPHIC PROFILE OF VENOM AS A POSSIBLE TAXONOMICAL TOOL

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ABSTRACT: Two new species of the *Tityus* genus are described. *T. isabelceciliae* n. sp. lives on the northern central slope of the Cordillera de la Costa. It belongs to the *discrepans* group and is dangerous to man due to its high number, aggressive behavior, domiciliary habits, and high toxicity of its venom. *T. isabelceciliae* venom is similar to other *Tityus* in relation to the molecular weight range and the biological activity of its components. However, the proportions of each fraction in the venom pooled from many *T. isabelceciliae* differ from the proportions in other *Tityus*, indicating that these proportions may have a taxonomical value. The venom LD₅₀ is 38.1 (36.3, 39.9) µg/g mouse (Death in 30 min, Dixon and Mood (14) sequential method, median and 95% confidence interval, n=7). Venom production was 916 (625, 1213) µg protein per animal (n=38): females [944 (750, 1150) mg protein per animal, n=24] and males [824 (550, 112) mg protein per animal, n=14] did not differ in venom production (P > 0.05). There was no correlation between animal total weight and venom production. *T. rusemelyae* n. sp. from the *androcottoides* group lives near the town of Humocaro Alto in the Lara State, Venezuela. The male specimens have clearly defined keels and granules. It differs from other species of this genus in that the prominent characteristics are observed in male specimens.

KEY WORDS: arachnids, scorpions, Buthidae, *Tityus*, new species, venom chromatographic profile, Venezuela.

INTRODUCTION

One hundred and five species of scorpions distributed in 4 families and 18 genera are known to exist in Venezuela. The *Tityus* genus comprises 28 species (6,8,9). Description of new species from this genus is of great interest since they are known as dangerous to humans. This work describes two new species *Tityus isabelceciliae* n. sp. and *Tityus rusemelyae* n. sp. also from Venezuela. The *Tityus* species are spread all over Venezuela, except for the central plains, comprising grasslands with very high daily temperatures in the dry season; snowy areas; and the high páramo of the Venezuelan Andes mountains.

Some species of *Tityus* are particularly abundant in densely populated areas of Venezuela. This is the case of *T. funestus* in the Táchira and Southern Mérida states and *T. discrepans* (11) in the vicinity of the country's capital Caracas. *T. discrepans* is the best studied Venezuelan *Tityus* (1,2,3,4). These species are responsible for a high number of human envenomings. *Tityus isabelceciliae* n. sp. is perhaps the most aggressive *Tityus* known to the authors. It is abundant in populated areas near Caracas and has been responsible of many deaths, especially among children, from where its name originates.

The author penciled the figures on millimetric paper using a dissecting microscope with an eyepiece reticule to ensure the accurate proportions of the depicted parts. All figures are drawn to the same scale. The typical specimens described here are part of the Manuel Angel Gonzalez-Sponga (MAGS) collection located at 1298, Cuyuni Street, El Marqués, Caracas, Venezuela. This collection includes 372 Venezuelan arachnid types and is the largest in the country.

SYSTEMATICS

DESCRIPTION 1

FAMILY. Buthidae (6)

GENUS. *Tityus* Koch, 1836 (12,13)

GROUP. *discrepans*

Tityus isabelceciliae n. sp.

Holotype. adult male specimen MAGS-4345. Paratype. adult female specimen MAGS-3300; 14 male and 20 female specimens at different stages of development. Collected by Prof. Zweibelky Carrera (1982), Dr. M. A. Arellano Parra (1983), Dr. Gina D'Suze, Dr. Carlos Sevcik, and Dr. Pedro Parrilla (Instituto Venezolano de Investigaciones Científicas, IVIC) and by the people of the "Urbanización El Junco" (1997).

Typical locations. Urbanización El Junco, on the road between Caracas and El Junquito, in Venezuela, 1250 m above sea level.

Habitat. High tropophilous forests and grasslands.

Climate. Awⁱ of Koeppen

Microhabitat. Human dwellings, under fallen leaves, in or under rotting wood and stone walls.

Etymology. *In memoriam* of Isabel Cecilia Itriago Viso, a girl who died from envenoming by this species.

Diagnosis. *Tityus isabelceciliae* n. sp. is distinguished by the following: a) dark brown color, more intense on segment V and telson; b) total length: male 86.75 mm, female 65.62 mm; c) length of caudal segment V: male 11.35mm, female 8.45 mm; d) telson form. These are the characteristics distinguishing *T. isabelceciliae* n. sp. from *T. discrepans* Karsch (11), *T. pittieri* Gonzalez-Sponga (G-S) (5), *T. arellanoparrai* G-S (7), *T. osmanus* G-S (8), species from the same group as *T. isabelceciliae* n. sp. Differences between these species are described by González-Sponga (1996) (8).

CHARACTERISTICS OF MALE SPECIMENS

Color. dark brown body, more marked on segment V and telson. Carapace. anterior border is slightly curved and the sides are divergent and sinuous; posterior border slightly concave in the middle and with almost straight sides. Furrows. anterior median is broad and flat, coriaceous with small dense granulation; posterior median wide, with a more or less deep pit in the middle and small granules on the bottom and sides; posterior marginals are shallow and covered with small dense granulation; posterior laterals are flat, coriaceous, with few granules. Carinae. anterior marginal carina has small granules in the middle, and its sides are the continuation of the anterior paramedian carinae. The anterior paramedian and supra-ocular keels are not prominent and have irregularly spread granules of different sizes; posterior paramedian keel is very irregular and has small elongated granules. Posterior paramedian carinae are not prominent and have small granules forming a sinuous line. The marginal posterior carina is densely populated with minute granules. The anterior oblique carinae have granules of different sizes. The rest of the tegument is coriaceous and has a few granules of different sizes. The granules are more abundant behind the lateral eyes and at the posterior lateral angle. The median eyes are separated at their diameter and the lateral eyes have a few very small granules.

Pedipalps. [Figure 1](#), orthobotriotoxic (15). Chela. [Figure 2](#). The chela is big and ovoid, all carinae except the exterior ventral are not prominent with few vestigial granules. The digital chela is broken in the middle. The exterior ventral is sinuous and delineated only by the angle of the manus. The inner ventral carina is absent. The intercarinal tegument has very small and spread vestigial granules. The fixed finger is spanned by the dorsal, lateral dorsal, and digital carinae. All these carinae are smooth. The inner border of the movable finger has a scoop and 14 to 15 denticle lines at the base. The fixed finger has a smooth external carina, an inner basal tubercle and 15 to 18 denticle lines. Tibiae. dorsal, lateral, external, and exterior ventral carinae, formed by regularly aligned poorly marked granules. The inner ventral carina has bigger granules than the other carinae and the granules are irregularly aligned. The inner median carina is formed by two pyramidal and obtuse tubercles, with basal and pyramidal granules, which diminish in size towards the distal extreme of the segment. The intercarinal tegument has abundant and very small vestigial granules. Femora. the dorsal carina has spread and regularly aligned granules of moderate size. Pyramidal obtuse granules form the inner lateral carina. The lateral external carina has one pyramidal granule at the base and the remaining granules are not prominent. Small irregularly aligned granules form the inner ventral carina. The intercarinal tegument resembles the tibial tegument. Tergites. the sagittal carina is formed by two granules near the posterior border on tergites I and II; the remaining tergites have some small granules which are larger at the distal extreme; lateral carinae are vestigial on tergites I and II and from tergites III to VI these carinae have granules of different sizes and irregular alignment. Tergites I to VI are densely covered by small granules, with some larger granules in the middle posterior central half. Tergite VII has a thick sagittal carina with some dorsal granules; the paramedian and lateral carinae are traversed by a row of granules of moderated size; the remaining tegument is densely populated with minute granules. Sternites. I is coriaceous, II through IV with vestigial granules, larger on the sides. Sternite V has two irregular and non-prominent paramedian carinae, the median basal half has abundant small granules. The distal carina has larger well-aligned granules. The lateral carinae occupy the middle third of the sternite and their granules are flat; there is a dense and small intercarinal granulation.

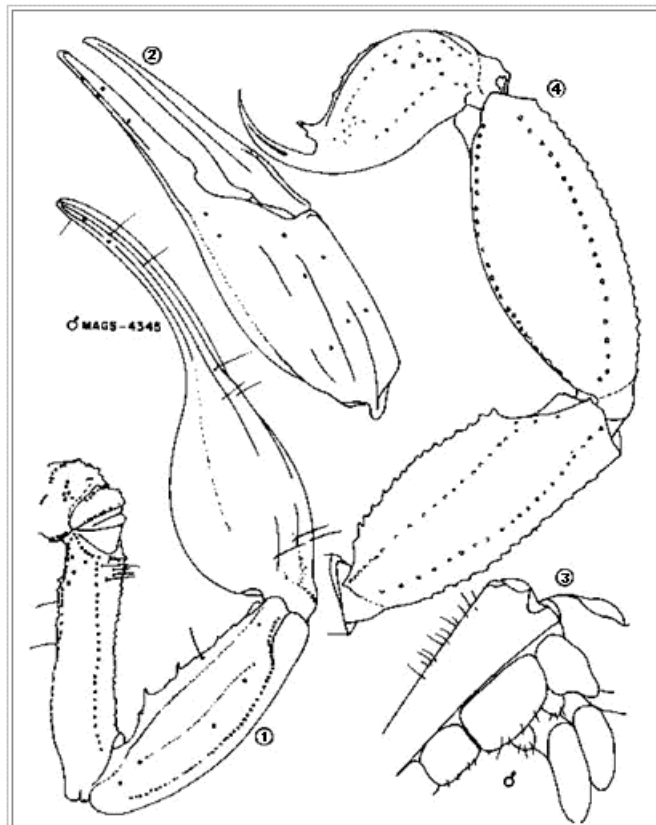


Figure 1. Right pedipalp dorsal view of *Tityus isabelceciae* n. sp. male specimen.

Figure 2. External side of chela of *Tityus isabelceciae* n. sp. male specimen.

Figure 3. Pecten basal lamellae of *Tityus isabelceciae* n. sp. male specimen.

Figure 4. Ventral side of caudal segments IV to V and telson of *Tityus isabelceciae* n. sp.

Walking legs. the femora exhibit the following carinae: dorsal, lateral dorsal, ventral, and lateral ventral carinae, all with vestigial granules. The tibiae have dorsal, lateral dorsal, external lateral, lateral ventral, and ventral carinae, all with vestigial granules and few spiny granules over the distal extreme of the ventral carina. Angulated pretarsus and basitarsus with smooth carinae, the inter-carinal tegument is coriaceous with abundant macrochetae on the ventral border of all segments.

Pectinal denticles. 18 to 19 pectinal denticles. The basal lamellae are shown in [Figure 3](#). Caudal segments. segment I has eight carinae, with homogenous granules. External lateral carinae are complete and ventral carinae slightly convergent at their distal end. The dorsal carinae on segments II to IV have larger distal granules, with no external lateral carinae but a single ventral median carina. Segment V has five carinae with small, spread and well-defined granules, the intercarinal tegument of segments I to IV is coriaceous and small. Segment V has abundant vestigial granules. Telson. dorsally flattened, the vesicular tegument is coriaceous; the lateral furrows are vestigial, the ventral furrows are prominent with inconspicuous granules, larger at the telson venter; the aculeus is short, with subaculear tubercle bent downwards with two dorsal granules ([Figure 4](#)).

DIFFERENTIAL CHARACTERISTICS OF FEMALES

Carapace. prominent carinae with large granules, the intercarinal and furrow granules are more abundant and larger. Pedipalps. [Figure 5](#). Chela. narrow, with well-defined carinae formed by small and long granules ([Figure 6](#)). The movable finger has 14 to 15 denticle alignments and the fixed finger 16 to 17. Tibia. abundant denticles in the median internal carina. Tergites. large granules on the carinae and in the middle of the tergite posterior part, interspersed with small dense granules. Pectinal denticles: 16-16. Pectineal basal lamellae are shown in [Figure 7](#). Caudal segments are shown in [Figure 8](#).

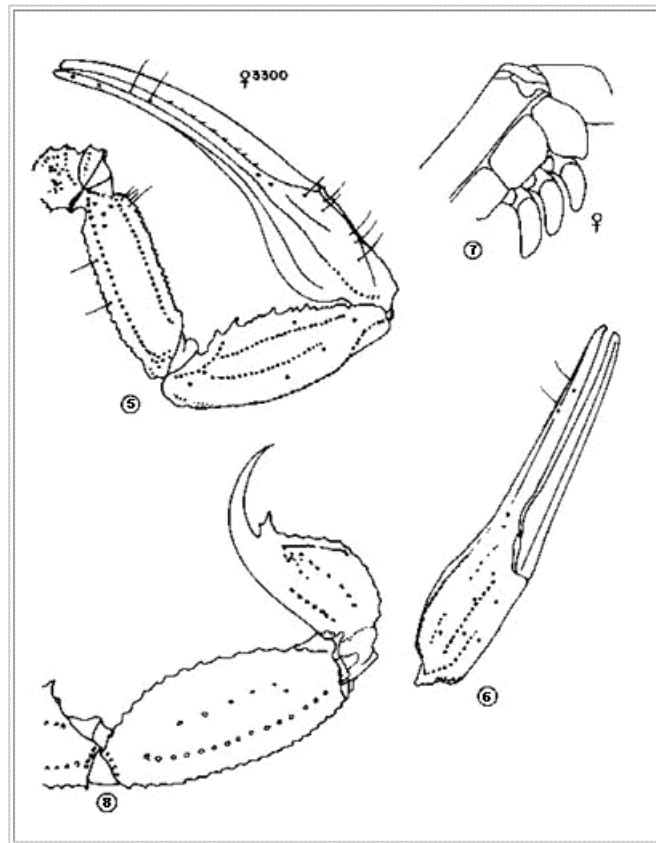


Figure 5. Right pedipalp dorsal view of *Tityus isabelceciae* n. sp. female specimen.

Figure 6. External side view of chela of *Tityus isabelceciae* n. sp. female specimen.

Figure 7. Pecten basal lamellae view of *Tityus isabelceciae* n. sp. female specimen.

Figure 8. Ventral side of caudal segments V and telson view of *Tityus isabelceciae* n. sp. female specimen.

Dimensions (mm) - [Table 1](#).

Table 1. Dimensions (mm) of Holotype (male) and Paratype (female) of *Tityus isabelceciliae* n. sp and *Tityus rusemeyae* n. sp.

	<i>T. isabelceciliae</i> n. sp		<i>T. rusemeyae</i> n. sp	
	Holotype	Paratype	Holotype	Paratype
Length of Carapace	8.70	7.30	6.73	6.30
Anterior Width of Carapace	4.50	4.42	4.20	4.12
Median Width of Carapace	3.00	2.50	2.40	2.40
Posterior Width of Carapace	9.00	8.40	7.20	6.80
1st Lateral Eye to Median Eye	3.25	2.80	2.48	2.32
Median Eyes to Anterior Margin	3.25	2.70	2.27	2.16
Width of Median Ocular Tubercle	1.90	1.64	1.55	1.50
Length of Fixed Finger	9.15	9.30	6.78	6.80
Length of Manus	9.70	4.63	4.37	4.20
Width of Manus	4.80	3.00	2.78	2.70
Height of Manus	4.74	3.00	2.74	2.50
Straight Exterior Length of Manus	7.80	6.00	5.40	5.63
Length of Movable Finger	10.95	10.07	7.63	7.90
Length of Tibia	9.42	8.10	7.36	6.35
Length of Femur	9.65	7.55	6.00	5.92
Length of Pedipalp	37.82	34.72	25.36	25.80
Length of Mesosomal	18.60	15.20	13.10	12.54
Width of Genital Operculum	2.50	2.50	2.35	2.22
Height of Genital Operculum	1.20	1.20	1.17	1.05
Length of Pecten	5.80	4.05	4.20	4.36
Length of Caudal Segment I	8.00	5.70	4.50	4.16
Width of Caudal Segment I	4.20	3.80	3.76	3.60
Length of Caudal Segment II	9.90	6.42	5.10	4.93
Width of Caudal Segment II	4.20	3.75	3.60	3.43
Length of Caudal Segment III	9.50	7.08	5.70	5.40
Width of Caudal Segment III	4.20	3.60	3.60	3.45
Length of Caudal Segment IV	11.25	8.10	6.70	6.10
Width of Caudal Segment V	4.80	3.90	3.33	3.30
Length of Telson	9.45	7.37	6.38	5.85
Width of Vesicle	4.50	3.00	2.70	2.60
Height of Vesicle	3.45	3.00	2.55	2.30
Length of Metasomal	59.45	43.12	35.45	33.14
Total Length	86.75	65.62	55.28	51.98

VENOM CHARACTERISTICS

Venom source and extraction. A colony of *Tityus isabelceciliae* fed with *Tenebrio* sp. larvae was kept in laboratory. The animals were anesthetized with CO₂ prior to venom extraction. Venom was milked every three weeks by electric stimulation of the telson. The venom was dissolved in double distilled water and centrifuged for 30 min at 27,200 g in a refrigerated centrifuge (Sorvall RC 28S). The supernatant protein content was spectrophotometrically determined, assuming one unit of absorbance at 280 nm (1 cm path length) is equal to 1 mg protein/ml. The freeze-dried supernatant was stored at -80°C. Some scorpions were individually milked, the venom of each animal was dissolved in 1 ml double distilled water, and centrifuged in an Eppendorf centrifuge (Model 5415C) at 4°C for 30 min at 13,000 g. The venom supernatant protein content in individual milking was determined, ranging between 0.2 and 1.8 mg of protein per animal. There was no correlation between the animal weight and successive milking with venom production. Venom production was 916 (625, 1213) µg protein per animal (median and 95% confidence interval, Hodges and Lehman method, n=38, see reference 12 for details), females [944 (750, 1150) µg protein per animal, n=24] and males [824 (550, 112) µg protein per animal, n=14] did not differ in venom production (P>0.05) (Figure 9A).

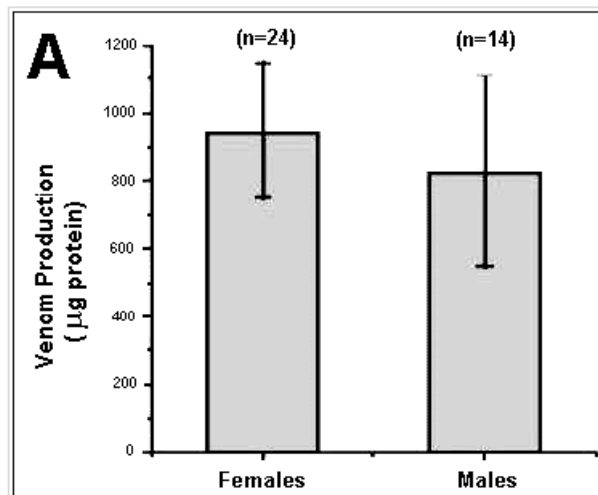


Figure 9. A: *T. isabelceciliae* male and female individual venom production. Gender indicated in the abscissa. Ordinate is in mg of protein per specimen, the protein content was estimated measuring absorbance at 280 nm.

Determination of the chromatographic profile. Lyophilized venom pools were dissolved in 20 mM CH₃.COOH at pH 4.85. Protein separation by molecular exclusion ([Figure 9B](#)) was achieved by high-performance liquid chromatography (HPLC) [Protein-PakTM 125 column (7.8 x 300 mm, Waters)] eluting at 0.5 ml/min with 20 mM CH₃.COOH at pH 4.85.

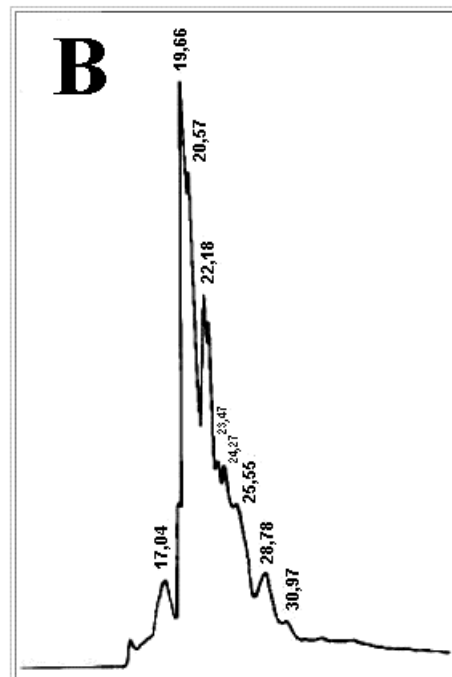


Figure 9.B: Chromatographic profile of pooled *T. isabelceciliae* venom. The trace is a plot of absorbance at 280 nm in arbitrary units, numbers near peaks indicate retention time in minutes. Soluble venom was applied to a Protein-PackTM 125 HPLC (7.8 x 300 mm, Waters) eluting isocratically at 0.5 ml/min during 40 min with 20 mM CH₃.COOH at pH 4.85 at room temperature.

The typical chromatographic profile of *T. isabelceciliae* venom is shown in [Figure 9B](#). Ten fractions were separated, with retention times ranging from 12.83 to 30.97 minutes. *T. isabelceciliae* venom has similar molecular weight range and component biological activity to other *Tityus*. We have found (unpublished results) that although the chromatographic profile of individual venom milkings from the same species varies considerably, the profile of venom pooled from a large number (>50) of specimens is remarkably constant and different from the pattern found in other *Tityus*, indicating that the elution profile may have a taxonomical value.

LD₅₀ determination. LD₅₀ was determined by a modified Dixon and Mood method (14). Venom toxicity was determined by IP injection in mice (white, IVIC strain, male, 20 g). LD₅₀ value was found as 38.09 (36.25, 39.92) µg/g mice (median and 95% confidence interval calculated using Hodges and Lehmann procedure (see reference 10 for details).

DESCRIPTION 2

FAMILY. Buthidae (6)

GENUS. *Tityus* Koch, 1836 (12,13)

Tityus rasmelyae n. sp.

Holotype. adult male specimen, MAGS-5239. Paratype. adult female specimen MAGS-5240; three adult female specimens collected by Enrique Yustiz, K. Mujica, E. Colmenarez and M. Torrealba, (03/27/95).

Typical Location. Cerro El Pajón, Humocaro Alto, Municipio Morán, Lara State, Venezuela.

Distribution. It is known only in its typical locality.

Habitat. Humid tropophilous forest, at 1900 m above sea level.

Climate. Aw"(s") i of Koeppen.

Microhabitat. In or under rotting wood.

Etymology. Professor Enrique Yustiz dedicates this species on request to Rasmely López, his former student and field collaborator.

Diagnosis. *Tityus rasmelyae* n.sp. is distinguished by the fact that the characteristics of male specimens are more prominent than in female specimens. This distinguishes this species from all others with convergent median ventral carinae on caudal segments II through IV. Differences between these species are described by González-Sponga (1996) (8).

CHARACTERISTICS OF MALE SPECIMENS

Color. anterior border of the carapace, fingers of the chelae, tergites I to VI, V caudal segments, and telson are dark brown; the remaining tegument is light brown. **Carapace.** anterior border is slightly obtuse in the middle, straight and divergent on anterior lateral angles, lateral borders are sinuous, posterior border sub-straight. **Furrows.** wide and flat, with many small granules, larger granules near the carapace border. The inter-ocular furrow is wide and shallow. Lateral ocular and posterior median furrows are narrow, with the latter being deeper at the distal half, with abundant granules of different sizes in their depth and at the sides. Posterior marginal furrows are straight, short, and densely populated with minute granules. The posterior lateral furrows are double, smooth in their depth and separated by some different-sized granules. **Carinae.** irregularly aligned granules of different shapes and sizes form the anterior marginal carina; some of these granules are coalescent. The anterior paramedian carinae are poorly defined, formed by irregularly aligned granules of different sizes, some are similar to the granules of the adjacent tegument. The supra-ocular carinae are smooth, with coalescent granules. The posterior paramedian carinae are poorly defined, with small closely packed granules. The anterior transverse carinae are short and with small scarce granules. The rest of the tegument is densely covered by small granules, with some larger irregularly scattered granules. The median eyes are separated by their diameter; there are three small lateral eyes with some small granules at their dorsum. **Pedipalps.** [Figure 10](#), orthobotriotaxic (15). **Chela.** [Figure 11](#). The dorsal carina with small granules spread at the median basal half, close to each other at the distal median half. Elongated granules form the external dorsal carina, smaller at the base of the fixed finger. The digital carina is non-prominent in the middle, where the granules are small and irregularly aligned, and it is inconspicuous at the base of the fixed finger. The external lateral carina has granules of different sizes at both extremes, it is raised in the middle with large coalesce granules. The external sub-median carina is short with poorly prominent granules. The external ventral carina is narrow and sharp, with closely packed elongated granules. The internal dorsal carina has vestigial granules. There is a granulose basal carina, which bridges the external lateral dorsal and the external ventral carinae. The intercarinal tegument is coriaceous and has abundant vestigial granules. The carinae on the fixed finger are smooth. **Tibiae.** all carina are prominent with well-defined, regularly spread, round granules. The exception is the internal lateral carina, which has a pyramidal tubercle and spread raised granules. The external and internal intercarinal dorsal tegument has abundant small granules with only vestigial granules on the ventral side. The movable finger denticle alignments: 15-15, fixed finger denticle alignments: 14-14. **Femora.** regularly aligned granules form the dorsal carinae. The external lateral carina is irregular with granules of different sizes. The internal lateral carina with denticulate granules of different heights and irregular alignment. The internal ventral carina is not prominent with granules of different sizes. Intercarinal tegument is thinly granular, with granules more prominent at the dorsum.

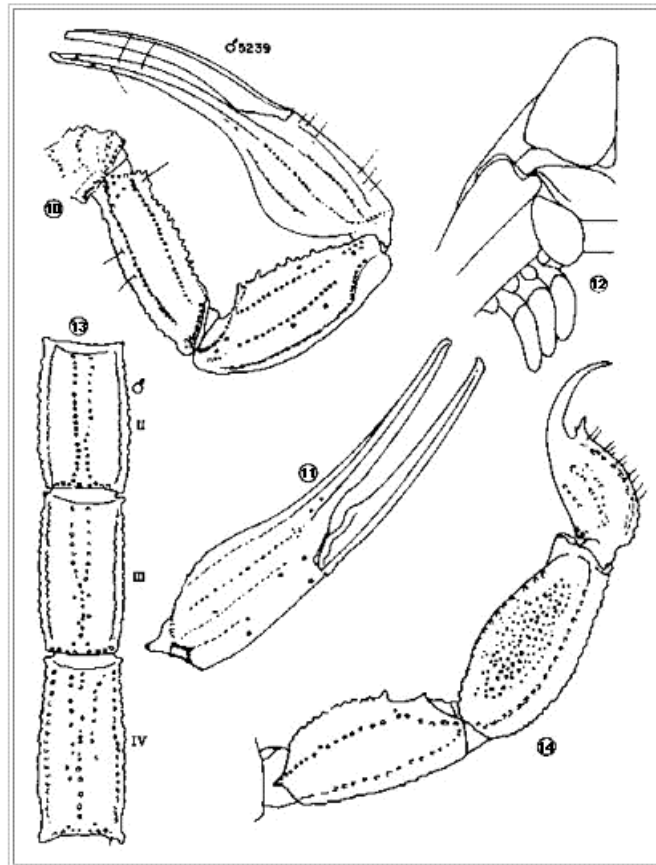


Figure 10. Right pedipalp dorsal view of *Tityus rusemelyae* n. sp. male specimen.

Figure 11. External side of chela of *Tityus rusemelyae* n. sp. male specimen.

Figure 12. Pecten basal lamellae of *Tityus rusemelyae* n. sp. male specimen.

Figure 13. Ventral side of caudal segments II to V of *Tityus rusemelyae* n. sp. male specimen.

Figure 14. External side of caudal segments IV to V and telson of *Tityus rusemelyae* n. sp. male specimen.

Walking legs, the granules and carinae are progressively more defined towards the posterior legs. The granules are in regular alignment; there are abundant intercarinal granules on the femora. The carinae are smooth except on the pretarsus, where they are granulose. Tergites, the sagittal carina becomes progressively larger towards the posterior tergites but is not present on tergite I. Tergites are formed by large granules, some of them coalescent. The granules on the transversal carina and posterior border of the tergite are big, round, irregularly distributed, and interspersed by a small dense and varying granulation. Tergite VII has a wide and raised sagittal carina covered with abundant small granules. Paramedian carinae occupy the whole segment with regularly aligned granules, except for their proximal extreme; this is formed by a group of granules. Lateral carinae are located in the median distal half. The intercarinal tegument is densely granulose. Stermites: stermite I is coriaceous, stermites II to V have dense and small granularity, the latter having a vestigial sagittal carina. The paramedian carinae occupy the distal half of the segment and their granules are progressively larger towards the posterior border. The lateral carinae occupy only one median third. The sides and posterior border of this stermite have large granules. Pectinal denticles: 15-16 (Figure 12). Caudal segments, the carinae of all segments have defined and prominent granules. The dorsal carinae segment granules increase in size towards their distal end, but on segments II to IV these carinae terminate at a large pyramidal granule. The lateral dorsal carinae are complete, with spherical and aligned granules. The lateral external carinae are complete at segment I but are vestigial in the distal end of segment II. The lateral ventral carinae are prominent and complete with big well-defined granules on segment IV. The ventral carinae are complete and parallel on segment I; on segment II they are double and slightly convergent in the distal third of this segment. Segments III and IV show double ventral carinae, which are parallel at the basal medial region; there are some aligned and some irregularly distributed granules in the distal half. The intercarinal tegument has abundant small granules, less marked on the dorsal furrow. Segment V has five carinae with irregularly distributed granules. The dorsal carina have small granules and the remaining carinae have large granules. Lateral and ventral teguments have flat granules of different sizes; the dorsal furrow is coriaceous with small and vestigial granules on the sides. Telson, the lateral and ventral furrows and dorsum are coriaceous. There are some small granules and a lateral dorsal furrow on the dorsal border. The sides have abundant granules and one ventral line that extends up to the base of the sub-aculear tubercle, which is sharply pointed with two dorsal granules (Figure 13 and Figure 14).

DIFFERENTIAL CHARACTERISTICS OF THE FEMALE

Their granules and carinae are less prominent, and the chelae are slightly narrower (Figure 15 and Figure 16). The denticle lines of the mobile finger: 15-16; the denticle lines of the fixed finger: 14-16. The basal lamellae media of the pectens are different (Figure 17). Pectinal denticles: 18-19. The caudal segment V and telson are shown in Figure 17. Table 1 lists the dimensions (mm).

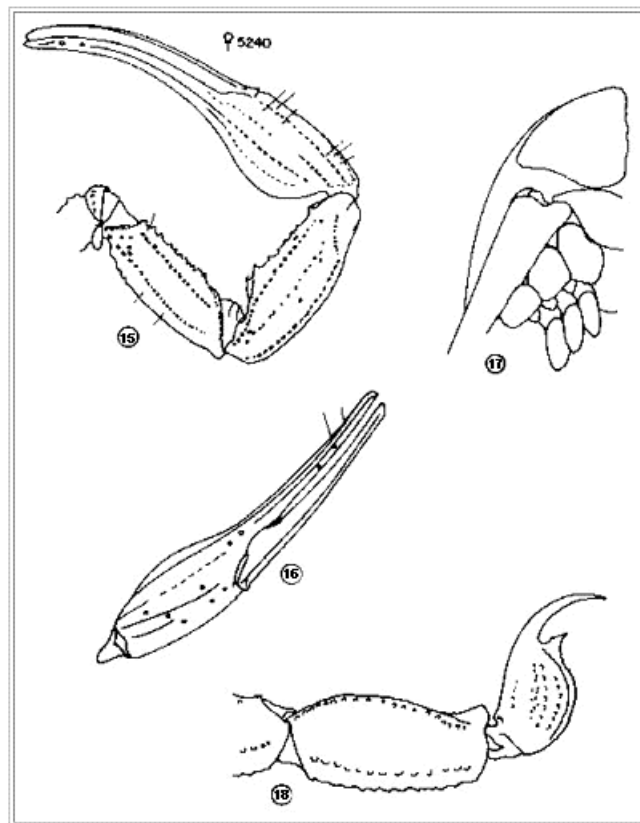


Figure 15. Right pedipalp dorsal view of *Tityus rusemelyae* n. sp. female specimen.

Figure 16. External side of chela of *Tityus rusemelyae* n. sp. female specimen.

Figure 17. Pecten basal lamellae of *Tityus rusemelyae* n. sp. female specimen.

Figure 18. External side of caudal segments V and telson of *Tityus rusemelyae* n. sp. female specimen.

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