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DEVELOPMENTAL STAGES AND REPRODUCTIVE BIOLOGY IN *TITYUS CONFLUENS* BORELLI, 1899 AND *TITYUS OCELOTE* (FRANCKE & STOCKWELL, 1987) (SCORPIONES, BUTHIDAE)

Seiter Michael

Department of Integrative Zoology, Faculty of Life Science, University of Vienna, Austria – seitermichael@hotmail.com

Abstract: The life cycle and postembryonic development of *Tityus confluens* Borelli, 1899 and *Tityus ocelote* (Francke & Stockwell, 1987) are for the first time described. The duration of embryonic development was estimated to range from 86 to 184 (N = 11) days in *T. confluens* and 68 to 72 (N = 4) days in *T. ocelote*. The molts between juvenile instars and adult stage took place at the average ages of 5, 38, 106 and 157 days in *T. confluens* and 4, 60, 62 and 97 days in *T. ocelote*. Compared with other species of the family Buthidae in the same range of size the figures are similar. The morphometric growth factor (Dyar's constant) are higher than those observed for other scorpions of the genus *Tityus* of the same size. Parthenogenesis is confirmed for *T. confluens* based on captive bred females raised in isolation since offspring (N = 156). No males were observed over a period of 3 generations.
Key words: Scorpiones, Buthidae, *Tityus confluens, Tityus ocelote*, life history, parthenogenesis.

Biología reproductiva y estadios del desarrollo de *Tityus confluens* Borelli, 1899 y *Tityus ocelote* (Francke & Stockwell, 1987) (Scorpiones, Buthidae)
Resumen: Se describen por primera vez el ciclo de vida y el desarrollo postembrionario de los escorpiones *Tityus confluens* Borelli, 1899 y *Tityus ocelote* (Francke & Stockwell, 1987). La duración de este último fue de 86-184 días en *T. confluens* (N = 11) y de 68-72 días en *T. ocelote* (N = 4). Las ecdisis sucesivas hasta la adultez ocurrieron a una edad promedio de 5, 38, 106 y 157 días en *T. confluens* y de 4, 60, 62 y 97 días en *T. ocelote*, valores similares a los registrados para otras especies de la familia Buthidae. El coeficiente de crecimiento (Constante de Dyar) de ambas fue mayor que el documentado en otros *Tityus* de talla similar. Se confirmó la ocurrencia de partenogénesis en hembras de *T. confluens* criadas en cautividad desde su nacimiento (N = 156): no se produjeron machos durante tres generaciones consecutivas.
Palabras clave: Scorpiones, Buthidae, *Tityus confluens*, *Tityus ocelote*, ciclo de vida, partenogénesis.

Introduction

Biological observations on the life cycle started early in the history of scorpiology (Polis & Sissom, 1990). However, the developmental stages of most species are not described and detailed data are missing. Some species belonging to the genus *Tityus* C.L. Koch, 1836 are already investigated (e.g. Lourenço & Cloudsley-Thompson, 1998; Lourenço & Cloudsley-Thompson, 2010). Nevertheless, here is a lack of observations on the entire life cycles of many species of this genus.

In general parthenogenesis was reported in different orders of Arachnida: mites (Acari), harvestmen (Opiliones), scorpions (Scorpiones) and true spiders (Araneomorphae). Parthenogenesis has been observed in 12 species of scorpions, belonging to 2 families and 4 genera. In the year 2010 the number of known scorpions reached 1922 species (Dupré, 2010). From this number of scorpion species only 0.57 % reproduce by parthenogenesis. Ten of the known parthenogenetic species belong to the family Buthidae, i.e.: *Centruroides gracilis* (Latreille), *Tityus columbianus* (Thorell), *Tityus metuendus* Pocock, *Tityus serrulatus* Lutz & Mello, *Tityus stigmurus* (Thorell), *Tityus trivittatus* Kraepelin, *Tityus uruguayensis* Borelli, *Ananteris coineaui* Lourenço, *Hottentotta hottentotta* (Fabricius), *Hottentotta caboverdensis* Lourenço & Ythier[1], *Tityus neblina* Lourenço and one member of the family Liochelidae, i.e. *Liocheles australasiae* (Fabricius) (Lourenço, 2008; Lourenço & Cloudsley-Thompson, 2010).

Most of the species are thelytokous parthenogenetic (all-female broods) (Lourenço, 2008). The only noted exception is *T. metuendus*. There arrehnotky parthenogenetic (all-male broods) exist (Lourenço & Cuellar, 1999). The morph of *T. serrulatus* (=*confluenciata*) reproduce by parthenogenesis (Matthiesen, 1962; Lourenço & Cloudsley-Thompson, 1999a). However, this was never documented and reported in captivity for *T. confluens*. For further information on the important topic "parthenogenetic in scorpions" the reader may refer to read Lourenço (2008), who summarizes the present knowledge and gives a list of references.

The aim of this study is to investigate the juvenile instars and reproduction biology of two Neotropical species of the genus *Tityus, i.e.*: *Tityus confluens* Borelli, 1899 and *Tityus ocelote* (Francke & Stockwell, 1987). After intensive biological studies on their reproductive biology the capacity of the species *T. confluens* to reproduce asexually by parthenogenesis is discussed.

Characteristics of *T. confluens*

T. confluens (fig. 1-3) is moderately sized compared to other species of the family Buthidae, adults range from 52 to 53 mm in total length. The general coloration is on the prosoma and mesosomal tergites I–VI almost uniform yellowish or blackish brown. The tergites VII are yellowish with a single median blackish spot, metasomal segments I to III are light yellow- brown, IV and V light brown to brown. The pedipalps are yellowish, with yellowish to brown fingers. The legs are

[1] *H. caboverdensis* was formerly synonymized with *H. hottentotta (*Kovařík, 2007). Nevertheless, *H. caboverdensis* was also reported to reproduce by parthenogenesis (Lourenço *et al*., 2007). However, Lourenço (2008) mentioned it again as a valid species and Ythier (2010) confirmed the validity of the species status.

yellowish at all. The fixed and movable fingers of pedipalps have 17-16 rows of granules. The pectinal tooth count in females is 23-24. A precise description has been published several times in recent history (Borelli, 1899; Maury, 1974; Lourenço, 1980; Bertani *et al*., 2005). Differences in colorations and morphological variation have been observed among the populations distribution in Argentina, Paraguay, Bolivia and Brazil.

Characteristics of *T. ocelote*

T. ocelote (fig. 4-5) is a small species, the total body length is under 40 mm. It has a mottled pattern and the ground coloration is yellowish to reddish- brown. The keels are well constituted and a lot of granules are present. It has 13 rows of granules on the fixed finger of the pedipalp and 11-14 pectin tooths (Francke & Stockwell, 1987). A sexual dimorphismus is visible as in many other buthid scorpions. Males have a longer V metasoma segment and this segment is more swollen and the pedipalp manus is distinct bulbous than in females. Their distribution ranges from the North Atlantic side in Costa Rica to some small populations in the southern part of this country in Central America. They also occur in Panama, where they are only known from northwestern part of the country, from the Bocas del Toro archipelago (Teruel & Cozijn, 2011). Their typical habitat is arboreal. This species is mostly found among dry and living bushes near the underground, as well in medium sized plants, like ferns or palms and in the cup of different tropical plants. They have been never observed higher than 5 meters above the ground (Víquez, 1999).

Material and methods

T. confluens were collected in Corrientes district (near Corrientes city) in Argentina and *T. ocelote* were captive breed species from Puerto Viejo de Sarapiquí, Provinz Heredia in Costa Rica. They have been reared in plastic terraria of different sizes using standard methods. These contained a layer of soil (2 cm deep) and some pieces of bark to hide. Food consisted of crickets (*Acheta domestica*) and cockroaches (*Shelfordella tartara*). They were feed under the same conditions and in the same interval every 7 days. Temperatures ranged from 26 to 29 °C, with humidity between 65-75 %. Individuals were raised under the same conditions and completely isolated after leaving the back of the females (instar II). In *T. confluens* a natural diapause was simulated once a year (temperature 20 °C during the daytime). After each molt the exuvia were removed and stored in 70 % ethanol. All dead specimens were stored. In this way the morphometric growth factors were measured both in dead specimen and exuvia. Specimens were studied, measured and photographed under a NOVEX FL-100 microscope, equipped with a Samsung Digimax V50 digital camera, and a Samsung Lens Adapter SLA-3537.

Digital images were processed using Adobe Photoshop® 8.0 to optimize contrast feature of micrographs. The prepared images were measured with the computer program ImageJ Version 1.43u.

The following three characters are measured: Carapace length, the length of the metasomal segment V, and length of the movable finger (Lourenço, 1979a; Lourenço, 1979b). Nomenclature and measurements follow Stahnke (1970).

The morphometric growth factor for arthropods, as defined by Dyar (1890) and Prizbram & Megusar (1912) is 1.26. The growth factor (Dyar's constant) between every instar was determined from each of these three structures by dividing the dimension at one instar by the dimension of the previous instar. Instar I can be assumed as atypical during the postembryonic development in scorpions and so the morphological differences of instar I and the all following instars are majority. Measurements are given in millimeters [mm]. The average growth factor for each molt was then calculated from all the data. Laboratory reared specimens are now deposited in the author's personal collection (SM)[2]. In *T. confluens* a total amount of 60 specimens were measured and 156 individuals are isolated raised and studied, and in *T. ocelote* a total amount of 66 specimens were studied.

Results

Developmental period of *T. ocelote*

The laboratory raised specimens were mated and four of them gave birth to an average of 9 neonates per female (ranged from 7 to 12 neonates). The duration of embryonic development averaged of 71 days (ranged from 68 to 72 days) (N = 4). The first instar specimens needed an average of 4 days for their first molt (ranged from 3 to 5 days) (N = 52). Juveniles began to disperse from their mother's back at the age of 7 to 9 days. The following molts took place at different ages: the second molt (reached instar III) took an average of 60 days (ranged from 44 to 72 days) (N = 37); the third molt (reached instar IV) took an average of 62 days (ranged from 42 to 105 days) (N = 18); the fourth molt (reached instar V, both females and males got adult at this stage) an average of 97 days (ranged from 71 to 119 days) (N = 9). This means an average of 223 days postembryonic development (Table I). So the total development to reach adulthood had an average of 294 days.

The growth parameters of *T. ocelote* based on measuring of both dead species and exuvias are shown in Table II.

Developmental period of *T. confluens*

The laboratory reared specimens gave birth to an average of 6 neonates per female (ranged from 2 to 9). The duration of embryonic development averaged of 124 days (ranged from 86 to 243 days) (N = 8). The first instar specimens needed an average of 5 days for their first molt (ranged from 4 to 5 days) (N = 25). Juveniles began to disperse from their mother's back at the age of 9 to 11 days. The following molts took place at different ages: the second molt (reached instar III) took an average of 38 days (ranged from 20 to 73 days) (N = 25); the third molt (reached instar IV) took an average of 106 days (ranged from 43 to 215 days) (N = 24); the fourth molt (reached instar V, adult) an average of 157 days (ranged from 117 to 199 days) (N = 24).

This results are an average of 306 days postembryonic development (tab. I). The total development time had an average of 430 days. The total life span of this scorpion is at least 1425 days, but cannot be calculated up to the end in total now, because some of them are still alive during the

[2] An abbreviation for the repositories of the specimens mentioned herein is SM: Seiter Michael's personal collection, Pottendorf, Austria.

Fig. 1: *Tityus confluens* (adult female).

Fig. 2: *Tityus confluens* with 1st instar brood.

Fig 3: *Tityus confluens*, adult. **A**, female dorsal view; **B**, female ventral view. Scale bare = 10 mm.

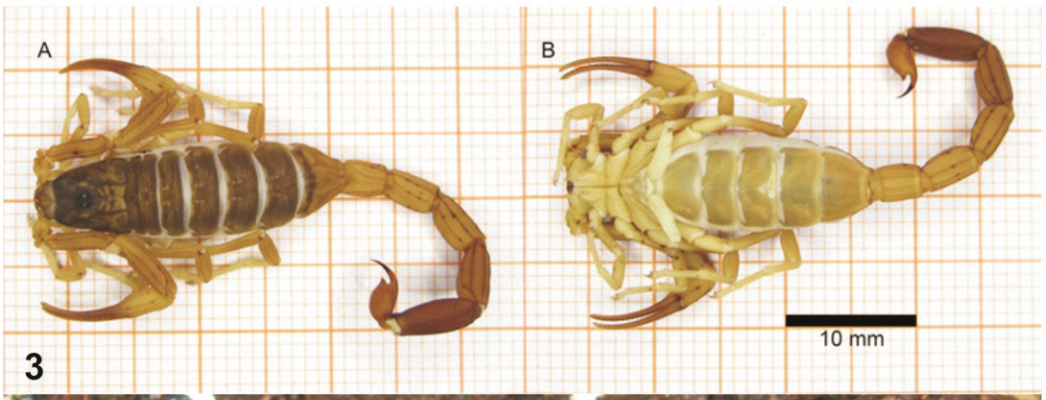

Fig. 4: *Tityus ocelote* male (left) and female with 2nd instar brood (right). Note: male specimen has distinct swollen pedipalps then female (sexual dimorphismus).

Fig 5: *Tityus ocelete*, adult. **A**, female dorsal view; **B**, female ventral view; **C**, male dorsal view; **D**, male ventral view. Scale bare = 10 mm.

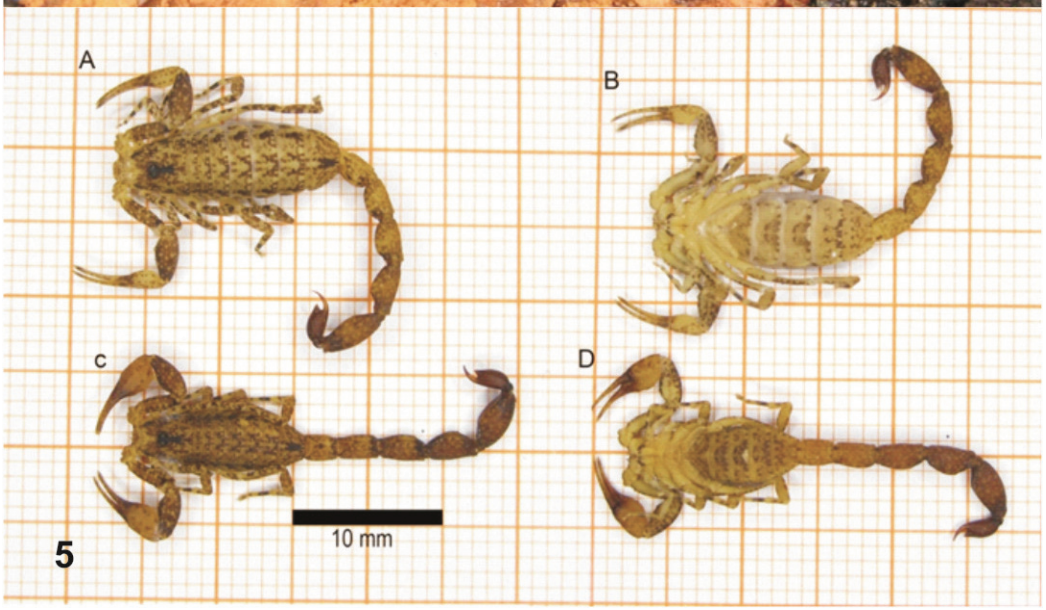

Table I: Average from one instar to the previous instar (in days) for *Tityus ocelote* and *Tityus confluens*. ∑ = sum of days.

	Instar I-II	Instar II-III	Instar III-IV	Instar IV-V	∑
Tityus ocelote	4	60	62	97	223
Tityus confluens	5	38	106	157	306

Table II: Average morphometric values for juvenile and adult instars of *Tityus ocelote*. Car.L. = carapace length. M.S.V.L. = metasomal segment V length. M.F.L. = moveable finger length. ϭ = standard deviation. N = number of specimens.

	Instar II (N = 37)	Instar III (N = 18)	Growth factor (Instar III)	Instar IV (N = 9)	Growth factor (Instar IV)	Adult male (N = 3)	Growth factor (Instar V male)	Adult female (N = 6)	Growth factor (Instar V female)	Mean of growth factors
Car.L.	1.47 mm	1.87 mm	1.27	2.27 mm	1.21	3.10 mm	1.36	3.34 mm	1.47	1.33
ϭ	± 0.11 mm	± 0.08 mm		± 0.17 mm		± 0.19 mm		± 0.91 mm		
M.S.V.L.	1.49 mm	1.97 mm	1.32	2.53 mm	1.28	3.82 mm	1.50	3.72 mm	1.47	1.39
ϭ	± 0.09 mm	± 0.86 mm		± 0.16 mm		± 0.13 mm		± 0.12 mm		
M.F.L.	1.56 mm	1.97 mm	1.26	2.65 mm	1.34	3.34 mm	1.26	3.60 mm	1.35	1.30
ϭ	± 0.06 mm	± 0.09 mm		± 0.13 mm		± 0.17 mm		± 0.23 mm		

Table III: Average morphometric values for juvenile and adult instars of females of *Tityus confluens*. Car.L. = carapace length. M.S.V.L. = metasomal segment V length. M.F.L. = moveable finger length. ϭ = standard deviation. N = number of specimens.

	Instar II (N = 25)	Instar III (N = 25)	Growth factor (Instar III)	Instar IV (N = 24)	Growth factor (Instar IV)	Adult female (N = 24)	Growth factor (Instar V female)	Mean of growth factors
Car.L.	2.38 mm	3.06 mm	1.28	3.93 mm	1.28	5.27 mm	1.34	1.30
ϭ	± 0.23 mm	± 0.22 mm		± 0.28 mm		± 0.17 mm		
M.S.V.L.	2.33 mm	3.23 mm	1.38	4.30 mm	1.33	6.17 mm	1.43	1.38
ϭ	± 0.21 mm	± 0.26 mm		± 0.38 mm		± 0.44 mm		
M.F.L.	2.92 mm	3.85 mm	1.32	4.98 mm	1.29	6.77 mm	1.36	1.32
ϭ	± 0.26 mm	± 0.27 mm		± 0.38 mm		± 0.50 mm		

preparation of this paper. The wild caught adult specimens gave three times birth to a diminishing number of offspring.

The growth parameters of *T. confluens* based on measuring of both dead species and exuvias are shown in Table III.

The female scorpions were reared isolated from each other and gave birth without being inseminated by a male. Over a period from three years not any male was born from these females. It can be concluded that the Corrientes population in Argentinia of *T. confluens* is an obligate thelytokous (all-female broods) parthenospecies.

T. confluens have a high degree of tolerance vis-à-vis specimen of the same species. Several species in different instar levels (*T. confluens* and *T. trivittatus*) lived together and molted without any cannibalism. Cannibalism appears to be uncommon in parthenogenetic species, and was never observed among species of *T. serrulatus, T. stigmurus, T. trivittatus* and *T. confluens* in the laboratory, even when several individuals of different species were cultivated together in the same tank (numbers of individuals ranges from 10 to 25). Some food sharing between mother and the newborn offspring can be documented too.

Discussion

Few studies directly measure and compare life history and traits of scorpions (e.g. Francke, 1979; Francke, 1981; Polis & Sissom, 1990; Lourenço & Cloudsley-Thompson, 1999b). While several studies have explored embryonic and postembryonic development, as well as the growth factor (Dyar's constant) (e.g. Francke, 1984; Lourenço *et al*., 2008). The number of offspring is in this previous studies are much larger than in the present studies of *T. ocelote* and *T. confluens* (cf. (Rouaud *et al*., 2000): p.89, tab. I; cf. (Lourenço, 2002): p.75, tab. I). Studies which have focused on the growth factor across neotropical buthids in the same range of size have a lower factor than *T. ocelote* (tab. I, tab. II) (Lourenço, 1979b; Lourenço, 2002). The morphometric value in males and females of *T. ocelote* goes apart up to instar IV. Here males get a longer and more bulbous V metasomal segment than females, instead the females get a longer pedipalp manus (moveable finger) (fig. 4-5). These illustrate the widespread sexual dimorphismus in scorpions. The typical shorter, but a lot more bulbous and stronger moveable finger, and the longer V metasomal segment in males. A few species of the family Buthidae are able to store sperms in the body (glandular tissue in the female genital tract) (Kovoor *et al*., 1987) and produce several broods from a single insemination. This capacity was observed in *T. ocelote* too, even though the number of offspring decreased to 1 to 3 neonates for the 2nd brood. All specimens died before they were able to give birth a 3rd time.

Parthenogenetic broods are smaller than sexual broods (Lourenço, 2008; Lourenço & Cloudsley-Thompson, 2010). So the smaller number of offspring in *T. confluens* (ranged from 2 to 9 neonates) seems to be normal. The results obtained for morphometric growth factors of the different instars in *T. confluens* are very similar to the standard theoretical values, but bigger than those obtained for most other Buthidae (fig. 6) (Lourenço, 2002). Consequently the duration of life span with at least 1425 days (some adult females are still alive during the process of final corrections of this manuscript) are similar to many other buthids in the same body size range (cf. (Lourenço, 2002): p.75, tab. I).

T. confluens reached adulthood at instar V (tab. III). This is common in most of known scorpions, but there are also some larger species which need one or two extra molts. One of the largest species is *Tityus insignis* (Pocock, 1889). Here the average from one instar to the previous is much bigger than in the observed species. A major impact is the habitat and environment. It depends if the species distribution is only in lowland rainforest or also in dry land and

savannicolus landscape. *Tityus obscurus* (Gervais, 1843)[3] can reach instar VII (both males and females) and in smaller buthids like *Tityus bastosi* Lourenço, 1984 in instar V (like *T. ocelote* and *T. confluens*) (tab. I). Consequently, these results suggest it is unusual for Neotropical species to reach adulthood in a instar level higher then V. These dates, including the environment of their capturing locality shows, that a diapause impacts the growth factors and gestation time. For a comparative experiment, however, some females should be kept isolated all along the year in a warm room, and in the normal room (control group) with temperature averaged ≤ 20°C for some weeks (as done with the studied specimens of *T. confluens*). *Tityus neblina* Lourenço, 2008 shows similar data (Lourenço & Cloudsley-Thompson, 2010), depending on their environment.

All specimens of *T. confluens* gave birth without being inseminated, and were exclusively composed of females. So this population seems to be an thelytokous (all-female broods) parthenospecies and not a deutherotokous (male and female brood) species, like *T. neblina* (Lourenço & Cloudsley-Thompson, 2010) or *T. columbianus* (Lourenço *et al*., 1996). Over a period of three years 156 specimens are reared and without a single male, contrary to *T. trivittatus* from the same locality in Corrientes, Argentina. Here several males were born and produced spermatophores (fig 7-8). In *T. trivittatus* a sex ratio 1:118 (Maury, 1997) and 1:145 (Maury, 1970) (males:females) were already investigated. It was first suggested that this is a new parthenospecies and later in history specimens from Córdoba, Argentina was later confirmed to reproduce parthenogenetically (Toscano-Gadea, 2004). They also reach adulthood in instar V and had similar embryonic development periods then *T. confluens*. The postembryonic development was estimated from 59 to 73 days and 9 to 11 offspring. Also this data are similar to *T. confluens*.

T. confluens have a high degree of tolerance vis-à-vis specimen of the same species. Tolerance in the genus *Tityus* is already documented. This may be an effect of the absence of males in this special population. Some food sharing between mother and the newborn offspring is also well known from different Arachnid species, e.g. the Amblypygi (whip spiders) species *Damon diadema* (Simon, 1876) (Phrynoidea: Phrynichidae) shows a lot of tolerance and interaction between adults and juvenile offspring (Rayor & Taylor, 2006; Walsh & Rayor, 2008).

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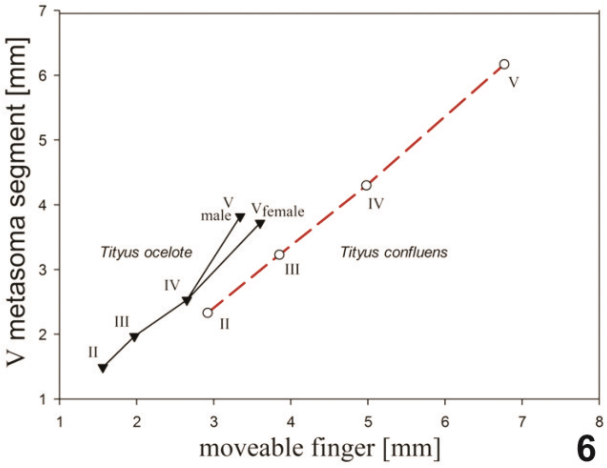

Fig. 6: Morphometric value in juvenile and adult females of *Tityus confluens* (○) (N = 24), females of *Tityus ocelote* (▼) (N = 6) and males of *Tityus ocelote* (▼) (N = 3). **Fig 7:** *Tityus trivittatus*, adult male. Scale bare = 10 mm. **Fig 8:** *Tityus trivittatus*, adult, right pedipalp. A, female; B, male. Note: male specimen has distinct swollen pedipalps then female (sexual dimorphismus). Scale bare = 5 mm

[3] *T. obscurus* was synonymized with *Tityus paraensis* and *Tityus cambridgei* (Lourenço & Leguin, 2008)

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