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URTICATING SETAE IN URUGUAYAN TARANTULAS: ACTIVE OR PASSIVE DEFENSE?

David Ortíz-Villatoro, Matías González-Barboza, Maite Hilario, Esteban Russi, Carlos Perafán, Fernando Pérez-Miles*

Universidad de la República, Facultad de Ciencias, Sección Entomología, Iguá 4225, 11400 Montevideo, Uruguay.

*Corresponding author: myga@fcien.edu.uy

ABSTRACT

Most of the New World tarantulas feature specialized setae as a defense mechanism. Two mechanisms of defense have been proposed for the urticating setae (US): active defense against potential predators, and passive defense against other arthropods by incorporating the US to the molting mat and egg sacs. Uruguayan tarantulas present three different US morphological types named: types I, III and IV. It has been proposed that type I is used in passive defense whereas type III serves its purpose in active defense. There are drastic differences of biological characteristics between adult females and males. Females live most of their entire life inside their burrows, while males wander when they reach adulthood, looking for females during the reproductive season. Considering these differences, diverse defense strategies should be expected. To assess the possible role of US in active/ passive defense strategies we have counted the number of US in the abdomen of individuals of four species while making comparisons between sexes. Significant differences were found between males and females of all sampled species, with females showing a predominance of types I (except subtype Ic) or IV setae over other types or subtypes, suggesting these type of US takes part in passive defense.

Key words: Theraphosidae, defense, urticating-setae, Uruguay

RESUMEN

Setas urticantes en tarantulas de Uruguay: ¿Defensa activa o pasiva? La mayoría de las tarántulas del nuevo mundo presentan setas especializadas como defensa. Dos mecanismos de defensa han sido propuestos para las setas urticantes (US): defensa activa contra potenciales depredadores y defensa pasiva contra otros artrópodos mediante la incorporación de US a las telas de mudas u ootecas. Las tarántulas uruguayas presentan tres tipos morfológicos de US llamados: I, III y IV. Se ha propuesto que el tipo I se utiliza principalmente en defensa pasiva mientras que el tipo III en defensa activa. Hembras y machos adultos presentan diferencias drásticas en su biología. Las hembras permanecen la mayor parte de su vida en sus cuevas mientras que los machos, una vez que se hacen adultos, salen y buscan activamente hembras durante la época reproductiva. Considerando estas diferencias se presumen diferentes estrategias defensivas entre los sexos. Para conocer el uso de los diferentes tipos de US en defensa activa o pasiva se estudiaron las dotaciones de US en individuos de cuatro especies de tarántulas de Uruguay, comparando machos y hembras. Se encontraron diferencias sexuales en todas las especies, las hembras muestran predominancia de US de los tipos I (excepto subtipo Ic) o IV sobre otros tipos y subtipos de US, lo que sugiere su participación en defensa pasiva.

Palabras clave: Theraphosidae, defensa, setas urticantes, Uruguay

INTRODUCTION

Theraphosid tarantulas are large sized spiders widely distributed in tropical and subtropical regions of the world. The family comprises about 1000 species, most of them found in the New World (WSC, 2020). Defensive mechanisms of the tarantulas when disturbed include behaviors such as palp, and forelegs raise, as well as chellicerae opened display. Old World tarantulas seem to be more aggressive and venomous than their New World counterparts and frequently perform defensive bites (Ezendam, 2007; Blatchford *et al.*, 2011; Fuchs *et al.*, 2014; Höfler, 1996 and pers. observ.). Noteworthy, New World tarantulas show an exclusive and conspicuous defensive mechanism: the use of urticating setae (Cooke *et al.*, 1972; Marshall & Uetz, 1990; Bertani & Marques, 1996; Pérez-Miles, 2002; Bertani *et al.*, 2003; Bertani & Guadanucci, 2013; Perafán *et al.*, 2016; Kaderka *et al*., 2019). These specialized setae are present in more than 90% of the New World tarantula species (Bertani & Guadanucci, 2013), corresponding to Aviculariinae *sensu lato* and Theraphosinae subfamilies, both endemic to the continent.

Urticating setae are located on the dorsal and/or lateral abdomen (Aviculariinae and Theraphosinae) or on the prolateral palpal femur (Type V) in *Ephebopus* Simon, 1892 (Psalmopoeinae). There are seven types of urticating setae (US) known to date with different morphology and release mechanisms. Aviculariinae present type II which is mostly transferred by direct contact (Bertani & Marques, 1996) with the exception of *Avicularia versicolor* (Linnaeus, 1758) which throws US by friction of the hindlegs against the abdomen (Bertani *et al*., 2003) as it occurs in Theraphosinae (Cooke *et al*., 1972). Urticating setae types I, III and IV are widespread in Theraphosinae (Pérez-Miles *et al*., 1996; Bertani & Guadanucci, 2013; Pérez-Miles & Perafán, 2015; Turner *et al*., 2018; Lüddecke *et al*., 2018; Kaderka *et al*., 2019; Foley *et al.,* 2019). Type VI is only present in *Hemirrhagus* Simon, 1903 and type VII only in *Kankuamo* Perafán *et al.* 2016; these Theraphosinae genera are endemic from Mexico and Colombia, respectively (Pérez-Miles, 1998; Pérez-Miles & Locht, 2003; Mendoza, 2014; Mendoza & Francke, 2018; Perafán *et al*., 2016). Recently, Kaderka *et al*. (2019) according with the results of Pérez-Miles (2002), reinterpreted type III US present in taxa with type I as a subtype of type I, called Ic.

Two mechanisms of defense were proposed for the US: active defense against potential predators (Torres, 1921; Bristowe, 1941; Gertsch, 1949; Bücherl, 1951; Cooke *et al*., 1972; Pérez-Miles & Prandi,1991; Bertani & Guadanucci, 2013), and passive defense against ants and phorid larvae (Marshall & Uetz, 1990; Bertani & Guadanucci, 2013). Passive defense consists in the incorporation of US to the silk mat where tarantulas lay on for molting and externally in egg sacs assembly (Marshall & Uetz, 1990; Pérez-Miles & Costa, 1994), depositing setae at a short range. Conversely, active defense would consist in US released farther to reach the target.

Bertani and Guadanucci (2013) have proposed that type III (and now also type Ic) setae are mainly involved in active defense whereas type I (now excepting Ic) are related with passive defense. The use of type IV US is enigmatic, but it is our hypothesis, based on personal observations, that these are mainly involved in passive defense.

Considering the differences in the lifestyle of adult males that actively walk searching for females during the reproductive season, in contrast with females that live almost their entire life inside their burrows (Costa & Pérez-Miles, 2002; Álvarez *et al*., 2016), different strategies of active or passive defense are expected to occur. Consequently, the proportion of setae involved in active and passive defense should vary between males and females. Following this reasoning, we expected that urticating setae used in active defense must be proportionally more abundant in males than in females; therefore, urticating setae used in passive defense would be more abundant in females. Our research question was how the proportion of setae used in active/passive defense would vary between males and females, observed in four Uruguayan species of Theraphosid spiders' representatives of groups with

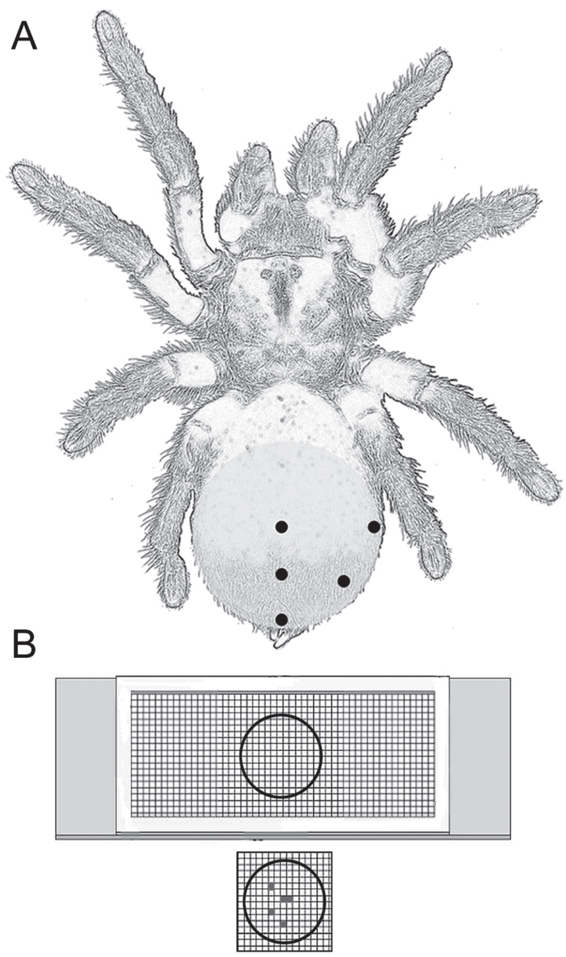

Fig. 1. Schematic representation of US sampling. A. Five fixed points on the abdominal patch where US samples were taken. B. Gridded rafter (grids = 1mm^2) and example of five squares where the US sampled were counted.

Table 1. Collection sites.

Species	Individuals	Location
Acanthoscurria suina	2 females, 3 males	Canelones, Ruta Interbalnearia, Km. 22-25 (34º48'S, 55º53'W)
	2 females	Montevideo, Cerro (35º54'S, 56º15'W)
	1 male	Montevideo, Melilla (34º47'S, 56º18'W)
	1 female	Montevideo, Mercado Modelo (34º54'S, 56º05'W)
	1 male	Montevideo, Paso de la Arena (34º50'S, 56º16'W)
Eupalaestrus weijenberghi	1 female	Canelones, Ruta Interbalnearia Km. 25.500 (34º48'S, 55º53'W)
	1 female	Colonia, Real de San Carlos (34º26'S, 57º53'W)
	2 females	Montevideo, Parque Lecocq (34º47'S, 56º20'W)
	5 males	Río Negro, Tres Arboles (32º26'S, 56º42'W)
	1 female	Rivera, Ruta 27 Km 86 (32º33'S, 56º04'W)
Homoeomma uruguayense	5 females	Maldonado, Sierra de las Animas (34º42'S, 55º19'W)
	5 males	Canelones, Pando (34º43'S 55º56'W)
Plesiopelma longisternale	1 male	Canelones, Piedras de Afilar (34º44'S, 55º35'W)
	3 males	Canelones, Salinas Norte (34º44'S, 55º52'W)
	5 females, 1 male	Lavalleja, Aguas Blancas (34º32'S, 55º24'W)

types I (including subtype Ic) and III+IV urticating setae.

MATERIAL AND METHODS

Four theraphosid species from Uruguay were examined: *Acanthoscurria cordubensis* Thorell, 1894 (previously known as *A. suina*), *Eupalaestrus weijenberghi* (Thorell, 1894), *Homoeomma uruguayense* (Mello-Leitão, 1946) and *Plesiopelma longisternale* (Schiapelli & Gerschman, 1942). Five females and five males of each species were used as samples, all collected in Uruguay; collection sites are provided in Table 1 and deposited in the Arachnological Collection of the Facultad de Ciencias, Uruguay. These species were selected given their different combinations of urticating setae types: I and Ic (ex III) in *A. cordubensis* and *E. weijenberghi*; III and IV in *H. uruguayense* and *P. longisternale*. Once examined at the stereomicroscope, only individuals with a full urticating setae patch were used. In this paper we refer as type I US all variants of this type except for the subtype Ic (see Kaderka *et al.*, 2019).

Three urticating setae (US) samples were removed from preserved specimens with precision forceps from each of five fixed points on the abdominal urticating patch (Fig. 1a). Each sample was placed on a gridded rafter (grids = 1mm^2) with a drop of ethanol. Once the urticating setae types were determined, they were counted in five squares of each sample (Fig. 1b). The numbers of setae were registered with the help of a manual counter. Considering Bertani & Guadanucci (2013) proposed that type III (and Ic) US are related with active defense while types I and IV with passive defense we used a ratio that we called «activity index (AI)»; it was calculated as the quotient between the number of US type III or Ic+1/ number of US type I or IV +1 (we added 1 to the numerator and denominator to avoid quotient with denominator 0). Comparisons between the amount of US of each type and AI were made separately for each species, between males and females and between setae types within each species and sex. Statistical analysis was done using PAST package v217b (Hammer *et al.*, 2013). The normality of the variables was analyzed with Shapiro-Wilk Test and the homogeneity of the variances with Levene's Test. Comparisons for each pair were made by Student's t test, and when not normal with Mann-Withney U; the limit of significance for all tests was p= 0.05.

RESULTS

In all species studied, the type III or Ic US were more numerous in males than in females (Fig. 2, Table

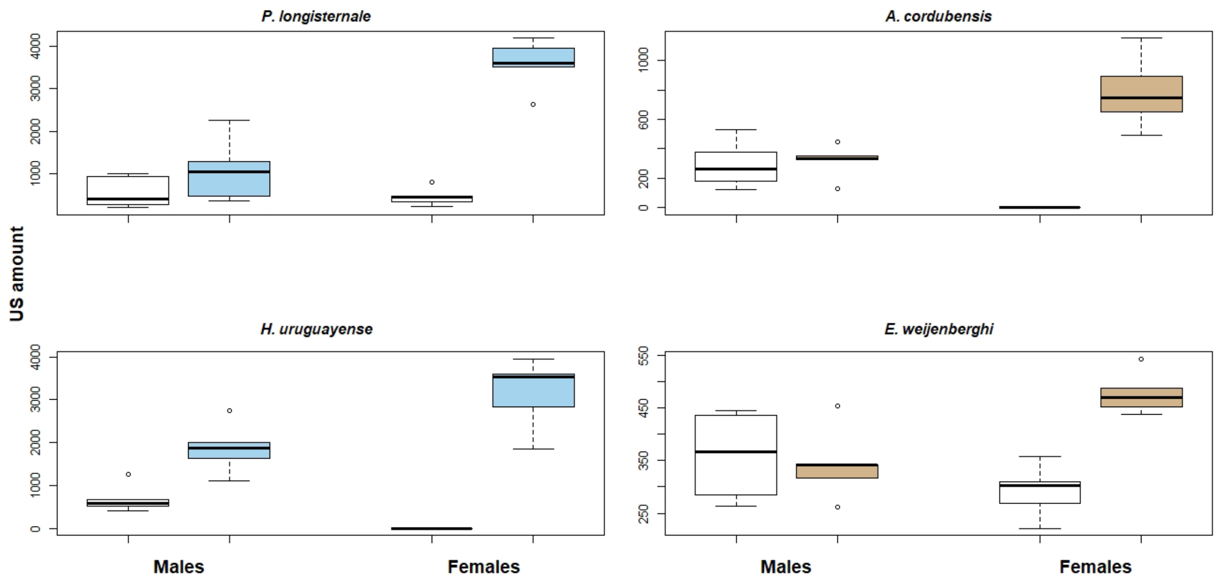

Fig. 2. Boxplots showing the amount of urticating setae (US) of males and females of the four species studied. In white types Ic or III US, in blue type IV US and in brown type I (except Ic) US.»

Table 2. Urticating setae type counts for the species studied and comparison between males and females, (mean±standard deviation), t= Student's t test, P = probability.

Species	Males	Females	t=	P=
		TYPES III or Ic SETAE		
A. cordubensis	295.8±72.28	3±1.76	4.05	0.004
E. weijenberghi	358.8±37.33	292.0±22.63	1.53	0.16
H. uruguayense	690.4±149.5	0	—	—
P. longisternale	585.2±170.58	455.8±95.06	0.56	0.59
		TYPE I SETAE		
A. cordubensis	319.6±51.44	786.4±112.06	3.78	0.0005
E. weijenberghi	342.8±31.44	478.2±18.25	3.72	0.005
		TYPE IV SETAE		
H. uruguayense	1874.4±266.17	3154.8±371.11	2.80	0.02
P. longisternale	1080.6±344.47	3584.6±266.86	5.75	0.0004

2). This difference was extreme in *H. uruguayense* which females completely lack type III setae. Differences between sexes were also high and significant in *A. cordubensis* and slight and not significant in *E. weijenberghi* and *P. longisternale*. Conversely, subtype Ic and type IV setae were significantly more numerous in females than in males (Fig.2, Table 2).

When comparing US types amounts within species and sexes, all of them showed slight to strong predominance of types I or IV setae over type III or Ic. However, in males, only *H. uruguayense* showed

Table 3. Activity index (proportion subtype Ic/other subtypes I or type III/IV urticating setae) and comparison between males and females (P= probability).

ACTIVITY INDEX

Species	Males	Females
A. cordubensis	1.34±1.53	0.004±0.01
E. weijenberghi	1.10±0.41	0.60±0.10
H. uruguayense	0.38±0.16	————
P. longisternale	078±0.82	014±0.09

significant differences (t=4.39, P= 0.01). All females showed a strong and significant predominance of types I and IV over type III or Ic (*A. cordubensis* t=6.94, P=0.002; *E. weijenberghi* t=10.42, P=0.004; *P. longisternale* t=8.78, P= 0.0009; in *H. uruguayense* females lack type III setae).

When comparing the activity index, all species showed significant differences between males and females with higher values in males than in females (Table 3).

DISCUSSION

Two defensive strategies have been proposed for tarantula's urticating setae: the active defense against potentially dangerous vertebrates, and the passive defense in which US are incorporated to the eggsac or the shed mat to protect against phorid larvae and probably ants as well (Torres, 1921; Bristowe, 1941; Gertsch, 1949; Bücherl, 1951; Cooke *et al*., 1972; Marshall & Uetz, 1990; Pérez-Miles & Prandi, 1991; Bertani & Guadanucci, 2013). The US does not occur in small spiderlings up to the fourth or fifth instar (Galiano, 1969, 1973). At these stages small spiders do not need releasing of US as a defensive strategy as they live inside the cocoon, and later in their mother's burrow; also this defensive strategy would be inefficient due to the small size and small amount of US (Kaderka *et al*., 2019). Types I and IV urticating setae occur earlier than type III or Ic during development (Pérez-Miles, 2002). Juveniles before dispersion and adult females have a similar lifestyle, remaining in their burrows most part of the time (Costa & Pérez-Miles, 2002; Álvarez *et al*., 2016). Furthermore, adult males leave the burrow and wander around searching for females (Costa & Pérez-Miles, 2002), increasing their exposure to potential enemies and predators. Bertani & Guadanucci (2013) suggested that type I US have a major involvement in passive defense than type III (and Ic) but nothing is known about the participation of type IV in active or passive defense. Considering that types I and IV are present in juveniles, they seem to be adaptive for the defense inside the burrow (passive defense), strategy expected to be also useful in adult females, since they remain most of the time in their burrows. However, we have not direct evidence of the incorporation of type IV urticating setae on eggsacs or shedding mats.

The above facts suggest that types I and probably IV are both more presumably involved in passive defense than type III. Our results clearly showed that adult females of the four species studied have a higher proportion of type I or type IV setae than type Ic or III and males have an inverse proportion. An extreme difference was confirmed in *H. uruguayense* in which adult females completely lack type III presenting only type IV US; in this species type III setae are lost during development (Pérez-Miles, 2002). A similar difference was found in adult females of *Bumba cabocla* (Pérez-Miles, 2000) presenting only type IV US (Pérez-Miles, 2000). Likewise, Bertani (1997) indicated that adult females of some Theraphosinae species only present type I setae, and completely lack type III (now Ic). All these facts support our hypothesis. However, the counting of setae showed that females of some species also present a significant amount of type III or Ic US and males have almost an equal amount of type III or Ic and type I or IV US. This result could suggest that all types of US have the potential to be used in both active and passive defense, in accordance with Bertani & Guadanucci (2013) at least for types I and III or Ic. At this point, a question that remains open and that should be explored in future research is the aerodynamics of these setae and determine how far they can be thrown by the spiders. The presence of type IV urticating setae on egg-sacs or shedding mats must be studied to obtain additional support for our hypothesis. In this sense, *H. uruguayense* would be a good candidate for this study considering the presence of setules on the axis of their urticating setae which was interpreted as a mechanism to improve adhesion (Perafán & Pérez-Miles, 2010).

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