2019. Journal of Arachnology 47:280–283

## SHORT COMMUNICATION

# Shape of male palpal hook affects female sexual cannibalism in *Leucauge mariana* (Araneae: Tetragnathidae)

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**Abstract.** In the Colombian orb-web spider *Leucauge mariana* (Taczanowski, 1881) (Tetragnathidae), both sexes contribute to mating plug formation. Males of this species show a conductor hook that could be involved in providing stability to the mating pair and used for mating plug removal. The aim of the study was to assess the relationship between the shape of the conductor hook in *L. mariana* and mating duration, the amount of sperm stored, and the occurrence of sexual cannibalism. We used geometric morphometric tools for studying the conductor hook shape. The width of the base of the conductor hook was negatively correlated with the probability of sexual cannibalism by females (PC2 = 0.017), but we did not find a significant relation with mating duration and the amount of sperm transferred. Female choice on male hook shape, or on the stability provided by the hook during the mating position, could be occurring in this species.

**Keywords:** Genital coupling, geometric morphometrics, genital trait, female sexual response

Different sexual selection mechanisms acting on several taxa including arachnids contribute to the rapid divergence of shapes and forms, particularly in sexual traits (Eberhard 1985, 1996; Arnqvist 1998). *Leucauge* White, 1841 is a genus of tetragnathid orb web spiders distributed worldwide, with an astounding biodiversity in tropic and temperate zones (World Spider Catalog 2018). One of the most striking characteristics of this genus is its variation in male genital traits (Álvarez-Padilla & Hormiga 2011; Barrantes et al. 2013). The form and function of male genital traits may be driven by different mechanisms of sexual selection such as sperm competition, sexually antagonistic coevolution and/or cryptic female choice (Parker 1970; Eberhard 1985, 1996; Johnstone 1995; Arnqvist 1998; Brennan & Prum 2015).

*Leucauge mariana* (Taczanowski, 1881) females produce mating plugs that totally or partially cover their epigynum (Eberhard & Huber 1998; Aisenberg et al. 2015a). Mating plugs in this species are substances provided by both sexes (Eberhard & Huber 1998). Female participation in plug formation has been suggested as evidence for cryptic female choice in this species due to the fact that females evaluate male sexual performance during courtship and mating, and supply—or not—a substance to the mating plug (Aisenberg & Eberhard 2009). This substance is necessary to form a plug that reduces the probability of future matings, favoring the deposited sperm over sperm of potential male suitors (Eberhard & Huber 1998; Aisenberg & Eberhard 2009). Males of this species possess a chitinized structure located beside the conductor process, the conductor hook (Fig. 1; Méndez & Eberhard (2014)). Its function has been related to plug removal, sperm transfer and it could be involved in stimulation to females during mating (Eberhard & Huber 1998; Méndez & Eberhard 2014).

In a population of *L. mariana* from Colombia (CO), females showed remarkable differences in sexual behavior compared with a population previously studied in Costa Rica (CR) (Aisenberg & Eberhard 2009; Hernández et al. 2018). *L. mariana* females from CO tended to be more aggressive at the end of the mating and on occasions cannibalized males, a fact which was related to male performance during mating (Hernández et al. 2018). Previous studies have shown that males' structures such as the conductor hook can play an important role to stabilize the couple during mating (Méndez & Eberhard 2014; Aisenberg et al. 2015a). In this study we tested if the male conductor hook shape in *L. mariana* from CO affects mating duration, sperm transfer, and female cannibalistic attempts. We predicted that wider male conductor hooks that provide more stability to the genital coupling would determine longer matings, with higher sperm counts and lower frequencies of cannibalistic attempts.

We collected 23 males and 23 sub-adult females of *L. mariana* between June and August 2015 in San Francisco, Pueblo Viejo, Cundinamarca Province, Colombia (4°56′30.8"N 74°16′45.9"W, elevation 1700 m). We carried out 20 male-female pairings, each one inside a terrarium measuring 25 cm long x 10 cm wide x 15 cm high, furnished with small wooden sticks to provide support for the web. The temperature during the trials averaged 23.4°C (± 0.2) and relative humidity was 53%. We used males 5 days after their collection from the wild. Trials started when we placed a male on the web at the opposite corner of the terrarium regarding the female. A trial finished when the male or the female remained motionless for 30 min, after 30 min of courtship but without mating, or after mating. All pairs mated on their first exposition. We considered as cannibalistic attempt when the female attacked the male resulting in male injury and/or cannibalization. We deposited voucher specimens at the Museo de Historia Natural de la Universidad de los Andes, Bogotá, Colombia.

We determined the shape of the conductor hook tip taking into account a previous study on *L. mariana* from CR (Méndez & Eberhard 2014). For that purpose, we took photographs of 20 right palpal organs oriented in lateral view and with the same inclination, incidence of light and magnification. We placed each male's hook in the same position using sand and alcohol to stabilize the structure. For each palpal hook, we took three pictures with a Nikon® Digital Sight DS Fi1 camera adapted to a stereomicroscope UNITRON Z850. We obtained hook shapes through standard geometric-morphometric methods (Adams et al. 2004). All morphometric measurements were performed on the same day. We placed all the landmarks (three) considering the homology of the structures; the first one corresponded to the tip of the hook, the second and third were located on the base of the hook. This allowed us to place all the semi-landmarks (seven) to follow the shape of the structure. We used tpsDig2 version 2.19 (Rohlf 2015) to place landmarks and semi-

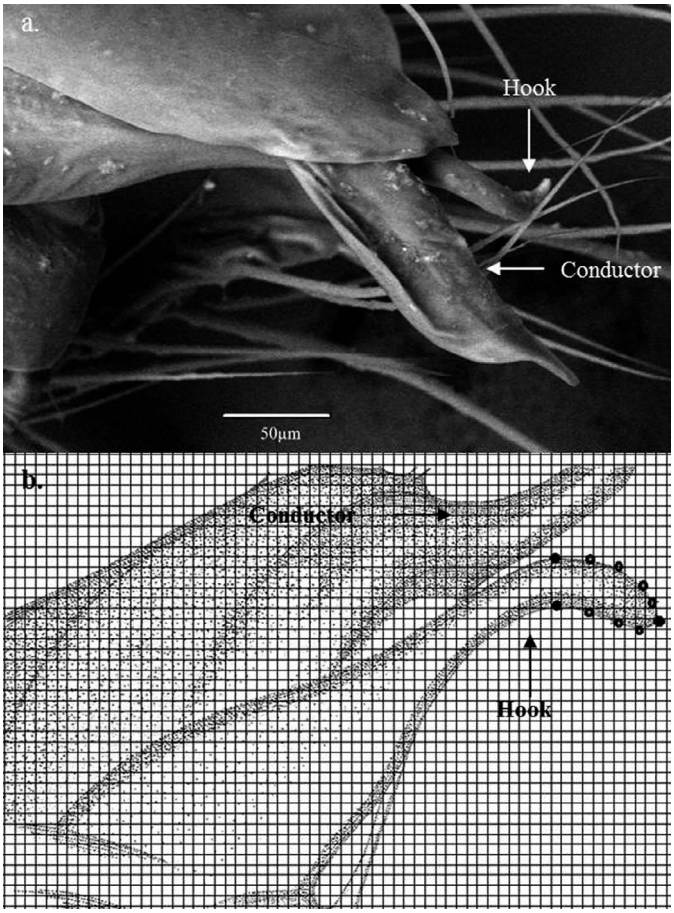


Figure 1.—a. SEM image of *L. mariana* male palp showing a close up view of conductor and hook. b. Illustration showing the position of landmarks (black points) and semi-landmarks (empty points).

landmarks. We placed semi-landmarks because it was not possible to find biologically constant landmarks along the hook. Therefore, we used a grid with the lines consistently separated by 0.01 mm (Fig. 1b). Finally, we used tpsRelw to obtain the uniform and non-uniform shape components, weight matrix, and the centroid size (square root of the sum of squared distances of all the landmarks of an object from their centroid) as a representation of the size component (Rohlf & Slice 1990; Swiderski 2003; Zelditch et al. 2004; Rohlf 2010). The carapace width was measured as a reference for male body size in spiders (Eberhard et al. 1998), and we quantified the amount of the sperm stored in female spermatheca following the modified protocol of Albo et al. (2013).

To determine if hook shape influences mating duration, cannibalism attempts and sperm counts, we ran linear models using lm or glm functions. We visually inspected model assumptions and the appropriate error distribution was chosen accordingly, as well as by comparing the Akaike information criteria (AIC). We used generalized linear models for analyzing sperm counts (Poisson) and cannibalistic attempts (binomial). We performed a multivariate regression between hook centroid size (CS) and shape matrix. To shape the weight matrix (matrix of partial warp scores together with the uniform component for a sample of shapes), we performed a principal component analysis (PCA) and found that the first three components explained 79% of total shape variation (PC1 = 43%, PC2 = 67%, PC3 = 79%). We carried out all statistical analyses in R, version 3.2.3 (R Development Core Team 2015).

We did not find a statistically significant correlation between hook centroid size and male body size (rho = 0.407, df = 1, $P = 0.683$), meaning that the size of the hook is not explained by body size. Also, we did not find a significant correlation between the hook shape and centroid size (Pillai = 0.872, $P = 0.477$), meaning that the shape of the hook is not changing with its size. In addition, we did not find any relationship between hook shape and mating duration ($r^2 = 0.074$, PC1: $P = 0.734$; PC2, $P = 0.294$; PC3: $P = 0.98$, df = 3). However, when we compared cannibalistic attempts (seven cases) with the hook shape matrix, we obtained a negative relationship with PC2, which represents the base of the hook (Logistic GLM $\chi^2 = 20.213$, df = 1; PC2: $P = 0.017$, Fig. 2). In this case, hooks with a wider base were related to an increase in cannibalistic attempts. Finally, when we compared sperm counts with hook shape, we did not find any

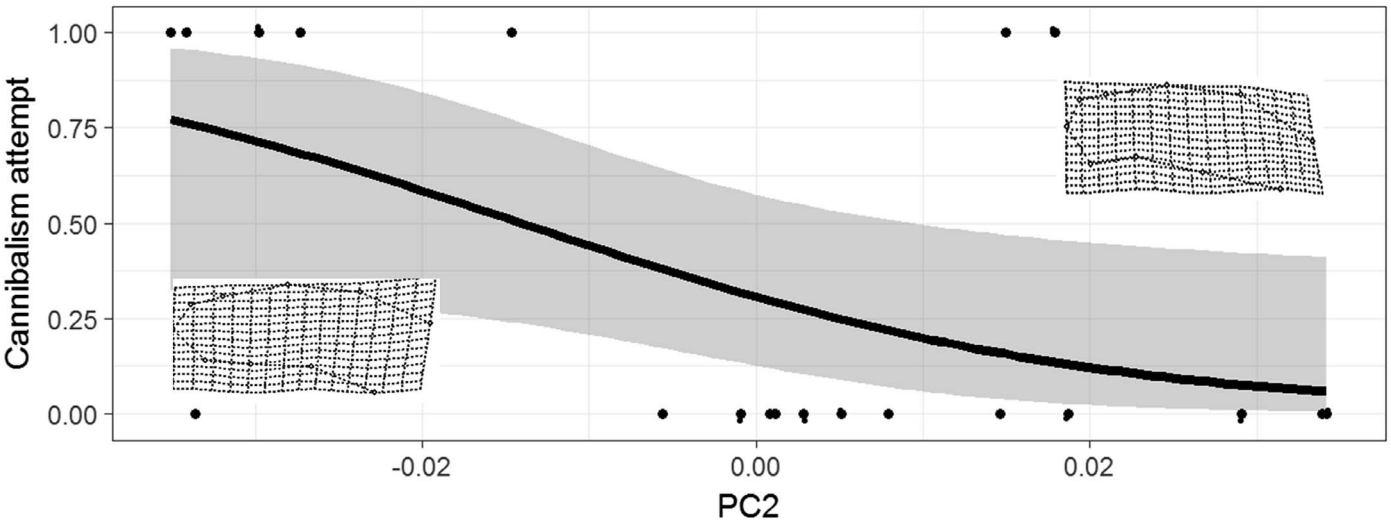


Figure 2.—Cannibalistic attempts by *L. mariana* females from Colombia and their relation to variation in the hook shape base (PC2). The PC2 shows the variable that explained the shape related to female cannibalistic attempts. A higher positive value in PC2 reflects a wider base of the hook. Conversely, negative values were related with hooks with a thinner base.

significant relationship between them (Poisson GLM $\chi^2 = 0.640$; PC1, $P = 0.865$; PC2 $P = 0.870$; PC3, $P = 0.908$, df = 3, $P = 0.511$).

In our study we found that the shape of the hook is related to female cannibalistic attempts. It is probable that a wider base of the tip hook prevents a better coupling of the palp during mating, which could be punished through female attacks (Barrantes et al. 2013) Similarly, in the spider *Hemicloea sundevalli* Thorell, 1870 (Trochanteriidae, formerly Gnaphosidae), the male tibial retrolateral apophysis is used to anchor the male palp to the epigynum to avoid rotation (Zakharov & Ovtcharenko 2013). It would be interesting to determine if males mechanically stabilize female position during mating depending on hook shape by performing reinforcing behaviors and/or intensifying female stimulation. It is important to highlight that only the base of the hook and not the centroid size was related to cannibalistic attempts. This would mean that the shape of the hook but not its size would have a relationship with cannibalistic attempts. The lack of correlation with male body size suggests that there is no allometric effect on the hook. Though some characteristics under sexual selection can show steep allometries, genital features in general show isometry or shallow allometries depending on the type of selection (Bonduriansky 2007; Eberhard 2009; Cayetano et al. 2011). It would be important to test if longer hooks could play a role in male copulatory courtship in a context of female choice, similarly as has been reported for other arthropod species (Arnaud et al. 2001; Rodriguez et al. 2004; Eberhard 2010; van Lieshout 2011; Dougherty et al. 2015).

Regarding hook shape and mating duration, some reproductive traits like copulation duration depend on the interaction between males and females and the influence of each sex over this (Bretman et al. 2013). For example, in the *L. mariana* pairs from CR, the duration of copulation is determined by the female and depends on male behaviors such as stimulation by cheliceral setae (Aisenberg et al. 2015b). Hernández et al. (2018) showed in the *L. mariana* population from CO that the amount of the sperm in female receptacles is correlated with the rate of long palpal insertions. However, we did not find a relationship between copulation duration, sperm counts and hook shape. Consequently, if the hook provides stability for the mating pair, this would not allow males to extend mating or increase sperm transfer in this species.

*L. mariana* females from CO perform post-copulatory sexual cannibalism (Hernández et al. 2018), unlike females from CR (Aisenberg & Eberhard 2009). This could function as a mechanism of cryptic female choice based on male genital traits and sexual performance. Similarly, in *Araneus diadematus* Clerck, 1757, females tend to cannibalize small males and those that mate but do not transfer sperm (Prenter et al. 2006). Although the conductor hook of *L. mariana* is not an intromittent structure directly involved with sperm transfer, the shape could play an important role in providing stability and allowing the embolus to be pushed inside the female duct (Eberhard & Huber 1998), as well as in puncturing or removing mating plugs (Aisenberg et al. 2015a). Future studies will analyze other structures involved in genital stability, as well as experiments modifying them and recording the consequences on female and male reproductive success. Moreover, histological procedures performed to male-female pairs during mating could be useful to determine how the male hook is coupled on female genitalia.

## ACKNOWLEDGMENTS

The capture of specimens for our study was performed under the permit resolution IDB0359. We thank Luis Hernández, Eudoro Mora, Valeria Ramirez, Iván Beltran, Alex Sabogal, and Francisco Quiroz for their help in fieldwork. We thank Alex Sabogal for his collaboration with equipment and Iván Beltran for his statistical advice. The suggestions of two anonymous reviewers improved the final version of the manuscript. This work was supported by "Proyecto Semilla" from the Faculty of Sciences, Universidad de Los Andes. A. Aisenberg acknowledges financial support by PEDECIBA (Programa de Desarrollo de Ciencias Básicas), and SNI (Sistema Nacional de Investigadores, ANII).

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*Manuscript received 26 June 2018, revised 25 January 2019.*