2021. Journal of Arachnology 49:257–261

SHORT COMMUNICATION

No strings attached: description of the sexual behavior in the Neotropical spider *Parabatinga brevipes* (Keyserling, 1891) (Araneae: Ctenidae)

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Abstract. In spiders, intersex communication during courtship is essential to avoid the risks of cannibalism due to lack of specific recognition. *Parabatinga brevipes* (Keyserling, 1891) is a Ctenidae spider with a distribution from Colombia to Uruguay. This study is the first to describe the sexual behavior of *P. brevipes*, and the fourth reported in the family. We introduced males to females in a cage and recorded their courtship and copulation behavior. Males began courtship after touching female silk, performing Leg-tapping of legs I and Palpal movements. We observed ten copulations that usually occur vertically, in the copulatory position reported for other ctenids, with the male on top of the female, oriented in opposite directions. Copulations usually involve the insertion of one male palp in a single female's genital opening and finish with the pair dropping from the vertical position. These sexual behaviors are compared with reports of other species in the family.

Keywords: Courtship, copulation, male silk-laying, spiderlings

https://doi.org/10.1636/JoA-S-20-027

Spiders present unique aspects in their reproductive biology that make them excellent models for investigating the evolutionary processes involved in the attraction of a mate, sexual encounters, and the maintenance of different reproductive strategies (Huber 2005). Spiders usually exhibit complex and ritualized behaviors, during which communication between both sexes commonly occurs through multimodal signals, mainly chemical, seismic, acoustic, tactile, and in some cases visual channels. These signals are fundamental for attracting, recognizing, and stimulating sexual behavior, and minimizing the risks of predation (Herberstein 2011; Schneider & Andrade 2011). However, knowledge of how sexual behavior occurs in some spider families is limited. An example of this occurs in the family Ctenidae, a group well represented in the Neotropics with members that inhabit forests, groves, and streams of Central and South America; some of them, mainly those belonging to the genus *Phoneutria* Perty, 1833, are of great medical importance (Merret 1988; Barth 2002; Folly-Ramos et al. 2002; Foelix 2011; Hazzi 2014; Trillo et al. 2019).

In Uruguay, four species of Ctenidae are currently known: *Guasuctenus longipes* (Keyserling, 1891); *Asthenoctenus borelli* Simon, 1897; *Parabatinga brevipes* (Keyserling, 1891) and *Ancylometes concolor* (Perty, 1833) (Pérez-Miles 1988; Aisenberg et al. 2011; World Spider Catalog 2020). However, only the sexual behavior of *G. longipes* (Trillo et al. 2019) has been described so far. *Parabatinga brevipes* is a small Ctenidae (1–1.5 cm) present in the southeast of Uruguay (Aisenberg et al. 2011). This species can be found in natural environments, in bush and grassland vegetation, woods in the proximity of streams, rocky and coastal areas, but also in peri-domiciliary areas profoundly modified by human activity (Simó et al. 2000; Aisenberg et al. 2011). In human modified environments, *P. brevipes* shelters during the day under rocks, fallen tree trunks, and the remains of construction materials. During the night, *P. brevipes* can be seen climbing medium-height vegetation (*Baccharis dracunculifolia* DC) or walking through the substrate. Although it is possible to find individuals throughout the year, their period of greater activity is during the summer, in the months of January to March (Simó et al. 2000; González & Toscano-Gadea, pers. obs.). In this work, we describe for the first time, the sexual behavior of *P. brevipes* in detail.

We collected sub-adult and adult individuals of *P. brevipes* during October 2016 and November 2017, in Villa Serrana (34°19′26.68″ S, 55°19′07.43″ W), Araminda (34°46′47.39″ S, 55°33′12.89″ W) and Santa Teresa (34°00′16.32″ S, 53°32′03.68″ W), Uruguay. The captures were performed manually during the night, using headlights that allowed us to see the spiders because of the brightness of the *tapetum lucidum* in their eyes (Foelix 2011). To assure that we used virgin individuals in this experiment, we reared the immature specimens under laboratory conditions (females ($n = 10$) and males ($n = 8$)) until the last molt. Nonetheless, we additionally collected two adult males from the field, to obtain data from 10 independent pairs. The spiders were housed individually in Petri dishes (diameter 9.5 cm, height 1.5 cm) with a thin layer of sand as substrate and a piece of cotton embedded in water. All the individuals were fed twice a week with *Tenebrio molitor* Linnaeus, 1758 (Coleoptera; Tenebrionidae); juvenile stages of *Blaptica dubia* Serville, 1839 (Blattodea; Blattellidae) and *Musca domestica* Linnaeus, 1758 (Diptera, Muscidae). Individuals were monitored daily to determine the exact date that they reached adulthood. Room temperature and humidity during breeding were (mean ± SD) 25.0 ± 1.3 °C, range 23–26.5 °C and 56.9 ± 6.9 %, range 50–71 %, respectively, and the photoperiod was 12:12 h light:dark approximately.

The pairs used for the trials were randomly selected. The specimens were presented for potential mating to an individual of the opposite sex 11.9 ± 8.0 days for females ($n = 10$) and 12.8 ± 4.7 days for males ($n = 8$), after the last molt. The remaining two adult males were kept 12 days in the laboratory before being presented to a female. The total body size of females was 4.71 ± 0.13 mm (range 4.2–5.3) and of males 4.19 ± 0.07 mm (range 3.67–4.47), females being larger than males ($t = 3.70$; $P = 0.002$).

We carried out a total of 10 experimental trials in glass cages of 15 cm of length, 15 cm width and 5 cm height, with sand as substrate, a piece of tree bark as a refuge and a small container with a cotton embedded in water. All the trials were performed between 10:00 am

Table 1.—Description of the behaviors performed by both sexes of *Parabatinga brevipes* during courtship and copulation, according to their temporal sequence.

Stage	Behavioral unit	Description	Duration (min ± SD)	Sex
Courtship	Leg-tapping	Touches the substrate with forelegs I, in some cases II, by alternate movements, with or without walking.	9.20 ± 10.3	Male
	Leg-tapping + Palpal movement	Touches the substrate as in 'Leg tapping' but at the same time moves his palps up and down while walking.	3.60 ± 1.50	Male
	Leg-palpal rubbing	Scrapes one palp over the other or a leg over a palp, without touching the chelicerae.	0.41 ± 0.60	Male
	Forelegs elevation	Raises legs I, and keeps them up, with or without walking.	2.45 ± 2.19	Both (mainly male)
	Threads adhesion	Touches the substrate with the spinnerets, and deposits silk threads with zigzag motion.	0.41 ± 0.24	Both (mainly male)
	Grooming	Rubs palps or legs using chelicerae.	3.40 ± 3.70	Male
	Slow leg-tapping	Touches the substrate by gentle and alternate movements of forelegs, with or without walking.	1.56 ± 1.02	Female
	Legs-drumming	Touches substrate or the female by alternate, energetic and quick movements of forelegs (mainly legs I, but may include legs II). This behavior occurs when both individuals are very close to each other, or have already touched.	1.50 ± 1.37	Male
	Silk-laying (i.e., Lays silk in Trillo et al., 2019)	Deposits an attachment disc and extends the silk thread immediately before trying 'Mounting'.	-	Male
Copulation	Mounting	Climbs on top of the female by the front and places the anterior part of his body over the female abdomen.	5.62 ± 10.06 (until dismount)	Male
	Leg-folding	Flexes legs around the body.		Female
	Pair shaking	Just before dismounting, the male and female vigorously move their bodies laterally.	2.14 ± 4.46	Both
	Pair drop	The pair abruptly drops from a vertical plane of copulation to the substrate.	-	Both
	Insertion	Introduces the embolus in one of the female genital openings. During each insertion the haematodocha expands.	8.27 ± 3.53	Male
	Palpal-drumming	Touches repeatedly with one palp on the abdomen or forelegs of the female, usually during the 'Insertion' of the other palp.	3.39 ± 3.17	Male
	Abdomen vibration	Moves his abdomen quickly up and down, generally between insertions.	0.21 ± 0.12	Male
	Dismounting	Descends from the female to the substrate abruptly and quickly. Immediately after the dismount the female usually chases the male.	-	Male
	Epigynum touch (ie. Touches epigynum in Trillo et al. 2019)	Passes the palps by the epigynum to take them to the chelicerae.	1.30 ± 0.60	Female

and 16:00 pm and natural light was blocked with curtains. Females were placed in sand 48–72 hours before each trial for the deposition of silk lines on the substrate. Males were carefully placed on the opposite side of the cage from the females. We registered courtship duration (from first courtship behavioral unit until mounting), copulation duration (from mounting until dismounting) and post copulation duration (from dismounting until the female began to move). The number of ejaculations was indirectly measured through the number of inflations of the hematodocha (see Trillo et al. 2019 for more details). We followed the terminology proposed by Trillo et al. (2019) to define the behavioral units. The temperature and humidity during the trials were (mean ± SD) 23.3 ± 2.0 °C, range 22.6–28.5 °C and 26.0 ± 8.0 %, range 18–39 %, respectively.

We video-recorded all the trials with a Sony DCR-SR45 camera with night shot mode. We used JWatcher software (Blumstein et al. 2000) to analyze the occurrences and durations of the behavioral units. All the interactions between sexes were analyzed to build an ethogram (Table 1) and a flow chart (Fig. 1). A detail of the male courtship, the behaviors prior to mounting, and during copulation are shown in the videos in Supplementary Materials (online at https://doi.org/10.1636/JoA-S-20-027.s1 and https://doi.org/10.1636/JoA-S-20-027.s2).

We performed Markov analyses to evaluate if there was statistical association between behavioral transitions. All statistical analyses were performed with the Past program (Paleontological Statistics Software 1.18; Hammer et al. 2003). Voucher specimens were deposited at the Colección de Entomología, Facultad de Ciencias (FCE), Montevideo, Uruguay.

The median duration of courtship was 15.8 minutes, (LQ, UQ 7.5, 27.7; range 0.5–53.7); median copulation was 0.9 minutes (LQ, UQ 0.3, 6.9; range 0.2–30.8), and median post-copulation was 15.3 minutes (LQ, UQ 8.7, 17.3, range 0.5–17.9). No differences were detected in these variables between males that reached adulthood in the laboratory, and those that were collected as adults in the field (courtship duration: $U = 32.50$, $P = 0.53$; copulation duration: $U = 39.5$, $P = 0.87$). Courtship began with a slow and cautious

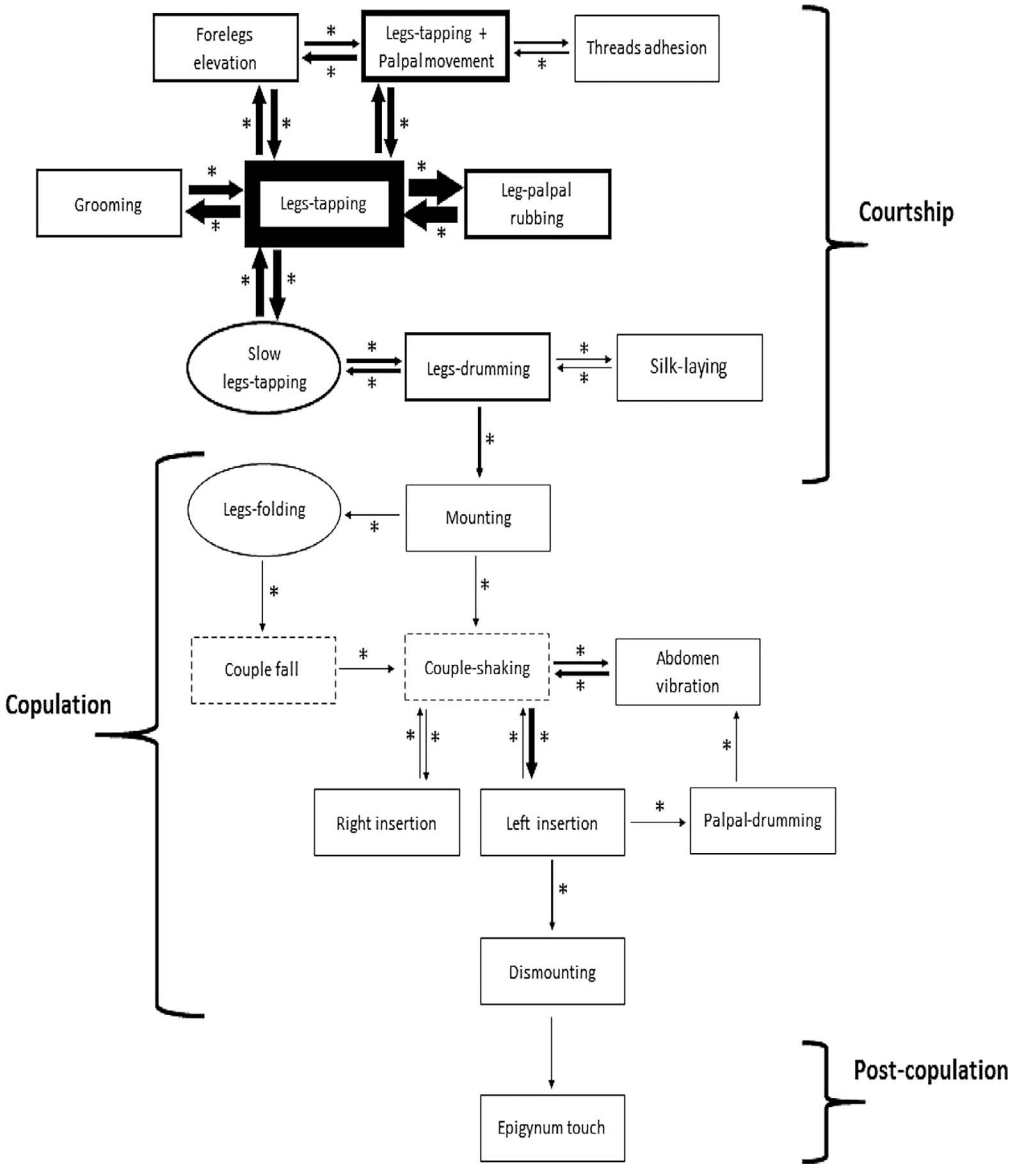

Figure 1.—Flowchart showing sequences of courtship and mating behavioral units in *Parabatinga brevipes*. The behaviors performed by the females are shown with ovals, male behaviors with rectangles, and combined male and female behaviors with dotted rectangles. Arrow thickness indicates the frequency of transitions. Asterisks indicate significant differences.

displacement of the male, interspersed with series of rapid movements raising legs I and touching the substrate with them (Leg-tapping) (Fig. 2A) combined with palpal rubbing and / or palpal rubbing with legs I (Leg-palpal rubbing), resuming rapid movements or remaining still. Throughout these behavioral patterns, males cautiously approached the females. In seven cases, the females moved directly to the males that were courting and slowly tapped the substrate with their legs I (Slow Leg-tapping). In the other three cases, the females remained immobile and the males began courtship only after touching the females. The male tended to drum his legs on the substrate and the female responded to the touch of the male with slow Leg-tapping. Just before mounting, six males attached a silk thread to the substrate (Silk-laying) (Fig. 2B). In two cases in which the female approached the courting male, she also attached a silk thread to the substrate.

The mounting occurred slowly and the copulatory position was similar to that observed in other species of Ctenidae; the male climbed on the back of the female and surrounded her body with legs III. The female allowed the male to mount by retracting her legs against the body (Leg-folding). Seven of the ten copulations observed occurred in a vertical position and, in these cases, the pairs dropped during them. In two cases, the first palpal insertion was performed after the pair dropped onto the substrate. All the males performed a variable number of circular movements of the palps on the epigyne prior to the insertion. However, the copulatory pattern was variable. Once the male was in the copulatory position, he could insert a palp, remove it and make a new insertion in the same female genital opening. Only in one case, the male inserted a palp and later changed side and performed a second insertion using the other palp in the remaining female genital opening. Six males made a single inflation of the hematodocha, one male performed two inflations in the same female genital opening, another male performed in total five inflations, using both palps and both female genital openings, and finally the remaining male performed in total six inflations using the same palp and in the same female genital opening. In these last three cases, the

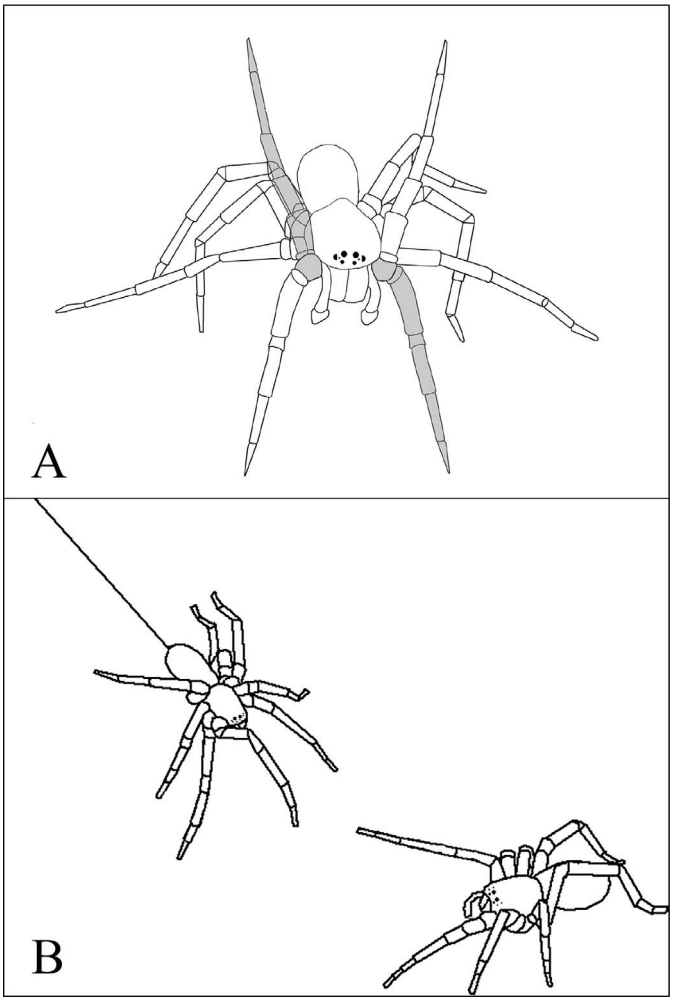

Figure 2.—(A) Schematic representation of Leg-tapping behavioral unit performed by the male during courtship. (B) Schematic representation of Silk-laying behavioral unit performed by the male immediately before mounting.

male slowly vibrated the abdomen between each insertion and moved the spinnerets. In-between the hematodochal inflations, and immediately before dismounting, all the pairs performed Pair shakings. The erection of the spines was scarcely visible; only the spines located in the metatarsus and tibia of hind legs were visible when hematodochal inflation occurred.

The dismounting was quick, and the males rapidly escaped from the female. In four cases, the female tried to capture the male but only in one case, and immediately after a second insertion, did she cannibalize the male. At the end of copulation, the female performed Epigynum touch ($n = 6$). In the remaining cases where the female attempted to capture the male, this behavior was not observed, but it may have happened later, after we stopped recording the trial. Therefore, it is possible that Epigynum touch occurrence was incompletely registered.

Approximately 30 days after copulation (median 26 days, LQ, UQ 24, 29, range 19–35) females built their first egg sac. Only six females built an egg sac, but three females sequentially constructed a second one (37 days, 35 days and 40 days after the first egg sac respectively) and one female constructed three (the second egg sac 39 days after the first and the third egg sac 35 days after the second). The egg sac was small ($\leq$ 1 cm length), with one face flattened and another face convex. Initially its coloration was white, but turned gray over time. It was common to observe debris of the substrate attached to the silk. The female held the egg sac with silk threads attached to the substrate or held it between her legs II, which enabled her to expose it to the light source (in our case, a window where sunlight entered). Spiderlings emergence happened during nighttime, and it was not observed if they had left the egg sac by their own effort, or with their mother's assistance. The median time until hatching was 61 days (LQ, UQ 40, 66, range 31–84).

As occurs with other species of Ctenidae (Merret 1988; Barth 2002; Hazzi 2014; Trillo et al. 2019), the courtship of *P. brevipes* began when the male touched the female's silk, which suggested the presence of pheromones associated with silk deposited by the female. The courtship behavior of the male after the detection of the female pheromone suggests a multimodal courtship. Both leg tapping and palpal movements suggested seismic communication through the substrate, as occurred in *Ancylometes bogotensis* (Keyserling, 1877), *Cupiennius coccineus* F.O. Pickard-Cambridge, 1901 and *Phoneutria boliviensis* (F.O. Pickard Cambridge, 1897) (Merret 1988; Barth 2002; Hazzi 2014). These behaviors could be a response to the environment inhabited by *P. brevipes*, where the visual channel did not seem to be the most relevant, at least at the beginning of a sexual encounter. It is very common to find both sexes in medium-height bushes, with abundant leaf cover which may prevent a first visual detection. Seismic communication may indicate to the female the presence and location of the male, but it is usually after the female goes towards the male and is close to him, when the visual behavioral communication between the sexes begins. Despite females being bigger than the males, there is no extreme sexual dimorphism which could explain the absence of bridal veil in this species, differing from reports in other species of Ctenidae (Trillo et al. 2019). The silk-laying performed by the male just prior mounting was also observed by Trillo et al. (2019) in *G. longipes*, and its function remains unknown.

The mating position of *P. brevipes* (Type III, Foelix 2011) is typical of other species of Ctenidae (Merret 1988; Barth 2002; Pellagati-Franco 2004; Hazzi 2014; Trillo et al. 2019) and of other modern wandering spider families (Foelix 2011; Hazzi 2014). However, the dropping observed in this species has not been reported before. Once the male mounted the female, she folded all the legs against the body, making the pair's vertical support more difficult. In addition, the male used his legs II, and sometimes also the III, to surround the middle part of the female's body. The additional effort performed at the time of sperm transfer and his weight could overcome the female resistance, causing the pair to drop and separate. Female Legs-folding has been reported also in *P. nigriventer* (Keyserling, 1891) (Folly-Ramos et al. 2002) and in *G. longipes* (Trillo et al. 2019); the shorter duration of the copula in the former species and the occurrence of bridal veil in the latter, can be related with the absence of pair drop. The low number of insertions in *P. brevipes* is similar to that of other Ctenidae species such as *P. boliviensis* (Hazzi 2014) and, despite the drop and separation of pairs, copulation durations were not shorter than in the other mentioned species. At the same time, copulating in the dense vegetation, as in *P. brevipes*, could be an adaptation to avoid predators, for example large wolf spiders such as *Lycosa poliostoma* (CL Koch, 1847), *L. erythrognatha* Lucas, 1836 and *Schizocosa malitiosa* Tullgren, 1905 that might prey on *P. brevipes* (Toscano-Gadea & González, pers. obs.). More studies will be necessary to clarify this hypothesis.

Future studies will seek to determine if this unique hematodochal inflation occurs in natural conditions, whether they cause a decrease in female receptivity to second males and if those second males prefer to insert in the female genital opening that has never been used. The shape and the number of the egg sacs, the number of spiderlings per egg sac and the dispersal following the second molt observed in this study coincide with Simó et al. (2000). However, the amount of debris

observed in the egg sacs silk of *P. brevipes*, suggests a camouflage like that observed in *C. medius* (Pellegatti-Franco 2004).

ACKNOWLEDGMENTS

M. González acknowledge financial support from PEDECIBA and SNI (ANII). We want to thank to Luiz E. Costa-Schmidt and Alexandra Elbakyan for the access to many of the bibliographic sources reviewed to carry out this work. We are also very grateful to Luis Aisenberg for reviewing and significantly improving the English. We specially thank two anonymous reviewers for their critical reading and valuable comments on the manuscript.

SUPPLEMENTAL MATERIALS

Supplementary video S1: Most representative behavioral units during courtship in *Parabatinga brevipes,* online at https://doi.org/10.1636/JoA-S-20-027.s1

Supplementary video S2: Behavioral pattern during pre-mounting and copulation in *Parabatinga brevipes*, online at https://doi.org/10.1636/JoA-S-20-027.s2

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Manuscript received 4 April 2020, revised 29 January 2021.