

## Sexual dimorphism and patterns of sexual behavior in *Lustrochernes argentinus* (Pseudoscorpiones: Chernetidae)

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**Abstract.** Sexual dimorphism between females and males can sometimes be related to particular mating behaviors. In pseudoscorpions, very little is known about the relationship between sexual size dimorphism (SSD) and reproductive behavior. This paper describes sexual dimorphism and the mating behavior patterns of *Lustrochernes argentinus* (Thorell, 1877), a Neotropical species of Cheliferioidea. We measured different body structures and appendages of 14 individuals of each sex, with the values compared with a T-test. We also analyzed the sexual behavior repertoire occurring during mating between nine couples. We found SSD for several structures: the fixed and movable finger width, and the angle between IV coxae are larger in females, in contrast with the gap between fingers which is bigger in males. The analysis of sexual behavior patterns in this species showed that the reproductive behavioral repertoire is structured in three phases: Phase I (examination), Phase II (spermatophore deposition) and Phase III (sperm transfer) with a mean duration of 2.2 min, 5 min, and 7.5 min, respectively. Some structures that showed SSD, and also the composition and duration of different behavioral phases, are different from those of other species of the family Chernetidae. We discuss the results based on current knowledge in pseudoscorpion biology with prospects of further studies studying sexual selection.

**Keywords:** Pseudoscorpions, sexual size dimorphism, courtship, mating behavior

Differences between sexes in morphology, physiology, and behavior are called sexual dimorphism (Fairbairn et al. 2007). One type is sexual size dimorphism (SSD), referring to differences in mean body size between males and females, the evolutionary causes of which are still subject to major controversies (Lovich & Gibbons 1992).

Many studies of these issues have been carried out in spiders due to their great diversity, wide distribution, and ease of collection, but also because of the great variety of reproductive strategies and patterns displayed before, during and after mating (Choe & Crespi 2004; Eberhard 2004; Huber 2005; McLean et al. 2018). Recently, these studies have extended to other arachnids that also present an abundance of behavioral patterns, such as scorpions (Peretti 2003, 2010; Peretti & Carrera 2005; Vrech et al. 2014; Olivero et al. 2015). Studies of sexual selection, sexual dimorphism, mating systems, life history, and sexual pheromones have also been carried out in harvestmen (Nazareth & Machado 2009; Munguía-Steyer et al. 2012; da Silva Fernandes & Willemart 2014; Machado et al. 2016), but other orders, such as pseudoscorpions, are still neglected.

Pseudoscorpions are arachnids with an evolutionary history that dates back to the Mid-Devonian period (390 Ma) (Harms & Dunlop 2017). Their morphology and adaptation to the environment have changed little during this time (Judson 1998, 2000).

Sexual dimorphism in pseudoscorpions is generally restricted to the genital area, although it is also found in other body parts, such as the coxal sacs, coxae, cheliceral galea and tarsal claws (e.g., Chamberlin 1931; Weygoldt 1969; Muchmore 1974; Zeh 1987; Sato 1991; Zeh & Zeh 2013). Females are generally larger than males (Zeh 1987), but exceptions to this are found within the family Chernetidae (Weygoldt 1969; Harvey 1995), where males of some species may have larger chelae than the females,

and some have keels on their chelae, as in *Semeiochernes* Beier, 1932 and *Mirochernes* Beier, 1930. There are extremely long pedipalps in *Metagoniochernes* Vachon, 1939, which are presumably used during courtship or in male-male combat (Chamberlin 1931; Vachon 1939; Weygoldt 1969).

All pseudoscorpions use indirect sperm transfer mediated by a spermatophore placed on the ground, as in other orders of Arachnida, such as Scorpiones, Amblypygi, Uropygi and some Acari (Zeh & Smith 1985). Pseudoscorpions are ovoviviparous and usually have three postembryonic stages before adulthood (Weygoldt 1969). Only species from the superfamily Cheliferioidea have seminal receptacles, a complex spermatophore, and a mating dance during their courtship (Harvey 1992). These spermatophores exhibit different degrees of complexity and generally consist of a stem and a sperm package. In Cheliferidae and Chernetidae, spermatophores also have a drop of fluid under the sperm package that acts as a trigger mechanism to help sperm enter the female genital atrium (Weygoldt 1966, 1969).

In some pseudoscorpions, the spermatophore is deposited in the absence of the female who locates it, presumably using chemical cues. This behavior is considered to be the ancestral condition within the order (Chamberlin 1931; Beier 1932; Weygoldt 1966, 1969, 1990; Schaller 1979; Thomas & Zeh 1984; Harvey 1992). In other families, spermatophores are deposited only in the presence of a female (Weygoldt 1966, 1969, 1990), while in derived taxa (Cheliferioidea), spermatophore deposition is preceded by courtship (Weygoldt 1966, 1969, 1990; Boissin 1973; Brach 1978; Thomas & Zeh 1984; Harvey 1992). These differences in the reproduction of pseudoscorpions could indicate an evolutionary transition from a dissociated model of sperm transfer to an undissociated

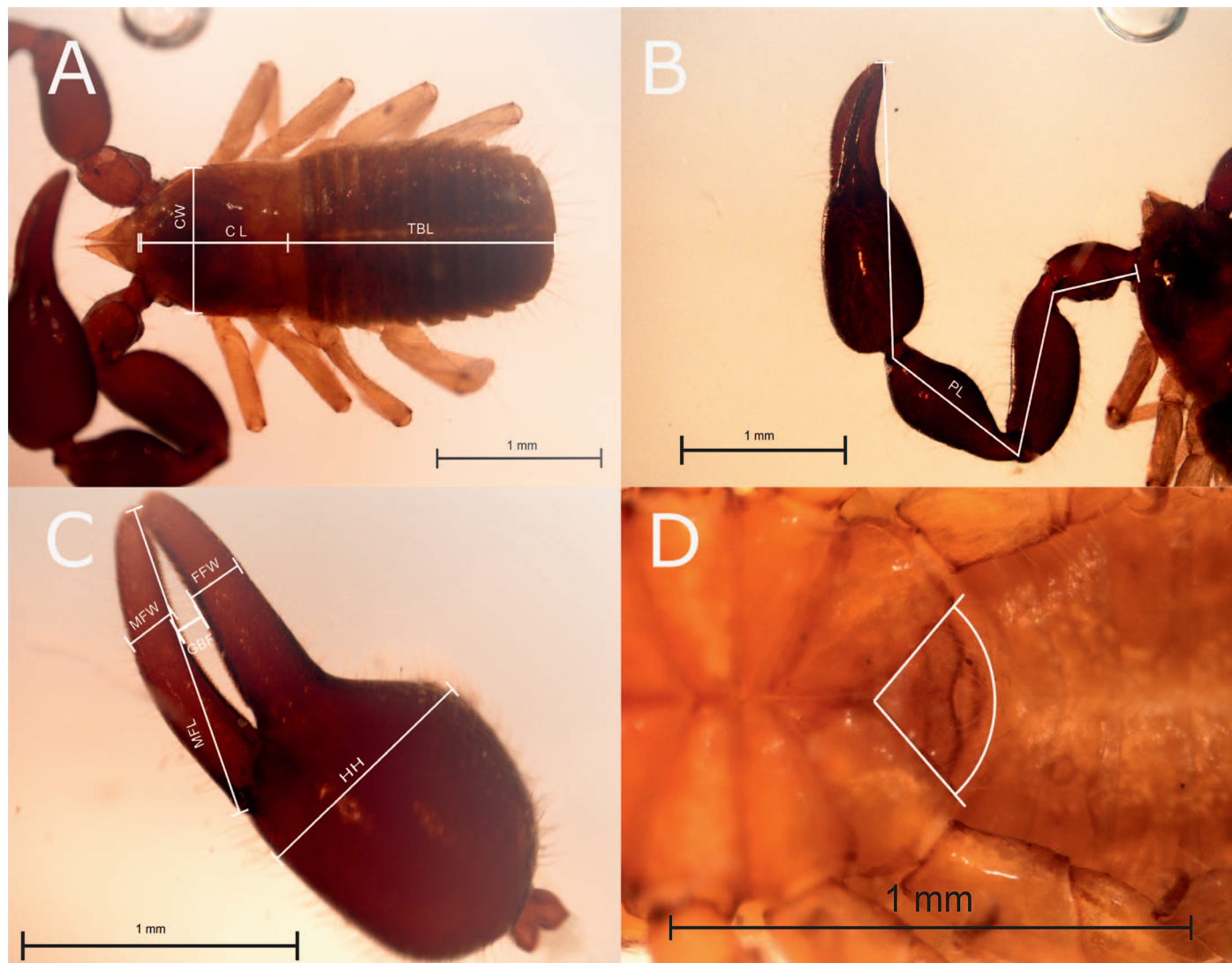


Figure 1.—Measurements of each structure for sexual dimorphism analysis. (All photos are of a male.) A. Total body length (TBL), carapace length (CL) and carapace width (CW); B. Pedipalp length (PL); C. Hand height (HH), fixed and movable finger width (FFW, MFW) and the gap between fingers (GBF); D. Angle between coxae IV.

one (i.e., there is temporary pair formation) (Weygoldt 1966; Harvey 1992).

Studies of the sexual behavior of pseudoscorpions are scarce worldwide, with only a few species studied by Vachon (1939), Weygoldt (1966, 1969), Zeh & Zeh (1991, 1997), and Andrade & Gnaspini (2003). *Maxchernes iporangae* Mahnert & Andrade, 1998 (Chernetidae), is the only South American species for which we have detailed knowledge of its courtship and mating (Andrade & Gnaspini 2003).

This paper describes sexual dimorphism and the mating behavior patterns of *Lustrochernes argentinus* (Thorell, 1877), a Neotropical species of Cheliferioidea, of which there are only preliminary studies of its sexual behavior (Ceballos 2008; Grimaldi et al., unpub. data; Cavigliaso et al., unpub. data). We expect that in the future this knowledge will allow for advances in experimental work in this species in order to explore functional aspects of sexual behavior and sexual size dimorphism.

## METHODS

Specimens of *Lustrochernes argentinus* were collected in Villa la Merced, Calamuchita Department, Córdoba, Argentina (31.82°S, 64.52°W) between September 2015 and March 2016. They were taken in the daytime from under the bark of *Eucalyptus* sp. and were placed individually in Eppendorf tubes. Once in the laboratory, individuals were sexed under a stereomicroscope (Nikon SMZ1500) using genital opercula coloration, the presence of apodemes and the chaetotaxy of the genital region according to Ceballos (2008). Afterward, they were placed individually in plastic containers (2.5 × 2.5 cm) with a piece of the bark from which each was collected. They were moistened and fed with *Drosophila melanogaster* larvae once a week.

To study the existence of sexual dimorphism, the genital region and various body structures (whole body, pedipalp, chelae, genital operculum, leg IV, among others) (Fig. 1) were photographed from individuals lodged in the CDA (Animal

Table 1.—Mean value in mm for each structure in males and females of *Lustrochernes argentinus*. FBL= Full body length. \* Indicates significant difference between males and females as determined by T-test or Mann-Whitney U test (depending on the normality of the data). † - T-test, T- value presented; ‡ - Mann-Whitney U Test, Z-value presented.

Structure	Female (N = 14)		Male (N = 14)		Statistic	p-value
	Mean	Structure/FBL	Mean	Structure/FBL		
Full body length	2.764		2.797			
Carapace length	0.955	0.350	0.877	0.335	0.904†	0.375
Carapace width	0.962	0.351	0.899	0.327	1.559†	0.132
Pedipalp length	4.524	1.664	4.644	1.730	-0.610†	0.548
IV leg length	2.319	0.854	2.379	0.887	-0.623†	0.539
Chelicera length	1.745	0.640	1.673	0.622	0.611†	0.547
Chelicera width	0.606	0.646	0.565	0.612	0.559 ‡	0.577
Movable finger width	0.801	0.294	0.772	0.288	0.366†	0.718
Fixed finger length	0.187	0.068	0.161	0.060	2.111 ‡	0.035*
Fixed finger width	0.222	0.081	0.173	0.064	3.856†	0.001*
Gap between fingers	0.032	0.012	0.058	0.021	-4.849 †	0.001*
IV coxae angle	109.89	40.067	95.305	35.449	3.066†	0.005*
Hand silhouette	1.058	0.388	0.953	0.353	1.354†	0.188

Diversity I Department Collection, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba) which are kept in 80% ethanol. Also we used individuals collected for the behavioral observation (preserved in 80% ethanol after their death). Fourteen individuals of each sex were photographed with a camera (Nikon Digital Sight DS-FI1-U2) attached to a stereomicroscope (Nikon SMZ1500). Images were taken over graph paper under different magnifications. These were analyzed using the ImageJ 1.45 (Schneider et al. 2012) photo analysis program. We measured the length and width of the carapace, total body length, chelicera length, left pedipalp and IV leg length, fixed finger length, fixed and movable finger width and the gap between them, the angle between the fourth coxae, all calculated following Chamberlin (1931), and the hand silhouette according to Zeh (1987). Finally, we calculated a proportion index for each structure ( $X$  structure/total body

length) in each specimen and then calculated the mean value, according to Olivero et al. (2012). A T-test or Mann-Whitney test (depending on the normality of data) was performed to compare the proportion index between males and females, using the program NCSS (© Hintze, 2015).

To observe mating behavior, nine random couples were placed in plastic capsules ( $2.5 \times 2.5$  cm) with a glass lid and a cork sheet at the bottom to simulate tree bark and to allow a better grip and locomotory movements. To be able to identify the male and the female in the videos, we added a tiny dot with non-toxic paint on the dorsum of one of them. The couple was filmed for 30 minutes or until the mating was finished, which was observed with a webcam (Logitech QuickCam pro 9000) attached to a stereomicroscope (Bausch & Lomb 0.7X-3.0X). Videos were analyzed using JWatcher software (Blumstein et al. 2007) to determine the sexual behavior repertoire occurring during mating. From these patterns, we analyzed common

Table 2.—Description of each behavioral unit observed during *L. argentinus* mating. For each unit, the phase to which it belongs is indicated: Ph I (examination), Ph II (spermatophore deposition), Ph III (sperm transfer).

Behavioral unit	Description	Phase
Chasing	Both male and female get closer and attempt to grasp each other while moving to different parts of the capsule.	Ph I
Touching with chelae	The male touches the female chela, leg I or leg II and on occasions, chelicerae.	Ph I
Chelae grasping	One of the individuals grasps the other one's chela.	Ph I
Walking	While gripped by one or both chelae, they change position inside the capsule.	Ph I
Pedipalps locking	With the male grasping both female chelae, they approach very closely (while still grasping), resulting in their chelicerae being close to each other. They retract their pedipalps on both sides of the body and remain in this position until spermatophore deposition is over.	Ph II
Chelicerae rubbing	Both individuals rub their chelicerae with the other without losing grip.	Ph II
Rubbing with legs	While being grasped, one of the individuals rubs a leg with the partner's leg I or leg II.	Ph II
Carapace movements	Female performs a slight lateral movement with her carapace.	Ph II
Body lifting	Male begins to raise the posterior part of his opisthosoma, still gripping the female chelae.	Ph II
Spermatophore deposition	With his opisthosoma raised, the male deposits the spermatophore and shakes his body to get rid of it, without letting the female go.	Ph II
Pedipalp pulling	Once the spermatophore is deposited, there is pedipalp pulling by both individuals.	Ph III
Separation	They lose grip, and the couple separates before the sperm transfer occurs but remain close.	Ph III
Sperm transfer	Female places herself over the spermatophore and rubs her genital operculum with the substrate to press the spermatophore and perform the sperm transfer.	Ph III
Walk away	Both individuals walk away from each other.	Ph III



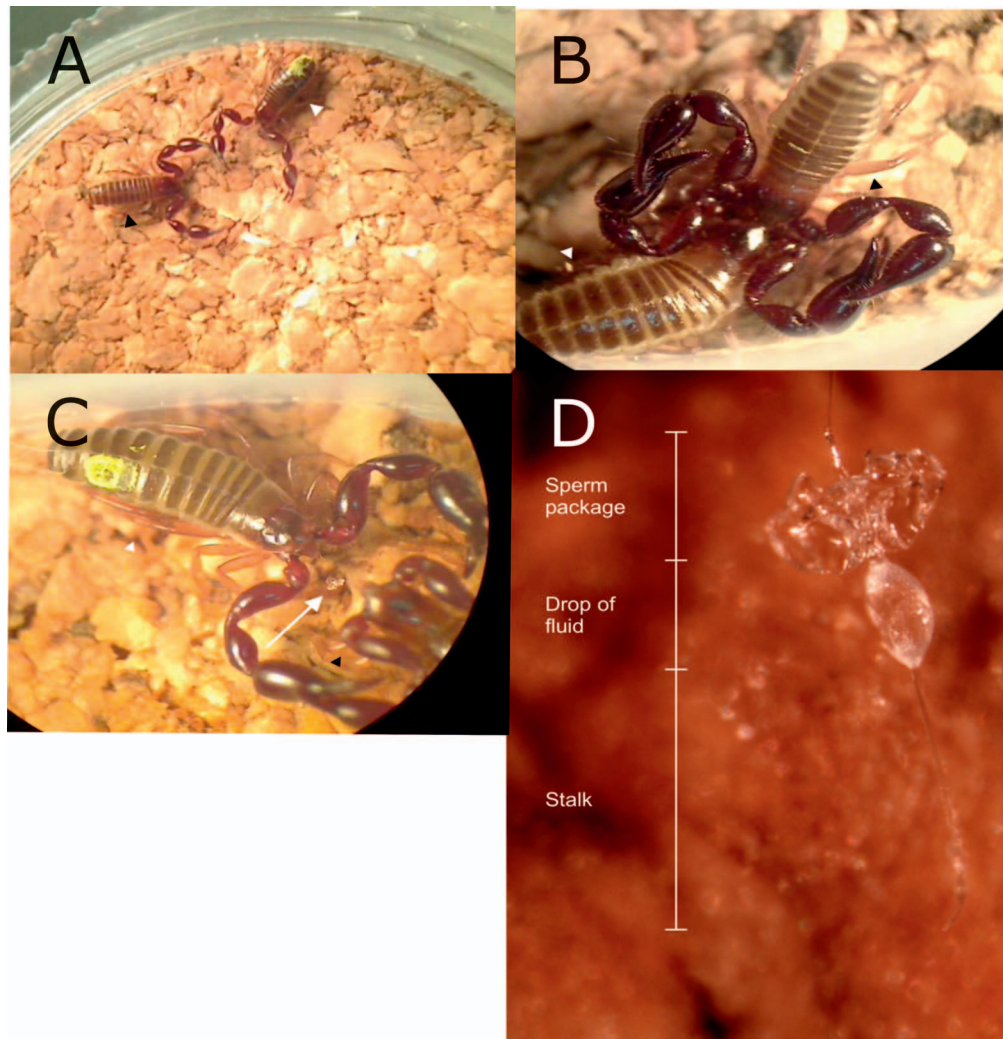


Figure 2.—Phases identified during mating. A. Phase I: examination—the male is grasping one of the female's chelae. B. Phase II: spermatophore deposition—the pedipalps in locking position. C. Phase III: sperm transfer—individuals are performing pedipalp pulling; the white arrow is pointing to the spermatophore between them. D. *L. argentinus* spermatophore in detail.  $\triangle$  Female,  $\blacktriangle$  Male

behaviors trying to identify possible phases and then calculating the duration and frequency of characteristic behaviors. The results were summarized in a flow diagram, showing the behavioral units of each phase and their relationships.

## RESULTS

The structures that showed sexual dimorphism were the fixed and movable finger width and the angle between IV coxae, which were larger in females. The gap between the fingers was significantly larger in males (Table 1).

The analysis of sexual behavior patterns showed that the reproductive behavioral repertoire was composed of the behavioral units listed in Table 2. Mating commenced when the male grasped the female's pedipalp and ended when the spermatophore was transferred. Three phases were identified:

Phase I (examination) (Fig. 2A): Chasing and constant chela touching characterized this phase. Generally, the male grasped the female by one chela and, in 20% of cases, with the other chela he rubbed leg I or II (in 10%, the chelicerae). This

grasping continued, and on some occasions, was also instigated by the female. The mean duration of this phase was  $133.4 \pm 115.8$  sec.

Phase II (spermatophore deposition) (Fig. 2B): This stage was initiated with pedipalp locking, remaining in this position until the finalization of spermatophore deposition. In 10% of the cases, cheliceral rubbing by both individuals or only by the female was observed. Then, in all 14 cases, the male raised the posterior part of his opisthosoma which indicated that he was depositing the spermatophore on the substrate (Fig. 2D). The deposition of the spermatophore constituted the end of this phase, which had a mean duration of  $275.6 \pm 52.2$  sec (approximately 5 minutes).

Phase III (sperm transfer) (Fig. 2C). This stage commenced when the pair began to pull each other's pedipalps, moving only a few millimeters. This pulling continued for a few seconds, and then the couple separated, the female approached the spermatophore and rubbed her genital opening over the sperm package and performed sperm transfer, indicating the end of

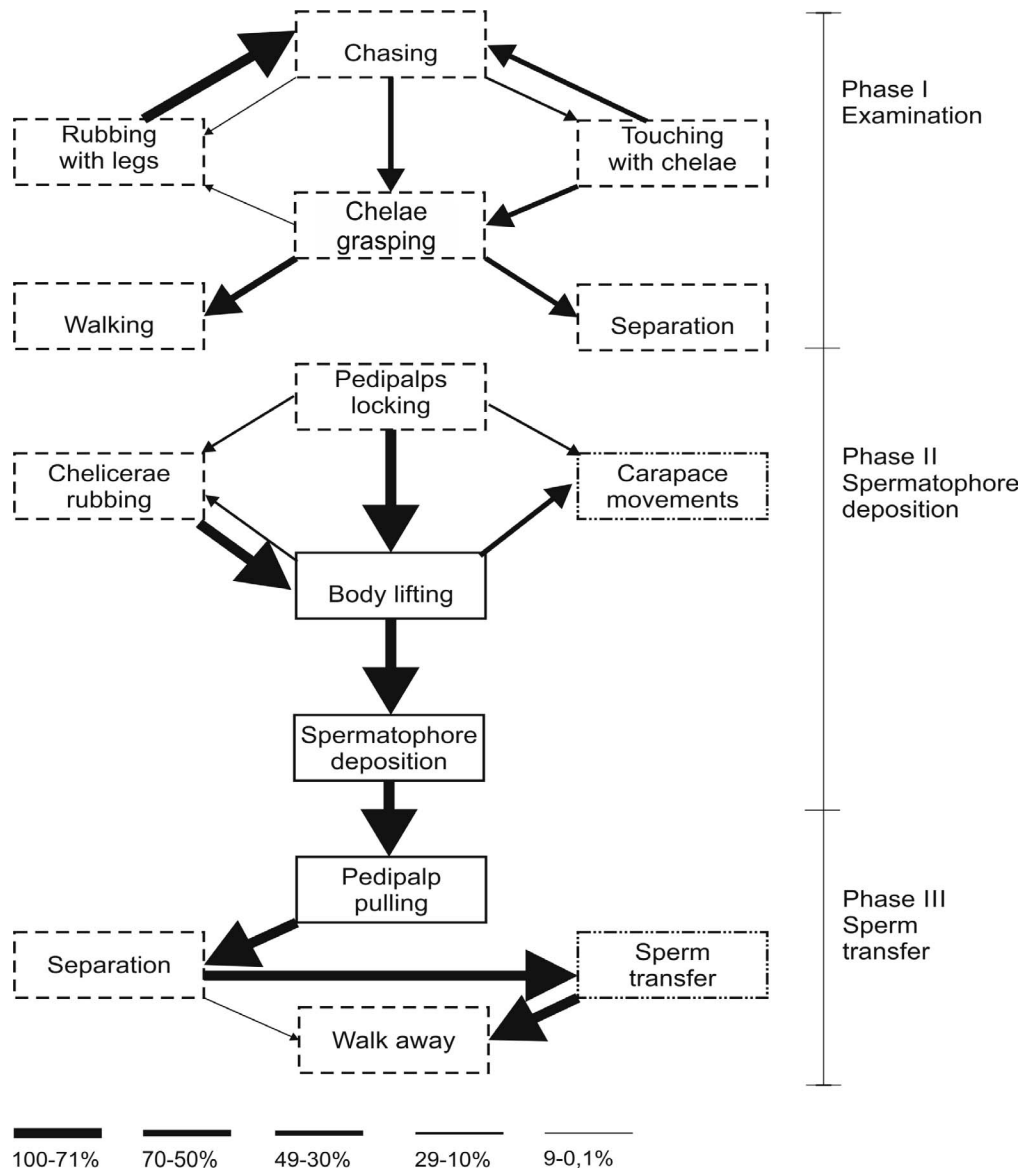


Figure 3.—Flow diagram showing the relationships between the behavioral units. The thickness of the arrows represents the frequency of transition between units. Boxes with continuous lines indicate behavioral units that were performed only by males, dashed lines represent units that were performed by females and mixed lines represent units that were performed by both individuals.

this phase and mating. The mean duration of this phase was  $10.1 \pm 6.4$  sec, the shortest of the three phases.

The mean duration of mating was  $418.0 \pm 146.9$  sec (approximately 7.5 min). All three phases and behavioral units are shown in a flow diagram (Fig. 3).

DISCUSSION

The chelae of female *Lustrochernes argentinus* are larger than those of males, as reported for most pseudoscorpions species (Zeh 1987). Some chernetid species, however, such as *Dinocheirus arizonensis* (Banks, 1901), *Barbaraella mainae* Harvey, 1995, *Cordylochernes scorpioides* (Linnaeus, 1758), among others, the male chelal size is 60%–150% larger than in females (Zeh 1987; Harvey 1995; Zeh & Zeh 2013). The chelal size in *L. argentinus* is 10% larger in females than in males.

Despite the larger chelae in females, the gap between the fingers is 40% wider in males, which could be explained by the male needing to provide a firm grip during mating, with the wider gap allowing better attachment to the female chelae. From a functional perspective of sexually dimorphic traits, similar situations in other arachnids have been observed. For example, in the scorpion *Zabius fuscus* (Thorell, 1877) males have gaps in the chelal fingers that are larger than those of the females, a feature that facilitates holding and moving together during mating (Peretti 1991, 2000, unpubl. data; Peretti & Carrera 2005).

In general, almost all chernetid species show common behavior patterns in their courtship. However, there are also small peculiarities that differentiate species from each other. For example, the behavioral phases described for the mating of *L. argentinus* partially match with those described for *M.*

*iporangae* by Andrade & Gnaspini (2003). As expected, we observed similarities in mating in both species (i.e., pedipalp grasping, locomotion and pedipalp moving, among other behavioral units) but there are other behaviors that are restricted to each species, such as the vibration of the forelegs in *M. iporangae* or cheliceral rubbing in *L. argentinus*.

Duration of the behavioral phases in *L. argentinus* is significantly different, even compared with other species of the family. In *L. argentinus*, what we called the “sperm transfer” phase includes the couple’s separation and the immediate use by the female of the deposited spermatophore, all with a mean duration of only nine seconds. In contrast, in *M. iporangae*, where these behaviors are divided into “spermatophore deposition and transfer” and “post-transfer” phases, the latter has a mean duration of 33–80 minutes, during which the male continues to hold the female’s pedipalps, and it is not until the end of the phase that the couple separates (Andrade & Gnaspini 2003).

On the other hand, another interesting difference is observed in *Epactiochernes tumidus* (Banks, 1895) with a mean mating duration of 15–30 minutes during which a new spermatophore is deposited by the male and transferred to the female every 5–7 minutes (Weygoldt 1966). These variations of duration between species could explain the differences in phase determination time, which is entirely subjective. However, as mentioned, sperm transfer and couple separation in *L. argentinus* occurs in only nine seconds, a rapid transition that would not be considered an exclusive “post-transfer” behavior. The mean duration of mating in *L. argentinus* is approximately seven minutes, which is considerably less than that of *M. iporangae* of  $63.3 \pm 13.9$  min, and even compared with other chernetid species, such as *Dendrochernes cyrneus* (L. Koch, 1873), 120–180 min (Kew 1912), *Lamprochernes minor* Hoff, 1949, 25–45 min (Levi 1953), and *Lasiochernes pilosus* (Ellingsen, 1910), with 15–60 min (Weygoldt 1969).

From a sexual selection perspective, female animals were traditionally thought of as passive participants during courtship and mating, but this could be due to a bias toward observing only male behavior in classic studies (Briceño & Eberhard 2017). Signaling in female arachnids and the effect that it has on the male is an essential feature in the intersexual interactions (Eberhard 2005; Peretti et al. 2006). In this context, the discovery that female *L. argentinus* actively performs the sperm transfer is a unique feature within pseudoscorpions, since it has not been observed in any other species. Preliminary studies also suggest that females may modulate this behavior and decide whether or not to use the spermatophore depending on the male parasitic burden (Palen-Pietri R. & A. Ceballos, 2017, unpubl. data). Hence, the female behavior of performing, or not, sperm transfer could respond to cryptic female choice (Eberhard 1996). In any case, as we mentioned, studies regarding mating behavior in pseudoscorpions are still scarce. It would be interesting to expand this line of knowledge in the group to determine if this behavior is observed in other species and what implication it has for sexual selection.

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