ZOOLOGY

Female genital morphology and mating behavior of *Orchestina* (Arachnida: Araneae: Oonopidae)

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ABSTRACT

The unusual reproductive biology of many spider species makes them compelling targets for evolutionary investigations. Mating behavior studies combined with genital morphological investigations help to understand complex spider reproductive systems and explain their function in the context of sexual selection. Oonopidae are a diverse spider family comprising a variety of species with complex internal female genitalia. Data on oonopid phylogeny are preliminary and especially studies on their mating behavior are very rare. The present investigation reports on the copulatory behavior of an *Orchestina* species for the first time. The female genitalia are described by means of serial semi-thin sections and scanning electron microscopy. Females of *Orchestina* sp. mate with multiple males. On average, copulations last between 15.4 and 23.54 min. During copulation, the spiders are in a position taken by most theraphosids and certain members of the subfamily Oonopinae: the male pushes the female back and is situated under her facing the female's sternum. Males of *Orchestina* sp. possibly display post-copulatory mate-guarding behavior. The female genitalia are complex. The genital opening leads into the uterus externus from which a single receptaculum emerges. The dorsal wall of the receptaculum forms a sclerite serving as muscle attachment. A sclerotized plate with attached muscles lies in the posterior wall of the uterus externus. The plate might be used to lock the uterus during copulation. The present study gives no direct evidence for cryptic female choice in *Orchestina* sp. but suggests that sexual selection occurs in the form of sperm competition through sperm mixing.

Introduction

Mating behavior observations in combination with a thorough investigation of the genital morphology have large potential consequences for the understanding of the reproductive biology of a particular species (e.g., Huber, 1994, 1995; Uhl et al., 1995; Huber and Eberhard, 1997; Burger, 2007). Especially fine genital morphological details help to understand the evolution of the genitalia and to explain their function in the context of sexual selection (Eberhard, 1985, 1996, 2004a,b; Galis, 1996; Hellriegel and Ward, 1998; Burger et al., 2003, 2006a,c; Huber, 2003, 2006; Alberti and Michalik, 2004; Burger and Kropf, 2007; Burger, 2008, 2009, 2010).

The unusual reproductive biology of many spider species makes them compelling targets for evolutionary studies (Eberhard, 2004a). According to the classification of Simon (1893), araneo-morph spiders were separated into the two groups Haplogynae and Entelegynae based on the gross morphology of their genitalia. In contrast to the more complex female genitalia of entelegynes, haplogynes are traditionally considered to have simple female genitalia (Wiehle, 1967; Austad, 1984; Uhl, 2002). Almost 3000 described species in 17 families belong to the Haplogynae (Platnick, 2009) and their monophyly seems to be well founded based on the origin of a cheliceral lamina, the basal fusion of the chelicerae, the fusion of the tegulum and subtegulum, and the loss of tartipores (Coddington and Levi, 1991; Platnick et al., 1991; Ramirez, 2000).

Investigations of genital morphology and copulatory mechanics in spiders have been carried out for entelegynes (e.g., van Helsdingen, 1965; Huber, 1993, 2004b; Uhl and Vollrath, 1998; Uhl and Gunnarsson, 2001; Berendonck and Greven, 2002, 2005; Dimitrov et al., 2007; Useta et al., 2007) and haplogynes (Huber, 1994, 1995, 1997, 1998, 2002, 2004a,b, 2006; Uhl, 1994, 1998, 2000, 2002; Uhl et al., 1995; Huber and Eberhard, 1997; Senglet, 2001; Burger et al., 2003, 2006a,c; Burger, 2007, 2008, 2009, 2010; Burger and Kropf, 2007; Fannes and Jocqué, 2008).

However, data on mating behavior and genital morphology are still missing for a majority of the haplogyne families.

Oonopidae are a highly diverse spider family belonging to the Haplogynae. They comprise a variety of species with rather complex internal female genitalia that do not correspond to the conventional type of haplogyne genitalia described by e.g. Wiehle (1967) (Dumitresco and Georgesco, 1983; Saaristo, 2001; Burger et al., 2003, 2006a; Burger, 2007, 2009, 2010; Fannes and Jocqué, 2008). The family is widespread in many habitats on all continents (except Antarctica) (e.g., Gerhardt, 1933; Chickering, 1951; Dumitresco and Georgesco, 1983; Harvey, 1987; Saaristo, 2001) and systematically placed within the group of the Dysderoidea (Coddington and Levi, 1991; Platnick et al., 1991). Especially the life history of most oonopids is unknown and descriptions of their mating behavior are very rare (Bristowe, 1929, 1930; Gerhardt, 1930, 1933; Burger, 2007). Relationships among oonopids are still largely unknown and many, perhaps most, of the genera have yet to be described. Two subgroups are conventionally recognized within Oonopidae (Simon, 1893) and often treated as subfamilies – the armored "loricati" or Gamasomorphinae and the soft-bodied "molles" or Oonopinae, which are presumably more basal oonopids. Chamberlin and Ivie (1945) highlighted the distinctiveness of the genus *Orchestina* from other oonopid genera by erecting the subfamily Orchestininae – a hypothesis which was not further considered in subsequent studies.

The present study reports on the copulatory behavior of a member belonging to the oonopine genus *Orchestina* for the first time. The female genitalia are described in detail by means of serial semi-thin sections and scanning electron microscopy. In addition, the male palp is briefly described. Mating behavior and functional aspects of the female genitalia are discussed in the context of previous studies on haplogynes.

Material and methods

Specimens

4 ♀ (PBI_OON 14890, PBI_OON 14905 MACN–Ar 17674, PBI_OON 14907 MACN–Ar 17675, PBI_OON 14908 MACN–Ar 17676), 4 ♂ (PBI_OON 14879 MACN–Ar 17718, PBI_OON 14882 MACN–Ar 17714, PBI_OON 14922 MACN–Ar 17678, PBI_OON 14924 MACN–Ar 17677), Argentina, Jujuy, Parque Nacional Calilegua, Seccional Aguas Negras, 23°45′43.3″S, 64°51′04.7″W (± 10 m, WGS84), elev. 605 m (GPS), col. C. Grismado, M. Izquierdo, F. Labarque, G. Rubio, M. Burger, P. Michalik, P. Carrera, A. Ojanguren, C. Mattoni, 6–11 December 2008, beating foliage; 2 ♀ (PBI_OON 14895 MACN–Ar 18016, PBI_OON 14896 MAC–Ar 18015), 2 ♂ (PBI_OON 14895 MACN–Ar 18016), same data, col. M. Izquierdo, L. Zapata, M. Akmentins, 27–31 January 2009. The material is deposited in the collection of arachnids of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN–Ar, Cristina Scioscia).

Mating behavior

The spiders were mature when collected and thus their mating history was unknown. The specimens collected on December 6–11, 2008 were brought to the Laboratorio de Biología Reproductiva y Evolución of the Universidad Nacional de Córdoba. The females were individually kept in plastic boxes (9 cm × 6.5 cm × 2.5 cm) with ground gypsum. A small plastic cap (2.5 cm diameter) containing a humid paper towel (5 cm × 10 cm) was added in each box. The towel was moistened every other day with a few drops of water. The males were individually kept in Eppendorf tubes (1.5 ml) together with a small paper towel moistened every other day. The spiders were not fed. Three females and three males were used for the mating behavior studies.

Copulations were observed with a binocular microscope (Nikon SMZ 1500, Nikon Instruments Inc., Tokyo, Japan) with attached digital camera. Photographs of the matings were taken at different focus levels, combined into a single shot using the software CombineZP, and partially edited using Adobe Photoshop Elements 2.0 (Adobe Systems Inc., San Jose, CA, USA).

For each pairing, the male was carefully removed from his tube and placed into the female's box. The first palpal insertion by the male was taken as the beginning of copulation. The end of copulation was defined as the moment when the spiders physically separated, which was also the moment when the copulatory organs were decoupled. In the results section, copulation durations are given as averages ± standard deviation. Post-copulatory behavior was observed for 15 min in each case before the spiders were separated. All females were mated three times in the lab (once with each male) and were given a resting period of 1–3 days between each copulation. The copulations are referred to as first, second, and third copulation, respectively. One female was mated twice on the same day with a resting period of 1 h between the copulations. The females were sacrificed one day after their third copulation using ethanol.

Light microscopy

The opisthosomas of the three females observed in the mating tests were dehydrated in a graded ethanol series, embedded in soft-grade acrylic resin (LR White; London Resin Co., London, UK) and semi-thin serially sectioned (1 μm) with a microtome (Sorvall JB-4, Thermo Fischer Scientific Inc., Waltham, MA, USA) using glass knives. The sections were stained with toluidine blue (1%) in an aqueous borax solution (1%) at approximately 90 °C for 30–60 s. Light microscopic studies were performed with an Olympus BH-2 (Olympus Corp., Tokyo, Japan) and a Leica DM2500 (Leica Microsystems GmbH, Wetzlar, Germany). The sections were photographed with a Leica DFC500 digital camera (Leica Camera AG, Solms, Germany) and partly edited using Adobe Photoshop Elements. The palp of one mated male (PBI_OON 14924) was detached, embedded in Hoyer's medium and slide-mounted. Drawings were made with an ink pen on finely granulated paper under an Olympus BH-2 light microscope with attached drawing tube and then shaded with a graphite pen. The drawings were scanned and edited using Adobe Photoshop Elements 2.0. The genitalia of one female (PBI_OON 14896) were embedded in clove oil, slide-mounted and observed under an Olympus BH-2 light microscope. Photographs were taken with a digital camera (Nikon DXM1200; Nikon Instruments Inc., Tokyo, Japan) and the focal planes were combined with Helicon Focus 3.10.3 (http://helicon.com.ua/heliconfocus/).

Scanning electron microscopy

The genitalia of two females (PBI_OON 14895) were dissected and kept in a borax–pancreatin solution for 1–2 h according to the protocol of Alvarez-Padilla and Hormiga (2007). After digestion the genitalia were flushed with a micropipette in distilled water until all the soft tissues were removed. The female genitalia and one male palp (PBI_OON 14924) were dehydrated in a graded ethanol series (80–100%), critical-point dried, and gold-sputtered. Scanning electron micrographs were taken under high vacuum with a FEI XL30 TMP (FEI Company, Hillsboro, OR, USA).

Results

Mating behavior

Pre-copulatory behavior

All of the females were sitting inside a little sheet web, which they had constructed on the side or in a corner of the box. When a male was placed in the female's box, he usually walked around. When he came into contact with the threads of a female's web, the male commenced searching for the female. He usually walked over the web for several times and searched for an opening. The female then reacted by turning (if necessary) and facing him. No male was ever seen filling his pedipalps with sperm prior to copulation. The male quickly advanced toward the female until both spiders touched each other's front legs (Fig. 1A). From there, the male either took the copulatory position directly by pushing the female back and creeping under her (Fig. 1B) or both palpated each other with their front legs first. In the latter cases, both spiders typically raised their cephalothorax and touched each others' metatarsi and tarsi of the first legs. The bouts lasted approximately 3–8 s. It could not be determined whether the palpating was initiated by the female or the male.

Special cases

(i) One male displayed a slightly different behavior prior to each of his three copulations: after coming in contact with the female (as described above), the male seemed to hesitate. He moved back and away from the female again. The female followed him and touched his first legs slightly. The male usually did not react and the female moved back again. This behavior could be repeated several times before the male finally made a quick advance toward the female and took the copulatory position. (ii) In one pairing [third pairing of the female; different male than in (i)], the female showed seemingly aggressive behavior. As soon as the male approached her, she scared him away with vigorous vibrations of the body, especially the front legs. The male moved back and stayed outside of the web for a few seconds. Then he approached the female again and she showed the same behavior. This behavior was repeated four times before the male managed to creep under the female and to take the copulatory position. The female moved back and tried to turn around but the male managed to copulate anyway. (iii) The female that was mated twice on the same day left the web after the second copulation and constructed a new web in another corner of the box. When a male was placed into the box the next day, he came in contact with the threads of the old web and commenced searching for the female there. He stayed inside the web. After approximately 15 min the male was removed from the web by the authors and brought close to the female. He immediately approached the female and displayed the pre-copulatory behavior described above.

Copulatory behavior

The first copulations lasted 18.11 ± 3.04 min, the second copulations 15.40 ± 3.35 min, and the third copulations 23.54 ± 5.29 min. In the copulatory position the male was under the female facing her sternum (Fig. 1C). The male palps were inserted simultaneously and moved alternately during the entire copulation. Only one palp moved at a time whereas the other remained motionless. The palp moved up and down and back and forth. The palp movements stopped approximately 2 min before the end of copulation. In some cases, the female slightly tapped the male with her prosoma approximately 30 s before separation. When the spiders separated, the male quickly moved back and both spiders palpated each other with the front legs for a few seconds. Sometimes, the female scared the male away with vibrations of her body and then turned around.

Post-copulatory behavior

After the separation, the male stayed close to the female's web (Fig. 1D) and showed intense self-grooming. He often ran his pedipalps through the chelicerae. The male kept walking over the female's web and spun threads over it. When he contacted the female by typically touching her front legs with his front legs, she scared him away with slight movements of the front legs and turned around. Sperm re-induction by the male could not be observed.

Morphology of the female genitalia

The slit-like genital opening (GO in Figs. 2A, B, 3A, B, 4A) of female Orchestina sp. lies in the epigastric furrow. It is bordered by a thick sclerotized ridge (Ri1 in Figs. 2A, B, 3A, D, 4A) anteriorly and paired short sclerotized ridges (Ri2 in Figs. 2A, 3A) posteriorly. Anterior of the genital opening, the cuticle of the opisthosoma forms paired semicircular apodemes (Ap in Figs. 2A, 3A, 4A, B) connected by a thin sclerotized ridge (Ri3 in Figs. 2A, B, 3A, 4B). The genital opening leads into the uterus externus (UE in Figs. 2B, 3B, 4D), which forms a median anterior fold (Fo in Figs. 2B, 3B). Laterally, the uterus externus is reduced to a short fold (arrowheads in Fig. 2A). A single bulge-like receptaculum (Re in Figs. 2A, B, 3A–D, 4C) emerges anteriorly from the uterus externus. The receptaculum is reduced to a small fold laterally (arrows in Fig. 2A). In all of the investigated females, spermatozoa (Sp in Fig. 3C, D) surrounded by secretion (Sec in Fig. 3C) were present in the receptaculum. On semi-thin sections, the spermatozoa appear as small dark particles (see Fig. 3C). The dorsal wall of the receptaculum forms a massive sclerite (Sc in Figs. 2A, B, 3A, B, D, 4B, C), which extends considerably toward anterior. The anterior part of the sclerite shows two lateral protrusions (Pr in Figs. 2A, C, 3A, E, 4B, C). Inside the anterior part of the sclerite, a large hollow space (see Figs. 2A, B, 3A, B, E) connects to the lumen of the receptaculum via a small circular cavity (Ca in Figs. 2A, B, 3A, E). The hollow space in two of the investigated females seemed to contain a small amount of secretion (Sec in Fig. 3D). The cavity continues into a curved slit (Sl in Figs. 2A, B, 3A, B, E) proceeding through the posterior part of the sclerite. The slit is connected with the lumen of the receptaculum (see Fig. 2B). A thin lamella (La in Figs. 2B, 3C) emerges posteriorly from the sclerite and reaches into the lumen of the receptaculum. A hook-like posterior extension (Ho in Figs. 2A, B, 3B) of the sclerite reaches into a depression (Dp in Figs. 2A, B, 3B) of a massive plate (Pl in Figs. 2A–C, 3A, B, D, E, 4B–D). The ventral extensions of the plate (Ex1 in Figs. 2A, 4B, D) are fused with the posterior wall of the uterus externus (see Fig. 4D). The plate is curved forward and expands considerably toward dorsal (see Figs. 2B, 3B, 4B, C). It forms massive lateral extensions dorsally (Ex2 in Figs. 2A, C, 3A, 4B, C).

The plate (Pl in Fig. 2C) serves as attachment site for various muscles. A paired muscle set (M1 in Fig. 2C) runs from the lateral parts of the plate toward ventral and attaches on the cuticle of the opisthosoma. Another muscle set (M2 in Figs. 2C, 3A, E) connects the lateral extensions of the plate (Ex2 in Fig. 2C) with the lateral protrusions (Pr in Fig. 2C) of the sclerite. The anterior part of the sclerite serves as attachment site for muscles (M3 in Figs. 2C, 3A, B, E) running to the ventral cuticle of the opisthosoma. Paired thin muscles (M4 in Figs. 2C, 3A) originate on the lateral extensions of the plate and are directed laterally, ending on the cuticle of the opisthosoma.

Morphology of the male palp

The tibia of the male palp of Orchestina sp. is swollen (see Fig. 5). The large, pyriform palpal bulb (PBu in Fig. 5)
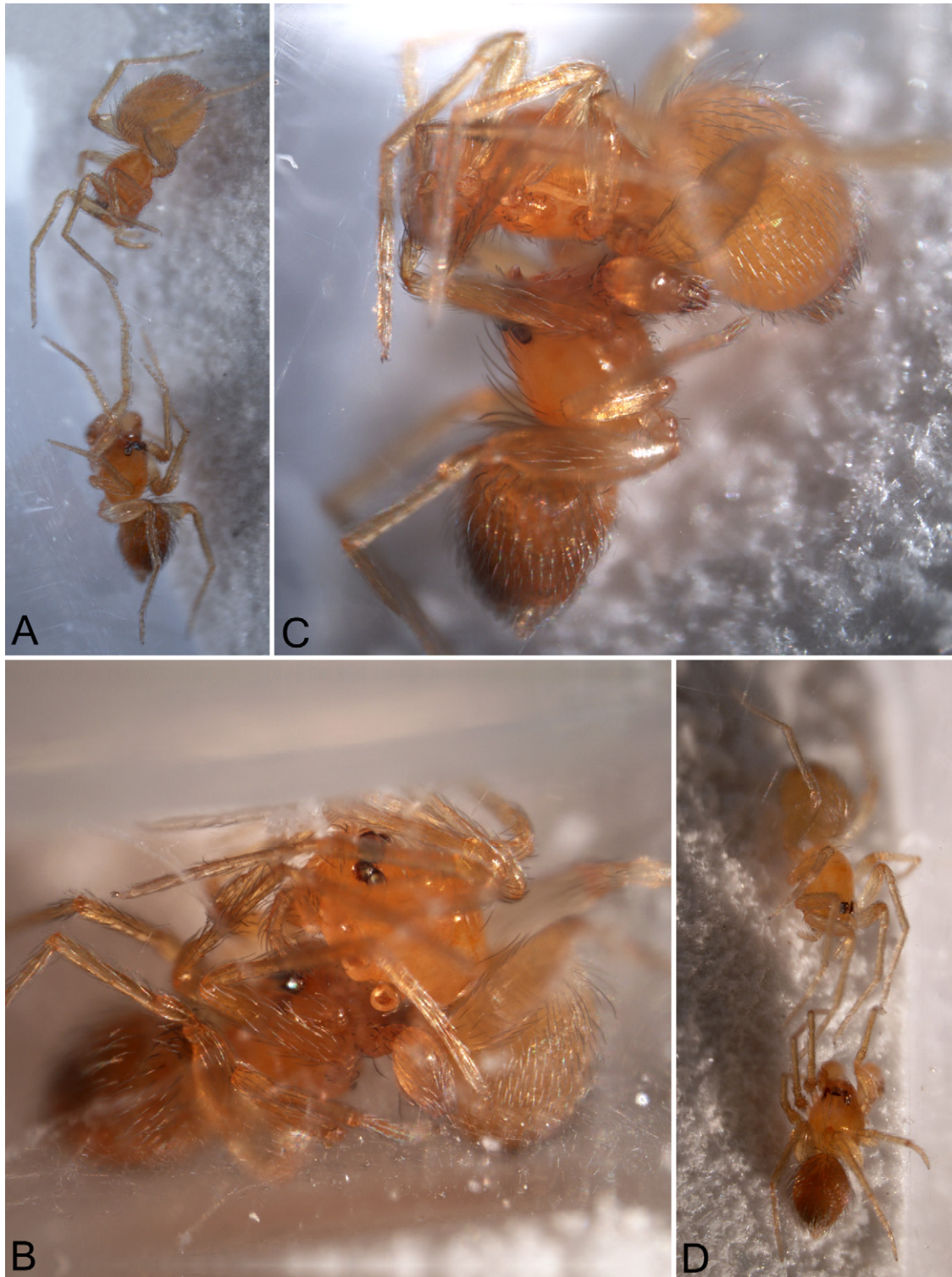
Fig. 1. Female and male of *Orchestina* sp. prior, during, and after copulation. Male at the bottom in (A), (C), (D), on the left in (B). (A) Male approaches female prior to copulation and both spiders touch each other's front legs. (B) Male pushes female back and creeps under her to take copulatory position. (C) Spiders in copula. (D) Male stays close to female after copulation.

continues into a short embolus (Em in Figs. 4E, F, 5) with the opening (EOp in Fig. 4E, F) situated at the tip. A small denticle (De in Figs. 4E, 5) is present at the base of the embolus on the prolateral side. The sclerotized sperm duct (SD in Fig. 5) is strongly curved and visible through the cuticle of the palpal bulb. Some areas of the sperm duct have pores (see arrowheads in Fig. 5) into which presumably gland ducts are leading.

Discussion

Mating behavior

Male spiders often display extensive courtship behavior prior to copulation (e.g., Maklakov et al., 2003; Cross et al., 2008; Gibson and Uetz, 2008). However, in haplogynes, the courtship by
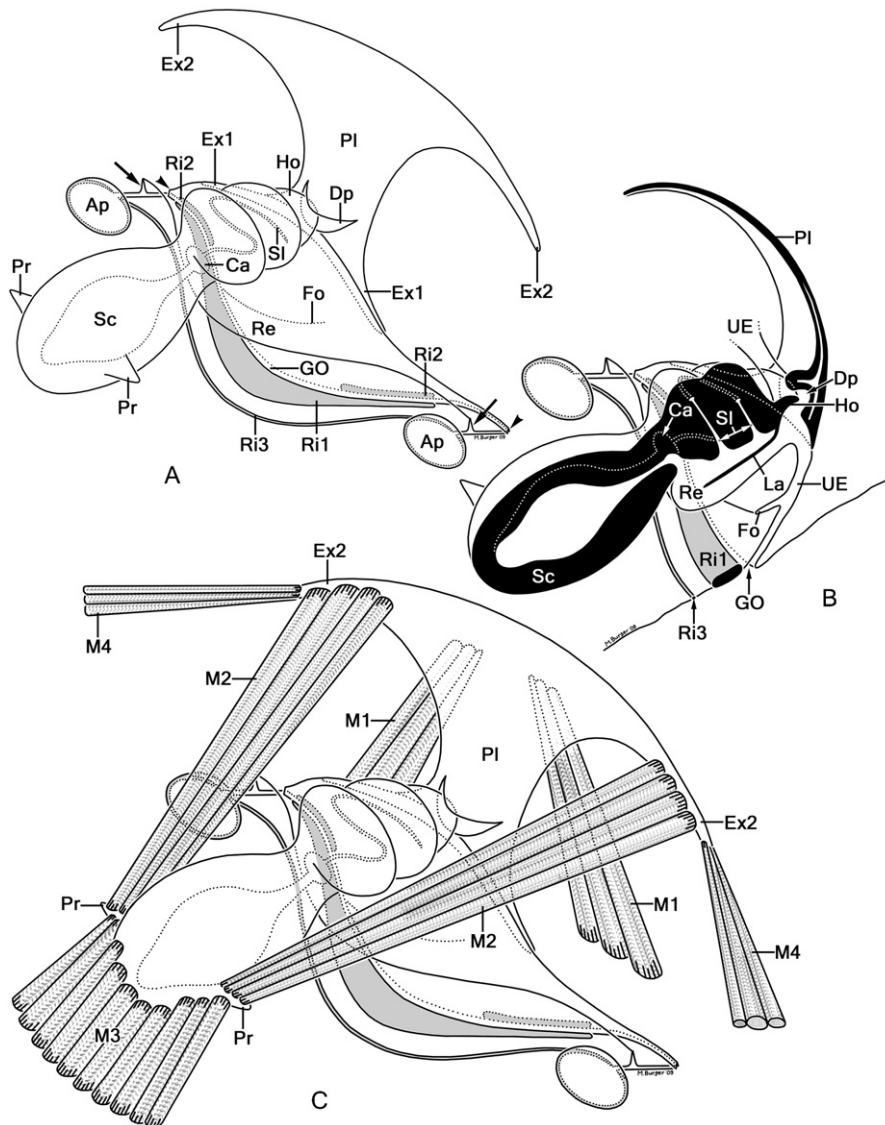
Fig. 2. Schematic drawings of female genitalia of *Orchestina* sp. (A) Overview. Arrowheads indicate lateral parts of the uterus externus, arrows point to lateral parts of the receptaculum (see text for details). (B) Median longitudinal section. (C) Genitalia with muscles. Abbreviations: Ap, apodeme; Ca, circular cavity; Dp, depression; Ex1, Ex2, lateral extensions of plate; Fo, fold; GO, genital opening; Ho, hook-like extension; La, lamella; M1–M4, muscles 1–4; Pl, plate; Pr, protrusion; Re, receptaculum; Ri1–Ri3, sclerotized ridges; Sc, sclerite; Sl, slit; UE, uterus externus.

males (if there is any at all) is restricted to vibrations of the opisthosoma or simple leg and palp movements (e.g., Bristowe, 1929; Gerhardt, 1929; Uhl et al., 1995; e.g., Bartos, 1998; Huber, 2002; Burger et al., 2006a). In the observed matings of *Orchestina* sp., male and female palpated each other with the front legs before copulation, which could represent some sort of pre-copulatory courtship behavior. Some of the variations – especially in the pre-copulatory behavior – described under "special cases" might be explained by the different mating history of the spiders. The palpation of the legs prior to copulation was also described for the oonopine *Oonops placidus* by Gerhardt (1930). Interestingly, Gerhardt (1930) also stressed the fact that the palps in *O. placidus* were inserted simultaneously but moved differently from other haplogynes that insert both palps at the same time such as certain dysderids (e.g., Jackson and Pollard, 1982) and pholcids (e.g., Huber, 1995; Uhl et al., 1995): only one palp moved at a time, whereas the other remained motionless. The present study shows that the same pattern of palp movements occurs in *Orchestina* sp.

In the most "primitive" copulatory position the male approaches the female frontally and creeps under her by pushing her back. The palps are inserted simultaneously or alternately (see von Helversen, 1976). This position is taken by most theraphosids (e.g., Gerhardt, 1929; Yáñez et al., 1999) and certain haplogynes such as *Segestria bavarica* (Gerhardt, 1929) and *Dysdera crocata* (Jackson and Pollard, 1982). The "primitive" copulatory position also occurs in members of the oonopine genera *Oonops* (Bristowe, 1929; Gerhardt, 1930; see also von Helversen, 1976) and *Orchestina* (as the present study shows), which are presumably more basal oonopids. A more "derived" copulatory position has evolved convergently in different spider groups: the male moves back and both spiders turn their ventral sides toward each other facing in the same direction (von Helversen, 1976). This position is adopted by certain gamasomorphine oonopids such as *Xestaspis nitida* (Gerhardt, 1933), *Grymeus robertsi* (Harvey, 1987), and *Silhouettella loricatula* (Bristowe, 1930; Burger, 2007), as well as by certain members of the family Tetrablemmidae (Burger et al., 2006a; Edwards and Edwards, 2006).
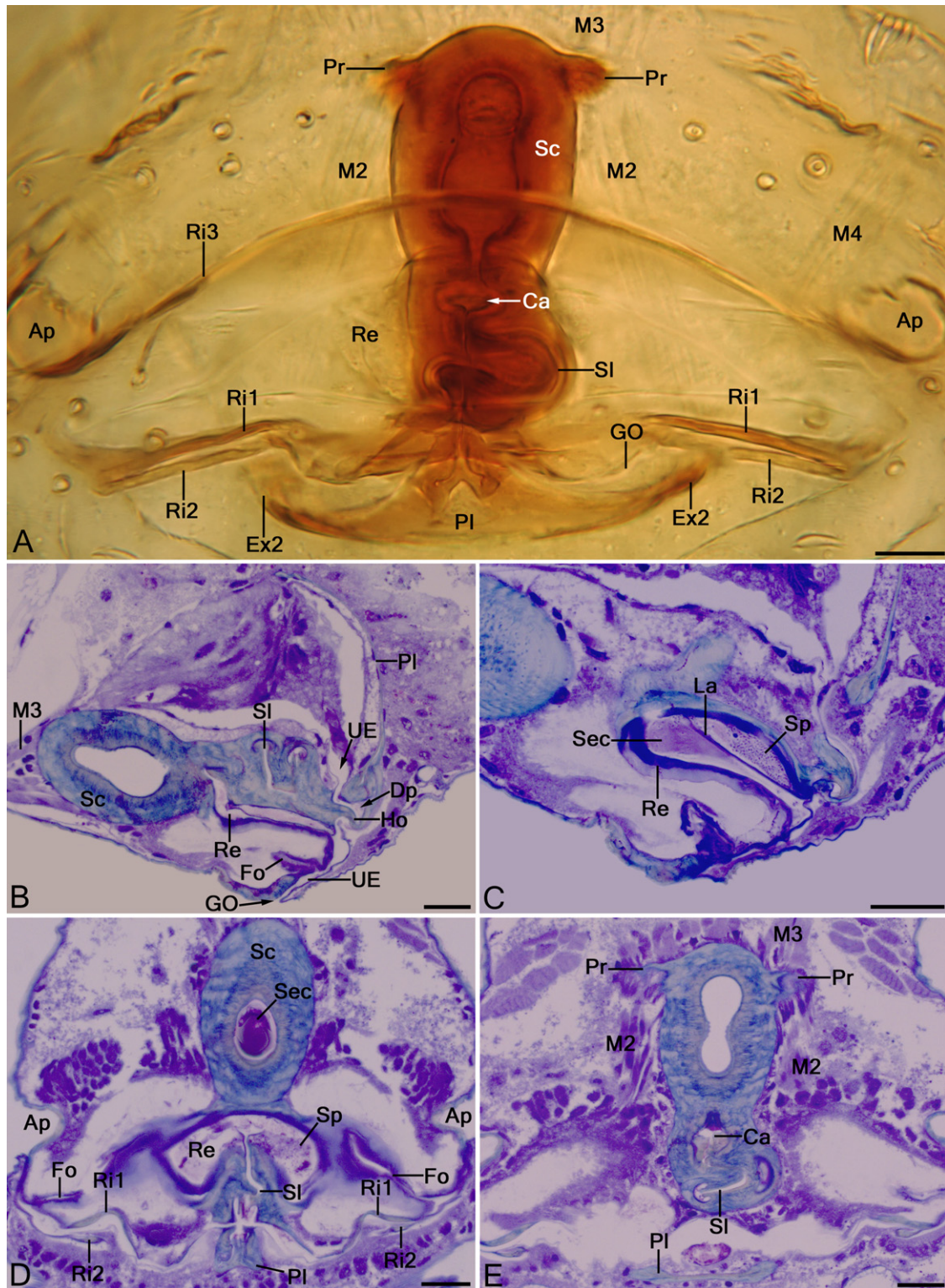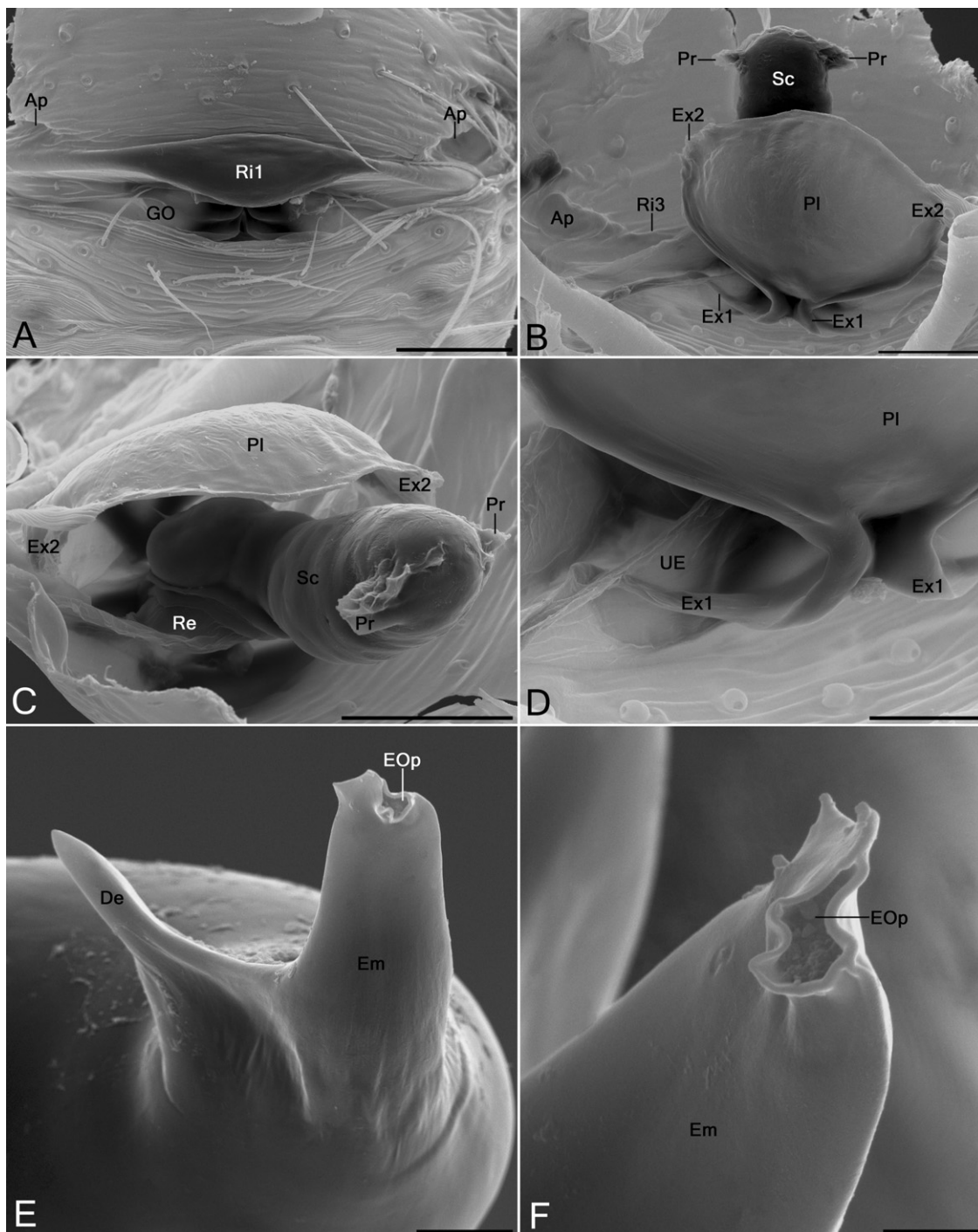
Fig. 3. Female genitalia of *Orchestina* sp., light microscopy. (A) Slide-mounted genitalia embedded in clove oil, dorsal view. (B) Median longitudinal section showing sclerite and plate. (C) Longitudinal section showing receptaculum that contains spermatozoa and secretion. (D) Horizontal section showing ventral part of receptaculum and sclerite. (E) Horizontal section showing dorsal part of sclerite with attached muscles. Scale bars: 20 µm. Abbreviations: Ap, apodeme; Ca, circular cavity; Dp, depression; Ex2, lateral extension of plate; Fo, fold; GO, genital opening; Ho, hook-like extension; La, lamella; M2–M4, muscles 2–4; Pl, plate; Pr, protrusion; Re, receptaculum; Ri1–Ri3, sclerotized ridges; Sc, sclerite; Sec, secretion; Sl, slit; Sp, spermatozoa; UE, uterus externus.

After copulation, the male of *Orchestina* sp. always stayed close to the female and sometimes spun threads over her web. Males accompanying females after copulation were observed in other haplogynes, such as the pholcid *Physocyclus globosus* (Eberhard, 1992; Huber and Eberhard, 1997). In some insects and spiders, the male guards the female prior to or after copulation in order to restrict access of other males to the female, thus guarding and protecting his own transferred ejaculate (e.g., Sillén-Tullberg, 1981; Schöfl and Taborsky, 2002; Prenter et al., 2003; Wynn and Vahed, 2004). However, whether the behavior displayed by male

Fig. 4. Female and male genitalia of *Orchestina* sp., scanning electron micrographs. (A) Female genital region, ventral view. (B) Internal female genitalia showing sclerite and plate, dorsal view. (C) Internal female genitalia showing sclerite and plate, antero-dorsal view. (D) Detail of plate, postero-lateral view. (E) Left embolus of male, antero-lateral view. (F) Left embolus of male with opening, apical view. Scale bars: 50 μm (A–C), 20 μm (D), 10 μm (E), 3 μm (F). Abbreviations: Ap, apodeme; De, denticle; Em, embolus; EOp, embolus opening; Ex1, Ex2, lateral extensions of plate; GO, genital opening; Pl, plate; Pr, protrusion; Re, receptaculum; Ri1, Ri3, sclerotized ridges; Sc, sclerite; UE, uterus externus.

Orchestina sp. serves as post-copulatory mate guarding remains unknown at this point.

Morphology of the female genitalia

Previous studies proved that the female genitalia of certain oonopids are astonishingly complex and clearly differ from the concept proposed for the group Haplogynae (Dumitresco and Georgesco, 1983; Burger et al., 2003, 2006a; Burger, 2007, 2009,

2010; Fannes and Jocqué, 2008). A widely accepted theory nowadays is that complicated genital structures have evolved under sexual selection by cryptic female choice (Thornhill, 1983; Eberhard, 1985, 1996), which is defined as female behavior, physiology or morphology that biases sperm precedence in favor of certain males against others after the beginning of copulation (Eberhard and Cordero, 1995).

The female genital structures of *Orchestina* sp. are complex and resemble the genitalia of the oonopine *Stenoonops reductus*
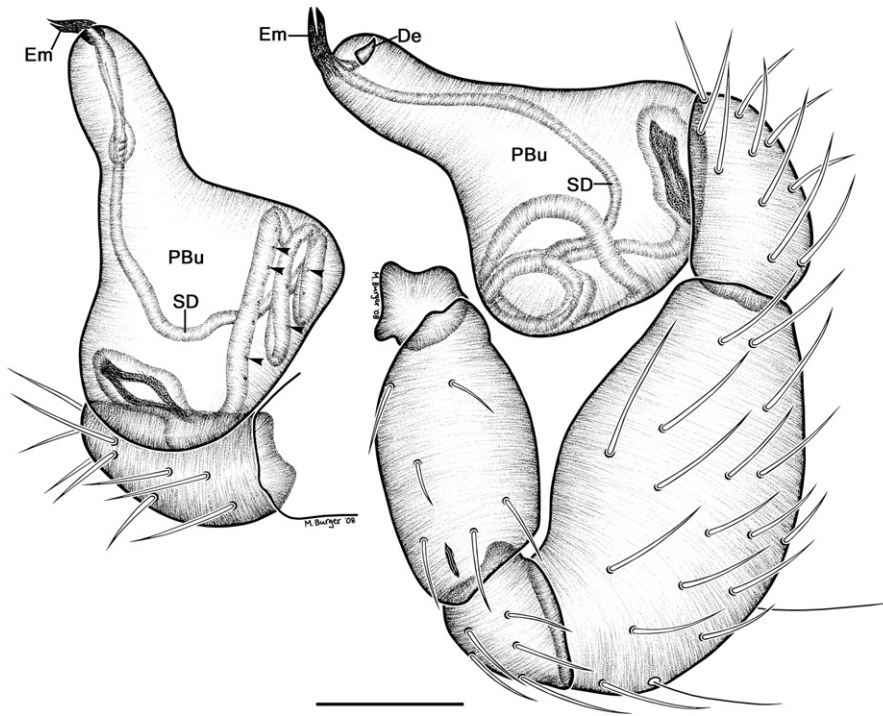
Fig. 5. Drawings of right palp of male *Orchestina* sp., prolateral view; retrolateral view of palpal bulb on the left. Arrowheads pointing to pores on sperm duct. Scale bar: 100 μm. Abbreviations: De, denticle; Em, embolus; PBu, palpal bulb; SD, sperm duct.

(Burger, 2009) and the dysderid *Harpactea lepida* (Burger and Kropf, 2007). In all three species, there is a massive anterior sclerite with an internal lumen and a large sclerotized plate with extensions situated in the posterior wall of the uterus externus. Both of these sclerotized structures serve as attachments for muscles (M1–M4 in Fig. 2C; M4 lacking in *H. lepida*) whose arrangement is also comparable in the three species. Interestingly, a number of gamasomorphine oonopids show a modified sclerite situated in the uterus wall. Apparently, the sclerite can be moved back and forth by muscle contractions, suggesting that the uterus externus can be locked, which might prevent sperm from getting into it during copulation (Burger et al., 2003, 2006b; Burger, 2010, 2009). The sclerotized plate (Pl in Fig. 2A–C) in *Orchestina* sp. might have the same function. However, for *S. reductus* and *H. lepida* it was suggested that the plate helps to move the spermatozoa into the receptacula (*S. reductus*; see Burger, 2009) and the uterus externus (*H. lepida*; see Burger and Kropf, 2007), respectively.

The present study shows that the anterior sclerite in *Orchestina* sp. extends from the sclerotized dorsal wall of the receptaculum where sperms are stored. A similar situation occurs in *H. lepida* where roundish sclerotized structures containing sperm are connected with the anterior sclerite (Burger and Kropf, 2007). The large sperm storage organs in *Stenoonops reductus*, however, have no connection to the anterior sclerite (Burger, 2009). Forster and Platnick (1985) proposed that the development of a posterior diverticulum as sperm storage organ represents a synapomorphy for the Dysderoidea (including the families Orsolobidae, Oonopidae, Dysderidae, and Segestriidae). According to these authors the primitive dysderoid genitalia consisted of an anterior receptaculum in association with a posterior secretory gland system, which was modified into a posterior diverticulum (or receptaculum) in certain dysderoid genera (Forster and Platnick, 1985). Apparently, in the investigated *Orchestina* sp. in the present study the posterior diverticulum is missing, which is also seen in orsolobids belonging to the genus *Subantarctia* (Forster and Platnick, 1985). In some gamasomorphine oonopids, however, the anterior receptaculum seems to be reduced (compare Burger et al., 2003, 2006b; Burger, 2010).

A study on the gamasomorphine *Silhouettella loricatula* showed that females of this species dump sperm of previous males during subsequent matings and thus might be able to exert cryptic female choice (Burger, 2007). Sperm dumping was suggested for other gamasomorphines according to the morphology of the female genital tracts (Burger et al., 2003; Burger, 2010). The present study gives no morphological or behavioral evidence for sperm dumping in *Orchestina* sp. However, sperm ejection during or after copulation cannot be ruled out completely. The female genital morphology of *Orchestina* sp. suggests that the ejaculates of different males mix inside the receptaculum as it occurs in the pholcid *Pholcus phalangioides* (Uhl, 1998; Yoward, 1998). In conclusion, the present study gives no direct evidence for cryptic female choice in *Orchestina* sp., but rather suggests that sexual selection occurs in the form of sperm competition through sperm mixing (see Birkhead and Møller, 1998), which could be influenced by post-copulatory mate guarding by the male.

Acknowledgements

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