NOTE

INTERNAL FEMALE GENITALIA OF THE ORB-WEAVER *Leucauge argyra* (ARANEAE: TETRAGNATHIDAE) AND ITS POSSIBLE INFLUENCE ON SPERM DYNAMICS

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ABSTRACT

We describe the genital morphology of females of the Entelegyne spider *Leucauge argyra* Walckenaer, 1842. Externally, *L. argyra* has a unique coned shaped epigynal process. On each side of the epigynum there is an atrium that leads internally into a copulatory fold-like-duct, which connects with the first of two chambers of the spermathecae. The second chamber is surrounded by glandular tissue, which may produce mating plugs. The conspicuous epigynum and the copulatory fold-like-duct may be unique traits in this genus.

Keywords: epigynum, genital morphology, mating plug, spermathecae.

RESUMEN

Genitalia femenina interna de la araña *Leucage argyra* (Araneae: Tetragnathidae) y su posible influencia sobre la dinámica del esperma. Externamente, el epigino está formado por una protuberancia única en forma de cono. Ambos lados del epigino presentan un atrio. Cada atrio dirige internamente hacia un ducto copulatorio en forma de pliegue, que conecta con la primera de dos cámaras de la espermateca. La segunda cámara está rodeada de tejido glandular. Proponemos que el tapón copulatorio podría ser producido en esta cámara y luego transportado al exterior del atrio. El epigino conspicuo y el pliegue copulatorio podrían ser rasgos únicos dentro de este género.

Palabras clave: epigino, espermateca, morfología genital, tapón copulatorio.

The morphology of the female genitalia in spiders is important because it can influence internal processes that may affect sperm usage during and after copulation. Sites of sperm deposition and storage can influence sperm competition, and can be essential for establishing sperm priority patterns (Austad, 1984; Uhl & Vollrath, 1998; Kaster & Jakob, 1997; Uhl, 2000; Berendonck & Greven, 2002; Danielson-Francois, 2002). Female internal genitalia can have an effect on cryptic female choice (Burger *et al.*, 2003; Berendonck & Greven, 2005), egg fertilization

(Eberhard, 2004) and even the sex ratio of the offspring (Uhl & Gunnarsson, 2001). Additionally, female morphology can influence the feasibility of plugs after copulation (Eberhard & Huber, 1998; Aisenberg & Eberhard, 2009; Uhl *et al*., 2010).

Genital morphology in spiders may also influence copulatory behaviors. Studies in the genus *Leucauge* White, 1841 attempt to relate the external morphology of the genitalia and its sexual behavior (Aisenberg & Eberhard, 2009; Aisenberg & Barrantes, 2011; Barrantes *et al*., 2013), but the internal genital morphology of females of the spider *Leucauge argyra* Walckenaer, 1842 is still undescribed. Mated females of this species produce a copulatory plug that covers a large portion of the epigynum. This plug is formed during copulation or in the next hours, which suggests it is produced by the female (Aisenberg & Barrantes, 2011; Barrantes *et al*., 2013). The plug serves as a sticky trap for cannibalizing the male (Aisenberg & Barrantes, 2011) or it prevents the intromission by a second male (Barrantes *et al*., 2013). Given the importance of increasing the knowledge in female genitalia and complementing the understanding of the sexual behavior of *L. argyra*, the aim of this study is to describe the genital morphology of females of this species and attempt to link it with possible sperm dynamics inside the female tract.

We captured non-virgin females, recognized by the presence of a mating plug on the epigynum, and penultimate female nymphs, recognized by being accompanied by mature males (Aisenberg & Barrantes, 2011; Barrantes *et al*., 2013), of *L. argyra* in a plantation of African oil palm (*Elaeis guineensis*) at Parrita, Puntarenas province, Costa Rica (09º30'N, 84º10'W, 10 masl), between 2010 and 2012. Penultimate females were used to obtain adult virgin females. We preserved all adult females in 70% ethanol.

For anatomical analyses, we removed the epigynum with the surrounding tissue and prepared serial thin sections. The epigyna of 14 copulated and three virgin females were dehydrated in a series of acetone concentrations, embedded in 100% Spurr´s Hard resin, and positioned in a Beem capsule (see Eberhard & Huber, 1998). Sections were cut using a Power Tome Pc RMC Products Ultramicrotome with diamond or glass blades. The thickness of the sections varied from 0.5 to 1 µm. From the hundreds of sections produced from each specimen, we mounted the first ten of every twenty sections on a glass slide and discarded the last ten because they were very similar. We stained the sections with Methylene blue and photographed them with an Olympus dp71 camera adapted to an Olympus IX51 inverted light microscope. To obtain additional details of female genitalia, we dissected and cleared three epigyna from mated females in 10% KOH.

Externally, *L. argyra* has a unique and easily distinguishable epigynum that consists of a large, conical process that extends ventrally (Barrantes *et al*., 2013; Fig. 1). The anterior surface of this ventral process has abundant thick, long setae, especially near its tip and around its base; the posterior surface is naked. On the anterior-lateral surface, near the base of the ventral process, each atrium opens laterally on each side (Fig. 1a). The atria and often the entire ventral process of copulated females were covered with a copulatory plug (Fig. 1b).

Internally, each atrium leads into a duct with a long and narrow entrance, having the overall appearance of a flattened tube or fold-like duct (not a cylindrical duct). This fold was connected to the opening of the first of two chambers of the spermathecae (Chamber I). The second chamber (Chamber II) was connected to Chamber I via a narrow passage that ranged from 5 to

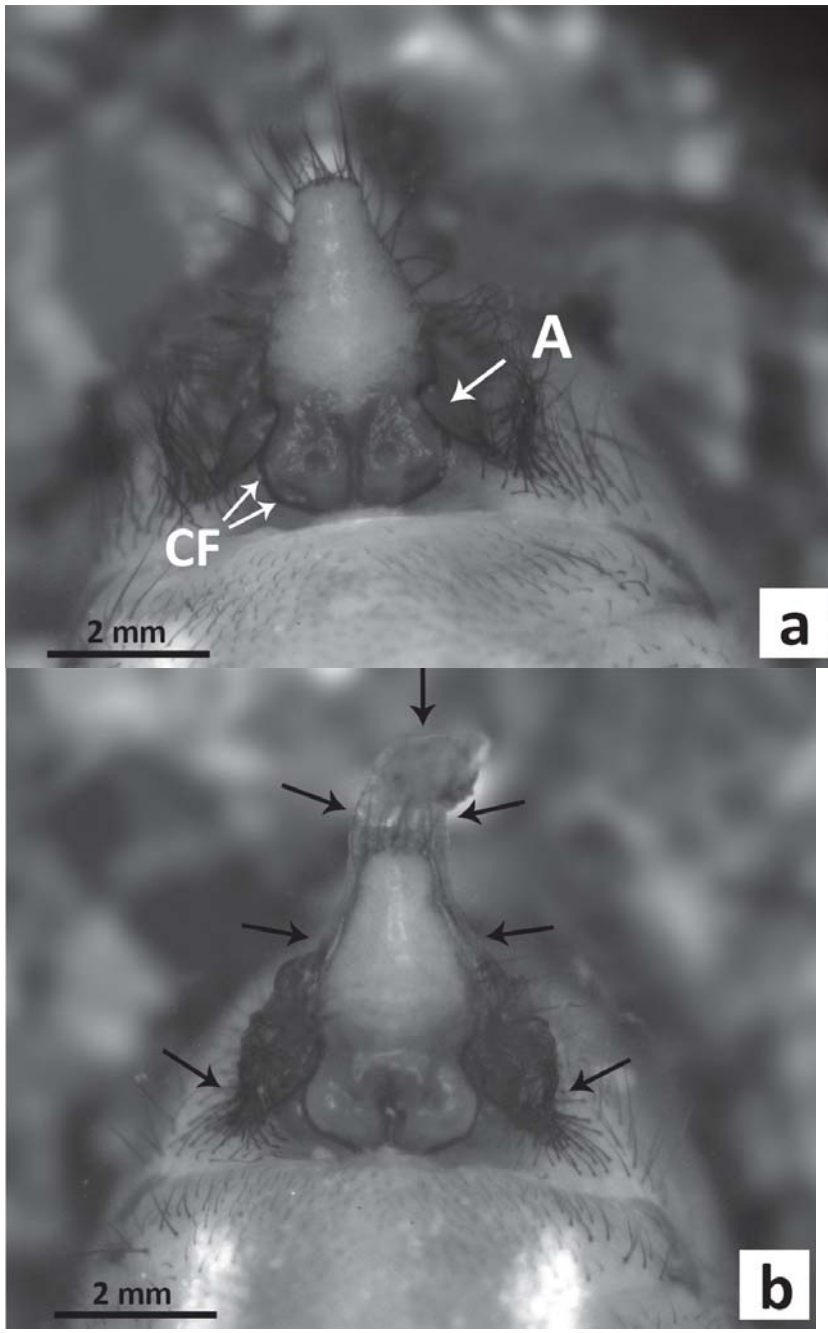

Figure 1. Posterior view of the epigynum of *Leucauge argyra*. (a) Virgin female without a mating plug. Notice the long setae present around the atria and on the anterior surface of the ventral process. A, atrium; CF, Copulatory fold. (b) Mated female largely covered with mating plug material. Black arrows indicate places where long setae are trapped in the plug substance.

70 µm approximately. This passage evidenced that Chamber I was not just the entrance of Chamber II. The walls of the copulatory fold-like-duct and Chamber I were a continuation of the abdominal cuticle and seemed to be formed by a thick sclerotized layer. Unlike Chamber I, Chamber II had membranous walls (Fig. 2a). The spermathecae were positioned near the base of the ventral process.

Chamber I was located at the base but to the side of Chamber II, was much smaller than Chamber II and had several small sclerotized branches and concavities (Figs 2a, 2c). The largest branch measured was about 80 µm long, but the size between branches seemed to vary. Chamber II was a long membranous sac about 300 µm long and 60 µm in diameter, that was empty and collapsed in virgin females (Fig. 2c). In copulated females, Chamber II was expanded and filled with sperm (Fig. 2b), and there were also sperm in Chamber I. The cells surrounding Chambers I and II were different. The tissue surrounding the membrane of the wall of Chamber II seemed to be glandular with large, long cells with large nuclei (Fig. 2d). The cells surrounding Chamber I did not seem to be glandular because they were small and rounded (Fig. 2c). In at least four copulated females there was a dark-stained homogeneous material inside both chambers, which was very similar to the plug material on the outside surface of the epigynum (Fig. 2a). We did not find any clear structure corresponding to the oviduct or the fertilization duct. We did find that after connecting with Chamber I of the spermatecae, the copulatory fold-like-duct continued and seemed to open very near the epigastric furrow, forming what could be a fertilization fold-like-duct.

Females of *L. argyra* are apparently unique among the genus in having a ventral process (Álvarez-Padilla & Hormiga, 2011; Barrantes *et al*., 2013) and the copulatory fold-like duct. Similar ducts occur in some Linyphiidae species (Saaristo & Tanasevitch, 1996; Uhl, 2000; Uhl & Gunnarsson, 2001). In *L. argyra*, the morphology of the fold leading to the first chamber suggests that the male conductor as well as his embolus could be inserted into this structure, acting as an insemination duct.

The total number of chambers in the spermathecae varies in *Leucauge* species. *Leucauge mariana* Taczanowski, 1881 has a three-chambered spermathecae (Eberhard & Huber, 1998) and *L. venusta* Walckenaer, 1842 also has more than two chambers, but the exact number is unknown (Danielson-Francois, 2002). In *L. argyra*, Chamber I is small and convoluted with a sclerotized wall, while Chamber II is large and has a membranous wall, rather than vice versa, as stated in Barrantes *et al.,* (2013). The presence of the membranous Chamber II in the spermathecae of *L. argyra* is in agreement with the phylogeny of Tetragnathidae, in which one synapomorphy of the subfamily Leucauginae Caporiacco, 1955 is a non-slerotized spermatheca with the appearance of a membranous sac (Álvarez-Padilla *et al*., 2009). Álvarez-Padilla & Hormiga (2011) also mention the weakly sclerotized spermathecal walls as diagnostic trait for *Leucauge* species. In *L. mariana* (Eberhard & Huber, 1998) and *L. venusta* (Danielson-Francois, 2002; Dimitrov & Hormiga, 2010), the membranous chambers correspond to the Chamber I, rather than to the Chamber II as in *L. argyra*.

One of the possible functions for the secretory products of spermathecal glands in spiders is the formation of mating plugs (Coyle *et al*., 1983; Danielson-Francois, 2002; Uhl *et al*., 2010). Previous studies with *L. argyra* suggest that the female rather than the male produces mating plugs because the plug material lacked sperm (Aisenberg & Barrantes, 2011). The internal

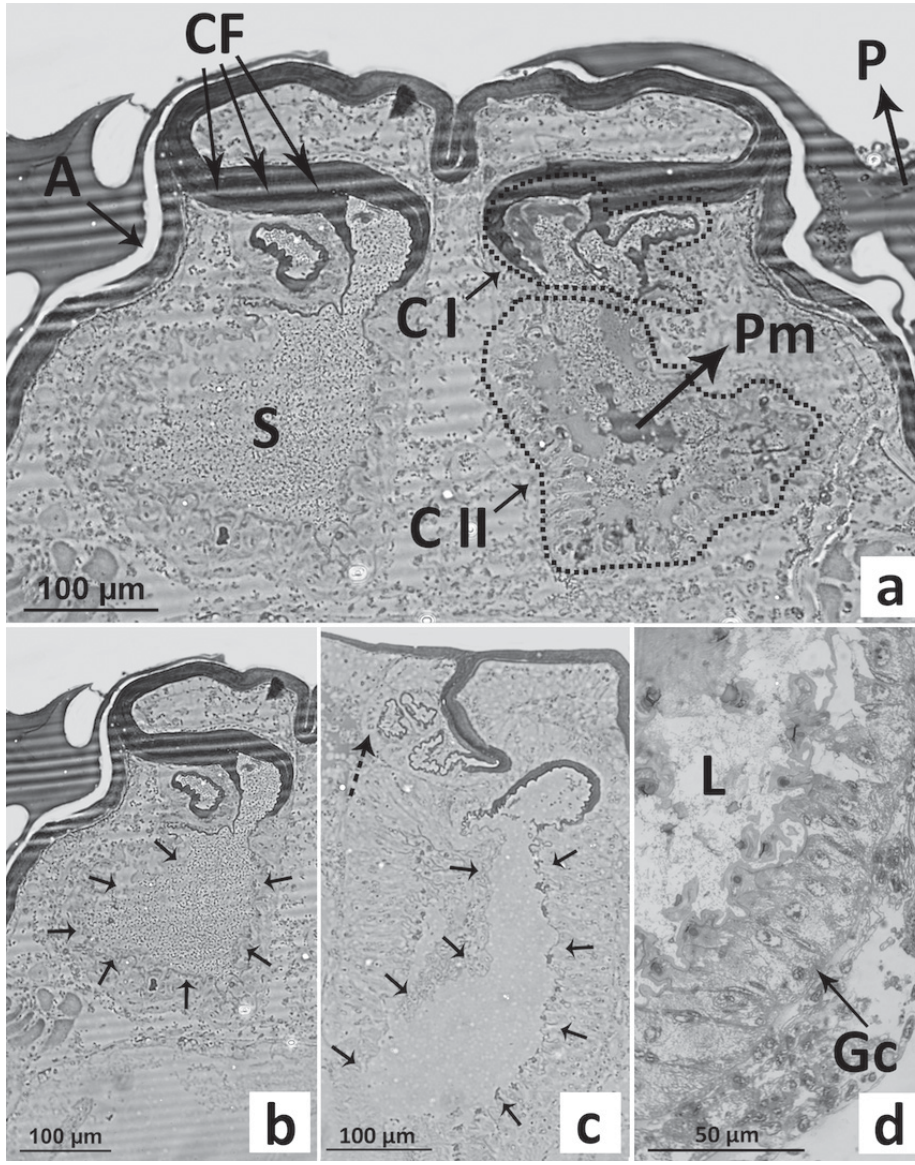

Figure 2. Transversal sections of the epigynum of *Leucauge argyra* showing the internal genital morphology. (a-b) Mated female. (a) A, atrium; CF, copulatory fold; S, sperm; C I, Chamber I; C II, Chamber II; P, plug; Pm, Plug material. (b) Arrows point Chamber II of the spermathecae expanded and filled with sperm. (c) Virgin female. Arrows point Chamber II of the spermathecae, which is empty and collapsed. Dashed arrow point non-glandular cells around Chamber I (d) Close-up of the glandular membrane of the walls of Chamber II of the spermathecae. L, lumen of the Chamber II of the spermathecae; Gc, glandular cells.

organization of the spermathecae in *L. argyra* shows that the glandular tissue surrounding Chamber II could be responsible for producing the plug substance in this chamber, even it would have to travel to the exterior of the epigynum by passing through Chamber I or at least through a part of it. The homogenous stained material found on both chambers and outside the epigynum could be mating plug material, supporting that the mating plug substance could be produced in Chamber II and move to the atria. As in *L. mariana*, this may represent selective female cooperation with some males (Eberhard & Huber, 1998), making mating plugs of different sizes and in different timing after copulation (Aisenberg & Barrantes, 2011; Barrantes *et al*., 2013). However, it is unclear how plug material from Chamber II would move throw the sperm just received from the male without expelling it.

Possible functions of external characteristics of the epigynum have been deduced previously from behavioral studies. The atria located on the anterior lateral surface of the base of the ventral process are employed when males couple the cymbial hook of their pedipalp on one side of the epigynum and their conductor on the other during insemination (Barrantes *et al*., 2013). The setae located at the tip and around the base of the cone-like epigynal projection could be used to sense stimuli from the male's pedipalp during copulation (Barrantes *et al.,* 2013). This use would resemble that of the cheliceral setae of the female during cheliceral clasping which influences mating processes (Aisenberg *et al.,* 2015). However, further studies are required to test this hypothesis.

We describe for the first time a copulatory fold-like duct instead of a close duct in the internal genital morphology of *L. argyra* females: a probable unusual trait within *Leucauge.* We also present new evidence that confirms that females and not males of *L. argyra* produce the mating plug material. In order to provide complementary information of how this substance travels to the outer surface of the epigynum, we suggest that future studies sample females at different times after copulation, attempting to track the path of the plug material to the epigynum surface.

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