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Loss of cheliceral clasping in *Leucauge* sp. (Araneae, Tetragnathidae)

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Summary

In male spiders, genitalia, sexual behaviour and secondary sex morphology tend to diverge rapidly across species, presumably as a result of sexual selection. In the three *Leucauge* species for which pre- and copulatory courtship behaviour is known, females clamp the male chelicerae prior to and during copulation. This brings the basal segment of the male's chelicerae into contact with the anterior surface of the female's chelicerae. The basal segment of male's chelicerae has also morphological features such as sturdy, abundant setae which are thought to have evolved to stimulate females, as well as other morphological features whose specific function is yet unknown. We show here that in a fourth species, *Leucauge* sp., the female does not clamp the male's chelicerae; as expected, this absence is associated with a lack of secondary sexual differences in the male chelicerae.

Introduction

Male genitalia as well as other sexually dimorphic male body parts that contact females during courtship and copulation often diverge rapidly relative to other body parts (Darwin 1879; West-Eberhard 1983; Eberhard 1985, 1996, 2010; Andersson 1994). In some cases the female body parts that are contacted by these male structures also change accordingly (Eberhard 2004). Discoveries in spider reproductive biology have that sexual selection has played a role in the evolution of genitalia, functional morphology and courtship behaviour. For instance, in jumping and wolf spiders, species-specific vibratory and visual sequence cues possibly play a dual role in species recognition and courtship behaviour (McClintock & Uetz 1996; Hebets & Uetz 1999). Similarly, copulatory position and temporal patterns of insertions and patterns of haematodochal inflations have also diverged across wolf spider species (Stratton *et al.* 1996).

The genital morphology of species in the large genus *Leucauge* (*c.* 170 species: Platnick 2014) diverge greatly across species (Levi 2007, unpublished figures), but divergence in cheliceral and other morphological traits that are in contact during courtship and copulation has not been studied in most species. Recent research has documented divergence in the morphology and behaviour of male genitalia and other morphological structures that contact with the female during pre-copulatory courtship and copulation in three species in the genus *Leucauge*, *L. mariana*, *L. argyra*, and *L. venusta*. Some of these traits are associated with the cheliceral clasping behaviour that occurs prior to and during copulation in these species (Castro 1995, Eberhard & Huber 1998, Aisenberg & Eberhard 2009, Aisenberg & Barrantes 2011, Barrantes, Aisenberg & Eberhard 2013, Aisenberg, Barrantes & Eberhard 2014, 2015). A female *Leucauge* clamps a male by closing her fangs around the distal portion of the basal segment of the male's chelicerae. In these three species the basal segment of the male chelicera differs from that of the female in having sturdy setae on the anterior surface, and experiments in *L. mariana* showed that these setae stimulate the female's chelicerae and induce her to respond in ways that are likely to favour the male's chances of paternity (Eberhard & Huber 1998; Álvarez-Padilla *et al.* 2009; Álvarez-Padilla & Hormiga 2011; Barrantes, Aisenberg & Eberhard 2013; Aisenberg, Barrantes & Eberhard 2014). In addition, the male of *L. mariana* has a ledge near the distal lateral border of the basal segment, while the basal cheliceral segment of the male of *L. argyra* has a rugose depression on the anterior surface of the distal portion. The functional significance of these morphological features is unknown.

This study presents detailed preliminary observations on a fourth *Leucauge* species (*Leucauge* sp.; Fig. 1) which support these interpretations of the function of the male cheliceral setae: the lack of cheliceral clasping during courtship and copulation is combined in *L.* sp. with a lack of secondary sexual modifications of the male's chelicerae. We also present some behavioural details of courtship and copulation to facilitate future comparisons with other *Leucauge* species.

Methods

We collected three males and four females of *L.* sp. (it is not currently possible to identify most *Leucauge* to species, due to lack of a recent taxonomic revision) in an oil palm plantation at El Silencio, Quepos, Puntarenas province, Costa Rica (09°24′N 84°01′W, 46 m a.s.l.), on July 2013. In Costa Rica this is an uncommon species (despite additional extensive efforts, we found only other 12 adult females and three sub-adult males) that inhabits early second-growth vegetation and forest edges along the southern Pacific lowlands. We kept each spider in a 10.0 cm high plastic cup 7.5 cm in diameter at the top and 5.5 cm at the bottom in the Universidad de Costa Rica, San José, Costa Rica (9°54′N 84°03′W; 1200 m a.s.l.). Each cup had a small twig for the spider to climb on, a small wad of wet cotton at the bottom, and a ring of paper at the top to which the spider could attach lines (Barrantes & Eberhard 2012). We fed spiders two *Drosophila* or other small flies every other day until

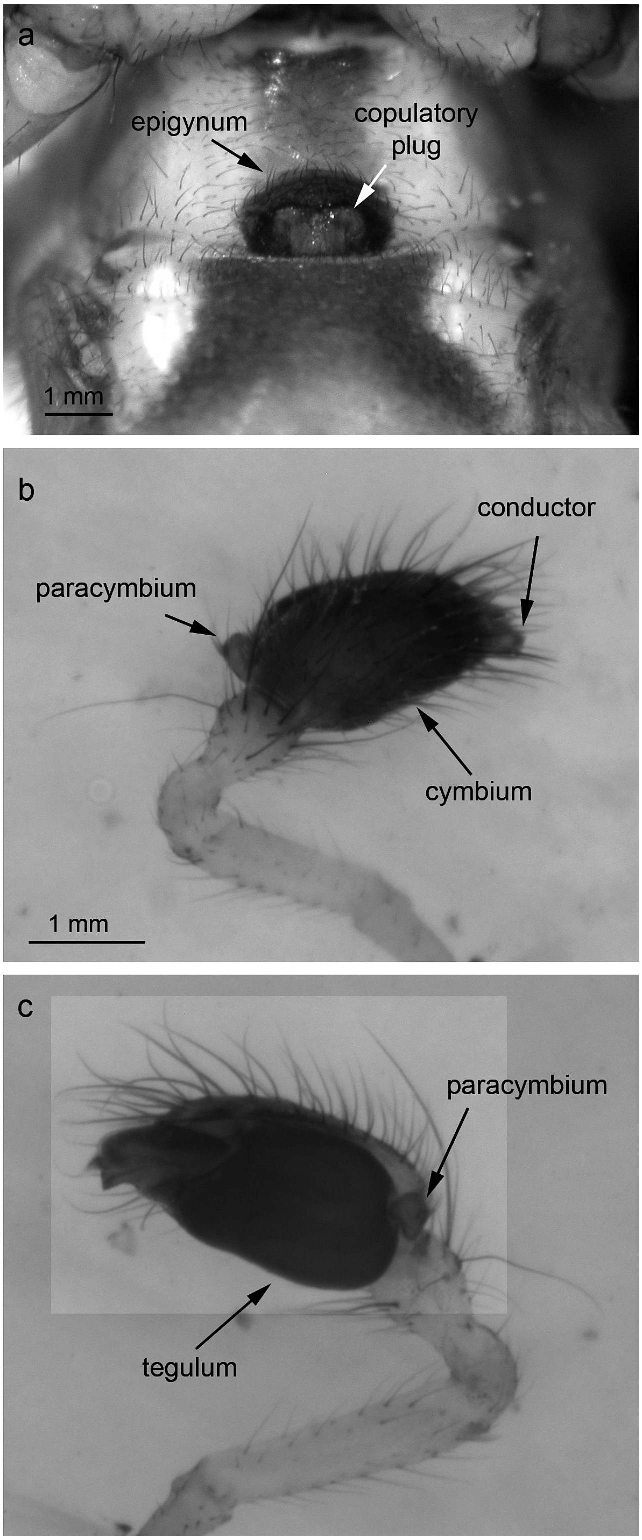

Fig. 1: Genitalia of *Leucauge* sp. **a** Female epigynum with copulatory plug; **b–c** two different views of male palp.

trials began. The temperature averaged during the trials 20.1 ± 0.5 (range: 19–21).

To obtain video recordings of courtship and copulation, we placed each female on a field-collected orb web built the same day by a mature female *L. mariana* (two webs were fresh and intact, the other partially destroyed). The web was taped to a plastic plate 22 cm diameter and about 2 cm deep. Each female was on the web for at least 10 min before an adult male was introduced onto the web about half way between the hub and the border of the web. We recorded courtship behaviour with a Dino-Eye Eyepiece digital color video camera (Model AM423X) attached to the ocular of a Wild M3Z dissecting microscope (Leica Microsystems). One female was removed because she did not react receptively (see results) to the male within 30 min, and was replaced by a different female. Each of the three males copulated once. Mean values are followed by ± 1 standard deviation. Voucher specimens of *L.* sp. were deposited in the Museo de Zoología, Universidad de Costa Rica.

Results

Behaviour of female on the web

Neither of the two females of *L.* sp. placed on an intact *L. mariana* web nor the other female on a partially destroyed orb removed lines or built any new lines, other than their drag lines when they walked to and from the hub. We fed one female a small fly about 3cm away from the hub. The spider rapidly attacked the fly and began to eat it at the attack site, without carrying it to the hub.

We could not exclude a possible effect of placing *L.* sp. on webs of *L. mariana* on male or female performance. However, if an effect is expected, this would be in the precopulatory behaviour, assuming that this behaviour may be affected by differences in web design or female pheromones impregnated on silk threads. Once the pair has engaged in copulation, it is less likely that the design of the web or the pheromones on the threads would affect the performance of either the male or female. We have also used the same approach in similar experiments with *L. argyra* in which the female often removes some threads of the *L. mariana* web and lays a few of its own silk threads but the precopulatory and copulatory courtship behaviours were not affected (Aisenberg & Barrantes 2011).

Precopulatory behaviour and copulatory position

In the case in which the female was feeding on a fly, as soon as the male was on the web, he moved towards the female and prey and when he was very close but not in contact with the prey the female moved to the hub. Then the male fed on the same prey for about 7 min before walking towards the female.

We did not observe the males twanging threads or any of the other pre-copulatory behaviour patterns that precede copulation in *L. mariana* and *L. argyra* (Eberhard & Huber 1998; Aisenberg & Barrantes 2011). We only observed the male prior to copulation in two of the three pairs, however; in the third pair the female was already in copulatory position and the male very close to her when we began observations. We did not see whether the male contacted any of the threads that the female had laid. Despite the lack of an apparent male pre-copulatory courtship, the female turned toward the male as he approached her in two pairs, and assumed a receptive posture. In the three pairs, she spread her legs I and II laterally, moved her cephalothorax downward, away from the web, and bent her abdomen ventrally.

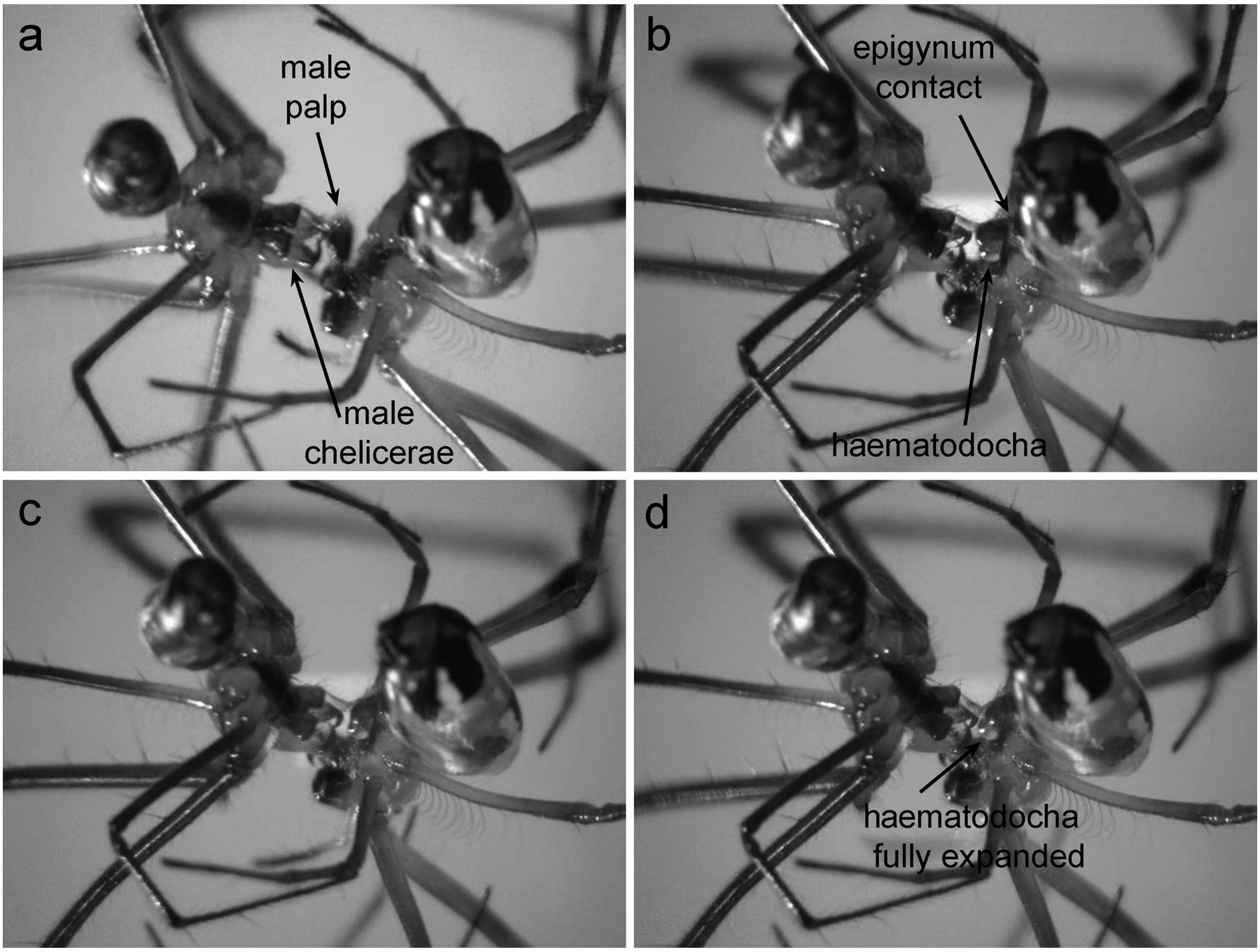

Fig. 2: Coupling of male genitalia. **a** the male approaches the female's epigynum; note that the basal segment of the male's chelicera is extended forward, his fangs are partially opened, and the flexion of the bulb; **b** a portion of the palpal bulb (possibly the conductor) contacts the epigynum; **c–d** expansion of the haematodocha.

The male contacted the female's legs gently, but did not perform sustained leg tapping, and in all three cases the female remained more or less immobile and gave no sign of resistance as he approached and contacted her.

To begin copulation, the male positioned his body at about 90° relative to the long axis of the female's body; he was above her, facing down towards her sternum, and his dorsal surface was oriented towards her anterior end (Fig. 2a). The male's chelicerae were far from those of the female, and the female's chelicerae made no move to clasp him (Fig. 2a).

Cheliceral movements, palpal extensions, and flubs

With both female and male in the copulatory position, the male moved the distal portion of the basal segment of his chelicerae anteriorly, approaching (but not contacting) the female's sternum (cheliceral extension hereafter). As the male extended his chelicerae, he opened his fangs partially (Fig. 2a), but they did not contact the female. He also extended the femur and tibia of the palp that was to be inserted anteriorly, toward the female's sternum. The palpal bulb was flexed medially *c.* 90° at the tibia–bulb joint so that its distal tip was directed toward the female's midline, Fig. 2a).

When the male first extended his chelicerae and palp, the tip of his palp was more than one and a half times the length of his body from the female epigynal area. The male then moved his body forward, so that he moved posteriorly with respect to the female, and brought the distal portion of his palp nearer to the female's epigynal area (Fig. 2a). The male then moved his palp laterally–medially several times over the female's epigynum, sometimes partially inflating the haematodocha and touching her epigynum (Fig. 2b). After each movement he moved his body backwards and then forwards, bringing his body slightly more posterior on the female each time. With each of these movements, his palpal bulb came closer to the female's epigynum. One male moved his palpal bulb laterally–medially eight times over the epyginal area just prior to genital coupling. In at least three cases the male partially inflated the haematocha, and in four the palpal bulb touched the female's epigynum without coupling his palp with her epigynum ("flubs" of Eberhard & Huber 1998). During the last of this series of eight movements the conductor hooked with the epigynum (Fig. 2b). The male chelicerae did not contact the female chelicerae before or during insertions (Fig. 2b–d).

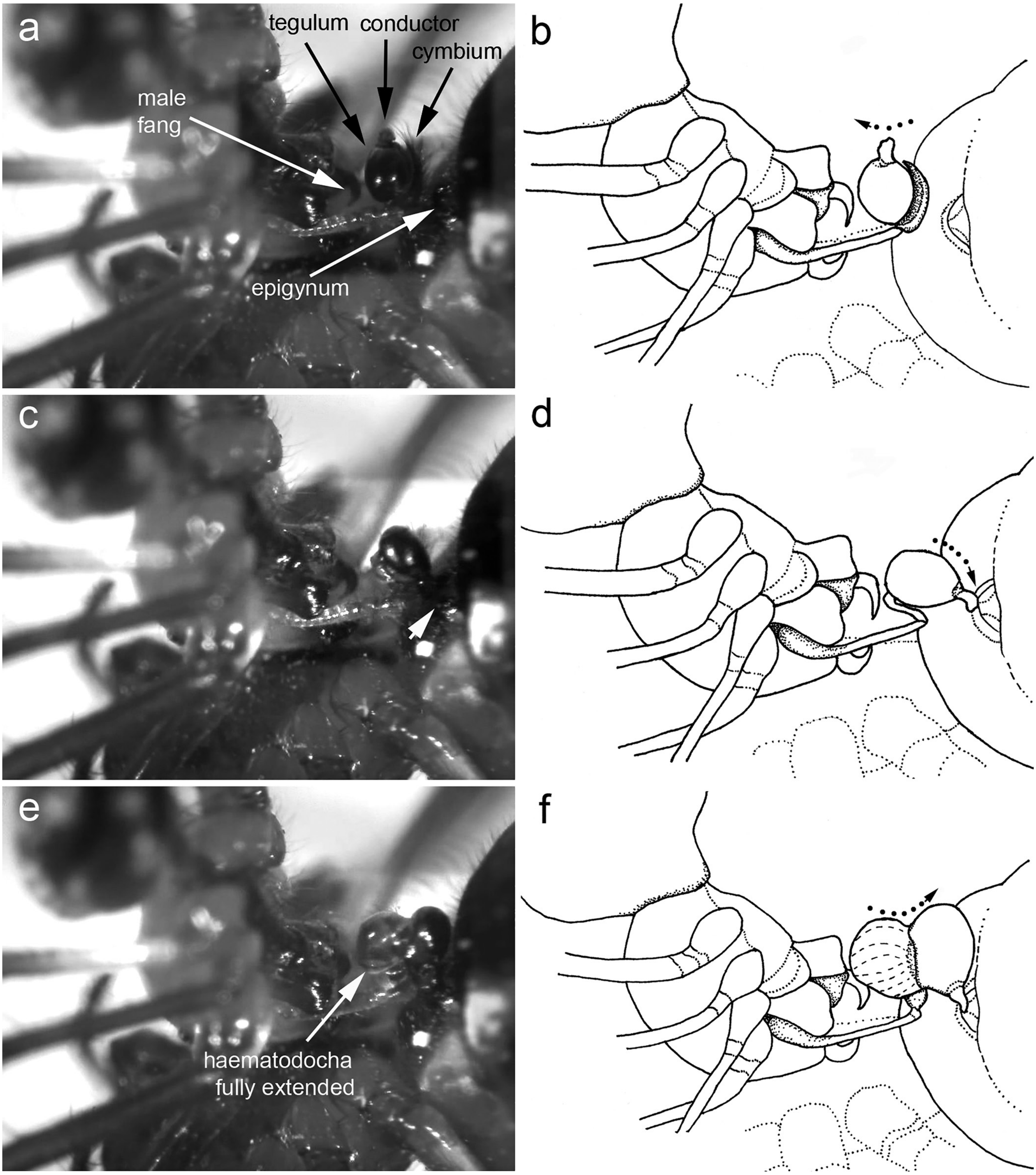

Fig. 3: Movements of sclerites of the male palpal bulb. **a** position of the sclerites when male palp has just contacted the epigynum; in **a** and **b** the tegulum and the conductor are displaced in a medial–lateral direction; **c–d** rotation of the tegulum *c.* 180°; **e–f** total expansion of haematodocha and rotation of tegulum toward a medial–anterior direction.

Movement of the male genitalia and possible male copulatory courtship

As soon as the conductor of the male palpal bulb hooked with the epigynum, he moved forward, further rearward on the female (Fig. 2). As he approached closer to the female, he bent his palpal tibia medially, so that the dorsal surface of the cymbium contacted the ventral surface of female's abdomen just anterior to her epigynum, as occurs in *L. mariana* prior to insertion (Eberhard & Huber 1998); at this point the haematodocha began to expand. We observed a total of four long insertions (multiple haematodochal inflations during a single insertion), and three short insertions (only one haematodochal inflation; Eberhard & Huber 1998). The durations of long insertions varied considerably (mean = 6.31 min ± 8.26, CV = 130%, n = 4 copulations from 3 pairs). In general they lasted several minutes; the longest was 18.28 min. The variation in the durations of short

Fig. 4: Chelicerae of females (left) and males (right) of three species of *Leucauge*: *L. mariana*, *L. argyra* and *L.* sp. Note the macrosetae and shape of the chelicerae of males in *L. mariana* and *L. argyra*.

insertions was lower (mean = 1.31 sec ± 0.65, CV = 49%, n = 3 inflations from 1 pair). The haematodocha inflated rhythmically during the long insertions; the mean rate was one every 2.9 sec ± 1.42 (n = 391 inflations, 3 pairs). In one pair the male inserted both palps consecutively without moving apart from the female between insertions.

The male's chelicerae moved in synch with the haematodochal movements. Each time the male fully inflated the haematodocha he extended his chelicerae and opened his fangs. In lateral view it was clear that his fangs often contacted the setae on the surface of the posterior half of female's sternum, each time they opened. The male then withdrew his chelicerae and closed his fangs each time his haematodocha partially collapsed. During copulation the male often vibrated his abdomen ("abdomen bobbing" of Eberhard & Huber 1998); the mean rate was once every 3.44 sec ± 4.71 (n = 53, 3 pairs) and he may have also shaken the web with his legs ("rocking").

Movement of male's genitalia

We recorded parts of one sequence of movements of the male's bulb during a long insertion. Prior to inflation of the basal haematodocha, the distal tip of the conductor was directed toward the midline of female's sternum (Fig. 3a). As the haematodocha began to inflate, the tegulum rotated in a medial–lateral direction (Fig. 3b) (i.e. the left bulb rotated to the right). Then the tegulum rotated nearly 180° so that the tip of the conductor was directed towards the female epyginun (Fig. 3c–d) (it was not possible to determine the site on the epigynum where the conductor hooked it). Finally, the full inflation of the haematodocha rotated the tegulum in a medial–anterior direction (Fig. 3e–f) (i.e. the left palp rotated toward the male's left and moved anteriorly).

Female movements

In two pairs the female tapped the body and first legs of the male with her palps during male flubs and for a few seconds after genital coupling. One of the females also repeatedly spread the basal segments of her chelicerae and opened and closed her fangs. In the third pair the female did not tap the male nor spread the basal segments of her chelicerae. During copulation the female's abdomen moved slightly ventrally in synchrony with each inflation of the male's haematodocha (n = 3 pairs). It was not clear whether these movements were an active response of the female, or an effect of the movements of the male's palp. In one pair the female brushed the setae of her palps repeatedly against the dorsal surface of the male's prosoma 0.77 times per sec (n = 100 times in 128 sec), but this behaviour was absent in the other two pairs.

Spider size and morphology of male chelicerae

Cephalothorax width of females (n = 4) *L.* sp. measured 1.49 mm ± 0.08 and 1.27 mm ± 0.12 in males (n = 3). The mean proportion of cephalothorax width (males/females) was 0.85, the same as the larger *L. mariana* (0.85; Aisenberg 2009), but smaller than the much larger *L. argyra* (0.98; Barrantes, Aisenberg & Eberhard 2013). The basal segments of the male and female chelicerae were very similar (Fig. 4), and we did not detect any sexually dimorphic structures in the basal segment.

Discussion

The lack of cheliceral clasps in *L.* sp. differs from the other three species of *Leucauge*, in which the female clasps the male's chelicerae (Aisenberg, Barrantes & Eberhard 2015). The male and female chelicerae also mesh in Tetragnathinae, a sister clade to Leucauginae, in which the chelicerae are also typically sexually dimorphic, but it is the male which clasps the female. The absence of a species-based phylogeny of the genus *Leucauge* makes it impossible to hypothesize on the relative apomorphy of the lack of cheliceral clasping that we have documented in females of *L.* sp. and the associated lack of sexual dimorphism on the anterior surface of male chelicerae. However, our findings are in accord with previous conclusions that some behavioural and morphological features in males of *L. mariana* and *L. argyra* (e.g. long-thick setae and morphological modifications on the anterior section basal segment of male chelicerae, Fig. 4) have been driven by sexual selection, (probably cryptic female choice) (Aisenberg, Barrantes & Eberhard 2015).

Despite the small number of matings of *L.* sp. that we observed, it is possible to make some general qualitative comparisons with previous observations of *L. mariana* and *L. argyra* (Eberhard & Huber 1998; Aisenberg & Eberhard 2009; Aisenberg & Barrantes 2011; Aisenberg, Barrantes & Eberhard 2014, 2015). Males of all three species flex the basal segment of the chelicerae anteriorly, perform flubs with their palps, make long insertions, and rock and bob their abdomens during copulation; males of both *L.* sp. and *L. mariana* also perform short insertions. The movements of the sclerites of the bulb during haematodochal inflation seem to be similar to those of males of *L. mariana*. In both species the inflation of the haematodocha produces a *c.* 180° movement of the tegulum that directs the conductor and embolus towards the epigynum (we did not observe the embolus, however, in *L.* sp.). The movements of the male bulb sclerites are quite different in *L. argyra*, and are possibly correlated with their strikingly different genital morphology (Barrantes, Aisenberg & Eberhard 2013; Aisenberg, Barrantes & Eberhard 2014). A larger sample of *L.* sp. behaviour will be needed to make quantitative comparisons with other species of *Leucauge*.

It has been proposed that cheliceral locking in tetragnatahids functions to anchor the male more securely to the female's body during mating, in order to facilitate the coupling of their relatively simple genitalia (Levi 1981; Kraus 1984; Aisenberg, Barrantes & Eberhard 2015). This hypothesis is not supported by the present study, because *L.* sp. does not have more complex genitalia (Fig. 1) but nevertheless lacks cheliceral locks.

The readiness of male *L.* sp. to court conspecific females that were on the webs of *L. mariana* was surprising. Castro (1995) found that males in mixed pairs of three other species of *Leucauge* generally showed no inclination to even initiate courtship. Further explorations of potential species recognition mechanisms would be interesting.

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