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RESEARCH ARTICLE

Tarantulas do not shoot silk from their legs: experimental evidence in four species of New World tarantulas

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SUMMARY

Theraphosid tarantulas, like all other spiders, secrete silk from spigots on the abdominal spinnerets. A few years ago, it was proposed that the large tarantula *Aphonopelma seemanni* could extrude silk from specialized spigots on the tarsi to help adhesion to vertical surfaces. This suggestion was later questioned because silk was not observed after the spinnerets had been sealed. Recently, experiments with the tarantula *Grammostola rosea* again suggested tarsal silk secretion. All observations of the supposed tarsal silk were made in spiders with functional spinnerets, thus contamination with silk coming from the spinnerets could not be excluded. Recent morphological arguments also questioned putative tarsal spigots and proposed that they are actually contact chemoreceptors. We here test the supposed tarsal silk secretion in *Aphonopelma seemanni*, *Avicularia avicularia*, *Brachypelma vagans* and *Grammostola mollicoma* using similar experimental conditions as the previous authors, but with sealed spinnerets. Our results clearly demonstrate that when spinnerets are sealed, tarantulas do not show any tarsal silk secretion. We reinterpret those putative tarsal spigots and discuss possible evolutionary implications of these findings.

Key words: New World tarantula, silk production, tarantula climbing, tarsal scopula.

INTRODUCTION

The most important synapomorphic characteristic of spiders is the secretion of silk, extruded from specialized abdominal appendages, the spinnerets (Coddington and Levi, 1991). Silk is released from spigots on the spinnerets. For more than two centuries, these features were considered to be unique for silk secretion in spiders; adult males also produce silk from epiandrous glands near the genital opening for the sperm web (Marples, 1967). More recently, Gorb et al. (Gorb et al., 2006) reported silk threads when the zebra tarantula *Aphonopelma seemanni* was climbing on vertical glass surfaces. These authors concluded that these fibers were secreted from spigots on the tarsi and claimed that this would help in adhesion. This constituted a novel and highly unusual finding.

Pérez-Miles et al. (Pérez-Miles et al., 2009) tested the same species walking on vertical and horizontal glass surfaces. When the spinnerets were sealed with paraffin, no silk was found. The authors concluded that silk was only secreted from spinnerets and probably was picked up by the legs. This could explain why Gorb et al. (Gorb et al., 2006) had found silk threads on the tarsi. A recent study (Rind et al., 2011) tested the theraphosid *Grammostola rosea* climbing on smooth vertical surfaces. After gently shaking the spider, silk threads were supposedly extruded from tarsal ‘spigots’. These authors also proposed that these fine silk threads would prevent falls when the spider was slipping. In all these experiments the tarantulas had normally functioning spinnerets. Peattie et al. (Peattie et al., 2011) studied the adhesion of spiders, solifugids and mites. They found fluid footprints when arachnids climbed on vertical surfaces. In experiments with the tarantula *G. rosea*, they also found threads, apparently originating from tarsal hairs, but were not sure of their silken nature. Most recently, Foelix et al. (Foelix et al., 2011; Foelix et al., 2012) provided good morphological evidence that the alleged tarsal spigots (Gorb et al., 2006; Rind et al., 2011) are sensory hairs, probably contact chemoreceptors.

Considering that silk is a light fiber that easily adheres to surfaces even without direct contact with spinnerets and that silk threads were only found in experiments using spiders with free spinnerets, the concept of tarsal silk secretion seems highly questionable. In fact, passive contamination with spinneret silk because of air currents or gravity could not be discounted in previous studies. Furthermore, adhesive setae (claw tufts and scopulae) of theraphosid legs were considered sufficient to ensure safe climbing on smooth vertical surfaces (Niederegger and Gorb, 2006). Another question that remains is how to explain evolutionary pressures for an additional adhesive mechanism in tarantulas. Here we test tarsal silk secretion in four species of tarantulas, one arboreal and three terrestrial, under extreme conditions of climbing on smooth surfaces with and without sealed spinnerets. Our study with confocal microscopy revealed the presence of fluid footprints but no silk traces when spider spinnerets were sealed. We reinterpret tarsal setae morphology and discuss possible phylogenetic implications of these findings.

MATERIALS AND METHODS

Experiments were performed on four tarantula species: *Aphonopelma seemanni* (F.O.P.-Cambridge 1897) (collected in Escuintla, Guatemala, route to Puerto San José, km 79); *Brachypelma vagans* Ausserer 1875 (born in captivity, mother from Alta Verapaz, San Pedro Carchá, Guatemala); *Grammostola mollicoma* Ausserer 1875 (from Durazno, Uruguay, Route 4, km 267); and the arboreal *Avicularia avicularia* (Linnaeus 1758) (from Pará, Belem, Brazil).

Fig. 1. Female Costa Rican zebra tarantula (*Aphonopelma seemanni*), showing the spinneret region of the abdomen sealed with paraffin for the trials.

To test any possible tarsal silk secretion, we used two individuals each of the three former species and one of *A. avicularia*, and performed two series of trials. In the first series, the tarantulas were tested with free spinnerets to allow spinneret silk secretion to occur. In the second series, we sealed tarantula spinnerets with paraffin (60°C) to prevent any secretion of spinneret silk (Fig. 1). The seal increased the spider mass by 1.01±0.41 g. We sealed the spiders 24 h before the trials to prevent silk transference to legs and removed the seal immediately after the trials using warm water (none of the specimens was injured by the manipulation).

For the trials, tarantulas were placed on a clean and dry glass surface (200×350 mm) covered by 12 pre-cleaned microscope slides. The spider was positioned on the slides in a horizontal position. After turning to a vertical position, the tarantula stood facing upwards (Fig. 2). We left the glass surface in a vertical position for 5 min and then we gently shook the glass slides to induce leg slip. We tried to reproduce the experimental conditions described in Rind et al. (Rind et al., 2011), with the exception of the second series of trials, in which we sealed the spider's spinnerets. We marked all the slides that contacted spider legs. After the trials, we removed all slides and examined the marked ones under a light microscope (Nikon, Tokyo, Japan). We also examined and photographed some selected slides with confocal microscopy using polarized light. The internal side of tarsal exuviae was studied by scanning electron microscopy (SEM).

Fig. 2. Female *Grammostola mollicoma* climbing on a vertical glass surface covered by 12 microscope slides (experimental device); dorsal view.

RESULTS

In both series of trials, tarantulas climbed on glass vertical surfaces (Fig. 3A) and all spider legs contacted the glass slides. When spiders climbed on vertical surfaces, we observed that the distal part of the ventral side of the leg, mainly the claw tufts, adhered to the glass (Fig. 3B).

In the first series of trials (free spinnerets), 48 of 72 slides had been in contact with spider legs. When these slides were examined, 24 of them exhibited silk threads and 37 showed urticating setae.

Fig. 3. Female *Grammostola mollicoma* climbing on a vertical glass surface. (A) Ventral view through the glass; all tarsi are in contact with the glass wall. (B) Close-up view of claw tufts and scopulae setae in contact with the wall. Photo credit: Gonzalo Useta.

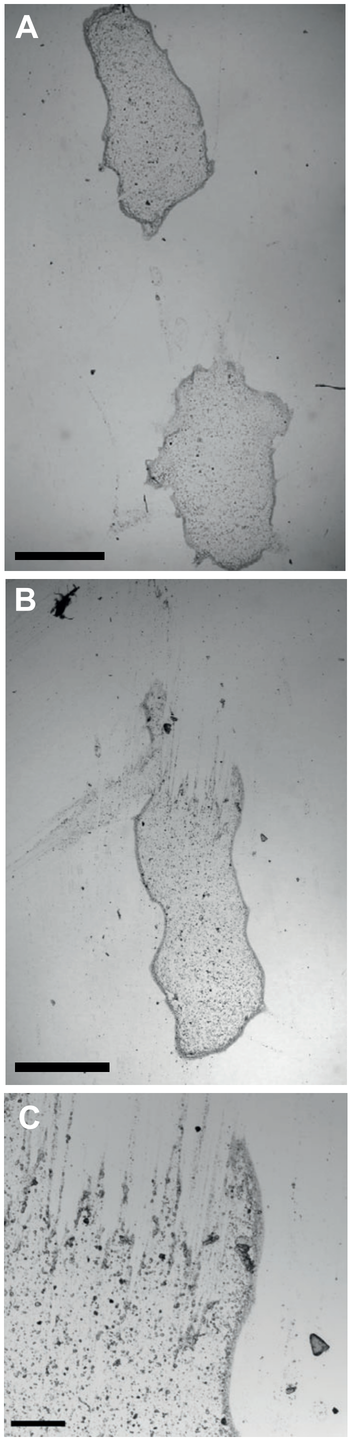

Fig. 4. Fluid footprints of a female *Grammostola mollicoma* that climbed the vertical glass surface with sealed spinnerets. (A) Two fluid droplet footprints. (B) Footprint in which the tarantula had slipped downwards, leaving lines of droplets above. (C) Close-up view of the upper region of the footprint of B (see the lines of droplets). Scale bars, (A,B) 500 μm; (C) 50 μm.

In the second series of trials (sealed spinnerets) 41 of 72 slides had been in contact with spider legs. None showed silk threads and 36 showed urticating setae.

In both series of trials, the slides showed fluid footprints. When we examined these footprints with the confocal microscope, we observed that they consisted of droplets (Fig. 4A). In *A. avicularia*, the pattern of the footprints showed two spots, corresponding to the lateral projections of tarsal scopulae and claw tufts, whereas in the other species they were ovoid spots of approximately 500 μm width. When the spiders slipped, we observed a pattern of parallel lines of droplets smeared in the opposite direction of leg slips (Fig. 4B,C). These lines were discontinuous and clearly differed from the pattern observed on spinneret silk threads (Fig. 5).

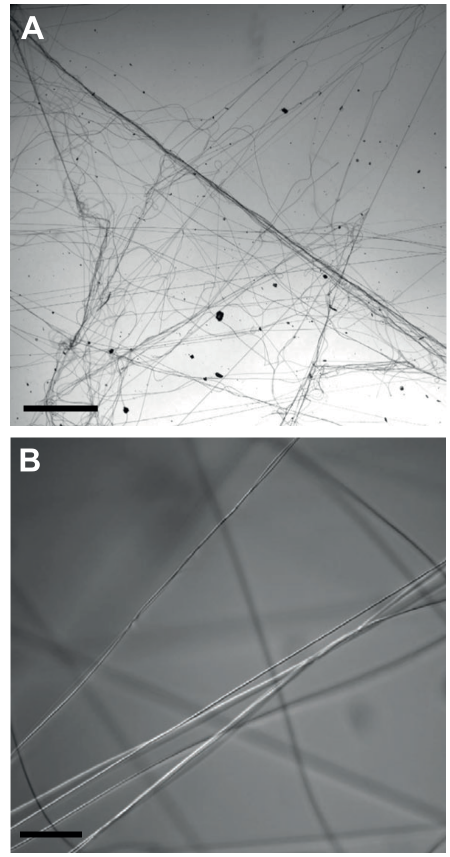

Fig. 5. Spinneret silk threads of *Grammostola mollicoma* after climbing on a vertical surface. (A) Silk threads at the same magnification of Fig. 4A,B to compare the differences from the footprints. Scale bar, 500 μm. (B) Close-up view of the silk threads. Scale bar, 50 μm.

The SEM examination of the internal side of leg exuviae in *A. seemanii* showed several orifices; the small ones are sockets of scopula hairs and the larger openings correspond to the sockets of sensory setae (Fig. 6). We found no traces of silk in any of these openings, whereas silk threads are commonly seen in abdominal spigots.

DISCUSSION

Our results disagree with silk secretion from theraphosid feet proposed by Gorb et al., Rind et al. and Peattie et al. (Gorb et al., 2006; Rind et al., 2011; Peattie et al., 2011). In all eight spiders of the four species with sealed spinnerets, no traces of silk were found, confirming the results of Pérez-Miles et al. (Pérez-Miles et al., 2009) in *A. seemanni.* The explanation by Rind et al. (Rind et al., 2011) as to why silk was absent in our previous study (Pérez-Miles et al., 2009) is not applicable here because we used larger vertical surfaces and also induced the tarantulas to slip.

All arguments used by Gorb et al., Rind et al. and Peattie et al. (Gorb et al., 2006; Gorb et al., 2009; Rind et al., 2011; Peattie et al.,

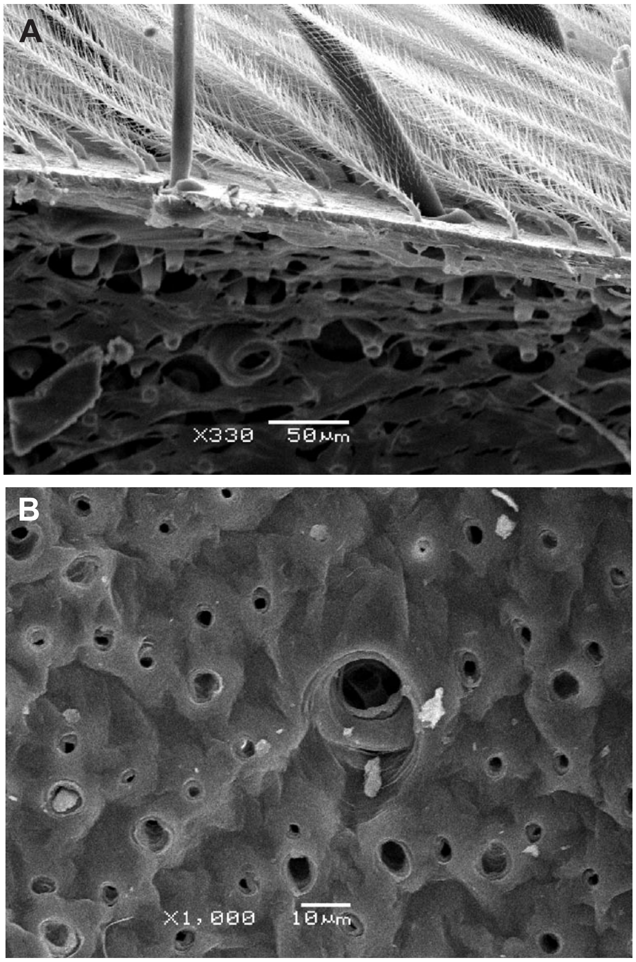

Fig. 6. Microphotograph (SEM) of the internal face of tarsal exuvia of female *Grammostola mollicoma*, showing no silk. (A) Lateral view of the exuviae, showing the internal face with setae sockets below and the ventral scopulae setae above. Scale bar, 50 µm. (B) Magnified view of the sockets. Scale bar, 10 µm. Photo credit: Cintya Perdomo.

2011) to support tarsal silk secretion were indirect; no SEM images of alleged tarsal spigots secreting silk were shown, whereas silk can be easily observed in SEM pictures of spinneret spigots (Rind et al., 2011; Foelix et al., 2012). Also, in all cases in which silk was found, the spiders had free spinnerets and contamination of slides with spinneret silk could not be excluded. Although Rind et al. (Rind et al., 2011) discarded slides that had been in contact with the abdomen, this is not sufficient to prevent contamination because silk could be transferred passively, without the need for contact with spinnerets. The morphology of the tarsal features that were interpreted by Gorb et al. (Gorb et al., 2006) and Rind et al. (Rind et al., 2011) as tarsal spigots clearly differs from that of spinneret spigots. Furthermore, there is now good morphological evidence that the alleged tarsal silk spigots are chemosensory setae (Foelix et al., 2011; Foelix et al., 2012). Chemosensory setae have a bent shape, lack a large bulbose base and possess a subterminal pore, whereas the spigots are typically straight and have a large bulbose base and a central terminal pore, often with extruding silk threads.

An additional adhesion mechanism for climbing, as supposed tarsal silk threads, could be expected mainly in arboreal tarantulas. The only arboreal species tested in this study (*A. avicularia*) did not secrete tarsal silk; in Aviculariinae, tarsal scopulae and claw tufts with adhesive hairs are more extended laterally than in other theraphosids (West et al., 2008). These scopular lateral extensions are probably related to the climbing habits of arboreal Aviculariinae (Bertani and Marques, 1996).

The fluid footprints observed in tarantulas with sealed and free spinnerets agree with the findings of Peattie et al. (Peattie et al., 2011) in *G. rosea.* They clearly differ from silk because they are made up of groups of droplets, as seen with the confocal microscope. These footprints may well originate from chemosensory setae and be composed of receptor lymph (Foelix et al., 2012). This exudate is probably sometimes fluid and sometimes dryer (filamentous lymph), depending on factors such as relative humidity, temperature and the hydration state of the spider, which could explain the confusion with fibers. These considerations agree with the idea that some spiders' abdominal silk glands may have evolved from chemosensory setae (Palmer, 1990). The importance of a thin fluid film between the scopula setae and the substrate for adhesion was proposed previously by Homann (Homann, 1957) and later by Roscoe and Walker (Roscoe and Walker, 1991). In the absence of a water film, as on Teflon foil, the spider slides and may fall off. Niederegger and Gorb (Niederegger and Gorb, 2006) demonstrated the diminishing adhesion in artificially dried spider scopulae. We did not find any evidence of tarsal silk secretion in Theraphosidae, but the presence of a thin fluid film between the scopula and the substrate seems to provide plenty of adhesion when climbing on vertical smooth surfaces.

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