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# Wing surface in the damselfly *Mecistogaster ornata* (Zygoptera, Pseudostigmatidae): interactions between nanoscale wax and sticky spider webs

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The representatives of the damselfly family Pseudostigmatidae are known for their ability to catch small orb web spiders, or in some cases small kleptoparasitic spiders in the webs of other spiders. In this paper, I demonstrate that the nanoscopic crystalline wax coverage of wings in the pseudostigmatid damselfly *Mecistogaster ornata* is partially altered due to the presence of fluid-contaminated spots corresponding in their shapes and distribution to the typical beads-on-a-string (BOAS) geometry of sticky threads of orb web spiders. The spider fluid has the ability to wet superhydrophobic crystalline wax coverage. Also residues of the sticky threads were revealed in high quantities on the wing surface. The data suggest that the pseudostigmatid damselflies, due to their specific prey capturing method, have some costs and risks of being trapped by the sticky spider webs. However, high resolution SEM images revealed that the crystalline wax coverage of wings, in spite of its wettability by the spider glue, functions as a sacrificial anti-adhesive layer protecting the damselfly surface against spider adhesive traps.

**Keywords:** spiders; Arachnida; sticky threads; scanning electron microscopy; SEM; wax crystals; adhesion; wing; Odonata; dragonfly

## Introduction

Damselflies from the family Pseudostigmatidae are known for their ability to catch small orb web spiders or in some cases, small kleptoparasitic spiders in the webs of other spiders (Calvert, 1923; Corbet, 1999; Fincke, 1992; Young, 1980). After detecting a spider, the damselfly typically moves back and, during a quick forward flight, catches the prey with its forelegs (Fincke, 1992; Rüppell, 1987a, 1987b; Rüppell & Fincke, 1989a, 1989b). During this action it can touch sticky threads of the spider web and even hang on the web for several seconds before returning. It was also observed that after such actions, damselflies often preened themselves of sticky threads by moving their long abdomen up and forwards between their wings, and by rubbing their head and eyes with their forelegs (Fincke, 1992). Pseudostigmatids are at risk of getting caught in spider webs – particularly the webs of large spiders, which is likely why they typically forage in full sun – conditions in which the spider webs are most visible. In spite of the high-speed video recordings and detailed description of feeding behavior (Fincke, 1992; Rüppell, 1987a, 1987b; Rüppell & Fincke, 1989a, 1989b), it remains unclear what effects on the body and especially on

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the wing surface the contact to the sticky threads has, and why the damselfly is not trapped by the efficient sticky fluid of orb web spiders.

It has been earlier reported that the crystalline wax of odonate cuticle (Gorb, 1995; Gorb, Tynkkynen, & Kotiaho, 2009) among numerous other functions can prevent wings from contamination by dust particles (Wagner, Neinhuis, & Barthlott, 1996). Due to the minute surface roughness, caused by the nanoscale wax structure, one may assume that adhesion of sticky material to the odonate cuticle is reduced, similar to the adhesion reduction of insect adhesive feet on plants that are covered with crystalline waxes (Gorb, 2001; Gorb & Gorb, 2002). It is known that surfaces covered with superhydrophobic nanostructures also might generally reduce adhesion of some sticky substances (Rakitov & Gorb, 2013). Taking all this into account, one may hypothesize that the wing wax coverage of pseudostigmatid damselflies may effect in adhesion reduction to the sticky capture threads of orb web spiders.

In this paper, I used high resolution scanning electron microscopy (SEM) to visualize the wing surface of the pseudostigmatid damselfly *Mecistogaster ornata*, in order to see the impact of the spider webs on the wing. The main goal was to understand nanoscopic interactions between sticky treads and crystalline wax layer of the wing. For comparison, I have also visualized sticky threads of orb web spiders in the cryo-SEM.

## Materials and methods

Individuals of the female damselfly *Mecistogaster ornata* Rambur originate from Venezuela (collection of J. DeMarmels). Desired pieces of wings were removed using forceps and fine scissors, mounted on aluminum holders, sputter-coated with gold-palladium (10 nm), and examined in a SEM Hitachi S-4800 (Tokyo, Japan) at 1–3 kV. Low accelerating voltage was used while operating at high magnifications, in order to prevent damage of fragile wax crystals by the focused electron beam.

To visualize the beads-on-a-string arrangement of an adhesive from spider orb webs, sticky threads of *Araneus diadematus* were mechanically fixed on small metal frames (1 mm gap), kept at 95% humidity for 10 min. frozen at the cryo-SEM stage (–140°C) and observed at –120°C in uncoated condition in a SEM Hitachi S-4800 at 1–3 kV.

## Results and discussion

The entire cuticle surface of the wings of *Mecistogaster ornata* was covered with crystalline wax of µm and sub-µm dimensions (Figure 1A). The crystals form rectangular structures made of nanoscopic platelets and at the corners of single rectangles, there are filamentous protrusions with the diameter of about 50 nm and length of 200 nm (Figure 1B, C). There is no difference in the structure, distribution and dimensions of individual crystals between the dorsal and ventral side of the wing. At the lower magnification images, the wax coverage is partially altered due to the presence of microscratches (Figure 2A, B) or contaminating fluids. Fluid contaminations were previously observed in the wax laxer of *Calopteryx splendens* damselflies (Gorb et al., 2009); however, in *M. ornata*, the arrangement of contaminated sites was not random, but rather corresponding to the typical beads-on-a-string (BOAS) geometry of sticky threads of orb web spiders.

More careful analysis of these sites revealed that the drops (3–30 µm in diameter) are indeed often (not always) interconnected by the sticky threads (about 1 µm in diameter) (Figure 2). It

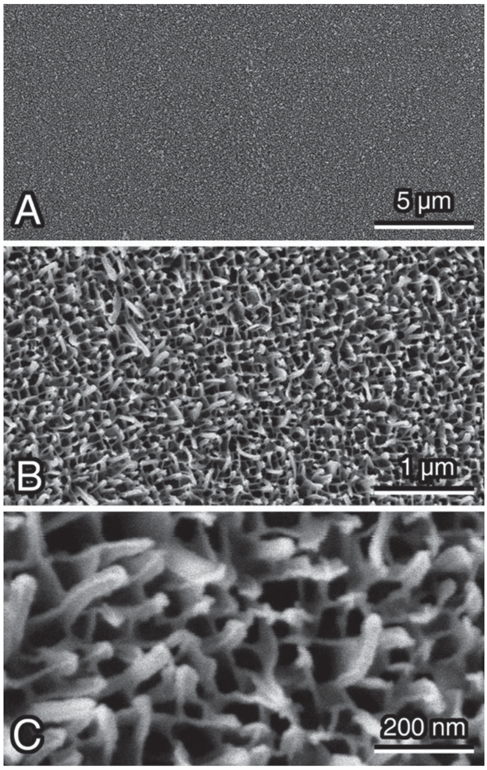


Figure 1. *Mecistogaster ornata*, SEM image of the ventral surface of the dry hind wing, intact areas covered with crystalline wax at three different magnifications.

is well known that crystalline wax coverage of dragonfly wings has superhydrophobic properties (Gorb et al., 2009; Kuitunen & Gorb, 2011), but surprisingly they were readily wetted by the spider adhesive fluid. It is interesting that due to the wetting and meniscus formation, the capillary forces at the perimeter of the fluid drop destroyed the wax arrangement by pulling crystals to the middle of the drop, and exposed the underlying epicuticle between the wetted and unwetted wax (Figure 2C, E). It is known that the spider adhesive fluid contains proteins (Amarpuri, Chaurasia, Jain, Blackledge, & Dhinojwala, 2015; Sahni, Blackledge, & Dhinojwala, 2011), which often act as surfactants reducing surface tension of water, where they are dissolved (Voigt & Gorb, 2010).

Threads between the drops were also covered by fluid and they stuck to the crystalline wax coverage as well (Figure 2C, D, F). There were many sticky spider threads with the BOAS or without them found on the wing surface (Figure 2A). The fluid and threads covered quite substantial areas of wings. When the thread and corresponding BOAS were removed from the wing surface by peeling off (presumably due to the active cleaning process by damselfly, see Introduction section), the wax coverage remained sticking to the thread or drop surface and was peeled off from the surface, exposing clean epicuticle surface penetrated by the nanoscopic pore channels (Figure 3A, B). Nanoscopic residues of the spider fluid were observed especially in the gaps generated by the capillary forces, at the perimeter of the BOAS meniscus (Figure 3C).

I studied the spider BOAS in the cryo-SEM (Figure 4). It is clearly seen that the fluid adhesive is not only situated within the drop, but spread over the “drop free” fiber. This might explain a good adhesion of the fibers to the wax surface of the damselfly and the peeling off the wax crystals, when the fiber is removed from the wing surface (Figure 3A, B). The importance of

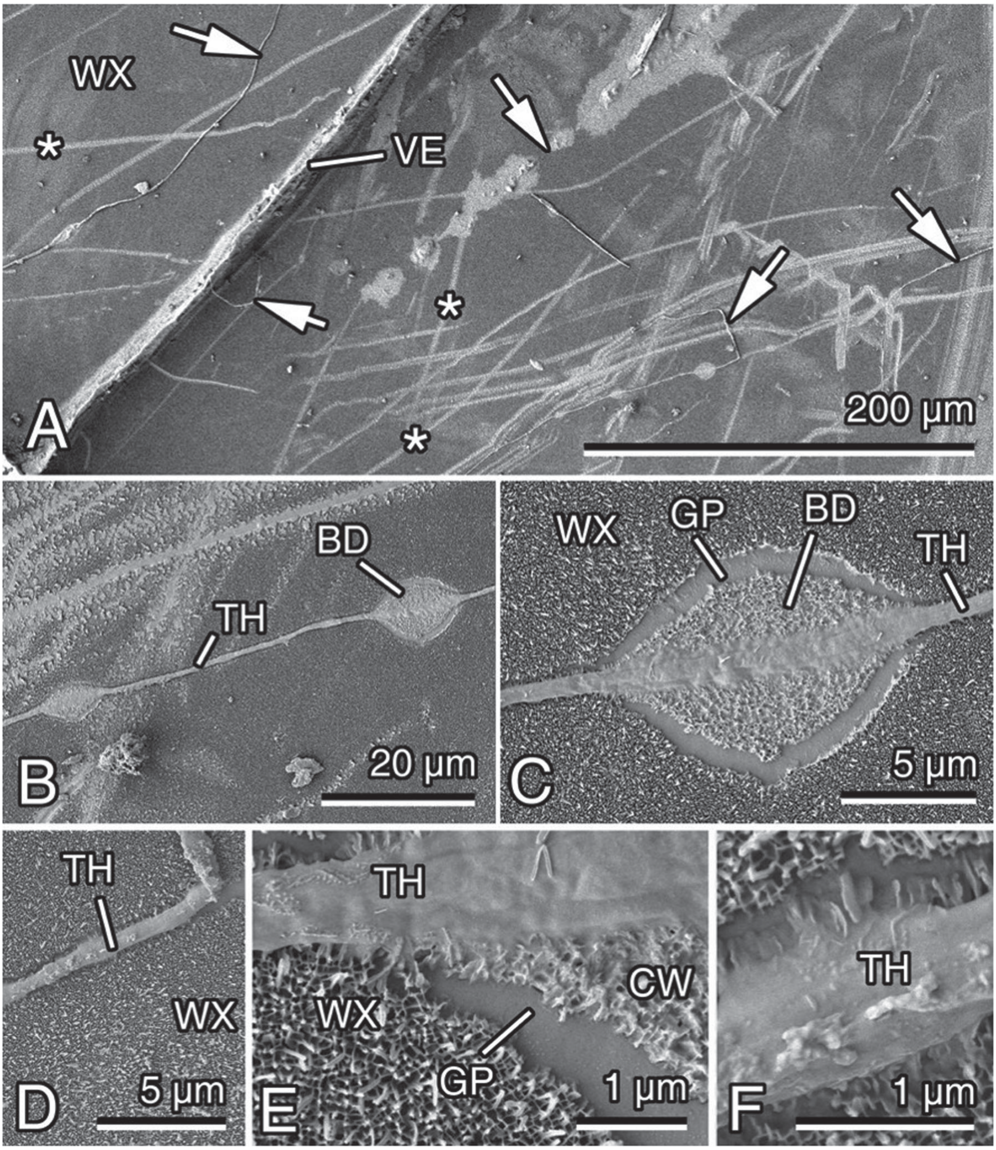


Figure 2. *Mecistogaster ornata*, SEM images of the ventral surface of the dry hind wing. (A) An overview of the wing area at low magnification of the SEM. (B, C) Beads-on-string spider adhesive residues after physical contact with the spider orb web. (D–F) Details of the spider sticky thread in contact with the wing surface. Abbreviations: BD, region, where the "bead" (adhesive drop) was in contact with the wing surface; CW, wax contaminated with the spider adhesive; GP, gap of the wax-free surface resulting from the action of capillary forces at the edge of the adhesive drop; TH, spider thread; VE, wing vein; WX, intact areas covered with crystalline wax. Asterisks indicate scratches in the crystalline wax layer. Arrows indicate spider threads in the overview image (A).

glycoproteins and salts is well described from the secretion of the aggregate silk glands of spiders that produce the tacky droplets on the capture threads in orb and cobweb spiders (Amarpuri et al., 2015; Sahni et al., 2014; Townley & Tillinghast, 2013). In these, the salts adsorb water, and thereby soften the glue and enhance adhesion by the solvation of the glycoproteins (Amarpuri et al., 2015; Sahni et al., 2014).

These data demonstrate that wax coverage of the damselfly wing is not repellent for the surfactant-rich adhesive fluid of spider webs. However, because of how the wax layer is readily

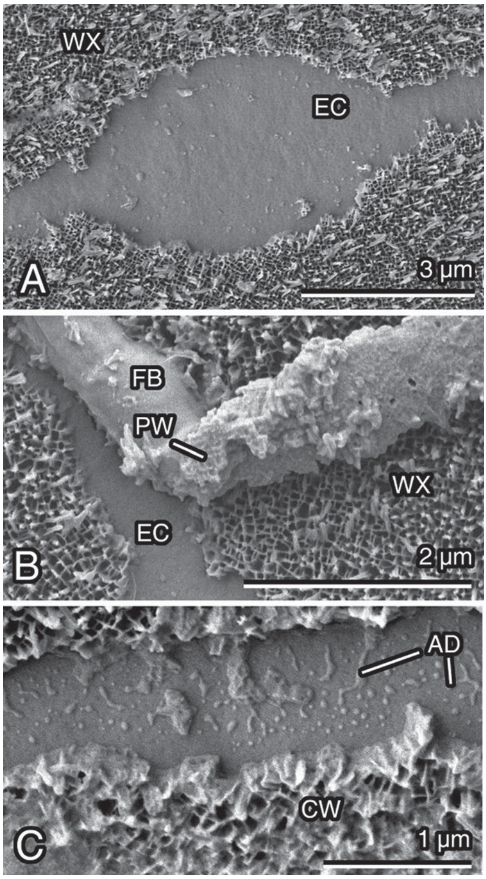


Figure 3. *Mecistogaster ornata*, SEM images of the ventral surface of the dry hind wing. (A) The wing region, where the spider sticky thread was initially in contact and was removed. Note that the crystalline wax layer is peeled off the wing surface, and an underlying epicuticle with minute nanoscopical pore channels can be seen. (B) The spider thread partially removed from the wing surface. Note that the wax remains attached to the adhesive thread and the wing epicuticle is free of wax crystals. (C) Details of the region at the circumference of the "bead" (adhesive drop). Abbreviations: AD, adhesive nanodrops (remains of the spider adhesive fluid); CW, wax contaminated with the spider adhesive fluid; EC, regions of the clean epicuticle, where the spider "bead" (adhesive drop) or adhesive thread were in contact with the wing surface; FB, spider adhesive fiber; PW, peeled off wax residues on the sticky thread; WX, areas covered with an intact crystalline wax.

wetted by the spider adhesive, and the wax crystals peel off easily, the wax layer still might have a protective function against spider webs due to the cohesive failure at the interface between wax crystals and the epicuticle. Thus the crystalline wax coverage might represent a kind of sacrifice layer, protecting damselflies from being captured by spider webs.

In general, three different types of damage in odonate wing wax coverage are known (Gorb, Kesel, & Berger, 2000; Gorb et al., 2009; Kuitunen & Gorb, 2011): wiping off, abrasion and contamination. Both wiping off and abrasion happen when an external force is applied (Figure 1A, B), and the wax layer can be smeared off or compressed, sometimes to such degree that the underlying cuticle becomes fully exposed. Wax layer contamination by amorphous substances of unknown nature was observed on the abdominal surface and partially on the head of *Calopteryx splendens* (Gorb et al., 2009). The contamination by spider adhesive fluid and by sticky threads of orb web spiders has not previously been described. It is especially important observation for the representatives of the damselfly family Pseudostigmatidae, which are known for their ability to capture orb web spiders from their webs. Given the data presented here, these slow, gliding

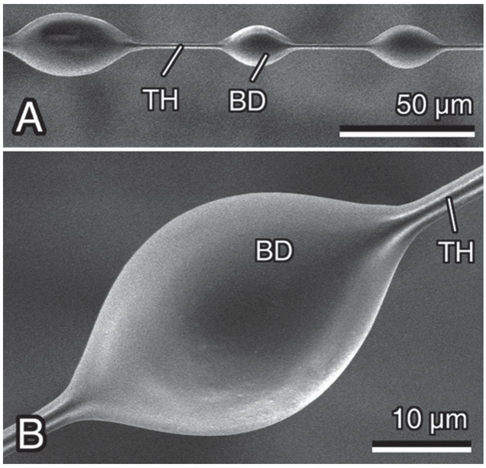


Figure 4. Cryo-SEM images of the beads-on-string adhesive threads of an orb-web spider. Abbreviations: BD, "bead" –adhesive drop; TH, thread.

damselflies (Dudley, 2000) are likely at risk of being trapped by the sticky webs of spiders. The crystalline wax coverage of the wings, which does not prevent adhesion of the sticky web strands, can be peeled away during wing cleaning. However, removal of this protective layer carries a cost in that it exposes the epicuticle, which in turn may increase the risk of entrapment.

As mentioned above, many odonate species have crystalline wax coverage that is rather similar to that of *M. ornata* (Gorb, 1995; Gorb et al., 2000, 2009). In this study, I did not find any morphological differences of the coverage from that of other previously studied odonate species. In general, odonate wax coverage decreases wettability of the body, which is essential for insects closely associated with water. Males of some Odonata may additionally benefit from this coverage during their floating displays, when courting females directly on the water surface (Gibbons & Pain, 1992), and both sexes may benefit from submerged oviposition (Fincke, 1986; Tsubaki, Kato, & Shintani, 2006; Corbet, 1999). Aside from pseudostigmatids, other odonates have occasionally been reported to also take spiders as prey (Fincke, 1992), or even insects from spider webs (Cham, 1992; Parr & Parr, 1996); one can hypothesize that the wax coverage of other Odonata may have similar functional significance for protection against spider sticky threads as reported here for *M. ornata*.

Given that the wax coverage in *M. ornata* does not show any differences from other previously studied odonates, from the evolutionary point of view its function in defense against spider webs is most likely secondary. The primary function is most probably the superhydrophobicity allowing male floating displays, female submerged oviposition and, more general, life in close vicinity to the water. Another important (and also likely secondary) function of the wax coverage is its use in sexual selection as previously shown for the pseudostigmatid *Megaloprepus caerulatus* (Schultz & Fincke, 2009) and two libellulids *Neurothemis tullia* (Gorb, Appel, & Kovalev, 2014) and *Zenithoptera lanei* (Guillermo-Ferreira, Bispo, Appel, Kovalev, & Gorb, 2015; Guilliermo-Ferreira, Appel, Urban, Bispo, & Gorb, 2017). The wax pigmented areas in these and some other odonate species are involved in mate recognition and courtship behavior.

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