Electronic Supplementary Material (ESI) for RSC Advances.


# Structural Characterization of Nanofiber Silk Produced by Embiopterans (Webspinners)

Supporting Information

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## Section 1: Mechanical Testing

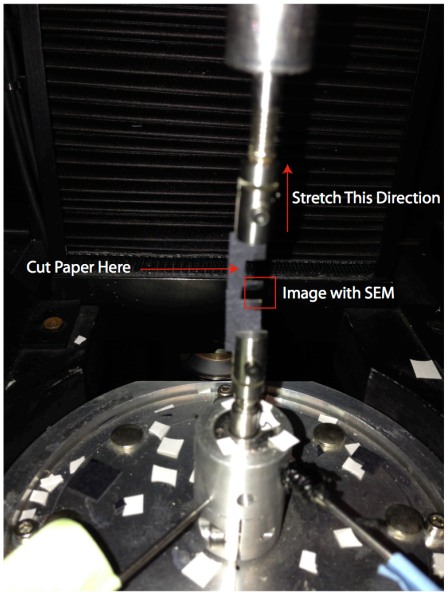


Fig. S1: Tensile properties for webspinner silks of the species *Antipaluria urichi* were investigated in an attempt to improve on suspect results from previous works. Fourteen stress-strain curves were obtained using a Nano Bionix tensile tester (MTS System Corp, Akron, OH, USA). Samples were prepared on E - shaped cards by first anesthetizing an adult female insect with $CO_2$ gas, then pinning the insect on her back using strips of Parafilm. Then under a dissecting microscope, the tips of the E card were brushed against the tarsus a couple times until a silk bundle was visible to the naked eye. The silk was then secured to the 3 tips of the E with superglue (cyanoacrylate). E-shaped cards were mounted on the Nano Bionix apparatus seen above for tensile testing. The card was secured, and one side of the E paper card was cut with scissors prior to testing. The other half of the E contains an untested silk bundle that was imaged with SEM to estimate the number of fibers present in the tested sample. Fiber bundles were extended at a rate of 1% strain per second until complete failure, and force versus extension curves were obtained for all samples.

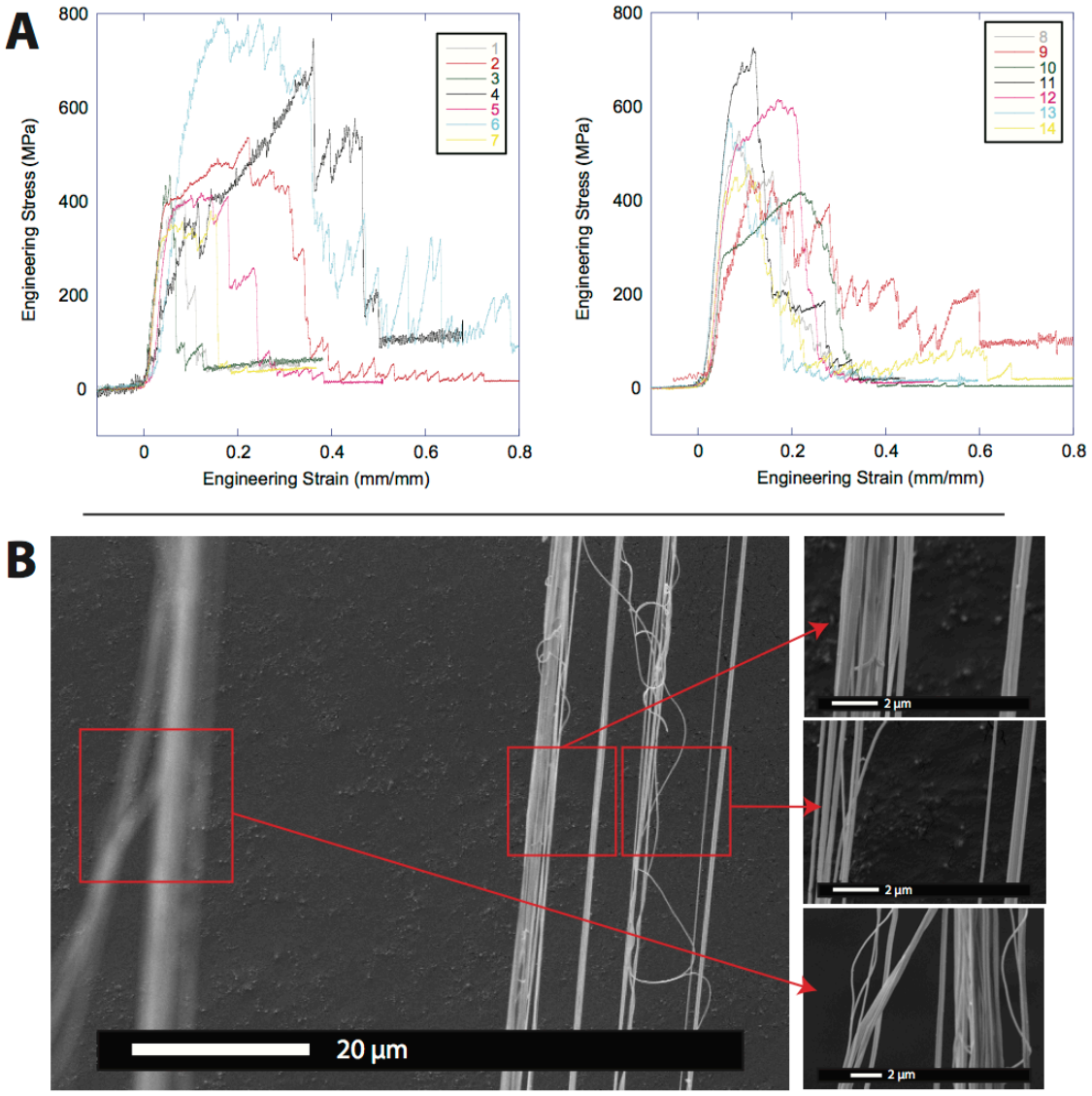


Fig. S2: (A) 14 stress-strain curves obtained from webspinner silk bundles of the species *Antipaluria urichi.* Raw force data was converted to engineering stress after accounting for the total silk cross sectional area as estimated through SEM imaging of the non-stretched portion of each sample. (B) Example SEM image corresponding to trace 13. Approximately 120 fibers are present in this case. The mean ultimate stress for the 14 fibers is 500 MPa, revealing that the webspinner silk is significantly stronger than previously reported.

| Sample | Number of Fibers | Ultimate Stress (MPa) |
|---|---|---|
| 1 | 25 | 380 |
| 2 | 150 | 480 |
| 3 | 30 | 425 |
| 4 | 20 | 660 |
| 5 | 100 | 400 |
| 6 | 40 | 760 |
| 7 | 100 | 350 |
| 8 | 200 | 430 |
| 9 | 30 | 430 |
| 10 | 300 | 410 |
| 11 | 80 | 680 |
| 12 | 240 | 590 |
| 13 | 120 | 530 |
| 14 | 120 | 430 |

Table S1: Approximate number of fibers present for each trace estimated from SEM images, and the ultimate stress (in MPa) for each trace. The mean ultimate stress from the 14 measurements is 500 MPa.

## Section 2: Fourier-Transform Infrared Spectroscopy

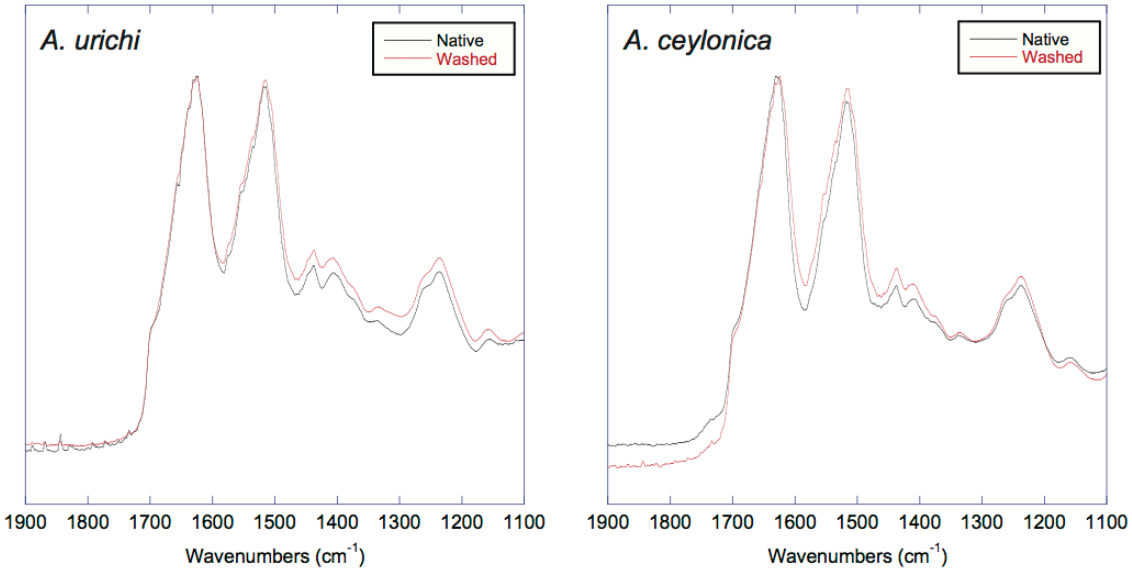


Fig. S3: Comparison of the FTIR absorbance profile before (black) and after (red) washing webspinner silks with 2:1 $CHCl_3$:MeOH to remove the surface lipid or alkane-rich layer. The amide I, II, and III bands are all essentially identical after washing, which suggests that the silk protein structure remains unchanged.

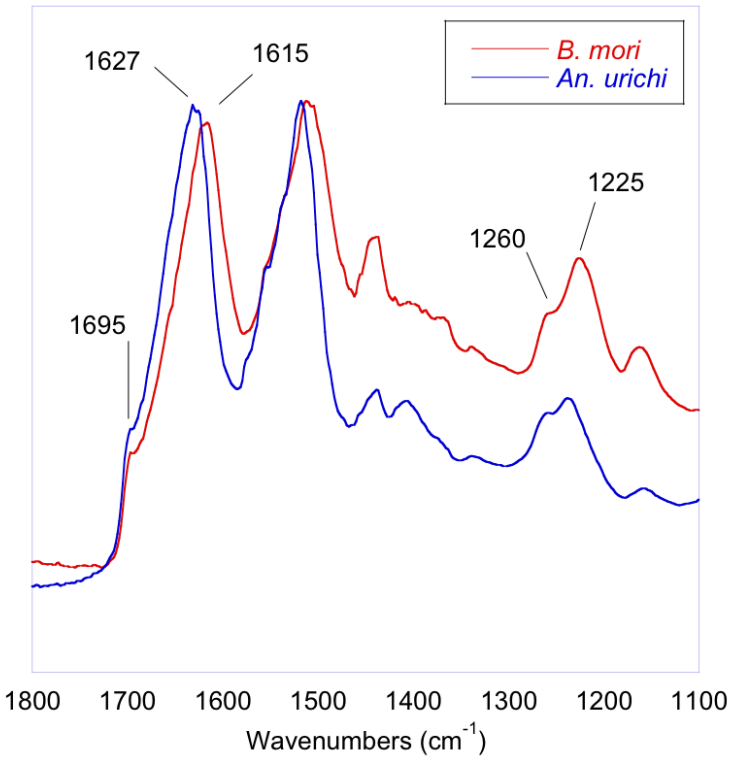


Fig. S4: Comparison of the FTIR absorbance profiles of degummed *Bombyx mori* silkworm silk (red) with native *Antipaluria urichi* silk (blue). Both silks are protein-based biopolymers rich in GAGAGS (*B. mori*) or GAGSGS (*An. urichi*) repetitive motifs. The profile for *An. urichi* silk is similar to that of *B. mori* silkworm silk, but the amide I, II and III bands are shifted towards slightly higher frequencies. We believe that the shift in frequency is a result of differing primary protein sequences. It is clear that both silks are dominated by β-sheet secondary structures.

## Section 3: Gas-Chromatography Mass-Spectroscopy

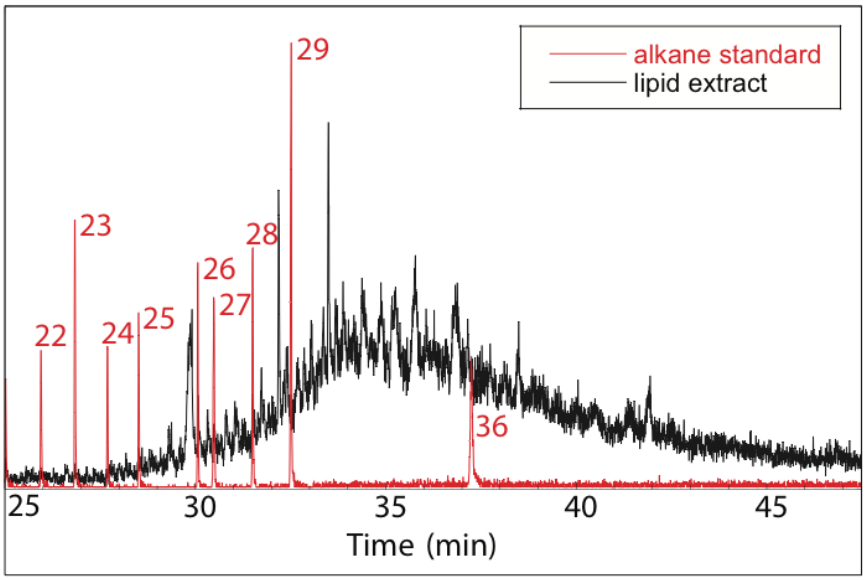


Fig. S5: Selected ion chromatogram at 99 m/z, a characteristic peak for alkanes, comparing lipids extracted from *Antipaluria urichi* silk (black) to an alkane standard (red). The sample was prepared by soaking 6 mg of *An. urichi* silk in 20 mL of 1:1 DCM:hexanes for 24 hours. The bulk silk was removed and the solvent was concentrated to dryness using a stream of $N_2$ gas. The residue was then dissolved in 250 µL of analytical grade DCM. The lipid extract was analyzed using an Agilent 6890N/5973 inert GC-MS, operated in electron ionization mode using a HP-5MS column (30 m × 0.250 mm × 0.25 µm) with splitless injection (10 psi) set at 300 °C. The helium carrier gas was set at a rate of 1.2 mL/min. The oven temperature was initially set to 65 °C for 10 min and then ramped to 300 °C at 10 °C/min and held for 20 minutes. The carbon chain-length of the eluting alkanes within the standard is indicated near each peak. A large hump and some poorly resolved peaks are seen for the extracted silk sample. This broad hump is known as the an unresolved complex mixture (UCM), which consists of a variety of straight and branched-chain alkanes of varying length and substitution position. The alkane species present in the extracted silk sample appear to span from less than 20 carbons to greater than 36 carbons in length.