Glowworm Capture Threads Studied by AFM

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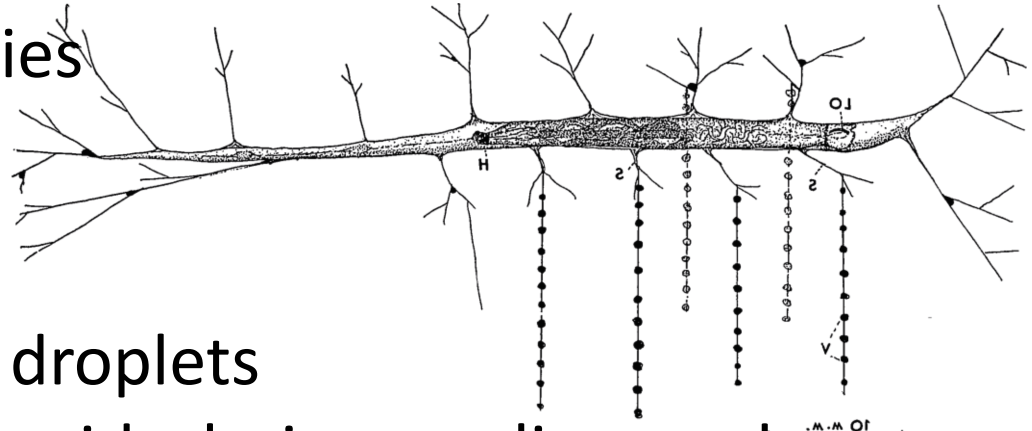
Glowworm Threads as a Material System

Environment: almost exclusively in the wet caves & temperate rainforests of NZ and Australia

GW - larvae of fungus gnat – nine known species

Arachnocampa luminosa, A. flava

A. richardsae, A. tasmaniensis



Threads: Silks (pair of side by side fibres) with droplets

Silk ~100% protein – rich in hydrophilic amino acids: lysine, proline, and thymine

Droplets ~99% water, small amounts urea, Na, S, K, Mg, O_2 & N_2

Synergistic system for prey capture providing:

1. Adhesion
2. Energy dissipation with minimal damage

Glowworm Snare/Trap

https://oreillys.com.au/uncategorized/secret-life-glow-worms/
Lamington National Park, Qld, *Arachnocampa flava*

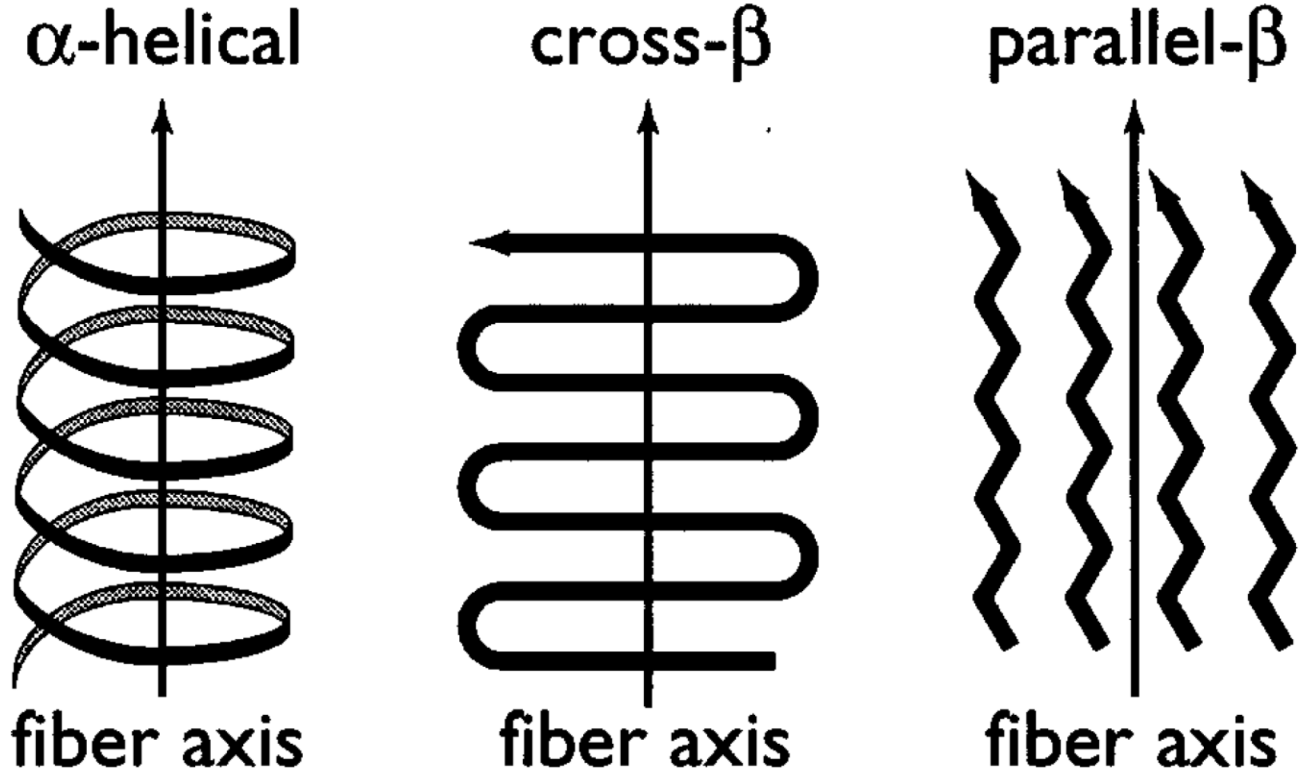
Arachnocampa tasmaniensis silk threads.
Photo credit: Joe Shemesh.

Glowworm Tourism

New Zealand photographer Joseph Michael, *Luminosity* series, 2015. Stamps issued 2 March 2016
https://www.joemichael.co.nz/

Arthropod Silks – Tough and Strong

Glowworm Silk: cross-β-rich (cf spider silk β-parallel and alpha-helix rich)



Catherine L. Craig, "Evolution of arthropod silks", *Annu. Rev. Entomol. 1997. 42:231–67*

This Study – Contrasting Stretched and Unstretched Silks

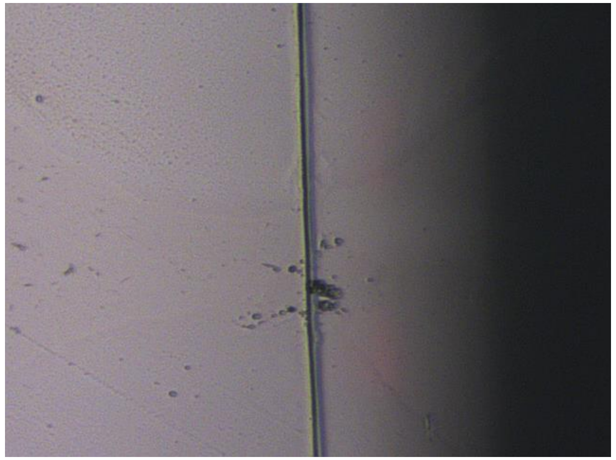
Aim – Is there any structural evidence of unravelling cross-β components in GW silk due to applied strain?

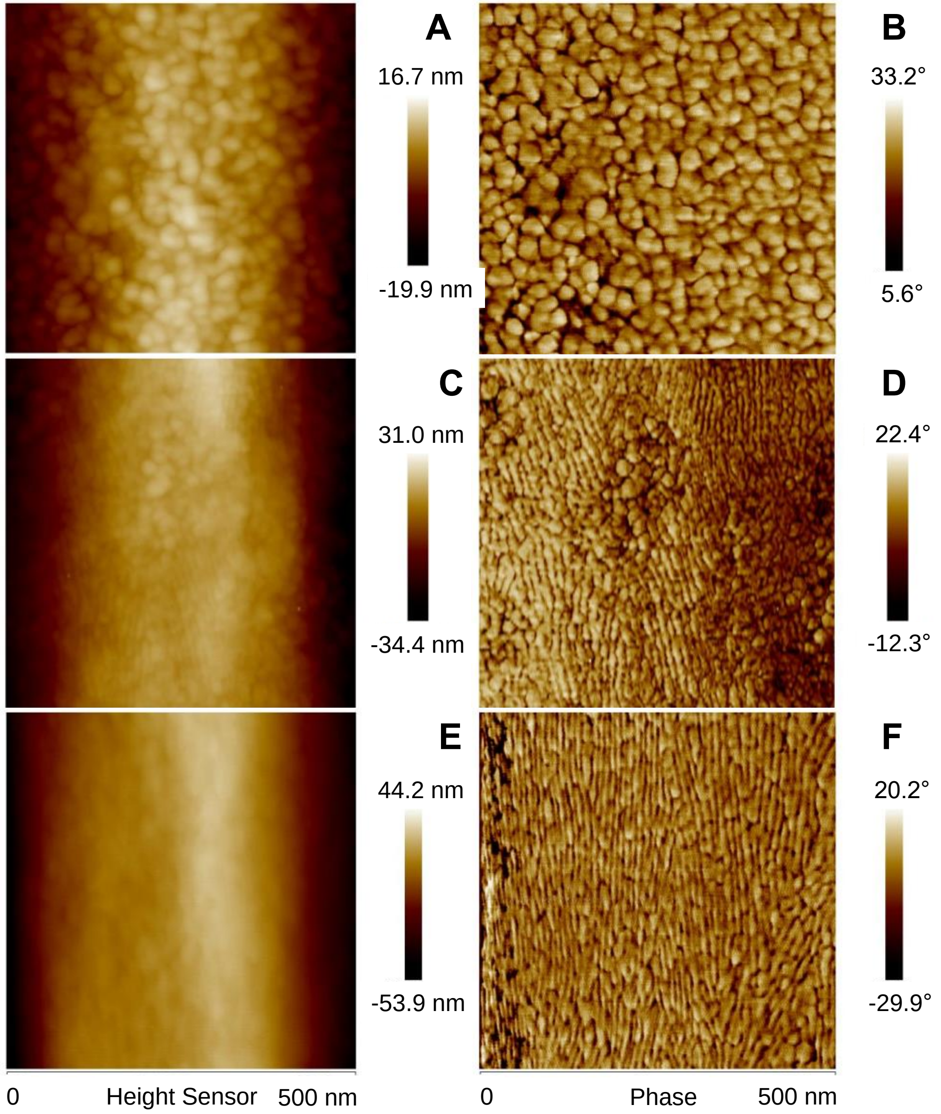
Species: *Arachnocampa tasmaniensis*

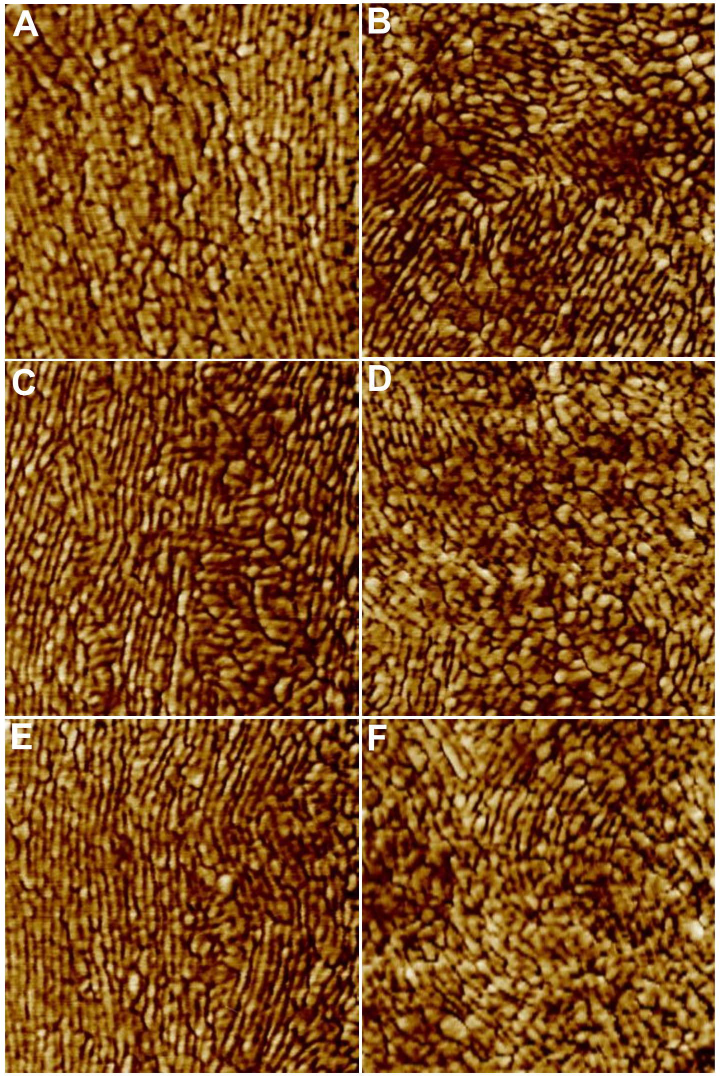
Samples collected from the entrance and twilight zones of the Bradley Chesterman caves in Southwest National Park, Tasmania, Australia, in Oct 2017, on glass slides

Tapping mode-AFM using Dimension Icon Bruker instrument
SSS-NCHR SuperSharp cantilevers, nominal spring constant (k) of 42 N/m & resonance frequency 330 kHz
Typical tip radius - 2 nm
Automatic amplitude setpoint 350–375 mV

Results







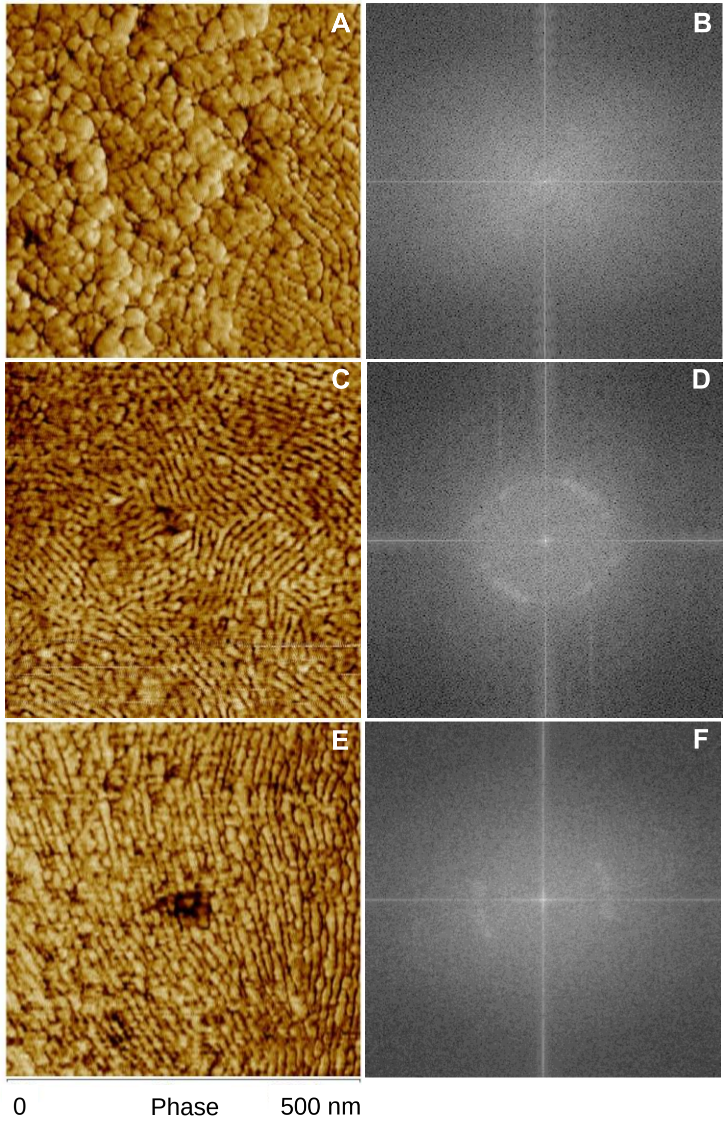
Stretched Unstretched

Fast Fourier Transformation - Results

500 nm x 500 nm images FFT processed: 40 Stretched silk samples; 40 Unstretched silk samples

1 μm x 1 μm images &
100 nm x 100 nm images, also in the image set

FFT yields size and orientation information about the inhomogeneities in stiffness of the material

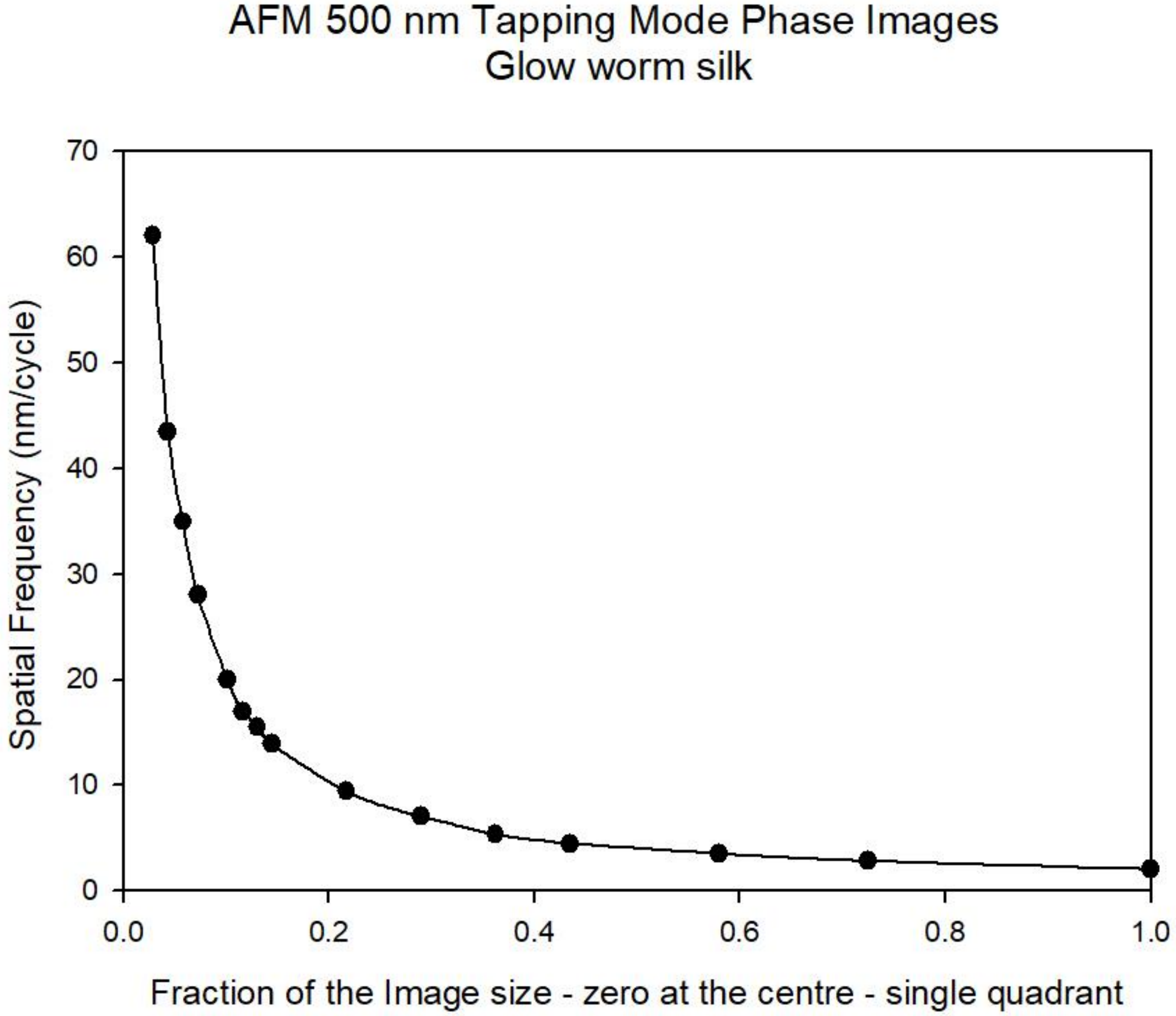


Unstretched

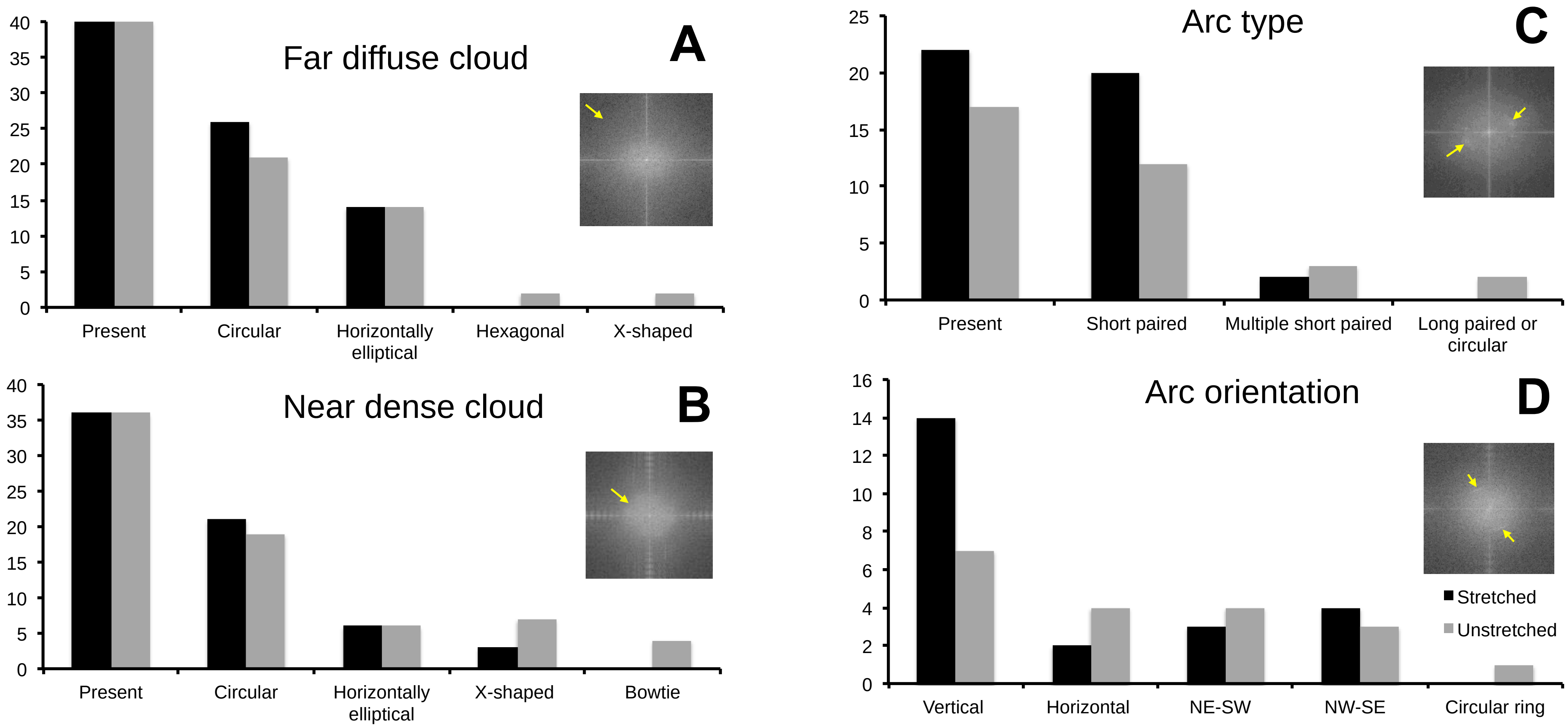
Unstretched

Stretched

Fast Fourier Transformation - Calibration

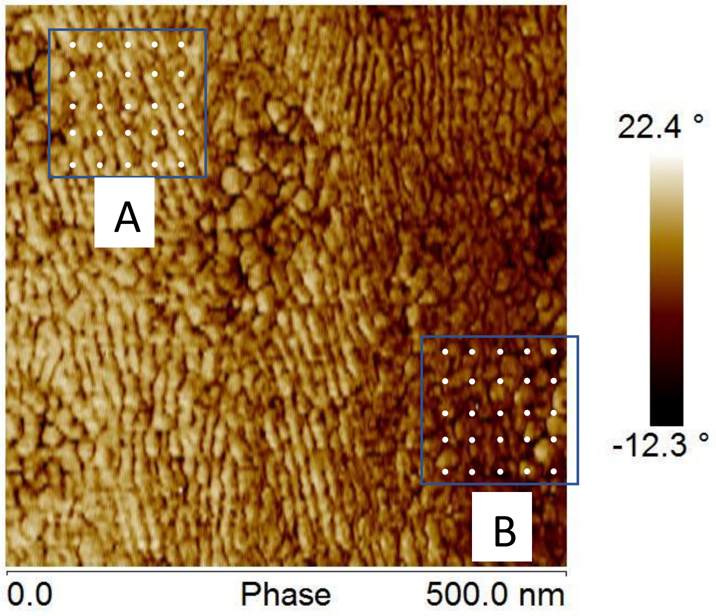


Collation of Fast Fourier Transformation 80 Images



Clear evidence of a more vertical, fibrillar features in the phase images of stretched cf unstretched GW silks

Unstretched silk - QNM mode



Indentation force	**10 nN**
Indentation velocity	3 um/s

	Region A		Region B	
	Young's modulus (MPa)	Reduced modulus (MPa)	Young's modulus (MPa)	Reduced modulus (MPa)
Average	1.91	2.11	3.05	3.35
St. Dev.	0.43	0.47	0.72	0.79
Max.	2.71	2.98	3.84	4.22
Min.	1.08	1.19	1.37	1.51

A Image File Name	Young's Modulus (MPa)	Reduced Modulus (MPa)
silk_500000.005	1.34	1.47
silk_500001.005	1.2	1.32
silk_500002.005	1.08	1.19
silk_500003.005	1.09	1.19
silk_500004.005	1.12	1.23
silk_500005.005	2.04	2.25
silk_500006.005	2.16	2.38
silk_500007.005	2.17	2.38
silk_500008.005	2.33	2.56
silk_500009.005	1.96	2.15
silk_500010.005	1.93	2.12
silk_500011.005	2.24	2.46
silk_500012.005	2.06	2.27
silk_500013.005	2.01	2.21
silk_500014.005	2.16	2.37
silk_500015.005	2.71	2.98
silk_500016.005	1.95	2.14
silk_500017.005	1.89	2.08
silk_500018.005	2.13	2.34
silk_500019.005	1.94	2.13
silk_500020.005	1.91	2.1
silk_500021.005	2.41	2.65
silk_500022.005	2.03	2.23
silk_500023.005	1.8	1.98
silk_500024.005	2.12	2.33
avg.	1.91	2.10
stdev.	0.43	0.47
max.	2.71	2.98
min.	1.08	1.19

B Image File Name	Young's Modulus (MPa)	Reduced Modulus (MPa)
silk_500025.005	3.19	3.51
silk_500026.005	3.23	3.55
silk_500027.005	3.36	3.69
silk_500028.005	2.52	2.77
silk_500029.005	2.57	2.82
silk_500030.005	2.42	2.66
silk_500031.005	2.94	3.23
silk_500032.005	3.47	3.81
silk_500033.005	3.4	3.73
silk_500034.005	3.49	3.84
silk_500035.005	3.3	3.63
silk_500036.005	3.8	4.17
silk_500037.005	3.84	4.22
silk_500038.005	3.52	3.87
silk_500039.005	1.37	1.5
silk_500040.005	1.47	1.62
silk_500041.005	3.32	3.64
silk_500042.005	3.64	3.99
silk_500043.005	3.57	3.92
silk_500044.005	2.99	3.28
silk_500045.005	1.64	1.81
silk_500046.005	3.46	3.8
silk_500047.005	3.68	4.04
silk_500048.005	3.73	4.1
silk_500049.005	2.36	2.6
avg.	3.05	3.35
stdev.	0.72	0.79
max.	3.84	4.22
min.	1.37	1.50

Elastic Modulus

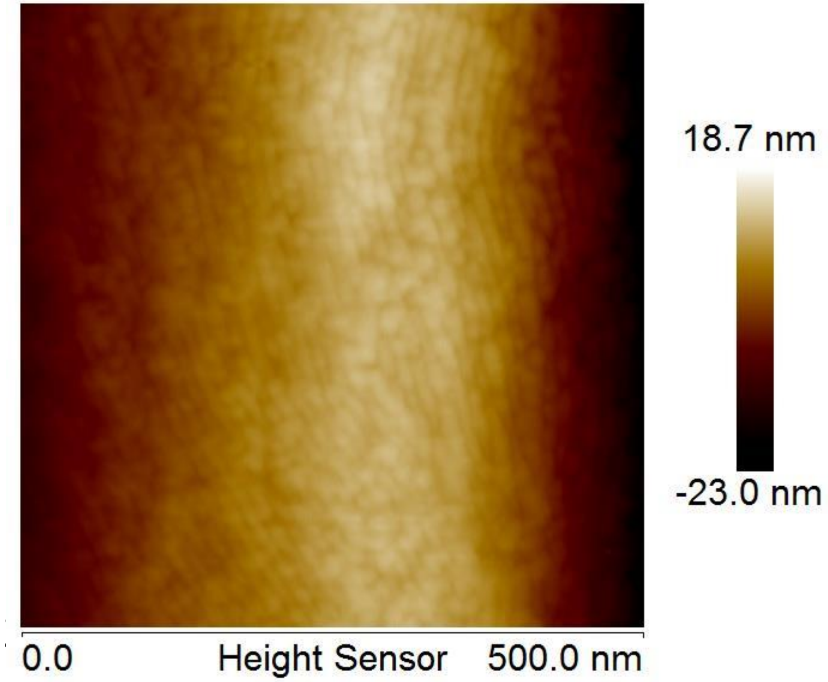
	Region A		Region B	
	Young's modulus (MPa)	Reduced modulus (MPa)	Young's modulus (MPa)	Reduced modulus (MPa)
Average	1.91	2.11	3.05	3.35
St. Dev.	0.43	0.47	0.72	0.79
Max.	2.71	2.98	3.84	4.22
Min.	1.08	1.19	1.37	1.51

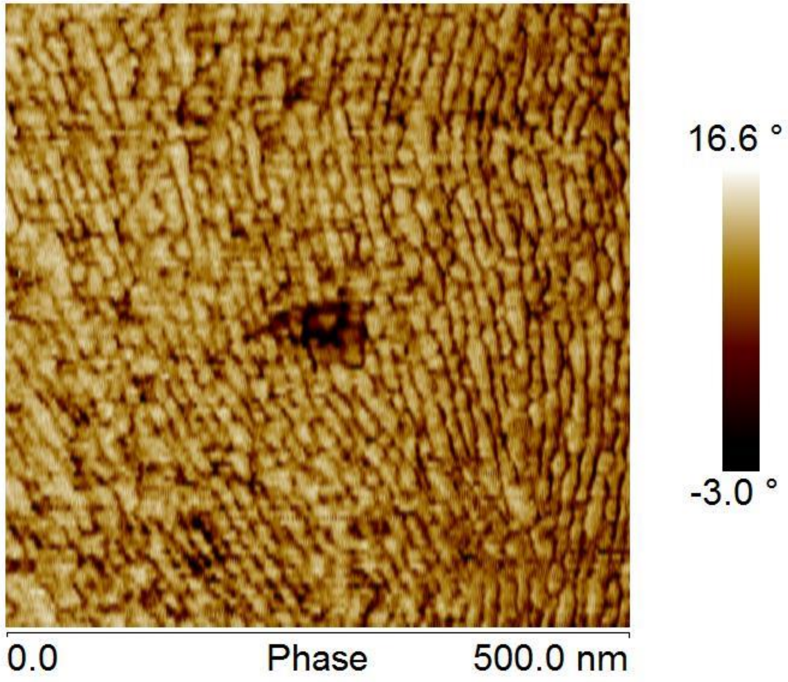
Standard tensile testing:

18.38 GPa at 30% RH

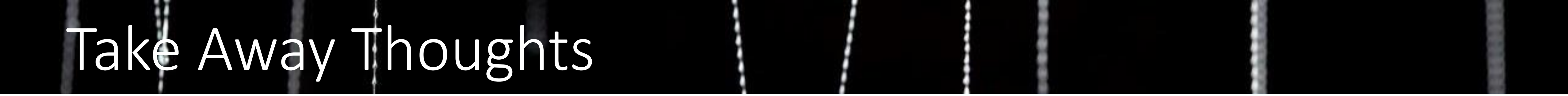
~100 MPa at >90% RH

Piorkowski, D.; Blackledge, T.A.; Liao, C.P.; Doran, N.E.; Wu, C.L.; Blamires, S.J.; Tso, I.M. Humidity-dependent mechanical and adhesive properties of *Arachnocampa tasmaniensis* capture threads. *J. Zool.* **2018**, *305*, 256–266





Stretched



Take Away Thoughts

Heterogeneous material morphology with size/width in the 10-15 nm range have been characterised.

Stretched silks show more fibrillar morphology than do unstrecthed silks, But both show variability in morphology

The physics of tapping mode AFM, and its potential to be applied in interrogating the modulus of soft, heterogeneous materials, on the nanoscale, has been known for more than 25 years. But it is under used.

A study, like the one here-in, undertaken with a team member with physics expertise on AFM involved from the outset, could have learned more about the material properties of the GW silks.

Thank you – contact deb.kane@anu.edu.au

Nanoscale Material Heterogeneity of Glowworm Capture Threads Revealed by AFM

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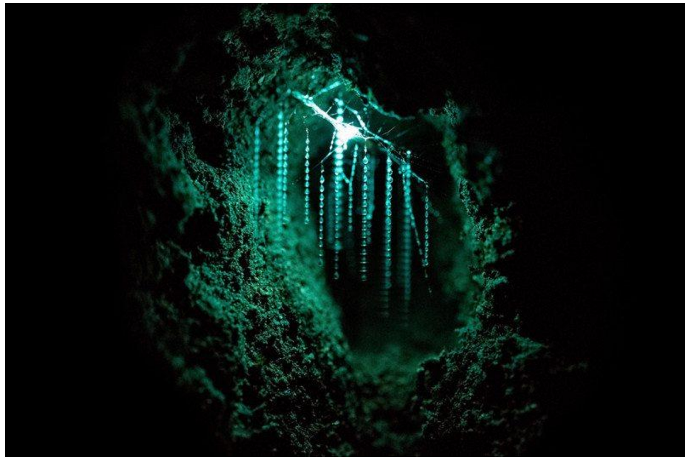

Abstract: Adhesive materials used by many arthropods for biological functions incorporate sticky substances and a supporting material that operate synergistically by exploiting substrate attachment and energy dissipation. While there has been much focus on the composition and properties of the sticky glues of these bio-composites, less attention has been given to the materials that support them. In particular, as these materials are primarily responsible for dissipation during adhesive pull-off, little is known of the structures that give rise to functionality, especially at the nano-scale. In this study we used tapping mode atomic force microscopy (TM-AFM) to analyze unstretched and stretched glowworm (*Arachnocampa tasmaniensis*) capture threads and revealed nano-scale features corresponding to variation in surface structure and elastic modulus near the surface of the silk. Phase images demonstrated a high resolution of viscoelastic variation and revealed mostly globular and elongated features in the material. Increased vertical orientation of 11–15 nm wide fibrillar features was observed in stretched threads. Fast Fourier transform analysis of phase images confirmed these results. Relative viscoelastic properties were also highly variable at inter- and intra-individual levels. Results of this study demonstrate the practical usefulness of TM-AFM, especially phase angle imaging, in investigating the nano-scale structures that give rise to macro-scale function of soft and highly heterogeneous materials of both natural and synthetic origins.

Keywords: biological material; height image; *Arachnocampa*; biofiber