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Short communication

The toughest recorded spider egg case silks are woven into composites with tear-resistant architectures

Parvez Alam [a,*], Danish Otieno [b], Matin Nuhamunada [a], Roselyn Anyango [b], Jared Odoyo [b], John Odhiambo [b], Kenneth Onyango [b]

[a] Laboratory of Paper Coating and Converting, Centre for Functional Materials, Abo Akademi University, Porthaninkatu 3, 20500 Turku, Finland
[b] Kisumu Insect Farms, Kenya

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A B S T R A C T

In this communication, we report important preliminary evidence for possibly the toughest egg case silk threads recorded to date spun by the hermit spider, *Nephilengys cruentata* ($\bar{G}$ =193 MJm^{-3}). We further elucidate that the egg case itself is woven with a specialised repeat cross-weave that when subjected to tension, drives perpendicular-to-force threads to *pile*. This *piling* of threads constrains damage to small areas and retains the architectural integrity of the surrounding egg case material. We deduce that by having ultra-tough threads coupled to a tear resistant architecture, *N. cruentata* is able to protect its eggs from predators with a considerable level of effectiveness.

Spiders increase the chances of spiderling hatching and survival by protecting their eggs with an egg case, which is built up predominantly of silk from the tubuliform (cylindriform) gland, though one type of aciniform gland silk (AC1) is also commonly used to construct the egg case architecture [1,2]. These, *cocoon silks*, are of similar strength to silks extruded from the minor ampullate glands, but are typically weaker than major ampullate silks [3], which are renown for high strength and toughness [4]. The egg cases are woven composite laminated structures [2,5,6], though less commonly the egg case may exist as a monolayer (non-laminated) woven [2]. The mechanical properties of egg case silks have not received as much attention as dragline silks, which have been painstakingly researched for a significant number of spider species [4]. Yet, it is fair to hypothecate that since a definitive role of egg cases are to protect unhatched spiderlings, they should display unique properties and characteristics that may be of interest to the scientific community. One recent example comes from the spider egg case stalk of *Meta menardi*, which has a reported measured extensibility of 751% [7]. When comparing egg case with dragline silks from the same spider species (*Argiope bruennichi*), egg case silks are found to strain twice as much as dragline silks to failure, though their strength values are discernably lower [8]. The characteristic of high elongation in spider silks is essentially a function of structural disorder and protein folding [9], which in turn has been shown to correlate with the glycine fraction in the silk proteins [10]. Cocoon silk proteins (tubuliform spidroin 1, TuSp1) have helical structures in solution, which develop β-sheets and β-turns when denatured during fibre forming processes [11]. β-sheets and β-turns contribute to nanocrytalline structures in silk and as such, egg case silks are indubitably able to develop crystallinity within its native, highly extensible molecular structure. Nanocrystals influence the overall mechanical performance of silk threads as a function of their size, stacking [12,13] and distribution [14,15,16] within the extensible amorphous matter. Furthermore, nanocrystal interfaces with amorphous matter give rise to metastable interfaces in silk that have semi-crystalline characteristics [17]. It is fair to presume that these semi-crystalline regions in silk help to circumvent stress-induced interfacial failure by promoting low stress-intensity stress transfer between the harder and softer phases of silk. Both egg case silks of different species and the architectures into which they are woven are still poorly researched. In this

* Corresponding author.
E-mail address: parvez.alam@abo.fi (P. Alam).

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communication, we highlight the properties of egg case silks, and the structure-property-function relationships of egg case segments from the hermit spider *Nephilengys cruentata*. Fig. 1 (a) shows *N. cruentata* perched upon its egg case and in Fig. 1 (b) we see the characteristic black and yellow pattern of *N. cruentata*, which allows for easy identification.

The egg case of *N. cruentata* consists of two separate layers of silk. The inner layer is light pink in colour and closely resembles thin tissue paper when observed with the naked eye. The outer layer is contrarily, visually web-like and consists of several interweaved fibres. In this communication, we lay focus on the outer web-like layer of the egg case. Egg cases were unwound judiciously to minimise damage to the individual fibres. Once unwound, the fibres were glued at either end between cardboard, which helped in gripping the fibres while relieving them of damage during loading into the mechanical test machine clamps. The cardboard grips were separated by a distance of 15 mm. Between 50 and 75 fibres were affixed unidirectionally to each set of cardboard grips and all fibres within one pair of cardboard grips had an equal gauge length of 15 mm. Five repeats were tested in an Instron-8872 at an extension rate of 10% strain per second. The fibres in each sample were counted using a stereo microscope with the aid of polarised light, which helped us to observe the fibres more clearly as they were semi-transparent. A mean fibre diameter was determined by measuring from scanning electron microscope (SEM) images for 150 fibres. Three measurements were made for each fibre using Image-J for image analysis and the platinum coating thickness (36.4 nm) deducted from each fibre to obtain the true fibre diameter. The mean fibre diameter was used to determine areas in the mechanical stress calculations, in similitude with Gnesa et al. [18], and single fibre mechanical properties calculated by normalising overall properties of each sample with the number of fibres within each. The egg case in its woven composite state was also tested in tension. The egg case was cut into sample length 40 × 5 mm (mechanical test gauge length = 15 mm), which are similar dimensions used previously for the mechanical characterisation of other egg cases or coccons [21–24]. The thickness of each sample (8 in total) was measured using a Lorentzen and Wettre Micrometer. Egg case fracture modes were observed by SEM, as was the original architecture of the egg case prior to mechanical stretching. To enrich our understanding of how the egg cases deform under loading, we developed elastic finite element models using electron micrographs to construct and idealise the geometries and the results from the unidirectional fibre tensile tests to approximate the elastic properties. These continuum mechanics models were fixed at one end with degrees of freedom, $U = 0$, and stressed to create tension from the opposing end with 0.5 kNm^{-2}.

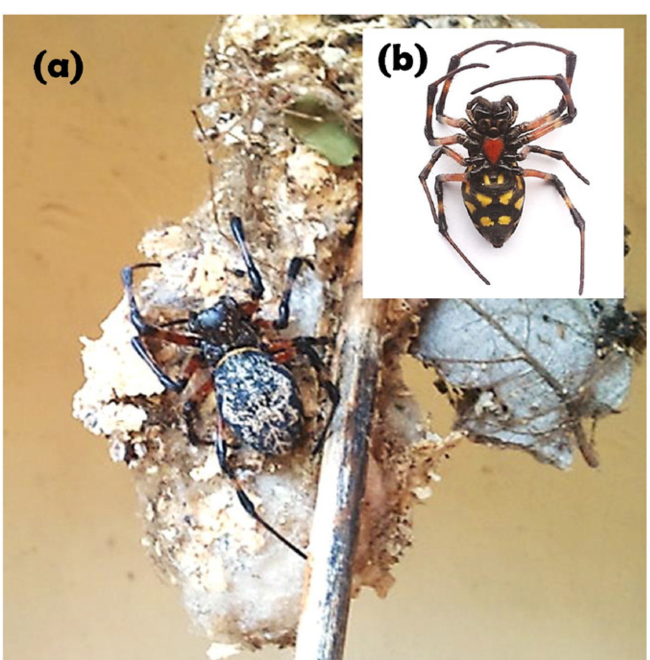

Fig. 1. (a) *N. cruentata* sitting on its egg case and (b) characteristic pattern on the underbelly of *N. cruentata*.

The egg case fibres were on average 5.14 μm (± 1.32 μm) in diameter. The fibre mean tensile strengths, σ, were 1.06 GPa (± 0.1 GPa), while the mean toughness, G, tensile modulus, E, and elongation, ε, values were 193 MJm^{-3} (± 29 MJm^{-3}), 4.9 GPa (± 2.0 GPa), and 0.3 (± 0.06), respectively. These are compared against other reported spider egg case silk properties in Table 1, and it is evident that of the different species, *N. cruentata* has, as far as we know, the toughest egg case silk fibres reported to date. The fibres are of similar toughness to many dragline silks, and in some cases are considerably higher. Though *N. cruentata* egg case silk has neither a particularly high nor low elongation as compared to the other egg case silks, it does exhibit considerable tensile strength, almost doubling the strength of the world's stretchiest egg case silk stalk spun by *M. menardi* [7].

Having evaluated the mechanical properties of *N. cruentata* silks against those of other spiders, our attention now turns to the way in which *N. cruentata* weaves its outer egg case. SEM micrographs reveal that the outer egg case has a primary thread cross-weave where threads intersect almost perpendicularly to each other, Fig. 2(a). These are in turn reinforced by thinner threads that appear to entangle at angles to the primary threads, Fig. 2(b), the angle often observed as being close to 45° from off the primary threads. These, being smaller threads are referred to henceforth as secondary threads. Both multi-layered (*Argiope amoena*, *Argiope aurantia*) and monolayer (*Nephila clavata*) egg cases have been previously reported [2,5,6]. Cutting through the egg case cross section, we notice the outer layer is in fact itself, a bilayer structure, though according to our observations, there is no difference in the orientation or architecture of threads in either layer. We furthermore note that the outer egg case under scrutiny here, is loosely wound, unlike the cocoons of e.g. *Bombyx mori*, which are comparatively more densely packed with fibres in a tight sericin glued weave [27]. We found that when trying to tear through the outer egg case by opening a hole and pulling, the egg case resisted tearing to the point where we were unable to open a large hole. To comprehend how the outer egg case weave may be functioning, we cut the egg case and performed mechanical tests, followed by SEM and image analysis.

Table 1
Comparison of spider egg case silk mechanical properties for different species of spider (mean values provided). σ unit in GPa, G units in MJm^{-3} (rounded to nearest integer), ε is dimensionless, E unit in GPa. [a] egg sac silk *stalk*. The results from our research on *N. cruentata* outer egg case silks are emboldened.

Species	σ	ε	G	E
A. gemmoides [3]	2.3	0.19	–	–
N. clavipes [3]	1.3	0.24	–	–
L. hesperus [18]	0.63	0.7	–	–
N. clavata [2]	0.73	0.26	107	11.3
A. argentata [19]	0.36	0.34	95	11.6
A. bruennich [8]	0.39	0.40	129	–
L. hesperus [20]	0.63	0.72	–	–
M. menardi [7] [a]	0.64	7.51	131	20.4
***N. cruentata* (this study)**	**1.06**	**0.3**	**193**	**4.9**

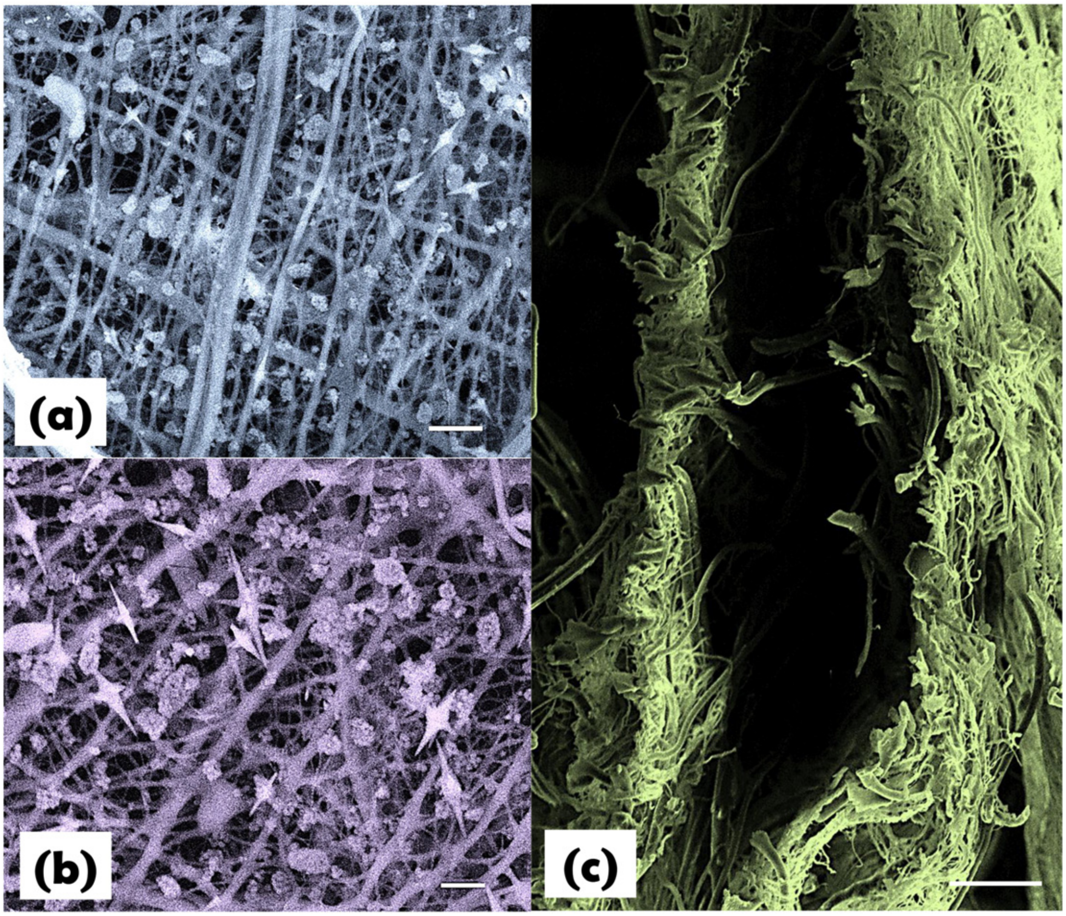

Fig. 2. SEM micrographs showing examples of (a) cross-weaved threads where primary threads intersect almost perpendicularly to one another (scale bar = 20 μm), (b) thinner threads reinforcing the primary threads at ca. 45° off the primary thread axes (scale bar = 10 μm) and (c) the (separated) bilayer structure of the egg case outer layer (scale bar = 50 μm).

The mechanical properties of the cut lengths of egg case silk are provided in Table 2 and these are compared to other arthropod cocoon silks [21–24]. Comparisons are made with non-arachnid cocoons because as far as we know, no arachnid cocoons have been mechanically characterised, though all the species in Table 2 belong to the same phylum (Arthropoda). It is evident from observing this table, that though *N. cruentata* is between 5 and 50 fold weaker than the other listed arthropod cocoons, it is considerably more stretchy, with a mean elongation measured at 66% (±37%). The fracture energy, G_c, of the *N. cruentata* egg case is calculated as $G_c = G \cdot w$, where $G = \int \sigma d\varepsilon$ and w is the width of the sample, corresponding to a horizontal crack opening under Mode I (tensile) conditions. For the cocoons, the mean fracture energy measured is 6.3 kJm^{-2} (±6.7 kJm^{-2}), which is higher than typical fracture energies of enamel, dentine and mollusc shells; and is in the range of values for bone, wood cell walls, polymer composites, high crystallinity polymers (e.g. polypropylene), skin and bone antler [25,26].

Table 2
Comparison of spider egg case mechanical properties for different species of cocoon weavers (mean values ± standard deviations). σ (strength) unit in MPa, ε (elongation) is dimensionless but expressed as a %. [a] depending on whether outer, middle or inner layer. Values are rounded to the nearest integer.

Species	σ	ε	Source
B. mori	17–54 (±1–11)	13–35 (±0.3–8)	[21,22,24,27]
H. cecropia [a]	16–47 (±7–12)	10–26 (±7–9)	[23]
S. cynthia	20 (±2)	21 (±5)	[21]
A. assamensis	23 (±6)	19 (±7)	[21]
A. pernyi	56 (±5)	41 (±6)	[21]
N. cruentata	3 (±1)	66 (±37)	This study

An SEM investigation reveals that the egg case primary threads oriented to the direction of loading stretch, while the cross threads (primary threads perpendicular to the direction of loading) pile into each other. We further notice that only the closest cross threads actually pile at the region of egg case tearing/fracture; however, below these threads the egg case microstructure remains unaffected. Piling tends to occur up to and not extending beyond ca. 1 mm from the fracture line (from the point of necking) and piles develop at intervals, which in turn appear to decrease in interval-length towards the fracture line, Fig. 3. Ordinarily primary threads are relatively evenly spaced at intervals of 20–40 μm (refer to Fig. 2). After deformation nevertheless, primary thread orientations can be seen to be somewhat less orderly and the distances separating them are smaller (and also less ordered). We interpret this piling of perpendicular primary threads as a mechanism by which means the egg case preserves its macro-architecture. This mode of fracture is entirely different to that of other silk cocoons such as those belonging to *Bombyx mori*, where more direct through fractures can be observed [27]. Simple finite element (FE) simulations, Fig. 4, bring to light that *N. cruentata* egg case cross threads closer to the loading edge experience greater displacement than those farther from the loaded edge. Moreover the FE simulations show that secondary threads

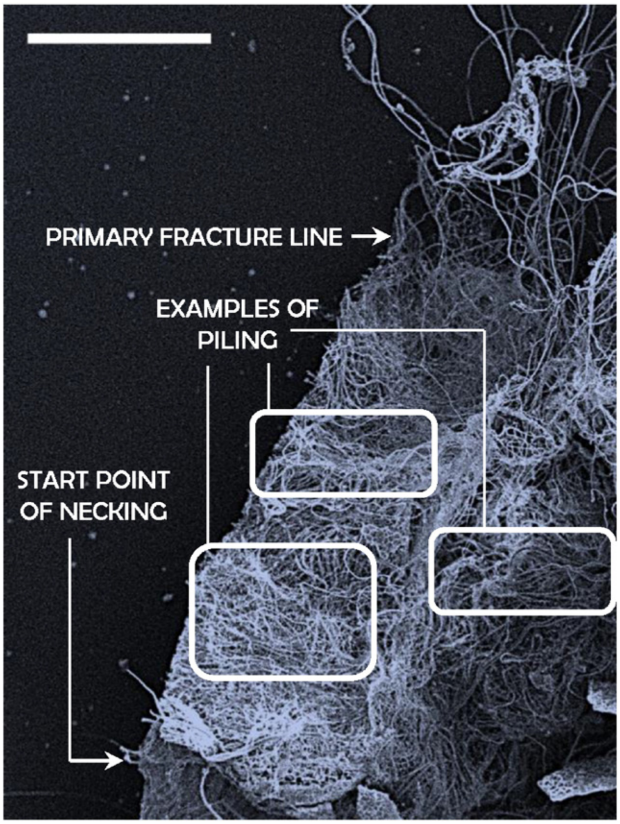

Fig. 3. SEM micrographs showing examples of (a) piling of cross threads at intervals to a distance no more than ca. 1 mm from the fracture line (scale bar = 0.5 mm).

(at angles to the primary threads) may instigate angular loading, which we presume cause primary threads to interlock when sufficiently displaced, thus giving rise to the formation of a pile. It would appear that the secondary (thinner) threads located between the primary threads play a role in pulling the parallel-to-loading primary threads closer to each other during deformation. This is evident as there is a differential build up of stress in both loading- and cross-directions at different connection points between the primary and secondary threads, logically resulting in local asymmetrical deformation. We are still unsure as to the mechanism behind piling at intervals, but hypothesise that certain geometrical patterns within the 3-dimensional architecture will influence load distribution about the web, permitting the interval piling observed in this study. The mechanisms controlling piling are important from a biomimetic perspective since they may provide excellent design ideas e.g. for tear resistant textiles. Tear resistant fabrics such as ripstop-materials, multilayer wovens and warp knits all show similar architectures to those of the *N. cruentata* egg case, [28]. Mimicry of the egg case technology of *N. cruentata* potentialises its utility in a diverse range of applications such as military fabrics [29], construction plies [30], permeable membrane-technology [31], aerospace wovens [32], reinforced biomedical sheets [33,34] and sports textiles [35–37], to name but a few.

In summary, we report preliminary research results into the mechanical properties of egg case silks from the hermit spider *Nephilengys cruentata*. We find that to the best of our knowledge, this spider has the highest recorded egg case silk toughness reported. We furthermore test egg case segments and find that the egg case has the highest elongation capacity recorded to date, and that the egg case seems to exhibit a piling mechanisms by which means it is able to localise the effects of tearing to a small region of the egg case.

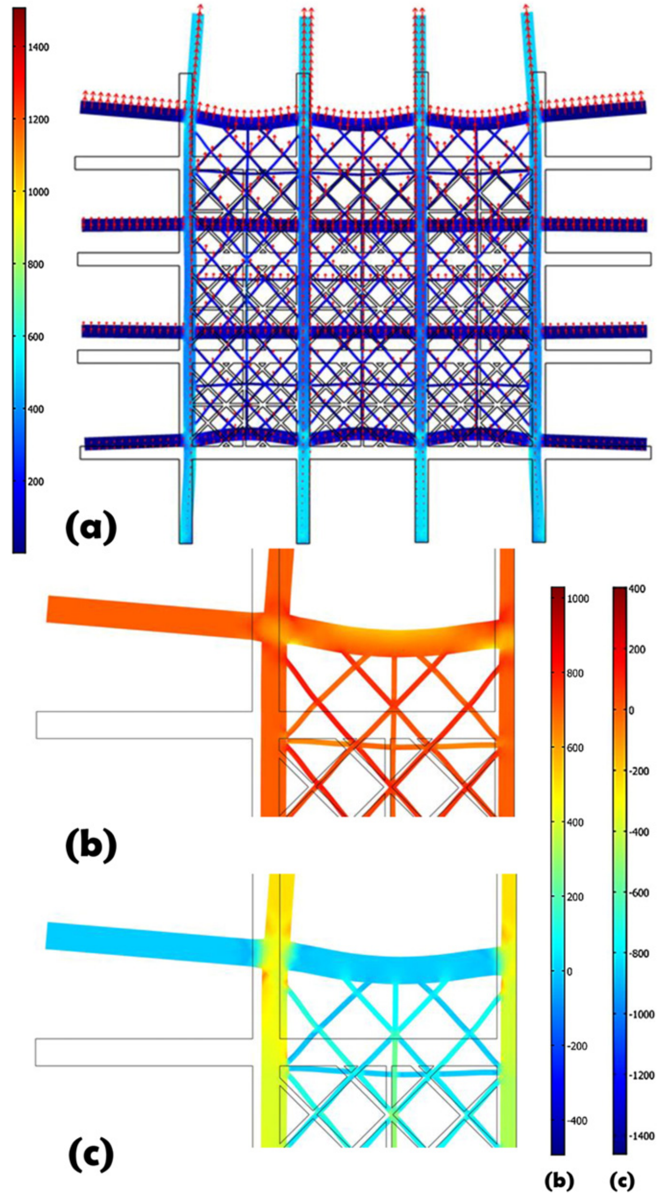

Fig. 4. Finite element model images of a geometrically approximated egg case web of *N. cruentata* showing (a) von Mises stress distribution surface plot with arrows showing the orientation of displacement (b) view of the top left corner of the model with stresses perpendicular to the loading direction and (c) view of the top left corner of the model with stresses parallel to the loading direction.

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