

# Structural and Mechanical Properties of Cocoons of *Antherina suraka* (Saturniidae, Lepidoptera), an Endemic Species Used for Silk Production in Madagascar

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## Abstract

*Antherina suraka* Boisduval (Saturniidae, Lepidoptera) produces a silken cocoon that has been the focus of efforts to create a commercial wild silk industry in Madagascar. In this study, structural and mechanical properties of the cocoon of *A. suraka* from two sites were measured and compared to the cocoon of *Bombyx mori* L. (Bombycidae, Lepidoptera) the world's most common source for silk. Results of environmental scanning electron microscopy and mechanical testing showed that the silk sheet of *A. suraka* cocoon is less compact, with greater thickness and lower tensile strength and stiffness than that of *B. mori*. Confirming these results, stiffness and cell and thread density were found to be negatively correlated with thickness, and the cell and thread volumes were positively correlated with thickness. *Antherina suraka* showed no major differences between silk sheets from Kirindy and Isalo sites in either structural or mechanical properties, except for mean cell volume, which was greater in cocoons from Kirindy. Comparison between the two layers forming the cocoon showed that the inner layer has greater elastic modulus, denser silk distribution and lower porosity. Cocoons from both Kirindy and Isalo are suitable for sericulture. Although the inner layer of cocoon silk is of higher quality than the outer layer, the fact that both layers are of great but lower tensile strength than *B. mori* silk suggests that the current practice of sewing the two layers together for making one single layer fabric should be continued in efforts to produce a commercially viable product.

**Key words:** Saturniidae, tensile strength, wild silk, Madagascar

Silk is a proteinaceous fiber composed primarily of fibroin that is produced by a wide variety of insects and spiders. Among its many functions, silk, in the form of a cocoon, is often used to protect the pupal stage, especially in Lepidoptera. This material produced by the domesticated mulberry silkworm *Bombyx mori* L. (Bombycidae, Lepidoptera) has long been a globally important article of commerce used for textiles and biomedical purposes (Altman et al. 2003; Sutherland et al. 2010). Production of non-mulberry silks from other lepidopterans (Peigler 1993), particularly in Africa and Asia (Jolly et al. 1979), differs from *B. mori* silk production in that the sources of silk fibers are wild or semi-domesticated. Comparisons of microstructures of cocoons constructed by *B. mori* and by species in different families of wild silk-producing caterpillars, namely Lasiocampidae, Notodontidae, and Saturniidae, also showed differences in arrangement and spacing of fiber layers (Teshome et al. 2012). Mechanical properties between wild silks also differ (Huang et al. 2008), although silks of some species, such as *Hyalophora*

*cecropia* L. and *Actias luna* L. (Saturniidae, Lepidoptera), possess tensile strength (TS) similar to that of *B. mori* (Reddy and Yang 2010, 2012).

The family Saturniidae is widely distributed in Madagascar, but silk from species in this family is not commonly used for sericulture there. This study focuses on silk made by *Antherina suraka* Boisduval (Saturniidae, Lepidoptera), one of the several species of wild silkworms being evaluated for their potential for commercial silk production (Razafimanantsoa et al. 2006). A non-profit organization (Conservation through Poverty ALleviation, International, CPALI) has started using *A. suraka* for silk rearing in Maroantsetra (north-east Madagascar), an alternative source of income for the local people who rely heavily on the remaining endangered forests in the region (Craig et al. 2012; Weber and Craig 2014). Although elucidating the structural and mechanical properties of this novel material is important for improving production, work on these properties has rarely been undertaken. Such work is a priority in

view of the fact that, according to Boulet-Audet et al. (2015), who used infra-red spectroscopy to analyze and compare the chemical composition of the cocoons of 35 species of Lepidoptera, the silk of *A. suraka* appears to be a relative outlier within the context of taxonomic expectations.

In this study, we have expanded the information available on the potentially commercially valuable silk of *A. suraka* by characterizing its physical and mechanical properties. In addition, we have examined whether these properties might be influenced by environmental factors by comparing the physical and mechanical properties from the inner and outer cocoons from two sites in Madagascar. In view of the fact that, at present, farmers use the non-woven cocoon sheet to make a silk patchwork for commercial purposes, our study focused on the TS, elastic modulus and the structural properties of the silk sheet instead of on individual fibers, which, in addition to being less relevant in the context of cocoon use, are highly non-uniform in cross-section and thus difficult to measure meaningfully.

## Materials and Methods

Cocoons of *A. suraka* and other saturniids, as well as *B. mori*, were used for the study. Cocoons of North American saturniids, *Antheraea polyphemus* (Cramer), *Hyalophora cecropia*, and *Actias luna*, were provided in February 2011 by breeders from New Hampshire (USA). Cocoons of the Malagasy saturniid *Argema mitrei* (Guérin-Méneville) were collected in Maroantsetra (north-east Madagascar) in 2010. Cocoons of *A. suraka* were collected in Maroantsetra (May 2010), Kirindy (west Madagascar—February 2011) and Isalo (south central Madagascar—March 2011). Cocoons of *B. mori* were obtained from eggs (Carolina Biological Supply Company, Burlington, NC, in July 2009) raised with leaves of white mulberry (*Morus alba* L.) in the laboratory (Department of Entomology) at the University of Illinois at Urbana-Champaign (UIUC).

To examine the micro-structural properties of the silks, pieces of silk sheets were sputter-coated with gold and palladium and images were collected with the use of an environmental scanning electron microscope (SEM) with a field-emission electron gun (ESEM-FEG; FEI Co., Hillsboro, OR) in HiVac mode at 5 kV and a spot size of 2.1 nm. This work was performed at the Beckman Institute at UIUC. The dimensions of the silk fibers were measured using ImageJ 1.49h (National Institutes of Health; Bethesda, MD). Vertical and horizontal chord lengths of the fiber cross-section were used as parameters to evaluate the fiber size. The chord length is the length across the centre of the projection area of the cross section. Any relationships between the fiber size and body size were studied by using adult wingspan and larval body length as reported in the literature.

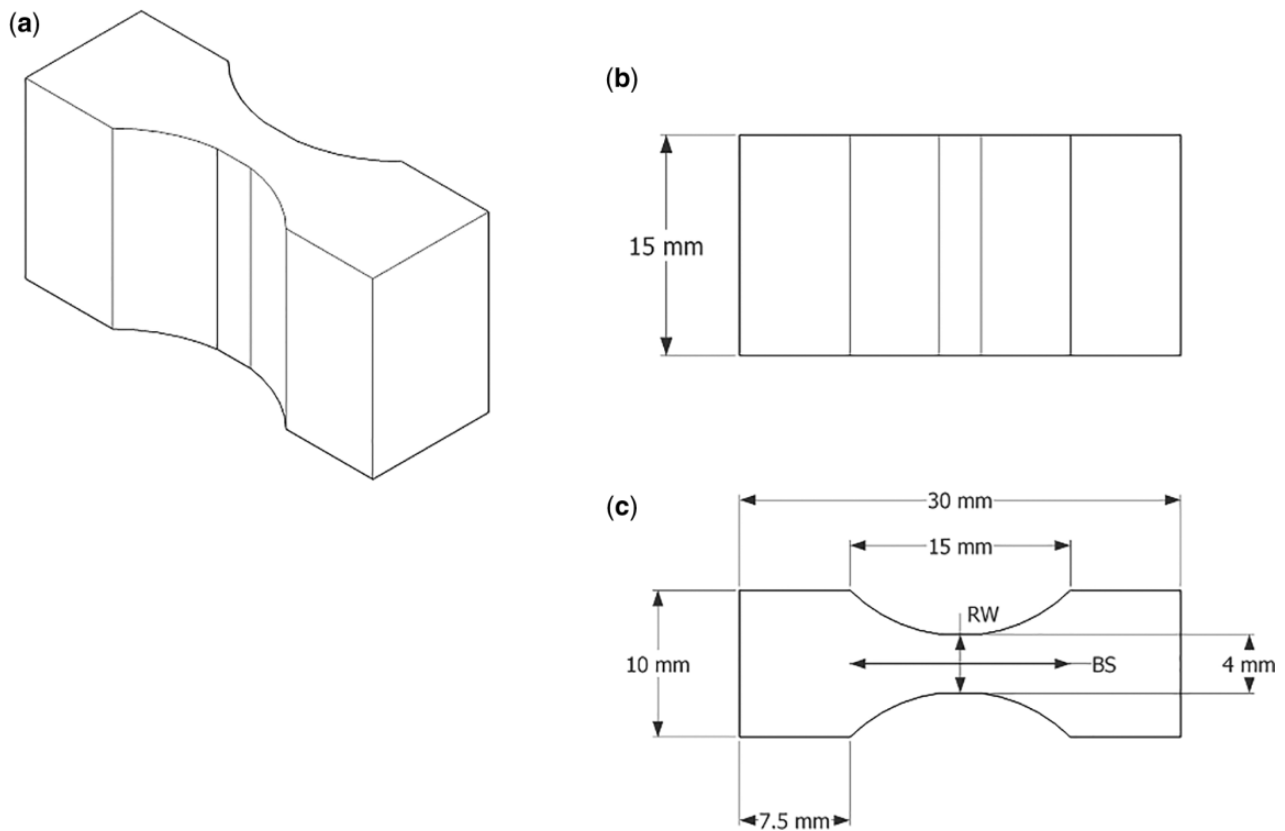
To test the mechanical properties of the silks, cocoons of *A. suraka* from Kirindy and Isalo were washed with a laboratory detergent to remove soil and other substances from the ground where the cocoons were collected, air-dried, and then cut longitudinally with a sharp scalpel. The external and internal sheets were separated manually. Each silk sheet was placed under a cotton cloth and then ironed on “cotton mode” for 10 s with a domestic electrical cloth iron (Model 0005087553275, Stanley Black & Decker, New Britain, CT) to remove wrinkles. The ironing temperature was lowered (due to the cotton cloth) to an average of 132° C, which was measured from 10 samples using a non-contact infrared thermometer (Model Lasergrip 774, Etekcity corporation, Anaheim, CA). The same method is used by the farmers in Maroantsetra, except the iron is not electrical but rather is powered by charcoal fuel. Cocoons of

*B. mori* were used as controls. The cocoons of *B. mori* possess many layers that could be separated manually in different thicknesses depending on the objectives of the experiment. They were not ironed because the cocoons of *B. mori* naturally have no wrinkles; ironing them would change the color of the fiber and other properties.

A dog-bone-shaped stencil (30 mm in length, 15 mm in height) was created with a shoulder at each end (10 mm width) and a most reduced width in between (RW: 4 mm); the distance between shoulders (BS) reached 15 mm (Fig. 1). The gauge length, ideally similar in size to BS, was measured for each sample, which was more or less larger than the stencil. The gauge width, ideally similar in size to RW, was measured at the shortest length of the dog-bone-shaped sample in the middle of the GL. The dog-bone-shaped stencil was designed to allow for uniform deformation and failure in the middle section of the sample due to maximum tensile loading (Roque et al. 2009; Brosch 2013), a standard model used in materials engineering. The stencil was placed on each ironed cocoon sheet of *A. suraka* and non-ironed cocoon sheet of *B. mori*. A permanent marker was used to trace around the stencil onto the cocoon sample. The cocoon was then cut around the tracing using a scalpel. Sample thickness was averaged by measuring in three locations on the GL with calipers.

Once each dog-bone-shaped sample was created, its TS was tested using an ElectroForce BioDynamic Test Instrument (Model 5100, Bose Corporation, Eden Prairie, MN; Fig. 2) with a displacement speed of 0.02 mm/s. The deformation rate (%/mn) could be determined by taking into consideration the displacement speed (in mm/mn) by dividing it with the corresponding GL of the sample and multiplying all with 100%. The load cell limit was 225 N. Two identical grips (40 mm wide and 38 mm tall) were used to hold the sample. The stencil and the grips were custom-designed using SolidWorks 2013 computer-aided design (CAD) software (Solidworks Corporation, Waltham, MA) and then fabricated with two 3D printers (Eden 350 and ABS-M30i, Stratasys, Ltd., Eden Prairie, MN), respectively, using VeroWhite polymer (Stratasys, Ltd., Eden Prairie, MN). The GL was parallel to the loading direction. WinTest 3.0 software (Bose Corporation, Eden Prairie, MN) was used to record load and displacement data. These data were converted to stress–strain data in order to calculate the peak strength, i.e., the TS, and the elastic modulus (E), a measure of stiffness. Stress is calculated by dividing the force by the cross-sectional area (Agnarsson et al. 2010), which is GW multiplied by average thickness. Strain is determined by calculating the change in length divided by the initial length for each displacement. The elastic modulus was determined by finding the slope of the linear part of the stress–strain curve before failure (Vollrath et al. 2001). Both TS and elastic modulus were measured in megapascal (MPa), equivalent to meganewton per square meter, or force per unit area. The failure mode of the samples of *A. suraka* (inner and out layers) and *B. mori* cocoons could be illustrated by representative stress–strain curves.

Images of the samples were obtained using a Canon EOS-5D Mark III or Mark II camera with remote switch and Canon 100 mm Macro IS lens (Canon U.S.A., Inc., Melville, NY). The image data were examined in the form of a binary image using LabVIEW 2013 (National Instruments, Austin, TX) to facilitate analyses of the fibers and the cells forming the cocoon sheets. A cell is defined as the empty closed space formed by at least three fibers crossing over at their ends. Five properties of the cocoon were then measured: density and volume of cells, density and volume of fibers, and the cell shape factor. The densities of the cells or the fibers (density of silk distribution) were determined by counting the numbers of cells or fibers per square millimeter. The cell volume was calculated by multiplying the cell area with the thickness of the cocoon sheet. The



**Fig. 1.** Stencil used to cut a piece of silk sheet for TS testing: isometric view (a), front view (b), and top view (c). BS, distance between shoulders (ideal sample gauge length); RW, reduced width (ideal sample gauge width).

thread volume was obtained by multiplying the component vector of the distance between nodes with the diameter of the thread and thickness. A node is defined as the crossover point between fibers (inter-fiber bonding). Thus, the distance between nodes is defined as the length of the thread, which can be represented by its component vector ( $d * \sin\alpha$ ), where  $\alpha$  is the angle of orientation of the fiber relative to the horizontal plane of the silk sheet. The use of a component vector ( $d * \sin\alpha$ ) instead of a simple distance ( $d$ ) is necessary in order to obtain uniform data because the fibers are oriented in different angles. The cell shape factor, with a formula  $(4 * \pi * Area)/(Perimeter^2)$ , approaches a value of 1 for a circle, 0.78 for a square, and 0 for a line.

All statistical analyses were performed using SPSS version 22 (IBM Corp. Released 2013, Armonk, NY). For each cocoon, values of each parameter were tested for a normal distribution. Values of parameters that were skewed were transformed to logarithm with base ten for normalization. Levene's test of equality of variances was performed followed by an independent  $t$ -test to compare parameters of inner and the outer layers of cocoons and of cocoons from two sites (Kirindy and Isalo). In cases where the equality of variances was not assumed when performing the  $t$ -test, the Welch-Satterthwaite method was used to adjust the degrees of freedom and the pooled estimate for the error term for the  $t$ -statistic was not used. Pearson correlations were used to determine pair wise relationships between the parameters.

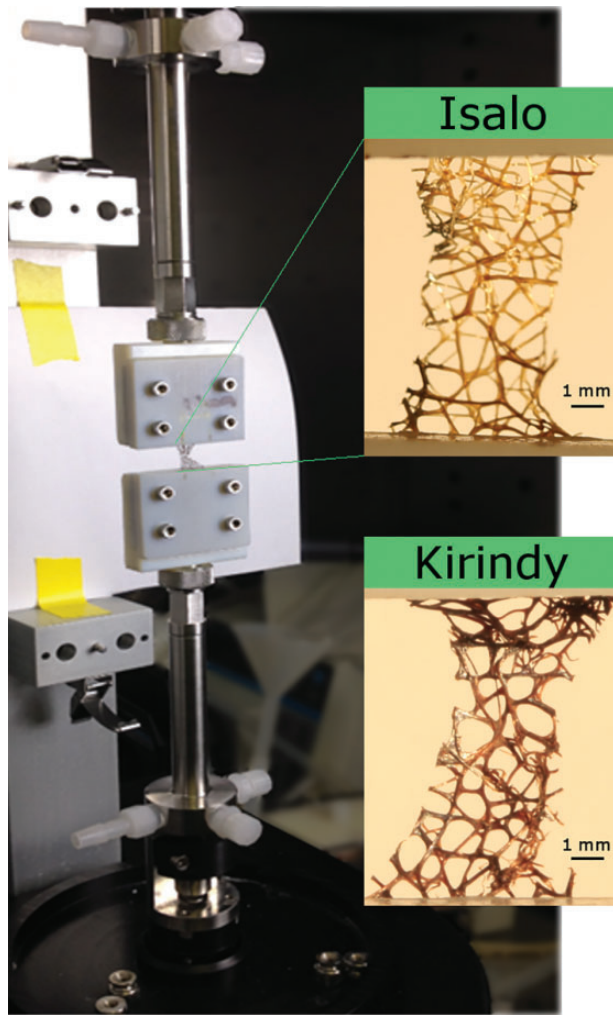
## Results

Evaluation of SEM microscope images revealed that cocoon sheets consisted of nodes of multiple threads (Fig. 3). The cross-section of the thread showed that it was composed of either two (*Bombyx*

*mori*) or multiple (saturniid species) strands that are themselves bundles of filaments. The double-stranded structure of *B. mori* (Fig. 4) is characterized by two strands of fibroin glued together by sericin (Sprague 1975).

The larger body size of Saturniidae, associated with larger spinnerets, or silk-spinning organs, in the larval stage, was reflected in the size of threads produced. The chord lengths of *A. suraka* threads were about five times greater than the threads produced by *B. mori* (Table 1). In species of saturniids, this body-spinneret ratio was not obvious when comparing the size of the thread that we measured and the wingspan reported in the literature. *Antherina suraka* (tribe Saturniini) showed similar thread size to two other species from the same tribe, *Antheraea polyphemus* and *Argema mittrei*, although *A. suraka* wingspan is approximately two-thirds the size of the two latter species. The threads of these three saturniid species were in turn three times greater than the threads produced by *Actias luna*, also belonging to the same tribe, Saturniini, and *Hyalophora cecropia*, belonging to another tribe, Attacini, although *Ac. luna* has a wingspan similar in size to that of *A. suraka*, and *H. cecropia* has a greater wingspan more similar in size to that of *An. polyphemus* (Table 1). The body-spinneret ratio was also not obvious when comparing the size of the thread that we measured and the larval body length of all the studied species reported in the literature. All the studied species in the tribe Saturniini (*A. suraka*, *Ac. luna*, and *An. polyphemus*), except *Ar. mittrei*, showed similar larval body length to *B. mori*, although the thread size and the wingspan of the latter were at least twice smaller (Table 1).

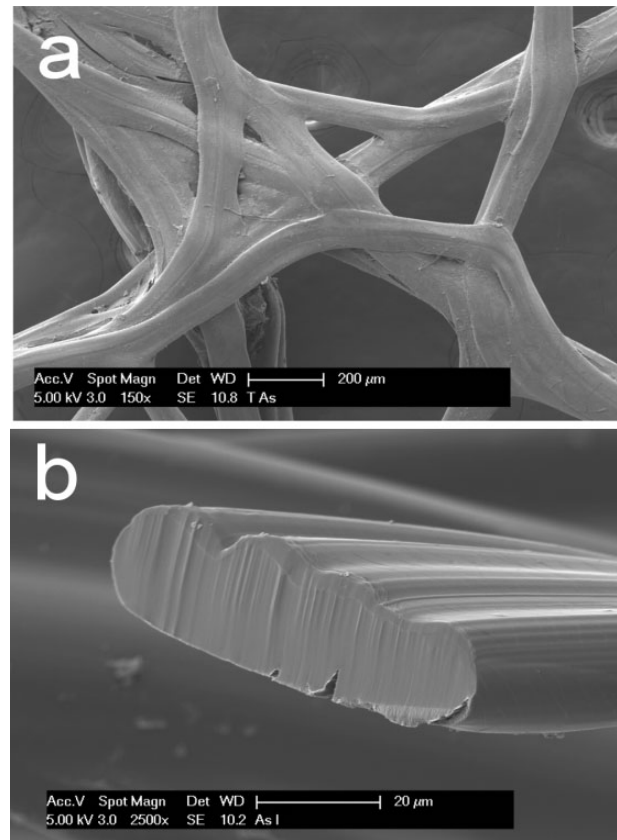
The ranges of GL (8–14.56 mm) and GW (3.06–5.24 mm) for *A. suraka* samples ( $n = 33$ ) differed from those of *B. mori* (GL: 9.5–12.63 mm;



**Fig. 2.** TS testing instrument where the dog-bone-shaped sample of *Antherina suraka* cocoon is held at their two extremities by two squared grips. Magnified view of intact samples (from Kirindy or Isalo, Madagascar) at the beginning of the TS testing is shown.

GW: 3.34–5.09 mm) samples ( $n = 11$ ). The displacement rate varied considerably for *A. suraka* samples ( $n = 33$ ), ranging from 8.24 to 15%/mm, in comparison with the more consistent rates for *B. mori* samples ( $n = 11$ ), which ranged from 9.50 to 9.64%/mm. The failure mode of the samples of *A. suraka* (inner and out layers) and *B. mori* cocoons were illustrated by the representative stress-strain curves. Stresses increased as strain increased, then peak stresses were followed by more or less abrupt failure depending on whether the sample was respectively *B. mori* or *A. suraka* (Fig. 5). Correlations between the mechanical and structural features of the silk sheet of *A. suraka* confirmed that the thickness was significantly negatively correlated with the elastic modulus and cell and thread density (Table 2). The cell and thread volumes were positively correlated with thickness. No correlation was found among the thickness, TS, and cell shape factor. The correlations showed that the thinner the silk sheets are, the stiffer they are. Thus, thinner silk sheets present more fibers and cells (more nodes or greater interfiber bonding), indicating higher cell and thread density, which form tighter meshes (lower porosity) as lower thread volume and smaller cell volume were observed.

Results of thickness measurements and mechanical testing showed that the *B. mori* cocoon sheet, although fourfold thinner,



**Fig. 3.** Images collected through environmental scanning electron microscope: a piece of cocoon of *Antherina suraka* (Saturniidae) with multiple threads forming nodes (a) and cross-section of a thread (c).

has fourfold higher peak strength and is fivefold stiffer (elastic modulus) than that of *A. suraka* (Table 3).

A comparison of cocoons of *A. suraka* from two different localities, Kirindy and Isalo, did not reveal substantial differences in terms of peak stress, elastic modulus, thickness, mean thread volume, or cell and thread density (Fig. 2 and Table 4). However, the mean cell volume was greater in cocoons from Kirindy than in those from Isalo (Table 4).

A comparison of inner and outer cocoons, irrespective of source, however, did reveal many differences. With respect to mechanical properties, the inner cocoons of *A. suraka* had significantly greater stiffness than the outer cocoons (Table 5). Most of the structural properties were significantly different, except for the cell shape factor where inner and outer cocoons had similar shape (Fig. 6). The inner cocoons had higher cell and thread density, and smaller mean cell and thread volume than the outer cocoons (Table 5). In other words, the inner layers of cocoons of *A. suraka* showed denser fiber distribution and lower porosity than the outer ones. These results were consistent with the correlations between mechanical and structural parameters analyzed previously (Table 2).

## Discussion

Differences in mechanical properties could be explained by taxonomic origins, fiber arrangement, protein composition, and structure of the silk fiber (Hayashi et al. 1999; Tokareva et al. 2014). Cocoons of *A. suraka* and other saturniids differ in microstructural properties in comparison with cocoons of *B. mori*. Environmental

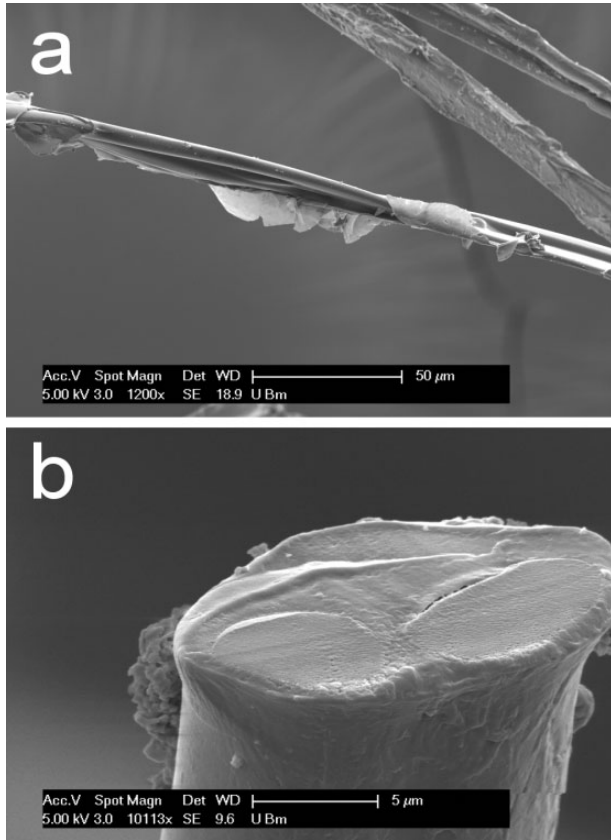
scanning electron microscope images showed that the cocoons of *A. suraka* resemble those of some *Caligula* spp. (Saturniidae, Lepidoptera) in showing a looser scaffold structure characterized by large pores supported by bundles of fibers; by contrast, the *B. mori* cocoon is characterized by high porosity and weak interlayer bonding (Chen et al. 2012a). Without magnification, cocoons of *B. mori*

seem compact because the pores are microscopic but the pores in *A. suraka* are visible to the naked eye.

Our study showed that body–spinneret ratio differs dramatically at the family level in comparisons of the thread size of *B. mori* with that of *A. suraka*. At the genus level, the thread size does not depend on body size (adult wing span and larval body length): thread sizes in species of the same tribe are not necessarily similar. In view of the process by which lepidopteran larvae spin silk, examining potential relationships between spinneret structures and silk thread size might be more informative than comparing relationships between fiber characters and body size.

The compact and thinner silk sheet of *B. mori* had greater TS and stiffness than the looser and thicker cocoon of *A. suraka*. These findings are consistent with those reported in a study on mechanical properties of *B. mori* cocoon, according to which thinner silk has proportionately higher elastic modulus and TS (Zhao et al. 2005). In *A. suraka* cocoons, the inner layers were thinner and stiffer with lower porosity and denser silk distribution (more interfiber bonding) than the outer ones; this finding, too, was confirmed for cocoon layers of *B. mori* (Zhao et al. 2005, Chen et al. 2012b) and species belonging to the same family as *A. suraka* (Saturniidae) and other wild silk moth families such as Lasiocampidae (Chen et al. 2012c). Chen et al. (2010) explained that the elastic modulus is controlled by the porosity of the silk composite according to the foam open cell model of Zhu (1997); the decrease in the elastic modulus is due to the gradual loss of connectivity of sericin bonding between the fibers forming the nodes. These nodes of the cocoon sheets that broke caused the failure shown in stress–strain curves (Chen et al. 2010). The general curve shapes of the samples of *A. suraka* (inner and out layers) and *B. mori* were typical cocoon sheets, consisting of a peak stress followed by a failure (Zhao et al. 2005; Chen et al. 2010; Chen et al. 2012a). Stress and strain values of the studied layers of cocoons of *B. mori* forming the curves were in the ranges indicated by Zhao et al. 2005.

*Antherina suraka* has been used for sericulture only recently (Razafimanantsoa et al. 2006; Craig et al. 2012) compared to the domesticated *B. mori*, which has been reared and subjected to artificial selection by humans for approximately 5,000 years (Kurin 2002). *Bombyx mori* produces high-quality silk that is woven worldwide to make fabrics primarily for clothing. As an entirely



**Fig. 4.** Images collected through environmental scanning electron microscope: a piece of cocoon of *Bombyx mori* (Bombycidae): degummed simple thread (a) and cross-section of a thread (b).

**Table 1.** Physical dimensions of silk threads of wild and domesticated silkworms measured from images obtained from a scanning electron microscope (one thread is composed of two or multiple strands that are themselves bundles of multiple filaments)

Species (number of individuals)	Vertical chord length Mean (SD) (in µm)	Horizontal chord length Mean (SD) (in µm)	Wing span (in mm)	Last instar larval body length (in mm)
Saturniidae (tribe Saturniini)				
<i>Antherina suraka</i> (5)	35.93 (13.81), <i>n</i> = 10	91.33 (35.76), <i>n</i> = 41	80–140 <sup>a</sup>	75 <sup>d</sup>
<i>Actias luna</i> (1)	13.07 (3.19), <i>n</i> = 4	40.03 (9.73), <i>n</i> = 9	95–135 <sup>b</sup>	75 <sup>b</sup>
<i>Antheraea polyphemus</i> (1)	32.44 (0.84), <i>n</i> = 2	87.54 (19.38), <i>n</i> = 11	110–150 <sup>b</sup>	75 <sup>b</sup>
<i>Argema mittrei</i> (1)	44.10 (36.09), <i>n</i> = 2	106.89 (23.94), <i>n</i> = 2	130–200 <sup>a</sup>	150 <sup>e</sup>
Saturniidae (tribe Attacini)				
<i>Hyalophora cecropia</i> (1)	11.00 (5.15), <i>n</i> = 5	34.69 (14.41), <i>n</i> = 5	110–150 <sup>b</sup>	100 <sup>b</sup>
Bombycidae				
<i>Bombyx mori</i> (1)	6.76 (0.01), <i>n</i> = 2	14.66 (3.58), <i>n</i> = 11	40 <sup>c</sup>	75 <sup>c</sup>

*n*, number of threads measured per sample.

<sup>a</sup> Griveaud (1961).

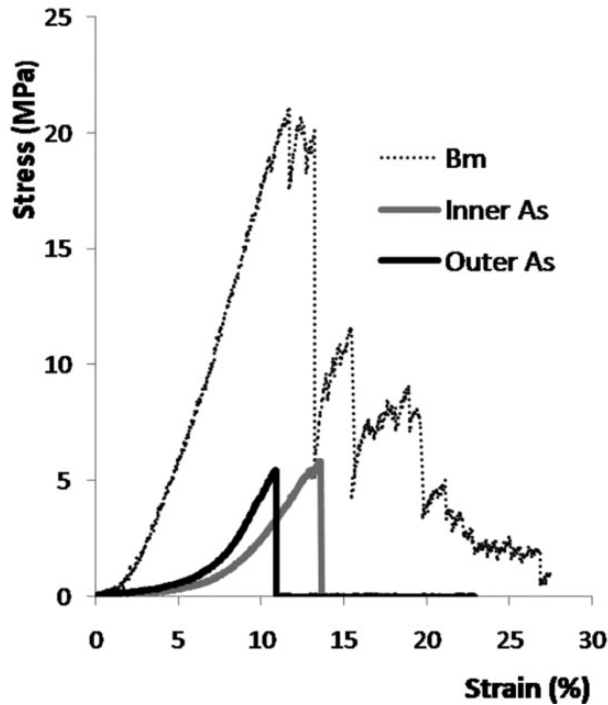
<sup>b</sup> Bouseman and Sternburg (2002).

<sup>c</sup> Pieprzyk-Kokocha and Burczyk (2006).

<sup>d</sup> Albano (1992).

<sup>e</sup> Lamour (1998).

domesticated species, *B. mori* spins its cocoon in artificial frames, four-walled wooden structures provided by humans that permit construction of compact cocoons. Thus, spacing available for spinning probably determines at least in part the form of cocoon. That physical space available during cocoon-spinning can influence compactness was demonstrated by Waldbauer and Sternburg (1967), who found compact cocoons of the North American saturniid



**Fig. 5.** Representative stress–strain curves of *Bombyx mori* (Bm) cocoon layers and *Antherina suraka* (As) inner and outer cocoon layers.

*Hyalophora cecropia* only on twigs or branches of trees or on higher parts of shrubs, where the larvae could find a three-dimensional support on a fork of twigs or could create a closed space for spinning by attaching silk to leaves. By contrast, cocoons formed on twigs of shrubs near the ground where leaves were absent were looser in structure because there were no space constraints on spinning cocoons.

Among all the structural properties analyzed in this study, only thickness was correlated with elastic modulus: the inner cocoon was thinner with greater TS and stiffness than the outer cocoon. A study on *B. mori* cocoon confirmed that the silk layer became thinner but retained a superior protective function when larvae experienced external disturbances and/or were forced to spin another cocoon when the first one was removed (Huang et al. 2008).

These mechanical properties of the *A. suraka* cocoon might be explained by its chemical composition, including the greater amount of polyalanine  $\beta$ -sheet nanocrystals present in the silk fibers of its inner layer when compared to its outer one (Boulet-Audet et al. 2015); these structures are indicative of the degree of crystallinity present (Porter et al. 2005).

External environmental conditions such as temperature, humidity, and rain may perturb the larvae when spinning cocoons (Ramachandra et al. 2001, Offord et al. 2016). Identifying environmental factors that influence both larval spinning behavior and silk attributes will be important in improving the *A. suraka* sericulture enterprise. In the lasiocampid wild silkworm *Gonomestica postica* Walker (Lepidoptera), physical properties of cocoons such as weight, size (length and width), and breaking energy (toughness) were significantly lower when the larvae were reared indoors than outdoors (Teshome et al. 2014), although no specific environmental factor was identified as being responsible for the differences. As well, in another species of Saturniidae, *Antheraea pernyi* (Guérin-Méneville), the silk undergoes glass transition at 140°C and there might be annealing by dehydration of the material at 100°C

**Table 2.** Pearson correlations between mechanical and structural features of *Antherina suraka* silk sheet

	Thickness	Elastic modulus	TS	Cell density	Thread density	Cell volume	Thread volume	Cell shape factor
Thickness	1							
Elastic modulus	-0.499**	1						
TS	-0.320	0.844**	1					
Cell density	-0.390*	0.112	0.141	1				
Thread density	-0.381*	0.091	0.147	0.991**	1			
Cell volume	0.657**	-0.302	-0.274	-0.873**	-0.879**	1		
Thread volume	0.897**	-0.306	-0.143	-0.638**	-0.618**	0.721**	1	
Cell shape factor	-0.162	0.078	0.033	-0.026	-0.033	0.065	-0.180	1

Values of elastic modulus, TS, cell volume, and thread volume were log-transformed to meet normality assumptions. An asterisk or a double asterisk indicates significant correlation ( $P < 0.05$  or  $P < 0.01$ , respectively).

**Table 3.** Features of cocoon sheets of *Antherina suraka* and *Bombyx mori*

Cocoon feature (unit)	<i>A. suraka</i> , mean (SD)	<i>B. mori</i> , mean (SD)	<i>t</i> -test
TS (MPa)	5.43 (2.93)	21.94 (6.07)	$t = -8.32$ , $df = 10.30$ , $P < 0.01$ $n_{A. suraka} = 33$ , $n_{B. mori} = 10$
Elastic modulus (MPa)	68.37 (44.50)	350.44 (114.82)	$t = -7.95$ , $df = 11.02$ , $P < 0.01$ $n_{A. suraka} = 33$ , $n_{B. mori} = 11$
Thickness (mm)	0.16 (0.06)	0.04 (0.02)	$t = -9.99$ , $df = 44$ , $P < 0.01$ $n_{A. suraka} = 34$ , $n_{B. mori} = 12$

*n*, number of cocoon sheets measured in that species.

$P < 0.01$  indicates significant differences between means in the same row.

**Table 4.** Mechanical and structural features of cocoon sheets of *Antherina suraka* collected in Kirindy and Isalo

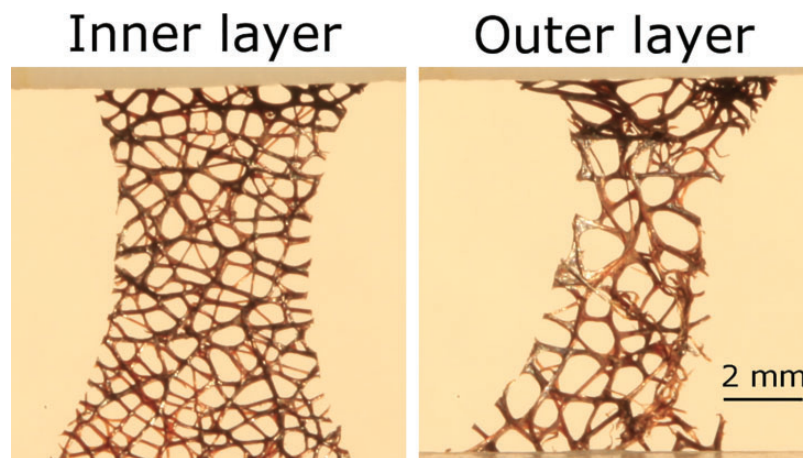
Feature (unit)	Kirindy mean (SD)	Isalo mean (SD)	<i>t</i> -test
TS	0.61 (0.26)	0.72 (0.25)	$t = -1.195$ , $df = 31$ , $P = 0.241$
Elastic modulus	1.77 (0.28)	1.73 (0.30)	$t = 0.414$ , $df = 31$ , $P = 0.682$
Thickness (mm)	0.15 (0.06)	0.17 (0.07)	$t = -0.703$ , $df = 31$ , $P = 0.488$
Cell density (cells/mm <sup>2</sup> )	4.0 (1.6)	4.7 (1.4)	$t = -1.316$ , $df = 31$ , $P = 0.198$
Thread density (threads/mm <sup>2</sup> )	8.8 (4.3)	11.1 (3.9)	$t = -1.654$ , $df = 31$ , $P = 0.108$
Cell volume	-1.71 (0.35)	-1.96 (0.31)	$t = 2.172$ , $df = 31$ , $P = 0.038^*$
Thread volume	-2.11 (0.40)	-1.98 (0.30)	$t = -1.062$ , $df = 31$ , $P = 0.296$
Cell shape factor	0.68 (0.04)	0.66 (0.03)	$t = 1.773$ , $df = 31$ , $P = 0.086$

Values of TS, elastic modulus, cell volume, and thread volume were log-transformed to meet normality assumptions; an asterisk (\*) indicates significant differences ( $P < 0.05$ ) between means in the same row;  $n = 15$  for cocoons in Kirindy and  $n = 18$  for those in Isalo.

**Table 5.** Mechanical and structural features of the internal and external cocoons of *Antherina suraka*

Feature of cocoon layer (unit)	Internal mean (SD)	External mean (SD)	<i>t</i> -test
TS	0.75 (0.26)	0.59 (0.22)	$t = 1.892$ , $df = 31$ , $P = 0.068$
Elastic modulus	1.87 (0.27)	1.63 (0.25)	$t = 2.674$ , $df = 31$ , $P = 0.012^*$
Thickness (mm)	0.11 (0.04)	0.21 (0.04)	$t = -6.610$ , $df = 31$ , $P < 0.001^{**}$
Cell density (cells/mm <sup>2</sup> )	5.1 (1.2)	3.7 (1.5)	$t = 2.930$ , $df = 31$ , $P = 0.006^{**}$
Thread density (threads/mm <sup>2</sup> )	11.9 (3.3)	8.3 (4.2)	$t = 2.670$ , $df = 31$ , $P = 0.012^*$
Cell volume	-2.07 (0.23)	-1.63 (0.32)	$t = -4.502$ , $df = 31$ , $P < 0.001^{**}$
Thread volume	-2.30 (0.27)	-1.79 (0.20)	$t = -6.244$ , $df = 31$ , $P < 0.001^{**}$
Cell shape factor	0.68 (0.03)	0.67 (0.04)	$t = 0.945$ , $df = 31$ , $P = 0.352$

Values of TS, elastic modulus, cell volume, and thread volume were log-transformed to meet normality assumptions; an asterisk or a double asterisk indicates significant differences ( $P < 0.05$  or  $P < 0.01$ , respectively) between means in the same row;  $n = 16$  for internal cocoons and  $n = 17$  for external ones.



**Fig. 6.** Images of dog-bone-shaped samples of *Antherina suraka* cocoons collected in Kirindy (Madagascar): inner layer (left); outer layer (right). The images show the samples at the beginning of the TS testing.

(Guan et al. 2016). To reduce the risk of glass transition, we controlled the temperature of the iron at 130 °C by limiting its duration and using a cloth to cover the cocoon to prevent too much deformation of the silk; nonetheless, water might well have been driven out annealing the silk as the temperature was over 100 °C. Thus, the method of ironing used in this study and by the farmers, apart from removing wrinkles, possibly changes some mechanical properties of the cocoons such as toughness.

Like the saturniid fauna in other countries such as India, the saturniid species in Madagascar have great potential for wild sericulture. The cocoons of *A. suraka* do not possess the strength of that of *B. mori*, but they do possess mechanical and physical properties that

make them suitable for use as a raw material for jewelry and for use in sheet form as a patchwork fabric for curtains and lampshades. Turning *A. suraka* silk into fabric is far less labor-intensive and time-consuming than silk fiber production for weaving from *B. mori*, rendering *A. suraka* silk more suitable for rural communities. The shiny brown color of *A. suraka* silk has considerable potential for artwork and decorative accessories. The species has already been semi-domesticated in Maroantsetra (Craig et al. 2012; Weber and Craig 2014) and sericulture is currently expanding to other species of Saturniidae, such as *Argema mittreii*, with its silvery cocoon, and *Ceranchia appolina* (Butler), with its lighter brown cocoon, which would diversify the field of wild sericulture in Madagascar.

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