Hydro-Responsive Curling of the Resurrection Plant *Selaginella lepidophylla*

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S1. PREPARATION OF SPURR'S RESIN-EMBEDDED SECTIONS

Inner and outer stem sections were fixed in 3% glutaraldehyde in 0.1M PO_4 buffer (pH 7.0) for 16 hours at 4°C on a nutator. Samples were then washed 3×10 minutes in 0.05M PO_4 buffer. Samples were post-fixed in a 1% OsO_4 solution (in 0.05M PO_4 buffer) for 2 hours at room temperature. Samples were rinsed in deionised water and subjected to an ethanol series (10 minutes in 30% ethanol, 1 hour each in 50%, 70% and 85%, 95% and 2x 100% ethanol) at room temperature. Samples were washed in 100% propylene oxide for 30 minutes, after which they were changed into a mixture of propylene oxide and Spurr's resin (2 hours each of 3:1 PO:Spurr's, 1:1 PO:Spurr's, and 1:3 PO:Spurr's). Samples were left in 100% Spurr's overnight at room temperature. The next day, samples were changed into fresh Spurr's twice and left overnight. Samples were changed into fresh Spurr's the next day and were polymerized in open tubes at 60°C for 48 hours. Samples were sectioned using a Leica EM UC6 Ultramicrotome and were mounted on regular glass slides.

S2. PREPARATION OF PARAFFIN-EMBEDDED SECTIONS

Inner and outer stem sections were fixed in FAA (4% paraformaldehyde, 5% acetic acid and 50% ethanol) for seven days at 4°C on a nutator. The FAA solution was changed for fresh FAA on day 3 of fixation. Stems were then washed twice for 30 minutes each in 50% ethanol 4°C and left overnight in 70% ethanol at 4°C on a nutator. Samples were dehydrated through an ethanol series (1hr each 85%, 95%, 2× 100%) at room temperature. After dehydration, samples were transferred to a mixture of xylene and ethanol (1hr each: 75% ethanol:25% xylene, 50% ethanol:50% xylene, 25% ethanol:75% xylene, 2× 100% xylene). Samples were placed in fresh xylene in scintillation vials and paraplast chips were added (1/4 volume of xylene) overnight at room temperature. The next day, the vials were placed at 42°C for 30 minutes. Another 1/4 volume of paraplast chips was added and samples were incubated at 60°C for 6 hours. Paraplast:xylene solution was discarded and replaced by molten paraplast (i.e. paraplast chips that had been melted at 60°C for 24 hours). Samples were then left at 60°C for one week and the molten paraplast was changed for fresh solution twice a day. Samples were embedded in fresh paraplast in small Petri dishes and the paraplast was allowed to harden at room temperature overnight. Samples were sectioned using a Leica RM2245

Microtome and were mounted on positively charged slides. Samples were deparaffinised prior to staining with 2x 100% xylene (15 minutes each).

S3. DISCRETE CURVATURE CHARACTERIZATION

The curvature of the stems of *S. lepidophylla* (Fig. 2a and b) are characterized by accurate estimations of the curvature of a smooth curve from its discrete approximation. Fig. S1 shows a segment of a smooth curve which is represented by a polyline with five points P_1 to P_5, with the corresponding edges $\overrightarrow{P_iP_{i+1}}$ $(i = 1, 2, 3, 4)$ denoted by $\boldsymbol{c}$, $\boldsymbol{d}$, $\boldsymbol{e}$ and $\boldsymbol{f}$, and their lengths are c, d, e and f. The curve can be represented by a Taylor series expansion [1]. A linear approximation for the true curvature vector $\boldsymbol{\kappa}$ can be obtained by finite difference approach and if all edges have equal length, the convergence is even quadratic.

$$\boldsymbol{\kappa} = \frac{2}{d+e}\left(\frac{\boldsymbol{e}}{e} - \frac{\boldsymbol{d}}{d}\right) \tag{S1}$$

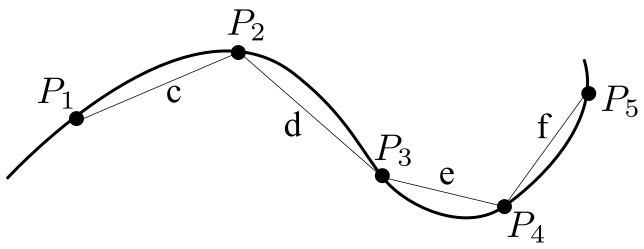

FIG. S1: **Discrete representation of a smooth curve.**

S4. GEOMETRICAL MODEL BASED ON EULER SPIRAL

Towards rationalizing the spiralling behaviour of the living stems, we adopt a geometrical model based on the definition of the normalized Euler (Cornu) spiral. By definition, an Euler spiral is a curve whose curvature κ changes linearly with its curve length s, i.e. $\kappa = a^2 s$ where a is a constant. This definition can be generalized to consider the role of material variation along the stem length. For a general class of power-law curvature (e.g. induced by the functionally graded hydro-actuation capacity of the tissue) defined by the Cesàro equation $\kappa = -a^{r+1}s^r$, the parametric equations for the spiral profile read:

$$x(\tilde{s}) = \frac{\tilde{s}^{2+r}}{(1+r)(2+r)\sqrt[r+1]{a}}\,{}_1F_2\left(\left\{\frac{1}{2}+\frac{1}{2(1+r)}\right\};\left\{\frac{3}{2},\frac{3}{2}+\frac{1}{2(1+r)}\right\};-\frac{\tilde{s}^{2(1+r)}}{4(1+r)^2}\right) \quad \text{(S2)}$$

$$y(\tilde{s}) = \frac{\tilde{s}}{\sqrt[r+1]{a}}\,{}_1F_2\left(\left\{\frac{1}{2(1+r)}\right\};\left\{\frac{1}{2},1+\frac{1}{2(1+r)}\right\};-\frac{\tilde{s}^{2(1+r)}}{4(1+r)^2}\right) \quad \text{(S3)}$$

where $\tilde{s} \in [0, a]$, $a \in [0, \eta]$ and ${}_pF_q(a_p; b_q; z)$ is the generalized hypergeometric function [2]. These equations were evaluated in the computational software Mathematica (Wolfram) using the built-in function `HypergeometricPFQ`. The above simplified model is used to investigate the role of material variation along the stem length as illustrated in Fig. 3a in the article.

S5. FINITE ELEMENT MODEL FOR BILAYER STEMS

Finite element (FE) simulations of bilayer stem models performed using the commercial package ABAQUS 6.11 (SIMULIA, Rising Sun Mills, Providence, RI, USA). A Python script is written to systematically create stem models. The bilayer is composed of a soft active (a) and a stiff passive (p) elastic layer, which have respectively the elastic moduli of E_a and E_p and the actuations strains of ε_a and ε_p. The Poisson's ratio for both layers is $\nu_a = \nu_p = 0.3$. The hydro-actuated strain is modelled with thermal expansion, where temperature represents moisture content. The stem is clamped at its base. Geometric nonlinearities are taken into account by activating `NLGEOM` option in ABAQUS which allows for large-deformation analysis. A mesh size sensitivity analysis is performed and based on that a mesh with about 7 elements along the stem thickness ($\sim$ 5000 triangular plane stress quadratic elements, CPS6) gives consistent results in the range of the parameters considered in this work. Fig. S2 shows the mesh for a bilayer stem in its deformed state. This mesh resolution allows us to characterize curvature smoothly along the stems centreline following the procedure introduced in S3.

In Fig. S3, the FE predictions of the normalized curvature of bilayer models for constant and varying thickness ratios are compared to those obtained by the Timoshenko bimetallic theory [3] at different actuation strains. For both cases, at small actuation strains, FE results are in very good agreement with theory; however, as the actuation strain increases the FE results deviates from the Timoshenko bi-metallic model which is derived based on

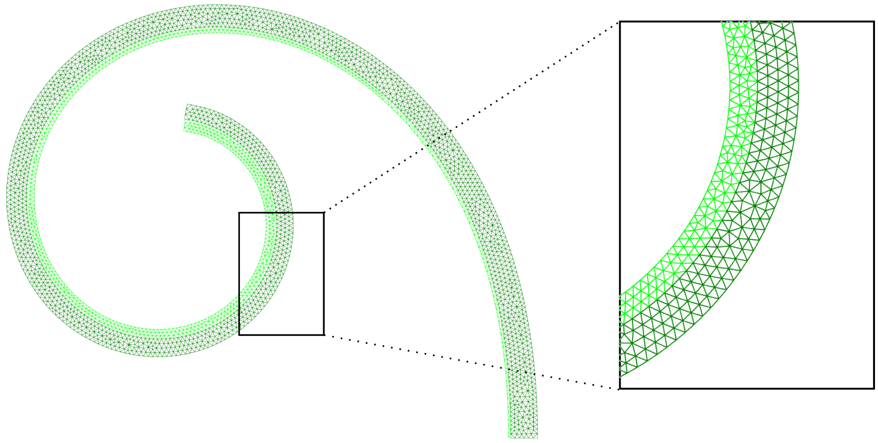

FIG. S2: **Finite element mesh for a bilayer stem.** A bilayer stem ($h/l = 0.02$, $h_a/h = 0.1$ at base and $h_p/h = 0.5$ at tip) is meshed with 4857 triangular plane stress quadratic elements (CPS6) in ABAQUS.

small deformation assumption. In FE simulations, we have taken into account geometric nonlinearities which allows nonlinear analysis of stems under large deformation, as observed in this work. Therefore, while Timoshenko model is still a fairly good model, it is not accurate for large actuation strains. According to Eq. (2) in the article, the predicted curvature of Timoshenko bi-metal model scales linearly with actuation strain. In contrast, FE results suggest that curvature does not magnify linearly with actuation strain and the location of maximum curvature shifts as the actuation strain increases. To summarize, to

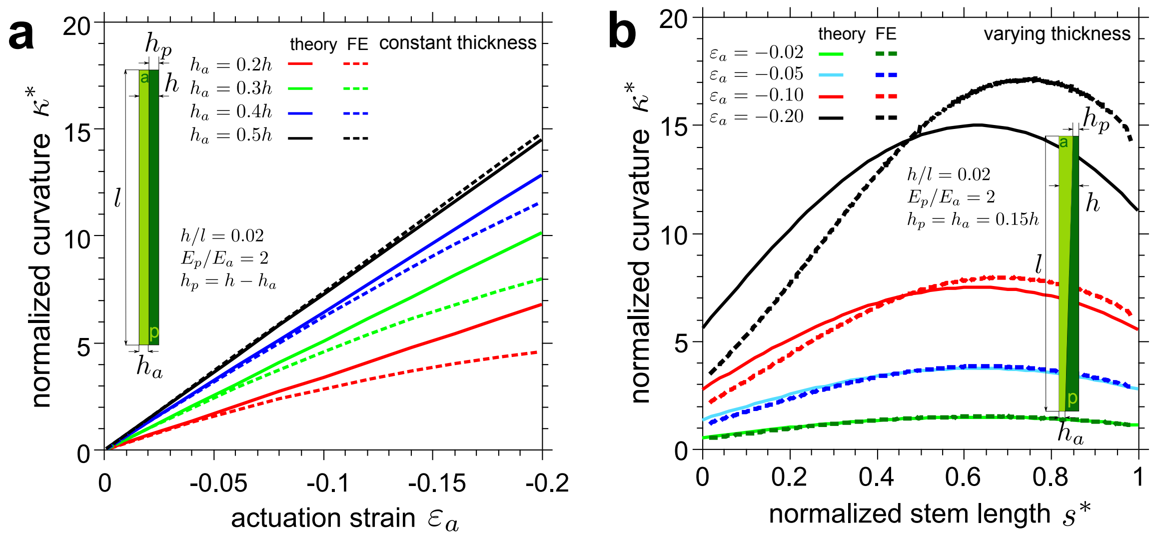

FIG. S3: **Comparison between FE simulations and theoretical Timoshenko bimetallic model for normalized curvature of bilayer stems.** (**a**) constant thickness and (**b**) variable thickness.

model the large deformation induced by stem curling upon hydration, we perform multiple simulations that account for geometric non-linearities. The bimetallic Timoshenko model was introduced as a limiting case, which - exact and sufficient for small deformation - still provides quite reasonable predictions for large strains.

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[3] Timoshenko, S. Analysis of bi-metal thermostats. *J. Opt. Soc. Am.* **11**, 233-255 (1925).