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Bouldering: an alternative strategy to long-vertical climbing in root-climbing hortensias

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1. Introduction

Climbing plants show a wide range of highly specialized anchoring structures such as hooks, spines, tendrils, twining stems and adhesive roots that allow them to climb on a range of natural and artificial surfaces [1,2]. Different attachment methods provide different degrees of anchoring and confer particular mechanical properties to the plant growth form and life history [2,3]. In general,
climbing plants are characterized by a decrease in stiffness during plant development (e.g. with increasing stem diameter), unlike for instance shrubs and trees that increase their stiffness towards older developmental stages [4]. Climbing plants that ascend by means of tendrils, twining stems and adhesive roots are often more firmly attached than for example those employing hooks or spines. Firmly attached plants usually develop more compliant tissues in older developmental stages than weakly attached ones; this is believed to be advantageous when coping with the potential stress produced by close contact with their supports (e.g. torsion, tensile or shear stress when a host tree swings with the wind [1,2]).

Root-climbers are plants that strongly anchor to their climbing surface by means of adhesive roots. These are either limited to certain positions along the stem, such as in the cheese plant (Monstera deliciosa), or all along the stem, as in the English ivy (Hedera helix). Root-climbers are also present in the popular ornamental hortensia family (Hydrangeaceae). Within this family, the root-climbing habit has independently evolved in the genera Decumaria, Hydrangea, Pileostegia and Schizaphygma [5]. Root-climbing Hydrangea species almost entirely belong to the nearly exclusive American Hydrangea section Cornidia ([5], hereafter shortened to Cornidia [6]). Adhesive roots in Cornidia species are closely positioned along one side of specialized anchoring stems, firmly attaching them to their support surfaces. During extensive fieldwork throughout the Neotropical distribution area of Cornidia, which ranges from North Mexico to central-south Chile and Argentina, we have never observed fully self-supporting juvenile or adult individuals. In general, mature individuals of Cornidia are long, lianescent climbers developing a major climbing portion, which is commonly secured to tree trunks and more rarely to tall vertical rock walls. Some Cornidia species, however, produce a robust self-supporting apical portion giving a ‘shrub-like’ appearance when climbing on short rounded boulders.

Phenotypic plasticity is the ability to express alternative phenotypes by a single genotype in response to different environmental conditions [7]. Exogenous conditions such as light incidence, forest openness, as well as availability and variability of supports are known to modulate climbing plant development [1,8–10]. Several climbing species vary according to the availability of supports, alternatively growing as self- or non-self-supporting individuals [1,8–13]. Studies combining plant biomechanics and anatomy offer a comprehensive method for characterizing growth form variation [3,4,14–18]. Plant architecture, moreover, provides a powerful method to assess endogenous developmental processes and isolate them from changes due to phenotypic plasticity triggered by environmental conditions [19,20].

Here, we investigate to what extent differences in support size and shape promote growth form plasticity in Cornidia species. We focus on the adult phase of development when many lianas experience a conspicuous shift in growth behaviour when reaching the canopy [12,13]. This change in growth behaviour is promoted by a series of covarying environmental conditions including loss of physical support and increased light exposure. Comparing long-vertical climbing surfaces (trees) with shorter rounded ones (boulders) could thus provide a reference framework to which growth form plasticity could be contrasted. Early increased light exposure and premature loss of physical support could lead to structural, mechanical and anatomical plastic responses.

We study Hydrangea seemannii, the only temperate Cornidia species of the northern hemisphere, which has frequently been reported in herbarium records both as a liana and a shrub. The species exhibits two contrasting climbing phenotypes: (i) long-vertical lianescent climbers on both coniferous trees (e.g. Cupressus and Pseudotsuga) as well as high vertical rock walls and (ii) short boulder-climbers that are basally anchored to small- or medium-sized boulders producing a ‘shrub-like’ form that protrudes well above the boulder surface. In order to address plastic responses to support variability in H. seemannii (e.g. differences in size and shape), we contrasted the architectural construction, stem bending properties, wood density and tissue organization of its climbing phenotypes. For comparison, mechanical properties of a second, strictly long-vertical tree-climbing Cornidia species were investigated. This species is new to science and is herein referred to as Hydrangea sp. 1. In contrast to H. seemannii and although rock outcrops are present in its habitat, Hydrangea sp. 1 climbs exclusively on trees.

Our study aimed to address three specific questions. First, do boulder-climbers and long-vertical tree-climbers of H. seemannii differ in their morphological, mechanical and anatomical architectures? If so, can this be considered growth form plasticity resulting from variability in support type? Second, what are the architectural, mechanical and anatomical traits underlying this plasticity? And third, how do mechanical properties of a taxon with ‘high’ phenotypic plasticity and climbing via both support types differ from the properties characterizing a taxon with a ‘low’ phenotypic plasticity and restricted to one type of climbing support?

2. Material and methods
2.1. Sampling and habitat description
Specimens of H. seemannii were collected in the Santa Bárbara canyon (Pueblo Nuevo, Durango) located in the northern Sierra Madre Occidental of Mexico (1050–2750 m elevation). This locality is a temperate sub-humid coniferous forest where H. seemannii is locally abundant and the only lianescent representative [21]. Frosts and occasional snowfalls occur in winter (annual mean temperature: 11.5°C, maximum 21.1°C, minimum 1.9°C; annual mean precipitation: 937.7 mm; 1951–2010). The Santa Bárbara locality comprises a relatively flat area at the top of a canyon and includes a complex range of habitats with variously sized igneous rock outcrops—including long-vertical rock walls and boulders, small waterfalls, perennial streams and a forest understory densely covered by a thick layer of moss. In total, four mature individuals, two per climbing phenotype, were sampled for mechanical, anatomical and architectural axis characterization.

Three specimens of the strictly long-vertical tree-climbing Hydrangea sp. 1 were collected from two different localities in the municipalities of San Andrés Tuxtla and Soteapan located in the Sierra de Los Tuxtlas, an isolated volcanic mountain range along the southeastern coast of the Gulf of Mexico in the state of Veracruz. The first of these localities is an extremely humid cloud forest with north-exposed slopes in the Sierra de Santa Marta—a mountain range of volcanic origin (ca 1300 m elevation). The second locality is located in a relatively flat rainforest area (ca 1050 m elevation) dominated among others by Lauraceae tree species (e.g. Persea and Nectandra). The Hydrangea sp. 1 collection zones are characterized by an average annual mean temperature of 24.3°C (maximum 28.4°C and minimum 20.2°C) and an average annual mean precipitation of 4183 mm (1951–2010). In contrast
to the *H. seemannii* habitat, the tropical forests of this region are characterized by numerous climbing taxa [22].

Owing to their root-climbing nature, portions of selected individuals were carefully detached by hand and cut at their base. Shoots growing along the forest floor and rhizomes were not collected. Higher portions of tree-climbing individuals were not accessible due to their high position on the supporting tree canopy. Although numerous localities of *H. seemannii* are known and despite its local abundance, it has been suggested to be at risk because of habitat destruction, timber extraction and climate change [23]. In addition, the habitat of *Hydrangea* sp. 1 is being continuously destroyed by human activities or local fires suggesting that the species is critically endangered (own observations). As biomechanical and anatomical measurements are necessarily destructive, only a minimal number of stems were sampled after careful evaluation of the local abundance of each species and in consideration of their ability to re-sprout from rhizomes or shoots present on the forest floor.

### 2.2. Architectural analysis

Plant axes of *H. seemannii* individuals were described morphologically following the revised architectural concepts and methods of Barthélémy & Caraglio [19]. All stems of each sampled individual were categorized in terms of: (1) axis habit, whether the stem was clining on trees or boulders (climbing), or standing independent of any surface (self-supporting); (2) branching pattern, considered as rhythmic when the stem branches were distributed in tiers or not branched; (3) growth direction, whether stems' growth direction followed (a) that of the contact surface (thigmotropic), (b) was generally vertical (orthotropic), (c) was horizontal (plagiotropic), (d) was mixed with a thigmotropic proximal portion and a plagiotropic distal end or (e) without precise growth direction; (4) presence or absence of adhesive roots; (5) ability to flower; (6) symmetry, whether the leaves and branches of a stem were disposed in all spatial directions (radial) or in one plane (bilateral); and (7) relative growth unit length. Number of axis categories differed among mechanically tested segments and processed for anatomical observations. These sections were sectioned transversely with a vibratome (Microtome Hyrax-V50, Carl Zeiss Micro-Imaging GmbH Jena, Germany) or with a standard sliding wood microtome. Sections (25–140 μm thick) were stained with carmine green or toluidine blue for distinguishing lignified from non-lignified tissues [25] and photographed with a digital camera (Olympus-DP71) mounted on a transmitted light compound microscope (Olympus-IX51) or a dissecting microscope (Olympus-SXZ9). Tissue transverse-sectional area outlines were analysed with the software package ImageJ v. 1.46r [26].

We differentiated three stem tissue categories: (i) parenchymatous pith, (ii) lignified pith along with wood and (iii) soft outer cortical tissues including cortex, secondary phloem and periderm. Then we determined the contribution of each tissue category to the total transverse-sectional area and second moment of area. A formula for an elliptical second moment of area $I = \left( \frac{\pi}{4} \times a^2 \times b \right)$ was applied for the parenchymatous pith as for the total transverse-section (see above), and a formula for elliptical rings $I = \left( \frac{\pi}{4} \times \left( a^2 - b^2 \right) \right)$ was used for wood along with lignified pith and outer cortical soft tissue rings (electronic supplementary material, figure S1). Additionally, we estimated the degree of pith eccentricity using the parenchymatous pith’s geometrical centre as the centre of area for the total transverse-section and the ellipses formed by the wood along with pith and the pith itself. Pith eccentricity was estimated by the distance between the parenchymatous pith and transverse-section’s geometrical centres ($\alpha$ and $\beta$, respectively), expressed as a percentage of the mean transverse-section’s radius (electronic supplementary material, figure S1).

### 2.4. Stem tissue organization

Seventeen to 18 segments per climbing phenotype were selected among mechanically tested segments and processed for anatomical observations. These sections were sectioned transversely with a vibratome (Microtome Hyrax-V50, Carl Zeiss Micro-Imaging GmbH Jena, Germany) or with a standard sliding wood microtome. Sections (25–140 μm thick) were stained with carmine green or toluidine blue for distinguishing lignified from non-lignified tissues [25] and photographed with a digital camera (Olympus-DP71) mounted on a transmitted light compound microscope (Olympus-IX51) or a dissecting microscope (Olympus-SXZ9). Tissue transverse-sectional area outlines were analysed with the software package ImageJ v. 1.46r [26].

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### 2.5. Stem developmental stages

Biomechanical and anatomical transitions at different stages of development were used to explore the changes underlying growth form variability [9,18]. Here, we identify two development stages of the stem distinguished by a specific anatomical change: a marked shift of the contribution of lignified pith along with wood and parenchymatous pith to the total cross-sectional area and second moment of area. This occurs when stems reach an approximate diameter of 7.4 mm (see the electronic supplementary material, figure S2).

### 2.6. Wood densitometry

Wood densitometry scans were performed with the Nanowood scanner [27] at the Centre for X-ray Tomography of Ghent University (UGCT, http://www.ugct.ugent.be). Ten segments above
Table 1. Summary of morphological features defining the five axis types composing *H. seemannii* architectural organization. These five axis types are classified in the functional categories for structural support and resource acquisition.

<table>
<thead>
<tr>
<th></th>
<th>structural support</th>
<th>resource acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>axis habit</td>
<td>climbing/self-supporting</td>
<td>climbing</td>
</tr>
<tr>
<td>branching pattern</td>
<td>rhythmic</td>
<td>rhythmic</td>
</tr>
<tr>
<td>growth direction</td>
<td>thigmotropic/plagiotropic</td>
<td>thigmotropic</td>
</tr>
<tr>
<td>adhesive roots</td>
<td>present/absent</td>
<td>present</td>
</tr>
<tr>
<td>flowering ability</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>symmetry</td>
<td>bilateral</td>
<td>bilateral</td>
</tr>
<tr>
<td>growth unit length</td>
<td>&lt;5 m</td>
<td>&lt;1 m</td>
</tr>
</tbody>
</table>

7.4 mm in diameter were selected, i.e. five segments for each climbing phenotype. One disc of uniform thickness of 0.5 cm was sectioned per segment, gradually dehydrated in a series of alcohol and subsequently air dried. Two helical scans (five discs per scan) were performed at 60 kV and 90 mA scanning for approximately 1 h with a rotation step size of 0.36°. Reconstructions were performed with Octopus, a tomography reconstruction package for parallel, cone-beam and helical geometry [28]. The reconstructed greyscale volumes were converted to specific gravity, further referred to as wood density (kg m⁻³) following the standard protocol adopted from De Ridder et al. [29]. The obtained scan resolution was 15 μm pixel⁻¹.

2.7. Statistical analyses

Normal distributions of variables and residuals were tested by Shapiro–Wilks tests, assuming normality at significance values more than 0.05. Non-normally distributed variables were normalized by transformation to specific gravity, further referred to as wood density (kg m⁻³) following the standard protocol adopted from De Ridder et al. [29]. The obtained scan resolution was 15 μm pixel⁻¹.

3. Results

3.1. Morphological architecture

The architectural unit of *H. seemannii* is composed of two main axis categories (table 1). The first consists of two axis types (C1 and C2) that together constitute the main anchoring and perennial structure of the plant (figures 1a,c, 2a, 3a,c and 4). These structural axes are mostly climbing and thigmotropic, with the exception of the apical self-supporting plagiotropic portions of C1 axes (figures 1c, 2a, 3a and 4a,c,d). They branch rhythmically, have a bilateral symmetry and support the remaining axis types (figures 1a,c, 2a, 3a,c and 4). C2 axes differ from C1 axes in their reduced secondary growth production, shorter lifespan and an entirely climbing habit. The second axis category is composed of three axis types (C3–C5) whose main function is for resource acquisition and/or flowering (figures 1a,c, 2a, 3 and 4). These three axes are entirely self-supporting and completely lack adhesive roots. C3 and C4 axes branch rhythmically and in addition to leaves, they can also expose the inflorescences (figures 1a,c, 2a and 3). By contrast, C5 axes are unbranched and exclusively produce leaves (figure 3). C3 axes differ from any other axis type in their orthotropic growth direction and radial symmetry (figures 1c, 2a and 3a,b). C4 axes have a plagiotropic growth direction (figures 1a,c, 2a and 3c), whereas C5 has no precise growth direction (figure 3), however, both have a bilateral symmetry.

When climbing on shaded, vertical and long tree trunks and rock walls, *H. seemannii* modules are very long and the structural axes C1 and C2 cling on their support along most of their length and for most of their lifespan (figure 1). Then the main structural C1 axis progressively changes its growth direction by laterally producing plagiotropic self-supporting modules that increase their frequency towards the end of the support (figures 1c and 4a). In a young tree-climber a main monopodial C1 axis rhythmically branches by mostly producing thigmotropic C2 axes, which distally bear abundant leafy C4 axes (figures 1a and 4b). At this early phase of plant development, few orthotropic C3 axes are produced (figure 4b). Symposial C1 axes of mature tree-climbing individuals laterally branch by regularly producing leafy C4 axes along most of their length, while orthotropic C3 axes become more abundant towards the apex of the plant (figures 1c and 4a). Conversely to young tree-climbers, mature tree-climbing individuals barely produce thigmotropic C2 axes (figures 1c and 4a,b). Plants climbing on boulders produce shorter modules during the climbing phase, tightly following the boulders’ uneven contour (figure 2a,c). Contrary to long-vertical climbers, main C1 axes of bouldering plants undergo a conspicuous change in their modules’ growth behaviour from thigmotropic on the boulder surface to plagiotropic above the boulder (figure 2a,b). Owing to their thigmotropic growth behaviour, C2 axes are then restricted to a short basal climbing phase of bouldering plants (figures 2a and 4c,d). Bouldering plants are characterized by an increased production of orthotropic C3 axes which develop almost entirely from the self-supporting and plagiotropic portion of the main C1 axes (figures 2a and 4c,d). Leafy C4 axes are
rather scarce and, if present, develop either from C1 or C3 axes (figures 2a and 4c,f).

3.2. Mechanical architecture

*Hydrangea seemannii* showed a general decrease in Young’s modulus towards large-diameter segments with Young’s modulus values ranging from ca 2300 to 560 MPa (figure 5). Segments belonging to the first developmental stage were characterized by a wide range of Young’s modulus values (ca 820–2300 MPa) largely overlapping among axis types and highly varying within them (figure 5). Segments corresponding to the second developmental stage, almost entirely constituted by the main structural C1 axis, showed a marked decrease of Young’s modulus from ca 1500–1400 MPa down to ca 560 MPa with increasing stem diameter (figure 5).

When considering the entire developmental range, both climbing phenotypes exhibited a general decrease in Young’s modulus with increasing stem diameter; Young’s modulus values ranged from ca 2300 to 560 MPa in tree-climbers and from ca 2300 to 820 MPa in boulder-climbers (figure 5). However, the nested ANOVA analysis showed no significant differences in Young’s modulus between climbing phenotypes in neither of the two stem diameter classes and only lower p-values were retrieved for stem segments more than or equal to 7.4 mm (figure 6). Within the first developmental stage, tree-climbers showed a mean Young’s modulus of 1432 MPa (s.d. ± 319) with Young’s modulus values ranging from ca 2300 to 870 MPa, whereas rock-climbers had a mean Young’s modulus of 1377 MPa (s.d. ± 332) with Young’s modulus values ranging from ca 2300 to 820 MPa (figure 6).

At the second developmental stage, Young’s modulus values of tree-climbers ranged from ca 1970 to 560 MPa and showed a mean Young’s modulus of 990 MPa (s.d. ± 419, figure 6). At this older developmental stage, boulder-climbers showed Young’s modulus values only ranging from ca 1000 to 990 MPa with a mean Young’s modulus of 1162 MPa (s.d. ± 215, figure 6).

*Hydrangea* sp. 1 exhibited a general decrease in Young’s modulus throughout development, but a steep increase in Young’s modulus for stem segments up to 10 mm in diameter with maximal Young’s modulus values at around 2600 MPa (figure 5). Above 10 mm in diameter, a sudden decrease in Young’s modulus occurred, followed by a more gradual
decrease towards larger diameter segments (figure 5). The
nested ANOVA analysis detected no significant differences in 
Young’s modulus between Hydrangea sp. 1 and H. seemannii
tree-climbers in neither of the younger (\(p = 0.708\)) nor older 
(\(p = 0.846\)) developmental stages. Similarly, no significant 
differences were obtained from the comparison of 
Hydrangea sp. 1 and H. seemannii boulder-climbers at the first developmen-
tal stage (\(p = 0.846\)) and lower, yet no significant 
\(p\)-values were 
retrieved in the older developmental stage (\(p = 0.348\)).

3.3. Stem anatomical organization and wood density
Considering the entire developmental range of H. seemannii,
contribution to the total cross-sectional area and second 
moment of area showed a marked decrease for the parenchy-
matous pith, a slight decrease for the soft outer cortical 
tissues and a marked increase for the lignified pith along 
with wood towards larger diameter segments, whereas pith 
eccentricity markedly increased towards older developmental 
stages (see the electronic supplementary material, figure S2).

The nested ANOVA analysis showed no significant differ-
ences between climbing phenotypes for any of the measured 
stem tissue traits in either of the two developmental stages. 
Values of mean plus 2 s.d. overlapped to different degrees 
between climbing phenotypes. This was observed in all 
tissue organizations and in both stem developmental stages, 
with the exception of pith eccentricity at the older developmen-
tal stage (figure 7; electronic supplementary material, table S3). 

Hydrangea seemannii tree-climbers showed significantly lower 
wood density than boulder-climbers for stems in the second 
developmental stage (\(p = 0.016\); electronic supplementary 
material, table S3; figure 8). Tree-climbers showed a mean 
wood density of 539.24 (s.d. ± 16.40), whereas boulder-climbers 
had a mean wood density of 663.93 (s.d. ± 64.09, electronic sup-
plementary material, table S3). Rings of eccentric climbing discs 
were narrower and compressed towards the abaxial position 
(the area close to the anchoring surface; figure 8), whereas 
rings of non-eccentric discs were more radially homogeneous 
(figure 8). Wider axes from both climbing phenotypes showed 
more dense wood around the pith periphery (figure 8).

4. Discussion
4.1. Do climbing phenotypes of Hydrangea seemannii 
 differ in morphological architecture?
In cultivation, seedlings of H. seemannii quickly become 
unstable in the absence of support (M. Libert, Ghent Univer-
sity, Belgium, 2013, personal communication) and, in their 
natural habitat, fully self-supporting individuals have not 
been observed. This suggests that the ability to produce an 
early self-supporting phase characteristic of other climbers 
such as M. aff. quinquepartita [1] or the ability to develop 
fully self-supporting phenotypes such as in western poison 
oak (Toxicodendron diversilobum [9]) are not present in this 
species. Both climbing phenotypes of H. seemannii instead 
resemble more the general architectural model of the English
ivy (*H. helix*), having a plagiotropic juvenile form and later climbing by means of adhesive roots [12,30].

In contrast to the long internodes and relatively straight stems in tree-climbers of *H. seemannii*, boulder-climbers of the species showed shorter internodes and skewed stems, which might represent morphological plastic responses to different climbing environments such as light availability and surface contour. Compared with boulder surfaces, light incidence at tree surfaces is potentially lower due to additional shading produced by the host tree branches and leaves. Stem lengthening has been reported as a frequent shade avoidance response in plants [31–34], supporting the idea that in *H. seemannii* this plastic response is related to light availability. In contrast to woody-climbers, trees and shrubs develop comparatively shorter internode lengths [13], suggesting that the shorter internodes of boulder-climbers are a morphological plastic response tending towards the production of a more self-supporting architecture. Moreover, the shorter, more rounded form of

**Figure 4.** (a,b) Schematic of tree-climbers and (c,d) boulder-climbers morphological architecture. Black and red axes represent the structural supporting axes C1 and C2, whereas green and blue axes denote the resource acquisition axes C3 and C4. Numbers along branches of the schematic correspond to measurements of Young’s modulus (MPa) graphed in figure 5. (Online version in colour.)
the boulders in the vicinity substrate compared with the linear form of trees probably underline the differences in shape (askew versus straight stems) representing a plastic response to the climbing surface contour. This might be expected and a characteristic of climbing plants that attach closely to the host surface.

The different proportions of orthotropic-radial, C3 axes and plagiotropic-bilateral, C4 axes between climbing phenotypes of *H. seemannii* may be an indication of architectural plasticity linked to a leaf display optimization in each climbing environment. Above the boulder’s surface, three-dimensional space is relatively open and light incidence is potentially higher than around vertical tree trunks. This possibly explains why boulder-climbers develop a high proportion of orthotropic-radial C3 axes that can maximize leaf exposure and why tree-climbers develop more plagiotropic-bilateral C4 axes that can facilitate leaf displaying among the host branches and leaves. One consistent finding was that, in the more open apical parts of tree trunks, the proportion of orthotropic-radial C3 axes gradually increased. The increased production of orthotropic and radial axes in open environments has been previously reported in other woody growth forms [31,35], supporting the idea that these plastic responses in *H. seemannii* are linked to resource acquisition and influenced by three-dimensional space and light availability.

The conspicuous change in stem growth direction and habit found in boulder-climbers might represent a plastic response to support length variability. In contrast to tree trunks, boulders are comparatively shorter promoting an earlier loss of physical support and increased light exposure. In many climbing plants,
Figure 8. Comparative wood density between climbing phenotypes, wood density colour scale values are shown in kg m\(^{-3}\). Pith and soft outer cortical tissues are shown in greyscale.

in magnitude to other climbing species with strong attachment systems (e.g. stem twiners and tendril climbers; [2]). Despite the difference in climbing support (boulders versus trees), our analysis identified no significant differences in mechanical properties in either of the two developmental stages (figure 6). Somewhat different values were found for the second developmental stage; however, high standard deviations likely caused the observed differences in mean values to be not significant. These involved older stages of boulder-climbers that tended to have higher values of Young’s modulus compared with older stages of tree-climbing stems. Several factors can explain the high variation in the obtained Young’s modulus values. First, segments belonging to the same developmental stage showed contrasting axis habits exposing them to different mechanical forces. Second, segments of similar stem diameter showed variation in their secondary growth production, most likely as a response to the climbing surface heterogeneity, as well as to the axis habit, directly influencing the stem mechanical properties. Third, boulder and tree surfaces are non-uniform exposing the climbing segments to a number of different mechanical forces.

Overall, boulder-climbers appear to be slightly stiffer than tree-climbers at the old developmental stage. The potentially different values of old stems in boulder-climbers could represent a fine plastic adjustment to a completely static climbing surface. In turn, climbing on tree trunks may require more compliant stems in order to endure for instance the continuous wind-induced movements of the host plants, which specially affect climbing plants, such as root-climbers, that closely and strongly attach to their climbing surfaces. Moreover, different degrees of attachment might result from climbing on the relatively smooth boulder surface compared with the rough tree surface, resulting in a lower strength of attachment in boulder-climbers than in tree-climbers. As detailed above (see also [1,2]), firmly attached climbers generally develop more compliant tissues in older developmental stages than weakly attached ones; also believed to be helpful when handling the stress produced by close contact with the host.

When compared with the strictly tree-climbing species *Hydrangea* sp. 1, climbing phenotypes of *H. seemannii* showed no significant differences in either of the two developmental stages. However, lower p-values were obtained in the comparison with *H. seemannii* boulder-climbers for the second developmental stage. This suggests that boulder-climbers present a slight, yet non-significant, deviation from the ‘typical’ tree-climbing mechanical behaviour, showing a fine plastic adjustment in a developmental stage that is highly affected by the close contact with the climbing surface.

### 4.3. Do climbing phenotypes of *Hydrangea seemannii* differ in their stem anatomical properties?

Anatomical development of *H. seemannii* involved a decrease of mostly non-lignified tissues (i.e. parenchymatous pith and soft outer cortical tissues), but an increase in contributions of lignified tissues (i.e. lignified pith along with wood). With the exception of pith eccentricity, all measured anatomical variables presented highly overlapping mean plus 2 s.d. ranges, especially at the first developmental stage, explaining the non-significant differences between climbing phenotypes (see discussion above). In the case of pith eccentricity, the non-significant p-values in addition might be the result of the nested factor design of the ANOVA analyses, because exclusion of this factor (data not shown) resulted in significantly different mean pith eccentricity values.

Although not statistically supported, somewhat higher contributions of lignified tissues and lower contributions of soft outer cortical tissues were observed in tree-climbers relative to boulder-climbers at the second developmental stage. Wood produced by tree-climbers at this older stage was significantly less dense than that of boulder-climbers. Wood density is known to be affected by the size and number of vessels, as well as by fibre wall thickness and therefore influences both stem conductance and stem stiffness [37–39]. Less dense wood, resulting from an increased vessel area fraction, is associated with a potentially higher conductance efficiency [15,38] and more compliant tissues [37,39]. Developmental plasticity resulted in a somewhat greater area of less dense wood in stems of old developmental stages of tree-climbers. This is consistent with higher hydraulic conductance capacities, which is particularly advantageous when water must be drawn to provision aerial shoots high in the canopy. In terms of mechanical properties, the somewhat different proportions of wood area could play a role in homogenizing mechanical properties between climbing phenotypes, despite significant differences in wood density. Given a fixed wood density and stem diameter, a lower proportion of wood would contribute to more compliant stem segments when compared with segments with a greater proportion of wood. Boulder-climbers present more dense wood, however, their wood proportion within the stem is lower, whereas tree-climbers show a greater area of less dense wood, possibly explaining why differences in wood density do not result in
statistically significant mechanical differences. A greater area of less dense wood is consistent with the mechanical demands of the tree-climbing habit, where close contact with the host tree and branches and swaying movement in the wind require flexibility rather than stiffness. In turn, the developmental plastic response of boulder-climbers is to produce a smaller area of more dense wood in old stem developmental stages necessarily to maintain the structural stability of the self-supporting habit of its axes without compromising its hydraulic demands.

*Hydrangea seemannii* and other *Cornulia* species develop ring-porous wood [40]. Ring-porous wood in self-supporting trees is typically characterized by growth rings with wide vessels (lower density) in the early wood and fibres and more narrow vessels (higher density) in the late wood. In trees, ring-porous wood is largely restricted to northern temperate species and shows the largest increase in stem conductivity with stem size, as well as an efficient conducting network compared with diffuse-porous wood or coniferous wood [41]. Both climbing phenotypes of *H. seemannii* develop ring-porous wood; however, tree-climbers present areas of low-density wood away from the anchoring side of the stem and relatively more dense wood close to the supporting surface (tension wood). In turn, boulder-climbers showed a more homogeneous distribution of wood with mostly higher density. Correspondingly, tree-climbers appeared to have more eccentric piths compared with boulder-climbers, although this does not reach statistical support. In *H. seemannii*, pith eccentricity appears to result from the cambium varying its activity in relation to the contact with the climbing substrate. In terms of strength of attachment, the limited inner wood production in tree-climbers maintains adhesive roots close together assuring an appropriated anchoring strength—an increased production of wood on the inner surface against the tree would instead prise attached roots away causing anchorage failure. In terms of structural stability, differences in wood density distributions (detected via cross sections) might represent a developmental plastic response acting towards providing more structurally stable tissue on the anchoring side of climbing stems, albeit compromising partially stem conductivity.

### 4.4. Can we assume growth form plasticity in *Hydrangea seemannii* resulting from variability in support type?

For phenotypic plasticity to take place, individuals with the same genotype, e.g. belonging to the same species and population, should show different observable characteristics, which can range from morphological or physiological to developmental and behavioural properties as a response to different environmental conditions [7,42]. Nevertheless, plant–environment interactions can be both complex and numerous, producing a continuum of growth form variations, where an absolute ‘description’ or ‘categorization’ is often difficult to provide [1,16,18,25]. While some plant species such as poison oak [9] can be clear-cut examples of phenotypic plasticity producing two contrasting growth forms (lianas versus shrubs), other species within the continuum of growth form variations can present finer plastic responses within individual growth form types. We investigated three critical aspects defining woody plant growth form variability including their morphological, mechanical and anatomical architectures.

Some of the overall characteristics of *H. seemannii* might at first sight suggest an absence or rather limited developmental plasticity at the growth form level—(i) the lack of entirely self-supporting individuals, (ii) the early dependence on the climbing habit, (iii) the marked decrease of stem stiffness during development and (iv) the general decrease in contributions of non-lignified tissues and increase in contributions of lignified tissues. However, other finer scale characteristics sustain the notion of yet finer plastic responses to support variability within the woody climbing growth form in which boulder-climbers tend to show a developmental plasticity towards a more self-supporting growth form. These plastic responses represent alternative root-climbing strategies that are potentially optimized for different climbing substrates and environmental conditions. One aspect of phenotypic plasticity is phenotypic accommodation which produces a buffering effect maintaining critical relationships among varying components and lessening the negative effects of change [43,44]. Evidence for phenotypic accommodation in morphological, hydraulic and mechanical stem traits has been provided in previous studies on woody plants [43,45,46]. In *H. seemannii*, this buffering effect could for example involve the production of smaller cross-sectional areas of more dense wood in boulder-climbers versus more area of less dense wood in tree-climbers, to equilibrate the mechanical and hydraulic constraints derived from their particular climbing environments.

Appropriate characterization of phenotypic plasticity is fundamental to studies such as evolutionary and conservational biology [7,31,32,47–49]. It has been advocated that plasticity conferring adaptive flexibility can influence patterns of species diversification (see review by [7]). Growth form evolutionary studies should therefore consider potential plasticity in growth form characterizations, because it might be influenced by natural selection. Conservation programmes should not only aim to preserve plant species in terms of their genetic diversity, but also maintain the environmental variation of their habitat. Being the only temperate *Cornulia* representative of the northern hemisphere, *H. seemannii* must be considered a priority for conservation programmes.

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### Data accessibility


### Funding statement

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