



HAL
open science

Scaling of petiole anatomies, mechanics, and vasculatures with leaf size in the widespread Neotropical pioneer tree species *Cecropia obtusa* Trécul (Urticaceae)

Sébastien Levionnois, Sabrina Coste, Eric-André Nicolini, Clement Stahl, Hélène Morel, Patrick Heuret

► To cite this version:

Sébastien Levionnois, Sabrina Coste, Eric-André Nicolini, Clement Stahl, Hélène Morel, et al.. Scaling of petiole anatomies, mechanics, and vasculatures with leaf size in the widespread Neotropical pioneer tree species *Cecropia obtusa* Trécul (Urticaceae). *Tree Physiology*, 2020, 40 (2), pp.245-258. 10.1093/treephys/tpz136 . hal-02464689

HAL Id: hal-02464689

<https://hal.umontpellier.fr/hal-02464689>

Submitted on 3 Feb 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **Original Article**

2 **Scaling of petiole anatomies, mechanics, and vasculatures with leaf size in the**
3 **widespread Neotropical pioneer tree species *Cecropia obtusa* Tr cul (Urticaceae)**

4 Running title:

5 S bastien Levionnois^{1*}, Sabrina Coste¹, Eric Nicolini², Cl ment Stahl¹, H l ne Morel¹,
6 Patrick Heuret^{1,2*}

7

8 1. UMR EcoFoG, AgroParisTech, CIRAD, CNRS, INRA, UA, UG, 97379 Kourou Cedex
9 France.

10 2. UMR AMAP, CIRAD, CNRS, INRA, IRD, Universit  de Montpellier, 34398 Montpellier,
11 France.

12

13 *Authors for correspondence:

14 S bastien Levionnois, PhD student

15 Postal address: UMR EcoFog, Campus Agronomique, Avenue de France

16 BP709 – 97387 Kourou Cedex

17 French Guiana

18 Tel: +33 760 26 73 83

19 Email: sebastien.levionnois.pro@gmail.com

20

21 Patrick Heuret, PhD

22 Postal adress: UMR AMAP, TA 1-51/PS2 Boulevard de la Lironde

23 34398 Montpellier Cedex 5

24 France

25 Tel: +33 467 61 55 14Email: patrick.heuret@inra.fr

26

27 Author contributions

28 P.H. conceived and designed the study; P.H., S.L., E.N., S.C., H.M. and C.S. collected field
29 samples and measured morphological traits; S.L. performed anatomical sections. S.L. and P.H.
30 performed images and data analysis; S.L. and P.H. wrote the manuscript; all authors discussed
31 the results and contributed valuable comments on the manuscript.

32

33 **ABSTRACT**

34 **1.** Although the leaf economic spectrum has deepened our understanding of leaf trait
35 variability, little is known about how leaf traits scale with leaf area. This uncertainty has
36 resulted in the assumption that leaf traits should vary by keeping the same pace of variation
37 with increases in leaf area across the leaf size range. We evaluated the scaling of
38 morphological, tissue-surface, and vascular traits with overall leaf area, and the functional
39 significance of such scaling.

40 **2.** We examined 1271 leaves for morphological traits, and 124 leaves for anatomical, and
41 hydraulic traits, from 38 trees of *Cecropia obtusa* Tr cul (Urticaceae) in French Guiana.
42 *Cecropia* is a Neotropical genus of pioneer trees that can exhibit large laminae (0.4 m² for *C.*
43 *obtusa*), with leaf size ranging by two orders of magnitude. We measured (i) tissue fractions
44 within petioles and their second moment of area, (ii) theoretical xylem hydraulic efficiency of
45 petioles, and (iii) the extent of leaf vessel widening within the hydraulic path.

46 **3.** We found that different scaling of morphological trait variability allows for optimisation of
47 lamina display among larger leaves, especially the positive allometric relationship between
48 lamina area and petiole cross-sectional area. Increasing the fraction of pith is a key factor that
49 increases the geometrical effect of supportive tissues on mechanical rigidity and thereby
50 increases carbon-use efficiency. We found that increasing xylem hydraulic efficiency with
51 vessel size results in lower leaf lamina area: xylem ratios, which also results in potential
52 carbon savings for large leaves. We found that the vessel widening is consistent with
53 hydraulic optimisation models.

54 **4.** Leaf size variability modifies scaling of leaf traits in this large-leaved species.

55

56 **Key words:** allometry, theoretical hydraulic conductivity, leaf size, petiole anatomy, scaling,
57 vessel widening, xylem.

58 INTRODUCTION

59 Leaf traits have received wide attention over recent years because of the major roles they play
60 in the plant carbon economy. A current, widely accepted paradigm exists as part of the global
61 spectrum of leaf economy (LES), which emphasizes the importance of “quick” to “slow”
62 returns on physiological investments (Wright et al. 2004). In this framework, coordination
63 between structural, chemical, and physiological traits has been convincingly described within
64 and between species (Poorter and Evans 1998, Reich et al. 1998, Hikosaka 2004, Shipley et
65 al. 2006, Niinemets 2015, Onoda et al. 2017). Some studies have also investigated some of
66 the anatomical and physiological changes underlying the variability of traits underpinning the
67 LES, such as leaf mass per area (LMA; Scoffoni et al. 2016, John et al. 2017, Onoda et al.
68 2017). Leaf size (i.e. petiole length and diameter, lamina length, and mainly lamina area), on
69 the other hand, has been generally studied as disconnected from the LES, or has been shown
70 to be decoupled from the LES (Baraloto et al. 2010), although it is a trait that can vary hugely,
71 encompassing six orders of magnitude across vascular plants (Niinemets et al. 2007, Milla
72 and Reich 2007, Wright et al. 2017). For instance, leaf size is certainly subject to strong
73 selective pressures such as light interception (Poorter and Rozendaal 2008, Smith et al. 2017)
74 or temperature regulation (Gates 1968, Parkhurst and Loucks 1972, Leigh et al. 2017). The
75 leaf size – stem size relationship and other related concepts (Corner’s rules; (Corner 1949,
76 White 1983a, 1983b, Ackerly and Donoghue 1998); leaf-stem allometry (Brouat et al. 1998,
77 Brouat and McKey 2001, Fan et al. 2017), the leaf size-twig size spectrum (Westoby et al.
78 2002) and the leptocaulis-pachycaulis spectrum (Hall  et al. 1978)) have been a fruitful
79 framework to tackle the within- and among-species leaf size variability (Westoby et al. 2002),
80 but the related studies remain mainly based on morphological traits (i.e. lamina area, stem
81 cross-sectional area, internode length, petiole length...), rather than anatomical or
82 physiological traits (however see Normand et al. 2008, Fan et al. 2017).

83 A key aspect of the knowledge gathered from leaf size – stem size relationships,
84 involves crucial questions about how and why leaf size varies across the plant kingdom. The
85 traits expressed in different lamina-petiole functional scaling (i.e. allometric vs isometric)
86 within and between species is likely of significance for these questions. Relationships
87 between two traits x and y can be described as: $y = ax^b$, such as: $\log(y) = \log(a) + b * \log(x)$,
88 where b is the slope (or allometric exponent) and a the intercept (allometric coefficient). An
89 isometric relationship, when $b = 1$, is a linear and proportionality relationship, implying no
90 change of organ or organism form and shape over ontogeny, or across species. An allometric
91 relationship, when $b \neq 1$, is a non-linear and disproportionality relationship, underlying

92 different pace of variation between two traits, and implying changes of organ or organism
93 form and shape. Discerning allometric vs isometric relationships between organ or organism
94 traits is an important priority, since different functional requirements can be reflected between
95 small vs large organs or organisms, and finally different responses to selective pressures
96 (Harvey and Pagel 1991, Brouat et al. 1998). The lamina-petiole relationship, or the
97 relationship between a given leaf trait with leaf size, has been little studied in this scaling
98 perspective. This gives scattered ideas if leaf size affects leaf functional requirements, and
99 further if sampling both small and large leaves make a big difference for mechanical,
100 hydraulic, and photosynthetic quantifications.

101 First, a set of studies have argued that larger leaves are disproportionately more carbon
102 expensive, based on the fact that the leaf area fails to keep pace with increases in leaf dry
103 mass, at both inter- (Niinemets et al. 2006, Niklas et al. 2007, Niinemets et al. 2007, Niklas
104 and Cobb 2008, Li et al. 2008, Niklas et al. 2009, Yang et al. 2010) and intraspecific levels
105 (Milla and Reich 2007, Sun Jun et al. 2017). The main explanation is that support
106 requirements increase disproportionately, as the bending moment of a cantilevered beam –as a
107 petiole- scales with the cube of its length (Gere and Timoshenko 1997, Niinemets et al. 2007),
108 in addition to the drag forces applied to the leaf. The leaf mass-leaf area scaling has been
109 relatively little studied at the intraspecific level (Sun Jun et al. 2017). Moreover, the leaf area-
110 petiole cross-sectional area scaling has received less attention. Fan et al. (2017) report an
111 isometric relationship across 28 *Ficus* species. Price and Enquist (2007)'s empirical data
112 support an isometric leaf area-petiole cross-sectional area relationship for 5 out of 18 species,
113 and an allometric relationship for 13 out of 18 species. Finally, for both leaf mass-leaf area
114 and leaf area-petiole cross-sectional area scalings, we do not know the size-related anatomy
115 underlying these scalings.

116 Mechanical measurements have been applied in relation to leaf area. Studies of Niklas
117 (1991, 1992) and Mahley et al. (2018) demonstrated that small vs large leaves are not
118 mechanically equivalent regarding flexural stiffness, both within and across species, with
119 petioles of large leaves being disproportionately stiffer. But the mechanical structure driving
120 the flexural stiffness between small vs large leaves remains poorly described (Niklas 1999).
121 Mechanical stability can be achieved through two properties: the material property and the
122 geometry. Modification of petiole tissue surfaces and fractions across a leaf size range can
123 determine the mechanical contribution through geometry of main supportive tissues such as
124 xylem, collenchyma, and sclerenchyma (Faisal et al. 2010, Niklas and Spatz 2012). Pith is
125 largely made of large parenchyma cells, and is a cheap due to weak cell wall lignification and

126 fine cell walls (Evert 2006). As the pith generally occupies the central position within an
127 organ, modifying pith area and fraction could be an efficient way to generate volume with
128 decreased carbon costs (Olson, Rosell, et al. 2018). Moreover, when pith fraction increases, it
129 mechanically shifts external and supportive tissues (xylem, sclerenchyma, collenchyma)
130 centrifugally, increasing the second moment of inertia of these tissues. Second moment of
131 area quantifies the distribution of mass in a cross-section with respect to the centre of inertia
132 of the cross-section, and this describes the important effect of size and geometry of the cross-
133 section in mechanics. The prevalent role of cross-sectional geometry in the stiffness of
134 petioles has been shown by Mahley et al. (2018), but only for ferns, which exhibit a very
135 different anatomy in comparison to flowering plants.

136 In comparison to the leaf dry mass-area scaling or petiole mechanics, little is currently
137 known on the link between leaf size, leaf hydraulic conductivity and vascular architecture.
138 Understanding size-related changes of leaf hydraulics and vasculature is important to address
139 size-independent variation, as pointed out for stems (Olson et al. 2009). The West-Brown-
140 Enquist model (WBE model) assumes that the variation of terminal (i.e. minor veins) conduit
141 size and number is independent of leaf size (West et al. 1999, Price and Enquist 2007).
142 Supporting this assumption, Sack et al. (2012) have shown that the global vein density across
143 species is independent of leaf size, even if major vein density decreases with leaf size. Fiorin
144 et al. (2016) suggest that the spatial organisation of stomata with veins is uniform across
145 leaves of different species. But it can also be hypothesised that small vs large leaves across
146 and within species have to deal with different selective pressures regarding conductive path
147 length and the water supply per unit leaf area. Indeed, across the leaf size range, plants should
148 maintain constant leaf conductance per unit leaf area (K_{leaf} , $\text{kg MPa}^{-1} \text{s}^{-1} \text{m}^{-2}$), or at least
149 minimise the loss of K_{leaf} , to sustain transpiration and carbon assimilation (Petit et al. 2016,
150 Pittermann et al. 2018, Echeverr a et al. 2019).

151 A longer leaf implies *de facto* a longer conductive path length that would thus increase
152 hydraulic resistance and likely affect K_{leaf} in the absence of appropriate vascular adjustments.
153 Theoretical models and empirical data support that the axial basipetal widening in vessel
154 diameter reduces the increase of hydrodynamic resistance with conductive path length (West
155 et al. 1999, Becker et al. 2000, Enquist 2003, Anfodillo et al. 2013, Olson et al. 2014). To our
156 knowledge only two studies measured vessel widening within leaves: Coomes et al. (2008) for
157 ten oak species, and Lechthaler et al. (2019) for one species of *Acer*. Yet, these studies
158 suggest higher rates of vessel widening within leaves, in comparison to stems. This is
159 consistent with the fact that leaves concentrate a significant part of the hydraulic resistance of

160 the plant (Sack and Holbrook 2006), owing to different vascular architecture between leaves
161 and stems.

162 A larger leaf also implies *de facto* a larger lamina area to supply with water, and thus
163 an appropriate and sufficient petiole conductivity is required. Increasing the xylem area to
164 increase the number of vessels, or increasing the vessel diameter are two non-exclusive ways
165 of reducing total resistance, and increase petiole and vein conductivity to achieve a sufficient
166 leaf water supply. However, an increase of the number of vessels comes with increasing
167 construction costs, because additional space is required (Banavar et al. 1999, McCulloh et al.
168 2003, Gleason et al. 2018). The conductivity of a vessel increases with the fourth power of its
169 diameter as predicted by the Hagen-Poiseuille law (Tyree and Zimmermann 2002). Thus, a
170 small increase in vessel diameter will drive an exponential increase in the vessel conductivity,
171 but potentially at the cost of increasing embolism risk. A positive allometric relationship of
172 vasculature with size should increase the xylem conductive efficiency (namely xylem-specific
173 conductivity, which is the xylem conductivity divided by the xylem surface), and therefore
174 should limit xylem volume and construction cost. Investigating the petiole xylem and
175 vasculature according to leaf size can be readily implemented, and should highlight some
176 hydraulic-related selective pressures correlating with the leaf size variability, at both inter-
177 and intraspecific levels.

178 We chose to investigate lamina-petiole traits at the intraspecific level as a first step.
179 We focussed this study on the genus *Cecropia*, which is known for its hyperdominant pioneer
180 trees that are pivotal in the initiation of tropical forest successions. We selected *Cecropia*
181 *obtusa* Tr  cul (Uticaceae), which is a widespread species from the Guiana shield, capable of
182 deploying large leaves with a large size range (100 – 4000 cm²), appropriate for addressing
183 the lamina-petiole scaling with petiole anatomical and vasculature correlates, at the
184 intraspecific level. We measured leaf morphology, tissue areas of the petiole, petiole vascular
185 and theoretical conductive traits. We built a dataset of 1271 leaves from 38 trees, with
186 anatomical and vascular data for 124 leaves. We aimed to address the following questions:

187 (i) Is the form of the lamina-petiole scaling isometric or allometric? We hypothesise to
188 find allometric lamina area-petiole cross-sectional area and lamina mass-petiole mass
189 relationships, with petiole mass increasing faster as mechanical requirements
190 disproportionately increase.

191 (ii) How does petiole anatomy determine petiole mechanics? We hypothesise that pith
192 fraction increases with leaf size, minimising volumetric construction cost, and enhancing
193 mechanical strength through geometry by shifting supportive tissues centrifugally.

194 (iii) How do longitudinal adjustments of xylem and vasculature variations limit loss of
195 K_{leaf} and construction costs? We hypothesise that K_{leaf} loss limitation for larger leaves is
196 allowed by vessel basipetal widening according to the leaf size and the conductive path
197 length. We hypothesise to find a higher rate of vessel widening within the leaf in comparison
198 to what is known for the stem, consistent with leaves supporting a large amount of the total
199 hydraulic resistance of plants. We also hypothesise that less construction cost is possible than
200 under the hypothesis of an invariable vessel diameter, thanks to a more efficient xylem due to
201 wider vessels, enabling more lamina area per xylem area.

202

203 MATERIALS AND METHODS

204 *Study site*

205 The study was conducted in French Guiana along a forestry road in Counami (N5.41430°,
206 W53.17547°, geodesic system WGS84) where the entrance to the road is located 5 km to the
207 east of Iracoubo municipality. The warm wet tropical climate of French Guiana is highly
208 seasonal due to the north-south movement of the Inter-Tropical Convergence Zone. Mean
209 annual rainfall is 2726 mm year⁻¹ and annual mean air temperature is 25.7 °C (Gourlet-Fleury
210 et al. 2004). There is a dry season lasting from mid-August to mid-November, during which
211 rainfall is < 100 mm month⁻¹.

212

213 *Plant material, sampling and morphological measurements*

214 Tree sizes ranged from 10.8 to 23.1 m in height, and 6.21 to 30.49 cm in diameter at breast
215 height. Trees were evenly felled in time from September 2014 to October 2016. All the leaves
216 in the crown were cut by cutting the petiole as close as possible to its insertion point with the
217 stem and placed immediately in plastic bags within coolers. One to five fully expanded leaves
218 per tree -hereafter called A-leaves - were kept for the anatomical studies.

219 *Cecropia* leaves are palmatilobate (Fig. 1b; Fig. S1) and so there is no single midrib
220 but the same number of midribs as there are lobes, i.e. a midrib departs into and supplies each
221 lobe. In the laboratory, all the leaves were processed within 4 hours after felling to keep them
222 as fresh as possible. Petioles were cut as close to the lamina as possible. Two orthogonal
223 diameters (mm) of each petiole, at the middle point of the petiole, were measured because of
224 the tendency of *Cecropia* petioles to exhibit an ellipse shape (Fig. S1; Table 1 for a list of
225 traits and abbreviation). From these two diameters, a cross-sectional area was derived in the
226 shape of an ellipse (A_{pet} , mm²; Fig. S1). For each lamina, the length of the main lobe (i.e. the
227 largest one in the continuation of the petiole; cm) was measured. In the A-leaves, two 1-cm-

228 long petiole segments in the median position was sampled (Fig. S1). The first segment was
229 used to measure their specific density, expressed as the ratio of dry mass to the fresh volume
230 (PD: petiole density; Williamson and Wiemann 2010; Table 1). The fresh volume of the
231 sample was calculated using an inverse Archimedes principle and a precision balance
232 (CP224S, Sartorius), based on the buoyancy (G) of the sample, i.e. $G = \text{fresh mass in air} -$
233 submerged mass (Lehnebach et al. 2019). Dry mass was derived after drying at 103 °C for
234 three days. PD was computed as the dry mass divided by G . The second segment was
235 preserved in 70% alcohol and used later for anatomical measurements (see the section headed
236 ‘*Anatomical measurements*’). Because we aimed at studying the effects of the fraction and
237 cross-sectional area of petiole tissues, we choose to focus on the mid-point segment of the
238 petiole between the connection to the stem and the leaf lamina. This point was selected so as
239 to avoid swelling effects at stem and lamina connections. At the base of the petiole, the wide
240 and triangular base is further modified by an external covering of trichilia and secretions of
241 M llerian bodies serving the nutrition of hosted ants. At the distal end of the petiole, at the
242 point of junction between petiole and lamina main veins, the petiole is more flexible in
243 torsion, which allows the lamina to rotate (pers. obs.). The distance between the anatomical
244 sampling point and the tip of the leaf main lobe was measured (conductive path length; PL,
245 cm) and used for the investigation of vessel widening. Since the vessel diameter – conductive
246 path length relationship is a power function, with rapid changes in vessel diameters close to
247 the conductive path tip, and slower nearly constant toward the base, we assume that
248 anatomical measurements at our sampling point are proportional to the petiole base.

249 Lamina dry mass (M_{lamina} , g) and petiole dry mass (M_{pet} , g) were measured with a
250 scale. The leaf dry mass (M_{leaf} , g) is the sum of M_{lamina} and M_{pet} . Lamina area (A_{lamina} , cm²;
251 Fig. S1) was measured with a planimeter (LiCor 3000A, LiCor Inc., Lincoln, NE, USA).

252 Data from 1271 mature fully developed leaves (A-leaves and all the other leaves) were
253 used for the characterisation of morphological and structural variability.

254

255 *Anatomical and vascular measurements*

256 We conducted our study on a subset of 124 A-leaves selected to represent the widest range of
257 petiole diameters (from 2.56 to 15.41 mm). Anatomical cross-sections (Fig. 1c), 20 to 50 μm
258 thick, were sampled from the petiole median segment with a manual microtome (Mikrot L,
259 Schenkung Dapples, Switzerland). All cross-sections were stained in a safranin/astra-blue
260 solution to stain unlignified cells blue and lignified cells red. Images of each petiole cross-
261 section were digitised with an optical microscope (Olympus BX60; Olympus Corporation;

262 Tokyo; Japan) with 50x magnification and a Canon camera EOS 500D (lens Olympus U-TVI-
263 X ; F 0.0; ISO 100; speed 1/25). Three or four close up images were taken of each petiole
264 section at different depths of focus and stacked with the Helicon Focus software (v.6.3.2.Pro,
265 <https://www.heliconsoft.com/>). Pictures were assembled in a panorama using Kolor
266 AutoPanoGiga software (v.3.0.0, <http://kolor.com/autopano/>) to obtain a complete picture of
267 the cross-section (Fig. 1c). The digitised cross-sections were processed with CS5 Photoshop
268 software (v.12.0, <http://adobe.com/products/photoshop/html>). We distinguished eight tissue
269 types that comprise the entire petiole anatomy (Fig. 1d, e). We manually delineated the tissues
270 on the photographs and created layer masks (Fig. 1e). The masks of these layers were used to
271 calculate the cross-sectional area of each tissue and the whole petiole cross-sectional area with
272 the ImageJ software (v.1.43u; <http://imagej.nih.gov/ij/>). We distinguished eight component
273 petiole tissues for further anatomical analysis: (i) the pith, (ii) the sclerenchymatous shield
274 associated with the vascular bundles, (iii) interfascicular parenchyma, (iv) primary xylem, (v)
275 secondary xylem, (vi) phloem (comprising primary and secondary phloem), (vii) cortical
276 parenchyma and (viii) cortical collenchyma (Fig. 1e).

277 As we knew the cross-sectional area of each petiole tissue, we calculated the second
278 moment of area (I , mm⁴) of the main supportive tissues (Table 1). I quantifies the distribution
279 of mass in a cross-section with respect to the centre of mass of the cross-section, and this
280 describes the important effect of size and geometry of the cross-section in mechanics, since
281 the flexural stiffness ($E*I$, where E is the elastic modulus) is directly proportional to I (Niklas
282 and Spatz 2012). As I is a fourth power function of the two radii delimiting the ring (Table 1),
283 slight increases in the ring diameter and/or cross-sectional area has dramatic consequences for
284 I , and thus its contribution to flexural stiffness. We focused on the sclerenchyma,
285 collenchyma and secondary xylem, since these tissues are well-known to be supportive tissues
286 in a section and exhibit lignified thick cell walls (Leroux 2012). We also estimated the
287 behaviour of the petiole flexural stiffness by calculating I for the total petiole section, and by
288 using the known petiole density as a proxy of E (Table 1). We assumed that this
289 approximation was appropriate, since it had been shown that the elastic modulus exhibits a
290 linear and positive relationship with the density for both wood (Chave et al. 2009, Dlouhá et
291 al. 2018) and bark (Rosell and Olson 2014).

292 For the xylem, the cropped part of the image in which the vessels were visible was
293 analysed with the ImageJ software to calculate theoretical xylem hydraulic properties
294 (Abramoff et al. 2004). For each vessel, we calculated its cross-sectional area (μm^2) and its
295 elliptical diameters. To study variations in the dimensions of the vessel, we used the mean

296 hydraulic diameter (D_h , μm , Table 1), i.e. the diameter that all vessels, considered as circles,
 297 in a given tissue would have to sustain exactly the same tissue hydraulic conductivity (Tyree
 298 and Zimmermann 2002). The number of vessels was counted for primary and secondary
 299 xylem. We also calculated the conductive area (mm^2) as the sum of the cross-sectional area of
 300 all vessels of the surrounding xylem. Knowing the dimensions of each vessel, the number of
 301 vessels, and the total petiole xylem area, a theoretical hydraulic conductivity (K_{th} , m kg MPa^{-1}
 302 s^{-1}) was estimated based on formulas in Table 1. To test the null hypothesis of a decreasing
 303 K_{leaf} ($\text{kg MPa}^{-1} \text{s}^{-1} \text{m}^{-2}$) across the leaf size range with no vessel widening occurring, we
 304 estimated a theoretical leaf conductance ($K_{leaf,null}$) by firstly dividing the K_{th} by the petiole
 305 length. We thus obtained a value for petiole conductance ($\text{kg MPa}^{-1} \text{s}^{-1}$) which we divided by
 306 the lamina area and obtaining $K_{leaf,null}$.

307 Previous studies have shown precise tapering rates of vessels along a leaf (Coomes et
 308 al. 2008; Lechthaler et al. 2019) or the entire tree (Bettiati et al. 2012, Petit et al. 2014) based
 309 on several anatomical measurements along the path of each individual leaf or individual tree.
 310 Here, we estimated a tapering rate (D_h/PL) based on a scaling exponent of the log-log
 311 relationship between D_h at the midpoint of the petiole, and the path length, across all
 312 measured petioles. Following past studies at the tree level for interspecific comparisons
 313 (Anfodillo et al. 2006; Olson et al. 2014), we assumed that vessel tapering is primarily
 314 determined by the distance from the leaf tip, assuming that all leaves display the same
 315 tapering rate.

316

317 *Data analysis*

318 Laminas used in anatomical studies were sometimes slightly damaged resulting in somewhat
 319 biased lamina area measurements. To correct this, we used a prediction model to calculate an
 320 estimated lamina area (A_{lamina} , cm^2), since the relationship between the length of the main lobe
 321 and the undamaged lamina area is very informative. We calculated the estimated lamina area
 322 as: *Estimated lamina area* = $0.701 * \text{Main lobe length}^{2.180}$ ($R^2 = 0.942$, $P < 0.001$, Fig. S2).
 323 This estimation of lamina area was used only to compare tissue cross-sectional area and
 324 vasculature traits with leaf area.

325 All statistical analyses were performed with R software (<https://cran.r-project.org/>).
 326 The relationship between each trait pair was determined with an SMA (Standardized major
 327 axis regression; Warton et al. 2006), which allows minimisation of the error on both the x -axis
 328 and y -axis (Harvey and Pagel 1991). These correlation relationships are described as: $y = ax^b$,
 329 such as: $\log(y) = \log(a) + b * \log(x)$, where b is the slope (or allometric exponent) and a the

330 intercept (allometric coefficient). A 95% confidence interval was used to decide whether it
331 was significantly correlated or not. A slope test was performed to determine if the slope
332 differed from 1 (H1: $b \neq 1$ for an allometric relationship) or not (H0: $b = 1$ for an isometric
333 relationship). SMA were carried out with the (S)MATR package (Falster et al. 2006). The
334 lamina area prediction from the main lobe length was modelled from a NLS (non-linear least
335 squares) with the STATS package.

336

337 **RESULTS**

338 *Leaf morphological variability*

339 A_{pet} and A_{lamina} were positively and allometrically correlated ($P < 0.001$; $R^2 = 0.779$; Fig. 2a;
340 Table 2, S1). A_{pet} and M_{lamina} were positively, and allometrically correlated ($P < 0.001$; $R^2 =$
341 0.898 ; Fig. 2b; Table 2). M_{pet} and M_{lamina} were positively, and allometrically correlated ($P <$
342 0.001 ; $R^2 = 0.947$; Fig. 2c; Table 2). A_{lamina} and M_{leaf} were positively, and isometrically
343 correlated ($P < 0.001$; $R^2 = 0.846$; Fig. 2d; Table 2). PD were negatively, and allometrically
344 correlated to A_{pet} ($P < 0.01$) but uncorrelated to A_{lamina} ($P < 0.05$)(Table S1).

345

346 *Petiole anatomy*

347 Petioles showed pronounced radial symmetry (Fig. 1c). The central parenchymatous pith
348 formed the main part of the cross-section. The numerous bundles (20 to 80) were arranged in
349 the pith periphery, mainly in only one circle, but in a few cases in two circles. The cambium
350 displayed a diversity of arrangement, with respect to the arrangement of the bundles. From a
351 strictly cyclic structure to a wavy one (Fig. 1f), and at the extremity, we observed isolated
352 bundles with complete cambium discontinuities in a more cortical position (Fig. 1g). Primary
353 and secondary xylem and secondary phloem were easy to identify (Fig. 1d). A sub-continuous
354 sclerenchymatous shield was present at the interface between the vascular bundles and the
355 pith. The vascular bundles were separated by interfascicular parenchyma. Occasional
356 sclerenchyma were present between the secondary phloem and cortical parenchyma.
357 Depending on the extent of secondary growth, the primary phloem was crushed between the
358 secondary phloem and cortical parenchyma. In the most external part, there was a ring of
359 collenchyma, between the epidermis and the cortical parenchyma. Laticiferous canals were
360 frequently visible, mainly in the cortex (Fig. 1f) but also in the pith, but were also sometimes
361 completely absent.

362

363 *Petiole anatomy and vascular architecture*

364 All tissue areas were significantly and positively correlated with A_{pet} and A_{lamina} ($P < 0.001$;
 365 Fig. S3a; Table S2), with most of these relationships being allometric. All tissue fractions
 366 relative to A_{pet} were correlated to A_{pet} ($P < 0.001$; Fig. S3b; Table S2). All tissue fractions
 367 relative to A_{pet} were correlated to A_{lamina} ($P < 0.001$; Fig. S3b; Table S2), except for the
 368 phloem ($P > 0.05$). Pith had the highest fraction ($44.78\% \pm 1.12$) with the highest effect on
 369 petiole size (27.02 to 59.41% of the cross-section; Fig. 3a). The lamina area allometrically
 370 and positively scaled with the number of vessels ($P < 0.001$; $R^2 = 0.596$; Fig. 3d; Table 2),
 371 such that large leaves were associated with disproportionately less vessels. The lamina area
 372 allometrically and positively scaled with conductive area ($P < 0.001$; $R^2 = 0.635$; Fig. 3c;
 373 Table 2), such that large leaves were associated with disproportionately less conductive area.
 374 The lamina area allometrically and positively scaled with xylem area ($P < 0.001$; $R^2 = 0.711$;
 375 Fig. 3b; Table 2), such that large leaves were associated with disproportionately less xylem
 376 area.

377 The second moment of area (I) for collenchyma, secondary xylem, and sclerenchyma
 378 were significantly and positively related to A_{lamina} ($P < 0.001$; $R^2 = 0.578, 0.712, \text{ and } 0.645$
 379 respectively; Fig. 4a,b,c). The approximated petiole flexural stiffness was positively and
 380 allometrically correlated to A_{lamina} with a slope significantly superior to 1 ($P < 0.001$; $R^2 =$
 381 0.653 ; Fig. 4d; Table 2).

382 D_h was significantly and positively related to A_{lamina} ($P < 0.001$; $R^2 = 0.502$; Fig. 5a;
 383 Table 2). D_h was positively and allometrically related to the conductive path length ($P <$
 384 0.001 ; $R^2 = 0.469$; Fig. 5b; Table 2). The estimated tapering rate (D_h/PL) was $0.549 \mu\text{m cm}^{-1}$
 385 (Fig. 5b). $K_{\text{leaf,null}}$ was significantly and negatively related to A_{lamina} and the conductive path
 386 length ($P < 0.01$; Fig. 5c,d).

387

388 DISCUSSION

389 Our results provide an understanding of the tissue-level and vascular adjustments
 390 characterising lamina-petiole scaling at the intraspecific level for a widespread Neotropical
 391 pioneer tree species, *Cecropia obtusa*. The results point out (i) an allometric scaling between
 392 lamina size and petiole size, such that larger leaves show a higher lamina area for a given
 393 petiole cross-section, (ii) a higher pith fraction, related to higher petiole flexural stiffness
 394 through the second moment of area (I) of the mechanically stiff tissues surrounding the pith,
 395 (iii) a vessel widening allowing for a reduced effect of increasing path length with leaf size
 396 and (iv) a higher lamina area per xylem area for larger leaves, due to relatively less
 397 conductive area for larger leaves.

398

399 *Morphological petiole-lamina scaling*

400 The finding of a positive and allometric relationship between petiole dry mass and lamina dry
401 mass (Fig. 2c), with a disproportionately higher petiole mass for a given lamina mass, is in
402 agreement with what has been previously described (Niinemets et al. 2006, 2007, Li et al.
403 2008). This pattern has been explained by the scaling of the bending moment of a cantilevered
404 beam with the cube of its length (Gere and Timoshenko 1997), in addition to drag forces
405 requiring a disproportionate mechanical reinforcement of the petiole with leaf size (Niinemets
406 et al. 2007, Li et al. 2008). However, the isometric scaling of lamina area with the leaf dry
407 mass (Fig. 2d) is not in agreement with the repeatedly shown diminishing return pattern,
408 predicting increasing costs for leaf support with increasing leaf size (Niinemets et al. 2006,
409 2007, Niklas et al. 2007, 2009, Milla and Reich 2007, Li et al. 2008b, Sun Jun et al. 2017).
410 Although the leaf area-leaf mass allometric scaling is significant at the interspecific level, the
411 allometric scaling is not ubiquitous at the intraspecific level, based on this present study for *C.*
412 *obtusa*, and Milla and Reich (2007) for 11 species.

413 We found a positive allometric relationship between lamina area and petiole cross-
414 sectional area (Fig. 2a), such that large leaves exhibit a larger lamina area for a given petiole
415 cross-sectional area. This change is in agreement with the allometric relationship between
416 lamina mass and petiole cross-sectional area we found (Fig. 2b), such that large leaves exhibit
417 larger lamina mass for a given petiole size. Larger petioles support a disproportionately larger
418 load. Therefore, we wondered what petiole anatomical and mechanical changes are associated
419 with this morphological pattern.

420

421 *Petiole tissue partitioning*

422 As pith fraction increases, the distance of a given tissue to the petiole cross-sectional centre of
423 inertia increases, as does the second moment of inertia to a fourth-power function of this
424 distance. The case of collenchyma is of primary importance since it is known to be a
425 supporting tissue, with small cells and relatively thick cell walls compared with other living
426 cells (Leroux 2012). The collenchyma is the most external tissue within the petiole section
427 with a ring-geometry, exhibiting the largest change in the second moment of area (Fig. 4a). In
428 the same line, the secondary xylem is also a well-known supporting tissue, exhibiting a ring-
429 geometry and which fraction increases with leaf size. Its increasing fraction occurs at the
430 same time as the distance to the cross-sectional centre of inertia increases with pith fraction,
431 and determines an overall increase of the second moment of area of the secondary xylem with

432 leaf size (Fig. 4b). This was also the case for the sclerenchyma (Fig. 4c), although
 433 sclerenchyma exhibited relatively low values of I (0.05 to 95 mm⁴). The allometric exponents
 434 of the supportive tissues according to the lamina area (Fig. 4a,b,c) indicate that I increases
 435 faster for the sclerenchyma, followed by the secondary xylem, suggesting that the relative
 436 geometrical contribution of these tissues to the petiole flexural stiffness increases across the
 437 leaf size range.

438 Increasing organ size and mass logically increases the mechanical load (Mahley et al.
 439 2018). Moreover, we found an allometric relationship between lamina dry mass and petiole
 440 cross-sectional area, with disproportionately higher lamina dry mass for leaves (Fig. 2b). This
 441 suggests a disproportionately higher mechanical load on petioles of large leaves. This is in
 442 agreement with the approximated petiole flexural stiffness allometrically related to the lamina
 443 area (Fig. 4d), such that petioles are disproportionately stiffer on large leaves to overcome the
 444 mechanical load. This suggestion is in agreement with past studies demonstrating
 445 disproportionately higher petiole stiffness with increasing petiole thickness (Niklas 1991,
 446 1992, Mahley et al. 2018). Therefore, our results suggest that the effect of geometry through
 447 pith fraction determines the overall increase in petiole flexural stiffness, since the decrease of
 448 petiole density with leaf size –and thus petiole elastic modulus- is balanced (Fig. 4d), as also
 449 shown by Mahley et al. (2018) for ferns. However, since the pith is often hollow in large
 450 structure, one may not exclude that the pressure of selection is actually on the surrounding
 451 tissues.

452 Changing petiole pith fraction and thus collenchyma, secondary xylem, and
 453 sclerenchyma I is clearly a cheap mechanism in terms of carbon allocation to balance the self-
 454 loading mechanical stress arising with leaf size. Moreover, we found a negative relationship
 455 between A_{pet} and PD, and no relationship of PD with A_{lamina} . These results suggest that
 456 volumetric construction cost can decrease with A_{pet} , or at least does not increase with leaf size.

458 *Petiole vascular architecture*

459 The estimation of $K_{\text{leaf,null}}$ (Fig 5a,b) confirms that K_{leaf} decreases with leaf size in the case of
 460 an absence of vessel widening, as the hydraulic resistance is well-known to be dependent on
 461 the path length (Tyree and Zimmermann 2002). Without vessel widening, large leaves cannot
 462 be selected since (i) the higher pressure drop that would be associated with such leaves would
 463 increase the risk of cavitation (Tyree and Ewers 1991, Cruiziat et al. 2002), and (ii) a lower
 464 K_{leaf} necessarily drives lower gas exchanges and carbon assimilation (Brodribb 2009, Scoffoni
 465 et al. 2016), and thus less efficient carbon payback. Therefore, this result suggests that K_{leaf} is

466 constant across leaves and not limiting for carbon assimilation (Echeverr a et al., Petit et al.
467 2016, Pittermann et al. 2018), even if water flux measurements would be required for
468 confirmation.

469 Even if such a pattern of vessel widening has been repeatedly shown along the stem of
470 adult trees for several species (Petit et al. 2008, 2010, Bettiati et al. 2012, Anfodillo et al.
471 2013, Olson et al. 2014), leaves have received less attention. The estimated widening rate
472 (0.55) was closely similar to those found by Coomes et al. (2008; mean of 0.54; a range of
473 0.42-0.73) across ten *Quercus* L. (Fagaceae) species, and not too far from those found by
474 Lechthaler et al. (2019; slope of 0.45) for one species of *Acer*. A striking feature here is the
475 convergence of widening rates between studies, despite contrasting ecology (i.e. temperate vs
476 tropical) and phylogenetic origins of the investigated species. Such a finding suggests that the
477 hydrodynamic resistance is such a strong selective pressure on hydraulic architecture that it
478 drives a single leaf vascular architecture across vascular plants. Hydraulic optimality models
479 (West et al. 1999) predict a minimum and convergent widening rate of 0.2, but only for stems,
480 to totally mitigate the hydrodynamic resistance. Indeed, this convergent widening rate is
481 found across most plants and trees when looking into the stem vasculature (Anfodillo et al.
482 2006, 2013, Petit et al. 2014, Olson et al. 2014, 2018). The vessel widening rate is not
483 constant through the total path length (Bettiati et al. 2012), with a scaling 2 to 3 times higher
484 in leaves (this study; Coomes et al. 2008, Lechthaler et al. 2019). This participates at
485 concentrating much of the hydraulic resistance in leaves –up to 50%- (Sack and Holbrook
486 2006), whereas leaves represent a small fraction of the total conductive path length, i.e. few
487 centimetres of few tens of centimetres for leaves against tens of meters for the entire tree. The
488 hydraulic segmentation hypothesis (Tyree and Zimmermann 2002, Pivovarovoff et al. 2014)
489 assumes that leaves should be hydraulically more resistant than stems, as “bottlenecks”, to
490 always preserve far lower water potentials in leaves, and promote drought-induced embolism
491 containment in easy-to-renew organs. These issues deserve more investigations, to link vessel
492 widening rates in leaves with the relative contribution of leaves in the hydraulic resistance of
493 plants.

494 The allometric relationship between the number of vessels and the supplied lamina
495 area suggests an increase in the number of vessels per leaf area, from the leaf base towards the
496 leaf tip (Fig. 3d). This therefore suggests vessel furcation. This contradicts the WBE model
497 (West et al. 1999, Rosell et al. 2017) which predicts an absence of furcation, even if the WBE
498 model does not initially integrate predictions for leaves. However, this result is in agreement

499 with the empirical test of Lechthaler et al. (2019), who also support the existence of conduit
500 furcation towards terminal parts within *Acer* leaves.

501 For *C. obtusa* leaves, vessel tapering and furcation towards the leaf tip leads to a
502 conductive area-decreasing architecture towards the leaf base, as supported by the allometric
503 relationship between the conductive area and the supplied lamina area (Fig. 3c). This supports
504 the prediction of the WBE model (West et al. 1999, Rosell et al. 2017). However, our result is
505 not in agreement with Lechthaler et al. (2019), who support a conductive area-preserving
506 architecture for *Acer* leaves. This discrepancy may come from the fact that Lechthaler et al.
507 (2019) analysed the vascular architecture across both the petiole and the midrib, whereas in
508 our study we only focused on the petiole. In the lamina, the strong furcation of the vein
509 system may lead to more numerous vessel furcations in comparison with the petiole.
510 Therefore, less vessel furcations within the petiole may allow for wider vessels, therefore
511 reducing the required conductive area, according to the Hagen-Poiseuille law.

512 The conductive area-decreasing architecture allows for a reduction of the xylem area
513 supplying the lamina for large leaves, as supported by the allometric relationship between
514 xylem area and lamina area (Fig. 3b). According to the Hagen-Poiseuille's law, a given
515 conductive area can determine different conductivities, from numerous but small conduits to
516 few but wide conduits. However, according to the packing rule, the building of numerous
517 small conduits would require more xylem area and volume. Therefore, this implies that
518 selection favours an even water supply to all parts of the leaf independently of leaf size, with
519 minimal carbon investment (Banavar et al. 1999).

520 Finally, disproportionately less xylem area for large leaves explains the allometric
521 relationship between the petiole cross-sectional area and the lamina area, with
522 disproportionately less petiole cross-sectional area for large leaves.

523

524 CONCLUSION

525 The different functional attributes linking flexural stiffness (elastic modulus vs I , and
526 allometric scaling of flexural stiffness), carbon cost (leaf mass and density), and vasculature
527 (vessel widening, xylem area, and conductive area) between large and small leaves, suggest
528 that these three functional dimension –mechanics, carbon allocation, and hydraulics
529 respectively- are selected for precise combinations, and further determine leaf size variation
530 possibilities. Environmental factors are known to shape leaf size diversity (e.g. annual
531 rainfall, temperature, solar radiation, soil nutrients...), but some other selective pressures are
532 intrinsically linked to the possibility of developing large leaves or not (i.e. return on invested

533 biomass, self-loading, conductive path length...). The contrasting mechanics, carbon
534 allocation, and vasculatures between large and small leaves shed light on these selective
535 pressures in our study. Indeed, we showed that large leaves exhibited (i) disproportionately
536 higher lamina area for a given petiole cross-sectional area, (ii) higher pith fraction with
537 disproportionately stiffer petioles, and (iii) disproportionately less xylem area for a given
538 lamina area, associated with a decreasing-area vascular architecture toward the leaf base. But
539 these selective pressures are currently not fully understood. Our study calls for more studies
540 on these selective pressures to better characterise and understand to what extent they are
541 independent or not. We argue that a better comprehension of these selective pressures should
542 extend the knowledge of drivers shaping leaf size variability, and, furthermore, permit us to
543 understand how and why leaf size seems disconnected from LES.

544

545 **ACKNOWLEDGEMENTS**

546 The authors thank Julie Bossu, Coffi Belmys Cakpo, Henri Caron, Jocelyn Cazal, Saint-Omer
547 Cazal, Aur lie Cuvelier, Bruno Clair, Aur lie Dourdain, Jean-Yves Goret, Marie Hartwig,
548 Ariane Mirabel, Audin Patient, Pascal Petronelli, Laurent Risser, Dylan Taxile, Val rie
549 Troispoux, Niklas Tysklind and Lore Verryckt for their assistance with field work and
550 measurement of leaf traits. We thank Jacques Beauch ne, Christine Heinz and Nick Rowe for
551 preliminary discussions around the project and first results. We thank Herv  Cochard for
552 fruitful discussions for data analysis. We thank Tancrede Alm ras, Camilo Zalamea and Nick
553 Rowe for critical and valuable comments on successive version of the manuscript.

554

555 **FUNDING**

556 S.L. was supported by a doctoral fellowship from CEBA (ref. ANR-10-LABX-0025). This
557 study benefited from an *Investissement d'Avenir* grant managed by the *Agence Nationale de*
558 *la Recherche* (CEBA, ref. ANR-10-LABX-0025).

559

560 **LITERATURE CITED**

- 561 Abramoff MD, Magalh es PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics*
562 Int 11:36–42.
- 563 Ackerly D, Donoghue MJ (1998) Leaf size, sapling allometry, and Corner's rules: phylogeny
564 and correlated evolution in maples (*Acer*). *Am Nat* 152:767–791.
- 565 Anfodillo T, Carraro V, Carrer M, Fior C, Rossi S (2006) Convergent tapering of xylem
566 conduits in different woody species. *New Phytol* 169:279–290.

- 567 Anfodillo T, Petit G, Crivellaro A (2013) Axial conduit widening in woody species: a still
568 neglected anatomical pattern. *IAWA J* 34:352–364.
- 569 Banavar JR, Maritan A, Rinaldo A (1999) Size and form in efficient transportation networks.
570 *Nature* 399:130.
- 571 Baraloto C, Timothy Paine CE, Poorter L, Beauchene J, Bonal D, Domenach A-M, Hérault B,
572 Patiño S, Roggy J-C, Chave J (2010) Decoupled leaf and stem economics in rain
573 forest trees. *Ecol Lett* 13:1338–1347.
- 574 Becker P, Gribben RJ, Lim CM (2000) Tapered conduits can buffer hydraulic conductance
575 from path-length effects. *Tree Physiol* 20:965–967.
- 576 Bettiati D, Petit G, Anfodillo T (2012) Testing the equi-resistance principle of the xylem
577 transport system in a small ash tree: empirical support from anatomical analyses. *Tree*
578 *Physiol* 32:171–177.
- 579 Brodribb TJ (2009) Xylem hydraulic physiology: The functional backbone of terrestrial plant
580 productivity. *Plant Sci* 177:245–251.
- 581 Brouat C, Gibernau M, Amsellem L, McKey D (1998) Corner's Rules revisited: ontogenetic
582 and interspecific patterns in leaf-stem allometry. *New Phytol* 139:459–470.
- 583 Brouat C, McKey D (2001) Leaf-stem allometry, hollow stems, and the evolution of caulinary
584 domatia in myrmecophytes. *New Phytol* 151:391–406.
- 585 Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a
586 worldwide wood economics spectrum. *Ecol Lett* 12:351–366.
- 587 Coomes DA, Heathcote S, Godfrey ER, Shepherd JJ, Sack L (2008) Scaling of xylem vessels
588 and veins within the leaves of oak species., Scaling of xylem vessels and veins within
589 the leaves of oak species. *Biol Lett Biol Lett* 4, 4:302, 302–306.
- 590 Corner EJH (1949) The Durian Theory or the origin of the modern tree. *Ann Bot* 13:367–414.
- 591 Cruiziat P, Cochard H, Améglio T (2002) Hydraulic architecture of trees: main concepts and
592 results. *Ann For Sci* 59:723–752.
- 593 Dlouhá J, Alméras T, Beauchêne J, Clair B, Fournier M (2018) Biophysical dependences
594 among functional wood traits. *Funct Ecol* 32:2652–2665.
- 595 Echeverría A, Anfodillo T, Soriano D, Rosell JA, Olson ME (2019) Constant theoretical
596 conductance via changes in vessel diameter and number with height growth in
597 *Moringa oleifera*. *J Exp Bot* 70:5765–5772.
- 598 Enquist B (2003) Cope's Rule and the evolution of long-distance transport in vascular plants:
599 allometric scaling, biomass partitioning and optimization. *Plant Cell Environ* 26:151–
600 161.
- 601 Evert R (2006) Parenchyma and Collenchyma. In: *Esau's Plant Anatomy*. Wiley-Blackwell,
602 pp 175–190.

- 603 Faisal TR, Khalil Abad EM, Hristozov N, Pasini D (2010) The impact of tissue morphology,
604 cross-section and turgor pressure on the mechanical properties of the leaf petiole in
605 plants. *J Bionic Eng* 7:S11–S23.
- 606 Falster DS, Warton DI, Wright II (2006) User's guide to SMATR: Standardised Major Axis
607 tests & routines Version 2.0, Copyright 2006. R Instr
- 608 Fan Z-X, Sterck F, Zhang S-B, Fu P-L, Hao G-Y (2017) Tradeoff between stem hydraulic
609 efficiency and mechanical strength affects leaf–stem allometry in 28 *Ficus* tree
610 species. *Front Plant Sci* 8
- 611 Fiorin L, Brodribb TJ, Anfodillo T (2016) Transport efficiency through uniformity:
612 organization of veins and stomata in angiosperm leaves. *New Phytol* 209:216–227.
- 613 Gates DM (1968) Transpiration and leaf temperature. *Annu Rev Plant Physiol* 19:211–238.
- 614 Gere JM, Timoshenko SP (1997) *Mechanics of materials*. PWS Publishing Company, Boston.
- 615 Gleason SM, Blackman CJ, Gleason ST, McCulloh KA, Ocheltree TW, Westoby M (2018)
616 Vessel scaling in evergreen angiosperm leaves conforms with Murray's law and area-
617 filling assumptions: implications for plant size, leaf size and cold tolerance. *New*
618 *Phytol* 218:1360–1370.
- 619 Gourlet-Fleury S, Guehl JM, Laroussine O (2004) *Ecology and management of a neotropical*
620 *rainforest : lessons drawn from Paracou, a long-term experimental research site in*
621 *French Guiana*. Elsevier, Paris.
- 622 Hallé F, Oldeman RAA, Tomlinson PB (1978) *Tropical Trees and Forests - An Architectural*
623 *Analysis*. Springer-Verlag Berlin Heidelberg.
- 624 Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford
625 University Press.
- 626 Hikosaka K (2004) Interspecific difference in the photosynthesis–nitrogen relationship:
627 patterns, physiological causes, and ecological importance. *J Plant Res* 117:481–494.
- 628 John GP, Scoffoni C, Buckley TN, Villar R, Poorter H, Sack L (2017) The anatomical and
629 compositional basis of leaf mass per area. *Ecol Lett* 20:412–425.
- 630 Lechthaler S, Colangeli P, Gazzabin M, Anfodillo T (2019) Axial anatomy of the leaf midrib
631 provides new insights into the hydraulic architecture and cavitation patterns of *Acer*
632 *pseudoplatanus* leaves. *J Exp Bot*.
- 633 Lehnebach R, Bossu J, Va S, Morel H, Amusant N, Nicolini E, Beauchêne J (2019) Wood
634 density variations of legume trees in french guiana along the shade tolerance
635 continuum: heartwood effects on radial patterns and gradients. *Forests* 10:80.
- 636 Leigh A, Sevanto S, Close JD, Nicotra AB (2017) The influence of leaf size and shape on leaf
637 thermal dynamics: does theory hold up under natural conditions? *Plant Cell Environ*
638 40:237–248.

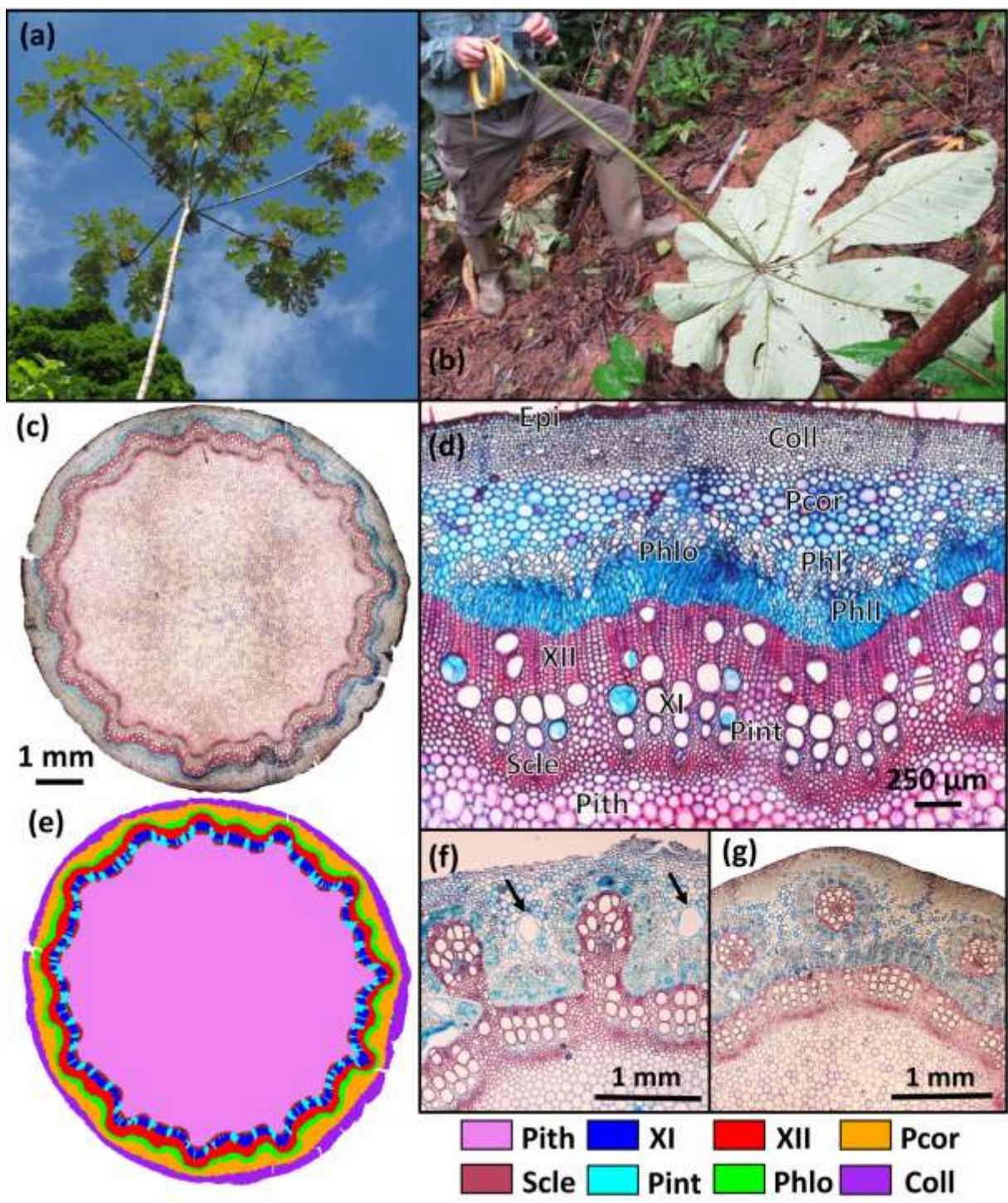
- 639 Leroux O (2012) Collenchyma: a versatile mechanical tissue with dynamic cell walls. *Ann*
640 *Bot* 110:1083–1098.
- 641 Li G, Yang D, Sun S (2008a) Allometric relationships between lamina area, lamina mass and
642 petiole mass of 93 temperate woody species vary with leaf habit, leaf form and
643 altitude. *Funct Ecol* 22:557–564.
- 644 Li G, Yang D, Sun S (2008b) Allometric relationships between lamina area, lamina mass and
645 petiole mass of 93 temperate woody species vary with leaf habit, leaf form and
646 altitude. *Funct Ecol* 22:557–564.
- 647 Mahley JN, Pittermann J, Rowe N, Baer A, Watkins JE, Schuettpelz E, Wheeler JK,
648 Mehlreter K, Windham M, Testo W, Beck J (2018) Geometry, allometry and
649 biomechanics of fern leaf petioles: their significance for the evolution of functional
650 and ecological diversity within the Pteridaceae. *Front Plant Sci* 9
- 651 McCulloh KA, Sperry JS, Adler FR (2003) Water transport in plants obeys Murray’s law.
652 *Nature* 421:939.
- 653 Milla R, Reich PB (2007) The scaling of leaf area and mass: the cost of light interception
654 increases with leaf size. *Proc R Soc Lond B Biol Sci* 274:2109–2115.
- 655 Niinemets Ü (2015) Is there a species spectrum within the world-wide leaf economics
656 spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll
657 *Quercus ilex*. *New Phytol* 205:79–96.
- 658 Niinemets Ü, Portsmouth A, Tena D, Tobias M, Matesanz S, Valladares F (2007) Do we
659 underestimate the importance of leaf size in plant economics? Disproportional scaling
660 of support costs within the spectrum of leaf physiognomy. *Ann Bot* 100:283.
- 661 Niinemets Ü, Portsmouth A, Tobias M (2006) Leaf size modifies support biomass distribution
662 among stems, petioles and mid-ribs in temperate plants. *New Phytol* 171:91–104.
- 663 Niklas KJ (1991) Flexural stiffness allometries of angiosperm and fern petioles and rachises:
664 evidence for biomechanical convergence. *Evolution* 45:734–750.
- 665 Niklas KJ (1992) Petiole mechanics, light interception by Lamina, and “Economy in Design”.
666 *Oecologia* 90:518–526.
- 667 Niklas KJ (1999) A mechanical perspective on foliage leaf form and function. *New Phytol*
668 143:19–31.
- 669 Niklas KJ, Cobb ED (2008) Evidence for “diminishing returns” from the scaling of stem
670 diameter and specific leaf area. *Am J Bot* 95:549–557.
- 671 Niklas KJ, Cobb ED, Niinemets Ü, Reich PB, Sellin A, Shipley B, Wright IJ (2007)
672 “Diminishing returns” in the scaling of functional leaf traits across and within species
673 groups. *Proc Natl Acad Sci* 104:8891–8896.
- 674 Niklas KJ, Cobb ED, Spatz H-C (2009) Predicting the allometry of leaf surface area and dry
675 mass. *Am J Bot* 96:531–536.

- 676 Niklas K, Spatz H-C (2012) *Plant Physics*.
- 677 Normand F, Bissery C, Damour G, Lauri P-É (2008) Hydraulic and mechanical stem
678 properties affect leaf–stem allometry in mango cultivars. *New Phytol* 178:590–602.
- 679 Olson ME, Aguirre-Hernández R, Rosell JA (2009) Universal foliage-stem scaling across
680 environments and species in dicot trees: plasticity, biomechanics and Corner’s Rules.
681 *Ecol Lett* 12:210–219.
- 682 Olson ME, Anfodillo T, Rosell JA, Petit G, Crivellaro A, Isnard S, León-Gómez C, Alvarado-
683 Cárdenas LO, Castorena M (2014) Universal hydraulics of the flowering plants: vessel
684 diameter scales with stem length across angiosperm lineages, habits and climates. *Ecol*
685 *Lett* 17:988–997.
- 686 Olson ME, Rosell JA, Zamora Muñoz S, Castorena M (2018) Carbon limitation, stem growth
687 rate and the biomechanical cause of Corner’s rules. *Ann Bot* 122:583–592.
- 688 Olson ME, Soriano D, Rosell JA, Anfodillo T, Donoghue MJ, Edwards EJ, León-Gómez C,
689 Dawson T, Martínez JJC, Castorena M, Echeverría A, Espinosa CI, Fajardo A, Gazol
690 A, Isnard S, Lima RS, Marcati CR, Méndez-Alonzo R (2018) Plant height and
691 hydraulic vulnerability to drought and cold. *Proc Natl Acad Sci* 115:7551–7556.
- 692 Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets Ü, Poorter H, Tosens T,
693 Westoby M (2017) Physiological and structural tradeoffs underlying the leaf
694 economics spectrum. *New Phytol* 214:1447–1463.
- 695 Parkhurst DF, Loucks OL (1972) Optimal leaf size in relation to environment. *J Ecol* 60:505–
696 537.
- 697 Petit G, Anfodillo T, Mencuccini M (2008) Tapering of xylem conduits and hydraulic
698 limitations in sycamore (*Acer pseudoplatanus*) trees. *New Phytol* 177:653–664.
- 699 Petit G, DeClerck FAJ, Carrer M, Anfodillo T (2014) Axial vessel widening in arborescent
700 monocots. *Tree Physiol* 34:137–145.
- 701 Petit G, Pfautsch S, Anfodillo T, Adams MA (2010) The challenge of tree height in
702 *Eucalyptus regnans*: when xylem tapering overcomes hydraulic resistance. *New*
703 *Phytol* 187:1146–1153.
- 704 Petit G, Savi T, Consolini M, Anfodillo T, Nardini A (2016) Interplay of growth rate and
705 xylem plasticity for optimal coordination of carbon and hydraulic economies in
706 *Fraxinus ornus* trees. *Tree Physiol* 36:1310–1319.
- 707 Pittermann J, Olson ME, Way D (2018) Transport efficiency and cavitation resistance in
708 developing shoots: a risk worth taking. *Tree Physiol* 38:1085–1087.
- 709 Pivovarov A, Sack S, Santiago L (2014) Coordination of stem and leaf hydraulic conductance
710 in southern California shrubs: a test of the hydraulic segmentation hypothesis. *New*
711 *Phytol* 203:842–850.
- 712 Poorter H, Evans JR (1998) Photosynthetic nitrogen-use efficiency of species that differ
713 inherently in specific leaf area. *Oecologia* 116:26–37.

- 714 Poorter, Rozendaal DMA (2008) Leaf size and leaf display of thirty-eight tropical tree
715 species. *Oecologia* 158:35–46.
- 716 Price CA, Enquist BJ (2007) Scaling mass and morphology in leaves: an extension of the
717 WBE model. *Ecology* 88:1132–1141.
- 718 Reich PB, Ellsworth DS, Walters MB (1998) Leaf structure (specific leaf area) modulates
719 photosynthesis–nitrogen relations: evidence from within and across species and
720 functional groups. *Funct Ecol* 12:948–958.
- 721 Rosell JA, Olson ME (2014) The evolution of bark mechanics and storage across habitats in a
722 clade of tropical trees. *Am J Bot* 101:764–777.
- 723 Rosell JA, Olson ME, Anfodillo T (2017) Scaling of xylem vessel diameter with plant size:
724 causes, predictions, and outstanding questions. *Curr For Rep* 3:46–59.
- 725 Sack L, Holbrook NM (2006) Leaf hydraulics. *Annu Rev Plant Biol* 57:361–381.
- 726 Sack L, Scoffoni C, McKown AD, Frole K, Rawls M, Havran JC, Tran H, Tran T (2012)
727 Developmentally based scaling of leaf venation architecture explains global ecological
728 patterns. *Nat Commun* 3:837.
- 729 Scoffoni C, Chatelet DS, Pasquet-kok J, Rawls M, Donoghue MJ, Edwards EJ, Sack L (2016)
730 Hydraulic basis for the evolution of photosynthetic productivity. *Nat Plants* 2:16072.
- 731 Shipley B, Lechowicz MJ, Wright I, Reich PB (2006) Fundamental trade-offs generating the
732 worldwide leaf economics spectrum. *Ecology* 87:535–541.
- 733 Smith DD, Sperry JS, Adler FR (2017) Convergence in leaf size versus twig leaf area scaling:
734 do plants optimize leaf area partitioning? *Ann Bot* 119:447–456.
- 735 Sun Jun, Fan Ruirui, Niklas Karl J., Zhong Quanlin, Yang Fuchun, Li Man, Chen Xiaoping,
736 Sun Mengke, Cheng Dongliang (2017) “Diminishing returns” in the scaling of leaf
737 area vs. dry mass in Wuyi Mountain bamboos, Southeast China. *Am J Bot* 104:993–
738 998.
- 739 Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New*
740 *Phytol* 119:345–360.
- 741 Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap, 2nd edn.
742 Springer-Verlag, Berlin Heidelberg.
- 743 Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for
744 allometry. *Biol Rev* 81:259–291.
- 745 West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of
746 plant vascular systems. *Nature* 400:664–667.
- 747 Westoby M, Daniel S. Falster DS, Angela T. Moles, Peter A. Vesk, Wright and IJ (2002)
748 Plant ecological strategies: some leading dimensions of variation between species.
749 *Annu Rev Ecol Syst* 33:125–159.

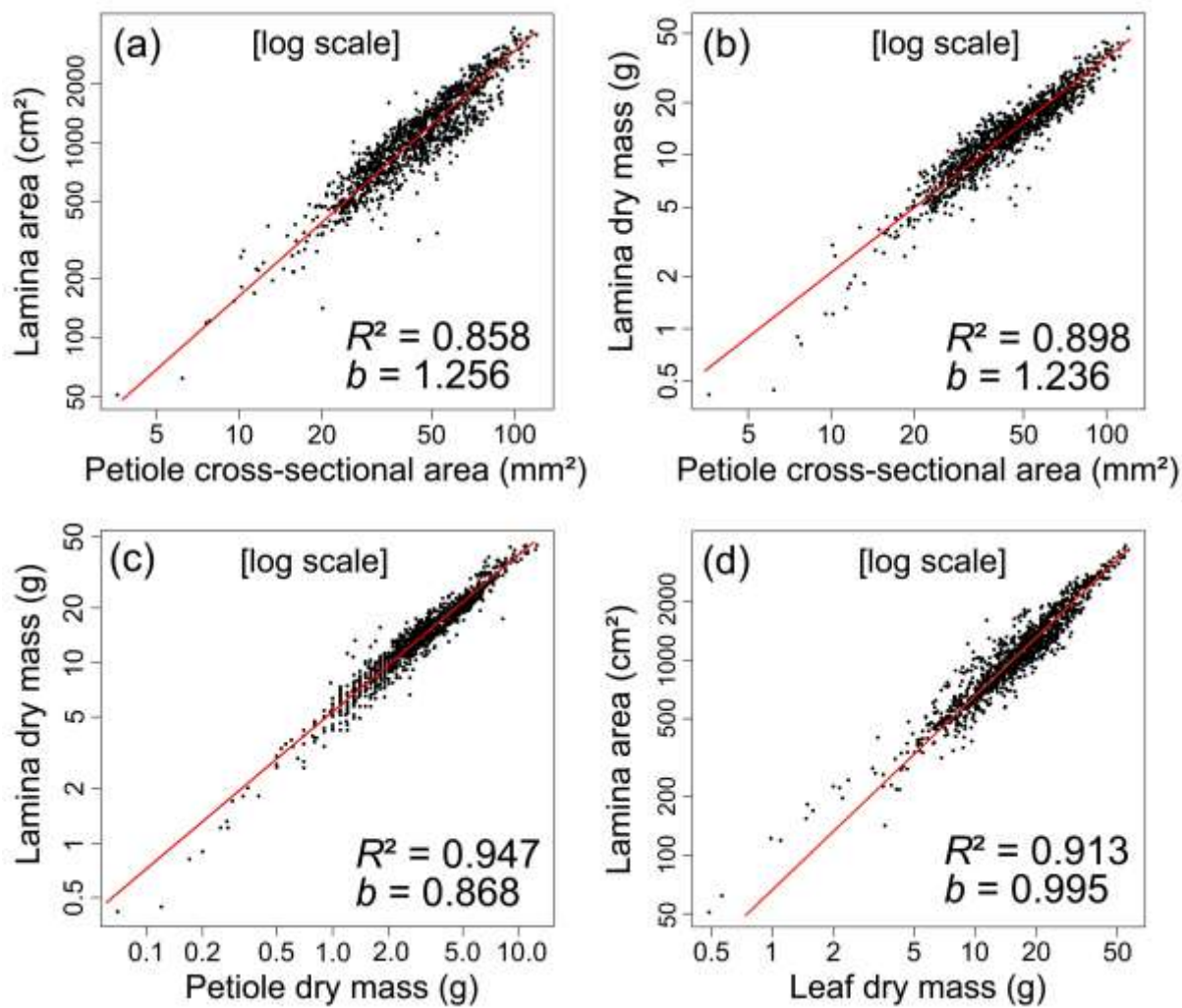
- 750 White PS (1983a) Corner's Rules in Eastern deciduous trees: allometry and its implications
751 for the adaptive architecture of trees. *Bull Torrey Bot Club* 110:203–212.
- 752 White PS (1983b) Evidence that temperate east north american evergreen woody plants
753 follow Corner's rule. *New Phytol* 95:139–145.
- 754 Williamson GB, Wiemann MC (2010) Measuring wood specific gravity...Correctly. *Am J*
755 *Bot* 97:519–524.
- 756 Wright IJ, Dong N, Maire V, Prentice IC, Westoby M, Díaz S, Gallagher RV, Jacobs BF,
757 Kooyman R, Law EA, Leishman MR, Niinemets Ü, Reich PB, Sack L, Villar R,
758 Wang H, Wilf P (2017) Global climatic drivers of leaf size. *Science* 357:917–921.
- 759 Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J,
760 Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J,
761 Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets
762 Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas
763 SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics
764 spectrum. *Nature* 428:821–827.
- 765 Yang D, Niklas KJ, Xiang S, Sun S (2010) Size-dependent leaf area ratio in plant twigs:
766 implication for leaf size optimization. *Ann Bot* 105:71.
- 767
- 768

769 **Fig. 1.** Habits, morphological and petiole-anatomical aspects of *C. obtusa* (Urticaceae). (a)
770 Habits of *C. obtusa*. (b) Leaf of *C. obtusa*. (c) Petiole cross-sectional anatomy of *C. obtusa* in
771 the middle part of the petiole. (d) Close-up of petiole constitutive tissues: pith (Pith),
772 sclerenchyma (Scle), interfascicular parenchyma (Pint), primary xylem (XI), secondary xylem
773 (XII), total phloem (Phlo), primary phloem (PhI), secondary phloem (PhII), cortical
774 parenchyma (Pcor), collenchyma (Coll) and epidermis (Epi). (e) Tissues and corresponding
775 layer masks studied. (f) “Wavy” cambium with a sub-bicyclic array of vascular bundles.
776 Arrows represent laticiferous canals. (g) Cambial discontinuities, island-like vascular bundles
777 and a bicyclic array of vascular bundles.
778



779
 780
 781
 782
 783
 784
 785
 786

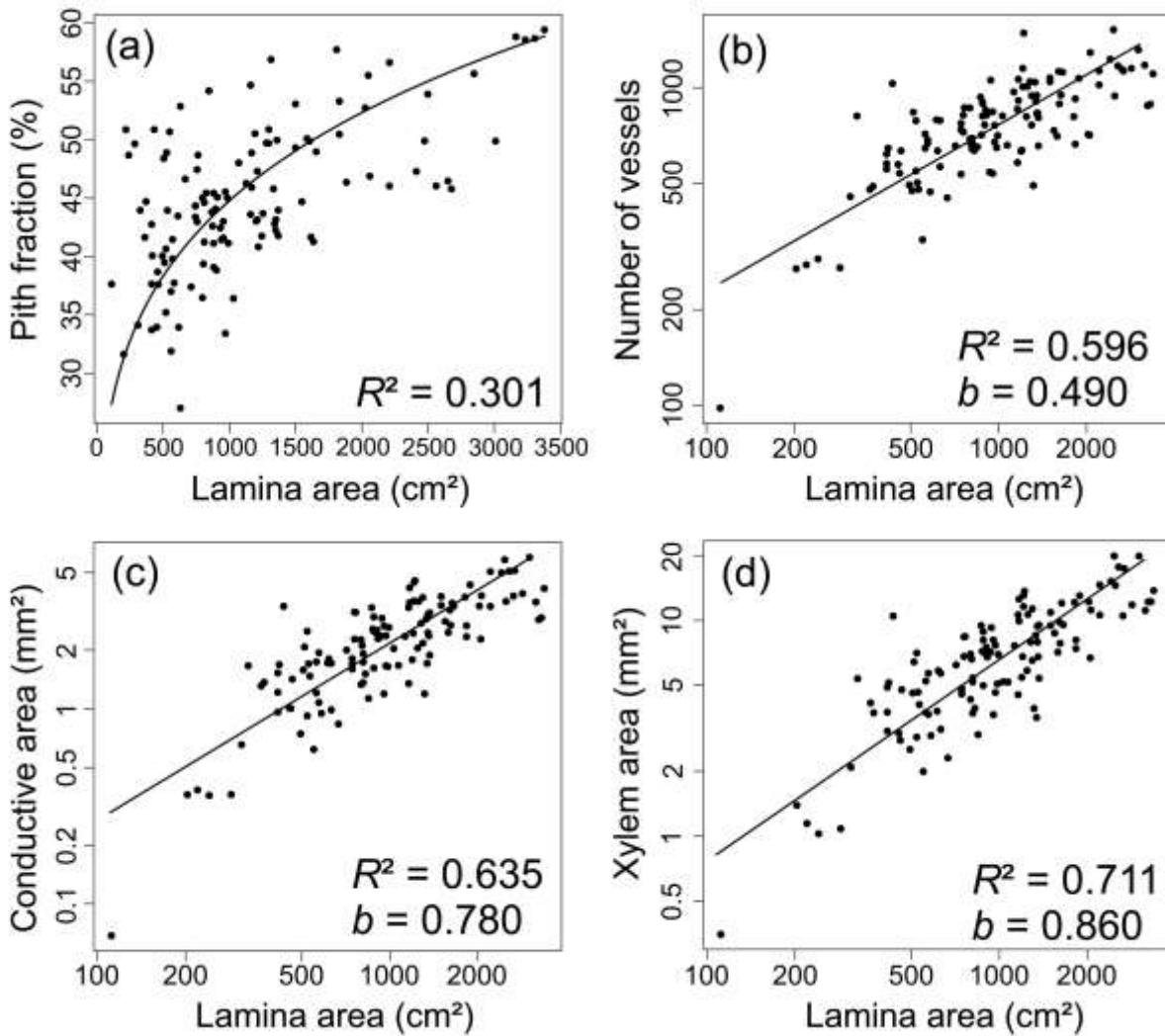
787 **Fig. 2.** Scaling of morphological traits with leaf size. (a) Lamina area according to petiole
 788 cross-sectional area. (b) Lamina dry mass according to petiole cross-sectional area. (c)
 789 Lamina dry mass according to petiole dry mass. (d) Lamina area according to leaf dry mass.
 790 All relationships are plotted on a log-scale. *b*: scaling exponent.
 791



792
 793
 794
 795
 796
 797
 798
 799
 800

801 **Fig. 3.** Scaling of petiole anatomy with leaf size. (a) Pith cross-sectional fraction according to
 802 lamina area. (b) Number of vessels according to lamina area plotted on a log-scale. (c)
 803 Conductive area according to lamina area, plotted on a log-scale. (d) Xylem area according to
 804 lamina area, plotted on a log-scale. *b*: scaling exponent.

805



806

807

808

809

810

811

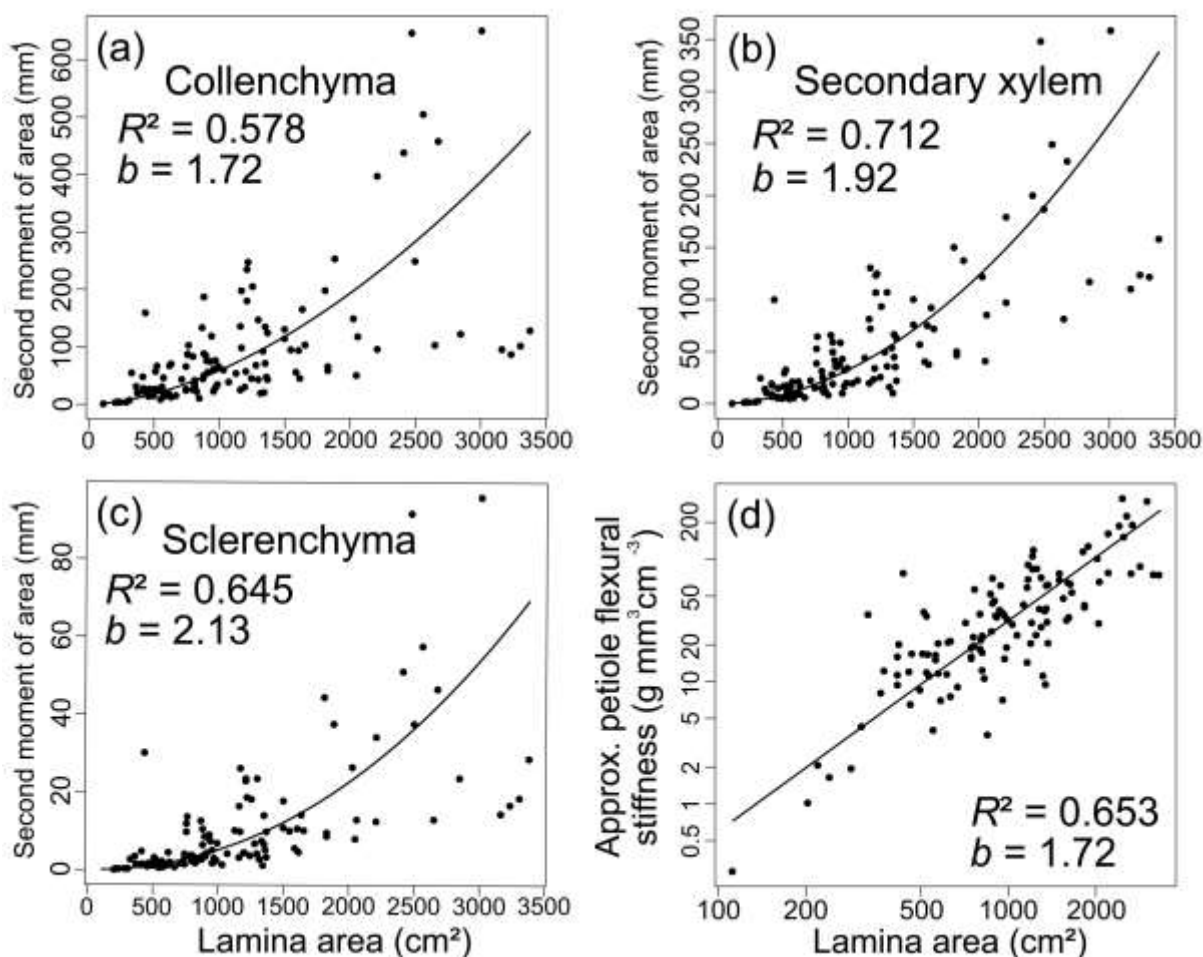
812

813

814

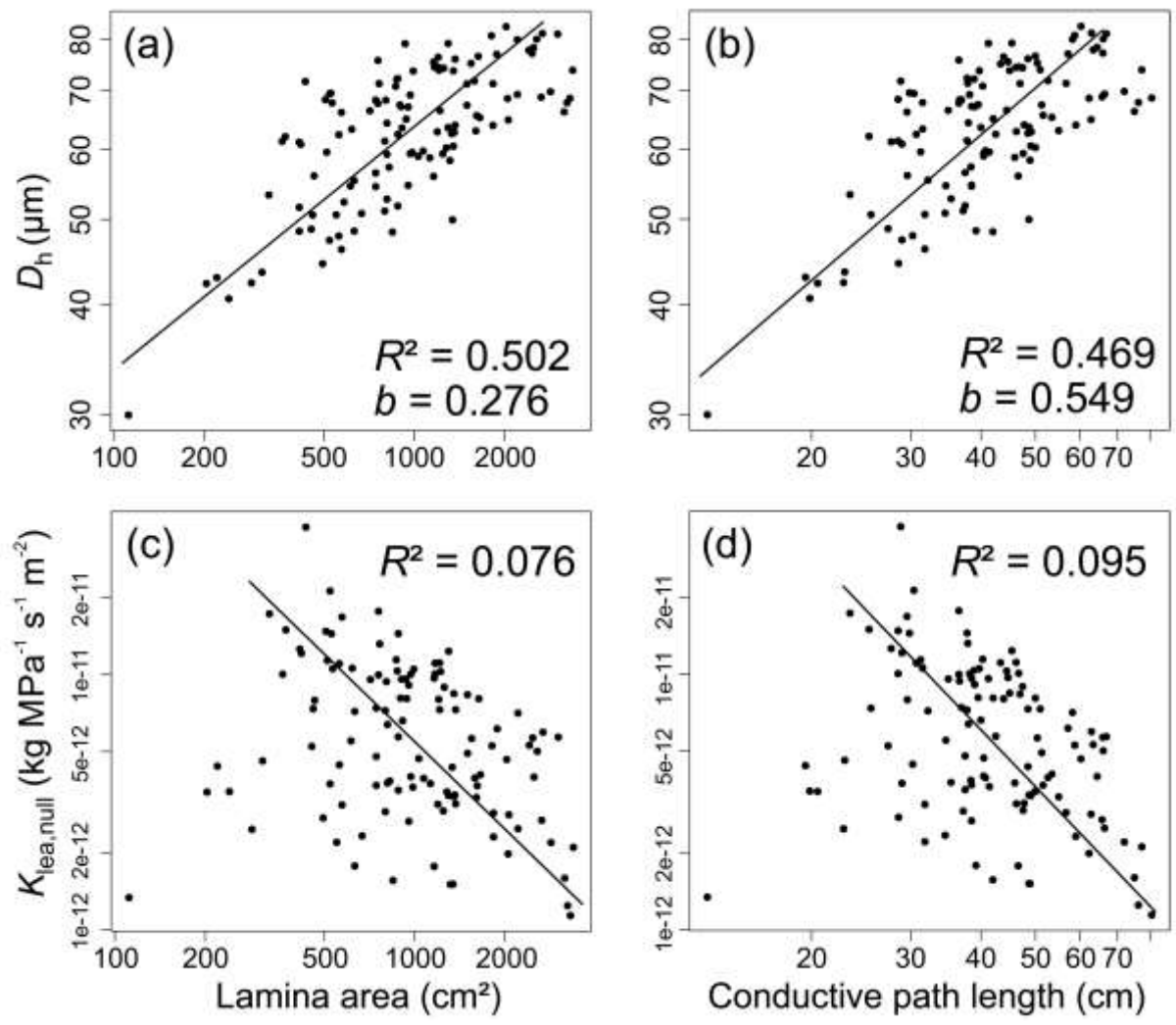
815

816 **Fig. 4.** Scaling of mechanical traits with leaf size. (a) Collenchyma xylem second moment of
 817 area according to lamina area. (b) Secondary xylem second moment of area according to
 818 lamina area. (c) Sclerenchyma second moment of area according to lamina area. (d)
 819 Approximated petiole flexural stiffness (product of petiole second moment of area with
 820 petiole density) according to lamina area, plotted on a log-scale. *b*: scaling exponent.
 821



822
 823
 824
 825
 826
 827
 828
 829
 830
 831

832 **Fig. 5.** Scaling of vascular traits with leaf size. (a) Mean hydraulic diameter according to
 833 lamina area. (b) Mean hydraulic diameter according to path length. (c) Theoretical leaf
 834 conductance under the hypothesis of no vessel widening according to lamina area. (d)
 835 Theoretical leaf conductance under the hypothesis of no vessel widening according to path
 836 length. All relationships are plotted on a log-scale. *b*: scaling exponent.
 837



838
 839

840 **Table 1.** List of measured traits and abbreviations.

841

Trait	Abbreviation	Unit	Formula
Petiole cross-sectional area	A_{pet}	mm ²	
Petiole dry mass	M_{pet}	g	
Lamina dry mass	M_{lamina}	g	
Leaf dry mass	M_{leaf}	g	$M_{leaf} = M_{petiole} + M_{lamina}$
Lamina area	A_{lamina}	cm ²	
Petiole density	PD	g cm ³	$PD = M_{dry} / (M_{fresh} - M_{immersed})$
Second moment of area of a ring-like tissue	I	mm ⁴	$I = \pi/4 * (r_{ext}^4 - r_{int}^4)$ with r_{ext} and r_{int} ring external and internal radii respectively
Approximated petiole flexural stiffness		g mm ⁴ cm ⁻³	$I * PD$
Conductive path length	PL	cm	
Mean hydraulic diameter	D_h	�m	$D_h = (\Sigma D_v^4 / N)^{1/4}$
Vessel hydraulic diameter	D_v	�m	$D_v = [32(ab)3/(a^2+b^2)]^{1/4}$ a and b major and minor ellipse diameters
Number of vessels	N_{vessel}		
Conductive area	CA	mm ²	$CA = \pi(D_h/2)^2 * N_{vessel}$
Theoretical hydraulic conductivity	K_{th}	kg s ⁻¹ MPa ⁻¹ m	$K_{th} = \Sigma K_{ellipse}$
Theoretical leaf conductance	$K_{leaf,null}$	kg s ⁻¹ MPa ⁻¹ m ⁻²	$K_{leaf} = (K_{th} / L_{pet} / 2) / A_{lamina}$ $K_{ellipse} = \pi a^3 b^3 / 64 \eta (a^3 + b^3)$
Ellipse conductivity	$K_{ellipse}$	kg s ⁻¹ MPa ⁻¹ m	with $\eta = 1.002 * 10^{-9}$ MPa s ⁻¹ at 20�C a and b major and minor ellipse diameters

842

843

844

845

846 **Table 2.** Main log-log linear relationships based on standardised major axis regression.

847

848

<i>Y</i>	<i>X</i>	<i>P</i>	<i>R</i> ²	slope	95% CIs (slope)
A_{lamina}	A_{pet}	< 0.001	0.858	1.256	1.229 – 1.283
M_{lamina}	A_{pet}	< 0.001	0.898	1.236	1.213 – 1.259
M_{lamina}	M_{pet}	< 0.001	0.947	0.868	0.857 – 0.881
A_{lamina}	M_{leaf}	< 0.001	0.913	0.995	0.977 – 1.012
D_{h}	PL	< 0.001	0.469	0.549	0.474 – 0.638
D_{h}	A_{lamina}	< 0.001	0.502	0.276	0.240 – 0.318
N_{vessel}	A_{lamina}	< 0.001	0.596	0.490	0.456 – 0.524
CA	A_{lamina}	< 0.001	0.635	0.780	0.685 – 0.875
Xylem area	A_{lamina}	< 0.001	0.711	0.860	0.772 – 0.978
Approx. EI	A_{lamina}	< 0.001	0.653	1.720	1.522 – 1.918

Bold values refer to significant correlation ($P < 0.05$). Table 1 for a list of abbreviations. CI: confidence interval.

849

850

851

852

853

854

855

856