

1 **FShort title: Dense venation and aridity-adapted photosynthesis**

2

3 **Apparent over-investment in leaf venation relaxes leaf morphological constraints on**
4 **photosynthesis in arid habitats**

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15 One sentence summary

16 Aridity-adapted *Eucalyptus* and *Corymbia* species invest in high leaf vein densities to offset the
17 negative effect of leaf thickness on photosynthesis.

18

19 List of author contributions

20 H.J.dB. and P.L.D. contributed equally as joint lead authors.

21 H.J.dB. prepared samples, measured leaf traits, constructed the hydraulics model with input from
22 P.L.D. and E.J.V. analysed data and drafted the article

23 P.L.D. prepared samples, measured leaf traits, derived the photosynthetic parameters, provided
24 input on modelling, analysed data and drafted the article

25 E.J.V. and H.J.dB. conceived the study and developed it with P.L.D.

26 E.J.V. oversaw measurements of leaf traits, provided input on modelling and supervised and
27 complemented the writing

28 E.W. prepared and measured leaves for vein density and stomatal morphological features

29 C.A.P. oversaw the vein measurements

30 E-D.S. conceived the original sample collection campaigns, measured several leaf traits and
31 commented on the manuscript

32 N.C.T. collected the leaf material and commented on the manuscript

33 D.N. located and identified trees and collected leaves during the sample collection campaigns

34

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43 **Abstract**

44 The primary function of leaf venation is to supply the mesophyll with water that evaporates when
45 stomata are open to allow CO₂ uptake for photosynthesis. Theoretical analyses suggest that water
46 is optimally distributed in the mesophyll when the lateral distance between veins (d_x) is equal to
47 the distance from these veins to the epidermis (d_y), expressed as $d_x:d_y \approx 1$. Although this theory is
48 supported by observations on many derived angiosperms, we hypothesise that plants in arid
49 environments may reduce $d_x:d_y$ below unity owing to climate-specific functional adaptations of
50 increased leaf thickness and increased vein density. To test our hypothesis we assembled leaf
51 hydraulic, morphological and photosynthetic traits of 68 species from the *Eucalyptus* and *Corymbia*
52 genera (termed eucalypts) along an aridity gradient in southwestern Australia. We inferred the
53 potential gas exchange advantage of reducing d_x beyond d_y using a model that links leaf
54 morphology and hydraulics to photosynthesis. Our observations reveal that eucalypts in arid
55 environments have thick amphistomatous leaves with high vein densities, resulting in $d_x:d_y$ ratios
56 that range from 1.6 to 0.15 along the aridity gradient. Our model suggests that as leaves become
57 thicker, the effect of reducing d_x beyond d_y is to offset the reduction in leaf gas exchange that
58 would result from maintaining $d_x:d_y$ at unity. This apparent over-investment in leaf venation may
59 be explained from the selective pressure of aridity, under which traits associated with long leaf
60 lifespan, high hydraulic and thermal capacitances, and high potential rates of leaf water transport
61 confer a competitive advantage.

62

63 **Keywords:** Australia, Eucalypts, hydraulic conductance, leaf thickness, LMA, VLA, vein
64 density, stomata

65 Introduction

66 A fundamental challenge for terrestrial plants is to balance the benefit of carbon uptake versus the
67 risk of desiccation resulting from concurrent transpirational water loss. To achieve this, water
68 transport processes in leaves are integrated such that leaf hydraulic properties determine the
69 supply of water to the mesophyll and transpirational losses are regulated by stomatal conductance
70 (g_s) (Sack and Scoffoni 2013). Water enters the leaf as a liquid and is distributed along the plane
71 of the leaf through xylem veins. After water exits the vein it moves transversally to the plane of
72 the leaf through the mesophyll toward the epidermis where it reaches the stomatal pore as water
73 vapour. Little is known about how exactly water moves from the veins to the stomatal pores and
74 where the liquid to gas phase transition exactly occurs (Sack and Holbrook 2006, Sack and
75 Scoffoni 2013), yet the length of the post-venous path (l_{H_2O}) shows a strong negative relationship
76 with leaf hydraulic conductance (k_{leaf}) (Brodribb et al. 2007, Buckley et al. 2015). As a result,
77 several leaf morphological traits such as leaf vein density, the depth of the veins inside the leaf
78 and leaf thickness (τ_L) influence leaf water transport (Noblin et al. 2008, Buckley et al. 2015). The
79 close relationship between leaf water status and g_s implies that the hydraulic architecture of the
80 leaf poses an important constraint on carbon uptake, specifically in environments with a high
81 evaporative demand.

82

83 Considering the dimensions of post-venous water transport led Zwieniecki and Boyce (2014) to
84 hypothesise that a functionally optimal vein placement would be achieved if the average distance
85 between neighbouring veins (d_x) were equal to the average distance from veins to the epidermis
86 (d_y), expressed by the ratio $d_x:d_y \approx 1$. This hypothesis is based on modelling and experiments with
87 artificial leaves (Noblin et al., 2008), which suggest that potential maximum transpiration rates
88 increase by increasing vein density when vein densities are relatively low (with $d_x:d_y \gg 1$),
89 whereas potential maximum transpiration rates become independent of vein density when vein
90 densities are very high (with $d_x:d_y \ll 1$). Zwieniecki and Boyce (2014) observed that derived
91 angiosperms achieve an optimal vein architecture (with $d_x:d_y \approx 1$) by combining high vein
92 densities with relatively thin leaves. This specific leaf morphology is unique to angiosperms (Feild
93 et al. 2011, de Boer et al. 2012) and allows high rates of transpiration and photosynthesis owing
94 to the short distances for post-venous water transport and subsequent high k_{leaf} (Brodribb et al.
95 2007). Some gymnosperms also achieve optimal vein placement with a contrasting morphology
96 that combines thick leaves with low vein densities, resulting in relatively long post-venous water
97 transport distances and subsequently low k_{leaf} . Deviations from this proposed hydraulically optimal
98 anatomy are observed for fern and basal angiosperms species that, owing to physiological or
99 developmental constraints, under-invest in veins by combining relatively thin leaves with relatively
100 low vein densities, resulting in $d_x:d_y > 1$ (Zwieniecki and Boyce 2014). This leaf morphology is
101 especially vulnerable to desiccation and therefore limited to environments with low light and/or
102 high atmospheric humidity (Feild et al. 2004). Zwieniecki and Boyce (2014) observed few species
103 that over-invest in veins (with $d_x:d_y < 1$) and argue that this leaf hydraulic architecture yields no
104 functional benefit over an optimal vein architecture ($d_x:d_y \approx 1$) because, like the theoretical
105 extreme ($d_x:d_y \ll 1$) exemplified by Noblin et al. (2008), the additional veins may not contribute
106 to additional leaf gas exchange capacity.

107

108 We argue that the proposed optimal leaf hydraulic architecture ($d_x:d_y \approx 1$) may not be the
109 phenotypic endpoint of evolution in environments that specifically select for traits associated with
110 thick leaves. Thick leaves are most common in warm, arid and high-light environments because of
111 advantages related to a longer leaf life span (Mott et al. 1982, Niinemets 2001) and a larger
112 hydraulic and thermal capacitance (Schymanski et al. 2013). Warm and dry climates also select for
113 high vein densities (Sack and Scoffoni 2013) because this morphology provides greater resistance
114 to loss of k_{leaf} during water stress and may facilitate high photosynthesis rates during (short) wet
115 periods (Grubb, 1998; Scoffoni et al., 2011). The morphological combination of thick leaves (with
116 subsequent long d_y) with high vein densities (with subsequent short d_x), implies that an apparent
117 over-investment in leaf venation (resulting in $d_x:d_y < 1$) may provide functional benefits under dry
118 and warm climates.

119

120 To understand how the selection pressure of aridity shapes leaf form and function, it is important
121 to not only consider the hydraulic aspects of leaf morphological adaptations, but also the
122 optimisation of photosynthetic biochemistry in relation to the prevailing climate. The fast-slow
123 continuum of the leaf economics spectrum has successfully predicted correlations between
124 photosynthetic capacity, g_s and leaf nitrogen on the one hand, and leaf lifespan and leaf mass per
125 unit area (LMA) on the other hand (Wright et al. 2004). Although these relationships emerge
126 independent of climate, leaves of drought-adapted plants tend to have a higher LMA and longer
127 lifespan (Wright et al. 2005). Given the requirement for plants to trade-off carbon uptake and
128 water loss, photosynthetic traits should also be coordinated with k_{leaf} . Some empirical evidence to
129 this effect has been presented (Franks 2006, Sack and Frole 2006, Brodribb et al. 2007, Brodribb
130 and Jordan 2008), but a theoretical proposition linking leaf economic traits to these processes (as
131 proposed by Blonder et al. (2011) for example) has yet to be widely accepted (Sack et al. 2014) or
132 supported experimentally. In contrast to the assertion of coordination, the recent work of Li et al.
133 (2015) indicates a decoupling of leaf economic and leaf hydraulic traits within tropical-subtropical
134 forests. The premise of Li et al. (2015) is that spatial compartmentalisation of traits in functionally
135 independent leaf internal structures may enable more nuanced adaptation in habitats that offer a
136 diversity of conditions. Despite potential compartmentalisation, leaf hydraulic traits are inherently
137 coupled to leaf economic and photosynthetic traits via constraints imposed by selection for the
138 most productive combinations of leaf life span and leaf morphology (Brodribb et al. 2010). In this
139 way, groups of functionally independent traits are expected to change concurrently to optimise the
140 overall performance of leaves in relation to the environmental pressure of aridity.

141

142 We aim to test the hypothesis that coordination between leaf hydraulic and photosynthetic traits is
143 achieved across mesic and arid environments by expressing leaf morphologies that deviate from
144 the theoretical optimal vein architecture with $d_x:d_y \approx 1$, as proposed by Noblin et al. (2008) and
145 Zwieniecki and Boyce (2014), resulting in $d_x:d_y$ ratios less than one. Hereto we assembled a
146 collection of leaf hydraulic and photosynthetic traits from the closely related genera *Eucalyptus*
147 and *Corymbia* (collectively referred to as eucalypts from here on) that are widely distributed
148 across the mesic-arid spectrum of Australian environments. Fossil evidence suggests that the

149 *Eucalyptus* and *Corymbia* clades evolved from a rainforest precursor during the Paleocene to Early
150 Eocene (Gandolfo et al. 2011). The subsequent diversification and radiation of these genera across
151 continental Australia through the Cenozoic took place against a backdrop of increasing aridity
152 (Ladiges et al. 2003, Martin 2006). This adaptive radiation required innovations in leaf traits that
153 are manifest in extant species. Work to classify parts of this group using morphological or
154 molecular approaches has generally observed more derived species in arid habitats (Steane et al.
155 2002, Hopper 2003, Ladiges et al. 2003, Steane et al. 2011). Within species, phylogeographical
156 patterns often indicate a complex population structure, indicative of multiple localised refugia
157 associated with historical shifts in aridity (Byrne et al. 2008).

158

159 In this study we draw upon a natural aridity gradient to test whether the leaf hydraulic and
160 photosynthetic traits of eucalypt leaves are coordinated across environments classified by the
161 supply and demand for water. Our analyses expand on the work of Schulze et al. (2006) who
162 sampled eucalypt leaves across a transect that spanned an aridity gradient from the wet coastal
163 region of southwestern Australia to the arid Australian interior near Kata Tjuta. From this collection
164 we selected three to five leaves per tree from 440 trees, representing 68 species occurring at 73
165 locations for analyses of leaf morphological and hydraulic traits along the aridity gradient (Fig. 1
166 and Table S1). From this subsample we selected three leaves from 107 trees (27 species, 30
167 locations) for measurements of stomatal traits. In our analyses we specifically focused on the
168 adaptation of the leaf morphological and hydraulic traits that influence the ratio $d_x:d_y$ (Fig. 1).
169 Based on these data sets we determined the adaptive innovations in leaf morphology and leaf
170 hydraulics that provide specific competitive advantage to eucalypts in arid environments.

171

172 **Results**

173 Our results show clear differences in eucalypt leaf morphology along an aridity gradient in
174 southwestern Australia. Regression with aridity index (AI) reveals that leaf vein density, expressed
175 as vein length per unit area (VLA), and T_L increase from the mesic to the arid sites ($r^2 = 0.44$, $P <$
176 0.001 and $r^2 = 0.11$, $P < 0.001$, respectively) (Fig. 2). The slopes and intercepts of the models
177 relating VLA to AI (Fig. 2) were not different between hypostomatous and amphistomatous species
178 ($P > 0.05$), hence a combined model is presented. The increase in T_L with aridity was only
179 significant for amphistomatous leaves and the combination of amphistomatous and hypostomatous
180 species and the slopes and intercepts of these models were not significantly different ($P > 0.05$).

181

182 As the leaf veins of the eucalypts studied here are positioned such that there is an equal distance
183 from the upper and lower epidermis (Fig. S1 and Table S2), increases in T_L are proportional to
184 increases in d_y . Furthermore, increases in VLA are closely linked to decreases in d_x ($r^2 = 1.0$, $P <$
185 0.001) (Fig. S2). Hence, the observed $d_x:d_y$ ratios decrease along the aridity gradient as a result of
186 the combined increases in VLA and T_L from mesic to arid sites ($r^2 = 0.41$, $P < 0.001$) (Fig. 2). This
187 pattern was evident regardless of stomatal distribution, and the slopes and intercepts of the
188 models describing these relationships did not differ between hypostomatous and amphistomatous
189 species. In our sample of eucalypts the $d_x:d_y$ ratios range between 0.15 and 1.6, and thereby
190 extend notably below the proposed theoretical optimum ratio $d_x:d_y \approx 1$. Our results also reveal a

191 shift from a hypostomatous to amphistomatous leaf morphology towards the more arid sites.
192 Consequently, the amphistomatous species in our dataset have leaves with a higher VLA than the
193 hypostomatous species ($16.0(\pm 3.7)$ mm mm⁻² and $9.0(\pm 2.1)$ mm mm⁻², respectively, $P < 0.001$)
194 and a larger T_L ($517(\pm 97)$ μ m and $350(\pm 75)$ μ m, respectively, $P < 0.001$). **These morphological**
195 **contrasts manifest as a lower $d_x:d_y$ in amphistomatous species compared to hypostomatous**
196 **species ($0.30(\pm 0.10)$ and $0.82(\pm 0.24)$, respectively $P < 0.001$, Fig. S3). Our species level**
197 **comparison of $d_x:d_y$ also reveals both the hypostomatous and amphistomatous eucalypts differ**
198 **from the derived angiosperms of Zwieniecki and Boyce (2014) (Fig. S3). Only the amphistomatous**
199 **eucalypts differ significantly from the proposed 1:1 ratio as hypothesised by Noblin et al. (2008).**
200 We note that our measurements of these leaf morphological and hydraulic traits were performed
201 on dried and rehydrated leaves. Our analyses to quantify the potential error arising from this
202 approach indicate that leaf area and T_L , affecting d_x and d_y respectively, may have been
203 underestimated by $2.2(\pm 2.7)\%$ and $8.9(\pm 4.2)\%$, respectively (Fig. S4). As the reduction in T_L is
204 larger than the reduction in leaf area, we argue that the reported values of $d_x:d_y$ may be
205 overestimated by approximately 5-10%.

206
207 A Principal Component Analysis (PCA) of the leaf morphological dataset indicates coupling of
208 hydraulic and photosynthetic traits (Fig. 3). The PC1 axis accounts for 59% of the variation and
209 this is dominated by loadings on $d_x:d_y$, LMA, L_T and k_{leaf} . For PC2, which accounts for 17% of the
210 variation, strong loadings are evident on the maximum carboxylation rate (V_{cmax}), VLA and k_{leaf} .
211 The PCA scores show clear separation in relation to aridity and stomatal distribution, which
212 highlights the dominant effect of aridity on leaf morphology within the eucalypts studied here. A
213 PCA of the stomatal morphological dataset also displays coupling of hydraulic and photosynthetic
214 traits (Fig. 3). The PC1 axis accounts for 64% of the variation and is dominated by loadings on
215 $d_x:d_y$, stomatal pore length (L_p), guard cell length (L_{gc}) and VLA. The PC2 axis accounts for 17% of
216 the variation and is dominated by loadings on the anatomical maximum stomatal conductance to
217 water vapour (g_{smax}) and stomatal density (D_s). As with the leaf morphological dataset, separation
218 in relation to aridity and stomatal distribution is evident.

219
220 Linear regression statistics further support the PCA-derived coupling of hydraulic and
221 photosynthetic traits in both the leaf morphological and stomatal data sets (Table 1 and Table 2,
222 respectively). In relation to our hypothesis, the leaf morphological data set reveals close
223 association between the ratio $d_x:d_y$ and a variety of leaf traits that span the hydraulic and
224 photosynthetic categories. Crucially, $d_x:d_y$ is negatively correlated with LMA ($r^2 = 0.57$, $P < 0.001$)
225 and leaf nitrogen per unit area (N_{area}) ($r^2 = 0.42$, $P < 0.001$), whereas both LMA and N_{area} are
226 negatively correlated with the leaf carbon isotope ratio (Δ_{leaf}) ($r^2 = 0.34$, $P < 0.001$ and $r^2 = 0.35$,
227 $P < 0.001$, respectively) (Table 1). The stomatal dataset reveals close association between leaf
228 hydraulic traits and stomatal morphology. Here, $d_x:d_y$ is positively correlated with both guard cell
229 length and stomatal pore length ($r^2 = 0.44$, $P < 0.001$ and $r^2 = 0.52$, $P < 0.001$, respectively)
230 (Table 2).

231

232 The leaf morphologies of the eucalypts studied here deviate from the analysis of Zwieniecki and
233 Boyce (2014), which shows that derived angiosperms closely coordinate d_x with d_y via changes in
234 VLA and the lateral position of veins inside the leaf to achieve $d_x:d_y$ ratios near unity (Fig. 4).
235 Unlike the relationship reported by Zwieniecki and Boyce (2014), our linear regression model
236 describing variation in d_y based solely on d_x gives only a weak fit (correlation $r^2 = 0.16$, $P < 0.001$
237 in Fig. 4 and adjusted $r^2 = 0.15$, $P < 0.001$ in Table S3 for the regression model). The inclusion of
238 AI in a multiple regression model substantially improves the model fit (adjusted $r^2 = 0.51$, $P <$
239 0.001 , Table S3). In spite of this result, the close relationship between aridity and the shift from
240 hypostomaty to amphistomaty (Fig S3) restricts our ability to statistically resolve the underlying
241 cause for a negative relationship between d_x and d_y in our eucalypt sample. Still, our observation
242 that the relationship between $d_x:d_y$ and AI is comparable across hypostomatous and
243 amphistomatous eucalypt species-site combinations (Fig. 2) suggests that aridity is shaping the
244 negative relationship between d_x and d_y and that amphistomaty is a specific adaptation associated
245 with the most arid sites.

246

247 The low values of $d_x:d_y$ observed in our selection of eucalypts are a result of the combined
248 increases in T_L and VLA along the aridity gradient. We hypothesised that these adaptations reflect
249 selection pressure of aridity on leaf morphology, whereby increases in leaf thickness and LMA
250 result from selection for a longer leaf life span, while the increases in VLA occur from selection for
251 enhanced water supply and the need to compensate for the potential loss in photosynthesis owing
252 to the increase in leaf thickness. This hypothesis is supported by our within-site comparisons of
253 species-level differences in T_L , $d_x:d_y$, time-integrated photosynthesis (\bar{A}) and VLA, relative to
254 differences in LMA (Fig. 4). The comparisons shows that, within sites, species with a higher LMA
255 have, on average, thicker leaves ($P < 0.001$, $N = 128$) with lower $d_x:d_y$ ($P < 0.01$, $N = 128$) than
256 species with lower LMA. Co-occurring species with higher LMA also have lower \bar{A} than species with
257 lower LMA ($P < 0.001$, $N = 178$). In contrast to the correlation between LMA and VLA that we
258 observed across sites ($r^2 = 0.22$, $P < 0.001$, Table 1), we found no within-site difference in VLA
259 between species with different LMA ($P > 0.05$, $N = 178$) (Fig. 4). This result suggests that the key
260 hydraulic trait VLA may be decoupled from LMA within sites, despite their correlated adaptation to
261 the common selection pressure of aridity across the sampled sites.

262

263 Although a complete phylogeny for the *Eucalyptus* and *Corymbia* genera is not available, we
264 estimated the effect of sampling bias and potential relatedness between species on our
265 observations by resampling several key traits at the taxonomic level of series (a subdivision of
266 genus) using the classification of Nicolle (2015) (Fig. S5). Our subsequent analysis shows that the
267 relationships between VLA and AI (bootstrapped $r^2 = 0.76$ and $P < 0.001$) and between $d_x:d_y$ and
268 AI (bootstrapped $r^2 = 0.67$ and $P < 0.001$) are robust to potential sampling bias. The relationship
269 between leaf thickness and AI (bootstrapped $r^2 = 0.20$ and $P = 0.16$) is not robust to potential
270 sampling bias, which reflects the weak association between leaf thickness and aridity index in the
271 raw data, as shown in Fig. 2 ($r^2 = 0.11$ $P < 0.001$).

272

273 The observation that our sample of eucalypts includes many species with $d_x:d_y$ ratios below unity
274 begs the question to what extent this leaf morphology conveys an advantage in terms of additional
275 gas exchange in comparison to a leaf morphology with $d_x:d_y \approx 1$. As the observed adaptations in
276 $d_x:d_y$ are closely linked to the photosynthetic and stomatal traits considered here (*c.f.* Table 1 and
277 Table 2), our empirical data cannot be used to directly test our hypothesis that these adaptations
278 provide an advantage in terms of additional transpiration and photosynthesis. We therefore used
279 the semi-empirical leaf hydraulic model of Brodrribb et al. (2007) and Brodrribb and Feild (2010) to
280 determine how changes in hydraulic architecture influence leaf gas exchange. We quantified the
281 potential gas exchange advantage of reducing $d_x:d_y$ below unity in terms of the modelled
282 difference in Rubisco-limited photosynthesis between the observed leaf morphology and a leaf
283 morphology with $d_x:d_y = 1$ as the percentage difference δA [%] for all species-site combinations.
284 This model approach is explained in detail in the Materials and Methods. A sensitivity and
285 uncertainty analysis of the model is provided in the Supplemental Information (Fig. S8 and Fig.
286 S9). Our model results show that the observed reduction in $d_x:d_y$ below unity reduces the post-
287 venous water transport path length l_{H_2O} and thereby increases the modelled k_{leaf} (Fig. 5). This
288 effect is most pronounced in thick leaves which suffer most from reduced k_{leaf} when the theoretical
289 optimal hydraulic architecture $d_x:d_y \approx 1$ is maintained. Our modelling results show that these
290 reductions in $d_x:d_y$ below unity provide a small but significant photosynthetic advantage δA of
291 $8.1(\pm 3.4)\%$ across all our species-site combinations (Fig. 5). The group of amphistomatous
292 species shows a larger average photosynthetic advantage of reducing $d_x:d_y$ below unity than the
293 group of hypostomatous species, with $\delta A = 8.9(\pm 2.5)\%$ for the amphistomatous species and $\delta A =$
294 $1.2(\pm 1.6)\%$ for the hypostomatous species.

295

296 Discussion

297 Our results reveal that in eucalypts, high vein densities occur in thick and amphistomatous leaves,
298 especially in species growing in the most arid localities. The consequence of this leaf morphology is
299 that the observed range in the ratio between the inter-vein distance d_x and the vein-epidermal
300 distance d_y extends to well below the proposed theoretical optimal ratio $d_x:d_y \approx 1$ (Noblin et al.
301 2008). Eucalypts thus form an exception to the strong 1:1 relationship reported for other derived
302 angiosperm species (Zwieniecki and Boyce 2014). The theoretical optimal vein placement requires
303 close coordination between leaf vein density, the position of veins in the leaf, and leaf thickness
304 that inadvertently requires leaves with highest k_{leaf} to be relatively thin. However, arid
305 environments select for thicker leaves owing to their longer life span (Wright and Westoby 2002),
306 larger thermal and hydraulic capacitance (Schymanski et al. 2013) and limited palatability (Chabot
307 and Hicks 1982, Choong et al. 1992). The result of selection for thicker leaves is a larger vein-
308 epidermal distance d_y and, all else being equal, a decrease in k_{leaf} and photosynthesis. Crucial is
309 that a decrease in k_{leaf} limits maximum rates of photosynthesis because it pertains directly to leaf
310 water status and the control of g_s . As water may be available during short intervals in arid
311 environments, there may be strong selection pressure for a higher k_{leaf} (Grubb, 1998; Scoffoni et
312 al., 2011). However, owing to the selection pressure for thicker leaves in arid environments, the
313 remaining option to increase k_{leaf} is to increase vein density and subsequently reduce the ratio
314 $d_x:d_y$. The coincidence of thick leaves and high vein densities in our study therefore does not point

315 to mechanistic coordination of these traits, as discussed between Blonder et al. (2014) and Sack et
316 al. (2014), but rather to parallel selection driven by common selection pressures associated with
317 aridity.

318

319 The VLA observed in our sample of eucalypts ranges from 6 mm mm⁻² in relatively mesic sites up
320 to 24 mm mm⁻² in the most arid localities. Our measurements of VLA are slightly overestimated as
321 our analyses were performed on dried and rehydrated leaves, which revealed an average
322 shrinkage of 2.2(±2.7)% compared to fresh leaves (see Fig. S4). This effect is of similar
323 magnitude as the shrinkage reported in rehydrated leaves by Blonder et al. (2012). Our
324 observation that VLA in eucalypts increases with increasing aridity is in-line with the global
325 compilation of vein density data by Sack and Scoffoni (2013), who show a strong correlation
326 between VLA and aridity across biomes. Their review includes only two eucalypt species (*E.*
327 *haemastoma* and *E. globulus* with VLA of 4.1 and 7.4 mm mm⁻², respectively), which are from
328 relatively mesic habitats and have vein densities comparable to our mesic species-site
329 combinations. The upper end of the vein density range of our eucalypt sample overlaps with the
330 highest vein densities of evergreen shrubs and trees reported in Sack and Scoffoni (2013). Boyce
331 et al. (2010) observed that these high vein densities (>20 mm mm⁻²) occur only in the rosoid clade,
332 which *Eucalyptus* and *Corymbia* belong to. Hence, this phylogenetic background may have allowed
333 eucalypts to develop the specific leaf morphology that combines relatively thick leaves with high
334 vein densities.

335

336 Mitigating the effect of leaf thickness on k_{leaf} by adjusting VLA and subsequent $d_x:d_y$ may be critical
337 for species in arid environments that must maximise carbon gain during short pulses of high soil
338 moisture content (Sack and Scoffoni 2013). This growth strategy has likely become relevant
339 during the evolution of eucalypts because major differentiation of the *Eucalyptus* and *Corymbia*
340 clades took place during a transition to greater aridity (Ladiges et al. 2003). This climatic shift
341 provided ecological opportunities, but the ensuing adaptive radiation of species formerly suited to
342 rainforests, the precursors of modern eucalypts, would have occurred in an environment
343 increasingly characterised by long dry periods punctuated by brief wet episodes (Martin 2006).
344 These novel environments would have ultimately encouraged the convergence of traits enabling a
345 long leaf lifespan and high thermal capacitance with traits supporting higher rates of leaf water
346 transport and photosynthesis (Givnish et al. 2005). Leaves would necessarily become thicker, but
347 at the same time selection for maximum photosynthetic yield would lead to the evolution of higher
348 vein densities. Zwieniecki and Boyce (2014) suggest that such an over-investment in veins may
349 result in a decrease in photosynthetic capacity due to a loss of internal space that could be
350 allocated to chlorophyll-bearing cells. However, in thick amphistomatous leaves of (semi)arid
351 biomes, photosynthetic capacity may not be limited by space constraints, but rather by the ability
352 of the hydraulic network to sustain liquid and vapour water transport.

353

354 Although the influence of adjusting inter-vein distance d_x on k_{leaf} is less than the effect of adjusting
355 vein-epidermal distance d_y (Noblin et al., 2008), our modelling results indicate that the eucalypts
356 studied here may still gain a small but significant photosynthetic benefit of reducing $d_x:d_y$ below

357 unity. Our model is based on the empirical relationship between k_{leaf} and the length of the post-
358 venous water transport $l_{\text{H}_2\text{O}}$ found by Brodribb et al. (2007). This relationship highlights that the
359 water transport pathway outside the xylem is the major constraint to k_{leaf} (Sack and Scoffoni,
360 2013; Buckley et al. 2015). According to our model, the photosynthetic advantage increases with
361 decreasing $d_x:d_y$ further below unity. Hence, the combined adaptation of increasing leaf thickness
362 and increasing VLA could have provided evolutionary advantage in terms of additional productivity
363 during (short) periods with sufficient water. As our measurements of $d_x:d_y$ may be overestimated
364 owing to the use of dried and rehydrated laves, the modelled advantage could be slightly
365 underestimated. Still, our interpretation of the modelling results appears at odds with the
366 conclusion of Zwieniecki and Boyce (2014) and the results from artificial leaves of Noblin et al.
367 (2008), who suggest that additional leaf veins do not contribute to additional leaf gas exchange in
368 the theoretical extreme situation with $d_x:d_y < 1$. However, the modelling results of Noblin et al.
369 (2008) also suggest that $d_x:d_y$ can extend to below unity before the response of increasing
370 transpiration rate with increasing vein density fully saturates, and this is especially so when the
371 vein-epidermal distance is relatively large and vein diameters are small. This interpretation of
372 Noblin et al. (2008) is consistent with our observations on eucalypt leaf morphology, which shows
373 that the lowest $d_x:d_y$ ratios occur in the thickest leaves with the highest vein densities. We
374 therefore argue for a more nuanced interpretation of the ratio $d_x:d_y$ by considering the specific
375 growth environments and leaf morphologies, in which small increments in leaf gas exchange may
376 yield specific competitive advantages.

377

378 Our results also reveal that various leaf morphologies co-exist in the same site and that thicker
379 leaves with a lower $d_x:d_y$ do not offer an absolute competitive advantage compared to thinner
380 leaves with a higher $d_x:d_y$. These within-site variations in $d_x:d_y$ and T_L could reflect the exploitation
381 of different niches, as proposed by Li et al. (2015) drawing on observations from a structurally
382 complex sub(tropical) environment, where light limitation is an important selection pressure. For
383 the eucalypts of our study, where light is not limiting, niche differentiation is likely to manifest as
384 different rooting depths and soil preferences (Drake et al. 2011, Poot and Veneklaas 2013),
385 resulting in differences in water availability, which in turn will influence stomatal opening and
386 carbon gain over the lifespan of a leaf. Thus species with thick leaves may co-occur with thin-
387 leaved species due to morphological specialisation to specific niches in relation to leaf economic
388 trade-offs.

389

390 A specific additional benefit of high VLA in arid environments may also lie in the selection for
391 longer-lived leaves (Wright and Westoby 2002) as leaf aging will inevitably lead to some loss of
392 hydraulic continuity when the cumulative exposure to water stress increases. The vascular
393 redundancy of leaves with high vein densities of a reticulate nature may reduce the risk of
394 complete hydraulic failure under these circumstances (Scoffoni et al. 2011, Price and Weitz 2014)
395 by providing alternative parallel flow paths for water transport (Sack et al. 2008). Thus selection
396 for elevated vein densities in arid biomes may occur to both mitigate the effect of leaf thickness
397 and to reduce the risk of hydraulic failure due to water stress. Although our observations are

398 limited to the eucalypt group, we expect that similar morphological trends may be present in other
399 (semi)arid evergreens.

400

401 Our results confirm the well-known link between leaf thickness, aridity, and amphistomaty (Mott et
402 al. 1982), as thicker amphistomatous leaves were found more commonly in eucalypts growing in
403 the most arid environments. Amphistomaty has different implications for the leaf internal
404 conductance of water (liquid or vapour) than for the leaf internal conductance of CO₂. Our results
405 indicate that amphistomaty does not reduce the path length from vein to stomata, as both hypo-
406 and amphistomatous leaves have their veins at approximately mid-depth. However, amphistomaty
407 does reduce the average path length for CO₂ diffusion to the mesophyll in thick leaves. Although
408 other factors, including mesophyll cell surface area, cell wall thickness and chloroplast distribution
409 are also strong determinants of mesophyll conductance to CO₂ (Flexas et al. 2013), it would seem
410 that amphistomaty tends to favour water-use efficiency due to a larger positive effect on CO₂
411 conductance than hydraulic conductance. Hypostomatous leaves are more common at mesic
412 locations in our study, where leaves are also thinner. The shorter pathways for water and CO₂
413 transport in thin leaves of mesic environments may favour the presence of stomata solely on the
414 abaxial surface (de Boer et al. 2012). The close relationship between aridity and stomatal
415 distribution challenges our ability to disentangle the effect of leaf type versus the specific adaption
416 of leaf morphology to aridity as an underlying cause for the negative relationship between d_x and
417 d_y . Although we infer that aridity is shaping this relationship in eucalypts, further work focusing on
418 thick hypostomatous leaves of arid environments would offer additional insight.

419

420 The observed adaptations in leaf morphology described here create a more complete picture of
421 eucalypt adaptations to their environments. Of particular interest is the fact that the high-LMA
422 amphistomatous leaves of (semi)arid habitats tend to have near-vertical angles. High leaf mass
423 may contribute to that trait, but the near-vertical leaf angle in eucalypts is generally interpreted as
424 enabling greater carbon capture when the sun is at low angles, and temperature and VPD are
425 lower (King 1997). At such steep leaf angles, the amphistomatous and isobilateral form (Gillison
426 1981) presumably enables light entry into the leaf from either or both sides. In contrast, in mesic
427 environments, vegetation tends to have higher leaf area indices, and most light enters leaves from
428 above, *i.e.* from the adaxial surface of the hypostomatous leaves that are common in such
429 environments. Thinner and less dense leaves (with lower LMA) tend to have a higher
430 photosynthetic light use efficiency than thicker and denser leaves (Reich et al. 1992, Niinemets
431 2001). In arid habitats, where light is not limiting (Bloom et al. 1985), the selection pressure for
432 high light use efficiency is presumably weak. Hence the relatively thick eucalypt leaves of
433 (semi)arid habitats may confer little, if any, negative consequences for photosynthesis, due to
434 relaxation of the morphological constraints on leaf venation.

435

436 **Conclusions**

437 We conclude that arid environments select for thicker amphistomatous leaves in eucalypts. Thick
438 leaves challenge the underlying selective pressure to distribute water efficiently to the sites of
439 evaporation because their morphology increases the distance for vein-to-stomata transport.

440 Eucalypts have, to some extent, overcome this problem by developing leaves with a high vein
441 density, thereby reducing the inter-vein distance. In this way, deviation from the theoretical
442 optimal vein placement can be considered a response to the selective advantage of producing thick
443 leaves in arid environments.

444

445 **Material and Methods**

446

447 **Plant material**

448 In 2003 and 2004 leaves of *Eucalyptus* species and closely related *Corymbia* species were sampled
449 from 971 trees of 65 distinct species across a transect that spanned 73 locations along an aridity
450 gradient from the wet coastal region of southwestern Australia to the arid Australian interior near
451 Kata Tjuta (Schulze et al. 2006). From this collection we selected three to five leaves per tree from
452 440 trees, representing 68 species occurring at 73 locations for analyses of leaf morphological
453 traits along the aridity gradient (Fig. 1 and Table S1). From this subsample we selected three
454 leaves from 107 trees (27 species, 30 locations) for measurements of stomatal traits. Our analyses
455 were performed at the species by site level. This approach allows for phenotypic or genetic
456 differences to influence the observed relationships with aridity. We deviate from this approach
457 when analysing the relationship between the inter-vein distance (d_x) with the vein-epidermal
458 distance (d_y), where we aggregate our data to the species level to match the data of Zwieniecki
459 and Boyce (2014). To compare the sensitivity of these different averaging approaches, we
460 analysed two critical results, the relationship d_x and d_y and the relationship between the $d_x:d_y$ ratio
461 with aridity index (AI) (Table S3). This analysis revealed that our results are insensitive to level of
462 averaging.

463

464 **Climate variables**

465 Climate variables for each location were obtained from the Atlas of Living Australia, a repository
466 for large environmental and ecological datasets (Atlas of Living Australia website
467 at <http://www.ala.org.au>. Accessed 15 December 2015). We selected the climatological variables
468 that were representative of gridded data at a resolution of approximately 1 km. We integrate
469 water supply and demand for each location as annual aridity index (AI), the average of monthly
470 ratios of precipitation to potential evaporation (pan, free-water surface). This is an adaptation of
471 the index proposed by the United Nations Environmental Programme and cited by Middleton and
472 Thomas (1997). Additional climate variables obtained from this repository were mean annual
473 daytime vapour pressure deficit (VPD), mean annual daytime temperature and mean annual
474 rainfall. We are aware that these climate variables, while representative of long term trends, may
475 not reflect the exact conditions under which individual leaves developed. However, as the
476 emphasis of our study is to demonstrate and interpret leaf morphological changes along an aridity
477 gradient rather than to study the relationships between climate variables and water relations of
478 individual leaves, we argue that the climate data provides sufficient detail for our analyses.

479

480 **Leaf morphological traits**

481 Fresh leaf area, leaf dry weight and leaf mass per unit area (LMA [g cm^{-2}]) were determined for
482 three to five leaves from each of the 440 trees sampled in this study. To determine leaf thickness
483 (T_L [μm]), we randomly selected one leaf per tree ($N = 270$ trees of the 68 species) and cut out a
484 1 cm^2 fragment at approximately the midpoint of the leaf long axis. Since the leaves had been
485 oven dried, these subsamples were first immersed in dilute detergent for one hour to soften the
486 highly sclerophyllous tissue. Cross sections were then taken from each subsample using a
487 microtome set to a thickness of $15 \mu\text{m}$. The cross sections were then stained in 5% toluidine blue
488 for 10 seconds, rinsed in dH_2O , then mounted on clear microscope slides using Kaiser's jelly:
489 1:5:4:1 gelatin:glycerol: dH_2O :antibacterial solution (Listerine™). We measured T_L at 100x
490 magnification using a compound light microscope (Olympus BX51, Olympus, Tokyo), $N = 3$
491 measurements for each cross section. The presence of stomata on either the abaxial surface
492 (hypostomatous) or both the abaxial and adaxial surfaces (amphistomatous) was determined
493 during these microscopic analyses for all the species considered. The error in T_L resulting from the
494 use of dried and rehydrated leaves was quantified on a set of fresh eucalypt leaves, which
495 revealed a $8.9(\pm 4.2)\%$ residual reduction in T_L (Fig. S4). The vein-epidermis distance (d_y [μm])
496 was derived from leaf thickness as $T_L/2$. This simplification was justified by regression analyses of
497 the relationship between d_y and $T_L/2$ from cross-sections of a random selection of 43 species ($r^2 =$
498 1.0 , $P < 0.001$, $N = 111$ leaves) (Supplementary material, Fig. S1 and Table S2). For this sample
499 d_y was measured with respect to the abaxial (lower) epidermis.

500

501 **Leaf venation traits**

502 The leaf vein density was expressed as vein length per unit leaf area (VLA [mm mm^{-2}]) and
503 measured by sampling $1\text{-}2 \text{ cm}^2$ sections from three leaves per tree from each of the 440 trees at
504 approximately the midpoint of the long axis while avoiding the mid rib. The sample was placed in a
505 5% sodium hydroxide solution until the cuticle had dissolved to reveal the vein network (2-3
506 weeks) (Brodribb and Feild 2010, Sommerville et al. 2012). The samples were then rinsed in dH_2O ,
507 stained in a solution of safranin in 5% ethanol for 1 hour, then rinsed in dH_2O . Three images of
508 each leaf were then obtained using a dissecting microscope (Model: SMZ800, Nikon Instrument
509 Inc., NY, USA) at 40x magnification and the vein network traced by hand using Paint© (Microsoft
510 Inc. WA, USA). In light of the high VLA values of our collection, we confirmed that the minor veins
511 of cleared leaves were conductive by drawing dye through fresh leaves via transpiration and
512 observing the vein network from paradermal images (Fig. S6). Cross sections of these leaves
513 confirm the positions of these numerous small veins, as well as the presence of several small
514 chlorenchyma cells in the mesophyll tissue of the inter-vein space (Fig. S6). Also we quantified the
515 amount of leaf shrinkage from drying and the subsequent expansion after rehydration on a set of
516 fresh eucalypt leaves, which revealed a $2.2(\pm 2.7)\%$ residual reduction in leaf area (Fig. S4).

517

518 The traced vein network of each image was further analysed in Matlab (version R2015b). The
519 average VLA of each traced image was obtained following Blonder et al. (2011). First, each set of
520 traces was smoothed and reduced to the width of a single pixel using the skeletonising algorithm
521 of the function 'bwmorph'. Total vein length was calculated from the skeletonised vein network

522 using the function 'bwarea'. The VLA was calculated as the quotient of total vein length and total
523 image area.

524

525 The inter-vein distance (d_x [μm]) was obtained from the skeletonised vein network images by
526 automatically drawing 10.000 random cross-section across each traced image. For each cross-
527 section, we mapped the points where the cross-section intersected with a traced vein and
528 subsequently calculated the average distance between the veins in each cross-section. This
529 automated method resembles the manual cross-section approaches of Blonder and Enquist (2014)
530 and Zwieniecki and Boyce (2014). The grand mean distance between veins in all randomly drawn
531 cross-sections was then calculated per image to obtain an average d_x for each sampled leaf.

532

533 **Stomatal morphological traits**

534 Three leaves were selected for each of the 27 species chosen for determination of stomatal traits.
535 For each leaf, a 1 cm^2 section was sampled and placed into a 2 ml Eppendorf tube. Each tube
536 received a 1:1 solution of 80% ethanol and 30% hydrogen peroxide. The tubes were left unsealed
537 and placed into a water bath set at 60°C until the cuticles and epidermis began to detach from the
538 underlying mesophyll and vascular tissues (after approximately 48 hours). The samples were then
539 removed from the tubes and rinsed under tap water. The cuticle and epidermis of both the abaxial
540 and adaxial surfaces were then carefully detached from the remaining tissue with fine forceps,
541 stained in 5% safranin for 30 seconds then rinsed in deionised water. The 'peels' were then
542 transferred to microscope slides and mounted using Kaiser's jelly. Stomatal density, i.e. number of
543 stomata per unit epidermal area (D_s [mm^{-2}]) was calculated for each stomata-bearing leaf surface
544 as the mean of five fields of view at 400 \times magnification using a compound light microscope
545 (Olympus BX51, Olympus, Tokyo). Stomatal morphological parameters were measured as the
546 mean of 20 stomatal complexes (guard cell pairs) for each stomata-bearing surface at 1000 \times
547 magnification using the same light microscope. Stomatal morphological parameters were: guard
548 cell length (L_{gc} [μm]), guard cell width (W_{gc} [μm]) and stomatal pore length (L_p [μm]) (Franks et
549 al. 2009). The anatomical maximum stomatal conductance to water vapour ($g_{s\text{max}}$ [$\text{mol m}^{-2} \text{s}^{-1}$]) of
550 the stomata-bearing leaf surface was estimated using (Franks and Farquhar 2001, Franks et al.
551 2009):

552

$$553 \quad g_{s\text{max}} = \frac{D_s d a'}{V \left(l + \frac{\pi}{2} \sqrt{\frac{a'}{\pi}} \right)} \quad (1)$$

554

555 where d is the diffusivity of water in air ($\text{m}^2 \text{s}^{-1}$), a' is the mean maximum stomatal pore area (m^2)
556 2), which was estimated as $L_p^2 \times \pi/4$, V is the molar volume of air ($\text{m}^3 \text{mol}^{-1}$), and l is the depth
557 of the stomatal pore (m), which was assumed equal to W_{gc} . For hypostomatous leaves, the
558 estimate of $g_{s\text{max}}$ is based on the stomatal morphology of a single leaf side, whereas $g_{s\text{max}}$ for
559 amphistomatous leaves is the sum of both leaf surfaces based on the assumption that gas
560 exchange from the abaxial and adaxial surfaces occurs in parallel.

561

562 Leaf nitrogen and leaf carbon isotopic composition

563 Schulze et al. (2006) collected a subsample of oven-dried leaves from each of the 440 trees. The
564 leaves were finely ground and part of this material used to determine total leaf nitrogen (% by
565 mass) using a nitrogen analyser (Vario EL II, Elementar, Hanau, Germany). Leaf nitrogen as a
566 percentage of mass (N_{mass}) was converted to an area basis as: N_{area} (g cm^{-2}) = N_{mass} (%) x LMA x
567 100. The leaf carbon isotope ratio ($\delta^{13}\text{C}_{\text{leaf}}$) of the remaining ground material was determined
568 following combustion using an isotope ratio mass spectrometer (Finnigan Delta + XL, Bremen,
569 Germany) in an on-line continuous flow system. $\delta^{13}\text{C}_{\text{leaf}}$ was converted to Δ_{leaf} according to
570 Farquhar et al. (1989) taking atmospheric $\delta^{13}\text{C}$ as 8.1 ‰, the average value recorded at the Cape
571 Grim Air Pollution Station, Tasmania, in the six months prior to each sample collection campaign.

572

573 Deriving photosynthetic rate from carbon isotopic composition

574 We estimated the maximum carboxylation capacity at 25°C ($V_{\text{cmax}25}$ [$\mu\text{mol m}^{-2} \text{s}^{-1}$]) according to an
575 empirical model which was derived from a meta-analysis of V_{cmax} , N_{area} and LMA determined for 27
576 eucalypt species reported in 10 studies (Table S4):

577

$$578 V_{\text{cmax}25} = 10^{1.7 - 0.5 \times \log N_{\text{area}} - 0.1 \times \log \text{LMA} - 0.4 \times \log N_{\text{area}} \times \log \text{LMA}} \quad (2)$$

579

580 $V_{\text{cmax}25}$ was then converted to the actual velocity of Rubisco for carboxylation at ambient
581 temperature (V_{cmax} [$\mu\text{mol m}^{-2} \text{s}^{-1}$]) according to Bernacchi et al. (2001) and the average daytime
582 temperature of each site.

583

584 A time-integrated measure of leaf intercellular (and mesophyll) CO_2 mole fraction (\bar{c}_i [$\mu\text{mol mol}^{-1}$])
585 was then estimated according to Farquhar et al. (1982):

586

$$587 \bar{c}_i = c_a \left(\frac{\Delta_{\text{leaf}} - 4.4}{22.6} \right) \quad (3)$$

588

589 where the atmospheric CO_2 mole fraction (c_a) was taken as 380 $\mu\text{mol mol}^{-1}$, reflecting the
590 atmospheric partial pressure of CO_2 in 2003 (Keeling and Whorf, 2004). We assumed that
591 photosynthesis was predominantly limited by carboxylation, and therefore the modelling
592 constraints of Farquhar et al. (1982) and Sharkey et al. (2007) could be applied to yield an
593 expression for time-integrated photosynthetic rate \bar{A} ($\mu\text{mol m}^{-2} \text{s}^{-1}$):

594

$$595 \bar{A} = \left(1 - \frac{\Gamma^*}{c_i} \right) \frac{V_{\text{cmax}} \bar{c}_i}{c_i + K_c (1 + \frac{\Gamma^*}{K_o})} - R_d \quad (4)$$

596

597 Here, the mitochondrial respiration rate (R_d) was taken as $0.01 V_{\text{cmax}}$, Γ^* is the CO_2 compensation
598 point in the absence of R_d , K_c and K_o are the Michaelis constants of Rubisco for carbon dioxide and

599 oxygen, respectively, and O is the atmospheric partial pressure of oxygen. For values of these
 600 parameters and their temperature dependencies we followed Bernacchi et al. (2001). We note that
 601 since \bar{c}_i represents the mole fraction of CO_2 within both intercellular spaces and the mesophyll,
 602 the expression for \bar{A} incorporates the draw-down in CO_2 at the sites of assimilation.

603

604 **Statistical analyses**

605 Multiple relationships between traits were determined by principal component analysis (PCA) using
 606 OriginPro 2015 (Northampton, MA, USA). PCAs were conducted on both the larger dataset
 607 comprising leaf morphological and hydraulic traits and the subset of data which included stomatal
 608 morphological traits. Data were log-transformed to meet the assumption of normality and z-scores
 609 calculated.

610

611 Standardised Major Axis (SMA) regression analyses using R (version 3.0.2) and the SMATR
 612 package (Warton et al. 2006) were used to describe specific relationships between VLA, T_L and
 613 $d_x:d_y$ and the aridity index, based on the Atlas of Living Australia. Pair-wise interactions between
 614 traits in the leaf morphological and stomatal morphological datasets were similarly quantified with
 615 SMA regression analyses. Data were log-transformed prior to these analyses. These analyses were
 616 performed at the species-site level. We estimated the effect of sampling bias and potential
 617 relatedness between species in the relationships between VLA, T_L and $d_x:d_y$ and the aridity index by
 618 resampling the data the taxonomic level of series (a subdivision of genus) using the taxonomic
 619 classification of Nicolle (2015). Our data set contains species from 11 distinct taxonomic series in this
 620 classification. We used a bootstrap resampling strategy with replacement to obtain distributions of
 621 the correlation r^2 and P-value as well as the SMA from 10,000 random subsamples. Each subsample
 622 contains 11 data points, with each data point being one species-site average, with the species
 623 belonging to one of the 11 series.

624

625 We further tested our hypothesis on the coordination of aridity-induced changes in leaf
 626 morphological, hydraulic and photosynthetic traits by analysing the relative differences in several
 627 key traits between species that co-occur in the same site. Hereto we first quantified the difference
 628 (Δ) in T_L , $d_x:d_y$, \bar{A} and VLA relative to the difference in LMA (ΔLMA) between all different species
 629 that co-occur at each site:

630

$$631 \frac{\Delta\text{Trait}_x(i, j, n)}{\Delta\text{LMA}} = \frac{\text{Trait}_x(\text{species}_i, \text{site}_n) - \text{Trait}_x(\text{species}_j, \text{site}_n)}{\text{LMA}(\text{species}_i, \text{site}_n) - \text{LMA}(\text{species}_j, \text{site}_n)} \quad (5)$$

632

633 In which Trait_x represents T_L , $d_x:d_y$, \bar{A} and VLA, respectively, and the index $i = 1 \dots j-1$ and the
 634 index $j = 2 \dots k$, with k being the number of species that co-occur at each site, and the index $n=1$
 635 $\dots m$, with m being the number of sites studied.

636

637 The observed differences in T_L , d_x , d_y , \bar{A} and VLA relative to LMA were interpreted based on the
 638 sign of their average value. For example, if the average $\Delta T_L / \Delta LMA > 0$ it means that species with
 639 thicker leaves have, on average and within sites, higher LMA. We tested whether the means of
 640 these relative differences deviated from zero using a two-tailed Student's t-Test at a 5%
 641 significance level, including a Bonferroni correction considering four comparisons. We excluded
 642 comparisons of species with a difference in the denominator LMA of less than $2 \mu\text{g cm}^{-2}$, which is
 643 equal to 5% of the total observed range in LMA.

644

645 **Modelling leaf hydraulics and photosynthesis**

646 The potential advantage of reducing d_x ; d_y below unity in terms of additional leaf gas exchange was
 647 quantified with a semi-empirical model that relates the length of the post-venous water flow path
 648 ($l_{\text{H}_2\text{O}}$) to leaf hydraulic conductance (k_{leaf}) and subsequent transpiration and photosynthesis
 649 following Brodrribb et al. (2007) and Brodrribb and Feild (2010). Brodrribb et al. (2007) provide an
 650 empirical expression for k_{leaf} that, given atmospheric humidity and temperature are known, can be
 651 related to a transpiration flux and subsequent photosynthesis following Brodrribb and Feild (2010).
 652 Principally, the model from Brodrribb et al. (2007) relates leaf morphology and subsequent $l_{\text{H}_2\text{O}}$
 653 [μm] to k_{leaf} [$\text{mmol MPa}^{-1} \text{m}^{-2} \text{s}^{-1}$] as:

654

$$655 \quad k_{\text{leaf}} = 12,674 \cdot l_{\text{H}_2\text{O}}^{-1.26} \quad (6)$$

656

657 in which

658

$$659 \quad l_{\text{H}_2\text{O}} = \tau \sqrt{dm_x^2 + d_y^2} \quad (7)$$

660

661 where τ is the curvature (or tortuosity) of the flow path through the leaf interior and assumed $\pi/2$,
 662 following Brodrribb and Feild (2010), and dm_x is the inter-vein distances as defined by Brodrribb and
 663 Feild (2010). We note that dm_x differs from the inter-vein-distance d_x in our study. Brodrribb et al.
 664 (2007) defined dm_x as the furthest equidistant point from surrounding veins. To apply our
 665 measurements of d_x to the model from Brodrribb et al. (2007) we use the conversion $dm_x =$
 666 $0.56 \cdot d_x$. This conversion was based on the difference in the relationships between dm_x and VLA as
 667 reported by Brodrribb et al. (2007) and between d_x and VLA as obtained from our traced vein
 668 networks (Fig. S2).

669

670 Brodrribb and Feild (2010) use the expression for k_{leaf} to model the steady-state transpiration rate
 671 by assuming that the flow of (liquid) water into the leaf (E_l) equals the (gaseous) transpiration flux
 672 (E_g):

673

$$674 \quad E_l = k_{\text{leaf}} \cdot \Delta \psi_{\text{leaf}} \quad (8)$$

675

$$676 \quad E_g = g_{\text{sw}} \cdot (w_i - w_a) \quad (9)$$

677

678 where $\Delta\psi_{\text{leaf}}$ [MPa] is the water potential gradient across the leaf, g_{sw} [$\text{mol m}^{-2} \text{s}^{-1}$] is the stomatal
679 conductance to water vapour, and $w_i - w_a$ [-] is the atmospheric humidity gradient between the leaf
680 interior and the leaf exterior.

681

682 Brodrribb and Feild (2010) approximated $\Delta\psi_{\text{leaf}}$ as constant (0.4 MPa) and based $w_i - w_a$ on the
683 typical growth conditions of their species. With these assumptions, the Equations (8) and (9) can
684 be equated and rearranged to yield an expression for g_{sw} as a function of d_x and d_y .

685

686 For our analyses we modified the geometrical relationships in the model framework from Brodrribb
687 and Feild (2010) and expressed k_{leaf} in terms of the traits $d_x:d_y$ and T_L . Hereto we invoked the
688 assumption that $d_y = T_L/2$ (see Supplementary material, Fig. S1 and Table S2, for empirical
689 support). For d_x we inserted its relationship with the ratio $d_x:d_y$ and the expression $d_y = T_L/2$ so
690 that $d_x = \frac{d_x}{d_y} \frac{T_L}{2}$. Inserting both expressions in Equation 7 yields:

691

$$692 \quad l_{\text{H}_2\text{O}} = \frac{1}{2\pi} \sqrt{\left(\frac{T_L^2}{4} + 0.078 \cdot \left(\frac{d_x}{d_y}\right)^2 \cdot T_L^2\right)} \quad (10)$$

693

694 Inserting this expression for $l_{\text{H}_2\text{O}}$ in Equation 6 yields, after rearranging, a semi-empirical
695 expression for k_{leaf} in terms of the leaf morphological traits $d_x:d_y$ and T_L :

696

$$697 \quad k_{\text{leaf}} = \frac{7140}{\left(\frac{T_L^2}{4} + 0.078 \cdot \left(\frac{d_x}{d_y}\right)^2 \cdot T_L^2\right)^{0.63}} \quad (11)$$

698

699 The expression above is subsequently used in an inverse approach to quantify the effect of changing
700 $d_x:d_y$ on photosynthesis as explained in the following.

701

702 We rearranged the Fick's law expression for stomatal diffusion of CO_2 to estimate the time
703 integrated stomatal conductance to CO_2 ($\overline{g_{\text{sc}}}$):

704

$$705 \quad \overline{g_{\text{sc}}} = \overline{A} \cdot (\overline{c_a} - \overline{c_i}) \quad (12)$$

706

707 Our measurements of $\delta^{13}\text{C}_{\text{leaf}}$ together with our approximation of $V_{\text{cmax}25}$ provide information on $\overline{c_i}$
708 and \overline{A} (Equations 3 and 4), which allow us to calculate $\overline{g_{\text{sc}}}$ for all our species-site combinations
709 individually.

710

711 The time-integrated transpiration (\overline{E}) of each species-site combination was subsequently
 712 calculated as:

$$714 \quad \overline{E} = 1.6 \cdot \overline{g_{sc}} \cdot (w_i - w_a) \quad (13)$$

715
 716 For w_i we assumed saturation at the annual average temperature for each site, and for w_a we used
 717 the annual average humidity for each site, based on the climate data from Atlas of Living Australia.
 718

719 With \overline{E} and k_{leaf} known, we subsequently approximated the time-integrated hydraulic pressure
 720 gradient across the leaf ($\Delta \overline{\Psi}_{leaf}$) for each species-site combination as:

$$722 \quad \Delta \overline{\Psi}_{leaf} = \frac{\overline{E}}{k_{leaf}} \quad (14)$$

723
 724 We then calculated the leaf hydraulic conductance of the theoretical optimal hydraulic architecture
 725 (k_{leaf_opt}) with $d_x:d_y = 1$, in combination with the observed T_L using Equation 11. The transpiration
 726 of this theoretical optimal hydraulic architecture (E_{opt}) was then calculated assuming that $\Delta \overline{\Psi}_{leaf}$
 727 remains unchanged from the value obtained using Equation 14:
 728

$$729 \quad E_{opt} = k_{leaf_opt} \cdot \Delta \overline{\Psi}_{leaf} \quad (15)$$

730
 731 The stomatal conductance to water vapour associated with this theoretical optimal leaf morphology
 732 (g_{sw_opt}) was calculated as:
 733

$$734 \quad g_{sw_opt} = \frac{E_{opt}}{(w_i - w_a)} \quad (16)$$

735
 736 The Rubisco-limited photosynthesis associated with the theoretical optimal leaf morphology (\overline{A}_{opt})
 737 is modelled from Fick's law assuming that $g_{sc_opt} = g_{sw_opt}/1.6$:
 738

$$739 \quad \overline{A}_{opt} = g_{sc_opt} \cdot (c_a - c_{i_opt}) \quad (17)$$

740
 741 In Equation 17, the leaf interior CO₂ concentration c_{i_opt} is unknown, but a second expression for
 742 steady-state photosynthesis as a function of c_i is obtained from the biochemical model of Farquhar
 743 et al. (1980) for Rubisco-limited photosynthesis while accounting for photorespiration (von
 744 Caemmerer, 2000) and mitochondrial respiration in light, as described in Equation 4. The two

745 expressions for steady-state photosynthetic rate $\overline{A_{opt}}$ were iteratively solved for $c_i = c_{i_opt}$ with a
746 prescribed c_a of $380 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, reflecting the atmospheric partial pressure of CO_2 in 2003
747 (Keeling and Whorf, 2004), using Matlab (version R2015b).

748

749 The above approach allows us to quantify the relative benefit of reducing $d_x:d_y$ below unity on
750 Rubisco-limited photosynthesis (δA [%]) for each species-site combination as:

751

$$752 \quad \delta A = \frac{\overline{A} - \overline{A_{opt}}}{\overline{A}} \cdot 100 \quad (18)$$

753

754 We tested whether the average δA of our species-site combinations deviated from zero using a
755 one-tailed Student's t-Test at a 5% significance level.

756

757 A sensitivity and uncertainty analysis of this model is provided in the Supplemental Material by
758 exploring the effect of variability and uncertainty in model input parameters (Table S5) on
759 modelled photosynthesis (Fig. S8) and δA (Fig. S9).

760

761 Supplemental Material

762 Table S1. Overview of the species, location, stomatal distribution and climate variables.

763 Figure S1. Relationship between vein depth and leaf thickness

764 Table S2. Standardised major axis (SMA) regression statistics for Figure S1

765 Figure S2. Relationship between vein length per unit area and the inter-vein distance

766 Figure S3. Inter-vein distance to vein-epidermal distance ratio and site-specific aridity index of
767 hypostomatous versus amphistomatous species.

768 Table S3. Regression models for the relationship between the vein-epidermal distance (d_y) and the
769 inter-vein distance (d_x)

770 Figure S4. The effect of artificial aging on eucalypt leaf area and thickness.

771 Figure S5. Statistics with correction for the classification based on Nicole (2015)

772 Figure S6. Paradermal image and cross section of a eucalypt leaves

773 Figure S7. Cleared leaf images of four species with high vein densities

774 Table S4. Data used to model V_{cmax} from N_{area} and LMA

775 Table S5. Parameter ranges used in the model sensitivity and uncertainty analyses

776 Figure S8. Model sensitivity analysis

777 Figure S9. Model uncertainty analysis

778

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785 **Tables**

786 Table 1. Standardised major axis linear regression results of functional traits from all sampled leaves

Trait	Abbrev	VLA	$d_x:d_y$	N_{area}	Δ_{leaf}	LMA	L_T
Vein density	VLA	-	***	***	***	***	***
Ratio of inter-vein to vein-epidermis distance	$d_x:d_y$	0.76(-)	-	***	***	***	***
Area-based nitrogen concentration	N_{area}	0.17(+)	0.42(-)	-	***	***	***
Leaf carbon isotope fractionation	Δ_{leaf}	0.10(-)	0.18(+)	0.35(-)	-	***	***
Leaf dry mass per area	LMA	0.22(+)	0.57(-)	0.63(+)	0.34(-)	-	***
Leaf thickness	L_T	0.13(+)	0.58(-)	0.44(+)	0.14(-)	0.73(+)	-

787 $P < 0.001$ ***788 r^2 values are shown in the bottom half of the table

789 + Positive correlation, – negative correlation

790 Data were log-transformed prior to analysis.

791 Table 2. Standardised major axis linear regression results of functional traits from leaves sampled for stomatal morphology

Trait	Abbrev	VLA	$d_x:d_y$	L_{gc}	L_p	D_s	g_{smax}	L_T
Vein density	VLA	-	***	***	***	*	**	**
Ratio of inter-vein to vein-epidermis distance	$d_x:d_y$	0.81(-)	-	***	***	*	*	***
Guard cell length	L_{gc}	0.33(+)	0.44(-)	-	***	***	NS	***
Stomatal pore length	L_p	0.49(+)	0.52(-)	0.80(+)	-	***	NS	***
Stomatal density	D_s	0.13(-)	0.12(+)	0.57(-)	0.60(-)	-	**	NS
Anatomical maximum stomatal conductance	g_{smax}	0.20(+)	0.12(-)	0.00	0.03	0.14(+)	-	NS
Leaf thickness	L_T	0.26(+)	0.68(-)	0.32(+)	0.29(+)	0.06	0.01	-

792 $P > 0.05$ NS, $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***

793 r^2 values are shown in the bottom half of the table

794 + Positive correlation, – negative correlation

795 Data were log-transformed prior to analysis.

796 **Figures and legends**

797 Figures and figure legends are submitted as separate files

798

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