Investigating the functional and adaptive significance of leaf size and shape variation in *Jamesbrittenia* (Scrophulariaceae (s.s). tribe Manuleae). An experimental and comparative approach.

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Abstract

The adaptive significance of variety in leaf forms remains a mystery for many plant groups. This study aimed, using a combined experimental and comparative approach, to investigate the functional significance of leaf size and shape variation, as well as the patterns of leaf form variation in the genus *Jamesbrittenia* within the context of phylogenetic history. Leaf sizes 63-measured *Jamesbrittenia* varied between 0.006cm^2 (in *J. microphylla*) and 6.52cm^2 (in *J. megaphylla*). Correlations between leaf form and the environment suggest leaf size and shape are primarily adapted to water availability, with only leaf dimension being significantly associated with temperature, while, soil fertility shows no relationship with leaf size. Life history is important, however, as broader leaved annuals and species with shorter-lived leaves are associated with more arid habitats. Results of the experimental trials suggest that the primary function leaf size and shape reduction is to reduce water loss, and not to increase heat shedding. While larger leaves transpire more on a leaf-by-leaf basis, transpiration may be higher in broader leaved species at the whole-plant due to higher total plant transpiration. Thus it is suggested a reduction in leaf size and dimension in *Jamesbrittenia* is an adaptation to more arid environments. Alternatively a change in life history may enable a plant to escape harsh periods and capitalise on favourable times. A small- to intermediate-leaved, perennial ancestor is inferred for *Jamesbrittenia*, which was associated with arid regions in either the summer or winter rainfall regions of southern Africa. Shifts to an annual life history in *Jamesbrittenia* are associated with a shift to drier habitats, particularly in the arid winter rainfall region of South Africa.

Introduction

South Africa is renowned for its high specific and functional diversity (Goldblatt and Manning, 2000). Associated with this large variety of life forms is a large range of climatic and environmental conditions. How this high habitat heterogeneity has influenced the proliferation of species and functional types in southern Africa is largely still a mystery (Verboom *et al* 2003, Hardy and Linder, 2005). It has been suggested that an understanding of the evolution of the broader southern African flora might best be gained through studying individual taxa and lineages that make up this diverse flora (Linder, 2005).

Jamebrittenia (Kuntze O.) is a diverse genus in the family Scrophulariacea, that is widely distributed throughout South Africa, Namibia, and neighbouring countries (Hilliard, 1994). *Jamesbrittenia* species are generally associated with nutrient poor soils, in a diversity of habitats, ranging from coastal scrub to grasslands, savannahs and arid deserts. The genus comprises mostly perennial shrubs; with the few annual herb species being confined to the arid, winter rainfall region of northern Namaqualand and southern Namibia (Hilliard, 1994). *Jamesbrittenia* displays wide variety in leaf morphologies. Leaf sizes range from small, pseudofasciculate to large, broad leaves; larger leaves generally associated with an annual life history. Leaf shape also varies greatly among species. The variation in leaf form in *Jamesbritteia* provides a unique opportunity for testing the adaptive significance of morphological variation and how this morphological variation may have arisen. Little work however, has been done to characterise the variation in leaf form in the genus, or understand the functional significance of this variation in leaf form in relation to the environments they occur in.

As Winn (1999) points out the functional fit between leaf form and the environment remains unclear. Variation in leaf form may be adaptive for two, non-exclusive purposes. Firstly, the interaction between leaf size and shape and wind speed produce boundary layer resistance (Givnish and Vermeij, 1976, Martin et al, 1999). This resistance is proportional to the thickness of the boundary layer, which is calculated as:

$$B = \frac{4* \sqrt{d}}{ws} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eqn 1;}$$

where B is boundary layer, d is leaf dimension, or the diameter of the largest circle that can be fitted into the leaf, and ws is wind speed (Nobel, 1974).
Thicker boundary layers provide more resistance to the transfer of heat and water from a leaf to its environment (Martin *et al*, 1999). Thus leaves with larger dimensions (which are less dissected) have less capacity for heat shedding to the environment through convective and transpirative cooling. Also, more water can be exchanged through a thinner boundary layer, allowing smaller leaves to lose more water through evapo-transpiration. Since large leaves may generally be expected to have lower boundary layer conductance, due to their larger dimensions, their capacity for heat shedding may be less than that of smaller leaves. Similarly, reduced water transfer across a thicker boundary layer may enhance water efficiency. Although larger leaves may be more water efficient on a per-leaf basis, if total leaf area on large-leaved plants is greater than individuals on with smaller leaves (Cunningham and Strain, 1969; Evenari *et al*, 1971) larger-leaved plants may lose more water on a per-plant basis. Leaf form may also be adaptive in the role transpiration plays in nutrient uptake. Because smaller leaves should transpire more, they may be able to access more nutrients by mass flow (Barber *et al*, 1962) due to greater pull of nutrients through the soil. In this case, temperature and water availability may be important -transpiration rates may differ with leaf size at different temperatures and water availability.

Leaves provide exceptional opportunities for comparative studies (Givnish, 1987). If a functional fit between leaf form and environment exists, then it is expected that leaf traits should correlate with the environmental conditions they occur in. These correlations provide tools for identifying current patterns of environment and morphological trait relatedness (Givnish, 1987). Consequently, numerous studies have attempted to relate observed variations in leaf form to specific environmental conditions, such as rainfall (e.g. Wolfe, 1995; McDonald *et al* 2003), temperature (e.g. Givnish, 1984; Núñez-Olivera *et al*, 1996) and soil fertility (e.g. McDonald *et al*, 2003). Results from these studies are mixed, but in general the trends are towards larger leaves in colder, wetter, more nutrient rich environments. Often these studies are limited by their capacity to account for fact that cross-species data sets generally do not comprise independent and identically distributed data points (Garland *et al*, 1999). Phylogenetic comparative methods (historical correlations) allow traditional topics in comparative biological relationships to be addressed with greater thoroughness, including whether physiological phenotypes, for example leaf traits, vary predictably in relation to environmental characteristics (Garland *et al*, 1999). Also, knowledge of the phylogenetic relationships between taxa allow inferences to be made about the evolution of morphological traits variation, at the same time providing an indication of the environments under which these traits have arisen. For example, shifts in life history have been associated with changes in rainfall and nutrient availability (e.g. Verboom *et al*, 2003; Verboom, 2004).

This paper set out to investigate the functional significance of leaf size and shape in *Jamesbrittenia*, and investigate patterns of leaf variation and function, using a joint experimental and correlative approach. This approach allowed for the empirical testing of leaf size and shape function, at the same time providing insights into the relationships between leaf form and the environment and, ultimately, what has driven the evolution of leaf size and shape in *Jamesbrittenia.*In the context of a phylogentic evaluation of past changes in leaf size and shape, as well as the environments in which these changes have occurred, three specific hypotheses will be tested. Firstly, that leaf size reduction is an adaptation for increased coupling of leaf temperature to air temperature. Smaller leaves will be better adapted to occurring in hotter, drier (less capacity for transpirative cooling) environments. Secondly, reducing leaf dimension is an adaptation for increasing transpiration, which in turn facilitates nutrient uptake by mass flow. Thus smaller leaves are expected in more nutrient-poor environments, or in environments with high temperatures during wet periods –as small leaves are expected to transpire more at low temperatures. Finally, a reduction in leaf area is associated with a reduction in total leaf area, and so is an adaptation for minimising water loss on a whole plant scale. Therefore plants with smaller leaves, and therefore less total leaf area, will be associated with more arid environments

Methods

Species selection and phylogeny

A phylogeny of 63 *Jamesbrittenia* species from Namibia and South Africa was obtained from Moncrieff (unpublished, Appendix 1), and was used for the reconstruction ancestral leaf and environmental states, and correlative analyses. The tree was based on two plastid markers (*rps16, psbA-trnH*) and one nuclear marker (*Gscp*). Because of the lack of climatic and other environmental data for Namibian species, only South African species were used for correlative analyses and environmental ancestral state reconstructions.

From the phylogeny, emerged the presence of two clades. The first clade is comprised of predominantly annual species from the winter rainfall region of northern Namaqualand, while the second is made up of predominantly perennial species from the summer rainfall region of eastern and southern South Africa. As a consequence, clades (the 'winter' and 'summer' rainfall clades, see Appendix 1) were evaluated separately during the correlative analyses.

Leaf morphology and life-history characterisation

Leaf morphological characters were measured for 67 *Jamesbrittenia* from specimens obtained from the following herbaria: NGB, PRE and BOL (see Appendix 2). Leaf size (leaf area) and shape (leaf dimension) were calculated by photographing a single, fully expanded leaf from three separate specimens. Where possible specimens collected from a range of localities were selected in order to account for geographic variation in leaf form within species. Leaf photographs included a reference square of known area ($1cm^2$) and a ruler for calculating leaf area and leaf dimension respectively.

Photographs were imported into Adobe Photoshop® 7.0 (Adobe Systems Inc, USA) where the 'Magic Wand Tool' was used to calculate the number of pixels in the reference square (i.e. the number of pixels in $1cm^2$) as well as in the leaf. Leaf area was then calculated by dividing the number of pixels in the leaf by the number of pixels in the reference square, to give leaf area in cm^2. Leaf dimension (cm) was measured by square-rooting the diameter of the largest circle that could fit within the margins of the leaf.

Specific Leaf Areas (SLA) were also calculated for leaves obtained from the un-mounted collections of G.A. Verboom. Leaves that had already been photographed for area and dimension calculations were weighed on a four-point Mettler AE200 electronic balance (Mettler-Toledo, Greinfensee, Switzerland).

Since species vary in their leaf phonologies and life histories (annual versus perennial), it is important to correlate leaf size and shape to the environmental conditions that relate to when leaves are present. Because of the relationship between SLA and leaf longevity (higher SLA-lower longevity), species where characterised as either leaf-deciduous or evergreen based on recorded and inferred SLA values. For the species classified as leaf-deciduous, as well as the annuals, flowerings and collection dates were used as proxies for the period of the year leaves were present. Leaf traits were then correlated with the environmental variables of that period.

Environmental trait characterisation and correlative analysis

Habitat parameters were estimated for South African species using GIS-based techniques. Geo-referenced species distributions, based on localities of herbarium specimen locations, were overlaid onto grid maps of agrohydrological data for South Africa (from Schultz, 1997). Using the Grid Analyst tool in Arcview© 3.3 (ESRI Inc), environmental data for each species was extracted. For annuals and putative leaf-deciduous species values of environmental variables were calculated based on the time of year leaves were expected to be present.

In total, six habitat parameters were considered. Firstly, to test the relationship between leaf traits and water availability, average mean annual rainfall (mm) and rainfall in the driest month (mm, represents the lowest limit of water availability) values were calculated for each species. Secondly, to test the relationship between nutrient availability and leaf size and shape, soil fertility, and maximum temperature in the wettest month (°C) were determined for each species. Finally, to test the relationship between temperature and leaf traits, values for the maximum temperature of the hottest month (°C) and rainfall in the hottest month (mm, indicates water available for transpirative cooling) were calculated.

Historical and ahistorical correlations between leaf traits and environmental conditions were determined using CACTUS 1.13 (Schwilk, 2001). Both forms of correlation were done to attempt to account for the non-independence of variables associated with ahistorical correlations. The winter and summer rainfall clades were analysed separately, due to the differences in life history between them, i.e. the winter rainfall clade comprised of predominantly annuals, while the summer rainfall clade comprised of mostly perennial species.

The two clades differed in the number of species they contained, the winter rainfall clade (9) being smaller than the summer rainfall clade (31). Sample size is important in determining the strength and significance of correlative relationships (ZAR, 1984). A Monte Carlo random sampling procedure was conducted to test for the effect of sample size on the strengths of the relationships obtained for significant historical correlations between the leaf and environmental traits of summer rainfall clade taxa. A distribution of R^2 values was obtained from a randomisation (1000 replicates) of subsample correlations of summer rainfall clade taxa (with a sample size equal to the sample size of the winter rainfall clade). A p-value was calculated for the probability of obtaining an R^2 value equal to or greater than the R^2 value obtained when all taxa in the summer rainfall clade were considered (i.e. with correlations based on larger sample sizes).

Ancestral state reconstructions

In order to infer the ancestral leaf form and associated historic environmental conditions in *Jamesbrittenia* ancestral leaf and environmental states were reconstructed using one of the trees obtained from Moncrieff (unpublished, see Appendix 1), using MESQUITE 2.0 (Maddison and Maddison, 2007). Continuous variables (leaf area and mean annual rainfall) were reconstructed using squared-change parsimony. Life history was coded discretely and optimised under maximum likelihood using a single-parameter Markov *k*-state model (Lewis, 2001). Branch lengths were assumed equal for all reconstructions

Experimental trials

On-plant leaf temperatures were measured for nine *Jamesbrittenia* species obtained from the greenhouse at the Kirstenbosch National Botanic Gardens, South Africa. The species were selected (Appendix 3) in order to test the effect of a range of leaf sizes on leaf temperature. Plants were kept in well-watered pots (all of the same size), in a phytotron growth chamber, set at 25°C and 50% humidity for 24 hours prior to measuring. The leaf temperatures of 20 fully expanded leaves per species were measured using an LS infrared temperature gun (Optris Inc. Berlin, Germany).

Measurements were taken on leaves as close to horizontal as possible, in order to reduce the variance in the amount of light absorbed between leaves. Mean individual leaf area was measured following the same procedure as above (see *Leaf morphology and life-history characterisation*)

In order to test for the effect of leaf size on transpiration rate, and leaf size, ten –six *Jamesbrittenia* and four other species (see Appendix 4)- species displaying a range of leaf sizes were selected. The four non-*Jamesbrittenia* species were included to increase the range of leaf sizes, towards the upper end of the scale. Plants were again obtained from Kirstenbosch National Botanic Gardens, South Africa and placed in a phytotron growth chamber. Transpiration measurements (on three shoots per species) were done in a Conifer-Chamber (LI6400-05) using a twin-channel LI-COR 6400 InfraRedGasAnalyser (IRGA, Li-Cor Inc, Lincoln, NB, USA). The air temperature of the conifer-chamber was altered to three different levels (18°C, 24°C and 32°C) to test for the effect of ambient temperature on leaf-transpiration rate. Mean individual leaf areas, as well as the amount of leaf area per shoot length were measured following the same procedure as above (see *Leaf morphology and life-history characterisation*) in order to test the relationship between transpiration rate and individual leaf area, as well as transpiration rate and leaf area per shoot length- as a measure of the overall transpiration by a plant.

Results

Leaf morphology and evolution in Jamebrittenia

Leaf areas and dimensions of the 67 extant *Jamesbrittenia* species that were measured varied greatly (Table 1). Leaf sizes ranged between 6.51cm^2 in *J. megaphylla* and 0.0063cm^2 in *J. microphylla*. In the same way, leaf dimension also showed large variation, raging between 2.63cm in *J. major* and 0.04cm in *J. microphylla*. In general leaf areas and dimensions were larger in species from the winter rainfall clade. A strong trend for leaf dimension to increase with increasing leaf area was also found (R^2= 0.89,p-value< 0.05; Figure 1).

The ancestral leaf size in *Jamesbrittenia* is small to intermediate (0.87cm^2, Figure 2). Shifts in leaf size are associated with shifts to higher rainfall (Figure 2 and 3).

Reconstruction of rainfall seasonality (Figure 4) is ambiguous, suggesting either a summer or winter rainfall origin. It does, however, generally support the division of the two major clades according to the season in which rain falls.

A perennial life history is reconstructed as ancestral in the genus (Figure 5), with annualness having arisen on at least three separate occasions. As with leaf size, life history shifts appear to be associated with shifts in rainfall, annuals having arisen in association with drier environments.

Correlations

The historical and ahistorical correlations show the relationships between the observed leaf sizes and shapes, and their associated environmental variables.

In general, there is consistency between ahistorical and historical correlations (Table 2). In the winter rainfall clade, incorporation of phylogenetic relationships tended to lower R^2 values. However the strength of the only significant relationship in the clade –between leaf dimension and mean annual rainfall- increased when phylogenetic history was accounted for (Table 2). For the summer rainfall clade, rainfall in the driest month, rainfall in the hottest month and mean annual rainfall correlated significantly with leaf dimension and leaf area, when phylogeny was taken into account, with relationships involving rainfall in the driest month being generally strongest.

It is important to note that the direction of the relationship between leaf traits and mean annual rainfall changed between clades (Figure 6). In the winter rainfall clade, rainfall declined with an increase in leaf size and dimension (R= -0.70, p-value= 0.053), while in the summer rainfall clade, the opposite trend was seen (R= 0.42 , p-value= 0.018).

Relationships in the summer rainfall clade were stronger than in the winter rainfall clade (Table 2). Considering history did strengthen some relationships, but weakened others. Both rainfall in the driest month and temperature in the hottest month became statistically significant when phylogenetic history was considered. Since the number of winter rainfall clade species sampled (nine in total) was less than in the summer rainfall clade (31), the differences in significance between these lineages could be a result of sample size differences. The results of the Monte Carlo (Table 3) test indicate that this possibility cannot be rejected at the $\alpha = 0.05$ level.

Multiple regression analysis for all environmental variables and leaf size and dimension did not yield a significant relationship for the winter rainfall clade. (p-values> 0.05) In the summer rainfall clade however, both leaf area ($R^2 = 0.57$, p-value<0.01) and leaf dimension ($R^2 = 0.44$, p-value< 0.02) correlated significantly with all environmental variables. Rainfall in the driest month was the only variable that correlated significantly with leaf area (when the variance of all other independent variables was held constant) and was the strongest predictor of leaf area (B= 0.099, p-value= 0.0048).

Experimental trials

The on-plant leaf temperatures of the eight *Jamesbrittenia* species measured using the Temperature Gun at 25°C differed significantly ($F_{17,162} = 16.26$, p-value<0.005, Figure 7). However, the relationship between leaf area and leaf temperature (Figure 9) was not significant.

Transpiration rates differed significantly across all three ambient temperatures between the ten species analysed (p<0.005). Transpiration decreased with an increase in leaf size, larger-leaved species, however -with more leaf area per shoot-length-transpired at higher overall rates than smaller leaved species.

Transpiration rate correlated significantly with individual leaf area ($R_{18}^2 = 0.62$, $R_{24}^2 = 0.46$, $R_{32}^2 = 0.44$, p-values< 0.05), at all three ambient temperatures, for the ten species of plants measured (Figure 9). The range of transpiration rates between ambient temperatures was greatest for smaller leaves. The transpiration rate of the smallest leaved species, *J. stellata*, increased by 154%, while transpiration rate of the largest species, *Barleria sp.*, only increased by 110%. There was a strong positive relationship, in the plants used for the transpiration measurements, between shoot area (leaf area per shoot) and individual leaf area ($R^2 = 0.94$, p-value= Figure 12). Also, transpiration rate per shoot length was significantly correlated with individual leaf area ($R_{18}^2 =$, $R_{24}^2 =$, $R_{32}^2 =$, p-values< 0.05), increasing as leaf area increased, across all three ambient temperatures(Figure 11).

Discussion

Previous studies on the role and evolution of leaf traits have focussed either on the empirical testing of the function of leaf form (Vogel, 1968; Parkhurst and Loucks, 1972; Givnish and Vermeij, 1976) or on attempting to relate patterns of leaf form variation to variation in environments (Givnish, 1984; McDonald *et al*, 2003). In order for a character to be considered adaptive, it must be shown to be functional in an organism's present environment and to have been generated through the action of natural selection for its current biological role (Baum and Larson, 1991). This study attempted a novel approach for the investigation of the adaptive significance of leaf form, particularly size and shape, and to explain patterns of leaf form variation in *Jamesbrittenia* in relation to the environment. It did this by combining a comparative and experimental approach. The results from both sets of analyses suggest an adaptive role for leaf size and shape, and suggest how these adaptations may have shaped the evolution of the genus.

The ancestor of *Jamesbrittenia* appears to have been found in the arid boundary zone between summer and winter rainfall regions in South Africa, possibly in northern part of Namaqualand or southern Namibia (Figures 3 and 4). This ancestor was more than likely a perennial plant (Figure 5), with a small to intermediate leaf size (Figure 2). As lineages split, small-leaved perennial species were favoured in more mesic environments, while –particularly in the arid winter rainfall region- broader-leaved annual species were favoured. Numerous other instances of annuals evolving in dry regions have been noted. Arcibald *et al* (2005) found the evolution of an annual life history in *Zaluzianskya* to be associated with more arid conditions, as did Verboom *et al* (2003) in the grass genus *Ehrhata*.

Not only does leaf form appear to correlate historically with the environment, the results of the comparative analyses suggest that certain environments favour certain leaf sizes and shapes. Correlative analyses suggest that both water availability (mean annual rainfall and rainfall in the driest month) and temperature are related to leaf size and shape (Table 2). The strengths and nature of these relationships differed between the summer and winter rainfall clades, also depending on whether phylogenetic history was considered. The influence of sample size on significance of the relationships cannot be discounted (Table 3). Interestingly, the largest leaves in *Jamesbrittenia* are found in the driest habitats (Figure 6). The strength direction of the relationship between water availability (mean annual rainfall and rainfall in the driest month) and leaf size and shape is different between the two clades studied here (Tables 2 and 4, Figure 6). The perennials of the summer rainfall clade fit the well-documented trend towards smaller leaves in drier environments (Givnish 1987; Cunningham, Summerhayes and Westoby 1999, McDonald *et al*, 2003). In contrast, the trend in the winter rainfall clade is for leaf size to increase with decrease in water availability (Table 2, Figure 6). This difference in relationship can be explained by the difference in the life history strategies associated with each clade. The large-leaved species in the driest areas are mostly the annual and putative leaf-deciduous perennial species found in the winter rainfall clade. One possible explanation as to why these species are able to survive in these arid regions is their ability to respond rapidly to changes on water availability. Annuals may persist in the environment for long periods of time as seeds, only producing leaves at times when enough water is available to do so. Leaf-deciduous species may be able to reduce total leaf area and thus reduce water loss sufficiently (see below) to cope with dry conditions. These comparative results highlight the importance of water availability, over temperature and nutrient availability, in determining leaf size and shape. The question remains, as to why, in the case of evergreen perennial *Jamesbrittenia* species, smaller leaves are adapted to drier environments.

Leaves in *Jamesbrittenia* display a wide range of sizes and shapes (Table 1). The fact that leaf size and shape in the genus are so closely linked (Figure 1), makes it is hard to evaluate the functional significance of each in isolation. The results of the experimental trials point towards leaf size and shape being driven primarily by their function of limiting water loss from a plant, while temperature may only play a minor role (Figure 8). The lack of a significant relationship between leaf area and leaf temperature (Figure 8) suggests that, in leaf size is not primarily adapted to temperature. This however, may be confounded by the biology of *Jamesbrittenia*. Hilliard (1994) has noted that many *Jamesbrittenia* tend to 'select' shaded places in their habitats. Vogel (1968) has shown that the differences in temperature between sun leaves and shade leaves can be large. Thus microhabitat selection by certain

Jamesbrittenia species might function as a mechanism to keep leaves cooler than ambient, thus allowing larger leaves in hotter environments than expected.
Water loss from plant leaves is governed by the operation of boundary layer conductance and stomatal conductance in series (Martin *et al* 1999). In this study, smaller leaves transpired more (Figure 9), as predicted based on the boundary layer equation (Eqn 1) and by the model developed by Givnish and Vermeij, 1976). This may suggest that larger leaves are more water-efficient, and thus better suited for dry environments. Preston and Ackerly (2003) found that mean individual leaf area did not correlate significantly with water availability. Rather, water availability may limit the total leaf area of a plant (Parkhurst and Loucks, 1972). The relationship between leaf size and shoot area (Figure 10) however, and the fact that transpiration per shoot length was greater for larger leaved individuals (Figure 11), suggest that larger-leaved individuals may lose more water at the whole-plant level. Thus while small leaves may lose more water through thinner boundary layers, the relationship found in this study between plant total leaf area (leaf area per shoot length) and leaf size means that smaller-leaved individuals will be better adapted to more arid environments.

All results suggest that the primary function of leaf size and shape in *Jamesbrittenia* is to reduce water loss in arid environments. The correlation between LA and $\sqrt{D}$ suggests that the mechanism of leaf size reduction in *Jamesbrittenia* is to decrease the dimension of a leaf, i.e. by increasing its dissectedness. However life history plays a role, as the trend for larger leaves in wetter environments is reversed in annuals and species with low leaf-longevity, associated with high SLA. An annual life history may thus represent an alternative strategy for coping with aridity. The ability to grow quickly and capitalise on episodic rainfall events may allow annuals to escape harsher periods as seeds. In terms of the role of leaf size and shape in leaf cooling, it is possible that this relationship is blurred due to the microhabitat selection displayed by some *Jamesbrittenia* species. This study also highlights the importance of an understanding of species relationships and phylogenetic history, without which the clear differences between the summer and winter rainfall clades may have blurred any patterns.

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Figures and Tables

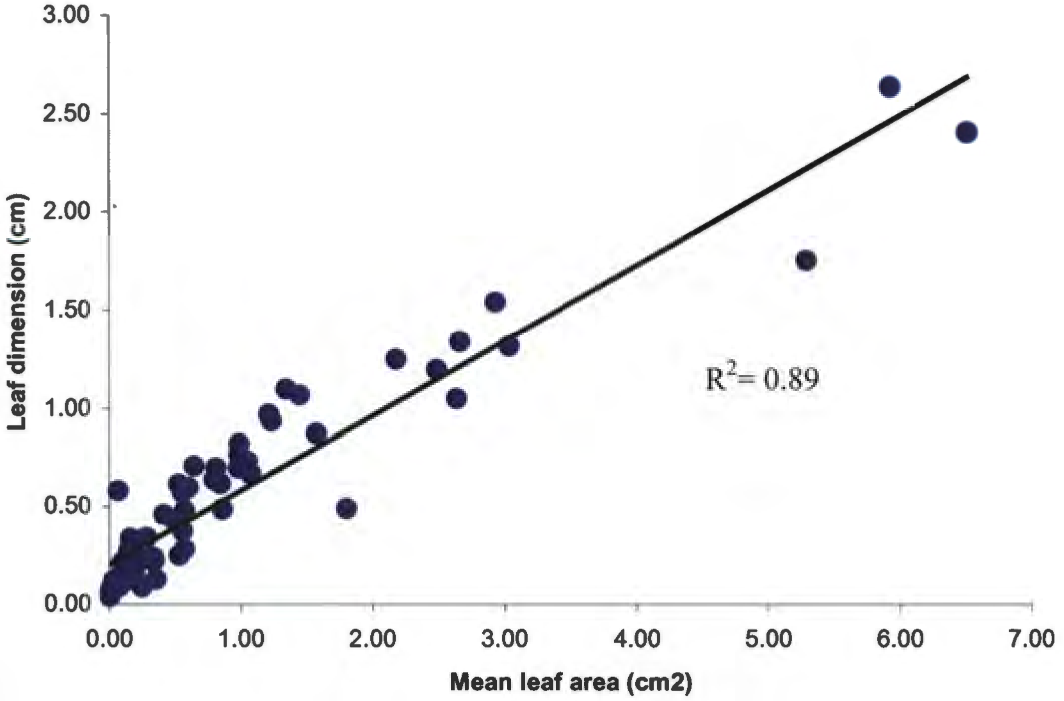


Figure 1. The relationship between the square of leaf dimension and mean leaf area, based on sampling of leaves from 67 *Jamesbrittenia* species.

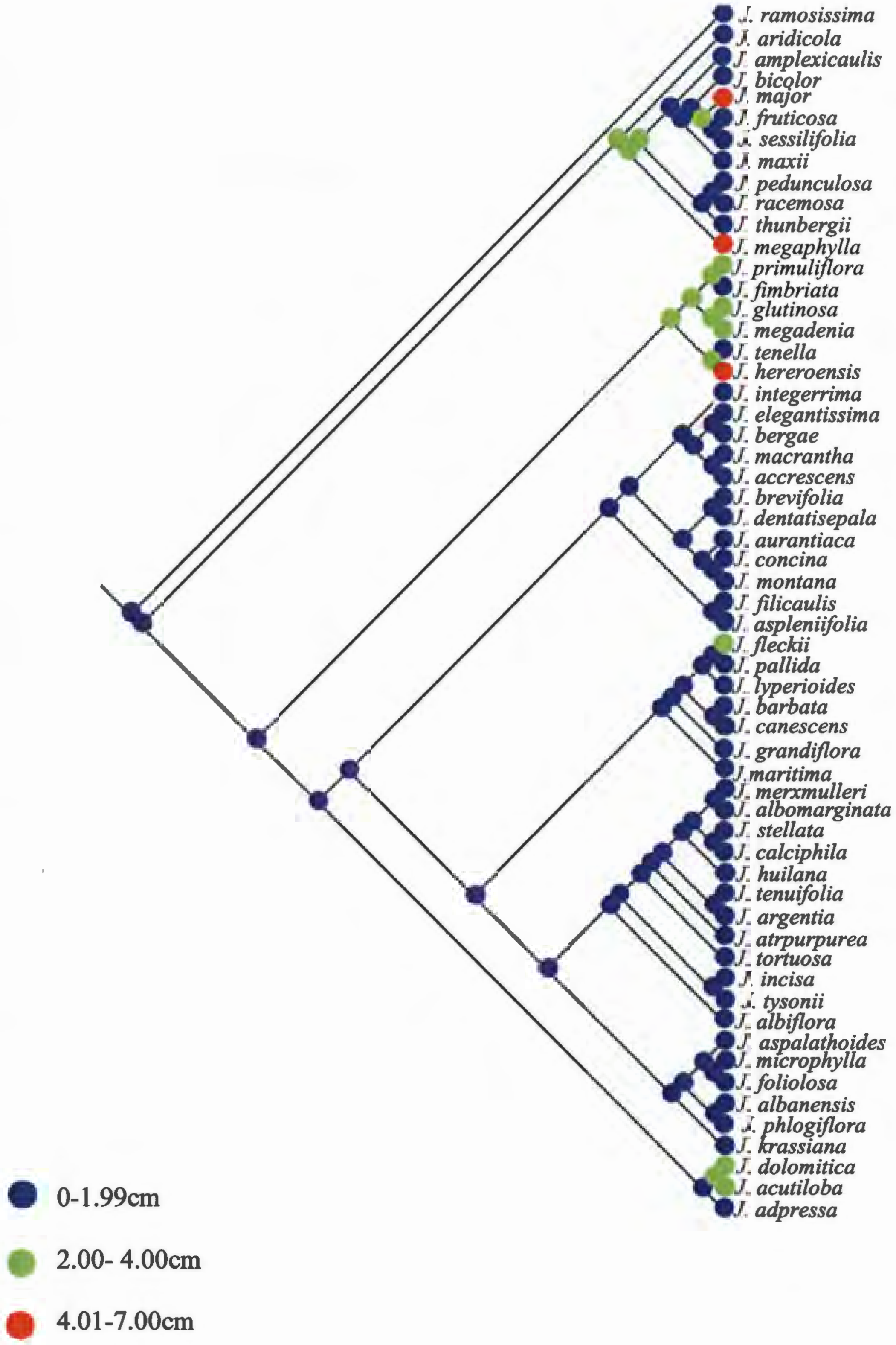


Figure 2 Parsimony reconstruction of leaf area onto one of 10 000 trees postrior distribution trees obtained from Moncrieff (unpublished). Characters were optimised using squared change parsimony in MESQUITE 2.0 (Maddison and Maddison, 2007). All branch lengths assumed equal.

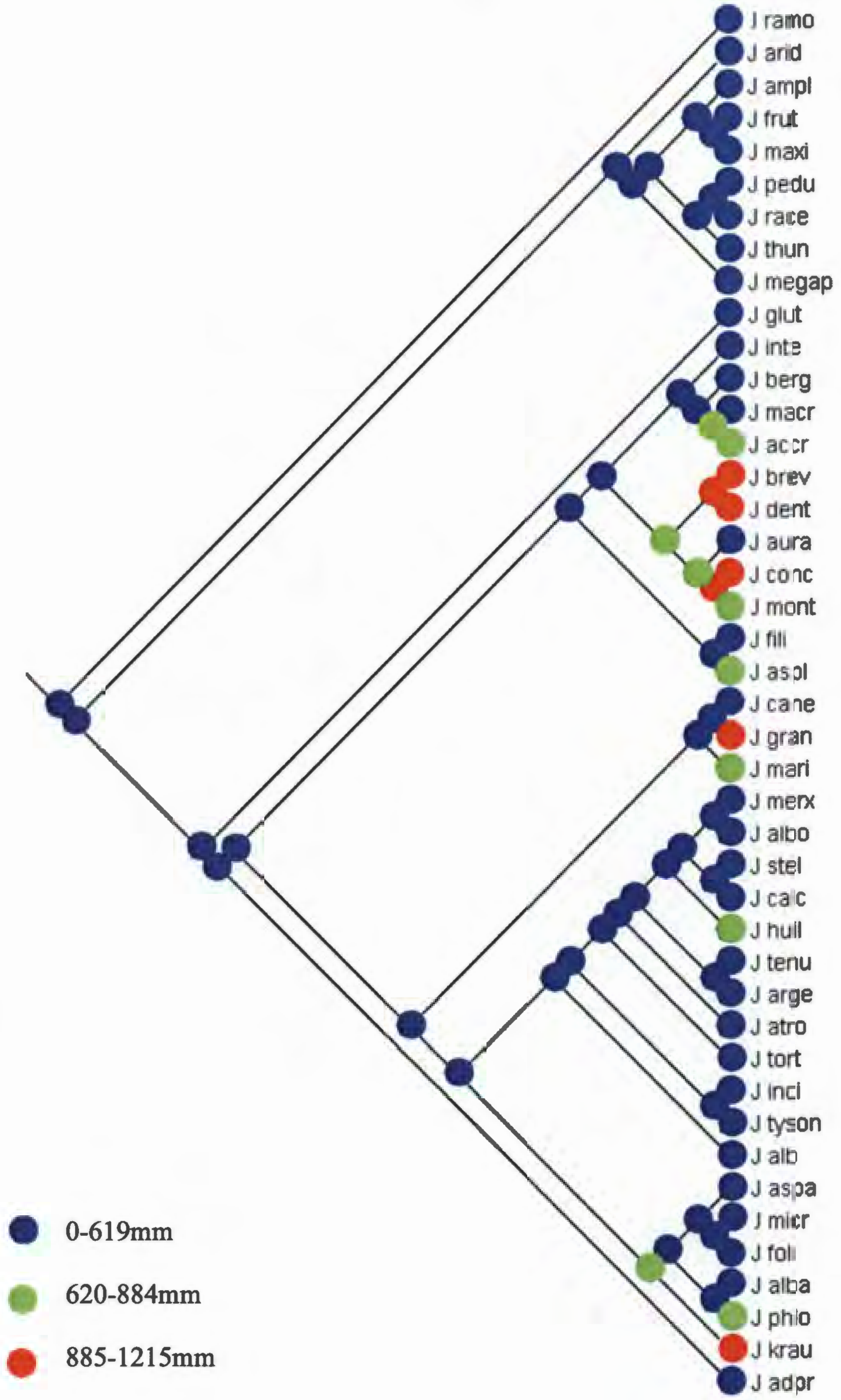


Figure 3. Parsimony reconstruction of Mean Annual Rainfall onto one of 10 000 trees posterior distribution trees obtained from Moncrieff (unpublished). Characters were optimised using squared change parsimony in MESQUITE 2.0 (Maddison and Maddison, 2007). All branch lengths assumed equal. (note:Abbriations correspond with full names in Figure 1 and Appendix 1)

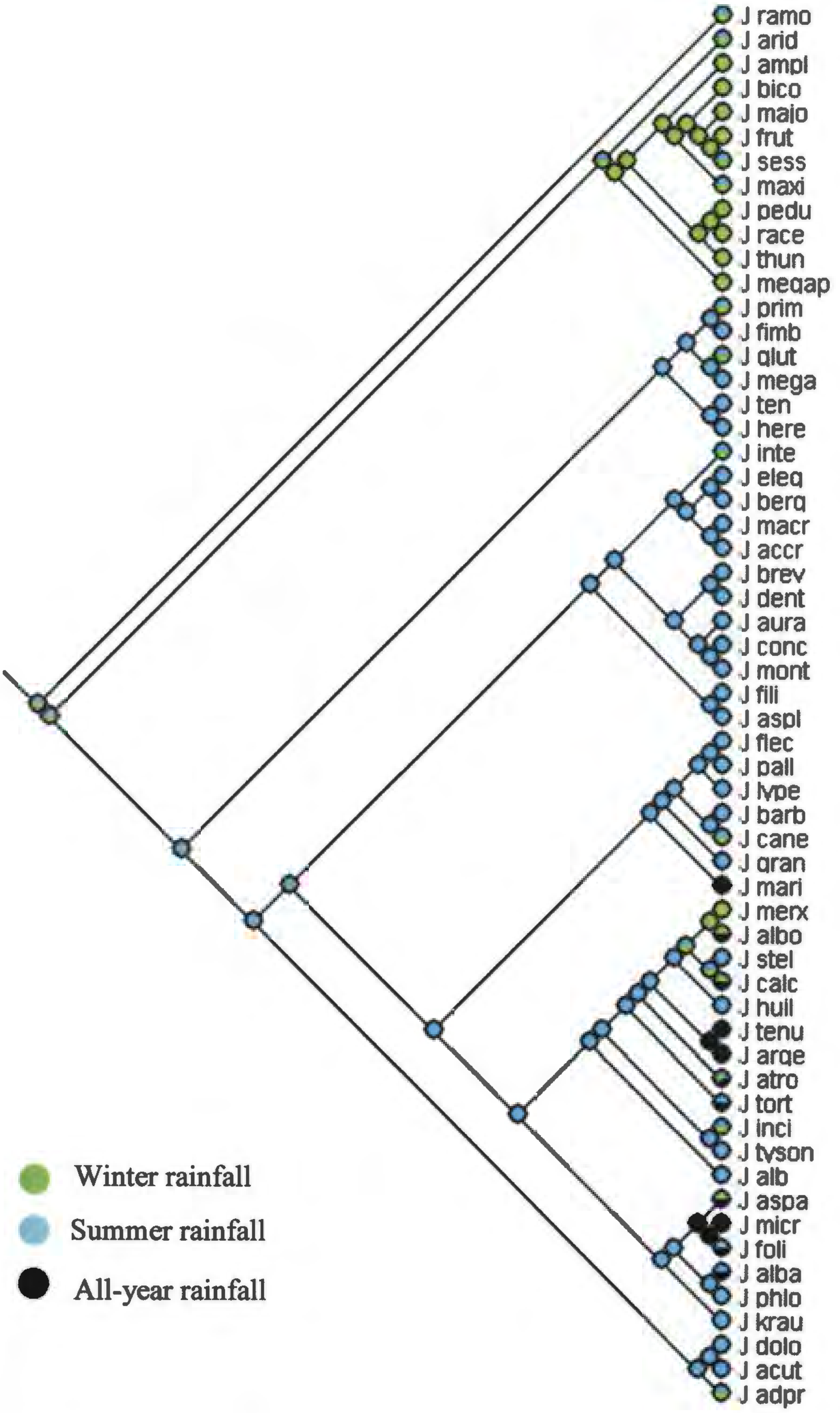


Figure 4. Parsimony reconstruction of rainfall seasonality onto one of 10 000 trees posterior distribution trees obtained from Moncrieff (unpublished). Characters were optimised using squared change parsimony in MESQUITE 2.0 (Maddison and Maddison, 2007). All branch lengths assumed equal. (note:Abbriations correspond with full names in Figure 1 and Appendix 1)

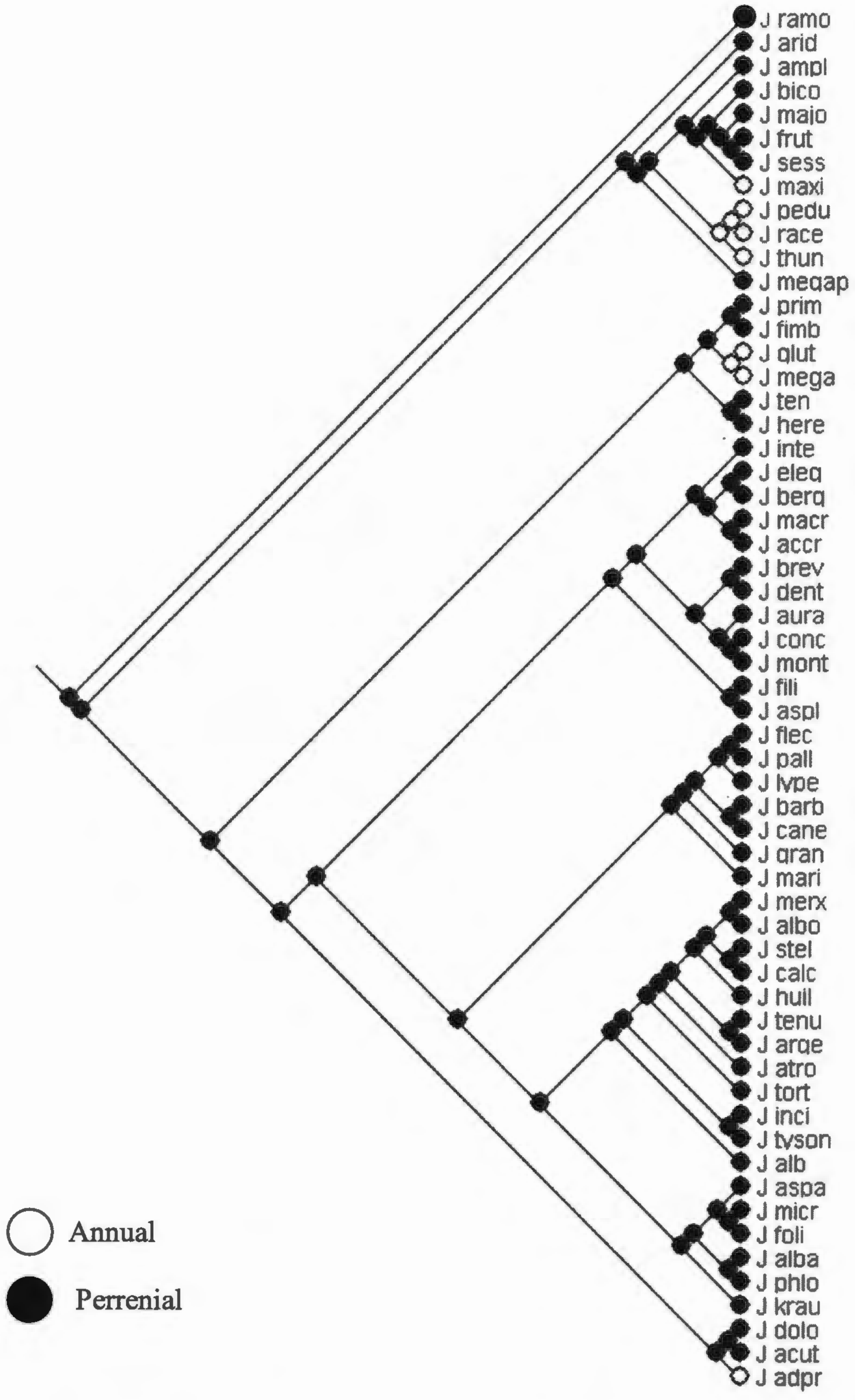


Figure 5. Maximum likelihood optimisation of life history onto one of 10 000 trees posterior distribution trees obtained from Moncrieff (unpublished). Optimised under maximum likelihood using a single-parameter Markov *k*-state model (Lewis, 2001). All branch lengths assumed equal. (note:Abbriations correspond with full names in Figure 1 and Appendix 1)

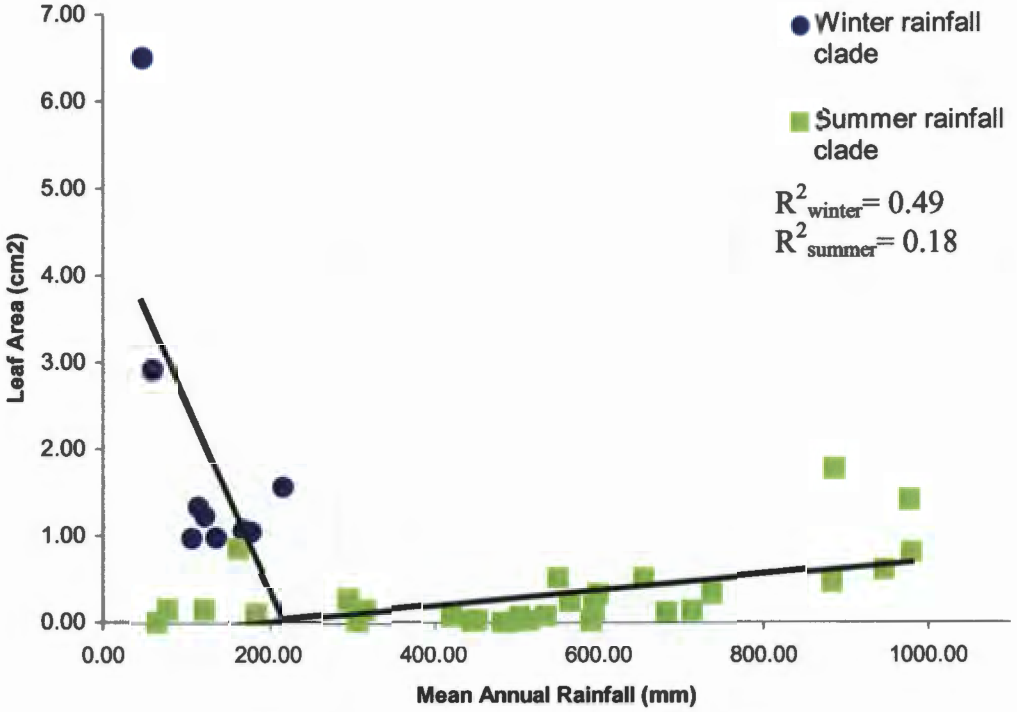


Figure 6. The importance of history. Relationship between leaf area and mean annual rainfall, highlighting the differences in relationship between clades. R_{winter}= -0.70, p= 0.053; R_{summer}= 0.42, p-value= 0.018)

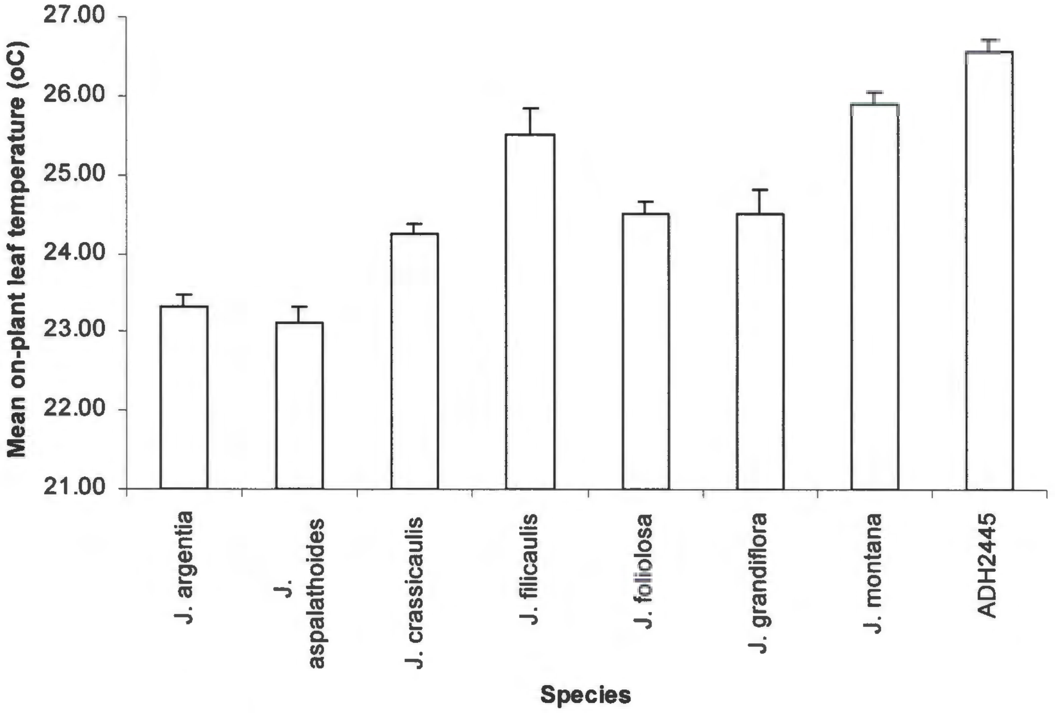


Figure 7. Mean-on plant temperatures for the eight *Jamesbrittenia* sampled (Appendix 3). Temperature measurements were taken with a temperature gun, with the plants housed in a phytotron unit set to 25°C and 50% humidity. Plants were watered prior to measurement being taken.

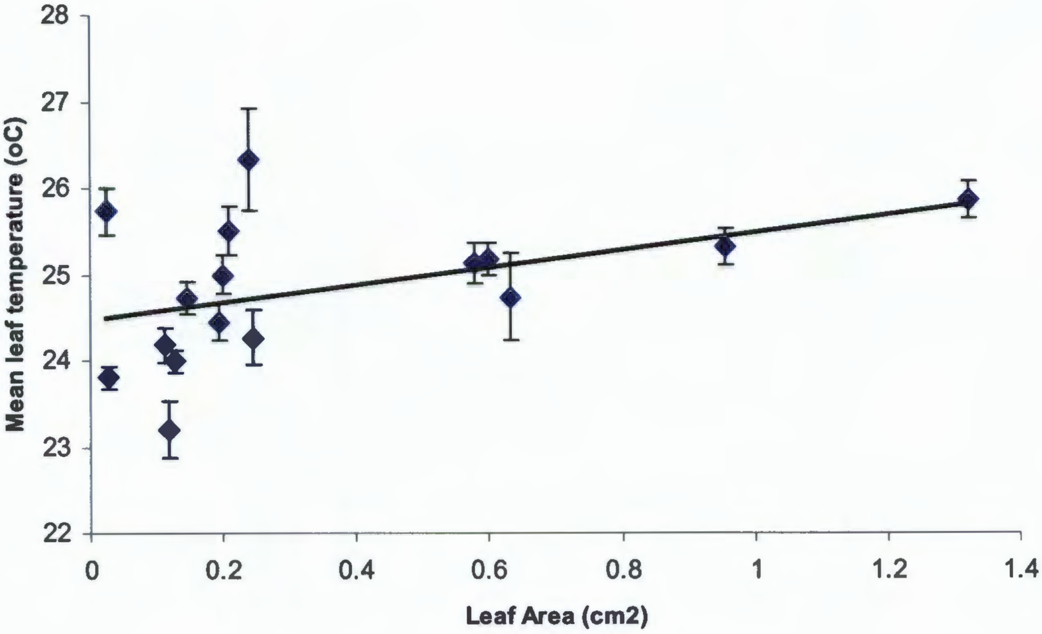


Figure 8. The relationship between leaf temperature and leaf size for the species used (Figure 7, Appendix 3). $R^2=0.20$, p-value>0.05.

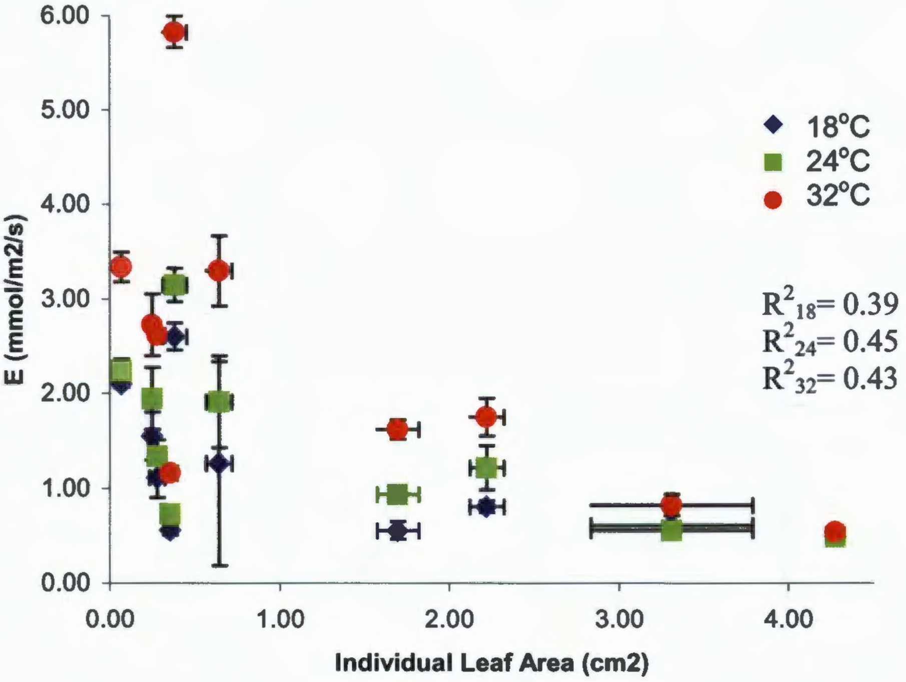


Figure 9. Transpiration rate (E) versus individual leaf area form ten species. Series correspond to temperature treatments, where the temperature in the Confiner Chamber (see methods) was set to, 18°C, 24°C and 32°C. All three relationships are significant (p-value< 0.05)

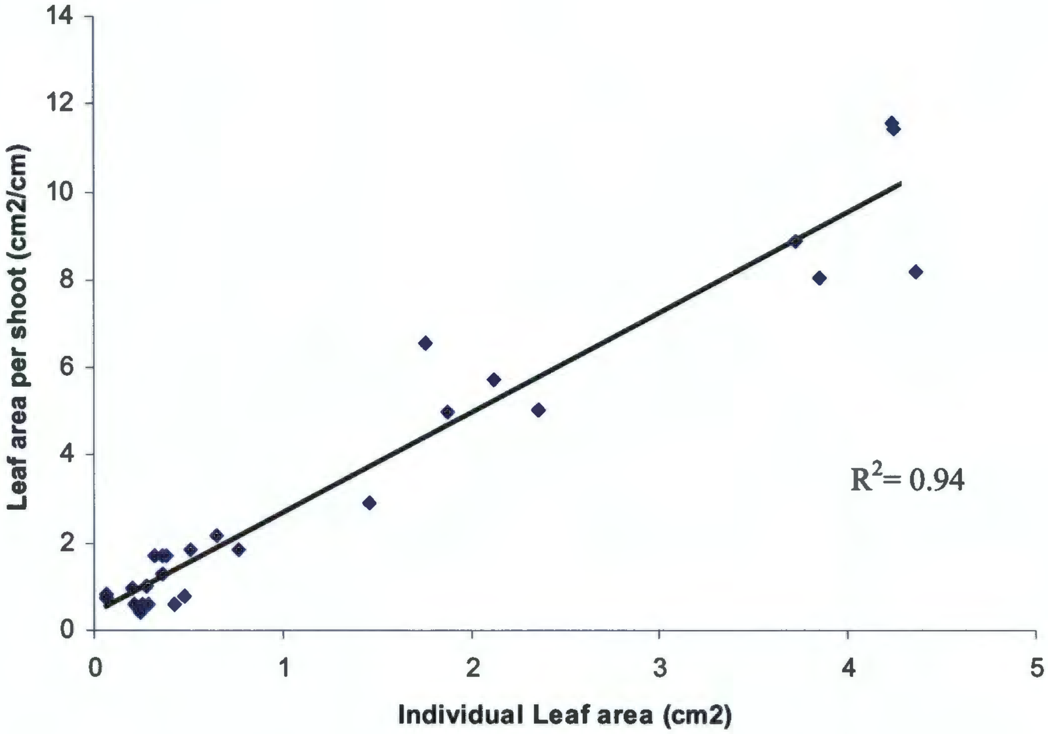


Figure 10. The relationship between leaf area per shoot (calculated at the total leaf area for each plant for the length of shoot that fitted into the Conifer Chamber (see methods) and individual leaf area ($p<0.005$).

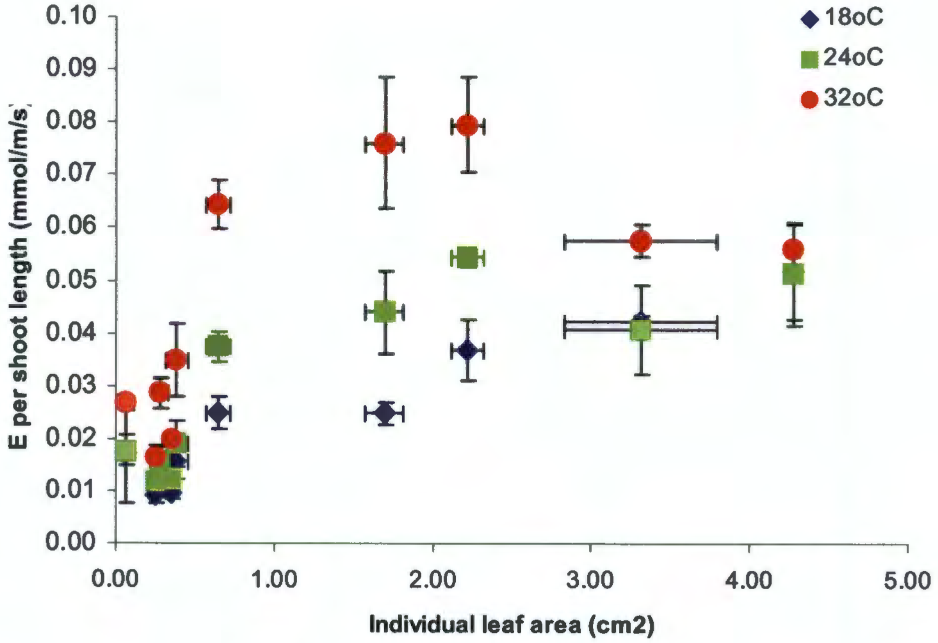


Figure 11. Relationship between transpiration (E) per shoot length (see Appendix 4 for shoot length values) and Individual leaf area

Table 1. Leaf morphological values for the *Jamesbrittenia* species sampled. Circle diameter is the diameter of the largest circle that can be fitted within the leaf margins, and its srare-root was used as a measure of leaf dimension (see in text). SLA values were only calculated for specimens from G.A.Verboom's collections (see Appendix 1).

Species	Mean Leaf Area	Mean Circle Diameter	SLA
J. accrescens	0.49±0.1	0.44±0.2	148.59
J. acutiloba	2.18	1.25	*
J. adpressa	0.16±0.05	0.13±0.06	90.05
J. albanensis	0.03	0.09	*
J. albiflora	0.07±0.04	0.14±0.03	37.06
J. albomarginata	0.03±0.02	0.12±0.01	*
J. amplexicaulis	0.99±0.14	0.82±0.24	*
J. argentia	0.15±0.05	0.28±0.06	*
J. aridicola	1.23±0.13	0.94±0.4	170.34
J. aspalathoides	0.02±0.02	0.09±0.01	*
J. aspleniifolia	0.15±0.07	0.17±0.03	50.75
J. atropurpurea	0.04±0.01	0.09±0.02	23.91
J. aurantiaca	0.25±0.01	0.09±0.05	143.16
J. barbata	0.60	0.60	146.10
J. bergae	0.53	0.25	91.72
J. bicolor	0.41±0.06	0.46±0.07	106.22
J. brevifolia	1.44±0.23	1.07±0.45	141.27
J. burkeana	0.18±0.02	0.32±0.05	108.92
J. calciphila	0.01±0.02	0.06	21.18
J. canescens	0.86±0.07	0.49±0.38	*
J. concinna	0.57±0.07	0.29±0.11	89.43
J. crassicaulis	0.22±0.01	0.12±0.03	*
J. dentatisepala	0.64	0.71	*
J. dolomitica	2.65	1.34	*
J. elegantissima	0.36	0.13	*
J. filicaulis	0.20±0.05	0.19±0.07	68.65
J. fimbriata	1.21	0.97	219.51
J. fleckii	2.48±0.29	1.20±0.89	171.90
J. foliolosa	0.080.02	0.09±0.02	156.63
J. fragilis	0.34	0.24	*
J. fruticosa	0.98±0.01	0.70±0.03	554.67
J. glutinosa	2.92±0.23	1.54±0.78	79.35
J. grandiflora	0.85±0.05	0.62±0.04	*
J. hereroensis	5.29±1.03	1.75±4.72	*
J. huillana	0.13±0.11	0.18±0.11	64.19
J. incisa	0.10	0.20	*
J. integerrima	0.280.03	0.34±0.02	68.86
J. kraussiana	1.80±0.2	0.49±0.75	261.22
J. lyperiodes	0.55±0.2	0.57±0.25	84.84
J. macrantha	0.33	0.22	85.31
J. major	5.92	2.63	363.27
J. maritima	0.53±0.14	0.62±0.15	102.40
J. maxii	1.34±0.12	1.10±0.29	163.92

J. megadenia	2.63±0.28	1.05±0.94	226.66
J. megaphylla	6.51±0.51	2.40±3.11	254.72
J. merxmuelleri	0.01±0.02	0.08	*
J. micrantha	0.55±0.06	0.38±0.12	*
J. microphylla	0.01±0.01	0.04	*
J. montana	0.34±0.01	0.22±0.06	*
J. namaquensis	0.03±0.03	0.11±0.02	137.96
J. pallida	0.57±0.21	0.48±0.42	210.29
J. pedunculosa	1.05±0.02	0.73±0.13	*
J. phlogiflora	0.20±0.02	0.26±0.02	73.72
J. pinnatiida	0.15	0.27	*
J. primuliflora	3.03±0.3	1.32±0.81	115.60
J. pristisepala	0.18±0.03	0.16±0.03	84.15
J. prixbrevi	0.79	0.64	141.69
J. racemosa	1.07±0.13	0.67±0.09	*
J. ramosissima	0.16±0.02	0.34±0.01	20.93
J. sessilifolia	0.98±0.17	0.77±0.36	165.17
J. stellata	0.10±0.07	0.21±0.06	*
J. stricta	0.07±0.05	0.59±0.01	*
J. tenella	0.81±0.05	0.70±0.22	*
J. tenuifloia	0.02±0.01	0.06±0.03	*
J. thunbergii	1.57±0.17	0.87±0.57	*
J. tortuosa	0.03±0.02	0.08±0.01	*
J. tysonii	0.17±0.05	0.22±0.03	*

Table 2. Ahistorical and historical correlations (from CACTUS 2.0) of leaf area and dimension and six independent variables. Clades were analysed separately on the basis of their differing life histories, i.e. the winter rainfall clade being made up of predominantly annuals and leaf deciduous perennials, while the summer rainfall clade comprised mostly of evergreen perennials

				Ahistorical			Historical	
	Dependent variable	**Independent variable**	**R**	**R 2**	**p-value**	**R**	**R 2**	**p-value**
Winter rainfall clade	leaf area	soil fertility	0.01	0.08	0.450	-0.08	0.04	0.654
		rainfall driest month	0.38	0.17	0.266	0.61	0.12	0.398
		temperature hottest month	-0.20	0.01	0.853	-0.19	0.02	0.730
		rainfall hottest month	0.57	0.25	0.175	0.38	0.21	0.257
		temperature wettest month	0.12	0.11	0.383	0.00	0.00	0.906
		mean annual rainfall	-0.66	0.43	0.054	-0.70	0.49	0.053
	leaf dimension	soil fertility	0.15	0.08	0.468	0.24	0.04	0.635
		rainfall driest month	0.17	0.35	0.096	0.49	0.22	0.239
		temperature hottest month	0.62	0.03	0.632	0.44	0.06	0.570
		rainfall hottest month	0.62	0.44	0.052	0.44	0.33	0.138
		temperature wettest month	0.00	0.10	0.399	-0.55	0.01	0.843
		mean annual rainfall	-0.76	0.58	**0.018**	-0.71	0.50	**0.049**
Summer rainfall clade	leaf area	soil fertility	0.01	0.00	0.973	-0.08	0.01	0.664
		rainfall driest month	0.38	0.15	**0.031**	0.61	0.37	**0.000**
		temperature hottest month	-0.20	0.04	0.285	-0.19	0.03	0.316
		rainfall hottest month	0.57	0.32	**0.001**	0.38	0.15	**0.034**
		temperature wettest month	-0.26	0.01	0.510	-0.36	0.00	0.985
		mean annual rainfall	0.55	0.30	**0.001**	0.42	0.18	**0.018**
	leaf dimension	soil fertility	0.15	0.02	0.408	0.24	0.06	0.191
		rainfall driest month	0.17	0.03	0.355	0.49	0.24	**0.006**
		temperature hottest month	-0.26	0.07	0.149	-0.36	0.13	**0.049**
		rainfall hottest month	0.62	0.39	**0.000**	0.44	0.20	**0.012**
		temperature wettest month	0.00	0.00	0.993	-0.27	0.07	0.145
		mean annual rainfall	0.57	0.33	**0.001**	0.53	0.28	**0.002**

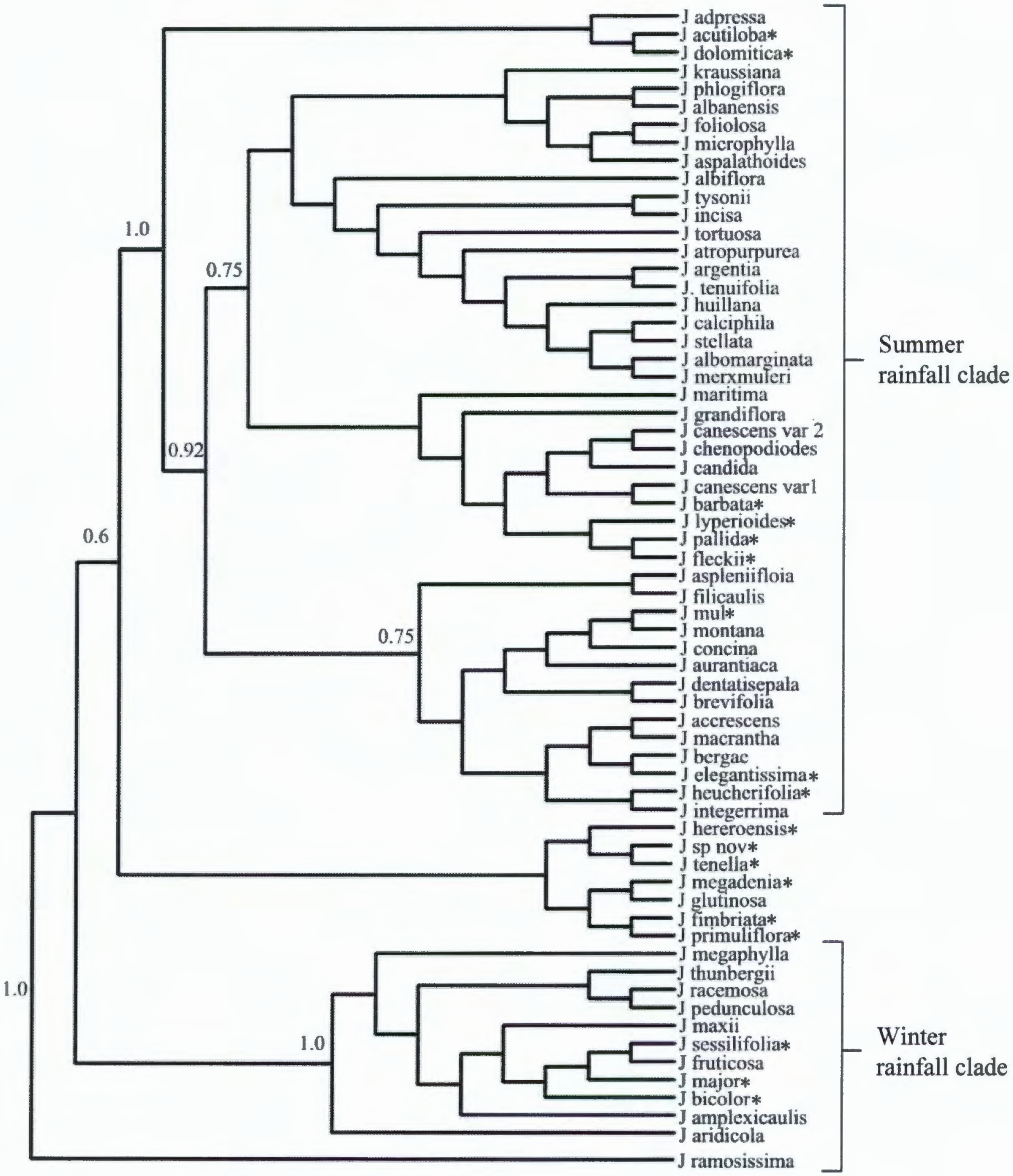
Table 3. Results of randomisation test for effect of sample size on R^2 values in winter rainfall clade. P-values represent the probability of obtaining and $R^2 \geq$ the observed R^2 value when all Clade 2 taxa are considered.

Dependent variable	Indepenent variable	p-value
leaf area	rainfall hottest month	0.336
	mean annual rainfall	0.398
leaf dimension	rainfall driest month	0.305
	temperature hottest month	0.311
	rainfall hottest month	0.41
	mean annual rainfall	0.118

Table 4. Regression summaries for the dependent variables LA of summer rainfall clade species. Beta is the relative contribution of each variable to the relationship. Marked tests significant at α= 0.05.

	B	Std.Err.	t(24)	p-level
Intercept	-1.6416	1.1237	-1.461	0.157
SF	-0.063	0.0651	-0.9675	0.3429
RDM	0.0987	0.0318	3.104	**0.0048**
THM	0.0339	0.0537	0.6308	0.5341
RHM	0.0063	0.0038	1.6565	0.1106
TWM	0.0242	0.0352	0.6869	0.4988
MAR	-0.0001	0.0008	-0.1391	0.8905

Appendix 1. Phylogeny of *Jamesbrittenia* based on three gene regions (*rps, psbA-trnH, Gscp*), from Moncrieff (unpublished). Tree inferred using a GTR + Γ model of sequence evolution. Numbers on nodes represent posterior probabilites. Namibian species, excluded from comparative analyses are indicated by a *.



Appendix 2. List of *Jamesbrittenia* Species. Accession numbers and Localities. Also shown. Collectors: DGE=D. Gwynne Evans; M or MH=M. Herron; V=G. A. Verboom; H=A. Harrower; B=N. Bergh.

Accession Number and Collector	*Jamesbrittenia* species	Locality
V808_ramo	*J. ramosissima*	Pella, N. Cape
V806_arid	*J. aridicola*	Aggeneys, N. Cape
V870_ampl	*J. amplexicaulis*	Okiep, N. Cape
V856_bico	*J. bicolor*	Witputs, Namibia
V815_majo	*J. major*	Ai-Ais, Namibia
V864_frut	*J. fruticosa*	Steinkopf, N. Cape
V854_sess	*J. sessilifolia*	Witputs, Namibia
V805_maxi	*J. maxii*	Aggeneys, N. Cape
V874_pedu	*J. pedunculosa*	Kamieskroon, N. Cape
V878_race	*J. racemosa*	Grootvlei Pass, N. Cape
V882_thun	*J. thunbergii*	Vanrhyns Pass, W. Cape
V859_mega	*J. megaphylla*	Vioolsdrif, N. Cape
V830_prim	*J. primuliflora*	Seeheim, Namibia
V847_fimb	*J. fimriata*	Sossusvlei, Namibia
V814_glut	*J. glutinosa*	Ai-Ais, Namibia
V823_mega	*J. megadenia*	Klein Karas, Namibia
V1102_ten	*J. tenella*	Windhoek, Namibia
V1108_spn	*J. sp nov*	Erongo Mts, Namibia
V1109_her	*J. hereroensis*	Bloedkoppie, Namibia
V851_inte	*J. integerrima*	Aus, Namibia
V1120_heu	*J. heucherifolia*	Epupa Falls, Namibia
V1124_ele	*J. elegantissima*	Popa Falls, Namibia
V1065_ber	*J. bergae*	Thabazimbi, ***
V1062_mac	*J. macrantha*	Roossenekal, ***
V1057_acc	*J. accrescens*	Sudwala, Mpumalanga
V1036_bre	*J. breviflora*	Sani Pass, Lesotho
V1030_den	*J. dentatisepala*	Garden Castle, Kwazulu-Natal
V1069_aur	*J. aurantiaca*	Jagersfontein, Free State
V1122_con	*J. concinna*	Tsumeb, Namibia
V1039_mon	*J. montana*	Dundee, Kwazulu-Natal
V1022_mul	*J. multisecta*	Engcobo, E. Cape
V1012_fil	*J. filicaulis*	Cathcart, E. Cape
V1018_asp	*J. aspleniifolia*	Clifford, E. Cape
V835_flec	*J. fleckii*	Kuiseb Canyon, Namibia
V1106_pal	*J. pallida*	Erongo Mts, Namibia
V1101_lyp	*J. lyperioides*	Windhoek, Namibia
V1112_bar	*J. barbata*	Swakop, Namibia
V833_cane	*J. canescens var seineri*	Kuiseb Canyon, Namibia
V1128_can	*J. canescens var laevior*	Otavifontein, Namibia
V1115_che	*J. chenopodioides*	Brandberg, Namibia
V817_cane	*J. canescens var canescens*	Ai-Ais, Namibia
V1048_gra	*J. grandiflora*	Barberton, Mpumalanga
V1002_mar	*J. maritima*	Alexandria, E. Cape
V866_merx	*J. merxmuelleri*	Alexander Bay, N. Cape
M36_albom	*J. albomarginata*	Locality uncertain
M38_stell	*J. stellata*	Cape Peninsula, W. Cape

H1679_cal	*J. calciphila*	Still Bay, W. Cape
V1056_hui	*J. huillana*	Barberton, Mpumalanga
V915_tenu	*J. tenuifolia*	Sedgefield, W. Cape
MH50_arge	*J. argentea*	Locality uncertain
V1070_atr	*J. atropurpurea*	Jagersfontein, Free State
TVPA2_tor	*J. tortuosa*	Prince Albert, W. Cape
V885_inci	*J. incisa*	Calvinia, N. Cape
DGE_tyson	*J. tysonii*	Locality uncertain
V1066_alb	*J. albiflora*	Jagersfontein, Free State
H1695_asp	*J. aspalathoides*	Locality uncertain
B1453_mic	*J. microphylla*	Sundays Mouth, E. Cape
H552_foli	*J. foliolosa*	Locality uncertain
V1008_alb	*J. albanensis*	Ecca Pass, E. Cape
V1011_phl	*J. phlogiflora*	Peddie, E. Cape
V1023_kra	*J. kraussiana*	Oribi Gorge, Kwazulu-Natal
V1125_dol	*J. dolomitica*	Otavi, Namibia
V1132_acu	*J. acutiloba*	Waterberg, Namibia
V829_adpr	*J. adpressa*	Seeheim, Namibia

Appendix 3. List of species used for on-plant temperature measurements. Two individual per species were used. Plants obtained from the greenhouse at the Kirstenbosch National Botanic Garden, South Africa.

Species	Mean leaf area
ADH2445	0.02
J. argentia	0.16
J. aspalationdes	0.11
J. crassicaulis	0.59
J. filicaulis	0.44
J. foliolosa	0.17
J. grandiflora	1.14
J. montana	0.22

Appendix 4. List of species used for transpiration measurements using the Gas exchange measurements were taken using a Conifer-Chamber (LI6400-05) attached to at a twin-channel LI-COR 6400 InfraRedGasAnalyser (IRGA, Li-Cor Inc, Lincoln, NB, USA). Plants obtained from the greenhouse at the Kirstenbosch National Botanic Garden, South Africa.

Species	Mean leaf area	Mean shoot length
J. grandiflora	2.22.0.01	4.850.35
J. huilana	0.360.02	6.20.49
J. stellata	0.070.00	4.670.12
J. foliolosa	0.280.04	5.70.56
J. pedunculosa	0.250.02	4.10.27
J. accrescens	1.70.12	51.26
Sutera sp.	0.650.08	5.10.38
Barleria sp	4.280.04	2.660.29
Barleria obtusa	3.310.48	2.430.20
Nemesia sp.	0.380.07	5.770.54