The Functional, Ecological and Evolutionary Significance of Culm Structures in the Cape Floristic Region

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CONTENTS

GENERAL INTRODUCTION

The Cape Floristic Region (CFR, Goldblatt & Manning 2000) lies in the southwestern Cape, South Africa, and is known for its high species richness and endemism (Goldblatt & Manning 2002). This Mediterranean-type climate region receives winter rainfall that averages between 250 and 650 mm annually over most of the region but reaches an average of more than 1000 mm in the mountains of the southwest and southern Cape (Cowling *et al.* 1992, Rebelo *et al.* 2006, Manning 2007). Average daily temperatures during midwinter vary between 7 and 15°C (Manning 2007). During midsummer, average daily temperatures vary between 15 and 25°C (Manning 2007), although temperatures frequently exceed 30°C. The mountains in the region consist mainly of erosion-resistant sandstones and quartzites, resulting in highly oligotrophic soils (Deacon *et al.* 1992). Heavy rain during winter causes further nutrient leaching (Deacon *et al.* 1992). The soils are generally acidic and coarse-grained and nitrogen and phosphorous are critical limiting nutrients in this system. In addition, the vegetation is fire-prone (Bond 1984), with fires being important for nutrient cycling in this highly oligotrophic environment (Stock & Lewis 1986a). The landscape of the CFR is highly heterogeneous, with variation in space and time of soil nutrient availability (Richards *et al.* 1997), rainfall, temperature, and disturbances such as fire, resulting in a diverse number of microhabitats. The summer drought, a transient period of water and nutrient availability in winter and spring as well as nutrient poor soils of the Cape Floristic Region pose complex physiological challenges for plants.

The CFR consists of a number of vegetation types, including fynbos, renosterveld, strandveld, karroid shrubland, forest and thicket (Rebelo 2006). The most common vegetation of the CFR is fynbos and is generally described as an evergreen, sclerophyllous shrubland because shrubs are common. Three growth forms are actually prevalent in the CFR: shrubs, graminoids and geophytes. Growth form diversity is not very high in fynbos, with many structurally and functionally similar species often belonging to the same genus (Cowling *et al.* 1996). Various plant adaptations have evolved that allow plants to cope with the harsh environmental conditions of the CFR.

Below-ground adaptations to nutrient-poor conditions in the CFR are common. Several families of plants native to this Mediterranean-type ecosystem, including Cyperaceae (dauciform roots), Restionaceae (capillaroid roots), Proteaceae and Fabaceae, produce root clusters during periods of high water availability that enhance nutrient uptake by increasing total root surface area and by releasing organic acids, which mobilise P from insoluble complexes thereby increasing its availability (Lambers *et al.* 2006). Symbiotic associations with mycorrhiza also facilitate nutrient uptake and are common in ericas in the Cape as well as other nutrient-poor heathland Mediterranean systems (Lamont 1982, Allsopp & Stock 1993). Legumes growing in this region form symbiotic associations with nitrogen-fixing bacteria in root nodules and thus obtain atmospheric-derived nitrogen (Stock & Lewis 1986b).

Above-ground adaptations to nutrient-poor conditions are also prevalent. Sclerophyllous leaves are a conspicuous feature of fynbos (Stock & Allsopp 1992) as well as other Mediterranean-type ecosystems of the world. Sclerophylly has advantages for nutrient use efficiency in nutrient-poor environments (Orians & Solbrig 1977, Stock *et al.* 1992), is correlated with other leaf characteristics, such as leaf longevity, and is believed to enhance carbon return per unit of nutrient invested (Orians & Solbrig 1977, Westoby 1998, Aerts & Chapin 2000). There is also considerable variation in leaf width and numerous narrow-leaved plants including members of the protea (Proteaceae), erica (Ericaceae), daisy (Asteraceae), legume (Fabaceae), jujube (Rhamnaceae), fibre-bark (Thyme-laeaceae) and blacktip (Bruniaceae) families. In Proteaceae narrow leaves transpired at faster rates than wider leaves, which may be important for enhancing nutrient mass-flow to plants during the wet winter and spring months (Cramer *et al.* 2008, Yates *et al.* 2010). Some plants, such as sundews and bladderworts, are carnivorous and obtain nutrients by trapping and digesting insects (Lamont 1982).

During summer in the CFR, rainfall is low and temperatures often exceed 30ºC. Furthermore, the hot, dry conditions are exacerbated on days when wind is negligible. Since water is scarce, latent heat loss is unfeasible and plants must rely on sensible heat loss for cooling. Narrow Proteaceae leaves shed more heat through sensible heat loss than wider leaves and may be adaptive for hot, dry conditions (Yates *et al.* 2010). Sclerophylly also has advantages for drought tolerance through its effect on xylem cavitation (Salleo *et al.* 1997). Geophytes and annuals employ an avoidance strategy to cope with summer drought in the CFR. Some shallow-rooted plants are thought to shut down physiological function during these hot, dry months. Importantly, the low water availability during summer drought also leads to nutrient-poor conditions because nutrients are not readily available.

The strong seasonality and winter rainfall of the CFR result in a transient period of nutrient availability in the wet winter and spring months during which capitalisation of resources is crucial for plants. Asynchronous growth patterns allow plants to cope with the variation of nutrients in time (Stock *et al.* 1987). Cluster roots are short-lived and coincide with the period of nutrient availability (Shane & Lambers 2005). Flowering times of certain plants result in seed germination coinciding with the wet winter months when germination is favourable. The fynbos is a fire-prone vegetation type and the post-fire environment is nutrient-rich with the added benefit of other vegetation being cleared (Bond 1984). Some plants, e.g. some Restionaceae, produce dormant seeds that respond to fire-linked environmental cues and germinate just after fire (Bond 1984). Other fire-survival strategies include resprouting and serotiny (Bond 1984). The summer drought, nutrient-poor soils and transient period of nutrient and water availability present challenges for plants and various structural and functional adaptations have evolved to enable plants to cope under these severe conditions.

While shrubs in the CFR tend to have fine, narrow, sclerophyllous leaves (Stock & Allsopp 1992, Yates *et al.* 2010), many graminoids have photosynthetic, cylindrical culms with no leaves, greatly reduced leaves or leaves that no longer function in photosynthesis. The prevalence of leafless photosynthetic culms is a striking feature of the CFR. Restionaceae, several Cyperaceae and some Poaceae, families that are well represented in this vegetation type, have cylindrical culms with no leaves, leaves that are greatly reduced or leaves which are present only at certain times of the year and during the post-fire succession sequence. A few species do produce leaves, in addition to culms, as part of their adult form. Other Fynbos genera that have photosynthetic branches and little or no leaf photosynthesis include *Psoralea* (Fabaceae), *Lebeckia* (Fabaceae), *Heliophila* (Brassicaceae), *Limonium* (Plumbaginaceae), *Polygala* (Polygalaceae), *Anginon* (Apiaceae), *Centella* (Apiaceae) and *Athanasis* (Asteraceae). In some species, such as *Erica* (Ericaceae) and *Gnidia*, *Lachnaea*, *Struthiola* and *Passerina* (Thymelaceae), leaves of some plants are tightly adpressed to the branches, seemingly mimicking a culm structure. Despite the abundance of these photosynthetic culm and culm-like structures in the CFR, the adaptive physiological significance of these structures in this region has not yet been investigated.

In most Cape Poales, culms carry out almost all of the photosynthetic function. Culm structures fulfil the role of photosynthesis in all Restionaceae and some Cyperaceae and Poaceae in the CFR, but certain species produce leaves when they are young, as part of their adult form and/or after fire. In Restionaceae leaves tend to be needle-like and considerably thinner than the culms. Species of Cyperaceae that produce leaves tend to have triangular-shaped (in cross-section), long leaves and Poaceae generally produce lanceolate, flat leaves. The prevalence of species with notable stem net photosynthesis is high in habitats with high PPFD (photosynthetic photon flux density) and high heat, such as tropical dry woodlands, tropical thorn woodland, deserts and chapparal (Gibson 1983, Nilsen 1992). Stem photosynthesis has been shown to be an adaptation for extended photosynthesis during stress conditions as it shows greater tolerance of water stress than leaf photosynthesis, although is limited to lower photosynthetic rates compared to leaves (Nilsen 1992). In addition, thick leaves are suggested to be adaptive under dry and/or infertile conditions, with a high cost of replacing transpirational water loss associated with thicker photosynthetic organs (Givnish 1978, 1979). Thus, culms may be adaptive for the summer drought conditions in the CFR, however, the significance of producing leaves at certain times is unclear. I was interested in investigating the evolutionary and ecological significance of culm photosynthesis as well as that of leaf production. Poales in the CFR provides a propitious group on which to work because the group is common in the CFR and displays a range of leaf and culm photosynthetic structures, from solely culm photosynthesis to predominantly leaf photosynthesis.

Approximately 841 Poales species occur in the CFR and occupy a diverse range of habitats (Goldblatt & Manning 2000). Poales are generally wind-pollinated with simple flowers. The CFR has been a hotbed of radiations, with radiations of many Cape floral clades dated to the Middle Miocene (Linder 2005, Verboom *et al.* 2009). Poales originated during the Cretaceous (Bremer 2002, Janssen & Bremer 2004, Linder & Rudall 2005), with the major lineages of Poales diversifying in the mid- to late Cretaceous and most Poales groups diversifying during the Cenozoic (Linder & Rudall 2005). During the Cretaceous, the climate was perennially wet and warm and ancestral Poales are suggested to have occupied wet, warm, nutrient-poor, sunny habitats (Linder & Rudall 2005). Since 65 MYA (million years ago), significant changes in climate have taken place (Zachos *et al.* 2001). During the Paleocene and late Eocene temperatures began to increase and the climate was less seasonal (Zachos *et al.* 2001). From 50 MYA (mid- to late Eocene) temperatures began to drop and climatic fluctuations occurred (Zachos *et al.* 2001). The climate during the Mid-Miocene was tropical to sub-tropical and mesic with no dry season (Zachos *et al.* 2001, Linder & Hardy 2004). Approximately 8-10 MYA (late Miocene) the glaciation of Antarctica and upwelling of cold waters near southern Africa brought about a highly seasonal climate with summer-arid and winter rainfall Mediterranean-type conditions in the Cape (Zachos *et al.* 2001, Linder & Hardy 2004). However, there is evidence that summer drought and the fynbos vegetation established 19.5 MYA (Early Miocene) and that the fire regime set in approximately 15 MYA during a period of cooling following the Mid-Miocene Climatic Optimum when aridification and open habitats increased (Bytebier *et al.* 2011). Chase *et al.* (2009) provide evidence for a recent period of pronounced aridification 3500 to 300 years BP. One proposal for radiation in the Cape is that this climate change led to the extinction of tropical flora, allowing formerly heathy, montane vegetation to occupy those habitats (Linder & Hardy 2004). It is suggested that during the Neogene, Poales diversified into seasonally dry habitats as well as fire-prone vegetation (Linder & Rudall 2005). While the culm photosynthetic structure is common in this group and has been suggested to be adaptive for nutrient-poor soils and seasonal drought (Linder & Rudall 2005), studies assessing the origin and functional and ecological significance of this structure are lacking. Furthermore, the importance of producing leaves during certain periods is not clear.

Within Restionaceae, all species have evergreen, cylindrical, photosynthetic culm structures, with some species having additional leaf photosynthesis. The African Restionaceae are common in the CFR and often form the dominant element of the vegetation, occupying the niche normally filled by grasses in other biomes (Linder 1984). The family Restionaceae consists of ca. 490 species and is mostly spread through the southwestern tip of southern Africa and southern Australia (Linder 2000), with outliers north into Indochina, tropical Africa and South America. The African Restionaceae (subfamily Restionoideae, Briggs & Linder 2009) radiated since the Eocene in the GCFR and includes some 350 species (Linder *et al.* 2005), thus constituting one of the largest Cape clades. The term Greater Cape Floristic Region (GCFR, Born, Linder & Desmet 2006) is used to include both fynbos and succulent karoo biomes. Almost all of the species are endemic to this region (Goldblatt & Manning 2000). Restionoideae occupy a diverse array of habitats, from permanently waterlogged wetlands to dry karroid shrubland. In addition to relying on culms entirely for photosynthesis, there is considerable variation in the diameter of Restionoideae culms, ranging from 1mm to 3cm in diameter. Boundary layer thickness is dependent on culm diameter and influences gas and heat exchange (Nobel 2005). Yates *et al*. (2010) demonstrated the consequences of varying leaf width (and thus boundary layer thickness) for gas and heat exchange in Cape Proteaceae and found narrow leaves to transpire and lose heat more rapidly than broader leaves. It is unclear whether theoretical principals of boundary layer dynamics apply to culm structures, which would result in wide and narrow culms having different gas and heat exchange rates. High transpiration rates associated with structures having thin boundary layers, such as narrow culms, may be advantageous in nutrient-poor conditions because transpiration drives mass-flow of nutrients towards the roots (Cramer *et al.* 2008). Alternately, narrow culms and thin boundary layers may allow increased sensible heat loss and be adaptive for summer drought conditions.

Restionaceae are unusual in that they rely entirely upon evergreen culm structures for photosynthesis and display considerable variation in culm diameter. Furthermore, the culm anatomy of Restionaceae is highly diverse and rather unusual. The basic organisation of the various tissues is constant through the whole family. The Restionoideae, in particular, with ca. 354 species, have highly diverse culm anatomic forms including numerous forms of stomatal architecture, epidermal cell wall thickening, epidermal hairs and papillae, varying sizes of sub-stomatal chambers, thickening of the sclerenchyma and chlorenchyma cavities (Linder 2000). Radiations in the Restionoideae began during the Late Miocene and Late Oligocene, when the climate became more seasonal with summer drought and winter rainfall (Linder & Hardy 2004, Linder 2005). Adaptation to fire and drought may have given an advantage to the Cape clades over the tropical clades and rates of speciation were possibly limited by certain lineage-specific traits, such as morphological adaptations (Linder & Hardy 2004). Adaptation to particular microhabitats in the highly heterogeneous Cape may be a key driver of morphological diversification and speciation in this region. The remarkable diversity of Restionoideae is certainly suggestive of an adaptive role. Anatomical traits can function as innovations stimulating major radiations: the recent radiation of a very large ruschioid clade (Aizoaceae) of 1563 species in the Succulent Karoo has been linked to the evolution of wide-band tracheids, suggesting that they may function to withstand water stress (Klak *et al.* 2004). The evolution of wide-band tracheids may have facilitated invasion of the Succulent Karoo but other factors, such as limited dispersal of seeds probably led to massive speciation in this clade (Givnish 2010). Restionoideae anatomical traits have been interpreted as an adaptation to arid conditions (Gilg 1891) and may have contributed to the radiation of this group. However, evolutionary and functional tests of these ideas are still required.

The prevalence of photosynthetic culm structures is a striking feature of the CFR. Furthermore, variation in culm diameter and anatomical structure is substantial. Since these traits are suggested to be associated with the summer-arid, winter rainfall Mediterranean-type conditions in the Cape, three chapters were set up to address the functional, evolutionary and ecological significance of these different culm traits in this environment. Chapters have been written as papers and questions, aims and hypotheses of each chapter are summarised as follows:

1. Are culm structures adaptive for summer-dry conditions? I tested whether culm photosynthesis and associated leaf loss in Cape Poales was an adaptation to summer-dry climate conditions, which set in during the Mid-Miocene. I also assessed whether culms and leaves functioned differently and explored the adaptive significance of producing leaves at certain times. I hypothesized that culm photosynthesis is adaptive for summer drought, culm photosynthesis is different from leaf photosynthesis and that leaves are produced by species associated with high seasonality and may facilitate capitalisation of nutrients during periods of high nutrient availability.
2. What are the physiological consequences of variation in culm diameter within Cape Restionaceae? I explored the influence of culm diameter on gas and heat exchange using cylindrical culm replicas as well as Restionoideae. In addition, I tested whether the effects of variation in culm diameter on gas and heat exchange were evident at an environmental scale. To determine whether the environmental patterns and gas exchange correlates were purely a function of culm dimension, I also assessed the structural co-variants of culm diameter. I proposed that narrow culm diameters might facilitate water loss and carbon assimilation in the wet winter and spring and sensible heat loss in summer.
3. Did the evolution of novel anatomical features facilitate occupation of seasonally arid habitats brought about by mid-Miocene aridification? In addition, with reversion to wet habitat conditions are these derived traits lost? I used correlative and experimental methods to test which of the rich diversity of anatomical structures are linked to more arid conditions, thus aiming to test the theory of anatomical adaptation in increasingly arid conditions. Correlative methods were used over the whole Restionoideae clade to test for

functional association between aridity and the derived epidermal structures (thickened cuticle and massively enlarged lateral epidermal walls), the stomatal position variation, the variation in the development in the protective cells, the variation in the thickness of the sclerenchyma cell walls, and finally in the formation of scattered cavities and solid tissue, or indeed the loss of cavities, in the ground tissue. Secondly, I tested whether the totality of anatomical novelties might be linked to greater drought tolerance.

REFERENCES

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research,* **30**, 1–67.

Allsopp, N. & Stock, W.D. (1993) Mycorrhizal status of plants growing in the Cape Floristic Region, South Africa. *Bothalia,* **23**, 91–104.

Bond, W.J. (1984) Fire survival of Cape Proteaceae – influence of fire season and seed predators. *Vegetatio*, **56**, 65-74.

Born, J., Linder, H.P. & Desmet, P.G. (2006) The Greater Cape Floristic Region. *Journal of Biogeography*, **33**, 1-16.

Briggs, B.G. & Linder, H.P. (2009) A new subfamilial and tribal classification of Restionaceae (Poales). *Telopea*, **12**, 333-345.

Bremer, K. (2002) Gondwanan evolution of the grass alliance of families (Poales). *Evolution,* **56**, 1374-1387.

Bytebier, B., Antonelli, A., Bellstedt, D.U. & Linder, H.P. (2011) Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. Proceedings of the Royal Society B, **278**, 188-195.

Chase, B.M., Meadows, M.E., Scott, L., Thomas, D.S.G., Marias, E., Sealy, J. & Reimer, P.J. (2009) A Record of rapid Holocene climate change preserved in hyrax middens from southwestern Africa. *Geology*, **37**, 703-706.

Cowling, R.M., Holmes, P.M. & Rebelo, A.G. (1992) Plant diversity and endemism. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K. & Arianoutsou, M. (1996) Plant diversity in Mediterranean-climate regions. *TREE*, **11**, 362-366.

Cramer, M.D., Hoffmann, V. & Verboom, G.A. (2008) Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist,* **179**, 1048-1057.

Deacon, H.J., Jury, R.M. and Ellis, F. (1992) Selective regime and time. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Gibson, A. (1983) Anatomy of photosynthetic old stems of non-succulent dicotyledons from north American deserts. *Botanical Gazette,* **144**, 347-362.

Gilg (1891) Beitrage zur vergleichende Anatomie der xerophilen Familie der Restionaceae. *Botanische Jahrbucher*, **13**, 541-606.

Givnish, T.J. (1978) Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica*, **27**, 83-142.

Givnish, T.J. (1979) On the adaptive significance of leaf form. In: *Topics in Plant Population Biology* (Eds. O. T. Solbrig, S. Jain, G. B. Johnson & P. H. Raven), pp. 375-407. Columbia University Press, New York.

Givnish, T.J. (2010) Ecology of Plant Speciation. *Taxon*, **59**, 1326-1366.

Golblatt, P. & Manning, J. (2000) *Cape Plants. A conspectus of the Cape flora of South Africa*, National Botanical Institute.

Golblatt, P. & Manning, J. (2002) Plant diversity of the Cape Region of southern Africa. *Ann. Mo. Bot. Gard.* **89**, 281-302.

Janssen, T. & Bremer, K. (2004) The age of major monocot groups inferred from 800+ rbcL sequences. *Botanical Journal of the Linnean Society,* **146**, 385-398.

Klak, C., Reeves, G. & Hedderson, T. (2004) Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature*, **427**, 63-65.

Lambers, H., Shane, M.W., Cramer, M.D., Pearce, S.J. & Veneklaas, E.J. (2006) Root structure and functioning for efficient acquistion of phosphorous: matching morphological and physiological traits. *Annals of Botany*, **98**, 693-713.

Lamont, B. (1982) Mechanisms for enhancing nutrient uptake in plants, with particular reference to Mediterranean South Africa and Western Australia. *The Botanical Review*, **48**, 597-689.

Linder, H.P. (1984) A phylogenetic classification of the genera of the African Restionaceae. *Bothalia,* **15**, 11-76.

Linder, H.P. (2000) Vicariance, climate change, anatomy and phylogeny of Restionaceae. In: *Under the microscope*: plant anatomy and systematics. Eds: Rudall, P.J. and Gasson, P.

Linder, H. P. (2005) The evolution of diversity: the Cape flora. *Trends in Plant Science*, **10**, 536-541.

Linder, H.P. & Hardy, C.R. (2004) Evolution of the species-rich Cape flora. *Phil. Trans. R. Soc. Lond.* **359**, 1623-1632.

Linder, H.P. & Rudall (2005) Evolutionary history of Poales. *Annu. Rev. Ecol. Evol. Syst*. **36**, 107-124.

Linder, H. P., Hardy, C. R. & Rutschmann, F. (2005) Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution*, **35**, 569-582.

Nobel, P.S. (2005) Physicochemical and environmental plant physiology. Academic Press, San Diego, California.

Orians, G.H. and Solbrig, O.T. (1977) A cost-income model of leaves and roots with special reference to arid and semi-arid areas. *American Naturalist,* **111**, 677-690.

Manning, J. (2007) Field Guide to Fynbos. Struik, Cape Town.

Nilsen, E.T. (1992) The influence of water stress on leaf and stem photosynthesis in Spartiumjunceum L. *Plant, Cell and Environment,* **15**, 455-461.

Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L. & Rutherford, M.C. (2006) Fynbos biome. In: *The vegetation of South Africa, Lesotho and Swaziland*. Strelitzia, vol. 19 (eds. L. Mucina & M.C. Rutherford), pp. 52-219. Pretoria, South Africa: South African National Biodiversity Institute.

Richards, M.B., Cowling, R.M. & Stock, W.D. (1997) Soil nutrient dynamics and community boundaries in the Fynbos vegetation. *Plant Ecology*, **130**, 143-153.

Salleo, S., Nardini, A. & Lo Gullo, M.A. (1997) Is sclerophylly of Mediterranean evergreens an adaptation to drought? *New Phytologist*, **135**, 603-612.

Shane, M. and Lambers, H. (2005) Cluster roots: A curiosity in context. *Plant and Soil,* **274**, 101-125.

Stock, W.D. & Lewis, O.A.M. (1986a) Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. *Journal of Ecology*, **74**, 317-328.

Stock, W.D. & Lewis, O.A.M. (1986b) Atmospheric input of nitrogen to a coastal fynbos ecosystem of the south-western Cape Province, South Africa. *South African Journal of Botany*, **52**, 273-276.

Stock, W.D. and Allsopp, N. (1992) Functional Perspectives of Ecosystems: In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Stock, W.D., Sommerville, J.E.M. and Lewis, O.A.M. (1987) Seasonal allocation of dry mass and nitrogen in a fynbos endemic Restionaceae species *Thamnochortus punctatus* Pill. *Oecologia,* **72**, 315-320.

Stock, W.D., van der Heyden, F. and Lewis, O.A.M. (1992) Plant structure and function. In: The

Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F., Mummenhoff, K., Linder, H.P., Muasya, A.M., Oberlander, K.C., Savolainen, V., Snijman, D.A., van der Niet, T. & Nowell, T.L. (2009) Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, **51**, 44-53.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil,* **199**, 213-227.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010) Ecophysiological significance of leaf size variation in Proteaceae form the Cape Floristic Region. *Functional Ecology,* **24**, 485-492.

Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 689-693.

CHAPTER 1

THE SIGNIFICANCE OF CULM AND LEAF STRUCTURES IN POALES FROM THE CAPE FLORISTIC REGION

ABSTRACT

1. Photosynthetic culm structures are highly prevalent in the Cape Floristic Region (CFR) where they occur in Poaceae, Cyperaceae and Restionaceae. Many species have a perennial, photosynthetic culm structure and some produce leaves when they are young, as part of their adult form, during winter and spring and/or after fire. Despite culm abundance and associated leaf loss, in both number and species richness, in the CFR, the physiological and ecological advantages and evolutionary history of this type of structure are not known.
2. I proposed that culm photosynthesis was an adaptation for summer drought and would be associated with seasonal environments, thus explaining the ubiquity of culms in the CFR. Alternately, I hypothesized that culm structures would have lower photosynthetic and transpiration rates than leaves and that leaves would function in resource acquisition and be produced by species associated with high seasonality and transient periods of high soil moisture and nutrient availability.
3. To assess the evolutionary history of culms and leaves, a supertree was constructed for CFR Poales, using published phylogenies. Differences in form and function were compared between leaves and culms of *Ehrharta ramosa* (Poaceae), *Ficinia bulbosa* (Cyperaceae) and *Thamnochortus sporadicus* (Restionaceae) sampled at the same locality at the end of winter/early spring. Modelling was used to investigate differences in environment between culm- and leaf-photosynthetic species.
4. Leaves were narrower and had higher specific areas than culms of the same species. This was consistent among the three species of different families. Leaves had significantly higher photosynthetic rates than culms on a per area and per mass basis. In *Thamnochortus sporadicus*, leaves also transpired faster and had lower temperatures. Leaf-photosynthetic species had the highest probability of occurrence in areas of high seasonality, high temperature range, high summer temperature and high winter rainfall. Culm-photosynthetic species were associated with wet, cold, aseasonal environments.

Culm photosynthesis evolved prior to the Mid-Miocene and it is likely that the ancestral lineage of Poales had both culm and leaf photosynthesis.

5. Culms were associated with aseasonal, wet environments and were not adaptive for summer drought. We suggest that leaf photosynthesis facilitates effective capitalisation of resources during periods of high resource availability through their enhanced assimilation and transpiration. This may be an important strategy in highly seasonal areas in which periods of water and nutrient availability are transient. Culms have higher carbon return per nutrient invested and may represent a long-term, nutrient efficient structure that allows persistence and perennial growth.

Keywords: Cape Floristic Region, culm, leaf, Restionaceae, Poales, seasonality

INTRODUCTION

The Cape Floristic Region (CFR) lies in the southwestern Cape and is known for its high species richness and endemism (Goldblatt & Manning 2000). Three growth forms are prevalent in the area: shrubs, geophytes and graminoids. Graminoids are represented by Restionaceae, Cyperaceae and Poaceae, which generally have perennial above-ground parts. While shrubs in the CFR tend to have fine, narrow, sclerophyllous leaves (e.g. Yates *et al.* 2010), many graminoids have cylindrical, photosynthetic culms and no leaves or at least greatly reduced leaves. The leaves of species belonging to Restionaceae have been reduced to sheaths and the culms are the primary photosynthetic structure (Linder 1984). However, some Restionaceae produce cylindrical, needle-shaped, leaf-like structures when they are young, as part of their adult growth form and/or after fire. Similar photosynthetic culms and leaf-like structures are found in some Cyperaceae. There are also some grasses in the CFR that have leafless culms, but which produce lanceolate, flat leaves during winter or spring and/or after fire. In many plants in the CFR, culms or stems have taken over the photosynthetic function of the leaves. The functional link between plant form and environment is a recurrent theme in plant ecology and functional biology (Orians & Solbrig 1977, Givnish, 1979, Givnish 1987, Mooney 1982, Ackerly & Reich 1999, Ackerly *et al.* 2002), with leaf traits and their potential role in carbon assimilation receiving the most attention. Despite the abundance of photosynthetic culm structures in the Cape flora, their adaptive significance, and the associated absence of leaves remains to be investigated.

Species of Restionaceae are evergreen, sclerophyllous plants that occupy the niche normally filled by grasses in other biomes (Linder 1984). Some Cyperaceae and Poaceae possess similar plant traits. Sclerophyllous leaves are a conspicuous feature of fynbos as well as other Mediterranean-type ecosystems of the world (Stock & Allsopp 1992). Sclerophylly has advantages for nutrient use efficiency and drought-tolerance in nutrient-poor environments (Stock *et al.* 1992, Salleo *et al.* 1997). Sclerophylly is correlated with other leaf characteristics, such as leaf longevity, and is believed to enhance carbon return per unit of nutrient invested (Westoby 1998). Specific leaf area (SLA, the ratio of leaf area to leaf dry mass) is associated with sclerophylly and represents the trade-off between growth rate and leaf longevity, with species with lower SLA having a s lower relative growth rates but greater leaf life spans and enhanced nutrient conservation (Orians & Solbrig 1977, Westoby 1998). Lower SLA thus contributes to leaf longevity, nutrient retention and protection from desiccation and may be minimized by producing a culm rather than leaves. Thick leaves are also suggested to be adaptive under dry and/or infertile conditions (Givnish 1978, 1979). Thus, a culm structure may enhance longevity, function in drought tolerance and nutrient efficiency and allow these plants to persist during the dry summer months in the CFR.

While the sclerophyllous nature of the Restionaceae culm may enhance drought tolerance and nutrient use-efficiency, the significance of leaf absence is not clear. The prevalence of species with notable stem net photosynthesis is high in habitats with high PPFD (photosynthetic photon flux density) and high heat, such as tropical dry woodlands, tropical thorn woodland, deserts and chapparal (Gibson 1983, Nilsen 1992). Stem photosynthesis has been shown to be an adaptation for extended photosynthesis during stress conditions as it shows greater tolerance of water stress than leaf photosynthesis, although the associated photosynthetic rates are low compared to those of leaves (Nilsen 1992). In several species, stems are the only photosynthetic organs active during the hot summer months (Yiotis *et al.* 2008). For example, leaves and stems of *Justicia californica* growing in the southern Sonoran desert, showed similar photosynthetic capacity and diurnal patterns of photosynthesis and stomatal conductance, but stem photosynthesis was less sensitive to water stress and was the only form of photosynthesis for seven months of the year (Tinoco-Ojanuren 2008). Leaf photosynthesis seems to be more sensitive generally to environmental stress than stem photosynthesis (Nilsen 1992, Tinoco-Ojanuren 2008). Thus, culms may perform the photosynthetic function of leaves due to their enhanced photosynthesis and greater tolerance of water stress during summer drought in the CFR.

Poales originated during the Cretaceous (Bremer 2002, Janssen & Bremer 2004, Linder & Rudall 2005), with the major lineages of Poales diversifying in the mid- to late Cretaceous and most Poales groups diversifying during the Cenozoic (Linder & Rudall 2005). Poales diversification into more seasonal climates and fire-adapted vegetation occurred during the Late Miocene (Linder & Rudall 2005). However, recent molecular evidence suggests that Poales diversification began prior to the end-Miocene climate changes when climates were more mesic and less seasonal than they are at present (Linder & Hardy 2004, Linder 2005, Verboom *et al.* 2009). The CFR has been a hotbed of radiations, with radiations of many Cape floral clades dated to the Middle Miocene (Linder 2005, Verboom *et al.* 2009). In the Cape, climatic changes during the Miocene resulted in a seasonal climate with winter rainfall and summer-arid climate conditions (Linder & Hardy 2004). One proposal for radiation in the Cape is that this climate change led to the extinction of tropical flora, allowing formerly heathy, montane vegetation to occupy those habitats (Linder & Hardy 2004). It is unclear whether culm or leaf photosynthesis was ancestral in Cape Poales. The culm structure may have evolved as an adaptation to summer-dry environments and could explain the success of Restionaceae, Cyperaceae and Poaceae in the CFR.

In the CFR, all Restionaceae and several Cyperaceae and Poaceae possess reduced photosynthetic leaves, or lack photosynthetic leaves, and are dependent entirely on culm fixation of carbon. Curiously, some Restionaceae, Cyperaceae and Poaceae produce leaves when they are young, as part of their adult form, during spring and/or after fire. Little is known about whether culm or leaf photosynthesis was ancestral in Cape Poales. I proposed that culm photosynthesis reflects adaptation to summer-dry conditions due to their sclerophyllous nature, and associated drought tolerance and nutrient efficiency. Since leaves are produced during spring and after fire, I proposed an alternate hypothesis that leaves function in resource acquistion during periods of high nutrient availability, such as spring or after fire. I tested these hypotheses in three different ways:

1) I mapped the evolution of these traits on a phylogeny of Cape Poales to determine whether leaf or culm photosynthesis was ancestral and to evaluate whether the appearance of culm photosynthesis coincided with the onset of summer-dry climates in the Cape towards the end of the Miocene.
2) I compared gas and heat exchange and traits of leaves and culms in one species of each family of Cape graminoids, with the expectation that leaves would be shorter-lived and transpire and photosynthesize at faster rates than culms.

3) I tested whether culm-photosynthetic species tended to be associated with summer-dry environments and whether species with leaves were associated with high rainfall during spring, when resource availability is high.

MATERIALS AND METHODS

CAPE POALES PHYLOGENY

Published phylogenies of Cape Poaceae, Cyperaceae and Restionaceae were used to generate a supertree relating as many Cape graminoid taxa as possible. An *Ehrharta* phylogeny was obtained from Verboom *et al.* (2004), while the phylogeny of danthonioid grasses was obtained from Galley & Linder (2007) and Pirie *et al.* (2008). A *Tetraria* phylogeny was obtained from Slingsby & Verboom (2006) while the placement of the other Cyperaceae was obtained from Muasya *et al.* (2001), Simpson *et al.* (2007) and Muasya *et al*. (2009). For Restionaceae, the Hardy *et al.* (2008) highest likelihood tree was used. Higher-level Poales relationships were obtained from Bremer (2002) and Bouchenak-Khelladi *et al*. (2008). Species were stitched together manually and branch lengths were not included. The placement of 460 species could be inferred on a phylogeny. The supertree phylogeny was built using Mesquite *V*2.72 (Maddison & Maddison 2010). The list of species can be found in Appendix A1.

CULM CHARACTER RECONSTRUCTION

We identified shifts between culm and leaf morphologies to determine whether these shifts occurred repeatedly, in which clades shifts occurred and when shifts occurred. However, the majority of Poales have neither solely photosynthetic culms nor leaves but a combination of the two. For this reason we scored leafiness as follows:

1. Culm only
2. Culm with narrower, culm-like, cylindrical leaves
3. Culm with a few large, broad, flat leaves
4. Thin culm with small, scarce, flat leaves
5. Mostly leafy, leaves broad and flat, thin non-photosynthetic reproductive culms

I found that this classification system sufficiently described all Poales species sampled. Herbarium samples (Bolus Herbarium, University of Cape Town) of all Poaceae and Cyperaceae species on the supertree were assessed and scored for leafiness. For Restionaceae, information on whether species produced leaves was obtained from "The African Restionaceae: an IntKey identification and description system" (Version 2, Linder 2004). Since we were interested in determining when the loss of leaves occurred, the classification system was simplified so that all species with leaves (categories 2-5) were separated from species possessing only a culm structure (category 1). Furthermore, we grouped species into functional categories; those having only culm photosynthesis (1), culm and leaf photosynthesis (2-4) and predominantly leaf photosynthesis (5). Character reconstruction was done using an ordered parsimony ancestral state model in Mesquite *V*2.72 (Maddison & Maddison 2010). To assess when the loss of leaves first occurred, we identified the node/s of leaf loss and then consulted dated Monocot phylogenies (Bremer 2002, Janssen & Bremer 2004, Christin *et al.* 2008) to approximate the date.

TRAIT AND FUNCTIONAL COMPARISON OF CULMS AND EPHEMERAL LEAVES IN POALES

During late Winter/early Spring (August 2010), plants of *Ehrharta ramosa* Thunb. (Poaceae), *Ficinia bulbosa* Nees. (Cyperaceae) and *Thamnochortus sporadicus* Pillans. (Restionaceae) (n = 3) co-occurring in Bainskloof, Western Cape, South Africa were found which had both photosynthetic culms and leaves. Plants were of similar size (20 cm in height) and flowering had not yet begun. Leaves were lanceolate, and flat in *E. ramosa* and cylindrical in *T. sporadicus* and *F. bulbosa* and, in all three species, were more fragile and shorter than the culms. Plants were growing in sandy soil in a rocky area at an altitude of 1500 m that had received rain the previous day. Culm and unrolled leaf diameters were measured using callipers. Culm and leaf samples were collected from each specimen. For all culms and leaves of *T. sporadicus* and *F. bulbosa*, photosynthetic area was estimated by measuring the length and diameter of the cylinders to calculate the area. For *E. ramosa*, photosynthetic leaf area was measured using an LI-3000 Area Meter (LICOR). Stomatal impressions were obtained by coating culm surfaces with clear nail varnish, which was peeled off and examined at 400X magnification on a transmission light microscope. Stomatal numbers in the field of view were counted expressed as stoma per mm^2. Samples were then dried in an oven at 80°C for 48 h and then weighed. Specific culm area (SCA) and specific leaf area (SLA) was calculated as the photosynthetic area (m^2) per dry weight (kg) of culm or leaf tissue. Gas exchange measurements were taken on culms and leaves of three specimens of each species between 10:00 h and 13:00 h. Culm and unrolled leaf photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) were measured using an LI-6400 Portable Photosynthesis System (LICOR, Lincoln, NE, USA). Measurements were taken at a saturating photon fluence rate of 1500 µmol quanta m^{-2} s^{-1} in a Licor LI-6400-02B cuvette set to 25ºC and a CO_2 concentration set to 400 µmol mol^{-1}. After ca. 3 min equilibration in the cuvette, measurements were recorded. Leaf and culm temperatures ranged from 20.2 to 22.4ºC. Vapour pressure deficit averaged 2.5 kPa. Temperature was calculated using energy balance equations.

CULM – ENVIRONMENT RELATIONSHIPS

To test whether leafy species were associated with different environmental conditions to species with only culms we performed boosted regression trees analysis, which allows non-linear modelling of a response variable to predictor variables. Distribution data of CFR Poaceae, Cyperaceae and Restionaceae taxa, obtained from collections in the Bolus Herbarium, University of Cape Town, were used to derive climatic variables. Distribution data were available for 218 of the 460 species included in the Cape Poales phylogeny. Distributions of each taxon were overlaid with 19 BIOCLIM climatic variables using the ARC Geographic Information System. Climatic variables were obtained for each distribution point and then averaged for each species. Environmental parameters included mean annual temperature, mean diurnal range (mean of all the weekly diurnal temperature ranges, isothermality (mean annual temperature/ mean monthly temperature range), temperature seasonality (temperature coefficient of variation - standard deviation of the weekly mean temperatures expressed as a percentage of the mean of those temperatures), maximum temperature of the warmest month, minimum temperature of the coldest month, mean temperatures of the warmest, coldest, wettest and driest months, annual precipitation, maximum precipitation of the wettest month, minimum precipitation of the driest month, precipitation seasonality (precipitation coefficient of variation - standard deviation of the weekly precipitation estimates expressed as a percentage of the mean of those estimates), and precipitation of the warmest, coldest, wettest and driest months. These variables are biologically meaningful as they include seasonality of climatic parameters, which is a key feature in the CFR.

We chose to use Boosted Regression Trees (BRT) analysis to determine the environmental correlates of culms as it allows non-linear modelling of a response variable to predictor variables, both of which can be either discrete or continuous (Elith ***et al.*** 2008). The response variable was binary, with species with only culms scored as 0 and species with leaves scored as 1. BRT analysis also allows identification of predictor variables that most explain the distribution of a response variable, which is extremely valuable in assessing environmental trait correlates (De'ath & Fabricius 2000, Hastie *et al.* 2001, Elith *et al.* 2008). The BRT analysis was implemented using the gbm package (version 1.6 -3.1, Ridgeway 2010) supplemented with the functions of Elith *et al.* (2008) in R (version 2.11.0, R Development Core Team 2010). During the analysis splitting events, described by a simple rule in a single predictor variable, create two homogenous groups (Hastie *et al.* 2001). Splitting is then applied recursively until a large tree is built (Hastie *et al.* 2001). Several trees are created using boosting techniques, with each successive tree concentrating on the variation not explained in the previous tree (De'ath & Fabricius 2000, Leathwick *et al.* 2006, Elith *et al.* 2008). We were interested in whether leafy species (2-5), which were scored as trait present, were associated with different conditions to culm species (1), which were scored as trait absent. In the model, a Bernoulli distribution (for the binary response variable) with a bag fraction of 0.75, a tree complexity of 5 and a learning rate of 0.001 was used. In our model, the optimal number of trees was found to be 2000. The number of splits in the tree as well as interactions between variables is determined by tree complexity (Elith *et al.* 2008). The learning rate determines the contribution of each tree to the model and thus the rate at which predictive performance improves with the number of trees (Elith *et al.* 2008). A cross-validation method in which the dataset is divided up into ten subsets, one of which is dropped before building a tree, is used to determine optimal tree complexity and learning rate (Hastie *et al.* 2001). The omitted dataset is used as a dataset on which to test the model and each of the ten subsets undergo this process, with the sum of deviances between subsets being used to evaluate the predictive deviance of the model (Hastie *et al.* 2001). The predictive deviance provides a goodness-of-fit measure between predicted and raw data and is expressed as a percentage of the null deviance (De'ath & Fabricius 2000, Hastie *et al.* 2001, Leathwick *et al.* 2008). The relative importance of predictor variables was assessed according to the method of Elith *et al.* (2008). Partial dependence plots were created to display the influence of a predictor variable on the response variable after accounting for the average effects of all the other predictor variables (Elith *et al.* 2008). Functions written by Elith *et al.* 2008 were used to assess the influence of interactions between predictor variables.

RESULTS

CULM/LEAF CHARACTER RECONSTRUCTION

When distinguishing between species with only culm photosynthesis (1) and those with leaf photosynthesis (2-5), the common ancestor of CFR Poaceae, Restionaceae and Cyperaceae was resolved as having leaf photosynthesis (Fig. 1). All species of Cape Poaceae and Cyperaceae are leaf photosynthetic to some degree. Within Restionaceae, leaf photosynthesis has evolved on at least ten independent occasions in four genera: *Restio*, *Rhodocoma, Thamnochortus* and *Elegia.* The state of the ancestral Restionaceae was ambiguous. When species were classified according to photosynthetic functionality (Fig. 2) the patterns became more complex. The ancestral Restionaceae was ambiguous, having either only culm photosynthesis or culm and leaf photosynthesis. Most *Ehrharta* species had a combination of culm and leaf photosynthesis, with predominantly leaf photosynthesis evolving in five taxa on at least three occasions. Photosynthesis in ancestral *Ehrharta* was ambiguous; either culm and leaf photosynthesis or primarily leaf photosynthesis. The danthonioid grass ancestor was unambiguously leafy and reversal to a combination of leaf and culm photosynthesis occurred in four *Pentaschistis* and two *Tribolium* taxa on at least five occasions. Both culm and leaf photosynthesis were present in the ancestral Cape Cyperaceae with predominantly leaf photosynthesis evolving in only 6 taxa: *Capeobolus brevicaulis*, *Cyathocoma hexandra*, *Ficinia trichodes*, *Ficinia ramosissima*, *Ficinia esterhuyseniae* and *Isolepis fluitans*. Overall, these results suggest that it is likely that the ancestral Poales had a combination of culm and leaf photosynthesis. However, our sample of Poales included only CFR taxa and is therefore biased by incomplete sampling of Poales.

The common ancestor of Poaceae, Restionaceae and Cyperaceae was reconstructed as leafy and the node has been dated to approximately 110 MY (Bremer 2002, Janssen & Bremer 2004). The common ancestor of Poaceae and Restionaceae, which has been dated to approximately 100 MY (Bremer 2002, Janssen & Bremer 2004), was reconstructed as having leaves. In Restionaceae, leaf photosynthesis was lost early on in the evolutionary history of the family, with almost all species having culm photosynthesis. Restionaceae originated approximately 65 MYA (Linder *et al.* 2003) and loss of leaves occurred near the basal split within Restionaceae, very early on in the evolutionary history of this family, indicating that culm photosynthesis was present prior to the onset of climate change during the Mid-Miocene. *Pentaschistis* and *Tribolium* originated approximately 14 MYA (Verboom *et al.* 2006, Galley & Linder 2007) and are predominantly leaf-photosynthetic. The *Ehrharta*, Shoeneae and *Isolepis/Ficinia* clades are older and have been dated to around 40-45 MYA (Verboom *et al.* 2003, Verboom 2006, Verboom *et al.* 2009). The majority of species in these clades had both leaf and culm photosynthesis.

STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN CULMS AND LEAVES

Leaves produced by *E. ramosa*, *T. sporadicus* and *F. bulbosa* during spring have markedly different structure and function compared with perennial culms of the same species. Furthermore these trends were consistent for all three species. Superficially, the leaves looked cylindrical in shape in *T. sporadicus* and *F. bulbosa* and flat in *E. ramosa* and were much smaller and more fragile than the culms. Leaves of *F. bulbosa* and *T. sporadicus* were cylindrical in shape but tended to be narrower in diameter and have higher specific area than culms of the same species (Fig. 3). Leaves of *E. ramosa* were flat but narrow and lanceolate and had higher specific area than *E. ramosa* culms. Leaves had higher photosynthetic rates than culms, although this was significant only in *E. ramosa* and *T. sporadicus* (Fig. 4). For *E. ramosa* and *F. bulbosa*, leaf and culm transpiration rates were similar. However, leaves of *T. sporadicus* transpired, on average, five times faster than culms. When transpiration and photosynthesis were compared on a per mass basis, significant differences between culms and leaves were still evident. Leaf and culm temperatures were similar for both *E. ramosa* and *F. bulbosa*, but *T. sporadicus* leaves were almost 4°C lower than culms, possibly due to evapotranspirational cooling. In all three species, differences in the number of stomata per area between culms and leaves were not evident.

ENVIRONMENTAL CORRELATES OF CULM TRAITS

The functions fitted by the BRT model were all non-linear. The predictive deviance of the BRT model, estimated using a cross validation process, was 71.8% indicating good fit between raw values and values predicted from independent data (Leathwick *et al.* 2008). The fitted functions show that leafy species occur most frequently in areas of moderate rainfall in the coldest quarter of the year (Fig. 5a), high rainfall seasonality (Fig. 5b), and high rainfall in the wettest month of the year (Fig. 5c). Leafy species were also mostly associated with areas where the temperature in the warmest quarter of the year is high (Fig. 5d), temperature in the wettest quarter of the year is high (Fig. 5e) and temperature range over the year is high (Fig. 5f). In contrast, leafless, culm species occur where rainfall in the coldest quarter is high, rainfall seasonality is low and rainfall in the wettest month is low. Areas in which culm species occur most frequently tend to have low temperatures during the warmest quarter of the year, low temperatures in the wettest quarter and a relatively low temperature range over the course of the year.

The strongest interactive effects were found between isothermality and rainfall seasonality (Fig. 6a) and between isothermality and temperature in the warmest quarter (Fig. 6b). Leafy species occurred most frequently in areas with both high isothermality and high rainfall seasonality. Leafy species were also associated with areas of high isothermality and high temperature in the warmest quarter of the year. By contrast culm species occur in areas with lower isothermality, low rainfall seasonality and cooler temperature in the warmest quarter.

DISCUSSION

These results show that species with only culm photosynthesis were associated with wet, cold, aseasonal environments. In addition, culm photosynthesis was present prior to onset of the mid-Miocene climate change. Based on this evidence, we suggest that culms are not adaptive for summer drought conditions. Culms are photosynthetic, evergreen structures that have low specific area, implying that they are long-lived and have higher carbon return per nutrient invested (Orians & Solbrig 1977, Westoby 1998). Sclerophylly is generally associated with drought tolerance and nutrient efficiency (Stock, van der Heyden & Lewis 1992, Salleo *et al.* 1997), but since these structures occur in mesic environments, nutrient efficiency seems a more likely explanation. Thicker photosynthetic structures often tend to be more sclerophyllous, having low area per unit mass. Thicker photosynthetic organs have been associated with infertile and/or dry conditions because transpirational costs are larger when photosynthetic rates on poor and/or dry soils are low (Givnish 1978, 1979). Culm structures may represent a thicker photosynthetic organ, and my data do show that culms tend to be more sclerophyllous, with lower photosynthetic area per unit mass. In Restionaceae, culms had significantly lower transpiration rates than leaves but occurred in mesic areas, thus supporting the economic findings of thicker photosynthetic organs in infertile conditions (Givnish 1978, 1979). The soils of the mountainous regions of the CFR are sandstone-derived and nutrient poor (Cowling & Holmes 1992) and culms may be an adaptation to nutrient-poor conditions. The higher carbon return per nutrient invested for culm structures compared to leaves may be advantageous in this nutrient-

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poor environment. Future studies assessing the evolutionary and ecological associations of culm photosynthesis and nutrients would be able to test this theory.

The conditions with which culms were associated are typical of high altitude areas. While most of the CFR receives high rainfall during winter, with little rain falling during the hot summer months, the seasonality in some areas is not so well defined. Verboom *et al.* (2009) highlighted that some high altitude environments receive moisture input from south-easterly winds during summer, resulting in reduced summer water deficits in these areas, and also tend to have the greatest species richness. These high altitude areas may act as refugia for taxa not well adapted to the summer-arid, seasonal conditions of the rest of the CFR. This may be the case for culm-photosynthetic species. Interestingly, culms are not as prevalent in the adjacent succulent karoo biome, which tends to be drier with a more extreme summer drought period (Cowling 1998).

From our CFR Poales supertree and by consulting dated Poales phylogenies, we found that a combination of culm and leaf photosynthesis may have been present in the common ancestor of Cape Poaceae, Cyperaceae and Restionaceae, which can be dated to the mid to late Cretaceous (Bremer 2002, Janssen & Bremer 2004). While our phylogeny was biased as it included only CFR taxa, this ancestral form may be likely. All Restionaceae were culm-photosynthetic. Restionaceae originated approximately 65 MYA (Linder, Eldenas & Briggs 2003) and loss of leaves appears to have occurred near the basal split within Restionaceae, very early on in the evolutionary history of this family. Intriguingly, tufty, leaf-like structures were subsequently regained on ten independent occasions in this family. Danthonioid grasses were predominantly leaf-photosynthetic and had a younger origin, while *Ehrharta* and Cyperaceae had mostly culm- and leaf-photosynthetic species and were older. In *Thamnochortus* (Restionaceae), the ancestral node was resolved as culm-photosynthetic and the combination of leaf and culm photosynthesis evolved on at least two occasions and was found in approximately 50% of species. Differences in gene function and/or regulation may explain the production of different leaf forms, and possibly culm structures, over evolutionary time (Hay & Tslantis 2006). In *Cardamine hirsuta* the presence of class I KNOTTED1-like homeobox (KNOX) proteins are required for the leaf to show a dissected leaf form, whereas absence of these KNOX proteins in *Arabidopsis thaliana*, a close relative of *C. hirsuta*, results in a simple leaf form (Hay & Tslantis 2006). Linder & Mann (1998) found evidence for an ancestral habitat with higher rainfall in the genus *Thamnochortus* and that coastal and summer-dry habitats were derived within the genus. This suggests that ancestral culm photosynthesis may have been associated with higher rainfall and the derived culm and leaf photosynthetic state may have been associated with summer-dry habitats. This provides evidence that evolution of leaf photosynthesis could have been in response to summer-arid, seasonal conditions. What are the advantages of leaf photosynthesis?

While all Restionaceae produce culms, approximately 9% of species produce leaf-like structures that are tufty, cylindrical and thinner than the culms. Leaf photosynthesis is common in Poaceae and Cyperaceae. In *T. sporadicus* (Restionaceae) and *F. bulbosa* (Cyperaceae), leaves were narrower, shorter and more fragile than culms. Leaves of *E. ramosa* (Poaceae) were flat and lanceolate. Compared to leaves of the same species, culms had lower specific area than leaves. This was consistent in all three species. Specific area is linked to longevity (Westoby 1998), and the high specific area of leaves is indicative of reduced longevity. The physiological functioning of culms and leaves was quite different. Leaves had significantly higher rates of photosynthesis than culms, both on a per area and a per mass basis. For *T. sporadicus*, leaves also had substantially higher rates of water loss and lower temperature (Fig. 4). Leaf-photosynthetic Poales were associated with warmer, more seasonal environments. In the Cape the mostly sandstone-derived soils are highly nutrient leached and winter rainfall causes further nutrient leaching (Cowling & Holmes 1992). Rain falls mostly during winter, creating a short window of opportunity at the end of winter and early spring in which surface water availability enhances nutrient availability. We suggest that leaf production represents a short-term nutrient acquisition strategy whereby leaves that are shorter-lived and have lower carbon return per nutrient invested are produced during periods of high resource availability. Despite the lower nutrient efficiency of leaf production compared to culm production, leaves facilitate efficient capitalisation of resources during these temporary conditions through their higher photosynthetic and transpiration rates. However, the cost of producing leaves is high and would only be advantageous when resources are not limiting. In *Thamnochortus punctatus*, root and rhizome development occurred during the wet winter months while culm elongation occurred in the spring to summer period (Stock *et al.* 1987). Interestingly, this species does produce leaf-like structures and while no mention of these was made in the study, this asynchronous growth pattern corroborates our findings.

Culm structures may represent a longer-term strategy in which the rigid, nutrient efficient structure with its high carbon return per nutrient invested of culms enables persistence and perennial growth. They may also fulfil the role of assimilation when resources are limiting and leaf production is not feasible. Culms may also function to provide structural support and increased height while simultaneously assimilating carbon. Many species with culm photosynthesis also occur in windy environments and are wind pollinated. The shape, rigidity and height of the culm structure may improve structural integrity during windy events and enhance pollen capture. Restionoideae culms tend to sway in the wind, which may prevent wind-induced cavitation. The Cape experiences very strong south-easterly winds during Spring that cause considerable damage. Thus, the significance of culm structures in the cape may be due to multiple factors. Further studies investigating the effectiveness of culms in relation to wind would be most interesting.

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REFERENCES

Ackerly, D.D. & Reich, P.B. (1999) Convergence and correlations among leaf size and function in seed plants: a comparative test using independent contrasts. *American Journal of Botany*, **86**, 1272-1281.

Ackerly, D.D., Knight, C.A., Weiss, S.B., Barton, K. and Starmer, K.P. (2002) Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. *Oecologia,* **130**, 449-457

Bouchenak-Khelladi, Y., Salamin, N., Savolainen, V., Forest, F., van der Bank, M., Chase, M. & Hodkinson, T.R. (2008) Large multi-gene phylogenetic trees of the grasses (Poaceae): Progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution,* **47**, 488-505.

Bremer, K. (2002) Gondwanan evolution of the grass alliance of families (Poales). *Evolution,* **56**, 1374-1387.

Christin, P., Besnard, G., Samaritani, E., Duvall, M.R., Hodkinson, T.R., Savolainen, V. & Salamin, N. (2008) Oligocene CO2 decline promoted C4 photosynthesis in grasses. *Current Biology,* **18**, 37-43.

Cowling, R.M. (1998) Extraordinary high regional-scale plant diversity in southern African arid lands: subcontinental and global comparisons. *Diversity and Distributions*, **4**, 27–36.

Cowling, R.M. and Holmes, P.M. (1992) Flora and vegetation. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

De'ath, G. & Fabricius, K.E. (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178-3192.

Elith, J., Leathwick, J.R. & Hastie, T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802-813.

Galley, C. & Linder, H.P. (2007) The phylogeny of the *Pentaschistis* clade (Danthonioideae, Poaceae) based on chloroplast DNA, and the evolution and loss of complex characters. *Evolution*, **61**, 864–884.

Gibson, A. (1983) Anatomy of photosynthetic old stems of non-succulent dicotyledons from north American deserts. *Botanical Gazette,* **144**, 347-362.

Givnish, T.J. (1978) Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica*, **27**, 83-142.

Givnish, T.J. (1979) On the adaptive significance of leaf form. In: *Topics in Plant Population Biology* (Eds. O. T. Solbrig, S. Jain, G. B. Johnson & P. H. Raven), pp. 375-407. Columbia University Press, New York.

Givnish, T.J. (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist*, **106**, 131-160.

Goldblatt, P. & Manning, J.C. (2000) *Cape Plants. A Conspectus of the Cape Flora of South Africa.* National Botanical Institute, Pretoria.

Hardy, C.R., Moline, P.M. & Linder, H.P. (2008) A phylogeny for the African Restionaceae, and new perspectives on morphology's role in generating complete species-level phylogenies for large clades. *International Journal of Plant Science*, **169**, 377-390.

Hastie, T., Tibshirani, R. & Friedman, J.H. (2001) *The elements of statistical learning: data mining, inference, and prediction*. Springer-Verlag, New York.

Hay, A. & Tslantis, M. (2006) The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. *Nature Genetics*, **38**, 942-947.

Janssen, T. & Bremer, K. (2004) The age of major monocot groups inferred from 800+ rbcL sequences. *Botanical Journal of the Linnean Society,* **146**, 385-398.

Leathwick, J.R., Elith, J., Francis, M.P., Hastie, T. & Taylor, P. (2006) Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. *Marine Ecology Progress Series*, **321**, 267-281.

Linder, H.P. (1984) A phylogenetic classification of the genera of the African Restionaceae. *Bothalia,* **15**, 11-76.

Linder, H.P. (2004) The African Restionaceae: an IntKey identification and description system, version 2. Contrib Bolus Herb 20. http://www .systbot.unizh.ch/datenbanken/restionaceae/restionaceae.php.

Linder, H.P. (2005) Evolution of diversity: the Cape flora. *Trends in Plant Science*, **10**, 536-541.

Linder, H.P. & Mann, D.M. (1998) Phylogeny and biogeography of *Thamnochortus* (Restionaceae). *Botanical Journal of the Linnean Society*, **128**, 319-357.

Linder, H.P. & Hardy, C.R. (2004) Evolution of the species-rich Cape flora. *Phil. Trans. R. Soc. Lond.* **359**, 1623-1632.

Linder, H.P. & Rudall (2005) Evolutionary history of Poales. *Annu. Rev. Ecol. Evol. Syst*. **36**, 107-124.

Linder, H.P., Eldenas, P. & Briggs, B.G. (2003) Contrasting patterns of radiation in African and Australian Restionaceae. *Evolution,* **57**, 2688-2702.

Maddison, W.P. & Maddison, D.R. (2010) *Mesquite: A Modular System for Evolutionary Analysis, Version 2.72*. Available at: http://mesquiteproject.org. Accessed 20 June 2010.

Mooney, H.A. (1982) Habitat, plant form, and plant water relations in Mediterranean-climate regions. *Ecol Mediterr* V: 481-488.

Muasya, A.M., Simpson, D.A., Chase, M.W. & Culham, A. (2001) Phylogeny of Isolepis (Cyperaceae) inferred using plastid rbcL and trnL-F sequence data. *Systematic Botany,* **26**, 342-353.

Muasya, A.M., Simpson, D.A., Verboom, G.A., Goetghebeur, P., Naczi, R.F.C., Chase, M.W. & Smets, E. (2009) Phylogeny of Cyperaceae based on DNA Sequence Data: Current Progress and Future Prospects. *Botanical Review,* **75**, 2-21.

Nilsen, E.T. (1992) The influence of water stress on leaf and stem photosynthesis in Spartiumjunceum L. *Plant, Cell and Environment,* **15**, 455-461.

Orians, G.H. and Solbrig, O.T. (1977) A cost-income model of leaves and roots with special reference to arid and semi-arid areas. *American Naturalist,* **111**, 677-690.

Pirie, M.D., Humphreys, A.M., Galley, C., Barker, N.P., Verboom, G.A., Orlovich, D., Draffin, S.J., Lloyd, K., Baeza, C.M., Negritto, M., Ruiz, E., Cota Sanchez, J.H., Reimer, E. & Linder, H.P. (2008) A novel supermatrix approach improves resolution of phylogenetic relationships in a comprehensive sample of danthonioid grasses. *Molecular Phylogenetics and Evolution,* **48**, 1106-1119.

R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ridgeway, G. (2010) GBM: Generalized boosted regression models. R package, version 1.6-3.1. http://www.i-pensieri.com/gregr/gbm.shtml.

Salleo, S., Nardini, A. & Lo Gullo, M.A. (1997) Is sclerophylly of Mediterranean evergreens an adaptation to drought? *New Phytologist*, **135**, 603-612.

Simpson, D.A., Muasya, A.M., Alves, M.V., Bruhl, J.J., Dhooge, S., Chase, M.W., Furness, C.A., Ghamkhar, K., Goetghebeur, P., Hodkinson, T., Marchant, A.D., Reznicek, A.A., Nieuwborg, R., Roalson, E.H., Smets, E., Starr, J.R., Thomas, W.W., Wilson, K. & Zhang, X. (2007) Phylogeny of Cyperaceae based on DNA sequence data – a new rbcL analysis. *Aliso,* **23**, 72-83.

Slingsby, J.A. & Verboom, G.A. (2006) Phylogenetic relatedness limits co-occurrence at fine spatial scales: evidence from the Schoenoid sedges (Cyperaceae: Schoeneae) of the Cape Floristic Region, South Africa.

Stock, W.D. and Allsopp, N. (1992) Functional Perspectives of Ecosystems: In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Stock, W.D., Sommerville, J.E.M. & Lewis, O.A.M. (1987) Seasonal allocation of dry mass and nitrogen in a fynbos endemic Restionaceae species *Thamnochortus punctatus* Pill. *Oecologia*, **72**, 315-320.

Stock, W.D., van der Heyden, F. and Lewis, O.A.M. (1992) Plant structure and function. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Tinoco-Ojanuren, C. (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran desert. *Journal of Arid Environments,* **72**, 127-140.

Verboom, G.A. (2006) A phylogeny of the schoenoid sedges (Cyperaceae: Schoeneae) based on plastid DNA sequences, with special reference to the genera found in Africa. *Molecular Phylogenetics and Evolution*, **38**, 79–89.

Verboom, G.A., Linder, H.P. & Stock, W.D. (2003) Phylogenetics of the grass genus Ehrharta: evidence for radiation in the summer-arid zone of the South African Cape. *Evolution,* **57**, 1008–1021.

Verboom, G.A., Linder, H.P. and Stock, W.D. (2004) Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus *Ehrharta* (Poaceae: Ehrhartoideae). *American Journal of Botany,* **91,** 1364-1370.

Verboom, G.A., Ntsohi, R. & Barker, N.P. (2006) Molecular phylogeny of African Rytidosperma-affiliated danthonioid grasses reveals generic polyphyly and convergent evolution in spikelet morphology. *Taxon*, **55**, 337–348.

Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F., Mummenhoff, K., Linder, H.P., Muasya, A.M., Oberlander, K.C., Savolainen, V., Snijman, D.A., van der Niet, T. & Nowell, T.L. (2009) Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, **51**, 44-53.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil,* **199**, 213-227.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010) Ecophysiological significance of leaf size variation in Proteaceae form the Cape Floristic Region. *Functional Ecology,* **24**, 485-492.

Yiotis, C., Psaras, G.K. and Manetas, Y. (2008) Seasonal photosynthetic changes in the green-stemmed Meditteranean shrub *Calicotome villosa*: a co mparison with leaves. *Photosynthetica,* **46**, 262-267.

FIGURE LEGENDS:

FIGURE 1: Reconstruction of culm photosynthesis (black) and evidence of leaf photosynthesis (red). Ambiguous branches are shown in orange. Species in the collapsed clades can be found in Appendix A1. The Restionaceae ancestor was reconstructed as ambiguous, but evidence of leaf photosynthesis was lost early in the history of Restionaceae, but subsequently evolved on a number of occasions.

FIGURE 2: Reconstruction of functional photosynthesis. Solely culm photosynthesis is show in black. A combination of culm and leaf photosynthesis is shown in blue. Species having predominantly leaf photosynthesis are shown in green. Where species are not shown, their names can be found in Appendix A1.

FIGURE 3: Structural differences between culms and ephemeral leaves of *E. ramosa*, *F. bulbosa* and *T. sporadicus*. The horizontal line shows the median, the bottom and top show the 25th and 75th percentiles and whiskers show the interquartile range. Significance is shown by ** for a = 0.05 and * for a = 0.1. Samples were collected in late winter/early spring.

FIGURE 4: Physiological differences between culms and leaves of *E. ramosa*, *F. bulbosa* and *T. sporadicus*. The horizontal line shows the median, the bottom and top show the 25th and 75th percentiles and whiskers show the interquartile range. Significance is shown by ** for a = 0.05 and * for a = 0.1. Measurements were taken at midday in late winter/early spring.

FIGURE 5: Partial dependence plots for the six most important environmental predictors in the boosted regression trees (BRT) model. These plots illustrate the frequency of occurrence of leafy species with particular environmental parameters while all other environmental predictors are held at their average values.

FIGURE 6: Plots of the two strongest interactions among environmental predictor variables. Plots show the interactive effects of these variables on the probability of occurrence of leafy species.

FIGURE 1

FIGURE 1 CONT.

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

APPENDIX PAGE 1

APPENDIX PAGE 2

APPENDIX PAGE 3

APPENDIX PAGE 4

APPENDIX PAGE 5

APPENDIX PAGE 6

CHAPTER 2

ECOPHYSIOLOGICAL CONSEQUENCES OF VARIATION IN CULM DIAMETER

ABSTRACT

1. Culms of varying diameter are highly prevalent in the Cape Floristic Region, where oligotrophic soils, fire, winter rainfall and summer drought present challenges for plant survival.
2. I proposed that narrow culm diameters might facilitate water loss in winter and sensible heat loss in summer and be adaptive for nutrient-poor and summer drought conditions. Alternately narrow culm diameters might reduce water flux and improve sensible heat loss and function solely in drought tolerance during summer. Since surface area: volume ratios increase with decreasing culm diameter, I expected narrow culms to have higher specific area and lower construction costs, whereas broad culms would have lower specific area, be more costly to produce and be longer-lived.
3. The influence of culm diameter on water and heat loss of filter paper cylinders and the culms of 16 Cape Restionaceae was explored. For filter paper cylinders I also investigated the influence of wind on water loss and cooling. In addition, I investigated culm allometric relationships for 39 Restionaceae species grown in a common garden. Correlations between specific area, stomatal and tissue density, plant height and culm diameter were assessed and compared. All correlations were assessed using phylogenetically independent contrasts. I then explored whether the consequences of culm diameter for water and heat loss were ecologically significant by evaluating the response of culm diameter to climate using boosted regression trees as well as phylogenetically independent contrasts.
4. Water loss decreased significantly with increasing filter paper cylinder diameter. For filter paper cylinders, the influence of culm diameter on water loss was evident at slow and faster wind speeds. When wind was absent, mixed and forced convection occurred and diameter influenced cooling. At winds greater than 0.5 m s^{-1}, cylinder diameter had little influence on cooling. Similar trends in water loss with increasing diameter were found for Restionaceae culm diameters, for both transpiration and photosynthesis. On average, narrow culms (*ca.* 0.1 mm) transpired and photosynthesized 8 and 9 times faster, respectively, than broad culms (*ca.* 4.5 mm). Culm temperature showed a positive

logarithmic relationship with culm diameter, however, the temperature difference between the narrowest and broadest culms was only 2ºC. Culm diameter was positively correlated with height and followed a negative power curve with specific area. Boosted regression trees analysis showed that narrow culms were associated with moderate minimum and maximum summer temperatures and received a very high proportion of their rainfall during spring, with little rain falling during winter and autumn.

5. Variation in culm diameter has important consequences for gas and heat exchange. Our data showed that narrow culms were associated with enhanced water loss as well as carbon assimilation. The prevalence of narrow culms in areas where most rain falls during spring suggests that narrow culms may be adaptive for seasonal conditions in which rapid transpiration and assimilation are advantageous. Rapid transpiration may be important for driving nutrient mass-flow to the roots of plants that take up most of their nutrients during spring from nutrient impoverished soils. While narrow culms had slightly improved sensible heat loss, they were associated with cool summers, indicating that the benefits of reduced diameter for cooling may not be substantial enough to influence physiological functioning.

Keywords: boundary layer, culm, diameter, photosynthesis, Restionaceae, specific culm area, temperature, transpiration.

INTRODUCTION

Gas and heat exchange influence the important physiological processes of photosynthesis, transpiration and temperature regulation, and may be key to understanding the adaptive significance of variation in culm diameter. Culm diameter has important consequences for gas and heat exchange through its effect on boundary layer thickness (Nobel 2005). The size of the still air boundary layer surrounding photosynthetic structures depends on the size of the plant structure, shape, wind speed and surface modifications (Schuepp 1993, Nobel 2005). The still air boundary layer influences the movement of air around the plant structure and the conductance of gases in and out of the structure (Nobel 2005). Although culms are photosynthetic structures that differ structurally from leaves, both are influenced by boundary layer dynamics. In Restionaceae, the leaves have been reduced to form sheaths and the culms are almost entirely responsible for photosynthesis (Linder 2000). The culms are evergreen and are mostly cylindrical. Within

Restionaceae, there is considerable variation in culm diameter, ranging from very narrow culms 1 mm in diameter, such as in *Elegia filacea* and *Restio gaudichaldianus*, to wide culms up to 3 cm in diameter, such as in *Cannamois grandis*, *Ceratocaryum argenteum* and *Rhodocoma gigantea*. While the influence of leaf size on plant physiological functioning has been assessed at both a leaf (Givnish, 1979, 1987, Parkhurst & Loucks 1972, Nobel 2005, Nicotra ***et al.*** 2008, Yates ***et al.*** 2010) and ecological (reviewed by Givnish 1987, MacDonald ***et al.*** 2003) level, culm diameter variation has received no attention. Empirical tests determining the physical principles of water loss from culms of varying size (Nobel 2005) to assess the influence of boundary layer on water loss and temperature as well as the adaptive significance of variation in culm diameter for plant ecophysiology are lacking.

For cylinders, a laminar boundary layer exists upwind of the structure, while downwind the air becomes turbulent, making evaluation of the boundary layer size and influence more challenging than for planar structures (Nobel 2005). Despite these complexities, boundary layer thickness (d) of a cylinder is approximated by d = a Öd/n, where a = 5.8, d is culm diameter in m and n is the wind speed in m s^{-1} (Nobel 2005). Wind thus affects boundary layer size, and at high wind speeds the thickness of the boundary layer is reduced, diminishing its influence on gas and heat exchange. While boundary layer thickness does influence gas movement into and out of the leaf by creating resistance, stomatal conductance also plays a crucial role in gas exchange (Martin ***et al.*** 1999, Nobel 2005). The relative extent to which stomatal conductance and boundary layer conductance control transpiration is evaluated using the Omega factor (Ω), which varies between 0 and 1 (McNaughton & Jarvis 1983). When boundary layer conductance is high, as for thin boundary layers, Ω is close to 0 and stomata are the principal controllers of the transpiration rate. Conversely, thick boundary layers greatly reduce potential conductance, such that changes in stomatal opening have little influence on the transpiration rate. By implication, structures with thin boundary layers facilitate tighter control of gas exchange through regulation of stomatal conductance.

Given the influence of culm diameter on boundary layer thickness and its consequences for gas and heat exchange, variation in culm diameter may be the consequence of adaptation to contrasting physical environments, analogous to the relationship between variation in leaf dimension (diameter of the largest circle that can be drawn in the leaf) and environmental conditions. The association of small-leaved species with arid environments has been attributed to their close coupling to ambient air temperatures (Gates *et al.* 1968). Within Cape Proteaceae, narrow leaves shed more heat through sensible heat loss and have the ability to transpire rapidly, features that are advantageous during hot dry summers as well as during wet winters (Yates *et al.* 2010). Leaf dissection in Cape *Pelargonium* was associated with high assimilation rates and low water use efficiencies, which are suggested to promote carbon gain and water delivery during periods of high soil moisture (Nicotra *et al.* 2008). At the environmental level, small leaves tend to occur at high altitudes and in areas with low mean annual precipitation, low mean annual temperature and poor soil fertility (reviewed by McDonald *et al.* 2003). Since boundary layer dynamics affect every structure, we expect to see similar physiological and environmental patterns with variation in culm diameter. The cylindrical shape of culms, however, implies different scaling relationships to those of leaves, which may complicate environmental patterns.

Since surface area: volume ratios increase with decreasing culm diameter, narrow culms would be expected to have higher specific area while broad culms would have lower specific area. Specific area is associated with sclerophylly, a trait that is common in Mediterranean-type ecosystems (Stock & Allsopp 1992). Sclerophylly has been linked with nutrient-limitation through its reduction in loss of nutrients at senescence (Orians & Solbrig 1977). Sclerophylly has also been shown to have advantages for drought tolerance through its effect on xylem cavitation (Salleo *et al.* 1997). Since culm diameter variation influences specific area, we may expect to see similar ecological patterns for culm specific area as those shown for leaf specific area. The relationship between specific area and leaf longevity represents a trade-off, with species with lower specific area having slower relative growth rates but longer leaf life spans and enhanced nutrient conservation (Givnish 1978, Westoby 1998, Aerts & Chapin 2000). Even though culms are cylindrical and have different scaling relationships to leaves, the underlying scaling principals and trade-offs between culm photosynthetic area and mass could be the same. Thus, functional correlates of culm diameter may be due to the relationship between culm diameter and specific area.

Restionaceae (subfamily Restionoideae, Briggs & Linder 2009), with variable culm diameters and occupying a diverse array of habitats, provides an appropriate system for examining the functional consequences of variation in culm diameter. The environmental conditions of the Cape Floristic Region pose complex physiological challenges for plants. The substrate in this area is highly heterogeneous and includes sandstone, quartzite, granite, shale and limestone, most of which tend to be highly oligotrophic. Furthermore, many parts of the region receive high precipitation during winter, which, combined with the skeletal soils, results in further nutrient leaching (Cowling 1992, Cowling & Holmes 1992). With most of the precipitation falling during the winter months, a narrow period of enhanced nutrient availability exists. During summer, high temperatures and low precipitation may produce substantial water and temperature stress in plants and low water availability impedes nutrient uptake. Summer drought, coupled with nutrient-poor soils and a limited period of nutrient availability pose complex challenges for plants and culm diameter variation may have adaptive significance in this region through its consequences for plant physiological functioning.

I investigated the functional consequences of variation in culm diameter within Cape Restionaceae. I hypothesized that higher transpiration and photosynthesis as well as enhanced sensible heat loss in narrow culmed species facilitates nutrient acquisition and assimilation when nutrients are available as well as cooling during the hot summer months. Since boundary layer dynamics strongly influence plant gas exchange, I expected to find similar physiological and environmental patterns with variation in culm diameter as has been shown for variation in leaf size. To corroborate theoretical perspectives on the influence of culm diameter variation on gas and heat exchange, I assessed the physical effects of diameter variation on evaporative water loss and cooling using filter paper cylinders. The influence of wind on water and heat loss was also explored using filter paper cylinders. I then tested whether the influence of culm diameter variation on gas and heat exchange could be detected in Restionaceae culms of varying diameter. In addition, I explored whether the effects of variation in culm diameter on gas and heat exchange were evident at an ecological scale. To determine whether the environmental patterns and gas exchange correlates were purely a function of culm diameter, I also assessed the structural co-variants of culm diameter.

MATERIALS AND METHODS

QUANTIFICATION OF THE RELATIONSHIP BETWEEN CYLINDER DIAMETER AND WATER AND HEAT LOSS AT VARYING WIND SPEEDS

I made culm replicas by wrapping pieces of filter paper around plastic irrigation cylinders of equal length (10 cm) and varying diameter (0.5 cm to 3 cm). The plastic inner tubes prevented water loss from the inner cylinder tube. Filter paper cylinders were saturated with water, placed vertically in water-filled petri dishes (1 cylinder per petri dish) and petri dishes and cylinders placed in a laminar flow chamber. The chamber was set to four different wind speeds; 0, 0.5, 1.0 and 1.5 m.s^{-1} but a number of wind speed measurements were taken for each sample and averaged. Cylinders and petri dishes were weighed at time intervals during drying and the rate of water loss per area (mmol m^{-2} s^{-1}) was plotted against both diameter (cm) and boundary layer thickness (mm). During the time intervals six measurements of wind speed were taken using an Anemometer (Airflow Instruments, TSI Instruments Ltd., UK). Surface temperatures of the cylinders were measured using an LS infrared thermometer (Optris, Berlin, Germany). Ambient temperature was 21.8 ± 0.16°C. Measurements were taken at very low irradiance (*ca*. 5 µmol m^{-2} s^{-1}). To assess cooling, the difference in cylinder temperature and ambient temperature (ΔT) was calculated. Using the Reynold's and Grashof numbers, we calculated the extent of free and forced convection occurring for each wind speed (Nobel 2005). The Reynold's number (Re) is a dimensionless number that describes the flow characteristics around an object of particular boundary layer thickness. The Grashof number (Gr) describes the tendency for free convection. The ratio Re^2/Gr indicates forced convection/free convection and experiments have revealed thresholds for free, mixed and forced convection (Nobel 2005). To compare evaporation for planar leaf-type surfaces and cylinders, water loss-dimension curves for flat filter paper leaf-replicas were obtained from Yates *et al.* (2010). Dimension directly correlates with boundary layer thickness and was used for this comparison. For cylinders, diameter is the same as dimension but for leaves dimension is the diameter of the largest circle that can be drawn in the leaf. While Yates *et al.* (2010) measured water loss at different temperatures and our study assessed water loss at varying wind speeds, differences in curves could be compared between cylinders at 20°C and minimal wind and leaf-replicas at 20°C and minimal wind.

PLANT CULTIVATION

Plants of 16 Restionaceae species (n = 3) of varying culm dimension were from Kirstenbosch Nursery, Cape Town. The plants were approximately 3.5 years old and had been grown from seed in 2 L plastic bags containing a 1:1 mixture of sand and compost. All species occur in the winter rainfall region of the Western Cape, South Africa. Species were selected for their variation in culm dimension and, where possible, variation in dimension within each of the four genera. Culm dimension ranged from 0.1 mm in the narrow *Restio similis* to 4.5 mm in the broad, hollow culms of *Elegia fistulosa*. Species and diameters can be found in Appendix A1.

GAS EXCHANGE AND TEMPERATURE MEASUREMENTS

Plants were maintained in a greenhouse with a temperature of 25ºC and moved to a temperature-controlled growth chamber set to 20ºC with a light : dark photoperiod of 14:10 h and an irradiance of 1000 µmol m^{-2} s^{-1} two weeks prior to gas exchange measurement. Measurements were taken two weeks prior to flowering. Culm photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and temperature were measured using an LI-6400 Portable Photosynthesis System (LICOR, Lincoln, NE, USA). A needle-leaf chamber (LI-6400-05 conifer chamber) was used to accommodate sufficient culm area and to allow the boundary layer effects on gas and heat exchange to be included. Measurements were taken at the base, mid and top of each culm. Measurements were taken at chamber irradiance of 1000 µmol quanta m^{-2} s^{-1}. The cuvette was set to a temperature of 25ºC and a CO_2 concentration of 400 µmol mol^{-1}. After ca. 3 min equilibration in the cuvette, measurements were recorded. Culm temperatures ranged from 24.1 to 26.6ºC. Vapour pressure deficit averaged 1.78 kPa. Culm temperature was calculated using energy balance equations.

CULM SAMPLE COLLECTION

Culm samples of 39 Restionaceae species (n = 3) of variable culm diameter were sampled from Kirstenbosch Botanical Gardens. Plants had been grown in a mixture of sand and compost in a common garden. All species harvested occur naturally in the winter-rainfall region of the Western Cape, South Africa. Where possible, both male and female specimens of each species were collected. Sampling took place during winter in 2009. These plants were sampled to include a larger number of species for assessing the structural co-variants of culm diameter.

CULM TRAIT MEASUREMENTS

Culm trait measurements were taken on the plants used for gas exchange as well as the samples collected at Kirstenbosch Botanical Gardens. Stem basal diameter and height of each culm were measured prior to harvesting. Culm sections approximately 2.5 cm in length were taken just above the base, at two-thirds of the culm and at the top (3 cm from the tip) of the culm. Length, diameter at the bottom and diameter at the top of each section were measured with a calliper. For hollow species, the diameter of the inner diameter was also measured. Length and diameter were used to calculate area. Stomatal impressions were obtained by coating culm surfaces with clear nail varnish, which was peeled off and examined at 400X magnification on a transmission light microscope. Stomatal numbers in three fields of view were counted, averaged and expressed as stoma per m^2. Stomatal density could not be obtained for *Thamnochortus cinereus* due to extensive hairs on the culm surface. Culm sections were dried in an oven at 80˚C for 48 h and then weighed. Tissue density (kg m^{-3}) was estimated using the weight of the section divided by its calculated volume. For hollow sections, the volume used excluded the inner hollow volume and thus constituted only tissue volume. Specific culm area (SCA, m^2 kg^{-1}) was calculated as the photosynthetic area per dry weight of culm. Species and average trait values can be found in Appendix A2.

STATISTICAL ANALYSIS

Phylogenetic trees describing the relationships among the 16 species used in the gas exchange experiment and the 39 species sampled at Kirstenbosch Botanical Gardens were obtained by pruning unsampled taxa from the Hardy *et al.* (2008) highest likelihood tree. Raw branch lengths were obtained from the highest likelihood tree. To control for phylogenetic trait covariance, relationships amongst traits were evaluated using phylogenetically independent contrasts (PIC, Felsenstein 1985) as implemented in the PDAP:PDTREE module (Version 1.15; Midford, Garland and Maddison 2009) of Mesquite (Version 2.72, Maddison & Maddison 2010). Since PIC correlations apply to linear relationships, non-linear curves were log-transformed prior to PIC analysis. For trait relationships, curve parameters were estimated using non-linear least squares regression in R (version 2.11.0, R Development Core Team 2010). For the gas exchange and trait data, measurements were taken at 3 places along each culm: base, mid and upper. Curves were initially fitted to base, mid and upper values separately and the curve parameters compared, using Student's t-tests, to assess whether they were significant differences in functions obtained from samples of different positions. Values from the base, mid and top of the culms were averaged for the PIC analysis.

CULM TRAIT-ENVIRONMENT CORRELATIONS IN RESTIONACEAE

Average basal diameter values were obtained for 248 species from The African Restionaceae: an IntKey identification and description system (Version 2, Linder 2004). Climatic variables were derived from distribution data of Restionaceae taxa, which were based on collections in the Bolus Herbarium, University of Cape Town. Distributions of each taxon were overlaid with climatic variables using the ARC Geographic Information System and filtered using a 1'x 1' grid to exclude redundancy. For each species, ranges of climatic variables were obtained from these grid cell values. Environmental variables included altitude, continentality (measure between continental and coastal climates, characterized by temperature range and indicates the tendency for the land to experience more thermal variation than water - moisture input moderates coastal climates and they have lower continentality), mean annual precipitation (MAP), A-pan evaporation (evaporation potential of open water, originally measured as evaporation from a Class A evaporation pan expressed over an average day), temperature in the hottest and coldest months and rainfall seasonality (proportion of rainfall during each of the four seasons). Minimum and maximum values are useful as they represent the extremes of the conditions experienced by each species and provide insights into climatic tolerances. Environmental variables for South Africa were obtained from the South African Atlas of Hydrology and Climatology, Computing Centre for Water Research (CCWR), University of Natal (Schultze 1997). Altitude was computed by a Digital Elevation Model obtained from CCWR, University of Natal.

To assess the correlations between basal diameter and climate, PIC analysis was performed using a tree relating the 248 species, obtained by pruning unsampled taxa from Hardy *et al.*'s (2008) published maximum likelihood tree. To assess the non-linear responses of culm diameters to environmental predictor variables, we used boosted regression tree analysis, implemented using the gbm package (version 1.6 -3.1, Ridgeway 2010) supplemented with the functions of Elith, Leathwick &Hastie (2008) in R (version 2.11.0, R Development Core Team 2010). We were interested in the response of culm dimension to environmental variables, and BRT analysis allows non-linear modelling of a response variable to predictor variables as well as identification of predictor variables that most explain the distribution of a response variable (De'ath &

Fabricius 2000, Hastie *et al.* 2001, Elith *et al.* 2008). To investigate the adaptive significance of culm diameter variation, we identified environmental variables that best explained the variation in culm diameter. In the model, a Gaussian distribution (for the continuous response variable) with a bag fraction of 0.75, a tree complexity of 5 and a learning rate of 0.001 was used. Predictive deviance of the model was used to provide a goodness-of-fit measure between predicted and raw data and is expressed as a percentage of the null deviance (De'ath & Fabricius 2000, Hastie *et al.* 2001, Leathwick *et al.* 2008). The relative importance of predictor variables was assessed according to the method of Elith *et al.* (2008). Partial dependence plots, which display the influence of a predictor variable on the response variable after accounting for the average effects of all the other predictor variables, were produced to visualise the results of the model.

RESULTS

EFFECT OF CYLINDER DIAMETER ON WATER AND HEAT LOSS OF FILTER PAPER CYLINDERS WITH VARYING WIND SPEEDS

Based on data from filter paper cylinders, the physical relationship between water loss and culm diameter followed a negative power relationship. Evaporation increased with increasing wind speed. With minimal wind (no detectable wind movement), there was 4.5-fold variation in evaporation across the range of diameters examined (Fig. 1a) whereas at the highest wind speed (1.3 m s^{-1}) the variation range was 4-fold. As wind speed increased, boundary layer thickness as well as variation in boundary layer thickness was reduced (Fig. 1b). With minimal forced convection, estimates of boundary layer thickness, based on the equations of Nobel (2005), varied from 1.3 mm to 3.2 mm while at the highest wind speed boundary layer thickness varies from just 0.4 mm to 0.9 mm. The large reduction in boundary layer thickness and variation with increasing wind speeds did not result in reduced effects of variation in boundary layer on evaporation. Heat loss of filter paper cylinders was greater for narrow cylinders, with narrow cylinders being just 1°C warmer than ambient while wide cylinders were 2 °C warmer than ambient (Fig. 1c). At higher wind speeds, the effect of boundary layer thickness on cooling was reduced. Although irradiation was minimal, the water used was slightly warmer than the ambient temperature, resulting in filter paper being warmer than ambient.

With no wind, six of the 18 samples had mixed convection, while for the other 12 samples forced convection occurred (Fig. 2). Free convection only occurs at Re^2/GR close to 0.1, and despite reducing wind movement in the chamber as far as possible, free convection did not occur. With increasing wind speed, the rate of water loss per unit diameter increased but then levelled off (Fig. 3). Thus the effect of wind speed increased up to a certain point, after which further increases in wind speed have little effect. This levelling off occurs at quite a low wind speed (*ca.* 0.4 m s^{-1}) and at this wind speed the effects of variation in boundary layer thickness on water loss were still evident. Boundary layers of cylinders differ from those of planar leaves due to their cylindrical shape. For the same dimension and wind speed (diameter for cylinders and for planar leaves the diameter of the widest circle that can be drawn in the leaf) cylinders tend to have thicker boundary layers (Fig. 4a). The difference in boundary layer size between leaves and cylinders increased with increasing dimension. Water loss curves were similar for filter paper cylinders and planar leaf-replicas and both were fitted with power functions (Fig. 4b). Since the ambient temperature was 20°C when measurements for cylinders were taken, the leaf-replica curve at 20°C and minimal wind and the cylinder curve at 0 m s^{-1} could most readily be compared. This cylinder water loss curve was steeper than the leaf-replica curve, indicating that water loss for culms of dimensions greater than 0.5 cm is extremely low and increases very rapidly for dimensions less than 0.5 cm. The leaf-replica curve levelled out more gradually, and the decrease in water loss with increasing dimension was less pronounced. With increasing wind speed (for leaf-replicas) and temperature (for cylinders), cylinder and planar leaf-replica water loss curves were remarkably similar.

GAS EXCHANGE AND CULM TEMPERATURE VARIATION WITH RESTIONACEAE CULM DIAMETER

For Restionaceae of varying diameter, transpirational water loss showed a negative power trend with culm diameter (Fig. 5a). This pattern was evident both within individuals and species and between species. Culm diameter decreased with increasing distance from the base, as did water loss. On average, the narrowest culms (0.02 cm) transpired eight times faster than did the widest culms (0.45 cm) (Fig. 5a). Water loss per stoma followed a similar negative power function, suggesting that the differences in water loss with different diameters are not a result of differences in stomatal control (Fig. 5b). Water loss from culms was lower than that of filter paper cylinders because filter paper water loss represents unhindered evaporation from a surface, whereas for culms water loss occurs only through stomata. The influence of boundary layer thickness was also evident for CO_2 exchange where the narrowest culms photosynthesized nine-fold faster than the widest culms (Fig. 6a). Very narrow culms also had significantly higher conductances compared to wide culms (Fig. 6b). Narrow culms had culm temperatures 2°C lower than broad culms (Fig 7a). There was no correlation between culm diameter and stomatal density (Fig. 7b), indicating that the gas exchange results were not due to differences in stomatal density, but rather the influence of boundary layer thickness on gas conductance.

CULM ALLOMETRY

Height was strongly correlated with basal diameter and followed a positive power curve (Fig. 8a). A doubling in height required a 2.5-fold increase in culm basal diameter. The relationship between basal diameter and SCA (taken near the base) followed a negative power curve, with narrow culms having very high SCA values (Fig. 8b). Above 5 mm basal diameter, SCA levels off and further increases in diameter produced little change in SCA. There was no correlation between culm dimension and stomatal or tissue densities nor SCA and stomatal or tissue densities. Correlations of samples taken at two-thirds the length of the culm and the top of the culm did not differ from those of samples taken at one-third the length of the culm.

ASSOCIATION OF CULM DIAMETER WITH CLIMATE

PIC analysis indicated that basal culm diameter was significantly correlated with maximum continentality, the proportion of rain in spring, autumn and winter as well as minimum altitude (Table 1). Basal diameter increased with increasing maximum continentality. Narrow culms tended to have a low maximum proportion of rain in spring and autumn and a high minimum proportion of rain falling in winter. Narrow culms were also correlated with high minimum altitude. The predictive deviance of the BRT model, estimated using a cross validation process, was 64.8 % indicating good fit between raw values and values predicted from independent data (Leathwick *et al.* 2008). Maximum continentality was the most influential predictor, with narrow culmed species occurring at low maximum continentality values, which tend to be nearer the coast (Fig. 9a). Species with very narrow culms tended to occur in areas with moderate maximum and low minimum summer temperatures (Figs. 9 b and c). These species received a large proportion of rainfall during spring (Fig. 9d), low amounts in winter and very little falling during autumn (Figs. 9 e and f). Since three of the six most influential variables were to do with what proportion of rain fell in which season, rainfall seasonality plays an important role in the response of basal culm diameter.

DISCUSSION

The African Restionaceae are widespread in the CFR and form a dominant structural component of the vegetation. The family includes approximately 350 species (Linder *et al.* 2005) that occupy a variety of habitats, from valley bottoms, streambanks, wetlands and high altitude habitats to dry karroid shrubland. While all Restionaceae produce evergreen, cylindrical culms, the variation in culm diameter is considerable, ranging from 1 mm to 3 cm. We investigated the functional consequences and adaptive significance of variation in culm diameter and found considerable evidence showing that variation in culm diameter significantly influences plant physiological functioning and may be adaptive for particular environmental conditions.

Cylinder diameter significantly influenced water loss, demonstrated for both filter paper cylinders as well as Restionaceae culms. The relationship between culm diameter and water loss was described by a negative power curve, with the narrowest culms (0.02 cm) transpiring eight times faster than the broadest culms (0.45 cm) (Fig. 5a). For culm diameters less than 0.1 cm, transpiration increased rapidly with decreasing diameter. However, for culms greater than 0.1 cm, variation in transpiration was not considerable. Thus, the strong influence of culm diameter on water loss is significant only for the narrowest culms. Both stomatal and boundary layer conductance determine transpiration rate, and despite stomatal control of transpiration, the influence of boundary layer on water loss was still evident (Fig. 5b). High transpiration rates could be dangerous for plants and could lead to reduced leaf water potential (Lambers *et al.* 1998). But narrow culms have an ability to lose water rapidly and also to tightly control their transpiration rate because for narrow culms stomatal conductance is less than boundary layer conductance and any change in stomatal conductance has an impact on transpiration. Wider culms have lower boundary layer conductances and changes in stomatal conductance have less of an influence on transpiration. Similar results have been reported for leaf dimension variation in Cape Proteaceae (Yates *et al.* 2010). What is the adaptive significance of high transpiration?

Cape Restionaceae are generally found on sandstone-derived soils which are critically deficient in plant nutrients, particularly nitrogen and phosphorous. In these environments, the ability to transpire rapidly when water is plentiful may have crucial consequences for nutrient uptake. Species with narrow culms received a very high proportion of rain during spring and rainfall seasonality was an influential predictor of culm diameter. The strong rainfall seasonality in the CFR results in a transient period of nutrient availability, because nutrient uptake is only possible when water is plentiful. Transpiration drives the movement of water and dissolved nutrients through the soil towards the roots by mass-flow (Barber 1995). Cramer *et al.* (2008) demonstrated a nutrient-transpiration link, where transpiration rates were found to be higher in nutrient-constrained *Ehrharta calycina* plants. The strong link between water and nutrient flux has been highlighted by Cramer *et al.* (2009). Recent research has revealed that nutrient availability may be a highly important environmental cue in regulating the degree of stomatal aperture (Desikan *et al.* 2002, Dodd *et al.* 2003). In *Thamnochortus punctatus*, root and rhizome development occurred during the wet winter months (Stock *et al.* 1987), demonstrating that nutrient uptake occurs during this time. Thus, the ability to transpire rapidly when water is plentiful may significantly enhance nutrient uptake and thus a plant's competitive advantage in this nutrient-poor, seasonal environment. We propose that narrow culm diameters facilitate high transpiration rates and are adaptive for highly seasonal, nutrient-poor environments that receive most of their rainfall during spring.

A negative power curve was also found for the relationship between culm diameter and photosynthesis (Fig. 6a). Since boundary layer thickness influences movement of gases into and out of the leaf, this result was not unexpected. However, the influence of boundary layer on CO_2 uptake was not found in Cape Proteaceae (Yates *et al.* 2010), which may have been because of complex leaf structures and also because photosynthesis is biochemically regulated. High carbon assimilation concomitant with rapid transpiration during transient periods of high water availability, such as spring, would enable efficient capitalisation of available nutrients. Excess carbon could be stored and used at a later stage. Additionally, during spring temperatures are favourable for photosynthesis. Thus, while water and nutrients may be available during winter, transpiration and photosynthesis are temperature-dependent processes and may not be favourable during the colder winter months. While temperatures may be favourable during summer, water is limited and high assimilation rates may not be feasible. In *Thamnochortus punctatus*, culm elongation occurred in the warmer, but drier, spring to summer period (Stock *et al.* 1987), demonstrating an asynchronous growth pattern that supports our theory of capitalisation of nutrients when nutrients are available, storage and use when needed. Nicotra *et al.* (2008) showed that leaf dissection in Cape *Pelargonium* was associated with high assimilation rates and low water use efficiencies, which are suggested to promote carbon gain and water delivery during periods of high soil moisture (Nicotra *et al.* 2008). We propose narrow culms facilitate high assimilation rates during spring, enabling efficient capitalisation of resources when they are available, which is an adaptive strategy in this highly seasonal environment.

Culm diameter influences cooling through its affect on boundary layer thickness in two ways; heat lost through evapotranspirational cooling (latent heat loss) and convective and conductive heat loss (sensible heat loss) (Nobel 2005). Both the filter paper cylinder experiment and the culm gas exchange measurements revealed that, in the absence of wind, heat loss was improved for narrow diameters. But in both cases this may have been largely due to evapotranspirational cooling rather than sensible heat loss. If evapotranspirational cooling, which would be higher for narrow culms, was excluded the differences in temperature between narrow and wide culms and cylinders would certainly be nullified. Unfortunately, assessing the contributions of latent and sensible heat loss to cooling is tricky and sensible heat loss may be important on hot windless days when transpirational cooling is not feasible. For Restionaceae culms, a difference of 2°C was shown between the narrowest and broadest culms when wind was minimal. An elevated leaf temperature 6ºC higher than ambient in *Abies amabilis*, a subalpine forest tree, was shown to have no significant impact on carbon gain during the growing season (Martin *et al*. 1999). The 2°C difference in temperature between the narrowest and the broadest Restionaceae culms is unlikely to significantly influence temperature-dependent plant physiological function. Furthermore, we have shown that narrow-culmed species were associated with cool summers (Figs 9b and c). The difference in temperature of 2ºC between broad and narrow culms and the evidence that they are associated with cooler summers suggests that narrow culms are not adaptive for hot summer conditions.

Boundary layer thickness has been proposed to play a role in cooling when wind is negligible and 'free convection', caused by density differences associated with the temperature gradient across the boundary layer, dominates heat loss (Roth-Nebelsick 2001). When wind is present, 'forced convection' dominates heat loss (Roth-Nebelsick 2001). When temperatures are high and wind is low, evapotranspirational cooling could be disadvantageous and the improved sensible heat loss of narrow culms may facilitate improved culm cooling. In the absence of wind, water loss from filter paper cylinders in the laminar flow chamber led to cases of mixed and forced (30 and 60 % of samples, respectively) convection (Fig 2.). Based on these results, we suggest that free, and even mixed, convection would be rare in nature, in agreement with Roth-Nebelsick (2001). As wind increases above 0.5 m s^{-1}, forced convection increases and variation in culm diameter becomes less significant for cooling. High wind speeds have also been proposed to nullify the effects of variation in boundary layer thickness on water loss by reducing the thickness of the boundary layer surrounding a structure. While increasing wind speed increased water loss, variation in water loss with diameter was still evident. Furthermore, as wind increases, water loss increased but then levelled off. From this evidence, we propose that increasing wind speed increases water loss to a certain point (0.4 m s^{-1} in our experiment), after which further increases in wind have no effect on water loss. In addition, we propose that wind does not nullify the effects of variation in diameter on evaporative water loss.

Variation in culm dimension influences the whole plant, through its effect on boundary layer conductance, which alters movement of gases in and out of the leaf as well as heat exchange. Narrow culmed species tend to be shorter than wider culmed species (Fig. 8a). These plants may occur in the vegetation understory where wind is negligible and the high boundary layer conductance associated with narrow culms may be crucial for transpiration and photosynthesis. Narrow culmed species also had higher SCA values. For leaves, specific area has been linked to relative growth rate, resource allocation and growth strategies (Poorter 1989). Species with high specific area tend to have a fast relative growth rate but are shorter-lived. The higher photosynthetic rates of narrow culmed species may also be linked to faster growth rates. It is not clear whether narrow and broad culmed species have vastly different life spans and life-history strategies. There are several other potential functions of culms that have not yet been explored. Restionaceae are wind pollinated and the culm structure may play a role in pollen collection from the wind. Restionaceae also occur in extremely windy environments and the culm structure may prevent damage, and potential loss of photosynthetic tissue, during windy periods.

Culms reflect adaptations to various ecological conditions, as do leaves (Givnish 1987). Exploring the biophysical consequences of plant structure helps us to understand how plants cope and thrive under particular environmental conditions. This study has shown that variation in culm diameter has important consequences for gas and heat exchange. Our data suggest that narrow culms function to enhance water loss and assimilation during spring when water and nutrients are available and temperatures are favourable for photosynthesis. While stomata control water and CO_2 exchange to a large extent, we suggest that reduced boundary layer resistance achieved through narrow culm dimensions confers a capacity for water loss and CO_2 uptake when water is plentiful. An important aspect of reduced boundary layer resistance is that it confers greater plant control on water and CO_2 exchange. This enhanced control facilitates water loss and assimilation when water is plentiful, but reduced water loss during summer and autumn when water availability is low, which may be particularly important in highly seasonal environments.

REFERENCES

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research,* **30**, 1–67.

Barber, S.A. (1995) *Soil Nutrient Bioavailability: A Mechanistic Approach, 2nd edition.* John Wiley & Sons, New York.

Briggs, B.G. & Linder, H.P. (2009) A new subfamilial and tribal classification of Restionaceae (Poales). *Telopea*, **12**, 333-345.

Cowling, R.M. & Holmes, P.M. (1992) Flora and vegetation. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Cowling, R.M. (1992) ***Preface.*** In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Cramer, M.D., Hoffmann, V. & Verboom, G.A. (2008) Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist,* **179**, 1048-1057.

Cramer, M.D., Verboom, G.A. & Hawkins, H.J. (2009) The importance of nutritional regulation of plant water flux. *Oecologia*, *161*, 15-24.

De'ath, G. & Fabricius, K.E. (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178-3192.

Desikan, R., Griffiths, R., Hancock, J. & Neill, S. (2002) A new role for an old enzyme: Nitric-reductase mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *PNAS,* **99,** 25.

Dodd, I.C., Tan, L.P. & He, J. (2003) Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following N deprivation. *Journal of Experimental Botany,* **54,** 1281-1288.

Elith, J., Leathwick, J.R. & Hastie, T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802-813.

Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist,* **125**, 1-15.

Gates, D.M., Alderfer, R. and Taylor, S.E. (1968) Leaf temperatures of desert plants. *Science,* **159**, 994-995.

Givnish, T.J. (1978) Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica*, **27**, 83-142.

Givnish, T. J. (1979) On the adaptive significance of leaf form. Pp. 375-407 In: Topics in plant population biology. Eds: Solbrig, O.T., Jain, S., Johnson, G.B. and Raven, P.H. Columbia University Press, New York, New York, USA

Givnish, T.J. (1987) Comparative structures of leaf form: assessing relative roles of selective pressures and phylogenetic constraints. *New Phytologist* **106**: 131-160

Hastie, T., Tibshirani, R. & Friedman, J.H. (2001) *The elements of statistical learning: data mining, inference, and prediction*. Springer-Verlag, New York.

Hardy, C.R., Moline, P.M. & Linder, H.P. (2008) A phylogeny for the African Restionaceae, and new perspectives on morphology's role in generating complete species-level phylogenies for large clades. *International Journal of Plant Science*, **169**, 377-390.

Leathwick, J.R., Elith, J., Francis, M.P., Hastie, T. & Taylor, P. (2006) Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. *Marine Ecology Progress Series*, **321**, 267-281.

Lambers, H., Chapin, F.S., and Pons, T.L. (1998) Plant Physiological Ecology. Springer-Verlag.

Linder, H.P. (2000) Vicariance, climate change, anatomy and phylogeny of Restionaceae. In: *Under the microscope*: plant anatomy and systematics. Eds: Rudall, P.J. and Gasson, P.

Linder, H.P. (2004) The African Restionaceae: an IntKey identification and description system, version 2. Contrib Bolus Herb 20. http://www .systbot.unizh.ch/datenbanken/restionaceae/restionaceae.php.

Linder, H. P., Hardy, C. R. & Rutschmann, F. (2005) Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution*, **35**, 569-582.

Maddison, W.P. & Maddison, D.R. (2010) *Mesquite: A Modular System for Evolutionary Analysis, Version 2.72*. Available at: http://mesquiteproject.org. Accessed 20 June 2010.

Martin, T.A., Hinckley, T.M., Meinzer, F.C. & Sprugel, D.G. (1999) B oundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. *Tree Physiology*, **19**, 435-443.

McNaughton, K.G. and Jarvis, P.G. (1983) Predicting effects of vegetation changes on transpiration and evaporation. In: *Water Deficits and Plant Growth*, Vol III. (ed. T.T. Kozlowski), pp. 1-47. Academic Press, Inc., San Diego.

McDonald, P.G., Fonseca, C.R., Overton, J.McC and Westoby, M. (2003) Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Functional Ecology,* **17**, 50-57.

Midford, P.E., Garland, T. & Maddison, W.P. (2010) PDAP:PDTREE: A Translation of the PDTREE Application of Garland et al.'s Phenotypic Diversity Analysis Programs. Available at: http://www.biology.ucr.edu/people/faculty/Garland/PDTREE_Mesquite.doc. Accessed 20 June 2010.

Nicotra, A.B., Cosgrove, M.J., Cowling, A., Schlichting, C.D. & Jones, C.C. (2008) Leaf shape linked to photosynthetic rates and temperature optima in South African Pelargonium species. *Oecologia*, **154**, 625-635.

Nobel, P.S. (2005) Physicochemical and environmental plant physiology. Academic Press, San Diego, California.

Orians, G.H. & Solbrig, O.T. (1977) A cost-income model of leaves and roots with special reference to arid and semi-arid areas. *American Naturalist,* **111**, 677-690.

Parkhurst, D.F. & Loucks, O.L. (1972) Optimal leaf size in relation to environment. *The Journal of Ecology*, **60**, 505-537.

Poorter, H. (1989) Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: Causes and consequences of variation in growth rate and productivity of higher plants. Eds: H. Lambers, M. L. Cambridge, H. Konings, and T. L. Pons, 45–68. SPB Academic Publishing, The Hague, Netherlands.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statsitical Computing, Vienna.

Ridgeway, G. (2010) GBM: Generalized boosted regression models. R package, version 1.6-3.1. http://www.i-pensieri.com/gregr/gbm.shtml.

Roth-Nebelsick, A. (2001) Computer-based analysis of steady-state and transient heat transfer of small-sized leaves by free and mixed convection. *Plant, Cell and Environment*, **24**, 631-640.

Salleo, S., Nardini, A. & Lo Gullo, M.A. (1997) Is sclerophylly of Mediterranean evergreens an adaptation to drought? *New Phytologist*, **135**, 603-612.

Schuepp, P.H. (1993) Leaf Boundary Layers. *New Phytologist*, **125**, 477–507.

Schulze, R.E. (1997) *South African Atlas of Agrohydrology and Climatology*. Water Research Commission, Pretoria, South Africa.

Shipley, B. (1995) Structured interspecific determinants of specific leaf are in 34 species of herbaceous angiosperms. *Functional Ecology*, **9**, 312–319.

Stock, W.D. and Allsopp, N. (1992) Functional Perspectives of Ecosystems: In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Stock, W.D., Sommerville, J.E.M. and Lewis, O.A.M. (1987) Seasonal allocation of dry mass and nitrogen in a fynbos endemic Restionaceae species *Thamnochortus punctatus* Pill. *Oecologia* **72**: 315-320.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil,* **199**, 213–227.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010) Ecophysiological significance of leaf size variation in Proteaceae form the Cape Floristic Region. *Functional Ecology,* **24**, 485-492.

FIGURE LEGENDS

FIGURE 1: Variation in water and heat loss with varying filter paper cylinder diameter and boundary layer thickness. a) the physical relationship between cylinder diameter and evaporation measured gravimetrically at four different chamber wind speeds in a laminar flow chamber. Evaporation was fitted with negative power functions using Non-linear Least Squares (NLS) Regression [0 m s^{-1}: 4.02 $x^{-0.77}$ r = 0.99, 0.5 m s^{-1}: 12.15 $x^{-0.76}$ r = 0.87, 1.0 m s^{-1}: 13.26 $x^{-0.69}$ r = 0.98, 1.3 m s^{-1}: 14.36 $x^{-0.71}$ r = 0.96 where x = cylinder diameter (cm). $P < 0.001$ for all slopes and intercepts] b) relationship between cylinder boundary layer thickness and evaporation, demonstrating the decrease in boundary layer thickness and variation with increasing wind speed. c) Variation in the difference between paper temperature and ambient temperature (DT) with cylinder diameter. DT was fitted with straight lines using NLS Regression [0 m s^{-1}: 0.25** (x) + 0.91*** r = 0.65, 0.5 m s^{-1}: 0.07 (x) + 0.75*** r = 0.23, 1.0 m s^{-1}: 0.004 (x) + 0.42** r = 0.01, 1.3 m s^{-1}: 0.005 (x) + 0.44** r = 0.95 where x = cylinder dimension (cm). For **: $P < 0.01$, ***: $P < 0.001$] d) Variation in DT with boundary layer thickness illustrating the decrease in boundary layer thickness and variation with increasing wind speed.

FIGURE 2: Slope of the ratio of forced convection to free convection, Re^2/Gr, with varying boundary layer thickness in minimal wind. The horizontal line, y = 1.0, indicates the threshold between mixed and forced convection. Free convection occurs only when Re^2/Gr is less than 0.1. A negative power curve was fitted using NLS regression [y = 114.56*** $x^{-2.50}$*** where x = boundary layer thickness (mm). r = 0.97. For *** $P < 0.001$].

FIGURE 3: Slope of evaporation per cylinder diameter increases with increasing wind speed but then levels off. A logarithmic function was fitted using NLS Regression [y = -1.16** log(x) - 8.0329*** where x = wind speed. r = 0.99. For ** $P < 0.01$, ***: $P < 0.001$]

FIGURE 4: a) Theoretical difference in boundary layer thickness between a leaf and a cylinder of the same dimension, calculated with a velocity of 1 m s^{-1}. While leaves and cylinders have the same scaling component for boundary layer thickness, cylinders have thicker boundary layers due to their shape. b) Comparison of dimension-water loss curves for filter paper cylinders and

planar leaf-replicas. Cylinder water loss was measured at 20°C. Water loss curves for leaf-replicas were obtained from Yates ***et al.*** (2010) and had been measured with minimal wind and irradiance. Culm replica curves: $4.02x^{-0.77}$, $12.15x^{-0.76}$,$13.26x^{-0.69}$,$14.36x^{-0.71}$ at 0, 0.5, 1.0 and 1.3 $m.s^{-1}$, respectively. Leaf-replica curves: $5.77x^{-0.43}$, $7.07x^{-0.68}$, $12.51x^{-0.74}$ at 8, 20 and 30°C, respectively.

FIGURE 5: Variation in evaporation with culm diameter. Measurements were taken at the base, mid and top of each culm. Curve parameters for each slope were compared using a Student's Test to assess differences. For both evaporation and stomatal water flux curve parameters did not differ significantly ($P > 0.05$) for the 3 curves and one curve, for all positions, was fitted (dashed line). a) Variation in transpiration (*E*) with culm diameter (CD) measured on intact, well-watered plants with the Licor infra-red gas analyser with block temperature set to 25°C. A negative power function was fitted [$E = 0.58^{***}\ CD^{-0.64***}$] b) Evaporation expressed per stoma. A negative power function was fitted [$E = 6.43^{***}\ CD^{-0.60***}$]. For *** $P < 0.001$. The ahistorical correlation coefficient, r, as well as the historical (PIC) correlation coefficient and *P*-value are shown.

FIGURE 6: Variation in photosynthesis and stomatal conductance with culm diameter (CD). Measurements were taken at the base, mid and top of each culm and then averaged for each species. Curve parameters for each slope were compared using a Student's Test to assess differences. For both photosynthesis and stomatal conductance, curve parameters of the 3 curves did not differ significantly ($P > 0.05$) and one curve, for all positions, was fitted (dashed line). a) Variation in photosynthesis (*A*) with culm diameter (CD) was fitted with a negative power function [$A = 1.61^{***}\ CD^{-0.67}$ ***]. b) Stomatal conductance variation with culm dimension. A negative power function was fitted [$\text{Conductance} = 0.03^{***}\ CD^{-0.71}$ ***]. For *** $P < 0.001$. The ahistorical correlation coefficient, r, as well as the historical (PIC) correlation coefficient and *P*-value are shown.

FIGURE 7: a) Variation in culm temperature with culm diameter (CD). Culm temperature was measured on intact, well-watered plants with the Licor infra-red gas analyser with block

temperature set to 25°C. Measurements were taken at the base, mid and top of each culm. Curve parameters for each slope were compared using a Student's Test to assess differences. Curve parameters of the 3 curves did not differ significantly ($P > 0.05$) and one curve, for all positions, was fitted (dashed line). A positive power function was fitted [Temperature = 27.2*** CD 0.023*** . For *** $P < 0.001$]. The ahistorical correlation coefficient, r, as well as the historical (PIC) correlation coefficient and P-value are shown. b) There was no significant correlation between culm dimension and stomatal density.

FIGURE 8: Culm structural relationships of 39 species collected from a common garden. a) The relationship between culm basal diameter and height was non-linear and followed a positive power curve. b) The relationship between culm basal diameter and SLA followed a negative power curve. Historical (PIC) correlation coefficients (r) and P-values are shown. Error bars indicate standard error.

FIGURE 9: Partial dependence plots for the six most influential variables on culm basal diameter in the BRT model. Functions illustrate the response of culm diameter to a particular environmental variable while all other predictor variables are kept at their average values.

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FIGURE 8

FIGURE 9

TABLE 1: PIC correlation results for the response of basal diameter to climate variables. Basal diameter values for 248 species were obtained from Intkey (Linder 2004). * indicates significance at the alpha = 0.05 level, *** at alpha < 0.001.

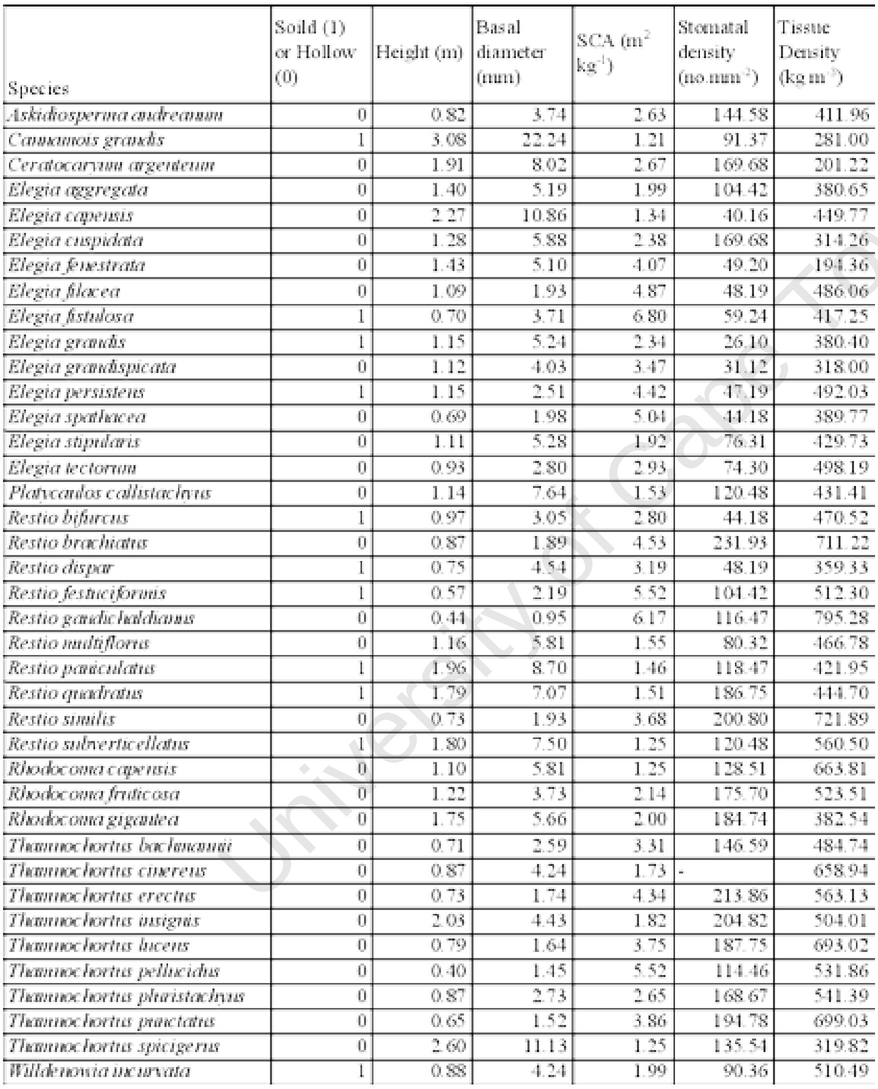
	r	*P-value*	
Minimum MAP	-0.023		0.359
Maximum MAP	0.069		0.139
Minimum Altitude	-0.114	*	0.036
Maximum Altitude	0.004		0.471
Minimum proportion of rain in Summer	0.022		0.361
Maximum proportion of rain in Summer	0.025		0.346
Minimum proportion of rain in Autumn	-0.007		0.454
Maximum proportion of rain in Autumn	0.267	*	0.000
Minimum proportion of rain in Winter	-0.194	***	0.001
Maximum proportion of rain in Winter	-0.071		0.131
Minimum proportion of rain in Spring	0.058		0.181
Maximum proportion of rain in Spring	0.144	*	0.011
Maximum temperature in the hottest month	-0.008		0.449
Minimum temperature in the hottest month	-0.054		0.195
Minimum temperature in the coldest month	-0.010		0.435
Maximum temperature in the coldest month	0.096		0.065
Minimum Apan	-0.084		0.093
Maximum Apan	0.014		0.410
Minimum Continentality	-0.047		0.229
Maximum Continentality	0.125	*	0.025

APPENDIX

A1. Species used for the gas exchange measurements. Gas exchange measurements were taken at the top, mid and base of the culm and diameters (cm) are shown here, in descending order of diameter at the base.

Species	Diameter base	Diameter mid	Diameter top
Elegia fistulosa	0.45	0.34	0.23
Elegia capensis	0.33	0.33	0.09
Thamnochortus spicigerus	0.32	0.30	0.14
Rhodocoma capensis	0.31	0.31	0.17
Elegia tectorum	0.30	0.20	0.12
Elegia cuspidata	0.30	0.25	0.12
Restio subverticellatus	0.27	0.24	0.04
Restio paniculatus	0.25	0.20	0.09
Restio tetragonus	0.24	0.21	0.14
Rhodocoma foliosa	0.22	0.18	0.05
Restio multiflorus	0.22	0.16	0.01
Restio quadratus	0.21	0.16	0.08
Thamnochortus fraternus	0.18	0.16	0.13
Thamnochortus insignis	0.16	0.14	0.09
Restio festuciformis	0.15	0.14	0.02
Restio similis	0.11	0.06	0.02

A2. Culm trait measurements for 39 Restionaceae grown in a common garden. Trait values are shown for samples taken at one-third of the length of the culm. Stomatal density could not be obtained for *Thamnochortus cinereus* due to extensive hairs on the culm surface.



Species	Soild (1) or Hollow (0)	Height (m)	Basal diameter (mm)	SCA ($m^2\ kg^{-1}$)	Stomatal density (no.mm^{-2})	Tissue Density ($kg\ m^{-3}$)
Askidiosperma andreaeanum	0	0.82	3.74	2.63	144.58	411.96
Cannamois grandis	1	3.08	22.24	1.21	91.37	281.00
Ceratocaryum argenteum	0	1.91	8.02	2.67	169.68	201.22
Elegia aggregata	0	1.40	5.19	1.99	104.42	380.65
Elegia capensis	0	2.27	10.86	1.34	40.16	449.77
Elegia cuspidata	0	1.28	5.88	2.38	169.68	314.26
Elegia fenestrata	0	1.43	5.10	4.07	49.20	194.36
Elegia filacea	0	1.09	1.93	4.87	48.19	486.06
Elegia fistulosa	1	0.70	3.71	6.80	59.24	417.25
Elegia grandis	1	1.15	5.24	2.34	26.10	380.40
Elegia grandispicata	0	1.12	4.03	3.47	31.12	318.00
Elegia persistens	1	1.15	2.51	4.42	47.19	492.03
Elegia spathacea	0	0.69	1.98	5.04	44.18	389.77
Elegia stipularis	0	1.11	5.28	1.92	76.31	429.73
Elegia tectorum	0	0.93	2.80	2.93	74.30	498.19
Platycaulos callistachyus	0	1.14	7.64	1.53	120.48	431.41
Restio bifurcus	1	0.97	3.05	2.80	44.18	470.52
Restio brachiatus	0	0.87	1.89	4.53	231.93	711.22
Restio dispar	1	0.75	4.54	3.19	48.19	359.33
Restio festuciformis	1	0.57	2.19	5.52	104.42	512.30
Restio gaudichaudianus	0	0.44	0.95	6.17	116.47	795.28
Restio multiflorus	0	1.16	5.81	1.55	80.32	466.78
Restio paniculatus	1	1.96	8.70	1.46	118.47	421.95
Restio quadratus	1	1.79	7.07	1.51	186.75	444.70
Restio similis	0	0.73	1.93	3.68	200.80	721.89
Restio subverticellatus	1	1.80	7.50	1.25	120.48	560.50
Rhodocoma capensis	0	1.10	5.81	1.25	128.51	663.81
Rhodocoma fruticosa	0	1.22	3.73	2.14	175.70	523.51
Rhodocoma gigantea	0	1.75	5.66	2.00	184.74	382.54
Thamnochortus bachmannii	0	0.71	2.59	3.31	146.59	484.74
Thamnochortus cinereus	0	0.87	4.24	1.73	-	658.94
Thamnochortus erectus	0	0.73	1.74	4.34	213.86	563.13
Thamnochortus insignis	0	2.03	4.43	1.82	204.82	504.01
Thamnochortus lucens	0	0.79	1.64	3.75	187.75	693.02
Thamnochortus pellucidus	0	0.40	1.45	5.52	114.46	531.86
Thamnochortus pluristachyus	0	0.87	2.73	2.65	168.67	541.39
Thamnochortus punctatus	0	0.65	1.52	3.86	194.78	699.03
Thamnochortus spicigerus	0	2.60	11.13	1.25	135.54	319.82
Willdenowia incurvata	1	0.88	4.24	1.99	90.36	510.49

CHAPTER 3

THE EVOLUTION OF NOVEL ANATOMICAL TRAITS IN RESTIONOIDEAE (RESTIONACEAE) FACILITATED OCCUPATION OF ARID HABITATS

ABSTRACT

1. Diverse culm anatomical features found in African Restionaceae, such as multiple forms of stomatal architecture, epidermal cell wall thickening, epidermal hairs and papillae, varying sizes of sub-stomatal chambers, thickening of the sclerenchyma and tannins, have been associated with xerophily, but the adaptive significance of these traits remains unknown.
2. I hypothesised particular anatomical traits are adaptations to xerophily and that the evolution of these novel anatomical traits facilitated movement to arid climate and habitat conditions. In addition, I postulated that with reversion to wet conditions, these derived traits would be lost.
3. I used correlative and experimental methods to test which of the rich diversity of anatomical structures were linked to more arid conditions and whether the evolution of particular traits was associated with arid conditions. Boosted regression trees were used to explore the correlation between the number of derived traits (as an indication of specialisation) and environmental variables and to identify the most influential environmental predictors of anatomical specialisation. In addition, I performed Fisher's Tests of association of each anatomical trait with 30 habitat types. Because our environmental data consisted of categorical habitat and continuous climate variables, two types of evolutionary test were used. Evolutionary associations of anatomical traits and habitats were tested using the Contingent States Test, as well as variations thereof. Phylogenetically Independent Contrasts (PIC) were used to evaluate the association of derived features with climate variables. The influence of anatomy on physiological functioning was investigated by taking gas exchange measurements of 16 Restionaceae species.
4. Anatomically specialised species (i.e. those with many derived anatomical traits) were associated with arid conditions, including high continentality, high A-pan evaporation and low rainfall, especially during autumn and spring.

Correlative tests showed that derived traits had evolved contingent upon and concurrent with shifts to arid habitats. Furthermore, derived traits were lost with movement back to wet habitats (three times more significant reversals in derived traits occurred with shift to wet compared to dry habitats). Phylogenetically Independent Contrast correlations also showed that derived traits, such as thickened lateral epidermal walls, ground tissue with scattered cavities and slightly sunken stomata, were correlated more with arid than wet climate conditions, further corroborating the evolutionary habitat tests. Anatomical traits influenced gas exchange and therefore play a role in plant physiological functioning.

5. The evolution of novel anatomical traits probably facilitated occupation of a wide range of habitats, particularly arid conditions created during the mid Miocene climate change, explaining the ubiquity of Restionaceae in the Cape. Furthermore, these adaptations have significantly contributed to the diversity in form and function of this clade. Similar trends have been found in Australian Proteaceae, suggesting that microevolutionary processes such as these may play a significant role in adaptive radiations.

Keywords: anatomy, adaptation, Cape flora, climate change, culm, Restionaceae, selective regime

INTRODUCTION

Traits are considered ‘adaptive’ to a particular selective regime when they enhance performance in a way that increases survival or reproduction within that selective regime and when their evolution has been driven by that selective regime (Gould & Vrba 1982, Baum & Larson 1991). Testing for function identifies a trait as aptive within a particular selective environment, but fails to distinguish whether the evolution of that trait was powered by that selective environment (i.e. it is adaptive to that selective environment) or by another (exaptive traits – aptive traits that differ in their historical genesis i.e. they evolved under a different selective regime) (Gould & Vrba 1982). Phylogenetic congruence between trait evolution and a measured performance advantage provides evidence that the trait has evolved through natural selection (Greene 1986) and the possibility of defining the selective regime, i.e. a set of factors that influence the action of natural selection on the trait (Baum & Larson 1991), makes this aspect of the study of adaptation feasible. Identifying adaptations and understanding their evolutionary history is important because it gives an indication of why the particular trait arose. Furthermore, these traits can lead to adaptive radiations and facilitate occupation of new habitats.

Radiations in a lineage are defined as adaptive when speciation is associated with phenotypic and ecological divergence (Schluter 1996). Thus, lineages in which adaptive radiations have occurred have highly diverse morphologies and functions (Losos & Miles 2002), compared to non-adaptively radiated lineages in which species are similar, and replace each other spatially rather than ecologically (Rundell & Price 2009). Adaptive radiations typically occur when new resources become available and novel adaptive traits facilitate utilization of these new resources. Novel traits can facilitate innovative exploitation of the environment and allow movement of lineages to a new adaptive zone (Baum & Larson 1991). While phylogenetic data are often used to provide evidence of adaptive radiation, assessing the evolutionary and functional links between phenotype and environment is a more direct, but less commonly used, method (Losos & Miles 2002, Harmon *et al.* 2003, Verboom *et al.* 2004). Verboom *et al.* (2004) provided evidence for an adaptive radiation in *Ehrharta*, a group of grasses that radiated in the arid Cape region, by identifying morphological forms that allowed the evolution of an annual habit on more nutrient-rich soils. Anatomical traits can also function as innovations stimulating major radiations: the recent radiation of a very large ruschioid clade (Aizoaceae) of 1563 species in the Succulent Karoo has been linked to the evolution of wide-band tracheids, suggesting that they may function to withstand water stress (Klak *et al.* 2004). It is also possible that the radiation of the PACCAD clade of grasses (Kellogg 2000, 2001) was linked to the repeated origin of C4 photosynthesis based on the curious grass leaf anatomy, and that this in turn drove the anatomical diversification of grass leaves (Metcalfe 1960). Thus, anatomical traits can play a significant role in adaptation to novel environments and may lead to adaptive radiations.

Adaptation to particular environments in the highly heterogeneous Cape may be a key driver of morphological diversification and speciation in this region. The Cape Floristic Region (CFR), which lies in the southwestern Cape, has an extraordinarily high number of species, 9000 in just 90 000 km^2, as well as high endemism (Goldblatt & Manning 2000). This region has been a hotbed of radiations, with radiations of many Cape floral clades dated to the Middle Miocene (Linder 2003, 2005, Verboom *et al.* 2009). While environmental correlates of some of these radiations have been studied, our knowledge of the underlying adaptive, or non-adaptive, process is limited. A study by Ellis, Weis & Gaut (2006), which found evidence that phenotypic divergence in *Argyroderma* (Aizoaceae) had arisen in response to habitat selection and flowering phenology, is one of the few studies investigating these underlying processes. More recently, Rymer *et al.* (2010) investigated mechanisms of speciation in the *Gladioulus carinatus* (Iridaceae) species complex by evaluating differences in spatial occurrence, phenology, floral morphology, genetic isolation and genomic selection. Furthermore, several Cape clades have unusual structures - ericoid leaves with rolled margins, hairy and sclerophyllous leaves, leaf succulence, bladder cells, needle-like or highly dissected leaves, tannins and other secondary metabolites – that are superficially treated as adaptations (Linder *et al.* 2010). In addition, there is a remarkable richness of geophytes, also attributed to their increased ability to tolerate the summer dry conditions (Proches *et al.* 2005a, Proches *et al.* 2005b). Despite the abundance of peculiar structures in the Cape and the phylogenetic evidence for adaptive radiations in many Cape clades, there have been few critical tests (but see Verboom *et al.* 2004, Yates *et al.* 2010) demonstrating the function and historic origins of these structures.

The African Restionaceae (subfamily Restionoideae, Briggs & Linder 2009) is a suitable group in which to investigate the potential role of anatomical adaptation in supporting an adaptive radiation. The subfamily includes some 350 species, which have radiated since the Eocene in the Cape Floristic Region (Linder *et al.* 2005), thus constituting one of the largest Cape clades. Almost all of the species are endemic to the Greater Cape Floristic Region (GCFR, Born *et al.* 2006), which includes both fynbos and succulent karoo biomes. The family Restionaceae consists of ca. 490 species and is mostly spread through the southwestern tip of southern Africa and southern Australia (Linder 2000), with outliers north into Indochina, tropical Africa and South America. The whole family is wind pollinated, with relatively simple flowers. The Restionoideae are typical of the common fynbos vegetation of the CFR, is often the dominant element (Taylor 1978, Rebelo *et al.* 2006), and occupy a diverse array of habitats, from permanently waterlogged wetlands to dry karroid shrubland. The culm anatomy of Restionaceae is rather unusual, as these are not only evergreen and persistent, but also the main photosynthetic organ in the family. The basic organisation of the culm (with a central ground tissue with embedded vasculature, a sclerenchymatous ring encircled by a parenchyma ring, and an outer green chlorophyllous ring) (see Fig. 1) is constant through the whole family. The Restionoideae, in particular, with ca. 354 species, have highly diverse culm anatomic forms ranging from the large, hollow culms of several *Elegia* species to the highly sclerified, narrow culms of *Restio karooicus*. Elaborate forms of stomatal architecture, epidermal cell wall thickening, epidermal hairs and papillae, varying sizes of sub-stomatal chambers, thickening of the sclerenchyma and chlorenchyma cavities (Van Greuning & Van der Schijff 1973) are just some of the curious anatomical features that have evolved within this group (Gilg 1891, Cutler 1969, Botha 1982, Linder 1984).

The remarkable culm anatomy of Restionaceae has generally been interpreted as a response to aridity (Gilg 1891, Cutler 1969, Linder 2000, Pfitzer 1870). Studies in the 19th century sought narrative explanations for these unusual structures, often with reference to functional investigations. Two sets of functions have been proposed. The first, most commonly invoked function is to reduce transpiration. Protective cells enclosing the substomatal cavity have been suggested to slow down water loss in arid conditions (Gilg 1891). Such substomatal cavities increase CO_2 uptake (Roth-Nebelsick 2007) by conducting the gas past the thick and impervious cuticle deep into the chlorenchyma. In the Restionoideae the protective cells lining the substomatal cavities are cuticle-lined (Linder 2000), which functions in reducing transpiration (Roth-Nebelsick 2007). The variety of stomatal architectural forms in Restionaceae, from stomata protruding on tubercles to sunken stomata, may also be associated with regulating transpiration rate (Jordan *et al.* 2008), but their effects are not always so obvious (Roth-Nebelsick 2007). A second function might be to provide mechanical strength, and this has been applied to the protective cells, ribs and false pillar cells, as well as the sclerenchyma sheath (Pfitzer 1870, Gilg 1891, Cutler 1969, Linder 2000). Ribs and pillar cells were proposed to prevent drought-induced damage from spreading through the culm (Pfitzer 1870) and to prevent loss of structure in arid conditions by providing mechanical strength (Gilg, 1891). In dry conditions, Restionaceae culms experience huge tensile forces (Moll & Sommerville 1985, van der Heyden & Lewis 1989) and mechanical strength may be crucial in maintaining culm structural integrity. A third set of explanations might be associated with the requirement to maximize transpiration when water is available, in order to optimize the mass-flow harvesting of soil nutrients (Cramer *et al.* 2009). Support for these explanations has been found in the convergent evolution of certain anatomical traits in African and Australian Restionaceae, associated with the occupation of more seasonally droughted habitats (Cutler 1972, Linder 2000). Thus, several of these anatomical traits have been speculated to function in drought tolerance, but correlative and experimental tests are lacking.

Here I used correlative and experimental methods to test which of the rich diversity of anatomical structures are linked to increasingly arid conditions, thus attempting to detail the proposal of Cutler (1972) and Linder (2000) of gradual adaptation in increasingly arid conditions. In particular, I used correlative methods over the whole clade to test for functional association between aridity and the derived epidermal structures (thickened cuticle and massively enlarged lateral epidermal walls), the stomatal position variation, the variation in the development in the protective cells, the variation in the thickness of the sclerenchyma cell walls, and finally in the formation of scattered cavities and solid tissue, or indeed the loss of cavities, in the ground tissue. I also directly tested the gas exchange rates of different culm anatomical types on a small subset of species. Secondly, I tested whether the totality of anatomical novelties might be linked to greater drought tolerance, and the extent to which this could be associated with the evolution and persistence of the species diversity within the Restionoideae. I hypothesised that particular anatomical traits are adaptations to xerophily and that the evolution of these novel anatomical traits facilitated movement to arid climate and habitat conditions. In addition, I postulated that with reversion to wet conditions, these derived traits would be lost. This reversal test is important because loss of a trait in response to a selective regime reversal corroborates the hypothesis that the trait is an adaptation to that selective regime.

MATERIALS AND METHODS

PHYLOGENY AND CLASSIFICATION

The Hardy *et al.* 2008 highest likelihood tree, which was based on plastid DNA sequence variation and morphological data, was used for ancestral state reconstruction of anatomical and habitat traits and historical tests of association. Taxonomy follows for the Restieae (Linder & Hardy 2010) and for the Willdenowieae (Linder 1985, except for additions). Species for which DNA sequence data were lacking were placed into resolved positions using morphology, a method that placed these taxa quite accurately (Hardy *et al.* 2008).

RESTIONACEAE CULM ANATOMICAL CHARACTERS

Detailed anatomical descriptions of each character were taken from the African Restionaceae Intkey identification and description system (Linder 2004). Each species was checked against the comprehensive slide collection at the Institute for Systematic Botany, University of Zurich. These sections had been prepared by boiling culm internodes in soapy water. Mid-internode transverse sections were cut by hand microtome at 10-20 µm, stained with safranin, which stains phenolics red, and Alcian blue, which stains cellulose blue, and mounted in DPX or Canada Balsam. Only one specimen was studied per species. Forty five anatomical characters which were variable were scored as discrete characters from culm cross-sections of 274 Restionaceae species (see Table 1 for a list of traits). The terminology for the various anatomical structures follows Cutler (1969). A description of the anatomical characters is also given online (http://www.systbot.unizh.ch/datenbanken/restionaceae/restionaceae.php). Derived anatomical features were identified by mapping anatomical traits over a rooted phylogeny, using ACCTRAN and DELTRAN parsimony reconstruction implemented in MacClade Version 4.08 (Maddison & Maddison 2005). Modifications ranged from the production of secondary metabolites in different tissues to drastic structural changes of the stomata, the epidermal layer as well as the shape of the culm (see Fig. 2).

HABITAT VARIABLES

Habitat characterizations for each species were based on field observations at numerous localities made by HPL over a period of close to three decades, as well as label information on some 10 000 herbarium specimens. Each species was scored as present/absent in a series of habitat types, as defined by the protocol of Linder (2005). Categorical habitat data simplifies the environmental conditions experienced by the plants substantially, particularly for testing the functional link between anatomy and environment. The different habitat types were grouped into categories (Table 2). Habitats in the water availability category were further simplified as dry or wet (Table 2). Fine-scale habitat categories may not be biologically meaningful for the plants and they create fewer cases, possibly causing statistical bias. Although our study focused on wet versus dry habitat variables, we ran analyses of correlated evolution on all habitat variables to assess whether water availability was a significant driver of anatomical adaptation compared with other habitat attributes.

CLIMATE VARIABLES

Climatic variables were derived from distribution data of Restionaceae based largely on the collections in the Bolus Herbarium, University of Cape Town. Using the ARC Geographic Information System, distributions of each taxon were overlaid with climatic variables and filtered using a 1’ x 1’ grid to exclude redundancy. For each species the minimum and maximum values for each climatic variable were obtained from these grid cell values. Environmental variables included altitude, continentality, mean annual precipitation (MAP), A-pan evaporation (evaporation potential of open water, originally measured as evaporation from a Class A evaporation pan over a period of one day), temperature in the hottest and coldest months and rainfall seasonality (rainfall during the four seasons). Minimum and maximum values are useful as they represent the extremes of the conditions experienced by each species and provide insights into species’ climatic tolerances. Environmental variables for South Africa were obtained from the South African Atlas of Hydrology and

Climatology, Computing Centre for Water Research (CCWR), University of Natal (Schultze 1997). Altitude was computed by a Digital Elevation Model and also obtained from CCWR. These variables were chosen *a priori* to include major environmental factors and to limit redundancy of factors, consequently potential correlation among the variables was not investigated.

STATISTICAL TESTS

The statistical analyses of the anatomical attributes was complicated by the need to include both categorical and continuous predictors and the incorporation of phylogenetic information. The tests described below were structured to explore the ahistorical and historical associations of derived traits as well as to accommodate both types of environmental data:

TABLE 3. Details of correlative tests of anatomical traits and environmental variables.

Test	Hypotheses	Phylogeny	Categorical	Continuous
Boosted Regression Trees	Species with unusually high numbers of derived traits are associated with arid conditions	No	Yes	Yes
Fisher's Exact	Specific anatomical traits are aptations to specific habitats	No	Yes	No
Fisher's Exact	The evolution of specific anatomical traits was contingent or concurrent upon arid habitat types	Yes	Yes	No
Independent Contrasts	Specific anatomical traits are associated with particular climate variables	Yes	No	Yes

We used boosted regression trees (BRT) to determine whether species with unusually high numbers of derived anatomical traits (as an indicator of the degree of anatomical specialisation) were more commonly found in arid habitats and climate. The majority of species had less than six derived anatomical traits (Fig. 3). BRT analysis was implemented using the gbm package of Ridgeway (2010 version 1.6 -3.1) and supplemented with the functions of Elith *et al.* (2008) carried out in R (version 2.11.0 R Development Core Team 2010). BRT analysis is a form of non-linear modelling used to identify the response of a variable to various predictor variables and to identify predictor variables that most explain the distribution of a response variable (De'ath & Fabricius 2000, Hastie *et al.* 2001, Elith *et al.* 2008). Advantages of applying this type of analysis include accepting both categorical and continuous variables that do not require data transformation, handling missing data points and outliers, fitting non-linear models and accounting for interactions between predictor variables (De'ath & Fabricius 2000, Friedman & Meulman 2003). We were interested in determining the response of derived anatomical traits to habitat and climate conditions (a total of 47 predictor variables) to explore in which habitat species with highly modified anatomies occurred. For each species, the numbers of derived traits were summed to give a response variable that indicated the degree of specialisation/derivation. In the model, a Gaussian distribution (for the continuous response variable) with a tree complexity of 5 and a learning rate of 0.001 was used. The relative importance of predictor variables was assessed according to the method of Elith *et al.* (2008). Partial dependence plots, which display the influence of a predictor variable on the response variable after accounting for the average effects of all the other predictor variables, were produced to visualise the results of the model.

We tested the relationship between specific derived anatomical traits and specific (categorical) habitats with a series of Fisher's Exact Tests, thus identifying potential aptations. BRT analysis indicated how the number of derived traits of each species was accounted for by the various environmental variables but failed to reveal the association of individual derived traits with the various habitat types. In this test we took neither phylogenetic effects nor interactions among the anatomical attributes into account (the latter could be detected with a regression type approach, but it would be infinitely complex). The numbers of significant associations with anatomical traits was counted for all habitat types to show which habitats had the most significant anatomical associations. In addition, the number of significant habitat associations for anatomical traits falling within each tissue type were counted to indicate which types of tissue had the strongest associations with habitat types.

We explored the evolutionary relationship between the categorical habitat characteristics and the anatomical traits with a series of tests constructed to determine which anatomical traits that had evolved in response to changes in selective regime, thus eliminating exaptations but including adaptations and nonadaptations. Nonadaptations were eliminated by assessing losses in response to reversals in selective regime, assuming that non-functional traits would be lost. The evolutionary association of the derived anatomical characters with habitat types can be reduced to three questions, each with a subtle variation in test:

1. Was the evolution of a particular anatomical character (0® 1) contingent upon the particular habitat (habitat state = 1) [Contingent States Test, (Sillén-Tullberg 1993)]
2. Did the evolution of a particular anatomical character (0® 1) occur concurrently with a shift in habitat (0® 1) [Concurrent Shift Test]
3. Was reversal of a particular anatomical character (1® 0) contingent upon a certain habitat (habitat state =1) [Contingent Reversal Test]

To answer these questions, ancestral states of categorical habitat and anatomical variables were reconstructed using parsimony optimization as implemented in MacClade v. 4.08 (Maddison & Maddison 2005), under both ACCTRAN and DELTRAN resolving options. Contingent States Tests were performed to assess whether a state transition in an anatomical character is equally likely in a certain habitat (Sillén-Tullberg 1993). For the Contingent States Test, the number of origins (0® 1) and the number of places with no change (0® 0) for branches with presence or absence of the habitat variable were counted (Sillén-Tullberg 1993) (Fig. 4). These counts were then tested for significance using Fisher's Exact Test, in which the null hypothesis stated that the evolution of state 1 (gain in an anatomical attribute) was equally likely to originate under either habitat condition i.e. presence or absence of a particular habitat (Sillén-Tullberg 1993). To test for concurrent shifts in states, the number of origins (0® 1) and the number of places with no change (0® 0) for branches with shift (0® 1) or no shift (0® 0) of the habitat variable were counted. To test whether reversals in anatomical characters were contingent upon particular habitats for the Contingent Reversal Test, the number of reversals (1® 0) and the number of places with no change (0® 0) for branches with presence or absence of the habitat variable were counted. For all habitat types the numbers of significant contingent states, concurrent shifts and contingent reversals of derived anatomical features within those habitats were counted. These counts were summed for habitat types falling under dry or wet, corrected for the number of habitat types within each category and were then compared. In addition, we counted the number of significant shifts, either contingent, concurrent or contingent reversals in all habitats for each tissue type to determine whether certain tissue types had been modified in response to water availability more than other habitat types over the evolutionary history of Restionaceae.

The main disadvantage of the Contingent and Concurrent States Tests was that only one tree topology could be used. Different topologies may produce different patterns of character evolution and the concurrent or contingent change with habitat may differ for some branches thus altering the results if a sufficient number of branches change state. Problems in statistical testing also arose for traits with either a very small or a very large number of changes on the tree. For traits with only one origin, statistical testing is not possible, while traits that are plastic and have had a large number of changes on the tree are more likely to yield statistically significant changes. However, traits with only one origin may also be lost several times, allowing the converse of the initial hypothesis to be tested in a statistically meaningful way (Galley & Linder 2007). Our modification of the Contingent States test, the Concurrent Shift Test, was less biased towards traits with several origins and identified very specific cases of shift in anatomical trait and habitat occurring simultaneously.

To assess whether the evolution of individual anatomical features was associated with particular types of change in continuous climate variables, independent contrast analyses (PIC, Felsenstein 1985) were performed using Phylocom v. 4.1 (Webb *et al.*

2008). For discrete anatomical traits, contrasts in the continuous climatic parameters could only be calculated on a limited set of nodes, i.e. those across which the state of the binary anatomical character changed. The sister-taxa (monophyletic) set of nodes was used. Statistical significance across multiple contrasts was evaluated using a one-sample t-test having a mean contrast = 0 as its null (Webb *et al.* 2008). Significant correlations were scored as arid or mesic, based on whether average environmental values (from the PIC analysis) were higher or lower for presence of the derived trait.

EXPERIMENTAL METHODS

Sixteen Restionaceae (three plants per species) with different culm anatomies were obtained from Kirstenbosch Nursery, Cape Town. The plants were of similar age and had been grown from seed in 2 L plastic bags containing a 1:1 mixture of a sand and compost. Species were selected for their variation in anatomical features. A list of species and their anatomical traits can be found in Appendix A1.

Plants were maintained in a greenhouse with a temperature of 25ºC and moved to a temperature-controlled growth chamber set to 20 ºC with a light : dark photoperiod of 14:10 h and an irradiance of 1000 µmol m^{-2} s^{-1} two weeks prior to gas exchange measurement. Photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and temperature of culms were measured using an LI-6400 Portable Photosynthesis System (LICOR, Lincoln, NE, USA). A needle-leaf chamber was used to accommodate sufficient culm area and to allow the boundary layer effects on gas exchange to be included. Measurements were taken near the base of the culm. Measurements were taken at chamber irradiance of 1000 µmol quanta m^{-2} s^{-1}. The cuvette was set to a temperature of 25ºC and a CO_2 concentration of 400 µmol mol^{-1}. After ca. 3 min equilibration in the cuvette, measurements were recorded. Culm temperatures ranged from 24.1 to 26.6ºC. Vapour pressure deficit averaged 1.78 kPa. Culm temperature was calculated using energy balance equations. Stomatal impressions were obtained by coating culm surfaces with clear nail varnish, which was peeled off and examined at 400X magnification on a transmission light microscope. Stomatal numbers in the field of view were counted five times for each individual, averaged and expressed as number per mm^2. Culm diameter was measured with callipers. Culm area was estimated using the diameter and height of a culm section from each individual. Culm sections were dried in an oven at 80 °C for 48 h and then weighed. Specific culm area (SCA) was calculated as the photosynthetic area (m^2) per dry weight (kg) of culm. Correlations between gas exchange and culm trait variables and derived culm anatomical features were assessed using phylogenetically independent contrasts for discrete and continuous variables (PIC, Felsenstein 1985) implemented in Phylocom v. 4.1 (Webb, Ackerly and Kembel 2008) (as above). A phylogenetic tree relating the 16 species used in the gas exchange experiment was obtained by pruning the Hardy *et al.* 2008 highest likelihood tree. Contrasts were between species with the anatomical trait and species lacking the trait and investigated whether species with or without the trait had higher or lower values of the continuous gas exchange or culm trait variable.

RESULTS

ANATOMICAL VARIATION

All culm tissue types (epidermis, chlorenchyma, parenchyma, sclerenchyma and central ground tissue) show variation within the Restionoideae (Table 1). Most traits are evolutionary labile: of the 34 derived traits, under ACCTRAN optimisation only five evolved just once, the average number of times they evolved is 14.3, with the most labile trait being the presence of sand in the central ground tissue, which evolved 40 times. There are also numerous reversals, under ACCTRAN: in total 745 reversals in 23 of the 45 traits, ranging from a minimum of 0 to a maximum of 44 (loss of tannin from the epidermis), giving an average per trait of 16.6 losses. Ancestral traits tended to be most common and had the highest number of trait reversals. Very few traits were genus specific, such as a double epidermal cell layer in *Elegia*, with most arising in a number of different genera. Culm shape shifted nine or ten times (ACCTRAN and DELTRAN resolving options, respectively) with reversals being uncommon (once under the ACCTRAN resolving option). The most modified tissue type is the epidermis, with variation in the epidermal cell layers, the outer epidermal walls thickened, the lateral cell walls wavy and thickened, and with variation in the surface ornamentation of the epidermal cells. The most labile anatomical attributes are the secondary metabolites, such as tannins, silica bodies and silica sand, which seemed to be readily gained or lost..

SPECIES WITH HIGH NUMBERS OF DERIVED TRAITS WERE ASSOCIATED WITH ARID CONDITIONS

The predictive deviance of the BRT model, estimated using a cross validation process, was 61.5%, indicating good fit between raw values and values predicted from independent data (Leathwick *et al.* 2008). Assessment of the contribution of predictor variables to the model shows that climate moisture parameters are the strongest correlates of the number of derived anatomical traits in a species (Fig. 5). Species with unusually high numbers of derived anatomical traits tended to occur either at very low or very high maximum continentality (Fig. 5A) and were associated with intermediate to high A-pan evaporation values (Fig. 5B). Rainfall seasonality was an influential predictor, with species with high numbers of derived traits receiving a high proportion of their rainfall in summer and very little in autumn and spring (Figs. 5C, D and F). Furthermore, these species were associated with very low maximum mean annual rainfall (Fig. 5E).

DERIVED TRAITS ARE ASSOCIATED WITH ARID HABITATS

Fisher's Exact Tests of association between each anatomical trait and all habitat types (aptations) showed that derived anatomical traits showed the highest number of significant associations with habitat types that influence water availability, followed by vegetation type and bedrock type, while least important were soil rockiness and the presence of south-easter clouds (Fig. 6, all associations shown in Supplementary Information S1). More derived features were significantly (Student's T-test, $P < 0.05$) associated with dry habitats than with wet habitats (Fig. 7). Lateral and outer epidermal wall thickening was associated with well-drained habitats. Hemispherical culm shapes were associated with stream banks and valley bottoms. Species with scattered cavities in the central ground tissue were also associated with mesic habitats such as marshes, streambanks and cliff seeps.

DERIVED TRAITS EVOLVED IN RESPONSE TO ARID CONDITIONS, WITH TRAIT REVERSALS OCCURRING IN RESPONSE TO SHIFT BACK TO WET CONDITIONS

Anatomical character evolution, under both ACCTRAN and DELTRAN resolving options, mostly occurred concurrently with shifts in water availability, bedrock type and vegetation type, with fewer shifts occurring concurrently with shift in soil rockiness and south-east clouds (Fig. 6). The Contingent States Test yielded slightly different results, with character evolution occurring mostly as a result of different habitat rockiness, with very few shifts occurring in the categories of water availability, bedrock type, vegetation type and south-east clouds. For both ACCTRAN and DELTRAN resolving options, reversals in derived traits were associated mostly with water availability.

When the number of significant shifts contingent on particular habitat types classified as dry or wet were counted for all derived anatomical traits, more shifts occurred under dry habitats (Fig. 7). By correcting for the number of habitats within dry and wet, we found that 0.5 shifts occurred per dry habitat while only 0.2 shifts occurred per wet habitat under both resolving options. The Concurrent States Test showed that under the ACCTRAN resolving option, 2.5 shifts per habitat occurred concurrently with shift to dry habitats, compared to only 1.2 shifts per habitat occurring concurrently with shift to wet habitats. Results under the DELTRAN resolving option conflicted with those under the ACCTRAN resolving option, with 2.2 shifts per habitat occurring concurrently with shift to wet habitat and only 2 shifts per habitat occurring concurrently with shift to dry habitats. Reversals occurred mostly with shift back to wet habitat conditions under both resolving options. Under the ACCTRAN resolving option, almost three times more reversals were contingent upon wet (1.4) compared to dry (0.5) habitat conditions. Under the DELTRAN resolving option, 1 reversal per habitat occurred in dry habitat conditions while 1.2 reversals per habitat occurred in wet habitats. Significant gains are detailed in Supplementary Information S2 and S3 for ACCTRAN and DELTRAN resolving options, respectively. Significant reversals are detailed in S4.

Modifications of the epidermis and the presence of tannins in different tissue types show the highest number of positive Fisher's associations (11 and 12, respectively) with different habitats, with modifications of culm shape, stomata, protective cells, central ground tissue, silica sand and silica having fewer significant habitat associations (Fig. 8). Anatomical modification contingent upon habitat only occurred in the epidermis and with tannins. Thus, shifts in traits falling under different categories of modifications were not contingent upon habitat type. Several anatomical tissue types were significantly modified concurrently with shifts in habitat, particularly stomata. A number of changes in culm shape, epidermis, tannin, silica sand and silica also occurred concurrently with shift in habitat. Changes in anatomical traits contingent upon reversal of habitat conditions occurred largely in the epidermal tissue and the stomata.

DERIVED ANATOMICAL TRAITS ARE ASSOCIATED WITH ARID CLIMATES

PIC correlations of derived, discrete anatomical traits and climate parameters showed that derived anatomical features had significant associations with rainfall, evaporation potential, altitude and temperature (Table 4). Of the total number of significant associations, eleven were indicative of mesic conditions, while thirteen were indicative of arid conditions. Lateral and outer epidermal cell wall thickening and scattered cavities in the ground tissue were unambiguously associated with arid conditions, while flat culms, thin sclerenchyma walls and solid central ground tissue were unambiguously associated with mesic conditions. Papillae and slightly sunken stomata were significantly correlated with both arid and mesic conditions. Although this test incorporated the effects of phylogenetic history, it is unable to identify the most influential variables and illustrate the response to various climate parameters as the BRT analysis did.

A FUNCTIONAL ROLE FOR ANATOMICAL TRAITS

Our assessment of the functional significance of derived anatomical features was limited to those features present in the 16 species included in our experiment. Hemispherical culms had smaller diameters, lower photosynthetic rates and lower culm temperatures than did cylindrical culms (Table 5). Culms with no or scattered cavities have lower SCA, transpiration and photosynthetic, but higher WUE and stomatal density than hollow culms. Species with unthickened sclerenchyma cells had higher stomatal densities and higher water use efficiencies. Unthickened outer epidermal walls were associated with narrower culms, higher transpiration, photosynthesis and stomatal density and lower WUE. Species with stomata sunken half way down the epidermal layer had significantly lower stomatal densities and slightly lower transpiration rates, although the latter was not significant. Species with protective cells longer than the chlorenchyma (but not reaching the parenchyma) (i.e. intermediate size substomatal cavity) had slightly lower transpiration rates compared to species with protective cells reaching the parenchyma and a large substomatal cavity, although this was not significant. Species with an intermediate size substomatal cavity had significantly lower photosynthetic rates and also had wider culms with lower SCA values. These phylogenetically corrected tests show that derived anatomical traits do influence culm gas exchange and therefore play a role in function.

DISCUSSION

Consistent with the interpretations of Gilg (1891), we found evidence both of a functional relationship between the anatomical attributes, and of adaptation to drought, in most tissue systems of the culms in Restionoideae. Modifications of the epidermis and stomata with change in habitat were most common. Three attributes of the epidermides could be adaptive. Species with thickened outer walls tend to have narrower culms, a higher stomatal density and, possibly because of that, higher transpiration and photosynthetic rates, and reduced WUE. This is indicative of an ability to control water loss, but an associated greater ability to maximize transpiration when water is available. Species with thickened outer walls were then also found in more arid climates, with a stronger continentality, indicative of drier summers, and lower mean annual rainfall. This trait is phylogenetically surprisingly conservative. The adaptive patterns in the epidermis are not surprising. This is the anatomical tissue that comes into direct contact with environmental conditions so we may expect a functional consequence of epidermis structure (Gilg 1891). However, few studies have assessed epidermal thickening in response to environmental conditions. Cunningham *et al.* (1999) reported epidermal thickening in perennial plants growing in low soil nutrients in southwestern Australia and suggested that it may reduce herbivory and thereby lessen resource demand in nutrient depauperate areas. Epidermal thickening can also be viewed as a strengthening strategy. The evolutionary habitat associations of this attribute are more difficult to interpret. Outer epidermal thickening evolved contingent upon habitats with no rock – these are deep sandy soils, often waterlogged in winter and very dry in summer, thus consistent with our model of maximizing transpiration when water is available, but controlling water loss in summer. Reversals occurred contingent upon shift to more forested vegetation, where summer drought stress is reduced. Habitat associations indicated that species with outer epidermal wall thickening occurred on bedrock and well-drained habitats. Further studies could assess the response of species with thickened outer epidermal walls to water stress. Gas exchange measurements were taken on well-watered plants and physiological function would be different under drought conditions and may shed light on the adaptive nature of this trait. While the gas exchange data suggests efficient functioning in arid conditions and habitat associations indicate that this trait is associated with dry conditions, the evolution of epidermal thickening was not correlated with dry habitats in African Restionaceae in general.

The patterns with the sinuose, thickened lateral walls are less clear, but also indicative of drought tolerance, and they show a functional correlation with well-drained soils. Maybe these curious structures are associated with mechanical strengthening. Evolutionary and ecological trends in epidermal ornamentation were more complex, and no clear patterns were evident. The vast majority of species have glabrous culms, hairy culms evolving only once (in *Thamnochortus*), and the species are found in most Cape habitats. Most species have more or less tuberculate (colliculate, rough) culms, and again no particular habitat associations have been traced.

Since stomata control stomatal conductance and their morphology and behaviour respond to a variety of environmental signals, literature has focused on this anatomical trait (see Zeiger *et al.* 1987). However, different stomatal architectural types affect stomatal conductance in different ways and influence not only transpiration but also assimilation (Roth-Nebelsick 2007). Sunken and encrypted stomata have conventionally been linked to dry climates due to their purported role in reducing in transpiration (Hill 1998, Roth-Nebelsick 2007, Jordan *et al.* 2008), however, crypts have been shown to have negligible influence on transpiration (Roth-Nebelsick *et al.* 2009). Furthermore, they are found in both arid and mesic areas and have been shown to allow CO_2 access deep in the mesophyll of the leaf (Hassiotou *et al.* 2009). In Restionaceae species with slightly sunken stomata (seated half way down the epidermal layer) had lower stomatal density, transpiration and assimilation and higher water use efficiency compared to species with non-sunken stomata (Table 5). This is more indicative of species tending to slow transpiration, and being more water-conservative, rather than optimising mass flow. Both slightly sunken stomata and fully sunken stomata evolved concurrent with movement to Karroid vegetation. These stomatal modifications and correlates are compatible with previous research into different types of stomatal architecture that suggest that sunken stomata are an adaptation to dry habitat conditions, which occur during the summer months in Mediterranean-type ecosystems (Jordaan *et al.* 2008).

Protective cells flank the substomatal cavity and may be derived from the epidermal or, most commonly, chlorenchyma tissue. Gilg (1891) suggested that these cells, which are common to the Restionaceae, function to reduce water loss. These cavities are cutinized. Roth-Nebelsick (2007) showed, using three dimensional computer simulations, that the presence of a cuticle in the substomatal chamber not only reduced transpiration, but also reduced conductance by 40%. Interestingly, this reduced conductance affected only water vapour and not CO_2. As all Restionaceae have protective cells, correlative approaches do not inform on about their function. However, there is variation in the relative length of these structures. Ancestrally in the Restionoideae they reach through the chlorenchyma, so that the substomatal cavity connects to the parenchyma ring. A reduction in the depth of the substomatal cavity evolved concurrent with move to basalt habitats. Reversal of this trait back to long protective cells that reach the parenchyma occurred with movement to marshes, valley bottoms and dune thicket. Thus, while patterns in trait evolution were not clear, reversals in protective cells tended to occur contingent on shifts back to wet habitats. Species with intermediate length protective cells (i.e. intermediate size substomatal cavity) had slightly lower transpiration rates compared to species with very long protective cells, although this was not significant. This may indicate that shortening of the protective cells, and thus the substomatal cavity, is linked to dry conditions and that long protective cells are adaptive for mesic conditions, where higher transpiration is not maladaptive. Species with intermediate length protective cells had significantly lower photosynthetic rates and also had lower SCA values, suggesting that they may be slower growing and are longer-lived. Gilg (1981) proposed an alternative function for protective cells: mechanical support of the epidermis in species with long protective cells that reach down to the parenchyma layer. While our study did not address this question specifically, the lower SCA value of species with intermediate length protective cells indicates higher tissue structural cost per unit area, which may reflect higher mechanical strengthening. This could be addressed by seeking correlations with other mechanical structural cells, such as the pillar cells or strengthened epidermal cells.

Ancestrally, Restionaceae have a sclerenchyma ring surrounding the central ground tissue that was already interpreted by Gilg (1891) as a mechanical strenghthening structure. For the Restionoideae the sclerenchyma cells were ancestrally very thick, and the thickening has been reduced between 33 and 36 times (ACCTRAN and DELTRAN, respectively). We show that the reduction in the thickening is significantly linked to a reduction in the Apan values and continentality, and an increase in the mean annual precipitation. Species with thin-walled sclerenchyma cells are associated with convex seeps, and inspection of the phylogeny suggests that this could be part of a general relationship to wetland habitats. Species with thin-walled sclrenchyma had higher specific areas than those with thick-walled sclerenchyma. Thus, the sclerenchyma ring may be a manifestation of sclerophylly. Sclerophylly is common in Mediterranean-type ecosystems and has advantages for nutrient use efficiency and drought-tolerance in nutrient-poor environments (Stock & Allsopp 1992, Stock, van der Heyden & Lewis 1992, Salleo *et al.* 1997). Sclerophylly has been linked with nutrient-limitation through its reduction in loss of nutrients at senescence (Orians & Solbrig 1977). The relationship between specific area, which is a measure of sclerophylly, and leaf longevity represents a trade-off, with species with lower specific leaf area having slower relative growth rates but longer leaf life spans and enhanced nutrient conservation (Westoby 1998, Aerts & Chapin 2000). The sclerenchyma ring may also be crucial for allowing the photosynthetic culm structure to grow, and remain, upright.

The central ground tissue of the Restionoideae ancestrally had a single central cavity. This was lost, leading to the absence of any cavity, some 25 times, and scattered cavities evolved some 17 times. Species with solid culms had significantly lower transpiration rates and higher water use efficiency than hollow culms. The manifestation of higher transpiration rates in ancestrally hollow-culmed Restionaceae, with derived traits being associated with lower transpiration, suggest that solid culms may be adaptive in arid conditions. Solid culmed species also had larger diameters than hollow culmed species. Interestingly, reversal of solid culms back to hollow culms occurred contingent upon movement back to wet habitat conditions. During summer drought, Restionaceae culms experience huge tensile forces (Moll & Sommerville 1985, van der Heyden & Lewis 1989). Hollow culms may not have been able to maintain structural integrity under these large forces and thus the evolution of solid ground tissue and ground tissue with scattered cavities may be adaptive in dry conditions.

The majority of African Restionaceae (74%) had up to six derived anatomical traits, with far fewer (26%) having more than six derived traits. Species with unusually high numbers of derived anatomical traits were most strongly associated with climate moisture variables (Fig. 5). These species tended to occur in areas characterised by particularly arid conditions, such as areas of high A-pan evaporation, low autumn and spring rain and low maximum mean annual rainfall. Furthermore, we provide different lines of evidence showing that the evolution of novel anatomic traits tended to occur more under arid than mesic conditions. Reversals in anatomic traits from the derived to the plesiomorphic condition occurred when habitat shifted from dry back to wet habitat conditions, suggesting that since these traits were lost under the new selective regime they are adaptive for the previous selective regime i.e. dry habitat.

Our results are consistent with previous suggestions that Restionaceae occurred ancestrally in wetland habitats that were not seasonally dry. Linder & Mann (1998) found evidence for an ancestral habitat with higher rainfall in the genus *Thamnochortus* and that coastal and summer-dry habitats were derived within the genus. For Restionaceae, a simple ancestor with plesiomorphic anatomical traits is suggested to have occurred in semi-aquatic habitats during the late Cretaceous, when the climate was more mesic (Cutler 1979, Deacon *et al.* 1992). Evidence from ancestral state reconstructions suggests a shift from wetlands to well-drained habitats (Linder 2000, Linder & Rudall 2005). Cutler (1979) postulated that similar anatomical adaptations had evolved in both African and Australian Restionaceae in response to increasing aridity, which was later corroborated using phylogenetic evidence (Linder 2000). There is also substantial evidence for not only an ancestral Poales in marshy, wet habitats (Linder & Rudall 2005) but also diversification of the commelinid group (Poales and related orders) in this type of habitat (Givnish *et al.* 1999). Aridification during the Mid Miocene brought about summer-arid conditions and Restionoideae, an ancestrally wetland lineage, evolved novel anatomical traits that allowed occupation of the newly created arid habitats.

Derived anatomical traits in African Restionaceae have mostly evolved concurrently with movement to arid habitats and there is substantial evidence suggesting that they function in drought tolerance. While our study focused on individual anatomical traits, the way in which these traits interact may be important in understanding their influence on physiology and overall plant functioning (Russo *et al.* 2010). We propose that diversification of culm anatomies has facilitated occupation of a diverse range of habitats, thus explaining the ubiquity of this lineage in the Cape. Anatomical trait evolution may thus have played an important role in the adaptive radiation of Restionaceae, which occurred during the Late Miocene and Late Oligocene (Linder & Hardy 2004), and gave Restionaceae a selective advantage over other groups. Although our results show that anatomical adaptation has facilitated occupation of novel habitats, these adaptations have not been linked to diversification rates. Certain anatomical attributes may have led to divergence in form and function, contributing to high speciation rates as well as diversity within this clade. Although phylogenies can provide evidence for adaptive radiations, identifying adaptive traits, their evolutionary history as well as their function is key to understanding plant radiations in response to changes in environment.

REFERENCES

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research,* **30**, 1–67.

Baum, D.A. & Larson, A. (1991) Adaptation Reviewed: A Phylogenetic Methodology for Studying Character Macroevolution. *Systematic Zoology*, **40**, 1-18.

Born, J., Linder, H.P. & Desmet, P.G. (2006) The Greater Cape Floristic Region. *Journal of Biogeography*, **33**, 1-16.

Botha, D.J. (1982) The stem anatomy of *Elegia stipularis* Mast. *Wetenskaplike Bydraes van Potchefstroom Universiteit*, **13**, 1-8.

Briggs, B.G. & Linder, H.P. (2009) A new subfamilial and tribal classification of Restionaceae (Poales). *Telopea*, **12**, 333-345.

Cunningham, S.A., Summerhayes, B. & Westoby, M. (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs*, **69**, 569-588.

Cutler, D.F. (1969) Juncales. In: Metcalfe C.R., ed. *Anatomy of the monocotyledons, volume 4*. Oxford: Clarendon Press, 1-357.

Cutler, D.F. (1972) Vicarious species of Restionaceae in Africa, Australia and South America. In: Valentine, D.H. (Eds.), *Taxonomy, phytogeography and evolution*. Academic Press, London, pp. 73-83.

Cramer, M. D., Hawkins, H. J. & Verboom, G. A. (2009) The importance of nutritional regulation of plant water flux. *Oecologia*, **161**, 15-24.

De'ath, G. & Fabricius, K.E. (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178-3192.

Deacon, H.J., Jury, R.M. and Ellis, F. (1992) Selective regime and time. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Elith, J., Leathwick, J.R. & Hastie, T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802-813.

Ellis, A.G., Weis, A.E. & Gaut, B.S. (2006) Evolutionary radiation of "stone plants" in the genus Argyroderma (Aizoaceae): Unravelling the effects of landscape, habitat, and flowering time. Evolution, 60, 39-55

Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist*, **125**, 1-15.

Friedman, J.H. & Meulman, J.J. (2003) Multiple additive regression trees with application in epidemiology. *Statistics in Medicine*, **22**, 1365-1381.

Galley, C. & Linder, H.P. (2007) The phylogeny of the Pentaschistis clade (Danthonioideae, Poaceae) based on chloroplast DNA, and the evolution and loss of complex characters. *Evolution*, **61**, 864-884.

Gilg (1891) Beitrage zur vergleichende Anatomie der xerophilen Familie der Restionaceae. *Botanische Jahrbucher*, **13**, 541-606.

Givnish, T.J., Pires, J.C., Sytsma, K.J. (1999) Polyphyly and convergent morphological evolution in Commelinales and Commelinidae: evidence from rbcL sequence data. *Molecular Phylogenetics and Evolution*, **12**, 360-385.

Goldblatt, P. & Manning, J.C. (2000) *Cape Plants. A Conspectus of the Cape Flora of South Africa.* National Botanical Institute, Pretoria.

Gould, S.J. & Vrba, E.S. (1982) Exaptation – A missing term in the science of form. *Paleobiology*, **8**, 4-15.

Greene, H.W. (1986) Natural history and evolutionary biology. In: Predator-prey relationships. Eds: M.E. Feder & Lauder, G.V. University of Chicago Press, Chicago.

Hardy, C.R., Moline, P.M. & Linder, H.P. (2008) A phylogeny for the African Restionaceae, and new perspectives on morphology's role in generating complete species-level phylogenies for large clades. *International Journal of Plant Science*, **169**, 377-390.

Harmon, L. J., Schulte, J. A., Larson, A. & Losos, J. B. (2003) Tempo and mode of evolutionary radiation in Iguanian lizards. *Science*, **301**, 961-964.

Hastie, T., Tibshirani, R. & Friedman, J.H. (2001) *The elements of statistical learning: data mining, inference, and prediction*. Springer-Verlag, New York.

Hassiotou, F., Evans, J.R., Ludwig, M. & Veneklaas, E.J. (2009) Stomatal crypts may facilitate diffusion of CO2 to adaxial mesophylls in thick sclerophylls. *Plant, Cell & Environment*, **32**, 1596-1611.

Hill, R.S. (1998) Fossil evidence for the onset of xeromorphy and scleromorphy in Australian Proteaceae. *Flora of Australia*, **16**, 21-30.

Jordan, G.J., Weston, P.H., Carpenter, R.J., Dillon, R.A. and Brodribb, T.J. (2008) The evolutionary relations of sunken, covered, and encrypted stomata to dry habitats in Proteaceae. *American Journal of Botany* **95** (5): 521-530.

Kellogg, E. A. (2000) The grasses: A case study in macroevolution. *Annual Review of Ecology and Systematics*, **31**, 217-238.

Kellogg, E. A. (2001) Evolutionary history of the grasses. *Plant Physiology*, **125**, 1198-1205.

Klak, C., Reeves, G. & Hedderson, T. (2004) Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature*, **427**, 63-65.

Leathwick, J.R., Elith, J., Chadderton, W.L., Rowe, D. & Hastie, T. (2008) Dispersal, disturbance, and the contrasting biogeographies of New Zealand's diadromous and non-diadromous fish species. *Journal of Biogeography*, **35**, 1481-1497.

Linder, H.P. (1984) A phylogenetic classification of the genera of African Restionaceae. *Bothalia*, **15**, 11-76.

Linder, H.P. (1985) Conspectus of the African species of Restionaceae. *Bothalia*, **15**, 387-503.

Linder, H.P. (2000) Vicariance, climate change, anatomy and phylogeny of Restionaceae. In: *Under the microscope*: plant anatomy and systematics. Eds: Rudall, P.J. and Gasson, P. pp. 159-177. The Linnean Society of London

Linder, H.P. (2003) The radiation of the Cape flora, southern Africa. *Biological Reviews*, **78**, 597–638.

Linder, H.P. (2004) The African Restionaceae: an IntKey identification and description system, version 2. *Contributions of the Bolus Herberium*, **20**. http://www.systbot.unizh.ch/datenbanken/restionaceae/restionaceae.php.

Linder, H.P. (2005) A protocol for the systematic documentation of the ecology of Cape plants. *South African Journal of Botany*, **71**, 201-210.

Linder, H. P. (2005) The evolution of diversity: the Cape flora. *Trends in Plant Science*, **10**, 536-541.

Linder, H.P. & Mann, D.M. (1998) Phylogeny and biogeography of *Thamnochortus* (Restionaceae). *Botanical Journal of the Linnean Society*, **128**, 319-357.

Linder, H.P. & Hardy, C.R. (2004) Evolution of the species-rich Cape flora. *Phil. Trans. R. Soc. Lond.* **359**, 1623-1632.

Linder, H.P. & Rudall (2005) Evolutionary history of Poales. *Annu. Rev. Ecol. Evol. Syst*. **36**, 107-124.

Linder, H.P. & Hardy, C.R. (2010) A generic classification for the Restioneae (Restionaceae), southern Africa. *Bothalia*, **40**, 1-35.

Linder, H. P., Hardy, C. R. & Rutschmann, F. (2005) Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution*, **35**, 569-582.

Linder, H. P., Johnson, S. D., Kuhlman, M., Matthee, C. A., Nyffeler, R. & Swartz, E. (2010) Biotic diversity in the Southern African winter-rainfall region. *Current Opinion in Environmental Systainability*, **2**, 109-116.

Losos, J.B. & Miles, D.B. (2002) Testing the hypothesis that a clade has adaptively radiated: iguanid lizard clades as a case study. *American Naturalist*, 160, 147-157.

Metcalfe, C. R. (1960) *Anatomy of the Monocotyledons I. Gramineae.* Clarendon Press, Oxford.

Moll, E.J. & Sommerville, J.E.M. (1985) Seasonal xylem pressure potentials of two South African coastal fynbos species in three soil types. *South African Journal of Botany*, **51**, 187-193.

Orians, G.H. and Solbrig, O.T. (1977) A cost-income model of leaves and roots with special reference to arid and semi-arid areas. *American Naturalist,* **111**, 677-690.

Pfitzer, E. (1870) Beiträge zur Kenntnis der Hautgewebe der Pflanzen. II. Über das Hautgewebe einiger Restionaceae. *Pringsheim's Jahrbücher für wissenschaftliche Botanik*, **7**, 561-584.

Proches, S., Cowling, R. M. & du Preez, D. R. (2005a) Patterns of geophyte diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity and Distributions*, **11**, 101-109.

Proches, S., Cowling, R. M., Goldblatt, P., Manning, J. C. & Snijman, D. A. (2005b) An overview of the Cape geophytes. *Biological Journal of the Linnean Society*, **87**, 27-43.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statsitical Computing, Vienna.

Rebelo, A. G., Boucher, C., Helme, N. A., Mucina, L. & Rutherford, M. C. (2006) Fynbos biome. South African National Biodiversity Institute, Pretoria.

Ridgeway, G. (2010) GBM: Generalized boosted regression models. R package, version 1.6-3.1. http://www.i-pensieri.com/gregr/gbm.shtml.

Roth-Nebelsick, A. (2007) Computer-based studies of diffusion through stomata of different architecture. *Annals of Botany*, **100**, 23-32.

Roth-Nebelsick, A., Hassiotou, F. & Veneklaas, E.J. (2009) Stomatal crypts have small effects on transpiration: A numerical model analysis. *Plant Physiology*, **151**, 2018-2027.

Russo, S.E., Cannon, W.L., Elowsky, C., Tan, S. & Davies, S.J. (2010) Variation in leaf stomatal traits of 28 tree species in relation to gas exchange along an edaphic gradient in a Bornean rain forest. *American Journal of Botany*, **97**, 1109-1120.

Rundell, R.J. & Price, T.D. (2009) Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution*, **24**, 394-399.

Rymer, P. D., Manning, J. C., Goldblatt, P., Powell, M. P. & Savolainen, V. (2010) Evidence of recent and continuous speciation in a biodiversity hotspot: a population genetic approach in southern African gladioli (Gladiolus; Iridaceae). *Molecular Ecology*, **19**, 4765-4782.

Salleo, S., Nardini, A. & Lo Gullo, M.A. (1997) Is sclerophylly of Mediterranean evergreens an adaptation to drought? *New Phytologist*, **135**, 603-612.

Schluter, D. (1996) Ecological causes of adaptive radiation. *American Naturalist*, **148**, S40-S64.

Schulze, R.E. (1997) South African Atlas of Agrohydrology and Climatology. Water Research Commission, Pretoria, South Africa.

Sillén-Tullberg, B. (1993) The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. *Evolution*, **47**, 1182-1191.

Stock, W.D. and Allsopp, N. (1992) Functional Perspectives of Ecosystems: In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Stock, W.D., van der Heyden, F. and Lewis, O.A.M. (1992) Plant structure and function. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Taylor, H. C. (1978) Capensis. Junk, The Hague.

Van der Heyden, F & Lewis, O.A.M. (1989) Seasonal variation in photosynthetic capacity with respect to plant water status of five species of the Mediterranean climate region of South Africa. *South African Journal of Botany*, **55**, 509-515.

Van Greuning, J.V. & Van der Schijff, H.P. (1973) Chlorenchyma and sclerenchyma in the culms of *Willdenowia* Thunb. and *Hypodiscus*. *Berichte der deutschen botanischen Gesellschaft*, **86**, 537-550.

Verboom, G.A., Linder, H.P. and Stock, W.D. (2004) Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus *Ehrharta* (Poaceae: Ehrhartoideae). *American Journal of Botany* **91,** 1364-1370.

Verboom, G.A., Archibald, J.K., Bakker, F.T., Bellstedt, D.U., Conrad, F., Dreyer, L.L., Forest, F., Galley, C., Goldblatt, P., Henning, J.F., Mummenhoff, K., Linder, H.P., Muasya, A.M., Oberlander, K.C., Savolainen, V., Snijman, D.A., van der Niet, T. & Nowell, T.L. (2009) Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? *Molecular Phylogenetics and Evolution*, **51**, 44-53.

Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008) Phylocom: software for the analysis of phylogenetic community structure and character evolution. Version 4.0 URL: http://phylodiversity.net/phylocom/.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil,* **199**, 213-227.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010) Ecophysiological significance of leaf size variation in Proteaceae form the Cape Floristic Region. *Functional Ecology,* **24**, 485-492.

Zeiger, E., Farquhar, G.D. & Cowan, I.R. (1987) *Stomatal function*. Stanford University Press, Stanford, California.

FIGURE LEGENDS:

FIGURE 1: Light microscope images of cross-section through Staberoha ornate showing the basic organisation of the culm. This basic organisation of tissues is constant through the whole Restionaceae.

FIGURE 2: Light microscope images of cross-sections through Restionaceae culms showing some of the intriguing anatomical features. a. *Calopsis marlothii* with stomata sunken halfway down the epidermal layer and thickening of the outer epidermal cell wall. b. *Restio occultus* with thickened outer epidermal cell walls, large substomatal cavities, solid central ground tissue and tannin scattered in the sclerenchyma and central ground tissue. c. *Restio saxatilis* with stomata born on elongated epidermal cells, forming tubercles. d. *Restio karooica* with thickened sclerenchyma cell walls and silica sand in the central ground tissue. e. The extremely flattened culm of *Platycaulos cascadensis*. f. *Rhodocoma arida*, with thickened outer and lateral epidermal cell walls and stomata sunken halfway down the epidermal layer. g. *Thamnochortus acuminatus* with prominent epidermal hairs. h. *Staberoha ornata* with epidermal papillae and deep substomatal cavities.

FIGURE 3: Histogram showing frequency of derived anatomical traits in African Restionaceae. This is a skewed distribution, with fewer species having more than six derived anatomical traits.

FIGURE 4: Diagram illustrating the various tests of association. Fisher's Exact Test was used to test the ahistorical association of anatomical traits with different habitats. Counts for the three evolutionary tests of association were obtained from the reconstructions of anatomical and habitat traits at each node of the Restionaceae phylogeny. Fisher's Exact Test was also used to test for significance of the three evolutionary tests.

FIGURE 5: Partial dependence plots for the six most influential variables in the BRT model. Functions illustrate the response of species with highly modified anatomies to

a particular environmental variable while all other predictor variables are kept at their average values.

FIGURE 6: Histograms showing the numbers of significant ahistorical (aptation) and evolutionary associations found under each habitat category, using both ACCTRAN (a) and DELTRAN (b) resolving options. Numbers represent the total number of significant ($a < 0.05$) associations (for the functional test) or shifts (for the evolutionary tests) of derived anatomical traits with habitat types that fall into the above habitat categories. Currently, derived traits tend to be strongly associated with water availability. Evolution of derived traits tended to be contingent mainly upon habitat rockiness. The evolution of derived anatomical traits mostly occurred concurrently with shift in bedrock type, water availability and vegetation type. Reversals in derived traits were predominantly dependent on water availability.

FIGURE 7: Histograms showing the number of significant associations of derived anatomical features (ahistorical), concurrent shifts in states and contingent gains and reversals in dry and wet habitat types. Numbers represent the number of significant associations (for the ahistorical test) or shifts (for the evolutionary tests) of derived anatomical traits with habitats that fall into the wet and dry water availability, weighted according to the number of habitats within the wet and dry water availability categories. For the historical tests, results for ACCTRAN (a) and DELTRAN (b) resolving options are shown. More changes are currently associated with, and historically arose contingent upon, dry habitats. For both ACCTRAN and DELTRAN resolving options, reversals of derived features tended to be contingent upon wet habitat conditions.

FIGURE 8: Histograms showing the numbers of significant ahistorical and evolutionary associations found under each anatomical tissue type, using only the ACCTRAN resolving option (DELTRAN results were similar). Numbers represent the total number of significant ($a < 0.05$) associations (for the ahistorical test) or shifts (for the evolutionary tests) of derived anatomical traits with habitat types that

fall into the above anatomical categories. High numbers of evolutionary shifts suggest high plasticity of the particular tissue.

FIGURE 1

FIGURE 2

FIGURE 3

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FIGURE 8

TABLES

TABLE 1

TABLE 2

TABLE 4

TABLE 5

SUPPORTING INFORMATION

S1

S2

S3

S4

APPENDIX A1

GENERAL DISCUSSION

Plant morphology and ecophysiological function vary considerably and influence plant growth rate, population dynamics, distributions and ecosystem functioning (Givnish 1978, 1979, 1987, Ackerly *et al.* 2000). Also, ecophysiological function may be influenced by morphology (Nobel 2005) and plant traits and function tend to be correlated with particular environmental conditions (Reich *et al.* 1997). A variety of plant traits, from biochemistry and gas exchange to structure, growth and allocation potentially influence fitness and may undergo natural selection (Ackerly *et al.* 2000). The study of adaptation involves firstly determining whether the trait has a function within a particular selective environment and secondly determining whether the evolution of the trait occurred within the particular selective environment (Gould & Vrba 1982, Baum & Larson 1991). When traits enhance performance in a way that increases survival or reproduction and when they have evolved through natural selection for that particular function within the particular selective environment they are considered 'adaptive' (Gould & Vrba 1982, Baum & Larson 1991). However, interactions between traits can create complexities in studies of adaptation. The importance of viewing plant phenotypes and adaptation as a combination of anatomy, morphology, growth, physiology and life-history has been highlighted by this study. Since culm structures are so prevalent in the CFR, I proposed that they were adaptive for seasonal environments with winter rainfall and summer drought. I investigated three different culm traits; culm shape, variation in size and anatomy. I found functional, ecological and evolutionary evidence that culm structure, variation in size of structure as well as culm anatomy were adaptive for particular environmental conditions, but not highly seasonal environmental conditions in all cases.

Cylindrical, culm photosynthetic structures were associated with wet, aseasonal environments, and tended to have lower photosynthetic and transpiration rates than leaf photosynthetic structures. Culms had higher carbon return per nutrient invested and represented a long-term, nutrient-efficient structure allowing persistence and perennial growth. Thicker photosynthetic structures are generally associated with infertile conditions because transpirational costs are higher when photosynthetic rates on nutrient-poor soils are lower (Givnish 1978, 1979). There are other potential functions of culms that have not been explored. Restionaceae are wind-pollinated and the cylindrical culm structure may be important for pollen capture. The culm structure may also allow vertical growth while simultaneously assimilating carbon and transpiring. Restionaceae are found in windy environments, and the culm structure may lessen wind-induced damage. The culm structures tend to bend in the wind and this may prevent damage. Leafy species may loose leaves in windy events, which may severely impact the plants' carbon assimilation. I found evidence that leaf photosynthetic structures facilitate effective capitalisation of resources during periods of high resource availability through their enhanced assimilation and transpiration. While leaves may be costly to produce, the benefits of their higher assimilation and transpiration may outweigh their production costs during periods of high nutrient and water availability. Additional studies on leaf loss in other systems may enhance our understanding of the adaptive significance leaf and culm photosynthetic structures.

The shape as well as the size of plant structures has important consequences for plant physiological functioning through their effects on boundary layer conductance. Models of stomatal conductance, photosynthesis and transpiration that include a laminar boundary layer have been developed that demonstrated the significance of boundary layer layers in influencing feedback loops which affect the regulation of stomatal conductance and latent heat flux (Collatz *et al.* 1991). Furthermore the effects of boundary layer conductance on transpiration, photosynthesis and heat exchange are known from a theoretical perspective (Nobel 2005). Despite this, the significance of boundary layer on plant function, evolutionary history and ecology has not received adequate attention. Enquist (2002) provided theory linking plant form and function using allometric scaling. While this theory takes size into account, it ignores the influence of size and thus boundary layer, on plant physiological functioning. The significant influence of boundary layer dynamics on plant gas and heat exchange certainly deserves further attention and inclusion in studies of plant function and adaptation.

I demonstrated enhanced cooling, transpiration as well as carbon assimilation in narrow culms with thinner boundary layers compared to broader culms with thicker boundary layers. Narrow-culmed species were associated with areas that received a high proportion of rainfall in winter and autumn. The ability of narrow culms, and thus thin boundary layers, to transpire rapidly may be particularly advantageous in nutrient-poor conditions because transpiration drives mass-flow on nutrients towards the roots (Barber 1995). A nutrient-transpiration link was demonstrated for *Ehrharta calycina*, where nutrient-constrained plants had higher transpiration rates than those with adequate nutrient supply (Cramer *et al.* 2008). Cramer *et al.* (2009) highlighted the strong links between plant water and nutrient flux. Several environmental cues regulate stomatal conductance, one of which is nutrient availability. Recent research has indicated that this cue, in particular, may be significant in regulating stomatal aperture. Dodd *et al.* (2003) showed that stomatal closure was induced in nitrogen-deprived plants due to changes in xylem sap concentration. Variation in Proteaceae leaf dimension in the CFR has been shown to significantly influence transpiration, with narrow leaves transpiring at faster rates than broad leaves (Yates *et al.* 2010). Plants with narrow leaves are found on sterile podsols, in montane and cloudforest with highly leached soils and in bogs (Givnish 1987) and variation in leaf width may be an adaptation to nutrient-poor conditions (Yates *et al.* 2010). In the CFR, nitrogen and phosphorous are critical limiting nutrients. Because phosphorous is sparingly soluble, mass flow probably increases inorganic nitrogen uptake. The higher assimilation rates of narrow culms may help fund nutrient and water uptake during transient periods of high water and nutrient availability. Narrow culmed-species also tended to be short and the enhanced transpiration and photosynthesis of narrow culms may be crucial for survival in an understory where wind movement is low. While narrow culms did have improved cooling, the temperature differences were not substantial enough to be of major physiological and adaptive consequence. Studies assessing correlations of culm width and environmental conditions at a global scale may find similar trends to support these hypotheses.

While rapid transpiration may have benefits for nutrient acquisition, it can be risky. High transpiration when water is scarce can lead to reduced leaf water potential (Lambers *et al.* 1998). The ability to transpire only when water is plentiful, such as the wet spring months, and thus the ability to tightly control transpiration is crucial. When boundary layers are thin, as for narrow culms, stomatal conductance is less than boundary layer conductance and stomata are the overriding regulator of transpiration (McNaughton & Jarvis 1983). For larger culms, stomatal conductance is more than boundary layer conductance, and changes in stomatal conductance tend to have a smaller effect on transpiration. The ability to tightly control transpiration would certainly be advantageous during the hot, dry summers when water is scarce. The influence of leaf resistance (i.e. boundary layer conductance) on stomatal control of photosynthesis has been demonstrated experimentally for orange leaves (*Citrus sinensis*). At lower leaf resistance (high boundary layer conductance) the ratio of transpiration to photosynthesis decreased with decreased stomatal aperture, indicating tighter control over water vapour loss than photosynthesis (Kriedemann 1971). Correlation between dimension and photosynthetic rate was not found in Cape Proteaceae (Yates *et al.* 2010), but was present in Restionaceae. Lack of correlation in Cape Proteaceae may have been because of biochemical regulation of photosynthesis and because photosynthesis is less tightly controlled than transpiration.

I found evidence for both a functional relationship between the anatomical attributes, and of adaptation to drought, in most anatomical tissue systems of the culms in Restionoideae. Anatomically specialised species (those with many derived traits) were associated with arid conditions, including high continentality, high A-pan evaporation and low rainfall during spring and autumn. Correlative tests showed that many derived traits had evolved both contingent upon and concurrent with shifts to arid habitat types. Furthermore, reversals in derived traits tended to occur with shifts back to wet habitats. Occupation of a wide range of habitats, particularly arid conditions created during the mid-Miocene climate change, may have been facilitated by the evolution of novel anatomical traits. These adaptations have significantly contributed to the diversity in form and function of this clade and may explain its ubiquity in the CFR. This study has shown that anatomical traits can influence physiological functioning. Further studies of individual traits are necessary. In addition, tests of function under different environmental conditions e.g. water stress, would provide knowledge of how particular traits may be important in coping with certain conditions. This thesis suggests that anatomical adaptation may have played a significant role in adaptive radiations.

Culm photosynthetic structures were present before the mid-Miocene climate change that created more seasonal conditions in the Cape. Culm photosynthetic structures were also found to be associated with aseasonal conditions. Narrow culms were associated with seasonal habitats that receive a high proportion of rain during winter and autumn. Derived anatomical traits were associated with arid and seasonal conditions. Both decrease in culm width and anatomical modifications may have facilitated occupation of seasonal and/or arid environments created during the mid-Miocene climate change. While leaf production could have facilitated effective capitalisation of resources during periods of high resource availability through their enhanced assimilation and transpiration, they may have been too costly to produce or there may have been genetic constraints to producing leaves after they had been lost. This demonstrates that a combination of traits may contribute to occupation of particular habitats. Further evolutionary studies assessing changes in culm width over time could shed light on this theory.

In all three tests of adaptive traits (culm shape, variation in culm diameter and anatomical variation), the most influential environmental variable was rainfall seasonality. The CFR experiences a Mediterranean-type climate with dry summers and high winter rainfall (Cowling 1992). In addition, the substrates of this area tend to be highly oligotrophic, with rain causing further nutrient leaching (Deacon *et al.* 1992). Surviving the hot, dry summer months and capitalising on scarce nutrients when they are available, typically during spring, were thought to pose challenges for plants growing in this area. Leaf structures and narrow culms were strongly associated with rainfall seasonality, in particular high rain during winter and spring, suggesting that these traits are not adaptive for hot, dry summer drought conditions but rather for nutrient and water acquisition when these resources are available. I suggest that traits that allow efficient capitalisation of resources when they are available are strongly selected for in this Mediterranean-type system. These traits may, in addition, be exaptive for summer drought conditions. For example, the enhanced transpirational control and sensible heat loss of narrow culms may also be advantageous during hot, dry summers. Anatomical specialisation was associated with arid conditions and certain anatomical traits were found to be adaptive for drought conditions. Culm anatomical modification may have allowed culm photosynthetic species to occupy more seasonal, arid habitats formed during the mid-Miocene climate change. Further studies testing the adaptive nature of traits in other Mediterranean-type areas could corroborate these theories. In addition, investigation of the link between nutrients and culm diameter and shape is necessary.

Leaves often reflect adaptations to various ecological conditions (Givnish 1987) and I have shown that culm structures too display patterns with environmental conditions. Furthermore, I have shown that adaptation may occur at various levels of culm structural organization, from shape, variation in size of shape to anatomy. Rainfall seasonality influenced the shape of the structure, the variation in size of the structure as well as anatomy of these structures. However, complex interactions between these various structural components may interact to influence plant physiological functioning and thus patterns with ecological conditions. There are many other aspects of the plants biology that we have not yet explored, such as rooting depth, which may also contribute to the way these plants function within this region.

Phylogenies are often used to provide evidence for adaptive radiations, but I suggest that identifying adaptive traits, their evolutionary history as well as their function is key to understanding plant radiations in response to changes in environment. With the increasing number of available phylogenies, testing both trait function and evolutionary history within a particular selective environment becomes feasible. The CFR is a species-rich region, where complex topography, heterogeneous substrates and harsh climatic conditions may have played a role in trait evolution and radiation. Understanding the evolutionary processes that led to the patterns in plant traits that we see today enhances our understanding of radiation and niche evolution in the CFR and contributes to understanding the extraordinarily high endemism in this area.

REFERENCES

Ackerly, D.D., Dudley, S.A., Sultan, S.E., Schmitt, J., Coleman, J.S., Linder, C.R., Sandquist, D.R., Geber, M.A., Evans, S.A., Dawson, T.E. & Lechowicz, M.J. (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *BioScience*, **50**, 979-995.

Baum, D.A. & Larson, A. (1991) Adaptation Reviewed: A Phylogenetic Methodology for Studying Character Macroevolution. *Systematic Zoology*, **40**, 1-18.

Barber, S.A. (1995) Soil Nutrient Bioavailability: A Mechanistic Approach, 2nd edition. John Wiley & Sons, New York.

Collatz, G.J., Ball, J.T., Grivet, C. & Berry, J.A. (1991) Physiological and environmental regulation of stomata conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology*, **54**, 107-136.

Cowling, R.M. (1992) Preface. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Cramer, M.D., Hoffmann, V. & Verboom, G.A. (2008) Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist,* **179**, 1048-1057.

Cramer, M.D., Verboom, G.A. & Hawkins, H.J. (2009) The importance of nutritional regulation of plant water flux. *Oecologia*, **161**, 15-24.

Deacon, H.J., Jury, R.M. and Ellis, F. (1992) Selective regime and time. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK.

Dodd, I.C., Tan, L.P. & He, J. (2003) Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following N deprivation. *Journal of Experimental Botany* **54,** 1281-1288.

Enquist, B. (2002) Universal scaling in tree and vascular plant allometry: toward a general quantitative theory linking plant form and function from cells to ecosystems. *Tree Physiology*, **22**, 1045-1064.

Givnish, T.J. (1978) Ecological aspects of plant morphology: leaf form in relation to environment. *Acta Biotheoretica*, **27**, 83-142.

Givnish, T.J. (1979) On the adaptive significance of leaf form. In: *Topics in Plant Population Biology* (Eds. O. T. Solbrig, S. Jain, G. B. Johnson & P. H. Raven), pp. 375-407. Columbia University Press, New York.

Givnish, T.J. (1987) Comparative structures of leaf form: assessing relative roles of selective pressures and phylogenetic constraints. *New Phytologist,* **106**, 131-160.

Gould, S.J. & Vrba, E.S. (1982) Exaptation – A missing term in the science of form. *Paleobiology*, **8**, 4-15.

Kriedemann, P.E. (1971) Photosynthesis and Transpiration as a Function of Gaseous Diffusive Resistances in Orange Leaves. *Physiologia Plantarum*, **24**, 218-225.

Lambers, H., Chapin, F.S., and Pons, T.L. (1998) Plant Physiological Ecology. Springer-Verlag

McNaughton, K.G. & Jarvis, P.G. (1983) Predicting effects of vegetation changes on transpiration and evaporation. In: Water Deficits and Plant Growth, Vol III. Ed. Kozlowski, T.T. Academic Press, Inc., San Diego, pp. 1-47.

Nobel, P.S. (2005) *Physicochemical and environmental plant physiology*. Academic Press, San Diego, California.

Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the Natural Academy of Science USA*, **94**, 13730-13734.

Yates, M.J., Verboom, G.A., Rebelo, A.G. & Cramer, M.D. (2010) Ecophysiological significance of leaf size variation in Proteaceae form the Cape Floristic Region. *Functional Ecology,* **24**, 485-492.

APPENDIX

Table A1. List of species in CFR Poales tree, listed in order of appearance in trees in Figs 1& 2. Species in collapsed clades have been listed with the clade name.

Anthochortus crinalis		*Thamnochortus karooica*	
Anthochortus graminifolius		*Thamnochortus muirii*	
Nevillea obtusissima		*Thamnochortus spicigerus*	Thamnochortus Clade
Anthochortus singularis		*Thamnochortus paniculatus*	
Hypodiscus aristatus	Cannamois/Ceratocaryum Clade	*Thamnochortus fraternus*	
Cannomois schlechteri		*Thamnochortus pluristachyus*	
Cannomois saundersii		*Thamnochortus glaber*	
Cannomois parviflora		*Thamnochortus papyraceus*	
Cannomois arenicola		*Thamnochortus acuminatus*	
Ceratocaryum fistulosum		*Thamnochortus fruticosus*	
Ceratocaryum fimbriatum		*Thamnochortus rigidus*	
Ceratocaryum xerophilum		*Thamnochortus cinereus*	
Ceratocaryum caespitosum		*Thamnochortus guthrieae*	
Ceratocaryum pulchrum		*Thamnochortus erectus*	
Ceratocaryum argenteum		*Thamnochortus insignis*	
Willdenowia glomerata		*Thamnochortus arenarius*	
Willdenowia arescens		*Thamnochortus pellucidus*	
Hypodiscus montanus		*Thamnochortus stokoei*	
Hypodiscus squamosus		*Thamnochortus dumosus*	
Mastersiella purpurea		*Thamnochortus lucens*	
Mastersiella spathulata		*Thamnochortus schlechteri*	
Cannomois scirpoides		*Thamnochortus platypteris*	
Cannamois dol		*Thamnochortus punctatus*	
Soroveta ambigua		*Thamnochortus bachmannii*	
Platycaulos galpinii	Platycaulos Clade	*Thamnochortus obtusus*	
Platycaulos mlanjinensis		*Thamnochortus sporadicus*	
Platycaulos mahonii subsp. mahonii		*Thamnochortus nutans*	
Platycaulos acutus		*Thamnochortus gracilis*	
Platycaulos cascadensis		*Thamnochortus pulcher*	
Platycaulos callistachyus		*Thamnochortus levynsiae*	
Platycaulos anceps		*Restio perseverans*	Restio Clade 1
Platycaulos compressus		*Restio filiformis*	
Platycaulos major		*Restio perplexus*	
Platycaulos depauperatus		*Restio aureolus*	
Platycaulos subcompressus		*Restio bolusii*	
Staberoha stokoei	Staberoha Clade	*Restio bifurcus*	
Staberoha remota		*Restio strobilifer*	
Staberoha aemula		*Restio nodosus*	
Staberoha cernua		*Restio pachystachyus*	
Staberoha distachyos		*Restio capillaris*	
Staberoha banksii		*Restio cymosus*	
Staberoha multispicula		*Restio patens*	
Staberoha ornata		*Restio occultus*	
Staberoha vaginata		*Restio brachiatus*	

Restio brunneus	Restio Clade 1	*Restio sieberi*	Restio Clade 4
Restio insignis		*Restio monanthos*	
Restio obscurus		*Restio parthenocarpos*	
Restio inveteratus		*Restio vilis*	
Restio burchellii		*Restio gossypinus*	
Restio pulvinatus		*Restio aridus*	
Restio fusiformis		*Restio capensis*	
Restio acockii		*Restio curvibracteatus*	
Restio praeacutus		*Restio wittebergensis*	
Rhodocoma vleibergensis		*Restio ocreatus*	
Rhodocoma fruticosa		*Restio elsiae*	
Rhodocoma alpina		*Restio hystrix*	
Rhodocoma foliosa		*Restio eleocharis*	
Rhodocoma gigantea		*Restio subverticellatus*	
Rhodocoma arida		*Restio triflora*	
Rhodocoma capensis		*Restio esterhuyseniae*	
Rhodocoma gracilis		*Restio leptoclados*	
Restio micans		*Restio setiger*	Restio Clade 5
Restio egregius		*Restio venustulus*	
Restio levynsiae		*Restio distractus*	
Restio rigidus		*Restio unispicatus*	
Restio andreaeanus	Restio Clade 2	*Restio gaudichaldianus*	
Restio tenuispicatus		*Restio nubigenus*	
Restio calcicola		*Restio caespitosus*	Restio Clade 6
Restio muirii		*Restio cincinnatus*	
Restio albotuberculatus		*Restio curviramis*	
Restio rigoratus		*Restio duthieae*	
Restio ramosissimus		*Restio nanus*	
Restio vimineus		*Restio pratensis*	
Restio rudolfii		*Restio sporadicus*	
Restio adpressus		*Restio pygmaeus*	
Restio durus		*Restio macer*	
Restio longiaristatus		*Restio anomalus*	Restio Clade 7
Restio sabulosus	Restio Clade 3	*Restio femineus*	
Restio rottboellioides		*Restio wallichii*	
Restio paludosus		*Restio tenuissimus*	
Restio papillosus		*Restio rivulus*	
Restio marlothii		*Restio pillansii*	Restio Clade 8
Restio schoenoides		*Restio corneolus*	
Restio constipatus		*Restio saroclados*	
Restio virgeus		*Restio leptostachyus*	
Restio laniger	Restio Clade 4	*Restio ejuncidus*	
Restio karooicus		*Restio nudiflorus*	
Restio coactilis		*Restio clandestinus*	

Restio triticeus	Restio Clade 8	*Restio quinquefarius*	Restio Clade 9
Restio strictus		*Restio similis*	
Restio verrucosus		*Restio distans*	
Restio pumilus		*Restio debilis*	
Restio zwartbergensis		*Askidiosperma insigne*	Askidiosperma Clade
Restio sejunctus		*Askidiosperma longiflorum*	
Restio scaberulus		*Askidiosperma capitatum*	
Restio stokoei		*Askidiosperma delicatulum*	
Restio multiflorus		*Askidiosperma albo-aristatu*	
Restio tuberculatus		*Askidiosperma nitidum*	
Restio festuciformis		*Askidiosperma chartaceum*	
Restio zuluensis		*Askidiosperma andreaeanum*	
Restio peculiaris		*Askidiosperma paniculatum*	
Restio arcuatus		*Askidiosperma alticolum*	
Restio inconspicuus		*Askidiosperma rugosum*	
Restio vallis-simius		*Askidiosperma esterhuysenia*	
Restio secundus		*Elegia mucronata*	
Restio decipiens		*Elegia cuspidata*	
Restio fragilis		*Elegia acockii*	
Restio implicatus		*Elegia deusta*	
Restio colliculospermus		*Elegia microcarpa*	Elegia Clade 1
Restio dodii var. dodii		*Elegia verreauxii*	
Restio dodii var. purpurascens		*Elegia recta*	
Restio ingens		*Elegia nuda*	
Restio purpurascens		*Elegia tectorum*	
Restio involutus		*Elegia elephantina*	
Restio dispar		*Elegia glapinii*	
Restio paludicola		*Elegia thyrsoidea*	
Restio communis		*Elegia asperiflora*	
Restio rarus		*Elegia glomerata*	
Restio degenerans		*Elegia caespitosa*	
Restio alticola		*Elegia intermedia*	Elegia Clade 2
Restio distichus		*Elegia fucata*	
Restio parvispiculus		*Elegia fistulosa*	
Restio asperus		*Elegia coleura*	
Restio pulcher		*Elegia grandispicata*	
Restio harveyi		*Elegia thyrsifera*	
Restio hyalinus		*Elegia spathacea*	
Restio versatilis		*Elegia rigida*	
Restio impolitus		*Elegia extensa*	
Restio paniculatus		*Elegia amoena*	Elegia Clade 3
Restio tetragonus		*Elegia racemosa*	
Restio quadratus		*Elegia persistens*	

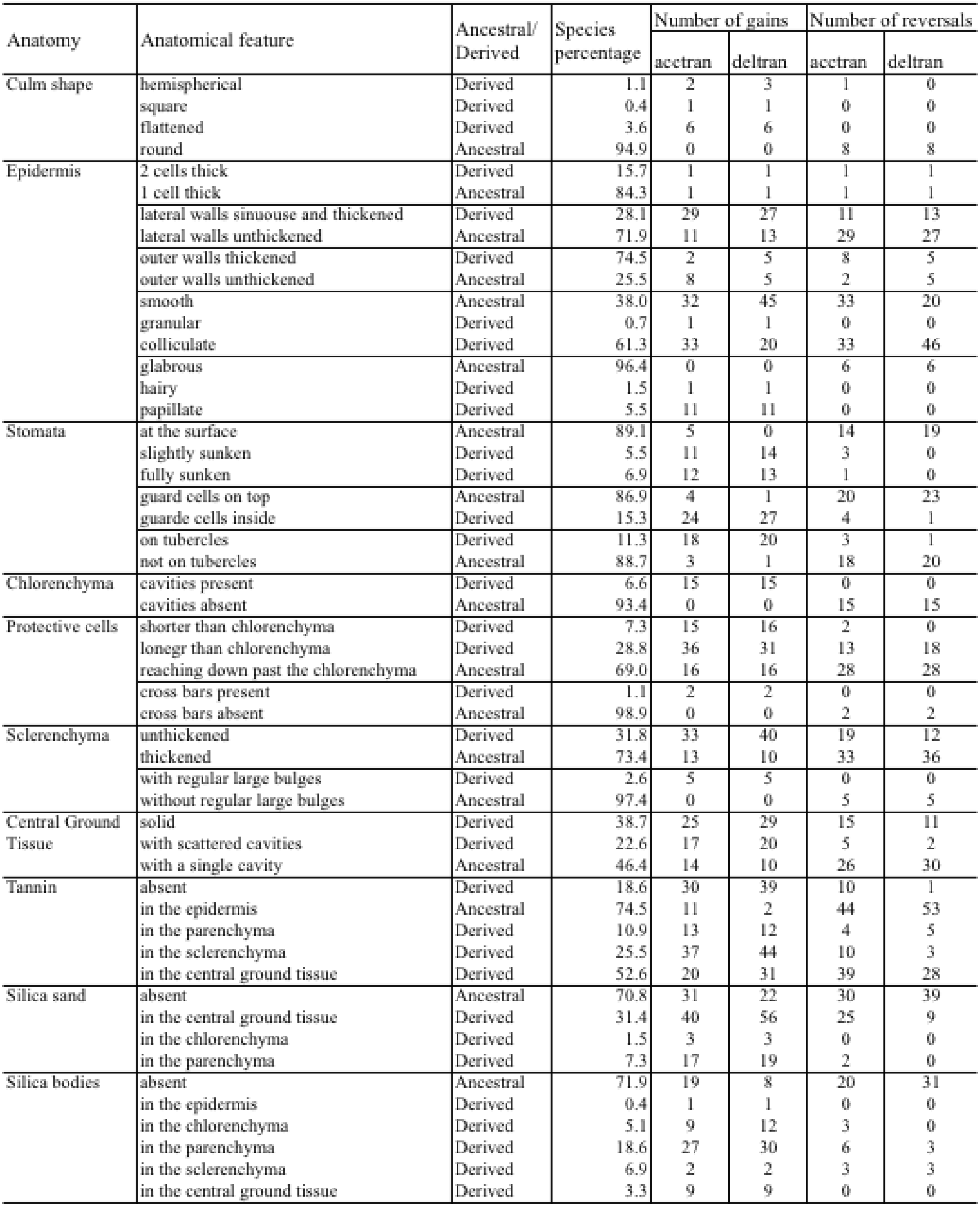
Elegia stipularis	Elegia	*Ehrharta longiflora*	POACEAE
Elegia stokoei		*Ehrharta delicatula*	
Elegia squamosa		*Ehrharta triandra*	
Elegia atratiflora		*Ehrharta eburnea*	
Elegia fenestrata		*Ehrharta barbinodis*	
Elegia vaginulata		*Ehrharta thunbergii*	
Elegia filacea		*Ehrharta villosa*	
Elegia juncea		*Ehrharta longigluma*	
Elegia equisetacea		*Ehrharta mellicoides*	
Elegia macrocarpa		*Ehrharta calycina*	
Elegia esterhuyseniae		*Ehrharta pusilla*	
Elegia capensis		*Ehrharta brevifolia*	
Elegia hutchinsonii		*Merxmuellera arundinaceae*	
Elegia prominens	Elegia Clade 4	*Merxmuellera decora*	
Elegia muirii		*Merxmuellera lupulina*	
Elegia neesii		*Merxmuellera rufa*	
Elegia grandis		*Merxmuellera cincta cincta*	
Elegia aggregata		*Merxmuellera setaceae*	
Elegia ebracteata		*Pentaschistis elegans*	
Elegia decipiens		*Pentaschistis alticola*	
Elegia hookeriana		*Pentaschistis tortuosa*	
Restio pedicellatus		*Pentaschistis colorata*	
Restio sp. aff pedicellatus		*Pentaschistis rigidissima*	
Restio subtilis		*Pentaschistis viscidula*	
Restio confusus		*Pentaschistis pusilla*	
Restio miser		*Pentaschistis aurea subsp. au*	
Restio distylis		*Pentaschistis ampla*	
Restio nuwebergensis		*Pentaschistis caulescens*	
Restio bifarius		*Pentaschistis scandens*	
Restio bifidus		*Pentaschistis acinosa*	
Restio echinatus		*Pentaschistis capensis*	
Restio monostylis		*Pentaschistis argentea*	
Restio papyraceus		*Pentaschistis malouinensis*	
Ehrharta dura	POACEAE	*Pentaschistis pungens*	
Ehrharta microlaena		*Pentaschistis pyrophila*	
Ehrharta rupestris		*Pentaschistis eriostoma*	
Ehrharta setaceae		*Pentaschistis rosea rosea*	
Ehrharta rehmanni		*Pentaschistis pallescens*	
Ehrharta ramosa		*Pentaschistis pallida TVN32*	
Ehrharta capensis		*Pentaschistis aristidoides*	
Ehrharta bulbosa		*Pentaschistis triseta*	
Ehrharta longifolia		*Pentaschistis velutina*	
Ehrharta ottonis		*Pentaschistis calcicola calcic*	
Ehrharta erecta		*Pentaschistis densifolia*	

Pentaschistis ecklonii	POACEAE	*Rhynchospora brownii*	CYPERACEAE
Pentaschistis aspera		*Capeobolus brevicaulis*	
Pentaschistis patula		*Cyathocoma hexandra*	
Pentaschistis barbata		*Tetrariararia nigrovaginata*	
Pentaschistis pallida CG545		*Tetrariararia fimbriolata*	
Pentaschistis reflexa		*Tetraria pillansii*	
Pentaschistis rupestris		*Tetraria microstachys*	
Pentaschistis papillosa		*Tetraria burmanii*	
Pentaschistis cirrhulosa		*Tetraria crinifolia*	
Pentaschistis glandulosa		*Tetraria fasciata*	
Pentaschistis montana		*Tetraria ustulata*	
Pentaschistis airoides		*Tetraria capillacea*	
Pentaschistis tomentella		*Tetraria flexuosa*	
Pentaschistis veneta		*Tetraria thermalis*	
Pentaschistis pseudopallescens		*Tetraria triangularis*	
Cortaderia selloana		*Tetraria compressa*	
Pseudopentameris caespitosa		*Tetraria involucrata*	
Pseudopentameris brachyphylla		*Tetraria bromoides*	
Pseudopentameris macrantha		*Neesenbeckia punctoria*	
Merxmuellera disticha		*Schoenus nigricans*	
Merxmuellera stricta		*Fimbristylis complanata*	
Schismus barbatus		*Bolboshoenus maritimus*	
Schismus scaberrimus		*Cyperus tenellus*	
Tribolium brachystachyum		*Scirpoides thunbergii*	
Tribolium uniolae		*Hellmuthia membranacea*	
Karroochloa tenella		*Isolepis marginata*	
Tribolium obtusifolium		*Ficinia trichodes*	
Tribolium obliterum		*Ficnia pinguior*	
Tribolium pusillum		*Ficinia tristachya*	
Karroochloa curva		*Ficinia rigida*	
Schismus pleuropogon		*Ficinia repens*	
Tribolium acutiflorum		*Ficinia ramosissima*	
Tribolium utriculosum		*Ficinia polystachya*	
Tribolium hispidum		*Ficinia indica*	
Tribolium cilliare		*Ficinia gydomontana*	
Tribolium echinatum		*Ficinia laciniata*	
Chrysitrix capensis	CYPERACEAE	*Ficinia esterhuyseniae*	
Cladium mariscus		*Ficinia bergiana*	
Carpha glomerata		*Ficinia distans*	
Schoenoxiphium ecklonii		*Isolepis sepulcralis*	
Schoenoxiphium sparteum		*Isolepis hystrix*	
Schoenoxiphium lehmanii		*Isolepis tenuissima*	
Trianoptiles solitaria		*Cyperus laevigatus*	
Ficinia paradoxa		*Cyperus longus*	

Pycreus mundtii	CYPERACEAE
Isolepis ludwigii	
Isolepis striata	
Isolepis fluitans	
Isolepis rubicunda	
Isolepis prolifera	
Isolepis digitata	
Isolepis setacea	
Isolepis diabolica	
Isolepis venustula	
Isolepis verruculosa	
Isolepis cernua	
Tetrariararia picta	
Tetraria compar	
Tetraria sylvatica	
Tetraria brachyphylla	
Epischoenus quadrangularis	
Tetraria bolusii	
Tetraria compacta	
Tetraria crassa	
Tetraria exilis	
Tetraria cuspidata	

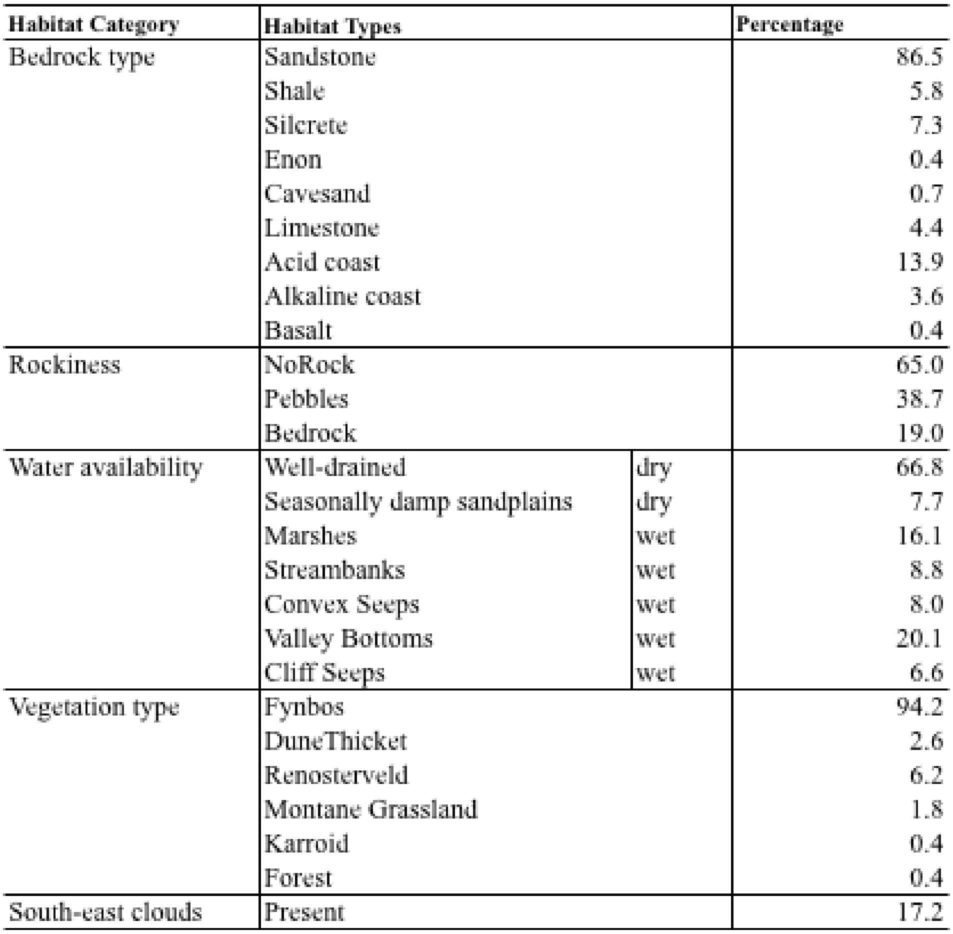
TABLES

TABLE 1: Modifications of the different tissue types showing whether they were derived, the percentage of species in which they occur and the number of times they were independently gained or lost.



Anatomy	Anatomical feature	Ancestral/ Derived	Species percentage	Number of gains		Number of reversals	
				acctran	deltran	acctran	deltran
Culm shape	hemispherical	Derived	1.1	2	3	1	0
	square	Derived	0.4	1	1	0	0
	flattened	Derived	3.6	6	6	0	0
	round	Ancestral	94.9	0	0	8	8
Epidermis	2 cells thick	Derived	15.7	1	1	1	1
	1 cell thick	Ancestral	84.3	1	1	1	1
	lateral walls sinuouse and thickened	Derived	28.1	29	27	11	13
	lateral walls unthickened	Ancestral	71.9	11	13	29	27
	outer walls thickened	Derived	74.5	2	5	8	5
	outer walls unthickened	Ancestral	25.5	8	5	2	5
	smooth	Ancestral	38.0	32	45	33	20
	granular	Derived	0.7	1	1	0	0
	colliculate	Derived	61.3	33	20	33	46
	glabrous	Ancestral	96.4	0	0	6	6
	hairy	Derived	1.5	1	1	0	0
	papillate	Derived	5.5	11	11	0	0
Stomata	at the surface	Ancestral	89.1	5	0	14	19
	slightly sunken	Derived	5.5	11	14	3	0
	fully sunken	Derived	6.9	12	13	1	0
	guard cells on top	Ancestral	86.9	4	1	20	23
	guarde cells inside	Derived	15.3	24	27	4	1
	on tubercles	Derived	11.3	18	20	3	1
	not on tubercles	Ancestral	88.7	3	1	18	20
Chlorenchyma	cavities present	Derived	6.6	15	15	0	0
	cavities absent	Ancestral	93.4	0	0	15	15
Protective cells	shorter than chlorenchyma	Derived	7.3	15	16	2	0
	lonegr than chlorenchyma	Derived	28.8	36	31	13	18
	reaching down past the chlorenchyma	Ancestral	69.0	16	16	28	28
	cross bars present	Derived	1.1	2	2	0	0
	cross bars absent	Ancestral	98.9	0	0	2	2
Sclerenchyma	unthickened	Derived	31.8	33	40	19	12
	thickened	Ancestral	73.4	13	10	33	36
	with regular large bulges	Derived	2.6	5	5	0	0
	without regular large bulges	Ancestral	97.4	0	0	5	5
Central Ground Tissue	solid	Derived	38.7	25	29	15	11
	with scattered cavities	Derived	22.6	17	20	5	2
	with a single cavity	Ancestral	46.4	14	10	26	30
Tannin	absent	Derived	18.6	30	39	10	1
	in the epidermis	Ancestral	74.5	11	2	44	53
	in the parenchyma	Derived	10.9	13	12	4	5
	in the sclerenchyma	Derived	25.5	37	44	10	3
	in the central ground tissue	Derived	52.6	20	31	39	28
Silica sand	absent	Ancestral	70.8	31	22	30	39
	in the central ground tissue	Derived	31.4	40	56	25	9
	in the chlorenchyma	Derived	1.5	3	3	0	0
	in the parenchyma	Derived	7.3	17	19	2	0
Silica bodies	absent	Ancestral	71.9	19	8	20	31
	in the epidermis	Derived	0.4	1	1	0	0
	in the chlorenchyma	Derived	5.1	9	12	3	0
	in the parenchyma	Derived	18.6	27	30	6	3
	in the sclerenchyma	Derived	6.9	2	2	3	3
	in the central ground tissue	Derived	3.3	9	9	0	0

TABLE 2: The different habitat types, grouped into categories, with the percentage of species that occur in that habitat type. Within the water availability category, habitat types were classified as dry or wet.



Habitat Category	Habitat Types		Percentage
Bedrock type	Sandstone		86.5
	Shale		5.8
	Silcrete		7.3
	Enon		0.4
	Cavesand		0.7
	Limestone		4.4
	Acid coast		13.9
	Alkaline coast		3.6
	Basalt		0.4
Rockiness	NoRock		65.0
	Pebbles		38.7
	Bedrock		19.0
Water availability	Well-drained	dry	66.8
	Seasonally damp sandplains	dry	7.7
	Marshes	wet	16.1
	Streambanks	wet	8.8
	Convex Seeps	wet	8.0
	Valley Bottoms	wet	20.1
	Cliff Seeps	wet	6.6
Vegetation type	Fynbos		94.2
	DuneThicket		2.6
	Renosterveld		6.2
	Montane Grassland		1.8
	Karroid		0.4
	Forest		0.4
South-east clouds	Present		17.2

TABLE 4: Traits that showed significant associations with climate variables in the PIC analysis. ★ indicates that higher or lower values for presence of that anatomical trait are associated with arid conditions, while ◆ indicates that they are associated with mesic conditions. ⊙ indicates that the environmnetal variable, whether higher or lower, is not indicative of arid or mesic conditions. All correlations were tested at the alpha = 0.05 level of significance.

Tissue	Trait	Apan min	Apan max	Continentality min	Continentality max	Temperature min	Temperature max	MAP min	MAP max	Altitude min	Altitude max	SUM arid ★	SUM mesic ◆
Culm shape	Flat						◆					0	1
Epidermis	Lateral wall thickened		★		★	⊙	★		★			4	0
	Outer wall thickened				★				★		⊙	2	0
	Papillate	★	◆			⊙						1	1
Stomata	Slightly sunken	★			★	⊙	◆				⊙	2	1
	On Tubercles					⊙				⊙		0	0
Sclerenchyma	Thin	◆			◆				◆			0	3
Central ground tissue	Scattered cavities		★	★		⊙	★	★		⊙		4	0
	Solid	◆		◆	◆	⊙		◆	◆	⊙	⊙	0	5
												13	11

TABLE 5: Phylogenetically independent contrast analysis results indicating whether the trait or gas exchange variable is higher (up arrow) or lower (down arrow) when the particular anatomical trait is present (i.e. gain in the trait). Asterisks indicate significant associations (alpha = 0.05).

	Culm shape hemispherical	Culm solid	Lateral walls thickened	Outer walls thickened	Sclerenchyma unthickened	Stomata slightly sunken	Protective cells longer than chlorenchyma
Diameter (cm)	↓ *	↑ *	↓	↓ *	↑	↑	↑ *
SCA ($m^2 kg^{-1}$)	↓	↓	↓	↓	↑	↓	↓ *
Stomatal density (no. per mm^2)	↑	↑	↑	↑	↑ *	↓ *	↓
Photosynthetic rate (mmol m-2 s-1)	↓ *	↓	↑	↑	↓	↓	↓ *
Transpiration rate (mmol m-2 s-1)	↑	↓ *	↑	↑ *	↓	↓	↓
Water Use Efficiency	↓	↑ *	↑	↓ *	↑ *	↑	↑
Culm temperature (˚C)	↓ *	↑	↓	↓	↑	↑	↑

SUPPLEMENTARY INFORMATION

S1. Significant ahistorical habitat association for the 34 derived anatomical characters using Fisher's Exact Test

Anatomy	Feature	Habitat Associations		
Culm shape	hemispherical	Streambank	Valley bottom	
	square			
	flattened	Marshes	Streambank	SE Clouds
Epidermis	2 cells thick	Acid coast	Dune thicket	
	lateral walls sinuouse and thickened	Pebbles	Well-drained	
	outer walls thickened	Silcrete	Bedrock	Well-drained
	granular			
	colliculate			
	hairy			
	papillate	Silcrete	Montane grassland	
Stomata	slightly sunken	Montane grassland		
	fully sunken			
	guarde cells inside	Sandstone	Pebbles	Fynbos
	on tubercles	Convex seep		
Chlorenchyma	cavities present			
Protective cells	shorter than chlorenchyma	Marshes	Dune thicket	
	longer than chlorenchyma	SE Clouds		
	cross bars present	SE Clouds		
Sclerenchyma	unthickened	Convex seep		
	with regular large bulges			
Central Ground Tissue	solid			
	with scattered cavities	Marshes	Streambank	Cliff seep
Tannin	absent	Sandstone	Suurvlakte	Convex seep
	in the parenchyma	Pebbles	SE Clouds	
	in the sclerenchyma	Limestone	Well-drained	Dune thicket
	in the central ground tissue	Well-drained	Renosterveld	
	in the central ground tissue			
	in the chlorenchyma	Renosterveld		
	in the parenchyma	Marshes	SE Clouds	
	in the epidermis	Limestone	Well-drained	
	in the chlorenchyma			
	in the parenchyma	SE Clouds		
	in the sclerenchyma	Bedrock	Renosterveld	
	in the central ground tissue			

S2. Table showing only habitats in which there occurred a significant (alpha <0.05) shift (concurrent or contingent) for each anatomical character under t
resolving option. Anatomical characters are grouped according to tissue type.

Anatomy	Feature	Contingent States Test	Concurrent Shifts Test
Culm shape	hemispherical		Valley bottom
	square		Silcrete Renosterveld
	flattened		SE Clouds
Epidermis	2 cells thick		
	lateral walls sinuouse and thickened		Pebbles
	outer walls thickened		
	granular		
	colliculate	Sandstone Pebbles Bedrock Valley bottom	Bedrock
	hairy		
	papillate		Silcrete Rock absent Suurvlakte Forest
Stomata	slightly sunken		Alkaline coast Pebbles Karroid
	fully sunken		Suurvlakte Karroid
	guarde cells inside		
	on tubercles		Renosterveld
Chlorenchyma	cavities present		Alkaline coast DuneThicket
Protective cells	shorter than chlorenchyma		Basalt
	longer than chlorenchyma		
	cross bars present		
Sclerenchyma	unthickened		Rock absent
	with regular large bulges		
Central Ground Tissue	solid		
	with scattered cavities		
Tannin	absent		Acid coast Suurvlakte
	in the parenchyma		Enon Pebbles Renosterveld
	in the sclerenchyma		Renosterveld
	in the central ground tissue	Sandstone Pebbles Well-drained Renosterveld SE Clouds	
Silica Sand	in the central ground tissue		Silcrete Well-drained Suurvlakte Convex seep Valley bottom Renosterveld
	in the chlorenchyma		Marshes
	in the parenchyma		
	in the epidermis		
	in the chlorenchyma		Convex seep Forest
	in the parenchyma		Acid coast Bedrock
	in the sclerenchyma		Enon
	in the central ground tissue		Silcrete Convex seep

S3. Table showing only habitats in which there occurred a significant (alpha <0.05) shift (concurrent or contingent) for each anatomical character under the deltran resolving option. Anatomical characters are grouped according to tissue type.

Anatomy	**Feature**	**Contingent States Test**			**Concurrent Shift Test**				
Culm shape	hemispherical				Enon	Bedrock			
	square				Silcrete	Silcrete	Renosterveld		
	flattened								
Epidermis	2 cells thick								
	lateral walls sinuouse and thickened				Pebbles				
	outer walls thickened	Rock absent	Montane grassland		Montane grassland				
	granular								
	colliculate	Sandstone	Pebbles	Bedrock					
		Well-drained	Renosterveld	SE Clouds					
	hairy								
	papillate				Silcrete	Bedrock	Forest		
Stomata	slightly sunken				Limestone	Alkaline coast	Pebbles	Dune thicket	Karroid
	fully sunken				Basalt	Karroid	SE Clouds		
	guarde cells inside				Limestone	Dune thicket	Renosterveld		
	on tubercles				Renosterveld				
Chlorenchyma	cavities present				Alkaline coast	Marshes	Dune thicket		
Protective cells	shorter than chlorenchyma				Basalt				
	longer than chlorenchyma								
	cross bars present								
Sclerenchyma	unthickened								
	with regular large bulges				Marshes				
Central Ground Tissue	solid								
	with scattered cavities								
Tannin	absent				Acid coast	Suurvlakte	Convex seep	Valley bottom	
	in the parenchyma				Shale				
	in the sclerenchyma				Streambank	Valley bottom	Renosterveld		
	in the central ground tissue	Limestone	Streambank		Limestone				
Silica Sand	in the central ground tissue				Silcrete	Silcrete	Bedrock	Suurvlakte	Convex seep
					Renosterveld				
	in the chlorenchyma				Marshes				
	in the parenchyma				Shale	Silcrete	Suurvlakte	Renosterveld	
	in the epidermis				Suurvlakte				
	in the chlorenchyma				Marshes	Streambank	Forest		
	in the parenchyma								
	in the sclerenchyma				Enon				
	in the central ground tissue				Silcrete	Convex seep			

S4. Table showing only habitats in which there occurred a significant (alpha <0.05) Contingent Reversal for each anatomical character under acctran and deltran resolving options. Anatomical characters are grouped according to tissue type.

Anatomy	**Feature**	**Acctran**			**Deltran**			
Culm shape	hemispherical	Silcrete	Renosterveld					
	square							
	flattened							
Epidermis	2 cells thick							
	lateral walls sinuouse and thickene	Silcrete	Acid coast	Renosterveld	Silcrete	Acid coast	Renosterveld	
	outer walls thickened				Forest			
	granular							
	colliculate	Streambank	SE Clouds		Bedrock	Streambank	CliffSeep	
	hairy							
	papillate							
Stomata	slightly sunken							
	fully sunken	Silcrete	Montane grassland					
	guarde cells inside	Acid coast	Dune thicket					
	on tubercles	Suurvlakte			Suurvlakte			
Chlorenchym	cavities present							
Protective cell	shorter than chlorenchyma							
	longer than chlorenchyma	Marshes			Shale	Acid coast	Valley bottom	Dune thicket
	cross bars present							
Sclerenchyma	unthickened							
	with regular large bulges							
Central Groun Tissue	solid	Convex seep	Valley bottom	Forest	Pebbles	Valley bottom		
	with scattered cavities							
Tannin	absent				Suurvlakte			
	in the parenchyma							
	in the sclerenchyma							
	in the central ground tissue	Streambank			Pebbles			
Silica Sand	in the central ground tissue	Convex seep			Pebbles	Suurvlakte	Streambank	Convex seep
	in the chlorenchyma							
	in the parenchyma	Marshes						
	in the epidermis							
	in the chlorenchyma							
	in the parenchyma							
	in the sclerenchyma							
	in the central ground tissue							

APPENDIX

A1. Gas exchange measurements were taken for the following species to assess whether different anatomical structures influenced physiology. Anatomical traits in which they differed are shown.

Species	Culm Shape Hemispherical	Culm solid or with scattered cavities	Lateral epidermal wall sinuose and theikened	Outer epidermal wall thickened	Sclerenchyma thin	Stomata slightly sunken	Protective cells longer than chlorenchyma (not not reaching parenchyma)
Elegia capensis	0	1	0	0	0	0	0
Elegia cuspidata	0	1	0	0	1	0	1
Elegia fistulosa	0	0	0	0	1	0	0
Elegia tectorum	0	1	0	0	0	0	1
Restio festuciformis	0	0	0	1	0	0	0
Restio multiflorus	0	0	0	1	0	0	0
Restio paniculatus	1	0	0	1	0	0	0
Restio quadratus	1	1	0	1	0	0	1
Restio similis	0	0	1	1	0	0	0
Restio subverticellatus	0	0	0	1	0	0	1
Restio tetragonus	0	0	0	1	0	1	1
Rhodocoma capensis	0	1	0	1	0	0	0
Rhodocoma foliosa	0	1	0	1	0	0	0
Thamnochortus fraternus	0	1	1	1	1	0	0
Thamnochortus insignis	0	1	1	1	0	0	0
Thamnochortus spicigerus	0	1	1	1	1	1	0

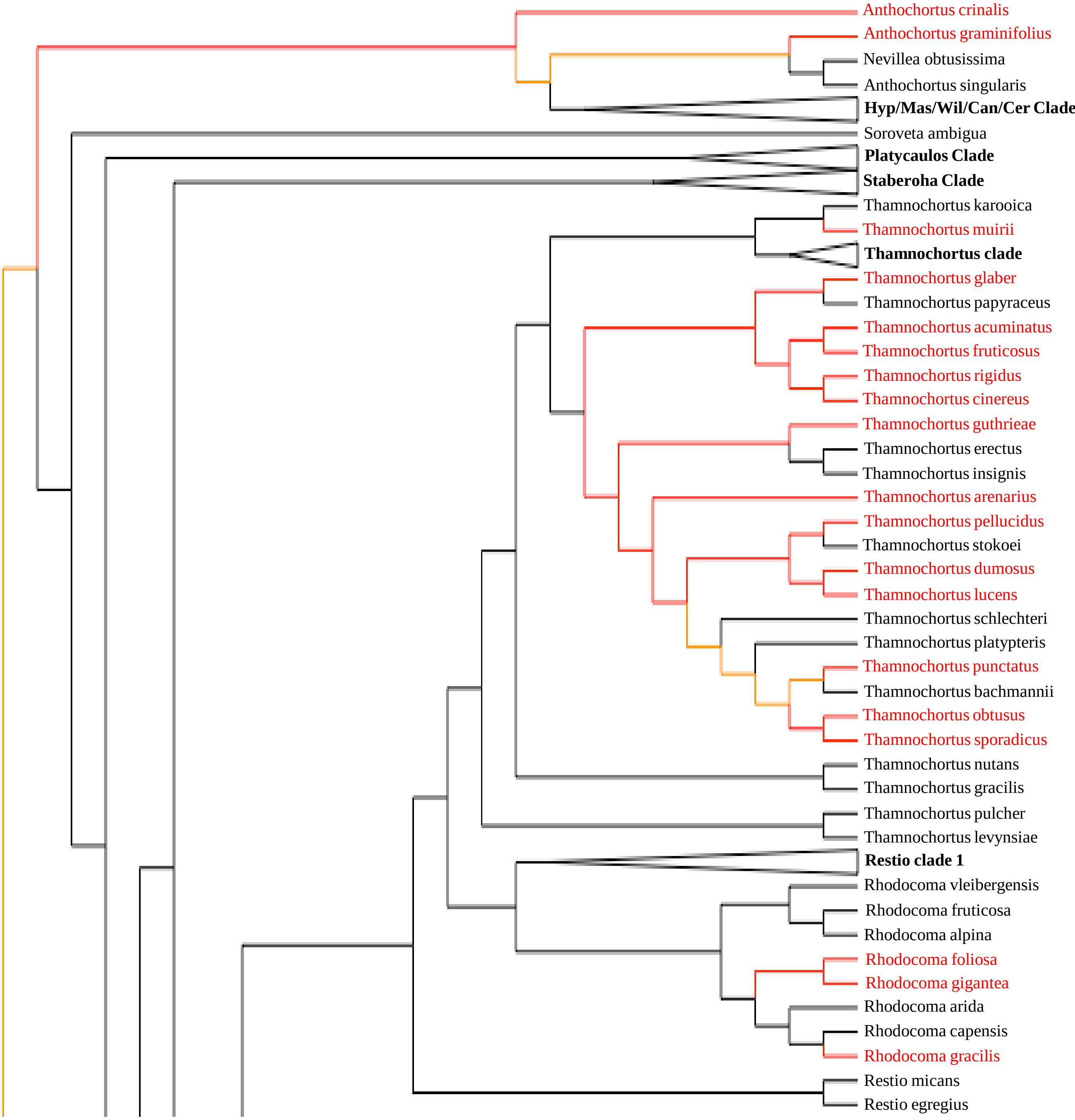

Anthochortus crinalis
Anthochortus graminifolius
Nevillea obtusissima
Anthochortus singularis
Hyp/Mas/Wil/Can/Cer Clade
Soroveta ambigua
Platycaulos Clade
Staberoha Clade
Thamnochortus karooica
Thamnochortus muirii
Thamnochortus clade
Thamnochortus glaber
Thamnochortus papyraceus
Thamnochortus acuminatus
Thamnochortus fruticosus
Thamnochortus rigidus
Thamnochortus cinereus
Thamnochortus guthrieae
Thamnochortus erectus
Thamnochortus insignis
Thamnochortus arenarius
Thamnochortus pellucidus
Thamnochortus stokoei
Thamnochortus dumosus
Thamnochortus lucens
Thamnochortus schlechteri
Thamnochortus platypteris
Thamnochortus punctatus
Thamnochortus bachmannii
Thamnochortus obtusus
Thamnochortus sporadicus
Thamnochortus nutans
Thamnochortus gracilis
Thamnochortus pulcher
Thamnochortus levynsiae
Restio clade 1
Rhodocoma vleibergensis
Rhodocoma fruticosa
Rhodocoma alpina
Rhodocoma foliosa
Rhodocoma gigantea
Rhodocoma arida
Rhodocoma capensis
Rhodocoma gracilis
Restio micans
Restio egregius

Figure 1

Figure 1 continued

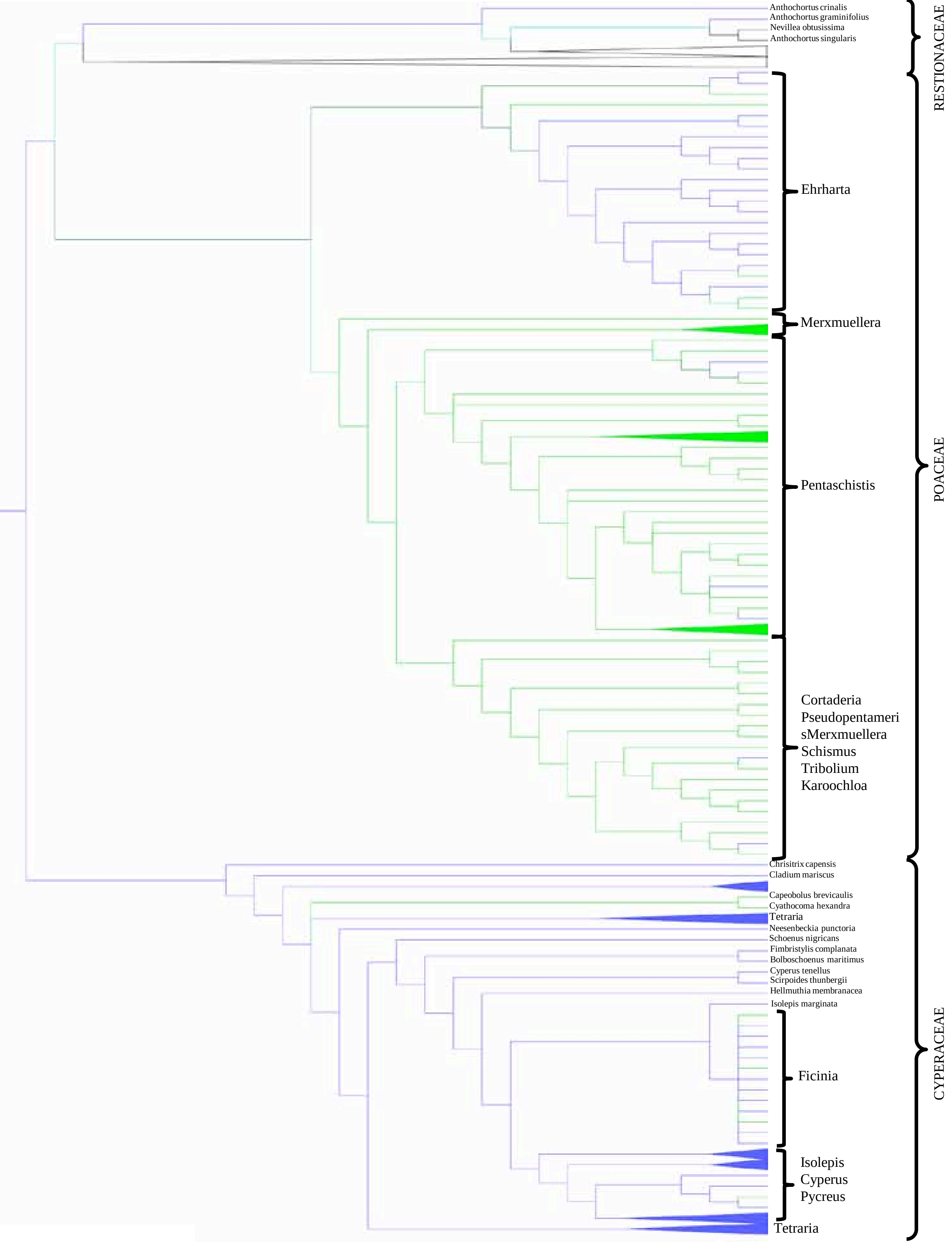

Anthochortus crinalis
Anthochortus graminifolius
Nevillea obtusissima
Anthochortus singularis
RESTIONACEAE
Ehrharta
Merxmuellera
Pentaschistis
POACEAE
Cortaderia
Pseudopentameri
sMerxmuellera
Schismus
Tribolium
Karoochloa
Chrisitrix capensis
Cladium mariscus
Capeobolus brevicaulis
Cyathocoma hexandra
Tetraria
Neesenbeckia punctoria
Schoenus nigricans
Fimbristylis complanata
Bolboschoenus maritimus
Cyperus tenellus
Scirpoides thunbergii
Hellmuthia membranacea
Isolepis marginata
Ficinia
CYPERACEAE
Isolepis
Cyperus
Pycreus
Tetraria

Figure 2

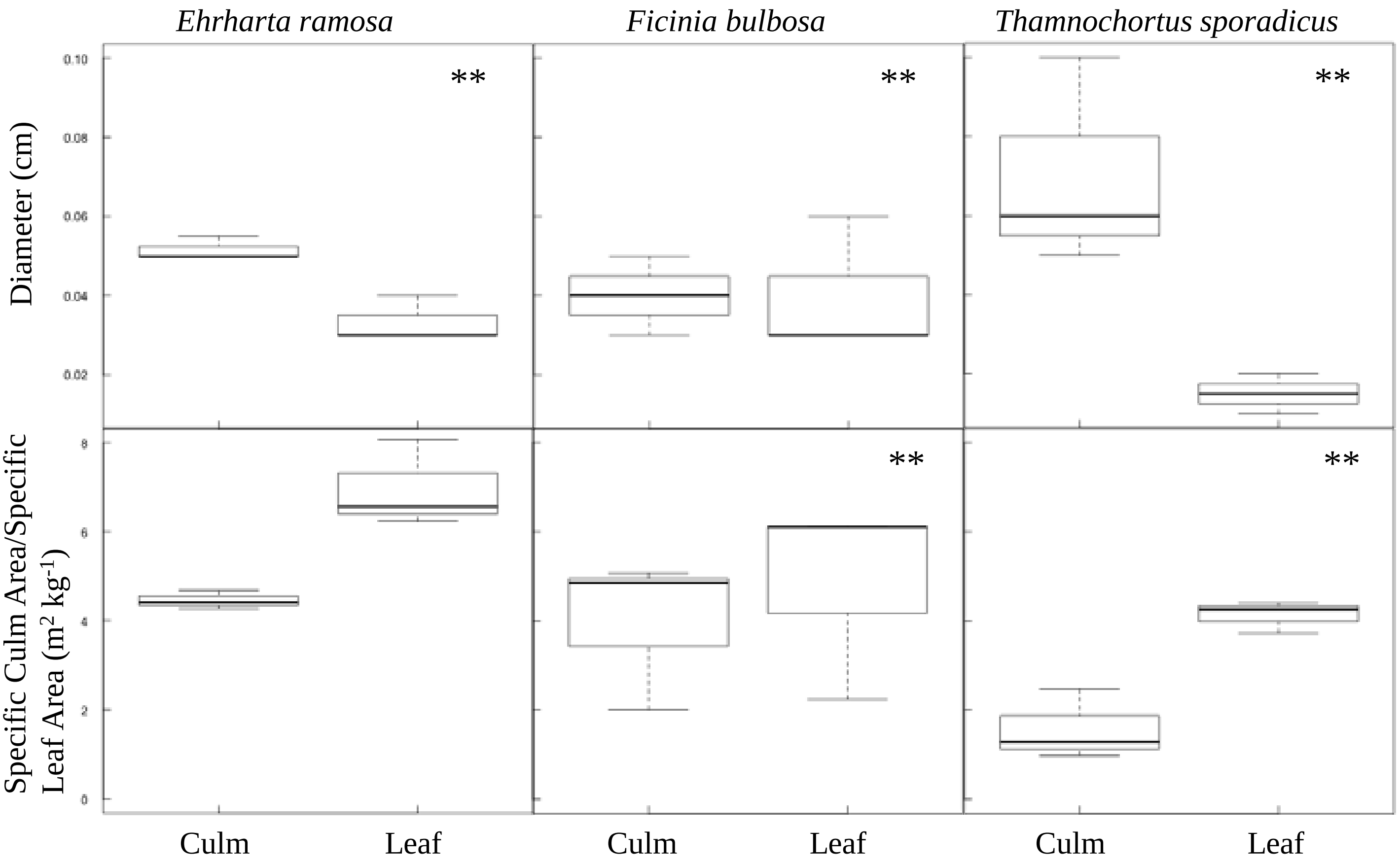
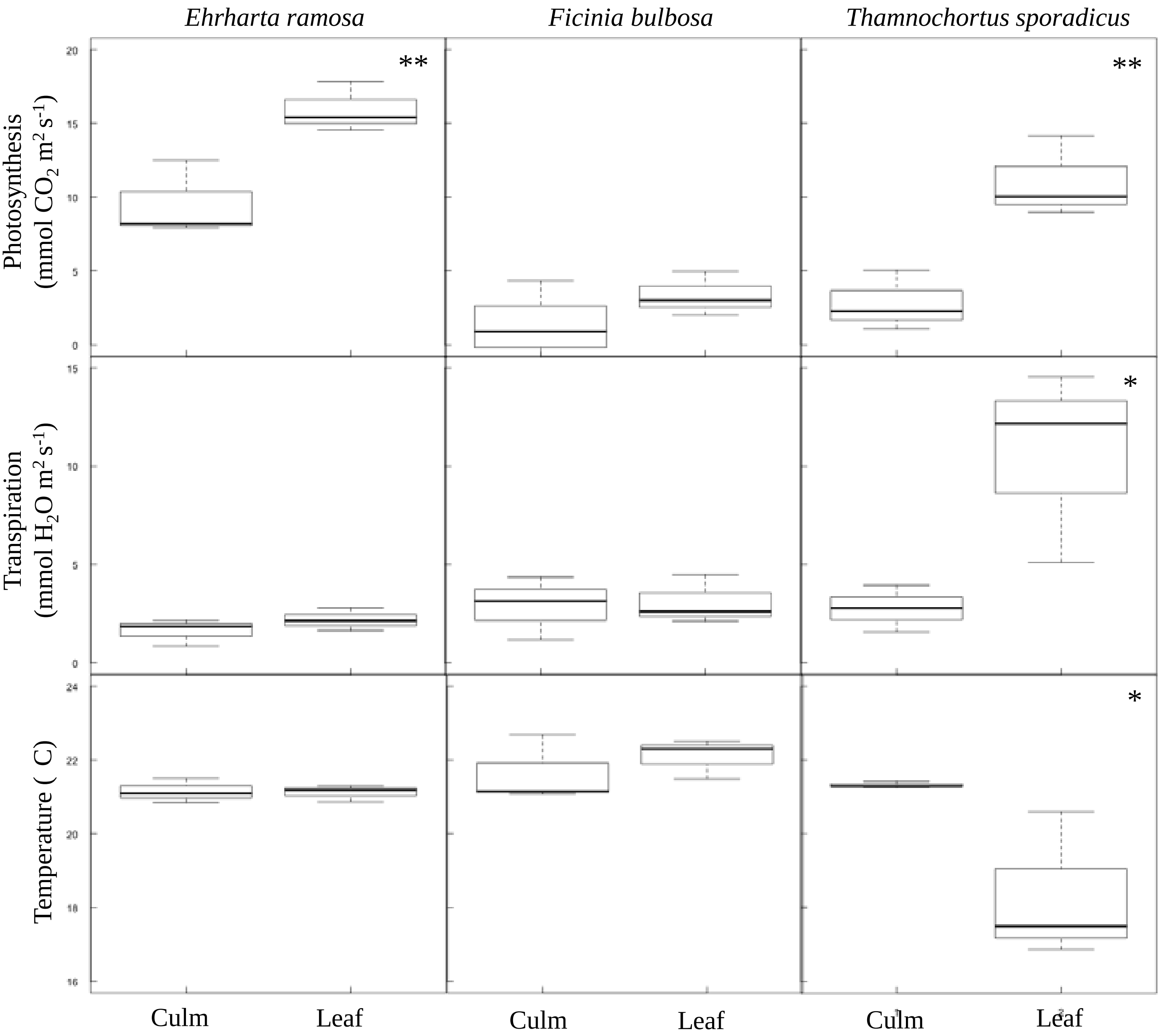

Ehrharta ramosa
Ficinia bulbosa
Thamnochortus sporadicus
Diameter (cm)
0.10
0.08
0.06
0.04
0.02
**
**
**
Specific Culm Area/Specific Leaf Area (m^2 kg^{-1})
8
6
4
2
0
**
**
Culm
Leaf
Culm
Leaf
Culm
Leaf

Figure 3



Ehrharta ramosa
Ficinia bulbosa
Thamnochortus sporadicus
Photosynthesis
(mmol CO$_2$ m^2 s^{-1})
Transpiration
(mmol H$_2$O m^2 s^{-1})
Temperature (C)
20
15
10
5
0
15
10
5
0
24
22
20
18
16
**
**
*
*
Culm
Leaf
Culm
Leaf
Culm
Leaf

Figure 4

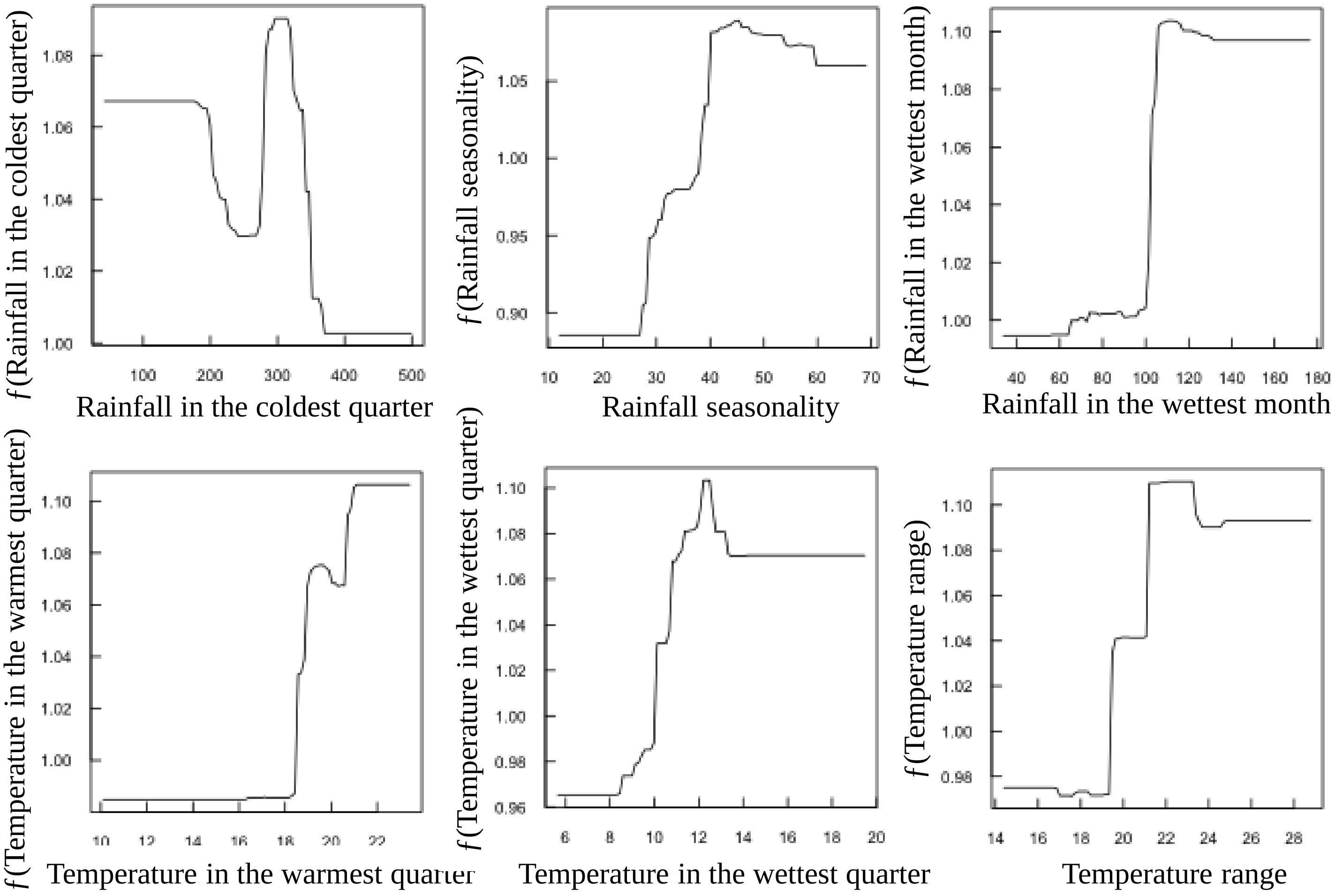
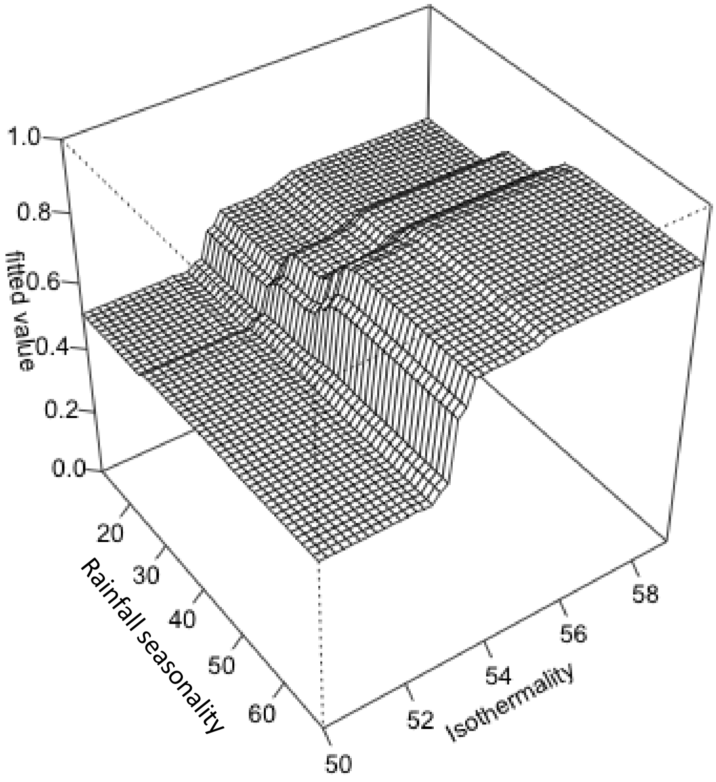
f(Rainfall in the coldest quarter)
1.08
1.06
1.04
1.02
1.00
100
200
300
400
500
Rainfall in the coldest quarter
f(Rainfall seasonality)
1.05
1.00
0.95
0.90
10
20
30
40
50
60
70
Rainfall seasonality
f(Rainfall in the wettest month)
1.10
1.08
1.06
1.04
1.02
1.00
40
60
80
100
120
140
160
180
Rainfall in the wettest month
f(Temperature in the warmest quarter)
1.10
1.08
1.06
1.04
1.02
1.00
10
12
14
16
18
20
22
Temperature in the warmest quarter
f(Temperature in the wettest quarter)
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
6
8
10
12
14
16
18
20
Temperature in the wettest quarter
f(Temperature range)
1.10
1.08
1.06
1.04
1.02
1.00
0.98
14
16
18
20
22
24
26
28
Temperature range

Figure 5


1.0
0.8
0.6
0.4
0.2
0.0
fitted value
20
30
40
50
60
Rainfall seasonality
50
52
54
56
58
Isothermality

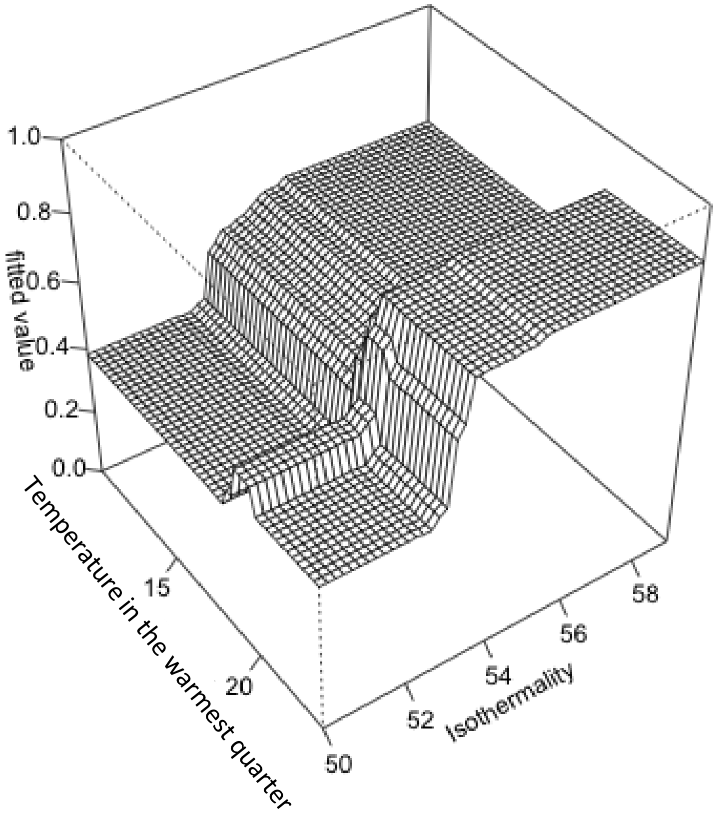

1.0
0.8
0.6
0.4
0.2
0.0
fitted value
15
20
Temperature in the warmest quarter
50
52
54
56
58
Isothermality

Figure 6

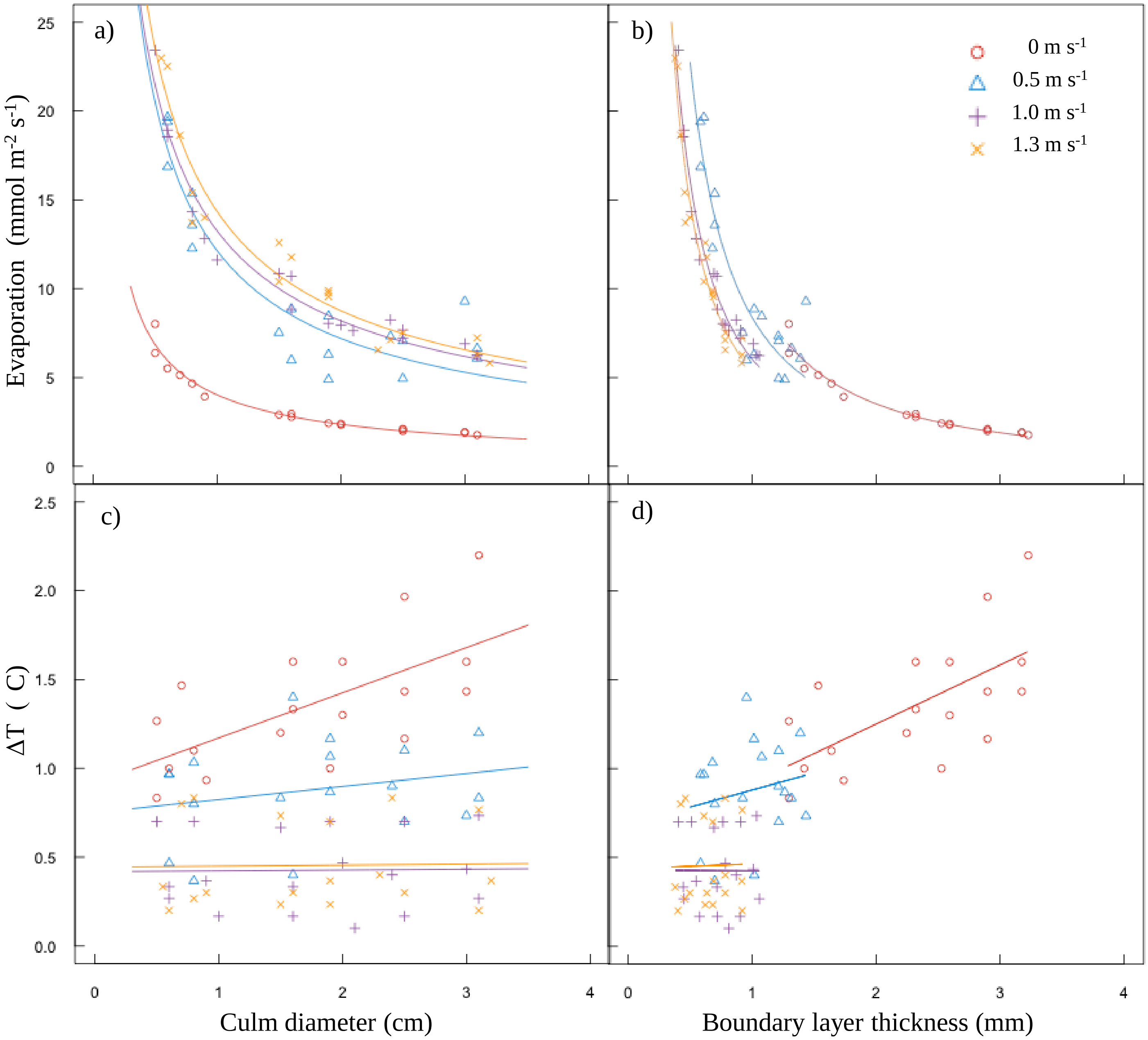

a)
b)
c)
d)
Evaporation (mmol m-2 s-1)
ΔT (C)
0 m s-1
0.5 m s-1
1.0 m s-1
1.3 m s-1
25
20
15
10
5
0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
0
1
2
3
4
Culm diameter (cm)
Boundary layer thickness (mm)

Figure 1

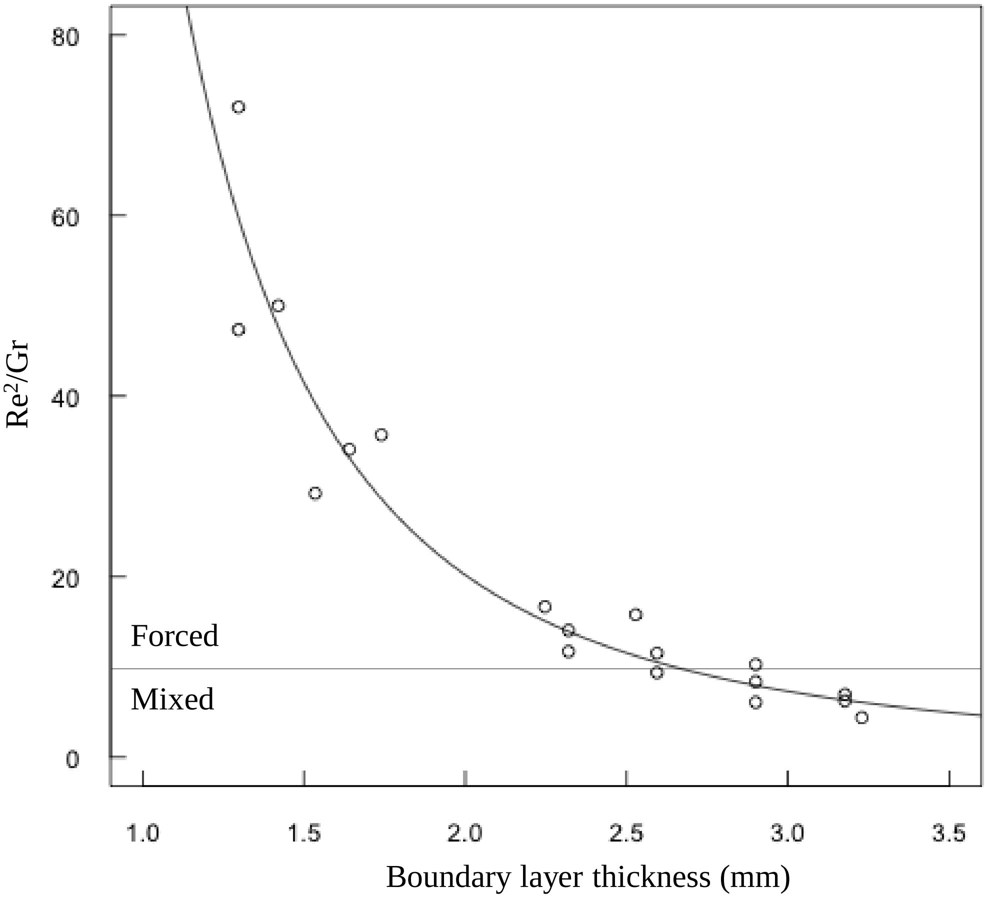

Re^2/Gr
80
60
40
20
0
Forced
Mixed
1.0
1.5
2.0
2.5
3.0
3.5
Boundary layer thickness (mm)

Figure 2

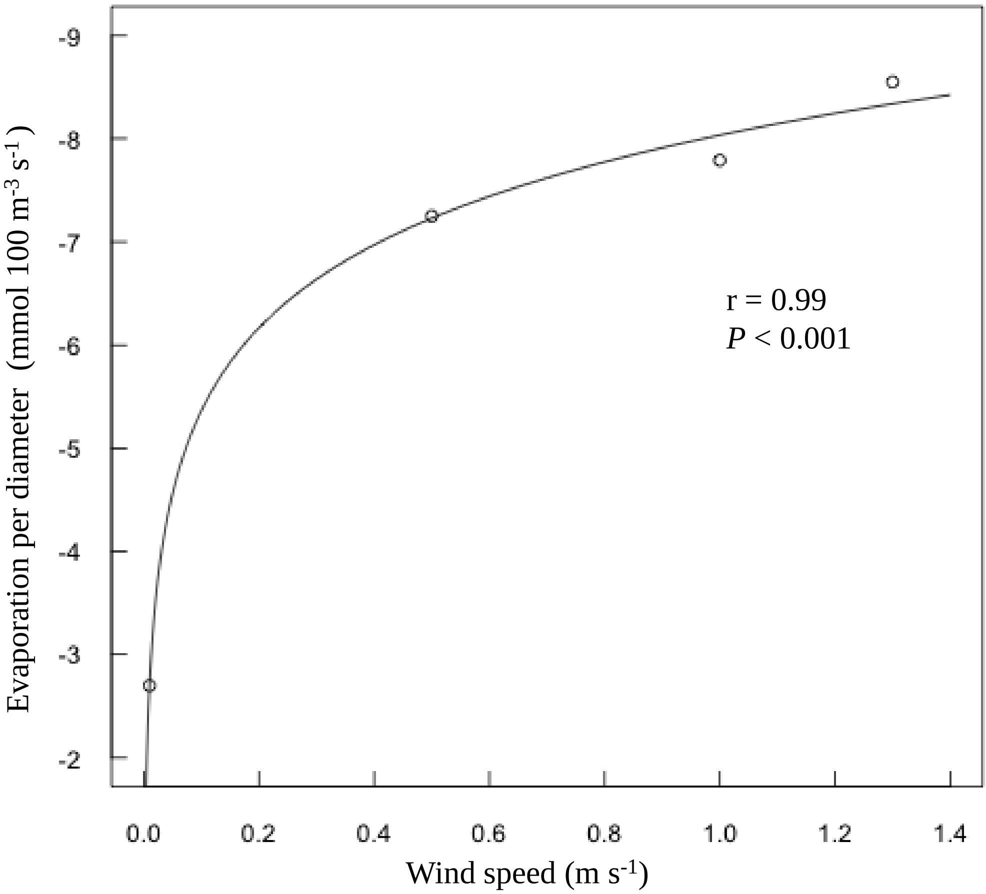

-9
-8
-7
-6
-5
-4
-3
-2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Evaporation per diameter (mmol 100 m^{-3} s^{-1})
Wind speed (m s^{-1})
r = 0.99
$P < 0.001$

Figure 3

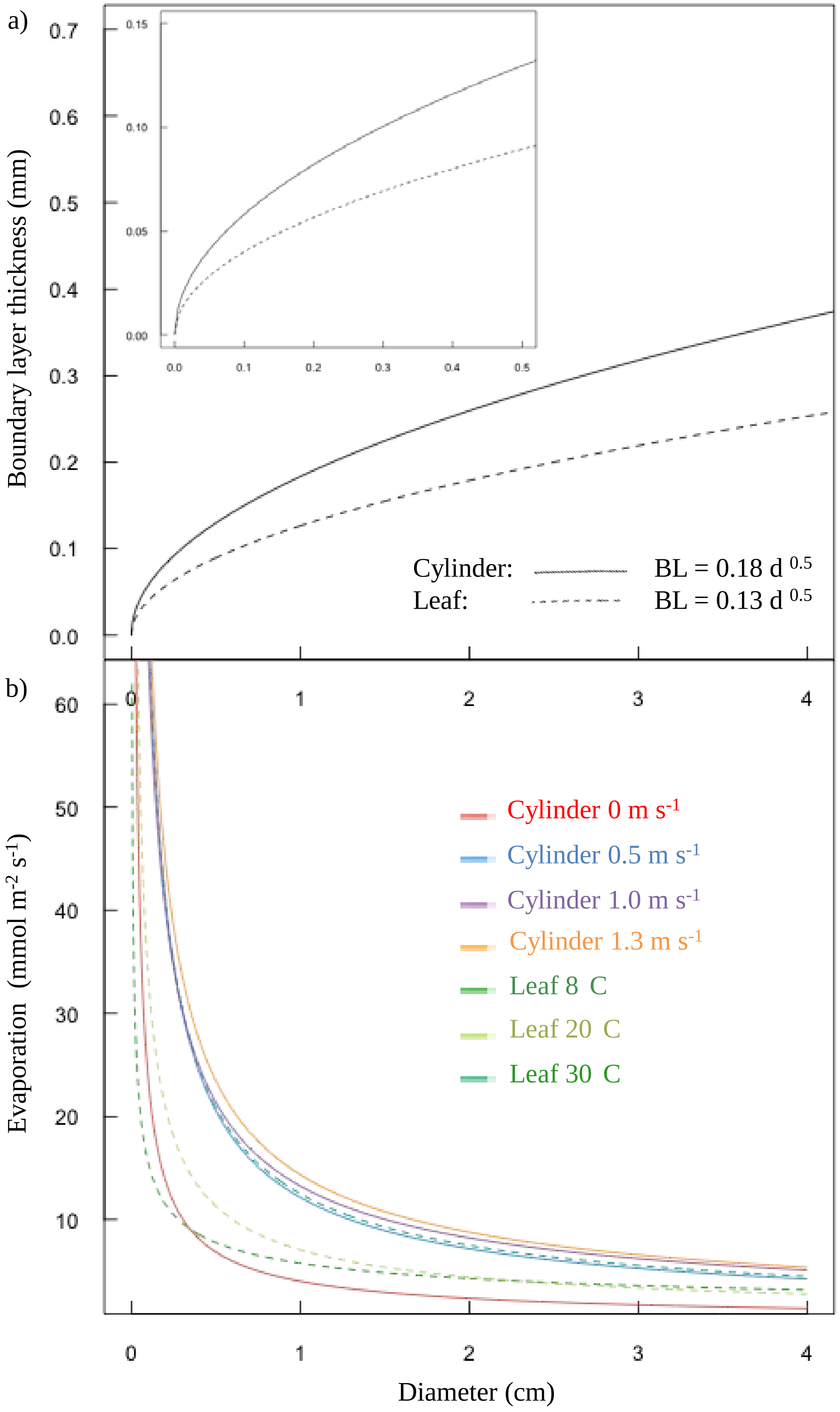

a)
Boundary layer thickness (mm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.00
0.05
0.10
0.15
0.0
0.1
0.2
0.3
0.4
0.5
Cylinder:
BL = 0.18 d $^{0.5}$
Leaf:
BL = 0.13 d $^{0.5}$
0
1
2
3
4
b)
Evaporation (mmol m^{-2} s^{-1})
10
20
30
40
50
60
Cylinder 0 m s^{-1}
Cylinder 0.5 m s^{-1}
Cylinder 1.0 m s^{-1}
Cylinder 1.3 m s^{-1}
Leaf 8 C
Leaf 20 C
Leaf 30 C
0
1
2
3
4
Diameter (cm)

Figure 4

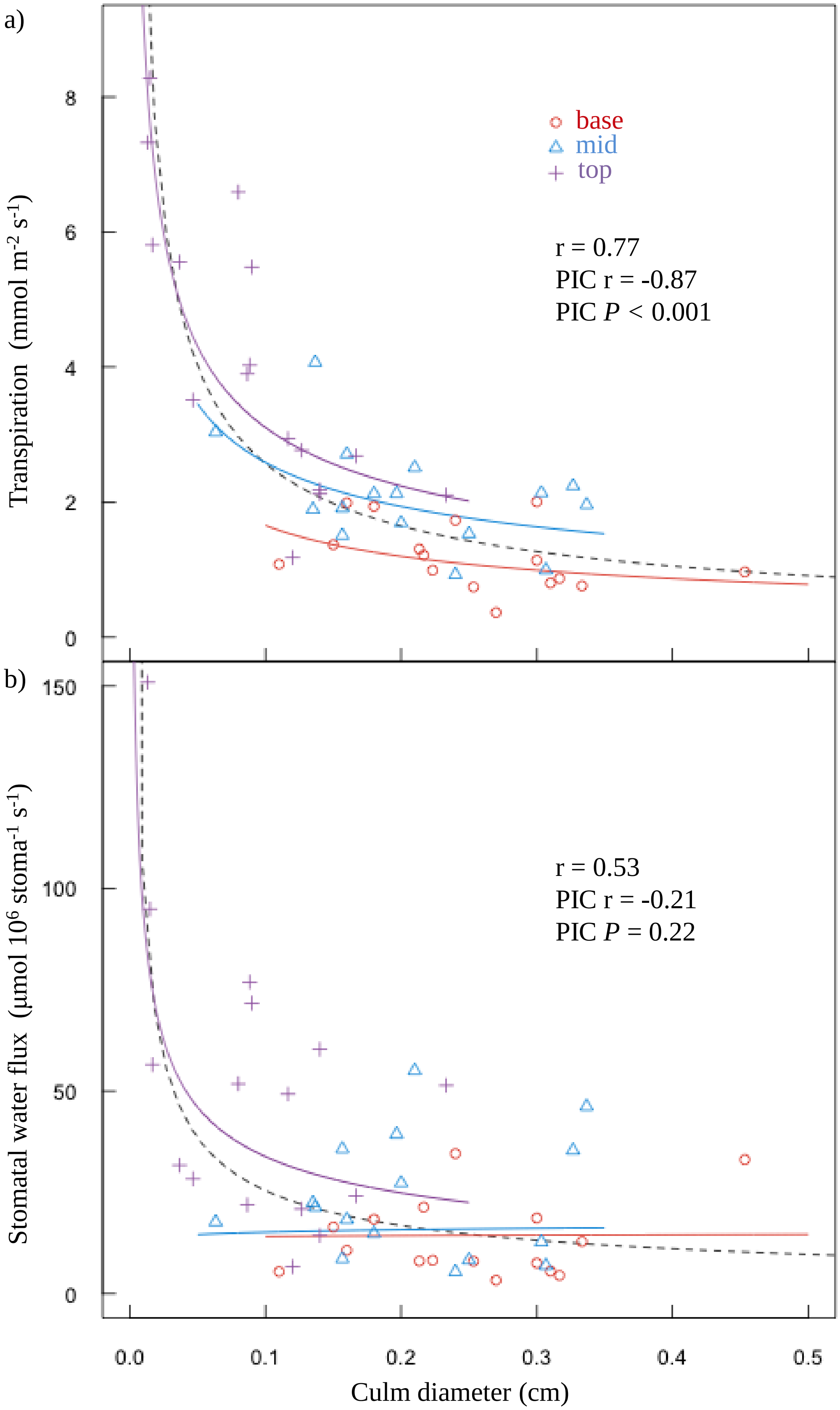

a)
base
mid
top
r = 0.77
PIC r = -0.87
PIC P < 0.001
Transpiration (mmol m^{-2} s^{-1})
0
2
4
6
8
b)
r = 0.53
PIC r = -0.21
PIC P = 0.22
Stomatal water flux (µmol 10^{6} stoma^{-1} s^{-1})
0
50
100
150
0.0
0.1
0.2
0.3
0.4
0.5
Culm diameter (cm)

Figure 5

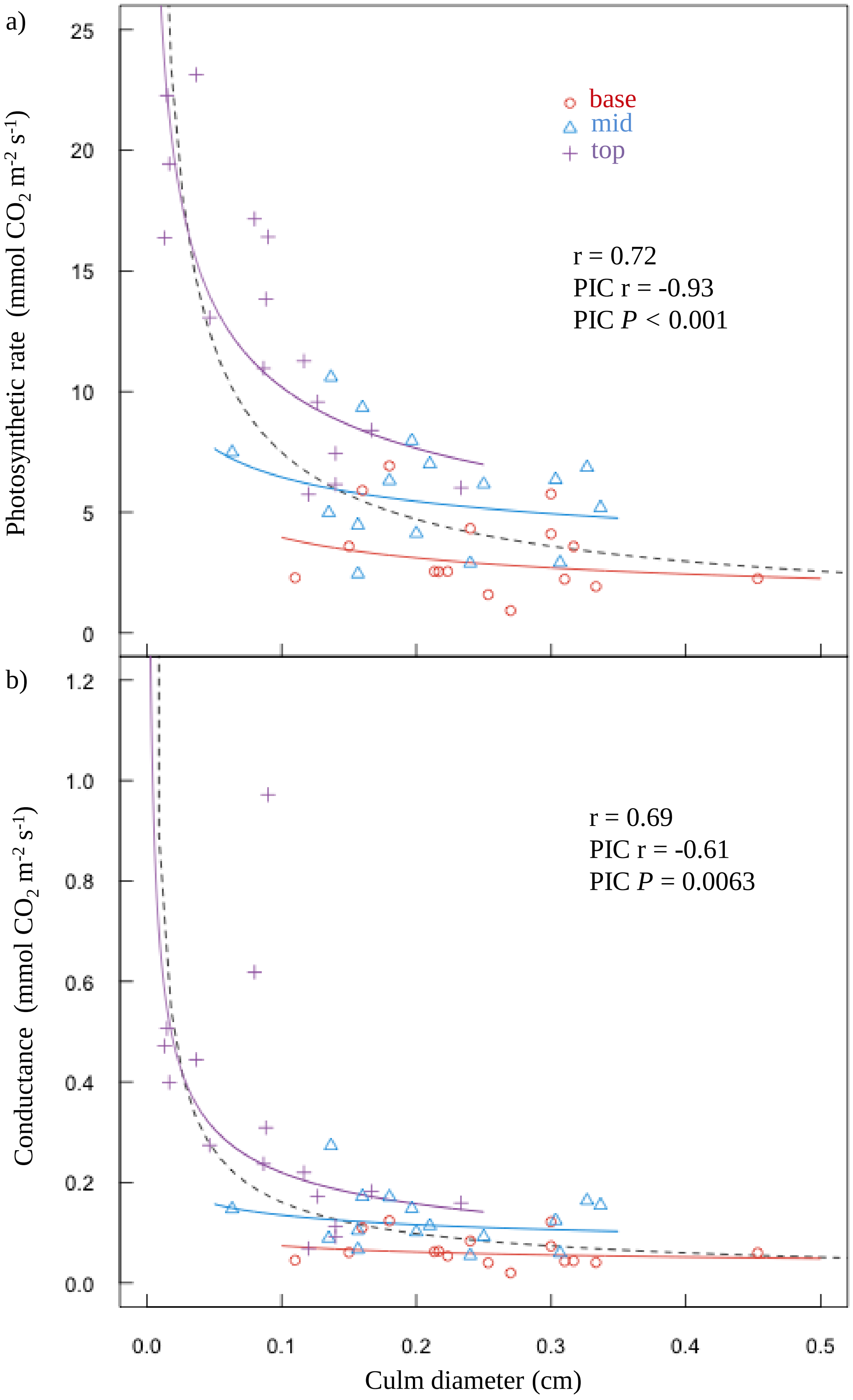

a)
Photosynthetic rate (mmol CO_2 m^{-2} s^{-1})
25
20
15
10
5
0
base
mid
top
r = 0.72
PIC r = -0.93
PIC P < 0.001
b)
Conductance (mmol CO_2 m^{-2} s^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
r = 0.69
PIC r = -0.61
PIC P = 0.0063
0.0
0.1
0.2
0.3
0.4
0.5
Culm diameter (cm)

Figure 6

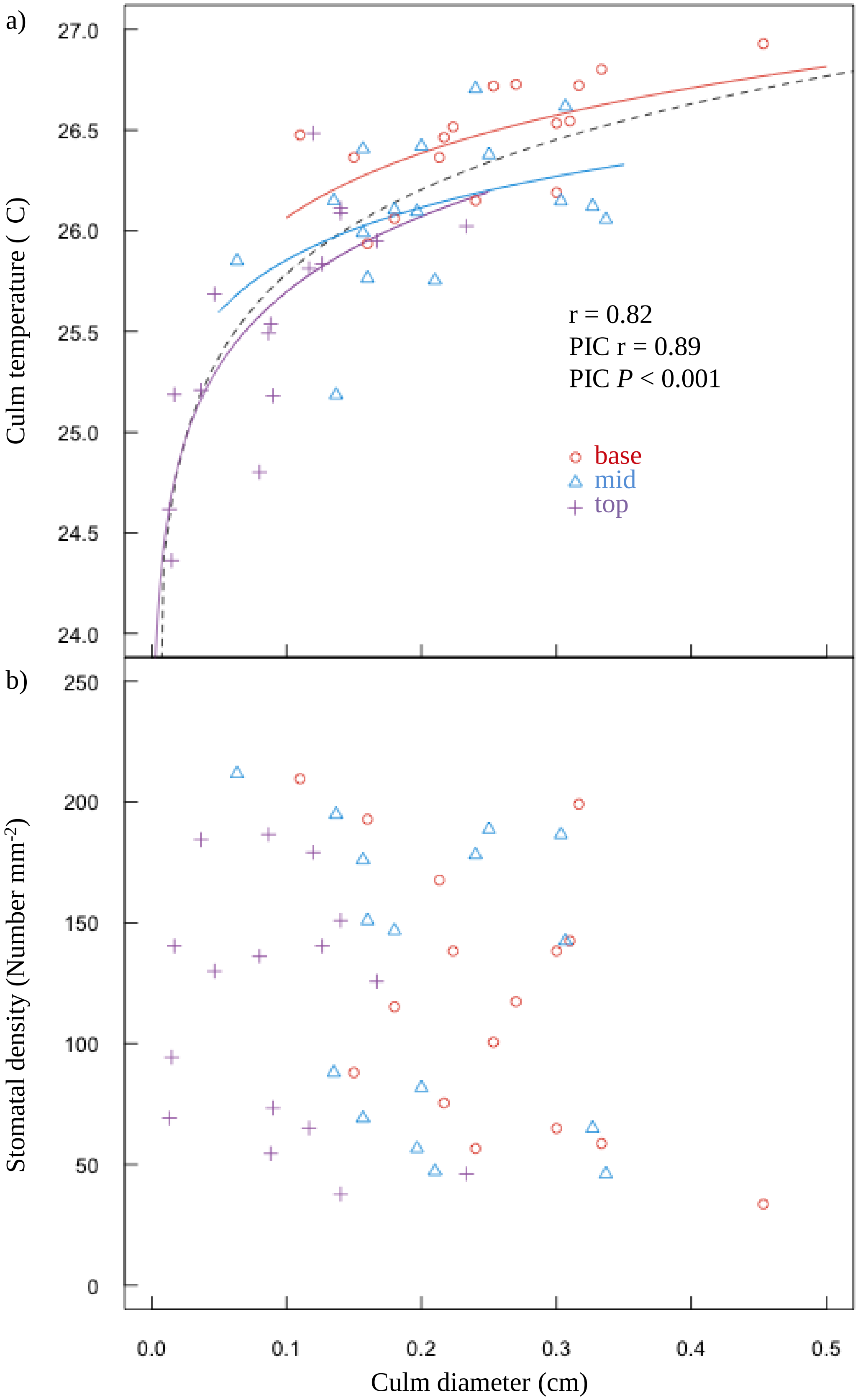

a)
27.0
26.5
26.0
25.5
25.0
24.5
24.0
Culm temperature (C)
r = 0.82
PIC r = 0.89
PIC $P < 0.001$
○ base
△ mid
+ top
b)
250
200
150
100
50
0
Stomatal density (Number mm^{-2})
0.0
0.1
0.2
0.3
0.4
0.5
Culm diameter (cm)

Figure 7

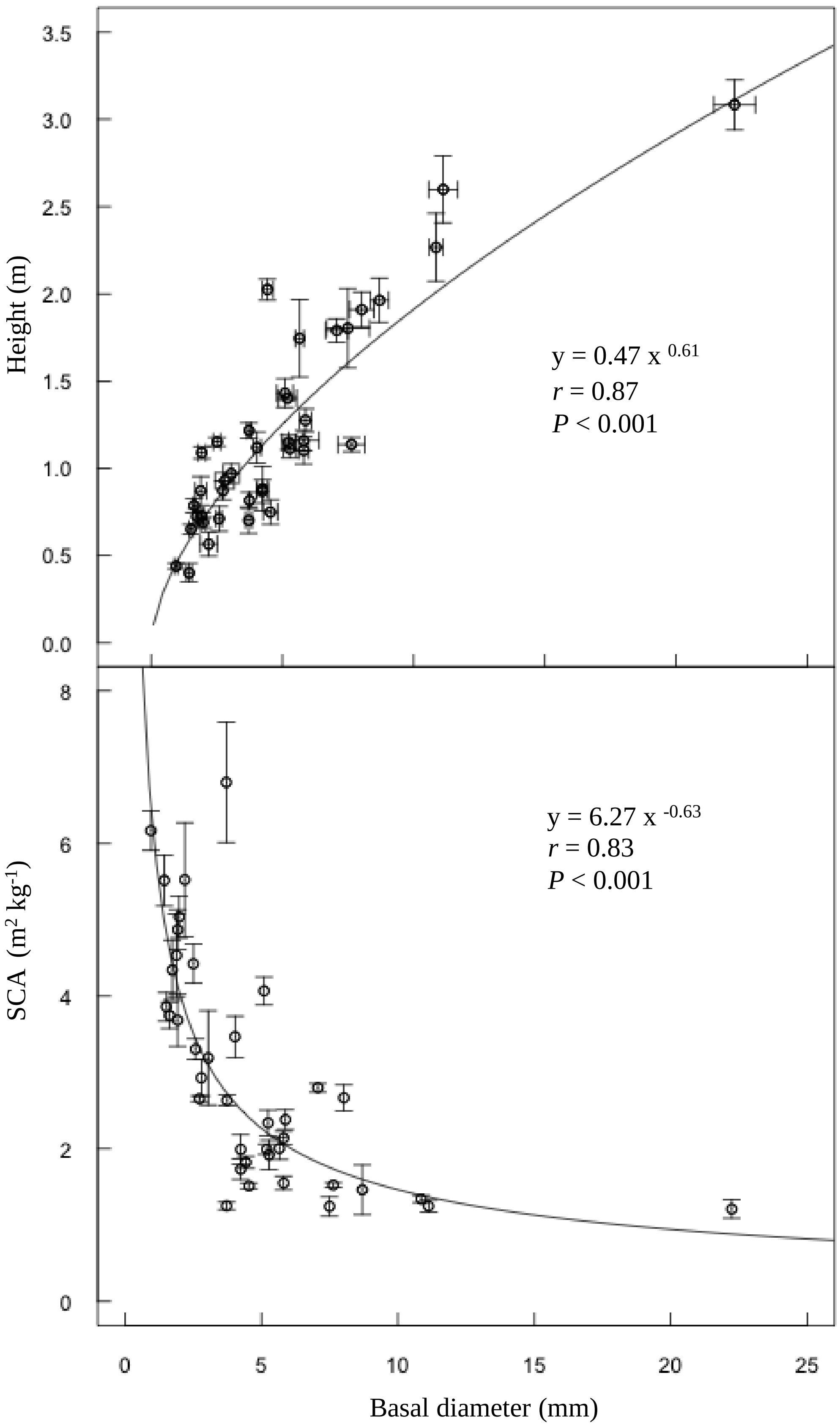

Height (m)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
y = 0.47 x 0.61
r = 0.87
P < 0.001
SCA (m2 kg-1)
8
6
4
2
0
y = 6.27 x -0.63
r = 0.83
P < 0.001
0
5
10
15
20
25
Basal diameter (mm)

Figure 8

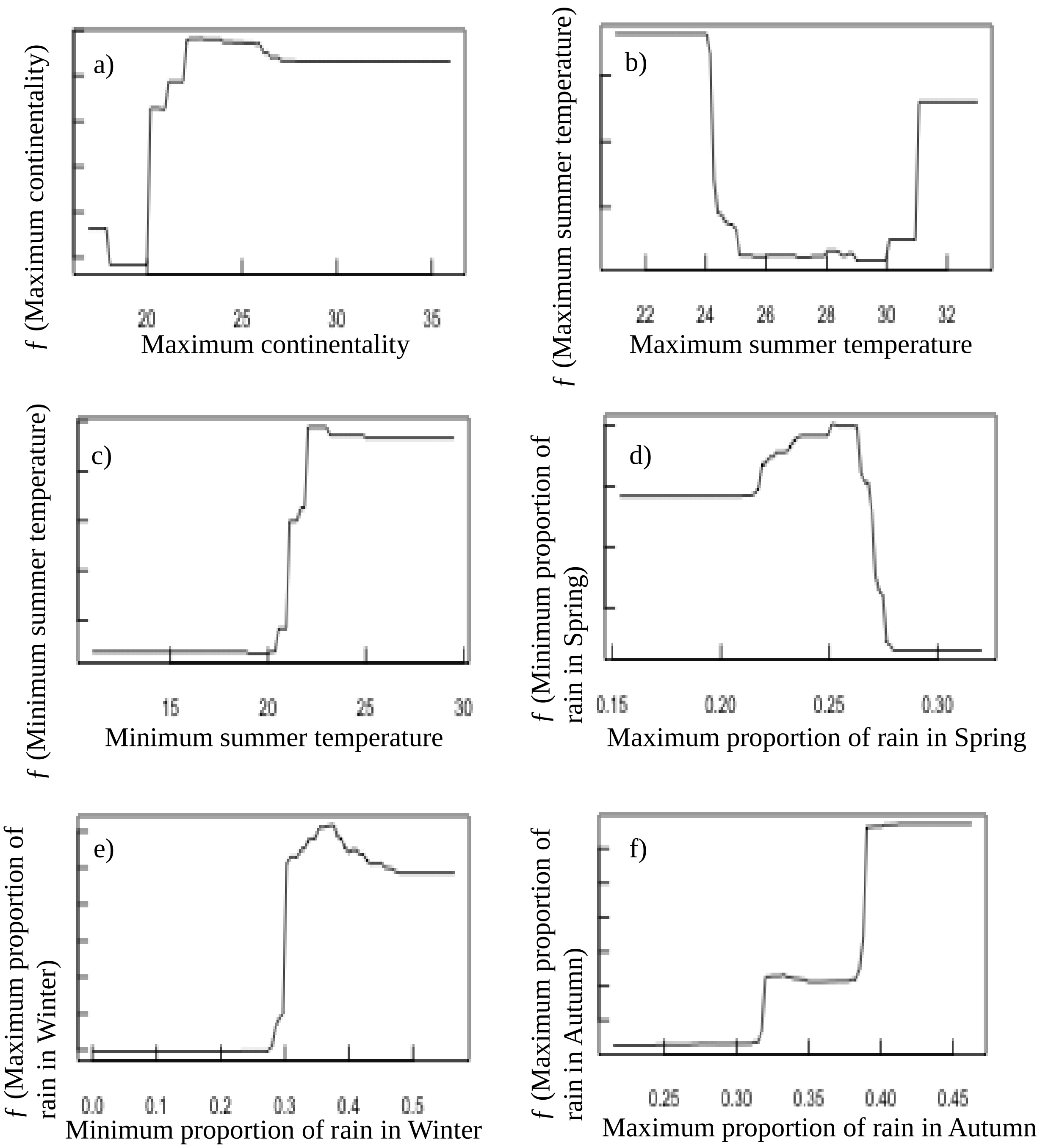

a)
f (Maximum continentality)
20
25
30
35
Maximum continentality
b)
f (Maximum summer temperature)
22
24
26
28
30
32
Maximum summer temperature
c)
f (Minimum summer temperature)
15
20
25
30
Minimum summer temperature
d)
f (Minimum proportion of rain in Spring)
0.15
0.20
0.25
0.30
Maximum proportion of rain in Spring
e)
f (Maximum proportion of rain in Winter)
0.0
0.1
0.2
0.3
0.4
0.5
Minimum proportion of rain in Winter
f)
f (Maximum proportion of rain in Autumn)
0.25
0.30
0.35
0.40
0.45
Maximum proportion of rain in Autumn

Figure 9

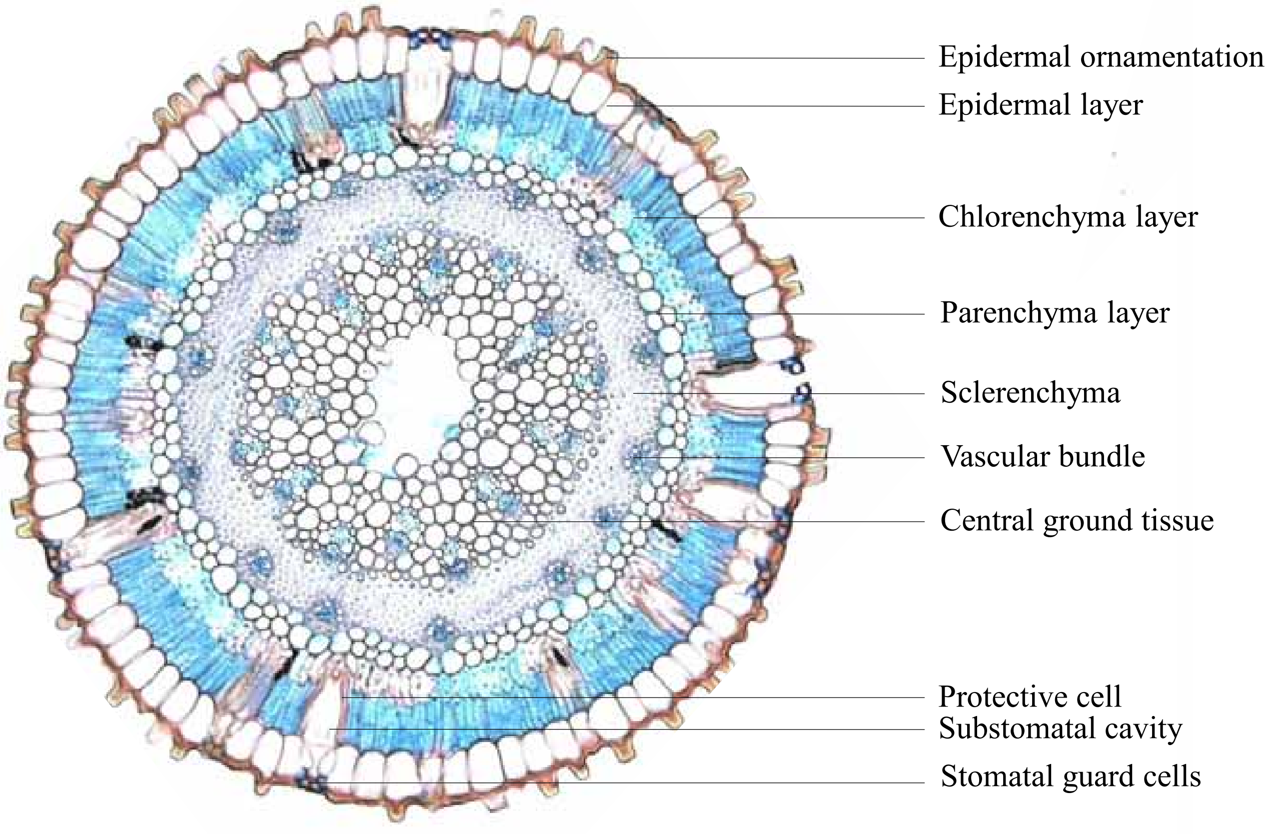

Epidermal ornamentation
Epidermal layer
Chlorenchyma layer
Parenchyma layer
Sclerenchyma
Vascular bundle
Central ground tissue
Protective cell
Substomatal cavity
Stomatal guard cells

Figure 1

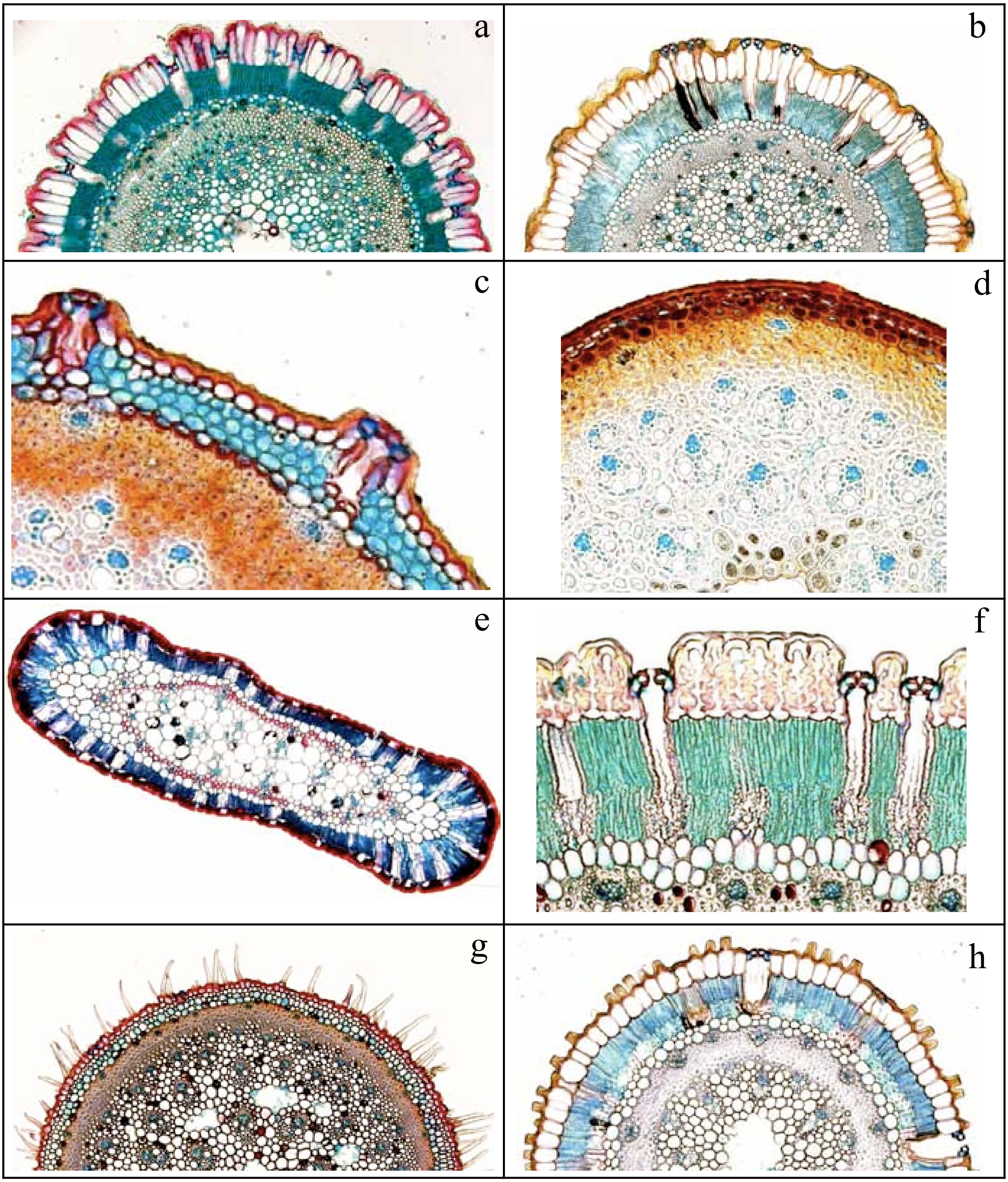


Figure 2

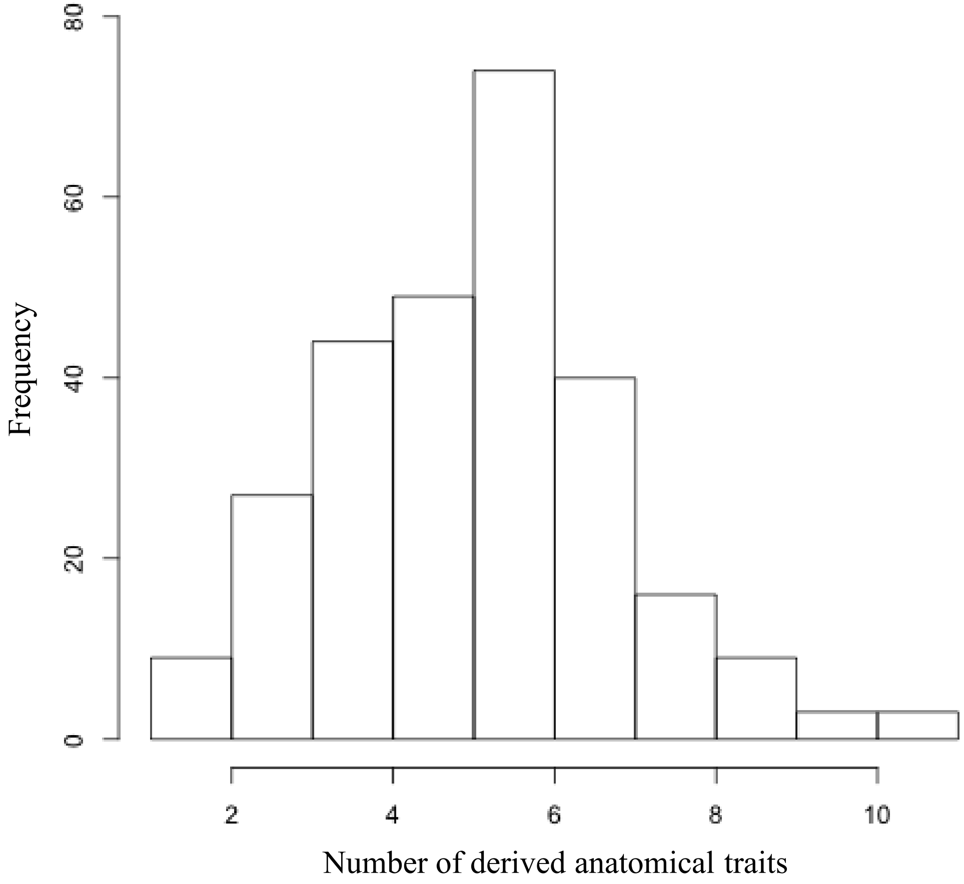
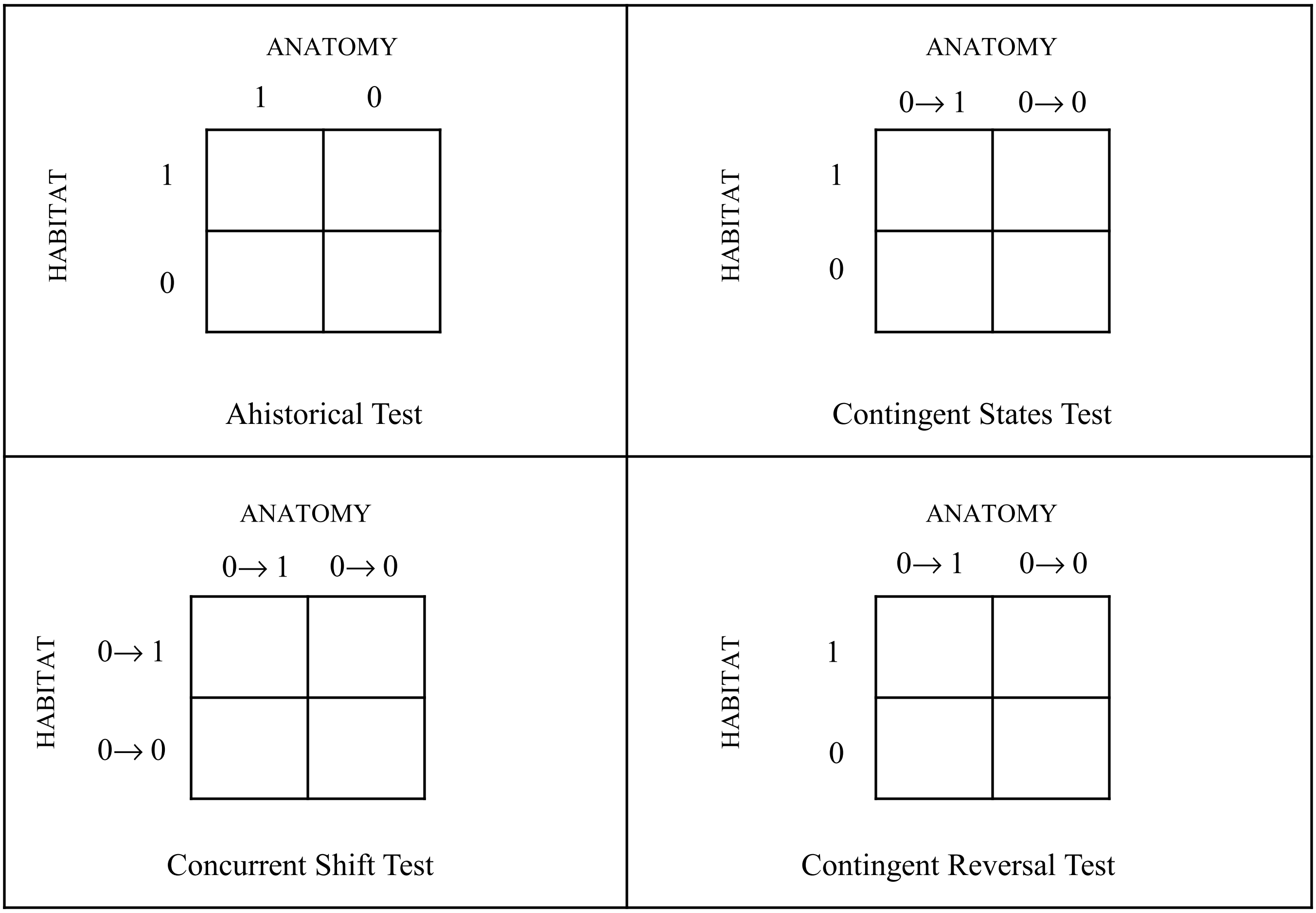

Frequency
0
20
40
60
80
2
4
6
8
10
Number of derived anatomical traits

Figure 3



Ahistorical Test

Contingent States Test

Concurrent Shift Test

Contingent Reversal Test

Figure 4

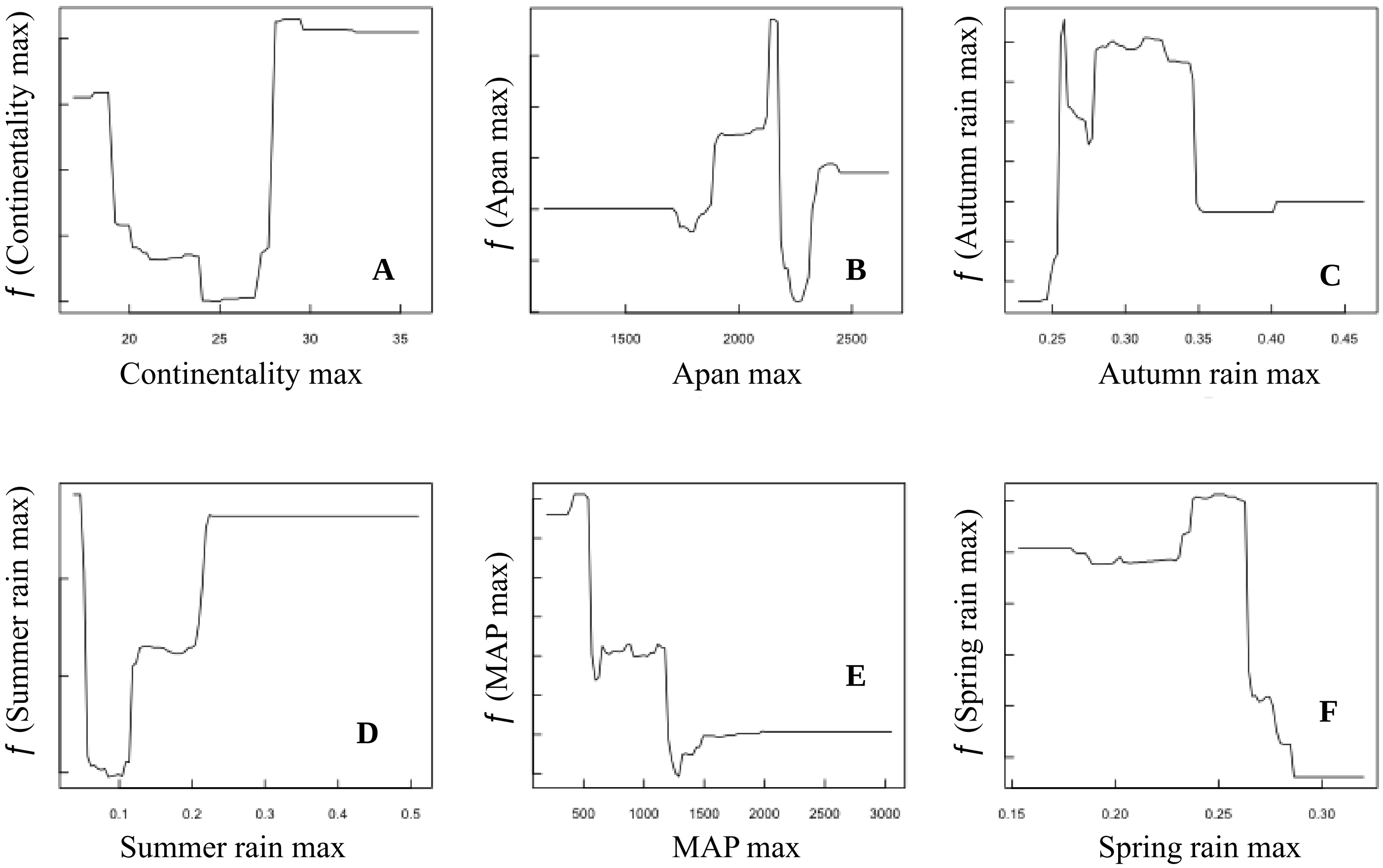

f (Continentality max)
A
20
25
30
35
Continentality max
f (Apan max)
B
1500
2000
2500
Apan max
f (Autumn rain max)
C
0.25
0.30
0.35
0.40
0.45
Autumn rain max
f (Summer rain max)
D
0.1
0.2
0.3
0.4
0.5
Summer rain max
f (MAP max)
E
500
1000
1500
2000
2500
3000
MAP max
f (Spring rain max)
F
0.15
0.20
0.25
0.30
Spring rain max

Figure 5

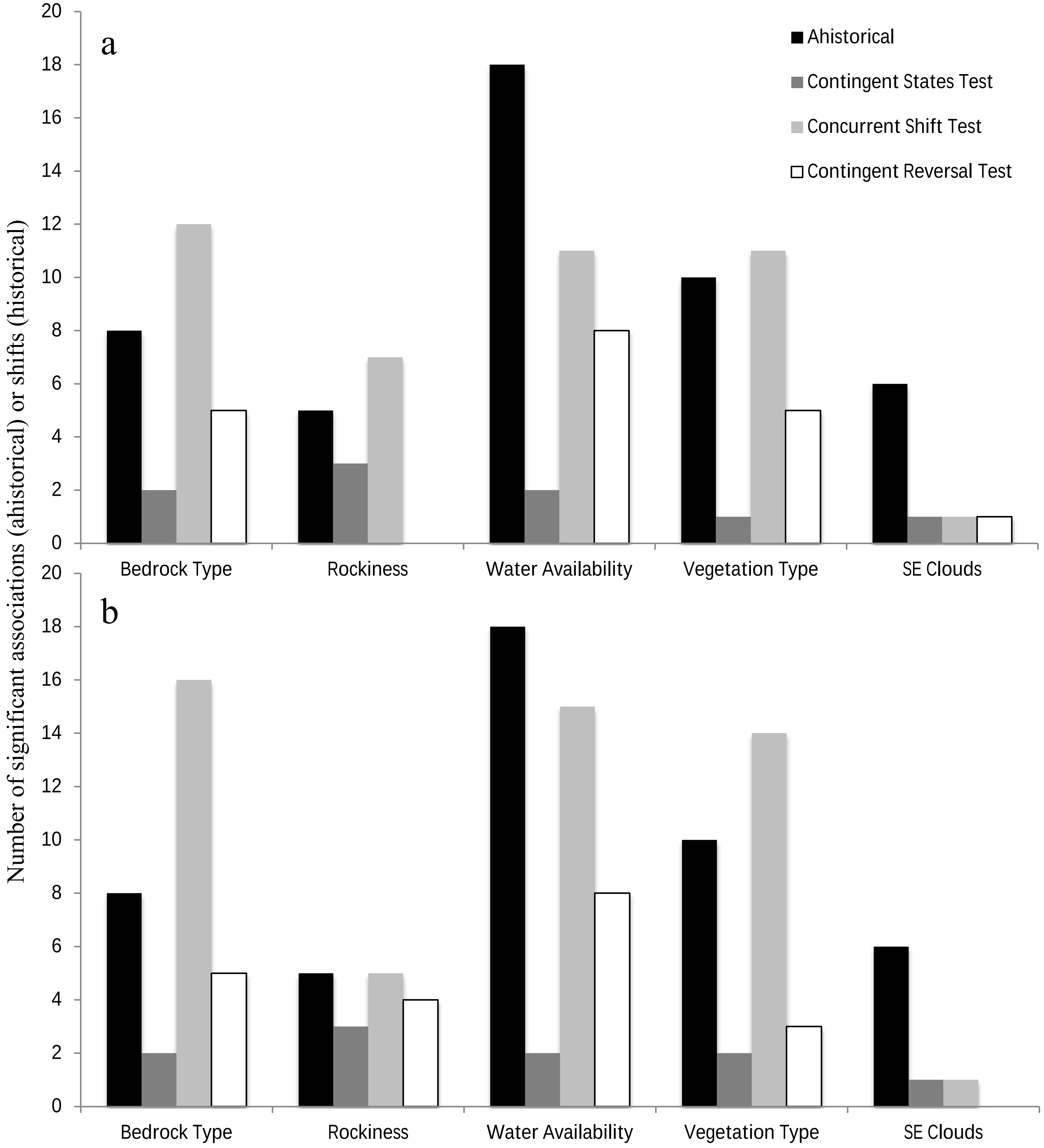

a
b
Ahistorical
Contingent States Test
Concurrent Shift Test
Contingent Reversal Test
Number of significant associations (ahistorical) or shifts (historical)
0
2
4
6
8
10
12
14
16
18
20
Bedrock Type
Rockiness
Water Availability
Vegetation Type
SE Clouds

Figure 6

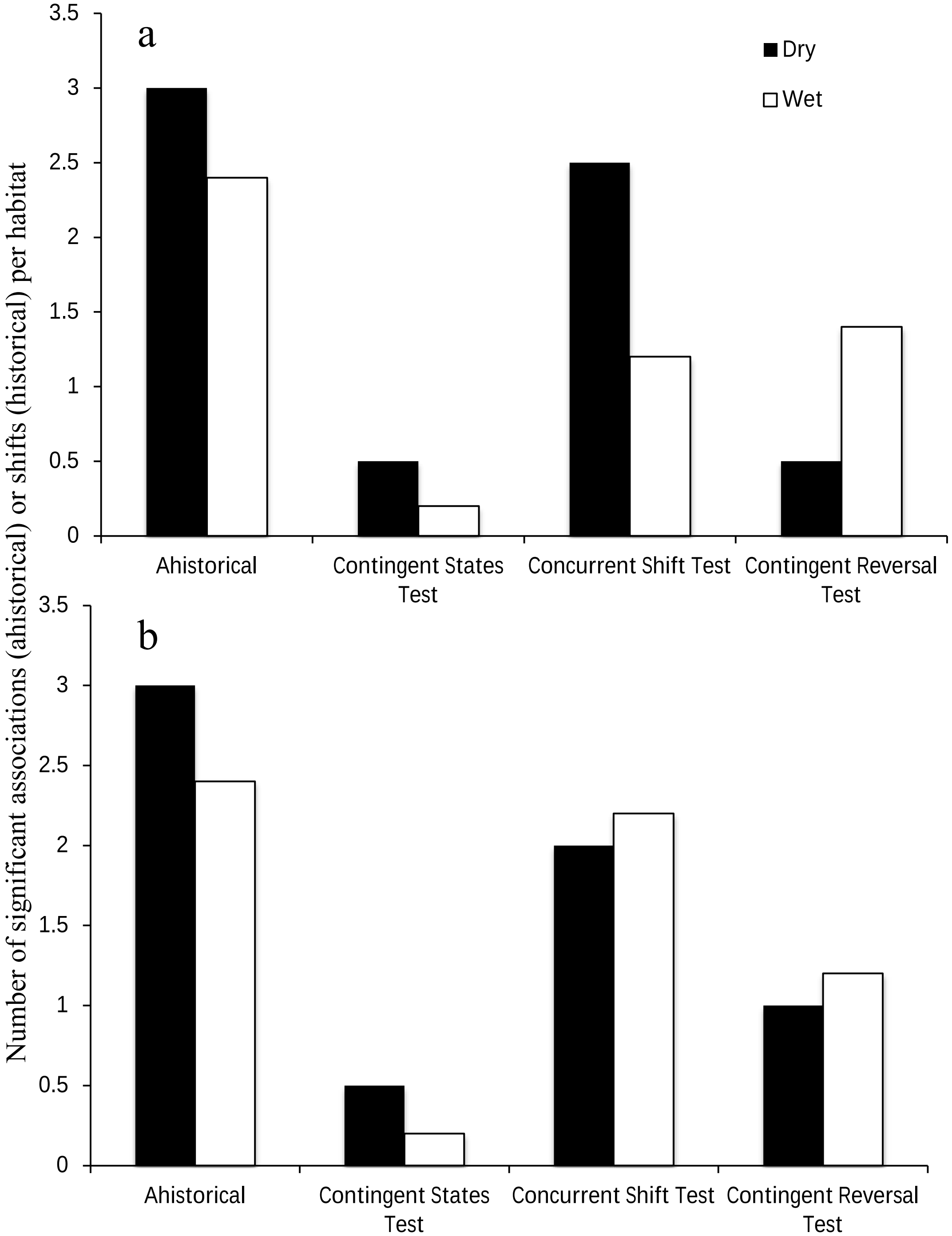
a
b
Number of significant associations (ahistorical) or shifts (historical) per habitat
Dry
Wet
3.5
3
2.5
2
1.5
1
0.5
0
Ahistorical
Contingent States Test
Concurrent Shift Test
Contingent Reversal Test

Figure 7

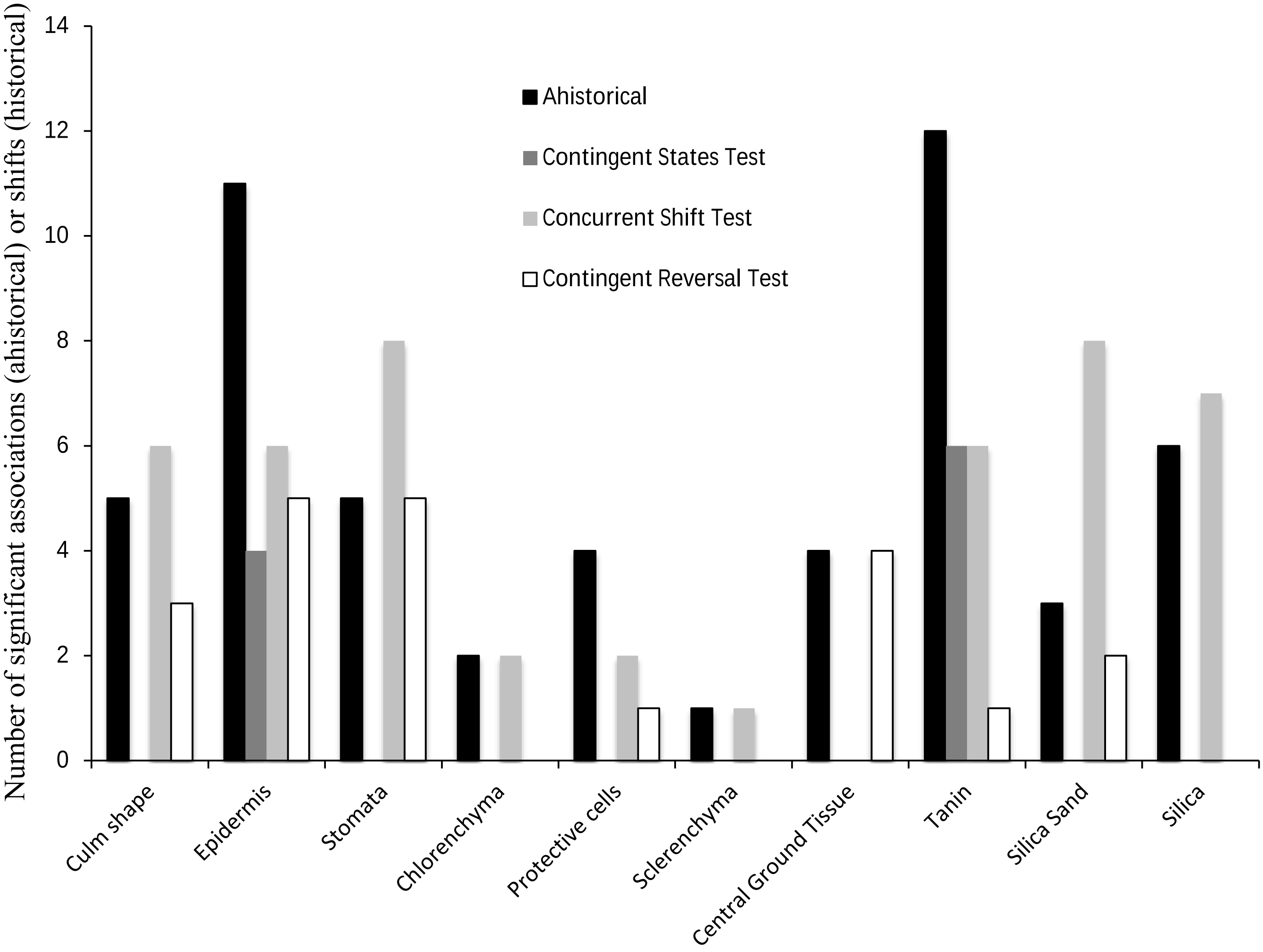

Number of significant associations (ahistorical) or shifts (historical)
14
12
10
8
6
4
2
0
■ Ahistorical
■ Contingent States Test
■ Concurrent Shift Test
□ Contingent Reversal Test
Culm shape
Epidermis
Stomata
Chlorenchyma
Protective cells
Sclerenchyma
Central Ground Tissue
Tanin
Silica Sand
Silica

Figure 8