SYSTEMATICS OF *FICINIA* CLADE (CYPERACEAE)

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DECLARATION STATEMENT

I know the meaning of plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own.

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TABLE OF CONTENT

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

CHAPTER 2: PHYLOGENETIC RELATIONSHIPS, MACROEVOLUTION AND CLASSIFICATION IN *FICINIA* CLADE

CHAPTER 3: THE ROLE OF ECOLOGY IN PROMOTING THE RADIATION OF *FICINIA*

CHAPTER 4: MORPHOMETRIC STUDY OF THE *FICINIA INDICA* COMPLEX

CHAPTER 5

List of Figures

List of Tables

Appendices

ABSTRACT

Cyperaceae is a cosmopolitan graminoid family comprising about 110 genera and over 5500 species, represented in Southern Africa by 30 genera and over 500 species. The family is an integral part of wetland and grassland vegetations, a number of species have various local uses, and several species are notorious weeds in agriculture. The *Ficinia* clade includes five genera, namely *Isolepis, Ficinia, Scirpoides, Dracoscirpoides* and *Hellmuthia.* The clade has a centre of diversity in the Cape Floristic Region (CFR), where over 70% (113 species) of the species occur, with several taxa dispersed into alpine areas of Africa, and to Australasia, Europe and the Americas. This study aims to infer the phylogenetic relationships and patterns of macroevolution in *Ficinia* clade; to estimate the divergence dates and investigate the role of ecology the speciation of sister species of the genus *Ficinia;* and to revise the taxonomy of the species in the *Ficinia indica* complex (*F. indica, F. dunensis, F. elatior, F.* aff. *indica* R and *F.* aff. *indica* MF). Phylogenetic relationships in *Ficinia* clade were reconstructed based on ITS and *rps*16 sequence data analysed using both parsimony and Bayesian methods. There is strong support for the *Ficinia* clade, excluding *Ficinia clandestina,* which is embedded withinthe C_4 *Cyperus, Isolepis* is paraphyletic, including the genus *Ficinia.* Key taxonomic characters, both qualitative and quantitative, were scored to reconstruct patterns of macroevolution of both vegetative (e.g. length of culm and leaf blade, ligule type) and reproductive features (e.g. inflorescence and spikelet types), where most genera lack unambiguous synapomorphies, except for the presence of the gynophore in *Ficinia.* Divergence times and speciation patterns in the genus *Ficinia* were investigated by conducting a molecular dating analysis of a densely sampled matrix (ITS and *rps*16 sequence data) of *Ficinia.* The clade has a stem age of about 30 Mya and the genus *Ficinia,* comprising over 75 species, originated about 13 Mya but rapidly diversified only in the last 5 Mya. Speciation has involved shifts in substrate type, geography, altitude and other ecological parameters among sister-species pairs. Univariate and multivariate approaches were used for the analysis of the morphological and ecological data in *Ficinia indica* complex. Species boundaries and name application within *Ficinia indica* complex were revised and two species new to science were recognized. This study adds to the systematic knowledge of one of the Cape clades, and reveals the evolution of the fynbos genus *Ficinia* to be contemporaneous with other fire-adapted taxa such as *Babiana*.

CHAPTER 1
GENERAL INTRODUCTION AND LITERATURE REVIEW

1.0. Problem statement

Plant species are not evenly distributed in nature. There are a number of factors that influence the population dynamics and patterns of plant distribution, such as differences in environmental conditions, competition and disturbance (Barbour et al., 1987). Different sets of environmental conditions not only modify the distribution and abundance of individuals but are likely to change the growth rate, seed production, branching pattern, leaf area, root area and size of the individuals. Plant distribution, survival, and patterns of growth and reproduction reflect the plant's adaptations to a particular environmental regime and thus are a critical part of plant ecology (Barbour et al., 1987).

The question 'What is driving speciation?' has been one of the core questions of biology since the publication of Charles Darwin's *The Origin of Species* (Darwin, 1859). His work has been of major influence on the life and earth sciences and on modern thought in general. Darwin laid the foundation of modern evolutionary theory with his concept of the morphological change of all forms of life through the process of natural selection (Bynum, 2006). Many causes have been tested for their influence and the amount of literature on this subject is vast (e.g. Coyne & Orr, 2004*)*. Ever since Darwin (1859), evolutionary biologists have suggested that ecology, among other factors such pollination and geographical barriers, plays an important role in the origin of new species, and research on the relationship between ecology and speciation has intensified some years back (e.g. Orr & Smith, 1998; Schluter, 2001; Schemske, 2000; Via, 2002). According to Schluter, 1998, 2000, 2001, ecology is important in speciation because incipient species often occur in different environment or utilize different resources (e.g. microhabitats). Previous studies (e.g. Orr & smith, 1998; Schluter, 1996; Schemske, 2000) on various morphologically diverse taxa suggest that natural selection caused by shifts in ecology or invasions of new habitats can cause extremely rapid divergence, and might play a prominent role in speciation. Wiens (2004a, b) argued that ecology can play an additional role in the origin of species that is very different from the one described in most of the recent literature on ecology and speciation. He outlined that natural selection acts to maintain the ecological niches of lineages over time by limiting dispersal across geographic barriers during vicariance, and adaptation may prevent the initial stage of speciation by allowing species to persist in or invade the novel ecological setting of a potential geographic barrier and thereby maintain gene flow between otherwise isolated populations..

1.1. Overall diversity in Cyperaceae

Cyperaceae, commonly called sedges, is the third largest family among monocotyledons, among the 10 largest families of angiosperms (Stevens, 2001), and the second largest family in Poales (Goetghebeur, 1998). They are a cosmopolitan graminoid family comprising about 110 genera and over 5500 species, represented in southern Africa by 30 genera and over 500 species (Archer, 2000; Goetghebeur, 1998). They are a specialized group of plants, particularly in relation to their reproductive structure and mostly wind-pollinated (anemophilous) flowers. The majority of sedges are perennials while annuals are limited to a small number of genera. They are adapted to open, sunny areas with reduced competition from taller shading trees and shrubs. Such habitats are often dependent upon natural or artificial disturbance. A variety of plants, including many sedges, have intrinsic characteristics that promote population expansion after disturbance, e.g. high reproductive output, rapid growth, vegetative proliferation and extended seed dormancy, and probably originally evolved as colonizers of disturbed habitats (Baker, 1965, 1974; McNaughton & Wolf, 1973). Sedges are distributed worldwide and are abundant in wet, marshy areas of tropical to temperate and subarctic zones and some are also found in fynbos, grassland and on mountain slopes. Cyperaceae have their greatest diversity in the humid and semi-humid tropics but they are also dominant in temperate and cold-temperate regions of the world (Goetghebeur, 1998).

1.1.1. Morphological diversity

(a) Below-ground morphology

The Cyperaceae, like most monocots, have sympodial growth with each culm forming a single flowering episode and new culms formed by buds located at base of plant. Culms are produced in clumps (tufts) or may be separated by stolons or rhizomes. A rhizome is a horizontal, underground part of the culm which is covered by tough scales. A lateral shoot can originate in the axil of a scale and grow out upwards into a new culm and downwards into a root. Stolons are lateral stem shoots, mostly underground and creeping near the surface, forming adventitious roots, in the nodes of which the shoots originate (Goetghebeur, 1998).

(b) Culms

Each stem normally ends in a peduncle carrying an inflorescence. The basal internodes of the culms may be very short so that the leaves are set basally, in which case the culm is usually elongate and without nodes above the base, as in most *Cyperus* species. Some culms may be leafy along their whole length with several relatively short internodes. The culms are usually triangular, more rarely, terete, flattened. In sedges, the culm is set with minute spine-like teeth, at least on the major ribs below the inflorescence, though some species have a completely glabrous culm while others have a culm with scattered long, whitish hairs (Haines & Lye, 1983).

(c) Leaves

A typical leaf has a closed tubular sheath ending in a throat and a flat blade. The leaf sheaths in sedges are usually closed and rarely open, sometimes succulent or spongy, and with or without ligules. The leaf blades are linear and rarely oblong, terete, filiform, ovate, elliptic and rarely pseudopetiolate (Goetghebeur, 1998). Sometimes the margin of the sheath mouth is thickened opposite the blade and raised to form a ligule that does not split, or the margin may be continued between the sheath and blade as a ligule, in the form of a simple membrane, a thickened rim, or a ciliate membrane (Haines & Lye 1983). There are often two keels on the upper surface of the leaf in large sedges with broader leaves, giving a girder-like M-section.

(d) Inflorescence

The morphology of the inflorescences and the establishment of homologies among different types of inflorescences is one of the main concerns of cyperologists (Raynal, 1971). The flowers of Cyperaceae are organized into spikelets with one or many glumes. The spikelets are furthermore arranged into spicate, paniculate, capitate or umbelliform inflorescences, which are not rarely reduced to a single spikelet that is open. These spikelets normally arise from the axil of normal bracts, and rarely from the axil of the prophylls. The prophyll encloses the rachilla and is rarely fertile or rather utriculiform. These two types differ in the degree of development of their internodes, the basic plan being common to both. Both types may become strongly contracted, then forming a dense head of crowded spikelets. The spikelets are made of glumes that are usually spirally or distichously arranged. The lowermost glumes of the spikelet are often empty but most usually subtend flowers, or with aborted flowers.

1.1.2. Phylogenetic relationships and classification in family Cyperaceae

According to the molecular phylogenetic study of Simpson et al. (2007) and Muasya et al 2009a, the family Cyperaceae can be subdivided into two subfamilies, the Mapanioideae and the Cyperoideae. However, the previous classification by Goetghebeur (1998), based on morphology, subdivided Cyperaceae into four subfamilies: Mapanioideae (140 species), Cyperoideae (2380 species), Sclerioideae (340 species) and Caricoideae (2150 species). In that classification, a combination of morphological data, mainly flower, inflorescence and embryo structure, was used to subdivide these families. Species in Mapanioideae have a peculiar floral structure. The subfamily Mapanioideae (Goetghebeur, 1998) is subdivided into two tribes, that is, Hypolytreae (9 genera, 130 species) and Chrysitricheae (4 genera, 13 species).

1.1.3. Economic uses of Cyperaceae

Cyperaceae are generally overlooked as plants of economic importance due to their localized uses, where they play a vital part in many local economies at regional or local level. About 45 genera and 502 species were identified to have economic significance worldwide, with plants used for different purposes by different communities (Simpson & Inglis, 2001) Table 1.1.

Table 1.1. Examples of species in Cyperaceae and their uses

Species	Distribution area and Ecology	Uses	References
Ficinia spiralis	New Zealand; coastal, on sand dunes	Animal food: Heavily grazed by rabbits and hares Environmental uses: erosion control, soil binder Revegetators: after mining Ornamentals	Partridge, 1992 Grounds, 1989; moores &Edgar, 1970 Partridge, 1992 Ground, 1989
Ficinia nodosa	Wide spread in South African hemisphere, coastal dunes and stream sides	Materials: Cane / reeds used: Culms used to make pack saddles	Pittier, 1935
Isolepis cernua	Wide spread, but excluding tropical Africa and SE Asia, damp and wet places	Medicinal: unspecified medicinal disorder Ornamental	Cauis & Banby, 1935 Ground, 1989 & Huxley, 1992
Isolepis prolifera	S. Africa, Australia, damp sand	Environmental: Ornamental	Huxley, 1992
Isolepis setacea	Most of Europe, Africa, N & W Asia, Australia, damp places	Environmental: Ornamental	Huxley, 1992
Kyllinga Polyphylla	Native to E. Africa, introduced into SE Africa and Pacific, often seasonally wet grassy places	Weed: unspecified weeds, ditches and road sides Cultivation: roots crops, coconut and sugar plantations Rice fields Gardens: Lawns pasture	Parsons & Cuthbertson, 1992 Partridge, 1992 Partridge, 1992

1.2. Classification of the genera within the *Ficinia* clade of tribe Cypereae and their taxonomic history.

1.2.1. *Ficinia* Schrad.

Ficinia comprises c. 80 species of tufted perennials with either rhizomes or stolons. They have culms with one internode but a few are noded; they have ligulate leaves and blade sometimes with margins or sheath. The inflorescences are usually pseudolateral and consist of spikelets with bisexual flowers with spirally or distichous arranged glumes. The fruit has the *Cyperus*-type embryo and can be diagnosed by the presence of a gynophore (Goetghebeur 1986, 1998; Vrijdaghs et al., 2005; Muasya et al., 2009; Muasya & de Lange, 2010). *Ficinia* species mostly occur in sub-Saharan Africa, but *F. nodosa* (Rottb.) Goetgh. et al. is nearly circum-antarctic (Muasya et al., 2000) and *F. spiralis* (A.Rich.) Muasya and de Lange is endemic to New Zealand (Muasya & De Lange, 2010). A number of *Ficinia* species are found in Africa outside the Fynbos biome, in the Greater Cape Floristic Region (GCFR) areas that share the winter rainfall, and some also extend into afroalpine zones in tropical Africa and Madagascar (Muasya et al., 2012).

1.2.2. *Isolepis* R.Br.

Isolepis comprises about 76 species of annuals or short-lived perennials, lacking both leaf ligules and gynophores, even though occasional specimens of *I. marginata* have seeds with gynophores. *Isolepis* mostly occurs in temperate regions of the southern hemisphere (Govaerts et al., 2007). The genus has its highest species diversity in the winter rainfall area of southern Africa, where annual species occur in ephemeral wetlands while perennials frequently form mats at the edges of wetlands or float in shallow freshwater (Archer, 2000; Muasya & Simpson, 2002). Their underground system consists of minute roots or rhizomes. Species with minute roots or ascending rhizomes grow in tufts with up to 50 culms consisting of one or more internodes which may be so short that the leaves are set basally consisting of only an elongated peduncle. Some taxa have a horizontal, whitish to yellowish rhizome up to 2 cm long. Above-ground rhizomes (runners) are found in the mat-forming *I. fluitans* and allied species (subgenus *Fluitantes*). Some taxa have one to four elongated internodes, with a leaf at each node. The elongation of the basal internodes appears to be influenced by growing conditions (Goetghebeur, 1998). Spikelet morphology has been used to separate *Isolepis* and *Ficinia* from *Cyperus: Isolepis* and *Ficinia* have terete spikelets with a spiral glume arrangement whereas *Cyperus* has flattened spikelets with distichously arranged glumes (Goetghebeur, 1998).

1.2.3. *Hellmuthia* Steud.

Hellmuthia is a tufted perennial with scapose culms and eligulate leaves, with lower leaves often reduced to the sheath. The inflorescences are capitate, where the lower primary bracts have a leaf-like appearance. It has one to ten spikelets with many densely, spirally arranged deciduous glumes, each subtending a bisexual flower. The style base is deciduous neither thickened nor distinct. The only species, *H. membranacea,* occurs in South Africa where it grows on coastal sands in the Western Cape (Goetghebeur, 1998). Muasya et al. (2000) suggested that *Hellmuthia* is phylogenetically closer to *Ficinia, Isolepis* and *Scirpoides* and eventually it has been classified in the Cyperoideae tribe Cypereae within the *Ficinia–Isolepis* clade (Simpson et al., 2003, Muasya et al., 2001). The floral ontogeny in *Hellmuthia* studied by Vrijdaghs et al. (2006) revealed that there are some unique features in its floral development and morphology, such as that it has three perianth scales in lower flowers.

Simpson et al. (2007) reported that Mapanioideae was resolved as a strongly supported group with an outlying member, *Hellmuthia,* placed as sister to the three Cypereae clades. A similar pattern was observed in other morphological and DNA studies (e.g., Muasya et al. 1998, 2000a, 2001; Simpson et al., 2003). *Hellmuthia* has been variously associated with *Desmoschoenus* Hook. f*., Ficinia, Isolepis,* and *Scirpoides* and it had been included in Chrysitricheae by Haines & Lye (1976) and Goetghebeur (1998) based on an interpretation of its floral morphology. Simpson et al. (2007) proposed the transfer of *Hellmuthia* to Cypereae because it is morphologically and genetically similar to *Ficinia,* and is endemic to the sand dunes of the Western Cape of South Africa, an area that is the centre of diversity for the *Ficinia–Isolepis* group. However, Mapanioideae is a monophyletic clade sister to the rest of Cyperaceae and Cyperoideae and other subfamilies are not monophyletic and are embedded in the Caricoideae clade (Simpson et al., 2007).

1.2.4. *Scirpoides* C.B.Clarke

Scirpoides species are tufted or shortly rhizomatous perennials with the scapose culms that are thickened at the base. The inflorescences are pseudolateral, anthelate or capitate with numerous spikelets and leafy bracts with the lowermost bracts erect. The branches of the partial inflorescences are conspicuously crescentiform in cross-section, bear spikelets with many spirally arranged, long, persistent glumes each subtending a bisexual flower and having 2 to 3 stamens with the style deeply trifid and the style base not distinct. About five species are found in seasonally wet areas of South Africa and warmer parts of Eurasia (Goetghebeur, 1998).

1.3. The Cape Floristic Region as a center of diversity for the *Ficinia* clade

Almost 70% (113 species) of the species in the *Ficinia* clade occur in the CFR and they form part of the Cape floral clades. In the next sections, a review of the characteristics and evolutionary processes associated with this diverse region are presented, with an emphasis on the major drivers of diversification.

1.3.1. Level of endemism and species richness in the Cape Floristic Region (CFR)

The Cape Floristic Region (CFR) is located at the southwestern tip of Africa, which is characterized by unique levels of species richness and endemism, reflected in the region's inclusion of two (CFR and Succulent Karoo) of the 34 hotspots of biodiversity (Mittermeier et al., 2004). The CFR contains more than 9000 plant species of which almost 70% are endemic in an area of about 90,000 km^2 (Goldblatt & Manning, 2000; Goldblatt et al., 2005). The CFR represents one of the most diverse temperate floras of the world and is substantially richer than other Mediterranean-type climate regions (Cowling et al., 1996). These features have led to the recognition of the CFR (Goldblatt & Manning, 2000) as one of the world's six floral kingdoms (Takhtajan, 1986). These other kingdoms are the Holarctic, Neotropics, Paleotropics, Cape, Australian and Holantarctic. (Takhtajan, 1986). The CFR extends along the west coast of South Africa and the Succulent Karoo also harbours an exceptional number of plant species (Driver et al., 2005; Mittermeier et al., 2004), including 30% of the world's 10,000 succulents. The close floristic affinities between the two regions have led to proposals to unify them as the Greater Cape Floristic Region (GCFR; Jurgens, 1997; Born et al., 2007), which provides an excellent model system for studying the causes of plant diversification because of its remarkable diversity.

Overall, orographic effects on rainfall are massive and the associated gradients are extremely steep in the CFR (Campbell, 1983; Deacon et al., 1992). The winter-rainfall component is significantly more reliable in the west than in the east, while the opposite holds true for summer rainfall (Proches et al., 2005). Most parts of the CFR receive an annual rainfall of between 300 and 2000 mm yr^{-1}, although some montane sites in the west receive as much as 3000 mm yr^{-1} (Deacon et al., 1992). In the west, rainfall, which is associated with cold fronts budded off from the circumpolar westerly system, is concentrated in the winter months (i.e. Mediterranean-climate conditions prevail). However, climate alone cannot explain species diversity in the CFR, as the region contains more than twice as many species as expected by global environmental models (Kreft & Jetz, 2007). Species richness in the CFR is thought to be a result of an interplay between a complex of diverse habitats and steep ecological gradients against a background of relatively stable climate and geology after the establishment of a Mediterranean climate since the beginning of the Pliocene (5 Ma) (Goldblatt & Manning, 2000). One of the major roles of the Cape botanical researchers is to better understand the processes driving the observed patterns of extreme species richness and endemism (Linder, 2006).

1.3.2. Estimation of the diversification rates in CFR clades

To explain why some taxa and geographic regions contain more species than others is an important goal of evolutionary biology (Valente et al., 2009). Phylogenetic approaches have been increasingly used to explore the timing and rates of diversification, in terms of the accumulation of speciation through time (Barraclough & Vogler, 2002; Rabosky, 2006; Ricklefs, 2007). It is now clear that the potential for clades to diversify depends on the geographic region they inhabit (Losos & Schluter, 2000; Ricklefs, 2003; Davies et al., 2004; Phillimore et al., 2006), when incorporating earlier ideas from island biogeography and ecological studies of diversity patterns (MacArthur & Wilson, 1967). The diversification rate of a clade is often estimated as the log of the number of species in the clade divided by the age of the clade, with some variations; e.g. Magallón & Sanderson, 2001 designed to better account for the possible effects of extinction (Ricklefs, 2007). The estimates of diversification rate are typically used as estimates of the net diversification in a clade over time, which are then used to compare it with other clades. It is clear that species do not necessarily accumulate at a constant rate over time within a clade, as speciation and especially extinction may be infrequent, episodic, and stochastic (Wiens, 2011).

Modern species diversity in the CFR is not necessarily the result of Paleocene diversification even though some clades might be relatively ancient within Southern Africa (Linder, 2003). The first date given to the radiation of the Cape floral clades was presented by Axelrod and Raven (1978) who claimed that radiation would have been the indirect result of climate change resulting from glaciation of Antarctica. However, the estimations of these dates are not supported by current studies. It has been stated that radiation commenced more or less at the Miocene–Pliocene boundary (Goldblatt & Manning 2000a; Cowling & Pressey, 2001). Another explanation that had been used to explain the radiation of the Cape floral clades is that species richness accumulated gradually through the Tertiary (Linder, 2003). However, most of the factors important for the isolation of the Cape flora were already in place by the beginning of the Tertiary but they were poorly developed (Linder, 2003). For example, Scotese (1997) stated that geographically, the southern oceans already existed at the beginning of the Cretaceous, but they were somewhat narrower. By Oligocene times, offshore particulate sedimentation in post-Gondwanan fault basins had virtually ceased (McMillan, 2003) and geographic senility in the Cape was far advanced. The fold mountains had already developed, but were much eroded and it implies that more of the relatively easily eroded shales were exposed, and these produce a more nutrient rich soil that sandstone. The shales were stripped off the mountains through the Cenozoic (Tertiary), leaving the erosion-resistant sandstones behind and these erode into a coarse, silicaceous, oligotrophic soil (Cowling et al., 2009). Seemingly, this means that the proportion of oligotrophic soils in the CFR increased through time and the coastal lowlands were capped with hardpans during much of the Tertiary. Modern relicts of these carry Cape floral elements (Linder, 2003). It seems therefore that the geographical distinction between the CFR and the rest of southern Africa may have evolved gradually through the Cenozoic (Linder, 2003).

1.3.3. Drivers of speciation in the CFR

Geographical (allopatric and parapatric) speciation, arising when populations are separated by physical barriers to gene flow, is thought to be a major mode of speciation, especially in the CFR (Linder, 2005). Van der Niet & Johnson (2009) showed that speciation events in the CFR are very frequently associated with ecological shifts, which brings researchers one step closer towards unravelling the underlying causes of the extreme species richness in the CFR. They elucidated that it is not just one ecological factor associated with speciation, but that different factors operate independently, that this pattern is not lineage-specific, and that it is found at similar frequencies in regions adjacent to the CFR. This means that plant speciation may have a strong ecological component, but that at the same time it is extremely variable in its nature. Ecological factors such as soils, climate and topography have been suggested as major drivers of speciation (Linder & Vlok, 1991; Linder, 2003, 2005; Van der Niet & Johnson, 2009). Another factor suggested to cause speciation in the CFR is adaptation to different pollinators, although the speciation model and the exact role that pollinators play a role in speciation process is still controversial (Goldblatt & Manning, 1996; Johnson 1996a, van der Niet, 2008). Another factor that has been suggested to be a driver of speciation in the CFR is fire adaptation. For example many species of orchids are rarely found in unburnt areas (Linder & Kurzweil, 1999) and it has been suggested that they would not exist without fire (Linder, 2003). Regular fires create habitats for whole growth-form groups, especially geophytic herbs such as Iridaceae and Orchidaceae. Alternatively, fire may not have played a role in driving the speciation in these clades, but it creates the ecological space for these groups of plants (Linder & Kurzweil, 1999). It was also stated in Bytebier et al. (2010) that many species are obligately fire-dependent and will only flower in the first year after fire, although the mechanism or potential triggers for this are not understood.

By studying sister taxa, this project will explicitly investigate the role of ecology (soil, altitude and phenology) in the speciation of CFR sedges in *Ficinia* clade. Members of *Ficinia* clade show fire adaptations (resprouting immediately after fire and persisting within the first year(s) after fire). We hypothesize that sister species occur in habitats with different ecological factors to indicate that ecology plays a major role in speciation in the clade

1.4. The concepts and data in delimitation of plant genera and species

Species are the primary objects of study in many fields of biology and most commonly used for conservation planning and management (Marguls & Pressey, 2000). The most important discovery that a biologist can make is to find a new species (Wilson, 1998) and to determine its phylogenetic relationship with other species. Wiens (2007) described species delimitation as a process by which species boundaries are determined and new species are discovered. It depends on having some ideas on what species are. There is a general consensus among biologists that species are lineages (e.g. de Queiroz, 2007; Knowles & Carstens, 2007; Shaffer & Thomson, 2007; Raxworthy et al., 2007; Rissler & Apodaca, 2007; Wiens, 2007), but there are still challenges on how to go about delimiting those lineages. Although most biologists probably hold a similar idea of what a species is (Mayr, 1942; Hey, 2001; Agapow et al., 2004; De Queiroz, 2005a), usually, no particular concept is explicitly adopted in most publications and, certainly, not in the vast majority of taxonomic ones. Many biologists who study speciation have adopted the theoretical framework of the biological species concept (BSC). Such biologists have argued that BSC is the best concept to facilitate the study of speciation (e.g. Coyne & Orr, 1998; Futuyma, 1998; Noor, 2002, Via, 2002). A view on how speciation occurs depends on the concept of what species are and how species originate. The BSC-based speciation research elucidates how species diverge and remain distinct from other species, but Wiens (2004b) illustrated that allopatric speciation does not require the populations to already have reproductive isolation. Another traditional criterion that is important in biology is the species delimitation using morphological differences based on one or more qualitative or quantitative morphological characters that do not overlap with other species (Wiens, 2007).

The issue of species delimitation has long been confused with the species conceptualization, leading to a half century of controversy concerning both the definition of the species category and method of inferring the boundaries and the numbers of species. The most important benefit of a unified species concept is that it clarifies the issues of species delimitation by clearly separating the conceptual problem of defining the species category from the methodological problem of inferring the boundaries and number of the species (de Queiroz, 2007).

As the consequence of there being several ways in which the observed patterns of variation in nature can be conveyed in the generic classification, most long-recognized genera have changed in size over time. In a survey of contemporary generic delimitation practice, Humphreys & Linder (2009) found a significant dichotomy between the studies that incorporate molecular data and those that rely exclusively on morphological data. The new data sources, including morphology (anatomy and cytology) and chemical (amino acid and DNA sequence data), that have been introduced have brought conceptual developments to evolution and monophyly (Humphreys & Linder 2009). Monophyly is important in that it increases the chances of a genus being predictive in the sense of predicting the true pattern or of predicting the genus of a new species.

The question of ‘‘What is a genus?’’ is among the fixed points to spring to biologists minds when carrying out identification or biodiversity-related work (Oberwinkler, 1994) before they could even ask themselves what a species is. Several studies stated that some genera are groups of species, in rare cases single species, that in some respects may exist in nature and others exist simply by means of definition (e.g. Anderson, 1940; Greenman, 1940; Stebbins, 1956; Walters, 1986). Therefore, the grouping of species into genera allows the representation of the recognized patterns (Jeffrey, 1987) in that the genera serve as the memory device (Raven et al., 1971; Clayton, 1983; Stevens, 1997) and as units for information storage and retrieval (Cronk, 1990; Barkley et al., 2004).

1.5. Use of the multivariate approach in analyzing morphological and ecological data

Multivariate analysis is mostly used to study morphology, ecology and other forms of data that require grouping and discrimination. Morphometrics can be defined as the quantitative analysis of morphological data. It has been widely used in a variety of disciplines including systematics. The data that are used for morphometric studies include qualitative and quantitative variables (Nixon & Wheeler, 1990). This study (Chapter 4) conducted morphometric analysis to revise the taxonomy of a problematic species complex using morphological data, and more information about that data will be discussed in Chapter 4. Some of the most commonly used morphometric multivariate techniques are Cluster Analysis (CA), Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). These methods were also used to analyze the ecological data.

1.5.1. Cluster Analysis

Cluster analysis is an exploratory tool where there are no statistical assumptions about the data. It is an example of Q-mode type of analysis, in which the association amongst the specimens is being assessed (Legendre & Legendre, 1998). The main purpose of conducting the CA is to group similar species (or other operational taxonomic units) together in a hierarchical manner and the results from the CA are mostly presented in the form of dendrograms. The most commonly used clustering algorithm in systematics is the hierarchical, agglomerative algorithm using averages, that is, UPGMA (unweighted pairwise method using arithmetic averages), but the association matrix is produced either by a similarity or distance coefficient (Barker & Zona, 2006).

1.5.2. Principal Component Analysis

Principal Component Analysis is an exploratory tool in systematics that involves rotating the axes of the coordinate system such that the first principal component passes through the greatest dimension of a swarm of data points and thus accounts for the greatest amount of variance of any possible axis. The second principal component is orthogonal to the first and it accounts for the greatest amount of residual variance. The scores of each specimen are mostly summarized by the first two components and usually these can be plotted on bivariate scatterplots (Barker & Zona, 2006).

1.5.3. Discriminant Function Analysis

Discriminant Function Analysis (DFA) is also known as Canonical Variate Analysis or Discriminant analysis. It comprises a group of methods rather than a single procedure (Pimentel, 1979) and it uses the same kind of data that is used in the PCA. The first step in DFA is to test the hypothesis that the centroids of a priori group are the same using multivariate analysis of variance (MANOVA). If MANOVA supports that the group centroids are significantly different, then classification and discrimination follows. For classification, the DFA produces identification functions that are used to determine which group a specimen belongs to. For discrimination, the discriminant functions best discriminate among the predefined groups by maximizing the differences among the groups while minimizing variation within the groups (Barker & Zona, 2006).

1.6. Sources of data for phylogenetic inference

The sources of information about organisms that are used by biologists to reconstruct their phylogenies or to derive classification systems are mostly morphological, anatomical, embryological, chromosomal, palynological, secondary metabolites, proteins and nucleic acid sequences (RNA and DNA). The use of DNA data for phylogenetic inference has become prominent in the last two to three decades with the development of several molecular techniques such as the polymerase chain reaction (PCR), the automation of DNA sequencing and the accessibility of efficient computer software used to analyse the sequence data.

The sources of DNA sequence data for phylogenetic studies in plants are from the plastid, the mitochondrial and the nuclear genomes. The mitochondrial genome is inherited through the maternal lineage (Ankel-Simons & Cummins, 1996; Mogensen, 1996), while the nuclear genome is inherited biparentally (Petit et al., 2005). It has been shown that, although the plastid genome is maternally hereditary for the majority of angiosperms, it is biparentally inherited in some angiosperm species (Tilney-Bassett, 1976; Hu et al., 2008). The plastid genome is the smallest of the three genomes and ranges from 135 to 160 kilobasepairs (kbp). It has received massive exploration for phylogenetic inference because it is quite stable both within cells and species, and rearrangements of genomes are rare enough in evolution that they are useful in demarcating major groups (Palmer, 1987). The mitochondrion is not as commonly used in plant studies as it is in animal studies such as on birds (Sturmbauer, 1998), fish (Bargelloni, 2000) and baboons (Newman, 2004). This is partly due to the evidence that mitochondrial genes evolve slowly in plants (Crochet & Desmarais, 2000) and therefore may only be more useful for assessing ancient events, yet most studies on plants tend to focus on recent speciation events. Other reasons include frequent genomic rearrangements, the incorporation of foreign DNA from the nuclear and chloroplast genomes, and the disruption of gene continuity in introns or exons (Knoop, 2004). Parts of the nuclear genome commonly used as the source of data for phylogenetics are arranged in tandem arrays of several hundred to several thousand copies and they include the small subunit 18S and the large subunit 26S of the ribosome separated by the small 5.8S gene. Nuclear genes with a copy number high enough for easy study are those encoding ribosomal RNA (Baldwin *et al*., 1995). There are short internal transcribed spacers (ITS) between the three genes, and the large spacer, the intergenic spacer (IGS) separates each set of three genes from the next set (Judd *et al*., 2008). The ITS evolve faster than the widely used chloroplast regions, which makes it useful for deducing relationships among closely related species (Álvarez & Wendel, 2003).

The use of morphological data in phylogeny reconstruction has been critisised by some authors who advocate a purely molecular approach (e.g. 2003; Scotland et al., 2003) due to the fact that most morphological characters are ambiguous, and that character coding and homology assessment is difficult and can be inaccurate. Felsenstein (1988) and Wiens (2001) postulated that ambiguities in morphological data can be resolved by treating morphological characters as continuous quantitative traits and the problem of coding morphological data can be addressed by comparing how well different methods of analysing morphological data recover clades that are strongly supported by independent, non-morphological data sets (Wiens, 1998).

1.7. Phylogenetic reconstruction

A phylogeny can be defined as the evolutionary history of a group of entities which is used to describe evolutionary relationships in terms of relative recency of common ancestors. These relationships are represented as a branching diagram or tree, with branches joined by nodes and leading to terminals at the tips of the tree (Harrison & Langdale, 2006). Monophyly, paraphyly and polyphyly are the three main types of relationships that are distinguished on trees (Hennig, 1966). Monophyletic and paraphyletic groups have a single evolutionary origin whereas polyphyletic group result from convergent evolution with the characters supporting the groups absent in the most recent common ancestor (Kitching et al., 1998). Monophyletic groups include all the descendants from the single ancestor and if one lineage from a monophyletic group is removed, a paraphyletic group remains. In gene families, these principles approximate to orthology and paralogy (Fitch, 1970).

1.8. Summary of research questions

This MSc project aimed to study the systematics of the *Ficinia* clade, focusing on phylogenetic reconstruction; macroevolution of morphological characters; estimation of dates of lineage divergence; investigating drivers of speciation between sister species; as well as revising the taxonomy of the *Ficinia indica* complex. Therefore, the specific objectives were as follows:

1. To infer the phylogenetic relationships within *Ficinia* clade
2. To reconstruct the evolution of the key diagnostic characters within *Ficinia* clade
3. To investigate the timing of the divergences and diversification rates in *Ficinia* clade

4. To test whether ecology played a role in speciation in *Ficinia*
5. To revise the taxonomy of the *Ficinia indica* species complex.

1.9. Outline of dissertation

Chapter two aims to infer the phylogenetic relationships and to reconstruct the evolution of the key diagnostic characters within *Ficinia* clade. Molecular data was used to achieve objective one, whereas objective two was achieved by scoring morphological characters and reconstructing their macroevolutionary patterns. Chapter three investigates the timing of the speciation of *Ficinia* clade and tests whether ecology played a role in the speciation of species in *Ficinia.* Objective three was achieved by dating the phylogeny, while objective four was achieved by analysing the ecological attributes such as soil nutrient levels, flowering times and altitude. Morphometric studies were done in Chapter four to achieve objective five, which was to revise the taxonomy of the *Ficinia indica* complex. The discussion and the summary will be presented in chapter five.

CHAPTER 2

PHYLOGENETIC RELATIONSHIPS, MACROEVOLUTION AND CLASSIFICATION IN *FICINIA CLADE*

2.1. Introduction

Species belonging to the *Ficinia* clade (sensu Muasya et al., 2009a) have a history of being misclassified. In Linnaeus's work *Species Plantarum* (1753) all known Cyperaceae bearing bisexual flowers and terete spikelets were placed in the genus *Scirpus* L. Using this broad taxonomic concept, a heterogeneous assemblage of lineages as amalgamated by other authors, notably Boeckeler (1870). Over time, several segregate genera were recognized, the largest being *Isolepis* R.Br. (Brown, 1810) and *Ficinia* Schrad. (Schrader, 1832). *Ficinia* and *Isolepis* together with *Scirpus* were classified in the tribe Scirpeae (e.g. Bruhl, 1995), but more recently these genera were transferred to the tribe Cypereae (Goetghebeur, 1998). Inclusion of *Ficinia* and *Isolepis* into Cypereae was initially based on embryological characteristics, with the shared presence of the *Cyperus*-type embryo (versus *Scirpus*-type embryo in Scirpeae; Haines & Lye, 1983; Goetghebeur, 1998). This classification has been further supported by molecular phylogenetic studies (Muasya et al., 1998; 2001; 2009a, b).

Muasya et al., (2009a) showed that Cypereae are resolved into the *Cyperus* and *Ficinia* clades (Figure 2.1). The *Cyperus* clade has *Cyperus* sensu stricto as the core genus, in which the thirteen derived genera (*Alinula, Androtrichum, Ascolepis, Courtoisina, Kyllinga, Kyllingiella, Lipocarpha, Oxycaryum, Pycreus, Queenslandiella, Remirea, Sphaerocyperus*, and *Volkiella*) are embedded (Muasya et al, 2009a). *Courtoisina, Kyllingiella* and *Oxycaryum* were recently sunken into the C_3 *Cyperus* group (Larridon et al., 2011a, b). The *Ficinia* clade sensu Muasya et al. (2009a) includes the Cypereae with predominantly terete spikelets, i.e. the genera *Ficinia*, *Hellmuthia* Steud., *Isolepis*, and *Scirpoides* Ség. Members of the *Ficinia* clade are predominantly characterized by inflorescences being pseudolateral with the lower-most primary bract erect and stem-like, or occasionally anthelate with leafy bracts; deciduous, subequal glumes, usually each subtending a bisexual flower; and style base neither distinct nor thickened (Goetghebeur, 1998). *Ficinia* (ca. 80 species; including the former monotypic genus *Desmoschoenus*; Muasya & de Lange, 2010) is easily recognized by its perennial habit, capitate inflorescences, and nutlets borne on a basal cuplike gynophore. *Isolepis* (ca. 76 species) contains both annuals and perennials that lack a gynophore, but some annual species (e.g. *I. marginata*) have well developed gynophores, thereby blurring the delimitation between the genera *Ficinia* and *Isolepis*. *Scirpoides* (five species) are perennials with a general *Ficinia* habit but with anthelate inflorescences and nutlets lacking a gynophore. The monotypic genus *Hellmuthia*, with capitate inflorescences and nutlets lacking a gynophore, has short bisexual flowers bearing three scale-like perianth parts (Vrijdaghs et al., 2006). In addition, the two aberrant *Scirpus* species (*S. falsus* and *S. ficinioides*; Figure 2.1), with an overall morphology similar to *Ficinia* but missing a gynophore and having distinct bristle-like perianth parts have been transferred into their own genus *Dracoscirpoides* Muasya (Muasya et al., 2012). As the genera in the *Ficinia* clade are diagnosed based on a single or combination of variable characters, there is a need to investigate patterns of evolution of the key characters used in generic classification.

A number of molecular phylogenetic studies have focused on the Cypereae. The tribe has been investigated in family-level studies (e.g. Muasya et al., 1998, 2009b), aiming at understanding the broad phylogenetic relationships and classification at a high phylogenetic level. Both plastid and nuclear markers have been used to study the evolution of the *Cyperus* clade (e.g. *rbcL/rps16/trnL–F*, Muasya et al., 2002; *rpl32–trnL/trnH–psbA/*ETS, Larridon et al., 2011). For the *Ficinia* clade, studies on *Isolepis* have used plastid sequence data (*rbcL/trnL–F*, Muasya et al., 2001), while the most advanced molecular study on *Ficinia* is based on both plastid and nuclear sequence data (*rps16/*ITS, Muasya and de Lange, 2010). However, fewer than a quarter of *Ficinia* species have been included in previous phylogenetics studies.

Among the two largest genera in the *Ficinia* clade, a recent monographic study of *Isolepis* (Muasya & Simpson, 2002) investigated the phylogenetic relationships at species level and the infrageneric boundaries and classification. On the other hand, *Ficinia* has only been studied in local floras (e.g. Levyns, 1950; Gordon-Gray, 1995), and in taxonomic papers describing new taxa (e.g. Arnold and Gordon-Gray, 1978; Lye, 1996; Muasya 2005; Muasya et al. 2012). The genus *Ficinia*, as currently accepted, comprises about 80 species, occurring predominantly in the winter rainfall area of Southern Africa (Archer, 2000; Govaerts et al., 2007). Some of these species have been previously treated as separate genera or subgenera of *Ficinia* (Table 2.1). The most extensive taxonomic treatment of *Ficinia* to date (Clarke, 1898) recognized five infrageneric taxa, namely subgen. *Sickmannia* (genus *Sickmannia* Nees), subgen. 2 (*F. ixioides*), subgen. *Ficinia* (genus *Ficinia* sensus stricto), subgen. *Acrolepis* (genus *Acrolepis* Schrad.), and subgen. *Hemichlaena* (genus *Hemichlaena* Schrad.). Pfeiffer

(1921) modified Clarke's classification by recognizing a number of sections and subsections in subgen. *Ficinia*, but merged subgen. *Sickmannia* and *Acrolepis* into subgen. *Ficinia*, and recognized subgenera *Pseudoficinia* and *Hemichlaena*. On the other hand, Levyns (1950) recognized *Sickmannia* as a separate genus and two subgenera in *Ficinia* (*Ficinia* and *Hemichlaena*). The difference among the infrageneric treatments of the above authors was mostly due to emphasis on different diagnostic characters for genera and infrageneric ranks.

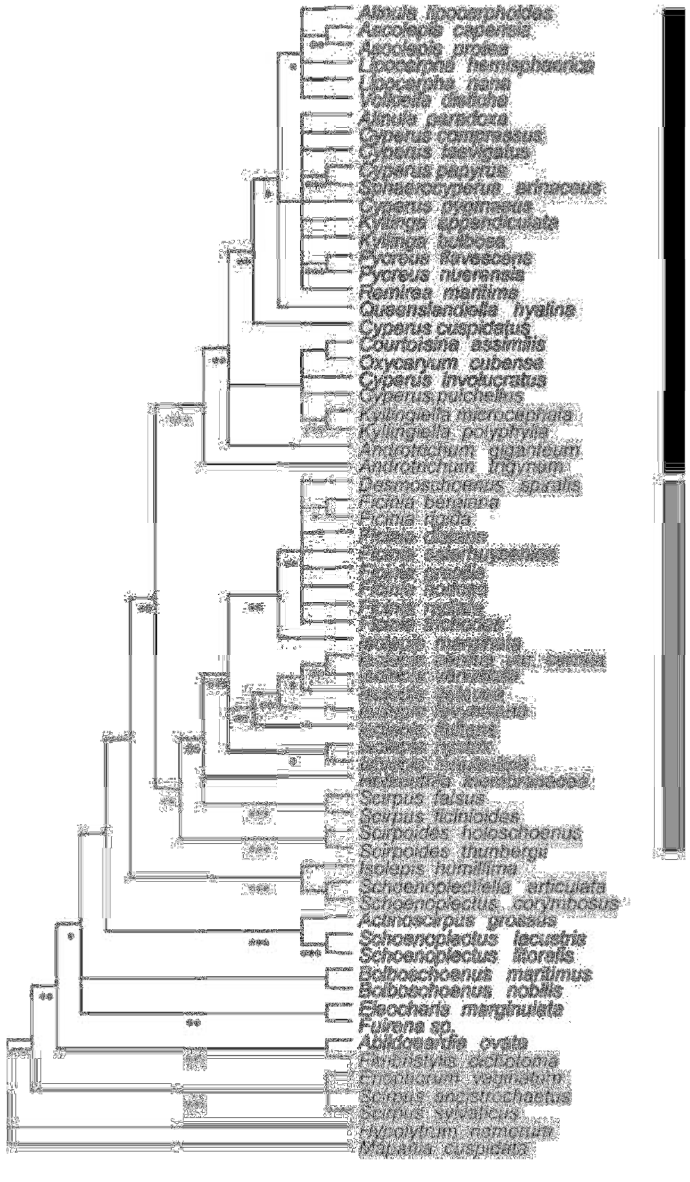

Figure 2.1 Maximum parsimony strict consensus tree of Cypereae based on heuristic analysis of plastid DNA sequence data (specify the genetic markers). *Cyperus* and *Ficinia* clades are marked by black and grey bars respectively. Bootstrap support values shown as * for 50–74%, ** for 75–89% and *** for 90–100% (Muasya et al, 2009a).

The gynophore as a synapomorphy of *Ficinia* is debated in taxonomic treatments. The gynophore has evolved independently in the *Cyperus* clade (in *Alinula lipocarphoides*; Muasya et al., 2009a), rejecting the utility of this morphological trait. Furthermore, a number of species with typical ficinioid characters, including the type species for the genus (*F. filiformis* (Lam.) Schrad.), lack a gynophore, thereby blurring the generic boundary with *Isolepis*. At the same time, the genus *Ficinia* was previously thought to be restricted to Africa, and ficinioid species bearing gynophores and occurring outside the continent (*F. nodosa* and *F. spiralis*) were excluded (Goetghebeur, 1998).

2.1.1 Objectives

This chapter provides a phylogenetic framework and a solid basis to investigate the relationships of described and putative new taxa in *Ficinia* clade. By constructing a robust phylogeny and studying the evolutionary patterns of key characters used in generic delimitation and infrageneric classifications, I will investigate the utility of these characters in a taxonomic context. In addition, I will investigate the taxonomic position of poorly known and atypical taxa, including the Ethiopian highland hemicryptophyte *Ficinia clandestina* (Steud.) Boeck. (formerly described as *Cyperus clandestinus Steud.*).

The more specific objectives of this study are:

1. To infer the phylogenetic relationships within *Ficinia* clade
2. To reconstruct the evolution of the key diagnostic characters within *Ficinia* clade.

Table 2.1. Infrageneric classification of *Ficinia*

Clarke (1898)	**Pfeiffer (1921)**	**Levyns (1950)**
Subgenus I – Sickmannia *F. radiata* ***Subgenus II*** *F. ixioides* ***Subgenus III – Eu-ficinia*** *F. acuminata , F. albicans , F. anceps, F. bergiana , F. brevifolia, F. bulbosa, F. cinnamomea, F. compasbergensis, F. ecklonea, F. fascicularis , F. fastigiata, F. filiformis, F. gracilis, F. laciniata , F. laevis F. lateralis, F. macowanii, F. micrantha, F. monticola, F. paradoxa, F. pinguior, F. praemorsa F. pygmaea, F. quinquangularis, F. repens, F. secunda, F. stolonifera, F. sylvatica, F. tristachya, F. truncata, F. zeyheri* ***Subgenus IV – Acrolepis Schrader*** *F. ramosissima, F. trichodes* ***Subgenus V - Hemichlaena* C.B.Cl.** *F. anguistifolia, F. capillifolia.*	***Subgenus I - Eufilicinia* H.Pfeiff.** **Section 1 – *Isolepiformes* H.Pfeiff.** 1. ***Graciliflorae* H.Pfeiff.** *F. albicans, F. acuminata, F. bergiana, F. cinnamomea, F. filiformis, F. quinquangularis, F. macowanii, F. micrantha, F. stolonifera, F. tenuifolia, F. tristachya* 2. ***Efoliatea* H.Pfeiff.** *F. lateralis, F. repens, F. trispicata* 3. ***Capitulae* H.Pfeiff.** *F. fastigiata, F. gracilis, F. brevifolia, F. ecklonea, F. laciniata , F. laevis, F. paradoxa, F. praemorsa F. truncata,* **Section 2 – *Bracteosae* H.Pfeiff.** 4. ***Sickmannia*** Nees *F. radiata* 5. ***Seticulmes* H.Pfeiff.** *F. anceps, F. compasbergensis, F. indica, F. ixiodes, F. fascicularis, F. montícola, F. pinguior* 6. ***Arboriculmes* H.Pfeiff.** *F. clandestine, F. pygmaea* **Section 3 – *Acrolepiformus* H.Pfeiff.** *F. ramosissima, F. trichodes , F. zeyheri* **Subgenus II – *Pseudofilicinia* H.Pfeiff.** *F. bulbosa, F. secunda* **Subgenus III – *Hemichlaena* C.B.Cl.** *F. angustifolia, F. capillifolia* **Species of uncertain position** *F. distans, F. micrantha, F. mucronata*	***Sickmannia*** *F. radiata* ***Ficinia*** *F. acuminata, F. anceps, F. anguistifolia, F. capillifolia, F. deusta, F.fastigiata, F. filiformis, F. indica, F. ixioides, F. micrantha, F. paradoxa, F. pinguior, F. polystachya, F. ramosissima, F. rigida, F. stolonifera, F. trichodes , F. tristachya, F. zeyheri*

2.2. Materials and methods

2.2.1. Taxon sampling

In total, 188 samples representing all major lineages of the genus *Ficinia* were sequenced (Table 2.2). About 80% of the species from the genus *Ficinia* were collected for phylogenetic analyses and macroevolutionary character reconstruction. One to three replicates for each species within *Ficinia* were included, covering morphologically variable species such as *F. indica*. The outgroup (i.e. *Cyperus*, *Kyllinga, Kyllingiella and Lipocarpha)* consisted of species belonging to *Cyperus* clade. A list showing the species that were analysed together with provenance and voucher information is presented in Table 2.2.

Table 2.2. Taxa and voucher information used for this study. Specimens were obtained from the Royal Botanic Gardens, Kew (K) and Bolus Herbarium (BOL). New sequences obtained are indicated with +, whereas - denotes sequences not obtained. * denotes old sequences obtained from Muasya and ** denotes the sequences obtained from genebank.

Species	**Author**	**Country**	**Collector**	**Voucher Number**	**ITS**	***rps16***
*Cyperus involucratus***	Rottb.	Madagascar	Kew Acc	61316603 (K)	+	+
*Cyperus kerstenii***	Boeckeler	Kenya	Muasya	984 (EA)	-	+
*Cyperus papyrus***	L.	Chad	Hepper	4213 (K)	+	+
Dracoscirpoides falsa	(C.B.Clarke) Muasya	South Africa	Muasya & Tshiila	4948(BOL)	+	-
Dracoscirpoides falsa	(C.B.Clarke) Muasya	South Africa	Muasya	4935(BOL)	+	-
*Dracoscirpoides falsa**	(C.B.Clarke) Muasya	South Africa	Hilliard & Burtt	13609(BOL) (GENT)	+	+
Dracoscirpoides ficinioides*	(Kunth) Muasya	South Africa	Hilliard & Burtt	16095(BOL) (GENT)	+	+
Ficinia acuminate	Nees	South Africa	Muasya	3247(BOL)	+	+
Ficinia acuminate	Nees	South Africa	Muasya	3796(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4610(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4653(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	82(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4514(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4612(BOL)	+	-
Ficinia aff. *Indica**		South Africa	Muasya	2372(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4581a(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4583(BOL)	+	+
Ficinia aff. *Indica*		South Africa	Muasya & Tshiila	4594(BOL)	+	+
Ficinia albicans	Nees	South Africa	Muasya	2953(BOL)	+	-
*Ficinia anceps**	Nees	South Africa	Muasya & Tshiila	4525(BOL)	+	-
*Ficinia angustifolia**	Schrad	South Africa	Muasya	2202(BOL)	+	+
*Ficinia anysbergensis**	Muasya	South Africa	Muasya	2363(BOL)	+	+
*Ficinia anysbergensis**	Muasya	South Africa	Tshiila & Muasya	111(BOL)	+	+
*Ficinia arenicola***	T.H.Arnold & Gordon-Gray	South Africa	Sonneberg	BS347 (GENT)	-	+
Ficinia sp. nov B.*		South Africa	Muasya	3751(BOL)	+	+

Ficinia arenicola **	T.H.Arnold & Gordon-Gray	South Africa	Clark	C97 (BOL)	+	+
Ficinia argyropa	Nees	South Africa	Muasya & Tshiila	4611(BOL)	+	+
*Ficinia argyropa**	Nees	South Africa	Muasya	2233(BOL)	+	-
*Ficinia bergiana**	Kunth	South Africa	Tshiila & Muasya	34(BOL)	+	+
Ficinia bergiana	Kunth	South Africa	Muasya	2337(BOL)	+	+
Ficinia bergiana	Kunth	South Africa	Tshiila & Muasya	35F(BOL)	+	+
Ficinia brevifolia	Nees ex Kunth	South Africa	Tshiila & Muasya	47F(BOL)	+	+
*Ficinia brevifolia**	Nees ex Kunth	South Africa	Muasya	2205(BOL)	+	-
Ficinia bulbosa	(L) Nees	South Africa	Tshiila & Muasya	57(BOL)	+	-
Ficinia bulbosa	(L) Nees	South Africa	Tshiila & Muasya	55(BOL)	+	-
Ficinia bulbosa	(L) Nees	South Africa	Tshiila & Muasya	10(BOL)	+	-
*Ficinia bulbosa**	(L) Nees	South Africa	Muasya	2359(BOL)	+	+
*Ficinia capillifolia**	(Schrad) C.B Clarke	South Africa	Muasya	2229(BOL)	+	+
*Ficinia capitella**	(Thunb.) Nees	South Africa	Muasya & Tshiila	4643(BOL)	+	-
Ficinia capitella	(Thunb.) Nees	South Africa	Tshiila & Muasya	6(BOL)	+	+
*Ficinia cedarbergensis**	T.H. Arnold & Gordon-Gray	South Africa	Muasya	2313(BOL)	-	+
*Ficinia clandestina**	(Steud.) Boeckeler	Ethiopia	Sebsebe & Muasya	6852 (BOL)	-	+
Ficinia compasbergensis	Drège ex Steud.	South Africa	Clark	171(BOL)	+	+
Ficinia deusta	(P.J. Bergius) Levyns	South Africa	Tshiila & Muasya	5(BOL)	+	-
Ficinia deusta	(P.J. Bergius) Levyns	South Africa	Tshiila & Muasya	2(BOL)	+	-
Ficinia deusta	(P.J. Bergius) Levyns	South Africa	Tshiila & Muasya	14(BOL)	+	+
*Ficinia deusta**	(P.J. Bergius) Levyns	South Africa	Muasya	2214(BOL)	+	+
*Ficinia dunensi*s*	Levyns	South Africa	Muasya	2217(BOL)	+	+
Ficinia dunensis	Levyns	South Africa	Muasya & Tshiila	51(BOL)	+	-
Ficinia dunensis	Levyns	South Africa	Muasya & Tshiila	54(BOL)	+	-
Ficinia dura	Turrill	South Africa	Tshiila & Muasya	19(BOL)	+	-
Ficinia dura	Turrill	South Africa	Tshiila & Muasya	12(BOL)	+	-
*Ficinia ecklonea**	(Steud.) Nees	South Africa	Muasya	2345(BOL)	+	+
Ficinia ecklonea	(Steud.) Nees	South Africa	Muasya & Tshiila	17(BOL)	+	-
*Ficinia elatior**	Levyns	South Africa	Muasya & Tshiila	69(BOL)	+	-
*Ficinia elatior**	Levyns	South Africa	Muasya	2215(BOL)	+	+
*Ficinia elatior**	Levyns	South Africa	Muasya	3235(BOL)	+	+

*Ficinia esterhuyseniae**	Muasya	South Africa	Muasya	2312(BOL)	+	+
*Ficinia fascicularis**	Nees	South Africa	Muasya	3825(BOL)	+	+
*Ficinia fascicularis**	Nees	South Africa	Muasya	2966(BOL)	+	+
Ficinia fastigiata	(Thunb.) Nees	South Africa	Muasya & Tshiila	64(BOL)	+	-
*Ficinia fastigiata**	(Thunb.) Nees	South Africa	Muasya	2230(BOL)	+	+
Ficinia fastigiata	(Thunb.) Nees	South Africa	Muasya & Tshiila	22(BOL)	+	+
Ficinia filiformis	(Lam.) Schrad.	South Africa	Muasya & Tshiila	16(BOL)	+	+
Ficinia filiformis	(Lam.) Schrad.	South Africa	Muasya & Tshiila	53(BOL)	+	+
Ficinia gracilis	Schrad.	South Africa	Muasya & Tshiila	4937(BOL)	+	+
*Ficinia gracilis**	Schrad.	South Africa	Muasya	2355(BOL)	+	+
*Ficinia gydomontana**	T.H. Arnold	South Africa	Muasya	2333(BOL)	+	+
Ficinia indica	(Lam) H.Pfeiff.	South Africa	Muasya & Tshiila	68(BOL)	+	-
Ficinia indica	(Lam.) H.Pfeiff.	South Africa	Muasya	2909b(BOL)	+	+
Ficinia indica	(Lam.) H.Pfeiff.	South Africa	Muasya & Tshiila	4351(BOL)	+	-
Ficinia indica	(Lam.) H.Pfeiff.	South Africa	Muasya & Tshiila	4598(BOL)	+	-
Ficinia ixioides	Nees	South Africa	Muasya & Tshiila	4626(BOL)	+	-
*Ficinia ixioides**	Nees	South Africa	Muasya	2207(BOL)	+	+
*Ficinia laciniata**	(Thunb.) Nees	South Africa	Muasya	2340(BOL)	+	+
Ficinia laevis	Nees	South Africa	Muasya & Tshiila	4608(BOL)	+	+
*Ficinia lateralis**	(Vahl) Kunth	South Africa	Muasya	2940(BOL)	+	+
Ficinia lateralis	(Vahl) Kunth	South Africa	Muasya & Tshiila	100(BOL)	+	+
Ficinia latifolia	T.H. Arnold & Gordon-Gray	South Africa	Muasya & Tshiila	18(BOL)	+	-
*Ficinia levynsiae**	T.H. Arnold & Gordon-Gray	South Africa	Muasya	2261(BOL)	+	-
*Ficinia macowanii**	C.B. Clarke	South Africa	Muasya	2249(BOL)	+	+
Ficinia macowanii	C.B. Clarke	South Africa	Muasya & Tshiila	50(BOL)	+	+
*Ficinia micrantha**	C.B. Clarke	South Africa	Muasya	2268(BOL)	+	+
*Ficinia minutiflora**	C.B. Clarke	South Africa	Muasya	2257(BOL)	+	+
*Ficinia monticola**	Kunth	South Africa	Muasya	2287(BOL)	+	+
Ficinia nigrescens	(Schard) J. Raynal	South Africa	Muasya	3036(BOL)	+	-
Ficinia nigrescens	(Schard) J. Raynal	South Africa	Muasya & Tshiila	30(BOL)	+	-
Ficinia nigrescens	(Schard) J. Raynal	South Africa	Muasya & Tshiila	31(BOL)	+	-
Ficinia nigrescens	(Schard) J.	South Africa	Muasya & Tshiila	33(BOL)	+	+

	Raynal					
Ficinia nigrescens	(Schard) J. Raynal	South Africa	Muasya & Tshiila	40(BOL)	+	+
Ficinia nodosa	(Rottb.) Goetgh., Muasya & D.A.Simpson	South Africa	Muasya & Tshiila	4522(BOL)	+	-
Ficinia nodosa	(Rottb.) Goetgh., Muasya & D.A.Simpson	South Africa	Muasya	3299(BOL)	+	+
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	11(BOL)	+	-
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	15(BOL)	+	-
*Ficinia oligantha**	(Stued.) J. Raynal	South Africa	Muasya	2204(BOL)	+	+
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	3(BOL)	+	+
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	4518(BOL)	+	+
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	4672(BOL)	+	+
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	52(BOL)	+	-
Ficinia oligantha	(Stued.) J. Raynal	South Africa	Muasya & Tshiila	66(BOL)	+	-
Ficinia pallens	(Schrad.) Nees	South Africa	Muasya & Tshiila	4509(BOL)	+	-
*Ficinia pallens**	(Schrad.) Nees	South Africa	Muasya	2225(BOL)	+	+
Ficinia paradoxa	(Schrad.) Nees	South Africa	Muasya & Tshiila	13(BOL)	+	+
*Ficinia petrophila**	T.H. Arnold & Gordon-Gray	South Africa	Muasya	2364(BOL)	+	+
*Ficinia petrophylla**	T.H.Arnold & Gordon-Gray	South Africa	Muasya	2364(BOL)	-	+
Ficinia pinguior	C.B. Clarke	South Africa	Muasya & Tshiila	1(BOL)	+	-
*Ficinia pinguior**	C.B. Clarke	South Africa	Muasya	1183(BOL)	+	+
*Ficinia pinguior**	C.B. Clarke	South Africa	Muasya	2218(BOL)	+	+
*Ficinia polystachya**	Levyns	South Africa	Muasya	2330(BOL)	+	+
*Ficinia polystachya**	Levyns	South Africa	Muasya & Tshiila	4637(BOL)	+	-
Ficinia polystachya	Levyns	South Africa	Muasya & Tshiila	120(BOL)	+	+

Ficinia praemorsa	Nees	South Africa	Muasya & Tshiila	56(BOL)	+	+
Ficinia praemorsa	Nees	South Africa	Muasya & Tshiila	49(BOL)	+	-
Ficinia praemorsa	Nees	South Africa	Muasya	2348(BOL)	+	+
Ficinia pygmaea	Boeck.	South Africa	Muasya	2296(BOL)	-	+
Ficinia quinquangularis	Boeck.	South Africa	Muasya & Tshiila	4127(BOL)	+	+
Ficinia radiata	(L.F.) Kunth	South Africa	Muasya & Tshiila	4580(BOL)	+	-
Ficinia radiata	(L.F.) Kunth	South Africa	Muasya & Tshiila	75(BOL)	+	+
*Ficinia ramosissima**	Kunth	South Africa	Muasya	2288(BOL)	+	+
*Ficinia repens**	(Nees) Kunth	South Africa	Muasya	2347(BOL)	+	+
*Ficinia rigida**	Levyns	South Africa	Muasya	2319(BOL)	+	+
Ficinia secunda	(Vahl) Kunth	South Africa	Muasya & Tshiila	27(BOL)	+	-
Ficinia sp. nov. A		South Africa	Muasya	3804(BOL)	-	+
Ficinia spiralis	(A.Rich.) Muasya & de Lange	New Zealand	HBUG	2003-0699 (GENT)	+	+
Ficinia sp. nov. A		South Africa	Muasya & Tshiila	4354(BOL)	+	+
Ficinia sp. nov. C		South Africa	Muasya & Tshiila	4592(BOL)	+	+
Ficinia sp. nov. C		South Africa	Muasya & Tshiila	4593(BOL)	-	+
*Ficinia stolonifera**	Boeck.	South Africa	Muasya	2221(BOL)	+	+
Ficinia stolonifera	Boeck.	South Africa	Muasya	3037(BOL)	+	-
Ficinia stolonifera	Boeck.	South Africa	Muasya	3771(BOL)	+	+
*Ficinia stolonifera**	Boeck.	South Africa	Muasya	2715(BOL)	-	+
Ficinia trichodes	(Schrad.) Benth. & Hook.f.	South Africa	Muasya & Tshiila	63(BOL)	+	-
*Ficinia trichodes**	(Schrad.) Benth. & Hook.f.	South Africa	Muasya	2328(BOL)	+	+
*Ficinia trispicata**	(L.F.) Druce	South Africa	Muasya	2252(BOL)	+	+
*Ficinia tristachya**	(Rottb.) Nees	South Africa	Muasya	2255(BOL)	+	+
*Ficinia truncata**	(Thunb.) Schrad.	South Africa	Muasya	2361(BOL)	+	+
*Ficinia zeyheri**	Boeck.	South Africa	Muasya	2208(BOL)	+	-
Ficinia zeyheri	Boeck.	South Africa	Muasya & Tshiila	4159(BOL)	+	-
Ficinia zeyheri	Boeck.	South Africa	Muasya & Tshiila	62(BOL)	+	+
Hellmuthia membranacea	(Thunb.) R.Haines & K. Lye	South Africa	Muasya	3081(BOL)	+	+
Isolepis sp.		South Africa	Muasya & Tshiila	4597d(BOL)	+	+
Isolepis sp.		South Africa	Muasya & Tshiila	4646b(BOL)	+	+

Isolepis expallescens	Kunth	South Africa	Muasya	3067(BOL)	+	+
Isolepis bicolor	Carmich.	Tristan da Cunha	Richardson	105 (K)	+	+
Isolepis antarctica	(L) Roem. & Schult	South Africa	Muasya	3007(BOL)	+	+
Isolepis aucklandica	Hook.f.	Australia	Wilson & Muasya	9492 (NSW)	+	+
Isolepis brevicaulis	(Levyns) J. Raynal	South Africa	Muasya	3003(BOL)	+	+
Isolepis capensis	Muasya	South Africa	Muasya	3078(BOL)	+	+
Isolepis cernua	(Vahl) Roem. & Schult	South Africa	Muasya	3073(BOL)	+	+
Isolepis costata	Hochst. ex A.Rich.	South Africa	Muasya & Tshiila	4941(BOL)	+	-
Isolepis costata	Hochst. ex A.Rich.	Kenya	Muasya	1109(BOL)	+	+
*Isolepis diabolica**	(Steud.) Schrad.	South Africa	Muasya	1163(BOL)	+	+
*Isolepis digitata**	Schrad.	South Africa	Muasya	1230(BOL)	+	+
*Isolepis fluitans**	(L.) R.Br.	Kenya	Muasya	1007(BOL)	+	+
*Isolepis habra***	(Edgar) Soják	Australia	Coveny &Muasya	17480 (NSW)	+	+
*Isolepis hemiuncialis**	(C.B.Clarke) J.Raynal	South Africa	Muasya	2895(BOL)	+	+
*Isolepis hystrix**	(Thunb.) Nees	South Africa	Muasya	2334(BOL)	+	+
*Isolepis inconspicua**	(Levyns) J. Raynal	South Africa	Muasya	2897(BOL)	+	+
*Isolepis inconspicua**	(Levyns) J. Raynal	South Africa	Muasya	2972(BOL)	+	+
Isolepis inundata	R.Br.	Australia	Wilson & Muasya	9493 (NSW)	+	+
*Isolepis inyangensis**	Muasya & Goetgh.	Zimbabwe	Muasya	2025(BOL)	+	+
Isolepis karroica	(C.B. Clarke) J. Raynal	South Africa	Muasya	3064(BOL)	+	+
*Isolepis keniaensis**	Lye	Kenya	Muasya	2552(BOL)	+	+
Isolepis leucoloma	(Nees) C. Archer	South Africa	Muasya	2976(BOL)	+	+
*Isolepis ludwigii**	(Nees) Steud.	South Africa	Muasya	1138(BOL)	+	+
Isolepis marginata	(Thunb.) A. Diert.	South Africa	Tshiila & Muasya	20(BOL)	+	-
Isolepis montivaga	(S.T.Blake)	Australia	Wilson & Muasya	9489 (NSW)	+	+

	K.L.Wilson					
*Isolepis namaquana***	Muasya & J.Viljoen	South Africa	Muasya	2891(BOL)	+	+
Isolepis pellocolea	B.L.Burtt	South Africa	Muasya	44694	+	+
*Isolepis prolifera***	R. Br.	Australia	Wilson & Muasya	9510 (NSW)	+	+
*Isolepis prolifera***	(Rottb.) R.Br.	Australia	Coverny &Muasya	17487 (NSW)	+	+
*Isolepis rubicunda**	Kunth	South Africa	Muasya	1221(BOL)	+	+
*Isolepis sepulcralis**	Steud.	South Africa	Muasya	1165(BOL)	+	+
*Isolepis setacea***	(L) R. Br.	Tanzania	Faden & Muasya	96/417 (K)	+	+
*Isolepis striata**	(Nees) Kunth	South Africa	Muasya	1180(BOL)	+	+
*Isolepis subtilissima***	Boeckeler	Australia	Coverny &Muasya	17475 (NSW)	+	+
*Isolepis tenuissima**	(Nees) Kunth	South Africa	Muasya	2369(BOL)	+	+
*Isolepis trachysperma**	Nees	South Africa	Muasya	2893(BOL)	+	+
*Isolepis venustula**	Kunth	South Africa	Muasya	1189(BOL)	+	+
Isolepis wakefieldiana	(S.T.Blake) K.L.Wilson	Australia	Neish & Muasya	110(BOL)	+	+
*Kyllinga bulbosa**	P.Beauv.	Kenya	Muasya	1020 (EA, K)	-	+
*Kyllingiella microcephala***	(Steud.) R.W.Haines & Lye	Zimbabwe	Muasya et al.	1118 (K)	-	+
*Kyllingiella polyphylla***	(A.Rich.) Lye	Tanzania	Wingfield	497 (K)	+	+
*Lipocarpha hemisphaerica***	(Roth) Goetgh.	Thailand	Muasya	1217 (K)	+	+
Scirpoides burkei	(C.B.Clarke) Goetgh., Muasya & D.A.Simpson	South Africa	Muasya	4949(BOL)	+	-
Scirpoides dioecus	(Kunth) Browning	South Africa	Muasya	3062(BOL)	+	+
*Scirpoides thunbergii***	(Schrad.) Sojak	South Africa	Muasya	51163(BOL)	+	+

2.2.2. DNA extraction, amplification and sequencing

DNA was extracted from freshly collected plant material. Alternatively, leafy shoots or photosynthetic stems were dried in silica gel, and other samples were obtained from mounted herbarium specimens (Table 2.2).

(a) Extraction

DNA extraction was carried out following the standard hexadecyltrimethy lammonium bromide (CTAB) method of Doyle & Doyle (1993). A master mixture containing 2× CTAB extraction buffer (700 µl) and 2-mercaptoethanol (1 µl) per sample was prepared and incubated in a pre-heated water bath at 65 ºC. Pestle and mortar were used to grind 20 mg of silica-dried or 40 mg of fresh plant material mixed with acid-washed sand and polyvinylpolypyrrolidone (PVPP). Liquid nitrogen was poured into the mortar during grinding to ensure that the samples remained frozen throughout. Each ground sample was transferred into a labelled microcentrifuge tube. 700 µl of the preheated CTAB was added to the ground plant material and mixed using a vortex. Samples were incubated in a water bath (65 ºC) for about 60 minutes. A chloroform: isoamyl alcohol mix (24:1, v/v; 600 µl) was added to each tube and mixed by repeated inversion for about 5 minutes. The samples were centrifuged for 5 minutes (12 000 rpm). The supernatant was pipetted out and transferred into a clean 1.5 µl microcentrifuge tube. An equal volume of ice-cold isopropanol (about 500–550 µl) was added to the supernatant and mixed by inversion. The samples were refrigerated overnight at –20 ºC to obtain higher DNA yield. After 24 hours, the samples were centrifuged at 12 000 rpm for 5 minutes. The isopropanol was then tipped out of the tube carefully and the tube was inverted open onto tissue paper for about 10 minutes to allow the residual liquid to drain out. 250 µl of 75% ethanol was then added to the tube to wash the DNA pellet. The tube was agitated to dislodge the pellet and to rinse any isopropanol remaining from the sides of the tubes. The samples were centrifuged for about 2–3 minutes at 12 000 rpm. The ethanol was then discarded and the tubes were inverted onto the tissue paper to allow them to dry. The sample tubes were left open to air-dry for about 30 minutes. The DNA samples were then re-suspended in 70 µl of sterile distilled water and stored at –20ºC.

(b) Screening of molecular markers, DNA amplification and sequencing

Several markers were tested and screened for successful amplification, good sequencing output and high level of sequence variation between closely related species in the *Ficinia* clade. The following genetic markers were tested in this study: *trnL–F*, *trnQ–rps16*, *ndhF–*

rp132, trnD–trnT, atpI–atpH (Shaw et al., 2005; 2007), ITS (White et al., 1990) and *rps16* intron (Oxelman et al., 1997). The plastid *rps16* intron and the nuclear ribosomal Internal Transcribed Spacer (ITS) were selected (Table 2.3) based on their high genetic variation and universal amplification success compared to the other genetic markers. The other markers that were screened had either low variation as in the case of atpI–H, or did not amplify due to suspected primer mismatches, e.g. *ndhF–rpl32*.

Genomic DNA was amplified using a standard polymerase chain reaction (PCR). A master PCR mix (30 µl) was prepared consisting of 18.6 µl of the PCR water (double-distilled water), 3 µl buffer, 3 µl $MgCl_2$, 1.2 µl dNTPs, 1.0 µl forward primer and 1.0 µl reverse primer, 0.2 µl *Taq* polymerase and 2.0 µl template DNA. All primers used for ITS and *rps16* amplification are listed in Table 2.3. Reactions were performed using a GeneAmp PCR system 2700 thermo cycler (Applied Biosystems) under the following conditions: pre-denaturation at 94 ºC for 2 min, followed by 33 cycles of denaturation at 94 ºC for 1 min, annealing at 52 ºC for 1 min, and elongation at 72 ºC for 2 min, and final elongation at 72 ºC for 7 min.

PCR amplicons were visualized by running the PCR products on a stained 1% agarose gel made by dissolving 0.25 g of agarose in 25 ml of 0.5 × TBE buffer at pH 8.3by heating it in the microwave for 2 min.. After cooling down to room temperature, 1.25 µl Goldview^{TM} nucleic acid stain was added and the gel was poured into a tray with a comb set. The gel was left to set for about 30 min at room temperature and loaded into an electrophoresis apparatus with 0.5 × TBE buffer. 3 µl of the PCR products were loaded into the wells. Electrophoresis of the PCR products was allowed to run for 13–15 min at 100 V current. Under UV light, the gel was checked and photographed with a UV light camera at an exposure of 0.2, 0.4 or 0.6 s to facilitate detection of bands. A reaction was considered to be successful if a single strong band was visible. The successful amplicons were sent to the University of Stellenbosch DNA sequencing facility for sequencing, using the same primers that had been used for amplification but now diluted with distilled water to a concentration of 1.1 µM.

Table 2.3: List of the primers used in this study

Name	Primer sequence (5′–3′)	References
*rps*16 (Forward)	GTG GTA GAA AGC AAC GTG CGA CTT	Oxelman et al., 1997
*rps*16 (Reverse)	TCG GGA TCG AAC ATC AAT TGC AAC	Oxelman et al., 1997
ITSL (Forward)	GGA AGT AAA AGT CGT AAC AAG G	Hsiao et al., 1994
ITS4 (Reverse)	TCC TCC GCT TAT TGA TAT GC	White et al., 1990

2.2.3. DNA sequence editing and alignment

The Staden package version 1.60 (Staden et al., 1998) was used to edit and to assemble the sequences. This package consists of Gap4.10 and Pregap4 version 1.5 which are used to assemble sequences. The preassembling process was done in Pregap4 which handles any number of sequences in a single run. Pregap4 creates an initial file for each reading which is used as input data in Gap4. 'Manual checking and editing of contigs is kept to a minimum by directing the users' attention only to consensus bases that are not determined to the required level of accuracy' (Staden *et al*. 1998).

The consensus sequences (reverse and forward sequence combined in Staden package) were imported into Bioedit version 7.0 (Hall, 1999) where they were first automatically aligned using the ClustalW multiple alignments and then remaining residues were aligned manually. Gaps were inserted for the regions where the taxa did not have the sequences and the gaps were treated as missing data. The sequences for *rps16* and ITS were combined manually in Notepad++. The alignment of the *rps16* and ITS matrices for the *Ficinia* clade and outgroups was fairly unambiguous.

2.2.4. Phylogeny reconstruction

The ITS and *rps16* intron data sets were analysed as a concatenated matrix using both maximum parsimony and Bayesian inference methods. The datasets for ITS and and rps16 were analysed separately to test the congruency. The pattern was more or less the same more especially on the node that were supported. The full data matrix (Matrix A) consisted of 188 sequences (see Table 2.2), which included sequences from multiple populations of the widespread or polymorphic species. The second analysis involved a pruned data set (Matrix B) comprising 93 taxa with each species represented by a single sequence entry. The taxon with most complete DNA sequence for two markers was selected to make the pruned dataset.

Some of the duplicate species (Eg. *F. arenicola and F. elatior*) were included in the pruned dataset because they were previously identified as different species.

Parsimony phylogenetic hypotheses were reconstructed in PAUP* (Phylogenetic analysis using parsimony, version 4.0b10, Swofford, 2002) using a heuristic search with 100 000 random addition replicates and tree bisection reconnection (TBR) branch swapping and saving five trees per replicate. Branch lengths were computed for all trees and the strict consensus of all most parsimonious trees was calculated for each analysis. Support for groups was evaluated by analysing 1000 bootstrap replicates using 10 random sequence addition and TBR, but saving one tree per replicate.

Bayesian inference (BI) analyses were performed for the combined ITS and *rps16* intron data sets. The best model of DNA evolution for the ITS and *rps16* intron were determined using Modeltest version 3.7 (Posada & Crandall, 1998). The nucleotide substitution model was selected using the Akaike information criterion (AIC; Akaike, 1974). The GRT+I+Γ (general time-reversible model with gamma-shaped rate variation and with a proportion of invariable sites) and GRT+Γ (general time-reversible model with gamma-distributed rate variation) models were the best-fitting models of DNA substitution for ITS and *rps16,* respectively. The same models were used for the analyses of the large and pruned data sets. The Bayesian inference analysis was performed in MrBayes (Huelsenbeck & Ronquist, 2003), using two simultaneous Metropolis-coupled Monte Carlo Markov chain (MCMCMC) runs, each with four chains of two million generations, sampling every 100th generation. All trees obtained before stationarity were discarded as burn-in trees and posterior probabilities (PP) were calculated. The stationarity was determined by plotting the log-likelihoods against the generation time and identifying the number of generations after which the likelihood stopped increasing.

2.2.5. Ancestral state reconstruction

A total of nine vegetative and floral morphological characters were scored based on observations from ten specimens of each species in the tree. Evolution of these morphological characters was reconstructed on the Bayesian tree (Matrix B) using the maximum-likelihood criterion in Mesquite version 2.71 (Maddison & Maddison, 2006). The characters and character states are summarized in Table 2.4.

Table 2.4. List of characters studied for ancestral trait reconstruction.

Character	States
Habit	0= perennial, 1= annual
Underground structure	0= stolon, 1= long rhizome, 2= short rhizome
Leaf sheath	0= papery, 1= not papery
Culm length	0= (< 30 mm), 1= (> 30 mm)
Culm internode	0= single, 1= multiple
Leaf blade length	0= (< 5 mm), 1= (> 5 mm)
Glume arrangement	0= spiral, 1= distichous
Inflorescence type	0= capitate, 1= spike, 2= anthelate
Gynophore	0= present, 1= absent

2.3. Results

2.3.1. Data matrices

ITS and the *rps16* intron were selected as useful DNA markers to reconstruct phylogenetic relationships in *Ficinia* clade. One hundred and eighty eight (188) samples were sequenced for the combined data set (Matrix A) and Matrix B consist of 93 sequences from B of which a single species if represented in the *Ficinia* clade. There are several species of *Ficinia* that could not amplify at all using these two successful and effective markers. For example, several attempts were made to amplify samples of *F. pygmaea* using ITS primers, but none of them were successful. Similarly, *F. laevis* could only be amplified for ITS but not for *rps16*. This observation suggests that primers were not universal for all the lineages of *Ficinia* due to mutations at the primer bindingsites.

Analyses of the complete matrix (Matrix A, 188 taxa) and pruned matrix (Matrix B, 93 taxa) were used to infer different aspects of the evolution of *Ficinia* clade. Matrix A was used to reconstruct phylogenetic relationships within *Ficinia* clade, while Matrix B was used for reconstructing character evolution and also to infer divergence patterns and identify sister-group relations (see Chapter 3). The information obtained from parsimony analysis of both matrices is presented in Table 2.5.

Table 2.5: Matrix and tree statistics for the parsimony analysis.

	Matrix A	Matrix B
Number of taxa	188	93
Constant position	840	929
Parsimony-informative characters	448	356
Parsimony-uninformative characters	211	214
Sequence length	1499	1499
Shortest tree length	2798	2349
Consistency index (CI)	0.64	0.45
Retention index (RI)	0.62	0.55

2.3.2. Phylogenetic relationships

Matrix A, including new samples from own field collections, was used to recover relationships in *Ficinia* at inter- and intraspecific level. A limited number of sequences were added from GenBank. Phylogenies were reconstructed using parsimony and Bayesian analysis (Figures 2.2 and 2.3). The trees obtained from matrix A and B consisted of two clades, *Ficinia* and *Cyperus* clades, as also found in the trees of matrix B. There is strong support for these clades in both parsimony (BS0.96) and Bayesian (PP99%) analyses. The duplicates for the majority of species were grouped together, with the exception of *F. indica* and *F. oligantha,* samples of which were placed at multiple positions in the phylogenetic trees. *Isolepis* contains two sub-clades in the Bayesian tree (i.e. the first clade of *I. sepulcralis* to *I. subtilissima* resolved by PP77% and the second supported by PP62% (Figure 2.3). Resolution within *Isolepis* is very high with each node resolved to PP>0.50 on the Bayesian tree. On the other hand, genus *Ficinia* was highly unresolved (Figure 2.2 and 2.3). The phylogeny obtained from the parsimony analysis had a similar pattern as the Bayesian tree. The parsimony tree consists of two main clades, (i.e. *Cyperus* and *Ficinia* clade) with *Ficinia* resolved by BS79% and *Cyperus* by BS0.1 (Figure 2.2). The sub-clade *Ficinia* was unresolved. However, the parsimony analyses resulted in a better resolved tree with nodes supported by higher posterior probability (PP) values. The ingroup is resolved by (PP=97%, BS0.76, excluding *Ficinia clandestina* which is resolved as part of *Cyperus* clade. Relationships at the base of the ingroup are poorly resolved, with *Hellmuthia, Dracoscirpoides* and the rest of *Ficinia* clade forming a polytomy and *Scirpoides* poorly supported as sister to the core *Ficinia* clade. *Dracoscirpoides* forms a strongly supported clade that includes *Scirpoides burkei* (PP99%, BS0.96). Among the remaining taxa, two clades are resolved, one comprising *Isolepis hemiuncialis* and *I. incomtula* and the other of the rest of *Isolepis* and *Ficinia*. The second clade further resolves into a core *Isolepis* clade (PP65%, BS0.62; *I. sepulcralis to I. subtilissima*) and a core *Ficinia* clade which includes the *I. marginata* clade. *Ficinia* + *Isolepis marginata* clade (comprising *I. capensis, I. leucoloma, I. minuta,* and *I. antarctica*) is sister to the Eastern Cape species *Ficinia arenicola*. The core *Ficinia* clade was strongly supported (PP95%), but has a large polytomy with only a few strongly supported sister species pairs. Taxa previously segregated as genera separate from *Ficinia* (*Desmoschoenus, Hemichlaena, Sickmannia*) are part of the *Ficinia* core clade in our results.

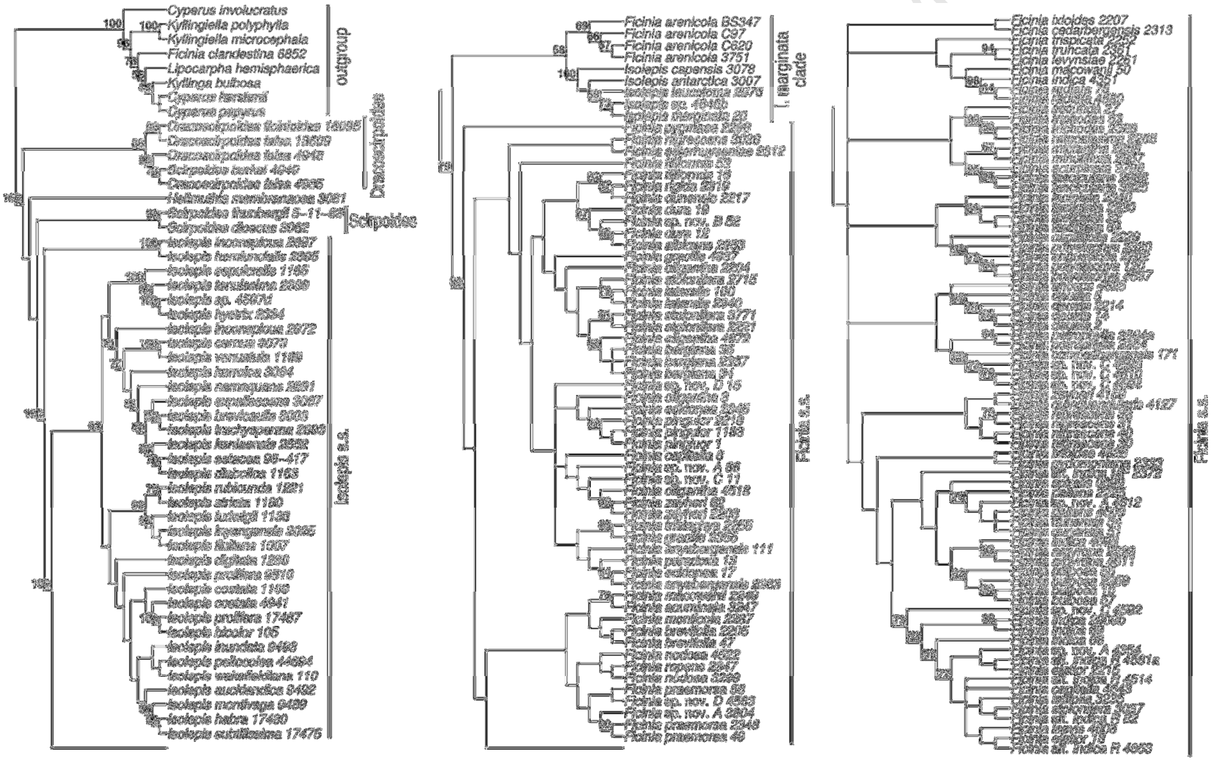

Figure 2.2. Parsimony consensus tree of the combined DNA dataset (ITS and *rps16*, 188 taxa) for the *Ficinia* clade. Bootstrap proportions ≥ 50% are shown on branches

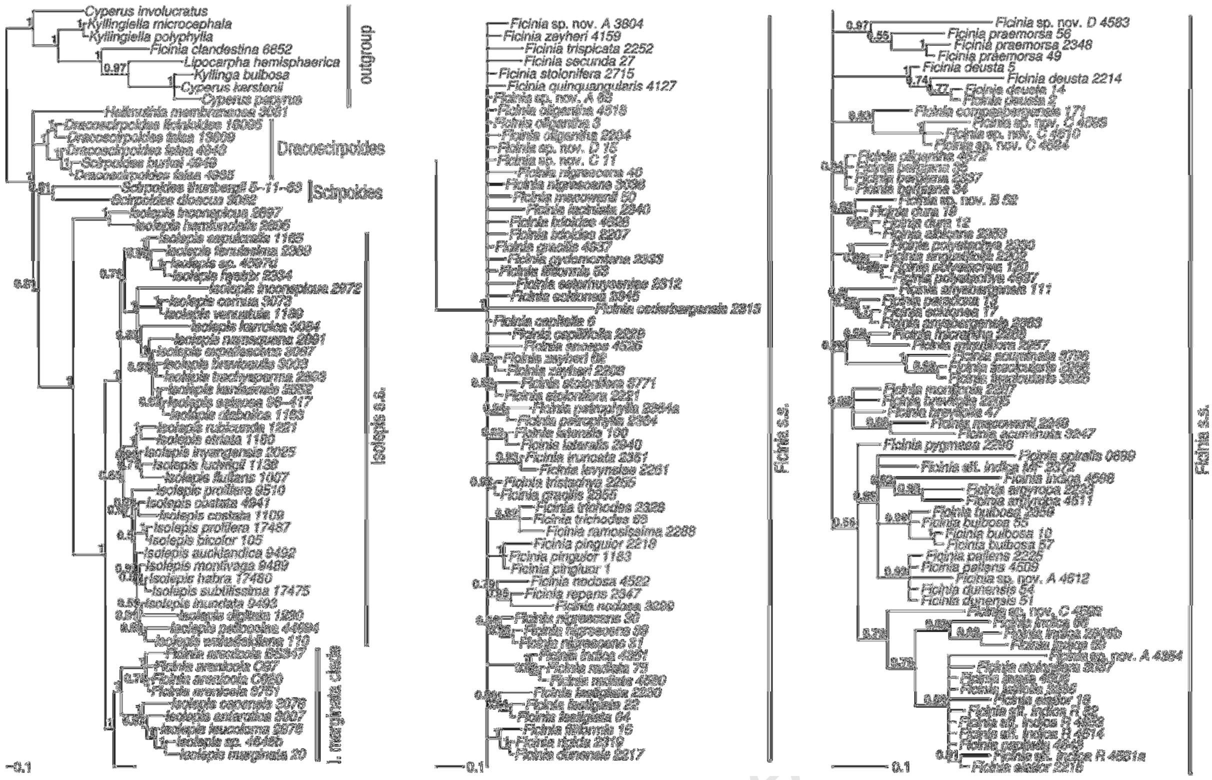

Figure 2.3. Bayesian tree of the combined DNA dataset (ITS and *rps16*, 188 taxa) for the *Ficinia* clade. Posterior probabilities (PP) of nodes are shown on branches.

The parsimony and Bayesian analyses of matrix B (93 taxa) produced phylogenies with similar topologies to the trees for matrix A. However, the Bayesian phylogeny is better resolved than the parsimony phylogeny. Two groups were supported, i.e. i) the *Ficini*a clade consisting of *Isolepis, Scirpoides, Hellmuthia* and *Ficinia*, ii) the *Cyperus* clade comprising *Cyperus* and allied genera (*Kyllingiella, Lipocarpha*) (Figures 2.4 and 2.5). *Ficinia clandestina* is resolved in the *Cyperus* clade in all phylogenies. The *Ficinia* clade includes *Dracoscirpoides* species (i.e. *D. falsa* and *D. ficinioides*) which share a similar morphology with the species of the genus *Ficinia*. Their sister relationship with *Scirpoides burkei* was confirmed by BS0.77 (Figure 2.4) on the parsimony tree and PP99% (Figure 2.5) on the Bayesian tree. Within the core *Ficinia* clade, two resolved (BS0.77 and PP71) clades are observed. Other strongly supported clades include *I hemiuncialis/ incomtula* clade*,* a second clade comprising most of the *Isolepis s.s* species (*I digitata, I. prolifera, I. ludwigii, I. striata, I. costata, I. hystix, I. brevicaulis, I. inconspicua* and *I. cernua* (BS0.60 and PP99% Figure 2.4 and 25, respectively)). Furthermore, the sister group relationship between *Ficinia arenicola,* collected from the Eastern Cape, and the *Ficinia* + *Isolepis marginata* clade *(I. marginata, I. antarctica, I. capensis* and *I. leucoloma*) was also recovered. The relationship between *I. marginata* clade and *F. arenicola* is moderately supported in the parsimony analyses (BS56; Figure 2.4), but highly resolved in the Bayesian analyses (PP98% and PP90%, Figure 2.5). *Ficinia* s.s. consists of the remaining *Ficinia* species and is largely unresolved, with a large number of taxa found in a polytomy.

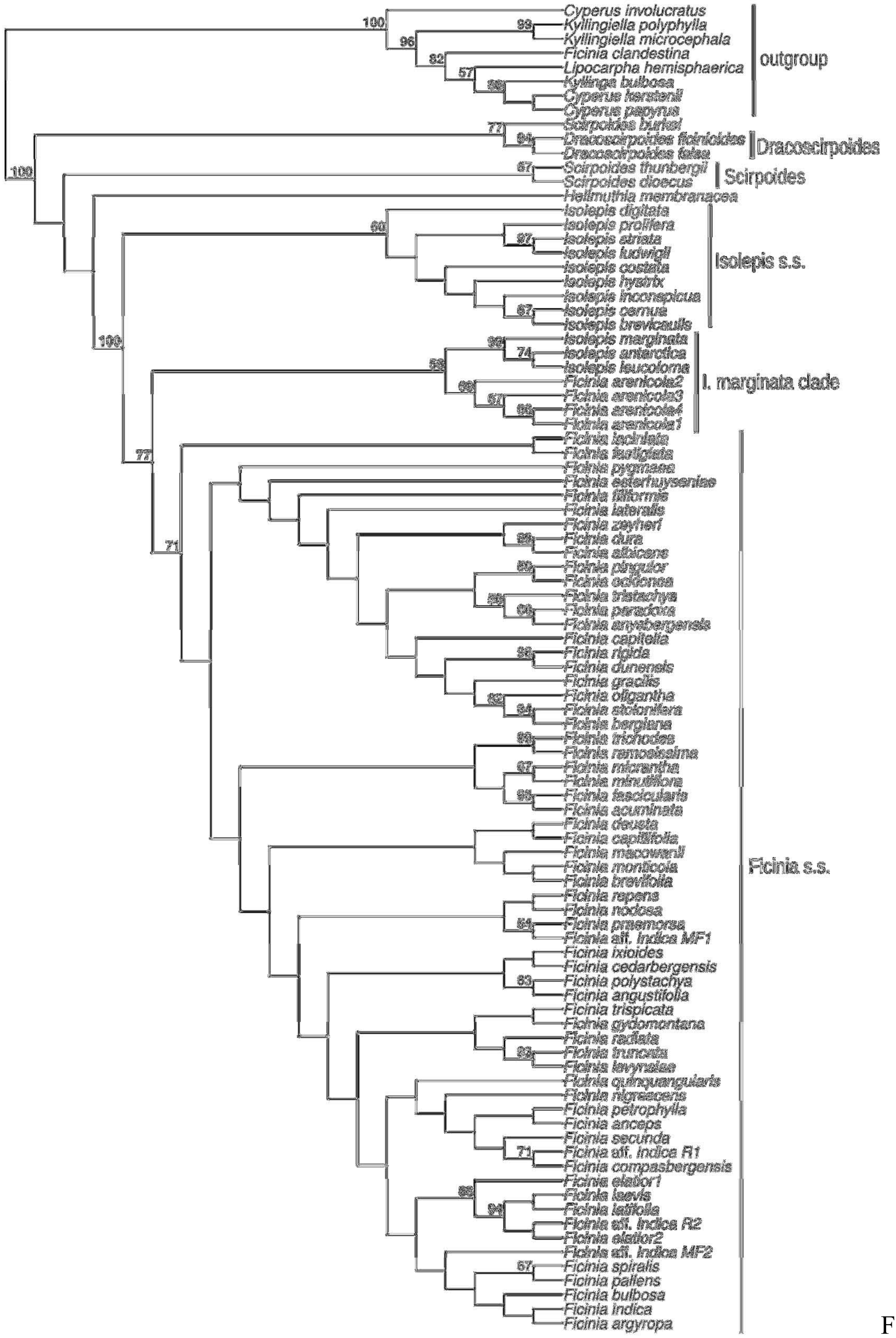

Figure 2.4. Parsimony consensus tree of the combined analyses based on ITS and *rps16* data (93 taxa) for the *Ficinia* clade. Bootstrap proportions ≥ 50% are shown on branches.

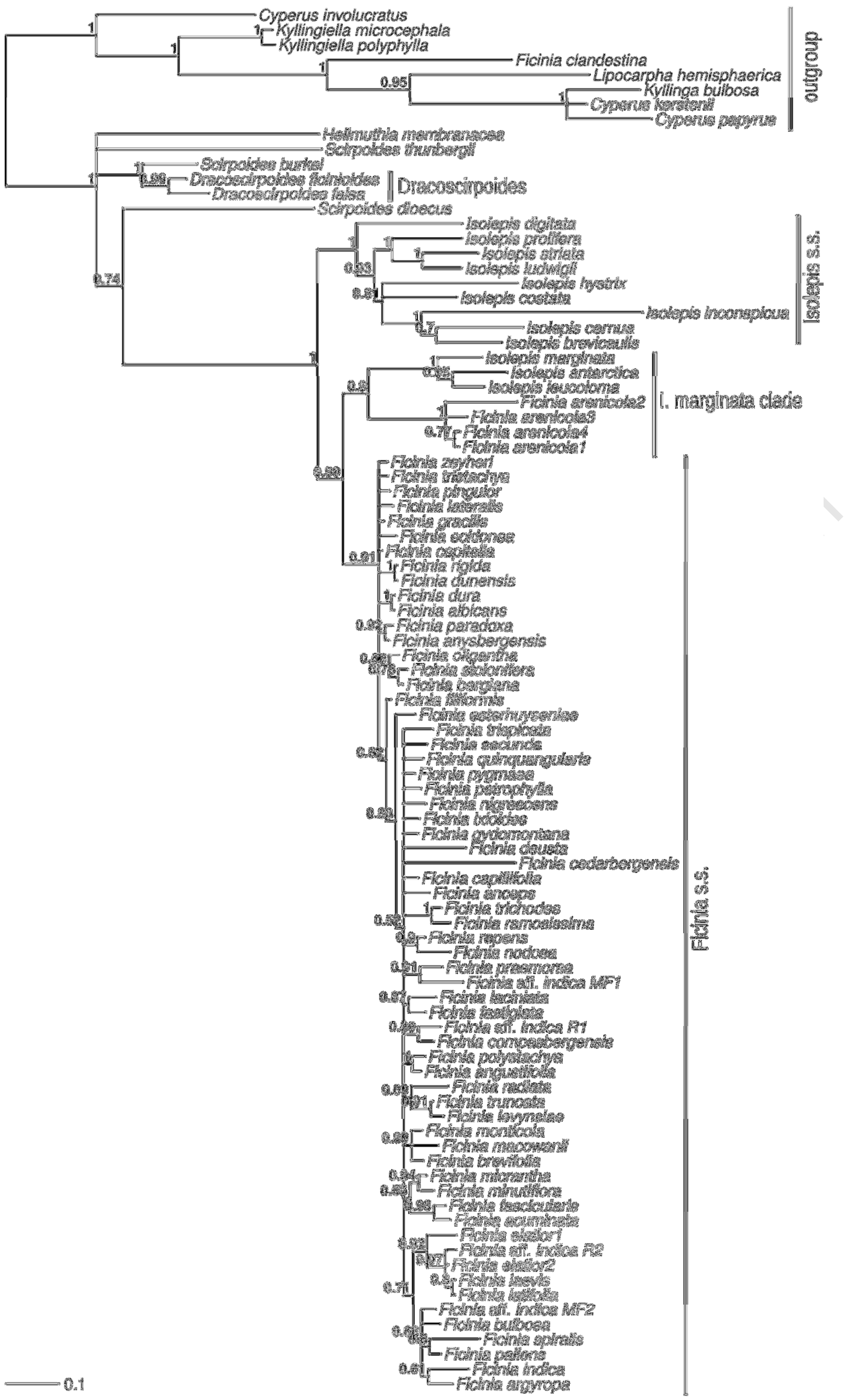

Figure 2.5. Bayesian tree of the combined analysis based on ITS and *rps16* data (93 taxa) for *Ficinia* the clade. Posterior probabilities (PP) of nodes are shown on branches.

2.3.3. Ancestral trait reconstruction

Figures 2.6a to 2.6i show how the observations of different morphological characters varied across the taxa and the reconstructed ancestral states for these characters. The majority of the taxa studied were perennials, with annuals only encountered in *Isolepis*. Annual life form was found in two clades, but one clade is characterized by a polytomy including *I. hystrix* (Figure 2.6a).

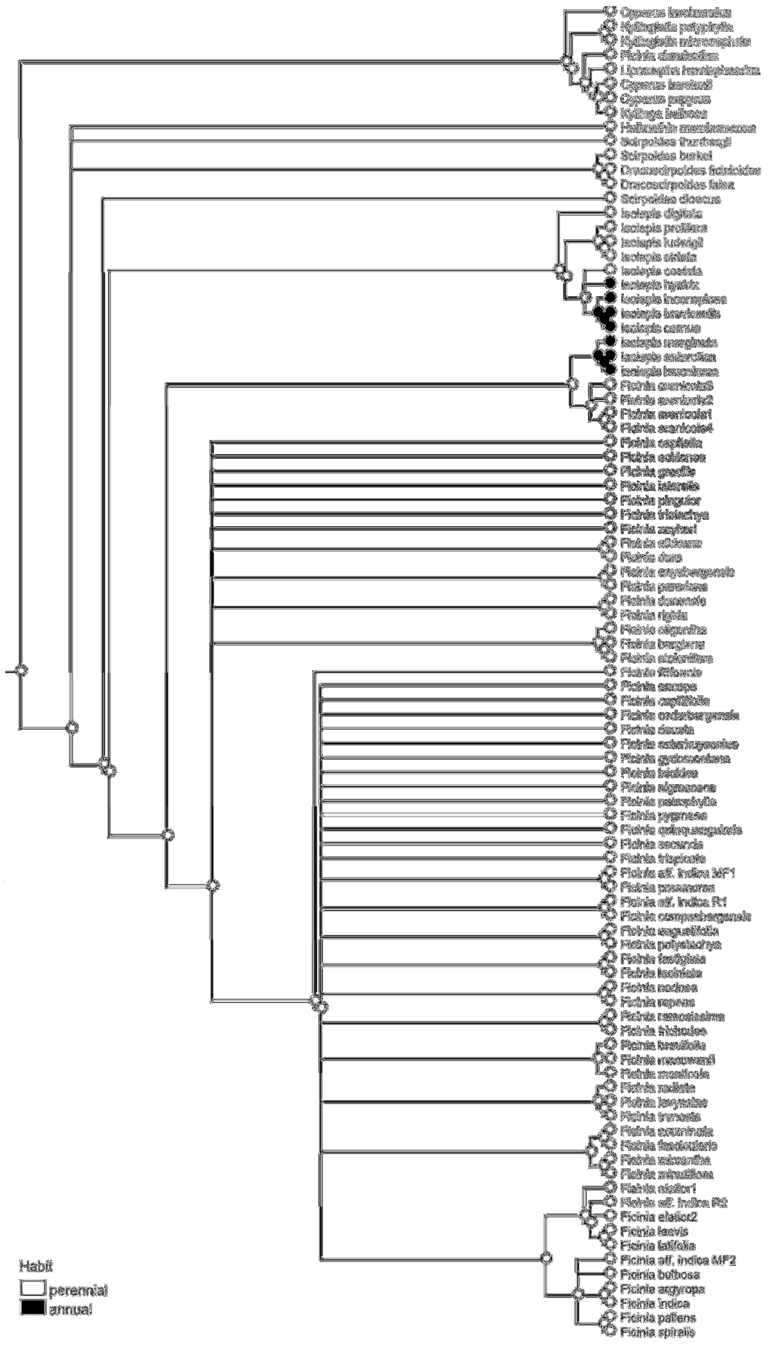

Figure 2.6a: Reconstruction of life form. Black represents the annual habit and White represents the perennial habit.

The life form is suspected to correspond to the size of the plants. For example, species from the genus *Isolepis* that are annuals mostly have very short culms whereas perennials, mostly from the genus *Ficinia,* possess longer culms (Figure 2.6b). However, there are some species of *Ficinia* (e.g. *Ficinia clandestina, F. radiata* and *F. pygmaea*) which are very short and perennial. This was observed when reconstructing macroevolutionary patterns for the culm length (Figure 2.6b).

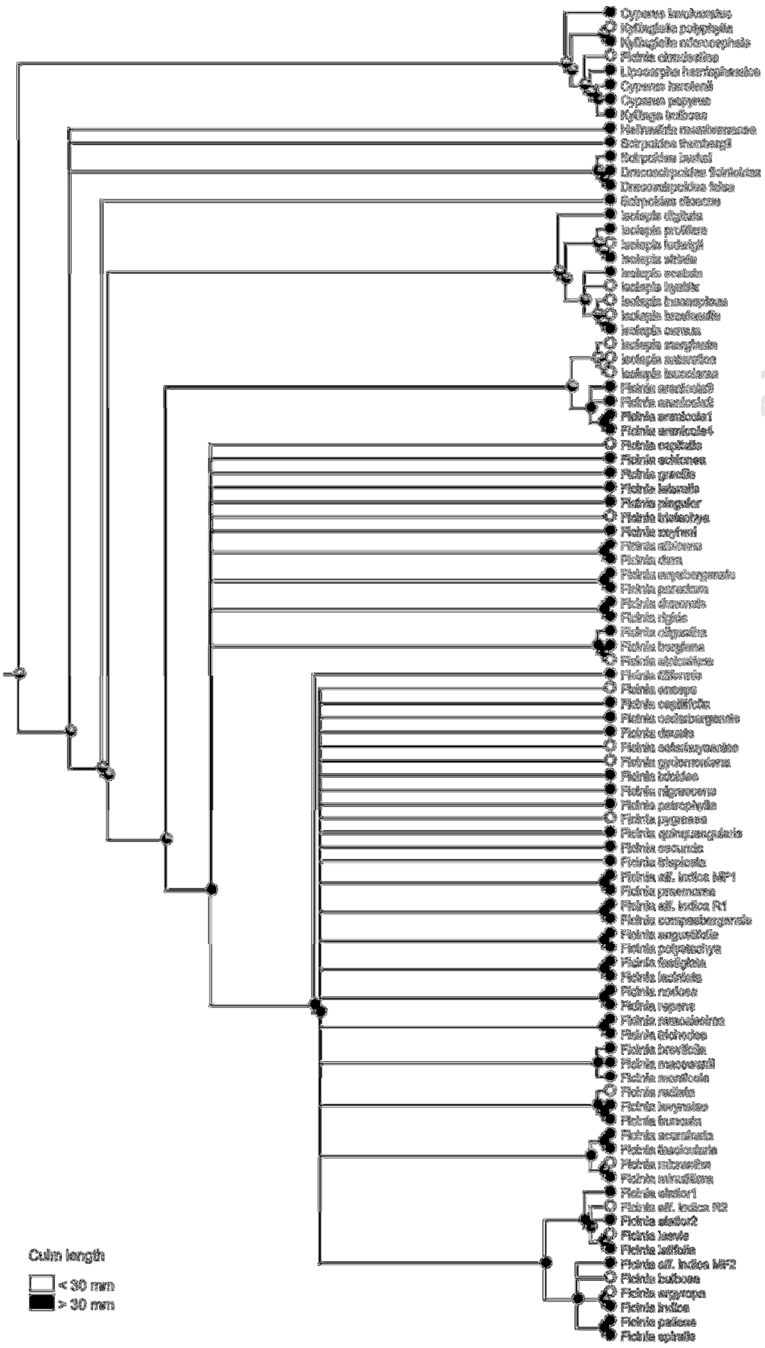

Figure 2.6b: Reconstruction of culm length. White represents culm length less than 30 mm and black represents culm length greater than 30 mm.

A number of *Ficinia* species bear a rhizome whereas others have stolons, and short rhizomes are observed in annual species of *Isolepis* (Figure 2.6c). For example, *F. stolonifera* and *F. bergiana* are sister species based on the molecular data but they differ morphologically: *F. stolonifera* possesses a stolon and *F. bergiana* possesses a long rhizome. However, the stoloniferous root is an ancestral character that can be used to diagnose some species in of the genus *Ficinia*.

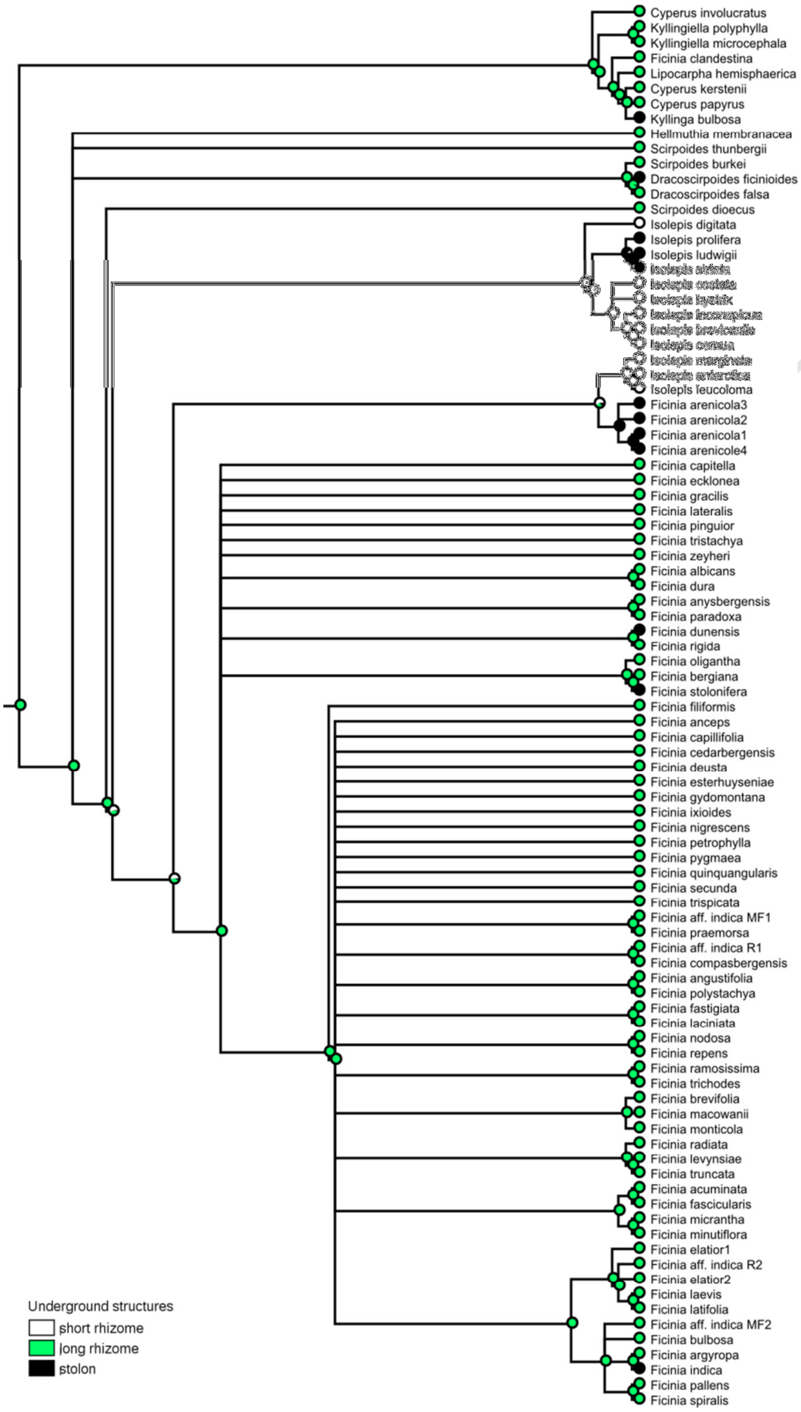

Figure 2.6c: Reconstruction of the underground structure. White represents the minute root structure, green represents rhizomes and black represents stolons.

There are a number of species in the *Ficinia* clade that possesses a papery leaf sheath appearance. The papery leaf sheaths are found only in *Ficinia* and not in any of the *Cyperus* species studied. Even *Ficinia* species with papery sheaths are scattered in the phylogeny (Figure 2.6d). The papery leaf sheath appearance can be used as a diagnostic character for the species within *Ficinia* since it only occurs in very few taxa (Figure 2.6d).

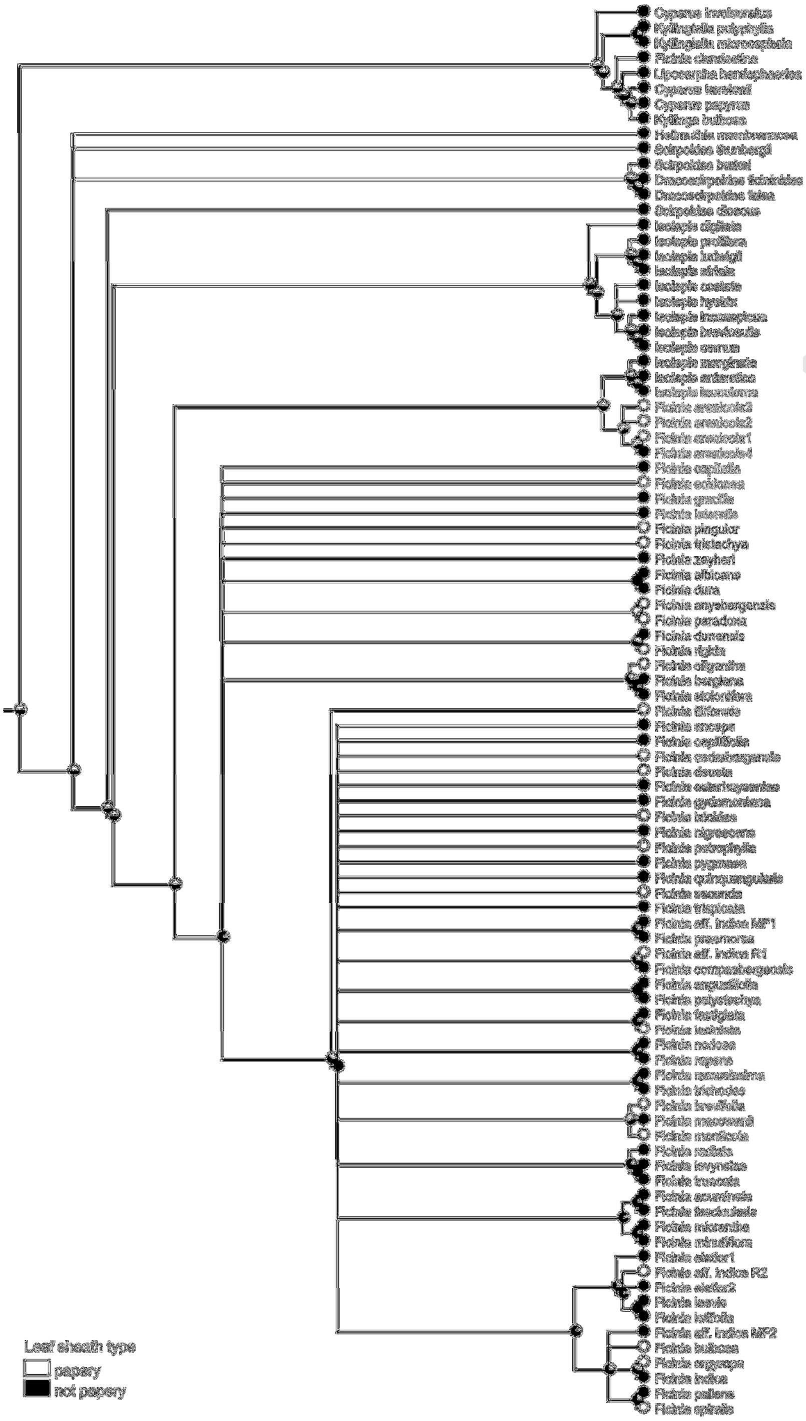

Figure 2.6d: Reconstruction of the leaf sheath appearance. White represents a papery leaf sheath and black indicates a leaf sheath that is not papery.

The presence of one internode is the ancestral character that is shared by species from the outgroup and ingroup (Figure 2.6e). Multiple internodes occur in *F. capillifolia*, *F. trichoides, F. ramosissima* and species of genus *Isolepis (I. striata)*.

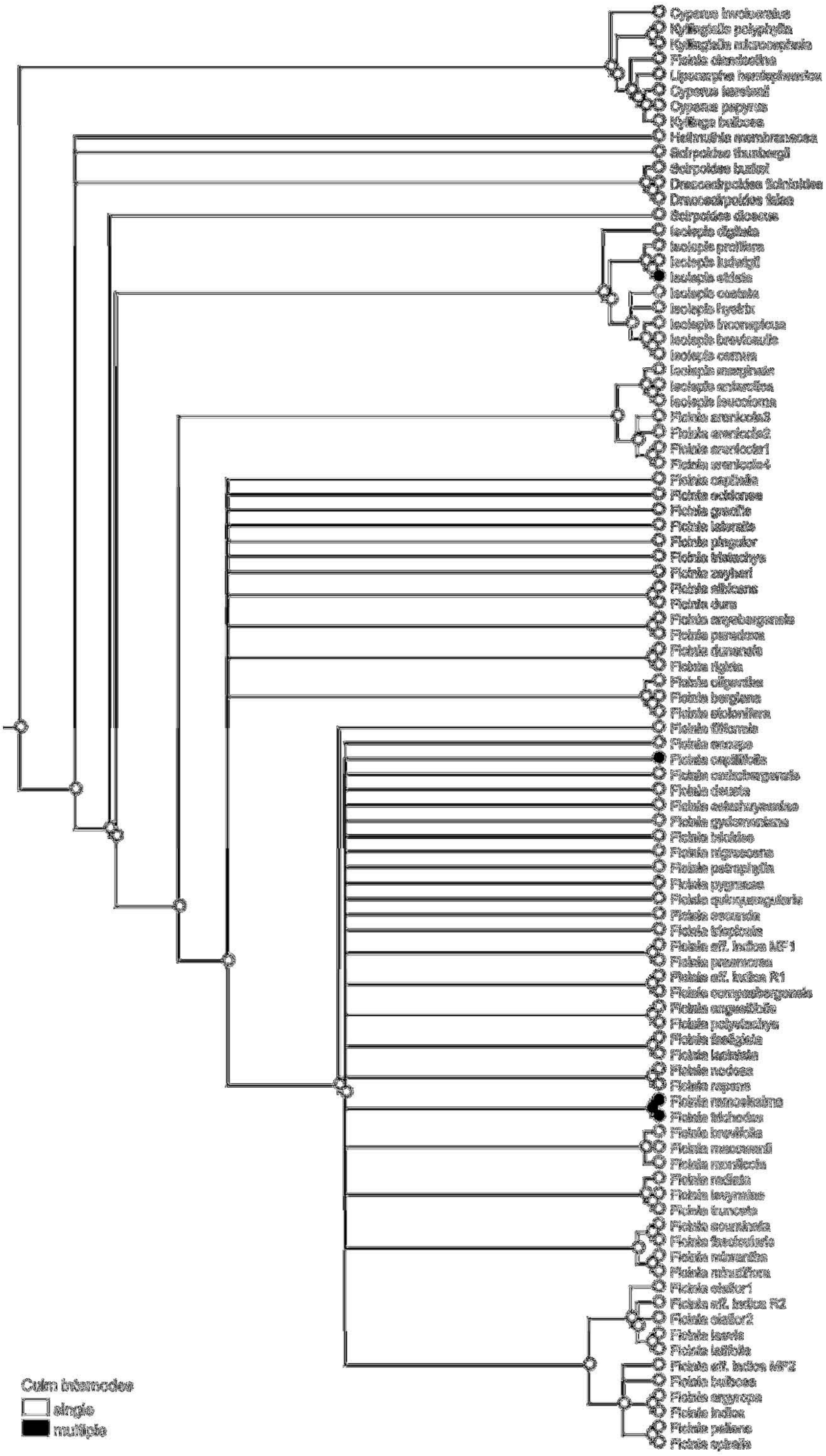

Figure 2.6e: Reconstruction of the number of internodes on the culm. Black indicates the species with multiple internodes and white represents the species with a single internode

Most of the taxa in the *Ficinia* clade exhibit a well developed leaf blade. In section Bracteosae (Arnold and Gordon-Gray, 1982), the absence of the elongated leaf blades occurs in *F. gydomontana*. Pfeiffer (1921) recognized a subsection of *Ficinia* (*Efoliatae*) comprising species that have reduced leaf blades. This study finds loss of leaf blade (under 5 mm long) to be a derived character in the *Ficinia* clade, evolving independently in *F. nodosa, F. repens, F. lateralis,* and *F. trispicata* (Figure 2.6f).

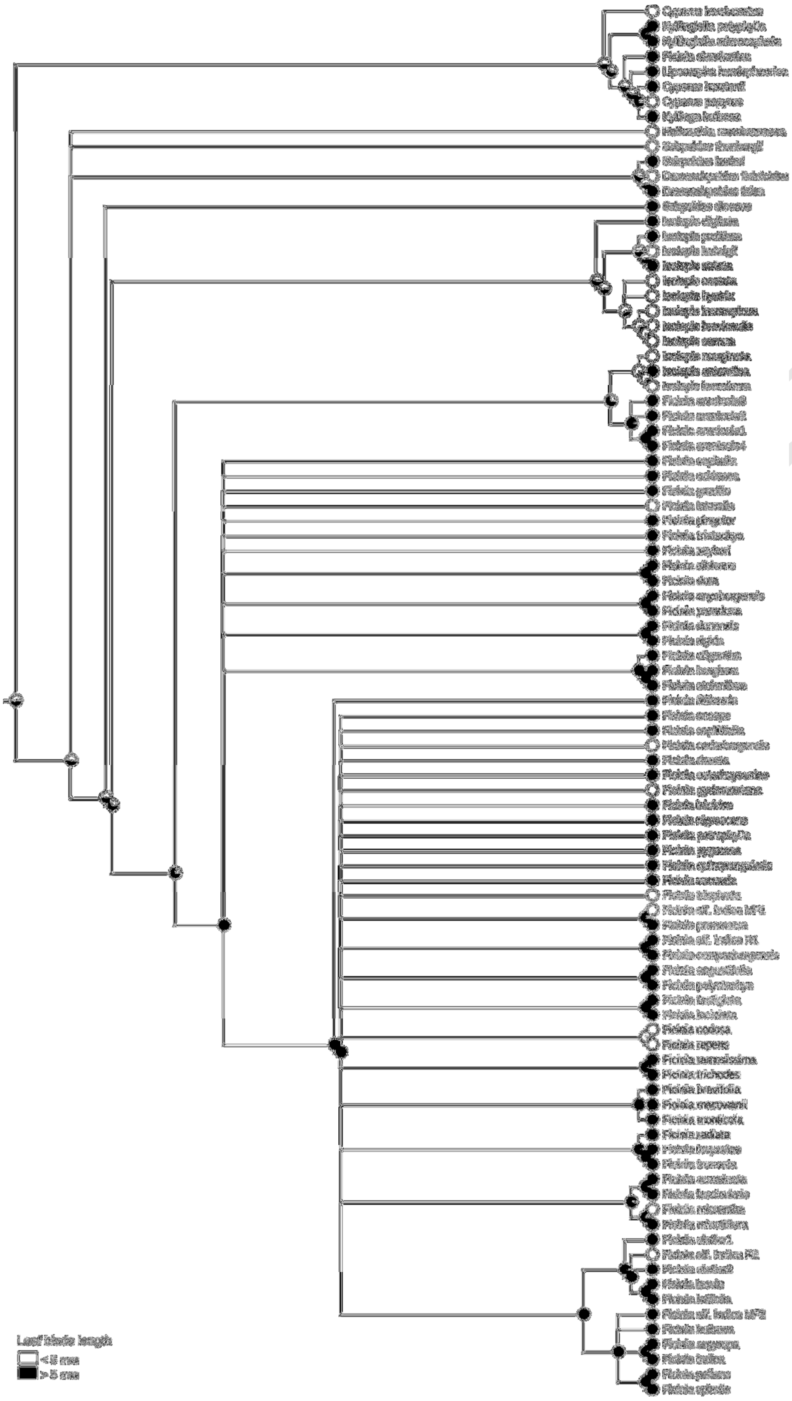

Figure 2.6f: Reconstruction of the leaf blade length. White indicates leaf blades less than 5 mm long and black indicates leaf blades longer than 5 mm.

Our results also confirmed capitate inflorescences to be ancestral in *Ficinia* (Figure 2.6g). In *Ficinia* clade, there is independent and multiple evolution of the spike, most notable in Pfeiffer's (1921) subgenus *Pseudoficinia* (*F. bulbosa, F. secunda*; not sister pairs). *Cyperus involucratus*, *C. papyrus, Scirpoides burkei* and *S. thunbergii* are the only species among the studied taxa with anthelate inflorescences, but anthelate inflorescences are more prevalent in the *Cyperus* clade.

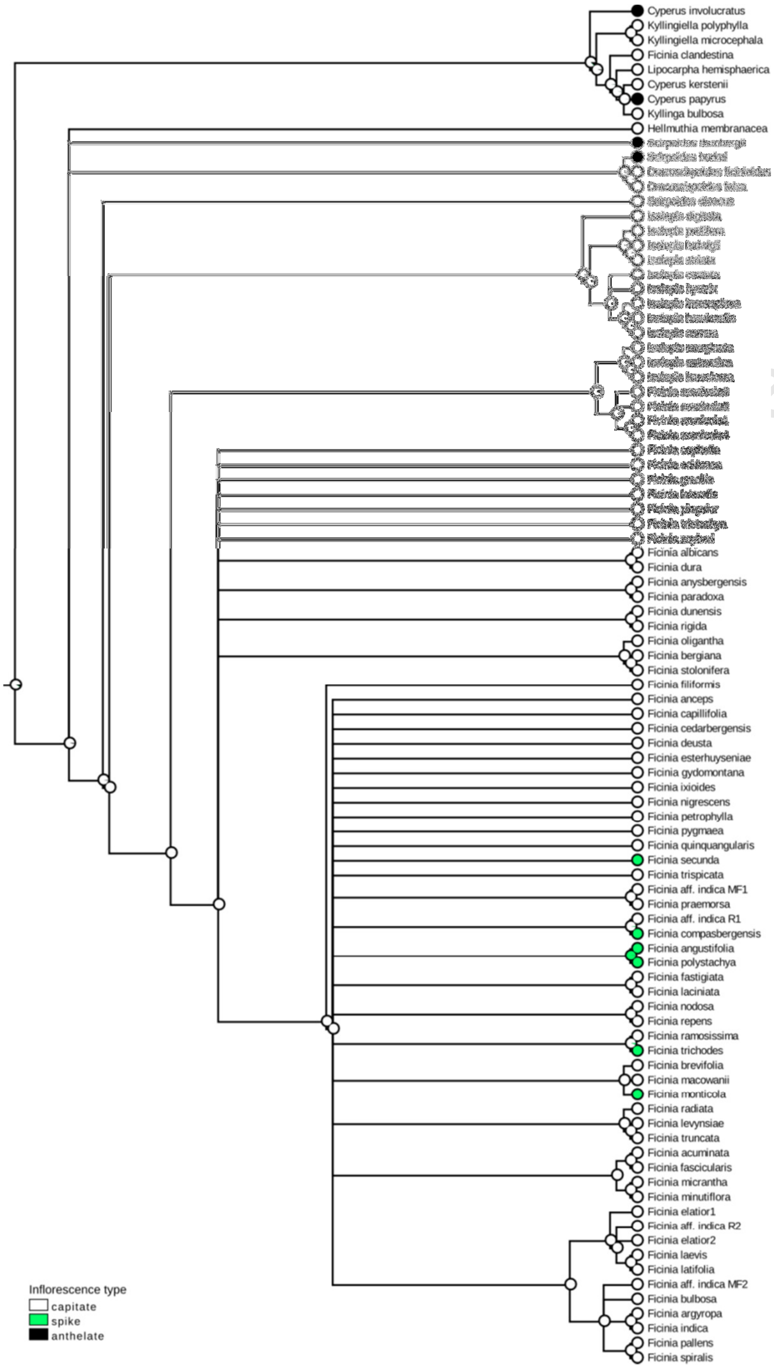

Figure 2.6g: Reconstruction of the inflorescence type. White indicates a capitate inflorescence, green indicates a spike and black is anthelate inflorescence.

Most *Ficinia* species possess glumes which are spirally arranged. Spiral glume arrangement (Figure 2.6h) is a plesiomorphic character for the *Ficinia* clade. Distichous glume arrangement has re-evolved independently in several species in the tree (*F. angustifolia, F. capillifolia, F. trispicata* and *F. trichodes*) and also in other species not represented in this study (*I. levynsiana* and *F. distans*). In the outgroup, distichous glume arrangement is widespread in *Cyperus* (e.g. *C. papyrus*) but spiral glume arrangement is found in *Ficinia clandestina* and *Kyllingiella* species.

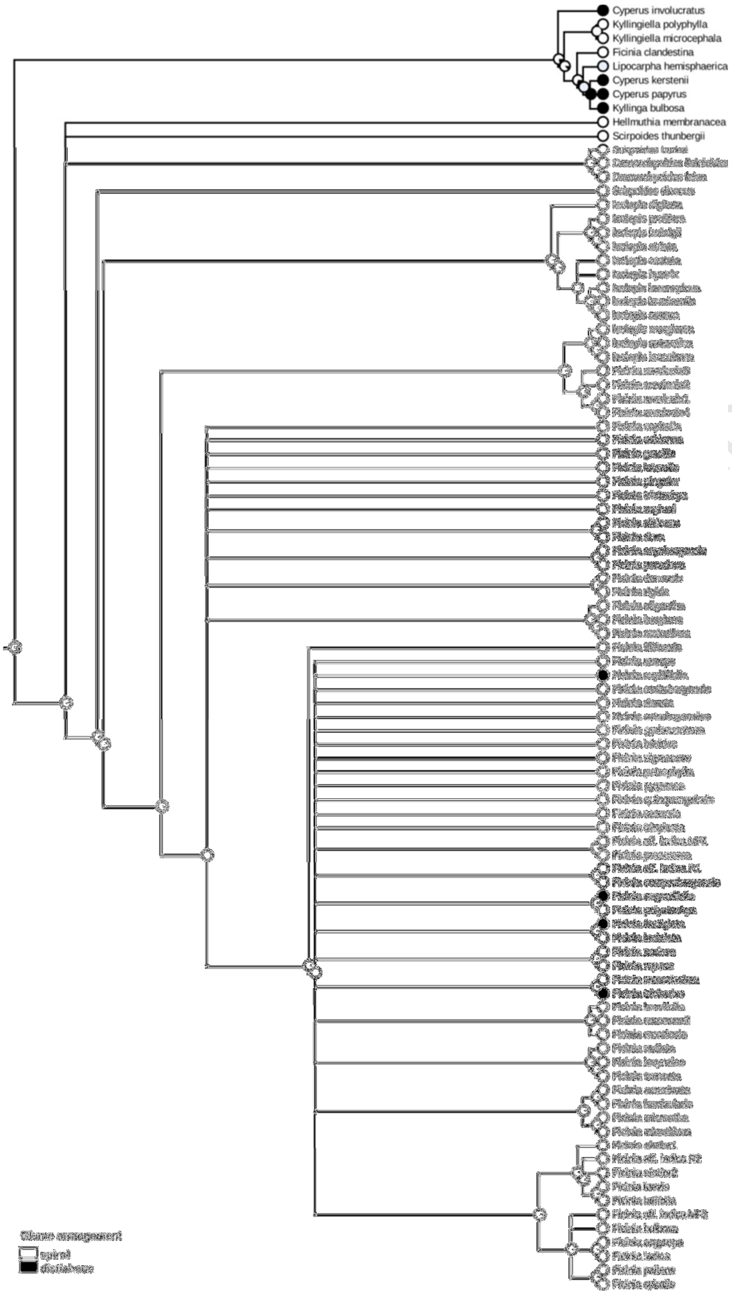

Figure 2.6h: Reconstruction of glume arrangement. White indicates spiral arrangement and black indicates distichous arrangement.

A gynophore, a short 'cup' of tissue enveloping the base of the young fruit, is a synapomorphy for the genus *Ficinia* (Figure 2.6i). Even though the species from the genus *Ficinia* have similar morphology to its sister *Isolepis* and *Scirpoides*, the presence of a gynophore is the diagnostic character for *Ficinia*. Absence of gynophore has secondarily evolved in *F. filiformis*. On the other hand, a gynophore is sporadically observed in specimens of *I. marginata*, thereby raising concerns that its evolution is labile.

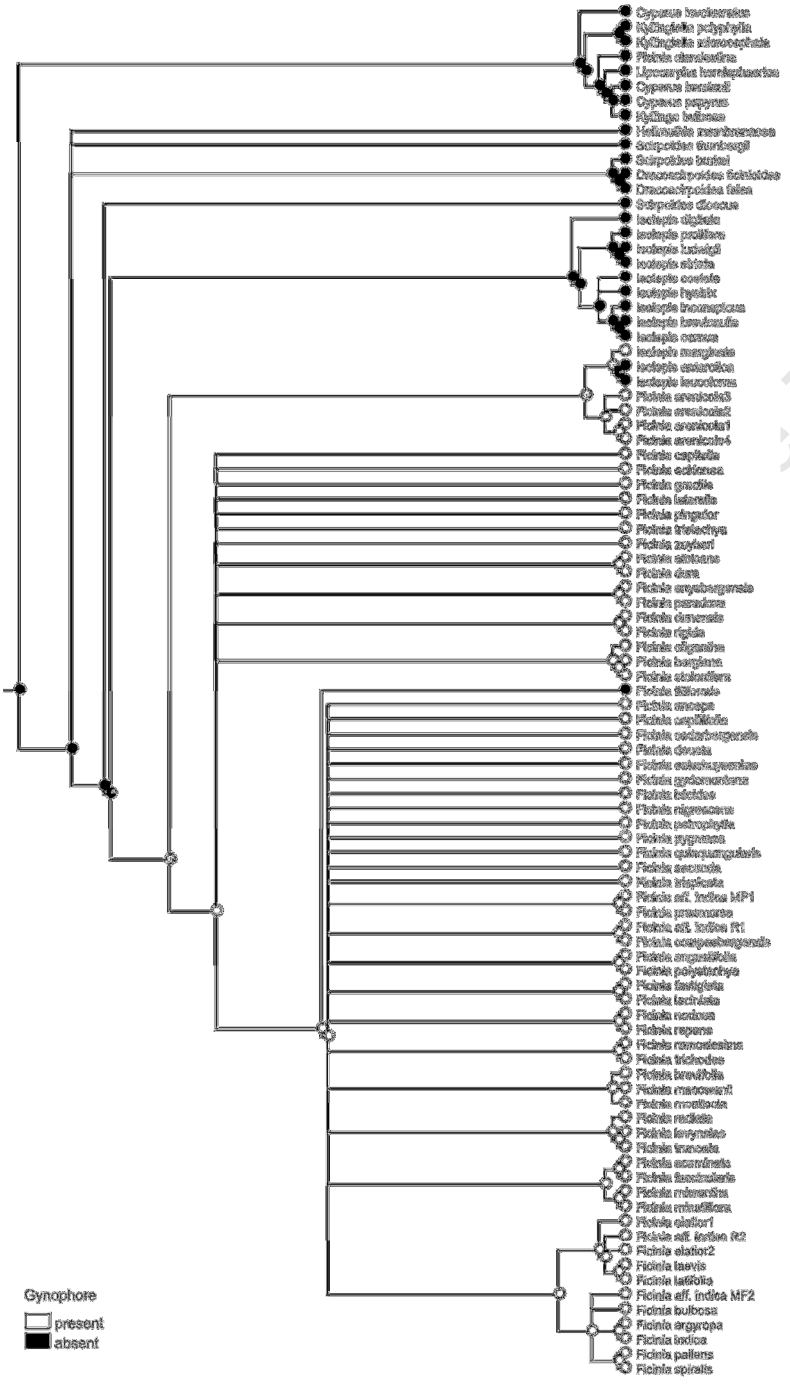

Figure 2.6i: Reconstruction of the evolution of the gynophore. White represents the species with the gynophore and black indicates the species without the gynophore.

2.4. Discussion

The current study employs a comprehensive taxon sampling to reconstruct the phylogenetic relationships in the *Ficinia* clade. Previous studies have focused mostly on the genus *Isolepis* (e.g. Muasya et al, 2001) and specific groups within the *Ficinia* complex (e.g. Muasya and de Lange, 2010). Overall, previous phylogenetic investigations were based on a low species sampling. Species sampling of all genera investigated in this study, however, is extensive, covering most important lineages within the genera *Dracoscirpoides* (66% of 3 species), *Ficinia* (75% of 80 species), *Hellmuthia* (100% of 1 species), *Isolepis* (52% of 76 species), and *Scirpoides* (60% of 5 species) for the complete dataset of 188 samples. This study uses plastid and nuclear DNA data and the markers resulted in congruent trees.

2.4.1. Phylogenetic reconstruction

Two DNA markers *(*ITS and *rps16*) were used to infer the phylogenetic relationships within the *Cypereae*. Two major clades (i.e. *Cyperus* and *Ficinia*) were recoverd from both the parsimony and Bayesian analyses, and our results corroborate the studies of Muasya and de Lange (2010) and Muasya *et al.* (2009a).

Overall, there were low levels of sequence variation in the DNA loci sampled resulting in poor node support values and several polytomies in the strict consensus trees. A large polytomy was observed in genus *Ficinia,* unlike in its sister clade (*Isolepis*). Based on the observation of low topological resolution and a low number of substitutions between closely related species of *Ficinia*, one may postulate that *Ficinia* is characterized by a recent rapid radiation while the main clades in *Isolepis* have older deeper nodes (Figure 2.4 and 2.5). There may have been morphological diversification in the absence of extensive accumulation of DNA mutations in the genus *Ficinia.* About 80% of *Isolepis* are annuals whereas *Ficinia* are perennial, and rates of molecular evolution in angiosperms are known to be linked to life history (Smith & Donoghue, 2010), hence deeper older nodes in *Isolepis* may be due to life history.

This study has shown that the Ethiopian species *Ficinia clandestina* belongs to the *Cyperus* clade, proven by both parsimony and Bayesian phylogenetic trees. Initially, *F. clandlestina* was described as a species of *Cyperus (C. clandestinus* Steud.), but Boeckeler (1887) transferred the species to *Ficinia*. Boeckeler interpreted the morphology of *F. clandestina* to be more similar to the Cape dune-slack species *F. pygmaea*. However, this interpretation has been disputed by later workers (e.g. Arnold & Gordon-Gray, 1978). A recent collection of a specimen with mature nutlets (Sebsebe Demissew and Muasya 6852), unknown until then, revealed that this taxon lacks a gynophore. Based on the absence of a gynophore (the synapomorphy for *Ficinia*) and their phylogenetic position, this study confirms Steudel's (1842) identification of the taxon as *Cyperus clandestinus*.

The base of the clade including the genera *Scirpoides, Hellmuthia, Dracoscirpoides* was not fully resolved (Figure 2.3). The lack of resolution might be partly due to some of the taxa being sequenced with only one marker. However, similar results were observed in previous studies (Muasya and De Lange, 2010, Muasya *et al.*, 1998, 2009). Thus, the poor resolution may be caused by slowly evolving lineages.

The strongly supported clade comprising *Isolepis/Ficinia* has four subclades (Figure 2.2: *I incomtula/I. hemiuncialis*; core *Isolepis; Isolepis marginata/F. arenicola*; core *Ficinia* clades). The genus (Figure 2.4, 2.5) *Ficinia* was embedded in a paraphyletic *Isolepis* (Figure 2.2, 2.3). One may also postulate that the genus *Ficinia* is paraphyletic, as the *Isolepis marginata* clade is sister to the *F. arenicola* clade forming a moderately supported node. This was consistent with the previous recent studies (eg. Muasya et al, 2009, Muasya *et al.* 2008). This study thereby confirms the results obtained from previous investigations where *Ficinia* and *Isolepis* formed a clade sister to *Hellmuthia* and *Scirpoides* (Muasya et al, 1998), and *Isolepis marginata* clade sister to the *Ficinia* s.s. clade (Muasya et al., 2009a, b; Muasya and De Lange, 2010). The close affinity between *Ficinia* and *Isolepis* is supported by morphological and embryological data (Goetghebeur, 1986, 1998), and these genera have consistently been classified in the same tribe.

The phylogenetic results obtained from this study did not support the infrageneric classifications by Clarke (1898), Pfeiffer (1921), or Levyns (1950). None of the previous classification of the species at subgeneric and sectional levels are supported as monophyletic gourps, and the segregate *Sickmannia* is embedded within *Ficinia*. There is partial support for Pfeiffer's (1921) section *Acrolepiformusis,* where *Ficinia trichodes/ F. ramosissima* are sister species but their clade does not include the third species included in section by Pfeiffer (*F. zeyheri*).

2.4.2. Ancestral state reconstruction

Among the taxa studied, *Isolepis* has been identified as the only genus with the annual life form. *Ficinia* exhibit a perennial life form and it helps to distinguish *Ficinia* from *Isolepis* (Figure 2.5e). Many perennial species in *Ficinia*, *Scirpoides* and *Hellmuthia* were found to be taller than the annual *Isolepis*. Most annuals have a minute root type whereas the perennials have rhizomes or stolons. Annual life form, a unique strategy whereby plants complete their life cycle in one growing season, is an uncommon life history in the CFR and contributes about 10% of vascular plants (Goldblatt & Manning, 2000). Among monocots, *Isolepis* is among the few lineages which have evolved this strategy in the CFR, which is completely absent among large families such as Iridaceae, Orchidaceae and Restionaceae (Goldblatt & Manning, 2000).

Rhizome presence is the ancestral character in *Ficinia* (Figure 2.8d) and stolons have evolved multiple times and are found in species like *F. stolonifera*, *F. indica and F. dunensis (*Figure 2.8d). The variations in the underground structure or derived stems are clearly well developed and can be used as a useful descriminating factor for some species within the clade. Two species, *F. dunensis* and *F. pallens,* show variation in the development of stolons (Arnold & Gordon-Gray, 1982).

Two distinct types of leaf sheath are presented among the *Ficinia* clade. This character was scored based on the absence or the presence of the papery leaf sheath. Papery leaf sheaths usually have the ligule which is well developed and up to 3 mm in length in some taxa. This form is very useful in separating some taxa. A papery leaf sheath is an autapomorphic trait that occurs in some species of *Ficinia (F. argyropa, F. anysbergensis, F. brevifolia, F. bulbosa, F. cedarbergensis, F. deusta, F. dura, F. ecklonea, F. grandiflora, F. ixioides, F. laciniata, F. monticola, F. oligantha, F. paradoxa, F. pinguior* and *F. stolonifera).* Considerable overlap or variations occur in the presence and degree of the development of leaf blade. One cannot completely rely on the leaf blade length as the distinguishing character. However, it is a very useful distinctive character for *F. arenicola* and *F. petrophylla* since they are the only *Ficinia* species with leaf blades that are longer than the culms.

Having multiple internodes on a culm is a derived trait that has evolved independently among species of *Isolepis (I. ludwigii* and *I. striata)* and *Ficinia (F. trichodes, F. capillifolia, F.*

ramossisima). The majority of Cyperaceae are scapose (Goetghebeur, 1998) and multiple internodes have arisen independently in many clades. Within the *Ficinia* clade, this trait is found among taxa growing as mats in wetlands (*Isolepis* subgenus *Fluitantes*, in this study represented by *I. ludwigii* and *I. striata*), and in taxa scrambling in fynbos vegetation (*F. trichodes*, *F. ramossisima* and *F. capillifolia*).

Muasya *et al.* (2009a) reported that a capitate inflorescence type is the ancestral state in *Ficinia*. The results in this study showed that most species possess a capitate inflorescence whereas few instances of the evolution to the spicate state occurred throughout *Ficinia*. Presence of stem-like involucral bract and pseudolateral capitate inflorescence is most common in *Ficinia* clade. However, *F. radiata* differs from other species of *Ficinia* by its multiple leafy bracts and it had been placed in a monotypic genus (*Sickmannia*; Levyns, 1950) or subgenus (Pffeiffer, 1921). *Cyperus involucratus*, *C. papyrus* and *Scirpoides burkei* exhibit the anthelate inflorescence, which makes them different from other species in the clade.

Ficinia possesses glumes which are spirally arranged and this relationship has also been reconstructed by Muasya *et al.* (2009a). Only few species from the genera *Ficinia* and *Isolepis* exhibit the distichous glume arrangement, where it appears to be autapomorphic. A study by Muasya *et al.* (2009a) used the distichous glume arrangement to diagnose *Cyperus* sensu lato and the spiral arrangement was described as a plesiomorphic state for the family as a whole. *Ficinia clandestina* was initially described as a *Cyperus* and subsequently transferred to the genus *Ficinia* using morphological data (including spiral glume arrangement). The DNA data used in this study confirms the morphological observations, showing 1) that *Ficinia clandestina* belongs to the genus *Cyperus*, and 2) that spiral glume arrangement is a secondarily derived character i.e. a reversion to the ancestral condition of the tribe. Spiral glume arrangement is recorded in a number of taxa in the *Cyperus* clade (e.g. species in *Kyllingiella*). Consequently, using glume arrangement as a single key trait to separate lineages within Cypereae should be avoided, although taxa with distichous glume arrangement are most likely to belong to the genus *Cyperus*.

Previous studies used the gynophore presence on *Ficinia* species as a key morphological character that distinguishes *Ficinia* from other perennial genera in the family Cyperaceae (Muasya et al, 2009a, b). The gynophore has been recognized in almost all *Ficinia* species sampled in this study with the exception of *F. filiformis*. The presence of the gynophore disk at the base of the nutlet on some specimens of *I. marginata* gives some evidence that these species have a closer affinity to *Ficinia.* Molecular data showed *I. marginata* to be closely related to the *F. arenicola* clade, which has a well developed gynophore.

2.5. Conclusions

Ficinia is a sub-Saharan African clade that has its centre of diversity in the CFR and about 90% of the species within the genus are endemic to the region. The current investigations have found the genus *Ficinia* to be paraphyletic with annual species of the clade (*I. marginata* and allied taxa) currently included in *Isolepis*. The research on whether there is support for inclusion of *I. marginata* and allied taxa into *Ficinia* based on molecular data need to be conducted. Our results obtained from the phylogenetic reconstruction confirmed previous classic taxonomic classifications carried out by Schrader, Clarke, and Levyns.

Our study includes a comprehensive sampling and the phylogenetic relationships provide novel insights in the evolution and classification of newly sampled *Ficinia* lineages. The use of the morphological characters such as leaf sheath type, growth habit, leaf blade length, and glume arrangement added a significant value in providing an overview in distinguishing most of the species occurring in the clade *Ficinia*.

CHAPTER 3

THE ROLE OF ECOLOGY IN PROMOTING THE RADIATION OF *FICINIA*

3.1. Introduction

The hyperdiverse Cape flora is a product of old radiations that have been preserved as well as recent rapid radiations of species (Linder, 2008). There is ample phylogenetic evidence that a number of Cape lineages started to radiate during the late Miocene and early Pliocene (Linder & Hardy 2004, Linder 2005a, Verboom et al., 2009). Radiation of lineages can be triggered as a consequence of key environmental deviations changing the selective regime and shaping a novel adaptive zone (Simpson, 1953). The advocated model of adaptive divergence of the Cape flora caused by major environmental variations offers two contending hypotheses that could enlighten declining speciation rates. The first hypothesis suggests that the main environmental changes during the Pliocene were followed by a period of environmental constancy (Cowling et al., 1996a, 2009, Linder 2003), resulting in Cape lineages showing decreasing rates of speciation subsequent to their early radiation. The second hypothesis proposes that diversification is characterized by an initial adaptive period, with species radiating into vacant ecological niches, followed by a declined speciation rate as a result of ecological niches gradually becoming saturated (Linder, 2003).

The diversity in the Cape Floristic Region (CFR) is mostly the result of the diversification of a limited number of clades that originated and radiated within the Cape (Linder 2003), with some estimates of speciation rates in the Cape being higher than in tropical rainforests (Latimer et al., 2005). The *Ficinia* clade is one of the important Cape floral clades and the largest among the sedges (Cypereceae), forming a conspicuous part of the fynbos. Consequently, the genus *Ficinia* provides an interesting study group to reveal the patterns of diversification, and to test the hypothesis that the clade radiated rapidly in the Miocene, contemporaneously with other Cape clades. Indeed, recent and rapid radiations have been validated for numerous plant groups in southern Africa (*Phylica*, Richardson et al., 2001; Ruschioideae; Klak et al., 2003) and it has been suggested that the whole flora might reflect such a recent burst of speciation (Levyns 1964; Linder et al., 1992; Sauquet et al., 2009).

Allopatric and parapatric speciation is thought to be a major mode of speciation, especially in the CFR (Linder, 2005; Goldbaltt and Manning, 1996; Johnson, 1996a; van der Niet et al., 2006; Van der Niet, 2008). Goldblatt (1978) highlighted allopatric speciation based on the observation of patterns of allopatry between groups of closely related plant species of Iridaceae (e.g. *Hexaglottis).* However, Goldblatt & Manning (2002) suggested a large role for parapatric speciation along habitat and soil boundaries which often occur in close proximity. However, the prediction from this model of parapatric speciation in a mosaic of microhabitats that species should have highly restricted distribution ranges and that closely related species occur in close geographical proximity (Linder, 2003) has not yet been formally tested.

Ecology has been long considered to be integral to divergence and speciation events (Dobzhansky, 1951; Simpson, 1953; Endler, 1997; Bush, 1994). However, its importance has often been eclipsed by other debates, such as whether reproductive isolation can evolve in sympatry and the relative importance of selection versus drift (Barton, 1996). These debates are fuelled by the fact that few empirical studies have distinguished the relative importance of natural selection versus drift in the evolution of reproductive isolation (Coyne, 1992). Previous studies on morphologically diverse taxa suggest that natural selection caused by shifts in ecology or invasions of new habitats can cause extremely rapid divergence (Losos et. al, 1997) and might play a prominent role in speciation (Schluter, 1996; Smith et.al 1997).

Ecological factors such as soil content (nutrients), climate and topography have been suggested as major drivers of speciation in the Cape flora (Linder & Vlok, 1991; Linder, 2003, 2005; Van der Neit & Johnson, 2009). The CFR is categorized by a mosaic of sharply different soil types, a complex landscape, and differences in rainfall and seasonality. Various combinations of these physical parameters result in a large number of distinct niches that are often in close geographical proximity. These observations led Linder (1985, 2003) and Goldblatt & Manning (2002) to propose a model of speciation across steep environmental gradients. The proposed model of speciation is supported by the observations that i) there is high species turnover along soil gradients (Cowling, 1990), ii) that closely related species often occur on different soils (e.g Goldblatt, 1982; Kurzweil et al., 1991; Schnitzler et al., 2011), and iii) that closely related species occurring on different soil types are differentially adapted (e.g. Verboom et al., 2004). For example, it has been suggested that the sandstones of the Cape Fold Mountain belt are readily leached. The shales, mudstones and granites, and the entire Namaqualand region produce soils that generally have a much higher pH and are more fertile than those derived from sandstones (Lambrechts, 1979). However, there is indication that closely related species that occur in close geographical proximity inhabit different climatic positions (e.g. Linder & Vlok, 1991) and that there is an adaptive element associated with habitat shifts. The association between plant functional traits and the ecological environment where the plant grows has been shown to occur between sites which differ only subtly, even amongst closely related taxa (e.g. Verboom et al., 2004). The Cape is topographically complex and, consequently, geographical separation by physical barriers caused by sharp altitudinal gradients might cause speciation (Cowling et al., 2009). Furthermore, the edaphic heterogeneity might also be important, providing a mosaic of divergent selection pressures promoting divergence and speciation (Linder 2003). The geographical separation and edaphic heterogeneity predict that sister species should tend to be isolated geographically and that recently diverged sister species should tend to occur in different edaphic environments (Schnitzler et al., 2011).

Adaptation to different pollinators is also suggested as a factor that promotes the speciation in the CFR, even though the speciation model and the exact role that pollinators play in the speciation process is still controversial (Goldblatt & Manning, 1996; Johnson, 1996a, 2006; van der Niet et al., 2006). Fire was also proposed to promote speciation in the CFR. However, the model to explain how fire would promote speciation is difficult to prove and the role of fire seems to be unforeseen, with ultimately geographical separation driving speciation (Cowling et al., 1992). Linder (2003) suggested that a shift in fire-survival strategy itself can drive speciation associated with differences in growth form, and phenological differences which indeed could isolate populations from each other. These strategies are coupled with different life histories which could result in a reduction of gene flow between populations and avoidance of competition for resources (Linder, 2003). The proposed hypothesis is that sister species should frequently have contrasting fire survival strategy, but it appears that reseeding lineages have diversified more than lineages that resprout (Schutte et al., 1995). However, the mechanism is not clear, but could encompass shorter generation time resultant in higher rates of molecular evolution in reseeders compared with resprouters (Cowling 1987). Van der Niet & Johnson (2009) found that sister species in the Cape frequently differ in general habitat, pollinators, and fire-survival strategy.

In this chapter, we estimate the timing of the origin of the *Ficinia* clade (*Ficinia, Isolepis, Hellmuthia* and *Scirpoides*) and patterns of speciation using both plastid and nuclear DNA sequence data. It is hypothesized that the *Ficinia* clade is a product of the increased speciation of the Cape flora associated with late Miocene aridification, and that sister species pairs in genus *Ficinia* occupy different ecological niches.

The key objectives are:

1. To investigate the timing on the divergence rates of *Ficinia* clade
2. To test whether ecology plays a role on diversification within the genus *Ficinia*.

3.2. Materials and Methods

3.2.1. Source of DNA data

DNA was extracted from freshly collected material. Alternatively, leafy shoots or photosynthetic stems were dried in silica gel, or samples were obtained from previous studies dealing with Cyperaceae. More complete details on the methodology (i.e. extraction, amplification and sequencing) and the sampling of taxa used were presented in Chapter 2 of this dissertation. I have used the same dataset as in chapter 3 (Matrix B).

3.2.2. Estimation of divergence dates, calibration points

The calibration points for the dating analysis were obtained from a previous family-level study (Besnard et al., 2009). We used minimum and maximum age constraints for different nodes (see Figure 3.1) to date the *Ficinia* clade. Calibration points were modelled as a normal distribution whose mean is equal to respective node age and the standard deviations shows upper and lower bounds. Calibration points were selected on only well supported clades from the Bayesian tree obtained in Chapter 2 (Figure 2.5). The crown group of *Isolepis–Ficinia–Hellmuthia* clade was set to 28.15 million years ago (Mya) with a standard deviation of 5.0. The most recent common ancestor (MRCA) of species occurring in the *Isolepis–Ficinia* clade has the mean of 18.6 Mya and the standard deviation of 4.0.

The dating analysis was carried out with a Bayesian relaxed clock analysis using BEAST v. 1.5.4 (Bayesian Evolutionary Analysis Sampling Trees) (Rambaut & Drummond 2007). The ages of the species within the clade were obtained by applying the Bayesian uncorrelated log-normal clock without employing topological constraints. The tree prior was modelled under the birth–death (constant speciation and extinction, extinction greater than 0) speciation process and the remaining priors were set at their default values. We applied the same substitution models used in the MrBayes analysis (Chapter 2) selected by Modeltest (i.e. ITS: GTR+I+Γ and *rps*16: GTR+Γ). The trees from the first 10% of samples were discarded as a 'burn-in' based on an assessment of convergence in TRACER v. 1.5 (Rambaut and Drummond 2007). The maximum-clade-credibility tree was built using TreeAnnotator v. 1.5.4 (part of the BEAST package) after removal of the appropriate number of burn-in generations and the tree was illustrated in the program FigTree v. 1.3 (Rambaut & Drummond, 2007).

3.2.3. Diversification rates

A lineage-through-time (LTT) plot was recovered from the dated tree in order to visualize the temporal dynamics of diversification in *Ficinia* clade using the *ape* (Paradis *et al.* 2004) package for R v. 2.11.0 (R Development Core Team, 2010). Relative cladogenesis tests were performed to detect rapid variations in the rates of species divergence through time (Purvis et al., 1995), and net diversification rates under a constant-rate lineage birth–death model were calculated for each tree and key clade using the method of Magallón & Sanderson (2005). This was done to allow the comparison to rates estimates for the members of *Ficinia* clade that occurs mostly in the CFR. The birth–death model (constant rate model) assumes that diversification rates do not vary through time (Nee, 2001).

3.2.4. Soil sampling and analysis

At each sampling locality (CFR, 21 localities), three replicates of soil samples were collected using an auger or a garden trowel taking a slice of soil down to 10–15 cm deep. Analyses of soil samples were restricted to species pairs that had medium to strong support (BS of more that 50%) in the dated phylogeny. The pairs that were selected are listed in Table 3.1. The samples were air-dried, sieved to pass through 2-mm pores and sent to BemLab Private Laboratory, Somerset, South Africa for analysis of the concentration of the following soil nutrients: phosphorus (Bray II P), total nitrogen (N), potassium (K), iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na), soil pH and the T-value, which is the sum of the concentrations of all exchangeable cations. The total N concentration was determined using the combustion method using a LECO FP-528 nitrogen analyser (Leco Corporation, St. Joseph, USA). Nitrogen concentration was measured by converting the soil N to N_2 that was measured by thermal conductivity cell (Yeomans & Bremner, 1991). Available P was determined by extracting 2 g of soil in Bray II solution. The samples were filtered through a

Whatman No. 2 filter paper and diluted to 200 ml before concentration of K, Ca and Mg were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista MPX, Mulgrave, Australia).

Table 3.1: List of sister species pairs selected

Sister pairs	
A.	*F. levynsiae* vs *F. Truncata*
B.	*F. brevifolia* vs *F. macowanii*
C.	*F. angustifolia* vs *F. polystachya*
D.	*F. anysbergensis* vs *F. paradoxa*
E.	*F. bergiana* vs *F oligantha*
F.	*F. elatior* vs *F. Laevis*
G.	*F.* sp. nov. D (4583) vs *F. praemorsa*
H.	*F. dunensis* vs *F. rigida*

3.2.5. Statistical analysis of ecological data for selected sister pairs

Each variable was log-transformed using Microsoft Excel before performing statistical analyses in order to avoid disproportionate effects on the results. All the variables were approximately normally distributed and bivariate relationships showed no significant departure from normality after transformation. The box-and-whisker plots were produced and *t* tests for each variable in turn were used to test the null hypothesis of equality of mean nutrient concentration between sister species of *Ficinia,* in order to identify the elements influencing the separation among sister pairs.

Evidence for geographical speciation was assessed by determining cooccurrence of sister species of *Ficinia* in quarter degree squares (QDS), together with flowering times and altitude, based on extensive specimen locality data (own observations and specimens at Bolus and SANBI Herbaria). The cooccurrence of sister species across QDSs was calculated as Jaccard's similarity index, *J,* for each species pair. The null distribution was created for each pair by removing QDS where neither member of the pair occurred, randomizing the occurrence of each species 1000 times within the QDS that remained (thereby keeping the species range size constant), and recalculating the *J* index on each replicate. The observed *J* (based on the actual data) was then compared with that distribution of 1000 random *J* values to get the *P* value, that is, the probability of getting a value less than or equal to the observed value by chance.

3.3. Results

3.3.1. Phylogenetic relationships and molecular dating of *Ficinia* clade

The BEAST analysis recovered a similar topology (Figure 3.1), but a better resolved tree, compared to the BI from MrBayes presented in Chapter 2 (Figure 2.5). There is higher node support, with strong posterior probabilities (*PP*>0.95) for the inclusion of *Ficinia clandestina* in the *Cyperus* clade. Within the *Ficinia* clade, there is strong support for i) *Dracoscirpoides* as sister to the remaining taxa, ii) for the sister-group relationship between *Isolepis* and the *Ficinia* clade including *I. marginata* group, iii) for *I. marginata* (and allied species) as sister to the Eastern Cape-distributed *F. arenicola*, and iv) for two clades in the core *Ficinia*. The sister-species pairs with PP > 0.60 were identified from the dated phylogeny (Figure 3.1). Although 16 sister-species pairs were identified, only eight pairs were assessed for similarity of means, due to logistical challenges such as species occurring in localities far from Cape Town.

Cypereae are resolved into the *Cyperus* and *Ficinia* clades, with a stem node age in the early Oligocene (33.29 Mya). The sampling in the *Cyperus* clade is too sparse to allow meaningful interpretation of the ages, but *Ficinia clandestina* is part of the C_4 *Cyperus* clade, which has a crown age of 13.3 Mya. The *Ficinia* clade has a crown age of 24.75 Mya, while the species-rich *Isolepis*/*Ficinia* clade has a crown age of 14.86 Mya.

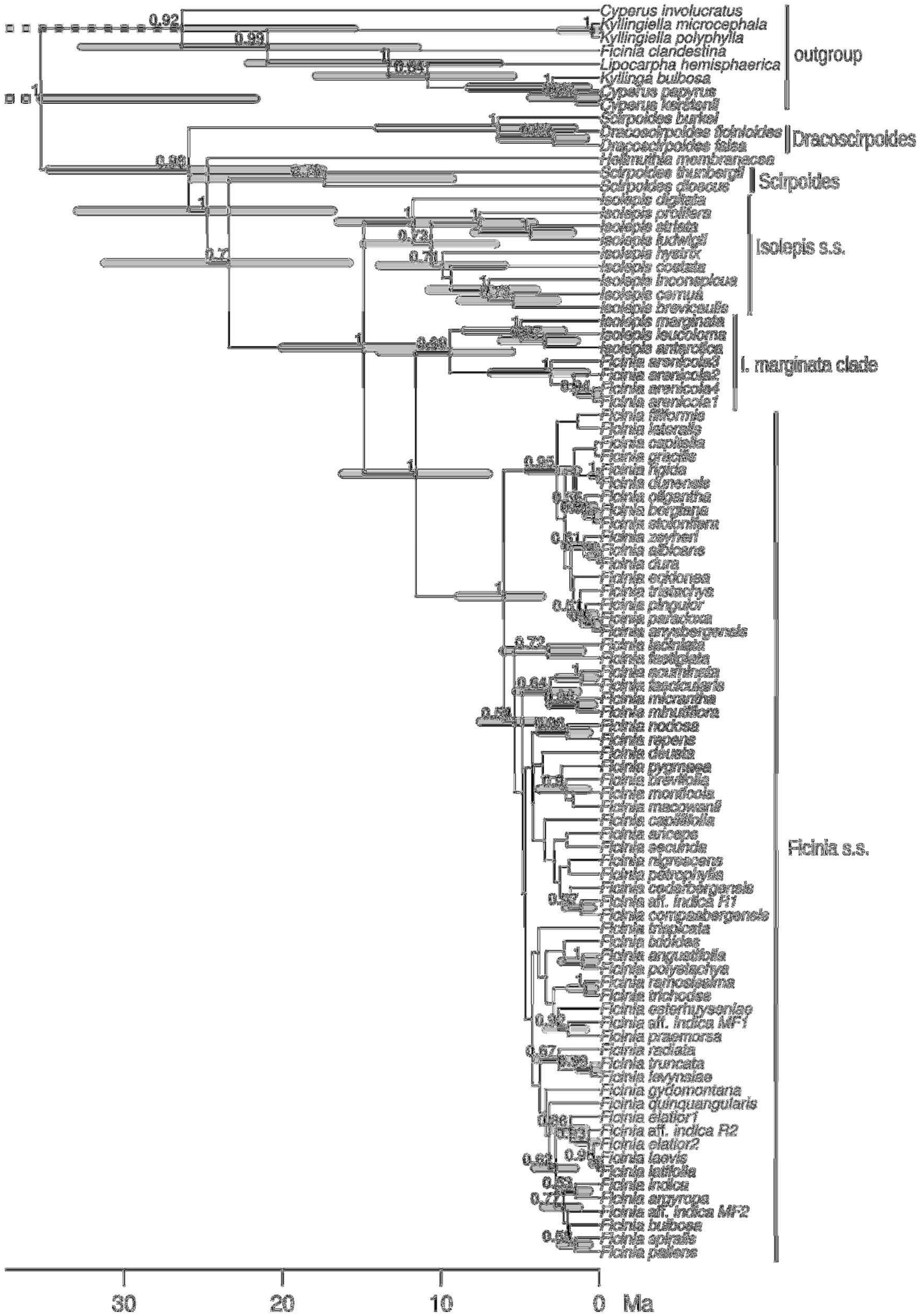

Figure 3.1: Chronogram of *Ficinia* clade. The numbers above the branches are the posterior probabilities (PP). The scale is in million years (Mya). Bars represent the 95% highest posterior probability density (HPD) intervals of age estimates.

3.3.2. The timing of divergence in *Ficinia* clade

The results show that the *Ficinia* clade has an origin within the Oligocene. The birth–death model was the best suited model selected, with the lineage through time (LTT) plot for *Ficinia* clade (Figure 3.2) that shows constant diversification up to about 6 Mya, followed by a shift to an exponential diversification. The most recent split between the sister taxa of the genus *Ficinia* is 0.09 Mya (*F. latifolia* vs. *F. laevis*). The observed accelerated diversification (Figure 3.2) is mostly contributed by rapid speciation in *Ficinia* (Figure 3.1).

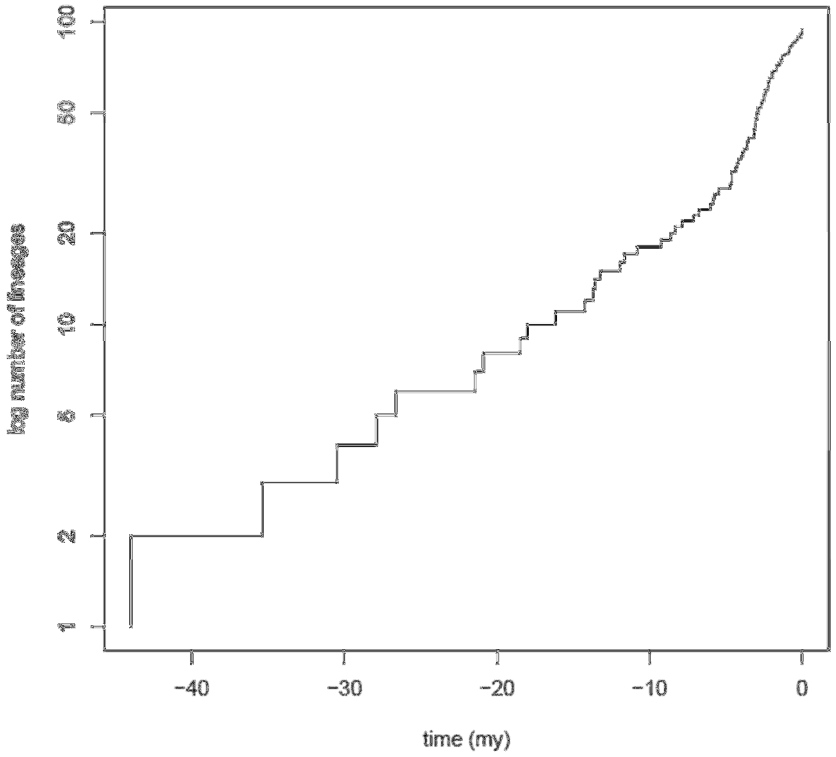

Figure 3.2: Lineage through time plot for *Ficinia* clade, based on the maximum credibility tree from the Bayesian analyses (Figure 3.1).

3.3.3. Comparison of soil nutrients between sister species

Table 3.2 presents a summary of the ecological parameters studied for the eight species pairs. The results showed that Pairs A, D, F, G and H do not overlap at all in geographical distribution for any recorded specimens (at QDS scale), whereas there is a partial overlap in the distribution ranges in Pairs B, C and E. There were significant differences in the soil nutrient regime within sister pairs A, D, E, F and G (Table 3.2, Figure 3.3), and separation in the multivariate analysis (PCA) within all pairs species except for B (Table 3.2). The results also show that there is a lack of overlap between the altitude ranges (except Pairs A, D, F and G) and the flowering times (except Pairs D and F) within the sister pairs of *Ficinia* (Table 3.2).

Table 3.2. Ecology of selected sister species pairs of the genus *Ficinia.* Altitude (m) A = 0–500, B = 501–1000, C = 1001–1500, D = 1501–2000, E = > 2000. Flowering times (months) A = DEC, JAN, FEB; B = MAR, APR, MAY; C = JUN, JUL, AUG; D = SEP, OCT, NOV. **Shared** = number of QDS where both species occur. **Not shared** = number of QDS where sister species do not both occur. ***J*** = Jaccard's similarity index: proportion of overlap.

Sister pairs	**Altitude**	**Flowering times**	**Sites not shared**	**Sites Shared**	***J***	***P***	**Nutrients differed**
A. *F. levynsiae* vs *F. truncata*	BE vs A	CD vs CD	31	0	0	0.00	pH, Ca, Mg, B, N
B. *F. brevifolia* vs *F. macowanii*	CDE vs BCE	BC vs BCD	30	7	0.19	0.0045	
C. *F. angustifolia* vs *F. polystachya*	AB vs AB	CD vs CD	18	7	0.28	0.0515	
D. *F. anysbergensis* vs *F. paradoxa*	B vs A	D vsABD	24	0	0	0.00	P, B, Fe, N
E. *F. bergiana* vs *F oligantha*	AB vs ABC	ACD vs BCD	37	3	0.08	0.0135	pH, Mn
F. *F. elatior* vs *F. Laevis*	A vs BC	B vs CD	12	0	0	0.00	pH
G. *F.* sp. nov. D (4583) vs *F. praemorsa*	DE vs A	ABCD vs ABCD	23	0	0	0.00	pH, B, N
H. *F. dunensis* vs *F. rigida*	A vs A	BC vs ABD	18	4	0.18	0.500	

Pair A (*F. levynsiae* vs *F. truncata*)

There is no overlap in distribution and altitude between the two species, as *F. levynsiae* grows on mountain slopes above 501 m whereas *F. truncata* grows on coastal limestone outcrops below 500 m. In addition, *F. levynsiae* grows on acidic sandstone soils (pH 4.27) and the sister species *F. truncata* grows on alkaline soils (pH 7.68) (Figure 3.3a), and there are differences in other soil nutrients. Other nutrients that are significantly different are Ca (Figure 3.3e) and B (Figure 3.3j) with the $P < 0.001$ and $P < 0.01$, respectively. However, both species flower in the period July to November.

Pair B (*F. brevifolia* vs *F. macowanii*)

There is partial overlap in flowering time, altitude and distribution between *F. brevifolia* and *F. macowanii* (Table 3.2). *F. brevifolia* is widespread in the Western Cape, occurring at mountain streams and commonly on wet rock faces of road cuttings. On the other hand, *F. macowanii* is a narrowly distributed taxon, only occurring in fynbos in the Langeberg Mountains around the Marloth Nature Reserve. While the two taxa overlap in their soil nutrients (Figure 3.3 a–l), they differ in the microhabitat regime and do not co-occur.

Pair C (*F. angustifolia* vs *F. polystachya*)

There are overlaps in altitude, flowering time and soil nutrients between these species (Figure 3.3 a–l). *Ficinia angustifolia* is more widespread at lower altitudes, where it occurs in seasonally wet sandstone fynbos, while *F. polystachya* is more common at higher altitudes, especially in bogs. The two species can be unambiguously separated based on morphology where they differ in glume arrangement, *F. angustifolia* being distichous and *F. polystachya* terete.

Pair D (*F. anysbergensis* vs *F. paradoxa*)

Ficinia anysbergensis is morphologically very similar to *F. paradoxa*. The two species do not overlap in distribution and altitude, as *F. anysbergensis* is restricted to Klein Karoo mountain slopes (above 501 m) whereas *F. paradoxa* grows outside the Klein Karoo on coastal flats (below 400 m). In addition, species pair D is growing on slightly different substrates based on Fe (Figure 3.3 k) and N (Figure 3.3 l), but their flowering times overlap.

Pair E (*F. bergiana* vs *F. oligantha*)

There are overlaps in the altitude and flowering times in *F. bergiana* and *F. oligantha*. These taxa have significantly different distributions ($P < 0.001$) with only three sites shared and 37 sites not shared (Table 3.2). Their soil nutrient characteristics are mostly similar, differing only in pH and Mn (Figure 3.3 a–l).

Pair F (*F. elatior* vs *F. laevis*)

Ficinia elatior is a species occurring in the Southwest and Agulhas Plain areas of the Western Cape, where it occurs on mountain slopes and flats in areas below 500 m. On the other hand, *F. laevis* occurs in the Northern Cape and the Northwest past of the Western Cape. Therefore, these two species do not overlap in distribution, and they are also– separated in flowering time and in a number of soil parameters (Table 3.2, Figure 3.3 a-l).

Pair G (*F.* sp. nov. D vs *F. praemorsa*)

These sister species differ in altitude, distribution and substrate, but overlap in flowering time (Table 3.2, Figure 3.3 a–l). *Ficinia praemorsa* is a limestone endemic occurring on the Agulhas Plain (pH 7.63) at an altitude below 500 m. The undescribed species, *F.* sp. nov. D, is restricted to sandstone mountain habitats (pH 4.53) above 1000 m.

Pair H (*F. dunensis* vs *F. rigida*)

Ficinia dunensis and *F. rigida* grow on sites with the same altitude (< 500 m) and their flowering times overlap. Their distributions overlap and both species occur on soils with similar nutrient regimes (Figure 3.3 a–l). Despite a strong overlap in geography and ecology, these species are morphologically very distinct and there is no evidence of individuals with intermediate morphology in their areas of co-existence. However, the soil nutrients for this last pair does not show any significant difference (Figure 3.3 a–l).

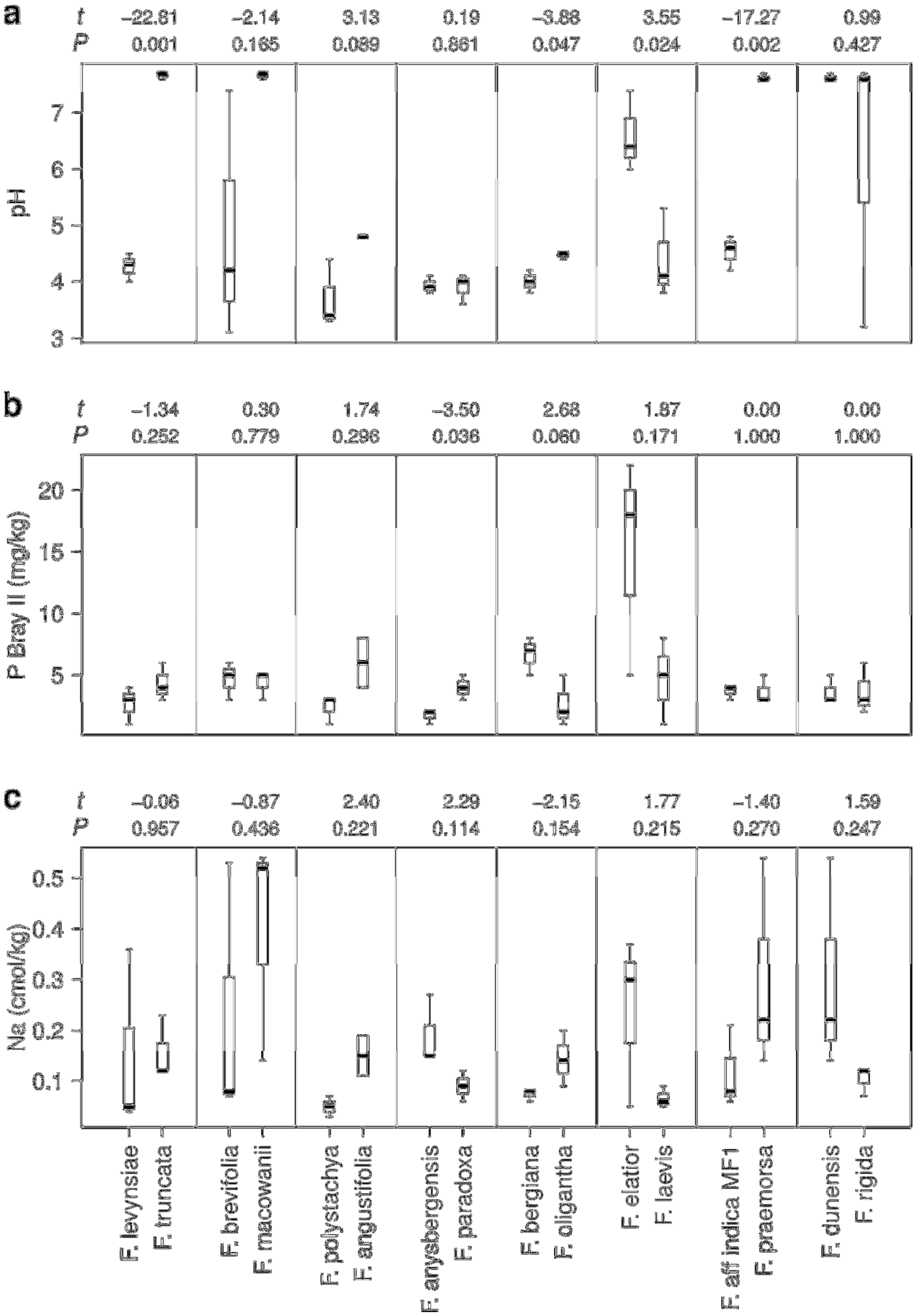

Figure 3.3a–c: Box-and-whisker plots showing the actual means of soil nutrients characteristics of the sister species in selected species pairs within the genus *Ficinia.* The *P* values and the *t*test results are shown above the plots for each variable.

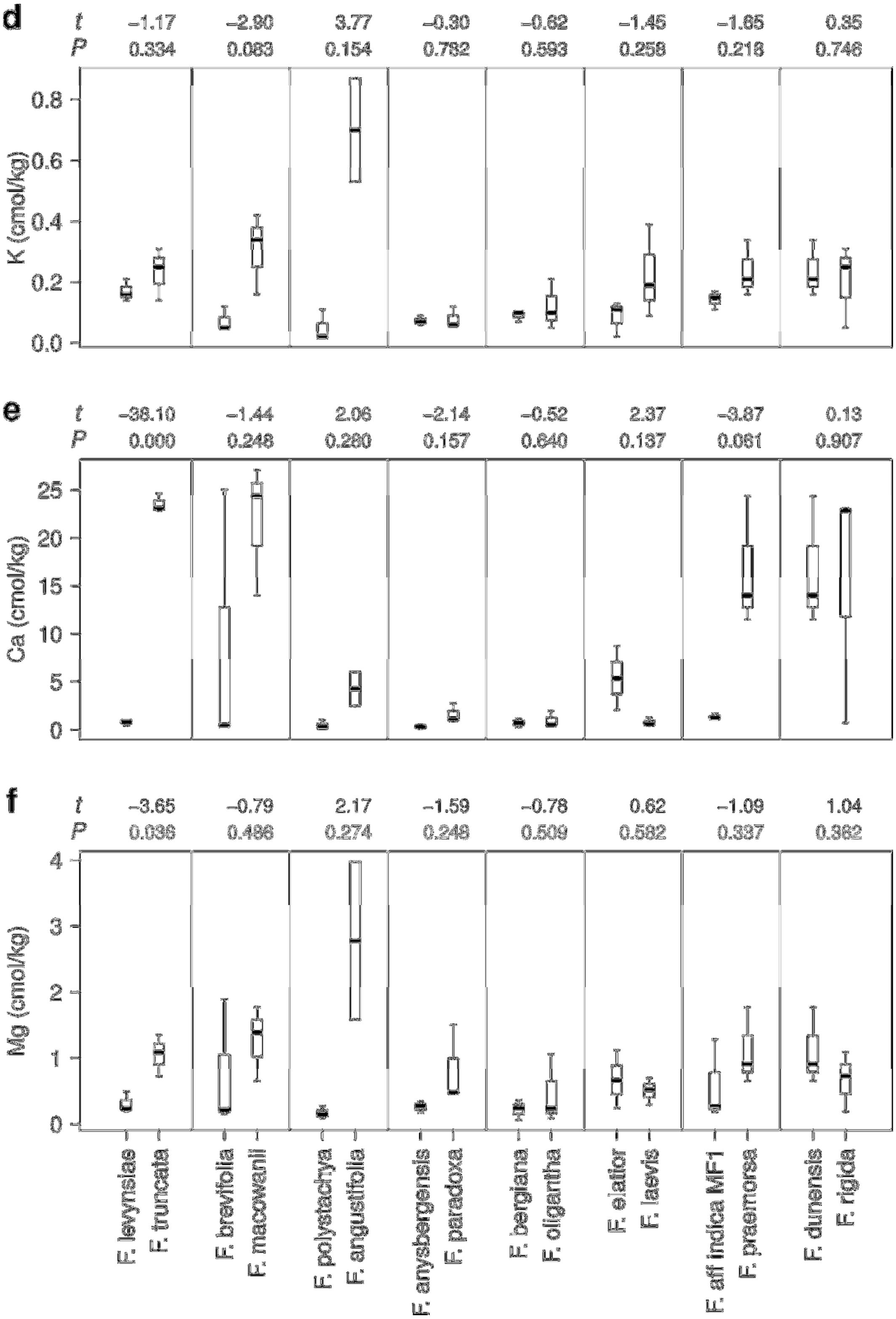

Figure 3.3d–f: The Box-and-whisker plots showing the means of soil nutrient concentrations of the sister species. The *P* value and the *t* test results are shown above the plots for each variable.

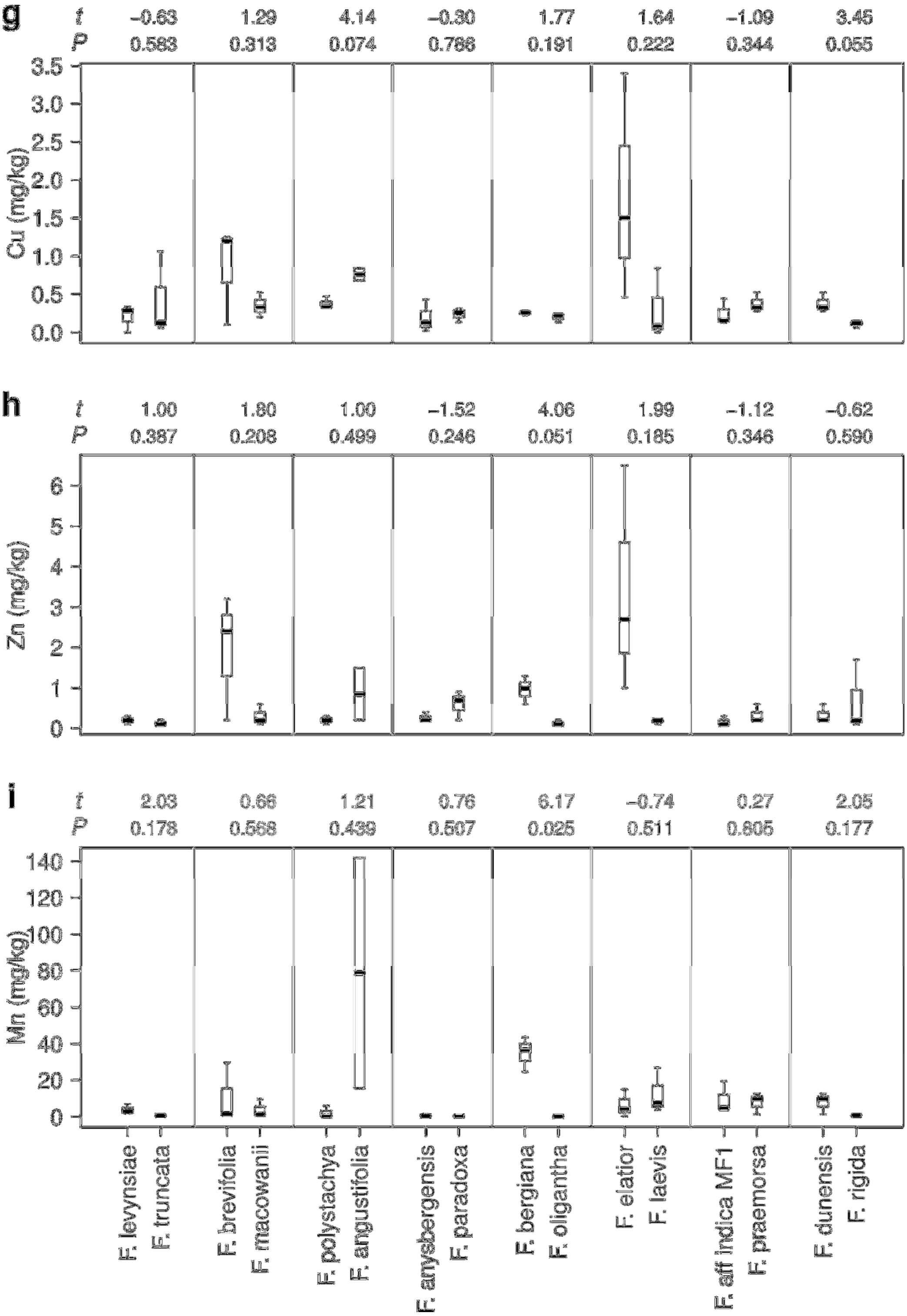

Figure 3.3g–i: The Box-and-whisker plots showing the actual means of soil nutrients characteristics contributed on the distribution of the sister species of the genus *Ficinia*. The numbers P value and the t-test are shown above the plots for each variable.

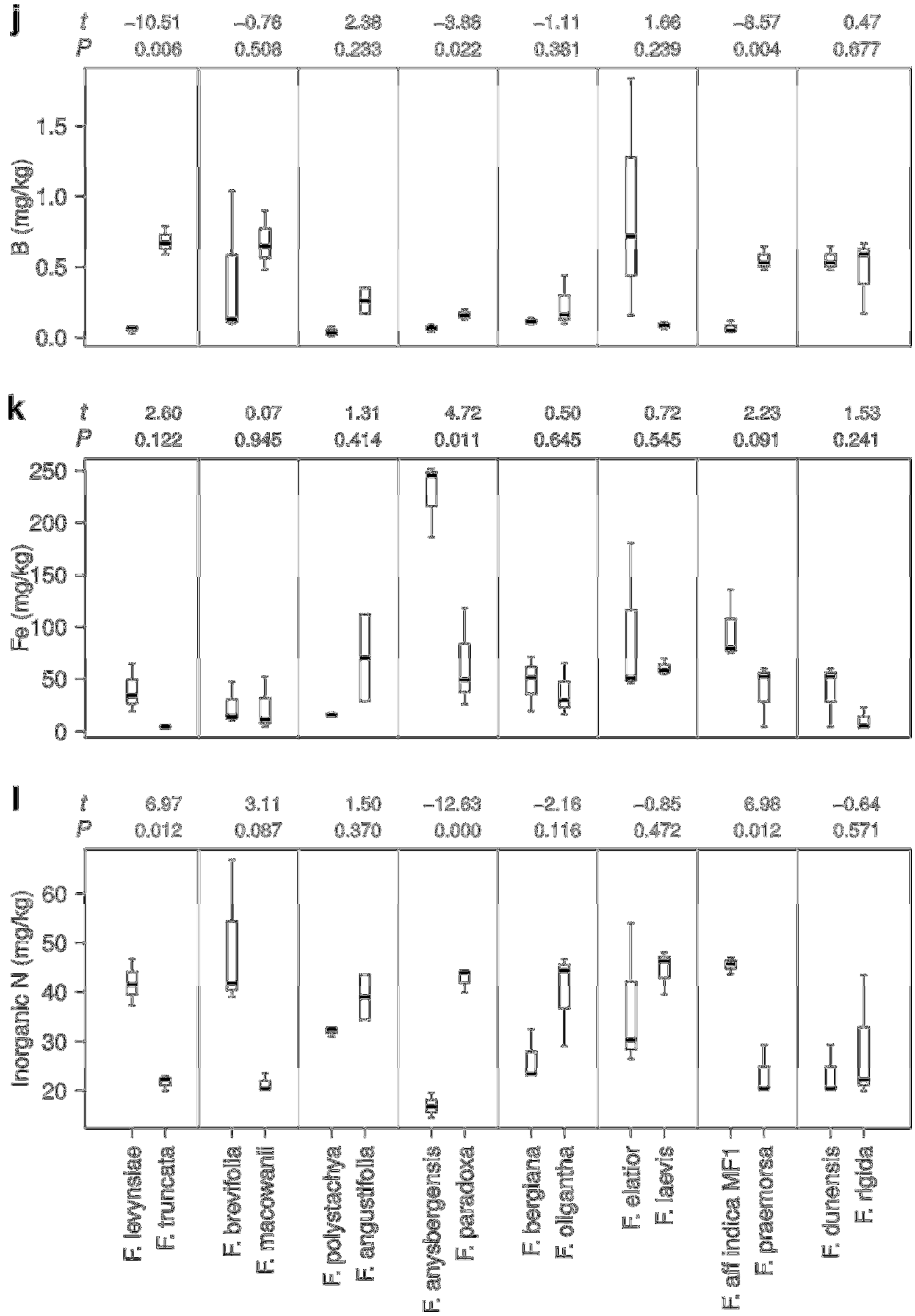

Figure 3.3j–l: The Box and Whisker plots showing the actual means of soil nutrients characteristics contributed on the distribution of the sister species of the genus *Ficinia*. The numbers P value and the t-test are shown above the plots for each variable.

3.4. Discussion

3.4.1. Diversification rates in the *Ficinia* clade

Linder (2003) stated previously that our understanding of the timing and drivers of diversification of the Cape flora could be significantly improved by thoroughly investigating lineages that best epitomize the flora. In this chapter, we estimated the timing and rates of lineage diversification in the *Ficinia* clade (Cyperaceae), which is one of the major Cape floral clades. Furthermore, this study investigates whether diversification was triggered by environmental change in the late Miocene/early Pliocene. We estimated the diversification rates of the *Ficinia* clade under a constant-rate lineage birth–death model. Similarly to the genus *Moraea,* the *Ficinia* clade occurs mostly in fynbos and afroalpine areas and never in the tropical forests or savannas that cover most of sub-Saharan Africa, with the majority of the species in the Cape of South Africa (Goldblatt et al., 2008).

The *Ficinia* clade sensu lato has a stem node in the Oligocene and the first lineages of the genus *Ficinia* originated in the early Miocene. The speciose clade contains the genera *Isolepis* and *Ficinia* (160 species) having a mid-Miocene stem age (Figure 3.1). The lineage through time plot (Figure 3.2) shows a constant rate from the Oligocence through the mid-Miocene, but there is a sudden increase in rate with no plateau from the late Miocene. This abrupt increase showed by the LTT plot reflects the explosive increase in *Ficinia* species, which continued into the Pliocene. Therefore the *Ficinia* clade is a relatively old clade in the CFR, with stem age comparable to Proteaceae (Schnitzler et al. 2011). Similar to other lineages in the CFR, the diversity of the *Ficinia* clade is a result of a long-term accumulation of species, but has Pliocene accelerated diversification similar to *Babiana* (Schnitzler et al. 2011) and other species which rapidly diversified after the shift into the fynbos biome associated with Miocene aridification. *Ficinia* clade is frequently found in the fynbos, colonizing a wide variety of substrates, wetness gradients and soil depths. Annual life form has evolved in *Isolepis* species growing in ephemeral wetlands on shallow soils, while the majority of *Ficinia* species resprout after fire and are common in post-fire vegetation. The age and ecology of the *Ficinia* clade is comparable to other well-known Cape clades such as *Disa* (Bytebier et al., 2011).

Our results on the diversification rates for *Ficinia* clade are consistent with previous estimated rates for Cyperaceae/ Poales as a whole and slightly faster than those for angiosperms as a whole (Magallon & Sanderson, 2005) when estimated under a constant-rate on lineage birth–death model. The rates estimated for *Ficinia* clade are lower than estimated rates within most Cape clades, but they do not resemble those clades within the slowly diversifying Cape Restionaceae (Warren & Hawkins, 2006). The diversity in the *Ficinia* clade is the result of recent diversification with shifts towards accelerated speciation in the last 5 Mya, which is similar to characteristic fynbos species in genus *Babiana*. The LTT results from this study corroborate the study of Schnitzler et al. (2011), who showed the substantial spread of the onset of diversification started in the early Oligocene. In the study of Schnitzler et al. (2011) the diversification rates in the *Ficinia* clade showed a decrease toward the present within the genus *Ficinia* and *Isolepis*. The ecological traits in *Ficinia* can be speculated to be associated with the burst of radiation in the Cape.

3.4.2. Do sister species differ in ecology?

To my knowledge, this is the first study that quantifies the pattern of speciation of the species within the family Cyperaceae using the sister species pairs identified from the phylogeny. The results in this study show that the ecological shift could be a phenomenon in speciation of sister species of genus *Ficinia* and that is equivalent to the frequent ecological shift in Cape sister clade (Van der Niet, 2009). When looking at the distribution patterns of the selected pairs per QDS, with the exception of two of the sister pairs (C and H), there was significant ($P<0.05$) lack of overlap in their distribution (Table 3.2). This means that the sister species are significantly less likely to co-occur than predicted by chance and it suggests that speciation in these six pairs has involved allopatry. In addition to geographical separation, five of these six pairs occupy different soil types and some at different altitudes. This study points to the possibility that habitat shift is frequent among the sister species of genus *Ficinia*, in essence, both allopatry and habitat shifts are associated with speciation in the clade.

The flowering times between the sister species investigated exhibited a high degree of overlap. The majority of *Ficinia* species flower in winter to early summer season, and plants exhibit typical features for wind pollination (flowers highly reduced, lack colourful parts, no scent, anthers extruded outside scales, plumose stigmas). Among sister species flowering at the same time, there is little chance for viable pollen to survive extensive distances separating sister taxa for pollination to occur. The studies of Friedman & Barrett (2008) did not include putative transitions from wind pollination to insect pollination in sedges, which lack nectar and often occur in open habitats, in their analysis that concluded that transitions from wind pollination to insect pollination were more likely in nectariferous lineages of closed habitats.

As *Ficinia* fruits (nutlets) are dispersed by ants (presence of elaiosomes), there is reduced chance for sister pairs separated by distance to disperse and there has been no experimental evidence that insects contribute substantially to pollination of any sedge lineage.

Two of the sister pairs have an overlap in distribution, flowering time and occupy similar soils. For the case of *F. brevifolia* and *F. macowanii*, these taxa are separated at ecological niche level with *F. brevifolia* growing on wet rocks and stream banks whereas *F. macowanii* is restricted to mountain slope habitats and the two never co-occur. On the other hand, *F. dunensis* and *F. rigida* occur together, and are perhaps separated by intrinsic mechanisms.

From the number of pairs that were sampled on this study, the results showed that the speciation of sister species of *Ficinia* is driven by the ecological factors such as soil type, altitude and the edaphic niche. However, previous studies (Goldblatt & Manning, 2000; Barraclough, 2006; Linder & Hardy, 2004, van der Niet & Johnson, 2009) found that there is a small proportion of ecological shifts in CFR taxa involving shift in soil type, but such observations could have been biased by the biology of lineages included in studies. For *Ficinia*, a wind-pollinated taxon whose fruits are dispersed over short distances by ants, speciation has involved geographical and ecological (including edaphic, altitude) niche shifts. Edaphic shifts are thought to be the major driver of speciation in general (Rajakaruna, 2004) and for the CFR in particular (e.g. Rourke, 1972; Goldblatt, 2002; Barraclough, 2009).

3.5. Conclusions

One of the objectives of this chapter was to examine whether there were ecological differences in sites where sister species of *Ficinia* were growing. The results obtained from this study showed that ecology plays a role in speciation in the clade. About 50% of the sister pairs were observed to be growing on different substrates and shifts in soil were mostly accompanied by separation in distance. In other cases where sister species had a similar substrate type, such pairs were separated by altitude or in distribution. This study therefore indicates the importance of ecological shifts in Cape clades, especially in wind-pollinated lineages with overlapping flowering periods. There is a constrasting pattern of diversification between the *Isolepis* and *Ficina* clades, The *Isolepis* group has several deep nodes whereas *Ficinia* has most rapid diversification in the late Miocene (since 6 Mya).

CHAPTER 4

MORPHOMETRIC STUDY OF THE *FICINIA INDICA* COMPLEX

4.1. Introduction

The history of morphometrics is, inevitably, entangled with the much wider issue of attempts to classify animals and plants. It antedates classificatory attempts but the basic principle in morphometrics is to measure the degree of similarity of two forms (Blackith & Reyment, 1971). Evolutionary and systematic biologists are concerned with the discovery and explanation of differences and similarities among organisms. The discrimination of taxa, description of ontogenetic or evolutionary change, and testing of evolutionary hypotheses require the analyses of the morphological patterns. Current methods for comparing biological forms range from classic verbal and pictorial representation to lists of the measured distances between the identifiable points on an organism (Bookstein et al., 1985). Parsons et al. (2003) compared traditional methods and geometric morphometrics and they reported that geometric morphometrics could be a more effective way to analyse and interpret body form, but also that traditional methods could be relied upon to provide statistical evidence of shape differences and it could be used for distinguishing species. It is already known that differences in morphology frequently indicate important developmental evolutionary changes in plant organs (Piazza et al., 2005; Shipunov & Bateman, 2005) and that the morphological traits are closely linked with other known adaptive traits (eg physiological traits). The goals of evolutionary biology are to facilitate the ongoing quest to understand the genetics and evolution of adaptations and the establishment of new species (Barton et al., 2007). Biologists often measure many morphological variables when describing organisms from different localities or to compare organisms that may or may not be taxonomically different. For the study of the *Ficinia indica* complex, the Taxonomic Species Concept were the evolutionary charactors were used to distinguish the species.

4.1.1. Morphology of the genus *Ficinia* Schrad.

The genus *Ficinia* consists of tufted perennials that possess rhizomes or stolons, have mostly scapose stems, and their spikelets have spirally arranged glumes enclosing bisexual flowers whose nutlets have a gynophore (Goetghebeur, 1998). Various species are recognized on the basis of a single or a combination of characters, including underground organs (e.g. rhizomes, stolons), stems (e.g. height, scapose or with multiple internodes), leaves (e.g. leaf sheath papery or not, ligule presence and shape, leaf blade size), inflorescence type (e.g. capitate, spike or pseudolateral), spikelet (e.g. spiral or distichous glume arrangement, size), and nutlet morphology (e.g. size, shape, surface ornamentation, gynophore size and shape). Ligule morphology is thought to be an important but under-utilized character (Gordon-Gray, 1995). A number of *Ficinia* species lack a ligule while other species have ligules that vary in size and shape (Figure 4.1).

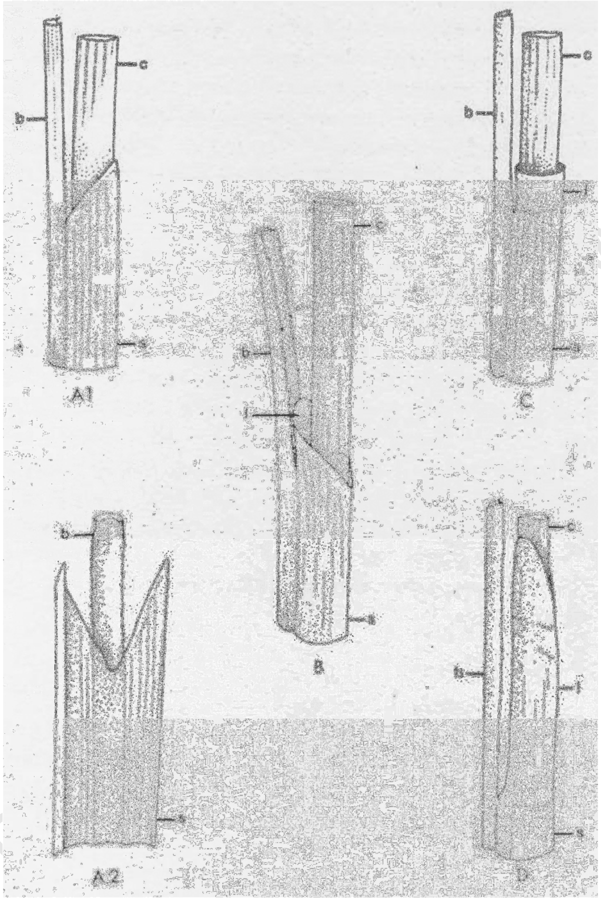

Figure 4.1: *Ficinia* leaf sheath apices and ligules in lateral view. **A1**, sheath mouth sloping inwards and downwards to leaf blade, ligule lacking; **A2**, sheath of A1, interior view showing absence of ligule; **B**, sheath mouth sloping downwards away from leaf blade, ligule a membranous outgrowth with in leaf blade; **C**, sheath mouth truncate, ligule a scuff-shape collar; **D,** sheath mouth sloping downwards away from blade, ligule a long, stalking-like enclosing membrane, fragmenting early. c, culm; l, ligule; b, leaf blade; s, membranous sheath (Gordon-Gray, 1995).

4.1.2. The *Ficinia indica* complex

There are a number of species complexes in *Ficinia* that require revision to clarify species boundaries and name application. Such complexes may occur in species groups with wide morphological variation to encompass population(s) with particular traits, while excluding other populations as different species though they share the same characters. The complex of interest in this chapter is the *Ficinia indica* complex consisting of five taxa, namely, *F. indica* (Lam.) Schrad., *F. elatior* Levyns, *F. dunensis* Levyns, a form of *F. indica* occurring in the renosterveld (*F.* aff. *indica* R) and another form occurring on sandstone at an altitude above 1000 m, where it forms mats on shallow soil (*F.* aff. *indica* MF). Herbarium folders of the named species in this complex contain misidentified species, and it is evident that many botanists cannot unambiguously identify specimens in the complex. Possibly, there are no discrete characters to discriminate among the different species within the *F. indica* complex, or the characters currently used are not suitable to separate the entities. This chapter aims to revise the taxonomy of the *Ficinia indica* complex and to investigate the role of ecology in the distribution of the species in the complex.

Ficinia indica was described by Lamarck (1785) as *Schoenus indicus,* based on a mislabelled collection thought to be from the East Indies but now shown to have originated in the Cape (Raynal, 1958). Later, workers described material of this taxon as different species, the most widely applied names being *Ficinia setiformis* (Schrader, 1832) and *Ficinia lithosperma* (Boeckeler, 1871), which mostly refers to *F. pallens* but also includes *F. indica*. The name *F. setiformis* was applied by Clarke (1898) for an entity including *Schoenus indicus* Lam., but Pfeiffer (1921) made the valid combination (*F. indica* (Lam.) Pfeiff.) in current use. Levyns (1947) described two species, *Ficinia dunensis* Levyns (more similar to *F. indica*) and *F. elatior* Levyns (more similar to *F. pallens*). However, looking through herbarium folders at Bolus, one of the largest collections of *Ficinia,* which includes specimens annotated by Levyns, there is ambiguity in the identity of *F. indica* and these species described by Levyns. These taxa, hereafter referred to as the *F. indica* complex, are misidentified and there appears to be no clear boundaries between the widespread *F. indica* and the two narrowly distributed *F. dunensis* and *F. elatior.* Moreover, there are specimens that do not fit into the morphological limits of the known species, with specimens collected from high-altitude rock substrate having a mat-forming habit (*F.* aff. *indica* MF) and specimens from shale soils from the renosterveld (*F.* aff. *indica* R) lacking the stolons that appear to be a distinctive character of *F. indica.*

In this chapter, the five entities in the *F. indica* complex are studied to evaluate whether they are morphologically and ecologically distinct. The five taxa differ in underground structure, with *F. indica* and *F. dunensis* having well developed stolons and the other three species missing a stolon structure. A short woody rhizome is seen in *F. elatior* and *F.* aff. *indica* R, while *F.* aff. *indica* MF is tufted (Figure 4.2). There are also differences in plant height, leaf and inflorescence size (Figure 4.2). In addition, these taxa appear to differ in distribution and ecological preferences, most notably *F.* aff. *indica* R being restricted to shale substrate while *F.* aff. *indica* MF is restricted to high altitude habitats with mostly sandstone substrate.

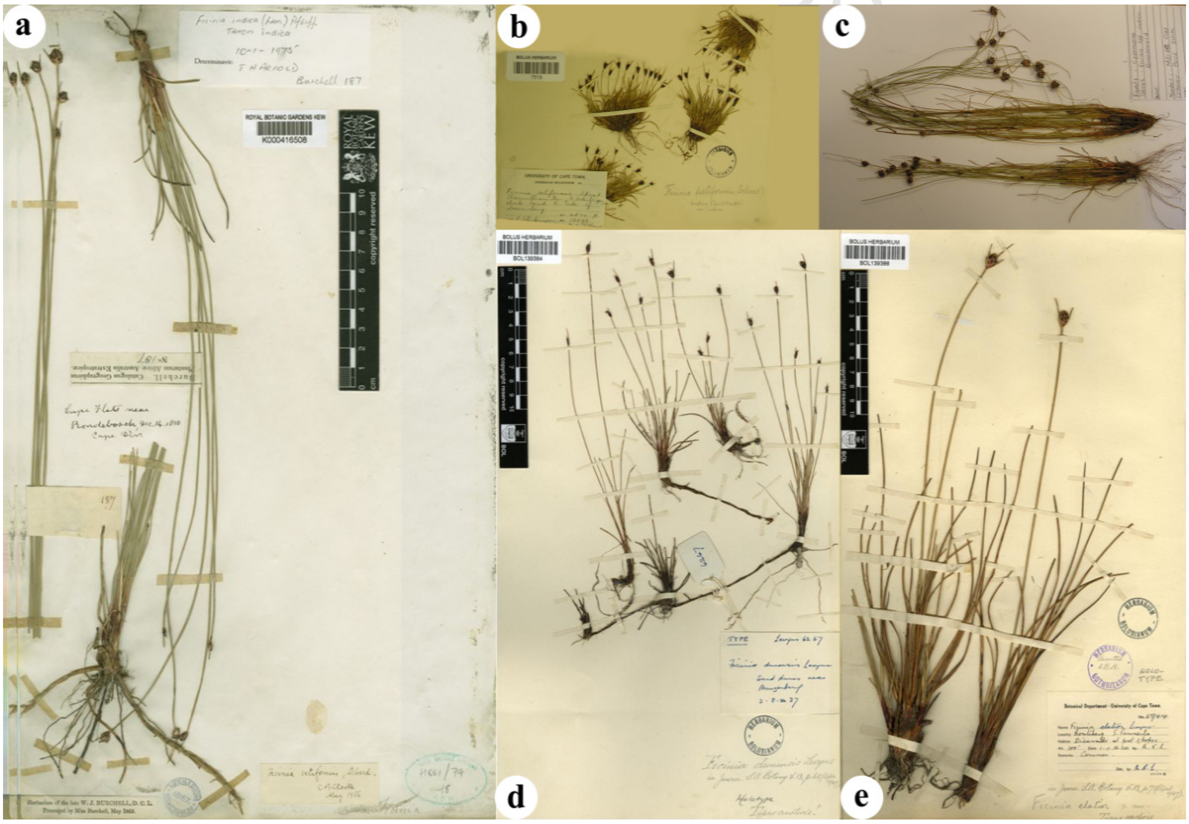

Figure 4.2: Species in *Ficinia indica* complex. a, *F. indica,* Burchell 187 (K, Isotype); b, *F.* aff. *indica* MF, Levyns 13093 (BOL, Type); c, *F.* aff. *indica* R, Dludlu 022 (BOL, Type). d, *F. dunensis*, Levyns 6267 (PRE, Isotype); e, *F. elatior,* Levyns 5944 (BOL, Isotype).

4.1.3 Objective

The main objective of this chapter is to revise the taxonomy of the *F. indica* complex.

4.2. Materials and Methods

4.2.1. Morphometric studies

(a) Specimens examined

Morphological characters were scored on the three named and two putative species of *F. indica* complex using herbarium specimen from Bolus Herbarium (BOL) and supplemented with specimens collected in this study. Specimens that contained vegetative parts and mature nutlets were prioritized, so as to avoid having many missing data and to ensure that the study included comparable mature individuals only. A total of 87 specimens were examined (see Appendix 1).

(b) Characters studied

A dissecting microscope with a measuring eye-piece was used to measure the following quantitative characters: spikelet length (SL) and width (SW), glume length (GL) and width (GW), and nutlet length (NL) and width (NW). A millimetre ruler was used to measure leaf sheath length (LSL), leaf blade length (LBL) and width (LBW), and involucral bract length (IBL). The following meristic and qualitative characters were also recorded: number of culms per tuft, spikelets per peduncle, glumes per spikelet, inflorescence type, leaf sheath type, root type, ligule type and glume arrangement. Each morphological character was measured three times on each specimen and the mean was calculated for further data analysis.

4.2.2. Soil sampling

For each location where the species within the *F. indica* complex was collected, three representative soil samples were collected and analysed for nutrient concentrations. Soil samples were collected using an auger or a garden trowel, taking a slice of soil down to 10–15 cm deep at the locations where the plant samples were collected. The samples were air-dried, sieved to pass through 2-mm pores and sent to BemLab Private Laboratory (Somerset-West, South Africa) for analyses of the following soil nutrients: phosphorus (Bray II P), inorganic nitrogen (N), potassium (K), iron (Fe), calcium (Ca), magnesium (Mg), exchangeable sodium (Na), T-value (summation of exchangeable cation concentrations) and soil pH. The means of the three samples were calculated for each specimen for further analysis.

4.2.3. Statistical analysis

Before conducting statistical analyses, each variable was log-transformed in (Statistica v. 9.0) to improve the normality and linearity of the data (Sokal & Rohlf, 1981). All the variables were approximately normally distributed and did not show any significant departure from normality after transformation.

(a) Analysis of variance (ANOVA)

Univariate analysis of variance (ANOVA) was performed to test the null hypothesis of equality of means among putative species groups for each of the morphometric characters and soil nutrient variables.

(b) Cluster analysis (CA)

Cluster analysis (CA) was used in this study to investigate if there were any clustering patterns in our dataset. This analysis was done before testing how well the data were able to discriminate between the proposed groups. It is useful in separating organisms into groups that may be used in classification and has been widely used to examine geographical patterns of variation (Thorpe, 1983). This method imposes a hierarchical structure on the data, but has the disadvantage that the analysis may show distinct clusters even if the variation is clinal (Thorpe, 1983). The CA brings together groups that are closely associated into a cluster (Blackith & Reyment, 1971) and such a cluster is then considered to be differentiated from other associations that form separate clusters, thus dividing a data set into *a priori* unknown subgroups (Flury & Riedwyl, 1988). Euclidean distances were used as a measure of distance. The cluster analysis was done in both R (R Development Core Team, 2010) and Statistica v. 9.0. Similar results were obtained from these two analyses and the phenogram from R is presented.

(c) Discriminant function analysis (DFA)

A multivariate study of the overall morphology was undertaken to test the distinctness of the species within the complex. DFA was used to test whether the species can be separated on overall morphology and also to find the predetermined groups with two or more response variables recorded for each observation. It generates a linear combination of variables that maximizes the probability of correctly assigning observations to their predetermined groups and can also be used to classify new observations into one of the groups (Flury & Riedwyl, 1988; Quinn & Keough, 2002). It is done in order to maximize the correlations between the CVs and group membership, and to maximize the ratio of between- to within-group variance (Sneath & Sokal, 1973; Krzanowski, 1990).

4.3. Results

4.3.1. Morphological studies of *Ficinia indica* complex

A total of 87 specimens were studied, representing *F. indica* (37 specimens), *F. dunensis* (15), *F. elatior* (7 specimens). *F.* aff. *indica* R (11 specimens) and *F.* aff. *indica* MF (17 specimens). The measurements of the eleven characters are presented in Appendix 4.1. These data were analyzed using various approaches and results are presented below.

(a) Cluster analysis

The cluster analysis results from R are presented in Figure 4.3. At an Euclidean distance of 5000, two clusters are evident. Cluster A consists of *F.* aff. *indica* MF specimens. Cluster B consists of *Ficinia indica, F. dunensis, F. elatior,* and *F.* aff. *indica* R specimens. The separation of the *F.* aff. *indica* MF cluster from all other groups is very clear.

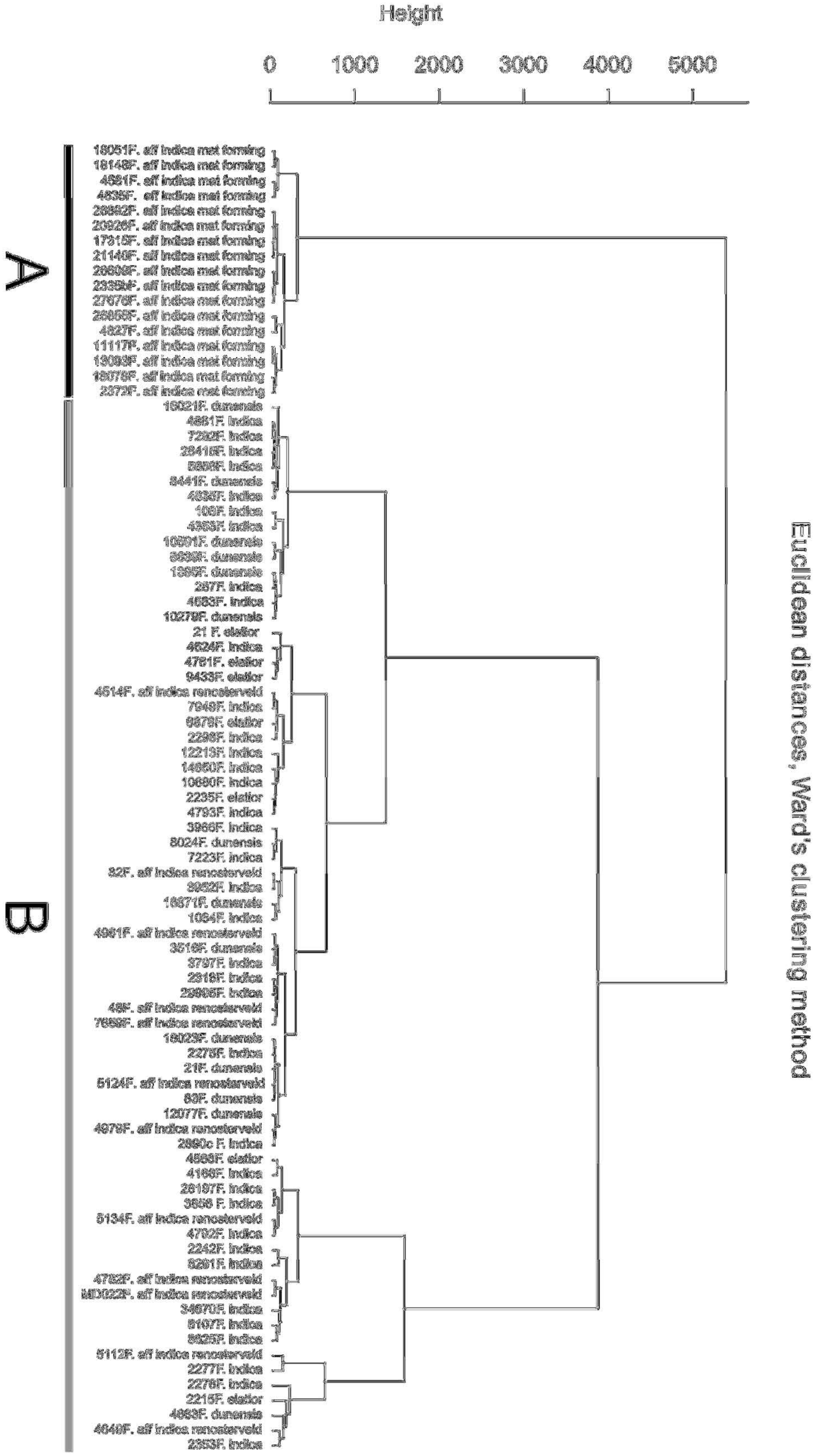

Figure 4.3: Phenogram of *Ficinia indica* complex.

(b) Discriminant Function Analysis (DFA)

The variability in the morphological data is mostly summarized by the first two DFA roots (93%), with the first root (Root 1) contributing 74% and the second root (Root 2) contributing 19% (Table 4.1). The remaining variability (7%) is explained by Roots 3 and 4, which are not included in the results table because of their very low contribution. A cut-off of 0.2 was used when deciding on the significance of the contribution of each variable within Roots 1 and 2. The first root is largely correlated with the culm length (1.05), leaf sheath length (–0.35) and the nutlet length (0.36). The second root was largely correlated with seven of the eleven characters that were measured, that is, nutlet length and width (–0.71 and 0.34, respectively), leaf blade width (0.55), glume length and width (0.47 and 0.21, respectively), the involucral bract length (0.26), and culm length (–0.28) (Table 4.4).

Table 4.1: Canonical vectors (CV) for morphological data. The values in brackets show the percentages which each root contributed. The highlighted values are for variables with high contribution (≥ 0.2) to the separation of the taxa within the complex.

Variables	**Root 1 (74.1%)**	**Root 2 (18.5%)**
Culm length (CL)	**1.05**	**–0.28**
Leaf blade length (LBL)	0.16	0.11
Leaf blade width (LBW)	0.02	**0.55**
Leaf sheath length (LSL)	**–0.35**	0.14
Involucral bract length (IBL)	0.17	**0.26**
Spikelet length (SL)	–0.17	–0.17
Spikelet width (SW)	–0.01	**0.47**
Glume length (GL)	0.16	**0.21**
Glume width (GW)	–0.13	0.12
Nutlet length (NL)	**0.36**	**–0.71**
Nutlet width (NW)	–0.11	**0.34**
Eigenvalue	3.46	0.86
Cumulative Proportion	0.74	0.92

Figure 4.4 shows the separation of taxa within the complex along Roots 1 and 2, according to the DFA of the morphometric data. There was a considerable overlap among taxa within the complex, except for *F.* aff. *indica* MF, which is nearly completely separated from the rest of the taxa along Root 1. The DFA gives similar results to the cluster analysis, where *Ficinia indica, F. dunensis, F. elatior* and *F.* aff. *indica* R clustered together.

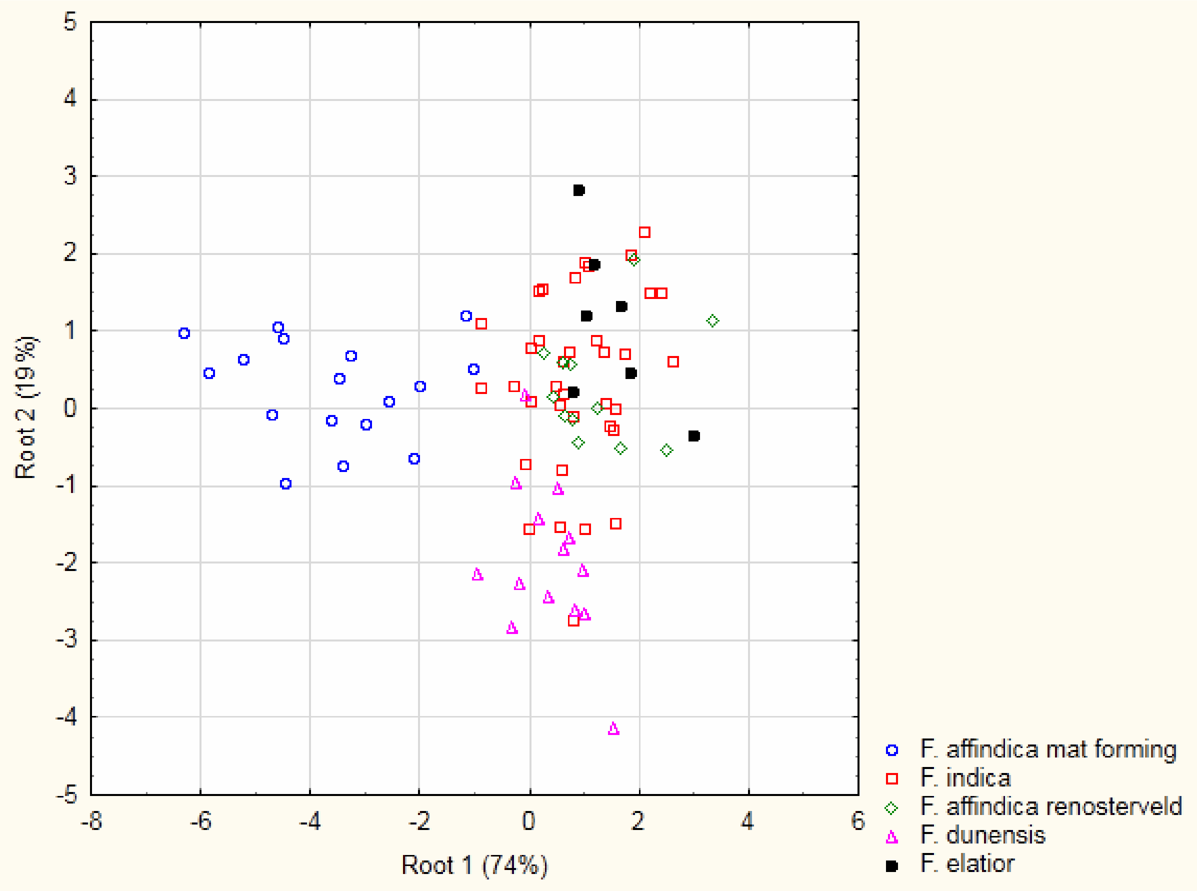

Figure 4.4: Ordination of *F. indica* complex taxa based on morphology.

(c) Variation in morphological characters within *F. indica* complex (ANOVA)

A summary of the morphometric characters that show significant differences ($P < 0.05$) among taxa studied is presented in Figure 4.5 and these characters are shown in Table 4.2. There is an overlap between some of the taxa for the characters that were measured, but the univariate analysis of variance (ANOVA) showed that some characters differ significantly among the taxa. The culm length, leaf sheath length and nutlet length were significantly shorter in *F.* aff. *indica* MF than in the other taxa (Figure 4.5). In addition, *F.* aff. *indica* MF possesses the highest number of culms per tuft (usually more than 16 culms), compared to the other taxa which have 3–12 culms per tuft (see Appendix 4.1). The culm length of the taxa within the complex ranged from 25–124 mm for *F.* aff. *indica* MF compared to 70–600 mm for *F. indica*, 70–1000 mm for *F.* aff. *indica* R, 245–900 mm for *F. elatior* and 102–207 mm for *F. dunensis*. The leaf sheath length ranges from 8–25 mm for *F.* aff. *indica* MF, 32–123mm for *F.* aff. *indica* R, 20–65 mm for *F. dunensis*, 17–90 mm for *F. elatior* and 17–90mm for *F. indica*. Another contributing factor that is showing a significant difference between the *F.* aff. *indica* MF and other taxa within the complex is the nutlet length, which ranges from 0.7–1.7 mm in *F.* aff. *indica* MF, 1.3–2.0 mm in *F.* aff. *indica* R, 1.0–2.0 mm in *F. elatior*, 0.8–1.8 mm in *F. dunensis* and 0.8–2.8 mm in *F. indica*.

There is a large overlap between the leaf blade, involucral bract and spikelet lengths within the complex. These ranged between 17–245 mm in leaf blade length, 3–100 mm for involucal bract length, and 2.5–6.8 mm for spikelet length (see Appendix 4.1). While the spikelet width of *F. dunensis* overlaps with that of *F.* aff. *indica* MF, these differ significantly from the other taxa in the complex (Figure 4.5). The glume length of *F.* aff. *indica* MF differs significantly from the other taxa (Table 4.2). All the members of the complex have a gynophore at the base of the nutlet.

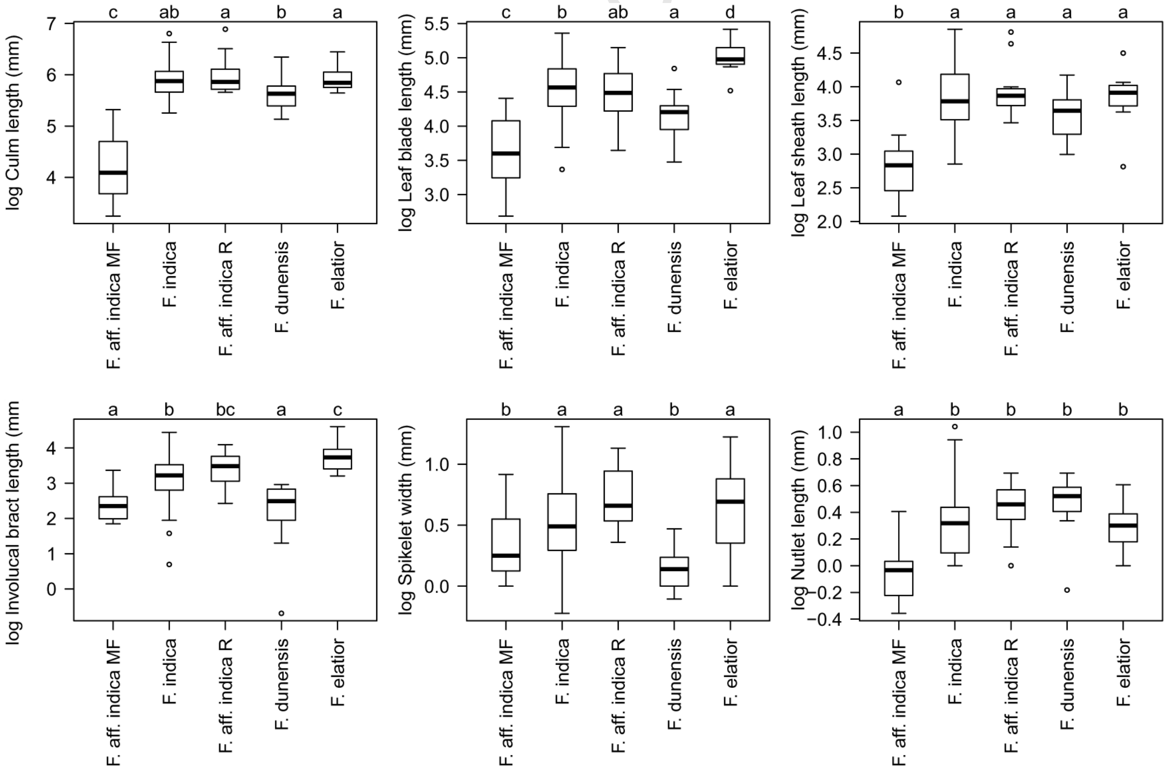

Figure 4.5: Box-and-whisker plots for the morphological characters in the *F. indica* complex that showed significant differences. The letters above the plots indicate significant differences between species

4.3.2. Soil nutrients analysis

(a) Analysis of variance on soil nutrient data

Analysis of variance (ANOVA) was used to evaluate if there were any differences in soil nutrients among the species' habitats. The means (± SE) for the soil substrate of each of the species are shown in Figure 4.6. The results showed that species within *Ficinia indica* complex are growing on soil substrates that are different ($P < 0.05$) in their chemistry for pH, Na, Ca, Mg, Mn and B. For instance, *F. dunensis* and *F. elatior* were growing in soils with higher Mg in comparison with *F.* aff. *indica* MF, *F. indica* and *F* aff. *indica* R. Furthermore, soil substrate of *F. dunensis* contained significantly lower levels of Mn, Fe, B and Cu than that of *F. elatior* (Figure 4.6). However, the soil chemistry of the habitats for *F.* aff. *indica* MF, *F. indica* and *F* aff. *indica* R was similar in pH, Na, Mn, but *F.* aff. *indica* MF soil contained higher levels of Mg, Ca, Fe and B than those of *F. indica* and *F* aff. *indica* R (Figure 4.6).

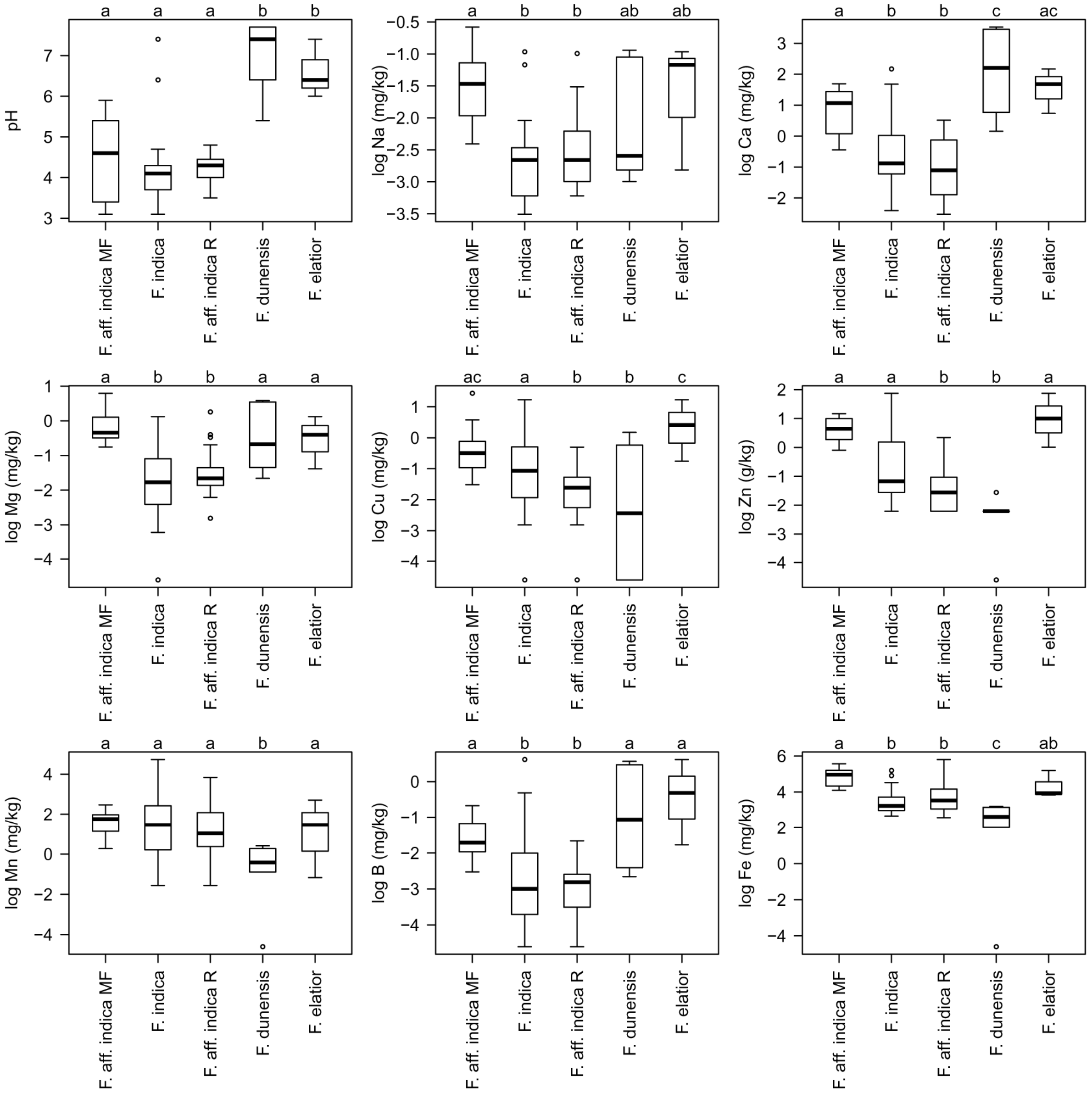

Figure 4.6: Box-and-whisker plots for the soil nutrient characteristics of *F. indica* complex showing significance differences in ANOVA. The letters above the plots indicate significant differences between species.

(b) Discriminant Function Analysis (DFA) on soil nutrients

The discriminant function analysis on the soil nutrient characteristics resulted in four roots with the first root contributing 68% and the second root contributing 18% (Table 4.3). The canonical values (CV) with high contributions (> 0.4) are highlighted in bold. The first root is largely correlated with pH (0.59), Bray II P (0.41), K (-0.82), Na (-0.63), Ca (0.63), B (0.99) and Fe -(0.47). Root 2 is largely correlated with pH (-0.54), K (0.44) and Ca (-1.44) (Table 4.3).

The plot of the first two roots of the DFA showed a clear separation of *F. dunensis* and a cluster of *F. indica* and *F.* aff. *indica* R (Figure 4.6). Furthermore, the soil of *F.* aff. *indica* MF separated completely with that of *F.* aff. *indica* R, but there was a mixture of sites between *F.* aff. *indica* MF and *F. indica* (Figure 4.6).

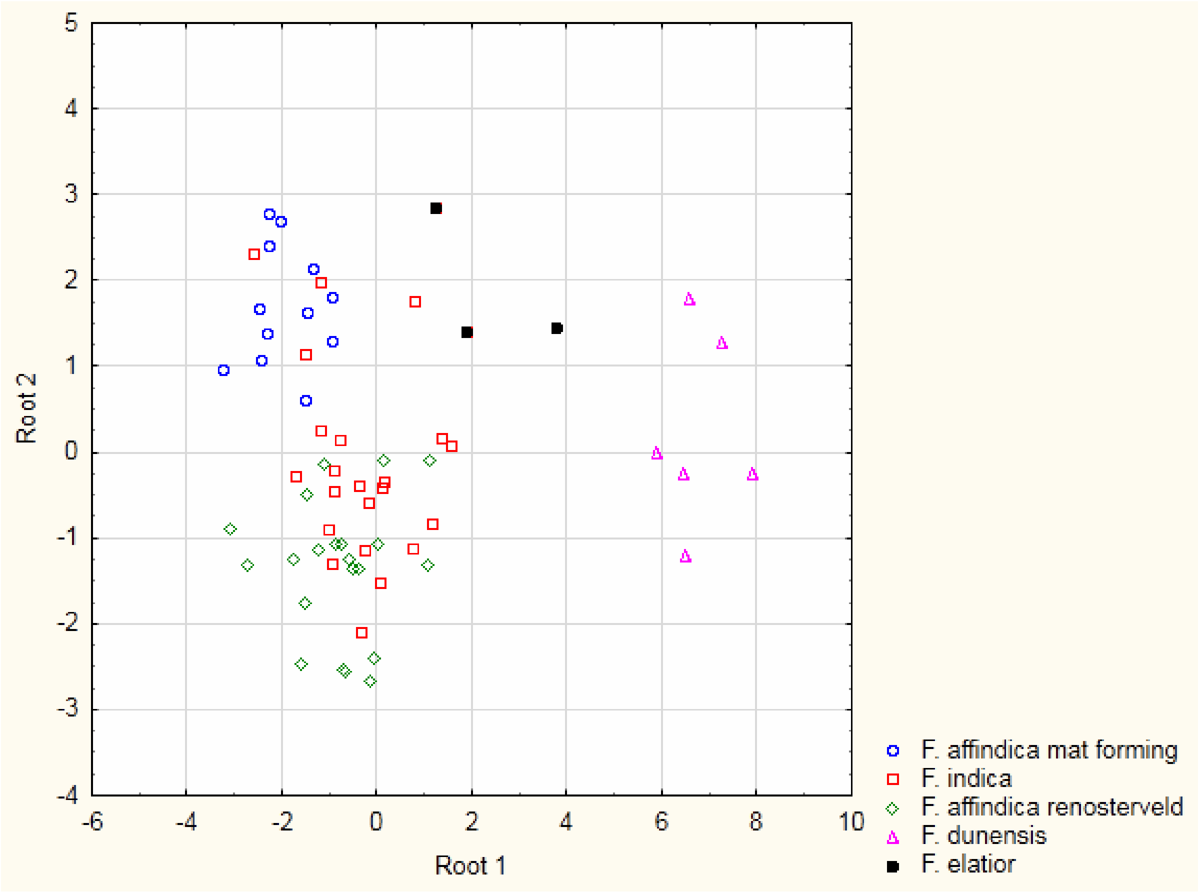

Figure 4.7: The ordination of the first and the second roots extracted in the discriminant analysis of soil nutrients of *Ficinia indica* complex.

Table 4.2: The canonical vectors for soil nutrient data. The values in brackets show the percentage that each root contributed. The highlighted values represent the nutrients contributing (> 0.4) to the separation of species within the complex.

Variable	**Root 1 (68%)**	**Root 2 (18%)**
pH (KCI)	**0.59**	**–0.54**
P Bray II (mg/kg)	**0.41**	0.21
K (mg/kg)	**–0.82**	**–0.44**
Na (mg/kg)	**–0.63**	0.39
Ca (mg/kg)	**0.63**	**1.44**
Mg (mg/kg)	–0.15	–0.39
Mn (mg/kg)	–0.38	–0.22
B (mg/kg)	**0.99**	–0.19
Fe (mg/kg)	**–0.47**	0.25
Inorganic N (mg/kg)	**0.44**	–0.27
Eigenvalue	5.52	1.46
Cumulative Proportion	0.68	0.86

4.4. Discussion

4.4.1. The morphological differences and similarities between the species in the *F. indica* complex

The main objective of this chapter was to revise the taxonomy of the species in the *F. indica* complex and to investigate whether the two putative new species in the complex (*F.* aff. *indica* MF and *F.* aff. *indica* R) are distinct. Both morphological (quantitative and qualitative characters) and ecological data were used to investigate the differences and similarities between the species of the complex. Cluster analysis resulted in two main clusters at a Euclidean distance of 5000 (Figure 4.3), where Cluster A comprised specimens of *F.* aff. *indica* MF while all the other taxa formed a separate single cluster (Cluster B). This observation is also supported by the ordination analysis of the first two roots of the DFA, with specimens in Cluster A (*F.* aff. *indica* MF) separated from the remaining species along Root 1 (Figure 4.4). Root 1 is mostly influenced by lengths of culm, leaf sheath and nutlets.

These results were further confirmed by univariate (ANOVA) analysis showing that these parameters were significantly shorter in *F.* aff. *indica* MF compared to the other species. Therefore, one can use culm length in combination with other characters to diagnose *F.* aff. *indica* MF. Similarly, Gordon-Gray (2008) used the culm length in taxon diagnoses in the *F. gracilis* complex, but this study cautioned that culms may elongate with maturation of fruits, which does not take place until late in the growing season.

Although there is considerable overlap between characters among Cluster B taxa (Figure 4.3; *F. indica, F.* aff. *indica* R, *F. dunensis* and *F. elatior*), there are several differences in qualitative characters that were not included in the morphometric analysis. These include differences in underground organs, where some taxa have stolons (*F. indica, F. dunensis*) whereas others possess rhizomes (*F. elatior* and *F.*aff. *indica* R). Typical *F. indica* has a well developed stolon and plants are robust with large inflorescences (≥5.6 mm). *Ficinia dunensis* was described by Levyns (1947) to include material which is more slender, bearing dark, wiry stolons with smaller inflorescences (4.3 mm). *Ficinia elatior* comprises robust plants that lack stolons and instead have rhizomes covered with firm scales. In addition, the taxa differ in the leaf sheath and ligule type, where *F. elatior* possesses a leaf sheath with the mouth sloping inwards and downwards to the leaf blade and lacking a ligule, whereas *F. indica* has wide variation in leaf sheath shape and various ligule types with no obvious distinction from *F.* aff. *indica* R. *Ficinia dunensis* possess a truncate sheath mouth and a ligule that has a cuff-shaped collar (Figure 4.1c) whereas the other taxa possess a leaf sheath with the mouth sloping inwards and downwards to the leaf blade and lacking a ligule. The shape of leaf sheath apex and presence or absence of the ligule were used to delimit *Ficinia* in Natal (Gordon-Gray, 1995). Therefore, the taxa in the *F. indica* complex can be separated using both qualitative and quantitative characters.

4.4.2. The ecological distributions within the *F. indica* complex

Analyses of soil nutrient data showed some separation of the taxa in nutrient regimes (Figure 4.6). The most distinct substrate was for *F. dunensis*, a taxon which grows on sand dunes with neutral to alkaline soils (with low concentration of Mn and Fe), whereas the other four taxa occur on acidic substrates. However, the ANOVA analysis of the species habitats showed that the soil substrate for *F. dunesis* and *F. elatior* were similar in pH, Mg, Na, and Ca despite the fact that the *F. elatior* site of collection was on the Cape Flats (Kenilworth), an area expected to be dominated by acid sands. Deviations of the *F. elatior* site may be due to burning of the vegetation in the season prior to sampling, as ash deposits are known to increase soil pH (Gupta et al., 2012).

Among the taxa on acidic substrates, the substrates supporting *F.* aff. *indica* MF recorded higher levels of Ca, Mg, Fe, Zn than that of *F.* aff. *indica* R (Figure 4.6), but *F. indica* occupies habitats that overlap with these two species. This is consistent with the observation from the ordination analysis (Figure 4.7) showing the substrate of *F.* aff. *indica* MF is different from that of *F.* aff. *indica* R. Futhermore, the *F.* aff. *indica* MF samples were collected from mountainous areas dominated by sandstone, whereas the *F.* aff. *indica* R samples were from Renosterveld areas dominated by shale-derived soils. In addition, taxa in the *F. indica* complex are separated by geography and altitude, with *F.* aff. *indica* MF occurring at high altitude (over 1500 m), *F. dunesis* in coastal areas (below 100 m), *F. elatior* on the Cape Peninsula, but *F. indica* more widespread and overlapping in geography and altitude with *F. dunensis* and *F.* aff. *indica* R.

4.5. Conclusions

This chapter investigated whether the taxa of *F. indica* complex are morphologically or ecologically distinct based on univariate and multivariate analyses. The discriminating power of the morphometric analyses and ecological data provided support that the taxa within *F. indica* complex are distinct and should not be merged into one species. They are separated by their morphology, habitat preference and the geographic distribution in the greater Cape Floristic Region. Therefore, *F. indica*, *F. elatior* and *F. dunensis* can continue to be recognized as previously circumscribed. Furthermore, this study provides evidence for the recognition of two additional taxa at the species rank. We, therefore, proceed to provide names for these taxa, with the mountain mat-forming taxon provisionally named *Ficinia montana* and the renosterveld taxon provisionally named *Ficinia rhinocerotis*. Formal publication of the new species in accordance to the *International Code of Nomenclature for algae, fungi, and plants* (*ICN*) will be pursued elsewhere.

4.5.1. Taxonomic treatment

(a) Key to the species of *Ficinia indica* complex

1a Mat-forming perennial, culms shorter than 200 mm, leaf sheath shorter than 25 mm; growing on mountains above 1500 m .... 1. *Ficinia montana*

1b Erect perennial, culms taller than 300 mm, leaf sheath longer than 49 mm; growing below 1000 m 2

  2a Plants forming stolons, inflorescence not enveloped by bract 3

    3a Stolons robust, more than 500 mm long; culm lenghth of 170-900mm long; leaf sheath apex lacking ligule; spikelet 10 mm long; leaf sheath papery .... ……………………………………………………………5. *Ficinia indica*

    3b Stolons wiry, 330 mm long; culm length of 170- 570mm lolng, leaf sheath apex with collar-shaped ligule; spikelet 1–5 mm long; leaf sheath not papery .... …………………………………………………………3. *Ficinia dunensis*

  2b Plants forming woody rhizomes, closely branched with firm brown scales; inflorescences densely crowded, enveloped by the wide reddish bases of the lower sheathing leafy bract 4

    4a Occurring in low-lying sandy habitats; culm lengh of 282-600mm longleaf sheath, mahogany-red below, paler above, and truncate.............. 4. *Ficinia elatior*

    4b Occurring on mountain slopes on clay substrate; culm length of 280- 980mm long, leaf sheath red, not papery ....2. *Ficinia rhinocerotis*

4.5.2. Descriptions of the species in *F. indica* complex

(1) *Ficinia montana* Tshiila, Muasya and Chimphango, sp. nov. Similar to *F. indica,* differing in habit (mat-forming vs. erect) and plant height (124 mm vs. 900 mm). Type: South Africa, Western Cape, near Middelberg Hut, Cederberg, Clanwilliam, Levyns 13093 (BOL).

Diagnostic features: Can be diagnosed by the short culms ranging from 25 to 124 mm. The structure of *F. montana* resembles a mat with more than 12 culms per tuft. The sheath (6.7–25 mm) mouth slopes inwards and downwards to the leaf blade and the ligule is lacking. Leaf blade length ranges from 0.3 to 0.8 mm. Spikelet and glume lengths ranges from 2.1–4.7 mm, width ranges from 1.00 – 2.50 mm and 1.0– 1.4mm, respectively. The matured nutlets are short (0.7- 1.5 mm long) and the width is 0.5 – 0.83 mm. The involucal bract ranges from

6.33 – 27.33mm. *F. montana* is currently known to grow on top of mountains with an altitude of about 1500–2000 m.

(2) *Ficinia rhinocerotis* Tshiila, Muasya and Chimphango, sp. nov. Similar to *F. indica,* differing in underground structures (rhizomes vs. stolons), leaf sheath type (not papery vs. papery) and plant height (900 – 980 mm). The culms are covered with brown to reddish scales on the base extending to form the closed leaf sheath. Type: South Africa, Western Cape, Renosterveld, Malmesbury, Dludlu 022 (BOL).

Diagnostic features: Perennial tuft (90cm and above) with brown to reddish scales. Reddish leaf sheath not papery. Spikelets (1.43- 3.10 mm in diameter, width 1.53 – 2.4mm) and the inflorescences are capitate. The glume length and wifth ranges from 1.77 – 4.53mm and 1.0 – 2.1mm, respectively. The involuctral bract ranges from 11.33 – 60.0mm). The leaf blades are longer than 120mm. Like other species of *Ficinia*, *F. rhinocerotis* consists of the gynophore (it ranges from 1.0 – 2.0mm long and 0.53 – 1.03mm wide). It consists of spirally arranged glumes. Occurs in Renosterveld and also on Cape Granite Suite-derived soils.

(3) *Ficinia dunensis* Levyns, Journ. S. Afr. Bot. 13: 68 (1947). Type: Type: South Africa, Western Cape, Muizenberg (3418AB), 3 Aug 1937, Levyns 6267 (Holotype: BOL; isotypes: PRE).

Diagnostic features: A perennial about 20 cm high, stoloniferous, bearing leaves and aerial stems in small tufts growing from stolons covered with dark brown scales when young, and are at length naked and wiry. The leaves of *F. dunensis* are usually less than half as long as the flowering stems with firm sheaths that are deep red. The leaves are truncate and the blades are filiform. Comprises 1–3 spikelets that are borne in a compact terminal head that is 1–3 mm in diameter. Spikelets are enveloped by two sheathing mucronate bracts with wide, dark red-brown bases and leafy tips exceeding the spikelet; the obovoid fruits are bluntly trigonous, rough and dark brown with a well developed disc that is shorty lobed. Grows on coastal dunes or mountainous slopes from Cederberg to Port Elizabeth and flowers from August to September. The species is allied to *F. indica* (Lam.) Pfeiff., from which it differs in its much smaller heads and its dark wiry stolons. Figure 4.2d shows the holotype of *F. dunensis* that had been described by MR Levyns (1947).

(4) ***Ficinia elatior*** **Levyns,** Journ. S. Afr. Bot. 13: 68 (1947). Type: South Africa, Western Cape, flats between Bonteberg and Klasjagerberg, Cape Peninsula, Levyns 5944 (BOL, isotype).

Diagnostic features: a perennial of about 40 cm high, lacking stolons. Has a woody rhizome which is loosely branched and covered with firm brown scales. Possesses rigid leaves that are about half as long as the flowering stems with the sheaths that are usually mahogany-red below, paler above, and truncate. Does not have ligules and the blades are channeled with scabrous margins. Inflorescence consists of a densely crowded compound spike, ovoid, 1 cm wide or more, enveloped by the wide reddish bases of the lower sheathing leafy bracts. The tips of the lower mucronate, deep red bract exceed the spikelets. Obovoid, trigonous, deep brown fruits with the faces almost smooth and a well developed, shortly lobed disc. It flowers between August and December and it is found from the Cape Peninsula to Bredasdorp (Levyns, 1947).

(5) ***Ficinia indica*** **(Lam.)** ***H.Pfeiff.,*** Revis. Gattung *Ficinia,* 38 (1921).Type: South Africa, : Burchell187 (.(K Isotype).

Diagnostic features: A tufted perennial 10–40 cm tall, with chestnut-brown spikelets (2.53–66.25 mm long and 1.0–3.7 mm wide)usually flowers between July and November and grows on flats and lower slopes from Namaqualand to the Eastern Cape (Goldblatt and Manning, 2000). Like all other species of *Ficinia,* it has capitate inflorescences with glumes (2.2–6.0 mm long and 1.0–2.7 mm) that are spirally arranged. Figure 4.2a shows the isotype of *F. indica.* The nutlet length and width of *F. indica* ranges from 1.03–2.83 mm and 0.5–1.5 mm, respectively.

CHAPTER 5

SUMMARY, SYNTHESIS AND RECOMMENDATIONS

The study had five major objectives to achieve. The first objective was to reconstruct the phylogenetic relationships within the *Ficinia* clade. The second was to reconstruct the patterns of macroevolution of taxonomically important morphological characters for the clade. The third was to estimate divergence dates within the clade. The fourth was to investigate the role of ecology in the speciation of the genus *Ficinia*. Finally, the fifth objective was to revise the taxonomy of the *Ficinia indica* complex.

Phylogenetic relationships in *Ficinia* were studied because there has been misunderstanding and the misplacement of several species within *Ficinia* clade based on morphology. Previous studies (Muasya et al. 2009a, Muasya and de Lange, 2010) using molecular approaches only sampled few taxa. This study sampled about 70% of the species within the group, meaning that additional information was obtained since the previous reseachers sampled only few taxa.. In addition, the phylogenies were reconstructed based on a dataset with two combined gene regions (ITS and *rps*16). The increased sampling of taxa in this study has value in showing the relationships of the newly sampled species. It also confirmed the results of previous studies (e.g. Muasya and de Lange, 2010; Muasya et al., 2009a, b; Simpson et al., 2007) where Cypereae resolved into two clades (*Cyperus* and *Ficinia* clade). The addition of duplicate taxa in the larger matrix (results in Figures 2.2 and 2.3 in Chapter 2) also confirmed the results obtained in previous studies, but there are some taxa that need to be further investigated since they appeared in different subclades (e.g. *F. oligantha and F. indica*). Both *Isolepis* and *Ficinia* are paraphyletic, while *Ficinia clandestina* is confirmed to belong to *Cyperus*.

Using the molecular approach was key to achieving the other objectives in this study. From inferred phylogenies, I was able to reconstruct the macroevolutionary patterns of major morphological characters for the species in *Ficinia* clade. The data used for this study showed that there are several characters that can be used to diagnose the species in the *Ficinia* clade. For example, the main characters that can be used to diagnose the genus *Ficinia* are the gynophores and the fact that they are perennial, as opposed to the annual

Isolepis. Some characters that were used to distinguish the species within the *Ficinia* clade are: root type, leaf sheath type, culm length, leaf blade, spikelets and glume arrangement (Chapter 2, Figure 2.6 a–i). However, none of infrageneric groups suggested for *Ficinia* by previous workers (e.g. Clarke, 1898; Pfeiffer, 1921; Levyns, 1950) were supported as monophyletic in this study, and characters used to diagnose such taxa (e.g. distichous glume arrangement, highly reduced leaves, etc.) have evolved independently multiple times in *Ficinia.*

Using the same molecular data, a dated phylogeny was produced to estimate the divergence dates of the lineages within *Ficinia* clade. The results showed that the *Ficinia* clade originated in late Oligocene, 24.75 Mya, with the split between the species-rich genera (*Isolepis* and *Ficinia;* 160 species) occurring in the mid-Miocene (14.86 Mya). *Ficinia,* a genus of resprouting species common in post-fire fynbos ecosystems, has rapidly radiated in the Pliocene (chapter 3, Figure 3.1). *Ficinia* has a diversification pattern comparable to other Cape clades such as *Babiana* (Schnitzler et al., 2011).

Sister species in the genus *Ficinia* were also studied to investigate the role of ecology in their speciation. These sister pairs were selected from the dated phylogeny (chapter 3, Figure 3.1), and their ecological data (altitude, flowering times, soil type, and the distribution data in QDS) was collected during field and herbarium studies (Chapter 3, Table 3.2). About 50% of the sister pairs were observed to be growing on different substrates (Chapter 3, Figure 3.3 a–j), and shifts in substrate type were mostly accompanied by geographical separation (non-overlapping distributions). In other cases where sister species had the same substrate, such pairs were separated in altitude or in distribution. This study therefore indicates the importance of ecological shifts in Cape clades, especially in wind-pollinated lineages with overlapping flowering periods. On the other hand, the role of intrinsic mechanisms in speciation need to be investigated as the species in one of the species pairs were observed to be co-occurring yet morphologically distinct.

The last objective was to revise the taxonomy of the *Ficinia indica* complex, based on morphology and soil type. *Ficinia indica* is a morphologically variable species that is widespread in the GCFR, and that has previously been circumscribed to include taxa that are genetically, ecologically, and morphologically distinct. This study supports Levyns's (1947) recognition of *F. dunensis* and *F. elatior* as separate species, and further segregates two new species, *Ficinia montana* and *Ficinia rhinocerotis*.

I recommend that other DNA regions should be screened to improve the resolution of the phylogeny and also that additional morphological characters should be included to support the findings of the present study. Such a robust phylogeny will allow for inference of the speciation processes by studying more sister-species pairs in relation to geographic and ecological parameters. Mutiple samples of several species (*F. oligantha*) appeared in different clades in the phylogeny, implying that such species may be broadly circumscribed to include genetically divergent taxa and this will be cleared up by using more markers, samples and characters.

REFERENCES

Agapow P.M., Bininda-Edmonds O.R.P, Crandall K.A.,Gittleman J.L., Mace G.M., Marshall J.C, Purvis A. (2004). The impact of species concept on biodiversity studies. Rev. Biol. 79:161–179.

Akaike H. (1974). A new look at statistical-model identification. IEEE Transactions on Automatic Control AC19, 716–723. ISSN 0018-9286.

Alvarez I. & Wendel J.F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution, 29, 417-434.

Anderson E. (1940). The concept of genus II, A survey of modern opinion. Bull. Torrey Bot. Clubb67: 363-369.

Ankel-Simons F. & Cummins M.J. (1996). Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proceedings of the National Academy of Sciences of the United States of America, 93, 3859–13863.

Archer C. (2000). Cyperaceae. In: Goldblatt P., Manning, J. (Eds.), Cape Plants: A Conspectus of the Cape Flora of South Africa: Strelitzia 9: 81–92.

Arnold T.H. & Gordon-Gray. (1978). Notes on the genus Ficinia: F. pygmaea, F. limosa, F. clandestina and nine new taxa from the South Western Cape, South Africa. Bothalia 12: 405-420.

Baker W.J. & Zona S. (2006). Traditional morphometrics in plant systematics and its role in palm systematics. Botanical Journal of the Linnean Society 151: 103 – 111.

Baldwin B.G., Sanderson, M.J., Porter, M. & Wojciechowski, M.F. (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82 (2): 247-277.

Bargelloni L., Marcato S., Zane L., Patarnello T. (2000). Mitochondrial Phylogeny of Notothenioids: A Molecular Approach to Antarctic Fish Evolution and Biogeography. Systematic Biology 49:114-129.

Barkely T.M., DePriest P., Funk V., Kiger R.W., Kress, W.J. & Moore, G. (2004) Linnean nomenclature in the 21st Century: a report from a workplace on integrating traditional nomenclature and phylogenetic classification. Taxon 53:153-158.

Barker N.P., Linder H.P., Morton C.M. & Lyle M. (2003). The paraphyly of Cortaderia (Danthonioideae: Poaceae) evidence from morphology and chloroplast and nuclear DNA sequence data. Annals of the Missouri Botanical Gardens 90 (1): 1-24.

Baraclough T.G. and Volger A.P. (2002). Sequence- Based species delimitation for DNA Taxonomy of undescribed insects. Systematic Biology (4) 595-609

Barton N.H. (1996). Natural selection and random genetic drift as causes of evolution on islands, Philos. Trans. R. Soc. London Ser. (B)351: 785–795.

Besnard G., Muasya A. M., Russier F., Roalson E. H., Salamin N., & Christin P.A. (2009). Phylogenomics of C4 photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. Molecular Biology and Evolution 26(8): (1909-1919).

Bockeler O. (1870). Die Cyperaceen des Koniglichen Herhariums zu Berlin. Linnaea 36: 271-512.

Born J., Linder H.P., Desmet P. (2007). The Great Cape Floristic Region. J. Biogeography. 34:147-162.

Brown R. (1810). Prodromus florae Novae Hollandiae et insulae Van-Diemen vol. 1. London: Johnson.

Bruhl J.J. (1995) Sedge genera of the world: relationships and a new classification of the Cyperaceae. Australian Systematic Botany /8: 125_305.al Review /75: 52_66.

Burkill H. M. (1985). The Useful Plants of West Tropical Africa 1 (2). Royal Botanic Gardens, Kew.

Bush G.L. (1994). Sympatric speciation in animals: new wine in old bottles, Trends Ecol. Evol. 9: 285–288

Bynum W. "Darwin, Charles Robert." Microsoft® Encarta® (2006.) [CD]. Microsoft Corporation, 2005.

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 RoyalSociety B-Biological Sciences 278: 188–195.

Campbell B.M. (1983). Montane plant environments in the Fynbos Biome. Bothalia 14, 283–298.

Cauis J. F. & Banby J. (1935). The medicinal and poisonous sedges of India. J. Bombay Nat. Hist. Soc. 38 (1): 163 - 70.

Friedman J, Barrett S.C.H. (2008). A phylogenetic analysis of the evolutionof wind pollination in the angiosperms. International Journal of Plant Sciences 169: 49–58.

Clarke C. B. (1898). Cyperaceae. In Flora Capensis, Thiselton Eyer W.T. (ed) 7: 235-260.

Clayton W.D. (1974). The logarithmic distribution of angiosperm families. Kew Bull. 29: 271-279.

Cowling R.M. (1987). Fire and its role in coexistence and speciation in Gondwanan shrublands. South African Journal of Science 83: 106-111.

Cowling R.M. & Pressey R.L. (2001). Rapid plant diversification: planning for an evolutionary.

Cowling R. M., Cartwright C. R., Parkington J.E. & Allsopp J.C. (1999). Fossil wood charcoal assemblages from Elands Bay Cave, South Africa: implications for Late Quaternary vegetation and climates in the winter-rainfall fynbos biome. Journal of Biogeography 26: 367–378.

Cowling R. M., Proches S. & Partridge T. C. (2009). Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. Molecular Phylogenetics and Evolution 51: 64–74.

Cowling R.M., Rundel P.W., Lamont B.B., Arroyo M.K. & Arianoutsou M. (1996). Plant diversity in Mediterranean- climate regions. Trends Ecology and Evolution 11: 362–366.

Coyne J. A. & Orr, H. A. (2004). Speciation. Sinauer Associates, Sunderland, MA.

Coyne J.A. (1992) Genetics and speciation, Nature 355:511–515.

Crochet P. & Desmarais E. (2000). Slow rate of evolution in the mitochondrial control region of gulls (Aves: Laridae). Molecular Biology and Evolution 17 (12): 1797–1806.

Cronk Q.C.B. (1961). The name of the pea: a quantitative history of legume classification. New Phytology 116:163-175.

Davies T.J., Barraclough T.G., Chase M.W., Soltis P.S., Soltis, D.E. & Savolainen V. (2004). Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Sciences of the United States of America 101:1904–1909.

de Queiroz K. (1999). The general lineage concept of species and the defining properties of the species category. Pages 49–89 in Species: New interdisciplinary essays (R. A. Wilson, ed.). MIT Press, Cambridge, Massachusetts.

de Queiroz K. (2005a). Different species problems and their solution. BioEssays 26:67–70.

de Queiroz K. (2005b). Ernest Mayr and the modern concept of species. Proceedings of the national Academy of Sciences of the United state of America 102:6600–6607.

de Queiroz K. (2007). Species concept and species delimitation. Systematic Biology. 56(6): 879-886

Deacon H.J., Jury M.R., Ellis F. (1992). Selective regime and time. In: Cowling, R.M. (Ed.), The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, Cape Town 6–22.

Davies T.J., Barraclough T.G., Chase M.W., Soltis P.S., Soltis, D.E. & Savolainen V. (2004). Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proceedings of the National Academy of Sciences of the United States of America 101:1904–1909.

Dobzhansky T. (1951). Genetics and the Origin of Species, Columbia University Press.

Driver A, Desmet P.G., Rouget M, Cowling R.M., Maze K.E., (2003). Succulent Karoo ecosystem plan. Biodiversity component, Technical Report. Cape Town (South Africa): Cape Conservation Unit, Botanical Society of South Africa.

Endler J.A. (1977). Geographic Variation, Speciation, and Clines, Princeton University Press.

Felsenstein J. (1988). Phylogenies and quantitative characters. Annual Review of Ecology and Systematics 19: 445-471.

Fitch W. M. (1971). Toward defining the course of evolution: Minimum change for a specific tree topology. Systematic Zoology 20: 406.

Flury B.R. & Riedwyl, H. (1988). Multivariate statistics: A practical approach, Chapman and Hall. London. Proceedings of the National Academy of Sciences of the United States of America 98: 5452-5457.

Futuyma D.J. (1998.) Evolutionary biology. 3rd edition. Sinauer, Sunderland, Mass.

Goetghebeur P. (1986). Genera Cyperacearum Unpublished DSc thesis. Ghent, Belgium, State University.

Goetghebeur P. (1998). Cyperaceae. In: Kubitzki K ed. The families and genera of vascular plants 4, Springer, Berlin 141-190.

Goldblatt P. (1977). Systematics of Moraea (iridaceae) In Tropical Africa. Annals of the Missouri Botanical Garden 64: 243–295.

Goldblatt P. (1978). An analysiso f the florao f southern Africa: Its characteristics, relationships, and origins. Ann. Missour Biot. Gard.6 5: 369-436.

Goldblatt P. & Manning J. (2000). Cape Plants: a conspectus of the Cape Flora of South Africa, National Botanical Institute of South Africa. Strelitzia 9. Pretoria.

Goldblatt P., Savolainen V., Porteous O., Sostaric I., Powell M., Reeves G., Manning J.C., Barraclough T.G. & Chase M.W. (2002). Radiation in the Cape flora and the phylogeny of peacock irises Moraea (Iridaceae) based on four plastid DNA regions. Molecular Phylogenetics and Evolution 25: 341-360.

.

Gordon-Gray K. D. (1995). Cyperaceae in Natal. Strelitzia 2. National Botanical Institute, Pretoria.

Govaerts R., Simpson D.A., Goetghebeur P., Wilson K.L., Egorova T. & Bruhl J. (2007). World checklist of Cyperaceae. The Board of Trustees of the Royal Botanic Gardens, Kew.

Greenman M. (1940). The concept of genus III. Genera from the standpoint of Morphology. Bull. Torrey Bot. Club 67:363-369.

Grounds R. (1989) Ornamental Grasses. Christopher Helm, London.

Gupta A.K., Singh R.P, Ibrahim M.H & Lee B.K. (2012). Fly Ash for Agriculture: Implications for soil properties, Nutrients, Heavy Metalas, Plant Growth and Pest Control, Sustainable Agriculture review 8:269-286.

Haines R. W. & Lye K.A. (1983).The Sedges and Rushes of East Africa. East African Natural History Society, Nairobi.

Hall T.A. (1999). BioEdit: a user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. In: Nucleic acids symposium. Oxford University Press.

Harrison C.J & Langdale J.a. (2006). A step by step guid to phylogeny reconstruction. The plant Journal 45: 561- 572

HenningW. (1966). Phylogenetic Systematics, University of Il-linois Press, Urbana, Chicago, London.

Hermann F.J. (1970). Manual of the Carices of the Rocky Mountains and Colorado Basin. U.S. Department of Agriculture, Forest Service. U.S. Department of Agriculture Handbook no. 374.

Hey J., Waples R.S., Arnold L.M,Butlin R.K. and Harrison R.G. (2003). Understanding and confronting species uncertainty in biology and conservation. Trends Ecol. Evol. 18:597–603.

Hsiao C., Chatterton N.J., Asay, K H. & Jensen K.B. (1994). Phylogenetic relationships of 10 grass species: an assessment of phylogenetic utility of the internal transcribed spacer region in nuclear ribosomal DNA in monocots. Genome 37(1): 112-120. doi:10.1139/g94-014.

Hu Y., Zhang, Q., Rao, G. & Sodmergen. (2008). Occurrence of plastids in the sperm cells of Caprifoliaceae: biparental plastid inheritance in angiosperms is unilaterally derived from maternal inheritance. Plant Cell Physiology, 49 (6): 958-968.

Humphrys M. & Linder H.P. (2009). Concept versus data in delimitation of plant genera. Taxon 58 (4): 1054-1074.

Huxley A. (1992). The New Royal Horticultural Society Dictionary of Gardening. Royal Horticultural Society, Wisley.

Johnson S.D. (1996). Pollination, adaptation and speciation models in the Cape flora of South Africa. Taxon 45, 59–66.

Judd W.S., Campbell C.S., Kellogg E.A., Stevens P.F. & Donoghue M.J. (2008). Plant Systematics: a Phylogenetic approach Sinauer Associates inc. Massachusetts. USA.

Jurgens N. (1997). Floristic biodiversity and history of African arid regions. Biodiversity Conservation. 6:495-514.

Kitching I.J., Forey P.L., Humphries C.J. and Williams D.M. (1998). Cladistics (2nd edn): The Theory and Practice of Parsimony Analysis. Oxford, UK: Oxford University Press.

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

Knoop V. (2004). The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. Current Genetics 46: 123–139.

Knowles L.L & Carstens B.C. (2007). Delimiting Species without Monophyletic Gene Trees, Syst. Biol. 56(6):887–895.

Krzanowski W.J. (1990). Principles of Multivariate analysis: a user's perspective, Oxford University Press. Oxford.

Kurzweil H., Linder, H.P. & Chesselet, P. (1991). The phylogeny and evolution of the Pterigodium-Corycium complex (Coriciinae, Orchidaceae). Plant Systematics and Evolution 175: 162-223.

Lambrechts J.J.N. (1979). Geology, geomorphology and soils. Pages 16– 26 in Fynbos ecology:Apreliminary synthesis (J. Day,W.R. Siegfried, G. N. Louw, and M. L. Jarman, eds.). Council for Scientific and Industrial Research, Pretoria, South Africa.

Latimer A.M., Silander J.A. & Cowling R. M. (2005). Neutral ecological theory reveals isolation and rapid speciation in a biodiversity hot spot. Science 309: 1722–1725.

Legendre P. & Legendre L. (1998). Numerical ecology. Amstadam: Elsevier

Levyns M.R. (1950). Cyperaceae. Cape peninsula: 97- 132.

Levyns M.R. (1964). Migrations and origins of the Cape flora. Transactions of the royal society . South Africa 37: 85–107.

Linder H.P. (2003). The radiation of the Cape flora, Southern Africa Biological Reviews, 78: 597-638.

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

Linder, H. (2005b). A protocol for the systematic documentation of the ecology of Cape plants. South African Journal of Botany 71, 201–210. ISSN 0254-6299.

Linder H.P. (2008). Plant species radiations: where, when, why? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363:3097y? Phi

Linder H.P. & Hardy C.R. (2004). Speciation in the Cape flora: A macroevolutionary and macroecological perspective. In Plant species-level systematics: New perspectives on pattern and process (F. T. Bakker, L. W. Chatrou, B. Gravendeel, and P. B. Pelser, eds.). Koeltz, K¨onigstein.

Linder H.P. & Vlok J. (1991). The morphology, taxonomy and evolution of Rhodocoma (Restionaceae), Plant Systematics and Evolution, 175: 139-160.

Linder H.P., Meadows M. E. & Cowling R. M. Ed (1992). History of the Cape flora Oxford University Press, Cape Town.

Linnaeus C. (1753) Species plantarum: a facsimile edition of the first edition. Ray Society, London.

Losos J.B. and Schluter D. (2000) Analysis of evolutionary species: area relationship. Nature. 408: 847-850

Losos J.B., Warheit K.B. & Schoener T.W. (1997). Adaptive differentiation following experimental island colonization in Anolis lizards, Nature 387: 70–73

MacArthur R. H. & Wilson E. O. (1967). The Theory of Island Biogeography. Princeton, NJ: Princeton University Press.

Maddison W.P. & Maddison D.R. (2006). Mesquite: A modular system for evolutionary analysis. <http://mesquiteproject.org>.

Magallon S.A. & Sanderson, M.J. (2001). Absolute diversification rates in angiosperm clades. Evolution. 55(9): 1762-1780

Magallon S.A. & Sanderson, M.J. (2005). Angiosperm divergence times: the effect of genes, codon positions, and time constraints. Evolution, 59, 1653–1670.

Margules C.R. & Pressey R.L. (2000). Systematic conservation planning. Nature 405:243–253.

Mayr E. (1942). Systematics and the origin of species from the viewpoint of a zoologist, 3rd edition. 1999. Harvard University Press, Cambridge, Massachusetts

Mittermeier R.A., Gil P.R., Hoffmann M., Pilgrim J.D., Brooks T.M., Mittermeier C.G., Lamoreux J.E., da Fonseca G.A.B. (2004). Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions. Sierra Madre (CA): University of Virginia.

Moore L. E. & Edgar, E. (1970). Flora of New Zealand 2. A. R. Shearer, Wellington.

Muasya A.M. & De Lange P.J. (2010) *Ficinia spiralis* (Cyperaceae) a new genus and combination for Desmoschoenus spiralis, New Zealand Journal of Botany 48 (1):31_39

Muasya A.M. & Simpson D.A. (2002). A monograph of the genus *Isolepis* R.Br. (Cyperaceae). Kew Bull, 57: 257–362.

Muasya A. M., D. A. Simpson, M.W. Chase, & A. Culham. (1998). An assessment of the suprageneric phylogeny in Cyperaceae using rbcL DNA sequences. Plant Syst. Evol. 211: 257–271.

Muasya A.M, Simpson DA, Chase MW, Culham A. (2001). A phylogeny of Isolepis (Cyperaceae) inferred using plastid rbcL and trnL-F sequence data. Systematic Botany 26: 342-353.

Muasya A.M., Simpson A.D., Goetghebeur P. (2000). New Combinations in *Trichophorum, Scirpoides,* and *Ficinia* (Cyperaceae). Novon, Vol. 10, No. 2. (Summer, 2000), pp. 132-133.

Muasya A.M., Simpson D.A., & Chase M.W. (2002). Phylogenetic relationships in Cyperus s.l. (Cyperaceae) inferred from plastid DNA sequence data. Botanical Journal of Linnean Society. 138: 145–153.

Muasya A.M., Simpson D.A., Chase, M.W., Culham A., (1998) .An assessment of the suprageneric phylogeny in Cyperaceae using rbcL DNA sequences. Plant Systematics and Evolution 211: 257-271.

Muasya A.M., Simpson D.A., Chase M.W. & Culham A. (2001). A 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.

Muasya A.M., Vrijdaghs A., Simpson D.A. Chase M.W., Goetghebeur P. & Smets E. (2009) What is a genus in Cypereae: phylogeny, character homology assessment and generic circumscription. Botanical Review 75:52–66.

Newman T.K., Jolly, C.J. & Rogers, J (2004). Mitochondrial phylogeny and systematics of baboons (Papio). American Journal of Physical Anthropology 124: 17-27.

Nixon K. C., & Wheeler Q.D. (1990). An amplification of the phylogenetic species concept. Cladistics 6:211–223.

Noor M.A.F. (2002). Is the biological species concept showing its age? Trends in Ecology & Evolution 17:153–154.

Oberwinkler F. (1994). Genera in a monophyletic group: the Dacrymycetales. Mycol. Helv. 6: 35-72.

Oxelman B., Lidén M. & Berglund D. (1997). Chloroplast rsp16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). Plant Systematics and Evolution 206: 393-410.

Palmer J.D. (1987). Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. The American Naturalist 130: (Supplement) S6-S29.

Paradis E., Claude J. & Strimmer K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.

Parsons W. T. & Cuthbertson E. G. (1992). Noxious Weeds of Australia. Inkata Press, Melbourne.

Partridge T. R. (1992). Vegetation recovery following sand mining on coastal dunesat Kaitorete Spit, Canterbury, New Zealand. Biology and Conservation. 61 (1): 59 - 71.

Pfeiffer H. (1921). Revision der Gattung *Ficinia* Schrad. Bremen.

Phillimore A.B. & Price T.D. (2008). Density-dependent cladogenesis in birds. Plos Biology 6: 71.

Phillimore A.B., Freckleton R.P. and Orme C.D.L. (2006). Ecology predict large scale patterns of phylogenetic diversification in birds. American Naturalist 168(2)

Piazza P., Jasinski S. & Tsiantis M. (2005). Evolution of leaf developmental mechanisms. New Phytology. 167: 693–710.

Pimental R.A. (1979). A comparative study of dataand ordination technique based on a hybrid swarm of sand verbenas (Abronian Juss.). Sytematic zoology 30: 250-267.

Pittier H. (1971). Manual de las plantas usuales de Venezuela. Fundacion Eugenio mendoza, Caracas.

Posada D. & Crandall K.A. (1998). Model test: Testing the Model of DNA Substitution. Bioinformatics Applications Note. Volume 14 no 9:817-818

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

Rabosky D.L. (2006). Laser: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenetics. Evolutionary Bioinformatics 60(6): 1152-64

Rabosky D.L. & McCune A.R. (2010). Reinventing species selection with molecular phylogenies. Trends in Ecology and Evolution 25: 68–74. ISSN 0169-5347.

Rambaut A. & Drummond, A. J. (2007) Tracer v1.4. http://beast.bio.ed.ac.uk/Tracer.

Raven P.H., Berlin B. & Breedlove D.E. (1971). The origin of taxonomy. Science 174: 1210-1213.

Raynal J. (1977). Notes Cyperologiques: 32. Le genre Iso- lepis R.Br. 1. Quelques especes africaines. Adansonia 17: 49-57.

Raxworthy J.C., Ingram M., Rabibisoa N. and Pearson R.G. (2007). Application of ecological niche modelling for species delimitation:A review and empirical Evaluation using day geckos (*Phelsuma*) from Madagascar. Systematic Biology. 56 (6): 907-923)

Reznick, D.N. (1997). Evaluation of the rate of evolution in natural populations of guppies (Poecilia reticulata), Science 275: 1934–1937

Richardson J.E., Weitz F.M., Fay M.F., Cronk Q.C.B., Linder H.P., Reeve S.G. & Chase M.W. (2001) Rapid and recent origin of species richness in the Cape flora of South Africa. Nature 412: 181–183.

Ricklefs, R. E. (1977). Environmental Heterogeneity and Plant Species Diversity: A Hypothesis. American Naturalist 111: 76–381.

Ricklefs, R.E. (2007). Evolution and the latitudinal diversity gradients: species extinction and biogeography. Ecology letters 10(4): 315-331.

Rissler L.J. & Apadaco J.J. (2007). Adding more ecology into species delimitation:Ecological models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). 56 (6): 924-942

Sauquet H., Weston P.H., Anderson C.L., Barker N.P., Cantrill D.J., Mast A.R. & Savolainen V. (2009). Contrasted patterns of hyperdiversification in Mediterranean hotspots. Proceedings of the National Academy of Sciences of the United States of America 106, 221–225. ISSN 0027-8424.

Schemske, D.W. & Bradshaw Jr., H.D., (1999). Pollinator preference and the evolution of floral traits

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

Schluter, D. (2001). Ecology and the origin of species. Trends in Ecology and Evolution 16, 372–380. ISSN 0169-5347.

Schnitzler J., Barraclough T.G., Boatwright J.S. Goldblatt P., Manning J.C., Powell M.P., Rebelo T. & Salvolainen, V. (20011). Causes of plant diversification in the Cape biodiversity hotspot of South Africa. Systematic Biology 60: 343-357.

Schutte, A. L., Vlok, J. H. J. & van Wyk, B.-E. (1995) Fire-survival strategy - a strategy of taxonomic, ecological and evolutionary importance in fynbos legumes. Plant Systematics and Evolution 195, 243–259.

Scotese, C. R., L. M. Gahagan, and R. L. Larson. (1988). Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. Tectonophysics 155:27-48.

Scotland R.W., Olmstead, R.G. & Bennett, J.R. (2003). Phylogeny reconstruction: the role of morphology. Sytematic Biology, 52, 539-548.

Shaffer H.B. and Thompson R.C. (2007): Delimiting species in recent radiation. Systemati Biology. 56(6): 896-906

Shipunov, A.B. & Bateman, R.M. (2005). Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia. Biological Journal of the Linnean Society 85 (1): 1-12.

Simpson & Inglis A (2001). Cyperaceae of Economic, Ethnobotanical and Horticultural Importance: a Checklist. Kew Bulletin: 56: 257-360.

Simpson D.A, Furness C.A, Hodkinson T.R, Muasya A.M, Chase M.W. (2003). Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. American Journal of Botany 90: 1071–1086.

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

Simpson, G.G., (1953). The Major Features of Evolution. Columbia University Press, New York.

Smith S.A & Donoghue M.J. (2010). Combining historical Biogeography with Niche Modelling in the Caprifolium clade of Lonicera (Caprifoliaceae, Dipsacales). Systematic Biology 590: 322-341

Smith, T.B. et al. (1997). A role for ecotones in generating rainforest, Science 276: 1855–1857.

Sneath P.H.A. & Sokal, R.R. (1973). Numerical Taxonomy. W.H. Freeman and Company, New York, USA.

Sokal R.R. & Rohlf, F.J. (1981). Biometry, Freeman, New York.

Staden R., Beal, K.F. & Bonfield, J.K. (1998). The Staden Package In: Bioinformatics Methods and Protocols.

Stebbins G.L. (1956). Taxonomy and evolution of genera, with special reference to the family Gramineae. Evolution 10: 235-245.

Steudel E.F. (1855). Synopsis Plantaruin Gluiimacearini. 2. Cyperaceae. Stuttgart: Metzler.

Stevens P.F. (1997). How to interpret classifications- suggestion from history. Bioscience 47: 243-250.

Sturmbauer C., Berger B., Dallinger R. & Föger M. (1998). Mitochondrial phylogeny of the genus Regulus and implications on the evolution of breeding behaviour in sylvioid songbirds. Molecular Phylogenetics and Evolution 10: 144-149.

Swofford D.L. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4b10, Sinauer Associates, Sunderland, MA

Takhtajan A. (1986). Floristic Regions of the World. University of California Press, Berkeley

Thorpe R.S. (1983). A review of the numerical methods for recognising and analysing racial differentiation. In: Felsenstein J, ed. Numerical taxonomy. Berlin: Springer-Verlag.

Valente L.M., Reeves G., Schnitzler J., Mason I.P., Fay M.F., Rebelo T.G., Chase M.W. & Barraclough T.G. (2009). Diversification of the African genus Protea (Proteaceae) in the Cape biodiversity hotspot and beyond: equal rates in different biomes. Evolution 64(3): 745-760.

Van der Niet, T & Johnson, S.D., (2009). Patterns of plant speciation in the Cape floristic region. Molecular Phylogenetics and Evolution 51: 85–93.

Van der Niet, T. & Linder H.P. (2008). Dealing with incongruence in the quest for the species tree: a case study from the orchid genus *Satyrium*. Molecular Phylogenetics and Evolution 47: 154–174.

Verboom, G.A., Linder H.P, Stock W.D. (2004). Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus Ehrharta (Poaceae: Ehrhartoideae). Am. J. Botanic 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. et al. (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.

Via S. (2002). The ecological genetics of speciation. American Naturalist 159(suppl.):S1–S7.

Vrijdaghs A., Goetghebeur P., Muasya M.A., Caris P. and Smets E. (2005). Floral Ontogeny in *Ficinia* and *Isolepis* (Cyperaceae), with Focus on the Nature and Origin of the Gynophore. Annals of Botany 96: 1247–1262.

Vrijdaghs A., Goetghebeur P., Smets E., Muasya A.M. (2006). The Floral Scales in Hellmuthia (Cyperaceae, Cyperoideae) and Paramapania (Cyperaceae, Mapanioideae): An Ontogenetic Study, Annals of Botany 98: 619–630.

Walters S.M. (1963). Method of classical plant taxonomy Pp 1-5 in: Swain. T. (ed). Chemical plant taxonomy. Academic Press, London

.

Warren B.H., Bakker F., Bellstedt D. U., Bytebier B., ClaSZen-Bockhoff R., Dreyer L. L., Edwards D., Forest F., Galley C., Hardy C.R. (2011). Consistent phenological shifts in the making of a biodiversity hotspot: the Cape flora. BMCEvolutionary Biology 11:39.

White T.J., Bruns, T., Lee, S. & Taylor, J. Ed. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. Academic Press, San Diego.

Wiens J.J. (2001). Character analysis in morphological phylogenetics: problems and solutions. Systematic Biology 50: 688-699.

Wiens J.J. (2004a). The role of morphological data in phylogeny reconstruction. Systematic Biology, 53 (4): 653-661.

Wiens J.J. (2004b). What is speciation and how should we study it? The American Naturalist 163 (6): 914-923.

Wiens J.J. (2007). Species delimitation: New Approaches for Discovering Diversity, Systematics Biology, 56 (6): 875-878.

Wien J.J. (2011). The causes of species richness patterns across space, time, and clade and the role of 'ecological limits'. The quaterly review of Biology. 86(2)

Wilson E.O. (1998). Consilience. The unity of knowledge. Abacus Press, London.

Appendix 4.1 Morphological data used for the *Ficinia indica* complex.

Species	Collector number	Culm length (mm)	Leaf blade length (mm)	Leaf blade width (mm)	Leaf sheath length (mm)	Involucal bract length (mm)	Spikelet length (mm)	Spikelet width (mm)	Glume length (mm)	Glume width (mm)	Nutlet length (mm)	Nutlet width (mm)
F. aff. *indica MF*	2335b	39.33	23.67	0.43	9.00	6.33	3.13	1.43	2.73	1.50	1.03	0.60
F. aff. *indica* mat-forming	2372	76.00	35.33	0.53	21.67	13.67	3.30	1.73	3.00	1.43	0.97	0.47
F. aff. *indica* mat-forming	4581	124.67	67.00	0.53	17.33	24.67	4.67	2.03	4.47	1.70	1.50	0.83
F. aff. *indica* mat-forming	4583	204.33	81.00	0.60	58.33	29.00	5.03	2.50	2.73	1.20	1.03	0.57
F. aff. *indica* mat-forming	4827	93.33	77.67	0.53	16.67	13.17	5.50	1.83	4.03	1.40	1.40	0.83
F. aff. *indica* mat-forming	11117	62.33	30.67	0.37	11.67	7.33	2.57	1.17	2.60	1.10	0.97	0.57
F. aff. *indica* mat-forming	18051	110.00	33.33	0.50	25.33	12.33	2.57	1.30	2.40	1.40	0.97	0.60
F. aff. *indica* mat-forming	18078	92.33	25.67	0.57	21.00	10.33	2.57	1.13	2.07	1.07	0.97	0.63
F. aff. *indica* mat-forming	18148	114.00	47.67	0.50	19.00	7.33	2.47	1.17	2.17	1.17	1.03	0.60
F. aff. *indica* mat-forming	21140	39.33	39.00	0.37	16.00	7.33	2.57	1.33	2.33	1.00	0.73	0.57
F. aff. *indica* mat-forming	26609	25.67	14.67	0.43	8.00	7.00	2.33	1.37	2.23	1.33	0.70	0.53
F. aff. *indica* mat-forming	17315	44.00	41.00	0.60	15.00	13.00	2.80	1.00	3.00	1.50	0.80	0.50
F. aff. *indica* mat-forming	20926	47.33	33.00	0.57	15.67	14.00	2.70	1.03	2.90	1.43	0.80	0.50
F. aff. *indica* mat-forming	26855	52.00	82.00	0.37	18.67	10.67	2.90	1.27	4.50	1.10	0.97	0.53
F. aff. *indica* mat-forming	27676	39.67	18.33	0.37	9.67	6.67	2.27	1.13	2.40	1.10	1.10	0.63
F. aff. *indica* mat-forming	26892	30.67	38.00	0.37	18.33	8.67	2.37	1.03	2.30	1.10	0.80	0.63
F. aff. *indica* mat-forming	13093	57.33	25.00	0.50	8.00	10.00	2.87	1.13	2.27	1.00	0.83	0.53

F. aff. *indica* mat-forming	4635	143.33	59.00	0.73	26.67	27.33	4.63	1.83	4.00	1.27	1.27	0.40
F. aff. *indica* renosterveld	4514	340.00	112.00	0.50	40.00	32.00	5.40	2.40	3.70	2.10	1.50	1.00
F. aff. *indica* renosterveld	4649	670.00	84.33	0.67	103.33	33.00	4.27	1.90	3.60	1.37	2.00	1.03
F. aff. *indica* renosterveld	4782	446.67	122.33	1.10	50.00	55.67	5.63	3.10	4.23	1.53	1.57	0.83
F. aff. *indica* renosterveld	4961	306.67	73.33	0.57	32.00	11.33	4.43	2.00	4.13	1.47	1.73	1.00
F. aff. *indica* renosterveld	5112	980.00	172.00	0.90	123.00	47.50	5.75	2.75	4.35	1.50	1.60	0.85
F. aff. *indica* renosterveld	5124	290.67	63.33	1.03	51.67	18.33	4.37	1.77	4.53	1.50	1.80	0.97
F. aff. *indica* renosterveld	5134	413.33	76.00	0.93	45.67	14.00	4.57	1.67	4.33	1.73	1.83	0.67
F. aff. *indica* renosterveld	82	362.33	41.67	0.37	36.33	24.67	4.17	2.93	3.30	1.63	1.53	1.03
F. aff. *indica* renosterveld	MD022	453.33	145.00	0.37	51.67	60.00	2.77	1.43	1.77	1.00	1.00	0.97
F. aff. *indica* renosterveld	48	306.67	113.33	0.67	43.33	37.67	4.37	1.97	2.27	1.30	1.33	0.53
F. aff. *indica* renosterveld	4979	286.33	38.33	0.73	42.67	39.00	4.03	1.53	3.93	1.87	1.67	0.93
F. aff. *indica* renosterveld	7659	301.00	93.50	0.80	54.50	26.00	3.30	1.75	2.55	1.20	1.15	0.60
F. dunensis	10279	219.67	93.33	0.43	36.67	17.67	3.93	1.03	3.63	1.00	1.50	0.83
F. dunensis	10591	169.67	41.00	0.47	25.67	5.00	3.63	1.13	2.73	1.33	1.77	0.97
F. dunensis	12077	280.00	52.00	0.43	32.00	13.67	3.30	1.27	2.70	1.00	1.67	1.00
F. dunensis	1395	191.67	71.33	0.47	33.67	18.67	4.00	1.33	3.43	1.43	1.90	1.27
F. dunensis	16021	224.00	32.33	0.53	22.00	3.67	3.13	1.23	2.53	1.27	1.80	1.00
F. dunensis	16671	355.00	73.33	0.33	40.00	16.67	2.77	1.00	2.90	1.00	0.83	0.53
F. dunensis	18023	292.50	73.50	0.85	65.00	9.50	3.50	1.60	2.95	1.45	1.40	0.75
F. dunensis	21	278.00	63.33	0.40	55.00	10.67	4.33	1.17	3.60	0.93	1.47	0.47
F. dunensis	3516	323.33	82.00	0.33	45.00	17.00	3.73	1.23	3.50	1.33	1.70	0.80
F. dunensis	4663	570.00	126.67	0.37	49.33	0.50	4.50	1.00	3.37	1.40	1.53	0.53
F. dunensis	8024	336.67	49.33	0.30	44.67	14.00	3.60	1.33	2.87	1.20	1.80	0.90
F. dunensis	83	301.00	58.67	0.47	43.67	19.33	4.20	1.13	3.97	1.23	1.53	0.63

F. dunensis	8441	260.00	71.00	0.50	20.00	7.00	3.20	1.00	2.70	1.00	2.00	1.10
F. dunensis	8639	172.33	56.67	0.37	27.00	10.67	3.40	0.90	2.77	0.97	1.83	0.90
F. elatior	21	340.00	193.33	0.97	53.33	61.33	6.87	3.40	4.30	1.50	1.30	0.93
F. elatior	2215	630.00	225.00	0.70	90.00	100.00	4.00	1.00	3.70	1.00	1.50	0.80
F. elatior	2235	380.00	130.00	1.00	50.00	35.00	3.60	1.50	3.10	1.50	1.10	0.60
F. elatior	4566	475.00	91.67	0.97	58.33	24.67	5.43	2.27	4.23	1.57	1.83	0.90
F. elatior	4761	291.67	153.00	0.80	16.67	41.67	5.33	2.57	4.37	1.47	1.00	0.53
F. elatior	6878	345.00	140.00	2.05	37.50	45.00	4.25	1.35	3.30	1.10	1.45	0.65
F. elatior	9433	282.50	145.00	0.65	45.00	26.00	4.55	2.00	3.60	1.00	1.35	0.60
F. indica	4835	250.00	75.00	0.80	20.00	25.00	4.50	2.50	3.50	1.70	1.70	1.00
F. indica	4353	214.00	120.00	0.70	27.00	30.00	4.70	1.50	6.00	2.50	1.50	0.50
F. indica	4793	365.00	124.00	0.90	45.00	31.00	5.00	2.50	4.50	2.70	1.80	1.50
F. indica	2242	430.00	210.00	0.60	65.00	19.00	3.00	1.50	3.10	2.00	1.40	0.60
F. indica	2275	295.00	79.67	0.33	47.33	8.00	3.00	1.33	2.53	1.17	1.17	0.63
F. indica	2277	900.00	89.33	1.03	128.33	25.00	3.63	1.47	3.40	1.20	1.33	0.93
F. indica	10680	395.00	132.50	0.95	48.00	20.50	3.95	1.85	3.10	1.35	1.50	0.95
F. indica	108	191.67	128.33	0.73	17.33	12.00	4.00	2.50	3.50	1.50	1.63	1.13
F. indica	1084	363.33	98.33	0.63	21.00	34.00	2.53	1.30	2.20	1.00	1.17	0.93
F. indica	12213	406.00	143.33	0.87	21.00	31.67	2.97	2.97	2.70	1.47	1.20	0.93
F. indica	14650	378.33	110.00	0.23	69.00	35.00	2.97	1.47	2.57	1.10	1.00	0.70
F. indica	2276	760.00	65.00	1.10	90.00	85.00	3.40	2.00	2.40	2.00	1.40	1.00
F. indica	2298	320.00	115.00	0.80	40.00	60.00	3.20	2.00	2.90	1.20	1.00	0.60
F. indica	2318	285.00	93.50	0.80	28.50	23.50	3.10	1.70	2.45	1.00	1.10	0.65
F. indica	2353	650.00	150.00	1.30	72.00	52.00	3.00	1.70	2.90	2.00	1.10	0.80
F. indica	28197	410.00	110.00	0.40	66.50	2.00	2.85	1.25	3.40	1.50	1.35	0.90
F. indica	28415	230.00	78.00	0.87	25.67	23.00	3.23	1.37	2.83	1.07	1.10	0.77
F. indica	287	207.50	62.50	0.75	42.50	16.00	3.00	1.35	3.35	1.45	1.05	0.80
F. indica	2890c	290.00	40.00	1.00	44.00	25.00	4.00	0.80	3.70	1.30	2.30	1.10
F. indica	29995	300.00	90.00	0.40	30.00	34.00	3.00	1.00	2.50	1.00	1.00	0.70
F. indica	34670	470.00	140.00	0.55	117.50	34.00	3.35	1.90	2.85	1.35	1.80	0.95
F. indica	3797	311.33	94.00	0.87	42.33	9.67	2.63	1.27	2.83	1.30	1.30	0.77
F. indica	3856	400.00	108.50	1.05	61.00	17.00	6.25	3.35	4.60	2.15	1.85	1.35
F. indica	3952	373.33	41.33	0.50	40.00	28.33	4.83	2.10	3.57	1.67	1.50	1.00
F. indica	3966	310.00	29.00	0.47	41.67	4.83	4.60	1.57	4.10	1.33	2.83	1.47
F. indica	4166	456.50	54.50	0.70	36.50	11.00	5.90	2.40	5.05	2.25	1.60	0.80
F. indica	4624	303.33	137.00	0.63	72.33	42.67	4.83	2.67	3.90	1.47	1.07	0.47
F. indica	4792	431.67	85.33	0.47	48.33	7.00	5.57	2.17	4.37	2.00	2.57	1.50

F. indica	4881	233.33	73.67	0.87	40.00	26.67	4.70	3.70	4.47	1.77	1.40	0.43
F. indica	5856	228.67	64.00	0.57	30.67	20.33	2.33	1.17	1.83	1.00	1.33	0.97
F. indica	6291	487.50	212.50	0.85	84.00	27.00	2.450	1.90	3.65	1.10	1.10	0.55
F. indica	7223	326.67	56.67	0.53	53.00	14.33	2.53	1.30	2.67	1.23	1.07	0.90
F. indica	7292	239.00	72.33	0.67	37.00	22.33	3.03	1.47	2.33	1.07	1.03	0.60
F. indica	7949	350.00	110.00	2.00	50.00	33.00	2.40	1.00	2.10	1.20	1.40	0.70
F. indica	8107	510.00	112.00	1.50	44.00	45.00	3.20	2.00	2.80	1.00	1.40	0.80
F. indica	8625	490.00	137.50	1.00	75.50	37.50	3.40	1.50	2.65	1.00	1.40	0.95

Apendix 4.2. Soil data used for the *Ficinia indica* complex

Species	**Collector number**	**pH (KCI)**	**P Bray II (mg/kg)**	**K (mg/kg)**	**Na (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**Cu (mg/kg)**	**Zn (g/kg)**	**Mn (mg/kg)**	**B (mg/kg)**	**Fe (mg/kg)**	**Inorganic N (mg/kg)**
F. aff. *indica* mat-forming	4821b	5.9	6.01	56.01	0.14	3.90	0.61	0.69	2.21	4.21	0.14	148.32	16.57
F. aff. *indica* mat-forming	108a	5.4	7.01	58.01	0.30	4.23	0.56	0.61	2.91	5.81	0.18	181.98	18.75
F. aff. *indica* mat-forming	108a	5.4	7.01	58.01	0.30	4.23	0.56	0.61	2.91	5.81	0.18	181.98	18.75
F. aff. *indica* mat-forming	4760	3.1	12.01	34.01	0.16	0.65	0.82	0.25	1.11	1.61	0.15	103.65	29.38
F. aff. *indica* mat-forming	4762	3.1	9.01	23.01	0.09	0.96	0.71	0.22	1.31	7.21	0.08	77.36	28.01
F. aff. *indica* mat-forming	4762b	3.1	8.01	27.01	0.12	1.08	0.74	0.38	1.51	6.51	0.11	70.78	27.85
F. aff. *indica* mat-forming	4764	3.4	8.01	95.01	0.13	0.64	0.61	4.20	1.91	1.31	0.31	195.34	20.58
F. aff. *indica* mat-forming	5090a	5.6	24.01	163.01	0.32	2.91	1.11	0.56	2.71	11.81	0.38	60.04	35.76
F. aff. *indica* mat-forming	5090b	4.3	11.01	25.01	0.15	1.30	0.47	0.22	1.81	2.51	0.09	62.03	17.58
F. aff. *indica* mat-forming	5091	4.6	8.01	31.01	0.23	1.62	0.66	0.52	0.91	7.21	0.20	76.37	18.68
F. aff. *indica* mat-forming	RM1	4.7	8.01	89.01	0.56	4.30	1.85	0.89	1.11	3.11	0.51	143.55	34.04
F. aff. *indica* mat-forming	RM2	4.5	10.01	59.01	0.42	4.25	1.66	1.78	3.21	8.31	0.36	263.22	44.26
F. aff. *indica* mat-forming	RM3	4.6	11.01	100.01	0.47	5.43	2.21	1.37	2.61	8.41	0.29	209.73	75.14
F. aff. *indica* renosterveld	119	3.6	3.01	21.01	0.07	0.14	0.18	0.08	0.21	1.51	0.05	79.00	18.83
F. aff. *indica* renosterveld	120	3.9	15.01	30.01	0.09	0.33	0.23	0.15	0.21	9.81	0.07	66.06	22.97
F. aff. *indica*	120a	3.9	16.01	28.01	0.09	0.32	0.21	0.13	0.21	9.11	0.06	44.81	21.38

renosterveld													
F. aff. *indica* renosterveld	4794	4.5	4.01	83.01	0.11	0.88	0.67	0.23	0.41	46.51	0.19	59.18	15.86
F. aff. *indica* renosterveld	4794a	4.6	2.01	79.01	0.11	0.84	0.62	0.26	0.51	45.51	0.13	332.75	16.97
F. aff. *indica* renosterveld	4827	4.0	7.01	41.01	0.11	0.13	0.16	0.15	0.11	1.31	0.06	16.34	16.96
F. aff. *indica* renosterveld	4827a	4.0	6.01	50.01	0.15	0.17	0.19	0.74	0.71	1.81	0.07	22.21	16.00
F. aff. *indica* renosterveld	4827b	4.0	8.01	46.01	0.12	0.11	0.16	0.20	0.21	1.41	0.06	16.54	14.68
F. aff. *indica* renosterveld	4821a	4.4	1.01	14.01	0.05	0.18	0.15	0.11	0.11	2.81	0.03	16.81	10.37
F. aff. *indica* renosterveld	4821b	4.4	1.01	13.01	0.04	0.14	0.13	0.10	0.11	2.81	0.03	26.92	9.82
F. aff. *indica* renosterveld	4821c	4.4	1.01	14.01	0.05	0.15	0.14	0.06	0.11	2.71	0.02	26.81	10.36
F. aff. *indica* renosterveld	5133	4.3	4.01	17.01	0.05	0.34	0.17	0.21	0.31	11.11	0.03	26.26	17.27
F. aff. *indica* renosterveld	RC1	4.3	10.01	4.01	0.05	0.19	0.12	0.23	1.41	1.11	0.03	20.38	13.64
F. aff. *indica* renosterveld	RC2	4.1	7.01	4.01	0.04	0.15	0.11	0.27	1.41	0.91	0.01	25.53	13.57
F. aff. *indica* renosterveld	RC3	4.1	9.01	4.01	0.04	0.08	0.06	0.34	1.31	0.71	0.01	35.78	11.00
F. aff. *indica* renosterveld	4649	4.6	4.01	45.01	0.07	1.67	0.28	0.45	0.11	4.71	0.06	79.50	47.95
F. aff. *indica* renosterveld	4663	4.8	3.01	60.01	0.22	1.24	1.29	0.17	0.11	19.41	0.13	75.26	44.52
F. aff. *indica* renosterveld	4583	4.5	3.01	53.01	0.05	1.02	0.50	0.34	0.21	3.01	0.04	64.47	47.26
F. aff. *indica* renosterveld	4586	4.0	4.01	80.01	0.06	0.88	0.24	0.01	0.31	7.01	0.08	34.14	42.74
F. aff. *indica* renosterveld	4589	4.3	1.01	63.01	0.37	0.47	0.21	0.29	0.11	1.81	0.08	19.06	37.62
F. aff. *indica* renosterveld	4581	3.5	4.01	30.01	0.08	0.37	0.17	0.01	0.11	0.21	0.05	13.04	48.00
F. aff. *indica* renosterveld	4581	4.5	3.01	53.01	0.05	1.02	0.50	0.34	0.21	3.01	0.04	64.47	47.26

F. aff. *indica* renosterveld	4581	4.0	4.01	80.01	0.06	0.88	0.24	0.01	0.31	7.01	0.08	34.14	42.74
F. dunensis	84	7.7	0.01	27.01	0.07	23.37	0.93	1.19	0.21	1.51	1.08	0.01	4.16
F. dunensis	85	7.7	28.01	127.01	0.39	31.57	1.79	0.79	0.11	0.71	1.60	7.49	31.56
F. dunensis	86	7.6	32.01	82.01	0.35	33.95	1.72	0.01	0.11	1.31	1.76	14.04	59.30
F. dunensis	88	5.4	1.01	13.01	0.08	1.17	0.28	0.01	0.01	0.01	0.11	24.53	30.68
F. dunensis	90	6.4	2.01	8.01	0.05	2.15	0.19	0.76	0.11	0.41	0.07	13.21	47.54
F. dunensis	91	7.2	5.01	9.01	0.06	3.52	0.26	0.01	0.11	0.61	0.09	23.24	47.37
F. elatior	69	6.0	5.01	9.01	0.06	2.08	0.25	1.51	1.01	0.31	0.17	46.06	27.48
F. elatior	71	6.4	22.01	44.01	0.38	8.75	1.13	3.41	6.51	15.01	1.85	180.81	56.70
F. elatior	72	7.4	18.01	51.01	0.31	5.36	0.67	0.47	2.71	4.31	0.73	51.16	31.54
F. indica	4961	3.8	5.01	6.01	0.03	0.09	0.08	0.08	0.11	4.11	0.01	33.23	12.92
F. indica	4962	4.1	40.01	29.01	0.07	1.06	0.32	0.28	0.21	113.51	0.03	32.72	15.97
F. indica	4966	3.7	18.01	22.01	0.04	0.25	0.09	0.06	0.11	11.01	0.01	30.05	19.33
F. indica	4975	3.9	15.01	9.01	0.04	0.25	0.07	0.15	0.31	7.91	0.01	36.03	14.15
F. indica	4976	3.9	13.01	12.01	0.04	0.42	0.09	0.16	0.11	11.51	0.01	20.27	16.89
F. indica	4978	3.9	12.01	8.01	0.03	0.15	0.04	0.41	0.91	7.21	0.01	54.41	16.82
F. indica	5129	4.3	5.01	18.01	0.05	0.40	0.18	0.13	0.21	9.71	0.03	34.64	16.99
F. indica	5130	4.3	5.01	16.01	0.04	0.31	0.16	0.16	0.21	8.71	0.03	22.93	14.92
F. indica	4652	4.2	4.01	67.01	0.09	1.24	0.19	0.14	0.31	4.31	0.05	135.43	46.59
F. indica	79	3.3	1.01	21.01	0.07	0.68	0.24	0.01	0.21	2.21	0.05	24.62	35.38
F. indica	80	3.3	3.01	8.01	0.06	0.32	0.10	0.34	0.11	0.41	0.04	17.73	33.53
F. indica	82	3.4	1.01	10.01	0.04	0.28	0.16	0.35	0.31	0.21	0.02	14.46	31.52
F. indica	71	6.4	22.01	44.01	0.38	8.75	1.13	3.41	6.51	15.01	1.85	180.81	56.70
F. indica	72	7.4	18.01	51.01	0.31	5.36	0.67	0.47	2.71	4.31	0.73	51.16	31.54
F. indica	73	4.5	0.01	6.01	0.05	0.37	0.12	0.62	1.01	0.21	0.05	24.07	28.12
F. indica	45	3.4	4.01	40.01	0.13	2.25	0.85	0.75	4.71	4.21	0.12	91.48	72.91
F. indica	47	3.1	3.01	20.01	0.08	0.41	0.16	1.26	2.41	0.71	0.14	47.31	43.25
F. indica	48	4.2	6.01	20.01	0.09	0.50	0.23	1.21	3.21	29.51	0.11	14.25	67.81
F. indica	29	4.2	5.01	27.01	0.07	0.32	0.07	0.27	1.31	24.51	0.12	19.38	23.92
F. indica	30	4.7	9.01	39.01	0.08	0.98	0.39	1.29	0.31	81.21	0.16	21.70	36.91
F. indica	31	4.3	4.01	22.01	0.06	0.21	0.01	0.67	0.31	4.11	0.11	18.43	30.17
F. indica	19	4.1	5.01	22.01	0.07	0.96	0.47	0.14	0.91	0.41	0.17	25.75	40.71
F. indica	20	4.2	2.01	9.01	0.07	1.20	0.35	0.74	0.41	1.01	0.13	19.48	2.96
F. indica	21	3.7	1.01	11.01	0.09	0.71	0.19	1.40	1.11	1.51	0.17	19.08	2.73