Delimiting the genera of the Ficinia Clade (Cypereae, Cyperaceae) based on molecular phylogenetic data (#52103)

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Delimiting the genera of the Ficinia Clade (Cypereae, Cyperaceae) based on molecular phylogenetic data

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Generic delimitations in the Ficinia Clade of tribe Cypereae are revisited. In particular, we aim to establish the placement of annual species currently included in *Isolepis* of which the phylogenetic position is uncertain. Phylogenetic inference is based on two nuclear markers (ETS, ITS) and five plastid marker (the genes *matK*, *ndhF*, *rbcL* and *rps16*, the *trnL* intron and *trnL-F* spacer) data, analyzed using model based methods. Topologies based on nuclear and plastid data show incongruence at the backbone. Therefore, the results are presented separately. The monophyly of the smaller genera (*Afroscirpoides*, *Dracoscirpoides*, *Erioscirpus*, *Hellmuthia*, *Scirpoides*) is confirmed. However, *Isolepis* is paraphyletic as *Ficinia* is retrieved as one of its clades. Furthermore, *Ficinia* is paraphyletic if *I. marginata* and allies are excluded. We take a pragmatic approach based on the nuclear topology, driven by a desire to minimize taxonomic changes, to recircumscribe *Ficinia* to include the annual *Isolepis* species characterized by cartilaginous glumes and formally include all the *Isolepis* species inferred outside the core *Isolepis* clade. Consequently, the circumscription of *Isolepis* is narrowed to encompass only those species retrieved as part of the core *Isolepis* clade. Five new combinations are made (*Ficinia capensis, Ficinia heminucialis, Ficinia incomtula, Ficinia leucoloma, Ficinia minuta*). We present nomenclatural summary at genus level, identification keys and diagnostic features

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Abstract

Generic delimitations in the Ficinia Clade of tribe Cypereae are revisited. In particular, we aim to establish the placement of annual species currently included in *Isolepis* of which the phylogenetic position is uncertain. Phylogenetic inference is based on two nuclear markers (ETS, ITS) and five plastid marker (the genes *matK*, *ndhF*, *rbcL* and *rps16*, the *trnL* intron and *trnL-F* spacer) data, analyzed using model based methods. Topologies based on nuclear and plastid data show incongruence at the backbone. Therefore, the results are presented separately. The monophyly of the smaller genera (*Afroscirpoides*, *Dracoscirpoides*, *Erioscirpus*, *Hellmuthia*, *Scirpoides*) is confirmed. However, *Isolepis* is paraphyletic as *Ficinia* is retrieved as one of its clades. Furthermore, *Ficinia* is paraphyletic if *I. marginata* and allies are excluded. We take a pragmatic approach based on the nuclear topology, driven by a desire to minimize taxonomic changes, to recircumscribe *Ficinia* to include the annual *Isolepis* species characterized by cartilaginous glumes and formally include all the *Isolepis* species inferred outside the core *Isolepis* clade. Consequently, the circumscription of *Isolepis* is narrowed to encompass only those species retrieved as part of the core *Isolepis* clade. Five new combinations are made (*Ficinia capensis, Ficinia heminucialis, Ficinia incomtula, Ficinia leucoloma, Ficinia minuta*). We present nomenclatural summary at genus level, identification keys and diagnostic features.

Introduction

The paradigm shift towards recognition of genera as monophyletic entities has necessitated changes in generic circumscription (Humphries & Linder, 2009). Within Cyperaceae, a number of changes have been made within the last decade, for example the merger of segregate genera into the paraphyletic core in *Carex* L. (*GCG, 2015*) and *Cyperus* L. (*Larridon et al., 2011a, b, 2013, 2014; Bauters et al., 2014*). A number of genera have been found to be polyphyletic, especially in the tribe Schoeneae, resolved by reclassification of entities and naming of a number of lineages as new genera (e.g. *Elliot & Muasya, 2017; Larridon et al., 2018a, b; Barrett et al., 2019*). Each of the four Cyperaceae genera recognized by Linnaeus (1753) has been reclassified over the years, with Linnaeus' circumscription of *Scirpus* L. as encompassing species with bisexual flowers and spiral glume arrangement representing the most heterogenous assemblage. Embryo morphology data (*Van der Veken, 1965; Goetghebeur, 1986; Semmouri et al., 2019*) have unequivocally demonstrated that lineages with distinct morphology were included in *Scirpus*. In his seminal treatment of the family, *Goetghebeur* (*1998*) placed the 24 taxa previously named *Scirpus* by *Linnaeus* (*1753*) in the tribes Cypereae, Fuireneae and Scirpeae, with *S. sylvaticus* L. being the only species described by Linnaeus recognized as a true *Scirpus* and two of the species recognized as members of *Isolepis* R.Br.

Within tribe Cypereae, the Ficinia Clade (sensu *Muasya et al., 2009a*) comprises taxa whose placement has been most contentious. *Goetghebeur* (*1998*) diagnosed tribe Cypereae to include species characterised by either a Cyperus or a Ficinia type embryo, where glumes are arranged distichously (*Cyperus* and allies) or spirally (*Isolepis*, *Ficinia* Schrad., *Scirpoides* Ség). Lineages bearing perianth segments were added to the clade based on molecular phylogenetic data, moving *Hellmuthia* Steud. from Chysitricheae (*Vrijdaghs et al., 2006; Muasya et al., 2009a, b*), *Erioscirpus* Palla from Scirpeae (*Yano et al., 2012*), and recognizing southern African taxa previously placed in *Scirpus* as a distinct genus *Dracoscirpoides* Muasya (*Muasya et al., 2012*). Furthermore, the delimitation of *Scirpoides* has been altered to exclude *Afroscirpoides* García-Madr. & Muasya (*García-Madrid et al., 2015*) and the addition of two species that were ambiguously placed (*Browning & Gordon Gray, 2011; Reid et al., 2017*). These genera are annual to perennial herbs, have basal leaves which vary in blade development, have considerable variation in inflorescence and floral morphology, and are diagnosed by a combination of morphological features (see Table 2 in *García-Madrid et al., 2015*).

Generic delimitation between *Isolepis* and *Ficinia* is based on few morphological characters. *Isolepis* have a varied habit (annual to perennial) and are widespread, whereas *Ficinia* are perennial and predominantly occur within the Cape flora and in Africa (*Goetghebeur, 1998; Muasya & Simpson, 2002*). A further distinction is the presence of a gynophore in *Ficinia*, but several species having a gynophore and occurring outside Africa were previously excluded from the genus. For example, the New Zealand iconic sand dune taxon (*Desmoschoenus spiralis* (A.Rich.) Hook.f.) is embedded within core *Ficinia* (as *Ficinia spiralis* (A.Rich.) Muasya & de Lange; *Muasya & de Lange, 2010*), and the more widespread *Ficinia nodosa* (Rottb.) Goetgh., Muasya & D.A.Simpson was retained in *Isolepis* despite having a well developed gynophore (*Muasya et al., 2000*). Furthermore, *Isolepis* may not be monophyletic as *Ficinia* is one of the three to four clades recovered in *Isolepis* (*Muasya & de Lange, 2010; García-Madrid et al., 2015*; *Hinchcliff & Roalson, 2013; Spalink et al., 2016; Semmouri et al., 2019*). Challenges on distinguishing *Ficinia* from *Isolepis* have persisted over the last 200 years, as evident from at least one in six of the currently recognized *Ficinia* species having a validly published epithet in *Isolepis* (Govaerts et al., 2020).

We use an expanded molecular phylogenetic study to investigate the generic limits in the Ficinia Clade. We infer the phylogenetic relationships and placement of ambiguously placed *Isolepis* species, namely (1) *I. hemiuncialis* (C.B.Clarke) J.Raynal and *I. incomtula* Nees – which have been previously recovered as sister to the core *Isolepis*/Ficinia clade; (2) *I. marginata* (Thunb.) A.Dietr. and allies (*I. antarctica* (L.) Roem. & Schult., *I. capensis* Muasya, *I. leucoloma* (Nees) C.Archer, *I. minuta* (Turrill) J.Raynal). – previously recovered as sister to core clade of *Ficinia*. The aim is to establish whether the genera in the Ficinia Clade, particularly *Isolepis* and *Ficinia*, are monophyletic, and to evaluate what characters diagnose the inferred (sub)clades.

Materials & Methods

Ethics statement

Part of the specimens studied were collected during field expeditions predominantly in Western Cape province of South Africa funded by grants from the National Research Foundation and with additional support from the University of Cape Town. Permit to collect these specimens were issued by the Cape Nature authorities (CN35-28-5831). The other specimens studied are available in publicly accessible herbaria (BOL, K; *Thiers, continuously updated*).

Nomenclature and taxonomy

A nomenclatural study including the taxonomic history of the genus and its species, critical for the correct coining of the new names and the proper use of prior ones, was performed. The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants (ICN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work which have been issued with identifiers by IPNI will eventually be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix "http://ipni.org/". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

Molecular study

The Ficinia Clade, our focus group, includes the genera *Afroscirpoides* (1 species), *Dracoscripoides* (3 species), *Erioscirpus* (2 species), *Ficinia* (81 species), *Hellmuthia* (1 species), *Isolepis* (75 species) and *Scirpoides* (4 species). A total of 166 ingroup accessions were sequenced, representing: 1 species of *Afroscirpoides* (100 %), 3 *Dracoscripoides* (100 %), 1 *Erioscirpus* (50 %), 64 (plus 3 undescribed) *Ficinia* (78 %), 1 *Hellmuthia* (100 %), 57 *Isolepis* (plus some infraspecific taxa) (76 %) and 3 *Scirpoides* (75%). The outgroup taxa, selected based on *Semmouri et al. (2019)* and *Larridon et al. (in review)*, consist 114 accession representing the six genera of tribe Fuireneae and the genus *Cyperus*, the only genus of the Cyperus Clade of tribe Cypereae after *Androtrichum* was recently synonymysed with *Cyperus* (*Pereira-Silva et al., accepted*). The DNA extraction protocol, primers, and material and methods for PCR amplification and sequencing follow Viljoen et al., 2013.

Sequences were assembled and edited in Geneious R8 (http://www.geneious.com, *Kearse et al., 2012*), aligned using MAFFT 7 (*Katoh et al., 2009*; *Katoh & Standley, 2013*) with 'maxiterate' and 'tree rebuilding number' set to 100 (long run), afterwards, alignments were checked manually in PhyDE 0.9971 (*Müller et al., 2010*).

We first inferred the gene trees for each of the regions separately to identify potential incongruence. As there were no instances of conflict at well-supported nodes between the two nuclear markers, and between the five chloroplast makers, the matrices of the regions were concatenated into a nuclear dataset including ETS and ITS, and in a chloroplast dataset including the genes *matK*, *ndhF*, *rbcL* and *rps16*, the *trnL* intron and *trnL-F* spacer for the downstream analyses. PartitionFinder 2.1.1 (*Lanfear et al., 2012*) was used to determine an appropriate data-partitioning scheme from potential partitions that were defined *a priori* (in this case, each marker was treated as a separate partition), as well as the best-fitting model of molecular evolution for each partition, using the Bayesian Information Criterion. For the nuclear dataset, PartitionFinder confirmed the *a priori* data-partitioning scheme, and the GTR+I+Γ (invgamma) model of sequence evolution was determined to be the best-fitting model for the two nrDNA markers. For the chloroplast dataset, PartitionFinder suggested partitioning the data in four partitions (*matK*+*rps16*, *ndhF*, *rbcL*, the *trnL* intron and *trnL-F* spacer), and the GTR+Γ (gamma) model of sequence evolution was determined to be the best-fitting model for all partitions except for *rbcL* for which the GTR+I+Γ (invgamma) model was suggested.

Maximum likelihood (ML) analyses of the optimally partitioned data were performed using RAxML 8.2.10 (*Stamatakis, 2014*). The search for an optimal ML tree was combined with a rapid bootstrap analysis of 1000 replicates. Additionally, partitioned analyses were conducted using Bayesian Inference (BI) in MrBayes 3.2.6 (*Ronquist et al., 2012*). Rate heterogeneity, base frequencies, and substitution rates across partitions were unlinked. The analysis was allowed to run for 100 million generations across two independent runs with four chains each, sampling every 10,000 generations. Convergence, associated likelihood values, effective sample size (ESS) values and burn-in values of the different runs were verified with Tracer 1.5 (*Rambaut & Drummond, 2007*). The first 25% of the trees from all runs were excluded as burn-in before making a majority-rule consensus of the posterior distribution trees using the "sumt" function. All phylogenetic analyses were run using the CIPRES portal (http://www.phylo.org/; *Miller et al., 2011*), and were executed for both full and reduced sampling alignments. Trees were drawn using TreeGraph2 (*Stöver & Müller, 2010*).

Morphological study

Herbarium specimens of BOL, BR, GENT, K, NBG, PRE (*Thiers, continuously updated*) were studied morphologically using a Leica (Leica Microsystems, Wetzlar, Germany) binocular microscope. Measurements where made with a ruler and digital calipers (e.g. leaf and culm length), or using a binocular microscope with graticule (e.g. spikelet and glume length). When examining width, this was done near the middle of the organ (e.g. middle of the nutlet).

Results

Molecular study

Analyses of the individual markers show congruence within the nuclear and plastid markers, as well as congruence among the methods of analyses (Supplementary figures). However, the nuclear (Figure 1A) and plastid phylogenies (Figure 1B) had conflicting backbone topologies and are therefore presented separately. Regardless, there is strong support in both data sets for the tribe Cypereae with the Cyperus and Ficinia clades as sister. In the plastid topology (Figure 1B), *Afroscirpoides* diverged first, then strongly supported sister clades comprising i) *Erioscirpus* sister to *Scirpoides*, and ii) a clade comprising *Hellmuthia*, *Dracoscirpoides*, *Isolepis* and *Ficinia*. In the nuclear topology, there is a grade showing successive divergence starting with *Erioscirpus*, *Afroscirpoides*, *Dracoscirpoides*, *Scirpoides*, *Hellmuthia*, then *Isolepis* and *Ficinia*. In both analyses, there is strong support for the monophyly of the *Dracoscirpoides*, *Hellmuthia* and *Scirpoides*, but *Isolepis* is paraphyletic with *Ficinia* as one of the clades.

The position of *Isolepis hemiuncialis* and *I. incomtula* differed in the two analyses. These two species formed an early diverging grade leading to a polytyomy among core *Isolepis* in the plastid topology (Figure 1B), but were part of the *I. marginata* clade in the nuclear phylogeny (Figure 1A; Figure 2). On the other hand, *I. marginata* and allied species (*I. antarctica, I. capensis, I. leucoloma* and *I. minuta)* were consistently resolved as part of a clade including *Ficinia eligulata* and sister to the core *Ficinia* clade. The nuclear topology is better resolved, showing subclades in core *Isolepis* which coincide with current infrageneric groups, but these groups are not clearly discrenable in the plastid topology. In addition, the nuclear ITS alignment shows a three nucleotide insertion (ATA; position 1890–1892, Supplemental Information File Data S1), unique to the core *Isolepis* clade and lacking in the outgroup as well other *Isolepis* (*I. hemiuncialis*, *I. incomtula*, *I. marginata* and allies) and *Ficinia*.

Morphological study

Table 1 summarises the morphological diversity among genera in the ingroup. All Ficinia Clade taxa share the presence of Cyperus or a modified type (Ficinia) embryo. They are annuals or perennials; are mostly scapose, though multiple nodes are observed in *Ficinia* (e.g. *F. trichodes* (Schrad.) B.D.Jacks.); and have leaf blades well developed or reduced to a lobe, with or without ligule. The inflorescence is diverse (single terminal, e.g. *I. fluitans* (L.) R.Br.; capitate, e.g. *F. radiata* (L.f.) Kunth; pseudolateral, e.g. *Afroscirpoides*; to anthelate, e.g. *S. burkei* (C.B.Clarke) Goetgh., Muasya & D.A.Simpson). Glume arrangement is predominantly spiral, with distichous arrangement in some species of *Isolepis* and *Ficinia* (e.g. *I. levynsiana* Muasya & D.A.Simpson, *F. distans* C.B.Clarke). The flowers are bisexual and occurring in most florets, but dioecy is observed in *Afroscirpoides*. While majority of taxa lack perianth, these occur in *Erioscirpus* (large and plumose as seen in *Eriophorum* L.), *Dracoscirpoides* (bristles) and *Hellmuthia* (scales). Nutlets are trigonous to oval in cross section, with base extended to form gynophore in majority of *Ficinia*.

Species in the *Isolepis* and *Ficinia* clades vary in subtle morphological and ecological features (Table1). *Isolepis hemiuncialis* and *I. incomtula* are annual species, whose gross morphology and ecology is similar to annual species in the core *Isolepis* clade and *I. marginata* (and allies). *Ficinia* is unique in ecology, frequently growing as perennial in dry habitats and unlike perennial *Isolepis* species that are restricted to wetlands. An additional feature, the presence of gynophore is unique to *Ficinia*, eventhough several species (e.g. *F. filiformis* (Lam.) Schrad., *F. trollii* (Kük.) Muasya & D.A.Simpson) lack this feature and vestigial gynophore occasionally occur in *Isolepis marginata*. Glume texture is chartaceous in *Isolepis* (including *I. hemiuncialis* and *I. incomtula*), whereas it is cartilaginous in *Ficinia* (including *I. marginata* and allies).

Discussion

This study has inferred the phylogeny of the Ficinia Clade using a large species sample (78% of species) and Sanger sequencing of nuclear and plastid markers. The patterns observed are similar to previous studies (*Muasya et al., 2009a, b; Muasya and De Lange 2010; Hinchcliff & Roalson, 2013; Spalink et al., 2016; Semmouri et al., 2019*), confirming the monophyly of the smaller genera but recovering *Ficinia* to be nested in *Isolepis*. The backbone differs between the plastid and nuclear topology, especially relating to the position of *Dracoscirpoides* which is positioned between *Afroscirpoides* and *Scirpoides* (nuclear, Figure 1A) or between *Hellmuthia* and the *Isolepis*/*Ficinia* clade (plastid, Figure 1B). Similarly, these varied topologies have been observed in previous studies (e.g. *García-Madrid et al., 2015*).

The phylogenetic position of *Isolepis hemiuncialis* and *I. incomtula* is unstable, shifting based on the markers analyzed. These taxa form a lineage (or grade) separate from the core *Isolepis* and *Ficinia* clades based on plastid data in this and previous studies (*García-Madrid et al., 2015; Spalink et al. 2016*), and a similar pattern was observed in a combined plastid and nuclear analysis (rps16 & ITS; *Muasya & De Lange, 2010*). In addition, the nuclear markers differ in their placement of these samples, with ITS having a pattern similar to plastid markers (similarly observed in Figure S1, S2 of *García-Madrid et al., 2015*), but ETS placing these species as part of clade with *I. marginata* (similarly observed in Figure S3, S4 of *García-Madrid et al., 2015*). In the combined nuclear matrix, these two species are part of the *I. marginata* clade. In contrast, *I. marginata* (and allied species) have been consistently observed to be forming a clade sister to core *Ficinia* in separate and combined analyses, and these species are in same clade with *F. eligulata* Gordon-Gray ex Muasya from the Drakensberg Mountain.

A unique 3 base-pair insertion in ITS2 further supports the uniqueness of the core *Isolepis*. This insertion is missing in *I. hemiuncialis*, *I. incomtula* as well as the species in the *I. marginata* clade, and can therefore be used as a synapomorphy for the core *Isolepis* clade. Similar use of indels, located at the 5.8S gene of the nuclear ribosomal DNA, as synapomorphies has been suggested for the Cypereae (*Yano et al., 2012*) and Cyperaceae (*Starr et al., 2007*).

A number of the genera in the ingroup can be distinguished unambiguously based on one or few characters (Table 1). The presence and type perianth segments, even though perhaps arising independently, are unique in *Dracoscirpoides* (scabrid bristles; *Muasya et al., 2012*), *Erioscirpus* (cotton-like bristles; Yano et al., 2012) and in *Hellmuthia* (scale-like; *Vrijdaghs et al., 2006*). Among the taxa lacking perianth segments, *Afroscirpoides* and *Scirpoides* have densely tufted culms which have reduced leaf blades (>5 mm, but some *Scirpoides* have well developed leaf blades), with the former having dioecious individuals wherea the later has bisexual florets. *Ficinia* is most similar in gross morphology and ecology to *Afroscirpoides* and *Scirpoides*, diagnosed by the presence of a cupular disk (gynophore; *Vrijdaghs et al., 2005*) at the base of the nutlets (except in several species where the trait is lost; *Muasya et al., 2014*). *Isolepis* is most similar to *Ficinia*, sharing presence of bisexual florets and glumes with well defined parallel veins, but differing in *Isolepis* lacking the gynophore. The glume texture appears to offer additional separation, being chartaceous to hyaline (herbaceous; *Muasya & Simpson, 2002*) in *Isolepis* but cartilaginous (or coriaceious) in *Ficinia*.

Generic boundaries within *Isolepis* and *Ficinia* have been noted as problematic. *Eleogiton*, still recognized as distinct in some floras (e.g. Germany, *Kadereit et al., 2016*) based on possessing multiple internodes and peduncle termination in single terminal spikelet, is confirmed to be a clade in *Isolepis* (subgenus *Fluitantes*; *Muasya et al., 2001; Muasya & Simpson, 2002*). In *Ficinia, Sickmania* Nees has been previously recognized based capitate inflorescence with multiple leaf-like bracts (*F. radiata*) whereas *Desmoschoenus* has primary bracts adnate to axis and covering congested spikelets) (Goetghebeur, 1998). The phylogenetic inference showing *I. maginata* and other annual species that lack a gynophore being closer to *Ficinia* further blurs the generic boundaries.

Cyperaceae has experienced shifting generic classification in the last two decades. The paradigm shift to recognize monophyletic genera (*Humphries & Linder, 2009*) accompanied by the use of DNA sequence data have enabled disentangling phylogenetic relatedness of taxa obscured by extreme morphological modification. Several highly diversified lineages appear to have been split into genera based on one of few characters, at times such characters arising independently. This phenomenon was epitomized *Cyperus*, now recognized as a single genus (*Larridon et al. 2011a, b, 2013, 2014; Bauters et al. 2014*), where 13 segregate genera were diagnosed based on morphology of reproductive structures (spikelet size and organization, nutlet orientation, style branching; *Muasya et al., 2009a*). This study supports a further refinement within the Cypereae, recognizing the core *Isolepis* and an enlarged *Ficinia* at generic level.

We speculate that the Ficinia clade evolved in southern Africa, given that majority of lineages and species occur in the region. Diversification in *Isolepis* and *Ficinia* has occurred since the Miocene (*Besnard et al., 2009*), perhaps ecologically driven by aridification associated with onset of the Mediterranean climate (Linder & Verboom, 2015), where emerging traits include annual life form, colonization of permanently wet habitats, sprouting regeneration driven by the frequent fires in sclerophyllous habitats, and ant dispersal of seeds (gynophore in *Ficinia*; *Bond & Slingsby, 1983*). Within southern Africa, the Ficinia Clade members are predominantly occurring in the Greater Cape Flora and exhibit the typical diversification pattern whereby lineages in the Fynbos are older than those in the Succelent Karoo (*Verboom et al., 2009*). Dispersal out of the Cape appears to be predominantly to other similar habitats, especially in Mediterranean Eurasia (*Erioscirpus*, *Isolepis*, *Scirpoides*), within temperate zones of high mountains in tropical Africa (*Dracoscirpoides*, *Ficinia*, *Isolepis*, *Scirpoides*) and austral temperate areas (*Ficinia*, *Isolepis*). Dispersal to Australasia in *Isolepis* has been accompanied by hybridization in *Isolepis* (*Ito et al., 2016*).

Taxonomic treatment

The current generic classification is supported for the smaller genera (*Afroscirpoides*, *Dracoscirpoides*, *Erioscirpus*, *Hellmuthia*, *Scirpoides*). However, *Isolepis* is paraphyletic as *Ficinia* is one of its clades as well as *Ficinia* is paraphyletic if *I. marginata* and allies are excluded. We acknowledge the conflicting topology between the nuclear and plastid phylogenies, particularly regarding the position of *I. hemiuncialis* and *I. incomtula*, opting to follow the nuclear phylogeny. We take a phragmatic approach, to recognize clades that will minimize nomenclatural changes, by adopting a classification framework based on the nuclear phylogeny (Figure 2). We therefore recognize an expanded concept of *Ficinia*, to include annual species with mostly cartilaginous glumes and lacking gynophore. As a consequence, *Isolepis* is now considered in a narrower concept which encompasis the core *Isolepis* and excludes the seven annual species placed within the *Ficinia* clade (*I. antactica, I. capensis*, *I. hemiuncialis, I. incomtula*, *I. leucoloma, I. marginata* and *I. minuta)*. Subclades within *Isolepis* can be recognized as infrageneric groups, recognizing four subgenera where 3 are similar to classification by *Muasya & Simpson (2002)* but elevating sect. *Proliferae* to subgeneric rank. In *Ficinia*, previous infrageneric groups (*Clarke 1897-98; Pfeiffer, 1921*) are not supported, but the two clades each with subclades could be could form basis for future infrageneric classification. Formal taxonomic changes are made here, but we note need of a comprehensive taxonomic revision of *Ficinia*.

Key to the species of Ficinia clade genera

1 Plants perennial or annual; perianth segements absent. 4
1 Plants perennial; perianth segements present. 2
2 Perianth segment cotton-like, restricted to Asia ....1. *Erioscirpus*
2 Perianth segment bristle or scale-like; the restricted to southern Africa 3
3 Slender plants, culm < 4 mm diameter; perianth bristle-like; in Drakensberg and surrounding areas. ....2. *Dracoscirpoides*
3 Robust plant, culm > 5 mm diameter; perianth scale-like; in Cape area. .... 3. *Hellmuthia*
4 Perennial habit; inflorescence in globose clusters of over 10 spikelets; nutlet lacking a gynophore; embryo Cyperus-type. ....5.
4 Perennial or annual habit; inflorescence in mostly clusters mostly of under 10 spikelets; nutlet with or without a gynophore; embryo Cyperus- or Ficinia-type ....6.
5 Plants leafless; inflorescence pseudolateral, dioecious. ....4. *Afroscirpoides*.
5 Plants leafless or well developed blades; inflorescence with bisexual flowers, pseudolateral to anthelate ....5. *Scirpoides*.
6 Glumes chartaceous, nutlet lacking gynophore. ....6. *Isolepis*.
6 Glumes cartilaginous, nutlet mostly bearing gynophore. ....7. *Ficinia*

1. *Erioscirpus* Palla, Bot. Zeitung (Berlin) 54: 151 (1896). Type species – *Erioscirpus comosus* (Wall.) Palla, designated here.

Two species of perennial hemicryptophytes, diagnosed on presence of cotton-like perianth. Taxonomic revision as part of regional flora, e.g. Flora of Pakistan (http://www.tropicos.org/Project/Pakistan).

Distributed in Asia, from Iran to China, occurring in shallow soil and rocky crevices, at 700–2300 m.

2. *Dracoscirpoides* Muasya, S. African J. Bot. 78: 108 (2012). Type species – *Dracoscirpoides falsa* (C.B.Clarke) Muasya.

Three species of perennial hemicryptophytes or rhizomatous geophytes, taxonomy revised in *Muasya et al. 2012.*

Restricted to Southern Africa, occurring in montane grasslands.

3. *Hellmuthia* Steud., Syn. Pl. Glumac. 2: 90 (1855). Type species – *Hellmuthia membranacea* (Thunb.) R.W.Haines & Lye.

Monotypic, hemicryptophytes or rhizomatous geophytes, diagnosed by presence of scale-like perianth. Taxonomic studies in local flora (e.g. *Archer & Muasya, 2012*).

Restricted to South Africa, occurring coastal areas in calcareous sandy soils in the Cape Flora.

4. *Afroscirpoides* García-Madr. & Muasya, Taxon 64: 698 (2015). Type species – *Afroscirpoides dioeca* (Kunth) García-Madr.

Monotypic, densely tufted hemicryptophytes or rhizomatous geophytes, diagnosed by dioecious flowers borne in dense globose inflorescences whose bract terminates in a sharp-pointed tip.

Restricted to Southern Africa, occurring in seepages and streambeds in brackish habitats.

5. *Scirpoides* Ség., Pl. Veron. 3: 73 (1754). Type species – *Scirpoides holoschoenus* (L.) Soják, designated here.

Four species recognized in *Govaerts et al. (2020)*, but two additional species segregated from the widespread *S. holoschoenus* by *García-Madrid et al. (2015).*

Widespread in Mediterannean habitat in Mexico, Canary Is. through northern Africa and Eurasia to W. Himalaya, South Africa.).

6. *Isolepis* R.Br., Prodr. Fl. Nov. Holland.: 221 (1810). Type species – *Isolepis setacea* (L.) R.Br.

About 70 species recognized here, after moving seven species to *Ficinia*. Nearly a third of species are therophytes, rest are hemicryptophytes or rhizomatous geophytes. Most recent and comprehensive taxonomic revision in *Muasya & Simpson (2002).*

Nearly cosmopolitan distribution, with highest species densities in austral temperate southern Africa and Australasia.

7. *Ficinia* Schrad., Commentat. Soc. Regiae Sci. Gott. Recent. 7: 143 (1832). Type species – *Ficinia gracilis* Schrad.

About 90 species are recognized here, including the annual species transferred from *Isolepis*. Majority of species are perennial hemicryptophytes or rhizomatous geophytes, adapted to survive frequest fires in the Fynbos biomes, but also few annual and pyrophytic short-lived perrenials. The most comprehensive taxonomic study of *Ficinia* was part of the Flora Capensis (*Clarke, 1897-98*) and recent synopsis of the Cape Flora (*Archer & Muasya, 2012*). Ongoing studies reveal existence of undescribed species and the *Ficinia* is among the highest priority Cypeaceae for taxonomic revision in Southern Africa.

Predominantly occurring in southern Africa in the Cape Flora and extending into montane areas of tropical Africa. Two species occur in Australasia, among which *F. nodosa* is nearly circumpolar.

Species transferred from *Isolepis* to *Ficinia* in this study:

The annual *Isolepis* species forming a clade sister to *F. eligulata* (Figure 2) are here transferred into *Ficinia*.

Ficinia capensis (Muasya) Muasya, **comb. nov.**

Isolepis capensis Muasya, Kew Bull. 57: 305 (2002). [basionym]

Ficinia heminucialis (C.B.Clarke) Muasya, **comb. nov.**

Scirpus hemiuncialis C.B.Clarke in É.A.J.de Wildeman, Pl. Nov. Horti Then. 1: 23 (1904). [basionym]

Isolepis hemiuncialis (C.B.Clarke) J.Raynal, Adansonia, n.s., 17: 55 (1977).

Ficinia incomtula (Nees) Muasya, **comb. nov.**

Isolepis incomtula Nees, Linnaea 10: 154 (1835). [basionym]

Ficinia leucoloma (Nees) Muasya, **comb. nov.**

Cyperus leucoloma Nees, Linnaea 10: 133 (1835). [basionym]

Isolepis leucoloma (Nees) C.Archer, Bothalia 28: 42 (1998).

Ficinia marginata (Thunb.) Fourc., Trans. Roy. Soc. South Africa 21: 76 (1932).

Scirpus marginatus Thunb., Prodr. Pl. Cap.: 17 (1794). [basionym].

Isolepis marginata (Thunb.) A.Dietr., Sp. Pl. 2: 110 (1833).

There appears to be continuity in the number of spikelets per inflorescence, with materials at extreme ends recognized as *I. marginata* versus *I. antarctica*. We retain the two taxa as a single species, *Ficinia marginata,* and refrain from making a new combination pending a detailed taxonomic study of the complex.

Ficinia minuta (Turrill) Muasya, **comb. nov.**

Scirpus minutus Turrill, Bull. Misc. Inform. Kew 1925: 69 (1925). [basionym]

Isolepis minuta (Turrill) J.Raynal, Adansonia, n.s., 17: 56 (1977)

Conclusions

This study aimed to establish the phylogenetic position of contentious annual species currently placed in *Isolepis* and to test the monophyly of the genera. All the other smaller gener in the Ficinia clade (*Afroscirpoides*, *Dracoscirpoides*, *Erioscirpus*, *Hellmuthia* and *Scirpoides*) are monophyletic. There is unambiguous placement of *I. marginata* and allies (*I. antarctica, I. capensis, I. leucoloma* and *I. minuta*) as a clade within *Ficinia* and not part of the core *Isolepis* clade. Inclusion of *I. hemiuncialis* and *I. incomtula* into a clade including *I. marginata* is supported by the nuclear phylogeny, but these taxa are placed in a grade outside the core *Isolepis* clade. We propose the reclassification of these *Isolepis* species, resolved outside the core *Isolepis*, as species within *Ficinia*. The proposed classification will add taxa lacking the gynophore, the currently used diagnostic character for *Ficinia*, with the core *Isolepis* diagnosed by a combination of morphology (e.g. chartaceous glumes, no gynophore) and a unique indel in ITS.

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Table 1(on next page)

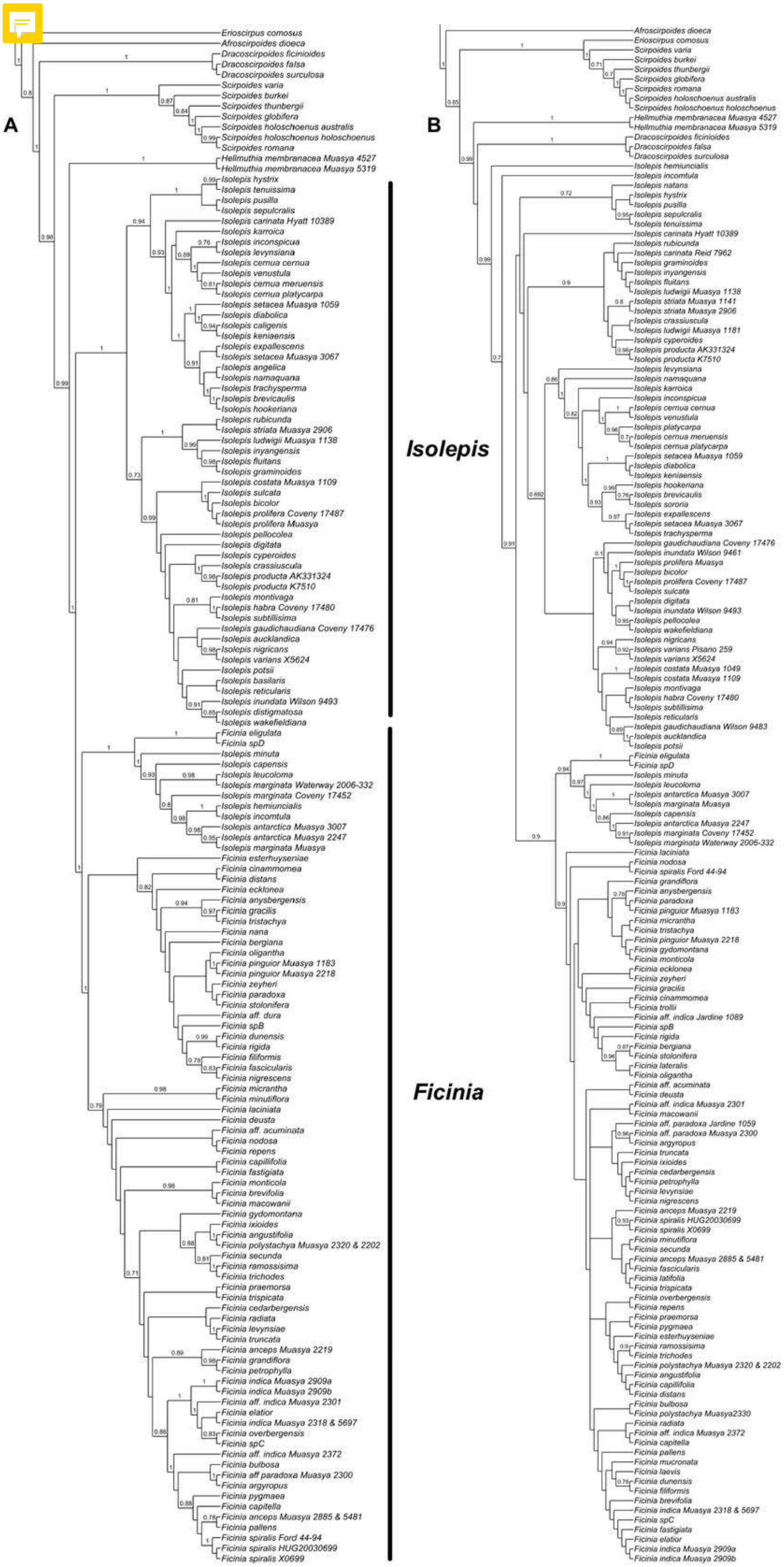
Comparison of genera in Ficinia clade, reflecting the revised classification.

	Erioscirpus Palla	*Afroscirpoides* García-Madr. & Muasya	*Dracoscirpoides* Muasya	*Scirpoides* Ség.	*Hellmuthia* Steud.	*Isolepis* R.Br.	*Ficinia* Schrad.
Life form	perennial	perennial	perennial	perennial	perennial	annual & perennial	perennial, few annual
Leaf blade	well developed	poorly developed (<5 mm long)	well developed	mostly poorly developed	mostly poorly developed	mostly well developed	mostly well developed
Inflorescence type	anthelate	capitate, pseudolateral	capitate, pseudolateral	capitate, anthelate, pseudolateral	capitate	capitate	capitate, spike, pseudolateral
Glume arrangement	spiral	spiral	spiral	spiral	spiral	spiral, few distichous	spiral, few distichous
Perianth type in fertile flowers	> 6 bristles, cotton-like	absent	6 (7) bristles, scabrid	absent	3 scales in lower flowers	absent	absent (single case recorded)
Gynophore	absent	absent	absent	absent	absent	absent	Mostly present
Embryo type	*Cyperus*	*Cyperus*	*Cyperus*	*Cyperus*	*Cyperus*	*Cyperus and Ficinia*	*Ficinia*
Number of species	2	1	3	4	1	70	~90

Distribution	Asia	Southern Africa	Southern Africa	Southern Africa, Eurasia, Americas	Southern Africa (Cape)	Southern & Tropical Africa, Australasia, Europe, Americas,	Southern & Tropical Africa, Australasia, circumpolar

Figure 1

The majority-rule consensus Bayesian Inference of the ingroup, showing posterior probabilities at nodes, based on MrBayes analyses. A) concatenated nuclear, B) concatenated plastid DNA sequence data.


A
Erioscirpus comosus
Afroscirpoides dioeca
Dracoscirpoides ficinioides
Dracoscirpoides falsa
Dracoscirpoides surculosa
Scirpoides varia
Scirpoides burkei
Scirpoides thunbergii
Scirpoides globifera
Scirpoides holoschoenus australis
Scirpoides holoschoenus holoschoenus
Scirpoides romana
Hellmuthia membranacea Muasya 4527
Hellmuthia membranacea Muasya 5319
Isolepis hystrix
Isolepis tenuissima
Isolepis pusilla
Isolepis sepulcralis
Isolepis carinata Hyatt 10389
Isolepis karroica
Isolepis inconspicua
Isolepis levynsiana
Isolepis cernua cernua
Isolepis venustula
Isolepis cernua meruensis
Isolepis cernua platycarpa
Isolepis setacea Muasya 1059
Isolepis diabolica
Isolepis caligenis
Isolepis keniaensis
Isolepis expallescens
Isolepis setacea Muasya 3067
Isolepis angelica
Isolepis namaquana
Isolepis trachysperma
Isolepis brevicaulis
Isolepis hookeriana
Isolepis rubicunda
Isolepis striata Muasya 2906
Isolepis ludwigii Muasya 1138
Isolepis inyangensis
Isolepis fluitans
Isolepis graminoides
Isolepis costata Muasya 1109
Isolepis sulcata
Isolepis bicolor
Isolepis prolifera Coveny 17487
Isolepis prolifera Muasya
Isolepis pellocolea
Isolepis digitata
Isolepis cyperoides
Isolepis crassiuscula
Isolepis producta AK331324
Isolepis producta K7510
Isolepis montivaga
Isolepis habra Coveny 17480
Isolepis subtilissima
Isolepis gaudichaudiana Coveny 17476
Isolepis aucklandica
Isolepis nigricans
Isolepis varians X5624
Isolepis potsii
Isolepis basilaris
Isolepis reticularis
Isolepis inundata Wilson 9493
Isolepis distigmatosa
Isolepis wakefieldiana
Ficinia eligulata
Ficinia spD
Isolepis minuta
Isolepis capensis
Isolepis leucoloma
Isolepis marginata Waterway 2006-332
Isolepis marginata Coveny 17452
Isolepis hemiuncialis
Isolepis incomtula
Isolepis antarctica Muasya 3007
Isolepis antarctica Muasya 2247
Isolepis marginata Muasya
Ficinia esterhuyseniae
Ficinia cinnamomea
Ficinia distans
Ficinia ecklonea
Ficinia anysbergensis
Ficinia gracilis
Ficinia tristachya
Ficinia nana
Ficinia bergiana
Ficinia oligantha
Ficinia pinguior Muasya 1183
Ficinia pinguior Muasya 2218
Ficinia zeyheri
Ficinia paradoxa
Ficinia stolonifera
Ficinia aff. dura
Ficinia spB
Ficinia dunensis
Ficinia rigida
Ficinia filiformis
Ficinia fascicularis
Ficinia nigrescens
Ficinia micrantha
Ficinia minutiflora
Ficinia laciniata
Ficinia deusta
Ficinia aff. acuminata
Ficinia nodosa
Ficinia repens
Ficinia capillifolia
Ficinia fastigiata
Ficinia monticola
Ficinia brevifolia
Ficinia macowanii
Ficinia gydomontana
Ficinia ixioides
Ficinia angustifolia
Ficinia polystachya Muasya 2320 & 2202
Ficinia secunda
Ficinia ramosissima
Ficinia trichodes
Ficinia praemorsa
Ficinia trispicata
Ficinia cedarbergensis
Ficinia radiata
Ficinia levynsiae
Ficinia truncata
Ficinia anceps Muasya 2219
Ficinia grandiflora
Ficinia petrophylla
Ficinia indica Muasya 2909a
Ficinia indica Muasya 2909b
Ficinia aff. indica Muasya 2301
Ficinia elatior
Ficinia indica Muasya 2318 & 5697
Ficinia overbergensis
Ficinia spC
Ficinia aff. indica Muasya 2372
Ficinia bulbosa
Ficinia aff paradoxa Muasya 2300
Ficinia argyropus
Ficinia pygmaea
Ficinia capitella
Ficinia anceps Muasya 2885 & 5481
Ficinia pallens
Ficinia spiralis Ford 44-94
Ficinia spiralis HUG20030699
Ficinia spiralis X0699
Isolepis
Ficinia
B
Afroscirpoides dioeca
Erioscirpus comosus
Scirpoides varia
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Isolepis pusilla
Isolepis sepulcralis
Isolepis tenuissima
Isolepis carinata Hyatt 10389
Isolepis rubicunda
Isolepis carinata Reid 7962
Isolepis graminoides
Isolepis inyangensis
Isolepis fluitans
Isolepis ludwigii Muasya 1138
Isolepis striata Muasya 1141
Isolepis striata Muasya 2906
Isolepis crassiuscula
Isolepis ludwigii Muasya 1181
Isolepis cyperoides
Isolepis producta AK331324
Isolepis producta K7510
Isolepis levynsiana
Isolepis namaquana
Isolepis karroica
Isolepis inconspicua
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Isolepis sororia
Isolepis expallescens
Isolepis setacea Muasya 3067
Isolepis trachysperma
Isolepis gaudichaudiana Coveny 17476
Isolepis inundata Wilson 9461
Isolepis prolifera Muasya
Isolepis bicolor
Isolepis prolifera Coveny 17487
Isolepis sulcata
Isolepis digitata
Isolepis inundata Wilson 9493
Isolepis pellocolea
Isolepis wakefieldiana
Isolepis nigricans
Isolepis varians Pisano 259
Isolepis varians X5624
Isolepis costata Muasya 1049
Isolepis costata Muasya 1109
Isolepis montivaga
Isolepis habra Coveny 17480
Isolepis subtilissima
Isolepis reticularis
Isolepis gaudichaudiana Wilson 9483
Isolepis aucklandica
Isolepis potsii
Ficinia eligulata
Ficinia spD
Isolepis minuta
Isolepis leucoloma
Isolepis antarctica Muasya 3007
Isolepis marginata Muasya
Isolepis capensis
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Ficinia ecklonea
Ficinia zeyheri
Ficinia gracilis
Ficinia cinnamomea
Ficinia trollii
Ficinia aff. indica Jardine 1089
Ficinia spB
Ficinia rigida
Ficinia bergiana
Ficinia stolonifera
Ficinia lateralis
Ficinia oligantha
Ficinia aff. acuminata
Ficinia deusta
Ficinia aff. indica Muasya 2301
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Ficinia aff. paradoxa Muasya 2300
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Ficinia ixioides
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Ficinia anceps Muasya 2885 & 5481
Ficinia fascicularis
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Ficinia repens
Ficinia praemorsa
Ficinia pygmaea
Ficinia esterhuyseniae
Ficinia ramosissima
Ficinia trichodes
Ficinia polystachya Muasya 2320 & 2202
Ficinia angustifolia
Ficinia capillifolia
Ficinia distans
Ficinia bulbosa
Ficinia polystachya Muasya2330
Ficinia radiata
Ficinia aff. indica Muasya 2372
Ficinia capitella
Ficinia pallens
Ficinia mucronata
Ficinia laevis
Ficinia dunensis
Ficinia filiformis
Ficinia brevifolia
Ficinia indica Muasya 2318 & 5697
Ficinia spC
Ficinia fastigiata
Ficinia elatior
Ficinia indica Muasya 2909a
Ficinia indica Muasya 2909b

Figure 2

A simplified majority-rule consensus Bayesian Inference of the ingroup based on the concatenated nuclear DNA sequence data