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RESEARCH ARTICLE

Monophyly and transoceanic dispersal in the widespread floating club-rush clade, *Isolepis* subgenus *Fluitantes* (Cyperaceae)

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Background and aims – Numerous lineages in the Western Cape of South Africa show affinities with the floras of tropical Africa and Australasia. *Isolepis* subgenus *Fluitantes*, comprising seven to nine species, includes the broadly-defined *I. fluitans*, which occurs throughout Africa into Europe and Asia, as well as on both sides of the Indian Ocean. Thus, it is well suited for testing the generality of both the Cape-to-Cairo pattern of dispersal and transoceanic dispersal between southern Africa and Australasia.
Material and methods – We inferred a dated population-level phylogeny based on new sequence data from the nuclear ITS and the chloroplast *atp*I–H gene regions. We constructed dispersal–extinction–cladogenesis models in Lagrange to infer ancestral areas and to compare the likelihoods of stepping-stone and long-distance modes of dispersal.
Key results – The *Fluitantes* originated in the Cape about 7 million years ago (mya). They spread stepwise onto the mountains of East Africa and thence into Europe and the islands of the Indian Ocean, seemingly tracking their ancestral habitat. Australasia was colonised by a single long-distance dispersal event ca 3 mya. Incongruence between the plastid and nuclear gene trees was apparent for the Australasian taxa, *I. crassiuscula*, *I. lenticularis*, and *I. producta*, with their *atp*I–H sequences placing them with *I. ludwigii* in the *Fluitantes* and the ITS nrDNA resolving them in the *Proliferae*. Furthermore, two African taxa (*I. graminoides*, *I. inyangensis*) diagnosed on unique morphology are resolved as part of the widespread *I. fluitans*.
Conclusion – This study supports and extends the northward migration model that accounts for the Cape element of the Afromontane flora. Australasia was colonised directly from southern Africa, perhaps assisted by wind or waterfowl. Despite ancient hybridization associated with dispersal, we recognise the three taxa in Australasia as distinct, but synonymise *I. graminoides* and *I. inyangensis* into the widespread *I. fluitans*.

Keywords – Gene tree incongruence; hybridisation; long-distance dispersal; phylogeny; phytogeography; stepping-stone dispersal; taxonomy.

INTRODUCTION

The Cape Floristic Region (CFR) has phytogeographical affinities with the high-altitude regions of the rest of Africa and with various parts of the southern Hemisphere, most notably Australasia (e.g. Linder 2005; Galley & Linder 2006; Moreira-Muñoz 2007; Sauquet et al. 2009). It was hypothesised by Levyns (1964) that the CFR lineages generally had their origins in tropical Africa, but more recent studies suggest otherwise. In order to determine the migration histories of vegetation elements shared between the CFR and the Afromontane regions, Galley et al. (2007) reconstructed the ancestral areas of clades in *Disa* P.J.Bergius., Irideae, *Pentaschistis* Stapf, and Restionaceae. Their results indicate that migrations have overwhelmingly been northward from the Cape into the tropics, in most cases over the Drakensberg Mountain range. Taxa that have colonised Africa from

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the north include *Carex* L., *Ranunculus* L., *Alchemilla* L. (Gehrke & Linder 2009), *Arabis alpina* L. (Koch et al. 2006), and *Lychnis* Tourn. ex L. (Popp et al. 2008), but migration from the African "sky islands" to the CFR has not been demonstrated (Galley et al. 2007).

Many important Cape groups, including members of the Aizoaceae, Asteraceae, Cupressaceae, Liliaceae, Myrtaceae, Poaceae, Podocarpaceae, Proteaceae, Restionaceae, and Rutaceae, are shared between the CFR and Australasia (Goldblatt 1978; Verboom et al. 2003). Although these two regions and Antarctica constituted adjacent parts of Gondwana, many of the lineages are too young for their current distribution to be the result of vicariance due to the breakup of Gondwana 165 million years ago (mya). For example, the Restionaceae are among the earliest clades to diversify in the Cape and they are < 50 mya old (Linder et al. 2003). Instead, more recent transoceanic dispersal between southwest Africa and southwest Australia has been invoked, e.g. for Proteaceae (Sauquet et al. 2009). Bergh & Linder (2009) summarise reports of nine further families, including the Cyperaceae, undergoing a total of 15 dispersal events (in both directions) between these two regions in the last 60 mya. The distribution of the sedges of tribe Schoeneae can only be accounted for by invoking at least five dispersal events between Africa and Australia in the last 30 mya (Verboom 2006; Viljoen et al. 2013). Furthermore, a densely sampled species-level study in *Schoenus* L. (Elliott et al. 2021) shows a Paleocene origin in Western Australia, followed by Miocene dispersal to southern Africa, but no dispersal in the opposite direction. Muñoz et al. (2004) hypothesised that the affinities among regions of the so-called Austral Kingdom (sensu Morrone 2002), which presently comprises Australasia, temperate South America, and the CFR, result from wind-assisted long-distance dispersal, with Antarctica as a possible stepping stone before it became glaciated in the Tertiary.

Isolepis R.Br. is a genus in the *Ficinia* clade of the Cyperaceae tribe Cypereae (Muasya & Larridon 2021) that has centres of diversity in the CFR and Australasia (WCSP 2021). Members of the *I. fluitans* group have been placed in the separate genus *Eleogiton* Link by some authors (e.g. Kadereit et al. 2016), but this clade is embedded within *Isolepis* according to DNA sequence data (Muasya et al. 2001, 2009, 2014; Muasya & Larridon 2021) and was named subgenus *Fluitantes* (C.B.Clarke) Muasya in a recent monograph of *Isolepis* (Muasya & Simpson 2002). This clade comprises seven to nine species, which have a distribution ranging from the Western Cape through Africa, Europe, and South Asia to Japan, Indonesia, and Australasia. Members are found submerged or floating in seepages, bogs, and shallow pools, with southern African taxa occurring in the distinct sclerophyllous wetland type (Sieben et al. 2017) found predominantly on nutrient-poor sandstone and quartzite substrates.

Isolepis fluitans (L.) R.Br. is one of the few plant species that naturally span the southern-temperate, afrotemperate, and northern-temperate regions. It occurs from southern Africa, through tropical Africa, into northern Europe in the north and the East African Islands, India, and southeast Asia in the east (Muasya & Simpson 2002). Until recently, *I. fluitans* was thought to occur in Australia and New Zealand as *I. fluitans* var. *lenticularis* (R.Br.) Muasya, but Ito et al. (2016) have reinstated this taxon as a separate species (*I. lenticularis* R.Br.). Three closely related species are found in the Western Cape: *I. rubicunda* (Nees) Kunth in low-altitude sandy (brackish) depressions; *I. striata* (Nees) Kunth floating in shallow water in mountain streams; and *I. ludwigii* (Steud.) Kunth occurring from the Western Cape to Natal on the edges of wetlands and ponds. Within tropical Africa, *I. inyangensis* Muasya & Goetgh. occurs in seepages and seasonally flooded montane grasslands from KwaZulu-Natal to Inyanga, Zimbabwe, whilst *I. graminoides* (R.W.Haines & Lye) Lye grows only in alpine bogs on Mt Elgon and Mt Ruwenzori. There is contention as to whether these two African taxa are distinct from the widespread *I. fluitans*, as *I. inyangensis* is considered by some sources (e.g. WCSP 2021) as a synonym of var. *fluitans*, and our recent field observations show a continuum in diagnostic characters especially in the KwaZulu-Natal midlands. In the Pacific, *I. crassiuscula* Hook.f. is found in Japan, Papua New Guinea, Australia, and New Zealand, while *I. lenticularis* R.Br. occurs in Australia and New Zealand, and *I. producta* (C.B.Clarke) K.L.Wilson is an Australian endemic. *Isolepis beccarii* (Boeck.) Goetgh. & D.A.Simpson is only found on the Indonesian island of Sumatra (Muasya & Simpson 2002). We hypothesise that the *Fluitantes* clade is, therefore, a further candidate for a sedge group showing this disjunct distribution due to transoceanic dispersal.

In this study, we examined whether *Isolepis* subgenus *Fluitantes* supports the general pattern of tropical clades embedded within, rather than sister to, the Cape clades, by reconstructing ancestral areas on a population-level phylogeny of the group. We also determined whether the *Fluitantes* clade colonised Eurasia from Africa or vice versa and inferred the scenario with the highest likelihood of explaining the transoceanic distribution. By sampling widely among the populations to capture taxonomic and ecological diversity, we evaluate the support for recognising the tropical African taxa (*I. graminoides*, *I. inyangensis)* as distinct from *I. fluitans*.

MATERIAL AND METHODS

DNA extraction, PCR amplification, and sequencing

Nucleotide sequences were collected for 2–37 accessions from 69 populations representing all putative *Fluitantes* species except *I. beccarii*, as well as for three species from *Ficinia* Schrad. and three from *Isolepis* subgen. *Isolepis* sect. *Proliferae* Muasya (supplementary file 1).

Total DNA was extracted using either the CTAB method (Doyle & Dickson 1987; Gawel & Jarret 1991) or the straight-to-PCR method of Bellstedt et al. (2010). The CTAB protocol was modified as follows: 0.02–0.04 g silica-dried material was ground in liquid nitrogen, mixed with 700 µl CTAB 2% extraction buffer containing 1 µl mercaptoethanol, and incubated at 65°C for at least an hour. DNA was extracted with 600 µl chloroform-isoamyl alcohol. It was left to precipitate at 4°C for at least 24 hours, washed

in 75% ethanol, dried over silica, and resuspended in 50 µl sterile double-distilled water.

Phylogeny reconstructions of *Isolepis* based on the commonly used *trn*L-F and *rps*16 regions (e.g. Muasya & Larridon 2021) failed to resolve relationships within the *I. fluitans* clade. A more rapidly evolving chloroplast marker was sought by screening the "Tortoise and Hare" markers of Shaw et al. (2007) for a subset of the DNA samples. The *atp*I–*atp*H intergenic spacer was selected as the chloroplast marker for this study on the basis of the number of variable sites and ease of amplification. The internal transcribed spacer (ITS) of the nuclear ribosomal gene region was the other marker used (primers ITS-4: White et al. 1990; ITS-L: Hsiao et al. 1994). Gel electrophoresis of PCR products did not reveal multiple bands, and the sequencing chromatograms did not show multiple peaks, indicating a lack of differentiated paralogues of this gene region within our study group. Thus, direct sequencing of PCR products was judged adequate and strategies like cloning were unnecessary.

Amplification of the ITS and *atp*I–H regions was performed with AB2720 thermal cyclers (Applied Biosystems, Inc., Foster City, California) in 30 µl reactions consisting of 1–2 µl DNA template in 3 µl buffer, 3 µl $MgCl_2$, 1.2 µl dNTPs, 1 µl of each primer, 0.6 µl DMSO, and 0.2 µl KAPA Taq DNA polymerase (KAPA Biosystems, Ltd., Cape Town, South Africa). Reaction conditions for ITS were as follows: initial denaturation at 94°C for 2 min; 33 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 2 min; and a final extension step at 72°C for 7 min. For *atp*I–H, we used the "slow and cold" program of Shaw et al. (2007). PCR products were cleaned and sequenced on ABI3730XL cycle sequencers at Macrogen, Inc. (Seoul, South Korea) or at the University of Stellenbosch DNA Sequencing Facility (Stellenbosch, South Africa).

Phylogeny reconstruction

Consensus sequences of forward and reverse sequencing runs were assembled using SeqMan v.7.0.0 (DNASTAR, Inc.). Muscle v.3.8.31 (Edgar 2004) was used with the default settings for sequence alignment. The resulting alignment was verified manually, and an ambiguously aligned region was removed from the *atp*I–H matrix.

The resulting matrices contained 78 accessions and 723 characters for ITS, of which 60% were variable, and 45 accessions and 1163 characters for *atp*I–H, of which 48% were variable. For estimating the *atp*I–H gene tree, 72 characters were added by coding the indels using the "simple indel coding" algorithm of Simmons & Ochoterena (2000) as implemented in SeqState v.1.4.1 (Müller 2006). A combined matrix was created by concatenating the DNA characters, partitioned by marker. Gene tree incongruence was apparent in the positions of the Australasian *I. crassiuscula*, *I. lenticularis*, and *I. producta.* The nuclear and chloroplast sequences for these taxa were thus entered into the concatenated matrix as separate terminals so as to unlink their inferred topologies (a strategy advocated by Pirie et al. 2009).

Model selection was done on the basis of AIC values calculated with MrModelTest v.2.3 (Nylander 2004), with maximum-likelihood trees optimised separately for each model using PhyML v.3.0 (Guindon & Gascuel 2003). The models chosen were K80+Γ for ITS and GTR+I+Γ for *atp*I–H. However, when analysing the combined matrix, the parameter estimates for the substitution rates and proportion of invariant sites in the *atp*I–H partition failed to converge, so the simpler HKY+Γ model was used instead.

Phylogenetic relationships in the *Fluitantes* clade were inferred using the Bayesian MCMC method implemented in MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). All parameters except topology and branch lengths were unlinked across partitions. The MCMC sampler was run for 10 million generations with four Metropolis-coupled chains at the default temperature setting and two simultaneous runs, sampling 10,000 sets of parameter estimates in each run. Tracer v.1.5 was used to confirm that the runs had converged and that sampling was sufficient. The first 10% of samples were discarded as burn-in and a majority-rule consensus tree was created from the post-burn-in parameter estimates in MrBayes. This was used as the starting tree for the dating analysis. The gene tree estimates converged more quickly, so they were only run for 3.15 million generations for ITS, discarding the first 0.15 million, and for 5 million generations for *atp*I–H, discarding the first 10%.

BEAST v.1.6.1 (Drummond & Rambaut 2007) was used to co-estimate the topology and the ages of the nodes in the *Fluitantes* tree. The data set was partitioned as above and analysed with the same substitution models. The uncorrelated log-normal relaxed clock (Drummond et al. 2006) was used as a rate model for both partitions, with a gamma-distributed prior (shape = 1, scale = 1). The birth rate in the Yule speciation model was parameterised with a gamma-distributed prior (shape = 1.5, scale = 1). The outgroup (*Ficinia*) and ingroup (*Isolepis* s.s.) were constrained to be reciprocally monophyletic and the prior probability distribution for the root height (the split between *Ficinia* and *Isolepis*) was set to a normal distribution centred on 10 mya with a standard deviation of 2 mya (Besnard et al. 2009) using BEAUTI v.1.6.1. The analysis was run twice for 20 million generations each, saving the parameter estimates and trees every 2000 generations. Tracer was again used to assess convergence and sampling. The first 5% of samples were discarded as burn-in. Post-burn-in tree samples were combined with LogCombiner v.1.6.1 and the maximum-clade-credibility tree was annotated with the posterior probabilities of clades (PP) and the 95% highest posterior density (HPD) intervals of clade ages using TreeAnnotator v.1.6.1.

Dispersal history

Dispersal events were reconstructed on the dated tree and the likelihoods of different dispersal scenarios were estimated using the dispersal–extinction–cladogenesis (DEC) model in Lagrange v.20171013 (Ree & Smith 2008), with the maximum range size set to 2. For this purpose, specimen localities were divided into five regions: Western Cape, Southeast Africa (Eastern Cape to Malawi), Tropical Africa

(Tanzania to Ethiopia, including Central Africa and East African islands), Europe, and Australasia. The Tristan da Cunha and Japan samples were removed from the tree prior to analysis. Lagrange was run via a custom Python script to generate more easily parsable output, and the results were summarised graphically by plotting the proportional likelihoods of each region in the estimated ancestral range at each node of the tree using the packages ape v.5.5 (Paradis & Schliep 2019), phyloch v.1.5.3 (Heibl 2008), ggtree v.3.0.3 (Yu et al. 2017), and tidyverse v.1.3.1 (Wickham et al. 2019) in R v.4.1.1 (R Core Team 2021).

We evaluated the likelihood of two dispersal-constrained models in comparison with the *Unconstrained* model: In the *Overland* model, dispersal to/from Australasia was constrained to be through Tropical Africa or Europe (in the absence of samples from South Asia), i.e. direct transoceanic dispersal from Southern Africa was disallowed. We also set up a *Stepping Stone* model, in which dispersal within Africa and Europe was limited to adjacent regions, in order to assess the prevalence of long-distance dispersal in the African *Fluitantes*.

Note that for the constrained models, dispersal between particular pairs of areas was set to 0, while the other transitions were all equally likely (set to 1). Thus, these dispersal rates are not free parameters, and the constrained and unconstrained models have the same number of parameters. This precludes the use of likelihood-ratio tests, so the model likelihoods were compared directly.

The analyses were run via Snakemake v.6.7.0 (Mölder et al. 2021). The workflow and custom analysis scripts are available at https://doi.org/10.5281/zenodo.5584964.

RESULTS

Dated phylogeny

The maximum sum of clade credibility tree summarised from the trees sampled in BEAST is shown in fig. 1. The topology is congruent with the MrBayes consensus tree at all nodes with PP ≥ 0.5, except that *I. bicolor* and *I. sulcata* in the *Proliferae* do not form a clade in the latter (supplementary file 4).

As expected from the conflict between the nuclear (supplementary file 2) and chloroplast (supplementary file 3) trees, the ITS samples of *I. crassiuscula*, *I. lenticularis*, and *I. producta* were resolved closer to the *I. prolifera* clade than to the *Fluitantes* (fig. 1; PP = 1.00), while the *atp*I–H samples are closest to *I. ludwigii* in the *Fluitantes* (PP = 0.94). The three species found in Australasia were reciprocally monophyletic according to the ITS samples but not according to the *atp*I–H ones. With exception of the ITS sequences of these species, the *Fluitantes* are strongly supported as monophyletic (PP = 1.00).

The most basal divergence (i.e. crown node) within the *Fluitantes* was between the *I. striata+rubicunda* (CFR; PP = 0.93) clade and the clade including *I. ludwigii* and *I. fluitans* (PP = 0.99), around 5 mya (HPD: 1.8–7.7 mya; excluding the Australasia nuclear data). *Isolepis fluitans* (IF; PP = 0.96) diverged from the *I. ludwigii* (IL; PP = 0.90) clade at ca 4 mya (HPD: 1.5–6.4 mya) and then split into three main clades (IF1, IF2.1, IF2.2; fig. 1) ca 3 mya (HPD: 0.9–5.2 mya). *Isolepis inyangensis* was found to be most similar to *I. fluitans* specimens from the IF1 clade but with weak support (PP = 0.70), while *I. graminoides* was resolved as part of the IF2.2 clade (PP = 0.86). Hence *I. fluitans* is not monophyletic as parts of the clades IF1 and IF2.2 are currently named as distinct taxa.

Reconstruction of dispersal history

The inferred dispersal events between the five main regions occupied by the *Fluitantes* are also shown in fig. 1. There was no trend regarding speciation in allopatry versus sympatry at this geographic scale, neither by clade nor by region.

The most likely area of origin of the *Fluitantes* clade was the CFR (pL = 0.85; or CFR and southeast Africa, pL = 0.12). The ancestor of the IL clade was reconstructed as occurring in both the CFR and Southeast Africa (pL = 0.80; or CFR alone, pL = 0.10), splitting into Eastern Cape *I. ludwigii* and a CFR clade spreading to Australia as *I. producta* and *I. crassiuscula.*

With the level of sampling in this study, the *Proliferae* were inferred to be of Cape origin (pL = 0.60; or CFR and Australasia, pL = 0.28), splitting into clades containing the Australasian ITS specimens of *I. producta*, *I. crassiuscula*, and New Zealand *I. lenticularis* on the one hand, and *I. prolifera*, found throughout the Southern Hemisphere, on the other hand. The ITS sequences of Australian (+Japan) and New Zealand *I. crassiuscula* diverged ca 1 mya. Direct dispersal between Australasia and Southern Africa (i.e. CFR or Southeast Africa) was much more likely than via Tropical Africa (*Unconstrained* lnL = −76.7; *Overland* lnL = −111.3).

The IF clade was found to have originated in Southeast Africa (pL = 0.88). It dispersed to the Comoro Islands in IF1; to Kenya and Madagascar in IF2.2; and to East, Northeast, and West Africa in IF2.1. Members of this last clade were also found in Europe and Réunion. The likelihood of the strict *Stepping Stone* dispersal model (lnL = −75.4) was slightly higher but within 2 units of the model allowing long-distance dispersal within Africa and to Europe (*Unconstrained* lnL = −76.7). (The *Stepping Stone* model is able to have a higher likelihood because, as noted in Methods, it does not have fewer parameters than the *Unconstrained* model.)

DISCUSSION

This study sampled multiple populations of *Isolepis* subgenus *Fluitantes* with the aim of understanding the temporal and geographical context of its evolution. There is support for the clade to have originated in the CFR in the late Miocene, dispersing through montane habitats of tropical Africa to Eurasia and across the Indian Ocean into Australasia. The transoceanic dispersal is accompanied by a hybridization event, with diversification and further dispersal within Australasia. *Isolepis fluitans* is shown to be a widespread species, occurring in Africa (including the Indian Ocean Islands), Europe, and Asia; some populations displaying unique morphology (e.g. short peduncle (*I. graminoides*) or

more than 10 florets in a spikelet (*I. inyangensis*)) have been named as distinct species.

Previous studies reconstructing the phylogeny of the *Fluitantes* clade (Ito et al. 2016; Muasya & Larridon 2021) observed that chloroplast gene trees have different topologies from the single nuclear locus sampled. Our study further shows this incongruence, where the Australasian taxa have their nuclear DNA matching members of section *Proliferae*, whereas their plastid phylogeny supports their placement in *Fluitantes*. At lower taxonomic levels, as in this study, such

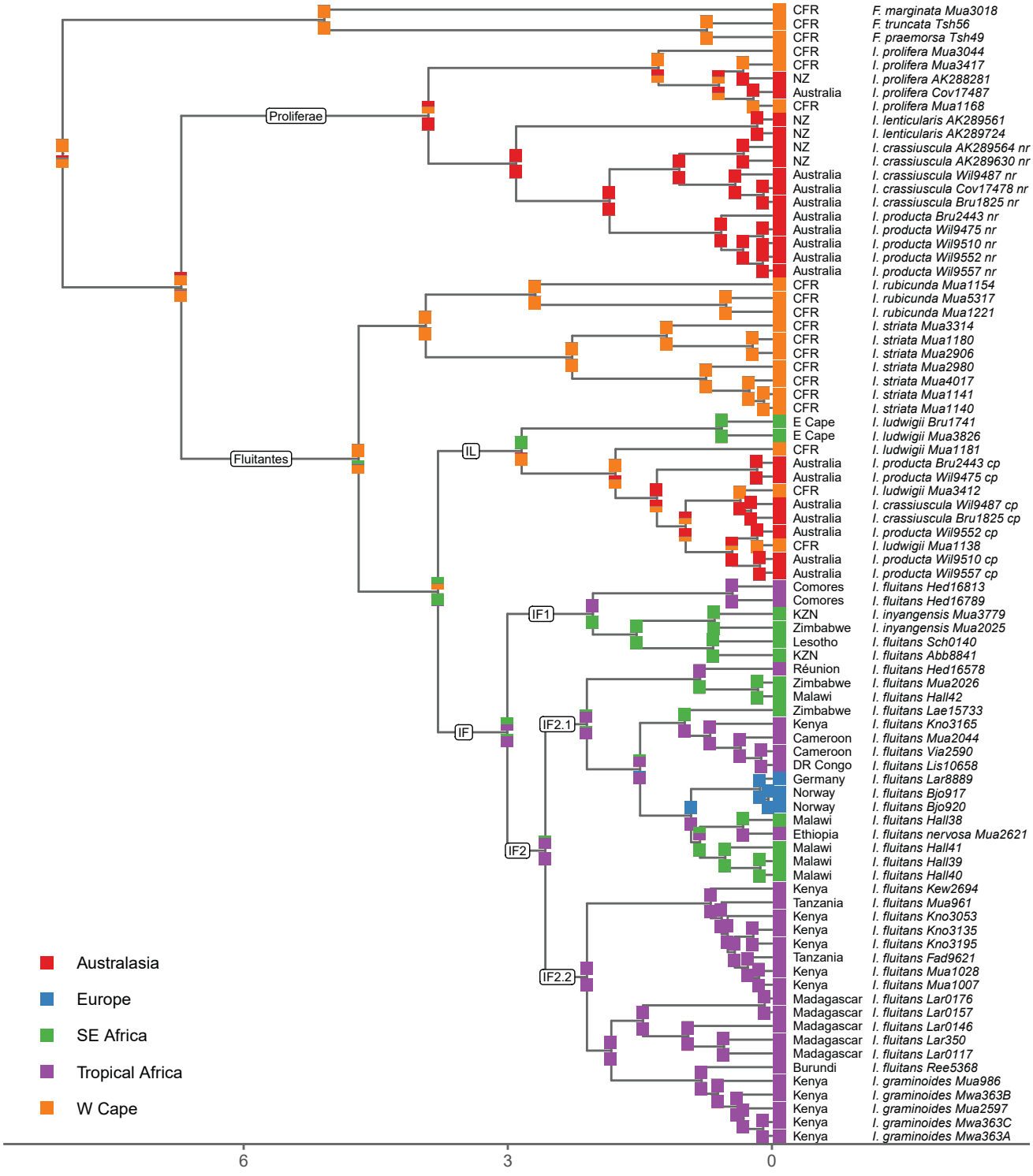

Figure 1 – Dated phylogeny of the *Fluitantes* clade showing proportional likelihood of ancestral areas of each lineage under the *Stepping Stone* model. The scale bar is in mya.

gene tree incongruence may be explained by three main processes: (i) divergence between paralogues (multiple gene copies) within a genome (Fitch 1970; Doyle 1991); (ii) incomplete lineage sorting, where the cessation of gene flow between populations is too recent (or has not yet occurred) for shared ancestral polymorphisms to have been differentially removed by genetic drift, or new mutations are still being shared among diverging populations (Pamilo & Nei 1988; Degnan & Salter 2005); or (iii) hybridization, where maternal and paternal genes have different histories and phylogenetic affinities (Soltis et al. 1996; Sang & Zhong 2000). See Ito et al. (2016) for a nuanced discussion on the possibility of the above three phenomena among the *Fluitantes*. As the Australasian taxa have some morphological similarity to the *Proliferae* (e.g. enlarged inflorescence bract) yet retain a plastid sequence and overall morphology of the *Fluitantes*, we interpret the topological incongruence to be caused by hybridization where the pollen originates from a member of sect. *Proliferae*. The nuclear sequences in the Australasia *Fluitantes* are more similar to the *Proliferae*, rather than intermediate or having multiple peaks at base positions, which we interpret as due to concerted evolution (Wendel et al. 1995).

Origin of *Fluitantes*

The *Fluitantes* were found to have originated in the CFR around 7 mya. In the last 5 mya, the clade spread eastwards, then northwards onto the mountains of tropical Africa. More recently, it also colonised Australasia, apparently directly from the CFR. The age of the split between *Ficinia* and *Isolepis* s.s. around 8 mya (95% HPD*:* 3.8–12.6 mya) agrees with estimates from previous studies (Besnard et al. 2009), and the t_{MRCA} of *I. bicolor* and *I. sulcata* (median: 0.41 mya, 95% HPD: 0.04–1.10 mya) is consistent with the geological age of the Tristan da Cunha islands in which they are endemic, i.e. 18, 3, and 0.5 mya for Nightingale Island, Inaccessible Island, and Tristan da Cunha, respectively (Gass 1967).

Since the *Proliferae* clade was not sampled extensively and the clade sister to the *Fluitantes* (containing *Isolepis cernua* and *I. hystrix*) was not sampled at all in this study, there is some uncertainty about the reconstructed ancestral areas of both the *Fluitantes* clade and of *Isolepis* as a whole. However, as most of the members of the *Proliferae* are Australasian, with some species found in the Cape, SE and tropical Africa, South America, and the subantarctic islands, greater sampling is not likely to qualitatively alter the reconstructed ancestral area of the *Proliferae* (i.e. Australasia + CFR). Similarly, for the rest of *Isolepis* (and *Ficinia*), we do not believe that the inferred ancestral area is substantially biased towards a CFR origin by our sampling, as the unsampled taxa are overwhelmingly CFR endemics (Muasya & Simpson 2002; Muasya & Larridon 2021).

African species

The different populations of *Isolepis fluitans* cover a wide geographic range despite relatively recent genetic divergence. The evolutionary history of the clade is, therefore, not necessarily an accurate reflection of its dispersal history, and we hesitate to draw inferences about events more recent than the divergence of the three main clades of this species. Wallis & Trewick (2009) had similar misgivings in their comparative phylogeographic study of New Zealand biota but successfully used a criterion of endemic lineages nested within paraphyla occupying the reconstructed source regions to interpret dispersal from the ancestral region into the region of the endemic clade. This is the approach we have emulated here, using the deeper, well-supported nodes of the *Fluitantes* tree.

In contrast to the African species of *Carex*, which have a Holarctic origin (Gehrke & Linder 2009; Larridon et al. 2021), the overall direction of dispersal of the African *Fluitantes* has been from the Cape, into eastern South Africa, and northwards from there, which Galley et al. (2007) termed the "Cape to Cairo" pattern. In addition to at least one southward dispersal event (the recolonization of southeast Africa from the tropics), several other events are apparent.

It appears that the Indian Ocean islands were colonised by members of three different clades of *I. fluitans:* from Southeast Africa to the Comoro Islands (IF1), from Tropical Africa to Réunion (IF2.1), and from Tropical Africa to Madagascar (IF2.2). The reconstructed timings of these divergences fall within the geologically determined age of Réunion of 2.5 mya (McDougall & Chamalaun 1969).

The rest of the IF2.2 clade (including *I. graminoides*) occurs only in tropical East Africa (including Madagascar). Clade IF2.1, on the other hand, is more widespread, occurring from southeast to northeast Africa, as well as in Cameroon and the DRC, and in Europe. There are no known phenotypic differences between these clades of *I. fluitans* to account for this difference in range size. It is interesting to note, however, that *Fluitantes* living in the tropics are only found at high altitudes, where conditions are more similar to the temperate habitats occupied by other members of the clade. This is in agreement with the hypothesis of niche conservatism (Wiens & Graham 2005), i.e. a lineage is more likely to track its ancestral habitat than to adapt to new environmental conditions. The African *Fluitantes* are unique in dispersing into the afrotemperate areas beyond South Africa, unlike the majority of lineages occurring in the sclerophyllous wetland type (Sieben et al. 2017), which are confined to nutrient-poor substrates of sandstone and quartzites.

Even though the tribe Cypereae is pantropical, where *Cyperus* L. species predominantly occur in the savanna and grassland biomes, the afrotemperate members of the *Fluitantes* are embedded within and derived from a southern-temperate fynbos lineage (*Ficinia* clade sensu Muasya et al. 2009; Muasya & Larridon 2021). Note, also, that the *Fluitantes* followed the "out of the Cape" (Galley et al. 2007) dispersal path of migration: first east out of the CFR, then north into southeast Africa. That they did not migrate directly over the semi-arid Kalahari is surprising, considering their apparent ability to disperse over long distances (e.g. across the Indian Ocean), and indicates a limitation on establishment and persistence, rather than on mere vagility. It is also puzzling why the *Fluitantes* are absent in South America, unlike savanna wetland plants (e.g.

Hydrocharitaceae, Chen et al. 2012) which are pantropical, perhaps pointing to lack of suitable niches.

The composition of the *Fluitantes* communities shows turnover along its path. The *Stepping Stone* DEC model fit the data as well as the *Unconstrained* one, suggesting that dispersal on the African continent was limited to adjacent regions. Thus, species turnover may be the result of restricted gene flow and isolation-by-distance. In the CFR, however, three closely related species co-occur, suggesting a role for adaptation to diverse microhabitats, with *I. rubicunda* occupying low-lying brackish depressions, *I. striata* occurring at higher altitudes floating in water, and *I. ludwigii* inhabiting the edges of streams and wetlands. Within tropical Africa, *I. fluitans* (IF1; fig. 1) shows successive dispersal to nearby Afromontane habitats, but the pattern is complicated by variation in the ages of the "sky islands". However, the occurrence of *I. fluitans* in India and South East Asia could not be rigorously evaluated due to lack of sampling in that region, but we speculate dispersal from tropical Africa based on morphological similarity in *I. fluitans* populations.

Australasian species

The *Unconstrained* DEC model fit the data much better than the *Overland* one, supporting long-distance transoceanic dispersal between the Cape and Australasia. The contrast with the short dispersal distances within Africa might be explained by the influence of Antarctic circumpolar wind currents, thought to be important in the dispersal of other plant groups across the southern oceans (Muñoz et al. 2004; Sanmartín et al. 2007; Sauquet et al. 2009; Ito et al. 2016). Species of Juncaceae (e.g. *Juncus* L.) and Cyperaceae (e.g. *Carex*, *Scirpus* L.) have also been reported to be dispersed in the gut and in mud on the feet of migrant water fowl (Hedberg 1970; Soons et al. 2016), providing a possible alternative mechanism for the dispersal of the *Fluitantes*.

The cpDNA sequences of the Australasian taxa do not resolve the relationships between *I. crassiuscula*, *I. ludwigii*, and *I. producta* (fig. 1), and no further details can be deduced from the dispersal and speciation history of the clade within Australasia. Although *atp*I–H is one of the most variable cpDNA markers (Shaw et al. 2007), we concur with Zeng et al. (2010)'s recommendation that it should be combined with other chloroplast markers for resolving relationships at lower taxonomic levels.

Two processes present themselves as possible explanations for the incongruence between the gene trees for the Australasian taxa: incomplete lineage sorting and lateral gene transfer (hybridization). With only one nuclear and one chloroplast marker, the former cannot be ruled out. However, incomplete lineage sorting seems unlikely given that the *Fluitantes* and section *Proliferae* diverged over 8 million years ago, and that only these three *Fluitantes* in Australasia have nuclear DNA similarity to the *Proliferae*. In addition, two previous studies (Ito et al. 2016; Yano et al. 2016) using non-overlapping samples and DNA regions have concluded that hybridization is most likely source of the incongruence. The phylogeny of the *Proliferae* clade will also need to be resolved in order to identify the species most closely related to *I. crassiuscula* and *I. producta* at the incongruent loci. On the other hand, morphological similarity of the Australasian *Fluitantes* to the *Proliferae* (presence of involucral bract equal or longer than spikelet; one to two spikelets) point to hybrid morphology. *Isolepis beccarii* is likely to have dispersed from Australasia to Sumatra, arising from such a hybrid ancestor, as it shows an enlarged involucral bract (Muasya & Simpson 2002) similar to the *Fluitantes* that have hybrid origin and whose spikelets are more similar to *I. prolifera*. Other instances of hybridization among closely and distantly related species have been reported in *Isolepis* (see Yano et al. 2016) but several of such putative hybrids have not been verified using molecular approaches.

Taxonomic implications

Despite the reticulate evolution of the Australasian *Fluitantes*, we recognise the taxa included in this study (*I. crassiuscula*, *I. lenticularis*, *I. producta*) as distinct species. A thorough study of *Isolepis* in Australasia may increase the number of species in the group, as Muasya & Larridon (2021) recovered *I. cyperoides* as part of the Australasian *Fluitantes* clade. However, the species status of the African taxa embedded in *I. fluitans* (*I. graminoides*, *I. inyangensis*) is not supported. We note that other sources have questioned the distinctness of *I. inyangensis*, with WCSP (2021) considering it as a synonym of *I. fluitans*, and our recent field observations in KwaZulu-Natal have revealed populations with morphological features (habit, floret number in spikelet) filling the continuum between the perceived discreet boundaries. We further view the distinctness of *I. graminoides*, whose inflorescences are borne on short peduncles and partially enclosed in the leaf sheaths, to be habit-driven (dwarfism) and a common phenomenon in high elevation (above 3500 m) bog sedges on Mt Elgon and in other Afromontane habitats. Furthermore, taxa previously recognised at varietal rank in Africa (var. *major*, var. *nervosa*) are resolved within the IF clade, and there is no morphological discontinuity nor genetic coherence to support such entities as distinct. Our study thus does not support recognition of infraspecific categories within *I. fluitans*, despite samples belonging to some of the previously recognized taxa (e.g. *I. graminoides*; fig. 1) forming distinct subclade derived out of *I. fluitans*, as there is evidently widespread dispersal and gene flow within tropical Africa.

Isolepis fluitans (L.) R.Br. (Brown 1810: 221) – *Scirpus fluitans* L. (Linnaeus 1753: 48) – Type: plate (Morison 1699: s. 8, t. 10, f. 31 "Gramen junceum clavatum minimum"; lectotype selected by Simpson et al. 2001)

Isolepis graminoides (R.W.Haines & Lye) Lye (Lye & Haines 1974: 525), **syn. nov.** – Type: KENYA • Trans Nzoia, Mt Elgon; 15 Dec. 1969; *A.M. Hamilton 1418*; holotype: MHU[MHU000062]; isotype: EA[EA000002577].

Isolepis inyangensis Muasya & Goetgh. (Muasya & Simpson 2002: 283). **syn. nov.** – Type: ZIMBABWE • Inyanga; 14 Nov. 1956; *E.A. Robinson 1889*; holotype: K; isotypes: B, BR[BR0000024914499], LISC, NRGH, PRE[PRE0574480], SRGH.

SUPPLEMENTARY FILES

Supplementary file 1 – List of studied taxa, showing voucher details, country of origin, and GenBank accession details. Samples sequenced in this study are submitted to GenBank, accession details provided.

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Supplementary file 2 – Phylogeny based on ITS (nrDNA) sequences.

https://doi.org/10.5091/plecevo.84466.supp2

Supplementary file 3 – Phylogeny based on *atp*I–H (cpDNA) sequences.

https://doi.org/10.5091/plecevo.84466.supp3

Supplementary file 4 – Phylogeny based on concatenated nrDNA and cpDNA sequence matrices.

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