Biogeography of *Isolepis* subgenus *Fluitantes* (C.B.Clarke) Muasya (Cyperaceae): niche conservatism, long-distance dispersal, and hybridization

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Abstract

Numerous lineages of the Western Cape of South Africa show affinities with the flora of tropical Africa and with Australasia. Recent work suggests that most migrations between the Western Cape and tropical Africa occur in a northward direction, and that connections between the flora of regions in the Southern Hemisphere are maintained by wind-assisted long-distance dispersal. The *Fluitantes* clade of *Isolepis* (Cyperaceae: Cypereae) is distributed throughout these areas and provides a useful study group to assess the general validity of published biogeographical trends. Furthermore, the cooccurrence of several closely related species in the Cape floristic region allows geographical and ecological patterns to be used for inference of speciation processes in the clade.

Sequence data of the ITS and *atpI–H* gene regions were collected for 82 specimens; these were used to construct haplotype networks and phylogenies. By using the Tristan da Cunha endemics in the genus, as well as results from higher-level studies, a dated phylogeny for the *Fluitantes* clade was constructed and allowed for ancestral character state optimization of distributions by maximum likelihood. Ecological data were extracted from geographic information systems map to test for environmental differentiation in the Cape taxa.

The *Fluitantes* were found to have originated in the Cape 7 million years ago. From there, they spread east and northwards onto the mountains of East Africa and to the islands of the Indian Ocean. Multiple dispersal events to Australia were recorded. Incongruence between the plastid and nuclear gene trees indicate hybridization to have taken place in Australasia, with possible subsequent speciation. Although the multivariate analysis found some ecological differentiation between the three Cape species, there was substantial overlap in all variables, and interpretation of habitat differences was difficult. It is suggested that, instead, differentiation may have taken place at the microhabitat scale, as *I. rubicunda* occupies low-lying sandy depressions, *I. striata* occurs at higher altitudes floating in water, and *I. ludwigii* inhabits the edges of streams and wetlands.

Introduction

Biogeography of the Western Cape

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., Moreira-Muñoz, 2007; Sauquet et al., 2009; Linder, 2005; Galley & Linder, 2006). It was hypothesized by Levyns and others that the CFR lineages generally had their origins in tropical Africa, but more recent studies suggest otherwise. Many important Cape elements including Proteaceae, Restionaceae, Rutaceae, and the gymnosperms *Podocarpus* and *Widdringtonia* are shared between the CFR and Australia (Galley & Linder, 2006). Other such groups include the grass genus *Ehrharta* (Verboom, 2000) and the sedge genera *Ficinia* and *Tetraria* (Muasya et al., 1998).

Although these two regions and Antarctica constituted adjacent parts of Gondwana, many of these lineages are too young for their current distributions to be the result of vicariance due to the breakup of Gondwana 165 million years ago (Mya) (e.g., Restionaceae are < 50 My old; Linder, 2003). Instead, the Proteaceae are thought to have undergone multiple dispersal events between southwest Africa and southwest Australia in the more recent past (Sauquet et al., 2009). Muñoz et al. (2004) hypothesized that the affinities within the so-called Austral Kingdom, 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 period.

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 the phylogenies of *Disa,* the Irideae, *Pentaschistis,* and the Restionaceae. Their results indicate that migrations have overwhelmingly been northward from the Cape into the tropics, in most cases over the Drakensberg mountain range. The present study aims to ascertain whether the dispersal of *Isolepis* subgenus *Fluitantes* (Cyperaceae) followed a similar pattern.

Study group

Isolepis R.Br. is a genus in the Cypereae clade of the Cyperaceae that has centres of diversity in the CFR and Australasia. Members of the *I. fluitans* group have been placed in the separate genus

Eleogiton Link by some authors, but this clade is embedded within *Isolepis* according to DNA sequence data (Muasya et al., 2001) and was named subgenus *Fluitantes* (C.B.Clarke) Muasya in the monograph of *Isolepis* (Muasya & Simpson, 2002).

This clade has a distribution ranging from the Western Cape, through Africa, Europe, and South Asia to Japan, Indonesia, and Australasia. *I. fluitans* (L.) R.Br. is the most widespread species, occurring in the Eastern Cape, the mountains of tropical Africa, Europe, the Indian Ocean islands, India, and Oceania. It is found submerged or floating in seepages, bogs, and shallow pools. Three species are found in the Western Cape: *I. rubicunda* (Nees) Kunth in low-altitude sandy depressions on the Cape Flats; *I. striata* (Nees) Kunth floating in shallow water; and *I. ludwigii* (Steud.) Kunth occurring from the Cape to Natal on the edges of wetlands and ponds. *I. inyangensis* Muasya & Goetgh. occurs in seepages and seasonally flooded grasslands from kwaZulu-Natal to Inyanga, Zimbabwe; *I. graminoides* (R.W.Haines & Lye) Lye only grows in alpine bogs on Mt. Elgon and Mt. Ruwenzori. In the Pacific, *I. crassiuscula* Hook. f. is found in Japan, Papua New Guinea, Australia, and New Zealand, while *I. producta* (C.B.Clarke) K.L.Wilson is an Australian endemic. *I. beccarii* (Boeck.) is only found on Sumatra (Muasya & Simpson, 2002).

In this study, the origin of the *Fluitantes* clade and its dispersal history through Africa and between the CFR and Australasia are reconstructed. Based on the results of previous biogeographic studies of Cape taxa, and the fact that *Isolepis* as a whole is thought to have originated in the CFR (Muasya & Viljoen, in preparation), it is hypothesized that the *Fluitantes* dispersed from the CFR into tropical Africa and Europe and that Australia was separately colonized by one or multiple long-distance dispersal events.

Determining the circumstances of speciation

The distributions of sister species have been used to infer the mode of speciation involved (e.g. Barraclough & Vogler, 2000; van der Niet & Johnson, 2009). However, the reasoning that currently sympatric species arose by sympatric speciation assumes that species ranges have not shifted over time. It is plausible that once-allopatric sister species have come into secondary contact, or, conversely, that sympatric species may have diverged to occupy disjunct ranges. The signal of

range overlap is soon lost and, with it, the ability to make straightforward inferences of speciation mode, e.g. by using age–range correlations (Losos & Glor, 2003; Fitzpatrick & Turelli, 2006).

In recently diverged taxa where reciprocal monophyly has not been reached, the circumstances of speciation may be elucidated by means of Bayesian phylogeographic tools, e.g. IMA (Hey & Nielsen, 2004). These involve modelling demographic parameters including migration rates between populations using DNA sequence data. Speciation due to geographical isolation (under the action of different selective pressures or by genetic drift) can be differentiated from sympatric speciation, e.g. by ecological divergence, as newly speciated sympatric populations are still expected to show small but non-zero levels of gene flow, which would be absent in the case of allopatry (Niemiller et al., 2008).

Alternatively, ecological niche modelling can be used to rule out the occurrence of sister taxa in the same area at the time of divergence. The niche of a species is modelled based on data describing its known localities. This is then projected onto geographical information system (GIS) maps to determine the areas that are potentially inhabitable by the species, since these areas have similar habitat characteristics to the current/sampled distribution range. Kozak & Wiens (2006) used this method to infer allopatric divergence in North American salamanders, as the potential ranges of several pairs of sister species were disjunct at the time of speciation. This method is based on the Niche Conservatism Hypothesis Wiens & Graham (2005), as it assumes that the niche of each species has not changed over time and that the common ancestor was constrained to occupying similar habitats as its descendent species (i.e., that it could not have inhabited the gap between the potential ranges).

The CFR is well known for its high species density, with ca. 9000 species in an area of ca. 90,000 km^2 (Goldblatt & Manning, 2002). Sympatric ecological speciation has therefore been hypothesized to be prevalent and to stem from high habitat heterogeneity in the region (Linder, 2003). The large number of potential niches is created by steep gradients in soil texture and fertility, water availability, and solar radiation due to complex geology and topography (Cowling et al., 2008), as well as by biotic factors including pollinator specificity and fire-adaptation strategies (van der Niet & Johnson, 2009).

In the present study, habitat data were extracted from GIS layers for the three Cape species (*I. ludwigii, I. rubicunda,* and *I. striata*) to investigate whether habitat differentiation played a role in their evolutionary divergence.

Methods

Primer selection

Phylogeny reconstructions of *Isolepis* based on the commonly used markers *trnL–trnF* and *rps16* intron failed to resolve relationships within the *I. fluitans* clade. Thus, a more rapidly evolving chloroplast marker was sought. PCR amplification of a subset of the DNA samples was attempted using the fastest "Tortoise and Hare" markers of (Shaw et al., 2007). Successfully amplified products were sequenced, and the sequences were aligned (see below for methods) and examined for variability in terms of the number of informative characters (Table 1).

Since incomplete lineage sorting may result in genes having different histories within a recently diverged set of lineages, it is desirable to reconstruct genealogies for several gene regions when attempting to infer the species trees of such lineages. Three nuclear markers were thus also screened for utility in this study, viz. ITS (primers ITS-L and ITS-4: Hsiao et al., 1994; White et al., 1990), ETS (Starr et al., 2003), and *5S*-NTS (Cox et al., 1992). Amplification of the ETS region was unsuccessful and alignment of *5S*-NTS sequences was problematic. ITS and *atpI–atpH* were chosen as the nuclear and chloroplast markers to be used due to their variability and ease of amplification, sequencing, and alignment.

DNA extraction, PCR amplification, and sequencing

All available silica-dried material of members of the *Fluitantes* clade was used in order to include multiple members of each sampled population (Table 2). Data were also collected for the following taxa to be used as calibration points and outgroup taxa: *Ficinia praemorsa, F. truncata, I. marginata, I. prolifera, I. sulcata,* and *I. bicolor.* In total, sequence data were collected for 82 specimens.

Total DNA was extracted using either the CTAB method (Doyle & Dickson, 1987; Gawel & Jarret, 1991) or the new straight-to-PCR procedure 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, thoroughly air-dried, and resuspended in 50–100 µl sterile double-distilled water.

Amplification of the ITS and *atpI–H* regions were 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 *atpI–H* they were: initial denaturation at 80 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 46 °C for 1 min, extension at 65 °C for 4 min; and a final extension step at 65 °C for 5 min (Shaw et al., 2007).

The success of amplifications was determined by means of gel electrophoresis, with PCR products run for ca. 10 min at 100 V in 1% agarose gels stained with 0.005% Goldview (Guangzhou Geneshun Biotech, Ltd., Guangdong, China) and viewed with a UVIdoc gel visualizer (UVItec, Ltd., Cambridge, England). 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).

Sequence alignment

Consensus sequences of forward and reverse runs were created using SeqMan v. 7.0.0 (DNASTAR, Inc.). A number of ITS sequences previously obtained by AM Muasya were included in the analyses (see Table 2). Muscle v. 3.8.31 (Edgar, 2004) was used for sequence alignment, with the resulting alignment verified manually.

The resulting matrices contained 74 taxa and 856 characters for ITS, of which 235 were variable and 116 were parsimony-informative; and 47 taxa and 1229 characters for *atpI–H,* of

which 248 were variable and 92 were parsimony-informative. In an attempt to increase resolution and recover members of a species in the same clade for the *atpI–H* haplotype assignments, 50 indels were recoded as substitutions using an R script by AJ Potts.

Haplotype networks

Haplotype networks were constructed by Statistical Parsimony using the packages APE v. 2.5.2 (Paradis et al., 2004) and PEGAS v. 0.3.2 (Paradis, 2010) in R v. 2.11.1 (R Development Core Team, 2010). The *Ficinia* outgroup taxa had to be removed from the network in order to reduce crowding in the diagram and to make relationships visible. The highly divergent sample *I. fluitans Henderson 16799* was removed for the same reason.

Phylogeny

The species phylogeny was reconstructed using the Bayesian MCMC method based on a model of sequence evolution. Gene tree incongruence was apparent in the clade comprising *I. crassiuscula, I. producta,* and the Australian/New Zealand varieties of *I. fluitans.* These taxa were thus duplicated when concatenating the two sequence matrices so that relationships in the chloroplast and nuclear lineages could be inferred separately.

The species tree was reconstructed in MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck, 2003) using the concatenated matrix with two partitions. The sequences obtained for other chloroplast markers during the primer screening phase were added to the *atpI–H* partition. Model selection was performed using MRMODELTEST v. 2 (Nylander, 2004); the models chosen were GTR+Γ for the ITS partition and GTR+I+Γ for the chloroplast one. Base frequencies, substitution probabilities, and gamma shape parameters were unlinked but a single topology and set of branch lengths was estimated in each generation. The analysis was run four times for two million generations, with one cold and three heated chains at the default temperature setting. TRACER v. 1.5 (Rambaut & Drummond, 2007) was used to calculate the effective sample size of each parameter; these were all near or above 200, indicating that the MCMC algorithm had been run long enough. Burn-in was assessed by inspecting the trace for each run; all runs were deemed to have reached stationarity after 300,000 generations. A 50% majority-rule consensus tree was created from the

post-burn-in parameter estimates in MrBayes, with posterior probabilities (*PP*) of nodes indicating clade support.

Tree Dating

A previous Bayesian dating of the phylogeny of all *Isolepis* species based on four calibration points (of which three were geological) found a 95% highest posterior density (*HPD*) for the age of the immediate ancestor (t_{MRCA}) of *I. sulcata* and *I. bicolor* of 0.5–2.6 My, and for all of *Isolepis s.s.* of 7.3–16.3 My (Muasya & Viljoen, in preparation). These date estimates were used as calibration points in a Bayesian estimation of the divergence times in the present phylogeny using Beast v. 1.5.4 (Drummond & Rambaut, 2007).

The prior probability distribution for the tree height was set as a normal distribution with mean 11.9 and s.d. 2.35 in Beauti v. 1.5.4. For the t_{MRCA} of *Isolepis s.s.,* the normally distributed prior was set to mean 9.8 and s.d. 2.2. The t_{MRCA} of the Tristan da Cunha taxa was calibrated with a uniform prior of 0–5 My to encompass the range expected from the full phylogeny of *Isolepis.* The data set was partitioned as with the MrBayes analysis and analysed with the same substitution models. The birth-death speciation model was used with the uncorrelated log-normal rate model (Drummond et al., 2006). The analysis was run twice for 15 million generations each, saving the parameter estimates every 1500 generations. Tracer was again used to assess sufficient sampling. Tree files were combined using LogCombiner v. 1.4.8, after discarding samples from the first 500,000 generations as burn-in. The tree with the highest total clade support was annotated with summary values of parameters in TreeAnnotator v. 1.4.8.

Verifying the identity of I. fluitans Hedderson 16799

This sample was found to be highly divergent from the other *I. fluitans* specimens in both its ITS and *atpI–H* sequences. In order to verify that it had been identified correctly, it was analysed with ITS sequences of other Cyperaceae taxa outside *Isolepis* (unpublished data) and with *atpI–H* sequences of the most closely related organisms available on GenBank. Parsimony reconstructions were performed in Paup* v. 4.0b10 (Swofford, 2002) using the TBR heuristic search method run for 2000 repetitions, and strict consensus trees were constructed.

Ancestral area reconstruction

Each sample was coded for region of origin and the regions of the ancestral nodes were reconstructed onto the dated phylogeny using the maximum likelihood criterion in MESQUITE v. 2.6 (Maddison & Maddison, 2007). The dated tree was used because the probability of changes in distribution along branches should be proportional to absolute time and not the amount of sequence evolution. All changes in character state were set as equally probable so as not to bias the reconstruction against long-distance dispersal events, since all the main clades of *Isolepis* have representatives on 2 or more continents (Muasya & Simpson, 2002).

Habitat inference using GIS

Using the package ADEHABITAT v. 1.8.3 in R (Calenge, 2006), the locality coordinates of all Cape *Fluitantes* specimens in the Bolus Herbarium were superimposed on a map of the CFR containing the 19 Bioclim layers of (Hijmans et al., 2005); 12 solar radiation layers derived from a minute-by-minute digital elevation model by J. Slingsby (University of Cape Town); and eleven categorical soil variable layers derived from the Council of Geosciences layers, coded on the basis of expert interpretations of geology types (Gelfand et al., 2005). This allowed the values of each variable at the different localities to be extracted and the habitat of each species to be characterized.

In the soil characteristics layers, cells were coded as 0 or 1 for each of Fertility 1–4, Texture 1–4, and pH 1–3. The soil data extracted from the *Fluitantes* localities were recoded as three variables – Fertility, Texture, and pH – such that they ranged from 1 to 4, 1 to 4, and 1 to 3, respectively.

ANOVA was applied as a univariate test for differences between species, with post-hoc Tukey HSD tests used to identify contrasting species pairs. Overall habitat differences were examined using discriminant function analysis (DFA) in the R package ADE4 v. 1.4.14 (Dray & Dufour, 2007). Since the influence of variables on the discriminant functions can be artificially skewed due to arbitrary differences in scale, all data were log-transformed prior to DFA to ensure that the ranges of the different variables did not differ by more than one order of magnitude. Due to the high degree of collinearity expected, e.g. between solar radiation values of successive months,

principal components were first extracted, from which the discriminant functions were then derived.

Results

Haplotype networks

In both the ITS and the *atpI–H* haplotype networks (Fig. 1), the connection with the root (*I. marginata*) was assigned with less than 95% probability. The connection of the nuclear *I. crassiuscula/I. producta* clade was also uncertain ($P < 95\%$). Each sample represented a unique haplotype in both gene regions, with the exception of Haplotype 47 (*I. fluitans Muasya 961* and *1028*) in the ITS tree.

Relationships in the ITS network (Fig. 1a) were fairly well resolved, except in the East Africa clade of *I. fluitans.* The root (Haplotype 1) fell in the Cape clade comprising *I. ludwigii, I. striata,* and *I. rubicunda. I. crassiuscula* and *I. producta* from Australia, New Zealand, and Japan formed a clade that had its greatest affinity with Haplotype 28 (*I. ludwigii Muasya 1181*). Also descended from this node was *I. fluitans,* which included *I. inyangensis* and *I. graminoides,* and comprised all African *Fluitantes* outside the Cape.

The *atpI–H* network was poorly resolved if only nucleotide substitutions are considered; including insertions/deletions improved the resolution (Fig. 1b). However, this tree does not show as apparent a geographic pattern as the ITS tree and several species are not resolved as clades. The root (Haplotype 29) was placed nearest the Australian/New Zealand group, with representatives of the three Cape taxa descended from them. The *I. inyangensis* specimens were also descendents of Haplotype 30, and the Madagascar *I. fluitans* were closely allied with this basal clade. The rest of the *I. fluitans* samples seem to be derived from the *I. graminoides* clade. The Kenyan *I. fluitans* were reconstructed as descendents of Tanzanian *I. fluitans* and were not closely related to the Kenyan *I. graminoides.*

In both networks, *I. fluitans Bruhl 1741* was placed close to the root: near *I. ludwigii* in the ITS tree and near *I. producta* and *I. striata* in the *atpI–H* tree. The Norwegian samples were most

closely related to the Madagascar *I. fluitans* in their nuclear DNA, while their *atpI–H* was closest to the Ethiopia sample.

I. fluitans samples from the Mascarene Islands were found in two parts of the tree, with the Réunion sample (*Hedderson 2007*) close to the Zimbabwe, Malawi, Cameroon, and Ethiopia samples in ITS and close to Malawi and Zimbabwe in *atpI–H,* while the ITS sequences of *Hedderson 16789* and *16813* placed them nearest to *Abbott 8841.13* from kwaZulu-Natal.

Phylogeny

The Bayesian consensus tree is shown in Figure 2. The nuclear genes of the Australian/New Zealand taxa were found to be more closely related to the *I. prolifera* clade (i.e. not in the *Fluitantes*) and they had 63% Bayesian support as a monophyletic group. The corresponding chloroplast sequences (labelled AUS) were poorly resolved as basal members of the *Fluitantes* clade, which itself did have high support (*PP* = 0.99). *I. striata* and *I. rubicunda* were reciprocally monophyletic and formed a clade with 75% support, while the position of the *I. ludwigii* clade (IL) was not well supported. Note that, as in the haplotype networks, the Australian *I. fluitans* sample was most closely related to the Eastern Cape sample of *I. ludwigii* (*PP* = 1.00).

The *I. fluitans* clade (IF) was well supported (*PP* = 0.99), with the Mascarene *I. fluitans,* Natal *I. fluitans,* and *I. inyangensis* from Natal–Zimbabwe as basal members. Two subclades were well supported: Clade IF1, which contains specimens from Malawi, Zimbabwe, Cameroon, Ethiopia, and Réunion; and Clade IF2, comprising *I. graminoides* (Kenya) and *I. fluitans* from Kenya, Tanzania, Madagascar, and Norway. Note, however, that one of the Kenyan samples, *Knox & Muasya 3165,* was found in Clade IF1.

Position of I. fluitans Hedderson 16799

The phylogeny based on *atpI–H* displayed in Figure 3a shows *Hedderson 16799* as being more closely related to members of *Isolepis* than to species of the Poaceae, confirming that it is in the Cyperaceae. Its position is further refined by the ITS phylogeny (Fig. 3b), in which it was placed in the Abilgaardieae clade of the Cyperaceae, following the classification of (Muasya et al. (2009)). It was therefore excluded from further analyses.

Dated tree

The dated tree (Fig. 4) shows a t_{MRCA} of *Isolepis s.s.* of 4.8–11.7 My, while the Tristan da Cunha clade is shown to be less than 1 My old. These dates are in accord with the geological evidence for the origins of the Tristan da Cunha islands ca. 18, 3, and 0.5 Mya (Gass, 1967; McDougall & Ollier, 1982), and fall within the ranges of the 95% *HPD* heights from the phylogeny of all *Isolepis* (Muasya & Viljoen, in prep.).

The age of the *Fluitantes* was estimated as ca. 6.8 My (95% *HPD* 3.3–10.1). The AUS clade was formed ca. 5 Mya; Clade IF+IL split from the IS+IR clade ca. 4.4 Mya; and *I. striata* and *I. rubicunda* split ca. 3.3 Mya. Clade IF diverged from *I. ludwigii* ca. 3.8 Mya, and the two sub-clades of *I. fluitans* (IF1 and IF2) split ca. 2.2 Mya. *I. graminoides* arose shortly thereafter, and *I. inyangensis* diverged from basal IF ca. 1.5 Mya.

Node support was higher in the BEAST result than in the results from MRBAYES for all labelled clades. The AUS taxa were resolved as monophyletic, as were the basal members of the *I. fluitans* clade (IF0). Nodes with *PP* < 0.5 remained unsupported.

Ancestral area reconstruction

The ancestral character state reconstruction of distribution (Fig. 5) showed high likelihood for a CFR origin of *Isolepis,* the *Fluitantes* clade, and the IF+IL clade (proportional likelihood, *prL* > 0.95). The AUS clade, which contains samples from Australia, New Zealand, and Japan, was reconstructed as originating in Australia, although relationships within the clade were unresolved and there were too few samples from New Zealand and Japan to be confident in this reconstruction.

Among the South African *Fluitantes,* the ancestors of both the IL and IR+IS clades were in the CFR, with a dispersal to the Eastern Cape and Australia apparent in IL. The ancestral region of the IF clade was unresolved, but the basal clade (IF0) occurred in kwaZulu-Natal (*prL* = 0.91) and dispersed to Zimbabwe and to the Mascarene Islands.

The ancestral region of Clade IF1+IF2 is reconstructed as either Malawi or Kenya, with the daughter nodes each occupying one of those regions. IF1 dispersed from Malawi to Cameroon, Ethiopia, and Réunion, while Madagascar, Norway, and Tanzania were colonized by members of the Kenyan IF2 clade.

Habitat inference using GIS

Figure 6 shows the localities of the herbarium specimens for which habitat data were extracted from GIS layers. With the exception of *I. fluitans,* these species were found to occur in the same general area in the Western Cape, where sampling density was highest, although the range of *I. ludwigii* extended much further east. Since there was only a single record for *I. fluitans* in the CFR, this species was excluded from the habitat analyses.

Summaries of the values extracted from the Bioclim, Solar Radiation, and Soil Characteristics layers are shown in Figure 7. Sample sizes for *I. ludwigii, I. rubicunda,* and *I. striata* were 15, 22, and 24 respectively. The three species had overlapping ranges in every variable.

The multivariate analysis shows the species clustering in different parts of the available niche space (Fig. 8), although some overlap was apparent. *I. ludwigii* occupied areas with less temperature seasonality than *I. rubicunda,* although its temperature range was greater, with hotter summers and cooler winters; it received more precipitation, but this was more seasonal, with less rain in the winter (Table 3). *I. ludwigii* generally had lower solar radiation than *I. striata,* lower and more seasonal temperatures but milder winters, and less precipitation, except during the wettest quarter. *I. rubicunda* had higher overall solar radiation, more temperature seasonality, milder winters, and less rain than *I. striata,* according to this analysis.

Discussion

Origin of the Fluitantes

The *atpI–H* haplotype network was rooted in the Australasian group (AUS), perhaps implying an Australian origin for the clade. However, relationships were poorly resolved when using this marker alone (Fig. 1; PAUP* tree, not shown). Zeng et al. (2010) investigated the use of multiple

chloroplast markers to improve resolution in a bambusoid clade of the Poaceae, and found that at least four other markers had to be used in addition to *atpI–H* to resolve relationships in the Arundinarieae. This explains the uncertainty in the position of the AUS taxa in the MrBayes phylogeny, although the use of a relaxed clock yielded increased certainty in their monophyly at the base of the *Fluitantes* (Fig. 4).

The ITS haplotype network and ACSR (Fig. 5) indicate that the *Fluitantes* first evolved ca. 7 Mya in the CFR. This is in accord with previous results showing that Isolepis as a whole originated in the Cape (Muasya & Viljoen, in preparation) and with the distribution of its sister clade, comprising *I. pusilla, I. hystrix,* and *I. sepulcralis,* all of which are African and mainly CFR endemics (Muasya & Simpson, 2002). Monophyly of the AUS clade suggests that they are the result of a single dispersal event from South Africa to Australia around 5 Mya, followed by further dispersal to New Zealand and Japan and local speciation.

There is, however, an Australian taxon (*Bruhl 1741*) in a different part of the tree, close to *I. ludwigii,* which has been identified as *I. fluitans.* Since it forms a well-supported clade with *I. ludwigii* from the Eastern Cape, it is probably the result of a separate migration event in the last 2 My and was misidentified on the assumption that *I. ludwigii* does not occur in Australia. This does, however, show that long-distance dispersal to Australia is not uncommon in the *Isolepis–Ficinia* clade and gives support to the views of Muñoz et al. (2004) and Sauquet et al. (2009) that the phytogeographical affinities between the two regions are due to recent and ongoing migration in many different plant groups.

Hybridization in Australia

Conflict in the positions of the ITS and *atpI–H* sequences of the AUS taxa, comprising *I. crassiuscula, I. producta,* and New Zealand members of *I. fluitans,* provides support for the prior suggestion of hybridization in this clade (AM Muasya, pers. comm.). The maternal ancestor (from which the chloroplast genome was inherited) was a basal member of the *Fluitantes* (Fig. 2), while the paternal ancestor is resolved in the *Proliferae* clade of Isolepis in the MrBayes phylogeny. The dated tree from Beast showed strong support for the AUS taxa as a monophyletic group containing both the plastid and nuclear material (Fig. 3). This difference may be due to the shared species

history of the two genomes influencing the placement of the nuclear material when using a relaxed clock model. Further analyses with greater sampling from the *Proliferae* clade should clarify the position of the nuclear material and make it possible to identify (the closest descendents of) the paternal taxon. It might then also be possible to infer whether multiple hybridization events took place, or whether the present four taxa resulted from subsequent in-situ speciation from a common hybrid ancestor.

Other plant groups have also hybridized in Australasia following dispersal from other continents, such as *Lepidium* in the Brassicaceae (Dierschke et al., 2009). Australasian members of this group contain material from Africa and California, and their nuclear genomes appear to have become homogenized to either the African or Californian type, with the chloroplast of Californian origin. However, the ambiguity in the position of the nuclear *Fluitantes* material makes it unclear whether the maternal contribution to this biparentally inherited genome has been purged, as is suggested by the MRBAYES phylogeny.

Dispersal to tropical Africa

The *I. fluitans* clade (IF) is well supported as monophyletic in the ITS haplotype network and the combined phylogeny. Relationships within the clade are also congruent between the phylogeny and the ITS haplotype network, indicating a greater influence of the ITS sequences on the phylogeny reconstruction than the *atpI–H* sequences. This is probably because of both the larger number and the higher variability in the ITS sequences.

Both regions reconstruct *I. inyangensis* as more closely related to the Eastern Cape *I. ludwigii* than the *I. fluitans* specimens (Fig. 1), and the combined phylogeny indicates that it is a basal member of the IF clade (Fig. 2) that evolved from the Natal *I. fluitans* (Fig. 4).

The IF1+IF2 clade from East or Southeast Africa underwent a divergence ca. 2.2 Mya (Fig. 3, 4) that resulted in one widely distributed subclade, IF1, occurring from Malawi to Cameroon, Ethiopia, and Réunion, and the other subclade, IF2, occupying the high mountains of Kenya, Tanzania, and Madagascar, as well as dispersing to Europe.

Among the Kenyan taxa are found two distinct, reciprocally monophyletic clades (IG and IFK), of which one has diverged sufficiently in its physical characteristics that it is recognized as a separate species, *I. graminoides:* its peduncles are 0.2–0.5 cm (cf. 1–13 cm) long, giving the inflo-

rescence a congested appearance when compared to *I. fluitans* (Muasya & Simpson, 2002). This divergence took place within Kenya ca. 1.8 Mya and Tanzania was subsequently colonized by Clade IFK within the last 1 My, although the phylogeny is not resolved well enough to determine the number of migration events (Fig. 5).

The Madagascar clade is well supported, indicating a single colonization event ca. 1.2 Mya (Fig. 3, 4, 5), even though the island has been in its current position for 121 My (Griffiths, 1993). The colonization history of the Mascarene Islands remains unclear, but probably involved at least two separate events (one from kwaZulu-Natal and one from Malawi) sometime in the last 1.5 My. This is consistent with the geological ages of these volcanic islands: 6.8 My for Mauritius and 2.5 My for Réunion (McDougall & Chamalaun, 1969).

This pattern is consistent with that found in other plant groups by Galley et al. (2007) of migration from the CFR to eastern South Africa and northward from there into tropical Africa. The *Fluitantes* are only found in or near streams and wetlands (Muasya & Simpson, 2002) and their dispersal was limited by these habitat requirements, as their migration into the tropics is characterized by the colonization of niches in high-altitude areas with similar climatic and hydrological attributes as their original habitat in the CFR. This follows the prediction of the Niche Conservatism Hypothesis that taxa would tend to track their ancestral conditions rather than adapt to novel ones (Wiens & Graham, 2005).

Speciation in the Cape

The ACSR results are unambiguous in reconstructing the Cape ancestors of Clades IR+IS and IL+IF as diverging in the CFR (Fig. 5), and *I. rubicunda* and *I. striata* also split within the same region. The fact that these three species are reciprocally monophyletic precludes the definitive test of sympatric (or parapatric) speciation – determining post-speciation gene flow (Niemiller et al., 2008) – as this can only be detected while lineage sorting is still incomplete (Freeland, 2005). Thus, to infer whether ecological divergence may have been responsible for speciation in these broadly sympatric taxa, their habitats characteristics were compared.

Although none of the variables tested showed disjunct ranges between any two species, they were somewhat separated in the multivariate analysis (Fig. 8). However, overlaps were still

apparent between all species pairs, indicating that ecological differentiation along these variables may not be sufficient to have prevented introgression in hybrid zones.

In addition, the eigenvector loadings give contradictory interpretations, e.g. Axis 1 showing lower rainfall and less seasonality overall, but also higher rainfall during the winter, for *I. rubicunda* compared to *I. striata* (Fig. 8, Table 3). The semantic relationship between these variables means that they should either be reduced (e.g. to principal components) on the basis of logical categories, or that model selection should be performed to eliminate an overabundance of variables leading to difficulty in interpretation.

The soil variables made no appreciable contribution to the disciminant functions (Table 3). Since the *Fluitantes* are associated with streams and wetlands, the substrate and its nutrient content seem to be less important habitat determinants than the climatic variables, which affect the water availability of a site. However, habitat characteristics other than these continuous climate variables may be effective in promoting niche partitioning and differentiation between these three species: *I. rubicunda* is only found in low-lying sandy depressions, while its sister species occurs in water at higher altitudes. *I. ludwigii* is found at a range of altitudes but occupies the edges of wetlands rather than floating in ponds and streams (Muasya & Simpson, 2002). Given their similar climatic and edaphic tolerances and the lack of geographical separation between these species (both currently and probably at the time of speciation), it is most likely these microhabitat differences that eventually led to their phylogenetic divergence.

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Table 1. Statistics used for primer selection

Region	No. of taxa	Total sites	Variable sites	Parsimony-informative sites	Point mutations	Indels
ITS	9	694	99	43	86	6
***5S*-NTS**	3	93	15		15	0
atpI–H	8	989	78	18	60	23
petA–psbJ	6	1089	29	8	26	5
ycf6–psbM	8	494	37	6	29	3
trnT–L	5	1232	45	4	24	6
trnV–ndhC	3	521	21		19	5

Table 2. Samples used in this study

Asterisks denote sequences collected by AM Muasya (University of Cape Town).

Species	Collector	Collection #	Region	Locality	Haplotype number	
					ITS	*atpI–H*
F. praemorsa	Muasya	49	CFR	Agulhas		
F. truncata	Muasya	56	CFR	Agulhas		
I. bicolor Carmich.	Richardson	105	Tristan da Cunha	Tristan da Cunha	*	
I. crassiuscula Hook. f.	Bruhl	1825	Australia	Australia	8	1
I. crassiuscula Hook. f.	Coveny et al.	17478	Australia	Australia	*10	
I. crassiuscula Hook. f.	GenBank DQ385578.1	AK289564	NZ	NZ	12	
I. crassiuscula Hook. f.	GenBank DQ385577.1	AK289630	NZ	NZ	13	
I. crassiuscula Hook. f.	GenBank AB261668.1	AB261668.1	Japan	Japan	9	
I. crassiuscula Hook. f.	Wilson et al.	9487	Australia	Australia	*11	2
I. fluitans (L.) R.Br. var. *fluitans*	Abbott	8841.13	KZN	Pt. Edward	31	
I. fluitans (L.) R.Br. var. *fluitans*	Bjorå	917	Norway	Norway	34	9
I. fluitans (L.) R.Br. var. *fluitans*	Bjorå	920	Norway	Norway	35	10
I. fluitans (L.) R.Br. var. *fluitans*	Bruhl	1741	Australia	Australia	27	11
I. fluitans (L.) R.Br. var. *fluitans*	Faden et al.	4 4/7	Tanzania	Tanzania	49	12
I. fluitans (L.) R.Br. var. *fluitans*	Hall	38	Malawi	Malawi	38	
I. fluitans (L.) R.Br. var. *fluitans*	Hall	39	Malawi	Malawi	42	
I. fluitans (L.) R.Br. var. *fluitans*	Hall	40	Malawi	Malawi	43	
I. fluitans (L.) R.Br. var. *fluitans*	Hall	41	Malawi	Malawi	36	
I. fluitans (L.) R.Br. var. *fluitans*	Hall	42	Malawi	Malawi		13
I. fluitans (L.) R.Br. var. *fluitans*	Hedderson	2007	Mascarenes	Reunion	37	14
I. fluitans (L.) R.Br. var. *fluitans*	Hedderson	16789	Mascarenes	Mascarenes	32	
I. fluitans (L.) R.Br. var. *fluitans*	Hedderson	16799	Mascarenes	Mascarenes		
I. fluitans (L.) R.Br. var. *fluitans*	Hedderson	16813	Mascarenes	Mascarenes	33	
I. fluitans (L.) R.Br. var. *fluitans*	Knox & Muasya	3053	Kenya	Nyandarua	45	15
I. fluitans (L.) R.Br. var. *fluitans*	Knox & Muasya	3135	Kenya	Kenya	*50	
I. fluitans (L.) R.Br. var. *fluitans*	Knox & Muasya	3165	Kenya	Kenya	44	
I. fluitans (L.) R.Br. var. *fluitans*	Knox & Muasya	3195	Kenya	Meru	51	16
I. fluitans (L.) R.Br. var. *fluitans*	Larridon et al.	2010-350	Madagascar	Madagascar	53	21
I. fluitans (L.) R.Br. var. *fluitans*	Larridon et al.	2010-0176	Madagascar	Madagascar	54	17
I. fluitans (L.) R.Br. var. *fluitans*	Larridon et al.	2010-0157	Madagascar	Madagascar	55	18
I. fluitans (L.) R.Br. var. *fluitans*	Larridon et al.	2010-0146	Madagascar	Madagascar	56	19
I. fluitans (L.) R.Br. var. *fluitans*	Larridon et al.	2010-0117	Madagascar	Madagascar	52	20
I. fluitans (L.) R.Br. var. *fluitans*	Muasya	961	Tanzania	Tanzania	*48	8
I. fluitans (L.) R.Br. var. *fluitans*	Muasya	1007	Kenya	Elgon	*46	3
I. fluitans (L.) R.Br. var. *fluitans*	Muasya	1028	Kenya	Timboroa	48	4
I. fluitans (L.) R.Br. var. *fluitans*	Muasya	2026	Zimbabwe	Zimbabwe	40	5
I. fluitans (L.) R.Br. var. *fluitans*	Muasya	2044	Cameroon	Cameroon	39	6
I. fluitans (L.) R.Br. var. *fluitans*	GenBank DQ385579.1	AK289724	NZ	NZ	2	
I. fluitans (L.) R.Br. var. *fluitans*	Kew	2694	Kenya	Kenya	1	

I. fluitans (L.) R.Br. var. *lenticularis* (R.Br.) Muasya	GenBank DQ385580.1	AK289561	NZ	NZ	2	
I. fluitans (L.) R.Br. var. *nervosa* Lye	Muasya	2621	Ethiopia	Ethiopia	*41	7
I. graminoides (R.W.Haines & Lye) Lye	Muasya	986	Kenya	Elgon	*57	23
I. graminoides (R.W.Haines & Lye) Lye	Muasya	2597	Kenya	Kenya	*58	22
I. graminoides (R.W.Haines & Lye) Lye	Mwachala et al.	363A	Kenya	Elgon	59	24
I. graminoides (R.W.Haines & Lye) Lye	Mwachala et al.	363B	Kenya	Elgon	60	
I. graminoides (R.W.Haines & Lye) Lye	Mwachala et al.	363C	Kenya	Elgon	61	25
I. inyangensis Muasya & Goetgh.	Muasya	2025	Zimbabwe	Zimbabwe	*29	26
I. inyangensis Muasya & Goetgh.	Muasya	3779	KZN	Utrecht	30	27
I. ludwigii (Steud.) Kunth	Muasya	1138	CFR	Cape of Good Hope Nature Reserve	*25	
I. ludwigii (Steud.) Kunth	Muasya	1181	CFR	Silvermine Nature Reserve	28	
I. ludwigii (Steud.) Kunth	Muasya	3412	CFR	Silvermine Nature Reserve	26	
I. ludwigii (Steud.) Kunth	Muasya	3826	ECape	Longmore, Pt. Elizabeth	24	28
I. marginata	Muasya	3018	CFR	Clanwilliam	*1	29
I. producta (C.B.Clarke) K.L.Wilson	Bruhl	2443	Australia	Australia		30
I. producta (C.B.Clarke) K.L.Wilson	Wilson	9475	Australia	Australia	*7	31
I. producta (C.B.Clarke) K.L.Wilson	Wilson	9510	Australia	Australia	*4	32
I. producta (C.B.Clarke) K.L.Wilson	Wilson	9552	Australia	Australia	5	33
I. producta (C.B.Clarke) K.L.Wilson	Wilson	9557	Australia	Australia	6	34
I. prolifera (Rottb.) R.Br.	Coveny et al.	17487	Australia	Australia	*	
I. prolifera (Rottb.) R.Br.	GenBank DQ385584	AK288281	NZ	NZ		
I. prolifera (Rottb.) R.Br.	Muasya	1168	CFR	Kirstenbosch	*	
I. prolifera (Rottb.) R.Br.	Muasya	3044	CFR	Calitzdorp		
I. prolifera (Rottb.) R.Br.	Muasya	3417	CFR	Groot Winterhoek, Cederberg		
I. prolifera (Rottb.) R.Br.	Muasya	4618d	CFR	Knolfontein, Ceres		
I. rubicunda (Nees) Kunth	Muasya	1154	CFR	Kenilworth	*14	
I. rubicunda (Nees) Kunth	Muasya	1221	CFR	Kensington	*15	35
I. rubicunda (Nees) Kunth	Muasya	5317	CFR	Rondevlei	16	
I. striata (Nees) Kunth	Muasya	1140	CFR	Cape of Good Hope Nature Reserve	17	
I. striata (Nees) Kunth	Muasya	1141	CFR	Cape of Good Hope Nature Reserve	18	
I. striata (Nees) Kunth	Muasya	1180	CFR	Silvermine Nature Reserve	*21	
I. striata (Nees) Kunth	Muasya	2906	CFR	unknown	*23	
I. striata (Nees) Kunth	Muasya	2980	CFR	Malmesbury	20	36
I. striata (Nees) Kunth	Muasya	3314	CFR	Van Rijn's Pass	22	37
I. striata (Nees) Kunth	Muasya	4017	CFR	Malmesbury	19	38
I. sulcata (Thouars) Carmich.	Richardson	80	Tristan da Cunha	Tristan da Cunha	*	

Table 3. Eigenvector loadings of the environmental variables on the discriminant function axes

Values outside the range of –5 to 5 are in bold.

Variable	Axis 1	Axis 2
Soil fertility	0.65	0.1
Soil texture	-0.02	-0.06
Soil pH	-0.69	0.09
Solar radiation–Jan	1.48	0.21
Solar radiation–Feb	-2.05	**-6.02**
Solar radiation–Mar	2.62	**7.29**
Solar radiation–Apr	2.47	**-6.59**
Solar radiation–May	0.06	3.81
Solar radiation–Jun	-2.09	-2.17
Solar radiation–Jul	3.71	3.55
Solar radiation–Aug	-0.99	-3.63
Solar radiation–Sep	**-11.9**	0.87
Solar radiation–Oct	**5.81**	2.03
Solar radiation–Nov	0.22	**-5.11**
Solar radiation–Dec	0.15	**6.2**
Annual mean Temp	4.88	**-15.99**
Mean diurnal Temp range	-3.06	-0.68
Isothermality	0.67	0.16
Temp seasonality	**-14.53**	**5.64**
Max Temp – warmest month	**-5.59**	3.46
Min Temp – coldest month	**5.55**	-4.71
Temp annual range	**8.98**	-3.95
Mean Temp – wettest season	-0.28	-0.6
Mean Temp – driest season	1.04	-0.89
Mean Temp – warmest season	**15.72**	1.73
Mean Temp – coldest season	**-23.66**	**19.78**
Annual precipitation	**13.68**	**-6.08**
Precip – wettest month	-1.77	-0.09
Precip – driest month	3.47	1.18
Precip seasonality	**9.75**	2.74
Precip – wettest season	5.21	**16.65**
Precip – driest season	-1.58	**8.8**
Precip – warmest season	-2.62	**-6.14**
Precip – coldest season	**-21.77**	**-14.48**
Eigenvalue	0.707	0.618

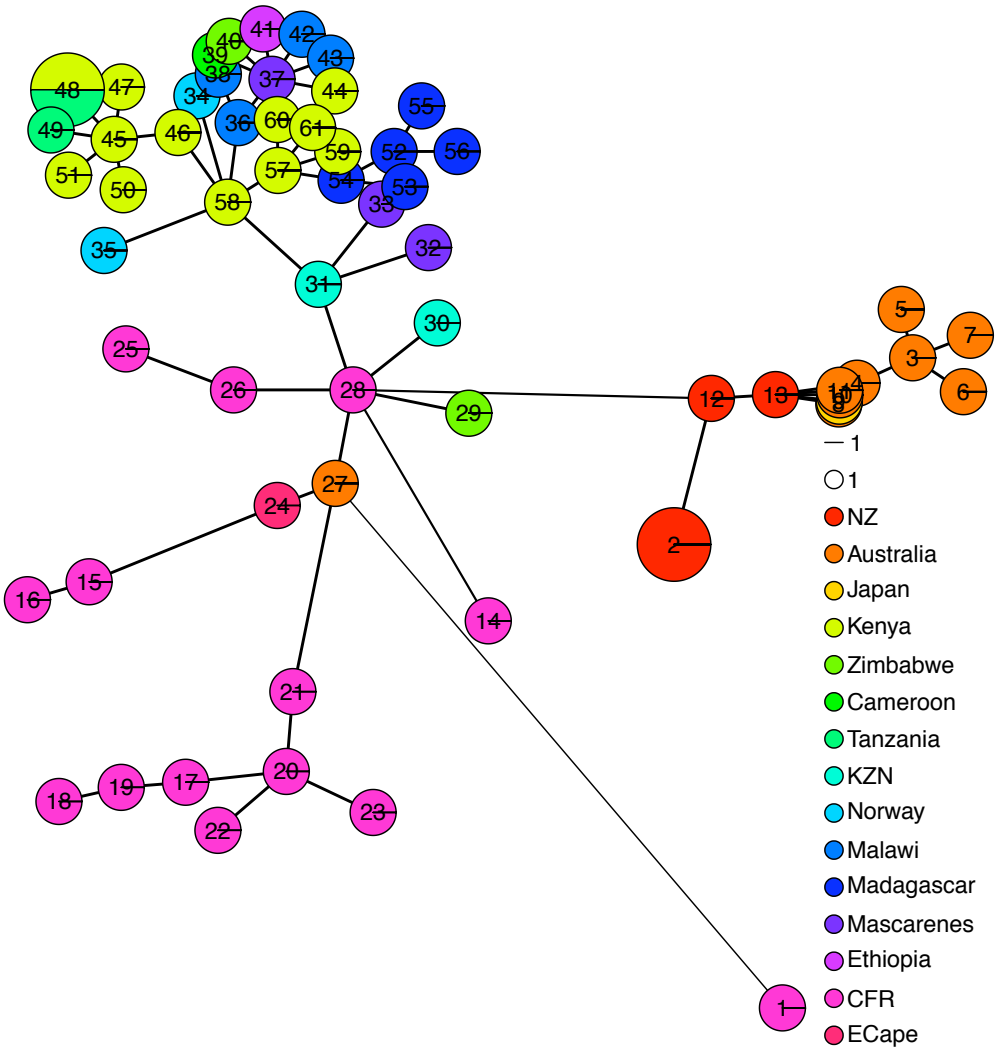
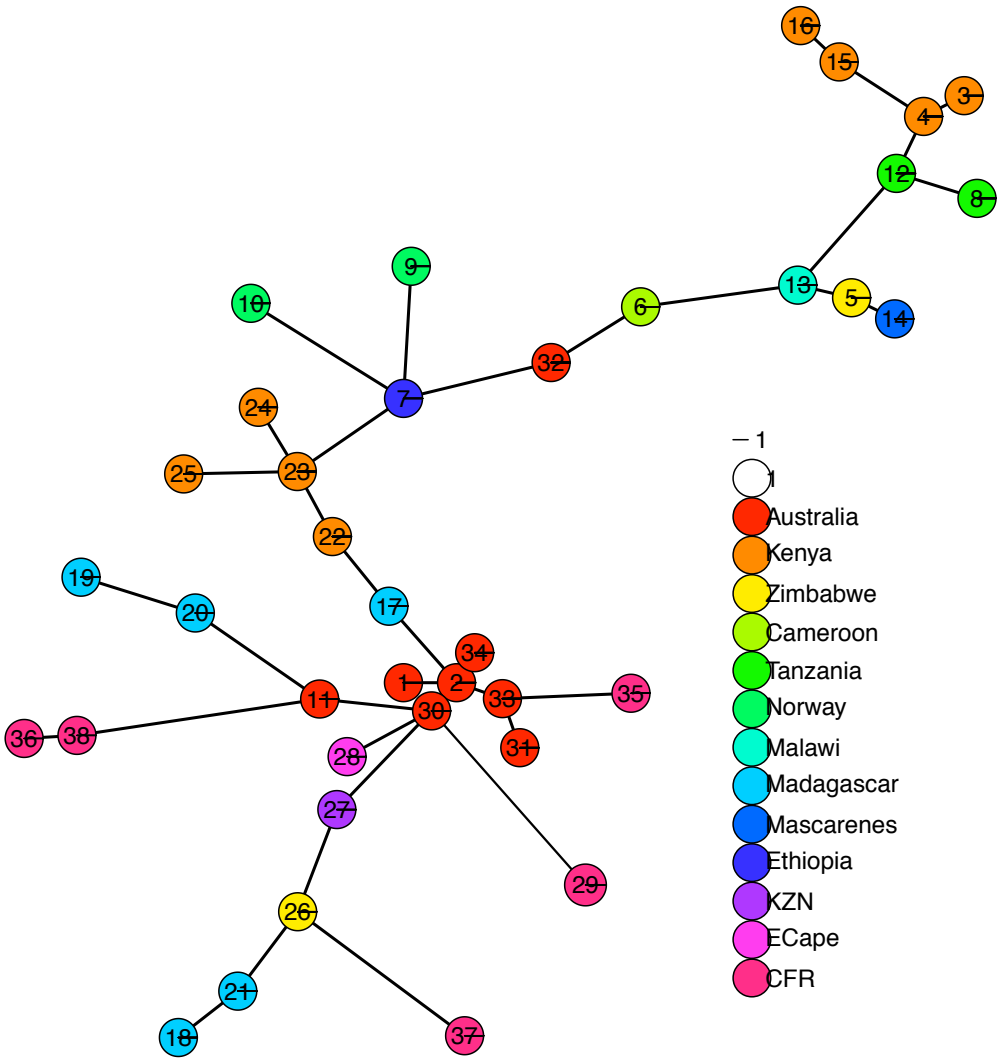


Figure 1. Haplotype networks

(a) ITS


16
15
3
4
12
8
9
10
13
5
6
14
32
7
24
25
23
22
19
20
17
34
1
2
33
35
11
30
36
38
31
28
27
29
26
21
18
37
1
1
Australia
Kenya
Zimbabwe
Cameroon
Tanzania
Norway
Malawi
Madagascar
Mascarenes
Ethiopia
KZN
ECape
CFR

(b) atpI–H

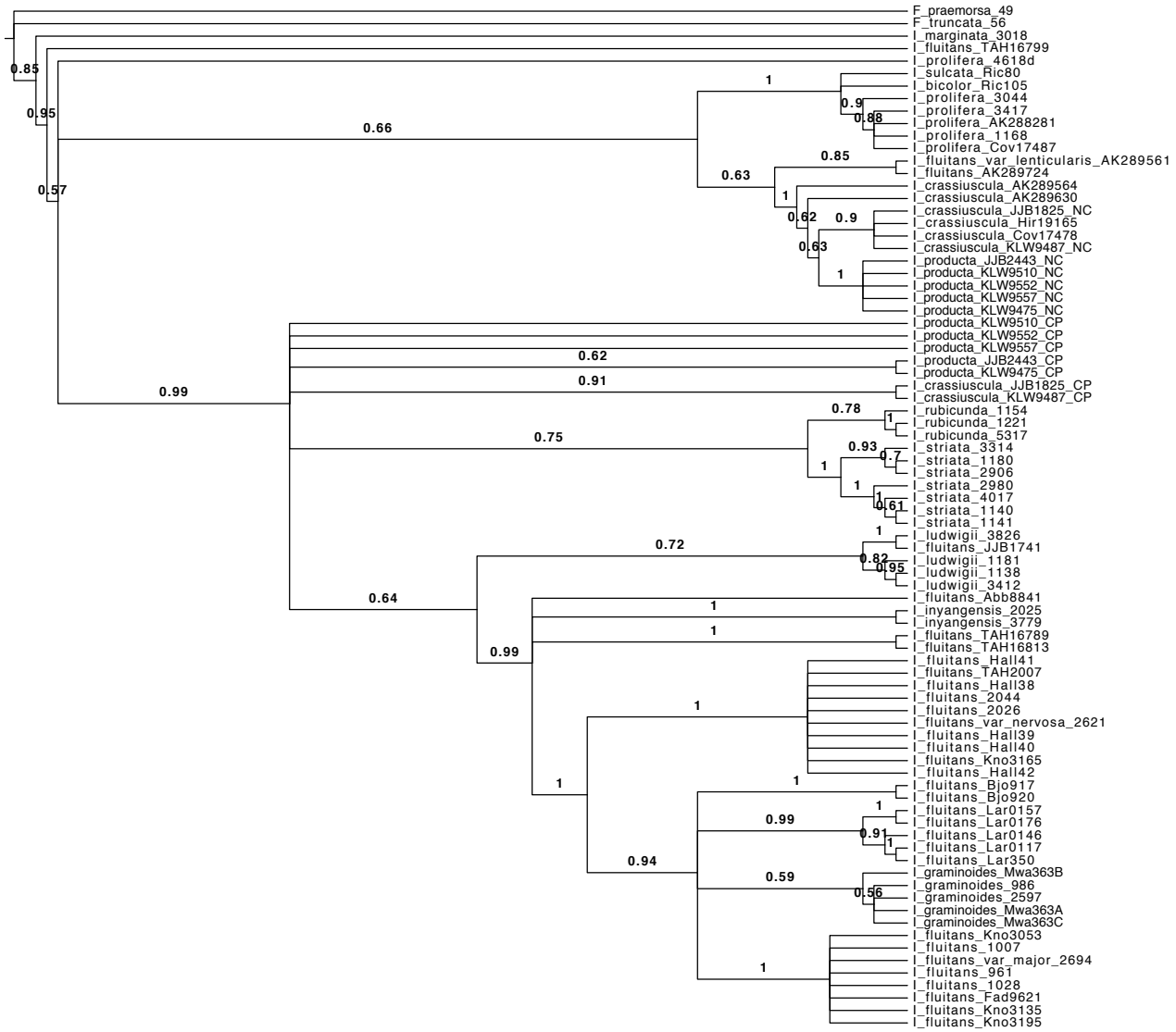


Figure 2. Bayesian phylogeny showing nodes with PP > 0.5

Values above branches denote posterior probabilities as measures of clade support. Tree is plotted as if ultrametric to improve legibility.

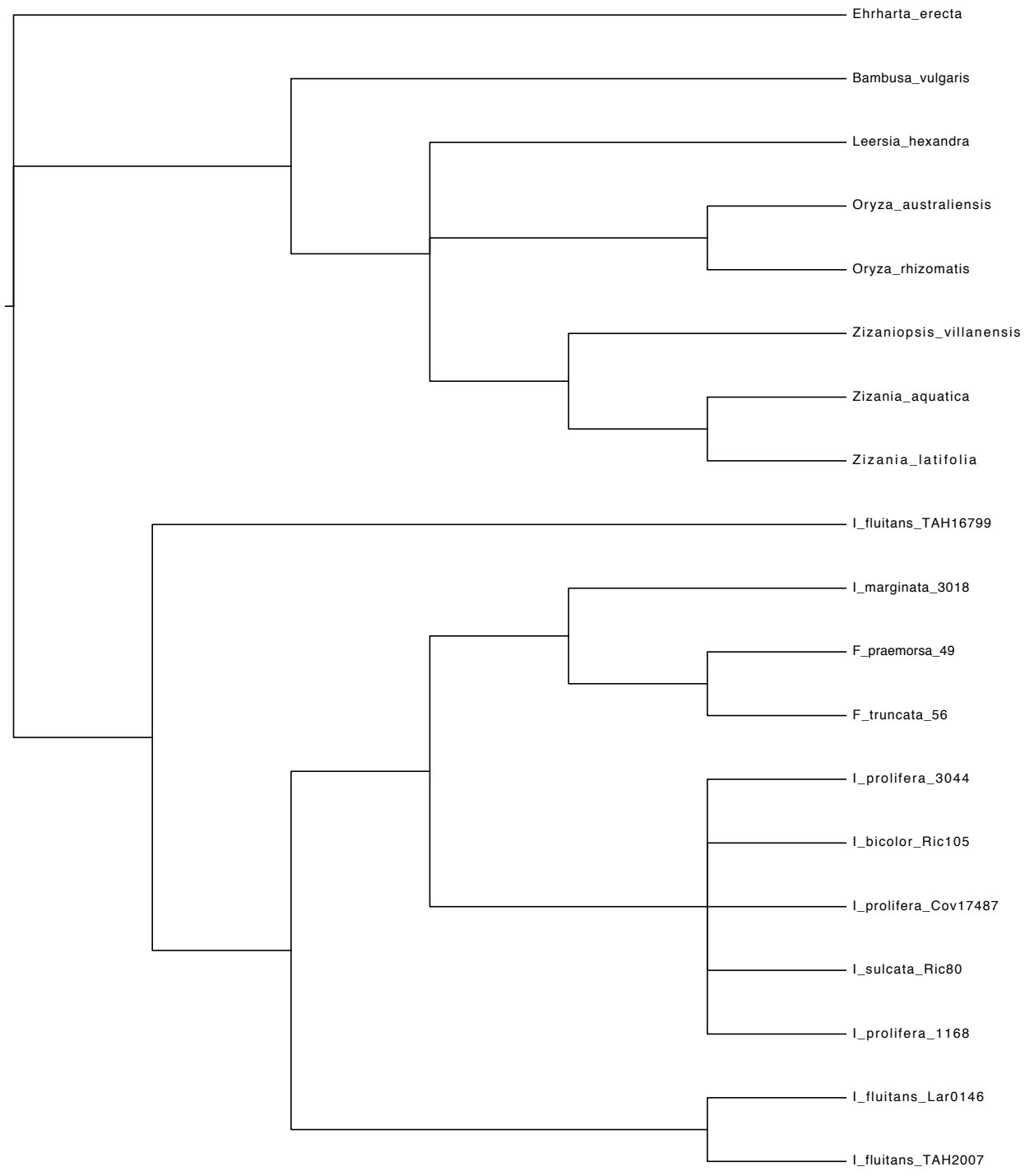
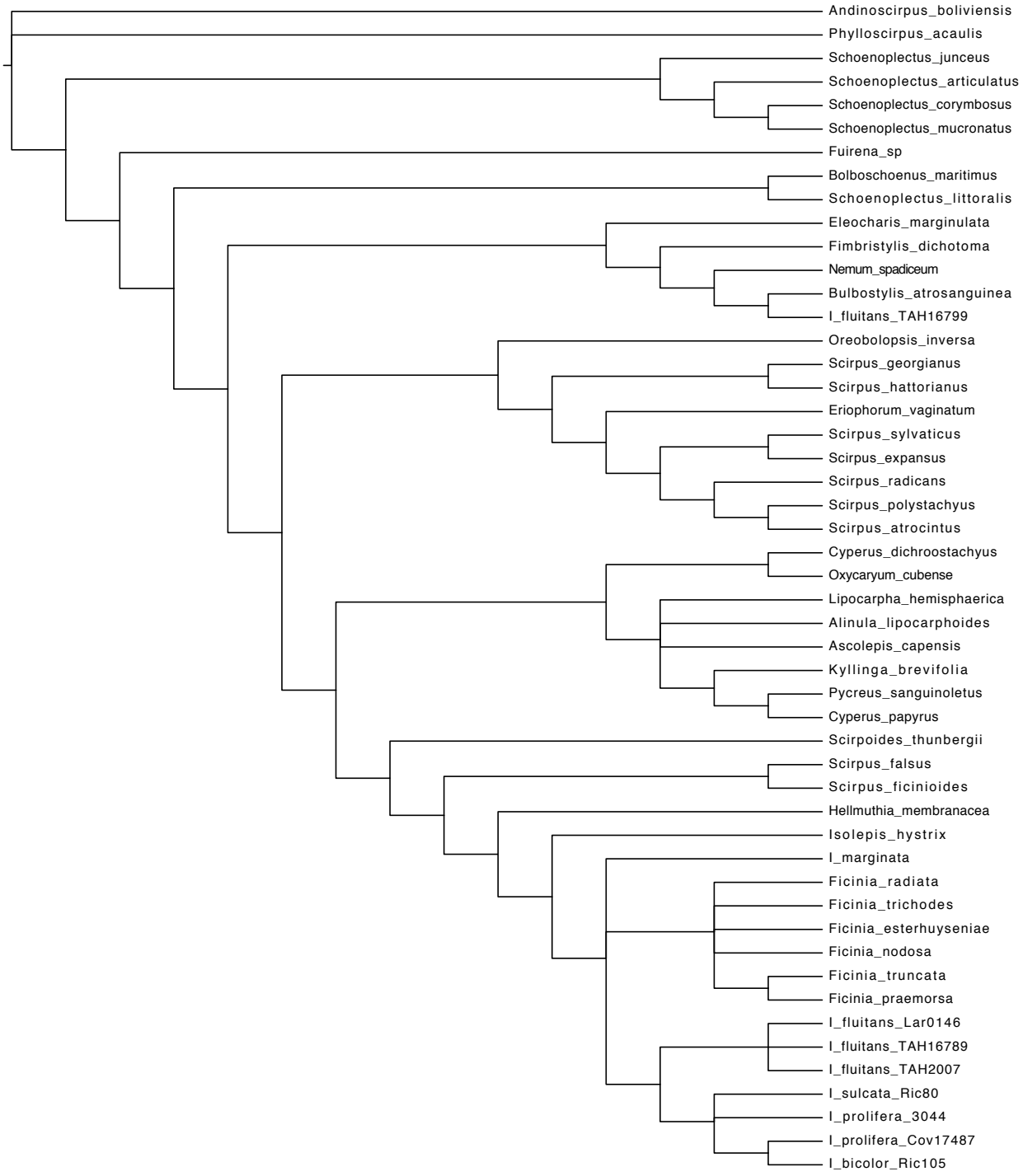


Figure 3. (a) atpI–H tree showing that I. fluitans Hedderson 16799 is in the Cyperaceae



(b) ITS tree showing I. fluitans Hedderson 16799 in the Abilgaardieae clade

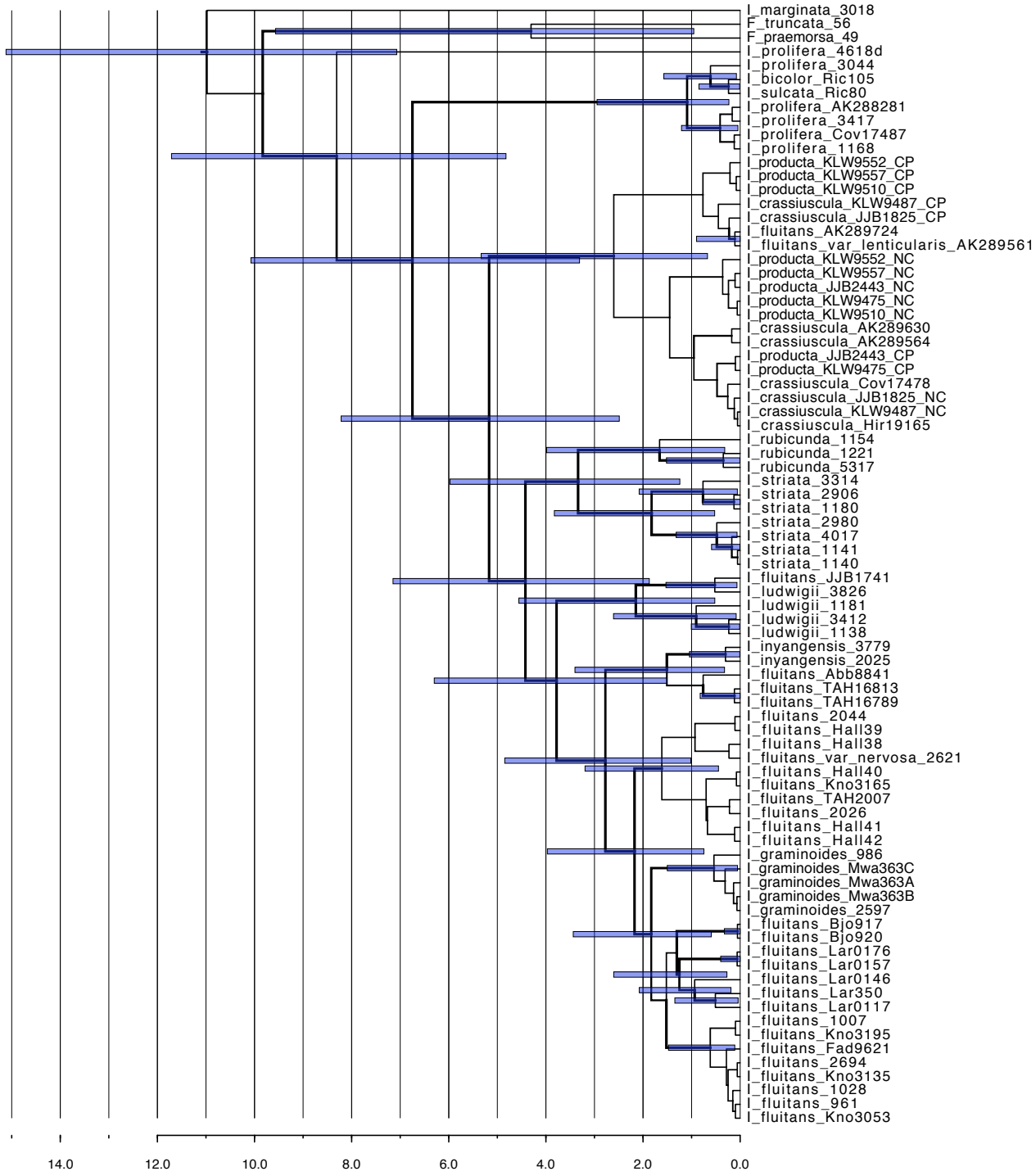


Figure 4. Dated phylogeny showing 95% HPD intervals of node heights.

Time scale is in million years.

Branch thickness is proportional to node support.

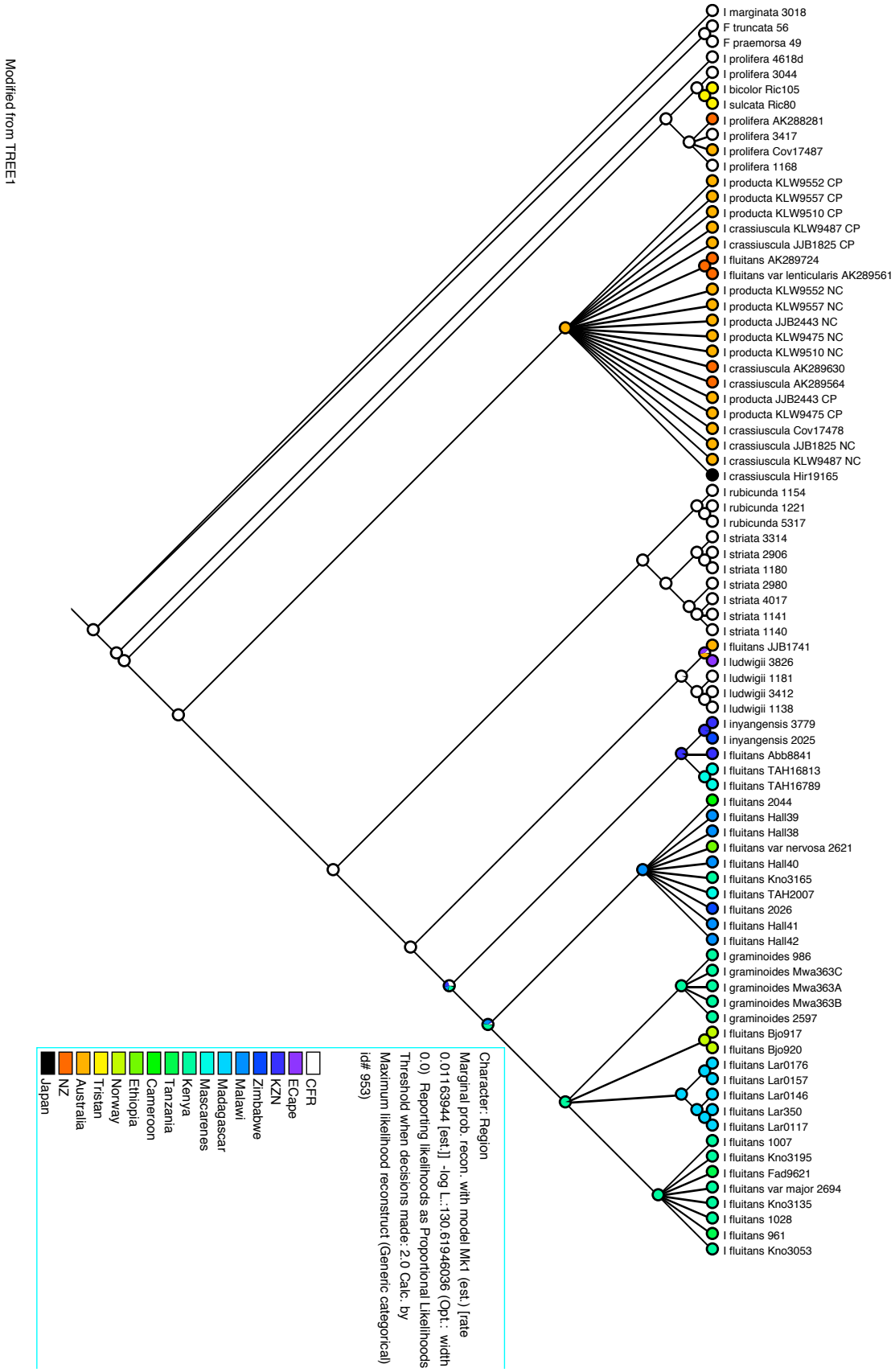

Modified from TREE1
I marginata 3018
F truncata 56
F praemorsa 49
I prolifera 4618d
I prolifera 3044
I bicolor Ric105
I sulcata Ric80
I prolifera AK288281
I prolifera 3417
I prolifera Cov17487
I prolifera 1168
I producta KLW9552 CP
I producta KLW9557 CP
I producta KLW9510 CP
I crassiuscula KLW9487 CP
I crassiuscula JJB1825 CP
I fluitans AK289724
I fluitans var lenticularis AK289561
I producta KLW9552 NC
I producta KLW9557 NC
I producta JJB2443 NC
I producta KLW9475 NC
I producta KLW9510 NC
I crassiuscula AK289630
I crassiuscula AK289564
I producta JJB2443 CP
I producta KLW9475 CP
I crassiuscula Cov17478
I crassiuscula JJB1825 NC
I crassiuscula KLW9487 NC
I crassiuscula Hir19165
I rubicunda 1154
I rubicunda 1221
I rubicunda 5317
I striata 3314
I striata 2906
I striata 1180
I striata 2980
I striata 4017
I striata 1141
I striata 1140
I fluitans JJB1741
I ludwigii 3826
I ludwigii 1181
I ludwigii 3412
I ludwigii 1138
I inyangensis 3779
I inyangensis 2025
I fluitans Abb8841
I fluitans TAH16813
I fluitans TAH16789
I fluitans 2044
I fluitans Hall39
I fluitans Hall38
I fluitans var nervosa 2621
I fluitans Hall40
I fluitans Kno3165
I fluitans TAH2007
I fluitans 2026
I fluitans Hall41
I fluitans Hall42
I graminoides 986
I graminoides Mwa363C
I graminoides Mwa363A
I graminoides Mwa363B
I graminoides 2597
I fluitans Bjo917
I fluitans Bjo920
I fluitans Lar0176
I fluitans Lar0157
I fluitans Lar0146
I fluitans Lar350
I fluitans Lar0117
I fluitans 1007
I fluitans Kno3195
I fluitans Fad9621
I fluitans var major 2694
I fluitans Kno3135
I fluitans 1028
I fluitans 961
I fluitans Kno3053
Character: Region
Marginal prob. recon. with model Mk1 (est.) [rate 0.01163944 [est.]] -log L:.130.61946036 (Opt.: width 0.0) Reporting likelihoods as Proportional Likelihoods Threshold when decisions made: 2.0 Calc. by Maximum likelihood reconstruct (Generic categorical) id# 953)
CFR
ECape
KZN
Zimbabwe
Malawi
Madagascar
Mascarenes
Kenya
Tanzania
Cameroon
Ethiopia
Norway
Tristan
Australia
NZ
Japan

Figure 5. Ancestral character state reconstruction by maximum likelihood of regions coded as unordered characters

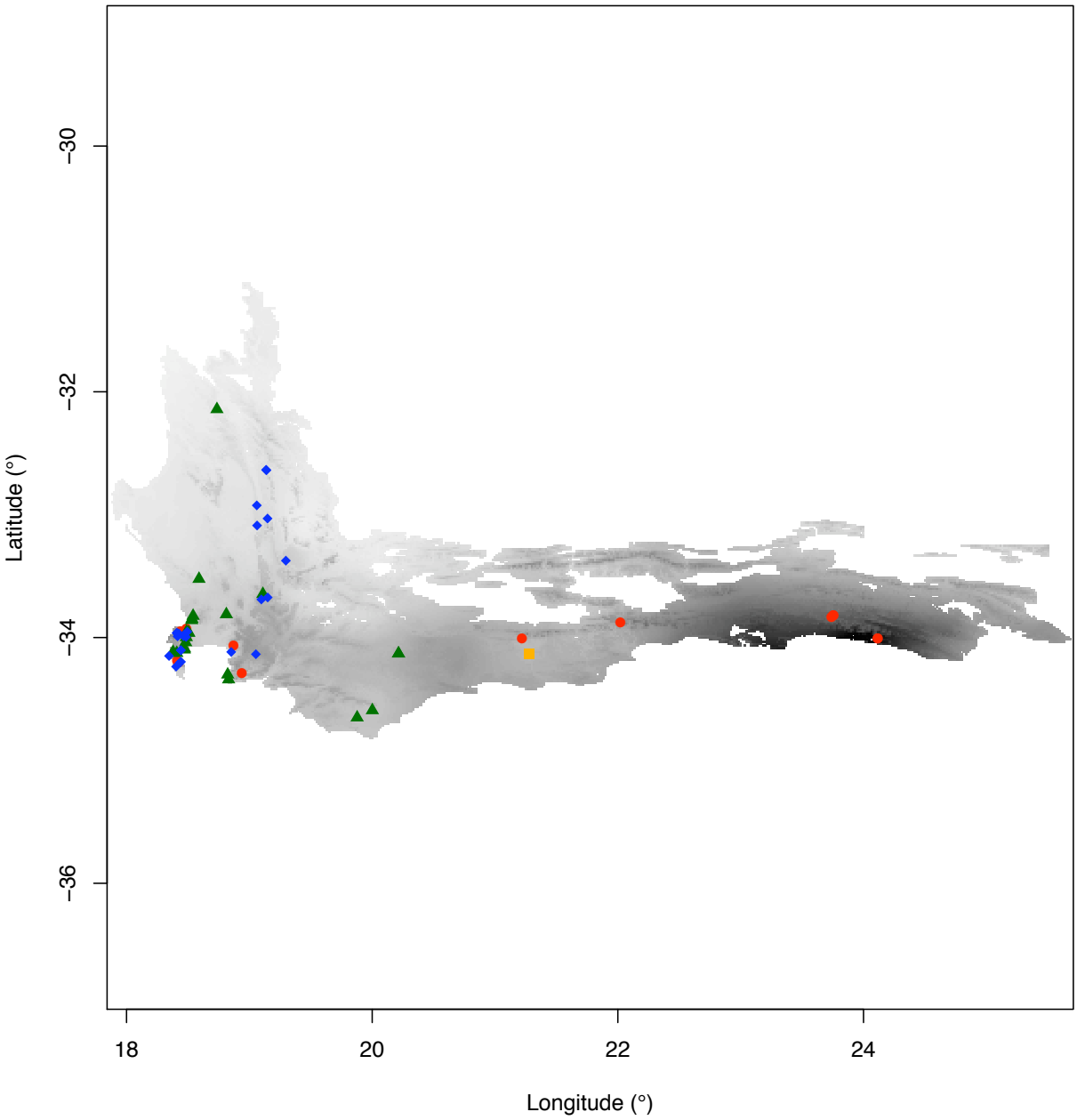


Figure 6. Localities of Bolus Herbarium specimens plotted on a representative Bioclim layer
Yellow square = *I. fluitans,* red circles = *I. ludwigii,* green triangles = *I. rubicunda,* blue diamonds = *I. striata*

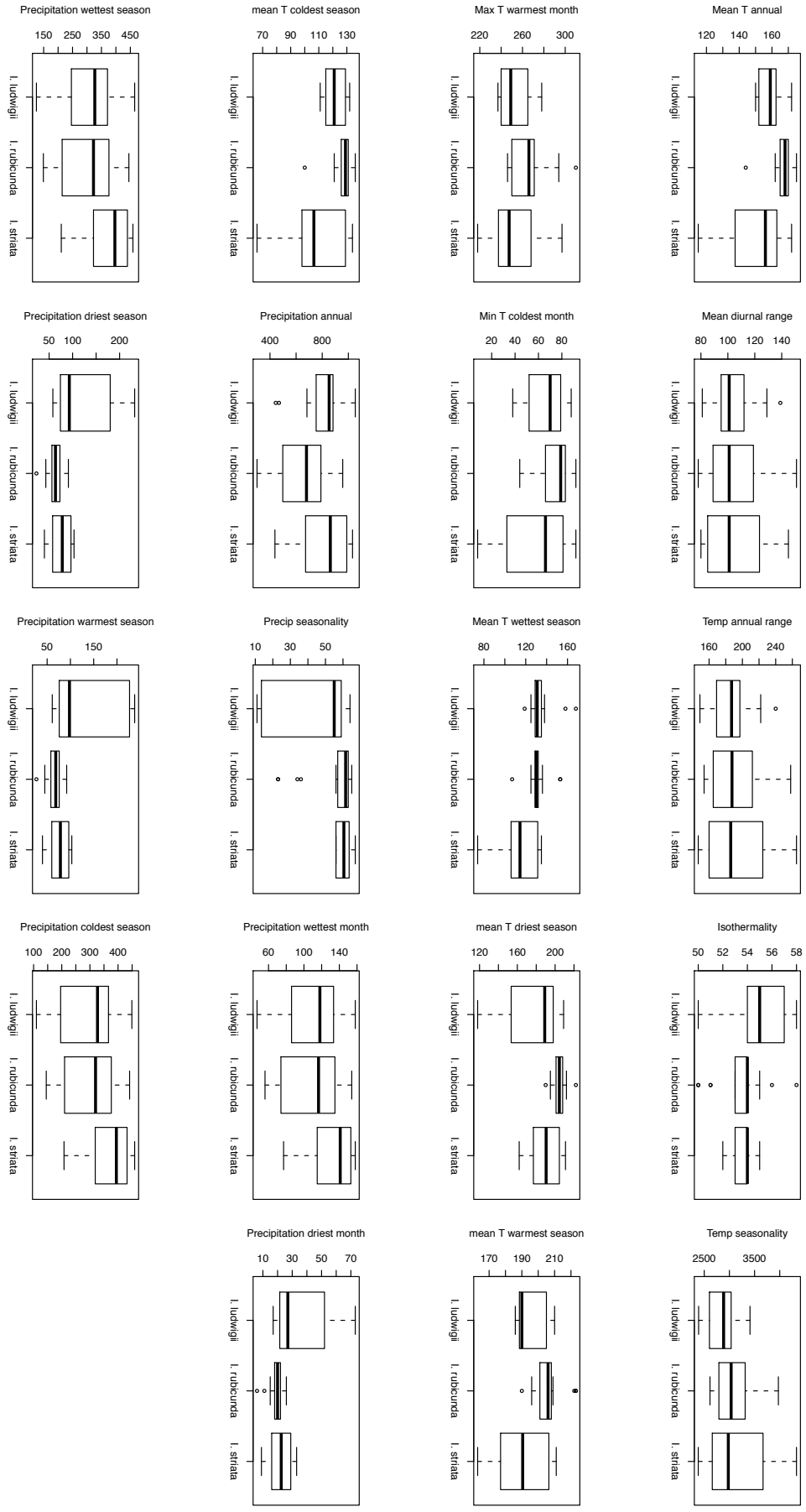
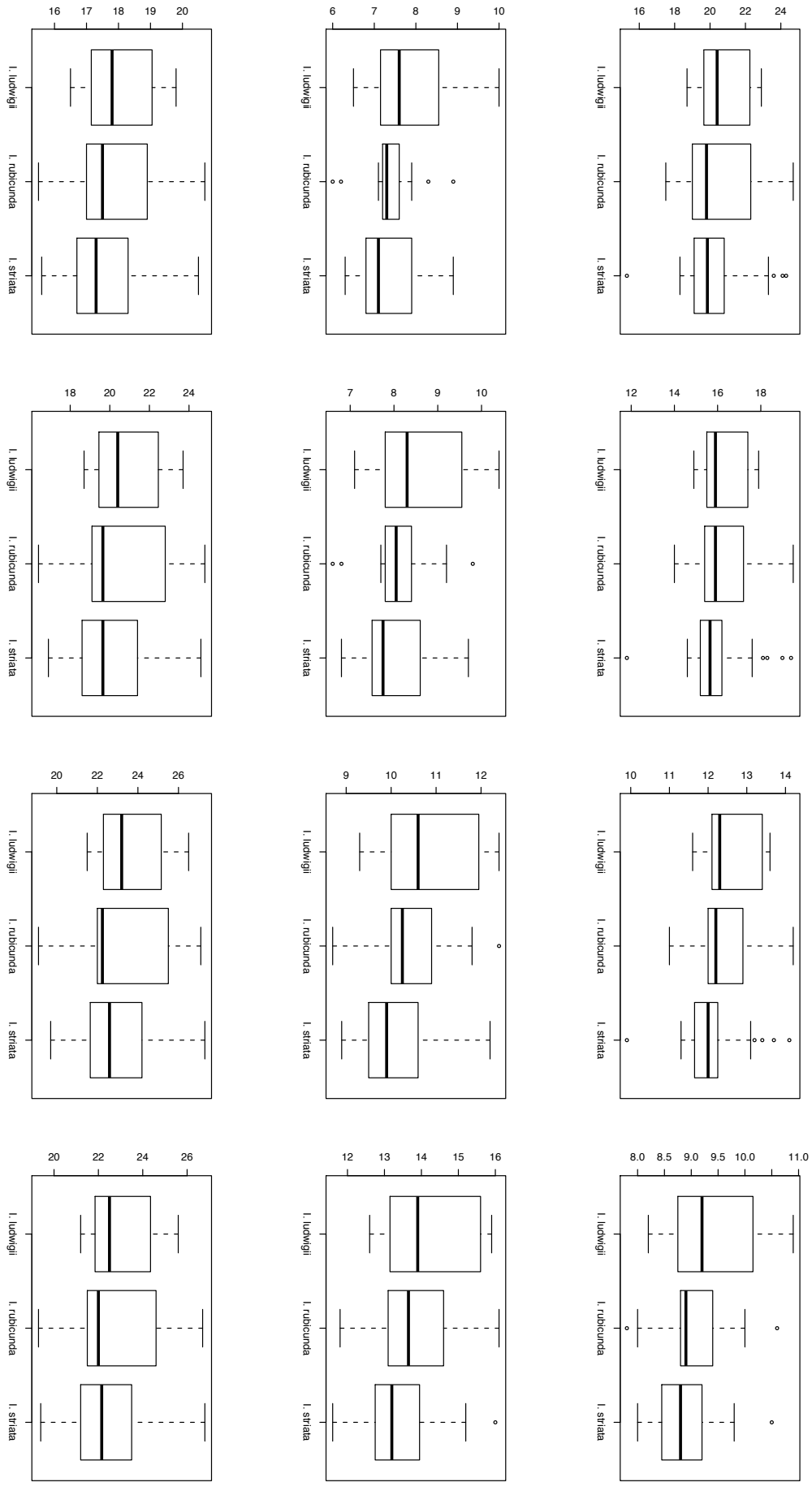
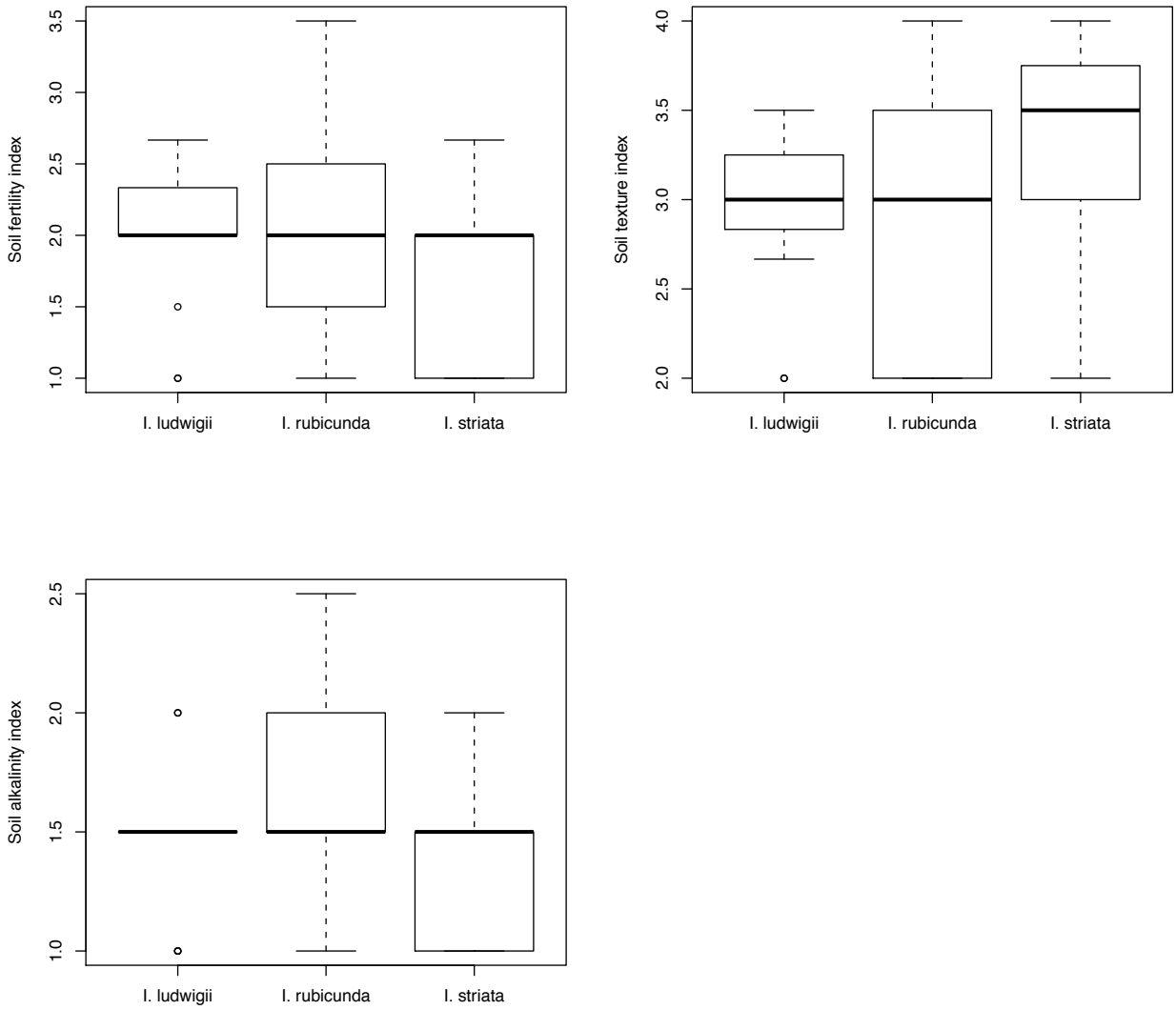


Figure 7. (a) Bioclim variables showing overlapping ranges between all species pairs



(b) Solar radiation values for each month of the year



(c) Recoded soil variables showing overlapping ranges between all species pairs

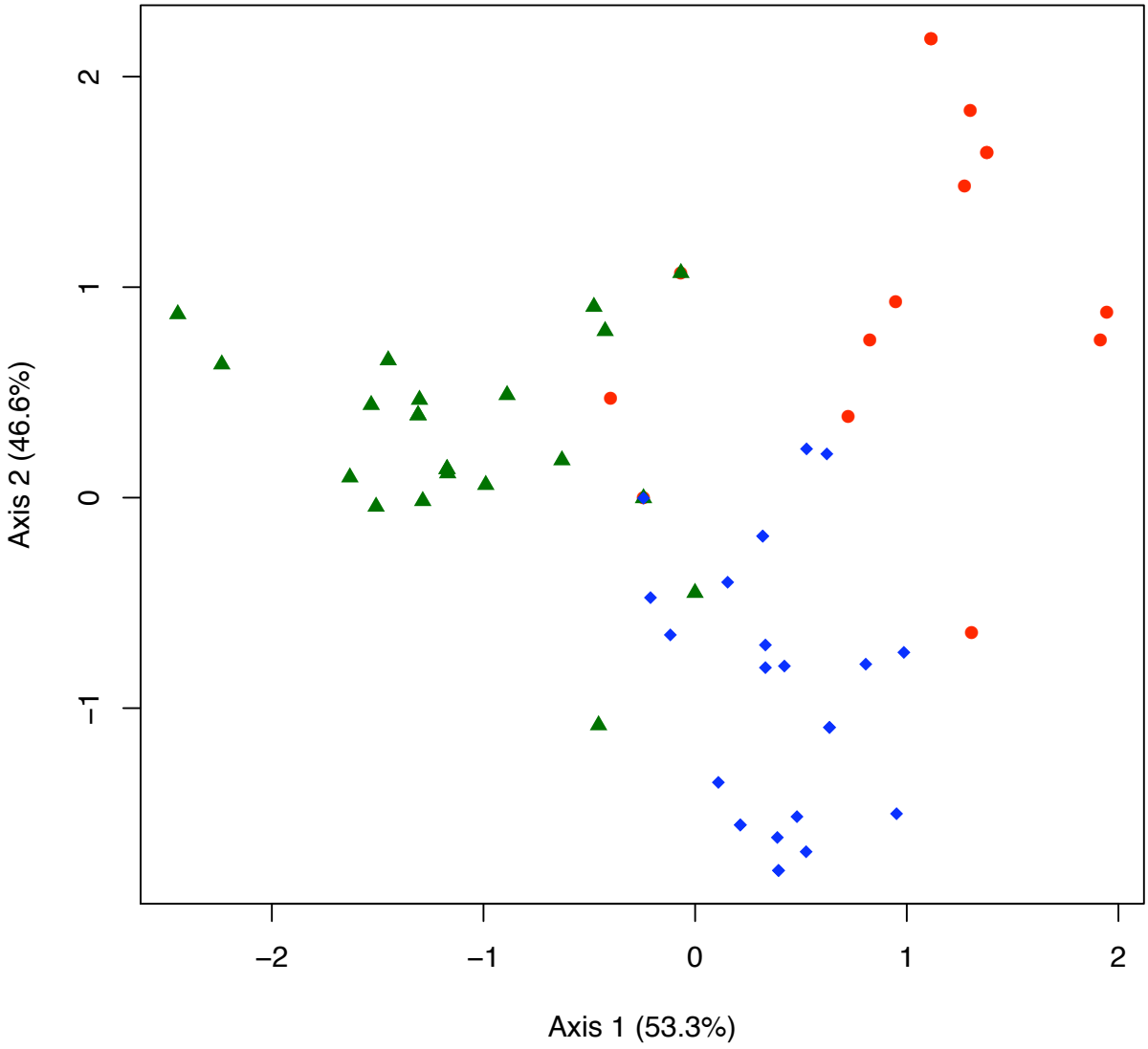


Figure 8. Discriminant function analysis showing some differentiation between species in multivariate niche space but no clear distinctions

Red circles = *I. ludwigii,* green triangles = *I. rubicunda,* blue diamonds = *I. striata*